Science of the Total Environment xxx (xxxx) xxx

Contents lists available at ScienceDirect

Science of the Total Environment

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

Anthropogenic stressors affect fungal more than bacterial communities in decaying leaf litter: A stream mesocosm experiment

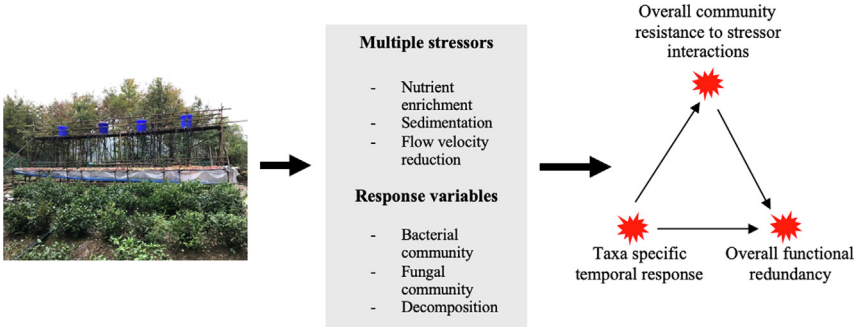
Noël P.D. Juvigny-Khenafou [a,b], Yixin Zhang [c,*], Jeremy J. Piggott [d], David Atkinson [a], Christoph D. Matthaei [e], Sunshine A. Van Bael [f], Naicheng Wu [b]

[a] Institute of Integrative Biology, University of Liverpool, Biosciences Building, Crown Street, Liverpool L69 7ZB, UK
[b] Department of Health and Environmental Science, Xi'an Jiaotong-Liverpool University, Suzhou, Jiangsu 215123, China
[c] Department of Landscape Architecture, Gold Mantis School of Architecture, Soochow University, Suzhou, China
[d] Trinity Centre for the Environment & Department of Zoology, School of Natural Sciences, Trinity College Dublin, The University of Dublin, College Green, Dublin 2, Ireland
[e] Department of Zoology, University of Otago, PO Box 56, Dunedin 9054, New Zealand
[f] Department of Ecology and Evolutionary Biology, Tulane University, 6823 St. Charles Avenue, Boggs 400, New Orleans, LA 70118, USA

HIGHLIGHTS

- Leaf litter decomposition rate changes paralleled fungal community changes.
- Stressor interactions were taxon-specific rather than affecting the overall community.
- Interactions changed with time, highlighting the role of exposure duration.
- The terrestrial microbial leaf litter community persisted after leaf submersion.
- Relative abundance shifts drove community responses to stress.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 August 2019
Received in revised form 15 October 2019
Accepted 16 October 2019
Available online xxxx

Editor: Sergi Sabater

Keywords:
16S rRNA
18s rRNA
Mesocosm
Decomposition
China
Multiple stressors

ABSTRACT

Despite the progress made in environmental microbiology techniques and knowledge, the succession and functional changes of the microbial community under multiple stressors are still poorly understood. This is a substantial knowledge gap as microbial communities regulate the biogeochemistry of stream ecosystems. Our study assessed the structural and temporal changes in stream fungal and bacterial communities associated with decomposing leaf litter under a multiple-stressor scenario. We conducted a fully crossed 4-factor experiment in 64 flow-through mesocosms fed by a pristine montane stream (21 days of colonisation, 21 days of manipulations) and investigated the effects of nutrient enrichment, flow velocity reduction and sedimentation after 2 and 3 weeks of stressor exposure. We used high-throughput sequencing and metabarcoding techniques (16S and 18S rRNA genes) to identify changes in microbial community composition. Our results indicate that (1) shifts in relative abundances of the pre-existing terrestrial microbial community, rather than changes in community identity, drove the observed responses to stressors; (2) changes in relative abundances within the microbial community paralleled decomposition rate patterns with time; (3) both fungal and bacterial communities had a certain resistance to stressors, as indicated by relatively minor changes in alpha diversity or multivariate community structure; (4) overall, stressor interactions were more common than stressor main effects when affecting microbial diversity metrics or abundant individual genera; and (5) stressor effects on microbes often changed from 2 weeks to 3 weeks of stressor exposure, with several response patterns being reversed. Our study suggests that future research should focus more on understanding the temporal dynamics

* Corresponding author.
E-mail address: yixin.zhang2019@suda.edu.cn (Y. Zhang).

https://doi.org/10.1016/j.scitotenv.2019.135053

of fungal and bacterial communities and how they relate to ecosystem processes to advance our understanding of the mechanisms associated with multiple-stressor interactions.

1. Introduction

Microbial communities drive stream biogeochemistry by playing a crucial role in ecosystem processes, such as ecosystem respiration, organic matter decomposition and nutrient cycling (Bruder et al., 2016; Kuehn, 2016; Manning et al., 2018). For example, microbial communities fix nutrients onto substrata and improve the palatability and quality of decomposing leaf litter, thus providing a high-nutrition food source for higher trophic levels (Kuehn, 2016). Their influence therefore starts at the bottom of the food chain, and then potentially shapes higher trophic-level communities along the river continuum. One of the central aims of microbial ecology is to understand how environmental changes drive the structure and function of communities (Herren et al., 2016). In ecology, 'stressors' can be defined as any anthropogenic activity resulting in environmental change that will take the studied system outside of its normal operational range (Sabater et al., 2019). Studies have previously revealed that microbial communities follow successional stages; however, the forces driving this progression are less well understood (Knelman et al., 2014). Indeed, community responses to environmental changes can either be stochastic or deterministic. Therefore, if the latter is true, then the microbial community's adaptation to stress can be predicted, always selecting for the species that are most adapted to the new conditions. However, the high functional redundancy within microbial species in a community (Bell et al., 2005) may lead to stochastic responses limiting the replicability of the observed patterns.

Stressors associated with agriculture and urbanization, for example sedimentation, reduced flow velocity and nutrient enrichment, are known to have significant effects on microbial assemblages, productivity and activities, and thus can lead to changes in ecosystem function such as altered decomposition rates (Pascoal and Cássio, 2004; Piggott et al., 2015a; Widder et al., 2014). Individually, sediment deposition and flow velocity reduction influence microbial community structure and diversity by creating physical barriers via an increase in boundary layer thickness (Barker Jørngensen and Des Marais, 1990; Stevens and Kurd, 1997) or a filtering action of the hyporheic zone (Cornut et al., 2014). Both processes limit the exchange of oxygen and nutrients between water column and biofilm but also modulate the dispersal-colonisation dynamics of the substratum (Besemer et al., 2007; Cornut et al., 2014). The effects of nutrient enrichment on litter-associated microbial communities are complex and can differ depending on microbial stoichiometry (Brosed et al., 2017), nutrient uptake rates (Gulis and Suberkropp, 2003), substratum stoichiometry (Manning et al., 2016), the environment's reference conditions and enrichment magnitude (Ferreira et al., 2015). In general, however, moderate nutrient enrichment tends to promote microbial growth and activity.

Stressor effects are known to vary along disturbance gradients, sometimes taking unforeseen directions regarding the microbial community's structure and function (Romero et al., 2019). For instance, stressors leading to changes in the physico-chemical (water chemistry, flow velocity, benthic substratum composition) and biological conditions along the river continuum are known to alter fungal presence, traits, life strategy and decay activities (Kuehn, 2016). Further, fungi and bacteria both co-habit the microbiome and play key roles in the decomposition of organic matter. During this process, complex biotic interactions between the two organism groups can occur. While it is generally agreed that both fungi and bacteria can influence each other's community structure, the questions of whether their interactions are predominantly facilitative or competitive, and how these interactions translate into ecosystem function, are still open and also context-dependent (Frey-Klett et al., 2011; Johnston et al., 2016; Romaní et al., 2006).

There has been some interest in the mechanisms which drive the spatial and temporal microbial community changes in response to stressors, including the interactions between bacteria and fungi. Several studies in terrestrial and aquatic environments have highlighted inter- and intra-kingdom interactions and dynamics (Gessner et al., 2010; Purahong et al., 2016). Nevertheless, how stressors may perturb these dynamics has been assessed less often in streams. Further, incubation time has also been shown to be a strong determinant of microbial community structure (Newman et al., 2015). Indeed, as decomposition advances, more recalcitrant compounds remain, favouring the development of microbial species capable of metabolising such compounds (Gessner et al., 2010). Stressors and stressor combinations have the potential to disrupt these processes by accelerating or slowing down decomposition rates as well as altering microbial succession and activity. Thus, a clearer understanding of these processes requires temporal studies at a fine taxonomical resolution for both bacteria and fungi.

Understanding and making sense of the microbial diversity and functionality under stress is a hard task to implement. Indeed, manipulative experiments on multiple stressors require considerable logistical investment to set up, and much of the research linking bacteria and fungi to litter decomposition in freshwater systems has been conducted on sterile (autoclaved) substrata in laboratory settings (Fernandes et al., 2014; Ferreira and Chauvet, 2012; Gulis et al., 2017). However, only a few microbes can be successfully reared and identified in laboratory conditions (Lloyd et al., 2018; Steen et al., 2019). Therefore, these studies provide a very simplified version of the natural environment (Johnston et al., 2016). Further, leaves falling into streams already come with their own set of microbes, as either endophytes (leaf interior) or phyllosphere (leaf surface) organisms, and several aquatic microbial genera are known to have a terrestrial stage in their life cycle (Mustonen et al., 2016; Röhl et al., 2017). Nevertheless, past research tends to overlook this initial community and how it could influence the successional trajectory of the microbial community during the leaves' aquatic decomposition. Recent advances in molecular techniques have enabled deeper investigation of the "microbial black box" in its natural environment (Cristescu, 2014). However, understanding global change effects on microbial communities and the resulting change in ecosystem function is still hindered by a lack of characterisation of communities (Antwis et al., 2017). Thus, questions about adaptation, functional redundancy, stochastic versus deterministic assembly patterns and temporal variations have been identified as some of the most prominent open questions in microbial ecology (Antwis et al., 2017).

Here we used field mesocosms to investigate the influence of nutrient enrichment, flow velocity reduction, increased sedimentation and their interactive effects on the microbial communities associated with decomposing leaf litter along a temporal gradient of three weeks. Through investigating the temporal dynamics of bacterial and fungal microbes, we aimed to show that stressor

Please cite this article as: N. P. D. Juvigny-Khenafou, Y. Zhang, J. J. Piggott et al., Anthropogenic stressors affect fungal more than bacterial communities in decaying leaf litter: A stream mesocosm experiment, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.135053

accumulation disrupts the natural microbial successional patterns, resulting in changed organic matter decomposition rates. We tested four specific hypotheses:

(1) By providing readily available extra resources, nutrient enrichment will enhance decomposition rates and enhance microbial diversity (Gulis and Suberkropp, 2004; Kerekes et al., 2013; Piggott et al., 2015a).
(2) Flow velocity reduction will slow decomposition rates and change microbial community composition, due to an increase in boundary layer thickness reducing O_2 and nutrient availability (Barker Jømgensen and Des Marais, 1990; Bruder et al., 2016; Stevens and Kurd, 1997).
(3) Fine sediment will also decrease decomposition rates, by changing the microbial community's functionality (e.g. by reducing efficiency in enzymatic activity of change in metabolic pathways; Tank et al., 2013).
(4) The positive nutrient effect on decomposition and microbial diversity will be counteracted by sedimentation and flow velocity reduction because both act as a physical barrier limiting resource exchange between microbiome and water column, resulting in antagonistic two-way and three-way interactions.

2. Methods

2.1. Study site

Our study occurred in a streamside mesocosm setup (ExStream System) fed by the Yinxi Stream originating from the Jiulongfeng Nature Reserve, Anhui Province, China. The mesocosm site is located just downstream of the reserve boundary (30°07′07″N, 118°01′24″E, 330 m a.s.l.). The reserve covers a 2720 ha area (98% forested, 80% native vegetation) on the west side of the main Huangshan Mountain massif. The reserve's vegetation distribution displays an altitudinal gradient of evergreen broad-leaved forest, evergreen deciduous broad-leaved forest, deciduous broad-leaved forest, alpine short forest and alpine meadows (Jiulongfeng Nature Reserve Director, Mr. X. H. Cao, personal communication). Mean annual temperature and precipitation are 15.4 °C and 1500–1600 mm, respectively (Huangshan District Government, http://www.hsq.gov.cn/; accessed August 2019).

Yinxi Stream is a near-pristine montane stream ($N\text{-}NO_3^-$ 0.39 ± 0.008 [SE] mg/L, $N\text{-}NH_4^+$ 0.26 ± 0.002 mg/L, $P\text{-}PO_4^{3-}$ 0.01 ± 0.001 mg/L, pH 7.87 ± 0.018, conductivity 46.45 ± 0.029 [SE] μS/cm; four measurements each collected with a YSI (Professional Plus, YSI Incorporated, Yellow Springs, OH, USA) at the mesocosm system's water intake point on September 24th 2018). The stream is bordered by steep slopes with dense forest shading the streambed. The only human impact on the stream is a small hydrologic dam located approximately 2.8 km upstream.

2.2. ExStream system and experimental design

The experiment ran for 42 days from October 1st to November 12th 2018. Since allochthonous carbon inputs can play an important role in stream dynamics (Gounand et al., 2018), the experimental period was chosen to include one autumn leaf senescence event to maximise microbial diversity and productivity.

The study was conducted using a 64-unit outdoor stream mesocosm system (ExStream Systems Ltd., Dunedin, New Zealand, Fig. 1) similar to mesocosms setups previously used in New Zealand (Piggott et al., 2015c), Germany (Elbrecht et al., 2016) and Ireland (Davis et al., 2018). Briefly, water and the associated drifting aquatic invertebrates, algae and microbes from the Yinxi Stream were continuously pumped (ACm150B2, Leo Group Co., LTD, Zhejiang, China) through a 4-mm mesh filter into four header tanks, each of which gravity-fed 16 circular mesocosms (outer diameter 24.5 cm, inner diameter 5.1 cm, volume 3 L, area 450 cm^2; Microwave Ring Moulds, Interworld, Auckland, New Zealand). The experiment comprised a 21-day colonisation period followed by a 21-day manipulation phase.

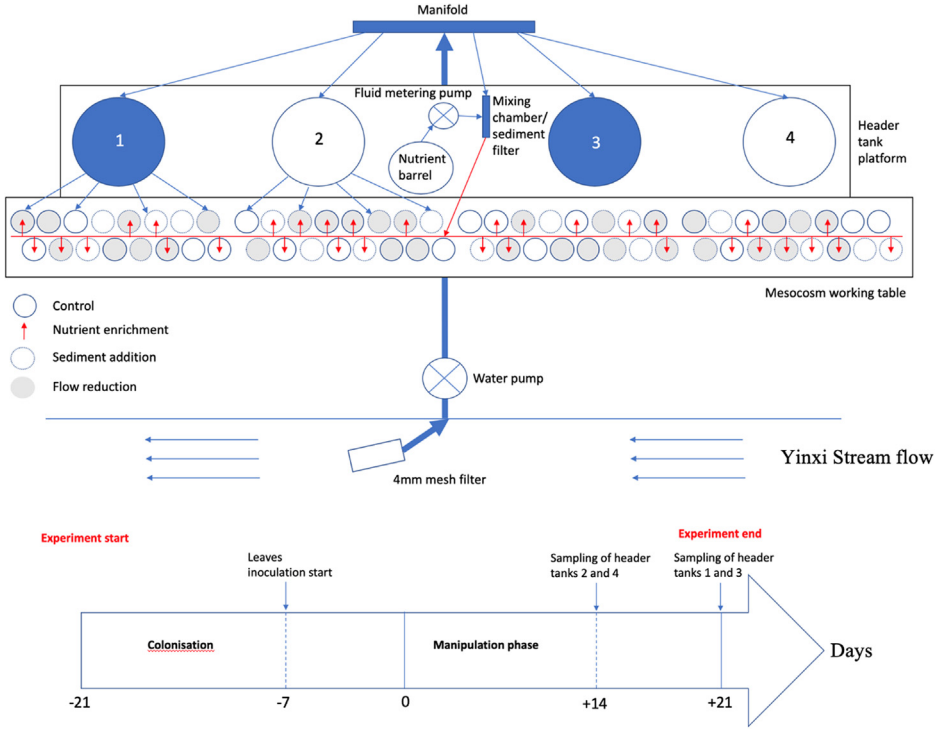


Fig. 1. Schematic of the experimental design and timeline of the experiment.

Please cite this article as: N. P. D. Juvigny-Khenafou, Y. Zhang, J. J. Piggott et al., Anthropogenic stressors affect fungal more than bacterial communities in decaying leaf litter: A stream mesocosm experiment, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.135053

We used a full factorial 2 × 2 × 2 design with eight replicates for each stressor combination: nutrients (ambient versus increased $N\text{-}NO_3^-$ and $P\text{-}PO_4^+$), flow velocity (control versus reduced) and fine sediment (control versus added). To assess temporal variation in the microbial responses, the system was sampled on two occasions (Days 14 and 21). On each occasion, 32 mesocosms from two randomly selected header tanks were sampled (four replicates per treatment combination). Each mesocosm was sampled only once during the experiment.

The water flow through each mesocosm was maintained at a constant rate of 2 L/min and recalibrated daily. Water leaving the mesocosms flowed through their inner circular opening, allowing natural emigration of stream invertebrates and microbes by drift. Temperature and light intensity were monitored every 5 min in one randomly selected mesocosm per header tank block using a HOBO pendant MX2202 data logger (Onset, USA). Mean water temperature over the length of the experiment was 16.14 ± 2.13 [SD] °C compared to 15.60 ± 1.72 [SD] °C at the pump intake in the river. Each mesocosm received 500 mL of coarse substratum (>2 mm), ten 3–4 cm surface stones and one large stone (>6 cm). This substratum composition represented similar habitat heterogeneity as reported in Chinese streams and rivers (Liu et al., 2016). The substratum was collected from a nearby, dry floodplain section of the Yinxi Stream.

Leaves from *Cinnamomum camphora* (camphor tree) were collected from a single street stretch in Suzhou, Jiangsu, China (31°16′17.96″N, 120°44′33.86″E) and air-dried in the laboratory for at least 2 weeks before being stored in the dark. *C. camphora* is an evergreen tree endemic to the southern Yangtze regions and one of the most widespread tree species across China. It is commonly used as an ornamental tree in cities and rural areas and is also being found near our study area (N.P.D. Juvigny-Khenafou, personal observation). Leaf bags consisted of 2.5 ± 0.01 [SD] g of dried leaf material placed into 4-mm mesh bags. To each mesocosm, two leaf bags containing dried *C. camphora* leaves were added on Day-7 to allow microbial colonisation. Leaf bags were pinned to the side of the mesocosms and kept flat on the substratum using surface stones, similar to real-life situations when leaves get trapped under surface rocks. An additional 5 g of the original litter, hereafter referred as terrestrial litter, was also stored at −20 °C to determine the original microbial community present in and on the leaves before the experiment started (Röhl et al., 2017).

Water flow and drift colonisation of the mesocosms started on 1st October 2018 (Day-21). On Day-4, macroinvertebrates were collected from the Yinxi Stream upstream of the pump intake from eight similar riffle environments using kick-net sampling for 3 min of a ~0.36 m^2 area (comparable to the benthic surface area of eight mesocosms). These invertebrates were added to the mesocosms to supplement natural colonisation by taxa underrepresented in the drift (Elbrecht et al., 2016; Piggott et al., 2015b). Following collection, each kick-net sample was divided into eight equal portions using a subsampler and then randomly distributed to individual mesocosms (one portion per mesocosm) following Elbrecht et al. (2016).

2.3. Stressor manipulation

Stressor combinations were randomly assigned within each block on 22nd October 2018 (Day-0). For the sediment treatment, flow in 32 mesocosms was interrupted for 5 min and 300 mL of fine sediment were added. This sediment had been collected from a dry floodplain downstream of the system, air-dried for one week and then sieved (mesh size 0.5 mm, D_{50} = 411.6 μm, Bettersize BT-2900, China) prior to addition. This treatment resulted in 100% sediment cover of the mesocosm substratum on Day-1, consistent with high sediment cover levels observed in catchment-scale stream and river surveys in China (Liu et al., 2016). However, due to the thickness of the leaf bags only the topmost leaves were fully covered by fine sediment, similar to leaves piling up and anchored by a few surface stones in real streams.

For the flow velocity reduction treatment, the inflow jets were removed and the inlet pointed downwards in 32 mesocosms, to decrease flow velocity whilst keeping identical discharge. This avoided confounding effects on nutrient concentrations and on unmanipulated physicochemical (e.g. water temperature, dissolved oxygen) and biological variables (e.g. drift of stream biota). Near-bed flow velocities in all mesocosms were recorded weekly (Days −20, −14, −5, 3, 10, 18) using an electromagnetic flow meter (MF Pro, OTT HydroMet GmbH, Germany). Achieved velocities were 0.10 ± 0.008 [SE] $m{\cdot}s^{-1}$ in the control treatment and zero (below the instrument's detection limit) in the reduced velocity treatment. Similar near-bed flow velocities have been obtained in previous experiments using the same mesocosm system in other countries (New-Zealand and Germany), and resulted in considerable differences for the measured biological response variables (Beermann et al., 2018; Bruder et al., 2016; Elbrecht et al., 2016).

Nutrient enrichment was achieved by continuously injecting a concentrated solution of $NaNO_3$ and KH_2PO_4 into 32 mesocosms using a fluid-metering pump (CK15, Kamoer, Shanghai, China). Nutrient concentrations were determined on Days 1, 8, 15 and 18 (n = 192, only 32 mesocosms remained on the last two dates) using standard methods (APHA, 1998). Sample aliquots were analysed with a Lachat flow injection analyser (QuickChem 8500, Hach, USA). Achieved concentrations were 2.19 ± 0.09 [SE] mg/L $N\text{-}NO_3^-$ and 0.12 ± 0.005 mg/L $P\text{-}PO_4^+$ in the enriched treatment compared to 0.57 ± 0.02 [SE] mg/L $N\text{-}NO_3^-$ and 0.01 ± 0.001 [SE] mg/L $P\text{-}PO_4^+$ in the ambient treatment. Enrichment levels were chosen to remain in the enriched water quality categories according to the 6-class water quality classification (GB 3838-2002) of the Ministry of Environmental Protection of the People's Republic of China (MEP, 2002), while also representing recognisably enriched levels according to other countries' frameworks (e.g. European Environment Agency, 2015). Further, they represented realistic enrichments in Chinese waterways following agricultural intensification; between 1970 and 2000 TDN and TDP increased 8- and 22-fold, respectively, at the river basin scale (Strokal et al., 2016).

2.4. Leaf bag processing

On Days 14 and 21, leaf bags were collected carefully, placed into individual ziplock bags and kept on ice before being frozen at −20 °C within 2 hrs of collection. Back in the laboratory, one leaf bag per mesocosm was thawed overnight at 2 °C and gently rinsed under running deionised water to remove sediments, invertebrates and other organic matter debris (Graça et al., 2005). All leaf materials were then freeze-dried for 48 hrs in sterile tubes and weighed to the nearest 0.001 mg to estimate the mass loss (Gessner, 1991) before being sent to Sangon Biotech Co., Ltd, (Shanghai, China) for downstream processing.

2.5. DNA extraction, PCR amplification and sequencing

The whole content of each leaf sample was homogenised in liquid nitrogen and 250 mg of the material was used for DNA extraction using the Mag-Bind Soil DNA Kit (Omega E.Z.N.A.™, Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's protocol. All samples were later processed by next-generation sequencing (Illumina MiSeq) using the PCR primer pairs (forward/reverse) 341F/805R (Du et al., 2018) and NS1/GCfung (Maza-Márquez et al., 2016), targeting the V3-V4 region of bacterial 16S rRNA and the 5′ end of the eukaryote 18S rRNA, respectively. Both forward and reverse primers were tagged with adapter sequences, pad and lin-

Please cite this article as: N. P. D. Juvigny-Khenafou, Y. Zhang, J. J. Piggott et al., Anthropogenic stressors affect fungal more than bacterial communities in decaying leaf litter: A stream mesocosm experiment, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.135053

ker regions and a unique barcode on the forward primer to permit the multiplexing of samples. All primers were provided by Sangon Biotech Co., Ltd., Shanghai, China. Nested PCRs were performed, following Du et al. (2018), and the samples were prepared for sequencing using a TruSeq DNA kit according to the manufacturer's instructions. The PCR-amplified products were examined by agarose gel-electrophoresis, purified using Agencourt AMPure XP beads (Beckman, Brea, CA, USA) and quantified with the Qubit 3.0 DNA test kit (Life Technologies, Carlsbad, CA, USA). Amplicons were then pooled in equimolar concentrations in the final mixture. The libraries were sequenced at 20 pmol/μL on MiSeq, 2 × 300 bp paired-end version 3 chemistry according to the manufacturer's specifications by Sangon Biotech Co., Ltd, Shanghai, China.

Sequences were processed and analysed using QIIME 1.8.0 based on sequence length, quality, primer and tag. The forward and reverse reads were joined with an overlap length of 150 bp. Following removal of the primer, all reads having a tail quality score below 20 (with a 10 bp window), containing ambiguous characters and less than 200 bp were removed. Chimeras were identified using the UCHIME software (Edgar et al., 2011). We did not rarefy the samples because of the associated issues concerning sample richness comparability (Chao and Jost, 2012; McMurdie and Holmes, 2014). The filtered reads were then clustered into OTUs (Operational Taxonomic Units) with USEARCH using a 97% similarity (Edgar, 2010) and OTUs found in only one read across the dataset were discarded. The average length of the remaining filtered sequences was 412 bp and 422 bp for the prokaryote and eukaryote dataset, respectively. The taxonomic assignment of OTUs was performed against the Silva database with the Ribosomal Database Project classifier and a minimal confidence score of 0.8 (Gustave et al., 2019; Purahong et al., 2016; Xiao et al., 2018). Coverage estimators were calculated for each sample to ensure their comparability prior to downstream analyses (Chao and Jost, 2012; Shimadzu, 2018). Raw prokaryotic and eukaryotic data were deposited in the NCBI Sequence Read Archive (SRA) under the BioProject ID PRJNA560484.

2.6. Statistical analysis

All statistical analyses were performed using R (version 3.5.2, R Core Team). The model structure used for all analyses (unless stated otherwise) was the following: intercept (d.f. 1) + nutrients (1) + sediment (1) + velocity (1) + time (1) + nutrients × time (1) + sediment × time (1) + velocity × time (1) + nutrients × sediment (1) + nutrients × velocity (1) + sediment × velocity (1) + nutrients × sediment × time (1) + nutrients × velocity × time (1) + sediment × velocity × time (1) + nutrients × sediment × velocity (1) + nutrients × sediment × velocity × time (1) + error (48; n = 64).

The significance level was set at $p < 0.05$, and all response patterns summarised in the Results were significant unless indicated otherwise. Standardised effect sizes (partial η^2 values, range 0–1; Garson, 2015), are presented for all p-values < 0.1 to allow our readers to evaluate the likely biological relevance of the results (Nakagawa, 2004), except for the PERMANOVA (see below). Following Nakagawa and Cuthill (2007), effect sizes can be classified as: <0.10 'very small', ≥0.10 'small', ≥0.30 'medium', and ≥0.50 'large'. Since all but two of the significant higher-order interaction terms had smaller effect sizes than the corresponding significant lower-order and/or main effect terms (see Results), the latter could mostly be interpreted reliably (Quinn and Keough, 2002).

We first generated relative abundance plots at the class level. When investigating taxon-specific response patterns, we adopted a slightly more conservative approach (genus level) than the OTU (species) level. This taxonomic resolution adopted the middle ground between the recommendations of a previous mesocosm study in a similar experimental system in New Zealand, which revealed that a resolution below order did not necessarily generate more accurate results when detecting stressor interactions (Salis et al., 2017), and most other recent microbial multiple-stressor studies which focused on OTUs. Bacterial and fungal alpha diversity, richness, Shannon index and Pielou's evenness were computed and analysed with the linear model detailed above. To investigate the total community response (including rare taxa) to the stressors, a PERMANOVA analysis (Bray-Curtis coefficient and 999 permutations) was performed, and a permutational analysis of multivariate dispersions (PERMDISP; Anderson, 2006) conducted to assess homogeneity. Since the bacterial community did not respond to any of the treatments or interaction combinations in the PERMANOVA, principal component analysis (PCoA) plots were made for the fungal community only to allow visual representation of significant PERMANOVA outputs. PCoA plots were based on Hellinger-transformed data to reduce the weights for genera with low read counts and to allow subsequent Euclidian representation of data based on Euclidian distances (Legendre and Gallagher, 2001). MANOVAs were then performed on the abundant fungal and bacterial taxa (>1% of the total reads, Baltar et al., 2015) to investigate taxon-specific responses. All multivariate analyses were performed using the R base functions and the *vegan* package (Oksanen, 2015).

3. Results

3.1. Stressor and time effects on leaf decomposition

Nutrient levels, flow velocity and exposure time all affected leaf decomposition (Fig. 2, Table 1); mean leaf mass loss across all treatments was 34% (range 28–42%) and 37% (31–47%) after two and three weeks, respectively. Leaf mass loss was higher in nutrient-enriched mesocosms and lower at slower flow, with similar response patterns after two and three weeks of incubation. No 2-way, 3-way or 4-way interactions between experimental factors were detected.

3.2. Microbial community composition in the different microhabitats post treatment exposure

The dataset had a total of 6,360,820 raw reads, 3,422,391 for prokaryotes and 2,938,429 for eukaryotes. Amongst the prokaryote and eukaryote datasets, a respective 2,956,912 and 2,025,927 reads were assigned to 23,141 bacterial and 7,792 fungal OTUs after dereplication, quality filtering and sorting. The Coverage estimator

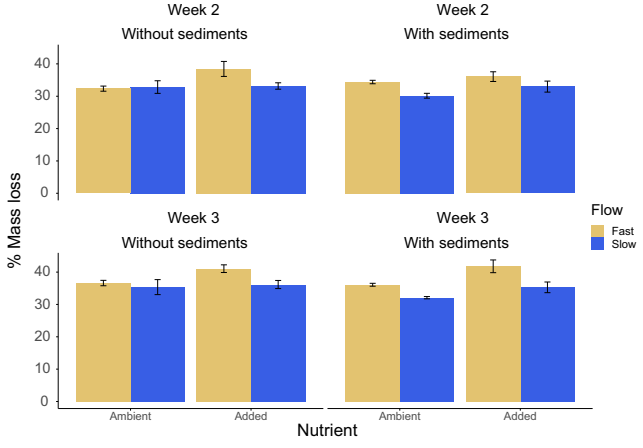


Fig. 2. Mean percentages of leaf litter mass loss (with standard errors) in the experimental treatments after 2 and 3 weeks of incubation.

Table 1
Summary (p-values and effect sizes) of LM results comparing microbial alpha diversity and leaf litter mass loss. For all manipulated factors, main effects are classified directionally as positive (+) or negative (−). P-values bolded where $p < 0.05$. Effect sizes (partial-η^2 values; range 0–1) are shown in parentheses for all cases where $p < 0.1$.

Response	Nutrient	Sediment	Flow	Time	Nutrient × Sediment	Nutrient × Flow	Nutrient × Time	Sediment × Flow	Sediment × Time	Flow × Time	Nutrient × Sediment × Flow	Nutrient × Sediment × Time	Nutrient × Flow × Time	Sediment × Flow × Time	Nutrient × Sediment × Flow × Time
Bacteria															
Richness	0.34	0.20	0.62	0.93	0.07 (0.06)	0.09 (0.06)	0.94	0.41	0.62	0.55	0.052 (0.08)	0.98	0.94	0.89	0.84
Shannon's	0.73	0.98	0.81	0.99	**0.049** (0.08)	0.58	0.89	0.97	0.72	0.32	0.41	**0.02** (0.10)	0.41	0.31	0.06 (0.07)
Pielou's	0.88	0.74	0.67	0.97	0.09 (0.06)	0.88	0.88	0.79	0.79	0.36	0.71	**0.01** (0.12)	0.39	0.24	**0.03** (0.09)
Fungi															
Richness	**0.001** (0.19) +	0.40	0.19	0.27	0.66	0.48	0.09 (0.06)	0.22	0.25	0.17	0.40	0.15	**0.04** (0.08)	0.77	0.51
Shannon's	0.08 (0.06)	0.83	0.08 (0.06)	0.34	0.12	0.47	0.13	0.54	0.74	0.06 (0.07)	0.58	0.20	0.14	0.30	0.053 (0.07)
Pielou's	0.32	0.97	0.11	0.46	0.10	0.52	0.20	0.29	0.44	0.08 (0.06)	0.39	0.30	0.29	0.27	**0.04** (0.08)
% Mass loss	**<0.001** (0.28) +	0.21	**<0.001** (0.34) −	**<0.001** (0.27) +	0.76	0.06 (0.05)	0.23	0.57	0.9	0.42	0.16	0.33	0.78	0.75	0.31

showed that similar degrees of completeness were achieved (16S Coverage range 0.97–0.99; 18S Coverage range 0.98–0.99) with sufficient sampling depth (16S: 45,491 ± 7,636 [SD]; 18S: 39,257 ± 1,643 [SD]) in each sample.

The post stressor exposure fungal community was dominated by organic-matter-decomposing Ascomycota (99.7% of all reads), amongst which Leotiomycetes (28.3% after 2 weeks of stressor exposure, 33.4% after 3 weeks), Sordariomycetes (28.4%, 20.2%) and Dothideomycetes (25.1%, 27.7%) were the most common classes. Changes in fungal community composition at the class level occurred across all treatment combinations, especially after 2 weeks of stressor exposure (Fig. 3).

The post stressors exposure bacterial community was dominated by Proteobacteria (83.3% of all reads), amongst which Alphaproteobacteria (28.8% after 2 weeks, 25.3% after 3 weeks), Betaproteobacteria (27.6%, 31.7%), Deltaproteobacteria (3.5%, 4.3%) and Gammaproteobacteria (22.6%, 22.1%) were the most abundant taxa (Fig. S1). At the class level, bacterial communities showed no clear patterns of change in response to the experimental treatments. Community change patterns at the genus level are presented in Section 3.4.

3.3. Alpha diversity patterns

Diversity metrics for bacteria and fungi responded relatively weakly to the stressors (Table 1; see Supplementary Material for bar graphs and 2-way interaction plots for all diversity metrics). Bacterial diversity and evenness showed a nutrient enrichment × sediment addition interaction which changed across sampling dates (Table 1, Fig. S4, Fig. S6.1-2). After two weeks of incubation, both diversity and evenness decreased with added sediment in nutrient-enriched mesocosms, but this pattern was reversed after three weeks (Fig. S6.1-2). Further, evenness displayed a 4-way interaction between all manipulated factors (Fig. S4). After two weeks, evenness increased slightly when only sediment was added. This increase turned into a decrease when sediment addition was combined with flow velocity reduction;

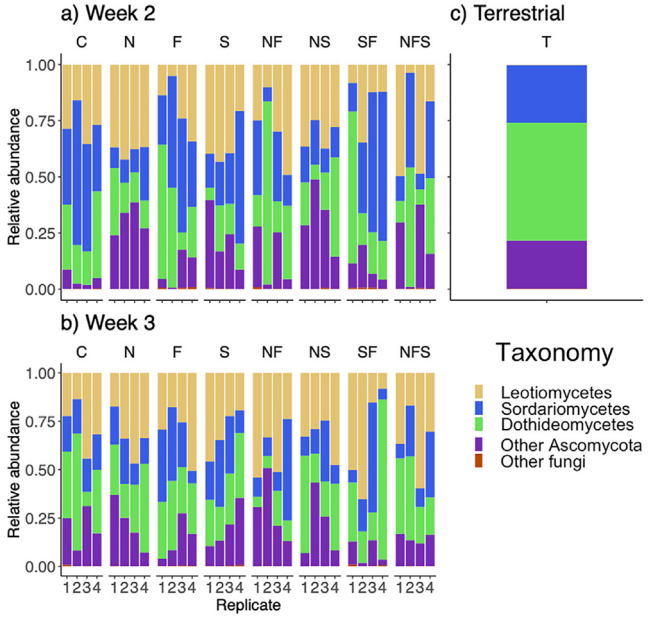


Fig. 3. Relative abundances of the fungal assemblages at the class level after (a) 2 weeks and (b) 3 weeks; (c) is the terrestrial fungal community before submersion. C: Control; N: Nutrient enrichment; F: Flow velocity reduction; S: Sediment addition; NF: Nutrients + Flow; NS: Nutrients + Sediment; SF: Sediment + Flow; NFS: All three stressors.

however, this negative effect of reduced velocity was moderated when nutrients were also added. After three weeks, sediment addition reduced evenness compared to control treatments, but this negative effect was mitigated by flow velocity reduction and further alleviated when both flow velocity was reduced and nutrients added.

For the fungal community, genus richness generally increased when nutrients were added, and a nutrients × flow × time interaction occurred (Table 1). After two weeks, the marked increase in richness associated with nutrient enrichment was weakened somewhat when combined with flow velocity reduction (Fig. S5, Fig. S6.3-4). After three weeks, richness increased much less with nutrients, and mainly at reduced flow velocity. Diversity was unaffected by the manipulated factors, and evenness displayed a 4-way interaction between all factors (Table 1). After two weeks, reduced velocity increased evenness slightly in all treatment combinations, except for when combined with nutrient enrichment and sediment addition, where evenness was lower at reduced velocity (Fig. S5). After three weeks, these patterns had been largely reversed, with evenness decreasing at reduced velocity in two of the four treatment combinations involving sediment addition and nutrient enrichment.

3.4. Community compositional changes

The multivariate PERMANOVA results (Table S1) showed that total fungal community composition (including rare taxa) changed due to nutrient enrichment ($F_{1,48} = 8.76$, $p = 0.001$) and flow velocity reduction ($F_{1,48} = 2.45$, $p = 0.02$). Further, both PERMANOVA and PCoA results suggested that nutrient enrichment and flow velocity reduction effects changed with time (Table S1, Fig. 4). Total bacterial community composition was unaffected by all four experimental factors or their interactions. Similar community-level response patterns were also found for bacterial and fungal community composition in the multivariate results of the MANOVAs based only on the abundant taxa (Table 2).

The univariate results of the MANOVAs indicated that most of the 17 abundant bacterial genera remained unaffected by the stressors, whereas most of the nine abundant fungal genera responded to either flow velocity reduction or nutrient addition (Table 2). Indeed, 55.5% of the abundant fungal genera showed significant main effects for nutrients (*Lunulospora* and *Pyrenochaeta* positive, *Bartalinia*, *Curvularia* and *Dothidea* negative), 22.2% for flow velocity reduction (*Bartalinia* and *Microdochium*, both negative), and 22.2% for sampling date (*Bartalinia* and *Lunulospora*, both becoming less prevalent after 3 weeks), whereas all nine genera were unaffected by sediment addition. Among the abundant bacterial genera, only *Actinoplanes* showed a main effect of flow velocity reduction (positive), and 11.7% of the genera displayed an effect of sampling date (*Aquabacterium* becoming more prevalent after 3 weeks, and *Gemmobacter* less prevalent).

Stressor interactions, including time variations, occurred more frequently in fungal rather than bacterial taxa (44.4% and 29.4% respectively) (see Supplementary Material for genus-specific bar graphs and interaction plots). Most bacterial interactive patterns changed through time; except for *Sphingorhabdus* in which nutrient addition moderated the negative effect of sediment and flow velocity reduction (Fig. S6.8). For *Acidovorax*, a positive effect of nutrient enrichment after 2 weeks of exposure became negative after 3 weeks (nutrients × time; Fig. S6.11). After 2 weeks, *Roseateles* and *Rhizobacter* increased in prevalence when sediment alone was added but decreased when nutrients were also added; after 3 weeks these patterns were reversed, and in both weeks the exact opposite patterns were observed for *Pantoea* – (sediment × nutrients × time; Fig. S6.16–21). Further, after 2 weeks *Roseateles* decreased under flow velocity reduction alone but increased when nutrients were added too, and these patterns were reversed after 3 weeks (flow velocity × nutrients × time; Fig. S6.14–15). Finally, *Pantoea* showed a weak, complex 4-way interaction among all manipulated factors (Fig. S6.10), which overlaid the stronger 3-way interaction described above.

Regarding interactive effects on abundant fungal genera, *Curvularia* increased in prevalence when sediment alone was added, whereas this genus decreased when nutrients were also added (sediment × nutrients; Fig. S6.12). The remaining interactions all involved temporal changes. *Goniopila* responded positively to sediment addition after 2 weeks but negatively after 3 weeks (sediment × time; Fig. S6.7), and *Pyrenochaeta* showed the same temporal change for reduced flow velocity (flow velocity × time; Fig. S6.9). Finally, the negative effect of nutrient enrichment was stronger after 2 weeks for *Bartalinia* and after 3 weeks for *Curvularia* (nutrients × time; Figs S6.5, S6.6).

3.5. Persistence of terrestrial microbes after submersion

Most of the terrestrial bacterial and fungal taxa were maintained in the experimental treatments after submersion and exposure to the stressors; however, their relative abundances were drastically altered, especially for fungi (Fig. 3, S2-S3). Terrestrial bacterial and fungal communities were composed of 280 and 56 genera, respectively. All these bacterial genera were detected in at least one treatment replicate on both sampling dates. For fungi, 88% of the terrestrial community was found in at least one treatment replicate after two weeks of incubation and 94% after three weeks.

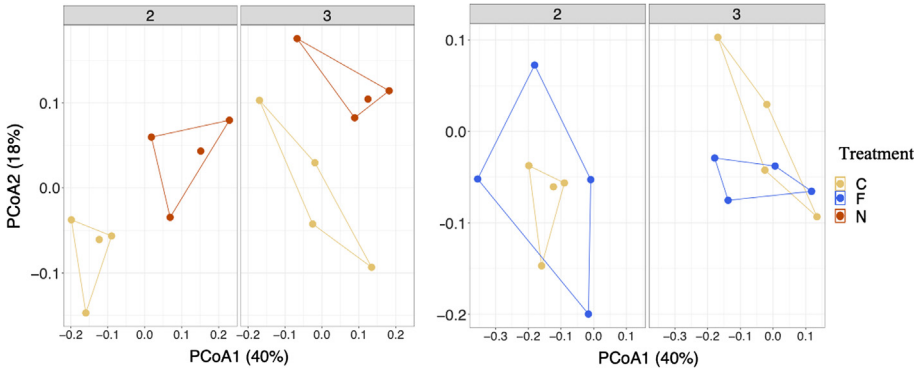


Fig. 4. PCoA graphical representation of the fungal communities for the nutrient and flow velocity treatments after 2 weeks and 3 weeks of stressor exposure. C is the control treatment, N the nutrient enrichment treatment (without any other stressors added) and F the flow reduction treatment (without any other stressors added).

Please cite this article as: N. P. D. Juvigny-Khenafou, Y. Zhang, J. J. Piggott et al., Anthropogenic stressors affect fungal more than bacterial communities in decaying leaf litter: A stream mesocosm experiment, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.135053

Table 2
Summary (p-values and effect sizes) of multi- and univariate LM results for the abundant taxa, with relative abundance by genera as the response variable. For all manipulated factors, significant main effects are classified directionally as positive (+) or negative (−). P-values in bold font where $p < 0.05$. Effect sizes (partial-η^2 values; range 0–1) are shown in parentheses for all cases where $p < 0.1$.

Response	Nutrient	Sediment	Flow	Time	Nutrient × Sediment	Nutrient × Flow	Sediment × Flow	Nutrient × Time	Sediment × Time	Flow × Time	Nutrient × Sediment × Flow	Nutrient × Sediment × Time	Nutrient × Flow × Time	Sediment × Flow × Time	Nutrient × Sediment × Flow × Time
Bacterial community	0.94	0.19	0.70	0.41	0.85	0.31	0.76	0.14	0.46	0.91	0.58	0.31	0.17	0.19	0.51
Acidovorax	0.42	0.93	0.56	0.41	0.56	0.70	0.16	**0.047** (0.08)	0.09 (0.06)	0.53	0.65	0.099 (0.05)	0.38	0.16	0.08 (0.06)
Actinoplanes	0.89	0.81	**0.04** (0.09) +	0.66	0.62	0.18	0.55	0.67	0.61	0.57	0.54	0.42	0.54	0.47	0.72
Aquabacterium	0.82	0.75	0.86	**0.01** (0.11) +	0.39	0.24	0.68	0.92	0.21	0.18	0.78	0.53	0.09	0.31	0.68
Conexibacter	0.88	0.26	0.73	0.11	0.20	0.62	0.48	0.92	0.07 (0.07)	0.25	0.23	0.54	0.65	0.37	0.32
Gemmobacter	0.84	0.35	0.19	**0.03** (0.09) −	0.82	0.65	0.50	0.14	0.28	0.60	0.08 (0.06)	0.55	0.053 (0.07)	0.10	0.48
Herbaspirillum	0.46	0.48	0.35	0.81	0.70	0.25	0.96	0.64	0.30	0.16	0.47	0.89	0.74	0.31	0.97
Lonsdalea	0.24	0.13	0.59	0.86	0.60	0.65	0.54	0.23	0.26	0.13	0.42	0.72	0.26	0.87	0.87
Novosphingobium	0.22	0.43	0.47	0.97	0.39	0.59	0.96	0.36	0.21	0.27	0.54	0.14	0.25	0.31	0.64
Pantoea	0.79	0.99	0.13	0.99	0.28	0.78	0.62	0.54	0.56	0.35	0.72	**0.01** (0.11)	0.28	0.69	**0.04** (0.08)
Pseudomonas	0.90	0.58	0.39	0.99	0.98	0.62	0.34	0.74	0.74	0.44	0.39	0.63	0.44	0.58	0.13
Rhizobacter	0.95	0.37	0.28	0.40	0.42	0.86	0.28	0.40	0.54	0.16	0.33	**0.02** (0.14)	0.92	0.36	0.38
Rhodoferax	0.40	0.20	0.65	0.18	0.92	0.73	0.80	0.31	0.53	0.08 (0.06)	0.40	0.90	0.39	0.30	0.94
Roseateles	0.10	0.79	0.40	0.43	1.00	**0.04** (0.08)	0.08 (0.06)	0.96	0.89	0.60	0.67	**0.02** (0.10)	**0.01** (0.12)	0.54	0.12
Sphaerotilus	0.58	0.24	0.27	0.28	0.53	0.40	0.35	0.75	0.76	0.56	0.90	0.81	0.54	0.15	0.59
Sphingobium	0.89	0.58	0.57	0.34	0.51	0.91	0.71	0.75	0.61	0.48	0.47	0.84	0.25	0.44	0.93
Sphingomonas	0.75	0.48	0.28	0.63	0.70	0.06 (0.07)	0.60	0.80	0.08 (0.06)	0.35	0.09	0.53	0.53	0.41	0.35
Sphingorhabdus	0.79	0.40	0.84	0.12	0.73	0.99	0.28	0.50	0.54	0.37	**0.03** (0.09)	0.08 (0.06)	0.26	0.22	0.90
Fungal community	**<0.001** (0.75)	0.74	0.10	**0.04** (0.34)	0.33	0.82	0.31	0.15	0.53	0.14	0.93	1.00	0.61	0.79	0.38
Amphisphaeria	0.62	0.31	0.20	0.94	0.99	0.30	0.052 (0.08)	0.81	0.34	0.45	0.99	0.86	0.72	0.46	0.52
Ascocoryne	0.15	0.94	0.51	0.18	0.40	0.51	0.80	0.68	0.84	0.06 (0.07)	0.70	0.98	0.71	0.99	0.37
Bartalinia	**<0.001** (0.25) −	0.89	0.16	**0.02** (0.11) −	0.52	0.68	0.39	**0.03** (0.09)	0.87	0.91	0.84	0.75	0.93	0.25	0.68
Curvularia	**0.005** (0.15) −	0.25	0.29	0.50	**0.04** (0.08)	0.75	0.88	**0.03** (0.09)	0.30	0.77	0.26	0.63	0.24	0.64	0.65
Dothidea	**0.04** (0.09) −	0.14	0.24	0.50	0.07 (0.06)	0.54	0.50	0.79	0.71	0.21	0.92	0.58	0.74	0.72	0.80
Goniopila	0.22	0.51	**0.03** (0.09) −	0.14	0.68	0.65	0.22	0.78	**0.04** (0.08)	0.71	0.29	0.88	0.58	0.64	0.09
Lunulospora	**<0.001** (0.26) +	0.35	0.52	**0.04** (0.08) −	0.38	0.71	0.64	0.08 (0.06)	0.80	0.30	0.76	0.72	0.06 (0.07)	0.72	0.86
Microdochium	0.06 (0.07)	0.61	**0.03** (0.09) −	0.07 (0.07)	0.70	0.32	0.40	0.25	0.88	0.65	0.64	0.92	0.43	0.41	0.75
Pyrenochaeta	**<0.001** (0.29) +	0.55	0.48	0.17	0.32	0.33	0.83	0.60	0.25	**0.04** (0.09)	0.88	0.79	0.17	0.36	0.70

Please cite this article as: N. P. D. Juvigny-Khenafou, Y. Zhang, J. J. Piggott et al., Anthropogenic stressors affect fungal more than bacterial communities in decaying leaf litter: A stream mesocosm experiment, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.135053

The terrestrial bacterial community was predominantly composed of *Actinoplanes*, *Sphingobium*, *Phenylobacterium*, *Novosphingobium* and *Rhizobacter* (Fig. S2), and these five genera also showed high mean relative abundances across treatments (except for *Phenylobacterium*). The terrestrial fungal community was dominated by *Knufia*, *Macrophomina*, *Corynespora*, *Wiesneriomyces* and *Macrodochium* (Fig. S3). Although these genera were still detected post submersion and treatment exposure, their relative abundances were much lower in all treatments.

4. Discussion

Predicting the composition of microbial communities following exposure to stressors and the functional impacts of any changes are important objectives of microbial ecology. Our experiment used culture-independent techniques, combined with a technologically advanced stream mesocosm system, to investigate the effect of multiple stressors on microbial dynamics involved with leaf litter decomposition in a semi-natural, multi-trophic context and along a short temporal gradient. Under nutrient enrichment, fungal community composition shifted due to changes in relative abundances of certain genera. These changes differed from those found when flow velocity was reduced. Fungal community changes were paralleled by acceleration of decomposition under nutrient enrichment, and deceleration under flow velocity reduction. Compositional changes were characterised by a steep increase in the relative abundances of pre-existing fungal genera such as *Ascocoryne* and *Bartalinia*, which had already colonised the litter in low abundances at the time of leaf senescence. The changes in the relative abundances rather than identity of the communities suggest that in the early stages of organic matter decomposition, instead of eliminating certain genera or encouraging new ones, our stressors induced a shift in genus prevalence patterns.

Summed across our diversity and common taxa response variables, significant interactions among nutrients, sediment and/or flow velocity (12 in total) were more common than the main effects of these three stressors (10 in total), highlighting the importance of using a full-factorial design in our experiment. This pattern was especially evident for the bacterial response variables, which were affected predominantly via stressor interactions rather than via main effects (9 versus 2), as discussed further below.

4.1. The three stressors compared

Our first and second hypotheses – higher decomposition with enriched nutrients and lower with flow velocity reduction – were supported by the observed decomposition rates in these treatments. Our finding for nutrient enrichment is in accordance with previous studies where enrichment increased the metabolic rate of the microbial communities (Manning et al., 2018; Piggott et al., 2015a). Indeed, microbes can easily assimilate resources directly available in the water column at a lower energetic cost (Fernandes et al., 2014; Gulis et al., 2017; Lin and Webster, 2014; Webster et al., 2009). These resources can then be re-mobilised to increase the activity of enzymes involved in the degradation of complex carbohydrates and phenolic compounds (Carreiro et al., 2000). At reduced flow velocity, an increase in the boundary layer thickness surrounding the microbiome is a likely driver of slower decomposition rates (Bruder et al., 2016; Mustonen et al., 2016; Piggott et al., 2015a). Thus, a thicker boundary layer has been associated with impeding the exchange of resources, such as nutrients and oxygen, between microbiome and water column, leading to reduced microbial activity (Barker Jørgensen and Des Marais, 1990; Lemly, 1982; Stevens and Kurd, 1997). It is unlikely that decreased physical abrasion was responsible for our observations as our normal flow velocity treatments were not fast enough to cause obvious loss of leaf material (N.P.D. Juvigny-Khenafou, personal observation) and decomposition did not advance to the later stages where leaves become easily friable (mass loss did not exceed 50% of the original mass).

The changes in decomposition rates observed in the flow velocity reduction and nutrient enrichment treatments were paralleled by diversity changes in the fungal community. We had hypothesised that changes in the identity of microbial genera and in their diversity would occur, but this was supported only for one diversity metric for the fungal community, where taxon richness increased when nutrients where added. Instead, the remaining stressor effects were mainly changes in relative abundances, creating distinct groups of dominant fungal genera in response to the flow velocity and nutrient manipulations. Species in microbial communities have a degree of functional redundancy (Bell et al., 2005; Gessner et al., 2010), and this redundancy may have allowed maintaining most genera across most of our experimental treatments. Moreover, our stream-connected system had a continual input of microbes which could have recolonised the mesocosm substrata, buffering losses of the original genera resulting from the different treatments. Overall, our results indicate that – in a 42-day experiment in a mesocosm system fed by a montane stream – abundance dominance patterns rather than richness drove microbial community shifts in response to stressors.

Our third hypothesis – decreased decomposition with increased sedimentation – was not supported: the sediment treatment affected neither decomposition rates nor microbial community structure. This result is largely divergent from previous studies which usually found a strong effect of sedimentation on decomposition rates and bacterial assemblages whether positive or negative (Bruder et al., 2016; Matthaei et al., 2010; Romero et al., 2019). We suspect the sedimentation level applied in our experiment was not high enough to create a strong barrier with the external environment for all the leaves in the packs (see Section 2.3). This idea is further supported by the lack of sediment main effects on microbial community alpha diversity and abundant genera (although several interactive stressor effects involving sediment occurred, see next section).

4.2. Stressor main effects and interactions across time

Stressor interactions were more common than stressor main effects, especially for the bacterial community. Most of the significant stressor interactions involved nutrients × sediment and nutrients × flow, often associated with changes from 2 to 3 weeks of stressor exposure. The observed interactive patterns largely supported our fourth hypothesis, that positive nutrient effects on decomposition rates and microbial diversity would be counteracted by sedimentation and flow velocity reduction (although no interactive effects were observed for decomposition rates). A nutrients × sediment × time interaction affected bacterial diversity, whilst a nutrients × flow × time interaction affected fungal richness and a 4-way interaction of all manipulated factors affected both bacterial and fungal evenness. As predicted, when combined with nutrient enrichment, sediment addition and flow velocity reduction had the opposite effects to that of only nutrient enrichment. We suspect that both sediment addition and flow velocity reduction create physical barriers in the boundary layer, which limits resource exchange between microbiome and water column and prevents a nutrient-enrichment effect. However, alpha diversity patterns were not matched by the total microbial genera turnover analysis where no interaction were found, suggesting a weak influence of community identity on the overall community.

Taxon-specific responses of the abundant microbial genera also followed similar patterns, with most significant interactions

Please cite this article as: N. P. D. Juvigny-Khenafou, Y. Zhang, J. J. Piggott et al., Anthropogenic stressors affect fungal more than bacterial communities in decaying leaf litter: A stream mesocosm experiment, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.135053

involving either nutrients × sediment or nutrients × flow, with a mitigating effect of nutrient enrichment counteracting negative effects of sedimentation or flow velocity reduction in several cases (except for *Curvularia*). Notably this mitigating effect was often inconsistent through time, either lagging (only observed after 3 weeks) or fading (ending after 2 weeks). Previous experiments in terrestrial microbiomes have found that nutrient enrichment accelerated microbial succession (Knelman et al., 2014); thus, the temporal taxon-specific interactive patterns observed in our study could be the result of changes in taxon-specific successional dynamics.

Similar to their interactive effects, stressor main effects on the microbial response variables often changed with time in our study, with several response patterns being reversed from 2 weeks to 3 weeks of stressor exposure. Such temporal effect reversals occurred for abundant individual bacterial (*Acidovorax*) and fungal taxa (*Goniopila*, *Pyrenochaeta, Bartalinia and Curvularia*). This finding is particularly interesting as temporal patterns are still largely unexplored in microbial community responses to multiple interacting stressors. We further speculate that taken into a community-network context, some biotic interactions among species confer a resistance level to individual microbial taxa, buffering their response to stressors (Tylianakis et al., 2010). However, biotic interactions change through time (Hutchinson et al., 2019), and new interactions can be created whilst others can be lost depending on other species' responses to stressors through time. We suggest that to better understand interactive stressor effects on microbial communities, future efforts should focus on biotic interactions, microbial succession and response thresholds to stressors.

4.3. Stochastic versus deterministic effects of stressors on microbial community composition

A previous experiment suggested that stressors, or disturbances, can mediate stochastic community assembly by filtering out unsuitable species (Herren et al., 2016). If stressor levels are strong enough to selectively prevent establishment of certain species, then specific groups of microbes should become associated with the different treatments, and consistent community structures should be found across treatment replicates. The close proximity of the different replicates in our PCoA analysis combined with the selection of specific fungal groups and the overall fungal community structural rearrangements under nutrient enrichment and flow velocity reduction suggests that both stressors can drive succession of the litter fungal community, thus implying deterministic changes. Microbial communities have been hypothesised to have a high functional redundancy because of their high diversity (Bell et al., 2005; Martínez and Canhoto, 2019). However, the lack of obvious selection patterns in most of our stressor combination treatments, combined with high variability between treatment replicates and unchanged decomposition rates, lean towards this functional redundancy hypothesis, indicating a resistance of the overall function of the decomposing litter microbial community to moderate stressors and stressor combinations.

4.4. The terrestrial microbial community matters

Our results also suggest that the microbial endosphere and phyllosphere of terrestrial leaves were not replaced during 3 weeks of submergence and stressor exposure. This result is in accordance with Röhl et al. (2017), who also found that a large proportion of the terrestial microbial community persisted in the first three weeks of submerged leaf litter decomposition. Rather, the community structural changes observed in our experiment were largely driven by rearrangements of relative abundances; distinct groups of minor genera present during the terrestrial stage (*Ascocoryne*, *Goniopila*, *Pyrenochaeta*, *Lunulospora*, *Microdochium*, *Amphisphaeria, Dothidea*, *Curvularia*, and *Bartalinia*) became dominant after two and three weeks of stressor exposure in stream water and displayed different responses to the stressor treatments. Despite evidence of terrestrial fungi in decomposing stream litter, the role of the initial microbial community has rarely been considered in multiple-stressor studies (Mustonen et al., 2016; Röhl et al., 2017). In our study, a large proportion of the bacterial and fungal genera involved in the variability of the microbial community across treatments were present at all time points of assessment (on Day-7, after 2 weeks and 3 weeks of stressor exposure). We therefore suggest that the original terrestrial microbial community that colonised leaf litter prior to senescence and submersion may be involved in the decomposition process. This finding is particularly interesting as it would provide a direct link between the terrestrial and aquatic environment. Because most related laboratory experiments involved sterilised leaves and relied on fungal sporulation to assess and/or identify the fungal community, they could not investigate this question (Artigas et al., 2008; Gardeström et al., 2016; Pascoal and Cássio, 2004; Suberkropp, 1998). This difference further highlights the need for more molecular studies on aquatic leaf litter decomposition to be conducted in mesocosms that can realistically simulate stream environments to better understand the mechanisms involved.

5. Limitations and conclusions

Our findings suggest that leaf-litter microbial communities have a tolerance level to moderate stressor addition. This point is interesting as our study was conducted in a pristine montane stream, implying that microbial decomposers may have a certain degree of natural resistance to stressor interactions displayed in both their community structure and their activities. Additionally, the absence of interactions among our manipulated stressors observed for leaf decomposition rates matches similar studies (Matthaei et al., 2010; Mustonen et al., 2016; Piggott et al., 2015a).

Our study has several limitations worth being aware of. We did not investigate any biological drivers that may have influenced the leaf litter microbial communities besides the three manipulated stressors, for example detritivorous stream invertebrates. Some invertebrates are known to selectively feed on microbes growing on submerged leaves and could have created varying levels of grazing pressure depending on their own density response to the stressors, thus potentially changing both microbial community composition and decomposition rates (Danger et al., 2016). Further, our experiment dealt only with relatively early stages of leaf decomposition, as can be seen in the moderate mean mass losses (34–37%) during our 4-week incubation period, perhaps due to the use of a tree species with waxy leaves which can prevent fast colonisation by aquatic fungi. Consequently, microbial community replacements patterns might have become stronger had we been able to continue our experiment for several more weeks. Finally, a better characterisation of the fungal community might have been obtained had we combined our 18S approach with ITS primers; this approach might have avoided biases associated with the different primers whilst following the commonly used primers for fungal communities assessments (De Filippis et al., 2017).

One previous study suggested that fungal biomass changes were more important than fungal identity in mediating litter decomposition (Ferreira and Chauvet, 2012), whereas others found relationships with microbial diversity (Costantini and Rossi, 2010; Duarte et al., 2006; Santschi et al., 2018). We did not determine microbial biomass so we cannot directly compare our results to Ferreira & Chauvet (2012), but our results do not support an effect of diversity. Such comparisons need to be made carefully, however,

Please cite this article as: N. P. D. Juvigny-Khenafou, Y. Zhang, J. J. Piggott et al., Anthropogenic stressors affect fungal more than bacterial communities in decaying leaf litter: A stream mesocosm experiment, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.135053

since these studies were conducted at different timescales. Additionally, working with relative rather than absolute microbial abundances may mean that some of the observed patterns in community composition can result from relic DNA that may linger in the aquatic system. Previous experiments in sediments have indeed shown that such relic DNA can influence relative abundance patterns of specific microbial taxa, whereas community structure remained unaffected (Gustave et al., 2019).

To conclude, further experiments combining high-throughput sequencing and metabarcoding with metabolic and enzymatic assays should be performed to better understand the microbial communities and their functional responses to multiple stressors in streams. Indeed, modern techniques, such as (meta)genomics, metabolomics, (meta)transcriptomics and (meta)proteomics, can offer unprecedented opportunities to investigate microbial communities' complexity and function *in situ*, greatly enhancing our knowledge beyond what can be acquired from laboratory experiments. When combined with ecosystem function metrics, these techniques have the potential to investigate leaf microbiome responses to stressors from molecules to species in their natural environments.

Funding

This work was supported by the Research Development Fund of Xi'an Jiaotong-Liverpool University (RDF-15-01-50) to Y.X. Zhang, D. Atkinson, and J.J. Piggott with a PhD scholarship to N.P.D. Juvigny-Khenafou, by the Natural Science Foundation of Jiangsu Province (BK20171238) to Y.X. Zhang, and by a University of Otago 'Priming Partnerships' grant to C.D. Matthaei, J.J. Piggott and Y.X. Zhang.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgments

We thank Qinsheng Zhu and the Jiulongfeng Nature Reserve director, Mr Xinhua Cao, and all his staff for their help and assistance during the setting-up of the ExStream system and the running of this project; Yili Chen, Xiao Zhou and Liangping Long for the logistical assistance as well as laboratory analysis; and Isabel Fernandes, Ferran Romero, Romana Salis, Eduardo Meldina for their valuable suggestions and comments during the writing of this manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.135053.

References

European Environment Agency, 2015. SOER 2015 Cross-country comparisons Freshwater quality — nutrients in rivers 1–4.

Anderson, M.J., 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62, 245–253. https://doi.org/10.1111/j.1541-0420.2005.00440.x.

Antwis, R.E., Griffiths, S.M., Harrison, X.A., Aranega-Bou, P., Arce, A., Bettridge, A.S., Brailsford, F.L., de Menezes, A., Devaynes, A., Forbes, K.M., Fry, E.L., Goodhead, I., Haskell, E., Heys, C., James, C., Johnston, S.R., Lewis, G.R., Lewis, Z., Macey, M.C., McCarthy, A., McDonald, J.E., Mejia-Florez, N.L., O'Brien, D., Orland, C., Pautasso, M., Reid, W.D.K., Robinson, H.A., Wilson, K., Sutherland, W.J., 2017. Fifty important research questions in microbial ecology. FEMS Microbiol. Ecol. 93, 1–10. https://doi.org/10.1093/femsec/fix044.

Artigas, J., Romaní, A.M., Sabater, S., 2008. Effect of nutrients on the sporulation and diversity of aquatic hyphomycetes on submerged substrata in a Mediterranean stream. Aquat. Bot. 88, 32–38. https://doi.org/10.1016/j.aquabot.2007.08.005.

Baltar, F., Palovaara, J., Vila-Costa, M., Salazar, G., Calvo, E., Pelejero, C., Marrasé, C., Gasol, J.M., Pinhassil, J., 2015. Response of rare, common and abundant bacterioplankton to anthropogenic perturbations in a Mediterranean coastal site. FEMS Microbiol. Ecol. 91, 1–12. https://doi.org/10.1093/femsec/fiv058.

Barker Jørngensen, B., Des Marais, D.J., 1990. The diffusive boundary layer of sediments: oxygen microgradients over a microbial mat. Limnol. Ocean. 35. 1343-135.

Beermann, A.J., Elbrecht, V., Karnatz, S., Ma, L., Matthaei, C.D., Piggott, J.J., Leese, F., 2018. Multiple-stressor effects on stream macroinvertebrate communities: A mesocosm experiment manipulating salinity, fine sediment and flow velocity. Sci. Total Environ. 610–611, 961–971. https://doi.org/10.1016/j.scitotenv.2017.08.084.

Bell, T., Newman, J.A., Silverman, B.W., Turner, S.L., Lilley, A.K., 2005. The contribution of species richness and composition to bacterial services. Nature 436, 1157–1160. https://doi.org/10.1038/nature03891.

Besemer, K., Singer, G., Limberger, R., Chlup, A.K., Hochedlinger, G., Hödl, I., Baranyi, C., Battin, T.J., 2007. Biophysical controls on community succession in stream biofilms. Appl. Environ. Microbiol. 73, 4966–4974. https://doi.org/10.1128/AEM.00588-07.

Brosed, M., Jabiol, J., Gessner, M.O., 2017. Nutrient stoichiometry of aquatic hyphomycetes: Interstrain variation and ergosterol conversion factors. Fungal Ecol. 29, 96–102. https://doi.org/10.1016/j.funeco.2017.04.008.

Bruder, A., Salis, R.K., McHugh, N.J., Matthaei, C.D., 2016. Multiple-stressor effects on leaf litter decomposition and fungal decomposers in agricultural streams contrast between litter species. Funct. Ecol. 30, 1257–1266. https://doi.org/10.1111/1365-2435.12598.

Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359–2365. https://doi.org/10.1890/0012-9658(2000) 081.

Chao, A., Jost, L., 2012. Coverage-based rarefaction and extrapolation: Standardizing samples by completeness rather than size. Ecology 93, 2533–2547. https://doi.org/10.1890/11-1952.1.

Cornut, J., Chauvet, E., Mermillod-Blondin, F., Assemat, F., Elger, A., 2014. Aquatic hyphomycete species are screened by the hyporheic zone of woodland streams. Appl. Environ. Microbiol. 80, 1949–1960. https://doi.org/10.1128/AEM.03024-13.

Costantini, M.L., Rossi, L., 2010. Species diversity and decomposition in laboratory aquatic systems: the role of species interactions. Freshw. Biol. 55, 2281–2295. https://doi.org/10.1111/j.1365-2427.2010.02433.x.

Cristescu, M.E., 2014. From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. Trends Ecol. Evol. 29, 566–571. https://doi.org/10.1016/j.tree.2014.08.001.

Danger, M., Gessner, M.O., Bärlocher, F., 2016. Ecological stoichiometry of aquatic fungi: current knowledge and perspectives. Fungal Ecol. 19, 100–111. https://doi.org/10.1016/j.funeco.2015.09.004.

Davis, S.J., Ó hUallacháin, D., Mellander, P.-E., Kelly, A.-M., Matthaei, C.D., Piggott, J.J., Kelly-Quinn, M., 2018. Multiple-stressor effects of sediment, phosphorus and nitrogen on stream macroinvertebrate communities. Sci. Total Environ. 637–638, 577–587. https://doi.org/10.1016/j.scitotenv.2018.05.052.

De Filippis, F., Laiola, M., Blaiotta, G., Ercolini, D., 2017. Different amplicon targets for sequencing-based studies of fungal diversity. Appl. Environ. Microbiol. 83, 1–9. https://doi.org/10.1128/AEM.00905-17.

Du, L., Trinh, X., Chen, Q., Wang, C., Wang, H., Xia, X., Zhou, Q., Xu, D., Wu, Z., 2018. Enhancement of microbial nitrogen removal pathway by vegetation in Integrated Vertical-Flow Constructed Wetlands (IVCWs) for treating reclaimed water. Bioresour. Technol. 249, 644–651. https://doi.org/10.1016/j.biortech.2017.10.074.

Duarte, S., Pascoal, C., Cássio, F., Bärlocher, F., 2006. Aquatic hyphomycete diversity and identity affect leaf litter decomposition in microcosms. Oecologia 147, 658–666. https://doi.org/10.1007/s00442-005-0300-4.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.

Elbrecht, V., Beermann, A.J., Goessler, G., Neumann, J., Tollrian, R., Wagner, R., Wlecklik, A., Piggott, J.J., Matthaei, C.D., Leese, F., 2016. Multiple-stressor effects on stream invertebrates: a mesocosm experiment manipulating nutrients, fine sediment and flow velocity. Freshw. Biol. 61, 362–375. https://doi.org/10.1111/fwb.12713.

Fernandes, I., Seena, S., Pascoal, C., Cássio, F., 2014. Elevated temperature may intensify the positive effects of nutrients on microbial decomposition in streams. Freshw. Biol. 59, 2390–2399. https://doi.org/10.1111/fwb.12445.

Ferreira, V., Castagneyrol, B., Koricheva, J., Gulis, V., Chauvet, E., Graça, M.A.S., 2015. A meta-analysis of the effects of nutrient enrichment on litter decomposition in streams. Biol. Rev. 90, 669–688. https://doi.org/10.1111/brv.12125.

Ferreira, V., Chauvet, E., 2012. Changes in dominance among species in aquatic hyphomycete assemblages do not affect litter decomposition rates. Aquat. Microb. Ecol. 66, 1–11. https://doi.org/10.3354/ame01556.

Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M., Sarniguet, A., 2011. Bacterial-Fungal Interactions : hyphens between Agricultural, Clinical, Environmental, and Food Microbiologists. Microbiol. Mol. Biol. Rev. 75, 583–609. https://doi.org/10.1128/MMBR.00020-11.

Gardeström, J., Ermold, M., Goedkoop, W., McKie, B.G., 2016. Disturbance history influences stressor impacts: effects of a fungicide and nutrients on microbial

diversity and litter decomposition. Freshw. Biol. 61, 2171–2184. https://doi.org/10.1111/fwb.12698.
Garson, D., 2015. MULTIVARIATE GLM, MANOVA, AND MANCOVA 2015 Edition.
Gessner, M.O., 1991. Differences in processing dynamics of fresh and dried leaf litter in a stream ecosystem. Freshw. Biol. 26, 387–398. https://doi.org/10.1111/j.1365-2427.1991.tb01406.x.
Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H., Hättenschwiler, S., 2010. Diversity meets decomposition. Trends Ecol. Evol. 25, 372–380. https://doi.org/10.1016/j.tree.2010.01.010.
Gounand, I., Little, C.J., Harvey, E., Altermatt, F., 2018. Cross-ecosystem carbon flows connecting ecosystems worldwide. Nat. Commun. 9, 4825. https://doi.org/10.1038/s41467-018-07238-2.
Graça, M.A.S., Bärlocher, F., Gessner, M.O., 2005. Methods to Study Litter Decomposition. Springer, Dordrecht The Netherlands.
Gulis, V., Kuehn, K.A., Schoettle, L.N., Leach, D., Benstead, J.P., Rosemond, A.D., 2017. Changes in nutrient stoichiometry, elemental homeostasis and growth rate of aquatic litter-associated fungi in response to inorganic nutrient supply. ISME J. 11, 2729–2739. https://doi.org/10.1038/ismej.2017.123.
Gulis, V., Suberkropp, K., 2004. Effects of whole-stream nutrient enrichment on the concentration and abundance of aquatic hyphomycete conidia in transport. Mycologia 96, 57–65. https://doi.org/10.2307/3761988.
Gulis, V., Suberkropp, K., 2003. Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. Microb Ecol 45, 11–19. https://doi.org/10.1007/s00248-002-1032-1.
Gustave, W., Yuan, Z.F., Sekar, R., Toppin, V., Liu, J.Y., Ren, Y.X., Zhang, J., Chen, Z., 2019. Relic DNA does not obscure the microbial community of paddy soil microbial fuel cells. Res. Microbiol. 170, 97–104. https://doi.org/10.1016/j.resmic.2018.11.002.
Herren, C.M., Webert, K.C., Mcmahon, D., 2016. Environmental Disturbances Decrease the Variability of Microbial Populations within Periphyton. mSystems 1, 1–14. https://doi.org/10.1128/mSystems.00013-16.Editor
Hutchinson, M.C., Bramon Mora, B., Pilosof, S., Barner, A.K., Kéfi, S., Thébault, E., Jordano, P., Stouffer, D.B., 2019. Seeing the forest for the trees: putting multilayer networks to work for community ecology. Funct. Ecol. 33, 206–217. https://doi.org/10.1111/1365-2435.13237.
Johnston, S.R., Boddy, L., Weightman, A.J., 2016. Bacteria in decomposing wood and their interactions with wood-decay fungi. FEMS Microbiol. Ecol. 92, 1–12. https://doi.org/10.1093/femsec/iw179.
Kerekes, J., Kaspari, M., Stevenson, B., Nilsson, R.H., Hartmann, M., Amend, A., Bruns, T.D., 2013. Nutrient enrichment increased species richness of leaf litter fungal assemblages in a tropical forest. Mol. Ecol. 22, 2827–2838. https://doi.org/10.1111/mec.12259.
Knelman, J.E., Schmidt, S.K., Lynch, R.C., Darcy, J.L., Castle, S.C., Cleveland, C.C., Nemergut, D.R., 2014. Nutrient addition dramatically accelerates microbial community succession. PLoS One 9,. https://doi.org/10.1371/journal.pone.0102609 e102609.
Kuehn, K.A., 2016. Lentic and lotic habitats as templets for fungal communities: Traits, adaptations, and their significance to litter decomposition within freshwater ecosystems. Fungal Ecol. 19, 135–154. https://doi.org/10.1016/j.funeco.2015.09.009.
Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271–280. https://doi.org/10.1007/s004420100716.
Lemly, A.D., 1982. Modification of benthic insect communities in polluted streams: combined effects of sedimentation and nutrient enrichment. Hydrobiologia 87, 229–245. https://doi.org/10.1007/BF00007232.
Lin, L., Webster, J.R., 2014. Detritus decomposition and nutrient dynamics in a forested headwater stream. Ecol. Modell. 293, 58–68. https://doi.org/10.1016/j.ecolmodel.2013.12.013.
Liu, S., Xie, G., Wang, L., Cottenie, K., Liu, D., Wang, B., 2016. Different roles of environmental variables and spatial factors in structuring stream benthic diatom and macroinvertebrate in Yangtze River Delta. China. Ecol. Indic. 61, 602–611. https://doi.org/10.1016/j.ecolind.2015.10.011.
Lloyd, K.G., Steen, A.D., Ladau, J., Yin, J., Crosby, L., 2018. Phylogenetically Novel Uncultured Microbial Cells Dominate Earth Microbiomes. mSystems 3. https://doi.org/10.1128/msystems.00055-18
Manning, D.W.P., Rosemond, A.D., Gulis, V., Benstead, J.P., Kominoski, J.S., 2018. Nutrients and temperature additively increase stream microbial respiration. Glob. Change Biol. 24, e233–e247. https://doi.org/10.1111/gcb.13906.
Manning, D.W.P., Rosemond, A.D., Gulis, V., Benstead, J.P., Kominoski, J.S., Maerz, J.C., 2016. Convergence of detrital stoichiometry predicts thresholds of nutrient-stimulated breakdown in streams. Ecol. Appl. 26, 1745–1757. https://doi.org/10.1890/15-1217.1.
Martínez, A., Canhoto, C., 2019. Functional redundancy in leaf-litter-associated aquatic hyphomycetes : fine sediment alters community composition but hardly decomposer activity Functional redundancy in leaf - litter - associated aquatic hyphomycetes : Fine sediment alters community comp. Int. Rev. Hydrobiol. https://doi.org/10.1002/iroh.201901997.
Matthaei, C.D., Piggott, J.J., Townsend, C.R., 2010. Multiple stressors in agricultural streams: Interactions among sediment addition, nutrient enrichment and water abstraction. J. Appl. Ecol. 47, 639–649. https://doi.org/10.1111/j.1365-2664.2010.01809.x.
Maza-Márquez, P., Vilchez-Vargas, R., Kerckhof, F.M., Aranda, E., González-López, J., Rodelas, B., 2016. Community structure, population dynamics and diversity of fungi in a full-scale membrane bioreactor (MBR) for urban wastewater treatment. Water Res. 105, 507–519. https://doi.org/10.1016/j.watres.2016.09.021.
McMurdie, P.J., Holmes, S., 2014. Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLoS Comput. Biol. 10. https://doi.org/10.1371/journal.pcbi.1003531.
Ministry of Environmental Protection, 2002. Ministry of Environmental Protection, 2002. Environmental quality standard for surface water (GB3838-2002). http://english. mep.gov.cn/standardsreports/standards/waterenvironment/qualitystandard/ 200710/t20071024111792.htm.
Mustonen, K.-R., Mykrä, H., Louhi, P., Markkola, A., Tolkkinen, M., Huusko, A., Alioravainen, N., Lehtinen, S., Muotka, T., 2016. Sediments and flow have mainly independent effects on multitrophic stream communities and ecosystem functions. Ecol. Appl. 26, 2116–2129. https://doi.org/10.1890/15-1841.1.
Nakagawa, S., 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. Behav. Ecol. 15, 1044–1045. https://doi.org/10.1093/beheco/arh107.
Nakagawa, S., Cuthill, I.C., 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol. Rev. 82, 591–605. https://doi.org/10.1111/j.1469-185X.2007.00027.x.
Newman, M.M., Liles, M.R., Feminella, J.W., 2015. Litter breakdown and microbial succession on two submerged leaf species in a small forested stream. PLoS One 10, 1–22. https://doi.org/10.1371/journal.pone.0130801.
Oksanen, J., 2015. Multivariate Analysis of Ecological Communities in R: vegan tutorial.
Pascoal, C., Cássio, F., 2004. Contribution of Fungi and Bacteria to Leaf Litter Decomposition in a Polluted River Contribution of Fungi and Bacteria to Leaf Litter Decomposition in a Polluted River. Appl. Environ. Microbiol. 70, 5266–5273. https://doi.org/10.1128/AEM.70.9.5266.
Piggott, J.J., Niyogi, D.K., Townsend, C.R., Matthaei, C.D., 2015a. Multiple stressors and stream ecosystem functioning: Climate warming and agricultural stressors interact to affect processing of organic matter. J. Appl. Ecol. 52, 1126–1134. https://doi.org/10.1111/1365-2664.12480.
Piggott, J.J., Salis, R.K., Lear, G., Townsend, C.R., Matthaei, C.D., 2015b. Climate warming and agricultural stressors interact to determine stream periphyton community composition. Glob. Change Biol. 21, 206–222. https://doi.org/10.1111/gcb.12861.
Piggott, J.J., Townsend, C.R., Matthaei, C.D., 2015c. Climate warming and agricultural stressors interact to determine stream macroinvertebrate community dynamics. Glob. Change Biol. 21, 1887–1906. https://doi.org/10.1111/gcb.12861.
Purahong, W., Wubet, T., Lentendu, G., Schloter, M., Pecyna, M.J., Kapturska, D., Hofrichter, M., Krüger, D., Buscot, F., 2016. Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. Mol. Ecol. 25, 4059–4074. https://doi.org/10.1111/mec.13739.
Quinn, G.P., Keough, M.J., 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, New York.
Röhl, O., Peršoh, D., Mittelbach, M., Elbrecht, V., Brachmann, A., Nuy, J., Boenigk, J., Leese, F., Begerow, D., 2017. Distinct sensitivity of fungal freshwater guilds to water quality. Mycol. Prog. 16, 155–169. https://doi.org/10.1007/s11557-016-1261-1.
Romaní, A.M., Fischer, H., Mille-Lindblom, C., Tranvik, L.J., 2006. Interactions of bacteria and fungi on decomposing litter: Differential extracellular enzyme activities. Ecology 87, 2559–2569. https://doi.org/10.1890/0012-9658(2006)87.
Romero, F., Sabater, S., Font, C., Balcázar, J.L., Acuña, V., 2019. Desiccation events change the microbial response to gradients of wastewater effluent pollution. Water Res. 151, 371–380. https://doi.org/10.1016/j.watres.2018.12.028.
Sabater, S., Elosegi, A., Ludwig, R., 2019. Multiple Stressors in River Ecosystems: Status Impacts and Prospects for the. Future. Elsevier.
Salis, R.K., Bruder, A., Piggott, J.J., Summerfield, T.C., Matthaei, C.D., 2017. High-throughput amplicon sequencing and stream benthic bacteria: Identifying the best taxonomic level for multiple-stressor research. Sci. Rep. 7, 44657. https://doi.org/10.1038/srep44657.
Santschi, F., Gounand, I., Harvey, E., Altermatt, F., 2018. Leaf litter diversity and structure of microbial decomposer communities modulate litter decomposition in aquatic systems. Funct. Ecol. 32, 522–532. https://doi.org/10.1111/1365-2435.12980.
Shimadzu, H., 2018. On species richness and rarefaction: size- and coverage-based techniques quantify different characteristics of richness change in biodiversity. J. Math. Biol. 77, 1363–1381. https://doi.org/10.1007/s00285-018-1255-5.
Steen, A.D., Crits-Christoph, A., Carini, P., DeAngelis, K.M., Fierer, N., Lloyd, K.G., Cameron Thrash, J., 2019. High proportions of bacteria and archaea across most biomes remain uncultured. ISME J. 1–5. https://doi.org/10.1038/s41396-019-0484-y.
Stevens, C.L., Kurd, C.L., 1997. Boundary-layers around bladed aquatic macrophytes. Hydrobiologia 346, 119–128.
Strokal, M., Ma, L., Bai, Z., Luan, S., Kroeze, C., Oenema, O., Velthof, G., Zhang, F., 2016. Alarming nutrient pollution of Chinese rivers as a result of agricultural transitions. Environ. Res. Lett. 11,. https://doi.org/10.1088/1748-9326/11/2/024014 024014.
Suberkropp, K., 1998. Effect of dissolved nutrients on two aquatic hyphomycetes growing on leaf litter. Mycol. Res. 102, 998–1002. https://doi.org/10.1017/S0953756297005807.
Tank, J.L., Rosi-Marshall, E.J., Griffiths, N.A., Entrekin, S.A., Stephen, M.L., 2013. A review of allochthonous organic matter dynamics and metabolism in streams. J. North Am. Benthol. Soc. 29, 118–146. https://doi.org/10.1899/08-170.1.

Please cite this article as: N. P. D. Juvigny-Khenafou, Y. Zhang, J. J. Piggott et al., Anthropogenic stressors affect fungal more than bacterial communities in decaying leaf litter: A stream mesocosm experiment, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.135053

Tylianakis, J.M., Laliberté, E., Nielsen, A., Bascompte, J., 2010. Conservation of species interaction networks. Biol. Conserv. 143, 2270–2279. https://doi.org/10.1016/j.biocon.2009.12.004.

Webster, J.R., Newbold, J.D., Thomas, S.A., Valett, H.M., Mulholland, P.J., 2009. Nutrient uptake and mineralization during leaf decay in streams-a model simulation. Int. Rev. Hydrobiol. 94, 372–390. https://doi.org/10.1002/iroh.200811158.

Widder, S., Besemer, K., Singer, G.A., Ceola, S., Bertuzzo, E., Quince, C., 2014. Fluvial network organization imprints on microbial co-occurrence networks. Proc. Natl. Acad. Sci. 111, 12799–12804. https://doi.org/10.1073/pnas.1411723111.

Xiao, X., Liang, Y., Zhou, S., Zhuang, S., Sun, B., 2018. Fungal community reveals less dispersal limitation and potentially more connected network than that of bacteria in bamboo forest soils. Mol. Ecol. 27, 550–563. https://doi.org/10.1111/mec.14428.

Please cite this article as: N. P. D. Juvigny-Khenafou, Y. Zhang, J. J. Piggott et al., Anthropogenic stressors affect fungal more than bacterial communities in decaying leaf litter: A stream mesocosm experiment, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.135053