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# Restriction of plant roots in boreal forest organic soils affects the microbial community but does not change the dominance from ectomycorrhizal to saprotrophic fungi

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**Abstract**

Boreal forest soils store significant amounts of carbon and are cohabited by saprotrophic and ectomycorrhizal fungi (ECM). The “Gadgil effect” implies antagonistic interactions between saprotrophic fungi and ECM. Plant photosynthates support the competitive fitness of the ECM, and may also shape the soil bacterial communities. Many “Gadgil effect” experiments have focused on litter layer ($O_L$) or have litter and root-fragments present, and thus possibly favor the saprotrophs. We compared how the restriction of plant roots and exudates affect soil microbial community structures in organic soil (mixed $O_F$ and $O_H$). For this, we established a three-year field experiment with three different mesh treatments affecting the penetration of plant roots and external fungal hyphae. Exclusion of plant photosynthates induced modest changes in both fungal and bacterial community structures, but not to potential functionality of the microbial community. The microbial community was resilient towards rather short-term disturbances. Contrary to the “Gadgil effect”, mesh treatments restricting the entrance of plant roots and external fungal hyphae did not favor saprotrophs that originally inhabited the soil. Thus, we propose that different substrate preferences (fresh litter vs. fermented or humified soil), rather than antagonism, maintain the spatial separation of saprotrophs and mycorrhizal fungi in boreal forest soils.

**Keywords:** boreal forest soil, Gadgil effect, saprotrophs, ectomycorrhiza, microbial community, functional gene profile

## Introduction

The boreal forests form globally stable and large carbon (C) pools. In the organic layers of boreal forest soils, carbon is stored in the form of complex polymers, including phenolic and humic compounds (DeLuca and Boisvenue 2012). In boreal forest ecosystems the lack of easily available nitrogen (N) in soils is considered to limit plant growth, since N is typically complexed with soil minerals or organic compounds, which are unavailable to the plants (Schulten and Schnitzer 1998; Näsholm, Kielland and Ganeteg 2009). To overcome the N limitation, plants form symbioses with mycorrhizal fungi (Smith and Read 2008), which are efficient scavengers of N and other nutrients from organic matter (Kohler *et al.* 2015; Shah *et al.* 2016).

The fungal and bacterial communities of boreal forest soils are interlinked and may either promote each other's growth and functioning or suppress it through exudation of secondary metabolites (de Boer *et al.* 2005). Mycorrhizal fungi can shape their associated bacterial community by carbon and nutrient flows (Izumi and Finlay 2011) and different mycorrhizal fungi harbor different bacterial populations (Skyring and Quadling 1969; Timonen and Sen 1998; Timonen *et al.* 1998). The fungal and bacterial communities participate together in SOM decomposition and nutrient cycling in boreal forest by producing various organic matter degrading enzymes and providing nutrients to each other (de Boer *et al.* 2005). In nutrient limited ecosystems microbes may also compete for nutrients with each other. Due to high diversity of microbes under different environmental conditions and niches, the extent and nature of the dynamic interaction between fungi and bacteria in soil is still largely unknown.

Although all fungal guilds can be found in all soil layers, the humus layers of the boreal forest ecosystem are dominated by ectomycorrhizal (ECM) fungi whereas saprotrophs dominate the litter layers (Lindahl *et al.* 2007; Santalahti *et al.* 2016). The growth and functioning of saprotrophic fungi in humus layers might be restricted by the limited availability of easily utilizable N (Lindahl, Taylor

and Finlay 2002) or by the depletion of the labile C pool in SOM. The dominance of ECM over saprotrophs in the boreal forest $O_F$ and $O_H$ layers is suggested to be maintained by the C flow from the plants. The restriction of the plant-derived C to ECM can shift this dominance to saprotrophs and other soil opportunists through a phenomenon called the Gadgil effect (Gadgil and Gadgil 1971, 1975; Lindahl and Tunlid 2015; Averill and Hawkes 2016), and possibly change the decomposition rate. However, contradictory results have been obtained and it is hypothesized that the role of ECM and saprotrophic fungi and their interplay in litter and SOM decomposition is complex and affected by various environmental conditions (Fernandez and Kennedy 2016). Since ECM fungi are supported by the plant-C flow, they generally produce enzymes in order to acquire N, rather than C, from the complex organic matter (Lindahl and Tunlid 2015; Bödeker *et al.* 2016). Scavenging for C from humus, where C is stored in the form of complex polymers, is suggested to be unbeneficial for saprotrophic fungi since the degradation process would require more energy than what could be gained from the degraded substrate (Baldrian 2009). Consequently, saprotrophs dominate the degradation of fresh plant litter.

Previous studies focusing on the effects of restricted plant carbon flow have been mainly conducted in microcosms (Lindahl *et al.* 1999) or as short-term field experiments which also have litter and root-fragments present (Gadgil and Gadgil 1971; Lindahl, de Boer and Finlay 2010; Bödeker *et al.* 2016; Fernandez and Kennedy 2016). Therefore, in these experiments, there has been plant biomass left in the soil to serve as an easy substrate for the saprotrophs. As proposed by Averill & Hawkes (2016) and Fernandez & Kennedy (2016), in these studies the magnitude and presence of the "Gadgil effect" might be mediated not only by the exclusion of the photosynthetic C flow but also by the increase of labile-C input to soils due to the disturbance of the treatment. It is proposed that the spatial separation of the ECM and saprotrophic guilds might also be due of different niche preferences (Fernandez & Kennedy 2016), which is further supported by the difference in the saprotrophic machineries between soil and litter saprotrophs and ECM fungi (Kohler *et al*. 2015).

To investigate the substrate dependence of the "Gadgil effect" we performed a field experiment in a boreal forest over three growing seasons to determine how the manipulation of plant photosynthetic C flow (1µm and 50 µm mesh treatments) affected the microbial communities and their potential functionality in organic soil from $O_F$ and $O_H$ layers. We assumed that $O_{F/H}$-soil contains limited amounts of labile-C, which is favored by saprotrophs. For this, we utilized a mesh bag approach where bags with three different pore sizes 1000 µm (allow penetration of both fungal hyphae and fine roots), 50 µm (allow fungal hyphae to penetrate but restrict tree-roots), and 1 µm (prevent the entrance of both external fungal hyphae and all plant roots) were filled with sieved, non-sterile soil from $O_F$ and $O_H$ layers. For studying the microbial community structures, we applied DNA-based MiSeq sequencing combined with a GeoChip microarray and multivariate statistical analyses. We hypothesized that the 1 µm mesh treatment restricting the entrance of plant photosynthates would: 1) according to the "Gadgil effect" theory favor saprotrophs and that this change would be detectable as increases in the relative abundance of saprotrophic taxa and in the potential functionality of these treatments compared to the 50 µm and 1000 µm treatments, 2) change the overall fungal and bacterial community compositions and their potential functionality compared to the 50 µm and 1000 µm treatments. And 3) these changes in microbial communities will become more pronounced in time, i.e. after 3$^{rd}$ growing season.

## Materials and methods

### Experimental design

The field experiment was conducted at Hyytiälä forestry field station (SMEAR II, University of Helsinki) located in southern Finland (61°51' N, 24°17' E). The forest at Hyytiälä field station is dominated by Scots pine (*Pinus sylvestris* L.) and the ground vegetation is dominated by shrubs and mosses. For the experimental setup, soil from the organic layer ($O_F$ and $O_H$) was collected from the

area surrounding Hyytiälä field station in autumn 2012. Prior to storing the collected organic soil at -20°C, it was homogenized and sieved (4 mm mesh) but left otherwise untreated. This sieved and homogenized non-sterile natural organic soil served later in the experiment as an initial fungal and bacterial inoculum for the microbial community and repository of the initial soil fauna in the mesh bags.

To manipulate the direct photosynthetic-C flows from the plants, mesh bags with three different pore sizes (1000 µm, 50 µm and 1 µm (Fig. 1)) were filled with sieved and homogenized organic soil from $O_F$ and $O_H$ layers corresponding to 14.2 g of dry weight. The different mesh sizes allowed penetration of both fungal hyphae and fine roots (1000 µm), allowed fungal hyphae to penetrate but restricted tree-roots (50 µm; the ericoid shrubs may have roots smaller in diameter than 50 µm), or prevented the entrance of both external fungal hyphae and all plant roots (1 µm) (Wallander *et al.* 2001). In contrast to the traditional use of sterilized material (usually sand) in mesh bags, we used unsterilized native organic soil for filling the mesh bags. Thus, the mesh bags contain also both living and dead microbes acting as the initial inoculum inside the mesh bags later in the experiment.

The mesh bags were buried between organic and mineral soil horizons in May 2013 randomly at three different sampling areas over 50 m apart from each other (described previously by Santalahti *et al.* (2016)). All the selected experimental sites in Hyytiälä field station had similar above ground vegetation and were visually uniform. One set of bags (n=15 for each mesh treatment, 3 sites with 5 replicates per site) were harvested yearly in late September after one, two and three growing seasons, resulting in a total of 135 samples. The collected mesh bags were transported from the field to the laboratory at +4 °C and divided into subsamples within the same working day as the harvest of the mesh bags. The external roots colonizing the mesh bags were picked away from the soil samples and their masses were recorded. Subsamples meant for molecular biology work were stored at -80 °C before further analysis.

**Determination of mass loss, soil moisture, soil pH and fungal biomass from the individual mesh treatments**

The soil moisture contents were determined from subsamples of each mesh bag by freeze drying the soil for 3 days. The mass losses of the soil placed inside the different mesh bags were determined by weighing the mesh bags at the time of the harvest and calculating the dry weight of the soil based on the known soil moisture contents. The soil pH was measured from subsampled soil in a soil water suspension of 1:2.5.

To assess the relative change in fungal biomass in each mesh treatment, ergosterol was extracted as described in Frostegard & Bååth (1996) and measured using HPLC with an UV detector (HP Agilent 1100). For the fungal biomass analysis, subsamples from each mesh size were pooled separately within each year and within each sampling site, resulting in total three replicates from each treatment in each sampling year (in total 27 samples). No initial time-point was analyzed for the ergosterol.

**DNA extraction**

The total DNA was extracted from the freshly frozen soil samples (amount corresponding to 50 mg of dry weight) using the commercially available NucleoSpin Soil DNA extraction kit (Macherey-Nagel GmbH & Co) with modifications described in Timonen et al. (2017). Extracted DNA was further purified using a PowerClean Pro DNA Clean-Up kit (MoBio Laboratories) according to the manufacturer's instructions. Concentrations of extracted dsDNA were measured fluorometrically using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific). Soil from initial time-point was not used for DNA extractions.

**Sequencing of the fungal ITS2 and bacterial V3–V4 regions**

For MiSeq sequencing 200 ng of extracted DNA was adjusted to a final concentration of 10 ng $\mu l^{-1}$. The fungal Internal Transcribed Spacer (ITS2) and bacterial V3–V4 regions of 16S rDNA from the extracted DNAs were sequenced using Illumina® MiSeq at the Institute of Biotechnology, University of Helsinki.

Prior to sequencing, a two-step-PCR was designed by and performed at the Institute of Biotechnology. In the first PCR, extracted DNAs were used as templates and the fungal-specific primers gITS7 and ITS4 (Ihrmark *et al.* 2012) or bacterial-specific primers f341 and r785 (Klindworth *et al.* 2013) containing partial TruSeq adapter sequences at the 5´ends (ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC T and GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC T, respectively) were used. In the second PCR, full-length TruSeq P5 and Index containing P7 adapters were used as primers and the products of the first PCR were used as templates.

**Processing of sequence data**

The raw ITS and 16S rDNA sequences were pre-processed at the Institute of Biotechnology and the general read quality was checked with FastQC software (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adapter and barcode sequences were cut away using Cutadapt software (Martin 2011). The nucleotide sequence data are available in the NCBI database under Bioproject number PRJNA454770.

The sequence data were further filtered and clustered to operational taxonomic units (OTUs) using mothur (version 1.39.5) (Schloss *et al.* 2009). For filtering, denoising and clustering the bacterial sequences to OTUs, the standard operating procedure ((Kozich *et al.* 2013), accessed 2017-01-15) was followed. For filtering, denoising and clustering fungal sequences to OTUs, the pipeline by Sietiö *et al*. (2018) was followed. For identification, bacterial sequences were aligned against the mothur-

formatted SILVA-database (release 123, (Quast *et al.* 2012; Yilmaz *et al.* 2014)) as described in the standard operating procedure ((Kozich *et al.* 2013), accessed 2017-01-15) and fungal sequences were aligned against the UNITE-database (UNITE+INSD version 7.1, (Kõljalg *et al.* 2013)) in mothur with classify.seqs.

For both bacterial and fungal communities, the sequence data were further de-noised in the R programming environment (R Core Team 2016) with the pipeline suggested by Callahan *et al.* (2016). During de-noising the threshold was set so that those taxa which were present in at least seven samples were kept in analysis and others were discarded. The diversity indices were calculated from the de-noised data with estimate_richness-function after randomly subsampling the data with rarefy_even_depth-function to 49 301 reads per sample with fungal sequences and 20 459 reads per sample with bacterial sequences (McMurdie and Holmes 2013). The obtained fungal taxa were assigned to functional guilds according to the FUNGuild database (Nguyen *et al.* 2016) with metagMisc package (Mikryukov 2017), and only those classifications which had a probability level of "Probable" or higher were kept in further analyses.

**GeoChip 5.0S microarray**

For the GeoChip 5.0S microarray, equal amounts of genomic DNA of each sample were pooled separately within each year and within each sampling site, resulting in a total of three replicates from each treatment in each sampling year (in total 27 samples). A total of 1000 ng of genomic DNA was used for GeoChip at Glomics Inc. There the genomic DNA was mixed with random primers and then labelled with 15 µl of labelling master mix, after which the labelled genomic DNA was purified, dried, and hybridized on the GeoChip 5.0S microarray as previously described (Van Nostrand *et al.* 2016). The obtained signal intensities were pre-processed at the Glomics Inc. The signal intensities of each spot were normalized by dividing the signal intensity of that spot with the mean of the whole

microarray and multiplied with a constant as described in (Liang *et al.* 2010). The probes were removed as negative if the signal-to-noise ratio was <2 or if the signal intensity was <200. The raw GeoChip microarray data is presented in Supplementary Table 1.

The GeoChip microarray data was organized to gene lineages and only fungal and bacterial genes were selected for further analysis, in which fungal and bacterial genes were analyzed separately. The gene diversities from GeoChip were calculated from the probe-level data with diversity and fisher-alpha functions from the vegan-package (Oksanen *et al.* 2017). Different probes from the same gene families were combined by summing them together. For clustering the genes into different subcategories based on their functionality, the preset categories by Glomics Inc were used.

**Statistical analyses**

All the statistical and descriptive analyses for the fungal OTUs were executed in the R programming environment (R Core Team 2016). The level of statistical significance of all analyses was set to $p \leq 0.05$. Significant differences in diversity indices were tested with the non-parametric Kruskal-Wallis test using kruskal.test-function from stats package (R Core Team 2016). Statistical significance of the changes detected between the different mesh treatments across the three growing seasons in overall fungal or bacterial communities as well as fungal functional guilds were tested with permutational multivariate analysis of variance (perMANOVA) using 999 permutations with adonis-function from vegan package (Oksanen *et al.* 2017). Prior to MANOVA the sequence counts were normalized with the library sizes and log-transformed, and in MANOVA, mesh size, growing season, soil pH, moisture and mass loss (average values represented in Supplementary Table 2) were used as explanatory variables. When performing MANOVA with mesh size as an explanatory variable, the growing season was set to grouping factor.

The differences in the soil fungal or bacterial communities between the 1 µm and 1000 µm mesh treatments across the three growing seasons were analyzed with partitioned canonical correspondence analysis (pCCA). The pCCA, where the effect of soil moisture content and sampling area was partitioned out, was conducted with the vegan package (Oksanen *et al.* 2017). For the pCCA, logarithmic transformed abundance data of all fungal OTUs (2566 OTUs) or 3000 most abundant bacterial OTUs were used as variables and mesh size, growing season and soil pH as explanatory variables. The pCCA was visualized with plot.cca-function, and the square root eigenvalues of species and site scores were scaled symmetrically using scaling "symmetric". The statistical significances of the CCA-axes and explanatory variables were determined with anova.cca-function from vegan package. The species scores of the 25% most abundant and 0.5% of the best fitting OTUs (Supplementary Table 3 for fungi and Supplementary Table 4 for bacteria) were selected with ordiselect-function from goeveg package (Goral and Schellenberg 2017) and visualized in the pCCA.

Fungal and bacterial orders, genera and species, and fungal functional guilds responding significantly to the treatment as well as their log2 changes were identified with DESeq2 package (Love, Huber and Anders 2014). In DESeq2, the level of significant log2 fold change value was set to ±1, adjusted p-value to 0.05, and in addition the change was considered significant only when the abundance of the species or genus was over 1% in at least one of the different mesh treatments. The log2 changes in DESeq2 were converted to fold changes by calculating 2 to the power of log2-value (i.e. $2^{\log 2 \text{ change}}$). The Spearman correlations between the bacterial genera or fungal species and the environmental variables, mass loss, soil pH and soil moisture content (Supplementary Table 2), were determined with rcorr-function from Hmisc package (Harrell 2017).

For the GeoChip microarray data, the gene diversities of each sample were calculated with R program (R Core Team 2016) using diversity and fisher.alpha-functions from the vegan package (Oksanen *et al.* 2017). The statistical differences in the diversity indices and in the signal intensities

between different gene families were determined with the non-parametric Dunn´s test (Dinno 2017). The level of statistical significance of the adjusted p-value was set to 0.05.

## Results

### The mesh treatment effect on mass losses, soil pH and fungal biomass

After the first growing season the pH of soils inside the mesh bags were at similar levels (pH 4.05-4.12) in all the mesh treatments (Supplementary table 2). After the second growing season the soil pH inside the 1000 µm mesh bags started to decline reaching pH 3.86 after the 3$^{rd}$ growing season whereas inside the 50 µm and 1 µm mesh bags the soil pH remained at pH 3.95-4.02 throughout the whole three-year experiment. The mesh size did not affect the soil moisture contents inside the different mesh treatments (Supplementary table 2). However, the soil moisture contents varied between the different growing seasons (Supplementary table 2) and experimental sites (data not shown).

During the experiment, the 1000 µm mesh bags had the highest measured mass losses (12 % of the total dry weight lost after 3$^{rd}$ growing season) whereas the mass loss of the 1 µm mesh bags was lowest (9 % of the total dry weight lost after 3$^{rd}$ growing season) (Supplementary table 2), indicating that the microbial SOM degradation activity was lowest in the 1um treatments. In addition, the fungal biomass (ergosterol) inside the 1 µm mesh treatments declined almost two-fold between the first and third growing seasons whereas in the 1000 µm mesh treatments the fungal biomass increased during the experiment (Supplementary table 2). Within the 1000 µm and 50 µm mesh treatments, plant fine roots grew inside the mesh bags, and after the 3$^{rd}$ growing season, the roots accounted for up to 2% of the total fresh weight of the 1000 µm mesh bags (Supplementary table 2). No root ingrowth was detected with the 1 µm mesh bags.

## Overall microbial community diversity

From samples describing the total microbial community of the mesh bags, a total of 16 848 032 raw fungal ITS2 sequences and 15 259 602 raw bacterial 16S rDNA sequences were obtained. After quality control and de-noising, 11 054 791 high-quality and non-chimeric fungal sequences and 11 321 704 bacterial sequences remained (Table 1 a, b). The fungal sequences were divided into 2566 fungal OTUs and the bacterial sequences were divided into 6537 bacterial OTUs. The alpha-diversities of neither fungal nor bacterial communities between 1000 µm, 50 µm and 1 µm mesh treatments differed significantly (Table 1 a,b), but based on the Fisher indices, both the fungal and bacterial species richness were the highest after the 1$^{st}$ growing season and decreased during the experimental time in all mesh sizes ($p \leq 0.01$, Kruskal-Wallis). In addition, based on the Shannon and Inverse Simpson indices, the detected species in the bacterial communities were more unevenly distributed after the 1$^{st}$ growing season than after 2$^{nd}$ and 3$^{rd}$ growing seasons ($p \leq 0.001$, Kruskal-Wallis) (Table 1 b).

## Changes in fungal community

The fungal community structures within each mesh size changed during the experimental time ($p \leq 0.001$ for each mesh treatment with perMANOVA). Differences in soil moisture content and soil pH explained the observed changes in the fungal community structures ($p \leq 0.001$ for each treatment with perMANOVA). Also, the fungal community structures between the three sampling areas differed from each other ($p \leq 0.001$ with perMANOVA, data not shown). Within each growing season, the fungal community structures between 1000 µm and 1 µm mesh treatments differed from each other ($p \leq 0.01$ with perMANOVA, Fig. 2a), but not from 50 µm treatment.

The fungal OTUs were divided into seven phyla: Basidiomycota (62% of fungal sequences), Ascomycota (29% of fungal sequences), Mucoromycota (5.4% of fungal sequences), Rozellomycota, Glomeromycota, Zoopagomycota and Chytridiomycota. The most abundant fungal phylum Basidiomycota harbored altogether 31 orders (Table 2). From these, Agaricales was more abundant in the 1 µm mesh treatment than in the 1000 µm treatment (p-adj ≤ 0.01 with DESeq2), whereas Boletales was more abundant in the 1000 µm mesh than in the other treatments (p-adj ≤ 0.001 with DESeq2). The abundances of ECM fungi from genera *Russula* (6.7% of all sequences), *Piloderma* (9.9% of all sequences) and *Tylospora* (9.2% of all sequences) increased during the experiment in all the mesh sizes (p-adj ≤ 0.001 for *Piloderma* with DESeq2, Table 2). In contrast, the abundances of ECM *Lactarius* (14.2% of all sequences) decreased during the experiment in all mesh sizes (Table 2).

Significant order, genus and species-level differences between the 1000 µm and 1µm as well as 1000 µm and 50 µm mesh treatments were also detected (Table 2). The ECM fungal genus *Suillus* (1.6% of sequences) was more abundant in 1000 µm mesh than in 50 µm or 1 µm mesh (p-adj ≤ 0.001 with DESeq2). Likewise, the ECM *Suillus bovinus* had a similar trend, and especially after the 3$^{rd}$ growing season it was more abundant in the 1000 µm mesh than in the other mesh sizes (p-adj ≤ 0.05 with DESeq2). Also, *Inocybe* and *Oidiodendron maius* were more abundant in the 1000 µm mesh than in 50 µm or 1 µm mesh treatments after the 3$^{rd}$ growing season (p-adj ≤ 0.01 with DESeq2). Contrarily, the ECM genus *Cortinarius* was more abundant in the 1 µm than in 1000 µm mesh treatment (p-adj ≤ 0.001 with DESeq2). In addition, the ECM *Russula decolorans*, was more abundant in 50µm and 1 µm mesh treatments than in 1000 µm (p-adj ≤ 0.05 with DESeq2). The abundance of the saprotroph *Sistotremastrum* increased between the 1$^{st}$ and 3$^{rd}$ growing seasons in the 1000 µm mesh treatment whereas in the 50 µm and 1 µm treatments its abundance decreased (p-adj ≤ 0.05 with DESeq2).

Functional distribution of the fungal community

A total of 1118 fungal OTUs (43.6% of all fungal OTUs and 68.6% of sequences) could be assigned to different trophic modes (Fig. 3). Symbiotrophs were the most abundant fungal trophic mode covering 44–60% of the assigned sequence reads. Symbiotrophs consisted mainly of ectomycorrhizal (ECM) fungi whereas ericoid mycorrhizal fungi were detected less, and as expected arbuscular mycorrhizal fungi were in minority across all the treatments. Saprotrophic activity possessing fungal OTUs covered 6.1–14% of the sequence reads of the different treatments. Pathotrophic activity possessing fungal OTUs covered 4.7–8.6% of the sequence reads and their proportion declined during the experiment in all the mesh treatments.

Although the amount of all fungal biomass declined in 1µm mesh treatment over the three growing seasons compared to 1000µm mesh treatment (Supplementary table 2), we detected also differences in the proportional distribution of all sequences per sample to different fungal functional guilds between the different mesh treatments. Overall, the percentage of plant pathogens, universal saprotrophs and endophytes from all sequences per sample was 1.1–2.4-fold higher (p-adj $\leq$ 0.05 with DESeq2, data not shown) in 1000 µm mesh treatment than in 1 µm mesh. The percentage of wood saprotrophs from all sequences per sample in 1000 µm mesh treatment was 1.5-fold higher (p-adj $\leq$ 0.05 with DESeq2, data not shown) than in 50 µm and 1 µm mesh. Contrarily, the proportion of "undefined saprotrophs" was 1.3-fold higher in 1µm mesh treatments (p-adj $\leq$ 0.05 with DESeq2, data not shown) than in 1000 µm mesh. The overall proportions of different fungal guilds from all the sequences per sample did not differ with statistical significance between the 50 µm and 1 µm mesh treatments.

**Changes in bacterial community**

The bacterial community structures within each mesh size changed significantly during the experiment ($p \leq 0.001$ for each mesh treatment with perMANOVA). Differences in soil moisture

content and soil pH influenced the bacterial community structures ($p \leq 0.001$ with perMANOVA). Within each growing season, the bacterial communities between 1000 µm and 1 µm treatments differed from each other ($p \leq 0.05$ with perMANOVA, Fig. 2b), but were similar with the 50 µm treatment. The bacterial community structures between the three sampling areas differed from each other ($p \leq 0.05$ with perMANOVA, data not shown).

The bacterial orders and genera correlating significantly with the soil properties (see Supplementary Table 2) belonged mainly to the classes Acidobacteria, Alphaproteobacteria, Deltaproteobacteria, Actinobacteria and Sphingobacteriia (Table 3). The abundances of class Sphingobacteriia (2.6% of sequences), orders Rhizobiales (15.7% of all sequence-reads), Caulobacterales (2.1% of sequences), as well as genera *Rhizomicrobium*, *Phenylobacterium*, and *Mucilaginibacter* correlated with the soil moisture content and the mass losses of the mesh bags with statistical significance (Table 3). The order Myxococcales (2.7% of sequences), genera *Haliangium* and *Acidobacterium* correlated with the soil moisture content and pH. In addition, the order Rhodospirillales (7.2% of the sequences) correlated significantly with the soil pH and mass losses of the mesh bags, whereas the order Acidobacteriales (15.9% of sequences) and the genus *Sorangium* correlated only with the soil pH with statistical significance.

**The potential functionality of the microbial communities**

A total of 2717 fungal gene probes and 30 183 bacterial gene probes were detected from the samples using the GeoChip 5.0S microarray. Gene probes originating from archaea and viruses were omitted from this analysis. The alpha-diversities between the different mesh treatments did not differ with each other (data not shown). The potential functional fungal and bacterial gene pools did not differ between the treatments, and the growing season was the most significant factor explaining the

differences in the potential functional gene pool structures of both fungi and bacteria ($p \leq 0.05$ with perMANOVA, Supplementary Table 5a,b).

## Discussion

We found that the three mesh treatments caused different changes to the fungal community structure inside the mesh bags and modulated also the bacterial community composition during three growing seasons. Contrary to our original hypothesis, in the 1 µm mesh bags, where access of plant roots and external fungal hyphae was restricted, the proportion of ECM fungi remained dominant when comparing to the total fungal community inside the mesh treatment. However, the total fungal ergosterol concentration declined inside the 1 µm mesh bags over the three growing seasons indicating a decline also in the fungal biomass (Supplementary table 2). Thus, despite of the relative dominance of ECM fungi in the 1 µm mesh treatments (Fig. 3), the absolute amount of ECM biomass likely declined when compared to other mesh treatments. As the changes in the fungal and bacterial community structures were modest (Fig. 2), our results indicate that members of the soil microbial community can survive from rather short-term environmental changes.

Both saprotrophs and ECM fungi can degrade plant biomass through Fenton chemistry (Lindahl and Tunlid 2015; Shah *et al.* 2016; Op De Beeck *et al.* 2018) and by producing plant cell wall degrading enzymes (Lundell, Mäkelä and Hildén 2010; Lindahl and Tunlid 2015). As more efficient degraders, true saprotrophs generally dominate the boreal forest litter layers whereas the $O_F$ and $O_H$ layers are dominated by ECM fungi (Lindahl *et al.* 2007; Santalahti *et al.* 2016). This separation is likely maintained by the competition for nutrients between saprotrophs and mycorrhiza and the ECM fungi are suggested to outcompete the saprotrophs via access to C provided by the plants (Gadgil and

396 Gadgil 1971). Based on the theory behind the “Gadgil effect”, we hypothesized that in the 1 µm
397 treatments the internal fungal community structure would change, the relative proportion of the soil
398 originally inhabiting saprotrophs would increase, and the proportion of mycorrhiza would decrease.
399 Contrary to our hypothesis and previous findings (Gadgil and Gadgil 1971; Lindahl, de Boer and
400 Finlay 2010; Bödeker *et al.* 2016), in 1 µm mesh bags internal community the proportion of
401 saprotrophs did not increase (Fig. 3). Contrary to previous experiments, in our experiment the soil
402 from $O_F$ and $O_H$ layers that was used for constructing the mesh bags was sieved to remove fine roots
403 and all particles larger than 4 mm leaving a limited amount of plant biomass to serve as a substrate
404 for the microbes. Thus, the microbes inside the 1 µm mesh treatment likely suffered from energy
405 depletion during the experiment. Furthermore, in the 1000 µm mesh treatment, the fine roots could
406 grow inside the mesh bags and the amount of fresh plant roots accounted for up to 2% of the total
407 fresh weight of the mesh bags after the last growing season (Supplementary table 2). Presumably, the
408 plant root exudates and litter had been used as easily-utilizable substrates by the saprotrophs, and thus
409 possibly explain the increase of saprotrophs in the 1000 µm mesh treatment.

410 In our study, the abundance of the ECM *Russula* and *Cortinarius* increased in the 1 µm mesh
411 treatment and decreased in the 1000 µm mesh treatment (Table 2). Most ECM are slow growing but
412 tend to dominate the decomposition of recalcitrant SOM (Fontaine, Mariotti and Abbadie 2003). Even
413 though the saprotrophic machinery of ECM is less efficient than that of true saprotrophs (Kohler *et*
414 *al.* 2015), this affinity towards recalcitrant SOM may have given some ECM fungi an advantage
415 against those saprotrophs that originally inhabited the organic soil placed inside the 1 µm mesh bags.
416 Although the ECM fungi scavenge SOM mainly for N (Lindahl and Tunlid 2015), based on the “Plan
417 B hypothesis” mycorrhizal fungi may decompose SOM to acquire C when the photosynthetic-C flow
418 is insufficient (Talbot, Allison and Treseder 2008). Also, other studies have indicated that the
419 outcome of competition between ECM and saprotrophic fungi is dependent on the quality of the
420 substrate (Lindahl *et al.* 1999; Lindahl, Stenlid and Finlay 2001), and in a field-study Bödeker *et al.*

(2016) showed that sterilized humus was colonized more eagerly by mycorrhizal fungi than saprotrophs after two growing seasons. In addition, the degradation of humus is suggested to be unprofitable for saprotrophs (Baldrian 2009). Thus, our results of ECM dominance in the 1 µm treatment combined with the previous studies indicate different niche and substrate preferences between mycorrhizal and saprotrophic fungi and support the hypothesis by Averill & Hawkes (2016) and Fernandez & Kennedy (2016) that the appearance of "Gadgil effect" is substrate dependent.

Since plant roots and their exudates as well as root associated fungi can affect the bacterial growth (de Boer *et al.* 2005), we hypothesized that the different mesh treatments would cause changes also to the soil bacterial communities. However, the bacterial populations were most affected by the soil pH and soil moisture content rather than the mesh treatments (Fig. 2, Table 3). These results are in line with previous findings that the bacterial communities in soils are sensitive to soil chemistry and pH (Männistö, Tiirola and Häggblom 2007; Jeanbille *et al.* 2016). However, previous studies have shown that the pH of organic soil from $O_F$ and $O_H$ layers is strongly influenced by the presence of plant roots and their exudates since the pH of unplanted soil is over pH 4.5 whereas in the presence of boreal plant roots the pH is typically around 4.0 (Adamczyk *et al.* 2016; Kieloaho *et al.* 2016). Also, in our study the pH of 1 µm mesh bags remained higher than the pH of 1000 µm bags across the three growing seasons (Supplementary table 2). The observed changes in soil pH and the significance of the pH to the bacterial community structures may be mediated by the exclusion of plant roots.

We hypothesized that the shift in microbial community structures would be also related to their potential functionality, and we investigated this with DNA-based GeoChip 5.0S. However, we did not detect significant differences between the treatments. Instead, inside each mesh treatment the functional gene pools changed differently during the three-year experiment. Since both bacteria and fungi typically contain multiple copies of genes encoding for enzymes related to cellulose, hemicelluloses and lignin degradation (Floudas *et al.* 2012; López-Mondéjar *et al.* 2016), it is

probable that only drastic changes in microbial community structures would affect the potential functionality of the community. However, the structure of the active microbial community in boreal forest soil can differ highly from that of the total microbial community (Baldrian *et al.* 2012) and the metabolic activity of microorganisms may decrease with decreasing nutrient availability (Stenström, Svensson and Johansson 2001; Wright *et al.* 2002; Nitsche *et al.* 2012). It is likely that in our study the plant biomass degradation related genes were differently expressed in the 1000 µm, 50 µm, and 1 µm treatments.

In this work, we assessed the total fungal and bacterial DNA from the mesh bags containing both living and dead microbes as well as spores. Based on the lower mass loss and lower fungal biomass in the 1 µm treatment compared to the other treatments (Supplementary table 2), it is probable that microorganisms have been less active or dead in the 1 µm treatment than in the other mesh sizes due to possible nutrient depletion. The depletion of nutrients and especially easily-utilizable C may have caused the soil microbes in 1 µm mesh bags to lower their metabolic rates. When encountering rather short environmental disturbances, such as nutrient depletion, microbes can adapt to it by going into a metabolically inactive state and recover from it when there are more nutrients available (Stenström, Svensson and Johansson 2001; Jones and Lennon 2010; Lennon and Jones 2011). In our experiment, the possibly dormant or metabolically less-active microbes, as well as spores, might have persisted longer inside the 1 µm mesh bags than they would normally in natural boreal forest soils since they were protected from soil macro- and meso-fauna. In addition, some microbes can produce propagules that are more resistant in the soil than others, and these may have contributed also in our experiment to the DNA-profiles of the total microbial communities detected in different mesh treatments. Thus, a prolonged mesh treatment could cause more drastic changes to microbial communities, and further investigations of shifts in both RNA- and DNA-profiles comparing active and total microbial communities are needed.

## Conclusions

The manipulation of the presence of plant roots and external fungal hyphae in mesh bags filled with homogenized soil over three growing seasons caused moderate changes to both fungal and bacterial community structures. Our results also indicate that the boreal forest soil microbial community can survive from relatively short-term environmental changes. Contrary to the theory behind the "Gadgil effect", the internal community of mesh bags restricting the entrance of both plant roots and external fungal hyphae were dominated by ECM fungi rather than saprotrophic fungi and the total fungal biomass declined significantly. It is noteworthy, that although the decline in ECM biomass was expected, saprotrophic fungi did not occupy the "free niche" in the 1 µm bags. Thus, it seems that appearance of the "Gadgil effect" is substrate dependent and might reflect the different niche preferences of the ECM and saprotrophic fungal guilds. However, to determine if the spatial separation of ECM and saprotrophs is due to antagonistic interactions or ecological niche preference, we propose that in future studies the long-term competitive interactions between ECM and saprotrophic guilds should be compared on different substrates containing for example sieved organic soil, organic soil mixed with litter, different litter qualities as well as root fragments. In addition, our results suggest that the plant roots and their exudates are important in shaping the microbial community structure and functionality in boreal forest soils. To confirm our findings, a more detailed study assessing the responses of both the total and active microbial community structures to long-term manipulations of the presence of plant roots in organic $O_F$ and $O_H$ soil layers is required.

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## Conflict of interest

There are no conflicts of interest related to this work.

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**Figures:**

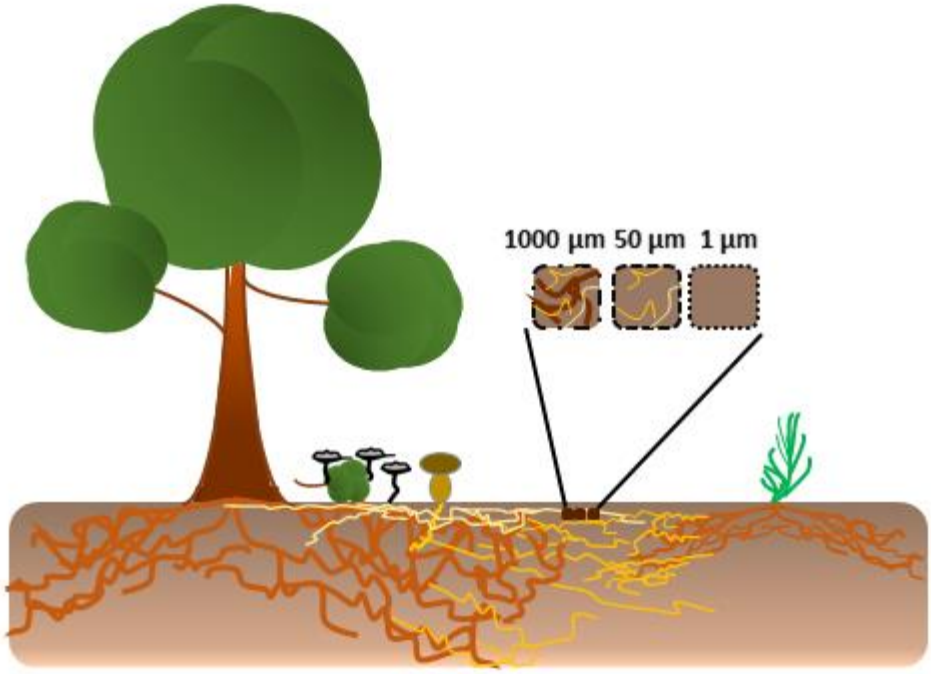


**Figure 1**. Schematic representation of the experimental setup where the mesh bags with three different pore sizes were buried between organic and mineral soil horizons. 1000 µm mesh size allowed penetration of both fungal hyphae and fine roots, 50 µm mesh size allowed fungal hyphae to penetrate but restricted plant roots, and 1 µm mesh size prevented the entrance of both fungal hyphae and plant roots. For determining the microbial community profiles, 18 mesh bags from each treatment were collected after 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ growing season.

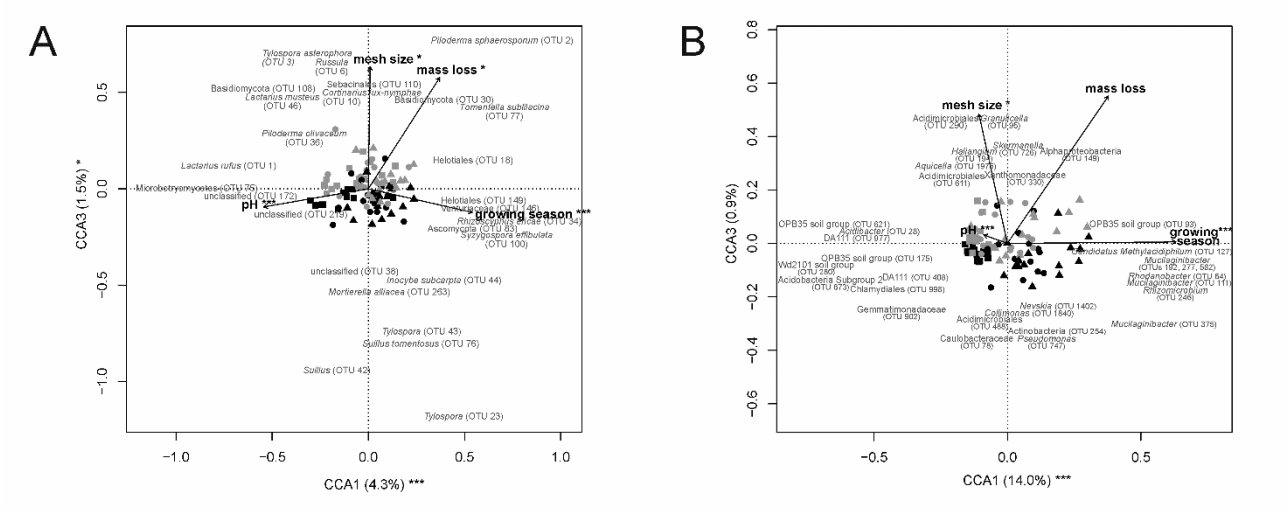


**Figure 2**. Partitioned canonical correspondence analysis, where the soil moisture content and sampling area have been partitioned out, representing the differences between a) fungal communities and b) bacterial communities of the 1000 µm and 1 µm mesh bags during the three growing seasons. The 1000 µm mesh treatment is visualized with gray and the 1 µm mesh treatment is visualized with black color. The different growing seasons are represented with square (■) for 1st growing season, circle (●) for 2nd growing season, and triangle (▲) for 3rd growing season. The species scores of the 25% most abundant and 0.5% of the best fitting fungal OTUs are visualized (Supplementary Table 3 for fungi and Supplementary Table S4 for bacteria). The statistical significances of the explanatory variables and axes are presented; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. The soil moisture content and sampling area explained 8.3 % of variation in case of fungal community and 10 % of variation in case of bacterial community. The fungal and bacterial community structures between 1000 µm and 1 µm mesh treatments differed from each other ($p \leq 0.01$ for fungal communities and $p \leq 0.05$ for bacterial communities with perMANOVA). Additionally, both the fungal and bacterial community structures within each mesh size changed significantly during the experiment ($p \leq 0.001$ with perMANOVA).

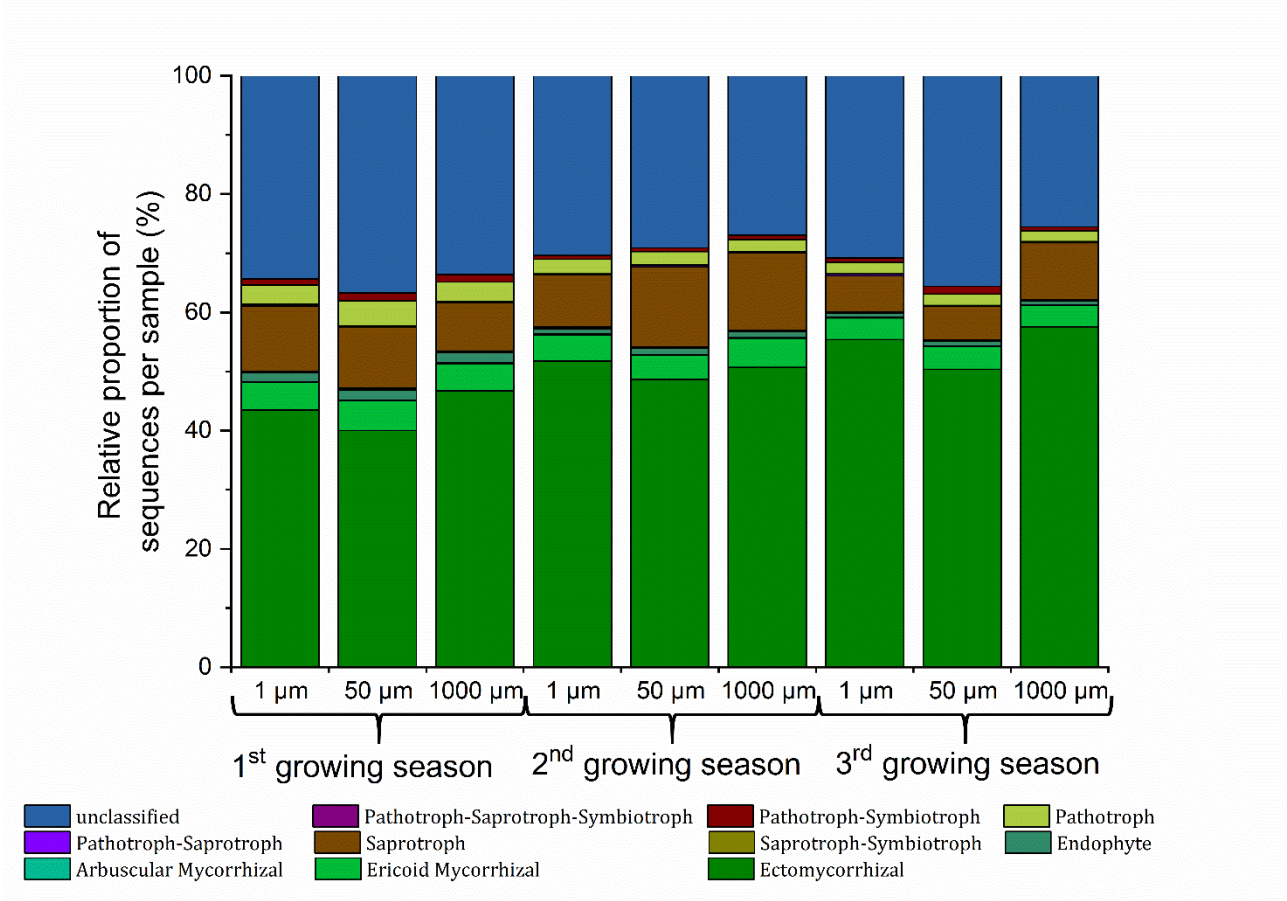


**Figure 3**. The relative proportion (percentages of the total sequence count per sample) of fungal OTUs in different trophic modes. Within the fungal functional guilds, the proportion of "plant pathogens", "universal saprotrophs" and "endophytes" were 1.1–2.4-fold higher in 1000 µm mesh treatment than in 1 µm mesh (p-adj ≤ 0.05 with DESeq2, data not shown), whereas the proportion of "undefined saprotrophs" was 1.3-fold higher in 1µm mesh treatments than in 1000 µm mesh (p-adj ≤ 0.05 with DESeq2, data not shown). In addition, the proportion of fungal guild "wood saprotrophs" was 1.5-fold higher in 1000 µm mesh treatments than in 50 µm and 1 µm mesh (p-adj ≤ 0.05 with DESeq2, data not shown).

**Tables:**

**Table 1.** Richness (observed and estimated OTUs), evenness and diversity indices for a) fungal ITS2 region libraries and b) bacterial 16S rDNA region libraries in the different mesh treatments across the three growing seasons. Average values and standard deviations are calculated with all the replicates (N=15). Statistical significance of the diversity indices was obtained with Kruskal-Wallis test. Levels of statistical significances ($p \leq 0.05$) within each mesh size between growing seasons are marked with letters.

a)

| | | Sequence count | Subsampled fungal OTUs | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Observed | Estimated | Shannon | InvSimpson | Fisher | Pielou´s eveness |
| 1st growing season | 1µm | 1 119 433 | 631 ± 69 | 707 ± 75 | 3.52 ± 0.92 | 15.05 ± 15.99 | 102.13 ± 13.17 | 1.25 ± 0.31 |
| | 50µm | 1 105 068 | 625 ± 69 | 710 ± 84 | 3.57 ± 0.82 | 15.25 ± 13.37 | 101.05 ± 13.33 | 1.28 ± 0.28 |
| | 1000µm | 1 095 877 | 626 ± 93 | 710 ± 110 | 3.41 ± 0.91 | 12.76 ± 11.64 | 101.36 ± 17.86 | 1.21 ± 0.30 |
| 2nd growing season | 1µm | 1 158 280 | 549 ± 42 | 628 ± 51 | 3.33 ± 0.47 | 8.87 ± 5.60 | 86.48 ± 7.74 | 1.21 ± 0.16 |
| | 50µm | 1 194 473 | 576 ± 55 | 667 ± 64 | 3.32 ± 0.72 | 9.72 ± 8.35 | 91.74 ± 10.29 | 1.20 ± 0.25 |
| | 1000µm | 1 294 510 | 561 ± 59 | 649 ± 68 | 3.22 ± 0.73 | 10.15 ± 11.47 | 88.95 ± 11.03 | 1.17 ± 0.25 |
| 3rd growing season | 1µm | 1 283 097 | 525 ± 51 | 598 ± 64 | 3.09 ± 0.49 | 7.12 ± 3.44 | 82.17 ± 9.38 | 1.13 ± 0.17 |
| | 50µm | 1 320 903 | 533 ± 47 | 624 ± 65 | 3.23 ± 0.60 | 9.47 ± 6.32 | 83.59 ± 8.75 | 1.18 ± 0.21 |
| | 1000µm | 1 483 150 | 524 ± 40 | 609 ± 43 | 3.04 ± 0.46 | 7.09 ± 6.04 | 81.94 ± 7.44 | 1.12 ± 0.16 |

b)

| | | Sequence count | Subsampled bacterial OTUs | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Observed | Estimated | Shannon | InvSimpson | Fisher | Pielou´s eveness |
| 1st growing season | 1µm | 1 605 945 | 1508 ± 57 | 2147 ± 75 | 5.78 ± 0.06 | 122.05 ± 7.90 | 375.74 ± 18.81 | 1.82 ± 0.01 |
| | 50µm | 1 520 772 | 1490 ± 64 | 2071 ± 133 | 5.76 ± 0.09 | 121.14 ± 10.33 | 369.59 ± 20.90 | 1.82 ± 0.02 |
| | 1000µm | 1 555 333 | 1481 ± 86 | 2064 ± 116 | 5.76 ± 0.11 | 121.03 ± 10.56 | 367.03 ± 28.15 | 1.82 ± 0.02 |
| 2nd growing season | 1µm | 1 365 140 | 1303 ± 204 | 1824 ± 266 | 5.46 ± 0.28 | 88.30 ± 18.33 | 311.79 ± 61.32 | 1.76 ± 0.05 |
| | 50µm | 1 304 927 | 1272 ± 191 | 1761 ± 260 | 5.43 ± 0.24 | 84.93 ± 14.96 | 301.97 ± 58.09 | 1.75 ± 0.04 |
| | 1000µm | 1 281 568 | 1342 ± 132 | 1869 ± 210 | 5.51 ± 0.18 | 89.58 ± 13.68 | 322.96 ± 40.84 | 1.76 ± 0.04 |
| 3rd growing season | 1µm | 879 696 | 1349 ± 87 | 1776 ± 152 | 5.55 ± 0.10 | 87.15 ± 6.54 | 324.49 ± 27.48 | 1.77 ± 0.02 |
| | 50µm | 922 043 | 1268 ± 182 | 1666 ± 285 | 5.43 ± 0.24 | 82.43 ± 14.59 | 300.56 ± 55.41 | 1.75 ± 0.04 |
| | 1000µm | 886 280 | 1331 ± 149 | 1793 ± 205 | 5.49 ± 0.20 | 84.62 ± 10.46 | 319.62 ± 45.92 | 1.76 ± 0.04 |

**Table 2.** The total number of OTUs, sequences and relative share of sequences (percentage of total sequence count) of fungal OTUs in different phyla, orders and genera, their relative abundances (percentages of the total sequence count per sample) and the standard deviation in different mesh treatments. The average values are calculated with all the replicates (N=15). The fungal genera and species did not correlate significantly with the environmental variables.

| Taxonomy | total % | number of sequences | number of OTUs | 1st growing season | | | 2nd growing season | | | 3rd growing season | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 µm | 50 µm | 1000 µm | 1 µm | 50 µm | 1000 µm | 1 µm | 50 µm | 1000 µm |
| Basidiomycota | 62.9 | 6 950 964 | 796 | 53.3 ± 15.1 | 50.8 ± 14.8 | 56.2 ± 13.8 | 63.3 ± 10.9 | 62.7 ± 12.8 | 66.3 ± 10.9 | 68.5 ± 7.7 | 64.8 ± 11.4 | 68.5 ± 12.4 |
| Agaricales | 5.5 | 609 096 | 144 | 7.2 ± 13.9 | 1.5 ± 1.0 | 3.7 ± 6.6 | 9.1 ± 16.3 | 5.2 ± 9.3 | 3.3 ± 5.2 | 7.5 ± 14.1 | 7.3 ± 9.9 | 2.7 ± 3.4 |
| Cortinarius | 2.7 | 298 180 | 64 | 0.95 ± 0.39 | 0.78 ± 0.22 | 0.73 ± 0.23 | 7.3 ± 16.6 | 3.3 ± 8.4 | 1.09 ± 1.09 | 5.55 ± 13.90 | 3.20 ± 7.42 | 1.16 ± 1.83 |
| Inocybe | 1.1 | 124 832 | 4 | 3.80 ± 13.28 | 0.00 ± 0.00 | 1.06 ± 4.01 | 0.40 ± 1.53 | 0.10 ± 0.37 | 0.38 ± 1.44 | 0.25 ± 0.96 | 1.72 ± 6.64 | 0.77 ± 2.85 |
| Atheliales | 19.1 | 2 108 101 | 152 | 4.35 ± 5.20 | 5.85 ± 7.02 | 6.87 ± 7.64 | 20.3 ± 16.7 | 17.9 ± 19.5 | 26.8 ± 20.0 | 20.4 ± 16.2 | 24.4 ± 17.9 | 29.2 ± 23.0 |
| Piloderma | 9.9 | 1 092 768 | 105 | 2.23 ± 4.92 | 2.83 ± 4.57 | 2.36 ± 4.19 | 5.7 ± 7.2 | 7.8 ± 10.4 | 14.8 ± 16.5 | 10.3 ± 11.1 | 14.7 ± 19.6 | 18.2 ± 21.3 |
| Tylospora | 9.2 | 1 012 782 | 43 | 2.11 ± 2.56 | 3.02 ± 5.26 | 4.51 ± 4.58 | 14.6 ± 14.4 | 10.0 ± 14.3 | 11.9 ± 16.0 | 10.1 ± 12.2 | 9.77 ± 8.91 | 11.0 ± 10.6 |
| Boletales | 2.4 | 265 572 | 29 | 1.05 ± 0.57 | 3.23 ± 5.31 | 4.04 ± 6.89 | 0.97 ± 0.55 | 1.27 ± 1.45 | 4.75 ± 7.42 | 1.12 ± 1.87 | 1.05 ± 1.52 | 4.62 ± 9.66 |
| Suillus | 1.6 | 175 044 | 21 | 0.13 ± 0.26 | 2.27 ± 5.23 | 3.17 ± 6.94 | 0.25 ± 0.49 | 0.58 ± 1.48 | 3.06 ± 6.66 | 0.56 ± 1.88 | 0.49 ± 1.53 | 4.17 ± 9.73 |
| Suillus bovinus | 0.8 | 91 725 | 2 | 0.07 ± 0.20 | 0.02 ± 0.06 | 2.67 ± 7.00 | 0.06 ± 0.15 | 0.38 ± 1.47 | 0.44 ± 1.19 | 0.54 ± 1.89 | 0.42 ± 1.53 | 3.12 ± 9.71 |
| Suillus tomentosus | 0.002 | 23 406 | 3 | 0.002 ± 0.00 | 0.009 ± 0.016 | 0.31 ± 0.82 | 0.07 ± 0.23 | 0.05 ± 0.11 | 0.99 ± 2.76 | 0.00 ± 0.00 | 0.01 ± 0.04 | 0.30 ± 1.15 |
| Russulales | 21.2 | 2 338 129 | 94 | 29.3 ± 27.3 | 26.4 ± 27.1 | 31.9 ± 27.5 | 17.6 ± 19.8 | 16.4 ± 18.3 | 11.9 ± 14.9 | 26.8 ± 25.0 | 17.5 ± 22.1 | 20.4 ± 21.8 |
| Lactarius | 14.2 | 1 572 147 | 46 | 24.8 ± 29.1 | 23.4 ± 26.8 | 28.6 ± 28.3 | 8.7 ± 13.9 | 7.2 ± 9.3 | 5.8 ± 10.8 | 15.2 ± 23.5 | 9.7 ± 17.9 | 10.6 ± 17.2 |
| Russula | 6.9 | 765 982 | 48 | 4.5 ± 11.8 | 2.9 ± 6.9 | 3.3 ± 12.3 | 8.9 ± 14.1 | 9.2 ± 16.3 | 6.1 ± 13.0 | 11.6 ± 18.7 | 7.8 ± 17.5 | 9.8 ± 18.6 |
| Russula decolorans | 2.3 | 260 994 | 3 | 2.11 ± 8.14 | 1.61 ± 6.18 | 1.24 ± 4.78 | 2.49 ± 9.61 | 3.26 ± 12.47 | 2.89 ± 11.15 | 3.98 ± 14.98 | 4.0 ± 15.4 | 4.0 ± 15.3 |
| Trechisporales | 3.1 | 346 768 | 35 | 1.67 ± 2.89 | 1.54 ± 4.10 | 0.57 ± 1.71 | 2.37 ± 8.00 | 6.08 ± 21.83 | 6.22 ± 20.76 | 0.51 ± 1.39 | 0.19 ± 0.43 | 3.80 ± 14.21 |
| Sistotremastrum | 3.0 | 326 751 | 11 | 1.53 ± 2.89 | 1.46 ± 4.06 | 0.49 ± 1.70 | 2.18 ± 8.01 | 6.01 ± 21.85 | 5.40 ± 20.85 | 0.47 ± 1.39 | 0.14 ± 0.43 | 3.73 ± 14.22 |
| Ascomycota | 28.9 | 3 193 635 | 1098 | 35.7 ± 11.7 | 39.2 ± 12.6 | 34.5 ± 10.8 | 29.3 ± 9.0 | 29.4 ± 10.1 | 26.2 ± 8.5 | 23.5 ± 5.5 | 27.5 ± 9.0 | 24.0 ± 8.7 |
| Chaetothyriales | 3.9 | 428 503 | 123 | 3.95 ± 1.67 | 4.77 ± 2.68 | 4.42 ± 2.25 | 4.32 ± 1.57 | 4.63 ± 2.10 | 4.28 ± 2.09 | 3.21 ± 0.90 | 3.36 ± 1.16 | 3.30 ± 0.81 |
| Helotiales | 9.3 | 1 030 661 | 245 | 12.5 ± 4.3 | 13.3 ± 4.4 | 11.7 ± 3.2 | 9.2 ± 3.8 | 8.8 ± 2.7 | 8.0 ± 2.1 | 7.4 ± 2.0 | 9.0 ± 4.2 | 7.0 ± 2.1 |
| Leotiomycetes order Incerta | 4.3 | 480 067 | 168 | 5.38 ± 1.76 | 5.68 ± 1.71 | 5.06 ± 1.82 | 4.49 ± 1.51 | 4.53 ± 1.54 | 4.29 ± 1.41 | 3.60 ± 0.85 | 3.91 ± 1.32 | 3.56 ± 1.10 |
| Oidiodendron | 3.2 | 359 211 | 108 | 4.00 ± 1.25 | 4.12 ± 1.24 | 3.78 ± 1.34 | 3.32 ± 1.19 | 3.39 ± 1.23 | 3.28 ± 1.19 | 2.69 ± 0.64 | 2.95 ± 1.01 | 2.74 ± 0.91 |
| Oidiodendron pilicola | 2.0 | 223 394 | 59 | 2.37 ± 0.75 | 2.55 ± 0.76 | 2.24 ± 0.68 | 2.16 ± 0.84 | 2.23 ± 0.91 | 1.90 ± 0.65 | 1.74 ± 0.36 | 1.93 ± 0.70 | 1.66 ± 0.56 |
| Oidiodendron maius | 0.4 | 41 887 | 13 | 0.54 ± 0.25 | 0.53 ± 0.25 | 0.51 ± 0.27 | 0.28 ± 0.15 | 0.30 ± 0.21 | 0.59 ± 0.53 | 0.15 ± 0.09 | 0.23 ± 0.22 | 0.42 ± 0.41 |
| Mucoromycota | 5.4 | 600 684 | 219 | 8.1 ± 2.2 | 7.3 ± 1.5 | 6.4 ± 1.8 | 5.2 ± 1.6 | 5.4 ± 1.5 | 5.0 ± 1.3 | 4.6 ± 0.8 | 4.7 ± 1.6 | 4.0 ± 1.4 |
| Mortierellales | 2.9 | 322 917 | 108 | 4.42 ± 1.91 | 4.09 ± 1.22 | 3.63 ± 1.68 | 2.60 ± 1.44 | 2.90 ± 1.22 | 2.77 ± 1.07 | 2.39 ± 0.58 | 2.46 ± 1.28 | 2.15 ± 1.16 |
| Mortierella | 2.7 | 301 765 | 99 | 4.15 ± 1.84 | 3.81 ± 1.18 | 3.27 ± 1.37 | 2.41 ± 1.42 | 2.72 ± 1.20 | 2.58 ± 1.06 | 2.26 ± 0.57 | 2.31 ± 1.28 | 2.04 ± 1.16 |
| Mucorales | 2.5 | 274 748 | 105 | 3.64 ± 1.10 | 3.23 ± 0.96 | 2.81 ± 0.77 | 2.64 ± 0.77 | 2.54 ± 0.91 | 2.19 ± 0.78 | 2.20 ± 0.50 | 2.29 ± 0.91 | 1.82 ± 0.70 |
| Umbelopsis | 2.1 | 232 126 | 85 | 2.84 ± 0.93 | 2.62 ± 0.78 | 2.35 ± 0.60 | 2.25 ± 0.68 | 2.18 ± 0.79 | 1.90 ± 0.63 | 1.92 ± 0.43 | 2.02 ± 0.80 | 1.62 ± 0.62 |
| Rozellomycota | 0.13 | 14 359 | 21 | 0.19 ± 0.08 | 0.17 ± 0.06 | 0.19 ± 0.07 | 0.14 ± 0.09 | 0.14 ± 0.06 | 0.11 ± 0.05 | 0.09 ± 0.04 | 0.11 ± 0.07 | 0.09 ± 0.06 |
| Glomeromycota | 0.11 | 11 885 | 9 | 0.18 ± 0.31 | 0.16 ± 0.35 | 0.20 ± 0.33 | 0.10 ± 0.13 | 0.17 ± 0.30 | 0.09 ± 0.11 | 0.04 ± 0.06 | 0.05 ± 0.11 | 0.05 ± 0.12 |
| Zoopagomycota | 0.001 | 73 | 2 | 0.04 ± 3.60 | 0.03 ± 2.56 | 0.03 ± 3.03 | 0.03 ± 2.62 | 0.03 ± 2.50 | 0.03 ± 1.91 | 0.02 ± 1.21 | 0.02 ± 2.62 | 0.02 ± 2.20 |
| Chytridiomycota | 0.002 | 229 | 4 | 0.004 ± 0.006 | 0.007 ± 0.008 | 0.005 ± 0.007 | 0.002 ± 0.005 | 0.001 ± 0.001 | 0.005 ± 0.005 | 0.002 ± 0.005 | 0.001 ± 0.002 | 0.001 ± 0.001 |
| unclassified | 2.6 | 282 892 | 417 | 2.5 ± 0.9 | 2.3 ± 0.8 | 2.5 ± 2.0 | 1.9 ± 0.6 | 2.0 ± 0.8 | 2.3 ± 1.5 | 3.3 ± 3.8 | 2.8 ± 1.6 | 3.4 ± 3.4 |

**Table 3.** The total number of OTUs, sequences and relative share of sequences (percentage of total sequence count) of bacterial OTUs in main phyla, classes, orders and genera, their relative abundances (percentages of the total sequence count per sample) and the standard deviation in different mesh treatments. The average values are calculated with all the replicates (N=15). The statistically significant ($p \leq 0.05$) R-values of Spearman correlations of the individual phyla, class, order and genus with the environmental variables (see Supplementary Table 2) are represented. ns=not statistically significant correlation.

| Taxonomy | total % | number of sequences | number of OTUs | 1st growing season | | | 2nd growing season | | | 3rd growing season | | | Spearman correlations | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 µm | 50 µm | 1 mm | 1 µm | 50 µm | 1 mm | 1 µm | 50 µm | 1 mm | pH | moisture | mass loss |
| Proteobacteria | 39.6 | 4 486 527 | 2783 | 38.5 ± 1.0 | 39.3 ± 1.9 | 38.6 ± 1.4 | 40.2 ± 2.3 | 40.3 ± 1.7 | 40.2 ± 1.8 | 40.1 ± 1.3 | 40.6 ± 1.9 | 40.0 ± 1.1 | ns | ns | ns |
| Alphaproteobacteria | 25.7 | 2 910 329 | 716 | 23.0 ± 0.9 | 24.2 ± 1.7 | 23.7 ± 1.1 | 27.1 ± 2.7 | 27.5 ± 1.8 | 27.1 ± 2.1 | 27.0 ± 1.3 | 27.4 ± 1.4 | 26.7 ± 1.2 | ns | -0.56 | 0.60 |
| Caulobacterales | 2.1 | 243 841 | 51 | 2.5 ± 0.3 | 2.6 ± 0.2 | 2.6 ± 0.3 | 1.8 ± 0.4 | 2.0 ± 0.3 | 2.1 ± 0.2 | 1.7 ± 0.3 | 1.8 ± 0.3 | 1.7 ± 0.2 | ns | 0.56 | -0.59 |
| Phenylobacterium | 1.4 | 163 364 | 24 | 1.6 ± 0.1 | 1.8 ± 0.2 | 1.7 ± 0.2 | 1.3 ± 0.3 | 1.4 ± 0.2 | 1.4 ± 0.1 | 1.1 ± 0.2 | 1.2 ± 0.2 | 1.1 ± 0.1 | ns | 0.52 | -0.61 |
| Rhizobiales | 15.7 | 1 753 630 | 167 | 14.0 ± 0.7 | 14.0 ± 0.5 | 13.9 ± 0.3 | 15.8 ± 1.6 | 16.7 ± 1.5 | 16.8 ± 1.4 | 16.9 ± 1.0 | 16.7 ± 1.3 | 16.6 ± 1.2 | ns | -0.60 | 0.55 |
| Rhizomicrobium | 2.1 | 237 665 | 37 | 2.6 ± 0.3 | 2.4 ± 0.2 | 2.5 ± 0.3 | 1.7 ± 0.3 | 1.8 ± 0.3 | 2.0 ± 0.4 | 1.7 ± 0.3 | 1.6 ± 0.3 | 1.9 ± 0.3 | ns | 0.57 | -0.52 |
| Variibacter | 4.3 | 487 414 | 15 | 3.5 ± 0.2 | 3.2 ± 0.3 | 3.3 ± 0.2 | 4.7 ± 0.9 | 4.9 ± 0.8 | 4.8 ± 0.7 | 5.6 ± 0.6 | 5.1 ± 0.8 | 4.9 ± 1.0 | ns | ns | 0.56 |
| Rhodospirillales | 7.2 | 796 766 | 198 | 5.3 ± 0.3 | 6.5 ± 1.7 | 6.1 ± 0.9 | 8.5 ± 3.6 | 7.9 ± 1.3 | 7.4 ± 2.0 | 7.4 ± 1.1 | 8.0 ± 1.8 | 7.5 ± 1.4 | ns | ns | ns |
| Acidicaldus | 0.9 | 97 103 | 8 | 0.6 ± 0.1 | 0.7 ± 0.1 | 0.6 ± 0.1 | 1.0 ± 0.2 | 1.0 ± 0.1 | 0.9 ± 0.1 | 1.1 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.2 | ns | ns | 0.63 |
| Acidocella | 1.2 | 136 844 | 17 | 0.8 ± 0.1 | 1.4 ± 1.2 | 1.0 ± 0.6 | 1.6 ± 2.6 | 1.3 ± 0.7 | 1.1 ± 1.3 | 1.0 ± 0.6 | 1.4 ± 1.3 | 1.3 ± 1.0 | ns | ns | ns |
| Gammaproteobacteria | 7.2 | 814 282 | 1090 | 7.7 ± 0.6 | 7.3 ± 0.6 | 7.5 ± 0.5 | 6.7 ± 2.0 | 6.3 ± 1.0 | 7.3 ± 1.7 | 7.3 ± 0.6 | 7.0 ± 1.2 | 8.2 ± 1.2 | ns | ns | ns |
| Deltaproteobacteria | 4.5 | 511 695 | 751 | 5.6 ± 0.3 | 5.2 ± 0.6 | 5.1 ± 0.6 | 4.3 ± 1.2 | 4.3 ± 0.8 | 3.7 ± 1.0 | 4.0 ± 0.5 | 3.9 ± 1.0 | 3.2 ± 1.1 | 0.62 | 0.75 | -0.59 |
| Myxococcales | 2.7 | 314 095 | 251 | 3.5 ± 0.3 | 3.2 ± 0.5 | 3.2 ± 0.6 | 2.5 ± 1.0 | 2.5 ± 0.8 | 2.2 ± 0.9 | 2.5 ± 0.5 | 2.4 ± 0.9 | 1.9 ± 0.9 | 0.60 | 0.67 | -0.49 |
| Haliangium | 0.9 | 104 757 | 36 | 1.3 ± 0.2 | 1.1 ± 0.3 | 1.1 ± 0.3 | 0.9 ± 0.4 | 0.8 ± 0.3 | 0.8 ± 0.4 | 0.7 ± 0.2 | 0.7 ± 0.4 | 0.6 ± 0.3 | 0.68 | 0.58 | -0.50 |
| Sorangium | 1.2 | 130 924 | 66 | 1.4 ± 0.1 | 1.4 ± 0.2 | 1.4 ± 0.2 | 1.0 ± 0.4 | 1.0 ± 0.3 | 0.9 ± 0.3 | 1.1 ± 0.2 | 1.0 ± 0.4 | 0.9 ± 0.4 | ns | 0.67 | ns |
| Acidobacteria | 26.6 | 3 013 927 | 397 | 24.3 ± 1.2 | 24.0 ± 1.5 | 23.9 ± 1.4 | 28.5 ± 3.4 | 28.2 ± 4.5 | 28.6 ± 4.8 | 28.1 ± 1.9 | 27.5 ± 3.6 | 29.5 ± 3.4 | -0.48 | -0.59 | 0.44 |
| Acidobacteria | 26.6 | 3 009 871 | 392 | 24.3 ± 1.2 | 24.0 ± 1.5 | 23.9 ± 1.4 | 28.5 ± 3.4 | 28.1 ± 4.5 | 28.5 ± 4.8 | 28.0 ± 1.9 | 27.4 ± 3.6 | 29.4 ± 3.4 | -0.48 | -0.58 | 0.44 |
| Acidobacteriales | 15.9 | 1 778 870 | 185 | 14.8 ± 0.9 | 15.1 ± 1.4 | 14.9 ± 1.3 | 16.4 ± 3.0 | 16.4 ± 2.4 | 16.4 ± 2.4 | 15.5 ± 1.3 | 15.9 ± 3.1 | 17.2 ± 4.0 | -0.50 | ns | ns |
| Acidobacterium | 2.7 | 301 713 | 28 | 2.5 ± 0.2 | 2.5 ± 0.3 | 2.5 ± 0.2 | 2.8 ± 0.7 | 2.9 ± 0.6 | 2.8 ± 0.6 | 2.6 ± 0.3 | 2.8 ± 0.5 | 3.0 ± 0.9 | -0.45 | -0.50 | ns |
| Granulicella | 5.0 | 562 965 | 44 | 4.7 ± 0.5 | 5.3 ± 1.2 | 4.9 ± 1.1 | 5.1 ± 2.9 | 5.0 ± 1.7 | 5.0 ± 1.5 | 4.4 ± 1.2 | 5.2 ± 2.3 | 5.6 ± 3.2 | ns | ns | ns |
| Telmatobacter | 1.7 | 189 802 | 18 | 1.6 ± 0.2 | 1.6 ± 0.3 | 1.6 ± 0.2 | 1.6 ± 0.4 | 1.6 ± 0.4 | 1.6 ± 0.3 | 2.0 ± 0.3 | 1.8 ± 0.4 | 1.9 ± 0.4 | ns | ns | ns |
| Actinobacteria | 16.5 | 1 872 279 | 462 | 17.2 ± 1.1 | 17.4 ± 1.3 | 17.9 ± 1.4 | 16.7 ± 3.2 | 16.3 ± 3.8 | 16.6 ± 4.1 | 14.6 ± 1.5 | 15.0 ± 1.3 | 14.8 ± 2.2 | ns | ns | ns |
| Actinobacteria | 9.4 | 1 064 727 | 207 | 9.7 ± 0.8 | 10.0 ± 0.8 | 10.3 ± 0.9 | 9.5 ± 1.5 | 9.3 ± 1.5 | 9.5 ± 2.0 | 8.1 ± 0.9 | 8.5 ± 0.9 | 8.5 ± 1.1 | ns | ns | ns |
| Acidimicrobiia | 3.5 | 393 103 | 78 | 3.5 ± 0.4 | 3.6 ± 0.4 | 3.4 ± 0.5 | 3.6 ± 1.1 | 3.6 ± 1.3 | 3.6 ± 1.3 | 3.2 ± 0.5 | 3.3 ± 0.5 | 3.0 ± 0.8 | ns | ns | ns |
| Planctomycetes | 5.2 | 583 176 | 530 | 5.1 ± 0.5 | 4.9 ± 0.4 | 5.0 ± 0.6 | 5.3 ± 0.9 | 5.5 ± 0.8 | 5.2 ± 0.7 | 5.1 ± 0.4 | 5.0 ± 0.8 | 4.6 ± 0.8 | ns | ns | ns |
| Planctomycetacia | 3.2 | 365 273 | 391 | 3.1 ± 0.3 | 3.0 ± 0.3 | 3.1 ± 0.5 | 3.4 ± 0.6 | 3.6 ± 0.7 | 3.4 ± 0.6 | 3.0 ± 0.3 | 3.2 ± 0.5 | 2.7 ± 0.5 | ns | ns | ns |
| Verrucomicrobia | 4.0 | 448 927 | 404 | 4.7 ± 0.3 | 4.6 ± 0.3 | 4.8 ± 0.6 | 3.0 ± 1.0 | 3.1 ± 0.9 | 3.0 ± 0.8 | 4.3 ± 0.6 | 4.2 ± 0.9 | 3.9 ± 0.7 | ns | 0.56 | ns |
| Bacteroidetes | 2.9 | 326 975 | 237 | 4.3 ± 0.6 | 3.9 ± 0.5 | 4.0 ± 0.5 | 1.8 ± 0.8 | 1.9 ± 0.9 | 2.2 ± 1.0 | 2.3 ± 0.3 | 2.3 ± 0.7 | 2.4 ± 0.6 | ns | 0.54 | -0.50 |
| Sphingobacteriia | 2.6 | 310 796 | 187 | 4.1 ± 0.6 | 3.7 ± 0.5 | 3.8 ± 0.5 | 1.7 ± 0.8 | 1.8 ± 0.8 | 2.1 ± 1.0 | 2.2 ± 0.3 | 2.1 ± 0.6 | 2.2 ± 0.6 | ns | 0.52 | -0.51 |
| Sphingobacteriales | 2.6 | 310 796 | 187 | 4.1 ± 0.6 | 3.7 ± 0.5 | 3.8 ± 0.5 | 1.7 ± 0.8 | 1.8 ± 0.8 | 2.1 ± 1.0 | 2.2 ± 0.3 | 2.1 ± 0.6 | 2.2 ± 0.6 | ns | 0.52 | -0.51 |
| Mucilaginibacter | 0.8 | 90 982 | 76 | 4.1 ± 0.6 | 3.7 ± 0.5 | 3.8 ± 0.5 | 1.7 ± 0.8 | 1.8 ± 0.8 | 2.1 ± 1.0 | 2.2 ± 0.3 | 2.1 ± 0.6 | 2.2 ± 0.6 | ns | ns | ns |
| others | 5.0 | 567 978 | 1 558 | 5.7 ± 1.2 | 5.7 ± 1.3 | 5.5 ± 1.2 | 4.3 ± 1.6 | 4.5 ± 1.8 | 4.1 ± 1.7 | 5.2 ± 1.6 | 5.3 ± 2.0 | 4.6 ± 1.9 | nd | nd | nd |
| unclassified | 0.2 | 21 915 | 166 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | na | na | na |