Fungal decomposition of river organic matter accelerated by decreasing glacier cover

Sarah C Fell, Jonathan L Carrivick, Sophie Cauvy-Fraunié, Verónica Crespo-Pérez, Eran Hood, Kate C Randall, Kirsty J Matthews Nicholass, Scott D Tiegs, Alex J Dumbrell, Lee E Brown

▶ **To cite this version:**

Sarah C Fell, Jonathan L Carrivick, Sophie Cauvy-Fraunié, Verónica Crespo-Pérez, Eran Hood, et al.. Fungal decomposition of river organic matter accelerated by decreasing glacier cover. Nature Climate Change, 2021, 11 (4), pp.349-353. 10.1038/s41558-021-01004-x . hal-03483668

HAL Id: hal-03483668
https://hal.inrae.fr/hal-03483668v1

Submitted on 16 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License

nature climate change

ARTICLES

https://doi.org/10.1038/s41558-021-01004-x

Check for updates

Fungal decomposition of river organic matter accelerated by decreasing glacier cover

Sarah C. Fell[1], Jonathan L. Carrivick[1], Sophie Cauvy-Fraunié[2], Verónica Crespo-Pérez[3], Eran Hood[4], Kate C. Randall[5], Kirsty J. Matthews Nicholass[5], Scott D. Tiegs[6], Alex J. Dumbrell[5] and Lee E. Brown[1]✉

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, but the effects of ice loss on other biological taxa and on whole-ecosystem functions are less well 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.

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 twenty-first 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, as well as 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 (that is, those that obtain nutrients from the 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 hypothesized 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 standardized 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]. The study sites spanned a gradient of catchment glacier cover from 85% to 0% (Fig. 1 and Supplementary Table 1). This approach allowed us to evaluate the influence of decreasing glacier cover on the decomposition of cellulose and the abundance (quantitative PCR (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 (ITS) region (a 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 consistent 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 the ITS NGS data, 1,063 unique fungal operational taxonomic units (OTUs), clustered at 97% sequence similarity[28], were identified by our standardized assay, with per-region maxima ranging from 150 (Southern Alps, New Zealand) to 603 (Eastern Alps, Austria). Reductions in catchment glacier cover were associated

[1]School of Geography & water@leeds, University of Leeds, Leeds, UK. [2]INRAE, UR RIVERLY, Centre de Lyon-Villeurbanne, Villeurbanne, France. [3]Limnology Laboratory, Zoology Museum, QCAZ I, School of Biological Sciences, Pontifical Catholic University of Ecuador, Quito, Ecuador. [4]Environmental Science Program, University of Alaska Southeast, Juneau, AK, USA. [5]School of Life Sciences, University of Essex, Wivenhoe Park, Colchester, UK. [6]Department of Biological Sciences, Oakland University, Rochester, MI, USA. ✉e-mail: l.brown@leeds.ac.uk

Content courtesy of Springer Nature, terms of use apply. Rights reserved

Fig. 1 | Global distribution and experimental details of glacierized 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. The cotton-strip images display examples of before versus after incubation. Further site information is provided in Supplementary Table 1. Photograph of Antisana, Ecuador, is adapted with permission from ref. [45], Springer Nature Limited.

with increased fungal ITS abundance (qPCR copy number) across all mountain river sites (Fig. 2a). This result was complemented by the NGS data, where increases in the relative abundances of subgroups of the fungal community were observed. These subgroups included those identified as saprotrophic[29] (such as *Lemonniera* and *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[30,31] (Supplementary Fig. 1). For saprotrophic taxa, this relationship was underpinned by positive correlations between the abundances of their OTUs and physicochemical parameters characteristic of decreasing glacier cover, including increased water temperature and channel stability (Supplementary Fig. 2 and Supplementary Table 2). The abundance (qPCR copy number) of the fungal *cbhI* gene increased significantly with reductions in catchment glacier cover (Fig. 2b) across sampling regions. This increase in gene abundance was more pronounced below approximately 30% glacier cover (Fig. 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. The abundances of some fungal species (OTUs) increased (such as *Lemonniera centrosphaera* and *Tetracladium marchalianum*) with decreasing catchment glacier cover, despite previously being identified as psychrophilic (cold adapted), whereas other taxa decreased (such as *Tetracladium* spp., *Leotiomycetes* sp. and *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—the biodiversity and community composition of some biofilm taxa are considered to be influenced by other environmental variables, including elevation and electrical conductivity[32].

We observed 294 fungal ITS OTUs exclusively in rivers with >52% catchment glacier cover. The fact that approximately 28% of the fungal community was restricted to high-glacier-cover sites highlights the potential vulnerability of fungal diversity to ice loss, as this habitat will be reduced with sustained glacier retreat[33]. While 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 adaptation in the fungal community may explain the consistent patterns spanning our multiple sampling regions (Supplementary Table 4). The observed fungal responses to decreasing glacier cover seem 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[3,34].

Increases in both fungal ITS and *cbhI* gene copy number were positively associated with OM decomposition rates (measured as the cellulose assay tensile-strength loss) across the multiple study sites (Fig. 2c,d). While the amplification of the *cbhI* gene cannot confirm its expression, its multiregional correlation with the 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 compared with fungal ITS copy number (Fig. 2c,d) indicates that fungal functional traits

Content courtesy of Springer Nature, terms of use apply. Rights reserved

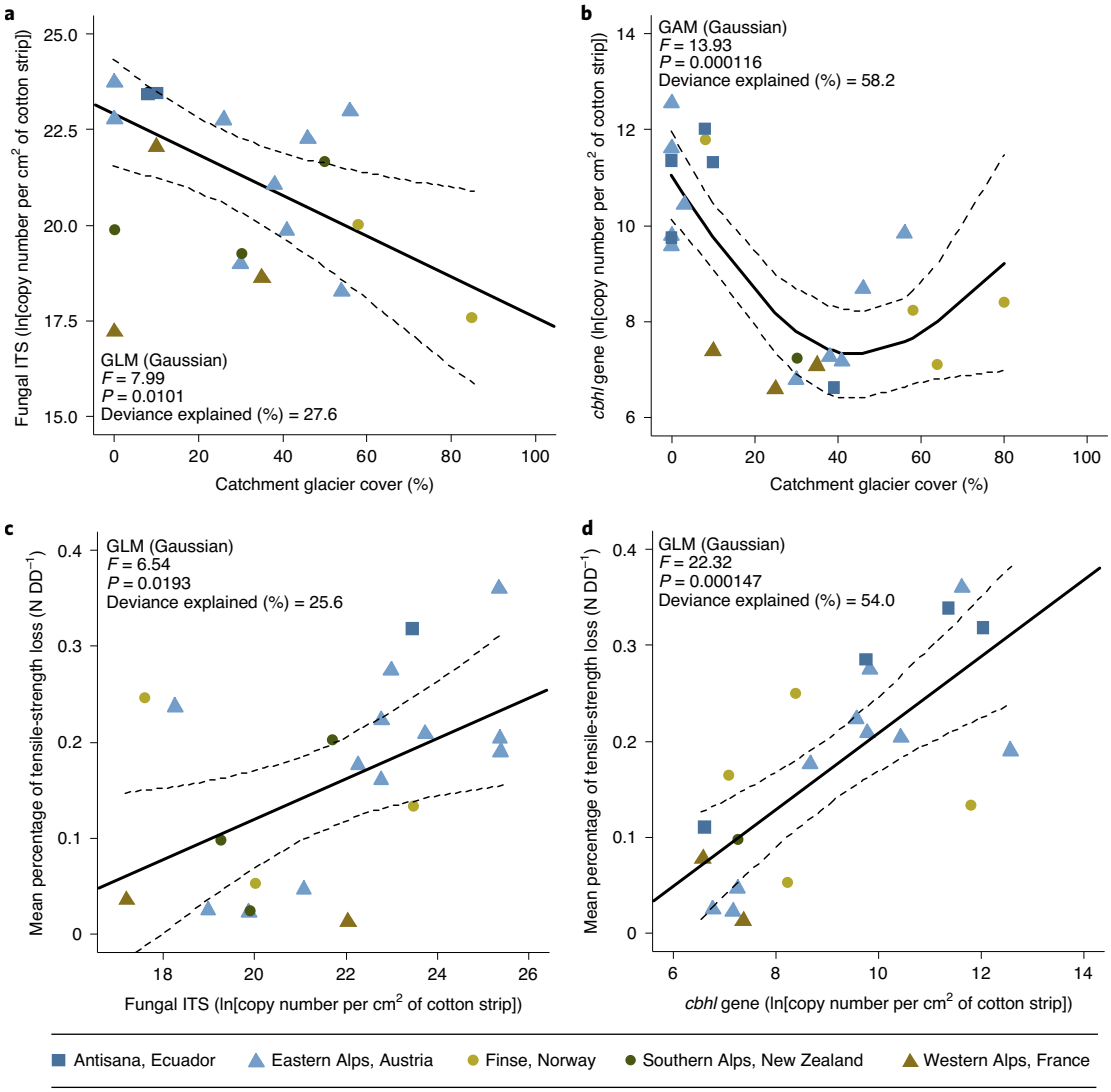

Fig. 2 | Globally consistent relationships between catchment glacier cover, abundance of fungal biomass from cotton-strip assay fungal communities and tensile-strength loss of river-incubated cotton strips. a,**b**, Increasing fungal ITS copy number (**a**) and increasing *cbhI* gene copy number (**b**) with declining catchment glacier cover. **c**,**d**, Increase in mean tensile-strength loss with increasing fungal ITS copy number (**c**) and with increasing *cbhI* gene copy number (**d**). For river sites in the Alaska Boundary Range, no amplification was detected. The sample numbers vary because the fungal ITS and *cbhI* gene did not amplify at all river sites. DD, degree-days; *F*, *F*-statistic. The solid lines represent generalized linear models (GLMs) or generalized additive models (GAMs), and the dashed lines represent 95% confidence intervals.

could be better predictors of decomposition than taxonomic measures[35]. Our findings thus suggest that the relative abundance of functional genes on standardized cotton strip assays could serve as a proxy to detect complex and difficult-to-measure changes in river ecosystem function. In our study, the 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 consistent across a wide range of biogeographic zones.

For the assays that were colonized 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 (Fig. 2 and Supplementary Fig. 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 Fig. 3a). This is because other processes can influence OM decay in glacier-fed rivers[19,36], including bacterial catabolism[8] and physical fragmentation due to abrasion[37]. We attributed this tensile-strength loss in cotton strips with no evidence of fungal colonization or *cbhI* presence (Supplementary Fig. 3a) primarily to dislodgement from their initial positioning on the riverbed. For cotton strips that 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 colonization from bed sediments or OM deposits and in turn limiting the accumulation of the fungal Cellobiohydrolase. Equally, the colonization of some assays that were not dislodged but still exposed to rapid velocity and turbulence could have been inhibited hydrodynamically[38,39]. A clearer direct relationship between tensile-strength loss and catchment glacier cover was evident when considering only those sites hosting *cbhI* gene amplification (Supplementary Fig. 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

Content courtesy of Springer Nature, terms of use apply. Rights reserved

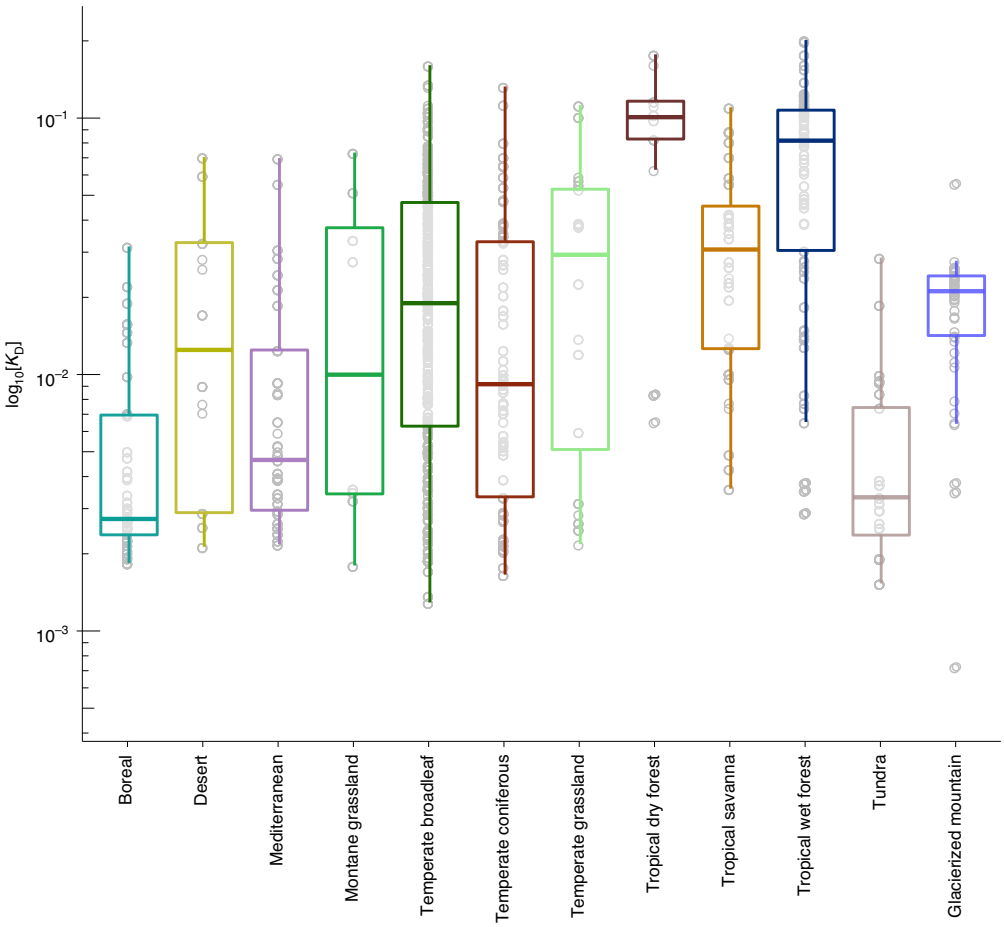

Fig. 3 | Comparison of glacierized mountain river cellulose decomposition rates with those in other biomes. Comparison of $\log_{10}$ daily cellulose decomposition rates (K_D) for rivers in glacierized mountain regions (0–85% catchment glacier cover) and rivers representing 11 other biomes. The K_D values indicate the mean daily decomposition rates of the cellulose fabric in each river ecosystem (grey open circles). In the box plots, the centre lines represent the medians, the box limits represent the interquartile ranges, the whiskers represent the quartiles plus 1.5× the interquartile range and grey circles represent outlying values. Figure adapted with permission from ref. [24], AAAS.

these processes. The clear increase in tensile-strength loss at <30% glacier cover for strips with biological colonization (Supplementary Fig. 3b) parallels the *cbhI* increase (Fig. 2b), providing further support for our findings that fungal-driven decomposition responds to decreasing glacier cover.

The standardized nature of the cotton-strip assay fabric enables the comparison of these mountain river cellulose decomposition rates with those in other biomes across the planet (Fig. 3). The 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 (Fig. 3). The lowest values from this study were recorded from two sites in Ecuador, both with zero glacier cover and high electrical conductivity compared with 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 those in 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 (Fig. 3). The high suspended sediment concentrations and flow variability characteristic of glacier-fed rivers[40] may have accelerated the physical fragmentation of the cotton strips, causing OM decomposition rates to be greater than in other cold-water river systems and consequently 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 that processes such as biotic interactions potentially influence OM decomposition rates[41]. Overall, our new data fit clearly into a global relationship observed between water temperature and decomposition[23] (Supplementary Fig. 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 seem to be globally consistent, 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 standardized 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 stabilize

Content courtesy of Springer Nature, terms of use apply. Rights reserved

and growing seasons lengthen[20,42]. In turn, the fungi-mediated breakdown of particulate carbon will provide dissolved organic carbon, for which processing and export are also expected to change with glacier shrinkage[43,44]. Where glacier retreat is occurring more rapidly than the colonization 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[44]. 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.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41558-021-01004-x.

Received: 12 March 2020; Accepted: 8 February 2021;
Published online: 15 March 2021

References

1. Huss, M. et al. Towards mountains without permanent snow and ice. *Earth's Future* **5**, 418–435 (2017).
2. Zemp, M. et al. Global glacier mass changes and their contributions to sea-level rise from 1961 to 2016. *Nature* **568**, 382–386 (2019).
3. Brown, L. E. et al. Functional diversity and community assembly of river invertebrates show globally consistent responses to decreasing glacier cover. *Nat. Ecol. Evol.* **2**, 325–333 (2018).
4. Cauvy-Fraunié, S. & Dangles, O. A global synthesis of biodiversity responses to glacier retreat. *Nat. Ecol. Evol.* **3**, 1675–1685 (2019).
5. Milner, A. M. et al. Glacier shrinkage driving global changes in downstream systems. *Proc. Natl Acad. Sci. USA* **114**, 9770–9778 (2017).
6. Huss, M. & Hock, R. Global-scale hydrological response to future glacier mass loss. *Nat. Clim. Change* **8**, 135–140 (2018).
7. Ren, Z., Gao, H., Elser, J. J. & Zhao, Q. Microbial functional genes elucidate environmental drivers of biofilm metabolism in glacier-fed streams. *Sci. Rep.* **7**, 12668 (2017).
8. Zhou, L. et al. Microbial production and consumption of dissolved organic matter in glacial ecosystems on the Tibetan Plateau. *Water Res.* **160**, 18–28 (2019).
9. Cavicchioli, R. et al. Scientists' warning to humanity: microorganisms and climate change. *Nat. Rev. Microbiol.* **17**, 569–586 (2019).
10. Timmis, K. et al. The urgent need for microbiology literacy in society. *Environ. Microbiol.* **21**, 1513–1528 (2019).
11. Hotaling, S., Hood, E. & Hamilton, T. L. Microbial ecology of mountain glacier ecosystems: biodiversity, ecological connections and implications of a warming climate. *Environ. Microbiol.* **19**, 2935–2948 (2017).
12. Aufdenkampe, A. K. et al. Riverine coupling of biogeochemical cycles between lands, oceans, and atmosphere. *Front. Ecol. Environ.* **9**, 53–60 (2011).
13. Raymond, P. et al. Global carbon dioxide emissions from inland waters. *Nature* **503**, 355–359 (2013).
14. Clark, D. R. et al. Streams of data from drops of water: 21st century molecular microbial ecology. *WIREs Water* **5**, e1280 (2018).
15. Zah, R. & Uehlinger, U. Particulate organic matter inputs to a glacial stream ecosystem in the Swiss Alps. *Freshw. Biol.* **46**, 1597–1608 (2001).
16. Singer, G. A. et al. Biogeochemically diverse organic matter in alpine glaciers and its downstream fate. *Nat. Geosci.* **5**, 710–714 (2012).
17. Uehlinger, U., Robinson, C. T., Hieber, M. & Zah, R. The physico-chemical habitat template for periphyton in alpine glacial streams under a changing climate. *Hydrobiologia* **657**, 107–121 (2010).
18. Robinson, C. T. & Gessner, M. O. Nutrient addition accelerates leaf breakdown in an alpine springbrook. *Oecologia* **122**, 258–263 (2000).
19. Robinson, C. T. & Jolidon, C. Leaf breakdown and the ecosystem functioning of alpine streams. *J. North Am. Benthol. Soc.* **24**, 495–508 (2005).
20. McKernan, C., Cooper, D. J. & Schweiger, E. W. Glacial loss and its effect on riparian vegetation of alpine streams. *Freshw. Biol.* **63**, 518–529 (2018).
21. Fellman, J. B. et al. Stream temperature response to variable glacier coverage in coastal watersheds of southeast Alaska. *Hydrol. Process.* **28**, 2062–2073 (2014).
22. Gessner, M. O. & Robinson, C. T. in *Aquatic Ecology Series: Ecology of a Glacial Floodplain* Vol. 1 (eds Ward, J. V. & Uehlinger, U.) 123–127 (Springer, 2003).
23. Tiegs, S. D., Clapcott, J. E., Griffiths, N. A. & Boulton, A. J. A standardized cotton-strip assay for measuring organic-matter decomposition in streams. *Ecol. Indic.* **32**, 131–139 (2013).
24. Tiegs, S. D. et al. Global patterns and drivers of ecosystem functioning in rivers and riparian zones. *Sci. Adv.* **5**, eaav0486 (2019).
25. Ward, N. D. et al. Degradation of terrestrially derived macromolecules in the Amazon River. *Nat. Geosci.* **6**, 530–533 (2013).
26. Colas, F. et al. Towards a simple global-standard bioassay for a key ecosystem process: organic-matter decomposition using cotton strips. *Ecol. Indic.* **106**, 105466 (2019).
27. Bayer, E. A., Shoham, Y. & Lamed, R. Cellulose-decomposing bacteria and their enzyme systems. *Prokaryotes* **2**, 578–617 (2006).
28. Lindahl, B. D. et al. Fungal community analysis by high-throughput sequencing of amplified markers—a user's guide. *N. Phytol. Trust* **199**, 288–299 (2013).
29. Nguyen, N. H. et al. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* **20**, 241–248 (2016).
30. Wang, M. et al. Psychrophilic fungi from the world's roof. *Persoonia* **34**, 100–112 (2015).
31. Zang, T. et al. Diversity and distribution of aquatic fungal communities in the Ny-Ålesund region, Svalbard (High Arctic). *Microb. Ecol.* **71**, 543–554 (2016).
32. Wilhelm, L., Singer, G. A., Fashing, C., Battin, T. J. & Besemer, K. Microbial biodiversity in glacier-fed streams. *ISME J.* **7**, 1651–1660 (2013).
33. Hotaling, S. et al. Microbial assemblages reflect environmental heterogeneity in alpine streams. *Glob. Change Biol.* **25**, 2576–2590 (2019).
34. Jacobsen, D. & Dangles, O. Environmental harshness and global richness patterns in glacier-fed streams. *Glob. Ecol. Biogeogr.* **21**, 647–656 (2012).
35. Green, J. L., Bohannan, B. J. M. & Whitaker, R. J. Microbial biogeography: from taxonomy to traits. *Science* **320**, 1039–1042 (2008).
36. Robinson, C. T., Gessner, M. O., Callies, K. A., Jolidon, C. & Ward, J. V. Larch needle breakdown in contrasting streams of an alpine glacial floodplain. *J. North Am. Benthol. Soc.* **19**, 250–262 (2000).
37. Ferreira, V., Graça, M., Pedroso de Lima, J. L. M. & Gomes, R. Role of physical fragmentation and invertebrate activity in the breakdown rate of leaves. *Arch. fur Hydrobiol.* **165**, 493–513 (2006).
38. Besemer, K., Singer, G., Hödl, I. & Battin, T. J. Bacterial community composition of stream biofilms in spatially variable-flow environments. *Appl. Environ. Microbiol.* **75**, 7189–7195 (2009).
39. Battin, T. J., Kaplan, L. A., Newbold, J. D., Cheng, X. & Hansen, C. Effects of current velocity on the nascent architecture of stream microbial biofilms. *Appl. Environ. Microbiol.* **9**, 5443–5452 (2003).
40. Fell, S. C., Carrivick, J. L. & Brown, L. E. The multitrophic effects of climate change and glacier retreat in mountain rivers. *BioScience* **67**, 897–911 (2017).
41. Cristiano, G., Cicolani, B., Miccoli, F. P. & Di Sabatino, A. A modification of the leaf-bags method to assess spring ecosystem functioning: benthic invertebrates and leaf-litter breakdown in Vera Spring (central Italy). *PeerJ* **7**, e6250 (2019).
42. Greenwood, S. & Jump, A. S. Consequences of treeline shifts for the diversity and function of high altitude ecosystems. *Arct. Antarct. Alp. Res.* **46**, 829–840 (2014).
43. Hood, E. & Berner, L. Effects of changing glacial coverage on the physical and biogeochemical properties of coastal streams in southeastern Alaska. *J. Geophys. Res. Biogeosci.* **114**, G03001 (2009).
44. Boix Canadell, M., Escoffier, N., Ulseth, A. J., Lane, S. N. & Battin, T. J. Alpine glacier shrinkage drives shifts in dissolved organic carbon export from quasi-chemostasis to transport limitation. *Geophys. Res. Lett.* **46**, 8872–8881 (2019).
45. Jacobsen, D., Milner, A. M., Brown, L. E. & Dangles, O. Biodiversity under threat in glacier-fed river systems. *Nat. Clim. Change* **2**, 361–364 (2012).

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2021

Content courtesy of Springer Nature, terms of use apply. Rights reserved

Methods

Study areas. Standardized 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 (Fig. 1 and Supplementary Table 1). The study sites were located on four continents, from 44° S to 60° N latitude. The assays were successfully incubated in 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 (Fig. 1 and 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[46] 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 and A13) (Supplementary Table 1) that hosted fungal amplification (fungal ITS or the *cbhI* gene) had upstream lakes; while previous studies have noted the influence of lake outflows on river OM decomposition rates[36,47,48], the measured relationships remained similar when river sites downstream of proglacial lakes were omitted from the analyses.

Environmental parameters. At each river site, pH was measured using a Hanna Instrument (HI98130) (Austria, New Zealand and Norway), a YSI Pro Plus water quality meter (Xylem) (Alaska), a HQ40D portable multimeter (HACH) (France) or an Extech meter (Extech) (Ecuador). Hourly water temperatures were recorded throughout the cotton-strip incubation periods using iButton Fobs (DS1990A-F5) (France), HOBO pendant data loggers (Onset) (Ecuador) or TinyTag Plus 2 data loggers (Gemini) (all other sites). The Pfankuch index[49] was used to estimate geomorphic river channel stability, and components designed to assess the stability of the channel bottom were 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 was assessed using a desktop turbidimeter (HACH 2100A) (Camlab). 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 that in Tiegs et al.[24]. The assay offered numerous advantages for our purposes, including its high degree of standardization, portability and direct comparability to other studies. The decomposition rates generated from this assay (as loss of the tensile strength of the 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[50]. Following the Cellulose Decomposition Experiment (CELLDEX) protocol[23,51], rectangular cotton strips (8 cm × 2.5 cm) were created from >95% cellulose artist's fabric (Fredrix Artist Canvas (unprimed 12-oz heavy-weight cotton fabric, style #548))[23,51]. The 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 minimize 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 the final analysis was conducted on data from 57 river sites. River sites were excluded if cotton strips were lost during incubation or found above the water level upon collection; or where temperature measurements suggested intermittent periods of low or no flow, as this prevented representative measurement of exclusively aquatic decomposition processes; or if representative tensile-strength measurements were not possible (for example, due to strips breaking incorrectly in the tensiometer). While cotton fabric may have a different nutrient content and physical structure from riparian and autochthonous inputs entering mountain rivers, the natural prevalence of cellulose in terrestrial and aquatic materials and the standardized form of the strips enabled between-site comparisons 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 the particulate OM supply was potentially naturally low and patchily distributed across channel microhabitats[16]. While the space-for-time substitution approach could not account for the potential confounding influence of natural variability in the OM supply and thus in existing fungal communities between study catchments, it enables the investigation of the impact of catchment glacier cover on the fungal catabolism of cellulose without real-time observation of glacier retreat. OM decomposition and fungal community response to glacier recession over long periods remain to be studied to allow cross-validation with spatial analyses, although studies of invertebrates in glacier-fed rivers have suggested that spatially distributed chronosequences can provide similar insights to observed site-specific successional time series[3].

At each site, four cotton strips were cable-tied to nylon cord (1 m long and 3 mm wide), which was staked to the riverbed in randomly selected locations at individual sites[23,51]. Rocks were placed upstream of each strip on the cord to keep them flat on benthic sediments and aligned to the current. Points of similar water depth and turbulence were selected to ensure that the strips were influenced by comparable environmental conditions[23]. A temperature logger recording hourly measurements was placed in a white plastic tube to shield it from solar radiation and abrasion and was cable-tied to one of the stakes at each site. For sites with high catchment glacier cover and highly unstable riverbeds, additional cotton strips (up to six) were incubated to increase the potential for some to remain in situ for the experiment duration.

The cotton-strip assays were incubated for 37 days, or as close to this duration as field logistics and weather conditions permitted (range, 31–39 days). This period was designed to maximize the potential of achieving 50% tensile-strength loss, the point of decay at which cotton strips are believed to be colonized by fungal and bacterial communities but not by shredding invertebrates[23]. The strips were cut from their cable binders and gently cleared of debris, and a 2 cm subsample of one cotton strip from each river site was detached using sterilized scissors and preserved in 1 ml of RNA*later* stabilization solution (Thermo Fisher Scientific)[52]. These subsamples were stored at 4 °C for transport and then −80 °C in the laboratory prior to molecular analysis. All remaining strips were submerged in 100% ethanol for 30 s on site to halt microbial activity[52].

Tensile strength determination. Tensile-strength loss of incubated cotton strips indicated the potential of a river ecosystem to decompose cellulose[26] and is a more sensitive metric than reductions in assay mass[53]. All cotton strips, minus the subsamples for fungal characterization, were oven-dried (40 °C, 26 h) and stored in a desiccator prior to tensile strength determination[54]. An advanced video extensometer (2663-821) (SN:5076) (Instron) was used to determine a single maximum tensile strength value for each incubated and control strip, extending at a consistent rate of 2 cm min^{-1}, with 1 cm portions of each strip end secured in the grips[23]. To calibrate the instrument, cotton strips constructed using the CELLDEX protocol but not transported or incubated were tested until their break points aligned to a consistent range and the machine jaws were sufficiently adjusted to minimize slippage. The sample order was randomized, with control strips processed throughout the sample run to identify instrument drift. Room temperature (19.5 °C) and humidity (60.7%) were kept constant across sample runs, and cotton strips from multiple regions were processed together to minimize the variability of instrumental and environmental conditions between testing. Strips that broke along the point of contact with the machine jaws were excluded from analysis ($n = 4$, 0.9%). For cotton strips whose maximum tensile strength was higher than the mean control strip value ($n = 24$, 5.2%), biological variation lay within the range of technical variation and the two could not be separated, so tensile-strength loss (decomposition) was recorded as zero for these strips.

Tensile-strength loss was calculated as a percentage of the initial strength lost for each cotton strip per DD, as adapted from Tiegs et al. (p. 134)[23].

$$\text{Tensile-strength loss} = \left(\left(1 - \left(\frac{\text{TSImax}}{\text{TSCmean}}\right)\right) \times 100\right) / \text{incubation period} \quad (1)$$

This equation uses the maximum tensile strength of each river-incubated strip (TSImax) and the mean tensile strength of all control strips (TSCmean). Temperature-adjusted DD were calculated by summing the mean temperatures recorded for each 24-hour period during the cotton-strip incubation. This enabled the temperature-normalized comparison of tensile-strength loss across regions, as mean river water temperature can vary dramatically on diurnal and seasonal timescales in glacierized catchments[21]. Percentage tensile-strength loss was averaged across all replicate strips to provide a mean value per river site. All reported tensile-strength values are calculated per DD unless stated otherwise. Non-temperature-adjusted cellulose decomposition rates were determined by representing the incubation period in days, in place of DD. These values were compared with those reported for 514 rivers in 11 other biomes[24]. An Arrhenius plot was constructed to correlate daily decomposition values with inverted relative mean water temperature[24].

Molecular methods. One 2 cm^2 subsample was taken from one cotton strip at each river site, and DNA was extracted from a standardized 1.5 cm^2 section of each subsample. The extraction followed a standard CTAB protocol[55] with DNA eluted in 50 µl of PCR-grade water (Invitrogen). The concentration of DNA in individual samples was quantified using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen) to enable the normalization of all samples to 1 ng $µl^{-1}$ for high-throughput preparation of NGS amplicon libraries. Total extracted DNA from each cotton-strip subsample from each river site was then used to quantify the absolute copy number of the fungal taxonomic (ITS)[56] and functional (*cbhI*)[57] marker genes via qPCR.

Absolute quantification of fungal ITS and cbhI copy number. qPCR was performed to determine the copy number (an estimation of abundance) of fungal ITS and *cbhI*[58]. qPCR DNA standards were created from end-point PCR amplification where the template DNA was 1 µl of DNA extract pooled from each sample. The resulting amplicons were purified using a QIAquick PCR purification kit (Qiagen)[58] and quantified using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen). qPCR was performed separately for each target gene, and the copy

Content courtesy of Springer Nature, terms of use apply. Rights reserved

number of that gene in each sample was then calculated per cm^2 of cotton strip and logged. qPCR amplification of fungal ITS and the *cbhI* gene was observed at 42% of the 57 river sites.

For each gene, all samples were run on a single 384-well plate and included a serial dilution of the purified standards ranging from 10^1 to 10^9, non-template (negative) controls and each sample, all of which were included in triplicate. The reagents and the determination of copy number for each sample for each assay followed McKew and Smith[58]. The qPCR reactions were performed in 10 µl reaction volume with 1 µl of DNA, 5 µl of SensiFAST Sybr Green (Bioline), 0.2 µl of each primer (10 µM) and 3.6 µl of PCR-grade water. To target the fungal ITS, the ITS2 region was amplified using the primer sets ITS3_KYO2 (5′-GATGAAGAACGYAGYRAA-3′)[56] and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′)[59]. The ITS2 region was targeted because it has good variability at the species level to aid taxonomic distinction[60] and is widely represented in the fungal databases used for genus/species-level taxonomic assignment[61]. The fungal *cbhI* gene was targeted using the primer sets fungcbhIF (5′-ACCAAYTGCTAYACIRGYAA-3′) and fungcbhIR (5′-GCYTCCCAIATRTCCATC-3′)[57]. The assays were run on a CFX real-time system (Bio-Rad). The qPCR conditions to amplify the fungal ITS2 and *cbhI* regions were the same, with an initial denaturation at 95 °C for 3 min, followed by 45 cycles at 95 °C for 5 s, 60 °C for 10 s and 72 °C for 20 s. This was immediately followed by melt-curve generation for one cycle at 95 °C for 5 s, 65 °C for 5 s and 95 °C for 5 s.

Library preparation for NGS of the fungal ITS and cbhI genes. Library preparation followed the protocol outline by Illumina[62] with the PCR conditions amended as a result of optimization for our two target genes. First-stage PCR reactions were performed in a 25 µl reaction volume with 3 µl of DNA template, 12.5 µl of appTAQ RedMix (2X) polymerase (Appleton Woods), 1 µl of each primer (4 µM) containing Illumina overhang adapters[62], 1.5 µl of 1% bovine serum albumin (BSA) and 6 µl of PCR-grade water. BSA was included to remove inhibitors and increase the yield of PCR amplification[63]. The fungal ITS region and *cbhI* gene were targeted using the same locus-specific primers as used for qPCR[56,57,59], but with the addition of Illumina overhang adapters to ensure compatibility with the sequencing platform[62]. PCR reactions were run on a 96-well Thermo Cycler (Applied Biosystems). The PCR conditions to amplify the fungal ITS2 region used an initial denaturation at 95 °C for 3 min, followed by 35 cycles at 95 °C for 15 s, 51 °C for 15 s and 72 °C for 30 s; and a final elongation of one cycle at 72 °C for 7 min. Despite multiple attempts to optimize the *cbhI* library preparation by changing the PCR annealing temperature, BSA addition, volume of DNA template and cycle number, insufficient samples amplified for the *cbhI* gene. As a result, the structure of the cellulose-degrading (*cbhI*) fungal community was not assessed. Samples where agarose gel electrophoresis indicated an absence of indexed and cleaned PCR amplicons were compared with the qPCR data.

After clean-up of the first-stage PCR reactions, amplicon libraries were indexed following the Illumina protocol[62] using the Nextera XT Library Prep Kit (Illumina). Annealing 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 the samples in equimolar concentrations. The final amplicon library concentration was then determined using a NEBNEXT Library Quant Kit for Illumina, before the 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-base-pair paired-end reads[62].

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 chimaeric PCR artefacts, following Dumbrell et al.[64] and Maček et al.[65]. 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[66]. The UNITE database enabled the 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[66]. This provided unambiguous species matches for our study through the generation of digital object identifiers[67]. 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 the OTU tables after 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 were rarefied to the smallest library size (10,543 reads). Sequence-based rarefaction was selected in preference to alternative procedures of normalization as an effective and ecologically meaningful method to standardize differential library sizes for fungal data[68,69]. OTU abundance therefore referred to the abundance of reads or 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[67]. 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 using the FUNGuild database[29]. Further subsets were created for members of the phylum Ascomycota and genus *Tetracladium*.

Statistical analysis. GLMs and GAMs 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 and 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. The models were computed in the mgcv package[70] of R v.3.3.2; model parsimony was evaluated using Akaike information criterion (AIC) values, and performance was assessed through the percentage of deviance explained. For GAMs, smoothing parameter selection followed Wood[71], with Gaussian and negative binomial distributions identified. The mgcv package was also used to construct generalized linear mixed models and generalized additive mixed models to incorporate the effect of absolute latitude (random effect). Mixed models had higher AIC values than fixed-effect-only models, but the values were similar (within two units). This suggested no obvious influence of latitude on the observed responses, although larger sample sizes from some regions would confirm this further. The manyglm function of the mvabund package[72] 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.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The raw demultiplexed sequence data have been uploaded to the NCBI Sequence Read Archive with BioProject accession number PRJNA684135. A dataset has been deposited with the NERC Environmental Information Data Centre at https://doi.org/10.5285/fec704d2-ee6a-427b-9345-850dd96ff1b4.

References

46. *GLIMS Glacier Viewer* (Global Land Ice Measurements from Space (GLIMS), 2018); http://www.glims.org/maps/glims
47. Robinson, C. T., Gessner, M. O. & Ward, J. V. Leaf breakdown and associated macroinvertebrates in alpine glacial streams. *Freshw. Biol.* **40**, 215–228 (1998).
48. Goodman, K. J., Baker, M. & Wurtsbaugh, W. Mountain lakes increase organic matter decomposition rates in streams. *J. North Am. Benthol. Soc.* **29**, 521–529 (2010).
49. Pfankuch, D. J. *Stream Reach Inventory and Channel Stability Evaluation* (Northern Region, Montana, US Department Forest Service, 1975).
50. Vizza, C., Zwart, J. A., Jones, S. E., Tiegs, S. D. & Lamberti, G. A. Landscape patterns shape wetland pond ecosystem function from glacial headwaters to ocean. *Limnol. Oceanogr.* **62**, S207–S221 (2017).
51. Tiegs, S. D. *CELLDEX Protocol Part 1* https://www.researchgate.net/publication/281243407_CELLDEX_Protocol_Part_1 (2015).
52. Tiegs, S. D. *Protocol for Microbial DNA/RNA Sampling—CELLDEX Protocol* https://www.researchgate.net/publication/281245895_Protocol_for_microbial_DNARNA_sampling_-_CELLDEX_Project (2015).
53. Tiegs, S. D., Langhans, S. D., Tockner, K. & Gessner, M. O. Cotton strips as a leaf surrogate to measure decomposition in river floodplain habitats. *J. North Am. Benthol. Soc.* **26**, 70–77 (2007).
54. Tiegs, S. D. *CELLDEX Protocol Part 2* https://www.researchgate.net/publication/283645782_CELLDEX_Protocol_Part_2 (2015).
55. Griffiths, R. I., Whiteley, A. S., O'Donnell, A. G. & Bailey, M. J. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. *Appl. Environ. Microbiol.* **66**, 5488–5491 (2000).
56. Toju, H., Tanabe, A. S., Yamamoto, S. & Sato, H. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PLoS ONE* **7**, e40863 (2012).
57. Edwards, I. P., Upchurch, R. A. & Zak, D. R. Isolation of fungal Cellobiohydrolase I genes from sporocarps and forest soils by PCR. *Appl. Environ. Microbiol.* **74**, 3481–3489 (2008).
58. McKew, B. A. & Smith, C. J. in *Hydrocarbon and Lipid Microbiology Protocols* (eds McGenity, T. J. et al.) 45–64 (Springer, 2017).
59. Gardes, M. & Bruns, T. D. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol. Ecol.* **2**, 113–118 (1993).
60. Nilsson, R. H. et al. Variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evol. Bioinform. Online* **4**, 193–201 (2008).

Content courtesy of Springer Nature, terms of use apply. Rights reserved

61. Nilsson, R. H., Ryberg, M., Abarenkov, K., Sjökvist, E. & Kristiansson, E. The ITS region as a target for characterization of fungal communities using emerging sequencing technologies. *FEMS Microbiol. Lett.* **296**, 97–111 (2009).
62. *16S Metagenomic Sequencing Library Preparation* (Illumina, 2013); https://ww.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf
63. Kreader, C. A. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Appl. Environ. Microbiol.* **62**, 1102–1106 (1995).
64. Dumbrell, A. J., Ferguson, R. M. W. & Clark, D. R. in *Hydrocarbon and Lipid Microbiology Protocols* (eds McGenity, T. J. et al.) 155–206 (Springer, 2017).
65. Maček, I. et al. Impacts of long-term elevated atmospheric CO_2 concentrations on communities of arbuscular mycorrhizal fungi. *Mol. Ecol.* **28**, 3445–3458 (2019).
66. Nilsson, R. H. et al. *UNITE Community: Communication and Identification of DNA Based Fungal Species* (UNITE, 2018); https://unite.ut.ee/search.php#fndtn-panel1
67. Nilsson, R. H. et al. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res.* **47**, D259–D264 (2018).
68. Weiss, S. et al. Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome* **5**, 2–18 (2017).
69. McKnight, D. T. et al. Methods for normalizing microbiome data: an ecological perspective. *Methods Ecol. Evol.* **10**, 389–400 (2019).
70. Wood, S. N. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J. R. Stat. Soc. B* **73**, 3–36 (2011).
71. Wood, S. N. Stable and efficient multiple smoothing parameter estimation for generalized additive models. *J. Am. Stat. Assoc.* **99**, 673–686 (2004).
72. Wang, Y., Maumann, U., Wright, S. & Warton, D. mvabund: Statistical methods for analysing multivariate abundance data. R package https://cran.r-project.org/package=mvabund (2018).

Acknowledgements

This research was funded by a Natural Environment Research Council Scholarship (no. NE/L002574/1) awarded to S.C.F. Additional financial support for laboratory overheads was provided to S.C.F. by the River Basin Processes and Management Cluster, School of Geography, University of Leeds. S.C.F., L.E.B. and J.L.C. received funding from INTERACT under the European Union H2020 (GLAC-REF, grant agreement no. 730938, Transnational Access) for fieldwork in Finse, Norway. Fieldwork in Ecuador was funded by the Pontifical Catholic University of Ecuador under project no. M13434 (PUCE 2016-2017). A.J.D. and K.C.R. were supported by a Natural Environment Research Council grant (no. NE/M02086X/1), and E.H. was funded by the Alaska Climate Adaptation Science Center. We thank L. Füreder (Austria); the Ecuadorian Ministry of the Environment (research permit no. MAE-DNM-2015-0030), the Reserva Ecológica Antisana, Public Metropolitan Company of Potable Water and Sanitation of Quito (EPMAPS) and Water Projection Fund (FONAG) (Ecuador); the Parc National de la Vanoise (France); the Department of Conservation (New Zealand); and U. Fjellstyre and T. Buttingsrud (Norway) for permission to access field sites and work within protected areas. We also thank the Finse Alpine Research Centre, Obergurgl Alpine Research Centre and the Design School of the University of Leeds for the use of their field and laboratory facilities, and N. Friberg for his hospitality. For their assistance and support in the field, we thank P. Andino, R. Espinosa, P. Rosero and J. Sutherland, and S.C.F. gives special thanks to C. Fell and N. Fell.

Author contributions

S.C.F. codeveloped 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 (except Figs. 1 and 3) and wrote the manuscript. J.L.C. completed fieldwork in New Zealand and Norway and created Fig. 1. S.C.-F. completed fieldwork in Ecuador and France. V.C.-P. completed fieldwork in Ecuador. E.H. completed fieldwork in Alaska. K.C.R. led the molecular sample preparation and PCR and qPCR analysis, and contributed text to the 'Molecular methods' section. K.J.M.N. assisted in the molecular sample preparation. A.J.D. developed the analytical protocol for the molecular sample analysis, ran the NGS, formatted the subsequent data for analysis, advised on statistical and ecoinformatic analysis and contributed text to the 'Molecular methods' section. S.D.T. developed and advised on the use of the cotton-strip assay protocol, provided data for Fig. 3 and Supplementary Fig. 4, and contributed text regarding the use of the cotton-strip assay. L.E.B. codeveloped the concept of the manuscript, completed fieldwork in Austria and Norway, advised on statistical analysis and the production of all figures, created Fig. 3 and provided detailed comments on the manuscript. All authors edited and revised the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41558-021-01004-x.

Correspondence and requests for materials should be addressed to L.E.B.

Peer review information *Nature Climate Change* thanks Luz Boyero and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Reprints and permissions information is available at www.nature.com/reprints.

Content courtesy of Springer Nature, terms of use apply. Rights reserved

nature research

Corresponding author(s): Lee Brown

Last updated by author(s): 26/11/2020

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection
Data analysis	Analysis used existing methods as described in the manuscript.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Raw demultiplexed sequence data has been uploaded to the NCBI Sequence Read Archive (SRA) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3013647/ with BioProject accession number PRJNA684135. A data set and its supporting documentation is currently in the process of being deposited in the NERC Environmental Information Data Centre https://eidc.ac.uk/ and will be available here before final acceptance of this manuscript.

Content courtesy of Springer Nature, terms of use apply. Rights reserved

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	We report new observations obtained using a standardised OM decomposition assay (the cotton-strip assay) from 56 mountain rivers spanning six countries on four continents. The dataset is unbalanced - 12 sites were planned for study in each country but some assays were lost and others showed no amplification of selected genes.
Research sample	The samples were standardised cotton-strip assays consisting of > 95 % cellulose, the key constituent of riparian and in-stream detritus. Samples were retrieved then analysed for microbial communities that had colonised the strips, plus their loss of tensile strength.
Sampling strategy	In each country 12 rivers were selected for study to provide coverage in each of four glacier cover bands (high, medium, low and zero glacier cover) to allow a consideration of responses to declining glacier cover.
Data collection	Following a standard procedure, cotton strips were deployed and retrieved, and associated environmental parameters measured, as follows: Alaska (Hood), Austria (Fell, Brown), Ecuador (Cauvy-Fraunie, Crespo-Perez), France (Cauvy-Fraunie), New Zealand (Fell, Carrivick) and Norway (Fell, Brown, Carrivick). Tensile strength analysis was completed for all samples in Leeds. Microbial analysis was completed for all samples in Essex.
Timing and spatial scale	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. Concurrent experiments were not possible due to constraints on field work timing in these remote environments, plus seasonal differences between northern and southern hemisphere countries. The selected countries were chosen to provide coverage on multiple continents and taking into account the availability of local collaborators.
Data exclusions	Some study sites that hosted fungal amplification (fungal ITS, cbhl gene) had upstream lakes (n = 2); and, while previous studies have noted the influence of lake outflows on river OM decomposition rates, measured relationships remained significant when river sites downstream of proglacial lakes were removed. 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). Exclusions are described in the manuscript.
Reproducibility	The study was a field experiment and has not been reproduced
Randomization	At each study site, four cotton strips were cable tied to nylon cord (1 m long, 3 mm wide) which was staked to the river-bed in randomly selected locations. Sample order for tensile strength analysis in the laboratory was randomised, with control strips processed throughout the sample run to identify instrument drift.
Blinding	Blinding was not relevant as the experiment did not involve participants

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Conditions such as water temperature, electrical conductivity, pH and turbidity are described as part of the analysis
Location	This information is provided in Supplementary Table 1 and the data files
Access and import/export	Permission to access field sites and work within protected areas was granted by the following groups/organisations: Obergurgl Alpine Research Centre, Austria, the Ecuadorian Ministry of the Environment (research permit number: MAE-DNM-2015-0030), the Reserva Ecológica Antisana, Public Metropolitan Company of Potable Water and Sanitation of Quito (EPMAPS) and Water Projection Fund (FONAG) (Ecuador), the Parc National de la Vanoise (France), the Department of Conservation (New Zealand) and Ulvik Fjellstyre and Trond Buttingsrud (Finse Alpine Research Centre, Norway).
Disturbance	The study did not cause any obvious disturbance to the selected sites.

Reporting for specific materials, systems and methods

Content courtesy of Springer Nature, terms of use apply. Rights reserved

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Content courtesy of Springer Nature, terms of use apply. Rights reserved

Terms and Conditions

Springer Nature journal content, brought to you courtesy of Springer Nature Customer Service Center GmbH (“Springer Nature”).
Springer Nature supports a reasonable amount of sharing of research papers by authors, subscribers and authorised users (“Users”), for small-scale personal, non-commercial use provided that all copyright, trade and service marks and other proprietary notices are maintained. By accessing, sharing, receiving or otherwise using the Springer Nature journal content you agree to these terms of use (“Terms”). For these purposes, Springer Nature considers academic use (by researchers and students) to be non-commercial.
These Terms are supplementary and will apply in addition to any applicable website terms and conditions, a relevant site licence or a personal subscription. These Terms will prevail over any conflict or ambiguity with regards to the relevant terms, a site licence or a personal subscription (to the extent of the conflict or ambiguity only). For Creative Commons-licensed articles, the terms of the Creative Commons license used will apply.
We collect and use personal data to provide access to the Springer Nature journal content. We may also use these personal data internally within ResearchGate and Springer Nature and as agreed share it, in an anonymised way, for purposes of tracking, analysis and reporting. We will not otherwise disclose your personal data outside the ResearchGate or the Springer Nature group of companies unless we have your permission as detailed in the Privacy Policy.
While Users may use the Springer Nature journal content for small scale, personal non-commercial use, it is important to note that Users may not:

1. use such content for the purpose of providing other users with access on a regular or large scale basis or as a means to circumvent access control;
2. use such content where to do so would be considered a criminal or statutory offence in any jurisdiction, or gives rise to civil liability, or is otherwise unlawful;
3. falsely or misleadingly imply or suggest endorsement, approval , sponsorship, or association unless explicitly agreed to by Springer Nature in writing;
4. use bots or other automated methods to access the content or redirect messages
5. override any security feature or exclusionary protocol; or
6. share the content in order to create substitute for Springer Nature products or services or a systematic database of Springer Nature journal content.

In line with the restriction against commercial use, Springer Nature does not permit the creation of a product or service that creates revenue, royalties, rent or income from our content or its inclusion as part of a paid for service or for other commercial gain. Springer Nature journal content cannot be used for inter-library loans and librarians may not upload Springer Nature journal content on a large scale into their, or any other, institutional repository.
These terms of use are reviewed regularly and may be amended at any time. Springer Nature is not obligated to publish any information or content on this website and may remove it or features or functionality at our sole discretion, at any time with or without notice. Springer Nature may revoke this licence to you at any time and remove access to any copies of the Springer Nature journal content which have been saved.
To the fullest extent permitted by law, Springer Nature makes no warranties, representations or guarantees to Users, either express or implied with respect to the Springer nature journal content and all parties disclaim and waive any implied warranties or warranties imposed by law, including merchantability or fitness for any particular purpose.
Please note that these rights do not automatically extend to content, data or other material published by Springer Nature that may be licensed from third parties.
If you would like to use or distribute our Springer Nature journal content to a wider audience or on a regular basis or in any other manner not expressly permitted by these Terms, please contact Springer Nature at

onlineservice@springernature.com