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Long-term changes and seasonal variability in the stream macrofauna of a Himalayan river system

Dean Jacobsen [ID],[a] Steve J. Ormerod [ID],[b] Ingrid Jüttner [ID],[c] Oscar Rojas [ID],[a] Kenneth Thorø Martinsen [ID],[a] Bhumika Thapa [ID],[a,d] Kumar Khatri [ID],[e] Pratima Paudel [ID],[f] Manita Rai [ID],[f] Nani Raut [ID],[f] and Smriti Gurung [ID][f]

[a]Freshwater Biological Section, Department of Biology, University of Copenhagen, Copenhagen, Denmark; [b]Water Research Institute, Cardiff School of Biosciences, Cardiff University, Cardiff, United Kingdom; [c]Department of Natural Sciences, Amgueddfa Cymru—Museum Cardiff, Cardiff, United Kingdom; [d]Nepal Water Conservation Foundation, Kathmandu, Nepal; [e]Central Department of Environmental Science, Tribhuvan University, Kirtipur, Nepal; [f]Department of Environmental Science and Engineering, Kathmandu University, Dhulikhel, Nepal

ABSTRACT

Interpreting long-term variability in mountain stream ecosystems is challenging where potential causes such as climate change and altered land use act simultaneously. The scarcity of historical data also limits research opportunity, especially at low latitudes. Here we assess the macroinvertebrate fauna of 12 Himalayan streams in Nepal covering an altitudinal gradient from 1500 to 3900 m a.s.l. using data from 1992, 2022, and 2023. We aimed to (1) compare long-term change in richness and community composition over 3 decades to short-term seasonal variation, and (2) analyse long-term changes in relation to catchment properties. Rarefied richness decreased equally with altitude in 1992 and 2022 but was greater at most sites in 2022, with several new families detected. By contrast, no clear seasonal pattern was found. The 2022:1992 ratio in rarefied richness was positively correlated with catchment area and weakly with maximum catchment altitude, whereas the 2 highest and glaciated sites gained relatively more taxa than other sites. Non-metric multidimensional scaling (NMDS) ordinations from rarefied Bray-Curtis dissimilarities showed considerable overlap in communities among the 3 datasets. Pairwise rarefied dissimilarities among all sites increased significantly with altitudinal distance between sites in 1992, but no such relationship was detected in 2022. Dissimilarities between 1992 and 2022 samples showed a near-significant correlation with increased catchment vegetation cover, as shown by the normalized difference vegetation index (NDVI). Our study is the first to document long-term variations in stream macroinvertebrate communities in Himalayan mountain streams, illustrating how some altitudinal patterns have remained stable through time while others seem to have weakened with links to global change.

Introduction

Mountain freshwater ecosystems contribute considerably to global freshwater biodiversity (Jacobsen and Dangles 2017) and provide multiple ecosystem services while also considered important indicators of climate change (Battarbee 2000, Hamerlík and Bitušík 2009, Shah et al. 2015). Global warming is particularly prominent at high altitudes—much faster than the global average—because of elevation-dependent heating (Pepin et al. 2015, Wu et al. 2023), affecting altitudinal species distributions worldwide (Freeman et al. 2018). In the aquatic environment, distribution models predict upward shifts in species distribution and changes in community composition with climate change (Domisch et al. 2011, Alba-Tercedor et al. 2017), but few such effects have so far been detected (Sheldon 2012, Giersch

et al. 2015), despite numerous effects of warming on alpine streams (Hotalling et al. 2017, Birrell et al. 2020).

Among the possible explanations for this mismatch between predication and observation is that global warming not only affects thermal regimes in streams, but also flow regimes and associated hydrodynamics through changing patterns in precipitation, meltwater dynamics, or glacial extent. Additionally, climatic change effects are sometimes confounded by quasi-natural climatic variations (Larsen et al. 2024) or by other sources of change, for example in catchment vegetation or land use and management practices that affect freshwater sediment regimes, nutrient flux, and organic or inorganic solutes (Wolf et al. 2012, Tasser et al. 2017, Schmeller et al. 2022). Macroinvertebrate distributional changes might also lag behind climate-based predictions

(Li et al. 2016) because of inertia in ecological systems as well as stochastic processes and natural spatiotemporal variability between locations.

For these reasons, long study periods are needed to diagnose, quantify, or even detect ecological changes in stream ecosystems (Gillette et al. 2022, Bailey and Reynoldson 2023). However, long time-series or comprehensive historic data that might allow the analysis of climate-mediated changes in biota are scarce, particularly from mountain streams (Finn et al. 2010, Milner et al. 2011, Robinson et al. 2024), and especially from low-latitude mountain systems (Murdoch et al. 2022) such as the Himalaya, where a range of stressors such as climate and land-use change could interact (Wester et al. 2019).

Nevertheless, some data from Himalayan streams and rivers allow historical comparison. As part of an extensive survey across a geographically extensive range in the 1990s (Manel et al. 2001), 14 tributaries of the Langtang River in Langtang National Park were sampled in November 1992 by Ormerod et al. (1994). Working along an altitudinal gradient from 1500 to 3900 m a.s.l., they collected samples of macrofauna, algae, and mosses from streams selected to reflect variations in stream and catchment properties, but with minimal human impact. We repeated this study in November 2022 and April 2023, resampling the same sites while also capturing seasonal variations expected to reflect the post- and pre-monsoon seasons that cause major changes in Himalayan stream ecosystems (Brewin et al. 2000). During the intervening 30 yr period, mean annual air temperatures in mountain areas of Nepal have risen by ∼2 °C (Karki et al. 2020), and by ∼3 °C in the higher part of the Langtang Valley (Pradhananga et al. 2014). Glaciers have retreated considerably (Ragettli et al. 2017), and runoff contributions from glacial meltwater have decreased (Immerzeel et al. 2012, Pradhananga et al. 2014).

Our specific objectives were to (1) report any changes in macroinvertebrate local richness and community composition over the intervening 30 years compared to short-term seasonal variation, identifying patterns in beta-diversity; and (2) assess long-term change in relation to catchment properties along the altitudinal gradient across the sites sampled. Although many such properties could be assessed (Soininen 2015), we focused on measurable factors most likely to have changed in the catchments over 3 decades, specifically native vegetation cover measured as normalized difference vegetation index (NDVI) and glacial cover obtained from satellite imagery. Stream macrofauna have previously been found to relate to NDVI measured at the catchment level (Griffith et al. 2002).

Macroinvertebrate communities in mountain streams also seem to respond to land use and vegetation cover in their catchment (Death and Collier 2010, Scotti et al. 2020, Palt et al. 2022). The ecological influence of glacial cover in alpine catchments is well documented (e.g., Jacobsen et al. 2012), as well as the effect of glacial retreat on downstream systems (Milner et al. 2017). We hypothesized that (1) taxonomic richness should increase through time alongside an upward shift in specific taxa, and (2) changes among communities would be most pronounced where catchment properties had changed most. Given that the altitudinal range of 2900–3500 m a.s.l. has been proposed as a particularly climate sensitive zone for macroinvertebrate communities in Nepal Himalayan streams (Shah et al. 2015), we expected the clearest long-term shifts in communities for sites within that altitudinal band.

Methods

Study area

The Langtang Valley is located in Nepal ∼80 km north of Kathmandu, within the 1710 km^2 Langtang National Park bordering Tibet (Fig. 1). The valley's main river, Langtang River, originates from the Langtang Glacier at 4500 m a.s.l. and joins the Trisuli River at 1400 m a.s.l., a major tributary to the Gandaki and subsequently the Ganges. Vegetation in the lower parts of the valley is subtropical montane forest that gradually turns into coniferous forest and tundra at higher altitudes. Barren land and glaciers cover a considerable proportion of the higher parts of the catchment. The entire region has minimal human impact because cultivation in the valley has not changed significantly through time and is limited to small areas of barley, buckwheat, and potatoes, and small numbers of livestock (e.g., yak hybrids, sheep, and mules) (Tuladhar et al. 2021). Other human land uses are linked to several small settlements that serve the large number of trekkers visiting the area. Consequently, observed ecological changes in stream communities should be mainly related to climate change effects alone. The region has a pronounced monsoon climate, with a warm and rainy season from June to September and a dry season from October to April (Brewin et al. 2000). The region was badly affected by the 2015 earthquake, resulting in massive landslides. We believe our study is the first record of aquatic fauna in the valley since the earthquake.

Fourteen streams along the Langtang Valley covering an altitudinal gradient from 1500 and 3900 m a.s.l. were originally sampled in November 1992 by Ormerod et al. (1994). Thirty years later we resampled 12 of these 14
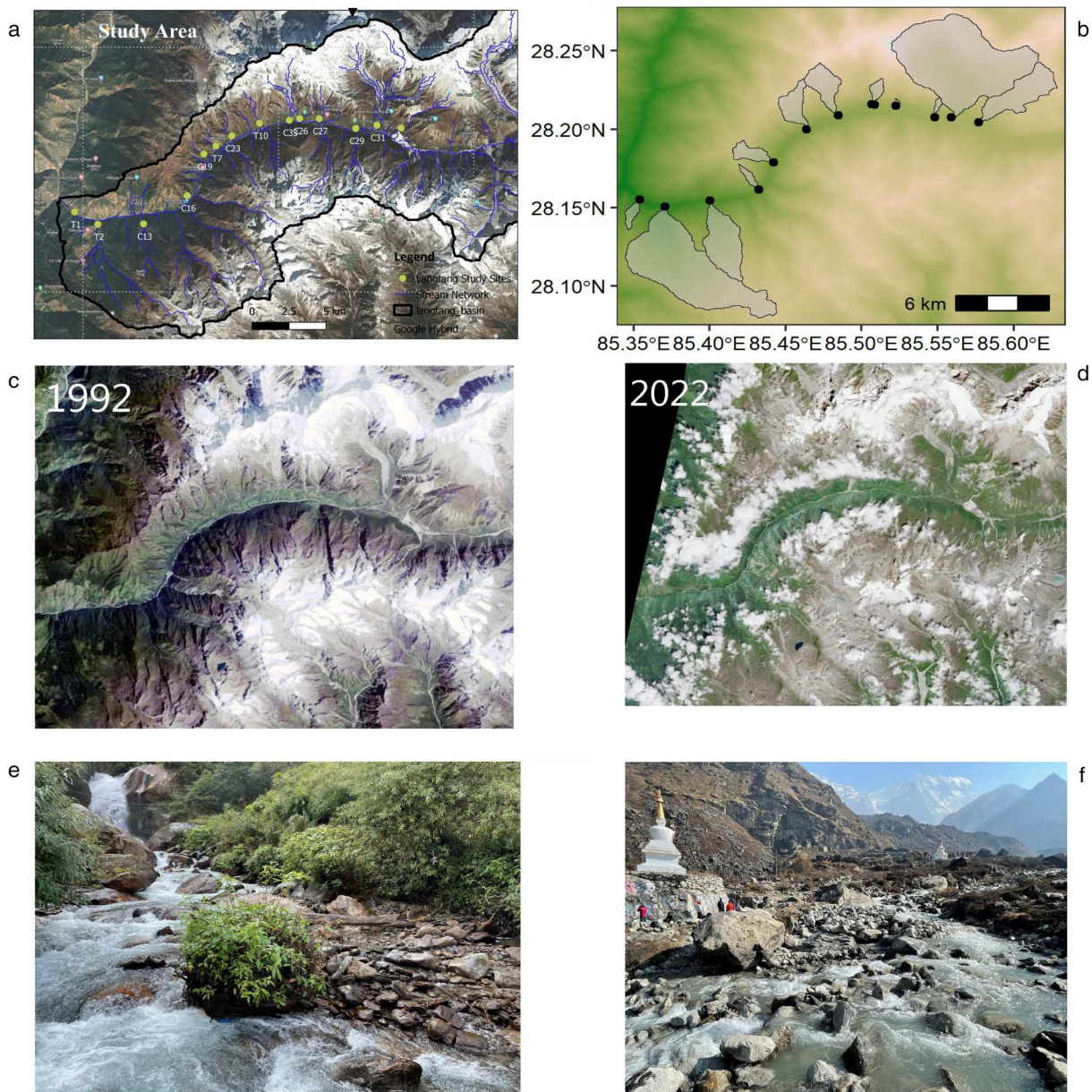
Figure 1. (a) Location and names of sampling sites, indicating the limit of the entire Langtang catchment (from Google Earth). (b) Catchments corresponding to each sampling site. (c–d) Landsat images of the Langtang Valley from 1992 and 2022, respectively. The decrease in ice cover and greening of the Langtang Valley from August 1992 to August 2022 is evident. (e) Sampling site T2 draining subtropical montane forest. (f) Sampling site C31 with considerable glacial meltwater influence.

tributary streams, omitting 2 sites (T7 and T10) that had dried up (Fig. 1), using the same nomenclature for the sampling sites as Ormerod et al. (1994). Because our main objective was to study any long-term changes in stream communities, our first field sampling campaign was 2–9 November 2022 at approximately the same post-monsoonal period of the year as Ormerod et al. (1994). To assess any seasonal variations, a second sampling campaign was conducted during the following pre-monsoon period, 15–22 April 2023. Fish were not observed in any of the streams in either 1992 or 2022–2023.

Macroinvertebrate sampling and field measurements

We collected macroinvertebrates with a 25 cm × 25 cm kick net, with net mesh size of 250 μm, as used by Ormerod et al. (1994). Samples were collected mainly from riffles by disturbing the substratum with the feet or hands in front of the net for ∼5 min. In the field, invertebrates were separated from mineral particles by repeatedly stirring in a bucket and decanting through a sieve that matched the mesh size of the net. Wet samples were preserved on-site in 96% ethanol prior to transport

to the laboratory for sorting and identification primarily to family level (as in Ormerod et al. 1994), referring to Merritt and Cummins (1996) and available regional literature. Treatment at this taxonomic level reflected the limited availability of local taxonomic information and keys, particularly during the 1992 survey.

In the field, pH, electrical conductivity (Lutron Electronics, Coopersburg, PA, USA) and turbidity (Eutech Instruments TN-100, Singapore) were measured using portable meters. Water samples were collected to estimate major ion concentrations and analyed following APHA (2012) protocols. Because these are all spot measurements of temporally highly variable parameters, they were performed to provide a basic description of the stream sites and were not used to analyze changes over time. Outline of physical features of the sites were evaluated visually.

Data treatment

Catchment delineation and properties

We delineated topographical catchment boundaries for each stream site using a digital elevation model (DEM) with a resolution of ~30 m (ESA 2021). The DEM was preprocessed using least-cost breaching (Lindsay and Dhun 2015) and filling depressions (Wang and Liu 2006). Flow directions were determined using the D8 algorithm (O'Callaghan and Mark 1984) and used to delineate catchment boundaries. We used the algorithms implemented in Whitebox Tools to process the DEM and catchment delineation (Lindsay 2016).

For each stream catchment we characterized geomorphometry and vegetation coverage, also determining summary statistics of elevation and slope from the DEM. For vegetation, we used satellite imagery to determine the mean NDVI in 1992 and 2023 calculated from surface reflectance for clear and cloud-free pixels using atmospherically corrected scenes. For 1992, we calculated NDVI at 30 m resolution using a single Landsat 5 scene with minimal cloud cover captured from 21 September (USGS 2020). For 2023, images were more cloudy, and thus we calculated NDVI using a median mosaic of 8 scenes collected in September 2023 from the Sentinel 2 constellation (Copernicus 2023).

We used the GDAL software (GDAL/OGR contributors 2023), R programming language (R Core Team 2022), and multiple packages (Padgham et al. 2017, Pebesma 2018, Baston 2022, Hijmans et al. 2023) for geospatial analysis.

Macroinvertebrates

Samples were generally larger in 2022–2023 compared to 1992, reflecting slightly increased sampling effort (2 vs. 5 min). We therefore standardized the sampling effort

by rarefying the abundance matrix ($n = 100$, permutation = 9999) with the function *rrarefy.perm* using the *EcolUtils* package (Salazar 2020), which generates one randomly rarefied community data for each sample with a fixed sample size. We calculated rarefied taxon richness with the function *rarefy* ($n = 100$) in the *vegan* package (Oksanen et al. 2022). To analyze the effects of time and season on these rarefied richness, we performed paired Brunner-Munzel tests as follows—time: Nov 1992 vs. Nov 2022; season: Nov 2022 vs. Apr 2023, using the *nparcomp* package (Konietschke et al. 2015). Additionally, we conducted pairwise PERMANOVA (using the pairwise *Adonis* package, Arbizu 2017) and 3D NMDS (within the *vegan* package) to compare communities over time and seasons. We also conducted independent 2D NMDS (using the Bray-Curtis dissimilarity index: B-C) for the community of each year and added the catchment variables and the significant taxa ($p < 0.05$) employing the *envfit* function (permutations = 999) in the *vegan* package. To test the relationship between pairwise B-C dissimilarities and altitudinal differences between individual sites in both 1992 and 2022, we performed Mantel tests with 9999 permutations using a Pearson's correlation analysis. Mantel tests are suitable for monotonic comparisons between the values of 2 distance matrices (Legendre and Fortin 2010). Long-term and seasonal beta diversity was partitioned into the turnover component, which refers to the replacement of taxa in a community, and the nestedness component, which indicates differences in richness resulting from species loss or gain (Legendre 2014). We calculated overall beta diversity (Sorensen), turnover (measured as Simpson dissimilarity), and nestedness (measured as the nestedness-resultant fraction of Sorensen dissimilarity) from the presence–absence matrix employing the package *betapart* (Baselga et al. 2021). Linear regressions were performed to analyze relationships between fauna variables (rarefied richness, dissimilarities) and site variables (altitude, NDVI) after the Shapiro-Wilk, Kolmogorov-Smirnov, and Anderson-Darling tests all showed nonsignificant departure from normal distributions. Because of the small number of sampling sites and the sporadic occurrence of some taxa, we were unable to analyze altitudinal shifts in the distribution of individual families. All statistical analyses were executed in R 4.2.2 (R Core Team 2022).

Results

Environmental properties: stream and catchment characteristics

The sampled stream sites varied considerably in terms of width and discharge from just a few tens of liters

Table 1. Selected environmental characteristics of the sampled stream sites measured and evaluated during the field campaigns in November 2022 and April 2023.

| Site name | Site altitude (m a.s.l.) | Coordinates | | Conductivity (µS cm⁻¹) | | pH | | Turbidity (NTU) | Mean width (m) | Current velocity | Dominant substratum | Comment |
		N	E	Nov	Apr	Nov	Apr	Apr				
T1	1504	28° 9′ 19.12″	85° 21′ 10.76″	91	104	8.3	8.8	2.7	1	Low	Pebbles/gravel	Just below waterfall before crossed by new dirt road
T2	1650	28° 9′ 3.40″	85° 22′ 10.56″	41	45	7.3	8.4	1.3	4	High	Boulders/cobbles	
C13	2012	28° 9′ 18,38″	85° 23′ 56.68″	46	18	8.9	8.5	11.1	7	High	Boulders/cobbles	
C16	2541	28° 9′ 45.55″	85° 25′ 52.11″	46	53	8.9	9.1	1.9	2	Moderate	Boulders/cobbles	In steep gorge. Step-pool type
C19	2785	28° 10′ 47.61″	85° 26′ 25.89″	57	72	8.4	8.3	1.4	1	Moderate	Boulders/cobbles	In unstable, steep gorge. Step-pool type
C23	3033	28° 12′ 04.70″	85° 27′ 43.00″	33	54	7.5	8.6	0.8	2	Moderate	Cobbles/pebbles	
C33	3442	28° 13′ 04.55″	85° 30′ 16.69″	51	nd	8.5	nd	nd	2	High	Boulders/cobbles	Was dry in April
C26	3455	28° 13′ 02,58″	85° 30′ 23,01″	59	62	7.1	8.3	0.6	2	Moderat	Cobbles/pebbles	Spring fed
C27	3560	28° 13′ 00.86″	85° 31′ 14.47″	33	22	8.4	9.2	3.8	1	Moderat	Cobbles/pebbles	Channel had been deviated recently
C29	3654	28° 12′ 35.50″	85° 32′ 47.05″	95	75	7.9	8.6	0.6	5	High	Cobbles/pebbles	Rheocrene spring
C31	3754	28° 12′ 36.85″	85° 33′ 26.43″	86	105	8.2	8.8	20.3	8	High	Boulders/cobbles	Glacial runoff
CC	3907	28° 12′ 25.64″	85° 34′ 31.73″	93	93	7.6	8.8	4.1	4	High	Boulders/cobbles	In a huge rockslide fan. Glacial runoff

per second (T1, C16, C19, C26, and C27) to cubic meters per second (T2, C13 and C31). All sampling sites (except T1) had a predominance of coarse substrata but also included areas with pebble and finer substrates appropriate for macroinvertebrate sampling. The main water source varied between sites, with 2 of the sites groundwater fed (C26 and C29) and 2 with considerable glacial meltwater contribution (C31 and CC; Table 1). These latter 2 also drained glaciers in 1992 and, despite overall glacial retreat over the 30 yr period, their glacial catchment in 2022 was still considerable at ~30% and ~15%, respectively. Other sites had undergone clear anthropogenic alterations (T1 and C27; Table 1). Catchments varied considerably with regard to mean altitude, NDVI, and area (Table 2), but the mean

NDVI correlated similarly with mean catchment altitude in 1992 (y = −0.0002x + 1.341, r^2 = 0.89) and in 2023 (y = −0.0002x + 1.294, r^2 = 0.83). Nevertheless, from 1992 to 2022 the mean NDVI increased in all but one of the catchments and proportionately more at higher sites as shown by an increase in the 2022:1992 ratio in mean NDVI with mean catchment altitude (p = 0.047, r = 0.58).

Long-term and seasonal changes in fauna richness and composition

From the 12 stream sites, 4440 individuals from 23 taxa were collected in the November 1992 samples, 10 522 individuals and 40 taxa in November 2022, and 29 287 individuals and 51 taxa in April 2023. The 2 most

Table 2. Characteristics of the catchments corresponding to the 12 sampled stream sites. NDVI = normalized difference vegetation index.
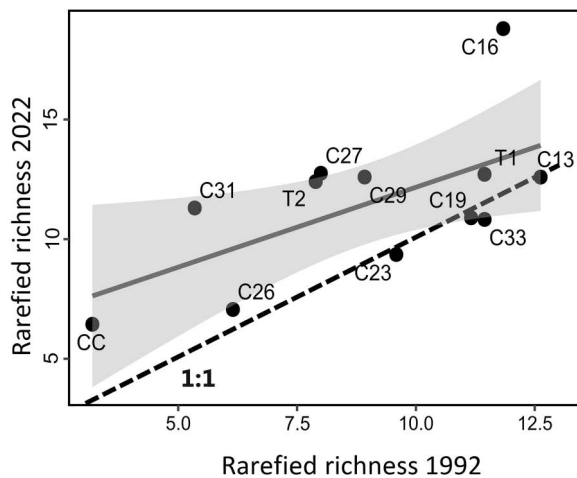
Site name	Max catchment altitude (m a.s.l.)	Mean catchment altitude (m a.s.l.)	Catchment area (km²)	Mean catchment slope (°)	Mean NDVI 1992	Mean NDVI 2022	Ratio NDVI 2022:1992
T1	2923	2293	1.03	34	0.75	0.76	1.01
T2	5132	3510	31.10	26	0.65	0.70	1.09
C13	4778	3530	8.96	29	0.67	0.73	1.09
C16	4171	3439	0.70	42	0.69	0.71	1.04
C19	4597	3948	2.16	36	0.59	0.62	1.05
C23	5555	4546	3.50	43	0.37	0.49	1.33
C33	5320	4480	0.97	46	0.38	0.54	1.43
C26	3832	3542	0.06	36	0.60	0.67	1.11
C27	4232	3771	0.10	46	0.54	0.65	1.20
C29	4798	4290	0.34	42	0.45	0.42	0.93
C31	7215	5113	37.75	34	0.27	0.32	1.16
CC	5664	4865	8.34	23	0.24	0.36	1.48

important families in 1992 were Baetidae (44% of individuals, present at 11 sites) and Chironomidae (15% individuals, 12 sites) with both abundant again in November 2022 (Chironomidae 26% individuals, 10 sites; Baetidae 13% individuals, 12 sites). Two taxa from 1992 were not detected in 2002–2023 (the trichopterans Lepidostomatidae and Leptoceridae) while a number of additional families were detected in November 2022, even in rarefied community data (Table 3). For the glacier-fed sites C31 and CC, which had the relatively greatest increase in richness (discussed later), the trichopteran Rhyacophilidae was abundant at both sites in 2022 but absent in 1992, and the plecopteran Nemouridae was abundant in C31 in 2022 but absent in 1992.

Rarefied richness at individual sites correlated significantly between 1992 and 2022 ($p = 0.031$, $r = 0.62$; Fig. 2), but 8 sites had greater richness in 2022, by 2.52 taxa on average. This apparent long-term increase was significant ($p = 0.018$, paired Brunner-Munzel test;

Fig. 3). By contrast, no systematic seasonal effects on richness ($p = 0.17$) were detected, with differences in rarefied richness at individual sites ranging from −4.2 to 6.3, with an overall average of 3.57 more taxa in November than April. No relationship in rarefied richness between November 2022 and April 2023 ($p = 0.182$, $r = 0.43$) was found, and no seasonal difference between these 2 sampling periods ($p = 0.086$, paired t-test; Fig. 3).

Community composition at individual sites varied considerably, both seasonally and long-term. Average rarefied Bray-Curtis seasonal dissimilarity (2022 vs. 2023) was 0.654 and 0.605, respectively, among and within sites. In 1992 versus 2022, the average dissimilarity among all sites was 0.644 and within sites was 0.546. No difference in seasonal (2022–2023) and long-term (1992–2022) rarefied Bray-Curtis dissimilarities was found within sites, and no difference in mean dissimilarity among all sites in 1992 (0.635) and 2022 (0.648). The NMDS ordination showed considerable overlap in community composition in the datasets from 1992, 2022, and 2023, and neither the 2022–2023 groups nor the 1992–2022 groups differed significantly ($p > 0.05$, pairwise PERMANOVA; Fig. 4).

At all sites, long-term beta diversity was consistently mainly composed of turnover (i.e., replacement of taxa) while the main component of seasonal beta diversity varied much more between sites, at some

Table 3. Occurrence of families in November 2022 not found at any of the 12 Langtang sites in November 1992.

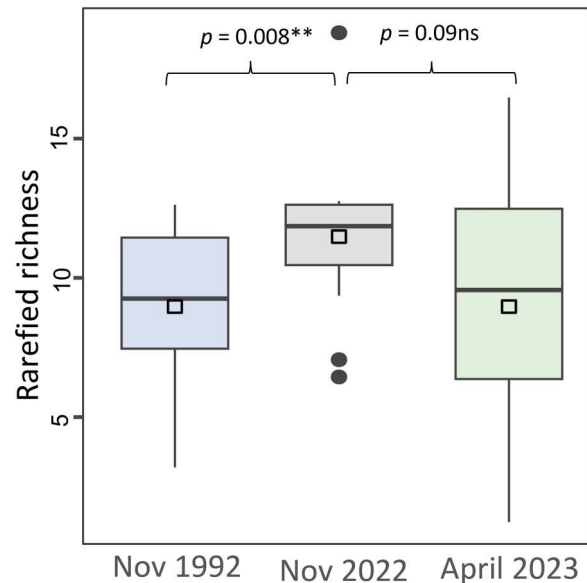|	Rarefied 2022 data ($n = 100$)		Crude 2022 data	
	Sites	Individuals	Sites	Individuals
Limoniidae (Diptera)	7	33 (1–14)	10	838 (1–756)
Nemouridae (Plecoptera)	6	37 (1–26)	7	209 (2–123)
Dixidae (Diptera)	5	6 (1–2)	6	26 (1–13)
Philopotamidae (Trichoptera)	4	9 (2–3)	6	55 (1–23)
Hydracarina (Aracnida)	4	4 (1–7)	5	413 (1–372)
Planaridae (Turbellaria)	3	12 (2–7)	5	389 (1–365)

Figure 2. Regression of rarefied richness of macroinvertebrates in November 2022 versus November 1992 in the 12 study streams compared to the 1:1 line (dashed). Shaded area denotes 95% confidence limits.
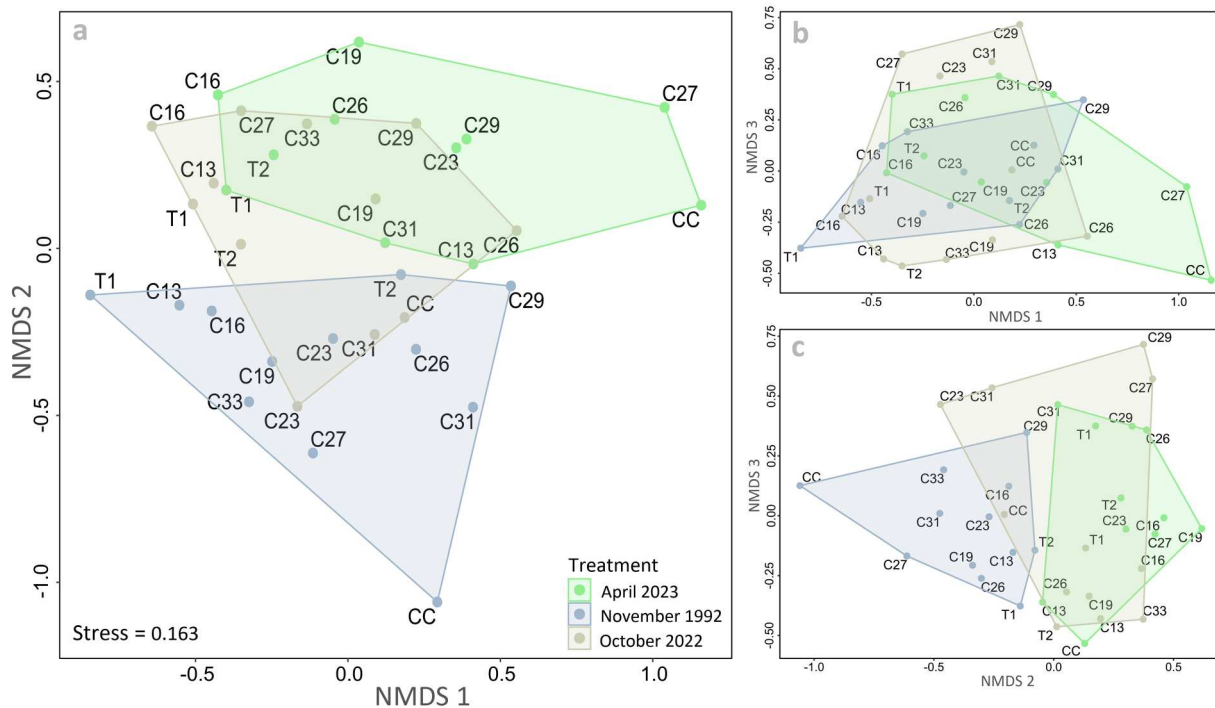

Figure 3. Boxplot comparing rarefied richness of macroinvertebrates in the 12 study streams in November 1992, November 2022 and April 2023. Horizontal lines are medians, dots in the boxes are means, limits of boxes are Q1 and Q3 quartiles, vertical lines are max-min values, and black dots are outliers. Statistical differences are indicated. ns = not significant.

Figure 4. Non-parametric multidimensional scaling ordinations (NMDS) based on Bray-Curtis dissimilarities of rarefied macroinvertebrate community data from November 1992, November 2022, and April 2023. (a) NMDS axis 1 and 2, (b) NMDS axis 1 and 3, and c) NMDS axis 2 and 3. Colored polygons are convex hulls.
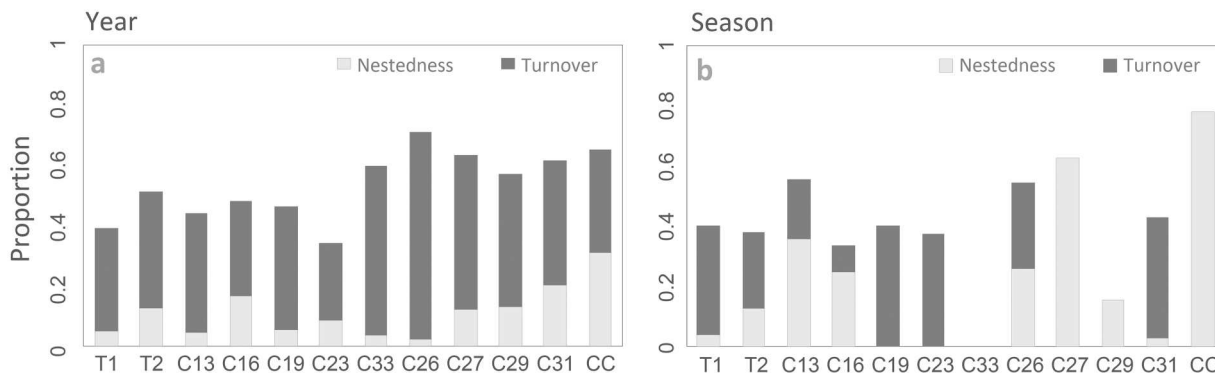
Figure 5. Overall beta diversity (Sørensen) partitioned into the turnover component (replacement of taxa) and nestedness component (richness loss or gain) for (a) long-term and (b) seasonal differences for each of the 12 study streams.

sites entirely due to either turnover or nestedness (Fig. 5).

### Long-term changes in fauna in relation to catchment characteristics and altitude

Rarefied richness decreased at a similar rate to sampling site altitude and mean catchment altitude in 1992 and in 2022 (Fig. 6). The overall increase in richness between 1992 and 2022 (expressed as a ratio 2022:1992) increased significantly with catchment area ($p = 0.048$, $r = 0.58$),

tending also to increase with maximum catchment altitude ($p = 0.098$, $r = 0.50$) and site altitude. These effects reflected considerably greater increase in richness by 2022 at the 2 glacially fed sites (C31 and CC) than at any other site (Fig. 7). No pronounced effect on richness was found at altitudes between 2900 and 3500 m a.s.l., and thus no signs of a climate sensitive zone.

In contrast to the consistency between 1992 and 2022 in altitudinal effects on rarefied richness, the effect of increasing altitudinal difference on pairwise rarefied dissimilarities between sites in 1992 ($p = 0.0002$, Mantel
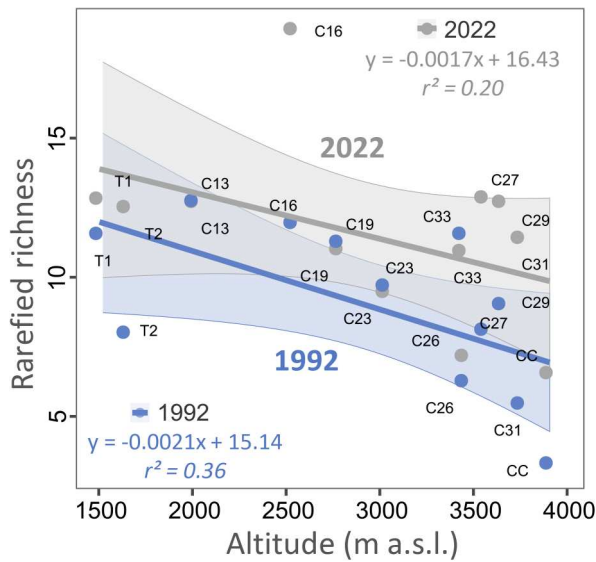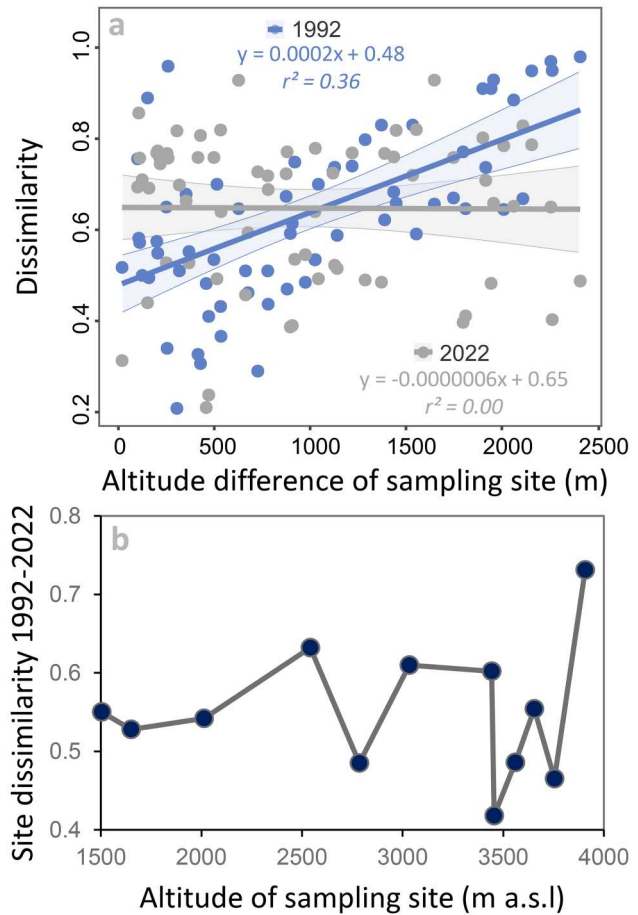
Figure 6. Linear regressions of rarefied richness of macroinvertebrates for the 12 study streams in November 1992 and November 2022 as a function of altitude of sampling site. Regression line equations are provided. Shaded area denotes 95% confidence limits.
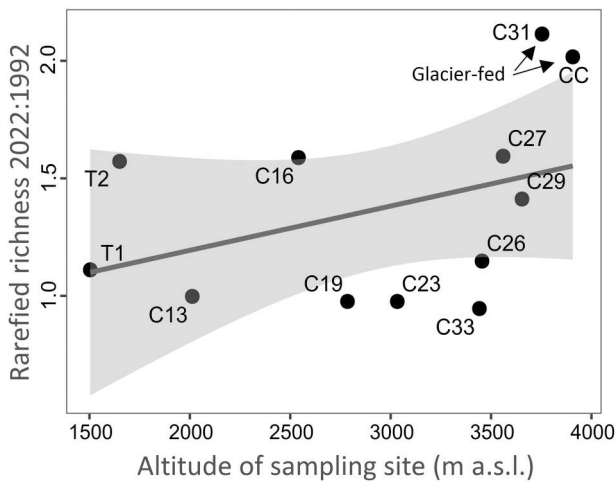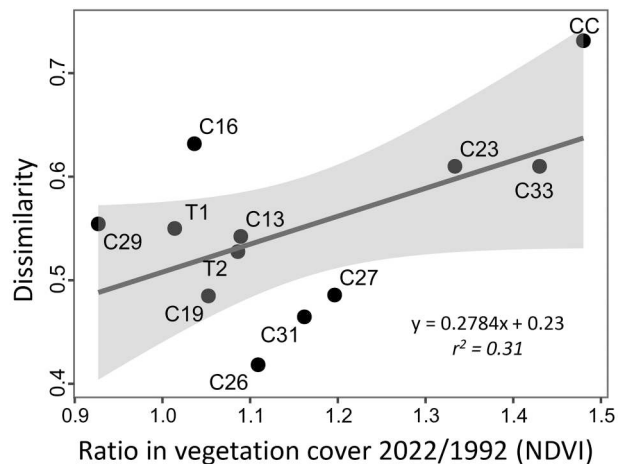

Figure 7. Linear regression of the 2022:1992 ratio of rarefied richness of macroinvertebrates for the 12 study streams as a function of altitude of sampling site. Shaded area denotes 95% confidence limits.
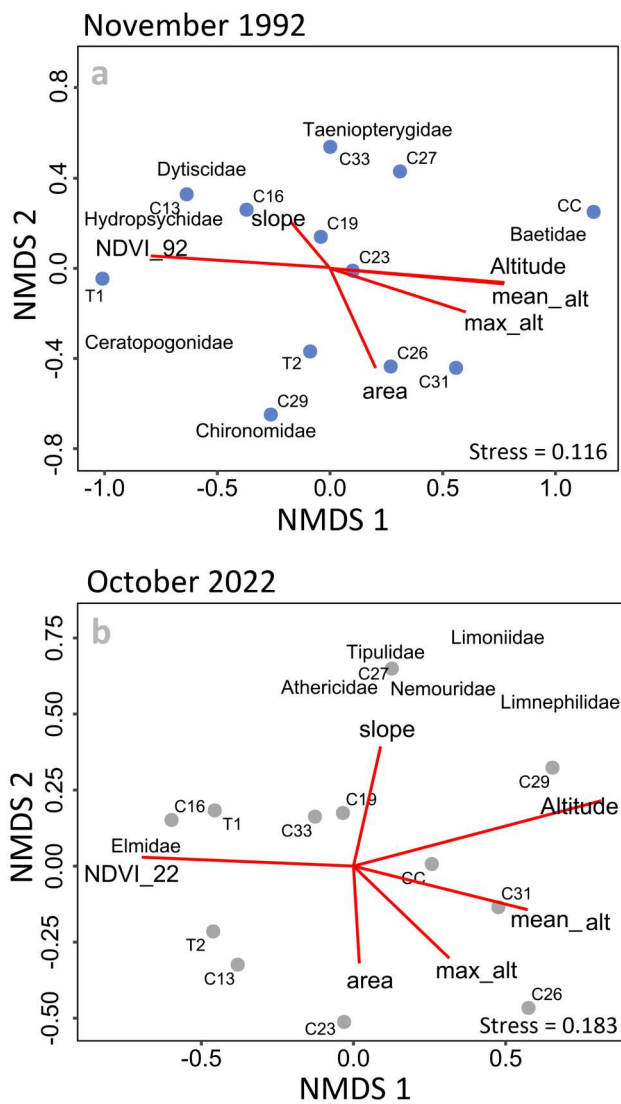

Figure 8. (a) Regression of pairwise Bray-Curtis dissimilarities in macroinvertebrate communities as a function of altitudinal differences between individual sampling sites in 1992 and 2022. Regression line equations are provided. Shaded areas denote 95% confidence limits. (b) Bray-Curtis dissimilarities between November 1992 and November 2022 at individual sites in relation to altitude of sampling sites.

Figure 9. Bray-Curtis dissimilarities in macroinvertebrate communities between November 1992 and November 2022 at individual sites as a function of the 2022:1992 ratio in catchment NDVI. Regression line equation is provided. Shaded area denotes 95% confidence limits.

test, $r = 0.60$) was no longer apparent in 2022 (Fig. 8a). Dissimilarities between 1992 and 2022 did not correlate significantly with altitude, and nor were there pronounced shifts in communities at sites between 2900 and 3500 m a.s.l., which might be expected from a climate sensitive zone (Fig. 8b). However, dissimilarities between 1992 and 2022 were nearly correlated with the 2022:1992 ratio in mean NDVI at $p = 0.061$ ($r = 0.55$), implying that ecological changes were greatest

Figure 10. Non-parametric multidimensional scaling ordinations triplots (NMDS) based on Bray-Curtis dissimilarities of rarefied macroinvertebrate community data, including sites, significant taxa, and catchment properties as environmental vectors from (a) November 1992 and (b) November 2022.

where catchments gained vegetation cover (Fig. 9). In both years, community composition was associated with the mean NDVI of the catchments and the altitude of the sampling sites (Fig. 10).

Discussion

Against the expectation of considerable dynamism in the world's mountain stream ecosystems linked to global change, the scarcity of long-term data has so far prevented a clear assessment of actual change (Domisch et al. 2011, Hotalling et al. 2017). Our datasets, separated by 30 years, illustrate that some patterns have been maintained, notably altitudinal effects on overall

richness and some degree of overlap in community composition. At the same time, however, these data provide evidence of long-term change, for example through increasing taxon richness particularly at the highest altitudes, turnover in community composition, and a weakening of altitudinal effects on site-to-site dissimilarity with upslope progression. Although relatively weak, the clearest explanation for these changes, based on our data, was increasing catchment cover by vegetation. Overall, the data supported the hypothesis of increasing richness through time, but they were not consistent with greatest sensitivity to change at intermediate altitudes. In the discussion that follows, we first outline some limitations in our study before considering variations between sampling occasions and appraising possible explanations for the observed long-term patterns.

While our data are among few to assess long-term changes in high mountain streams, especially in the Himalaya, there are inevitably caveats and weaknesses, including the effects of increased sampling effort in the 2022 collection compared with 1992 (5 vs. 2 min), some uncertainty over reidentifying locations sampled in 1992 before the advent of portable GPS systems, and the relatively small array of sites sampled in just one main river system. There is also an inevitable question about the extent to which long-term change can be inferred from just 2 sampling years as opposed to much stronger time-series data, especially given the magnitude of change in invertebrate communities shown by our data between 2 recent seasons. Family-level identification, limited by the availability of taxonomic information in 1992, might also mask important changes among genera or species. In addition, our explanatory power on faunal changes is limited by the lack of long-term, site-specific data on environmental variables such as water temperature and chemistry. Notwithstanding these issues, the study spans a much longer period (30 yr) than many other field studies on long-term changes in freshwater biodiversity (Murdoch et al. 2022), especially in this region of significant ecological interest. Further uniqueness is added by our ability to relate the observed invertebrate dynamics to changes in catchment greenness.

Long-term and seasonal changes in fauna richness and composition

Although our data were insufficient to test for actual altitudinal shifts in individual taxa, one of the clearest results was an increase in invertebrate richness at most sites from 1992 to 2022, an expected result because the predicted biological effects of climate warming

include increased alpha (local) diversity as species from more diverse assemblages colonize upstream locations (Hotalling et al. 2017). Other studies on alpine streams covering several years have also found increasing alpha richness among macroinvertebrates over time (Brown et al. 2006), especially where glacial retreat in the catchment is involved (e.g., Muhlfeld et al. 2020, Lencioni et al. 2022).

The overall seasonal difference in rarefied richness was actually greater than long-term change, with the highest average richness at the post-monsoonal samples from November compared to the pre-monsoon season sampling in April. The variability between sites was considerable in our data, however, rendering this seasonal change nonsignificant. We have no categorical explanation for this large seasonal variability between sites, but stochastic sampling effects could be involved. Pronounced short-term variability was also evident in greater seasonal than long-term dissimilarities, both overall and within sites. Previous Himalayan studies have linked seasonal changes in invertebrate communities and abundance to life cycle adaptations linked to the pronounced flow changes that accompany monsoonal effects (Brewin et al. 2000). These flow variations diminish with increasing altitude, however, which might explain some variation in seasonal invertebrate changes among locations.

We anticipated that long-term beta diversity would predominantly reflect the turnover of taxa, which is typically the main component of both temporal and spatial beta diversity among freshwater macroinvertebrates (e.g., Tonkin et al. 2018, Jacobsen et al. 2020, Paripatyadar et al. 2020, He et al. 2023) as well as other natural communities (Soininen et al. 2018, Khattar et al. 2021). In addition, we expected the contribution from the nestedness, or richness, component linked to changes in climate and land cover (Li et al. 2024) might be systematically more important in the long-term than seasonal changes because richness increased at most sites over the 3 decades. However, seasonal beta diversity varied over the entire range, from 100% turnover to 100% nestedness, therefore comparing long-term and seasonal nestedness was not possible.

Long-term changes in fauna in relation to catchment characteristics and altitude

As expected, taxonomic richness decreased with the altitude of sampling sites and the mean altitude of the catchments, both in 1992 and 2022. This altitudinal trend is well known from various regions (Suren 1994, Jacobsen 2004, Lujan et al. 2013) and is theorized to reflect the combined effects of reduced nutrients, reduced temperature, lower oxygen availability, reduced production, and altered sediment dynamics alongside other geophysical changes (Ward 1994, Jacobsen and Dangles 2017). Long-term differences between 1992 and 2022 in Langtang were apparently insufficient to disrupt this overall altitudinal pattern, although the increase in taxon richness through time tended to modify the precise slope and intercept in this relationship (Fig. 6). Whereas long-term and seasonal differences in rarefied richness were unrelated to catchment characteristics, the 2 sites with the greatest relative increase were the 2 streams with glacial meltwater inflow, which also had the highest contribution of nestedness to long-term beta diversity. Glacier-fed streams are harsh habitats with lower taxonomic richness than nonglacial streams, even at similar altitudes (Jacobsen and Dangles 2012, Lencioni 2018). However, macrofaunal alpha richness tends to increase as glacial meltwater contributions are replaced by groundwater and surface runoff (Brown et al. 2006, Jacobsen et al. 2012, Cauvy-Fraunié et al. 2016). Glacial meltwater runoff in the Langtang Valley has decreased during the 30-year study period (Immerzeel et al. 2012, Pradhananga et al. 2014), with likely consequences for thermal regimes, hydrological dynamics, solute concentrations, and other factors that could influence invertebrate colonization.

Patterns in beta diversity of stream macroinvertebrate fauna are often only weakly related to environmental and spatial factors (Heino et al. 2015a) but seem to be more so across catchments at intermediate dispersal levels (Heino et al. 2015b). The positive relationship in 1992 between pairwise dissimilarities among all sites and altitudinal difference (i.e., distance–decay relationship) was thus expected and confirm findings in other studies over altitudinal gradients (Harrington et al. 2016, Jacobsen et al. 2020), including one based on data from the Langtang catchment in 2012–1213 (Tonkin et al. 2017). Across the Himalayas as a whole, Gutiérrez-Cánovas et al. (2013) showed how beta diversity among macroinvertebrates reflected turnover as dissimilarity increased with increasing altitude. Against these previously established patterns, climate change is expected to produce some degree of homogenization in environmental conditions, and potentially also in natural communities, resulting in reduced beta diversity in alpine stream catchments as local niche filtering declines (Leibold et al. 2004, Jacobsen et al. 2012, Hotalling et al. 2017). However, overall dissimilarity among our sites was similar in 1992 and 2022, and actually slightly higher in 2022. The more surprising finding was that dissimilarity in 2022 did not reflect altitude and so was less clearly related to geographical or environmental distance (Fig.

8). Potential explanations include the growing importance of other environmental factors, for example growing human influences, impacts related to the 2015 earthquake, or effects of climate change that have been more context-dependent than altitudinal. We have insufficient data to address these or other explanations, but one important inference is that some altitudinal patterns in this catchment have weakened through time.

The NDVI of catchments and the altitude of sampling sites were the 2 characteristics most associated with the composition of the communities and were consistent across 1992 and 2022 (Fig. 9). Long-term dissimilarities among macroinvertebrate communities were positively related to increases in NDVI, which were clear in most catchments over the long-term (Fig. 8), although surprisingly no link was found between NDVI and long-term change in faunal richness. Nevertheless, the array of families gained in Langtang through time included nest-spinning trichopterans, leaf-shredding nemourid stoneflies, and several dipterans typical of wetted habits at the stream edge, all implying possible shift in basal resources towards allochthony, in turn potentially linked to resource subsidies from catchment vegetation (Larsen et al. 2016). In other regions, catchment values of NDVI were highly correlated with a range of local environmental and biotic variables in mid-US streams, including macroinvertebrate indices (Griffith et al. 2002). Processes mediated by catchment vegetation are complex, however; for example, vegetation cover can also influence channel stability (Asfaha et al. 2016), sediment dynamics, downslope hydrology through effects on evapotranspiration, and surface water chemistry through fluxes of nutrients and organic solutes (Soininen 2015). Our data show some potential for hydrochemical effects on dissimilarity given that the concentrations of most ions have increased during the study period (Tuladhar et al. 2015). In addition to these complexities, relationships between local stream conditions and NDVI might be scale dependent. While our data hinted at whole-catchment effects of NDVI, Dias-Silva et al. (2021) found that local NDVI values measured >30–300 m radii around sampling sites in Brazilian streams failed to explain any variation in local stream environmental variables. These variations suggest the need for further studies to assess the possible contribution of global change effects on catchment or riparian vegetation cover in high mountain regions and the downstream ecological consequences.

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Data availability

Data are available by request to the corresponding authors.

ORCID

Dean Jacobsen 🔘 http://orcid.org/0000-0001-5137-297X
Steve J. Ormerod 🔘 http://orcid.org/0000-0002-8174-302X
Ingrid Jüttner 🔘 http://orcid.org/0000-0003-0841-6206
Oscar Rojas 🔘 http://orcid.org/0000-0002-0176-9685
Kenneth Thorø Martinsen 🔘 http://orcid.org/0000-0001-8064-513X
Bhumika Thapa 🔘 http://orcid.org/0000-0002-3247-6519
Kumar Khatri 🔘 http://orcid.org/0000-0001-6582-9256
Pratima Paudel 🔘 http://orcid.org/0009-0007-0321-0272
Manita Rai 🔘 http://orcid.org/0009-0004-7915-0470
Nani Raut 🔘 http://orcid.org/0000-0003-0951-8896
Smriti Gurung 🔘 http://orcid.org/0000-0002-2975-3204

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