Fungal decomposition of river organic matter accelerated by decreasing glacier cover

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Abstract

Climate change is altering the structure and functioning of river ecosystems worldwide. In mountain rivers, glacier retreat has been shown to result in systematic changes in aquatic invertebrate biodiversity, yet the effects of ice loss on other biological taxa, and on whole ecosystem functions are less understood. Using data from mountain rivers spanning six countries on four continents, we show that decreasing glacier cover leads to consistent fungal-driven increases in the decomposition rate of cellulose, the world's most abundant organic polymer. Cellulose-decomposition rates were associated with greater abundance of aquatic fungi and the fungal cellulose-degrading Cellobiohydrolase I (*cbhI*) gene, illustrating the potential for predicting ecosystem-level functions from gene-level data. Clear associations between fungal genes, populations and communities, and ecosystem functioning in mountain rivers, indicate that ongoing global decreases in glacier cover can be expected to change vital ecosystem functions, including carbon cycle processes.

Main

The retreat of mountain glaciers is accelerating at an unprecedented rate in many parts of the world, with climate change predicted to drive continued ice loss throughout the 21st century[1,2]. Following peak glacier melt, sustained reductions in the volume, rate, and duration of ice contributions to proglacial river systems will alter their geomorphological and hydrological characteristics, with implications for freshwater biodiversity[3,4] and downstream ecosystem services[5,6]. However, the response of river ecosystem functions (such as nutrient and carbon cycling) to decreasing glacier cover, and the role of fungal biodiversity in driving these fundamental processes, remains poorly understood[7-11]. Organic-matter (OM) decomposition in aquatic environments is a key component of the global carbon cycle[12,13], and advances in next generation sequencing (NGS) and ecoinformatics[14] offer new possibilities to link OM decomposition rates to specific fungal taxa, saprotrophic groups, (i.e. those that obtain nutrients from decomposition of detritus) and key functional genes.

In high-mountain ecosystems, OM sources to freshwaters include riparian grasses, shrubs and wind-blown detritus, alongside autochthonous (originating in the river) macrophytes, algae and material released from melting ice and snow[15,16]. In some parts of the world, such as Alaska and New Zealand, glacier-fed rivers extend into forests that provide greater amounts of allochthonous (imported into the

river) OM inputs to freshwater food webs. As primary production of glacier-fed rivers is constrained by low water temperature, unstable channels and high turbidity[17], OM breakdown critically augments energy availability to these aquatic ecosystems[18,19]. As glaciers shrink, reductions in meltwater will increase river channel geomorphological stability, thus expanding habitat availability for riparian vegetation[20] and increasing OM inputs to rivers. Concurrent increases in river water temperature with deglaciation[21] are hypothesised to enhance biological decomposition processes, with fungal hyphomycetes previously identified as principal decomposers, especially during the initial stages of OM decay in mountain rivers[22].

In this study we report novel measurements of OM decomposition rates and associated fungal genes and community composition data, obtained using a standardised OM decomposition assay (the cotton-strip assay[23]), from 57 mountain rivers spanning six countries on four continents. The material used in this assay consists of > 95% cellulose, the key constituent of riparian and in-stream detritus[24], and the most abundant organic polymer on Earth[25]. The assay estimates the capacity of ecosystems to process organic carbon – their decomposition potential – and integrates the activity of microbes and environmental factors[23,26]. Study sites spanned a gradient of catchment glacier cover from 85% to 0% (Figure 1, Supplementary Table 1). This approach allowed us to evaluate the influence of decreasing glacier cover on the decomposition of cellulose and the abundance (quantitative Polymerase Chain Reaction (qPCR) copy number) of a cellulolytic fungal gene (*cbhI*) critical to the decomposition of cellulose[27]. Through the use of NGS to target the fungal Internal Transcribed Spacer region (ITS: DNA barcode used for the molecular identification of fungi[28]), we were further able to evaluate fungal community, population and functional responses to decreasing glacier cover. Our findings suggest a globally coherent response in fungal abundance and cellulose decomposition in mountain rivers experiencing reductions in glacier cover and provide key insights into how OM dynamics may shift in these ecosystems.

From ITS NGS data, a total of 1063 unique fungal Operational Taxonomic Units (OTUs), clustered at 97% sequence similarity[28], were identified by our standardised assay, ranging from per region maxima of 150 (Southern Alps, New Zealand) to 603 (Eastern Alps, Austria). Reductions in catchment glacier cover were associated with increased fungal (ITS) abundance (qPCR copy number) across all

mountain river sites (Figure 2a). This was complemented by the NGS data, where increases in the relative abundance of subgroups of the fungal community were observed. These subgroups included those identified as saprotrophic[30] (e.g. *Lemonniera*, *Davidiella*), alongside populations of the phylum Ascomycota (sac fungi) and more specifically the genus *Tetracladium*, both of which are thought to encompass saprotrophic aquatic hyphomycetes adapted to glacier-fed freshwater environments[31,32] (Supplementary Figure 1). For saprotrophic taxa, this relationship was underpinned by positive correlations between the abundance of their OTUs and physicochemical parameters characteristic of decreasing glacier cover, including increased water temperature and channel stability (Supplementary Figure 2, Supplementary Table 2). Abundance (qPCR copy number) of the fungal *cbhI* gene increased significantly with reductions in catchment glacier cover (Figure 2b) across sampling regions. This increase in gene abundance was more pronounced below approximately 30% glacier cover (Figure 2b).

Despite an overall increase in fungal (ITS) copy number with reductions in catchment glacier cover, population-level responses showed the potential for both 'winners' and 'losers' with decreasing glacier cover. For example, the abundance of some fungal species (OTUs) increased (e.g. *Lemonniera centrosphaera, Tetracladium marchalianum*) with decreasing catchment glacier cover, despite previously being identified as psychrophilic (cold adapted), whereas other taxa decreased (e.g. *Tetracladium* spp., *Leotiomycetes* sp., *Ascomycota* sp.) (Supplementary Table 3). These mixed taxonomic responses were observed across a relatively constrained range of mean water temperatures (0.7 – 9.7 °C) including sites without glacial influence. This suggests that the sensitivity of these taxa to river warming as ice is lost might not only be a function of temperature, with the biodiversity and community composition of some biofilm taxa considered to be influenced by environmental variables, including elevation and electrical conductivity[33].

We observed 294 fungal (ITS) OTUs exclusively in rivers with > 52% catchment glacier cover. As approximately 28% of the fungal community was restricted to high glacier cover sites, it highlights the potential vulnerability of fungal diversity to ice loss, as this habitat will be reduced with sustained glacier retreat[34]. Whilst larger sample numbers across some mountain ranges are needed to provide a more complete assessment of among-region differences, the potential widespread prevalence of

cold adaption within the fungal community may explain the consistent patterns spanning our multiple sampling regions (Supplementary Table 4). The observed fungal responses to decreasing glacier cover appear to be unrelated to latitudinal position (Supplementary Table 4), in contrast to previous studies showing strong effects of latitude on benthic macroinvertebrate communities in glacial rivers[3,35].

Increases in both fungal (ITS) and *cbhI* gene copy number were associated positively with OM decomposition rates (measured as the cellulose assay tensile-strength loss) across the multiple study sites (Figure 2c, d). While amplification of the *cbhI* gene cannot confirm its expression, its multi-regional correlation with decomposition rate suggested that increases in the fungal populations containing this gene were likely to be a key driver of increased cellulose-decomposition rates. The stronger relationship between decomposition rates and *cbhI* copy number in comparison to fungal (ITS) copy number (Figure 2c, d) indicates that fungal functional traits could be better predictors of decomposition than taxonomic measures[36]. Thus, our findings suggest that the relative abundance of functional genes on standardised cotton strip assays could serve as a proxy to detect complex and difficult to measure changes in river ecosystem function. In our study, amplification of the *cbhI* gene along the gradient of 0 to 80% catchment glacier cover in multiple mountain regions indicates that the response of fungal catabolism of OM to decreasing glacier cover is coherent across a wide range of biogeographic zones.

For the assays that were colonised by fungi, our findings suggest that links between reduced catchment glacier cover and tensile-strength loss are driven by increased abundance of aquatic fungi and their *cbhI* gene (Figure 2, Supplementary Figure 3b). Tensile-strength loss also occurred at 27 river sites despite no evidence of fungal-driven decomposition (fungal ITS and/or the *cbhI* gene were not detected) (Supplementary Figure 3a). This is because other processes can influence OM decay in glacier-fed rivers[19,37] including bacterial catabolism[38] and physical fragmentation due to abrasion[39.] We attributed this tensile-strength loss for cotton strips with no evidence of fungal colonisation or *cbhI* presence (Supplementary Figure 3a) primarily to dislodgement from their initial positioning on the river-bed. For cotton strips which remained fixed at river sites, the high water velocities and shifts in unstable bed sediments could have lifted them into the water column. This would expose them to

turbulent flows and enhanced physical forces, providing little opportunity for fungal colonisation from bed sediments or OM deposits and in turn, limiting the accumulation of the fungal Cellobiohydrolase. Equally, colonisation of some assays that were not dislodged but still exposed to rapid velocity and turbulence could have been inhibited hydrodynamically[40,41]. A clearer direct relationship between tensile-strength loss and catchment glacier cover was evident though when considering only those sites hosting *cbhI* gene amplification (Supplementary Figure 3b). High tensile-strength losses at some sites with > 50% glacier cover suggest that physical processes were acting in concert with fungal decomposition, and further controlled experiments are needed to separate these processes. The clear increase in tensile-strength loss < 30% glacier cover for strips with biological colonisation (Supplementary Figure 3b) parallels the *cbhI* increase (Figure 2b), providing further support for our findings that fungal driven decomposition responds to decreasing glacier cover.

The standardised nature of the cotton-strip assay fabric enables comparison of these mountain river cellulose-decomposition rates with those of other biomes across the planet (Figure 3). Observed daily cellulose-decomposition rates across the sampled rivers ($\log_{10}$ *mean*: -1.74, $\log_{10}$ *median*: -1.64, $\log_{10}$ *range*: -1.53 to -3.22) provided some of the lowest tensile-strength loss values reported to date (Figure 3). The lowest values from this study were recorded from two sites in Ecuador, both with zero glacier cover and high electrical conductivity compared to other nearby streams, highlighting a potential role for local factors such as geology (dissolved ions) in limiting fungal decomposition. Elsewhere, fungal communities also drove cellulose processing rates comparable to river systems of other biomes[24], with many tensile-strength losses similar to those of temperate broadleaf, temperate grassland and tropical savanna but largely in excess of values recorded for cold tundra and boreal zones (Figure 3). The high suspended sediment concentrations and flow variability characteristic of glacier-fed rivers[42] may have accelerated physical fragmentation of the cotton strips, causing OM decomposition rates to be greater than other cold water river systems and subsequently more comparable to those in temperate and tropical biomes. In addition, leaf-pack decay rates have previously been identified as comparable between cold freshwaters (~ 8 °C) and streams with higher temperatures, suggesting processes such as biotic interactions potentially influence OM decomposition rates[43]. Overall, our new data fit clearly into a global relationship observed between water temperature and decomposition[23] (Supplementary Figure 4). This provides evidence that

activation energy estimates, drawn from the metabolic theory of ecology, can inform predictions of river functional response to decreasing glacier cover.

Our findings demonstrate clear links between the abundance of fungal taxonomic and functional genes and OM decomposition rates. These mechanistic links spanning biological scales from genes to ecosystem function appear to be globally coherent, with reductions in catchment glacier cover accelerating the fungal decomposition of riverine OM across several mountainous regions. Sustained decreases in glacier cover are therefore likely to accelerate the fungal processing of particulate carbon in mountain rivers worldwide. The use of a standardised assay across a contemporary gradient of catchment glacier cover may provide only conservative insights into these effects, as climate change and decreasing glacier cover will potentially intensify OM provision to many mountain rivers due to uphill treeline migration, the development of soil organic carbon stocks in glacier forelands, changes to instream production and more terrestrial riparian vegetation growth as channels stabilise and growing seasons lengthen[20,44]. In turn, fungi mediated breakdown of particulate carbon will provide dissolved organic carbon, for which processing and export is also expected to change with glacier shrinkage[45,46]. Where glacier retreat is occurring more rapidly than the colonisation and succession of riparian vegetation these effects could be lagged, but ultimately we predict more particulate OM input to river systems that are themselves expected to experience warming[46]. These combined effects of changing OM provision, decreasing glacier cover and fungal community changes can be expected to alter the role of mountain rivers in the global carbon cycle.

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Methods

Study areas: Standardised cotton-strip assays were incubated in riffles of glacier-fed rivers, their tributaries and groundwater sourced streams of catchments across mountainous regions of Alaska, Austria, Ecuador, France, New Zealand and Norway (Figure 1, Supplementary Table 1). Study sites were located on four continents, from -44° to 60° latitude. Assays were successfully incubated in a total of 57 river sites (from initial deployments at 75 sites). River sites were selected with minimal anthropogenic influence and spanning an overall gradient of 0 to 85% catchment glacier cover (Figure 1, Supplementary Table 1). The percentage of each river catchment covered by ice was calculated by delineating watershed areas for individual river sites (filled 5 m to 30 m ASTER Digital Elevation Models) using manually refined watershed analysis functions of ArcMap™ 10.4 (hydrology tools) and calculating the regional ice area[47] within these boundaries. The experiments were performed during boreal and austral summer months (2016 and 2017) to capture the highest possible decomposition rates and reduce the influence of flow intermittency. Some study sites (A12, A13) (Supplementary Table 1) that hosted fungal amplification (fungal ITS, *cbhI* gene) had upstream lakes; and, while previous studies have noted the influence of lake outflows on river OM decomposition rates[48,49,50], measured relationships remained similar when river sites downstream of proglacial lakes were omitted from analyses.

Environmental parameters: At each river site, pH was measured using a Hanna Instrument (HI98130, Woonsocket, Rhode Island, USA) (Austria, New Zealand, Norway), a YSI Pro Plus water quality meter (Xylem, Yellow Springs, Ohio, USA) (Alaska), a HQ40D portable multi meter (HACH, Düsseldorf, Germany) (France) or an Extech meter (Extech, Nashua, New Hampshire, USA) (Ecuador). Hourly water temperatures were recorded throughout the cotton-strip incubation periods using iButton Fobs (DS1990A-F5, Foshan, China) (France), HOBO pendant data loggers (Onset, Bourne, Massachusetts, USA) (Ecuador) or TinyTag Plus 2 data loggers (Gemini, Chichester, UK) (all

other sites). The Pfankuch Index[51] was used to estimate geomorphic river channel stability, with components designed to assess stability of the channel bottom noted for all sites, except those in Alaska. Reciprocal values (1/Pfankuch Index) were calculated to enable higher scores to represent greater river channel stability. Water samples (100 mL) were collected and stored at 4°C and *ex-situ* optical turbidity assessed using a desktop turbidimeter (HACH 2100A) (Camlab, Cambridge, UK). All measurements and samples were collected at the beginning of cotton-strip incubation.

Decomposition assay: To quantify and compare decomposition rates across our sites we used an identical cotton-strip assay to Tiegs et al. (2019)[24]. For our purposes the assay offered numerous advantages including its high degree of standardisation, portability, and direct comparability to other studies. The decomposition rates generated from this assay (as loss of tensile strength of fabric, see below) correlate with fungal activity[23] and are sensitive to variation in environmental parameters such as water temperature[24], pH[26] and concentrations of dissolved nutrients[52]. Following the Cellulose Decomposition Experiment (CELLDEX) protocol[23,53], rectangular cotton strips (8 cm x 2.5 cm) were created from > 95% cellulose artist's fabric (Fredrix Artist Canvas, Georgia, USA (unprimed 12-oz heavy-weight cotton fabric, style #548))[23,53]. Strips comprised exactly 27 threads, with 3 mm of fray along each edge. A total of 460 cotton strips were stored in a dry environment and transported flat to minimise damage and fraying. Control strips were created and transported identically but were returned without river incubation. The number of control strips was approximately 15% of the deployed strips in each region. Initially, assays were deployed at 75 river sites but final analysis was conducted on data from 57 river sites. River sites were excluded if 1) cotton strips were lost during incubation, 2) or found above the water level upon collection, or where temperature measurements suggested intermittent periods of low/no flow, as this prevented representative measurement of exclusively aquatic decomposition processes, or 3) if representative tensile-strength measurements were not possible (e.g. due to strips breaking incorrectly in the tensiometer). While cotton fabric may have a different nutrient content and physical structure to riparian and autochthonous inputs entering mountain rivers, the natural prevalence of cellulose in terrestrial and aquatic materials and the standardised form of the strips enabled between-site comparison of decomposition rates and of fungal decomposers[26]. The cellulose assay also provided a locally unlimited carbon source throughout the incubation period, in rivers where particulate OM supply was potentially naturally low and patchily

359 distributed across channel microhabitats[16]. While the space-for-time substitution approach could not
360 account for the potential confounding influence of natural variability in OM supply and thus existing
361 fungal communities between study catchments, it enables investigation of the impact of catchment
362 glacier cover on the fungal catabolism of cellulose without real-time observation of glacier retreat. OM
363 decomposition and fungal community response to glacier recession over long time periods remain to
364 be studied to allow cross-validation with spatial analyses, although studies of invertebrates in glacier-
365 fed rivers have suggested spatially distributed chronosequences can provide similar insights to
366 observed site-specific successional time-series[54].
367
368 At each site, four cotton strips were cable tied to nylon cord (1 m long, 3 mm wide) which was staked
369 to the river-bed in randomly selected locations at individual sites[23,53]. Rocks were placed upstream of
370 each strip upon the cord, to keep them flat on benthic sediments and aligned to the current. Points of
371 similar water depth and turbulence were selected to ensure strips were influenced by comparable
372 environmental conditions[23]. A temperature logger recording hourly measurements was placed in a
373 white plastic tube to shield it from solar radiation and abrasion and cable tied to one of the stakes at
374 each site. For sites with high catchment glacier cover and highly unstable river-beds, additional cotton
375 strips (up to 6) were incubated to increase the potential for some to remain in-situ for the experiment
376 duration.
377
378 Cotton-strip assays were incubated for 37 days, or as close to this duration as field logistics and
379 weather conditions permitted (min. 31 to max. 39 days). This period was designed to maximise the
380 potential of achieving 50% tensile-strength loss, the point of decay at which cotton strips are believed
381 to be colonised by fungal and bacterial communities, but not shredding invertebrates[23]. Strips were
382 cut from their cable binders, gently cleared of debris, and a 2 cm subsample of one cotton strip from
383 each river site was detached using sterilised scissors and preserved in 1 mL of RNA*later*™
384 stabilization solution (ThermoFisher Scientific, Massachusetts, USA)[55]. These subsamples were
385 stored at 4 °C for transport and then -80 °C in the laboratory prior to molecular analysis. All remaining
386 strips were submerged in 100% ethanol for 30 s on site, to halt microbial activity[55].
387

388 **Tensile strength determination**: Tensile-strength loss of incubated cotton strips indicated the
389 potential of a river ecosystem to decompose cellulose[26] and is a more sensitive metric than reductions
390 in assay mass[56]. All cotton strips, minus the subsamples for fungal characterisation, were oven dried
391 (40 °C, 26 hrs) and stored in a desiccator prior to tensile strength determination[57]. An advanced video
392 extensometer (2663-821) (SN:5076) (Instron, High Wycombe, UK) was used to determine a single
393 maximum tensile strength value for each incubated and control strip, extending at a consistent rate of
394 2 cm/min, with 1 cm portions of each strip end secured within the grips[23]. To calibrate the instrument,
395 cotton strips constructed using the CELLDEX protocol but not transported or incubated, were tested
396 until their break points aligned to a consistent range and the machine jaws were sufficiently adjusted
397 to minimise slippage. The sample order was randomised, with control strips processed throughout the
398 sample run to identify instrument drift. Room temperature (19.5 °C) and humidity (60.7%) were kept
399 constant across sample runs, and cotton strips from multiple regions processed together to minimise
400 variability of instrumental and environmental conditions between testing. Strips which broke along the
401 point of contact with the machine jaws were excluded from analysis (n = 4, 0.9%). For cotton strips
402 whose maximum tensile strength was higher than the mean control strip value (n = 24, 5.2%),
403 biological variation lay within the range of technical variation and the two could not be separated, so
404 tensile-strength loss (decomposition) was recorded as zero for these strips.
405
406 Tensile-strength loss was calculated as a percentage of initial strength lost for each cotton strip per
407 degree-day, as adapted from Tiegs et al. (2013, p.134)[23].
408
409 $$\textit{Tensile-strength loss} = ((1-(\frac{TSImax}{TSCmean})) \times 100) / \textit{ incubation period (DD)} \quad (1)$$
410
411 This equation uses the maximum tensile strength of each river incubated strip (TSImax) and the mean
412 tensile strength of all control strips (TSCmean). Temperature-adjusted degree-days (DD) were
413 calculated by summing the mean temperatures recorded for each 24-hour period during the cotton-
414 strip incubation. This enabled temperature normalised comparison of tensile-strength loss across
415 regions, as mean river water temperature can vary dramatically on diurnal and seasonal timescales in
416 glacierised catchments[21]. Percentage tensile-strength loss was averaged across all replicate strips to
417 provide a mean value per river site. All reported tensile-strength values are calculated per degree-day

418 unless stated otherwise. Non-temperature-adjusted cellulose-decomposition rates were determined
419 by representing the incubation period in days, in place of degree-days. These values were compared
420 to those reported for 514 rivers in eleven other biomes[24]. An Arrhenius plot was constructed to
421 correlate daily decomposition values to inverted relative mean water temperature[24].
422
423 **Molecular methods**: One 2 cm^2 subsample was taken from one cotton strip at each river site, and
424 DNA extracted from a standardised 1.5 cm^2 section of each subsample. Extraction followed a
425 standard CTAB protocol[58] with DNA eluted in 50 µL of PCR grade water (Invitrogen, Waltham,
426 Massachusetts, USA). The concentration of DNA in individual samples was quantified using the
427 Quant-iT PicoGreen dsDNA assay kit (Invitrogen), to enable normalisation of all samples to 1 ng/µL
428 for high-throughput preparation of NGS amplicon libraries. Total extracted DNA from each cotton-strip
429 subsample, from each river site, was then used to quantify the absolute copy number of the fungal
430 taxonomic (ITS)[59] and functional (*cbhI*)[60] marker genes via qPCR.
431
432 Absolute quantification of fungal ITS and *cbhI* copy number
433 qPCR was performed to determine the copy number (an estimation of abundance) of fungal ITS and
434 *cbhI*[61]. qPCR DNA standards were created from end-point PCR amplification where the template DNA
435 was 1 µL of DNA extract pooled from each sample. Resulting amplicons were purified using a
436 QIAquick PCR purification kit (Qiagen, Hilden, Germany)[61] and quantified using the Quant-iT
437 PicoGreen dsDNA assay kit (Invitrogen). qPCR was performed separately for each target gene and
438 the copy number of that gene in each sample was then calculated per cm^2 cotton strip and logged.
439 qPCR amplification of fungi (ITS) and the *cbhI* gene was observed at 42% of the 57 river sites.
440
441 For each gene, all samples were run on a single 384 well plate and included a serial dilution of the
442 purified standards ranging from 10^1 to 10^9, non-template (negative) controls and each sample, all of
443 which were included in triplicate. Reagents and the determination of copy number for each sample for
444 each assay followed McKew and Smith (2017)[61]. qPCR reactions were performed in 10 µL reaction
445 volume with 1 µL of DNA, 5 µL of SensiFAST Sybr Green (Bioline), 0.2 µL of each primer (10 µM) and
446 3.6 µL of PCR grade water. To target the fungal ITS, the ITS2 region was amplified using the primer
447 sets ITS3_KYO2 (5'-GATGAAGAACGYAGYRAA-3')[59] and ITS4 (5'-TCCTCCGCTTATTGATATGC-

448 3')[62]. The ITS2 region was targeted because it has good variability at the species level to aid
449 taxonomic distinction[63] and is widely represented in the fungal databases used for genus/species
450 level taxonomic assignment[64]. The fungal *cbhI* gene was targeted using the primer sets fungcbhIF (5'-
451 ACCAAYTGCTAYACIRGYAA-3') and fungcbhIR (5'- GCYTCCCAIATRTCCATC-3')[60]. The assays
452 were run on a CFX real-time system (Bio-Rad, Hercules, California, USA). qPCR conditions to amplify
453 the fungal ITS2 and *cbhI* regions were the same, with an initial denaturation at 95 °C for 3 min,
454 followed by 45 cycles at: 95 °C for 5 s, 60 °C for 10 s and 72 °C for 20 s. This was immediately
455 followed by melt curve generation for one cycle at 95 °C for 5 s, 65 °C for 5 s and 95 °C for 5s.
456
457 Library preparation for NGS of the fungal ITS and *cbhI* genes
458 Library preparation followed the protocol outline by Illumina[65] with PCR conditions amended as a
459 result of optimisation for our two target genes. First stage PCR reactions were performed in a 25 µL
460 reaction volume with 3 µL of DNA template, 12.5 µL appTAQ RedMix (2X) polymerase (Appleton
461 Woods Ltd, Birmingham, UK), 1 µL of each primer (4 µM) containing Illumina overhang adapters[65],
462 1.5 µL of 1% bovine serum albumin (BSA) and 6 µL of PCR grade water. BSA was included to
463 remove inhibitors and increase the yield of PCR amplification[66]. The fungal ITS region and *cbhI* gene
464 were targeted using the same locus-specific primers as used for qPCR[59,60,62], but with the addition of
465 Illumina overhang adapters to ensure compatibility with the sequencing platform[65]. PCR reactions
466 were run on a 96 Well Thermo Cycler (Applied Biosystems, Warrington, UK). PCR conditions to
467 amplify the fungal ITS2 region used an initial denaturation at 95 °C for 3 min, followed by 35 cycles at
468 95 °C for 15 s, 51 °C for 15 s and 72 °C for 30 s for 35 cycles; 72 °C for 7 min. Despite multiple
469 attempts to optimise the *cbhI* library preparation by changing PCR annealing temperature, BSA
470 addition, volume of DNA template and cycle number, insufficient samples amplified for the *cbhI* gene.
471 As a result, the structure of the cellulose-degrading (*cbhI*) fungal community was not assessed.
472 Samples where Agarose gel electrophoresis indicated an absence of indexed and cleaned PCR
473 amplicons were compared with qPCR data.
474
475 Following clean-up of the first stage PCR reactions, amplicon libraries were indexed following the
476 Illumina protocol[65] using the Nextera™ XT Library Prep Kit (Illumina, Cambridge, UK). Annealing
477 temperature mirrored that used in the first stage PCR. For each amplicon library, cleaned and indexed

individual samples were then quantified using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen) before pooling samples in equimolar concentrations. Final amplicon library concentration was then determined using a NEBNEXT® Library Quant Kit for Illumina®, before samples were sequenced as a single run on an Illumina MiSeq platform at the University of Essex, using an Illumina MiSeq reagent kit v3 (600 cycles) generating 300 bp paired end reads[65].

Bioinformatic analysis: The raw ITS amplicon NGS reads were subjected to quality control, including sequencing trimming, error correction and the removal of poor-quality sequences and chimeric PCR artefacts, following Dumbrell et al. (2017)[67] and Maček et al. (2019)[68]. *De novo* clustering of fungal OTUs was performed at 97% similarity[28]. Taxonomic classifications were then assigned to each OTU determined from the amplicon libraries, using the RDP classifier and UNITE database[69]. The UNITE database enabled assignment of ITS sequences from the International Nucleotide Sequence Database Collaboration clustered to approximately the species level (97 – 100% similarity in steps of 0.5%) via a species hypotheses algorithm[69]. This provided unambiguous species matches for our study through the generation of digital object identifiers[70]. Data tables were produced detailing the abundance of OTUs per sample site and the taxonomic classification of each OTU. Additional tables were constructed to host associated environmental information.

Ecoinformatic analysis: Negative controls were removed from OTU tables following confirmation that contamination was negligible (fungi: 1 to 8 reads). Three sites containing very low numbers of sequences (1, 41 and 84 reads) were also removed from the fungal (ITS) OTU tables and all samples rarefied to the smallest library size (10,543 reads). Sequence-based rarefaction was selected in preference to alternative procedures of normalisation as an effective and ecologically meaningful method to standardise differential library sizes for fungal data[71,72]. Therefore, OTU abundance referred to the abundance of reads/sequences recorded for each OTU, relative to the minimum library size. Using the taxonomic identification of fungi in the NGS dataset, associated functions could then be confidently mapped, which is increasingly the norm in fungal research[70]. Subsets of the fungal (ITS) OTU table were created to represent only OTUs with saprotrophic trophic modes. Trophic mode (including taxa identified as saprotrophic) and a confidence ranking describing this assignment were

identified for each OTU utilising the FUNGuild database[30]. Further subsets were created for members of the phylum Ascomycota and genus *Tetracladium*.

Statistical analysis: Generalised linear models (GLM) and generalised additive models (GAM) were used to test relationships between catchment glacier cover (fixed effect) and the following response variables: percentage tensile-strength loss, the qPCR determined abundance of fungal (ITS) and *cbhI* gene amplicon copy number, the estimated abundance of fungal OTUs classified as Ascomycota, *Tetracladium* or saprotrophic and physicochemical variables (mean river water temperature, 1/Pfankuch Index, pH, optical turbidity) across the six study regions. GAMs were also constructed to test the relationship between fungal (ITS) and *cbhI* copy number on tensile-strength loss. Models were computed in the *mgcv* package[73] of R v. 3.3.2, with model parsimony evaluated using Akaike information criterion (AIC) values and performance assessed through the percentage of deviance explained. For GAM, smoothing parameter selection followed Wood (2004)[74], with Gaussian and Negative Binomial distributions identified. The *mgcv* package was also used to construct generalised linear mixed models (GLMM) and generalised additive mixed models (GAMM) to incorporate the effect of absolute latitude (random effect). Mixed models had higher AIC values relative to fixed-effect only models, but values were similar (within 2 units). This suggested no obvious latitude influence on observed responses, although larger sample sizes from some regions would confirm this further. The *manyglm* function of the *mvabund* package[75] of R was used to fit GLMs (Poisson) to individual OTU responses to catchment glacier cover and tensile-strength loss, with relationship significance determined from Wald statistics.

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Figure legends

Figure 1: Global distribution and experimental details of glacierised mountain river sampling sites. For each sampling region, the date and duration of cotton-strip incubation are displayed, alongside the number of river sites sampled and the range of percentage catchment glacier cover that they represent. Cotton-strip images display examples of before versus after incubation. The photograph accompanying the 'Antisana, Ecuador' site information is courtesy of Dean Jacobsen[29]. Further site information is provided in Supplementary Table 1.

Figure 2: Globally coherent relationships between catchment glacier cover, abundance of fungal biomass from cotton-strip assay fungal communities and tensile-strength loss of river incubated cotton strips. (a) increasing fungal (ITS) copy number and (b) increasing *cbhI* gene copy number with declining catchment glacier cover, (c) increase in mean tensile-strength loss with increasing fungal (ITS) copy number and (d) increasing mean tensile-strength loss with increasing *cbhI* gene copy number. For river sites in the Alaska Boundary Range no amplification was detected. Sample numbers vary as the fungal (ITS) and *cbhI* gene did not amplify at all river sites. DD = degree-days. Solid lines are GLMs or GAMs and dashed lines represent 95% confidence intervals.

Figure 3: Comparison of glacierised mountain river cellulose-decomposition rates with other biomes. Comparison of $\log_{10}$ daily cellulose-decomposition rates (K_D) for rivers in glacierised

mountain regions (0 – 85% catchment glacier cover) and rivers representing eleven other biomes. The K_D values indicate the mean daily decomposition rates of the cellulose fabric in each river ecosystem (grey open circles). Boxes represent the median and interquartile range, whiskers represent quartiles plus 1.5 multiplied by the interquartile range, and circles with black dots represent outlying values. Figure adapted from Tiegs et al. (2019)[22].

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Author contributions

SCF co-developed the concept of the manuscript, completed fieldwork in Austria, New Zealand and Norway, assisted with the molecular lab work, ran the statistical analysis, created the figures (with the exception of Figures 1 and 3) and wrote the manuscript. JLC completed fieldwork in New Zealand and

Norway and created Figure 1. SC-F completed fieldwork in Ecuador and France. VC-P completed fieldwork in Ecuador. EH completed fieldwork in Alaska. KCR led molecular sample preparation and PCR and qPCR analysis, and contributed text to the Molecular Methods section. KJMN assisted in molecular sample preparation. AJD developed the analytical protocol for molecular sample analysis, ran the next generation sequencing, formatted the subsequent data for analysis, advised on statistical and ecoinformatic analysis and contributed text to the Molecular Methods section. SDT developed and advised on use of the cotton-strip assay protocol, provided data for Figure 3 and Supplementary Figure 4, and contributed text regarding use of the cotton-strip assay. LEB co-developed the concept of the manuscript, completed fieldwork in Austria and Norway, advised on statistical analysis and production of all figures, created Figure 3 and provided detailed comment on the manuscript. All authors edited and revised the manuscript.

Competing Interests statement

The authors declare no competing interests.

Data availability statement

Raw demultiplexed sequence data has been uploaded to the NCBI Sequence Read Archive (SRA) with BioProject accession number PRJNA684135. A data set has been sent to the NERC Environmental Information Data Centre and this, alongside supporting documentation, are in the process of being deposited here.