Oikos

Appendix oik-04879

Pietsch, K. A., Eichenberg, D., Nadrowski, K., Bauhus, J., Buscot, F., Purahong, W., Wipfler, B., Wubet, T., Yu, M. and Wirth, C. 2018. Wood decomposition is more strongly controlled by temperature than by tree species and decomposer diversity in highly species rich subtropical forests. – Oikos doi: 10.1111/oik.04879

Appendix 1

Supplementary material to material and methods

Detailed description of fungal community composition analysis

For the analysis of fungal community composition we used wood samples harvested at the end of the experiment in 2012. For each species-by-treatment combination we analysed one out of four samples per plot. The samples used for this analysis have been frozen immediately after the harvest. For the analysis they were ground to fine powder. From this we extracted DNA using a ZR Soil Microbe DNA MiniPrep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's protocol. We then checked the presence and quantity of genomic DNA using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany). DNA extracts were then stored at –20°C for further analysis. We amplified fungal amplicon libraries for pyrosequencing using custom fusion primers.

We used 454 pyrotag sequencing for the analysis of the fungal community composition. We filtered for good quality sequences and used them for further analyses. Good quality sequences were characterised by holding one of the expected barcodes with at most one mismatch, a minimum length of 360 nt, a minimum average Phred score of 20, containing homopolymers with a maximum length of 15 nt and without ambiguous nucleotides. The quality-filtered reads were shortened to their first 360 bases and normalized to the smallest read per sample (3077). Potential chimeras were removed using UCHIME (Edgar et al. 2011) as implemented in MOTHUR. Unique sequences were sorted by decreasing abundances and were clustered into Operational Taxonomic Units (OTUs) using CD-HIT-EST (Fu et al. 2012) at a threshold of 97% pairwise similarity. Fungal ITS OTU representative sequences were first classified against the dynamic version of the UNITE fungal ITS sequence database (ver. 6, released 15.01.2014; Kõljalg et al. 2013). The sequences with fungi only identified were further classified against the full version of the UNITE database to improve their taxonomic annotation. Rare OTUs (singletons to tripletons) were removed from the dataset, as they could potentially originate from artificial sequencing errors (Kunin et al. 2010). Thus we used the

abundant OTUs (OTUs with > 3 reads) for further statistical analysis. To assess the role of the omitted rare OTUs, a Mantel test based on a Bray–Curtis distance measure with 999 permutations was applied to both the full matrix and the one excluding the rare OTUs (Hammer et al. 2001, Hoppe et al. 2016, Purahong et al. 2017). The result indicated that the removal of rare OTUs from the fungal communities had no effect on community composition (RMantel = 0.999, p = 0.001). The raw sequence datasets are available in the European Nucleotide Archive under the study number PRJEB89781. Fungal biome OTU table, OTU representative sequences and the bioinformatics scripts are available at Dryad (<https://datadryad.org/resource/doi:10.5061/dryad.54qr4>).

Our bioinformatics analysis includes procedures to eliminate biases and errors for richness estimation. These procedures include, 1) the quality-filtering (only good quality sequences are further analysed), 2) the normalization (i.e. all samples were normalized to the smallest read per sample), 3) the removal of potential chimeras and 4) the removal of rare OTUs (i.e. singletons to tripletons; removing all sequences potentially originating from artificial sequencing errors). With the high number of sequencing reads per samples (3077), the sample-based rarefaction curves indicated a saturation of fungal richness at the analysed sequencing depth for most samples (Fig xx). This indicates that the observed OTU richness is a suitable measure for fungal diversity. We calculated fungal richness from high quality datasets with the normalization step to reduce the biases in richness estimates due to variation in the number of sequence reads per sample. An in-depth description of the methods that were used to extract DNA and determine the fungal community composition in our dead wood samples is given in Purahong et al. (2017).

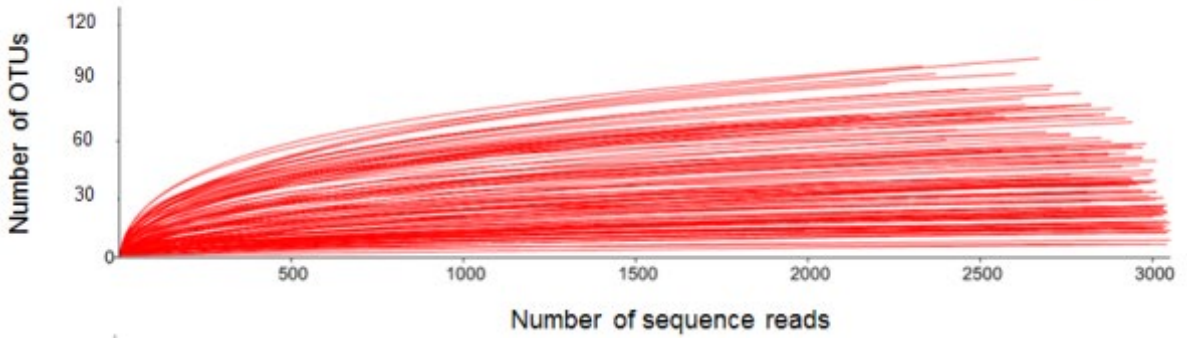

Figure A1. Individual rarefaction curves of wood-inhabiting fungi detected in each deadwood sample. The curves show a saturation of fungal richness at the analysed sequencing depth

Detailed description of invertebrate rearing

The chambers which we used to rear invertebrates were constructed of commercially available plastic boxes (~40 × 35 × 35 cm) with a lid. At the bottom in the middle close to one of the short sides we drilled a hole with 10 cm diameter to attach a funnel with a 100 ml plastic collection bottle. The bottle was filled with 50 ml of a glycol–water solution (1:1) as preservative. At the top of both short sides of the boxes we drilled three ventilation holes of 2 cm diameter. The holes were covered with a fine mesh to prevent invertebrates from escaping. During the rearing period, the microclimate should be similar to conditions in the forest. However we deliberately refrained from placing the emergence chambers directly in the forest because we could not insure their safety. Instead, the chambers were placed in a well ventilated barn room close to the study area in the forest. We used data loggers to constantly measure the temperature and humidity in the room and in randomly selected emergence chambers. Climatic conditions in the room were similar to the forest climate. Inside the chambers the humidity decreased slightly during the rearing. The chambers were installed for one year following each of the two wood retrievals. Reared invertebrates were collected from the catch bottles monthly and transferred to a solution of ethanol, acetic acid and water (7:1:2). The preservative solution was exchanged as required.

References

Edgar, R. C. et al. 2011. UCHIME improves sensitivity and speed of chimera detection. – Bioinformatics 27: 2194–2200.

Fu, L. et al. 2012. CD-HIT. Accelerated for clustering the next-generation sequencing data. – Bioinformatics 28: 3150–3152.

Hammer, Ø. et al. 2001. PAST. Paleontological statistics software package for education and data analysis. – Palaeontol. Electronica 4: 1–9.

Hoppe, B. et al. 2016. Linking molecular deadwood-inhabiting fungal diversity and community dynamics to ecosystem functions and processes in central European forests. – Fungal Divers. 77: 367–379.

Kõljalg, U. et al. 2013. Towards a unified paradigm for sequence-based identification of fungi. – Mol. Ecol. 22: 5271–5277.

Kunin, V. et al. 2010. Wrinkles in the rare biosphere. Pyrosequencing errors can lead to artificial inflation of diversity estimates. – Environ. Microbiol. 12: 118–123.

Purahong, W. et al. 2017. Characterization of unexplored deadwood mycobiome in highly diverse subtropical forests using culture-independent molecular technique. – Front. Microbiol. 8.