Methanotrophs are core members of the diazotroph community in decaying Norway spruce logs


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Short Communication

Methanotrophs are core members of the diazotroph community in decaying Norway spruce logs

Raisa Mäkipää, Sanna M. Leppänen, Sonia Sanz Munoz, Aino Smolander, Marja Tiirola, Tero Tuomivirta, Hannu Fritze

ABSTRACT

Dead wood is initially a nitrogen (N) poor substrate, where the N content increases with decay, partly due to biological N2 fixation, but the drivers of the N accumulation are poorly known. We quantified the rate of N2 fixation in decaying Norway spruce logs of different decay stages and studied the potential regulators of the N2 fixation rate. The average rate for acetylene reduction in the decaying wood was 7.5 nmol ethylene g−1 d−1, which corresponds to 52.9 μg N kg−1 d−1. The number of nifH copies (g−1 dry matter) was higher at the later decay stages, but no correlation between the copy number and the nd fixation rate was found. All recovered nifH sequences were assigned to the order Rhizobiales, and therein mostly (60%) to methane oxidizing archaeal 16S rRNA and bacterial nifH genes are listed in Supplementary Table 1. The average rate of the acetylene reduction was 7.55 nmol ethylene g−1 d−1, which gives 52.9 μg N kg−1 d−1. The number of nifH copies (g−1 dry matter) and detected DGGE bands correlated negatively with the wood density (r = −0.43, p = 0.014; and r = −0.52, p = 0.002, respectively) and positively with the wood moisture (r = 0.54, p = 0.002 and r = 0.44, p = 0.011). The measured rate of N2 fixation was higher at the intermediate stage of decay (Fig. 1). The number of nifH copies and sequenced DGGE bands was higher at the later decay stages, which suggests that the importance of the diazotrophs increases during the decay. However, no correlation between the nifH copy

ARTICLE INFO

Keywords:
Asymbiotic nitrogen fixation
Coarse woody debris
Dead wood
nifH
Picea abies

In boreal forests with nitrogen (N) supply deficiency, asymbiotic N2 fixation occurs in the dead wood (Brunner and Kimmins, 2003; Hicks et al., 2003; Rinne et al., 2017), the bryophyte (DeLuca et al., 2002; Leppänen et al., 2013) and the litter and soil layers (Todd et al., 1978; Vitousek and Hobbie, 2000). During the dead wood decay, where fungal communities proliferate, both the N concentration and the total amount of N in the wood increase along with the decay phase (Rajala et al., 2012) and the biological N2 fixation, which explains part of this increase (Rinne et al., 2017). In decaying wood, the N cycling processes and fungal-driven decomposition are tightly linked (Bebber et al., 2011), and the transfer of fixed N2 to the fungal biomass has been proven (Weishaupt et al., 2011). However, the relationship between the diazotroph community and the rate of N2 fixation in dead wood is poorly known. We hypothesize that the N2 fixation rate and the abundance of diazotrophic bacteria increase with the mass loss of decaying wood.

Our study site was an unmanaged Norway spruce dominated forest in Sipoo, Finland (60°28′N, 25°12′E). A full description of the study site, sampling, decay classification, acetylene reduction assay (ARA), and molecular analyses are given in Rajala et al. (2012) and in the Supplementary material. Briefly, wood discs were sampled from fallen dead trees representing different decay stages from recently fallen trees (decay stage 1) to late decay stage (5) when the logs were very soft and covered by bryophytes. The assessment of the N2 fixation by acetylene reduction assay (ARA) in 32 dead fallen Norway spruce (Picea abies L. (Karst)) logs (using four replicates per sample log) followed the methods described in Rinne et al. (2017). The methods of DNA extraction and purification, denaturing gradient gel electrophoresis (DGGE; gradient range 35–75%; 75 V; 60°C; 16 h), DGGE band excision and Sanger sequencing followed Rajala et al. (2012), except for that the DNA was extracted from 100 mg of lyophilized and milled material with the Nucleospin Soil extraction kit (Macherey-Nagel, Germany). The SL1 lysis buffer was used, but no SX enhancer. For DGGE, the nifH was first amplified for 40 cycles by qPCR was followed by rounds of end-point PCR (10–26 cycles depending on the previous Ct values) using the primers PolF with a GC-clamp and PolR (Paly et al., 2001). The primers and qPCR conditions used in quantifying the bacterial and archaegal 16S rRNA and bacterial nifH genes are listed in Supplementary Table 1.

The average rate of the acetylene reduction was 7.55 nmol ethylene g−1 d−1, which gives 52.9 μg N kg−1 d−1. The number of nifH copies (g−1 dry matter) and detected DGGE bands correlated negatively with the wood density (r = −0.43, p = 0.014; and r = −0.52, p = 0.002, respectively) and positively with the wood moisture (r = 0.54, p = 0.002 and r = 0.44, p = 0.011). The measured rate of N2 fixation was higher at the intermediate stage of decay (Fig. 1). The number of nifH copies and sequenced DGGE bands was higher at the later decay stages, which suggests that the importance of the diazotrophs increases during the decay. However, no correlation between the nifH copy

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https://doi.org/10.1016/j.soilbio.2018.02.012
Received 11 July 2017; Received in revised form 4 February 2018; Accepted 17 February 2018
0038-0717/ © 2018 Published by Elsevier Ltd.
numbers and the N₂ fixation rate by ARA was found (p = 0.249). Neither was there a correlation between the N₂ fixation and the bacterial and the archaean 16S rRNA gene copy numbers or the number of DGGE bands. The number of the bacterial copies correlated negatively with the wood density (r =−0.49, p = 0.004), but no correlation was found for the archaecal copies (p = 0.8254).

All recovered nifH sequences (representing 113 DGGE bands) from the decaying wood belonged to the Alphaproteobacteria, order Rhizobiales, and a major part (60%) of these were related to methane oxidizing bacteria, even though methanogenic Archaea have not contributed to the process (Auman et al., 2001). The ecological importance of methanotrophy in the N-cycle may be underestimated, since when using the conventional ARA method for measuring the N₂ fixation it dams the methane oxidation enzyme activity, and thus the activity of the methanotrophs is not easily detected (Flett et al., 1975) even though the method is used for this purpose (Auman et al., 2001). This may be the reason why no correlation between the nifH copy numbers and the N₂ fixation rate by ARA was found in our study. In a methanogenic Sphagnum dominated peatland ecosystem, the importance of methanotrophs in N₂ fixation was observed by using ^15N labelled N (Larmola et al., 2014), explaining over 30% of the peat N accumulation. Here, our results suggest that the methanotrophs also supply N to the decomposers in the N poor dead wood.

Hitherto, upland forests have not been classified as methanogenic environments, but recent findings of CH₄ emissions from shoots, tree trunks and canopies of Scots pine (Machacova et al., 2016; Halmeenmäki et al., 2017) show that we have to challenge this dogma even though methanogenic Archaea have not contributed to the process (Halmeenmäki et al., 2017). Furthermore, Lenhart et al. (2012) showed

Table 1
The closest matches for nifH nucleotide sequences. MOTU no. is the GenBank accession number for representative sequence of the molecular operational taxonomic unit derived from dead wood in this study, DC is occurrence in different decay stages (1–5), frequency is the number of similar sequences in the data set, organism is the closest matching bacterial species in the database and acc. no. refers to its accession number.

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that five different decomposer fungi produce variable amounts of CH₄ under aerobic conditions. As we have shown that methanotrophs are members of the nitrogen-fixing communities in all wood decay stages (Table 1), their abundance, community and contribution to the N-cycle ought to be studied using labeling techniques (¹³CH₄ & ¹⁵N₂) and new deep-sequencing methods in order to fully understand the role of the diazotrophs-methanotrophs consortium in the C/N cycle as well as their role in the decay process.

Our result show that methanotrophs are members of the *nifH* community in dead wood, where decomposition is driven by fungi, leads to a hypothesis for further research: Decomposer fungi that are capable of producing CH₄ feed methane-oxidising bacteria to fix atmospheric N₂. If this is the case, these fungi achieve a competitive advantage compared to other decomposers since N is the most important nutrient limiting the ecosystem processes.

Acknowledgements

Financial support was provided by the Academy of Finland (project 292899).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2018.02.012.

References


