

Soil trenching – are microbial communities alike in experimental peatland plots measuring total and heterotrophic respiration?

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ABSTRACT

Soil trenching is a generally applied method used to differentiate heterotrophic respiration (R_{HET}) from total respiration in soil CO_2 flux data collection. However, the soil microbial community composition may change due to trenching and estimates of the impacts of any human-induced disturbance on R_{HET} might be inflated if the microbial community involved was not the same as in the ambient untrenched environment. Here, we report that the bacterial and fungal community, as measured by amplicon sequencing, of 30 different research sites in peatland forests was mostly alike in trenched and untrenched plots still four years after trenching. Soil trenching thus seems to be a feasible method to study the R_{HET} from peatland forest soils from the overall microbial community composition point of view as no major changes were observed.

Soil total respiration is the sum of CO_2 evolving from microbial, faunal and root respiration, while the respiration by soil organisms is called heterotrophic respiration (R_{HET}) (Kuzyakov, 2006). R_{HET} is mostly microbial, and soil fauna impacts by ca 5 % of the CO_2 (Jiang et al., 2016). Root exclusion by trenching enables measuring R_{HET} using chamber techniques (e.g. Jovani-Sancho et al., 2018). The soil column isolated from living roots produces CO_2 emissions solely from decomposing organic matter, yielding soil C loss estimates for the soil C balance (Jauhiainen et al., 2019, 2023). However, cutting the roots changes the soil environment as reduced water uptake by plants increases soil moisture, and the C input changes due to the decomposition of killed roots and lack of new root exudates (Högberg and Read, 2006; Savage et al., 2018). Over time, these processes may affect the soil microbiome. However, it is uncertain if significant differences emerge over 0.2–4 years, the typical time span for soil CO_2 flux measurements. This knowledge is critical since testing the response of R_{HET} to environmental perturbances, like climate change (Lavigne et al., 2004), windthrow (Mayer et al., 2017), fire (Hu et al., 2021), and forest management (Wei et al., 2022), implicitly assumes that the microbiome responsible for CO_2 evolution is the same in trenched and untrenched measurement plots.

Upland forest soil trenching revealed no short-term changes in the microbial community when sampled over one to two years (Siira-Pietikäinen et al., 2003; Díaz-Pinés et al., 2010; Bluhm et al., 2019; Wu et al., 2024), but some contrasting results exist (Siira-Pietikäinen et al., 2003; Wang et al., 2013; Jin et al., 2023). Siira-Pietikäinen et al. (2003) studied a coniferous forest, where a trenching effect was observed in the organic humus layer but not in the mineral soil. Sampling later than two years from trenching can reveal a changed microbial community composition (Brant et al., 2006; Marañón-Jiménez et al., 2021). As scientific knowledge is still scarce and no data exists for peat soils, we analyzed the microbial community from 30 peatland forest sites across Finland, Estonia, Latvia, and Lithuania, where trenching was performed 5–51 months before sampling (Table S1). The sites had a variety of dominant tree species and management status (Table S1).

Trenched and untrenched treatments were replicated ($n = 3$) within each research site on subplots established at least 25 m apart from each other. In each subplot, trenching was performed down to 60 cm depth on a 60 × 60 cm measurement plot located up to 5 m apart from a respective untrenched plot. Non-woven polypropylene (150–220 g m⁻²)

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geotextile preventing root ingrowth was inserted on the sides (see Table S1 for the time elapsed since trenching in each site). Vegetation and litter from trenched surfaces were placed on geotextile sheet and kept in the original position between gas flux monitoring events. The untrenched surfaces were left intact. Surface peat samples sized $10 \times 10 \times 10$ cm were aseptically taken in September 2021, at 15 cm northeast from the flux monitoring areas located at the center of each plot. Sub-samples for DNA extraction were taken from the center of each sample, carried on ice within 24 h to the laboratory, and frozen to -20 °C. Details about DNA extraction, fungal ITS and bacterial 16S rRNA gene library preparation, sequencing, and bioinformatics are given in the Supplement. Alpha-diversity measures as Hill's numbers, HQ0 for species richness and HQ1 for the exponential of Shannon's entropy index, were obtained from bacterial and fungal OTU data with the 'hill_taxa' function in the 'hillR' R package (Chao et al., 2014). The major functional guilds: saprotrophic, symbiotrophic, and pathotrophic fungi, were obtained from the fungal OTU data using the 'FUNGuild.py' script (Nguyen et al., 2016).

We applied linear mixed models (LMM; estimated using REML and nlptwrap optimizer) using fungal ITS and bacterial 16S rRNA gene-derived alpha diversity measures (HQ0/HQ1 ITS/16S rRNA) (Fig. S1) and the relative abundance of fungal guilds (Fig. S2) as response variables (Table S2; see Supplement for choices of fixed and random effects in the models). Additional LMM was applied for alpha diversity from the combined bacterial 16S rRNA gene data from all countries using HQ0/HQ1_16S as response variable (Table S3). If the data did not follow the normal distribution, investigated with the 'shapiro.test', it was log-, sqrt- or Cox-Box-transformed to ensure normal distribution before modeling. LMMs were conducted using the 'lmer' function in the 'lme2' R package (Bates et al., 2015).

Prior to the permutational analysis of variance (PERMANOVA) to test the effect of trenching on beta-diversity, the bacterial and fungal OTU data were normalized using the pairwise ratios (GMPR) method (Chen et al., 2018) geometric mean to diminish the effect of variable sample library sizes and to increase the resolution for zero-inflated data. PERMANOVA was conducted with the 'adonis2' function in the 'vegan R package 2.6-4' (Oksanen et al., 2022). All analyses were conducted with

the R version 4.2.1 (R Core Team, 2022) and the R studio version 2022.7.1.554 (RStudio Team, 2022).

Trenching did not affect any bacterial or fungal alpha-diversity measures or relative abundances of fungal guilds except in Lithuania where it had an increasing impact ($p = 0.04$) on the fungal alpha-diversity; variation was very high, however (HQ0 ITS, HQ1 ITS; Table S2). Additional LMMs for Lithuanian fungal guild-derived OTUs indicated that trenching may have increased saprotrophic richness (HQ0) and pathotrophic diversity (HQ1), potentially explaining the increased alpha-diversity (Table S4). Trenching had a minor effect on fungal beta-diversity in Finland, explaining 2% of the variation, whereas it did not affect fungal ITS composition elsewhere (Fig. 1). Since the oldest trenching treatments were located in Finland (Table S1), the effect of time elapsed from trenching was analyzed separately for the Finnish data, showing no effect on the ITS composition (PERMANOVA $P = 0.63$; not shown). Bacterial 16S rRNA gene-derived beta-diversity was not influenced by trenching either (Tables S2 and S3; Figs. S3 and S4).

Trenching seems not to cause major changes in the soil microbial communities in the short term. This statement is based on i) the altogether missing trenching response of the bacterial community; ii) the fungal alpha-diversity reacted only in one data set (Lithuania); iii) the fungal beta-diversity (community composition) reacted only in one data set (Finland) explaining as little as 2% of the variation, and iv) the relative abundance of fungal guilds did not respond to trenching. As trenching interrupts the photosynthetic C flow to symbiotrophs, it could be expected that this guild would react first and decrease proportionally. Such a change was, however, not detected. The majority (90%) of the symbiotrophic fungi were ectomycorrhiza with mycelial cords enabling utilization seemingly of the soil resources in the trenched areas of the size used in this study. Overall, it thus seems that changes in the microbial community do not jeopardize the use of trenching for measurements of R_{HET} . The performed forest silvicultural practices did not influence the interpretation of our result. For further validation, transcriptomic techniques should be applied to evaluate if the same members of the soil microbiome actually respire in trenched and untrenched soil. Other confounding factors such as decomposition of the killed roots and potential soil temperature differences (e.g., Hermans et al., 2022)

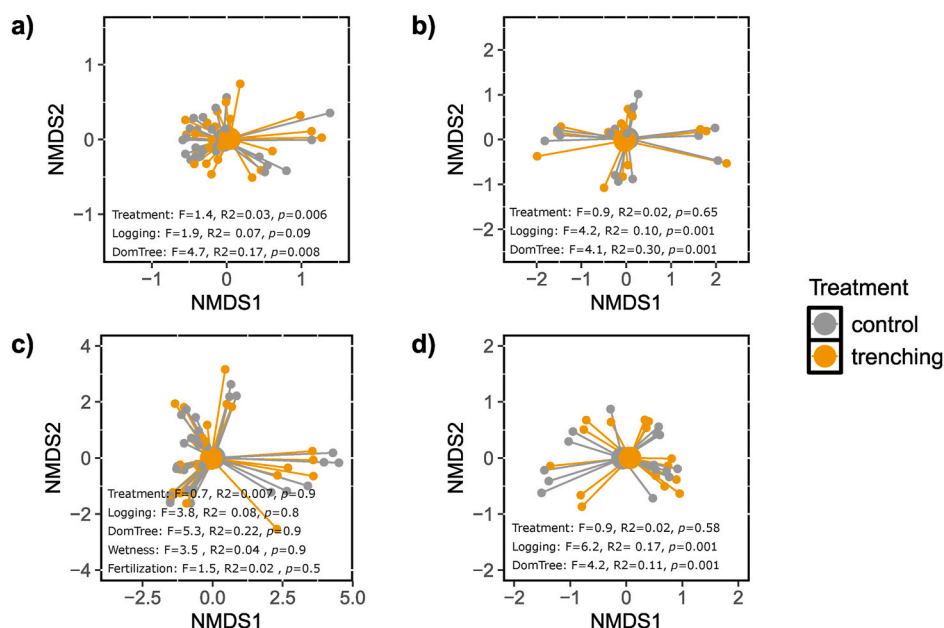


Fig. 1. Two-dimensional nonmetric multidimensional scaling (NMDS) plots with PERMANOVA results (F-value, R2, p-values) to test the Treatment (trenching), Logging, dominant tree of the site (DomTree), Wetness and ash fertilization effect on fungal beta-diversity (fungal ITS derived OTU composition) determined from samples of a) Finland ($n = 47$), b) Estonia ($n = 30$), c) Latvia ($n = 71$) and d) Lithuania ($n = 30$). p -values less than 0.05 are considered statistically significant. ITS data was analyzed separately for the countries (a–d) due to different sequencing batches (see Supplement). Lines connect each data point to the treatment centroid. The figure was created with the ggplot2 package (Wickham, 2016).

still need to be taken into account when applying trenching.

CRedit authorship contribution statement

Hannu Fritze: Writing – original draft, Visualization, Funding acquisition, Conceptualization. **Jyrki Jauhiainen:** Writing – review & editing, Visualization, Validation, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Arta Bårdale:** Writing – review & editing, Validation, Investigation, Funding acquisition. **Aldis Butlers:** Writing – review & editing, Validation, Investigation. **Dovilė Čiuldienė:** Writing – review & editing, Validation, Investigation. **Muhammad Kamil-Sardar:** Writing – review & editing, Validation, Investigation. **Ain Kull:** Writing – review & editing, Validation. **Raija Laiho:** Writing – review & editing, Validation, Funding acquisition, Conceptualization. **Andis Lazdiņš:** Writing – review & editing, Validation, Investigation, Funding acquisition. **Valters Samariks:** Writing – review & editing, Validation. **Thomas Schindler:** Writing – review & editing, Validation, Investigation. **Kaido Soosaar:** Writing – review & editing, Validation, Investigation, Funding acquisition. **Egidijus Vigrigas:** Writing – review & editing, Validation, Investigation. **Krista Peltoniemi:** Writing – review & editing, Visualization, Formal analysis, Conceptualization.

Data availability

Raw sequencing data from this study are deposited in the sequence read archive of the NCBI database under BioProject PRJNA1089491 with the accession numbers SAMN40541160 – SAMN40541337. Original data obtained from sequence reads (Hill's numbers, relative abundances of the fungal guilds) are deposited in the repository of zenodo under DOI 10.5281/zenodo.14229884.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2025.109747>.

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