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Saproxylic beetles in Populus tremula fauna depots - how do you construct the best depot?

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Abstract
The biodiversity of saproxylic organisms and in the case of this study, saproxylic beetles, is dependent on the amount and quality of course woody debris (CWD) in the environment. Over the past decades the quantity of CWD in Swedish forests has increased, yet the quality has decreased, forests are managed and exploited, and old-growth forests are rarer. To counteract this, fauna depots, artificial piles of dead wood, can be placed to increase the amount and quality of habitat for saproxylic organisms. This study measured fauna depots of aspen CWD placed in Uppsala municipality to find which combination of qualities resulted in the highest species richness and abundance (diversity). A total of 242 beetles across 26 species were sampled using a bark sifter and Tullgren extraction funnels. The CWD qualities; number of logs, log diameter, sun exposure and decomposition level were measured. Log diameter was confirmed to be positively correlated with species richness supporting previous research. Decomposition was also positively correlated with species richness, but this not supported by previous research. Some qualities, like sun exposure, were unexpectedly not correlated and number of logs was negatively correlated. Only one red-listed species was found across all samples. Overall results were somewhat inconclusive but provide hints towards better practice in saproxylic beetle conservation, such as placing larger logs in the depots.

Introduction
Dead wood has become of increasingly worse quality in the forest landscape since the inception of modern industrialised forestry (Berg *et al.* 1995, Jacobs *et al.* 2007). Commonly called coarse woody debris (CWD), it is made up of trees that lie or remain standing after death. CWD has critical functions in the forest ecosystem like the recycling of nutrients such as nitrogen, carbon and phosphorus locked in the wood (Fisk et al. 2002) and as habitat for organisms. CWD essentially forms a microhabitat for smaller organisms like fungi, plants, arthropods, small birds and mammals (Purahong *et al.* 2014, Jacobs *et al.* 2007, Jancewicz & Kielan 2017). Saproxylic beetles are one of these organisms and are important in the forest ecosystem because of their role in the degradation of CWD and as prey species (Hammond *et al.* 2001). These beetles depend on dead wood mainly for three reasons; as habitat, for reproduction and/or as feeding ground for fungivores (Hammond *et al.* 2001). Saproxylic beetles are closely linked with the fungus on the CWD they live on, and many have varying degrees of specialisation for certain fungi species (Persiani *et al.* 2010). This makes some species of fungus more important than others. One of the most important CWD trees in Sweden is *Populus tremula*. This is because some species of saproxylic beetle specialise in *P. tremula* and because the tree has become rarer due to modern forestry practices (Sahlin & Schroeder 2010).

The biotic and abiotic qualities of CWD also affect the diversity of the species living within them. Log diameter, sun exposure and amount of wood are examples of such factors (Ranius *et al.* 2011, Schroeder *et al.* 2011). And thus, as the availability and quality of CWD decreases, so does the diversity of saproxylic beetles (Siitonen & Martikainen 1994).

For these reasons improvement measures have been taken by conservation agencies around the world, and one method of conservation is the creation of fauna depots. A fauna depot is a pile of dead wood artificially placed to augment the increasingly rarer natural CWD. When comparing localities for beetle diversity, one of which had a pile of dead wood in it and the others had not, the locality with the dead wood had greater richness and abundance of red-listed species (Franc & Aulén 2008). A fauna depot of high quality should be a local biodiversity hotspot with high species richness and abundance (diversity). A single fauna depot, and natural CWD in general, is not a permanent fixture in the landscape, however. As the wood decomposes the habitat quality also changes. This leads to a succession where some species are replaced by others more favoured by the more decomposed wood (Ranius *et al.* 2011). This therefore necessitates that new depots be placed, or existing ones be “refilled” over time for maximum species retention.

The municipality of Uppsala has employed fauna depots of *P. tremula* to protect the beetle *Cucujus cinnaberinus*. The depots have been surveyed for *C. cinnaberinus* in years prior and have shown evidence of harbouring the threatened species (Jonsell 2014, unpub). This study was thus conducted to see which combination of quality factors of these fauna depots results in the greatest species diversity. The tested quality factors were number of logs in the depot, average log diameter, sun exposure and level of decomposition. A secondary goal of this study is to find if any rare or red-listed species currently inhabiting these fauna depots.

Method

Study site

The study took place in the western and southern parts of Uppsala, particularly in the nature reserves Håga-Nåsten, Stadsskogen and Kronparken (Figure 1). These nature reserves are in close proximity to the city and are and have historically been heavily affected by human activity such as hunting, agriculture, military exercises and recreation.

Figure 1. Map of the city of Uppsala, with nature reserves outlined in green. 1 Håga-Nåsten, 2 Stadsskogen and 3 Kronparken.

In these reserves fauna depots of dead *P. tremula* wood have been placed by the municipality of Uppsala. These depots consist of a number of dead *P. tremula* logs piled next to and on top of each other (Figure 2). All piles except one was located next to forest trails.

Field work and sampling

During a period of two weeks from the 10th to the 21st of September 2019 I collected bark samples from these piles. All 17 existing piles in sampleable condition (those with enough bark left to peel) were sampled. Three logs were randomly sampled from each pile for a total number of 51 samples. The diameter and number of logs was measured and counted. The decomposition was measured by plunging a knife into the wood and classifying the log into a decay class depending on the depth of the cut according to the scale by Siitonen & Saaristo 2000: (I) wood hard, phloem still fresh or currently used by primary scolytids, at most 1 year old, (II) wood hard, but more than 1 year old, (III) a knife can be pushed 0.5-2 cm, (IV) a knife can be pushed 2-4 cm, (V) a knife can be pushed >5 cm with the trunk retaining cylindrical form, (VI) a knife can be pushed >5 cm with the trunk disintegrating easily with flattened form and parts totally decomposed. Sun exposure was measured by approximating the amount of sunlight the pile would receive during a sunny day (depending on the surrounding foliage and positioning of the pile) and classifying it into one of three classes; Low exposure, Intermediate exposure and High exposure. The fruit bodies of fungi growing on sampled logs were identified as far as was possible.

Samples were collected by peeling off a 30x30 cm^2 square of bark from a log. The bark was then sieved through a sifter which extracts the fine fractions of the bark and most arthropods in the sampled square. Each sample was then put into a Tullgren funnel with a light source above, which forces the arthropods down the funnel and into a bottle with glycol, killing and preserving them. This extraction procedure lasted for at least 24 hours for each sample. The extracted beetles were then counted and identified. All non-saproxylic beetles (as according to literature once they were identified) were excluded from the analysis.

Figure 2. One of the sampled piles, this one was located in Håga-Nåsten nature reserve. The bark is visibly decomposing and fungi growth is evident.

Statistics

A paired Wilcoxon test was used to detect significances between species diversity and each quality factor one at a time for individual logs. This non-parametric test was chosen as the data do not fit the assumption of normality assumed in a parametric test. A Linear Mixed-Effects Model was used as a full model to analyse the effect of the factors on species diversity in each pile, with species richness and abundance as response, with all quality factors as operators and the pile sample number as a random effect. Chi-square tests were also used to test for correlations of quality factors with occurrence (presence/absence) of individual species, chosen based on abundance greater than 30 individuals, frequency above 10 occurrences and red-list status. Statistical significance was considered at $p<0.05$. R statistical analysis software was used, alongside the packages lme4 and lmerTest (R Core Team 2014, Bates *et al.* 2015, Kuznetsova *et al.* 2017).

Results

In all 51 samples a total of 26 different species were found, and a total of 242 individuals were identified (Table 1). The most abundant species was *Cis micans* closely followed by *Sulcacis nitidus*. The most frequent was *Homalota plana*. Only one redlisted species, *Agaricochara latissima*, was found across all samples.

Table 1. List of species found in the *P. tremula* fauna depots. The species marked with * were in larval or nymph stages of their lifecycles and thus unidentifiable. Species are listed in systematic order.

Species	Redlist status	Number of individuals	Number of occurrences
Agathidium nigripenne	LC	1	1
Nudobius lentus	LC	6	4
Gabrius splendidulus	LC	6	4
Bibloporus bicolor	LC	3	3
Acrulia inflata	LC	2	2
Agaricochara latissima	NT	22	3
Anomognathus cuspidatus	LC	10	6
Homalota plana	LC	35	20
Cartodere nodifer	LC	3	3
Rhizophagus dispar	LC	1	1
Cerylon deplanatum	LC	26	9
Sericoderus lateralis	LC	1	1
Orthoperus punctatus	LC	1	1
Bitoma crenata	LC	14	4
Salpingus planirostris	LC	2	2
Salpingus ruficollis	LC	1	1
Cis comptus	LC	1	1
Cis micans	LC	42	5
Sulcacis nitidus	LC	41	4
Octotemnus glabriculus	LC	6	2
Xyleborus cryptographus	LC	3	3
Trypophloeus asperatus	LC	1	1
Trachodes hispidus	LC	1	1
*Aradidae sp.*1*	N/A	1	1
*Aradidae sp.*2*	N/A	1	1
*Cerambycidae sp.**	N/A	11	8

The species richness analysis of all logs without pile association (the Wilcoxon tests) had positive correlation with the factor mean diameter of the logs (p<0,0001) (Figure 3), negative correlation with the factor number of logs (p<0,0001) (Figure 4), positive correlation with the factor decomposition level (p=0.0023) (Figure 5) and no correlation with the factor sun exposure (p=0.34) (Figure 6). The species richness of each pile (the Linear Mixed-Effects Model) had a trend to be correlated with the factor mean diameter of logs (p=0.08) and no correlation with the factors sun exposure (p=0.40), number of logs (p=0.30) and decomposition level (p=0.89) (Table 2). The abundance analysis of all logs without pile association (the Wilcoxon tests) had correlation with all factors (p<0.05). The abundance analysis of each pile (the Linear Mixed-Effects Model) had no correlation with any of the factors mean diameter (p=0.75), sun exposure (p=0.57), number of logs (p=0.11) and decomposition (p=0.154).

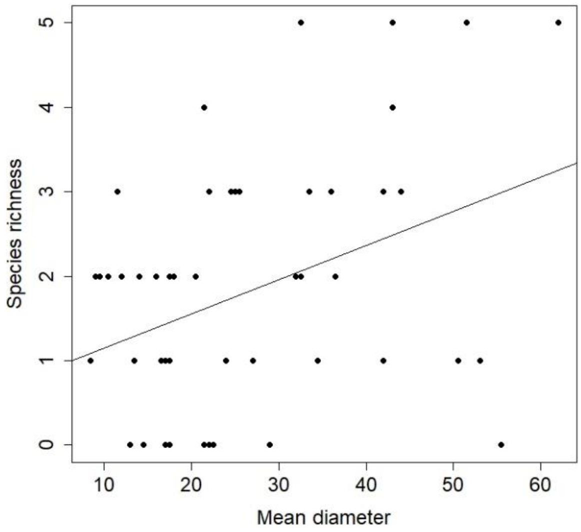

Figure 3. The relationship between mean diameter and species richness. A positive trend is evident.

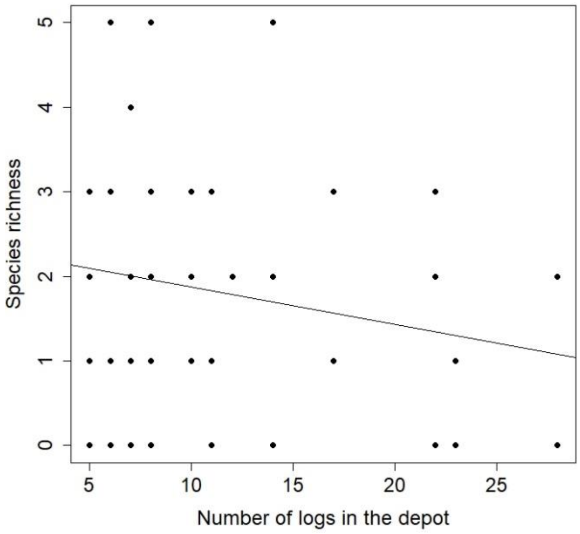

Figure 4. The relationship between the number of logs and species richness. A negative trend is evident.

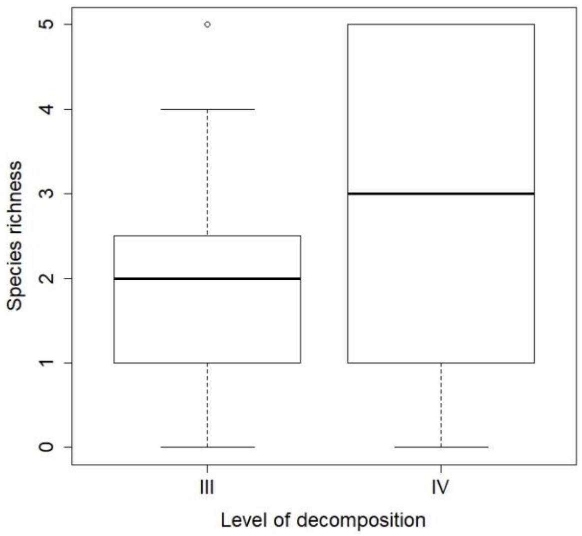

Figure 5. Boxplot of species richness in each class of decomposition. There is a great difference between the means, and greater variance in class IV.

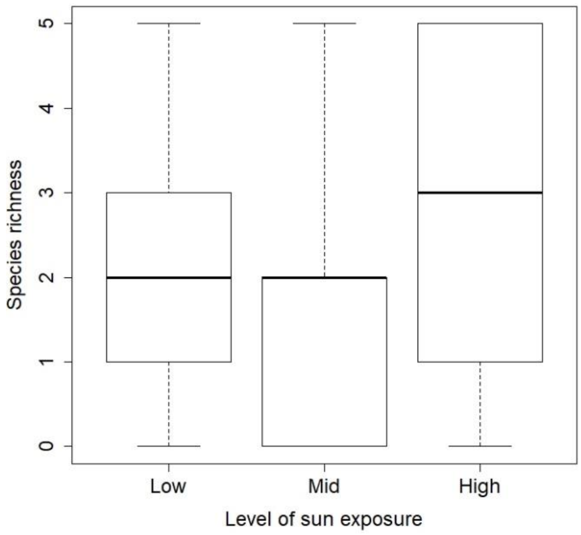

Figure 6. Boxplot of species richness in each class of sun exposure. There is no great difference between the means.

Table 2. Table of outputs of the summary of the full modelled linear fixed-effects model for pile-specific analysis of species richness.

Factor	Estimate	Std. Error	Degrees of freedom	t-value	p-value
Sun exposure	-0.42653	0.86680	2	1.866	0.4031
Number of logs	-0.04051	0.03943	17	-1.027	0.3098
Mean diameter	0.03503	0.31964	17	1.783	0.0814
Decomposition	0.75954	0.69375	1	1.095	0.2794

Table 3. Table of outputs of the summary of the full modelled linear fixed-effects model for pile-specific analysis of abundance.

Factor	Estimate	Std. Error	Degrees of freedom	t-value	p-value
Sun exposure	2.72907	4.85338	2	0.562	0.577
Number of logs	-0.36219	0.22716	17	-1.594	0.118
Mean diameter	0.03655	0.11458	17	0.319	0.751
Decomposition	6.40243	4.41126	1	1.451	0.154

The five species that were tested individually for association to the quality factors were; *Homalota plana* (the most frequent), *Cis micans* and *Sulcacis nitidus* (the most abundant), *Agaricochara latissima* (the only redlisted one) and *Cerylon deplanatum* (which has been on the redlist in the past). There were two significant positive correlations; *A. latissima* a nd high sun exposure (p=0.009) and *C. deplanatum* and high sun exposure (p=0.056). There were also two almost significances; *C. micans* and low sun exposure (p=0.056) and *A. latissima* and high mean diameter (p=0.077) (Table 4).

The fungi identified on the logs all belonged to the genus *Trametes*, with the species *Trametes betulina* growing on two.

Table 4. Results of the chi-square tests of individual species occurrence against the quality factors. Those marked * are significant and those marked (*) are almost significant.

Species	Mean diameter (p-value)	Number of logs (p-value)	Sun exposure (p-value)	Decomposition (p-value)
Homalota plana	0.5801	0.1776	0.1303	0.2994
Cis micans	0.4783	0.4609	0.05599(*)	0.2655
Sulcacis nitidus	0.6155	0.6472	0.6758	1
Agaricochara latissima	0.07733(*)	0.1883	0.008552*	0.8787
Cerylon deplanatum	0.1643	0.3924	0.04627*	0.177

Discussion

The factors that affected beetle species diversity in this study were log diameter and number of logs for species richness, and all factors but at the same time no factors for abundance. Log thickness is an established cause of species richness within them, as the larger the log is the higher the number of microhabitats it can fit (Schroeder *et al.* 2011).

The number of logs in a pile being negatively associated with species richness is a surprise, as it is an indicator of species richness and has been proven to be associated with species richness in the past (Persiani *et al.* 2010). After all, more logs equal more potential habitat which should increase species richness (Ranius *et al.* 2011). I suspect that my negative results for number of logs are this way due to an inappropriate sampling strategy for this factor as I only sampled three logs from each pile, regardless of how many logs there were in the pile. If

I had sampled a ratio of logs from each pile dependant on the number of logs in it, then the results may have been better sampled to test species richness in each pile. The number of logs in a pile does not necessarily increase the number of species per particular log, but in the pile over all (Victorsson & Jonsell 2013). However, if this sampling strategy had been employed a different issue would appear; the sampling precision and statistical power would be different for piles of different sizes.

Sun exposure not having an impact on species richness is strange, as sun exposure is usually associated with species richness (Ranius *et al.* 2011). This could be explained by the relatively rough and imprecise method used for determining it. The best way to measure it would be with a sophisticated lux meter, preferably over an entire day. Another issue is that only two piles of 17 had a high sun exposure, while the rest were split between low and intermediate. To study this the number of samples in each class should be balanced, which increases the potential to detect differences between them. The best way to achieve this would be a controlled experiment where an equal number of piles are placed in locations with varying lux levels instead of a field experiment.

Decomposition had a positive correlation with species richness, but that does not make as much sense as one would at first believe. The habitat inside the log changes as the log decays and lead to species succession over time (Jacobs *et al.* 2007). A log with a lot of decay and mulch-like wood could reach a stage where fewer species prefer it as habitat. The same goes for a log with little decomposition and mostly hard wood. Species richness is an uneven gradient across time, not ever-increasing as the wood rots (Jacobs *et al.* 2007). Another problem is that decomposition, just like sun exposure, did not have a balance of classes. Of all six classes in the system, only two turned up in this study, and the vast majority of those were of class (III). This is because the municipality's project to protect *C. cinnaberinus* with these depots has not been ongoing long enough for the wood to reach the higher classes of decomposition, while at the same time no very recently dead trees (class I and II) had been placed in the depots. It is likely therefore that this has led to a false positive because of an uneven distribution of classes, but it is also possible that *P. tremula* CWD simple is in a more species rich state at decomposition class (IV) than class (III).

There is also a statistical issue involved; some samples are not independent on each other. 17 piles were sampled for a total of 51 logs, which means three logs were sampled from each pile. These sets of three are interdependent, which makes the Wilcoxon-test an "ugly analysis" as it assumes that each sample is independent from the others. The Wilcoxon-test is thus not entirely correctly used, but there is no real way to go around the fact that the samples are dependent, apart from very advanced statistical models. The test does give a pointer towards an answer, however. The species richness of entire piles and the species richness of individual logs point in roughly the same direction for each quality factor.

The results of the analysis of abundance however is highly uncertain. The pile analysis returns no significances for any quality factor while the individual log analysis returns significances for all factors. As described above, the logs in the Wilcoxon-tests are dependent on each other and the tests not entirely reliable. This is the complete opposite of the species richness analysis, as the analysis of the piles and the analysis of the logs by themselves point in completely different directions. The results of the abundance analysis should thus be disregarded entirely.

The chi-square tests on individual species were done in an attempt to analyse the species composition, and how some species in particular are affected by the quality of CWD. An example would be if a red-listed and rare species is affected in a way by a quality factor that the total species richness is not. If that were the case, then a different conservation approach would need to be taken to protect the rare species and this could lead to a more species poor type of wood being worthier of protection than a species rich one. This all depends on which priorities a conservation program has. In my analysis the occurrence of *C. deplanatum*, a formerly redlisted species, and *A. latissima*, a currently redlisted one, were correlated with high sun exposure. The dataset of a single species is not large, only nine samples contained *C. deplanatum* and only three contained *A. latissima.* It is thus lacking in statistical power. But it also points to the fact that higher sun exposure could benefit certain species (Ranius *et al.* 2011). An alternative way to analyse species composition this would be using an NMDS ordination, but the dataset was not large enough for that kind of analysis.

Some results of the study I was unable to be studied statistically are also worth mentioning. I also looked at the species of fungi growing on each log. Two samples stood out from the rest with very high abundance of beetles. Those two samples had *Trametes betulina* growing on them. Other *Trametes* species were also found but did not have the same circumstantial abundance. Perhaps this particular species serves as an especially good food source or habitat for saproxylic beetles. Two samples out of 51 is however not enough power to test this statistically, but the presence of *T. betulina* could be interpreted as an indicator for some kind of species diversity, and something that could be studied in greater detail in the future.

In summary, the previously known fact that log thickness positively affects saproxylic beetle diversity is confirmed by this study, with the other factors being inconclusive. To positively identify the conditions of a high diversity fauna depot a larger more controlled experiment should be conducted to achieve greater parity of classes and a more control than a field experiment can provide.

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