Chapter V

Diversity of macro-detritivores in dead wood is influenced by tree species, decay stage and environment

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Abstract

Diplopoda (millipedes) and Isopoda (woodlice) are among the most abundant macro-detritivores in temperate forests. These key regulators of plant litter decomposition are influenced by habitat and substrate quality, including that of dead wood. Dead wood provides shelter and resources to macro-detritivores, but the relative effects of tree species, wood decay stage, forest environment and their interactions on macro-detritivore communities are poorly known. To unravel these effects, we combined a reciprocal field incubation experiment and direct field sampling to compare the Diplopoda and Isopoda communities in logs of silver birch (Betula pendula) and Norway spruce (Picea abies) in two contrasting sites in terms of soil texture, pH, fertility and microclimate. We found: (1) a curvilinear relationship between wood decay stage and abundance of Diplopoda and Isopoda, by using wood density as a measure for the decay stage; (2) the pH of dead wood was a good predictor of wood decay stage in a site with pH close to neutrality but not in an acidic site; (3) Diplopoda and Isopoda community composition on different tree species converged during the decay process, consequently tree species are more important in the substrate selection of macro-detritivores at the beginning of their dead wood decomposition; (4) tree species, the growing environment of the trees and the decomposition environment of the logs strongly determined Diplopoda and Isopoda community composition in dead wood, these drivers of macro-detritivore communities interacted with each other and with the wood decay stage. Thus, when trying to understand and predict future patterns of macro-detritivore diversity under regimes of changing land-use and climate, these interactions should be taken into account. An important next step will be to quantify the feedback of macro-detritivore community composition to dead wood decomposition itself. This feedback may be better understood from the combination of (1) the complex interactions of tree species, wood decay stage and forest environment on the macro-detritivore community and (2) the functional traits of these macro-detritivore species. A better knowledge about these feedbacks can help in predicting carbon storage and nutrient cycling functions of dead wood in forests differing or changing in tree species composition and abiotic environment.

Introduction

Woody debris is an important and abundant component of forest ecosystems where it fulfils crucial ecological functions, e.g. as a habitat for myriad organisms and energy flow and nutrient cycling (Harmon et al., 1986). Decomposition is one of the main loss pathways of woody debris, having a profound effect on the global carbon cycle (Cornwell et al., 2009). The presence of detritivore macro-fauna has been shown to accelerate the decomposition rate of plant leaf litter by 1.6-66% depending on litter type and climate (González and Seastedt, 2001; Vasconcelos and Laurance, 2005; Riutta et al., 2012). In a reciprocal incubation experiment, Anderson (1973a, b) found that leaf litter breakdown and leaching of chemical components to soil were influenced by tree species, soil animals and environment. Macro-fauna are also important for the decomposition of woody debris (Stokland et al., 2012; Ulyshen and Wagner, 2013), but this role has been less studied in quantitative terms than for leaf litter.
Diplopoda (millipedes) and Isopoda (woodlice) are among the most abundant macro-decomposers in temperate forests, and have been found to play a significant role in decomposition and nitrogen release (Wall et al., 2008; David and Handa, 2010; Vos et al., 2011). As saprophages, they participate in decomposition by consumption, fragmentation and transformation of dead organic material and by moving it to biologically more favorable microclimate conditions (Sutton et al., 1980; Rushton and Hassall, 1987; David and Handa, 2010), thereby stimulating microbial colonization, growth and activity and, hence, litter decomposition. By producing faeces, the substrate quality for microbes will be changed (usually improved), and macro-detritivores thereby influence microbial functioning (Teuben and Roelofsma, 1990). Macro-detritivores, especially Isopoda, may also feed on microbes including decomposing fungi, like other soil fauna groups such as Collembola and Acari, thereby also affecting wood debris turnover (Bradford et al., 2002; Crowther et al., 2011). In temperate woodlands macro-fauna have been shown to be most important for the breakdown of more recalcitrant litter types (Riutta et al., 2012), but they prefer easily decomposable litter, and so are thought to feed on recalcitrant litter only in later stages of decomposition (Paoletti and Hassall, 1999; Vos et al., 2011).

In forests the diversity of macro-detritivores is influenced by several factors (Figure 1). One is tree species, which determines microclimatic conditions and also the quantity and quality of dead organic substrate, i.e. leaf litter and dead wood, for macro-detritivores (Stasiov et al., 2012). While much is known about the effect of leaf litter quantity and quality on macro-detritivore communities (Zimmer et al., 2005; de Oliveira et al., 2010; Gessner et al., 2010), less is known about how differences in dead wood traits affect macro-detritivores. It is known that an increase in the amount of dead wood on the forest floor enhances macro-detritivore diversity and abundance (Jonsson et al., 2005; Stokland et al., 2012). Woody debris can be both a resource and a hiding place, which might be tree species specific. However, whether and how tree species differ in these effects on macro-detritivores is poorly known.

**Figure 1.** Conceptual scheme showing the relationship between tree species, wood traits, decay stage, soil environment and macro-detritivore community composition. The soil environment has a direct effect on community composition (via abiotic conditions; solid line), but also an indirect effect as it may determine forest tree species composition (dashed line) and wood trait values (dashed line). The trait box represents the hypothesis that community composition depends on wood traits. The trait values will change with wood decay and the trait variance will decrease with decay stage, leading to a convergence of community composition over time.
Physical and chemical traits of wood, such as wood density and nutrient content, have been shown to influence the composition of saprophagous insect assemblages (Stokland et al., 2012). These traits affect the accessibility of wood to saprophagous species. As traits differ between tree species, they may account for tree species-specific effects on macro-detritivore communities. During the first stage of decomposition bark traits are probably important as they determine the quality of the wood as shelter site and resource for macro-detritivores (Franceschi et al. 2005; Stokland et al., 2012). Wood properties that are important in explaining variation in associated community composition of invertebrates do not only cohere with tree species, but also with the stage of decay of wood and the environment in which decomposition takes place (Harmon et al., 1986; Stokland et al., 2012). Whilst previous studies on dead wood traits focussed mostly on their role in decomposition (Weedon et al., 2009; Van Geffen et al., 2010; Freschet et al., 2012), some studies indicated that the decay stage seems to influence macro-detritivore community composition (Grove, 2002; Jonsell et al., 2007; Ulyshen and Hanula, 2010). As wood decay progresses, the tree species identity seems to become less important for the substrate selection of macro-detritivores, presumably because of convergence of wood properties, and their community composition on different host trees becomes more similar (Grove, 2002; Jonsell et al., 2007).

Soil macro-fauna diversity is heterogeneously distributed and this spatial variation in species composition is partly caused by the spatial heterogeneity of environmental conditions (Ettema and Wardle, 2002; Berg, 2012). Among the environmental factors that affect the distribution of macro-detritivores, the predominant ones are thought to be soil type, which strongly affects soil moisture regime, soil chemistry (especially pH and Ca content) and overall substrate availability. These factors may influence the micro-environment in dead wood as well as the macro-faunal species pool at a site. Thus, we also expect the soil surface environment to be important in determining macro-fauna communities in dead wood (Figure 1). Based on these previous findings, it can be expected that the abundance and community composition of Diplopoda and Isopoda will differ between tree species, decay stage and forest soil environment, with possible interactions between these factors.

In this study we aim to experimentally unravel these interactions by comparing the macro-detritivore community composition in dead wood of two contrasting tree species, one deciduous species (Betula pendula) and one coniferous species (Picea abies), both in a range of decay stages and in two contrasting forest environments: a dry, acid sandy soil versus a moist, clay soil with a pH close to neutrality. Specifically, we hypothesize that:

(1) Different tree species, through variation in dead wood and bark traits, will host different Diplopoda and Isopoda communities.

(2) Diplopoda and Isopoda abundance and species composition in dead wood will change with the progression of its decomposition, as indicated by a decline in wood density (Harmon et al., 1986; Freschet et al., 2012). Their abundance will increase as more decayed, softer wood becomes accessible to the animals, with a higher moisture content and more available resources (nutrients, carbon and microbes). But as wood decay progresses, different tree species will become more similar in community composition, because structural and chemical components that inhibit access to wood will breakdown during decomposition
and microbes that are an important additional food source will increase in abundance.

(3) The environment in which dead wood decays will interact with the factors decay stage (see hypothesis 2) and tree species (see hypothesis 1) as trees and logs of different species may influence site conditions, such as temperature, moisture and nutrient availability, with consequences for the macro-detritivore species pool and feedback to log moisture and nutrition status and decomposition rate.

While some of these individual factors that influence community composition of Diplopoda and Isopoda in dead wood have been studied before, this is, to our knowledge, the first experimental study that combines and unravels all these factors simultaneously.

**Material and Methods**

*Study area, tree species and logs*

Two contrasting temperate sites were selected to represent two predominant forest types in the Netherlands: (1) the Hollandse Hout forest plantation in Flevoland, called Flevopolder (F) (52.46 N, 5.42 E) and (2) a forest estate in the Veluwe region, called Schovenhorst (S) (52.25 N, 5.63 E). Both located in the central part of the Netherlands. Site F was reclaimed from the former Zuiderzee in the 1960s. This relatively young soil consists of marine clay and is calcareous, moist and fertile, with a pH close to neutrality. This forest site mainly consists of monospecific plantations used for commercial forestry. In contrast site S has a sandy and podzolic soil that is well-drained. The soil is acidic and has low fertility. Site details are given in Cornelissen et al. (2012).

Two tree species were compared, the broad-leaved *Betula pendula* Roth and the conifer *Picea abies* (L.) H. Karst. Dead logs in different decay stages of these two species could be easily found and identified on the soil surface of monoculture plantations in each site. Thirty-five logs with a diameter between 8 and 10 cm (bark included) and a broad spectrum of decay stages were collected from each tree species in each location; 15 logs were 50 cm long, the other 20 logs were 90 cm long. In total 140 logs (35 logs × 2 species × 2 sites) were collected (Figure 2). Decay stage was coarsely estimated by eye during collection, in order to embrace the widest possible range of decay stages.

*Reciprocal incubation*

For 80 logs (20 logs × 2 species × 2 sites) each 90 cm long, each was sawn into two pieces of 40 cm and one piece of 10 cm. The 10 cm piece was used for pre-incubation wood trait analyses (see *Wood analyses*). One of the 40 cm pieces was incubated in the original site and the other simultaneously in the contrasting site. As a result 160 logs (20 logs × 2 species × 2 collection sites ×2 incubation sites) were incubated from the middle of March 2012 (Figure 2). The incubation plot at site F is a relatively light-open *Populus x canadensis* Moench stand with a sumptuous herb layer dominated by the nitrophilic herbs *Urtica dioica* L. and *Galium aparine* L. The incubation plot at site S is a *Larix kaempferi* (Lambert) Carrière stand that is also relatively light-open.
There is a low and dense ground layer of predominantly the acidophilic grass *Deschampsia flexuosa* (L.) Trin. intermingled with mosses and patches of the dwarf shrub *Vaccinium myrtillus* L. More information about the two incubation sites is given in Cornelissen et al. (2012).

The two 40 cm pieces from each log were carefully put in labelled plastic bags separately for transport to the incubation sites, thus at least some of the animals were assumed to still remain in the wood at the start of incubation. Bark that fell off the wood during transport was placed back on the wood. The logs were put in the plots in a random order, at the same compass orientation, approximately 30 cm apart and with good soil contact. The logs were incubated for 2 months in order to give animals the chance to colonize the wood (or for animals still in the wood to leave). Logs that were incubated at the same site made it possible to specifically study the effect of tree species and the collection site. Logs of the same tree and collection site that were incubated in two contrasting sites were suitable for comparing the effect of the incubation site (Figure 2). After 2 months (mid May 2012) the Diplopoda and Isopoda in the logs were sampled for identification by extraction from the wood (see *Animal extraction*). Animals present in wood and under the bark were sampled, those found on the ground and between bark and soil were excluded.

![Diagram of experimental setup](image)

**Figure 2.** Overview of the experimental setup. The reciprocal incubation is shown in the upper part of the figure. From each log one part was incubated in the site of origin, one part was incubated in the contrasting site, and the small disc was used to measure pre-incubation wood traits. Direct sampling is shown in the lower part of the figure. Black and white symbols represent the two tree species.

**Direct sampling**

For 60 logs (15 logs × 2 species × 2 sites) each 50 cm long, each was sawn by hand into pieces of 40 cm and 10 cm length (Figure 2). The 10 cm pieces were used for wood trait analyses (see *Wood analyses*). The 40 cm pieces were used for direct animal extraction (see *Animal extraction*). Sawing was done above a large tray to catch the animals that came out of the wood immediately.
**Animal extraction**

Percentages of moss- and bark cover of the logs were visually recorded in the field before animal extraction, as these parameters were considered potentially important co-variables influencing invertebrate communities. In the field, the log was put into a large tray, after which Diplopoda and Isopoda were collected alive using forceps and pooters. Peeling off the bark, and shaking and fragmenting the wood with a screwdriver helped to extract animals deeper inside the logs. The animals were transferred to vials with 70% ethanol for identification and counting later on. After transport to the laboratory at VU University, Amsterdam the logs were dissected and checked again thoroughly for any animals that had been missed in the field.

Hand sampling of the logs was very time consuming and had to be completed before the animals might die. Therefore, not all the 160 logs of the reciprocal incubation were processed for animal extraction: 34 B. pendula and 35 P. abies from site F, and 34 B. pendula and 36 P. abies from site S were randomly selected and processed.

**Wood analyses**

The 10 cm wood subsamples were taken to the laboratory for wood trait analyses.

Field moist weight of the wood pieces was determined shortly after they had been taken to the laboratory, using a Sartorius laboratory scale to the nearest gram. Water-saturated weight was measured by completely submerging wood pieces in a plastic bag filled with 1.5 L distilled water, separately. The bags were stored in a cool room (at ca. 5 °C) for three days to fully saturate, and were weighed after briefly blotting dry the outside with tissue paper.

Wood volume of a water-saturated wood piece was measured via water displacement in a water column in a 2L measuring cylinder after immersion to the nearest ml. The volume of water displaced equals the volume of the water-saturated wood piece. Wood dry weight was measured after drying the wood pieces in an oven at 65°C to constant weight. Wood density was calculated by dividing the wood dry weight by its volume.

Wood pH of subsamples was measured following Cornelissen et al. (2006; 2011). From both ends of the dry wood pieces fine wood powder was produced using a hand file and mixed with 1.2 mL demineralized water in a 2.5 mL Eppendorf tube (volume ratio 1:8). After 1 hour of shaking at 250 rpm the tubes were centrifuged for 5 minutes at 13,000 rpm and the supernatant measured using a narrow (5 mm diameter) SenTix Mic electrode connected to an Inolab Level 2 pH meter (both: WTW, Weilheim, Germany). We calibrated the pH meter against buffer solutions (pH 4 and 7) before each measurement series.

**Statistics**

To compare the difference in macro-detritivore community composition, we used the Bray-Curtis similarity index (Bray and Curtis, 1957):

\[ BC_{ij} = 2C_{ij} / (S_i + S_j) \]
where \( C_{ij} \) is the sum of the lesser values for only those species in common between both logs, \( S_i \) and \( S_j \) are the total number of specimens of Diplopoda and Isopoda counted in both logs. Thus, if two logs had no species in common, the index would be 0; if they shared all the same species in exactly the same numbers, the index would be 1. When two logs contained only zero values, the index could not make a reliable comparison. For this reason one individual of the isopod species \textit{Trichoniscus pusillus} was added to those logs. This small species had a really high abundance so adding one individual did not really affect the results, but resulted in a valid test without errors.

We employed a non-parametric multivariate analysis of variance (PERMANOVA) on Bray-Curtis distances when comparing Diplopoda and Isopoda community composition in logs of direct sampling (Anderson, 2001).

To test the effects and interactions of tree species, wood density, collection site and incubation site, we made a predictive model for differences in Bray-Curtis similarity patterns for animal composition on decaying logs under the null hypothesis of no interaction effects (Table 3a). All the comparisons take wood density (decay stage) into account. For the other factors, we assumed the effect of incubation site to be the strongest because of the expected strong influences of the regional species pool and microclimate on wood invertebrate composition. After that, we expected the effect of tree species (plant traits) to be stronger than that of log collection site on wood invertebrate composition (cf. Kattge et al. 2011). From dark to light shading (see Table 3a) the strength of the indicated factors on species composition are expected to increase, so Bray-Curtis similarity is expected to decrease. We combined the predictive model and actual results to test the effects of different combinations of drivers of invertebrate species composition, where strong deviations from the expectation were interpreted as (non-additive) interactions.

To test the hypothesis of convergence in species composition at late decay stage, the logs were classified into relatively early and late decay stage groups (based on wood density) using non-hierarchical clustering, which allows to split data into a predefined number (\( K \)) of clusters (Legendre and Legendre 1998). \( K \)-means clustering split the logs into a relatively early and a later decay stage cluster, with a higher (\textit{B. pendula} > 0.330 g/cm\(^3\), \textit{P. abies} > 0.285 g/cm\(^3\)) and lower (\textit{B. pendula} < 0.330 g/cm\(^3\), \textit{P. abies} < 0.285 g/cm\(^3\)) wood density, respectively. We quantified the similarity in macro-detritivore community composition between logs of (1) early decay stage of two tree species, (2) late decay stage of two tree species. We employed the Wilcoxon–Mann–Whitney U-test to compare the difference in Bray-Curtis similarity in species composition between early and late decay stage.

We performed a polynomial regression to test the relationship between abundance of Diplopoda and Isopoda in the wood (response or dependent variable) and wood density (independent variable). We compared the quadratic (second-power) regression model and cubic (third-power) regression model with an ANOVA table. As the cubic model did not significantly improve the fit (\( P > 0.05 \)), the quadratic regression was used.

To test the relationship between wood pH (response or dependent variable) and wood density (independent variable) we used linear regression.
Statistical analyses were performed using the R language version 3.0.3 (R Core Team, 2014). We used the kmeans function for cluster analysis, the adonis function in the vegan package for PERMANOVA (Oksanen et al. 2013), the metaMDS function in the vegan package (Oksanen et al. 2013) for non-metric multidimensional scaling (NMDS), the lm function for polynomial and linear regression and the wilcox.test function for the Wilcoxon–Mann–Whitney U-test.

Results

In total we observed 1015 individuals belonging to 10 species of Diplopoda and 4189 individuals belonging to 6 species of Isopoda. The Diplopoda and Isopoda species list and total abundance of each species are given in Table 1.

Table 1. Diplopoda and Isopoda species-list with total numbers sampled from logs collected at the two study sites Flevopolder (FLEVO) and Schovenhorst (SCHOV).

<table>
<thead>
<tr>
<th>Species</th>
<th>Collected in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FLEVO</td>
</tr>
<tr>
<td><strong>Isopoda</strong></td>
<td></td>
</tr>
<tr>
<td><em>Trichoniscus pusillus</em> Brandt 1833</td>
<td>2789</td>
</tr>
<tr>
<td><em>Oniscus asellus</em> Linnaeus 1758</td>
<td>283</td>
</tr>
<tr>
<td><em>Philoscia muscorum</em> (Scopoli 1763)</td>
<td>199</td>
</tr>
<tr>
<td><em>Trachelipus rathkii</em> (Brandt 1833)</td>
<td>114</td>
</tr>
<tr>
<td><em>Porcellio scaber</em> Latrell 1804</td>
<td>19</td>
</tr>
<tr>
<td><em>Armadillidium pulchellum</em> Zenker, 1799</td>
<td>0</td>
</tr>
<tr>
<td><strong>Diplopoda</strong></td>
<td></td>
</tr>
<tr>
<td><em>Cylindroiulus punctatus</em> Leach 1815</td>
<td>279</td>
</tr>
<tr>
<td><em>Julus scandinavius</em> Latzel 1884</td>
<td>151</td>
</tr>
<tr>
<td><em>Brachydesmus superus</em> Latzel 1884</td>
<td>105</td>
</tr>
<tr>
<td><em>Polydesmus denticulatus</em> C.L. Koch 1847</td>
<td>76</td>
</tr>
<tr>
<td><em>Cylindroiulus britannicus</em> (Verhoeff 1891)</td>
<td>38</td>
</tr>
<tr>
<td><em>Proteroiulus fuscus</em> (Am Stein 1857)</td>
<td>19</td>
</tr>
<tr>
<td><em>Brachyiulus pusillus</em> (Leach 1814)</td>
<td>7</td>
</tr>
<tr>
<td><em>Craspedosoma rawlinsi</em> Leach 1814</td>
<td>6</td>
</tr>
<tr>
<td><em>Polydesmus inconstans</em> Latzel 1884</td>
<td>5</td>
</tr>
<tr>
<td><em>Polydesmus angustus</em> Latzel 1884</td>
<td>1</td>
</tr>
</tbody>
</table>

Effect of tree species on macro-detritivores

There was a significant effect of tree species (*B. pendula* versus *P. abies*) on macro-detritivore abundance and community composition in the logs that were directly sampled (PERMANOVA, tree species *P* < 0.05, Table 2, Figure 3). In logs that were reciprocal incubated, Bray-Curtis similarity values for species composition were smaller in wood density plus tree species (D+SP) comparisons than in wood density only (D) comparison (Table 3b).
Table 2. Permutational Multivariate Analysis of Variance (PERMANOVA) on Bray-Curtis similarity distances for Diplopoda and Isopoda abundance and community composition in dead logs of different tree species, wood density and collection site in the logs that were directly sampled.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>1</td>
<td>0.460</td>
<td>0.460</td>
<td>2.119</td>
<td>0.038*</td>
</tr>
<tr>
<td>Wood density (WD)</td>
<td>1</td>
<td>1.205</td>
<td>1.205</td>
<td>5.556</td>
<td>0.001***</td>
</tr>
<tr>
<td>Collection site (CS)</td>
<td>1</td>
<td>4.026</td>
<td>4.026</td>
<td>18.56</td>
<td>0.001***</td>
</tr>
<tr>
<td>Species × WD</td>
<td>1</td>
<td>0.332</td>
<td>0.332</td>
<td>1.531</td>
<td>0.150</td>
</tr>
<tr>
<td>Species × CS</td>
<td>1</td>
<td>0.362</td>
<td>0.362</td>
<td>1.670</td>
<td>0.106</td>
</tr>
<tr>
<td>WD× CS</td>
<td>1</td>
<td>0.584</td>
<td>0.584</td>
<td>2.695</td>
<td>0.013*</td>
</tr>
<tr>
<td>Species × WD× CS</td>
<td>1</td>
<td>0.499</td>
<td>0.499</td>
<td>2.299</td>
<td>0.035*</td>
</tr>
<tr>
<td>Residuals</td>
<td>51</td>
<td>11.06</td>
<td>0.217</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>18.53</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Two-factor non-metric MDS plot of Diplopoda and Isopoda communities in dead logs collected at two sites (with direct sampling). Betula = *Betula pendula* Picea = *Picea abies*; F = site Flevopolder, S = site Schovenhorst.

Effect of wood decay stage on macro-detritivores

Wood density was used as a measure for the decay stage of dead wood. The lower the density of wood, the more it has been decomposed. The direct sampling showed a significant wood density effect on abundance and community composition (PERMANOVA, density $P < 0.001$, Table 2, Figure 3). However, wood density interacted with collection site, and the interaction between tree species and collection site was significant (Table 2, Figure 3).

A curvilinear relationship was found between the wood density and the total abundance of Diplopoda and Isopoda (Figure 4). There was a significant quadratic relationship between the wood density and the total number of Diplopoda and Isopoda in the logs of *P. abies* for both sites (Figure 4c, d). The relationship was not significant (at 0.05 probability threshold) for *B. pendula* (polynomial regression; logs from F: n = 15, $R^2 = 0.21$, $P = 0.097$; logs from S: n = 14, $R^2 = 0.03$, $P = 0.344$; Figure 4a, b).
At site F, similarity in fauna composition between tree species for late decay logs (0.320 ± 0.017, mean ± SE) was significantly higher ($P = 0.002$) than for early decay logs (0.164 ± 0.049). At site S, similarity between tree species for late decay logs (0.452 ± 0.040) was also significantly higher ($P = 0.046$) than for early decay logs (0.341 ± 0.025). Thus, the similarity of macro-detritivore composition between tree species was significantly higher for logs of late decay stage than of early decay stage, at both sites.

Wood pH changed with the decay process (Figure 5). The decrease in wood pH with decay stage was significant in logs from site F (Figure 5a, c), which has a soil pH close to neutrality. The wood pH range of the logs from the more acidic site S was much lower (Figure 5b, d) and was not related to wood density (linear regression; $B.\, pendula$: $n = 20$, $R^2 = 0.06$, $P = 0.296$; $P.\, abies$: $n = 20$, $R^2 = 0.01$, $P = 0.800$). The low pH of the sandy soil of site S was reflected in the logs found there. The pH of the logs here was already much lower than that at site F, and close to soil pH. Therefore, it seems that the more decayed logs could not become a lot more acidic, and as a consequence the range of pH variation during the decay process was much smaller at site S and decoupled from wood density.

Effect of the environment

The directly sampled logs showed a significant collection site effect on abundance and community composition of macro-detritivores (PERMANOVA, collection site $P < 0.001$, Table 2, Figure 3). However, wood density interacted with collection site, and the interaction of tree species, wood density and collection site was also significant (Table 2, Figure 3).
In the reciprocal incubation experiment, Bray-Curtis similarity values for fauna composition were smaller in logs that differed in wood density plus collection site (D+CS), and wood density plus incubation site (D+IS) than in logs that differed in wood density only (D) (Table 3).

The Bray-Curtis similarity patterns were not always consistent with the predictive model indicating that there were interactions of the four factors (tree species, wood density, collection site and incubation site). For example, we expected the smallest similarity values (the lightest shading) when logs were compared that varied in all four factors (D+CS+SP+IS). However two Bray-Curtis similarity values (0.247 and 0.159 in the four-factor variations) were higher than expected (Table 3).

Macro-detritivore species were specific or had a high preference for a particular site (Table 1). Abundance at site F was much higher than at site S for *T. pusillus*, *Philoscia muscorum*, *Trachelipus rathkii*, *Cylindroiulus punctatus*, *Julus scandinavius*, *Brachydesmus superus* and *Polydesmus denticulatus*. Abundance at site S was much higher than at site F for *Oniscus asellus*, *Porcellio scaber*, *Cylindroiulus britannicus* and *Polydesmus angustus*. The woodlouse *Armadillidium pulchellum* was only found at site S (Veluwe area). The millipedes *Brachyiulus pusillus*, *Craspedosoma rawlinsi* and *Polydesmus inconstans* were only found at site F.

**Discussion**

There were strong effects of tree species, wood decay stage and environment on macro-detritivore communities in dead wood. Together, our findings provided support for all three hypotheses: (1) different tree species hosted different Diplopoda and Isopoda communities, both in terms of abundance and species composition; (2) the differences in macro-detritivore composition between tree
species diminished with wood decay stage; and (3) there were two, three and four-way interactions between tree growing environment, tree species, wood decay stage and decomposition environment on macro-detritivore abundance and composition. Below we discuss these findings in some more detail and in the context of previous literature.

Table 3. Bray-Curtis similarity patterns model for animal composition in decaying logs, as a function of collection site, tree species and incubation site. (a) Predictive model under the null hypothesis of no interaction effects. From dark to light shading the strength of the factors that affect species composition are expected to increase, so Bray-Curtis similarity is expected to decrease. Sources of variation in species composition: D, Decomposition stage; IS, Incubation site; SP, Tree species; CS, Collection site. (b) Predictive model (shading) combined with actual results. Bray-Curtis similarity values indicate the comparisons between two groups of logs (mean ± SE). The values that do not match the predicted pattern reject the null hypothesis of no interaction effects. Values in bold and italic font indicate strong opposite deviations from the prediction, higher and smaller than expected respectively. Collect.Site=collection site, Incub.Site= incubation site; FLEVO= Flevopolder, SCHOV=Schovenhorst; BETULA= Betula pendula PICEA= Picea abies.

| Collect. Site | Tree Species | Tree Species |
|--------------|--------------|
| Collect. Site | FLEVO | SCHOV | FLEVO | SCHOV | FLEVO | SCHOV |
| BETULA | D | D+CS | D+SP | D+CS+SP | D+IS | D+CS+IS | D+IS+SP+IS |
| SCHOV | D | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS | D+IS+SP+IS |
| FLEVO | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS | D+IS+SP+IS |
| SCHOV | D+CS+SP+IS | D+IS | D+CS+IS | D+IS+SP+IS | D+IS |
| FLEVO | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS |
| SCHOV | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS |
| FLEVO | D+CS | D+SP | D+CS+SP+IS | D+IS | D+CS+IS |
| SCHOV | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS |
| FLEVO | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS |
| SCHOV | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS |
| FLEVO | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS |
| SCHOV | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS |
| FLEVO | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS |
| SCHOV | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS |
| FLEVO | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS |
| SCHOV | D+CS+SP | D+SP | D+CS+SP+IS | D+IS | D+CS+IS |

(a) Predictive model

| Collect. Site | Tree Species | Tree Species |
|--------------|--------------|
| Collect. Site | FLEVO | SCHOV | FLEVO | SCHOV | FLEVO | SCHOV |
| BETULA | 0.371 ± 0.018 | 0.278 ± 0.021 | 0.292 ± 0.033 | 0.143 ± 0.009 | 0.233 ± 0.009 | 0.164 ± 0.009 | 0.247 ± 0.013 |
| SCHOV | 0.269 ± 0.017 | 0.166 ± 0.016 | 0.095 ± 0.006 | 0.139 ± 0.007 | 0.066 ± 0.005 | 0.132 ± 0.010 | 0.132 ± 0.010 |
| FLEVO | 0.223 ± 0.014 | 0.180 ± 0.015 | 0.129 ± 0.007 | 0.159 ± 0.008 | 0.094 ± 0.004 | 0.166 ± 0.010 | 0.166 ± 0.010 |
| SCHOV | 0.173 ± 0.024 | 0.045 ± 0.005 | 0.061 ± 0.004 | 0.033 ± 0.004 | 0.090 ± 0.011 | 0.090 ± 0.011 | 0.090 ± 0.011 |
| FLEVO | 0.383 ± 0.030 | 0.381 ± 0.011 | 0.278 ± 0.013 | 0.205 ± 0.010 | 0.205 ± 0.010 | 0.205 ± 0.010 | 0.205 ± 0.010 |
| SCHOV | 0.397 ± 0.015 | 0.263 ± 0.011 | 0.230 ± 0.010 | 0.230 ± 0.010 | 0.230 ± 0.010 | 0.230 ± 0.010 | 0.230 ± 0.010 |
| FLEVO | 0.278 ± 0.012 | 0.143 ± 0.010 | 0.164 ± 0.009 | 0.247 ± 0.013 | 0.247 ± 0.013 | 0.247 ± 0.013 | 0.247 ± 0.013 |
| SCHOV | 0.278 ± 0.012 | 0.143 ± 0.010 | 0.164 ± 0.009 | 0.247 ± 0.013 | 0.247 ± 0.013 | 0.247 ± 0.013 | 0.247 ± 0.013 |

(b) Predictive model combined with actual results

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Trait effects on macro-detritivore abundance and community composition as related to tree species and wood decay stage

In our study, the Diplopoda and Isopoda abundance and community composition in dead wood differed between logs of *B. pendula* and *P. abies* at the same site. The differences are probably due to afterlife effects of tree species-specific traits, such as bark morphology, wood density, nutrient content and secondary metabolites such as terpenes (Cornwell et al., 2009; Stokland et al., 2012). Contrary to saproxylic insects (Jonsell et al., 1998), macro-detritivores are mostly not specialized in wood-feeding but feed on plant litter, mixed with fungi (David and Handa, 2010). They tend to be selective feeders, preferring organic matter with high nitrogen contents and low amounts of structural compounds, such as lignin and secondary metabolites (Hendriksen, 1990; Hättenschwiler and Bretscher, 2001; van Geffen et al., 2011). Besides chemical properties, micro- and macro-morphological traits, e.g. bark and wood surface features, may serve as ‘habitat’ traits to macro-detritivores (Cornwell et al., 2009). The two tree species differ in bark surface features, e.g. *Betula* has a more smooth bark compared to *Picea*, which in particular determines the quantity of micro-sites for shelter. Besides, moss cover in this study had a significant relationship with macro-detritivore abundance in the direct sampling of *B. pendula* logs at site F (*P* = 0.028, data not shown). Moss cover seemed to have a positive effect on the amount of animals associated with dead wood, providing safe, moist places to hide. This function differs between tree species (Humphrey et al., 2002) as related to tree bark traits (see above). These findings suggest that initial differences in resource quality between the two tree species can be one explanation of macro-detritivore community differences.

The abundance of macro-detritivores was influenced by decay stage as indicated inversely by wood density and, in the base-rich site F, by wood pH. Also we found a succession of macro-detritivore abundance (although only significantly in *P. abies*) and composition between early and late decay stages (see Introduction; Jonsell et al., 1998). Due to breakdown of structural components, such as lignin and cellulose, the wood loses its structure and becomes softer. Softer wood is more accessible to the animals, has a higher moisture content and contains more available resources (nutrients, carbon and especially fungal mycelium; Laiho and Prescott, 2004). More decayed wood provides additional elements of heterogeneity, for instance more space to shelter by the loosening-up of bark, and hence more niches for different species (Kruys et al., 1999). Besides, decay stage is related to time since death, the longer a piece of dead wood remains in situ, the more likely it is to be colonized (Crites and Dale, 1998).

The pH of dead wood was a good proxy for decay stage, at least in the logs from site F. At this site the soil pH was close to neutrality and the pH range of the logs during the decay process was quite large. With progression of decomposition the pH of the logs became lower, probably due to the production of oxalic (and/or other) acids by wood-decomposing fungi (Shimada et al., 1997). It remains to be studied whether and how pH itself might influence macro-detritivore composition.
Effect of environment on macro-detritivore community composition

The macro-detritivore abundance and community composition in dead wood was not only influenced by tree species and wood decay stage, but also by (1) the environment in which decomposition took place and (2) the site where a tree had grown before it produced the log. When comparing the logs from different collection sites or incubation sites, the average similarity of the macro-detritivore community was always less than the average similarity without site variation (i.e. values for D+CS and D+IS were always smaller than those for D, Table 3). This difference must have been caused by an effect of the environment, possibly due to differences both in the local to regional species pool and in soil moisture, pH and/or nutrient status.

Soil fauna community composition is strongly determined by soil type (Berg et al., 2008). The animal abundance at site F was higher than at site S, as was expected due to the moist and fertile soils with a neutral pH at site F. In general clay soils, with a higher pH and soil moisture during the drier months of the year are more favorable to macro-detritivores; they tend to have a higher diversity and abundance. Sandy soils are often too dry in summer, posing drought stress to soil fauna (Paoletti and Hassall 1999, Berg et al. 2008). Moreover, soil at site S is rather acidic. Especially Isopoda need enough Ca and Mg to build-up their exoskeleton. In acidic soils therefore they tend to have a lower abundance and diversity (Kappes et al. 2007).

Interactions between tree species, decay stage and environment

In the Bray-Curtis similarity patterns model, the null hypothesis predicted no interaction effects of different drivers of invertebrate community composition when comparing different groups of logs pairwise (Table 3a). Actual values indicated two strong and opposite deviations from the prediction of logs varying in three or four factors, presenting higher and smaller than expected values respectively (in bold and italic font respectively in Table 3b). Higher than expected Bray-Curtis similarity values might indicate convergent interactions due to different factors having contrary effects on macro-detritivore compositions. Smaller values than expected might indicate the opposite, i.e. divergent interactions between factors that determine fauna community composition. Thus, tree species, wood decay stage and environment not only had single effects on macro-detritivore diversity, but also interacted with each other in various, sometimes complex ways. Our study has shown that macro-detritivores are selective in their wood tree species choice, but this selectivity decreases as wood decay progresses. Indeed, macro-detritivore community composition on different tree species converged during the decay process. In early decay stages of dead wood, macro-detritivores are more confined to particular tree species with their different chemical and structural wood and bark properties. In later decay stages these properties probably converge, i.e. overall decrease in wood density (accessibility) and C/N ratio (quality) and increase in moisture (survival under dry conditions). Other factors become more important (Jonsell et al., 1998), e.g. increase in the presence and abundance of microbes, especially decaying fungi (Kaila et al., 1994). High abundance and diversity of especially fungi at later decay stages (Boddy, 2001) not only provides additional resources for macro-detritivores (Stokland et al., 2012) but contributes to decomposition and thereby trait convergence (see above). Wood traits probably affect the fungal community
composition, but this potential impact on macro-detritivores is probably overruled by the significant increase in the total amount of fungal mycelium. Consequently tree species properties are more important for the substrate selection of macro-detritivores at the beginning of their dead wood decomposition.

Bark change with decay is accompanied by an increase in the amount of loose bark and space to shelter. Also, under loose bark the feces of macro-detritivores accumulates, increasing the amount of soil organic matter and microbes. Some macro-detritivores are coprophagous, feeding on feces that contain a high amount of resources (David and Handa, 2010). So to find a relationship between bark cover and macro-detritivore diversity, the bark looseness (in other words the decay stage) has to be taken into account. Together, these various structural and chemical changes during decomposition can explain the convergence in community composition of macro-detritivores with decay stage for different tree species.

The difference of macro-detritivore community in dead wood of different tree species during wood decay also depends on the soil environment. Previous studies on leaf litter decomposition have shown that soil animal composition differed between tree species and sites during decomposition (Anderson, 1973a). Our findings suggest that the effects of wood traits owing to tree species and the logs’ original environment on macro-detritivore community, depend on the wood decay stage and the decomposition environment and on interactions between these factors. These interactions are consistent with the decomposition environment affecting the macro-detritivore species pool, microclimate and dead wood quality; the latter for instance by the decomposition environment influencing decay rate and the nutrient and moisture exchange between wood and environment.

Conclusions and outlook

Our results suggest that wood and bark traits play an important role in explaining macro-detritivore community differences in early decay stages, but the differences diminish between tree species with decay. Perhaps other characteristics, such as amount of loose bark, become more important then. Moreover, the decrease in wood density, accompanied by an increase in resource quality due to microbial growth positively affects macro-detritivore diversity. The change in these characteristics might of course indirectly be influenced by wood and bark traits. These relations suggest that dead wood of difference tree species in different decay stages is probably very important to sustain macro-detritivore diversity in forests (Jabin et al. 2004, Kappes et al. 2007).

The environment played a role in macro-detritivore community composition in wood and in tree species - decay stage interactions. This was mainly due to site differences in the species pool of macro-detritivore communities, due to the strong divergence in site characteristics, especially soil type, soil nutrient status and soil pH. Under global changes in land-use and climate, the soil environment will change. This in turn can influence the composition of tree species in forests, their growth conditions and the decomposition environment of the dead wood they produce, thereby influencing decomposition rates. As we have shown here, all these drivers of macro-detritivore community composition interact. Thus, when trying to understand and predict future
patterns of macro-detritivore diversity, these interactions should be taken into account.

An important next step will be to quantify the feedback of macro-detritivore community to dead wood decomposition itself. This feedback may be better understood from the combination of (1) the complex interactions of tree species, decay stage and forest environment on macro-detritivore diversity and (2) the functional traits of these macro-detritivore species with respect to their effects on wood carbon and nutrient dynamics. A better knowledge about these feedbacks can help in predicting carbon storage and nutrient cycling functions of dead wood in forests differing or changing in tree species composition and abiotic environment. This would contribute greatly to our predictive power of carbon and nutrient cycling through wood decomposition.
References


Macro-detritivore diversity in dead wood


