

# **Direct and indirect effects of forest management on tree-hole inhabiting aquatic organisms and their functional traits**

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## Abstract

Ecological communities in forests have been shown to be strongly affected by forest management but a detailed understanding of how different components of management affect insect communities directly and indirectly via environmental variables, how management influences functional trait diversity and composition, and whether these results can be transferred to other functional groups besides insects (e.g. bacteria or nematodes) is still missing. To address these questions we used water-filled tree holes, which provide habitats for insect larvae and other aquatic organisms in forests, as a model system. We mapped all water-filled tree holes in 75 forest plots (1 ha) under different management intensity in three regions of Germany. We measured structural and climatic conditions at different spatial scales, sampled insect communities in 123 tree holes and bacterial and nematode communities in a subset of these. We found that forest management in terms of harvesting intensity and the proportion of non-natural tree species (species not part of the natural vegetation at the sites) negatively affected tree-hole abundance. An increased proportion of non-natural tree species had a positive direct effect on insect richness and functional diversity in the tree holes. However, a structural equation model showed that increasing management intensity had negative indirect effects on insect abundance and richness, operating via environmental variables at stand and tree-hole scale. Functional diversity and trait composition of insect communities similarly responded to changes in management-related variables. In contrast to insects, bacterial and nematode richness were not directly impacted by forest management but by other environmental variables. Our results suggest that forest management may strongly alter insect communities of tree holes, while nematodes and bacteria seem less affected. Most effects in our study were indirect and negative,

indicating that management has often complex consequences for forest communities that should be taken into account in forest management schemes.

**Keywords**

Phytotelmata; aquatic insects; nematodes; bacteria; metacommunity; land-use intensity

## 1. Introduction

Humans have strongly modified forest ecosystems worldwide and this has caused a dramatic loss in biodiversity across spatial scales (Sala *et al.*, 2000; Newbold *et al.*, 2015). A recent meta-analysis (Paillet *et al.*, 2010) and several subsequent individual studies (Wells *et al.*, 2011; Gossner *et al.*, 2014; Petermann *et al.*, 2016; Elek *et al.*, 2018) have shown that an intensified management of forests may negatively affect various groups of animals and plants. However, different aspects of forest management may vary in intensity and direction of their effects. Harvesting, the direct removal of trees, can lead to increased habitat fragmentation as well as to a reduced availability of microhabitats such as tree cavities and crown deadwood, with most severe consequences expected for dispersal-limited species (Carvalho and Vasconcelos, 1999; Augustynczyk *et al.*, 2019). An additional effect of management is the modification of tree species composition of forest stands. In Europe, fast growing tree species such as spruce (*Picea abies* (L.) H. Karst.) are favored to increase timber production, even if they are not part of the natural vegetation at the site (“non-natural species”). These changes in tree species composition may alter habitat and resource availability as well as microclimatic conditions (Carnus *et al.*, 2006). Further effects of forest management practices on biodiversity may be mediated by changed forest structure and stand age, which may result in changes in leaf litter, understory vegetation, flowering plants (Maleque *et al.*, 2009) and local microclimate (Ehbrecht *et al.*, 2017). The resulting altered habitat and resource availability might affect many forest-inhabiting species. However, these indirect effects of forest management are often not studied explicitly.

One way to investigate the direct and indirect effects of environmental change such as intensified forest management is to use natural microcosms, for example water-filled tree holes (Srivastava and Lawton, 1998). Water-filled tree holes are inhabited by aquatic insect larvae,

protists, fungi, nematodes, aquatic mites and other microorganisms (Kitching, 1971) and these organisms may be particularly sensitive to forest management practices due to the rather small sizes of populations, reliance on detritus as the major resource (Yanoviak, 2001; Paradise, 2004) and in some cases low dispersal abilities (De Bie *et al.*, 2012). In addition to being a useful model system, water-filled tree holes are potentially important for forest nutrient cycling and provide habitat and resources for a number of often specialized species (Kitching, 1971; Blakely *et al.*, 2012). These habitats may also pose a potential risk for humans due to disease transmission of some inhabiting species such as mosquitoes (Becker, 2003).

Previous work on the impacts of forest management on tree holes is limited. The few existing studies have been mainly done in the tropics and primarily focused on tree holes as habitat for potential vectors of malaria. Yanoviak and colleagues (2006b) found that increases in timber harvesting resulted in increasing tree-hole abundance and mosquito populations. The rise in mosquito populations was due to both an increase in habitat availability as well as a decrease in predatory larvae, such as damselfly and dragonflies (Yanoviak *et al.*, 2006a; Yanoviak *et al.*, 2006b). In temperate forests, tree-hole communities have been shown to be negatively affected by forest management in natural tree holes through changes in detritus accumulation and altered water chemistry (Gossner *et al.*, 2016), as well as in artificial tree holes through changes in tree diameter (Petermann *et al.*, 2016).

In addition to these few results on forest management impacts on tree-hole communities, it is conceivable that further environmental variables in forests are affected directly or indirectly by forest management that may be important for tree-hole-inhabiting organisms. For example, dispersal abilities differ very much between different taxa inhabiting tree holes. Insects typically live in tree holes as larvae and are only able to leave the hole during adulthood when adults will

search for mates and locate new tree holes to lay their eggs in (Kitching, 1972; Jenkins *et al.*, 1992). Different insect species differ in their dispersal abilities and some species (for example short-winged ones) likely have low dispersal distances. In contrast, nematodes and bacteria typically disperse passively by wind, potentially resulting in a non-selective, random distribution (Cáceres and Soluk, 2002; Ptatscheck and Traunspurger, 2015). However, these organisms may also travel with insects and other animal hosts (Kiontke, 1996; Junqueira *et al.*, 2017). Unfortunately, little is known on dispersal distances of insects or other species in tree holes. Thus, it is unclear if habitat limitation (tree-hole abundance) which is potentially influenced by forest management, for example via harvesting, altered tree-species composition, reduced forest structural complexity, stand age and age variability, may affect communities in tree holes.

Tree-hole selection and successful colonization by these organisms may additionally depend on resource availability, both inside the tree hole and for insect adults also throughout the forest stand. Previous studies have found bottom-up processes (accumulation of leaf debris) to be a strong driver of insect and nematode communities in the tree-hole system (Paradise and Dunson, 1997; Kitching, 2001; Ptatscheck and Traunspurger, 2014; Gossner *et al.*, 2016). In this respect, rot holes may differ from pan holes (tree holes with an intact bark lining, for example in branch axils) as they have additional nutrients available from the decomposition of the dead wood (Kitching, 1971).

Tree holes are frequently ephemeral due to their small size, and the organisms colonizing them have evolved different traits and strategies to avoid or withstand drought (Kitching, 2001). However, drought and high water temperatures induced by low canopy cover or a dry microclimate, especially in holes with low water volume, a large opening area or for those

located high up on trees, may affect survival of insects and even of more drought tolerant organisms such as bacteria and nematodes.

In this study, we make use of a large-scale research platform with established plots in forests under different management intensity to investigate how different aspects of forest management, specifically the amount of biomass harvested and the proportion of non-natural tree species, affect the communities of insects (abundance, richness, species composition, trait diversity and composition), nematodes (abundance and richness) and bacteria (richness) in water-filled tree holes. We consider direct and indirect effects via changes in microclimate, habitat and resource availability at forest stand and tree-hole scales. We hypothesize that (1) tree-hole abundance and type (proportion rot holes vs. pan holes) are affected by forest management intensity, (2) species richness and composition of tree-hole insect communities are directly influenced by forest management and indirectly via changes in a number of environmental variables, (3) the diversity and composition of functional traits in tree-hole insect communities are impacted by forest management, (4) insect, nematode and bacterial communities in tree holes differ in their response to forest management and environmental drivers.

## **2. Material and Methods**

### **2.1 Study Regions**

The study was conducted within the Biodiversity Exploratories project ([www.biodiversity-exploratories.de](http://www.biodiversity-exploratories.de), Fischer *et al.*, 2010) in three different regions of Germany: the Biosphere Reserve Schwäbische Alb (ALB) in the southwest (48°34' - 48°53' N, 9°18' - 9°60' E), the Biosphere Reserve Schorfheide-Chorin (SCH) in the northeast (52°51' - 53°11' N, 13°36' -

14°01' E) and the National Park Hainich and the surrounding Hainich-Dün region (HAI) in the center (50°94'–51°38'N; 10°17'–10°78'E). The distances between ALB and HAI and between HAI and SCH are approx. 200km. ALB is a low mountain range with a mean annual precipitation of 700–1000 mm and a mean temperature of 6–7°C. The forest landscape, which primarily consists of *Fagus sylvatica* (Figure 1), is fragmented by agriculture and rural settlements. SCH is a post-glacial, flat landscape and one of the driest regions in Germany with a mean precipitation of 500–600 mm and a mean temperature of 8–8.5°C. HAI has an annual mean temperature of 6.5–8 °C and a mean annual precipitation of 500–800mm. HAI is a forested hill chain and comprises the largest coherent area of deciduous trees in Germany.

## 2.2 Forest sites and management

In each region 50 forest plots were installed by the Biodiversity Exploratories project in 2008 (Fischer *et al.*, 2010). Plot size is one-hectare (100 m x 100 m), each located within a larger management unit, and minimum distance between plots is 200 m. Plots represent the regional spectrum of the main forest management types and intensities, ranging from unmanaged beech forest, uneven- and even-aged managed beech forests to conifer plantations (spruce in ALB and HAI, pine in SCH). In each region, we selected 25 plots out of the 50 covering an even distribution across this spectrum of forest management types and intensities for our study (Table A1 in the Appendix).

We used a continuous measure of forest management intensity and its components for quantifying effects of forest management on tree holes and their bacterial and invertebrate communities. The forest management intensity index (ForMI; Kahl and Bauhus, 2014) considers



the management activities on each plot over the last 30-40 years and combines three different components of forest management – the proportion of harvested tree volume (Iharv, henceforth “proportion of harvested tree biomass”), the proportion of trees on the plots which are not part of the natural vegetation at the sites (Inonat, henceforth “proportion of non-natural tree species”) and the proportion of dead wood which shows saw cuts (Idwcut, Kahl and Bauhus, 2014). Management decisions by foresters are mainly made regarding the amount of biomass to be harvested and regarding the tree species planted. For this reason, we focused our analysis on the forest management components proportion of harvested tree biomass and proportion of non-natural tree species.

### **2.3 Tree-hole mapping**

All water-filled tree holes were mapped in each 1-ha plot in spring and early summer 2014 (between May and July). The mapping was done after considerable rain in each region so all holes that could hold water would have been wet. In order to locate all tree holes, we walked 5-meter wide transects through each plot. Tree holes at ground level were recorded directly, and those which were inaccessible from the ground were checked with a 15-meter long tree top peeper camera (Appendix, Figure A1). If it could not be determined with the camera whether the hole contained water the tree was climbed using single rope climbing technique (Perry, 1978).

During mapping, we measured the opening size (area) of the tree holes as the area of an ellipse, current and potential water depth, height from the ground of each tree hole and took the diameter at breast height (DBH) and the GPS coordinates for each tree with tree holes. Tree holes above a height of two meters were considered canopy holes, those below two meters ground holes following the classification of Kitching (1971). Tree holes were categorized by

hole type, distinguishing between pan holes (intact bark lining) and rot holes (wood exposed and rotting). Estimated potential volume was calculated for all holes using the measures for the opening size and the potential water depth of the hole and this measure was used to select tree holes for subsequent sampling (see *Sampling of organisms in tree holes* below). For tree holes that were checked with the tree top peeper camera only but not accessed by climbing (and also not sampled, see below) we could not assess the current and potential water depth but estimated the opening size.

## 2.4 Sampling of organisms in tree holes

Of the mapped tree holes, those to be sampled for organisms were chosen based on the following criteria. Two ground holes and two canopy holes were chosen from each plot to be sampled for bacteria and insect larvae; for nematodes one ground and one canopy hole were selected. Ground and canopy holes showed similar abundances in the plots (see *Results* below) and we thus attempted to sample equal numbers near the ground and in the canopy. However, in plots with fewer than four tree holes, all tree holes available were sampled for bacteria and insect larvae. Tree holes were selected for sampling by excluding extremely small ( $< 200\text{ml}$ ) and large holes ( $> 1000\text{ml}$ ) to reduce the variation in tree-hole size (potential volume). However, actual potential volume often differed from the estimated potential volume. Actual potential volume was measured after all contents were removed from the hole, by filling the tree hole with premeasured water until the water would overflow. Since our focus was on the aquatic communities we chose holes with a visible water column, or if such were not available, wet, water-saturated holes. Dry holes were not sampled. Rot holes were predominant in spruce plots; only one pan hole was found in all the spruce plots. In contrast, only 70 out of the 985 holes

found in beech plots were rot holes. To ensure comparable results between the conifer and broadleaf forests preference was given to sample from rot holes in beech plots if they were available.

#### **2.4.1 Insect larvae**

In total, 123 tree holes in 52 plots were sampled, 20 rot holes and 103 pan holes. Of the 123 holes, 45 holes were located in the canopy and 78 at ground level (Table A1). We did not find tree holes on all plots. In addition, some of the previously mapped tree holes had fallen dry by the time of sampling and thus, could not be sampled. Insect sampling was carried out once for each hole between the end of July and beginning of September 2014 following the guidelines in Yanoviak and Fincke (2005). In brief, canopy holes were accessed by single rope climbing technique. After initial water temperature, pH and dissolved oxygen (see *Water nutrient analysis and tree-hole environmental variables* below) were measured, water was removed from the tree hole using a turkey baster and pipettes and placed into labeled 120-ml containers (Sarstedt AG & CO – Roehre, 114mm x 44mm). Leaf debris and soil was then removed using tongs and stored in labeled Ziploc bags. Holes were flushed with water and checked over with a flashlight to ensure all organisms were collected. Samples were kept in a cooler in the field and transferred immediately into a fridge where they were kept at 4-8°C until they were sorted in the lab.

Samples were filtered using a 0.5-mm mesh to collect all insect larvae and to separate coarse and fine debris (see *Fine and coarse debris* below). Larger insect larvae were removed from the coarse debris and placed into 99.5% ethanol. A stereomicroscope was then used to locate all remaining macroinvertebrates from the sample which were subsequently transferred to

99.5% ethanol. Insect larvae were identified to species level using insect larval identification keys (Cranston *et al.*, 1987; Smith, 1989; Rotheray, 1993; Klausnitzer, 1996; Nilsson, 1997; Sundermann *et al.*, 2007; Thyssen, 2010). Species that we were unable to identify to species level we assigned to morphospecies. We only considered species or morphospecies that are known or assumed to develop in water-filled tree holes in further analyses.

### *Morphology and life history trait characterization*

Morphological and life history traits of the tree-hole insects found in our study were compiled from our own previous research (Gossner *et al.*, 2016) and the literature (see resulting trait Table A2) in order to investigate how forest management affects the functional diversity and trait composition of communities. From the literature, we collected information on feeding mode (particle feeder, filter feeder, predator/scavenger, shredder), respiration type (air, water), pupal location (inside tree hole, outside tree hole), maximum larval length, overwintering stage (larval, larval and egg, larval and pupal), movement within tree hole (swimming, crawling along bottom, crawling along surface), specialization (tree-hole specialist yes, no), egg formation (single, cluster) and adult food source of females (blood and nectar, nectar, insects). Images of adult insects available online were used to measure adult wing length relative to body length (head to end of abdomen, antennae not included) to calculate the relative wing length. Additionally, raw data from Gossner *et al.* (2016) and Schmidl *et al.* (2008) were used, in combination with data from this study (following this order) to assess the tolerance for different water chemical features (minimum pH, minimum dissolved oxygen, median depth/opening area ratio, maximum ammonium, maximum nitrate and maximum phosphorus).

### 2.4.2 *Bacterial communities*

Fifty-two tree holes were sampled from 34 plots (SCH: 5, HAI: 15; ALB: 14) for bacterial community analysis, including 11 rot holes and 41 pan holes. Thirty-four tree holes were located at ground level and 18 tree holes were canopy holes (Table A1). Bacterial samples were collected in parallel to the insect larvae samples, however, only holes containing at least 200ml of free standing water were selected (methodological restriction). This led to a reduction in the number of tree holes sampled, in particular in SCH where there was little rainfall over the summer. Sixty milliliters of water were directly removed from the tree hole using a sterile syringe and filtered through a sterile 0.1µm 47mm-diameter polycarbonate filter. The filter was stored at -80°C for DNA extraction. Details regarding the extraction of genomic DNA can be found in the Appendix (“Supplemental methods”).

Sequences were clustered into operational taxonomic units (OTUs) using a cutoff of 97% sequence identity employing QIIME V1.9.1 (Caporaso *et al.*, 2010) following an open reference OTU picking strategy with UCLUST (Edgar, 2010). Taxonomy was determined using hierarchical classification based on the SILVA Database (v.128) (Quast *et al.*, 2013; Yilmaz *et al.*, 2014) and employing UCLUST (Edgar, 2010). All Illumina datasets were submitted to the SRA database under accession number PRJNA529605. Alpha diversity of the bacterial communities was analyzed using the RAM R package (version 1.2.1.3) (Chen *et al.*, 2016) based on OTUs defined at 3% sequence dissimilarity.

### 2.4.3 *Nematode communities*

Nematode samples were collected in early spring (March 18 – 27), 2015 in SCH only. In total, 28 tree holes were sampled for nematodes from 15 different plots. Nine tree holes were sampled

in the canopy and 19 at ground level; 26 were pan holes and two were rot holes (Table A1). Initially, pH, dissolved oxygen and temperature were measured (see Appendix), a 15-ml water sample was collected by pushing a sterile pipette into the water and taking a sample from the bottom of the hole including coarse and fine debris, the habitat in which nematodes occur. The water-debris suspension was transferred to a 15-ml centrifuge tube and placed in a cooler. The extraction of nematodes from the samples in the lab is described in the Appendix (“Supplemental methods”). Nematodes were identified and counted by light microscopy and identified to family level using the key of Bongers (1994). The trophic level was assigned based on Yeates et al. (1993).

## **2.5 Environmental variables**

To study indirect effects of forest management intensity on tree-hole organisms, we measured or derived a number of additional environmental variables at forest stand scale (local climate, leaf fall forest structural complexity, diameter at breast height) and at tree-hole scale (fine and coarse debris, percent canopy cover, other tree-hole environmental variables) that are potentially influenced by forest management intensity and that may subsequently affect insect, bacterial or nematode communities in tree holes. For detailed descriptions of these measurements, please see the Appendix (“Supplemental methods”).

## **2.6 Statistical Analysis**

### ***2.6.1 General approach: Correlations, global model and multi-model inference***

All statistical analyses were performed in R version 3.3.1 (R Development Core Team, 2016). Depending on the data distribution of the response variable we used either linear mixed effects models (LMM: nlme package, Pinheiro *et al.*, 2014) or generalized linear mixed effects models (GLMM: lme4 package, Bates *et al.*, 2014). In all models, plot was specified as a random effect nested in region - except for models with either plot-based variables (only region as a random factor) or models regarding the nematode community (only plot as random factor because they were measured only in SCH).

To reduce the number of variables for a final structural equation model (SEM) we conducted a two-step approach. First, Pearson correlations were calculated between all predictor variables for the insect larval and bacterial communities and separately for the nematode communities as these were sampled in different holes and plots and different variables were measured. According to Tabachnick and Fidell (1996) independent variables with a bivariate correlation  $> 0.70$  should not be included in multiple regression analysis. Therefore, out of variables with a Pearson correlation coefficient  $> 0.70$ , the variables based on more accurate or more detailed measurements or those that we considered directly important for tree-hole organisms were included in further analysis (opening area was selected over potential volume, coarse debris was selected over potential volume, fine debris and total debris, proportion harvested biomass was selected over the complete forest management index ForMI). Second, we applied a multi-model inference approach as proposed by Grueber *et al.* (2011) to assess the importance of our predictor variables in explaining tree-hole abundance and type, insect larval, nematode and bacterial communities. A global model was created for each response variable we investigated (for details on the respective global models, see the following sections below). All continuous variables were scaled using the “standardize” method of the decostand function in the

vegan package (Oksanen *et al.*, 2017), which sets the mean to zero with unit variance. The dredge function (MuMIn package) (Barton, 2015) was used to test all possible model variations. Second-order Akaike information criterion for small sample sizes (AICc) was used to rank the models (Grueber *et al.*, 2011). A subset of all possible models was selected with those having a  $\Delta AICc < 2$  (Burnham *et al.*, 2011). Models with a  $\Delta AICc \geq 2$  are considered to have less support (Burnham and Anderson, 2004). We considered variables with a relative importance  $\geq 0.5$  to be of high importance and those  $\leq 0.2$  to be of low importance – variables that fell in between were considered of intermediate importance. Variables with a relative importance  $\geq 0.5$  were then included in the structural equation model.

#### ***2.6.2 Effect of forest management on tree-hole abundance and the proportion of pan holes***

The impact of forest management on tree-hole abundance and on hole type, i.e. the proportion of rot vs. pan holes (both stand-based variables) was assessed using two different global models as described above (one for each of the two response variables, see also 2.6.1), both containing the same predictor variables: the forest management components - proportion of non-natural tree species and proportion of harvested tree biomass, mean DBH, DBH variance and the forest structural complexity of each plot. An LMM was used to test for the drivers of tree-hole abundance (log-transformed). To test for the drivers of the proportion of rot vs. pan holes a binomial GLMM with a logit link was used.



### **2.6.3 Effect of forest management on insect communities**

As insect community attributes and hence as response variables, we used insect larval abundance, species richness, community composition and functional diversity (see *Functional diversity of tree-hole insect communities* below). To analyze the effects of forest management on insect larval abundance and species richness we used two LMMs (one for abundance and one for species richness). Abundance was log transformed and species richness was square-root transformed. To assess community composition, a non-metric multidimensional scaling analysis (NMDS) was performed on a Bray-Curtis dissimilarity matrix using the metaMDS function in the vegan package (Oksanen *et al.*, 2017) with 1000 permutations and two axes. The NMDS scores of the two axes were then used as response variables in two separate global models (LMMs), in order to test the effects of the predictor variables on community composition.

### **2.6.4 Direct and indirect effects of forest management on insect communities**

In order to assess the direct and indirect effects of forest management practices from the forest-stand scale to the local tree-hole scale on the tree-hole insect communities, we applied structural equation modeling (SEM). Analyses were conducted using the piecewiseSEM package, which allows random and non-linear effects to be included (Lefcheck, 2016). In this approach, goodness-of-fit tests are conducted using Fisher's C, AIC, AICc and p-values. Variables were selected to be included in the SEM, based on the results from the multi-model inference approach for the four response variables representing insect community attributes (abundance, species richness, NMDS1 and NMDS2, i.e. the scores from the two axes of the NMDS analysis) and tree-hole abundance. Pathways were tested between forest management components

(proportion of harvested tree biomass and proportion of non-natural tree species), environmental variables and response variables. Forest management variables were set as the source variables. Among the environmental variables, only those that had a relative importance above 0.5 for explaining one of the insect community variables or tree-hole abundance were included. Based on these variables we built a model including all expected direct and indirect relationships that showed high importance in the multi-model inference approaches. Missing links were identified with the SEM.fit function and added to the model when we could interpret the effect in a meaningful way. The final model was selected if no further significant missing links were identified and the p-value of the Fisher's C test was non-significant.

#### ***2.6.5 Functional diversity of insect communities***

The FD package was used to assess the functional diversity, represented by RaoQ (Botta-Dukát, 2005) and functional dispersion (FDis, Laliberté and Legendre, 2010) of the insect communities in each tree hole, two measures that are less strongly affected by species richness. All literature and measured traits (see above) were included. A Gower dissimilarity distance matrix (Gower, 1971) was created with different weights assigned to the traits and semi-quantitative variables treated as described by Podani (1999). Traits related to water chemistry (minimum DO and pH and maximum nitrogen, ammonium and phosphorus) were assessed for normality. These traits were down-weighted (half) because data were taken from two different studies and results strongly varied between them. The Gower dissimilarity matrix was then used in the function dbFD to compute the RaoQ and FDis. Two global models (LMMs) were created - one with RaoQ and one with FDis, both square-root transformed.

In order to investigate changes in functional trait composition, we created a functional trait matrix by (1) dividing all continuous traits in three categories of equal numbers; (2) calculating the number of species that were assigned to a particular trait category for each tree hole; (3) dividing each value by the number of species occurring in a particular tree hole to correct for differences in species numbers among tree holes; (4) scaling all trait categories to values between 0 and 100 to standardize them. A non-metric multidimensional scaling (NMDS) analysis was performed on the functional trait matrix using Bray Curtis distances. The trait NMDS scores were extracted to create two global models (LMMs), one with trait NMDS1 and one with trait NMDS2. Traits identified as having a high importance to the trait composition were fitted to the NMDS plot using the `envfit` function from the `vegan` package.

### ***2.6.6 Effect of forest management on bacterial communities***

Bacterial OTUs defined at 3% of 16S rRNA gene sequence dissimilarity (= species level) were used to assess the effect of forest management on bacterial richness. An LMM was used including the variables that remained after the correlation (see 2.6.1). The global model for the multi-model inference included the forest management components proportion of harvested tree biomass and proportion of non-natural tree species, the total number of tree holes, mean DBH, DBH variance, forest structural complexity, relative humidity, and leaf fall as stand-scale variables and opening area, coarse debris, percent canopy cover above the tree hole and hole type as tree-hole scale variables.

### ***2.6.7 Effect of forest management on nematode communities***

Nematode samples were collected in a different year and in different tree holes than the insect and bacterial communities and some environmental variables were not measured in all of these tree holes. Due to the reduced sample size for the nematode communities certain variables were selected for analysis and included in three separate global models. These were at stand-scale the total number of tree holes, mean DBH, forest structural complexity, forest management intensity components, leaf fall and at tree-hole scale opening area. To test the effects of these variables on nematode abundance a Poisson GLMM with a log link was used. Two global models (LMMs) were calculated for family richness, one model including and one model excluding nematode abundance (log-transformed) as an additional response variable.

## **3. Results**

### **3.1 Tree-hole abundance and tree-hole type**

In total, 1034 tree holes (SCH: 285, HAI: 357, ALB: 392) were found on the 75 plots (Table A1). The average number of tree holes per 1-ha plot was  $14 \pm 1$  SE with a wide range between plots (0-45 holes per plot). Pan holes ( $11 \pm 1.2$ ) occurred more frequently than rot holes ( $1 \pm 0.2$ ). Ground and canopy holes showed similar abundances (ground:  $7 \pm 0.9$ ; canopy:  $6 \pm 0.7$  per plot).

Following the multi-model inference approach, variables with a relative importance  $\geq 0.5$  were considered to be of high importance, those  $\leq 0.2$  to be of low importance, and those that fell in between were considered of intermediate importance. Only variables of high importance are described in the text. The variables that affected tree-hole abundance most strongly were the

forest management component “proportion of non-natural tree species” (negative effect: -, Table 1), forest structural complexity (-) and mean DBH (positive effect: +). The proportion of pan holes was most strongly affected by the proportion of non-natural tree species (-) and forest structural complexity (+).

### 3.2 Tree-hole insect communities

From the 123 tree holes sampled for insects, a total of 6660 insect larvae of 19 different morphospecies were collected (Table A3). Abundance was highest in ALB ( $98 \pm 20.1$  individuals per hole, range: 0 - 595) and the lowest in SCH ( $36 \pm 4.1$ , 0 - 116), HAI had a slightly higher abundance than SCH ( $38 \pm 8.9$ , 0 - 249). Species richness was similar in all three regions (ALB: mean:  $3 \pm 0.3$ , range: 0 - 9 morphospecies per hole, HAI:  $3 \pm 0.2$ , 0 - 6, SCH:  $2 \pm 0.3$ , 0 - 9). After the correlation approach (Table A4), 13 predictor variables remained and were used in the multi-model inference approach (Figure 2, Table A5). The most important predictors of insect abundance in tree holes were tree-hole abundance (+, Figure 2 A, Table A5) and coarse debris (+). The most important variables affecting insect species richness in tree holes were insect larval abundance (+, Figure 2 B, Table A5), the proportion of non-natural tree species (+), tree-hole abundance (-), relative mean DBH (+) and humidity (-). The most important variables shifting species composition were tree-hole abundance (NMDS1, Figure 2C, Table A5), mean DBH (NMDS1 and 2), forest structural complexity (NMDS2), coarse debris (NMDS2), opening area (NMDS1) and hole type (NMDS2).

### ***3.3 Direct and indirect effects***

The final structural equation model illustrates the direct and indirect pathways of the effect of forest management on insect communities (abundance, species richness, and community composition) via changes in environmental conditions at stand and tree-hole scale (Figure 3). According to this model, tree-hole insect communities were primarily influenced by indirect effects of forest management. Overall, insect abundance was reduced by both forest management components (total effect of proportion of harvested tree biomass: -0.066; proportion of non-natural tree species: -0.299; Table A6). The strongest negative effect of harvesting on larval abundance was caused by a reduction of coarse debris in individual tree holes. Increasing proportion of non-natural tree species resulted in a reduction of tree-hole abundance and forest structural complexity at stand scale, which decreased insect abundances in individual tree holes.

Species richness of insects in tree holes was positively influenced directly by the proportion of non-natural tree species (0.496; Table A6) and negatively influenced by indirect effects of both components of forest management. Proportion of non-natural tree species had a negative indirect effect on species richness (-0.397). This effect was mainly driven by reduced forest structural complexity and by the reduction in larval abundance caused by the reduced tree-hole abundance. The total effect including direct and indirect effects was positive (0.103). Increasing proportion of harvested tree biomass indirectly impacted species richness negatively (-0.030), primarily by a decreased amount of coarse debris and thus lower insect abundance at tree-hole scale.

Insect community composition (NMDS1) in the tree holes was altered indirectly by the proportion of non-natural tree species (0.339, Table A6), while the effects of harvested biomass on community composition were weak and cancelled out (0.007). The proportion of non-natural

tree species shifted species composition mainly by reducing tree-hole abundance and forest structural complexity.

### ***3.4 Functional traits***

The most important variables affecting functional diversity and functional dispersion were the proportion of non-natural tree species (+, Figure 2D, Table A7), tree-hole abundance (-) and mean DBH (+). Variables that were most important for trait composition were tree-hole abundance (NMDS1, Figure 2E, Table A7), mean DBH (NMDS2), forest structural complexity (NMDS1), coarse debris (NMDS1 and 2) and hole type (NMDS2).

Fitting the variables of high importance to the ordination and testing for significance by using permutation tests showed that trait composition was mainly affected by tree-hole abundance (1000 permutations,  $r^2=0.06$ ,  $p=0.02$ ) and mean DBH ( $r^2=0.05$ ,  $p=0.05$ , Figure 4). Two traits, adult relative wing length and overwintering stage of the insect species, showed a visible shift along the tree-hole abundance axis (Figure 4). Relationships appeared, however, to be nonlinear. Long-winged and short-winged species relative to their body size were more abundant in plots with low tree-hole abundance. Species with intermediate wing-size were more abundant in plots with high tree-hole abundance. Species that overwintered in the tree hole at larval stage were found more frequently in plots with higher tree-hole abundance, whereas species that overwintered as pupae or eggs, in addition to larvae, were found more frequently in plots with lower tree-hole abundance.

### ***3.5 Bacterial community***

In the 52 tree holes sampled for bacteria 499,959 different OTUs defined at 97% 16S rRNA gene sequence similarity were found ( $23800 \pm 1783$  per tree hole). The predictor variables that remained for the bacterial community analysis after the correlation approach (Table A4) were the same as for the insect communities. The richness of the bacterial communities was most strongly affected by forest structural complexity (+, Figure 5 Table A8) with intermediate importance of percent canopy cover (+).

### ***3.6 Nematode community***

In the 15-ml subsamples from the 28 sampled tree holes a total of 935 nematode individuals ( $33 \pm 18$  per tree hole) from 12 different families were found. Four trophic groups were observed: bacterivores, fungivores, herbivores and predators. All trophic groups were found in both ground and canopy holes, with the exception of predators for which only three individuals were found in ground holes and none in canopy holes. Based on correlation analyses excluding strongly correlated variables (Table A9) nine variables were used in the models (see Table A10). Proportion of harvested tree biomass (-, Table A10) and forest structural complexity (+) were the most important predictors of nematode abundance. None of the variables showed high importance as predictors of nematode family richness; tree-hole abundance (+) and height on the tree (+) had intermediate importance (Figure 5, Table A10).



## 4. Discussion

We found that forest management had various direct and indirect effects on tree holes and associated communities. First, an increased proportion of non-natural tree species reduced tree-hole abundance. Second, effects of management on insect communities were often strong, primarily indirect and negative. Only the proportion of non-natural tree species had a strong and positive direct effect on insect species richness. The indirect effects operated mainly through the reduction of tree-hole abundance, forest structural complexity and coarse debris. Third, the proportion of non-natural tree species showed a positive effect on insect functional trait diversity and dispersion. Tree-hole abundance, mean DBH, forest structural complexity, coarse debris and hole type were the most important drivers of trait composition. Fourth, while insect abundance, richness and community composition were strongly influenced by forest management, little effect was seen on bacterial and nematode communities. In the following we discuss possible mechanisms underlying these findings.

### 4.1 Drivers of tree-hole abundance and the proportion of rot and pan holes

Tree holes were most abundant in forests with low forest management intensity which supports the results of Gossner et al. (2016) who similarly found higher tree-hole densities under low-intensity forest management. In addition, our study revealed that it was primarily the shift in tree species towards an increased proportion of non-natural tree species that decreased tree-hole abundance. Tree species non-natural to the study sites were primarily conifers, which are preferred over naturally occurring beech by forest managers due to their higher growth rates (Dirk, 1998; Schelhaas *et al.*, 2003). Conifer species rarely produce tree holes (Rohnert, 1950;

Kitching, 1971) and mainly rot holes develop in stumps after harvesting, explaining the increasing proportion of rot holes with increasing proportion of non-natural tree species. This suggests a tree-species identity effect in these results. A higher mean DBH in the forest stand resulted in a higher abundance of tree holes (only shown in the multi-model inference approach). This was likely a result of the overall larger tree volume, increased wear on older trees leading to damage and subsequent rot-hole formation. Surprisingly, increased forest structural complexity reduced tree-hole abundance (only shown in the multi-model inference approach). This may have been due to changes in branching patterns caused by shifts in stand density (Juchheim *et al.*, 2017). For example, forest stands consisting of pole wood have a higher density of small branches, increasing the measure of forest structural complexity that we used (Puettmann *et al.*, 2012; Ehbrecht *et al.*, 2017), but have a low abundance of tree holes.

#### **4.2 Direct and indirect pathways of forest management effects on tree-hole insect communities**

The proportion of non-natural tree species had a direct positive effect on insect species richness, which is difficult to explain, especially since most tree holes in plots with high proportion of non-natural tree species were located in admixed deciduous trees. We can only suggest that water chemistry, detritus quality or habitats for adult insects (which were not measured in our study) were different in plots with high proportions of non-natural tree species and this may have increased species richness per tree hole. We suggest that future studies specifically target these variables to elucidate the positive effect of non-natural tree species on insect species richness in tree holes. However, the indirect effect of non-natural tree species on insect richness was significant and negative due to a significant and negative indirect effect via reduced tree-hole

abundance and forest structural complexity. In addition, the overall indirect effect of non-natural tree species on insect species abundance via the same pathway was significant and negative. Tree-hole abundance had a general positive effect on insect abundance and also affected species and trait composition. This may indicate that insect species with certain traits (for example those that are related to competitive ability, such as resource acquisition strategies or reproductive output) may outcompete others and are able to reach high abundances in plots with higher tree-hole abundance and therefore, higher habitat density. In contrast, mainly species with high dispersal ability (which can be related to wing length relative to the body, see 4.3) might persist in plots with low habitat density. Counter to the results from this study, Gossner et al. (2016) found that increased tree-hole abundance decreased insect larval abundance in individual tree holes. This discrepancy may be due to the snap-shot nature of studies in which certain species may be recorded during different periods of their phenology. Here, long-term studies are useful (Gossner, 2018) that take into account the life cycle of the organisms of interest. The negative indirect effects of the proportion of non-natural tree species on tree-hole communities were also mediated by a decrease in forest structural complexity. Other studies have also found a negative effect of non-natural tree species on forest structural complexity (Pretzsch *et al.*, 2016; Ehbrecht *et al.*, 2017) and this may affect insect species via reduced adult habitats or overwintering sites as well as through lower microclimate heterogeneity.

Harvesting intensity did not have a direct effect on the insect community. The indirect effects of harvesting intensity on insect larval abundance and subsequently on species richness were overall negative but weaker than those caused by the proportion of non-natural tree species. Harvesting reduced insect abundance and thus, species richness in individual tree holes through reduced coarse debris accumulation (see also Gossner et al. (2016)). High harvesting intensity

has been shown to reduce leaf fall (Inagaki *et al.*, 2008). In our multi-model inference approach leaf fall was tested but did not have high importance. Accumulation effects may be highly temporally and locally variable (for example, depending on the shape of the tree), and leaf fall data used in our study may not fully reflect this variation. The average opening area of the tree holes in our study did not vary with forest management intensity but tree holes with larger openings were able to accumulate larger amounts of coarse debris, influencing abundance, species richness and community composition, a result supporting previous studies (Paradise, 2004; Schmidl *et al.*, 2008; Gossner *et al.*, 2016). Decreasing mean DBH may additionally affect species richness and composition via reductions in adult and overwintering habitats.

#### **4.3 Forest management impact on the functional diversity and composition of insect traits**

Functional diversity (RaoQ and FDis) in individual tree holes showed similar results as species richness in the multi-model inference approach. This correspondence in results may be due to distinct traits of each individual species, especially since water-filled tree holes contain relatively species-poor communities with most species belonging to different major clades. This is supported by a study by Stuart-Smith *et al.* (2013) which found that in species-rich tropical regions, individual species contribute proportionally less to functional diversity at the community level than in temperate regions.

Changes in insect trait composition were mainly explained by habitat availability (tree-hole abundance), stand age (mean DBH), forest structural complexity, coarse debris and tree-hole type, closely following the results for community composition. We could identify some insect traits which were sensitive to our predictor variables. For example, tree holes in plots with

a lower tree-hole abundance contained species with a long relative wing length as adults. Wing length has been shown to impact dispersal of adult freshwater insects (Malmqvist, 2000), thus, dispersal distance may be limiting to some tree-hole inhabiting insect species. Overwintering stage as a trait was also influenced by tree-hole abundance. Insect species that overwintered in the tree holes during the pupal and egg stages, in addition to the larval stage, were found in plots with low tree-hole abundance and species which overwintered only as larvae were found in plots with high tree-hole abundance. Species with varying overwintering stages are typically more flexible in adapting to environmental conditions (see e.g. Nolte et al. 2017 for carabids) and thus might have an advantage in such ephemeral habitats as water-filled tree holes, leading to higher population densities. Species which overwinter as pupae, for instance, may be the first to emerge in the spring to then populate new tree holes and have thus an advantage as early colonizers (Miall and Hammond, 1900; Rozkošný *et al.*, 1997). In combination with better dispersal abilities these species might have an advantage over highly competitive species in forests with low tree-hole abundance.

#### **4.4 Differences between responses of insects, nematodes and bacteria**

Increased forest management intensity strongly affected insect species richness while no clear influence of either management component was shown on bacterial and nematode richness. Apparently, other variables exert more important effects on bacterial and nematode communities than forest management. We did not use a structural equation modelling approach on bacterial and nematode communities since we had limited data sets for those groups. It is well possible that they were also affected by indirect forest management effects via other environmental variables. Tree-hole abundance, had no direct impact on insect richness (a negative impact

showed in the multi-model inference approach, a positive indirect one via abundance in the structural equation model), no impact on bacterial richness and only a moderate positive impact on nematode richness. These different results between the organism groups may potentially be explained through differences in dispersal techniques and reproductive strategies. Dispersal limitation in insect communities may vary between species (e.g. depending on wing length) but is likely lower than in mostly passively dispersing organisms such as nematodes and bacteria (De Bie *et al.*, 2012). Nematodes, mainly reproduce bisexually and previous studies have also shown that they are somewhat limited by dispersal (Dümmer *et al.*, 2016). In contrast, bacteria have higher population sizes than nematodes and reproduce asexually, reducing the impact of dispersal limitation (Blackburn and Gaston, 1999).

Climate showed some importance for determining insect richness (negative effect of relative humidity - only shown in multi-model inference approach - and negative effect of percent canopy cover), intermediate importance for bacterial richness (positive effect of percent canopy cover) and no importance for nematode richness. Not all insect species are adapted to drought during their aquatic life stages and this climatic situation may reduce competition and increase insect species richness. In contrast, many bacteria and nematodes species are able to undergo anhydrobiosis (Giere, 2009). Previous studies have shown that bacterial communities can still be strongly influenced by environmental gradients. For example temperature extremes and fluctuations can negatively affect their survival (Ewert and Deming, 2014), but higher temperatures within a benign range can also increase their activity (Kaplan and Bott, 1989). Increased forest structural complexity had a positive influence on bacterial richness and insect richness (structural equation model), possibly through the provisioning of additional habitats. Generally, we conclude from our results that insect species in tree holes are more sensitive to

changes in forest management intensity and (micro-) climate than smaller-sized organisms such as nematodes and bacteria.

## 5. Conclusion

Our results show that tree-hole abundance, composition and environmental conditions change with increasing forest management intensity, in particular with an increasing proportion of non-natural tree species, and that these changes mostly indirectly and negatively influence insect abundance, species richness, functional diversity and community and trait composition in tree holes. Despite being complex and difficult to predict, these indirect effects should be taken into account as much as possible during the early planning stage of forest management actions. For example, lower tree-hole abundance, caused by increases in management intensity will strongly affect the species composition of tree-hole communities because some species, depending on their traits (such as wing length), will be affected by dispersal limitation but others may benefit from reduced competition with otherwise dominant species. As our results show, the effects of management intensity on tree-hole communities may vary between different taxa with different life-history strategies such as insects, bacteria and nematodes. Therefore, a range of functional groups need to be studied to fully understand the impacts of forest management intensity on communities (see also Seibold *et al.*, 2018). Since almost nothing is known on bacteria and nematodes in water-filled tree holes we strongly advocate the inclusion of those groups into future studies on tree holes.

In order to support biodiversity and probably also related ecosystem functions such as decomposition and nutrient cycling, we suggest increasing habitat availability by increasing the

abundance of natural tree species which may form tree holes and sustain high microhabitat availability, such as for example beech trees with big crowns and buttress roots. In intensively managed conifer forests that produce higher timber yields, tree-hole abundance, and thus the abundance and diversity of tree-hole organisms, could be increased by admixing natural tree-hole forming species, such as beech and hornbeam. Our results show that these intensively managed forests can then still sustain a high species richness and functional diversity of insects in each tree hole. These results might also hold for communities in other microhabitats such as crown deadwood and cracks since they may be similarly structured by potential dispersal limitation and habitat availability in managed forests. More detailed future analyses of beta and gamma diversity should test whether the effects of admixed trees remain positive on larger scales of forest management or the results rather call for a landscape-scale heterogeneity of management.



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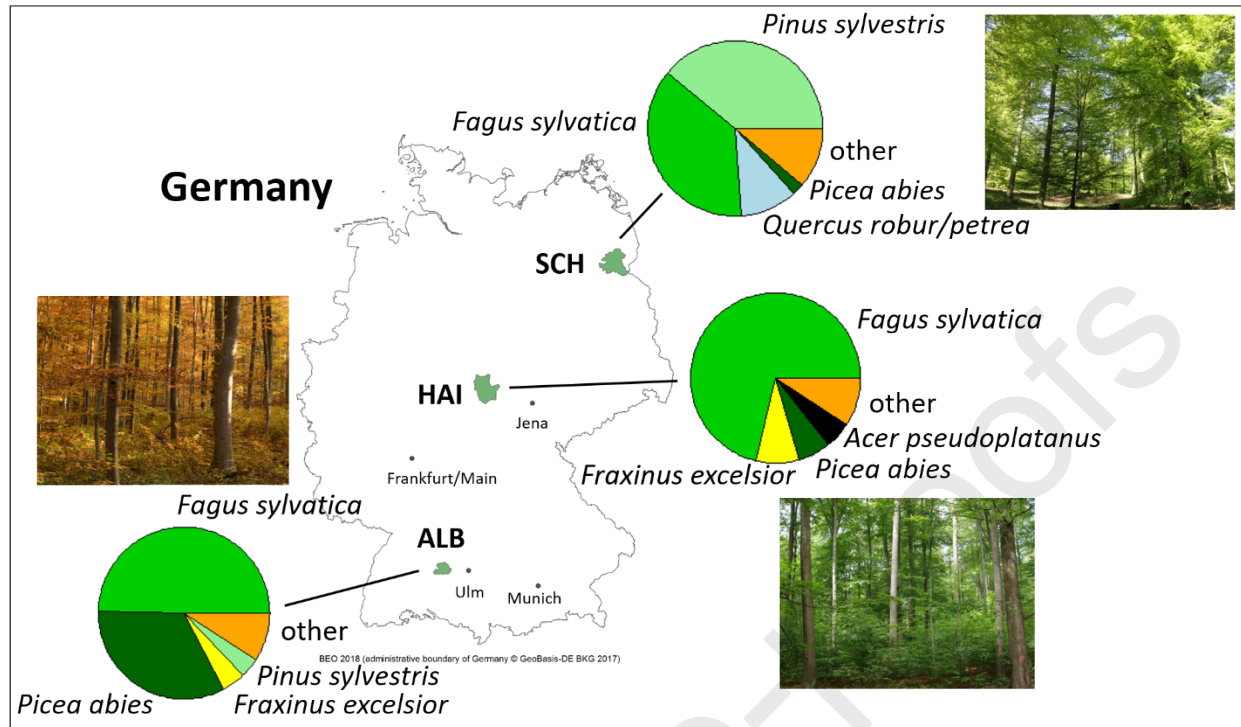


Figure 1: The three sites of the Biodiversity Exploratories in Germany ([www.biodiversity-exploratories.de](http://www.biodiversity-exploratories.de)) where this study was conducted: the Biosphere Reserve Schorfheide-Chorin (SCH), the National Park Hainich with the surrounding Hainich-Dün region (HAI) and the Biosphere Reserve Schwäbische Alb (ALB). Pie charts depict the species composition of forests in the area calculated based on forest inventories conducted on 500 grid plots of 100 x 100m in each region (see Fischer *et al.*, 2010). The pictures show beech (*Fagus sylvatica*)-dominated plots from each region. Fotos by J. Petermann (SCH), M. Gossner (HAI), M. Fellendorf (ALB).

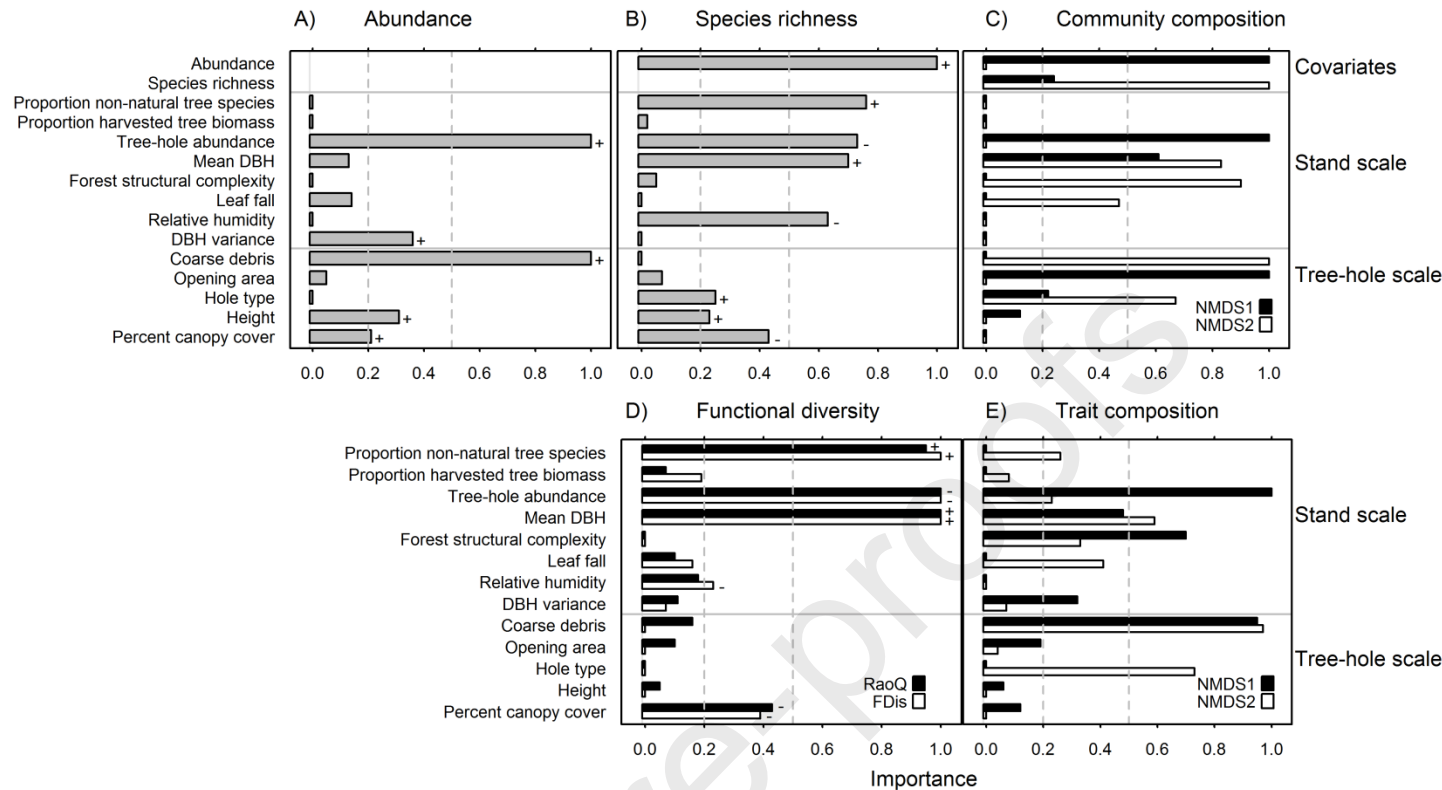


Figure 2: Relative importance of predictor variables for the response variables A) insect abundance, B) species richness and C) community composition (NMDS1 and NMDS2, shown as separate bars), as well as D) functional diversity (functional diversity RaoQ and functional dispersion FDis, shown as separate bars) and E) trait composition (NMDS1 and NMDS2, shown as separate bars) based on a multi-model inference with  $\Delta AIC_c < 2$  ( $n = 12$  abundance models;  $n = 48$  species richness models;  $n = 7$  NMDS1 models;  $n = 6$  NMDS2 models,  $n = 16$  RaoQ models;  $n = 9$  FDis models,  $n = 14$  NMDS1 trait composition models;  $n = 6$  NMDS2 trait composition models). Predictor variables are sorted into covariates, stand-scale and tree-hole scale variables, separated by horizontal grey lines. Importance of the variables is separated by grey dashed lines into low importance (below 0.2), intermediate importance (between 0.2 and 0.5) and high importance (above 0.5). Missing bars indicate an untested variable (NA), variables that were tested but had no importance are indicated with a bar that ends at 0. + and - next to bars with at least an intermediate importance ( $>0.2$ ) indicate positive or negative effects, respectively. Hole type affected insect richness: more insect species were found in rot holes compared with pan holes.

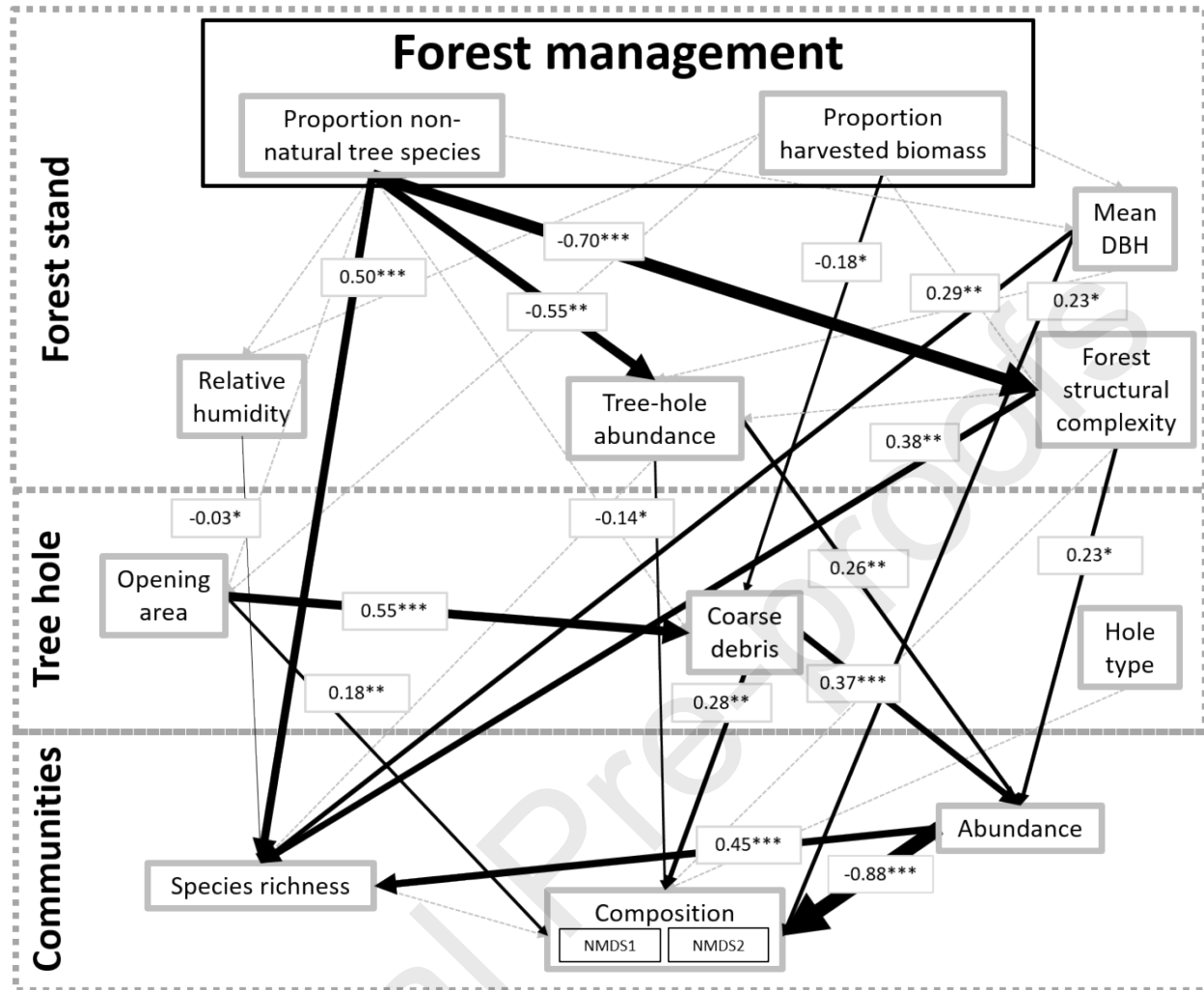


Figure 3: Final structural equation model testing for the direct effects of forest management and its indirect effects via environmental variables on tree-hole insect larval communities, specifically their abundance, species richness and community composition ( $p = 0.705$ , Fisher's  $C = 78.49$ , AIC = 220,  $df = 86$ ). Standardized path coefficients included in boxes along arrows give the direction and strength of the effect, stars represent the level of significance ( $*** = 0.001$ ,  $** = 0.01$ ,  $* = 0.05$ ). Community composition was analyzed as the two axes from an NMDS analysis: NMDS1 and NMDS2. Here, the coefficient shown is from the link with the stronger effect. Solid arrows indicate significant effects ( $p < 0.05$ ) and the thickness is scaled to the strength of the effect; dashed grey arrows indicate effects that were tested but were not significant ( $p > 0.05$ ). Variables are separated into three scales: forest-stand scale, tree-hole scale and community scale by dashed lines.

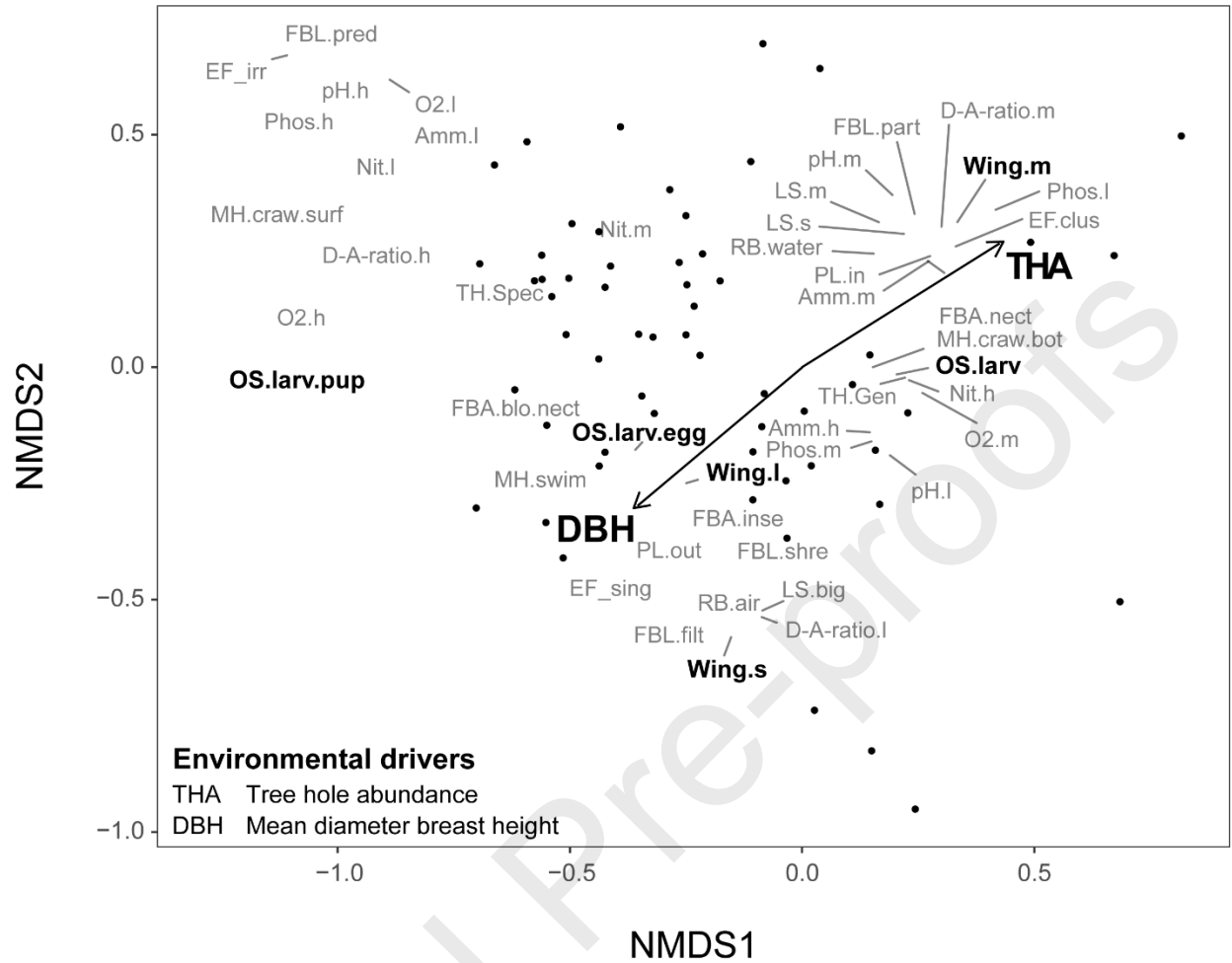


Figure 4: NMDS of traits (stress= 0.14) expressed by insect species in the tree holes, with main environmental variables (*Tree-hole abundance; Mean diameter at breast height*) plotted as arrows (rescaled for illustration). Traits used here were taken from the literature and own data (Table A2): *Phos.l*, *Phos.m*, *Phos.h*: maximum phosphorus content low, medium and high; *Nit.l*, *Nit.m*, *Nit.h*: maximum nitrate content low, medium and high; *O2.l*, *O2.m*, *O2.h*: minimum dissolved oxygen content low, medium and high; *Amm.l*, *Amm.h*: maximum ammonium content low and high; *D-A-ratio.l*, *D-A-ratio.m*, *D-A-ratio.h*: median depth:area ratio low, medium and high; *EF.sing*, *EF.clus*, *EF.irr*: egg formation as clusters, single or irregular; *Wing.l*, *Wing.m*, *Wing.s*: relative wing length long, medium and short; *TH.Spec*, *TH.Gen*: tree-hole specialist, generalist; *pH.l*, *pH.m*, *pH.h*: minimum pH low, medium or high; *MH.craw.bot*, *MH.craw.surf*, *MH.swim*: movement in tree-hole at bottom, surface or in water column; *LS.s*, *LS.m*, *LS.big*: Larval size small, medium or big; *OS.larv.egg*, *OS.larv*, *OS.lara.pupa*: overwintering stage larva and egg, larva, larva and pupa; *FBA.blo.nect*, *FBA.nect*, *FBA.inse*: feeding behavior of adults: feed on blood and nectar, nectar or insects; *FBL.filt*, *FBL.part*, *FBL.pred*, *FBL.shre*, feeding behavior of larvae: filter feeder, particle feeder,

*predator/scavenger or shredder; RB.air, RB.water: respiration behavior, air or water; PL.in, PL.out: pupa location, inside tree hole or outside tree hole.* Those traits that showed a visible shift along the tree-hole abundance axis in this plot are printed in bold and are described in more detail in the text.

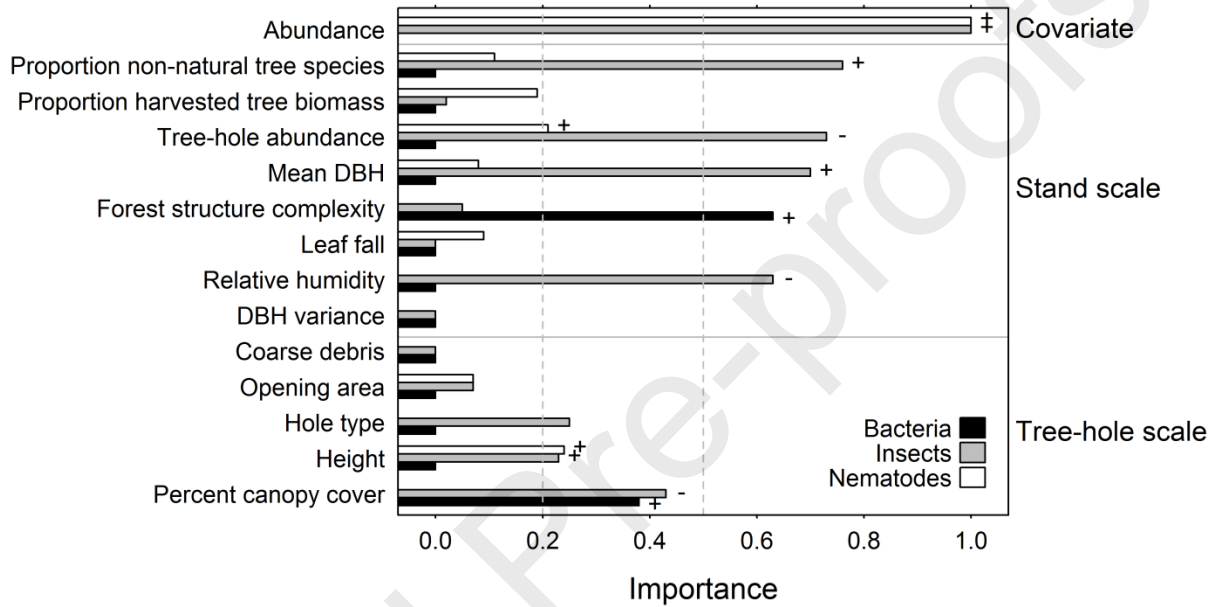


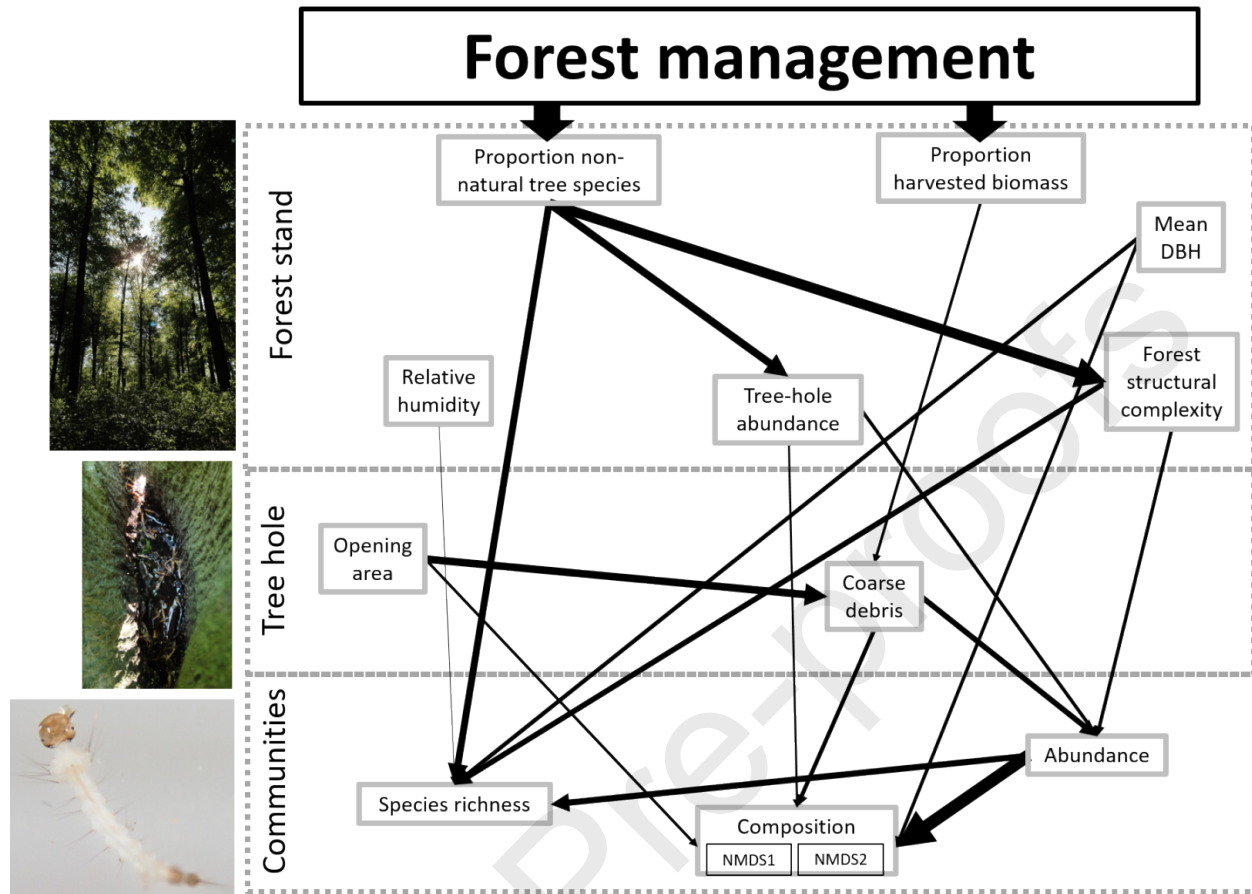
Figure 5: Relative importance of predictor variables for the response variables richness of bacteria, insects and nematodes based on a multi-model inference approach using all possible model combinations with  $\Delta AIC_c < 2$  ( $n = 14$ ,  $n = 148$ ,  $n = 10$  respectively). Predictor variables are sorted into covariate (only abundance), stand-scale and tree-hole scale variables, separated by horizontal grey lines. Importance of the variables is separated by grey dashed lines into low importance (below 0.2), intermediate importance (between 0.2 and 0.5) and high importance (above 0.5). Missing bars indicate an untested variable (NA). + and – next to bars with at least an intermediate importance ( $> 0.2$ ) indicate positive or negative effects, respectively. Hole type affected insect richness: more insect species were found in rot holes compared with pan holes.

Table 1: Relative importance of predictor variables (all at stand scale) for tree-hole abundance and the proportion of pan holes (vs. rot holes), based on a multi-model inference approach using all possible model combinations ( $n = 3$



for both response variables) with  $\Delta AIC_c < 2$ . The number of models (M#) each variable was present in and the direction of effect are given. DBH= diameter at breast height.

| Variable                                      | Tree-hole abundance |    |           | Proportion of pan holes |    |           |
|---|---------------------|----|-----------|-------------------------|----|-----------|
|   | Importance          | M# | Direction | Importance              | M# | Direction |
| <b>Proportion of harvested tree biomass</b>   | 0                   | 0  |           | 0                       | 0  |           |
| <b>Proportion of non-natural tree species</b> | 1                   | 3  | ↓         | 1                       | 3  | ↓         |
| <b>Forest structure complexity</b>            | 0.52                | 2  | ↓         | 0.76                    | 3  | ↑         |
| <b>Mean DBH</b>                               | 1                   | 3  | ↑         | 0                       | 0  |           |
| <b>DBH variance</b>                           | 0.19                | 1  | ↑         | 0.25                    | 1  | ↓         |



### Highlights

- Anthropogenic forest use and management may affect ecological communities
- Aquatic communities in water-filled tree holes were used as a model system
- Forest management showed strong, often negative and indirect effects on insects
- Nematode and bacterial communities were less affected
- Forest management impacts are complex but should be considered explicitly

### Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: