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ABSTRACT

Dead wood availability and the variability in dead wood quality, i.e. tree species and decay stages, are often low in managed forests, which negatively affects biodiversity of invertebrate species. Leaving more (coarse) dead wood can increase invertebrate richness, but it remains unclear how many and which combinations of tree taxa and decay stages are required to optimize niche heterogeneity in managed forests. We investigated the diversity of the main arthropod groups associated with dead wood, i.e. millipedes, centipedes, isopods and beetles, through the first four years of decomposition of logs of twenty common temperate tree species placed in the "common garden" experiment LOGLIFE. We hypothesized that (1) invertebrate richness for combinations of a given number of tree species would be promoted by mixing both tree species and decay period and that (2) invertebrate richness increases up to a saturation point with more tree species at different decay stages added. We also hypothesized that (3) an increase in phylogenetic distance among the tree species in combinations would promote their overall invertebrate diversity. We found that the better combinations, in terms of invertebrate richness, after one and two years of decay, but not after four years, consisted of a mix of gymnosperms and angiosperms, indicating that variation in tree species is especially important during the initial decomposition period. The best combinations in terms of invertebrate richness consisted of at least one tree species from each decay period, indicating that also variation in the decay stage of the tree is important to promote invertebrate diversity. We observed that at least four wood types were required to approach the 95% saturation point for species richness. The third hypothesis, that dissimilarity in phylogenetic position could be a predictive tool for increasing invertebrate richness in combinations of tree species, was not supported by our results. Thus, in order to maintain diversity of dead wood invertebrates in forests we recommend not only to provide richness in tree species, but also to plant particular combinations of trees (preferably angiosperm-gymnosperm combinations) that differ in the invertebrate communities they typically host and to temporally spread the logging of trees. This way the logging residues cover different resources and habitats at each moment in time, which is likely to result in a large diversity of dead wood invertebrates.

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1. Introduction

Dead wood is an important source of biodiversity in forests (Stokland et al., 2002; Harmon et al., 1986). Dead trunks and branches increase heterogeneity in microclimate, habitat and resources for many organisms, like bacteria, fungi and invertebrates (Seibold et al., 2015; Cornelissen et al., 2012; Grove, 2002), a large proportion of which play important roles in wood decomposition and nutrient cycling (Speight, 1989). Wood-associated invertebrates are essential for decomposition (Ulysen et al., 2016), since they are responsible for the mechanical breakdown of bark and wood. As such, they are important vectors and facilitators for the colonization of wood by microorganisms and other invertebrates (Zuo et al., 2016; Davies et al., 2008; Speight, 1989). Moreover, the mode of action of wood decomposers in the process of decomposition can be very diverse, and this makes maintaining the diversity of invertebrates in forest an important conservation target. However, worldwide the availability of dead wood in many managed forests has been declining and ecosystems are becoming less heterogeneous; this decline in resources results in a decrease in invertebrate diversity (Laussace et al., 2011; Sobek et al., 2009). In order to maintain invertebrate diversity it is essential to know not only how much dead wood should be present (see Della Rocca et al., 2014; Müller and Bütler, 2010), but also which tree species contribute most to diversity of invertebrates. However, there is very limited if any comprehensive, experimental information on which combinations of tree species and at which stages of wood decomposition, should be present in a forest to maintain or enhance dead wood invertebrate diversity for different groups of arthropods.

Multiple factors determine resource quality for invertebrates in dead wood, but the most important are tree species (Muller et al., 2015; Brändle and Brandl, 2001), stem diameter (Della Rocco et al., 2014; Grove, 2002), bark cover (Dossa et al., 2018), decay stages (Ulysen and Hanula, 2010; Vanderwel et al., 2006; Stokland et al., 2002) and the local abiotic environment in which wood decomposes (Muller et al., 2015; Chisholm et al., 2014). The species of tree, via for example its bark traits, is especially important in the early stages of decomposition (Zuo et al., 2016). The invertebrates colonizing fresh dead wood are mostly phloem feeders that consume carbohydrates in the secondary phloem inside the bark (Siitonen, 2001; Parisi et al., 2018). This makes the characteristics of the bark, like the surface of wood covered by bark, bark thickness and looseness, important determinants for the faunal species composition in the early stage of decomposition (Barbour et al., 2009; Zuo et al., 2016). Furthermore, the structural and chemical defences that protect the living trees against herbivores (Wainhouse et al., 1990; Franceschi et al., 2005) may have afterlife effects (Cornwell et al., 2009a) that influence dead wood inhabiting invertebrates. Due to these species-specific traits and afterlife effects of bark, many invertebrates colonizing fresh dead wood show a narrow host preference. These preferences are usually for a tree family rather than for one particular tree species (Tavakilian et al., 1997; Grove, 2002), which is consistent with the view that closely related tree species share similarities in traits (Pan et al., 2015) and have therefore a more similar invertebrate community composition than distantly related tree species. However, information on the importance of phylogeny in determining invertebrate community compositions in dead wood is largely lacking.

Species-specific wood and bark traits interact with decomposition stage, resulting in changes in invertebrate species composition in wood over time. As decomposition progresses and wood is colonised by microorganisms, bark will come loose from the wood due to the activity of microbes, phloem feeders and physical forces. At this stage of decomposition, the traits of the xylem and the ability of wood boring insects, fungi and microbes to infiltrate the dead wood will become more influential for invertebrate species composition compared to bark traits (Cornelissen et al., 2012; Zuo et al., 2016). Moreover, moss covering the logs may have a buffering effect on the microclimate as well as provide habitat irrespective of tree species traits. The species composition inside the decomposing logs will gradually change from mostly phloem feeders in the bark towards a community with xylo-detritophagous (xylem-feeding) and mycetophagous (fungus-feeding) species and their associated predators (Siitonen, 2001; Ulyshen & Hanula, 2010; Lee et al., 2014). At even later stages of decay, bark will often completely fall off and the traits of xylem of the different tree species are hypothesized to converge due to degradation by biota (Stokland et al., 2002; Gossner et al., 2016). So the tree species and the decay stage, as well as the decay environment interact with each other and in combination determine the species composition in dead wood (Zuo et al., 2014).

Most previous research on invertebrate species composition focused on the effect of forest management strategies and dead wood already present in the forest was sampled (Della Rocca et al., 2014; Seibold et al., 2015). The standardized comparison of invertebrate communities across many tree species of standard diameter and at different stages of decomposition in one and the same forest site, would provide valuable information on how to maintain or increase habitat and resource heterogeneity for invertebrates in forests. Combining wood types with different invertebrate species communities could provide an indication on the minimum level of heterogeneity, in terms of tree species and decomposition stage, required to retain species diversity in forests. However, such large scale experiments are rare (but see Gossner et al., 2016).

The aim of this study was to investigate, in coarse dead wood in temperate forest, which combinations of tree species and forest incubation periods (i.e. duration of decomposition) promote the highest diversity of invertebrates. With this information we assessed how many tree species are needed to reach a close to maximum richness of wood-associated invertebrates. We hypothesized (1) that invertebrate richness for a given number of tree species will be promoted by mixing different tree species with different forest incubation periods, providing a high dissimilarity in resources and microhabitats. Furthermore, we hypothesized that (2) invertebrate richness will increase up to a saturation point with more dead wood types added. Saturation of species richness will occur because with each tree species added, the statistical likelihood to add a species that significantly increases resource and/or microhabitat diversity will decrease. Additionally, we investigated whether phylogeny could be used to select the best combinations of tree species in terms of invertebrate richness. Thus, we hypothesized that (3) overall phylogenetic distance among the tree species in combinations will promote their overall invertebrate richness. We expect that phylogenetically distant tree species are more dissimilar in resource and microhabitat traits, which would increase invertebrate diversity. To test these hypotheses, we compared the invertebrate species richness and composition in logs of twenty taxonomically and ecologically wide-ranging temperate tree species, after one, two and four years of decomposition in a “common garden” experiment in a Dutch forest (Cornelissen et al., 2012). We focused on the most dominant macro-invertebrate groups, and examined which combinations of two or more tree species and decay periods, had the highest invertebrate richness, both in terms of families and species. This information provide insight into how to promote the diversity of invertebrates and, subsequently, ecosystem functions and services provided by them. Therefore our findings will advance forest management strategies.

2. Material and methods

2.1. Research site and tree species

The study site Hollandse Hout, province of Flevoland, the Netherlands (52.46 N, 5.42 E) was a *Populus x canadensis* Moench forest plantation with a discontinuous canopy on clay soil. The undergrowth consisted of *Urtica dioica* and *Galium aparine*; this herbaceous layer also covered the logs during the growing season. Trees of ten different species were extracted from mono-specific forestry plantations, in 2012. These plantations where either close to the study site or close to the

Schovenhorst Estate, province of Gelderland, the Netherlands (52.25 N, 5.63 E). The species collected for the study were *Betula pendula* Roth; *Fraxinus excelsior* L., *Populus x canadensis* and *Fagus sylvatica* L. The species extracted at site Schovenhorst were *Larix kaempferi* (Lamb.) Carr., *Pseudotsuga menziesii* (Mirb.) Franco, *Abies grandis* and *Populus tremula* L.. The remaining two species, *Picea abies* (D. Don) Lindl. and *Quercus robur* L., were extracted from each of the two sites in order to account for site-specific growing conditions and were treated as different species in the analyses. Ten additional tree species from the study site were added in 2013, namely *Acer pseudoplatanus* L., *Alnus glutinosa* (L.) Gaertn., *Carpinus betulus* L., *Castanea sativa* Mill., *Pinus nigra* J.F. Arnold, *Prunus avium* (L.), *Robinia pseudoacacia* L., *Salix alba* L., *Tilia cordata* Mill., and *Ulmus x hollandica* Mill.. In addition to these species, *F. excelsior*, extracted from the study site in 2012, was extracted from the same plot and also incubated for comparison between the different collection years. In total, twenty different tree species, of which fifteen angiosperms and five gymnosperm, were incubated and together they represent the most abundant tree genera in North West European forests. A detailed description of the study site can be found in Cornelissen et al. (2012).

2.2. Experimental design

Between mid-January and mid-February, in both 2012 and 2013, healthy trees with a trunk diameter of 25 cm (± 3 cm) were cut with a chainsaw. For each of the twenty species, five individual trees without visible damage were each cut into five logs of one meter length and transported to the study site. The five logs of one single tree were incubated in their own randomised block (Cornelissen et al., 2012). The five logs from a single tree were placed 30 cm apart from each other and logs of different trees species were at least 40 cm apart. The location and orientation (north-south or east-west) of the logs within a block were randomly selected. In total, 300 logs were incubated in February 2012, and 275 logs in February 2013 in the five forest incubation plots. The details of the full initial experimental design by 2012 can be found in Cornelissen et al. (2012).

2.3. Invertebrate sampling

After one, two, and four years of forest incubation one randomly selected log of each tree individual, e.g. five logs for each tree species, was sampled in February. In the field, the logs were sawn into two halves of 50 cm each. One half was taken for bark and wood trait measurements (Zuo et al., 2016), and the second half was carefully placed back in its own place in the block. These halves were collected around the 22th April, when the invertebrates living in the decomposing wood were assumed to be active again after the winter period. The logs were quickly extracted, placed into plastic bags and transported to the laboratory at Vrije Universiteit, Amsterdam, where they were stored at 3 °C. In year one of the 2012 batch, not all logs were collected and analysed. All five logs of *P. x canadensis*, *P. tremula* and *P. abies* were sampled, but just one or two logs were sampled for the other tree species, because field observations suggested they were still so hard, with bark firmly attached, that they had hardly or not been colonized at all. The bags were opened once every two weeks to let fresh air in.

To extract invertebrates, the bark was separated from the wood using chisels and fragmented by hand into smaller pieces when searched for animals. The macrofauna (body size approximately larger than 3 mm) living in the bark, moss and wood were manually extracted with forceps and placed in jars with 70% ethanol for later identification. In the first two years most invertebrates were found in the bark, while in year four of forest incubation also many invertebrates were extracted from the moss and the wood. The soft xylem around beetle-made holes was carefully cut away with chisels until all soft wood was removed to make sure that all invertebrates were collected. All the logs were processed within two months after collection. By then the invertebrates inside the logs were generally still alive. The invertebrates were counted and Chilopoda, Diplopoda and Isopoda were identified to species level and Coleoptera were only identified to family level, since identification to species level was often not feasible due to the high number of juveniles. Juveniles that could not be identified to the required taxonomic level, i.e. 1.2% of the collected individuals, were excluded from the analyses. Because Coleoptera were only identified to family level two datasets were created: one dataset including the four clades identified to family level and one dataset excluding Coleoptera identified to species level.

2.4. Statistical analyses

Both in 2012 and 2013, logs of *F. excelsior* were incubated for comparison between the two different starting years. To visualize the variance between the fauna community in *F. excelsior* between the collection years, nonmetric multidimensional scaling (NMDS) was performed using the metaMDS function from the R package 'vegan', version 3.4.4. The analyses were only performed with the family dataset for year two and year four of decay, respectively. The data from year one was not included due to the incomplete sampling of *F. excelsior* after one year of decay of the 2012 batch and the dataset at species level was too small to perform a reliable NMDS. An additional NMDS was performed with all 20 tree species of collection year two and four, respectively, to assess the difference in fauna community composition of the *F. excelsior* in 2012 and 2013 batch in the context of the other tree species. A PERMANOVA (Anderson, 2001) was performed to determine whether the fauna community of *F. excelsior* differed significantly between the two collection years.

To find the combinations of tree species and decay periods with the highest invertebrate richness, the species or family richness of single tree species was determined. This was done for each sampling year separately. The counts were transformed to presence and absence data.

A taxa was recorded as present, when at least two individuals were found in the five logs of a single tree species. This criterion prevented that a transient single individual that may not even have an actual association with the tree, could disproportionally affect the overall richness analyses across tree species. After transformation, we recorded in how many replicates invertebrates were present. The observed faunal richness per tree species was then used to calculate the overall richness when combining two or more (up to eight) tree species for each sampling year separately. A permutation test was used to calculate all possible combinations of tree species and then species richness was calculated for each combination, in which we corrected for overlapping species. Subsequently, the same analyses were carried out including all sampling years. These analyses only included up to five tree species, due to computational challenges, e.g. 6.5 billion calculations would have been needed for family richness with 6-species combinations. The observed richness for the calculated combinations was plotted. We also determined and plotted the richness at which 95% of maximum richness, as derived from all tree species taken together, was reached.

A phylogenetic tree was built using online phylomatic (V3) (http://phylodiversity.net/phylomatic/) with zanne2014 as a stored tree (Zanne et al., 2014). Subsequently, the phylogenetic tree with branch ages was visualized using the 'read.newick' function of the *phytools* package and the 'collapse.singles' and 'plot.phylo' functions of the R package '*ape*' (R core team, 2016). Based on their branch lengths, we calculated the phylogenetic distance for each combination of tree species using the 'cophenetic.phylo' function. The combined observed invertebrate family richness was calculated for each pair of tree species. The matrices with the phylogenetic distance and the observed family richness of the pairs of trees were compared with the mantel test (Legendre & Legendre, 1998) using a Spearman correlation. All analyses were performed in R 3.4.3 (www.R-project.org).

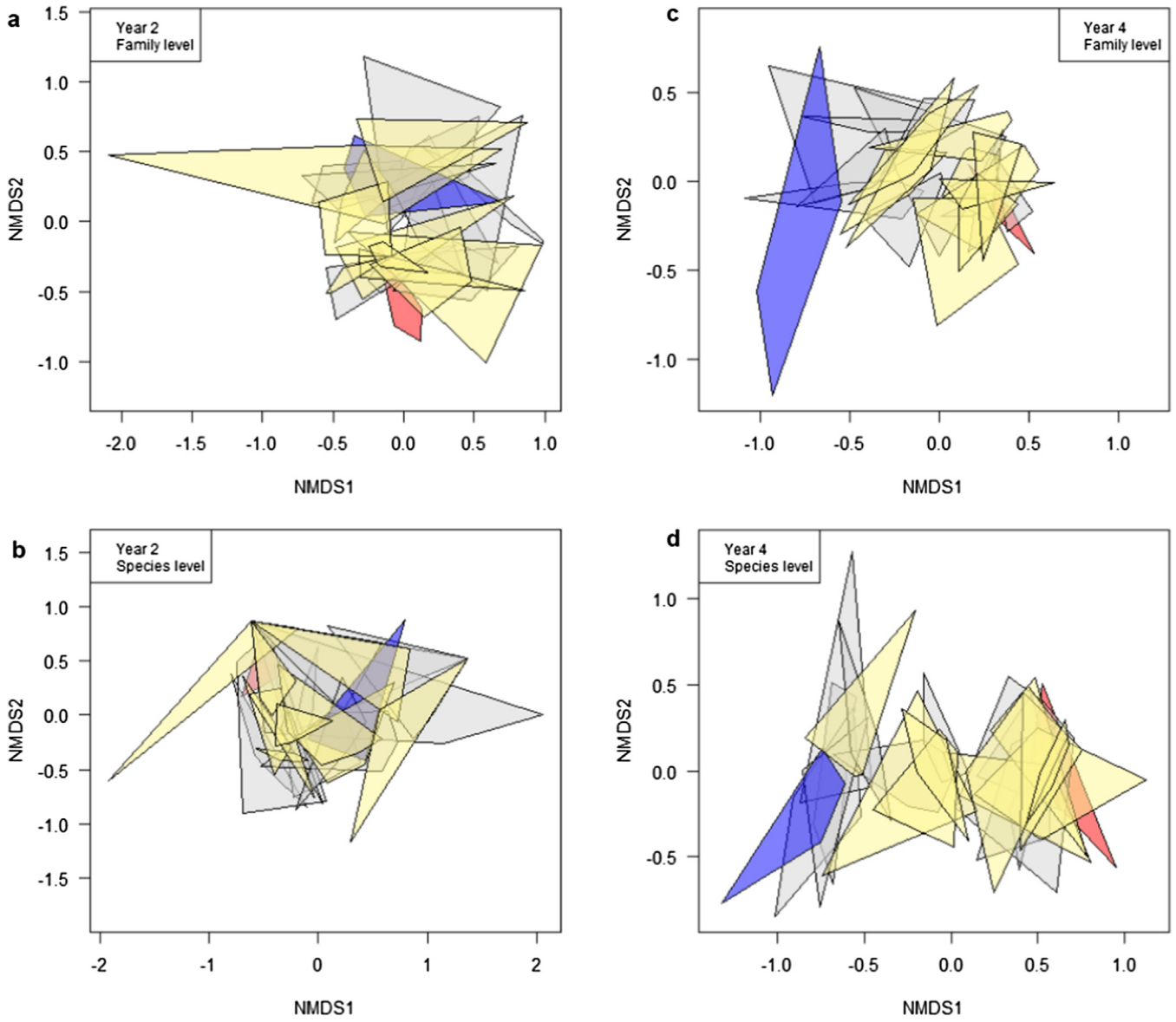
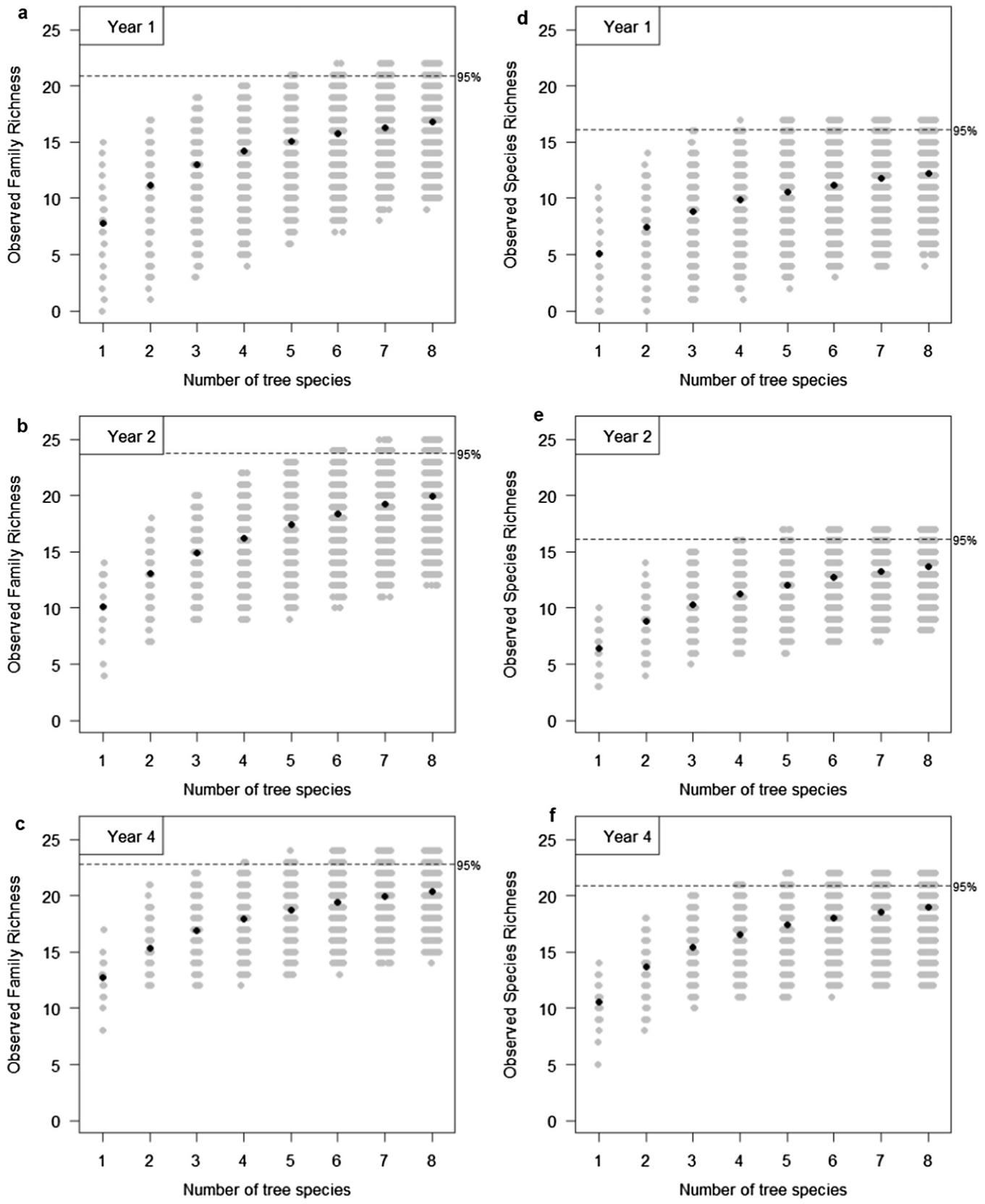


Fig. 1. nonmetric MDS plots of fauna community in logs of 20 different tree species after two (a and b) and four years (c and d) of decomposition. The fauna community consisted of centipedes, millipedes, isopods and beetles identified to family level (a and c) or centipedes, millipedes and isopods identified to species level (b and d). The polygons indicates the five samples of *F. excelsior* 2012 (red), *F. excelsior* 2013 (blue) tree species placed in the forest in 2012 (grey) and 2013 (yellow). At family level the stress values for the three-dimensional (k = 3) ordination are (a): 0.17 and (c): 0.17. At family level the stress values for the four-dimensional (k = 4) ordination are (b): 0.10 and (d): 0.11. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

In total 24,159 invertebrate specimens were collected of which 23,866 could be identified. The specimens belonged to 9 species of Chilopoda (5 families), 14 species of Diplopoda (4 families), 6 species of Isopoda (5 families) and 27 families of Coleoptera (see Supplementary Table s1). From the logs incubated in 2012, a total of 13,731 individuals were collected of which 7886, 1878 and 3967 individuals were extracted after one, two and four years of incubation, respectively. The large number of individuals found after one year of incubation can be explained by the large number of bark beetles (Scolytinae; 6757 ind.) found in that year, predominantly in *P. abies*. From the logs incubated from 2013, a total of 10,135 individuals were extracted of which 2788, 2164 and 5183 individuals after one, two and four years of incubation, respectively.

The NMDS including all tree species sampled showed that for harvest years two and four, both at family and species level, there was overlap in invertebrate composition between starting years 2012 and 2013 (see Fig. 1), even though the overlap was somewhat less for family level composition at year four. This indicates that year to year differences did not result in a very different community composition in the logs. However, the NMDS polygons of the logs of *F. excelsior,* which were placed in the forest both in 2012 and 2013, showed no overlap in community composition. When only comparing the community composition between the logs of *F. excelsior* incubated in 2012 and 2013 (see Fig. s1), we found a significant difference for year two with the invertebrates identified to family level (PERMANOVA, $F_{1,9} = 2.59$, $p < 0.05$) and for year four with the invertebrates identified to species level (PERMANOVA, $F_{1,9} = 2.92$, $p < 0.01$), but this was not the case for year two with the invertebrates identified to species level (PERMANOVA, $F_{1,7} = 1.76$, $p > 0.1$) and for year four with the invertebrates identified to family level (PERMANOVA, $F_{1,9} = 1.64$, $p > 0.1$). These



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results show that year to year differences in environmental regime and/or invertebrate species pool influence the arthropod community composition in the logs and may impact the results. Therefore, the combination with the best tree species was also calculated for the 2012 and 2013 dataset separately (data not included), to see how combining the dataset would impact the main conclusions. The results showed that

Fig. 2. Observed family richness (a–c) and observed species richness (d–f) of the tree species incubated in 2012 and 2013 for each tree species separate and when combining two up to eight tree species. Combinations were made with data from the logs after one (a and d), two (b and e) and four (c and f) years of decomposition. The black dots show the mean per tree species richness. The grey symbols represent multiple data points and these are getting wider as more and more data points, i.e. tree species combinations, are added when combining more tree species. The dotted line indicates the 95% saturation level of the richness. For the two letters behind the species name, FF means collected from and incubated in Flevoland, SF indicates collected from Schovenhorst (Veluwe) and incubated in Flevoland. 2a: the tree species with the highest richness was *P. nigra*; combinations with 2 tree species: *P. x canadensis* and *P. abies* (SF); 3 tree species: *P. x canadensis*, *P. abies* (SF) and *C. betulus*; 5 tree species: *P. x canadensis*, *P. abies* (SF), *C. betulus*, Q. robur (FF), P. tremula. 2b: the tree species with the highest richness was *C. betulus*; combinations with 2 tree species: *L. kaempferi* and *A. glutinosa*; 3 tree species: *P. abies* (SF), *A. glutinosa* and *A. pseudoplatanus*; 5 tree species: *P. abies* (SF), *A. glutinosa*, *A. pseudoplatanus*, *P. x canadensis* and *Q. robur* (SF). 2c: the tree species with the highest richness was *Q. robur* (FF); combinations with 2 tree species: *Q. robur* (FF); and *C. sativa*; 3 tree species: *Q. robur* (FF), *C. sativa* and *F. excelsior* (2012); 5 tree species: *Q. robur* (FF), *C. sativa*, *F. excelsior* (2012), *L. kaempferi* and *R. pseudoacacia*. 2d: the tree species with the highest richness was *S. alba*; combinations with 2 tree species: *S. alba* and *P. x canadensis*; 3 tree species: *S. alba*, *P. x canadensis* and *C. betulus*; 5 tree species: *S. alba*, *P. x canadensis*, *C. betulus*, *P. abies* (SF) and *B. pendula*. 2e: the tree species with the highest richness was *S. alba*; combinations with 2 tree species: *L. kaempferi* and *A. glutinosa*; 3 tree species: *L. kaempferi*, A. glutinosa and *F. excelsior* (2012); 5 tree species: L. kaempferi, A. glutinosa, F. excelsior (2012), A. pseudoplantanus and P. abies (FF). 2f: the tree species with the highest richness was *P. abies* (SF); combinations with 2 tree species: *S. sativa* and *F. excelsior* (2012); 3 tree species: *S. sativa*, *B. pendula* and *Q. robur* (FF); 5 tree species: *S. sativa*, *B. pendula*, *Q. robur* (FF), *P. avium* and *L. kaempferi*.

the general conclusion for the combinations for the separate datasets and the combined dataset were similar and based on this, the decision was made to combine the datasets. However, starting year was always indicated explicitly for *F. excelsior* in the various combinations reported below.

3.1. Tree species richness and combinations

After one year of incubation, the combination with the highest family richness using three tree species was made up of *P. x canadensis*, *C. betulus* and *P. abies* (from Schovenhorst) (Fig. 2a). Even though *P. nigra* had the highest family richness (see supplementary Table s2 and Fig. s2), it did not feature in any of the combinations with two, three or five tree species. The combination with the highest species richness consisted of *P. x canadensis*, *C. betulus and S. alba*, the latter of which had the highest single species richness (Fig. 2d). The 95% saturation level of invertebrate family and species richness was reached at family level when combining five tree species and was reached at species level when combining three tree species.

After two years, *C. betulus* had the highest observed family richness and *S. alba* remained the species with the highest species richness. The tree species combinations with the highest richness were made up of *P. abies* (from Schovenhorst), *A. glutinosa and A. pseudoplatanus* for family level (Fig. 2b) and *L. kaempferi, A. glutinosa and F. excelsior* incubated in 2012 for species level (Fig. 2e). We found that combining *L. kaempferi* and *A. glutinosa* resulted in a high observed invertebrate richness for both family and species level. Family richness reached 95% saturation when combining six tree species, while the 95% saturation for species richness was reached when combining four tree species.

After four years, the observed fauna family and species richness in angiosperm trees was higher compared to one or two years (Fig. 2), but this result was not always the case for the gymnosperms. The tree species incubated in 2013 showed a much lower amount of variation in family and species richness compared to the tree species incubated in 2012 (see supplementary Fig. s2). *Q. robur* collected from Flevoland had the highest estimated family and species richness after four years, while *Q. robur* collected from Schovenhorst had a much lower family richness (13.0 compared to 20.2) and species richness (9 compared to 17). The tree combinations with the highest invertebrate richness were made of *Q. robur* (from Schovenhorst), *C. sativa* and *F. excelsior* (2012) for family level and *C. sativa*, *B. pendula* and *Q. robur* (from Flevoland) for species level (Fig. 2c and f). The 95% saturation for family and species richness was reached when combining four tree species.

For years one and two, the combinations with the highest richness using two and three tree species consisted of a mix of gymnosperms and angiosperms, but this was not the case after four years, in which the richest combinations consisted only of angiosperms. All combinations with the highest richness using five tree species consisted of a mix of angiosperms and gymnosperms.

3.2. Combinations across all sampling years

When combining the data from all the harvest years, it became clear that variation in decomposition stage had an important influence on the invertebrate richness in the dead wood species combinations. The richest ones were made when combining trees from the different sampling years. The combinations using two or three tree species were made with logs of *P. nigra* after one year, *A. glutinosa* after two years of and *Q. robur* (from Flevoland) after four years. Surprisingly, when combining five tree species neither *Q. robur* (from Flevoland) after four years nor *P. nigra* after one year were selected. Instead a combination of *P. x canadensis* after one year, *P. abies* (from Schovenhorst) after one year, *A. glutinosa* after two years, *F. excelsior* (2012) after four years and *P. nigra* after four years resulted in the highest family richness (Fig. 3a).

At invertebrate species level, the most species-rich combinations with two tree species were made with logs of *P. x canadensis* after one year of incubation and logs of *P. abies* (from Schovenhorst) after four years of incubation. Neither *P. abies* (from Schovenhorst) nor *P. x canadensis* were used in the combination of five tree species with the highest combined species richness. This combination consisted of logs of *P. abies* (from Flevoland) after one year, *C. betulus* after one year, *Q. robur* (from Flevoland) after four years, *L. kaempferi* after four years and *C. betulus* after four years of incubation (Fig. 3b). Both at family and species level, the combinations consisted of a mix of angiosperms and gymnosperms.

3.3. Combinations based on phylogenetic distance

To test whether tree phylogeny (see supplementary Fig. s4) could be used as a predictive tool to form combinations of dead wood with high taxonomic invertebrate richness, we analyzed the relationship between phylogenetic distance and the combined family and species richness of a pair of tree species (see supplementary Fig. s3). The mantel test showed no relationship between the two factors for any of the years, neither at family nor at species level (see Table s3).

4. Discussion

The aim of this study was to investigate the relationship between dead wood and associated fauna, and especially which combinations of tree species and wood decay stages promote diversity within four predominant dead wood invertebrate clades. Our first hypothesis was that invertebrate richness for a given number of tree species would be promoted by mixing different tree species with different forest incubation periods. We did not find a particular tree species that always supported a high richness. The tree species combined in the best set with respect to invertebrate richness differed among the sampled years, invertebrate taxonomic level as well as the number of tree species considered. Nevertheless, the data showed that a mix of gymnosperms and angiosperms resulted in the richest combinations after one and two

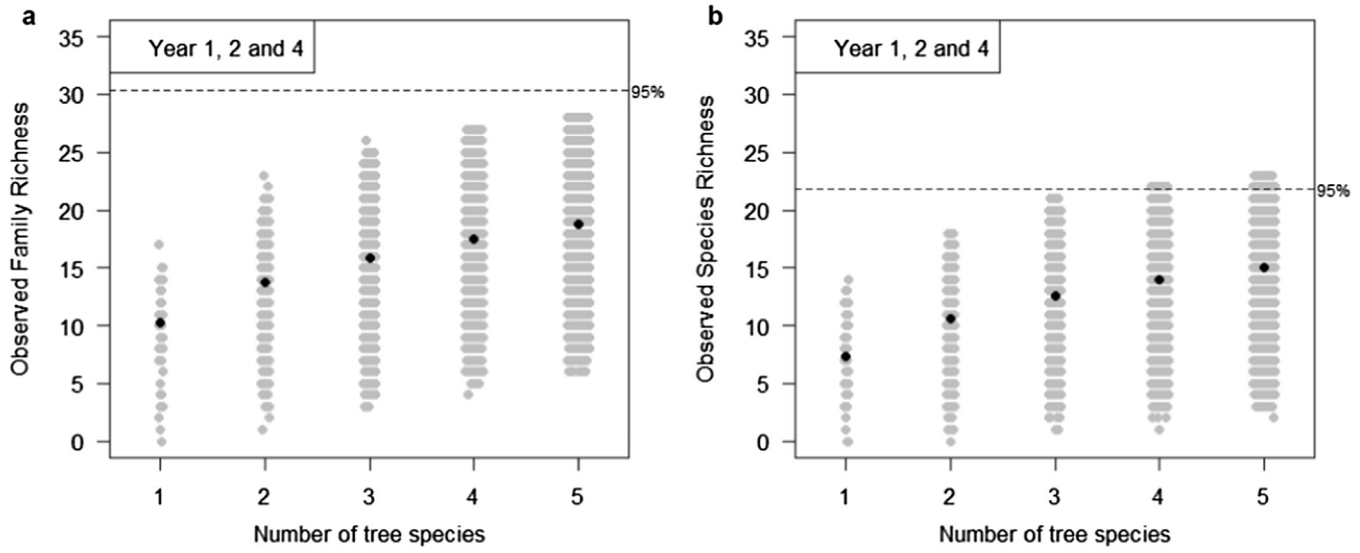

Fig. 3. Observed family richness (a) and observed species richness (b) of the tree species from the 2012 and 2013 batches for each tree species separate and when combining two up to five tree species. Combinations were made with data from the logs after one, two and four years of decomposition. The black dots show the mean species richness per tree. The grey symbols represent multiple data points and these are getting wider as more and more data points, i.e. tree species combinations, are added when combining more tree species. The dotted line indicates the 95% saturation level of the richness. For the two letters behind the species name, FF means collected from and incubated in Flevoland, SF indicates collected from Schovenhorst (Veluwe) and incubated in Flevoland. 3a: the tree species with the highest richness was *Q. robur* (FF) year 4; combinations with 2 tree species: *P. nigra* year 1 and *Q. robur* (FF) year 4; 3 tree species: *P. nigra* year 1, *A. glutinosa* year 2 and *Q. robur* (FF) year 4; 5 tree species: *P. x canadensis* year 1, *P. abies* (SF) year 1, *A. glutinosa* year 2, *F. excelsior* (2012) year 4 and *P. nigra* year 4. 3b: the tree species with the highest richness was *P. abies* (SF) year 4; combinations with 2 tree species: *P. x canadensis* year 1 and *P. abies* year 4; 3 tree species: *P. x canadensis* year 1, *P. abies* (SF) year 2 and *C. betulus* year 4; 5 tree species: *P. abies* (FF) year 1, C. betulus year 1, *Q. robur* (FF) year 4, *L. kaempferi* year 4 and *C. betulus* year 4.

years of decomposition. Besides, the data clearly showed that variation in length of forest incubation, a proxy for decay stage and associated differences in resources and microclimate, was important to promote diversity associated with dead wood in our forest site. When combining the data from all periods, the best combinations in terms of invertebrate family or species richness all consisted of at least one tree species from each period, which strongly supports our first hypothesis. Our second hypothesis was that invertebrate richness would increase up to a saturation point with more dead wood types added. Our results indeed showed 95% saturation in the invertebrate richness when increasing the number of trees to five species or more, included in the dead wood combination. However, our third hypothesis, that distance in tree phylogenetic position could be a predictive tool for increasing invertebrate richness in combinations of tree species, was not supported by our data. Together these results can be used to improve management strategies, i.e. increase resource and microhabitat heterogeneity for dead wood associated invertebrates.

The single tree species with the highest invertebrate richness was not necessarily selected in the richest dead wood species combinations. Instead, invertebrate-rich combinations consisted of tree species with the most contrasting single species compositions. After one and two years, combinations of angiosperms and a gymnosperm resulted in the highest invertebrate richness. These results confirm those of Gossner et al. (2016), for beetles only, who also showed that species rich combinations at an early stage of decomposition usually consist of an angiosperm and a gymnosperm. This may be explained by the large difference in resources and defenses of gymnosperms and angiosperms, which results in differences in community composition (Weedon et al., 2009; Kahl et al., 2017). Meta-analyses (Weedon et al., 2009; Pietsch et al., 2014) showed that in general gymnosperms have a lower decay rate compared to angiosperms, as related to higher overall differences in resource and defence traits between angiosperms and gymnosperms. This difference in decay rate may explain our result that invertebrate richness increased in richness over time within angiosperms, but not within gymnosperms. Other research also found that the species richness in angiosperms increased with decomposition (Hammond et al., 2004; Saint-Germain et al., 2007; David and Handa, 2010) and that this was not always the case for gymnosperms (Saint-Germain, et al., 2007; Ulysen and Hanula, 2010). As decay progresses, logs of different species tend to converge in trait composition (Zuo et al., 2014) and this convergence may also explain why the richest combinations after four years of decomposition did not consist of a mix of gymnosperms and angiosperms; however further research is required to test this hypothesis.

Tree species identity was not the only factor determining the highest invertebrate richness in tree species mixtures. Which invertebrate clades were considered and to which taxonomic level the invertebrates were identified also played a role. This indicates that a single tree species that provides a high variety of microhabitats and resources for beetle species may not necessarily provide this for other invertebrate groups that live in and around dead wood. Further research on improving the spatial heterogeneity of the forest floor for dead wood-associate species should therefore not be restricted to one particular group of invertebrates (Seibold et al., 2015). Besides, temporal variations in environmental conditions should also be taken into account. We found dissimilarity in the community compositions between *F. excelsior* logs of the same decay stage, i.e. the same duration of forest incubation, extracted from the same forest stand in 2012 versus in 2013. Differences in sun exposure, temperature and rainfall (Seibold et al., 2015 and reference therein, Gossner et al., 2016) between the sampling years may have caused differences in the arthropod population (i.e. “species pool”) in the forest and community composition inside the logs. These year-to-year abiotic differences may have influenced the colonisation order through priority effects (Weslien et al., 2011; Victorsson, 2012) and with that the abundance and community composition inside the logs. These differences in colonisation may not only influence the community composition in the first year, but may have an impact on the whole colonisation and community assembly trajectory, the growth and reproduction of the invertebrates and their interactions. This implies that, which combinations of tree species and decay stages are the richest or poorest in invertebrates, is variable in time. This suggests that, in management terms, spreading logging activity of a given

species over subsequent years would further add to stand-scale invertebrate diversity not only by mixing decay stages (see below), but also by embracing year-to-year environmental variation.

When combining the data from all 20 tree species from the three sampling years, the combinations with the highest invertebrate richness consisted of at least one tree species from each year. This clearly shows that besides variation in abiotic conditions between years, decay period is also important for promoting diversity associated with dead wood. Especially in logs lying on the ground for one year, we observed a high number of bark beetles, which feed on the phloem of the bark. It is generally known that this dominant group of invertebrates feeds predominantly on freshly fallen wood (Rose et al., 1994). Their presence also facilitates the colonisation by other invertebrates, making the bark accessible to other species due to their wood-boring behaviour (Zuo et al., 2016). This may partly explain the observed shift in species composition and increase in species richness for angiosperms after four years as compared to one year, which may be explained by changes in available habitat and resources (Zuo et al., 2014). The xylem, inaccessible to fauna in the first years of decay, was colonized by wood boring invertebrates, while the moss cover on the bark provided additional habitat for a variety of invertebrates and their associated predators (Siitonen, 2001). Furthermore, resource availability inside the logs changes due to the breakdown of recalcitrant material and the growth of fungi, which in turn attract mycetophagous beetle families (Siitonen, 2001; Ulyshen & Hanula, 2010; Lee et al., 2014). This succession in community composition shows the importance of an annual availability of different decay stages in forests (Grove, 2002; Lee et al., 2014). Dead wood in different stages of decay is therefore a critical factor to be considered when looking at which combinations of log types, via microhabitat heterogeneity, provide the highest invertebrate diversity (De Groot et al., 2016). While fast decomposing wood quickly provides new habitat for species, slower decaying wood provides habitat for a long period of time (Grove, 2002).

Our analyses showed that invertebrate species richness saturates quickly in dead wood mixtures when increasing the number of tree species. The 95% saturation point for the centipedes, millipedes and isopods was reached after combining three to four different tree species. When including the beetle families and calculating the best combination of dead wood, the saturation point was reached after four to six tree species. This shows that an increase in the number of taxa may also lead to an increase in the number of tree species required to reach the saturation point, since species differ in their (narrow) habitat and resources preferences. This indicates that the saturation point for invertebrate diversity depends on the species pool in the forest and the number of species. Furthermore, the combinations of tree species and decay stages that yield the highest invertebrate richness will probably also differ between regions, depending on the species pool. This means that, at this stage, it is uncertain how applicable the specific combinations of tree species and decay stage and saturation highlighted here are for forests outside Flevoland. Nevertheless, in general terms, our results and those of Gossner et al. (2016) together showed that, in the first two years, the best combinations of tree species were made when mixing gymnosperms and angiosperms. However, their combined absolute effect on invertebrate species richness strongly depends on which tree species are selected in the mixture.

Together the results of this study indicate that heterogeneity in dead wood on the forest floor, i.e. variation in tree species and the decay stages of single wood species, together with year-to-year variation in microclimate in a forest, all interact and contribute to the overall dead wood invertebrate richness. This adds to our current understanding of the role of dead wood for invertebrate (especially saproxylic beetle) diversity, as both the quantity (i.e. volume) of dead wood (Schiegg, 2000; Grove, 2002; Lassauce et al., 2011), its diameter (Schiegg, 2001; Grove, 2002) as well as its quality (Similä et al., 2003; Jacobs et al., 2007) including stage of decomposition (Lee et al., 2014) have been shown to be important drivers of arthropod diversity in a given forest environment. Our findings could help improve management decisions when it comes to dead wood availability and microhabitat heterogeneity in temperate forests around the world. For instance, an increase in the number of tree species in forest plantations will increase the variability in dead wood and associated resources and microhabitat, which will facilitate invertebrate richness. Moreover, not only would it be recommendable, in managed forest, to go for tree species mixtures of a particular combination based on information on dead wood characteristics, it would also be advisable to spread the logging of trees through the years. This way the dead wood in the forest will cover a variety of resources and habitats which will allow for natural succession of wood-associated invertebrates communities, by making sure that different microhabitat and resources are simultaneously available. However, the most important effect of forest management on invertebrate diversity will be the presence and volume of dead wood *per se*, as in many managed forests large amounts of dead wood are removed.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2019.03.029.

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