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Pedobiologia - Journal of Soil Ecology 97–98 (2023) 150875

Contents lists available at ScienceDirect

Pedobiologia - Journal of Soil Ecology

journal homepage: www.elsevier.com/locate/pedobi

# Taxonomical and functional responses of microbial communities from forest soils of differing tree species diversity to drying-rewetting cycles

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ARTICLE INFO

*Keywords:*
Soil microorganism functional and taxonomic diversity
European forests
Drought
Soil water availability
Community level physiological profiles

ABSTRACT

The predicted increases in drought in many forest ecosystems may alter soil microbial community diversity and activity, which may further depend on tree species richness. Shifts in microbial community composition and activity could engender changes in ecosystem function, notably, in soil greenhouse gas emissions and C storage. Using soils from mono-specific and mixed three-species forest stands from across Europe, we performed a microcosm experiment to test how soil microbial taxonomic and catabolic diversity are affected by repeated drying-rewetting (DRW) cycles and tree species mixing. We used Illumina sequencing and MicroResp™ analyses to explore community-level changes between microbial functional groups. DRW decreased bacterial richness and carbon substrate use diversity and increased fungal Shannon diversity. Additionally, microbial communities exposed to DRW changed their consumption of 11 out of 15 substrates significantly, suggesting microbial functional shifts. The legacy effect of tree species mixing influenced the structure of the microbial communities (i.e. taxonomic differential abundance) although, community weighted mean (CWM) values of absorptive root traits appeared to affect more strongly microbial richness, relative abundance, and Shannon diversity. No significant tree species mixing:DRW interaction was found for most microbial variables, except for the use of certain substrates and potentially differential abundance. Our data from a laboratory experiment with soils from different forest ecosystems underline that drought may cause shifts in microbial taxonomic and catabolic diversity, while tree species influences primarily taxonomic diversity through root traits.

## 1. Introduction

Forest ecosystems play a critical role in many of the planet's processes, such as water and biogeochemical cycling, which can be strongly impacted by shifts in forest structure and composition (Bonan, 2008; Keenan et al., 2015; Lladó et al., 2017). Climate models globally predict increasing evapotranspiration, decreasing soil water content, and increasing drought frequency and severity over the next century (Dale et al., 2001; Dai, 2013; Masson-Delmotte et al., 2021), which can have considerable effects on forest ecosystems such as increased susceptibility of trees to drought and pathogens leading to mortality (Desprez-Loustau et al., 2006; Thomas and Packham, 2007; Allen et al., 2010).

The soil microbial community, responsible for the majority of terrestrial ecosystem nutrient cycling (Lladó et al., 2017; Crowther et al., 2019), will also be susceptible to future droughts both directly and indirectly (e.g. altered environmental conditions caused by changing plant communities) (Waldrop and Firestone, 2006; Schimel et al., 2007; Fromin et al., 2020). Following drought, soil rewetting presents an equally consequential stress due to rapid osmotic change that can lead to cell lysing and microbial mortality (Schimel, 2018). Although drying and rewetting (DRW) is a stress commonly experienced by soil microbial communities in many habitats (Schimel et al., 2007), increased climate extremes may push even drought-adapted microbial communities beyond their tolerance ranges (Clark et al., 2009). Soil microbial activity

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https://doi.org/10.1016/j.pedobi.2023.150875
Received 7 December 2022; Received in revised form 5 March 2023; Accepted 23 March 2023
Available online 30 March 2023

generally decreases with repeated DRW cycles (Fierer and Schimel, 2002; Schimel et al., 2007), but soils with a DRW-legacy may become more resistant to DRW events due to the selection of resistant microorganisms (Evans and Wallenstein, 2012; Leizeaga et al., 2022).

Fast-growing microorganisms could recover more rapidly after rewetting, taking advantage of the carbon (C) released during DRW events (de Nijs et al., 2019; Leizeaga et al., 2022). However, oligotrophic microorganisms (e.g. many fungi and Gram-positive bacteria) often become more dominant in microbial communities with successive DRW cycles due to their more desiccation-resistant physiology (Uhlířová et al., 2005; Schimel et al., 2007; Bapiri et al., 2010; Wallenstein and Hall, 2012; de Vries et al., 2018; Preece et al., 2019), though not always (Liu et al., 2019). This is supported by recent works showing that Gram-positive taxa (e.g. Actinobacteria, Firmicutes) may be more tolerant to drought than Gram-negative bacteria (e.g. Proteobacteria, Verrucomicrobia) due to stronger, thicker peptidoglycan cell walls (Naylor and Coleman-Derr, 2018; Zhou et al., 2018; Jansson and Hofmockel, 2020). Differences in DRW tolerance within the fungal community is less studied, but a decline of Basidiomycota, Leotiomycetes, and Agaricomycetes and an increase in Ascomycota was seen with DRW, Ascomycota generally exhibiting a fast-growing copiotrophic life-strategy (Liu et al., 2019). A decrease of microbial activity after multiple DRW events has been speculated to be the result of selection for more resistant microorganism which, potentially due to the costs of water stress adaptation, have lower metabolic rates (Orwin and Wardle, 2005; Schimel et al., 2007; Wallenstein and Hall, 2012). Altogether, the literature suggests that repeated DRW events will select for desiccation-resistant but fast-growing organisms (Hicks et al., 2022), which is not easy to reconcile with the usual view the oligotrophs are more stress-resistant compared to copiotrophs.

Such a taxonomic shift, along with overall lower bacterial diversity (Preece et al., 2019), may engender changes in microbial community functioning due to the loss of specific functions represented by microorganisms that become less abundant or disappear (Strickland et al., 2009; Preece et al., 2019; Crowther et al., 2019). Liu et al. (2019) found microbial responses to DRW tied to C mineralization, with DRW selecting for microorganisms that use more easily available C sources (e.g. copiotrophic taxa). Indeed, the composition of the microbial community influences the consumption of different C sources due to different capabilities in obtaining and consuming simple (e.g. carbohydrates; usually associated r-strategy groups, e.g. copiotrophic taxa) or complex sources (e.g. phenolic acids; usually associated with k-strategy groups, e.g. oligotrophic taxa) (Gao et al., 2022). These shifts could have large consequences for ecosystem function, notably, in greenhouse gas emissions and C storage (Crowther et al., 2019).

Forest tree diversity may have a significant effect on microbial DRW responses due to the variation in tree morphological and physiological traits and their subsequent influence on soil chemical and physical properties (e.g. pH, water content, nutrient availability) (Bardgett and Wardle, 2010; Rivest et al., 2015; Hicks et al., 2018). Higher tree diversity generally appears correlated with higher primary productivity (Forrester and Bauhus, 2016; Liang et al., 2016), which, through increased root exudation and litter production, could induce higher nutrient concentrations available to soil microorganisms (Steinauer et al., 2016). A global meta-analysis found a positive plant diversity effect on microbial respiration and biomass over a wide range of habitats including forests (Chen et al., 2019). Whether microbial diversity increases with plant diversity is less clear, tree identity might be a more significant factor (Lange et al., 2014; Scheibe et al., 2015; Prada-Salcedo et al., 2021a). Tree species mixing, by displaying contrasted root traits and exudating different ions and organic compounds (Prescott and Grayston, 2013; McCormack et al., 2015), may also increase the probability of complementary plant traits that benefit soil microorganisms (Pretzsch, 2017). Studies are beginning to elucidate how tree species diversity can affect microbial responses to DRW events in forests, namely on microbial activity resistance and recovery (Rivest et al., 2015; Rahman et al., 2018; Gillespie et al., 2020), but knowledge on how it may influence microbial diversity (both taxonomic and functional) during these stress events is limited (Hicks et al., 2018).

In this study, we explored how DRW and tree species mixing affects soil microbial taxonomic and catabolic diversity in soil from 64 forest stands representing Mediterranean, montane temperate, hemiboreal and boreal ecosystems across a wide range in climate and with varying soil types (total of 13 species and 34 tree species combinations) as to explore general, oppose to site- or species-specific, effects. To allow proper comparison and in terms of feasibility across such a wide range of forests, we opted for a microcosm experiment under fully controlled conditions, in which we subjected soils, originating from forest stands composed of one or three dominant tree species, to DRW cycles and compared microbial community changes. We used Illumina sequencing and MicroResp™ analyses to explore bacterial and fungal communities and their functional response (i.e. soil respiration) in order to identify potential divergent changes between different microbial functional groups. Soil parameters and the community weighted mean of absorptive root traits were included as further variables that could influence the aforementioned effects. We hypothesized that (1) DRW cycles cause shifts in the microbial community taxonomic composition toward more DRW tolerant bacteria and saprotrophic fungi, (2) changes in microbial capabilities and catabolic diversity of soil microbial communities in the wake of a decrease in taxonomic diversity following DRW cycles, and (3) tree species mixture would support higher taxonomic and catabolic diversity following these DRW events. Indeed, resistance of microbial community activity (indicated by $CO_2$ and $N_2O$ production) during DRW cycles was found to be higher in mixed tree species soils, as compared to mono-specific soils, in a complementary study from the DRW experiment presented here (Gillespie et al., 2020). By comparing microbial taxonomic and functional diversity in the same experiment simulating DRW cycles on soils from multiple forest stands across Europe comprising different tree species combinations, our study provides insight in how drought and tree species mixing interactively affect soil microbial communities across distinct forest ecosystems.

## 2. Materials and methods

### 2.1. Initial soil sampling

We sampled soils from four European sites in different climatic zones, i.e. Mediterranean (Colline Metallifere, Italy), montane temperate (Râşca, Romania), hemiboreal (Białowieża, Poland), and boreal (North Karelia, Finland) that corresponded to four different types of forest ecosystems: Mediterranean thermophilous, mountainous mixed beech, hemiboreal mixed broadleaved-coniferous, and boreal forests, respectively (Table S1, Fig. S1). Within each site, we selected 30 m × 30 m plots in different forest stands that had either one dominant tree species (mono-specific stands) or three co-dominating tree species (mixed stands) (Table S1); dominance defined as non-target species not exceeding 5–10 % of total basal area (for 71 % of all plots non-target species contributed less than 5 % to basal area, Baeten et al., 2013). In other words, mono-specific stands were composed of a single target tree species at more than 90–95 % of total basal area, and stands with three co-dominating tree species were composed of the three species at more than 90–95 % of total basal area. Mixed stands were selected for the highest possible evenness (Baeten et al., 2013), and each of the three species contributed at least 15 % to total basal area (in most cases the proportion was close to 30 %). The sites and plots used are part of a permanent network of existing mature forest stands across Europe established in 2011–2012 (see Baeten et al., 2013 for plot selection methods and descriptions). At each site, there were two replicate mono-specific plots for each tree species, with some exceptions at the Białowieża, Poland site due to limited occurrence of these species in mono-specific plots: one replicate mono-specific plot for *Picea abies* and for *Quercus robur*, and no mono-specific plot for *Betula pendula*. Each site

had three replicate mixed plots; however, the tree species included in the three replicates were any of the site target species, i.e. potentially different species combinations. Tree species were selected because of their regional commonality and/or silvicultural importance. The large geographical distribution of our plots allowed for the primary thirteen European broadleaf and coniferous tree species to be represented in our experiment with 34 different combinations of mono-specific and mixed tree species stands (Table S1). This also allowed a large climate gradient and soil diversity to be included into our experiment.

We selected five tree triplets within each plot, a tree triplet defined as three tree individuals triangularly position within 8 m of each other that had no other tree individuals within the triangle or between the chosen three individuals. In mono-specific stands, the tree triplets were composed of one tree species, while in the mixed stands each triplet had one individual of each co-dominating tree species. We then sampled at the estimated center of the tree triplet, which was weighted by tree size (diameter at breast height), i.e. samples were taken closer to smaller trees to ensure their effects were represented. The litter layer was removed, and three to five core samples (5.3 cm diameter) were taken from the first 10 cm of the A horizon to obtain a total of 400 g of soil (the number of cores required depended on bulk density and soil texture) and sieved together (2 mm) and air dried for transportation. Soils were air dried, constituting an initial drought exposure for the microbial communities, because it was not possible to work with fresh soil due to the geographical spread of the sampling sites, time required to sample all plots, and constraints with sample shipping. Depending on soil type, air-drying can cause shifts in some microbial parameters, however the relative differences between carbon and nitrogen transformations between samples are typically preserved (Makarov et al., 2013). Moreover, air-drying is also considered one of the "least bad" soil storage options proceeding microbial catabolic measurements (Martí et al., 2012). This sampling method was repeated for all five tree triplets for all plots. The five tree triplet soil samples, corresponding to subplot samples were pooled per plot for the experiment, yielding a total of 64 soil samples for a total of 64 forest stands.

### 2.2. Initial soil parameters

Soil pH, bulk density, carbon:nitrogen (C:N) ratio, and carbon concentration were measured in 2012 during the FunDivEurope project on nine pooled mineral soil cores (3.6 cm diameter) taken from the first 10 cm of soil after litter removal from each plot (Baeten et al., 2013; Dawud et al., 2016). Because the plots of this study are in mature, established forests (average tree age >40-years-old) with limited disturbance (minimal management and no fire regime), pH, bulk density, and soil organic matter are unlikely to have changed noticeably within the five years interval (Wall, 2014). The samples were dried at 55ºC and sieved to 2 mm, then ground for 6 min at 280 rpm using a Planetary Ball Mill PM 400. The soil pH was determined using an 827 pH lab (Metrohm AG, Herisau, Switzerland) and a 0.01 M $CaCLl_2$ solution (1:2.5 ratio). Bulk density was calculated by dividing the soil weight by its volume. The soil carbon and nitrogen stock was measured by dry combustion method (Matejovic, 1993) and a FLASH 2000 Soil CN Analyzer (Thermo Fisher 171 Scientific, Milan, Italy). The C:N ratio was simply the division of the C stock by the N stock. Soil texture was measured using the soil sampled during the FunDivEurope project using laser granulometry (Malvern Mastersizer S, Malvern Instruments Limited, Worcestershire, United Kingdom) after an organic and carbonate removal pre-treatment (ISRIC and FAO, 2002) if necessary. Texture class cut-off levels were defined as following: sand 2.00–0.63 mm; loam 0.63 mm - 2 μm; and clay < 2 μm. Soil parameters are shown in Table S2. These soil parameters were then incorporated into a principal component analysis (PCA) with the R software (version 3.5.3; R Development Core Team, 2022) using the function 'prcomp' from the factoextra package (version 1.0.6, Kassambara and Mundt, 2019) and the first axis scores were extracted (explaining 52.5 % of the variance; Fig. S2a). This soil PC1 variable was then used in the statistical models.

### 2.3. Absorptive root traits of the tree communities

The absorptive root traits of the plots were characterized to consider tree community legacy effects on the soil microbial community. These traits were used as complementary tree variables in addition to tree species mixture effects. To do so, one soil core per sub-plot was sampled for root characterization at the same time as the other soil cores and frozen (−20 °C) until processed. Root sampling and measurements were conducted at the University of Freiburg, Germany and are described in detail in Wambsganss, Freschet, et al., (2021), including absorptive root diameter (mm), ectomycorrhizal colonization intensity (ECM; number $cm^{-1}$), nitrogen concentration (N; %), root length density (RLD; cm $cm^{-3}$ fine-earth volume), root tissue density (RTD; g $cm^{-3}$ root volume), specific root length (SRL; m $g^{-1}$), and surface area ($cm^2$). Analysis results are provided in Table S2.

In order to consider latitudinal intraspecific variability, we used tree species trait means at the site level. The basal area of the triplet tree individuals was used to determine the relative abundance, i.e. dominance. At each plot, the basal area of all tree individuals of the same species was then summed and reported to the sum of the basal area of all tree individuals, producing the relative basal area of each tree species. We then used this to calculate the community weighted mean (CWM; Garnier et al., 2004) of absorptive root traits at the plot level to test the mass-ratio hypothesis, which suggests that the influence species or traits exert depends on dominance within the community (Grime, 1998). The CWM weighs species traits based on their relative abundance. This CWM data was then incorporated into a PCA to synthesize the data, and the first axis scores were extracted (explaining 52.8 % of the variance; Fig. S2b). Although it is not clear whether a root economic spectrum exists for woody plants (Weemstra et al., 2016; Ma et al., 2019; McCormack and Iversen, 2019; Kong et al., 2019), we interpreted the first absorptive root CWM PCA axis (Root CWM PC1) as an acquisitive to conservative trait gradient (Bergmann et al., 2020), with higher scores representing conservative traits (higher tissue density and large diameter) and lower scores representing acquisitive traits (high SRL, N concentration, and ECM colonization intensity). This root CWM PC1 variable was then used in the statistical models.

### 2.4. Microcosm experiment design

The microcosm experiment was conducted at the Montpellier European Ecotron - CNRS in Montpellier, France. The soil sample from each of the 64 plots was divided between six replicate microcosms (250 ml glass plasma vials); three of the technical replicates were used as control treatments and the other three would be subjected to a 2-cycle drying and rewetting (DRW) treatment, yielding a total of 384 microcosms (Fig. S3a). Each microcosm contained 95 g of dry soil (soil volume 51–72 ml; air volume 259–279 ml), this shallow soil depth allowed better gas exchange and water infiltration throughout the soil. The 64 microcosms from each treatment were distributed, at the same height, equidistant from one another in a growth chamber (volume of 2 $m^3$ and a working area of 1 $m^2$) and were randomized weekly within the growth chamber. At the beginning of the experiment and before the DRW treatments were applied, al microcosms were incubated for 21 days at 80 % water holding capacity, 40 % relative humidity, 25 °C, and no light to allow the microbial communities to re-activate. The microcosms were covered with Parafilm® to prevent soil desiccation while still allowing gas exchange. The soil was rehydrated with deionized water, and a 20 ml syringe was used to ensure even distribution over the soil surface. We gravimetrically measured the water content 2–3 times a week over the duration of the experiment using the combined vial and dry soil weight as the reference weight.

The DRW treatment microcosms were subjected to two DRW cycles. Due to large climatic and soil composition variation between the sites

(Table S1), the drought duration and intensity (i.e. water potential level) were site-specific to consider microbial in situ drought exposure. Indeed, microbial community sensitivity to drought is tied to the soil drought legacy (Hawkes et al., 2017; Kaisermann et al., 2017; Meisner et al., 2018). To determine the drought intensity for each site, we measured the permanent wilting point using a pressure plate extractor (1500F2, Soilmoisture Equipment Corp., Santa Barbara, USA) at pF 4.2 (15.5 bar) in the fastest and slowest drying soils of each site (drying speed previously measured) and we then averaged the two values. The drought intensity threshold per site was as such: Colline Metallifere 11 % $H_2O$ $g^{-1}$ dry soil; Râşca 30 % $H_2O$ $g^{-1}$ dry soil; Białowieża 12 % $H_2O$ $g^{-1}$ dry soil; and North Karelia 12 % $H_2O$ $g^{-1}$ dry soil. The permanent wilting point was used as water stress indicator since no microbial equivalent is yet available to our knowledge. We considered the beginning of the drought to be when the soil dried to the drought intensity threshold. We again parafilmed the microcosm to keep the soil at this water content. To determine the drought duration, we used the forest drought history data from Grossiord et al. (2014) on soil drought conditions of the plots over a 14-year period (1997–2010) using wood cores. We measured the number of days the relative extractable water (REW) dropped below 0.2 per year and then averaged this number over the 14-year period for each site which was then used as the drought duration: Colline Metallifere 38 days; Râşca 12 days; Białowieża 8 days; North Karelia 0 days. Notably, the soil from North Karelia never saw drought conditions below 0.2 REW, but we consider the drying period from 80 % to 12 % water holding capacity (WHC), lasting from 7 to 12 days, an already substantial stress for these non-drought-adapted microbial populations. When the microcosms from the North Karelia forest plots dried to the drought intensity threshold, they were immediately rewetted to 80 % WHC. Once the microcosms from the remaining three sites arrived at their appointed thresholds, the soil was maintained at this water content for the previously determined drought duration. At the end of the drought duration, the soils were rewetted to 80 % WHC (80 % WHC being the minimum water requirement for all soils to be completely rewetted) and kept at this water content for seven days as to allow the microbial activity to stabilize after the Birch effect (Xiang et al., 2008; Schimel, 2018). The second DRW cycle was started following this rewetting period.

Owing to the different drought durations and varying drying speeds, the experimental length varied between and within sites. In order to have the microbial communities active for the same duration, we created eight groups based on site and drying duration (two groups per site) and staggered the beginning of the experiment of each group to synchronize the end of the experiment; we refer to this staggered period as the buffer period (Fig. S3b). There was still a very small difference in experimental durations of six days maximum because it was not feasible to measure all 384 microcosms on the same day. Control microcosms were watered every 2–3 days to be maintained at 80 % WHC for the duration of the experiment.

### 2.5. Bacterial and fungal taxonomic diversity

Total genomic DNA was extracted from 0.5 g of soil from each microcosm sample using the Power Soil™ DNA Isolation Kit (QIAGEN Laboratories Inc., Solana Beach, USA) following the manufacturer's instructions. The three DNA extracts (i.e. technical replicates) from the three replicate microcosm per treatment were pooled with equal DNA proportions. The primers used for the amplification of bacterial 16 S rDNA V4 and fungal internal transcripted spacer 2 (ITS2) regions were bacteria 515 f/806r, and fITS7/ITS4 respectively (Gardes and Bruns, 1993; Ihrmark et al., 2012; Leonhardt et al., 2019). Amplification and paired-end sequencing of 2 × 300 bp, using the MiSeq Reagent kit v3 on an Illumina with MiSeq platform was performed at the Department Soil Ecology Helmholtz-Centre for Environmental Research UFZ in Halle (Saale), Germany, as described in Prada-Salcedo et al. (2021b) and Prada-Salcedo, Wambsganss, et al., 2021.

Retrieved raw bacterial and fungal sequences were extracted based on their unique barcodes. The bioinformatics workflow was based on software package DADA2 and MOTHUR classifier (Schloss et al., 2009; Callahan et al., 2016; Weißbecker et al., 2021) implemented in the pipeline dadasnake (Weißbecker et al., 2021; https://github.com/a-h-b/dadasnake/). Primers were detected allowing maximum a 20 % mismatch and were cut using cutadapt 1.18. Bacterial sequences were filtered by adaptingtrunc_quality:11, trunc_length:0 and max_EE:0.5. During the filtering of fungal sequences, trunc_quality:11, trunc_length:0, max_EE:3, and minLen: 70, were adapted. Taxonomic annotation of the high quality reads per amplicon sequence variant (ASV) was assigned with Bayesian classifier against Silva 132 (Quast et al., 2013) and UNITE v2014 (Nilsson et al., 2019) data bases for bacteria and fungi, respectively. One ASV was therefore considered representative of one taxon (i.e. ASV is the lowest taxonomical unit). FUNGuild V1.0 tool was used to parse fungal taxonomy and establish ecological guilds (Nguyen et al., 2016), here called functional subgroups, and we classified bacterial taxa according to their potential capacity to cope with drought conditions as described in Naylor and Coleman-Derr (2018) (see Table S3 & S4).

Third, diversity and statistical analyses were performed using the software R and its packages phyloseq (version 1.16.2, McMurdie and Holmes, 2013) and vegan (version 2.5–6, Oksanen et al., 2019). Considering data type, data normalization was done by rarefying to 20,000 and 10,000 depth number of sequences among the bacterial and fungi samples, respectively and rarefaction curves confirmed a sufficient sequencing depth reaching the saturation for each sample (see Fig. S4 for rarefaction curves) (Weiss et al., 2017). Alpha diversity was explored using ASV richness and the Shannon diversity index (Shannon, 1948). After rarefaction, the multiple guilds were grouped by general lifestyles.

As an alternative method to evaluating microbial communities at a finer level (e.g. genus), the raw ASV data was used for differential abundance, this method determines which specific taxa are significantly differentially abundant between two ecosystems through statistical testing. Using DESeq2 (v1.26.0) (Love et al., 2014), we explored whether taxa were differentially abundant between DRW and control treatments and between mono-specific and mixed plots in relation to the mean abundance of each treatment. The differential abundance ASVs were define by a 0.05 p-value threshold.

### 2.6. Microbial catabolic diversity

In order to estimate the soil microbial catabolic diversity, we used the MicroResp™ method as described by Shihan et al. (2017), which measures variation in how the microbial community uses 15 carbon sources from five substrate groups: carbohydrates (D-glucose, xylan, cellulose), amino acids (L-asparagine, L-serine, L-lysine, L-glycine, L-glutamine), *N*-acetylglucosamine, carboxylic acids (oxalic acid, uric acid, malic acid), and phenolic acids (caffeic acid, syringic acid, and vanillic acid). Soil (dry weight equivalent of approximately 0.37 g, 0.34 g, 0.46 g, and 0.38 g for Italy, Romania, Polish, and Finland sites, respectively) was incubated in triplicate (i.e. three technical replicates) with 1.5 mg C $g^{-1}$ of dry weight soil of one of the carbon sources with enough solution to reach 80 % WHC in 96-DeepWell Microplates (Fisher Scientific E39199). Respiration rates were estimated from optical density measured in the detection plates over a 4 h period starting 2 h after the beginning of incubation and ending after 6 h. The global catabolic activity was estimated by summing the respiration rates of all 15 substrates (Sum15). If a substrate is used by the soil microorganisms, it should be indicated by higher microbial activity (i.e. higher soil respiration) than if no C substrate was added (i.e. just water). For each of the 15 carbon sources, we therefore considered the substrate 'used' when the respiration was 15 % higher (to be significantly different) than the respiration without a C addition. 'Not-used' respiration rates (i.e. <15 %) were replaced with zeros. Using these 'used' respiration rates, we calculated the catabolic Shannon diversity index using the 'diversity'

function in the vegan package.

### 2.7. Statistical analyses

All statistical analyses were run with the R software, figures were made using the 'pirateplot' function from the YaRrr! package (version 0.1.5, Phillips, 2018), the function 'fviz_pca_biplot' from the factoextra package (version 1.0.6, Kassambara and Mundt, 2019), and the ggplot2 package (version 3.2.0, Wickham, 2016). Permutational multivariate analysis of variance (PERMANOVA) were performed on the ASV richness, relative abundance, and *H'* of the bacterial functional groups and of the fungal functional groups as well as on the 'used' substrate respiration rates using the function 'adonis' in the vegan package to explore the influence of DRW, tree species mixing, their interaction, root CWM PC1, and soil PC1 at a multivariate level.

When PERMANOVA results showed significant (p-value $< 0.05$) explanatory variable effects at the multivariate level, we then ran univariate linear mixed-effects models (LMM) using the lme4 package (version 1.1–21; Bates et al., 2019) to test the effects of aforementioned explanatory variables on the microbial taxonomic and catabolic diversity variables. The soil microbial taxonomic diversity response variables included the ASV richness, relative abundance, and *H'* for: all bacteria, the bacteria functional groups, all fungi, and the fungi functional groups. LMM were also run for the ASV bacterial richness response variables, but not for the ASV fungal response variables since PERMANOVA results were not significant. The soil microbial catabolic diversity response variables included: Sum15, catabolic *H'*, and the substrate respiration for all 15 'used' substrates (GLU, XYL, CEL, ASP, SER, LYS, GLY, GLUT, *N*-AC, OX, UR, MAL, CAF, SYR, and VAN). Extreme values were removed (defined as ± 3 times the interquartile; number of removed data points never exceeded 6 % of the total number of data points) and random variables were transformed (log2) when necessary to meet normality assumptions of LMM. The plot nested within site and climate chambers were included as random variables. The LMMs were structured as such: response variable ~ Root CWM + Soil PC1 + Treatment*Tree species number + (1|Chamber) +(1|Site/-Plot). The most parsimonious model was identified using the 'dredge' function from the MuMIn package (version 1.43.6;(Bartoń, 2019) which ranks all possible models with all possible combinations of the explanatory variables included in the full model by the lowest Akaike Information Criteria (AIC). To correct for family-wise error rate, p-values were corrected for multiple comparisons using the Bonferroni correction method with the function 'p.adjust' in the stats package for all p-values together.

## 3. Results

The soil bacterial and fungal phyla are presented in Fig. S5. Across both experimental treatments (control and DRW) and types of forest stands (mono-specific or mixed tree species forests), we found 27,377 AVSs before and 14,211 bacterial ASVs after rarefaction. Bacteria were grouped into the functional subgroups: DRW tolerant (2925 ASVs), sensitive (2475 ASVs), dynamic (4680 ASVs; ASVs with no particular tolerant/sensitive affiliation); and undefined (4131 ASVs). The average bacterial ASV richness of samples from the studied groups ranged from 237 to 1246. The average bacterial ASV richness was higher in the DRW dynamic subgroup (range: 86–422) than in the sensitive (21−271) or tolerant (49−259) subgroups (Fig. 1a). The average bacterial relative abundance was highest in the DRW dynamic subgroup (range: 0.31–0.76), followed by DRW tolerant subgroup (0.08–0.53), and then DRW sensitive subgroup (0.02–0.17; Fig. 2a). The Shannon taxonomic diversity index (ASV *H'*) ranged from 4.3 to 6.6 for all bacteria. Moreover, the average ASV *H'* of the DRW tolerant (range: 2.9–5.0) and dynamic (3.1–5.5) subgroups were comparable and were higher than the DRW sensitive (2.1–5.1) subgroup (Fig. 3a).

For fungi, we obtained 6592 ASVs before and 2212 ASVs after rarefaction. Fungal ASVs were grouped into the functional subgroups: parasitic/pathogenic (67 ASVs), saprotrophic (645 ASVs), symbiotic (i.e. mycorrhiza, endophyte, epiphyte and lichenized; 293 ASVs), multi-lifestyle (349 ASVs; ASVs that shift between guilds during their life-cycle); and undefined (858 ASVs). The average richness of samples ranged from 23 to 156 across both experimental treatments (control or DRW) and types of forest stands (mono-specific or tree species mixture forests). In general, multi-lifestyle, saprotrophic, and undefined subgroups showed greater richness than other subgroups (Fig. 1b). For the five fungal subgroups, the ASV richness ranged from 3 to 29 for multi-lifestyle, 0–10 for parasitic/pathogenic, 12–63 for saprotrophic, and 0–26 for symbiotic, and 8–63 undefined. The average fungal relative abundance was highest for the saprotrophic subgroup (range: 0.28–0.83) and lowest in the parasitic/pathogenic subgroup (0–0.05; Fig. 2b). The average fungal ASV *H'* ranged from 1.5 to 3.9. In this case, the ASV *H'* of the symbiotic subgroup was similar to the multi-lifestyle, saprotrophic, and undefined subgroups (Fig. 3b). The ASV *H'* of five fungal subgroups ranged from 0.6 to 2.6 for multi-lifestyle, 0.0–1.8 for parasitic/pathogenic, 0.8–3.0 for saprotrophic, and 0.0–2.8 for symbiotic. Although included in the analyses, the DRW dynamic bacterial subgroup and the undefined fungal subgroup were not interpreted since little could be extrapolated from these groups.

The PERMANOVA results showed effects of the explanatory variables

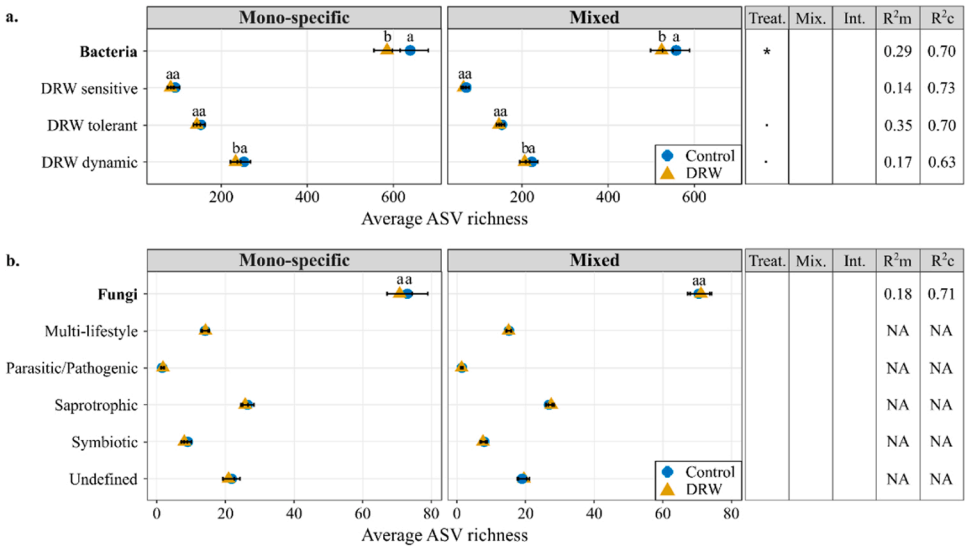


**Fig. 1.** *Average ASV richness in soils from mono-specific (left panel) and mixed species forest stands (right panel) in response to DRW (control treatment in blue and DRW treatment in orange) for* ***a.*** *bacteria and all subgroups and for* ***b.*** *fungi and all subgroups. Marginal $R^2$ ($R^2$m) and conditional $R^2$ ($R^2$c) values are from LMM results. Fungal subgroups were not analyzed at the univariate level because PERMANOVA results showed no significant explanatory variable effects at the multivariate level. Error bars indicate standard error (SE). Letters indicate statistically significant differences and asterisk indicate statistical significance between treatments (Treat.), forest stand type (Mix.), and the interaction between the two (Int.) for each microbial group individually. P-values are coded as: $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

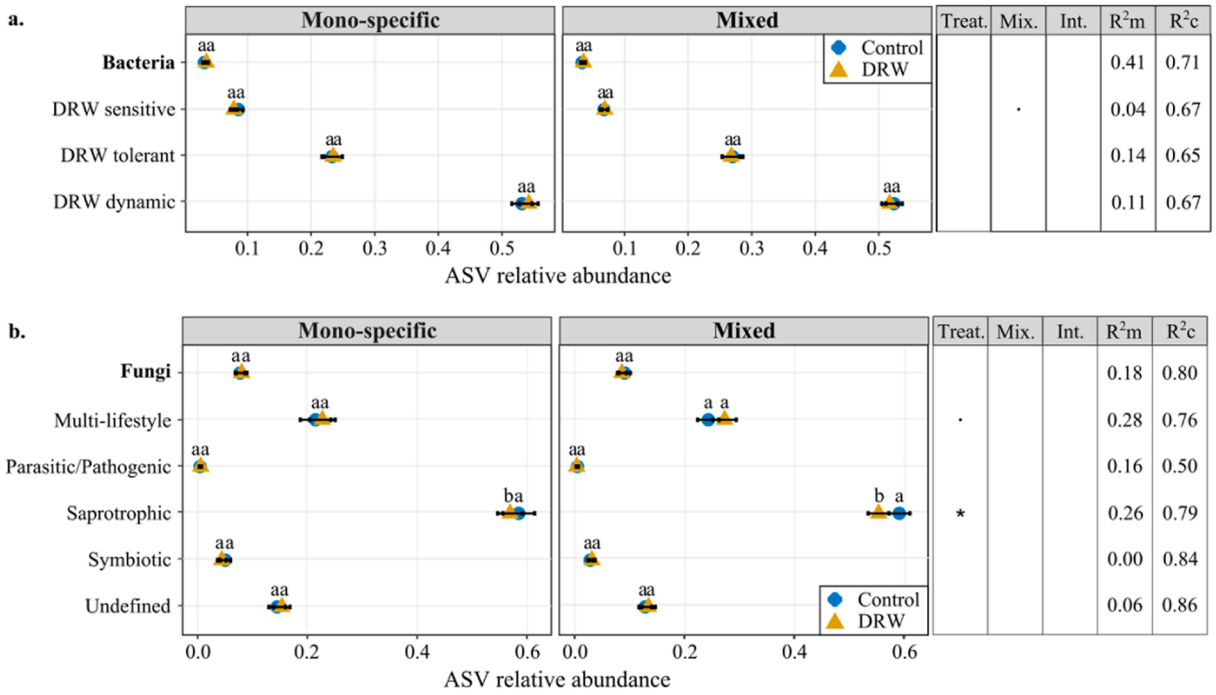


**Fig. 2.** *ASV relative abundance in soils from mono-specific (left panel) and mixed species forest stands (right panel) in response to DRW (control treatment in blue and DRW treatment in orange) for* ***a.*** *bacteria and all subgroups and for* ***b.*** *fungi and all subgroups. Marginal $R^2$ ($R^2$m) and conditional $R^2$ ($R^2$c) values are from LMM results. General bacteria and fungi relative abundances (in bold) are at the genus level, while the subgroups are at the phylum level. Error bars indicate standard error (SE). Letters indicate statistically significant differences and asterisk indicate statistical significance between treatments (Treat.), forest stand type (Mix.), and the interaction between the two (Int.) for each microbial group individually. P-values are coded as: p < 0.05*, p < 0.01**, p < 0.001***.

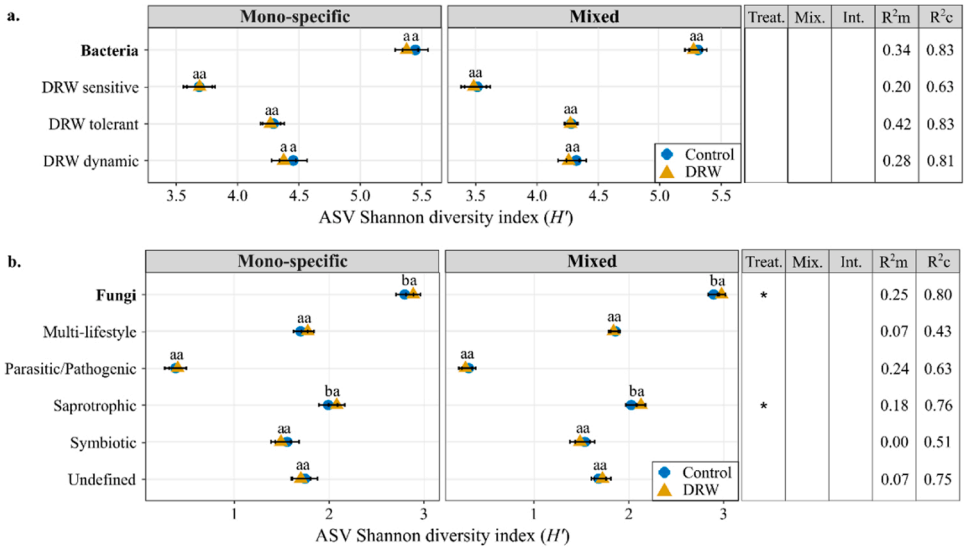


**Fig. 3.** *ASV Shannon diversity index (ASV H') in soils from mono-specific (left panel) and mixed species forest stands (right panel) in response to DRW (control treatment in blue and DRW treatment in orange) for* ***a.*** *bacteria and all subgroups and for* ***b.*** *fungi and all subgroups. Marginal $R^2$ ($R^2$m) and conditional $R^2$ ($R^2$c) values are from LMM results. Error bars indicate standard error (SE). Letters indicate statistically significant differences and asterisk indicate statistical significance between treatments (Treat.), forest stand type (Mix.), and the interaction between the two (Int.) for each microbial group individually. P-values are coded as: p < 0.05*, p < 0.01**, p < 0.001***.

for all groups and random variables except for fungi ASV richness (Table S5, S6), LMMs were therefore run with all random variables for all subgroups except the latter. For differential abundance, bacterial DRW tolerant and dynamic subgroups showed higher number of taxa with differential abundant ASVs than the DRW sensitive subgroup (Fig. 4, Table S7-10). In the case of fungi, more differential abundant taxa were enhanced in the saprotrophic and multi-lifestyle fungal subgroups.

The sum of the microbial catabolic respiration induced by 15 different C-sources (Sum15) ranged from 3.2 to 44.5 μg C-$CO_2$ $g^{-1}$ dry soil $h^{-1}$ (Fig. 5a). The 'used' substrate respiration rates, i.e. when the respiration was 15 % higher than the respiration without a C addition (i.e. just water added), ranged from 0.15 to 6.4 μg C-$CO_2$ $g^{-1}$ dry soil $h^{-1}$ for individual substrates (Fig. 6). On average, oxalic acid (OX) and L-lysine (LYS) were used to a larger extent for soil microbial respiration, as compared to the other C sources tested, and vanillic acid (VAN) the least. The catabolic *H'*, calculated using the 'used' substrate respiration rates, ranged from 0.7 to 2.6 (Fig. 5b).

### 3.1. DRW effect

ASV relative abundances for bacterial DRW tolerant and dynamic subgroups were higher in the DRW treatment than the control treatment, opposite of that found for the DRW sensitive subgroup (Table S3).

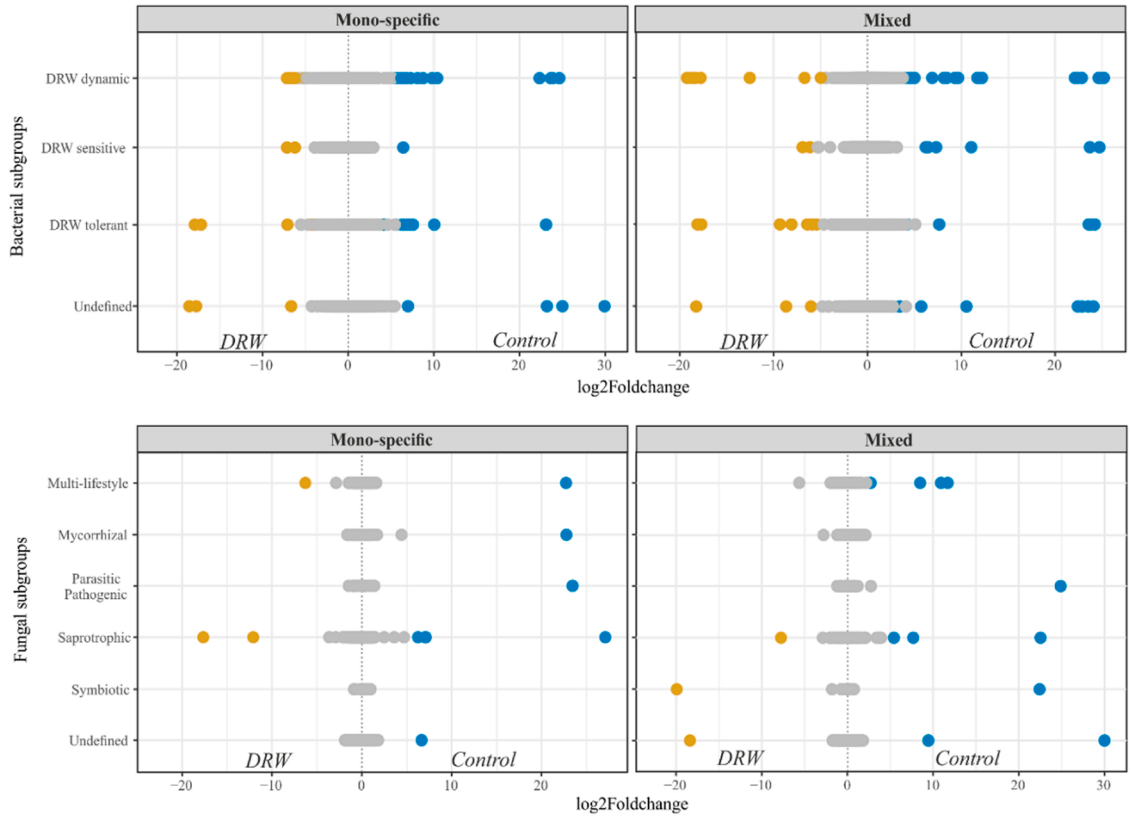


**Fig. 4.** Differential abundance analysis for bacteria and fungi communities between DRW and control treatments for mono-specific (left panels) and mixed stands (right panels). Gray circles around the zero line represent taxa that are not significantly different in abundance, blue circles represent taxa that are significantly (p-value $< 0.05$) more abundant in soils from the control treatment (positive relative difference on the right) and yellow circles represent taxa more abundant in soils from the DRW treatment (negative relative difference on the left). See Table S7 −10 for details on differential abundant ASVs).

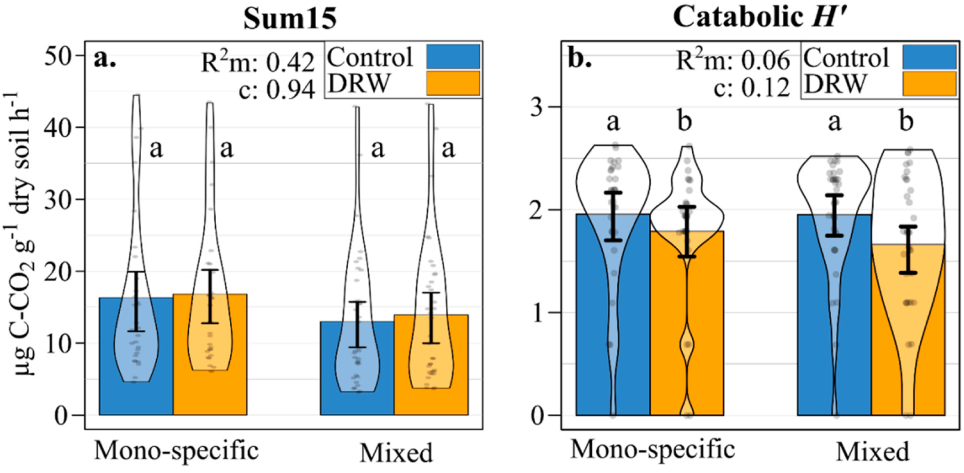


**Fig. 5.** *Microbial catabolic activity* ***a.*** *sum of the microbial catabolic respiration induced by 15 different C-sources (Sum15; µg C-$CO_2$ $g^{-1}$ dry soil $h^{-1}$), and* ***b.*** *catabolic Shannon diversity index of used substrates ('used' catabolic H'). Control treatment soils are in blue and DRW treatment soils in orange. Marginal $R^2$ ($R^2$m) and conditional ($R^2$c) values are from LMM results, letters indicate statistically significant differences, and error bars indicate standard error (SE). P-values are coded as: $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

The parasitic/pathogenic, saprotrophic, and symbiotic fungal subgroups showed higher ASV relative abundances in the control treatment compared to the DRW treatment, while the multi-lifestyle and undefined groups showed higher ASV relative abundances in the DRW than control treatment (Table S4).

The multivariate analyses on the bacterial and fungal subgroups showed no DRW effect except a marginally significant effect on bacterial ASV richness (Table S5). In univariate analyses, average bacteria ASV richness was lower in DRW treatment compared to control soils (average −7.9 %, $p < 0.05$; Table S12, Fig. 1). ASV richness of the DRW-tolerant bacteria was lower in DRW treated soils, while the effect was marginally significant. There was no effect of DRW treatment for total or subgroup bacterial ASV relative abundance or *H'* (Table S12, Figs. 2a, 3a). Total fungal ASV *H'* was slightly higher in soils subjected to DRW compared to control soils (average +2.9 %, $p < 0.01$; Table S12, Fig. 3b). This effect was mostly driven by the saprotrophic subgroup (+4.5 %). The relative abundance of the saprotrophic subgroup was concurrently lower in DRW subjected soils (−4.5 %).

The cumulated respiration rates of all 15 substrates (Sum15) from soils subjected to the DRW treatment did not differ from those in control soils (Table S6, Fig. 5a). The catabolic *H'* did vary between treatments, with values 11 % lower in DRW soils (Table S6, Fig. 5b). The

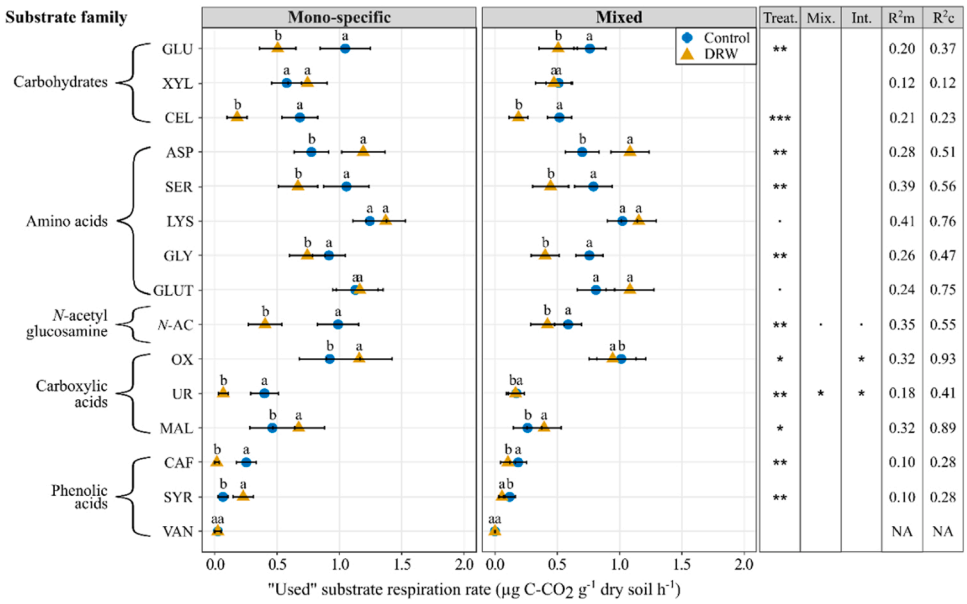


**Fig. 6.** "Used" respiration rates (µg C-$CO_2$ $g^{-1}$ dry soil $h^{-1}$), i.e. when the respiration was 15 % higher than the respiration without a C addition (i.e. just water added), for the fifteen substrates in soils from mono-specific forest stands (left panel) and mixed tree species stands (right panel) in response to DRW (control in blue and DRW in orange). Abbreviations: D-glucose (GLU), xylan (XYL), cellulose (CEL), N-acetyl-glucosamine (N-AC), L-asparagine (ASP), L-glutamine (GLUT), L-lysine (LYS), L-serine (SER), L-glycine (GLY), malic acid (MAL), oxalic acid (OX), uric acid (UR), caffeic acid (CAF), syringic acid (SYR), and vanillic acid (VAN). Error bars indicate standard error (SE). Letters indicate statistically significant differences between control and DRW treatments (Treat.) and between tree mixtures (Mix.), and the interaction between the two (Int.) from univariate LMMs for each substrate individually. P-values are coded as: $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

multivariate analysis of the 'used' substrate respiration rate also showed a significant treatment effect (Table S6). LMM analyses at the substrate level showed significantly higher respirations rates for some substrates (ASP, OX, MAL) and significantly lower for others (GLU, CEL, SER, GLY, *N*-AC, UR) in DRW compared to control soils (Table S13, Fig. 6).

### 3.2. Tree species mixture effect

The multivariate analyses on the bacterial and fungal subgroups showed no tree mixing effect (Table S5). This was mostly supported by the univariate LMM results, except for a marginally significant negative effect on DRW sensitive relative abundance (Table S12, Fig. 2). Tree species mixture also had no effect on Sum15 nor on catabolic *H'* (Table S13, Fig. 5a,b). For differential abundance, mixed forest induced higher differential abundances compared to mono-specific plots. The differential abundance results also showed differences between experimental treatments (control and DRW) between mono-specific and tree species mixtures (Tables S7-S10, Fig. 4). Among the 27,377 bacterial ASVs, the mono-specific plots showed 18 ASVs enriched under DRW and 31 ASVs under control treatment. Moreover, from the total 6592 fungal ASVs, the mono-specific plots showed 3 and 8 ASVs enriched under DRW and control treatments, respectively, while tree species mixtures showed 3 and 12 ASVs enriched under DRW and control treatments, respectively (Tables S7-S10, Fig. 4).

Mixing did negatively affect the respiration rates of the uric acid (UR) substrate and had a marginally significant effect on the 'used' *N*-acetylglucosamine (*N*-AC) substrate (Table S13, Fig. 6), and the use of UR, and marginally significant for *N*-AC, was also influenced by the interaction between tree species mixing and treatment. The respiration rate of the 'used' OX substrate was positively affected by tree species mixing and also affected by the mixture:treatment interaction.

### 3.3. Tree species absorptive root trait CWM effects

Multivariate analyses on bacterial and fungal subgroups showed no root trait community weighted mean PCA axis 1 (root CWM PC1) effect except a marginally significant effect on bacteria ASV relative abundance (Table S5), while LMM results showed significant subgroup effects. The bacteria DRW tolerant subgroup ASV richness, *H'*, and the relative abundance (marginally significant), were negatively influenced by root CWM, as was the ASV *H'* of the fungi saprotrophic subgroup (Table S12). This meant higher DRW tolerant ASV richness with higher absorptive root ectomycorrhizal colonization intensity (ECM colonization), nitrogen (N) concentration, and specific root length (SRL) and lower root tissue density (RTD) and diameter (Fig. S2b). The ASV relative abundance of the bacteria DRW dynamic subgroup and the fungi multi-lifestyle subgroup, and the saprotrophic subgroup (marginally significant), were positively affected by root CWM (Table S12).

Root CWM PC1 positively influenced Sum15 rates, with higher Sum15 correlated with higher root diameter and RTD and lower ECM colonization, N concentration, and SRL (Table S13, Fig. S2b). The 'used' substrate respiration rates were not significantly affected by root CWM PC1.

### 3.4. Soil effect

Soil parameters (Soil PC1) had a consistent influence on microbial taxonomic diversity. Higher average bacterial ASV richness was associated with higher soil PC1 scores (i.e. lower bulk density and higher pH, C and clay concentrations), with all the bacterial subgroups showing the same correlations (Table S12, Fig. S2a). This was identical for bacterial and fungal ASV *H'*, as well as for all bacterial and most fungal subgroups. ASV relative abundance was not as consistent between subgroups. DRW dynamic bacteria and saprotrophic fungi relative abundances were negatively correlated to soil PC1, while multi-lifestyle and parasitic/pathogenic fungi were positively correlated.

Sum15 rates and catabolic *H'* (marginally significant) were positively correlated with soil PC1, meaning they were higher in soils with lower bulk density and higher pH, C and clay concentrations (Table S13; Fig. S2a). All 'used' substrates were also positively correlated to soil PC1 scores, excluding VAN (Table S13) which was not run due to insufficient non-zero datapoints.

## 4. Discussion

In this study, we explored the influence of drying and rewetting (DRW) cycles on taxonomic and functional (based on carbon substrate use) diversity of microorganisms in soils from different mature, natural forests dominated either by one or by three tree species. We first hypothesized that DRW cycles would cause shifts in bacterial and fungal community diversity towards more DRW tolerant bacterial and fungal taxa (mostly saprotrophic). Our results partially supported this hypothesis: Bacterial ASV richness was indeed negatively affected by DRW cycles, while Shannon molecular diversity was not. This could be

explained by a decrease in ASV number with a concurrent non-linear increase in ASV evenness, possibly due to a loss of rare ASV. Such dissimilar DRW effects with decreased microbial community richness but stable evenness were previously found in sandy riparian soils in the Netherlands (Meisner et al., 2018). However, the non-significant treatment effect on the relative abundance we reported and the non-significant treatment effect on bacterial evenness (Table S11) do not support this idea. The diversity and relative abundance of the DRW sensitive bacterial group was unaffected by DRW cycles compared to the marginally significant DRW-associated decrease in ASV richness of the DRW tolerant and dynamic subgroups. The differential abundance also showed a more active response of DRW tolerant and dynamic bacterial taxa which reflect the adaptive physiological responses to DRW. The enriched taxa ("Candidatus Koribacter", "Candidatus Nitrocosmicus", *Phenylobacterium*, *Granulicella*, *Sphingomonas, Mycobacterium, Conexibacter, Tumebacillus*) are generally found under low-nutrient conditions and produce amorphous extracellular polysaccharide-like substances, which are traits that facilitate in coping with DRW cycles (Pankratov and Dedysh, 2010; Tóth et al., 2017; Naylor and Coleman-Derr, 2018; Na et al., 2019; Luo et al., 2019; Zhao et al., 2021). Overall limited effects on the drought-sensitive bacteria diversity were likely caused by an initial decline after the air-drying conducted for soil transportation, and a subsequent inability of these microbes to recover under the experimental conditions. This experimental artefact was inevitable, because soils had to be conserved until the beginning of the experiment, given the different sampling periods as a consequence of the large geographical range we covered with our study. However, air drying is a common method of soil pretreatment and is considered as one of the most gentle methods among the different procedures (Martí et al., 2012).

For fungi, we found an increase in taxonomic diversity with DRW cycles. The increase in ASV *H'* with no simultaneous change in ASV richness suggests a non-linear increase in fungal evenness. Most of the enriched fungi belong to the comparatively fast-growing Ascomycota and Mucoromycota, which is in line with our first hypothesis predicting an increase in fungal saprotrophs in response to drying-rewetting cycles. Guhr and Kircher (2020) reported a memory effect in saprotrophic filamentous fungi to previous stress events, which increases phenotypic plasticity in response traits without genetic changes (termed as "stress priming"). These fungi might therefore, play a prominent role in the cycling and movement of soil nutrients (de Vries et al., 2018; Guhr and Kircher, 2020). Although the fast-growing trait might be the more important factor than taxonomy per se (Hicks et al., 2022). Mortierellomycota and Basidiomycota had fewer taxa but were strongly stimulated following drying-rewetting cycles (see Fig. 4 and Tables S9 and S10). Within Basidiomyceta, some yeast (e.g. *Saitozyma* and *Solicoccozyma*) are considered to be specialized for simpler compounds and are more persistent in stressful environments (Treseder and Lennon, 2015; Frey et al., 2021). Others (e.g. *Geminibasidium*) are considered to be heat-resistant and xerotolerant, with a double-walled basidiospores that can confer these taxa their ecological advantage under dry conditions (Nguyen et al., 2013). The limited effects seen for the parasitic/pathogenic and symbiotic groups is likely influenced by the absence of hosts in our experimental set-up. A more detailed evaluation of how these groups respond to drought and tree species richness would require different experimental approaches including plants.

In our second hypothesis, we predicted changes in microbial capabilities and catabolic diversity of soil microbial communities following DRW cycles. Supporting this hypothesis, individual substrates were respired distinctively in response to DRW. Easily-available C sources such as carbohydrates were less respired under DRW compared to the control treatment, particularly for cellulose and D-glucose, while other C sources such as oxalic and malic acid were respired at higher rates. The generally clear differences in the use of the different substrates between microbial communities from the DRW vs. control treatment (a total of 11 out of 15 substrates showed significant differences, Fig. 6) suggest shifts in substrate use in response to DRW cycles that could have further effects on elemental cycling with increased drought frequencies. Indeed, a study in forest soils found that low water treatment impacts soil microbial (MicroResp™) profiles, especially the use of aromatic acids (e.g. syringic and caffeic acid) (Strukelj et al., 2021). This could engender changes in plant litter turnover, greenhouse gas emissions, and C sequestration (Sherman and Steinberger, 2012; Crowther et al., 2019).

Changes in taxonomic diversity with DRW concurrent with lower community-level catabolic *H'* in DRW soils does not support the functional redundancy hypothesis, stating that many taxa perform the same function therefore making the loss of species or species turnover less destabilizing for ecosystem functioning (Eisenhauer et al., 2010; Pretzsch, 2017; Bardgett and Caruso, 2020). However, we only assessed microbial use of C sources and results may differ for more specialized functions (e.g. methane and sulfur oxidization and nitrification) (Schimel et al., 2007). For example, in boreal forests in Sweden, Högberg et al. (2007) found a negative correlation between fungi:bacteria biomass ratio and N mineralization. This indicates that if DRW selects for fungi due to their higher drought resistance, N mineralization rates may subsequently decline. Indeed, as previously reported from the same experiment, the denitrification activity decreased during drought, leading to an overall decline in cumulative denitrification activity over two DRW cycles (Gillespie et al., 2020). Additionally, in dryland ecosystem soil, key microbial taxa were found to play a predominant role in ecosystem resistance to soil warming and DRW, more so than microbial richness, abundance, or fungal:bacterial (F:B) ratio (Delgado-Baquerizo et al., 2017). Our experiment was designed to follow relatively short-term responses of an existing microbial community; potential adaptations or immigrations from other types of ecosystems at a regional scale may modify the responses at larger temporal scales.

In our third hypothesis, we predicted that tree species mixture would support higher taxonomic and catabolic diversity following DRW events as compared to mono-specific stands. This hypothesis was generally not supported by our results, with the exception of a potential tree species mixture effect on DRW sensitive bacteria relative abundance. However, although tree species mixing generally did not influence taxonomic richness, relative abundance, nor *H'*, we did find that tree species mixture induced a higher enrichment of differential abundance of certain bacterial and fungi communities than mono-specific stands in both the DRW and control soils. That is to say, a greater number of microbial species with higher abundances. This suggests that tree species mixtures can induce higher abundances of specific taxa and potentially the diversity of disturbance responses. The study from Rivest et al. (2019) exploring the effects of tree diversity (1, 2, 4, and 12 species richness levels) on soil microbial diversity in a planted temperate forest in Western Canada, found tree species richness to have a weak significant effect on bacterial taxonomic diversity and no effect on fungal taxonomic diversity. Studies on soil from a temperate mixed broad-leaved forest in central Germany (Thoms et al., 2010; Scheibe et al., 2015), natural European forests (Prada-Salcedo et al., 2021a), and planted Chinese forests (Jiang et al., 2012) found microbial community structure to be more influenced by tree identity than by species richness per se. Microbial catabolic diversity was also not influenced by tree species mixtures in our study. This is broadly in line with the study by Jiang et al. (2012), who's findings suggest microbial catabolic diversity is driven by tree functional types (e.g. conifer effect) and not species richness. On the other hand, the study from Strukelj et al. (2021) did find that microbial functional identity (estimated by respiration of carboxylic and aromatic acids) was influenced by tree species richness. Additionally, our results showed an interaction between tree species mixing and DRW for the consumption of some substrates (*N*-AC, OX, and UR) and potentially taxonomic differential abundance, but a general trend was not apparent. This appears to partially support the findings of Strukelj et al. (2021) for tree species richness effects on microbial processes in low water conditions.

The inclusion of traits from tree fine roots and physical and chemical

parameters from the soils we sampled for our experiment provided further insight into the tree-associated drivers of soil microbial community characteristics. The influence of root trait community weighted mean PCA axis 1 (root CWM PC1) on microbial taxonomic diversity at the subgroup level indicated that DRW tolerant bacterial richness and diversity as well as saprotrophic fungal diversity were higher when traits associated with an acquisitive absorptive root strategy (i.e. higher ECM colonization intensity, N content, and SRL and lower diameter and RTD) were dominant within the stand (Prada-Salcedo et al., 2021a; Wambsganss et al., 2021b). More acquisitive root traits could be associated with more productive trees and thus higher rhizodeposition and litter production and nutrient turnover (de Vries et al., 2012; Bardgett et al., 2014). For microbial catabolic diversity, the summed microbial respiration Sum15 was potentially higher in stands dominated by species with conservative absorptive root traits (lower ECM colonization intensity, N content, and SRL and higher diameter and RTD). These traits were associated with mono-specific stands which also showed higher absorptive root biomass per unit of soil (Wambsganss et al., 2021a). Higher root biomass likely correlates with higher root inputs to the soil, which could therefore be a legacy effect supporting higher microbial activity. These results suggest again that tree species identity has a stronger influence than tree species mixing per se.

Finally, soil parameters had a strong and consistent effect on microbial taxonomic and catabolic diversity. Generally, higher microbial taxonomic diversity (richness and *H'* of all subgroups, and the relative abundance of some subgroups) and higher microbial catabolic diversity (summed respiration rates, respiration rates from all 15 substrates, and, potentially, catabolic *H'*) were associated with lower soil density and higher soil pH, and higher amounts of organic matter and clay. These microbial variables may be facilitated by less dense and acidic soils with higher organic matter and clay contents by imposing fewer constraints on the availability of certain easily available substrates.

In conclusion, our results showed that DRW treatment in our experiment had some substantial effects on microbial taxonomic and catabolic diversity. Tree species mixing generally had no effect on the microbial variables measured here, aside from an influence on taxonomic differential abundance and the use of certain C substrates, notably also showing a tree species mixing:DRW interaction. The effects of tree absorptive root CWM, in addition to that of tree species mixture, suggest tree diversity effects on microbial taxonomic and catabolic diversity are mostly determined by species identity within the community. Our data from 64 different forest stands of four different forest ecosystems ranging from Mediterranean to boreal forests support that increased drought frequency and severity predicted by climate models can cause shifts in microbial taxonomic diversity and further in the diversity and the C substrates used by these altered microbial communities. Changing substrate use may affect ecosystem C and nutrient cycling. Moreover, the influence of tree species mixing via tree community root traits suggests that climate change-induced shifts in forest tree composition can affect soil microbial-driven ecosystem functioning beyond the direct effects of changing climatic conditions.

## CRediT authorship contribution statement

L.M.G., L.D.P.S., N.F., S.H., A.M., and F.B. developed the study design, planned and assisted in field sampling, and participated in article redaction. S.H., N.F., A.M., and L.M.G. developed the experimental design and B.B. provided knowledge and experience for gas flux measurements. L.M.G. conducted the experiment, with assistance from A.S., and lead data analysis and manuscript redaction. K.G. provided knowledge and experience for the microbial taxonomic measurements and analyses, while L.D.P.S. performed them. A.S. performed the soil microbial activity measurements.

## 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.

## Data availability

Data will be made available on request.

## Acknowledgements

This research was part of the SoilForEUROPE project funded through the 2015-2016 BiodivERsA COFUND call for research proposals, with the national funders Agence Nationale de la Recherche (ANR, France), Belgian Science Policy Office (BELSPO, Belgium), Deutsche Forschungsgemeinschaft (DFG, Germany), Research Foundation Flanders (FWO, Belgium), and The Swedish Research Council (FORMAS, Sweden). We thank the site managers Leena Finér with the Finnish Forest Research Institute (Metla), Bogdan Jaroszewicz with the University of Warsaw, Olivier Bouriaud with the Forest Research and Management Institute (ICAS), and Filippo Bussotti with University of Florence and associates, as well as the SoilForEUROPE consortium for their assistance with the soil sampling campaign. This study benefited from the CNRS human and technical resources allocated to the ECOTRONS Research Infrastructure as well as from the state allocation "Investissement d'Avenir" AnaEE-France ANR-11-INBS-0001. We thank the Ecotron staff and in particular Sébastien Devidal for the technical support during the incubation period. We thank the experimental platform PACE at CEFE Montpellier, France for the gas flux measurements. We also thank Bart Muys and Karen Vancampenhout at KU Leuven University for the soil texture data, and Iulian Danila at the University of Suceava for the tree basal data. We thank Beatrix Schnabel and Anna Heintz-Buschart for their molecular and bioinformatics contributions. The sequencing data were analyzed at the High-Performance Computing (HPC) Cluster EVE, a joint effort between the Helmholtz Centre for Environmental Research - UFZ and the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.pedobi.2023.150875.

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