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The Impacts of Different Anthropogenic Disturbances on Macroinvertebrate Community Structure and Functional Traits of Glacier-Fed Streams in the Tianshan Mountains

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Abstract: Macroinvertebrates are sensitive to environmental disturbances, however, the effects of human activities on macroinvertebrate community structures and functional traits in glacier-fed streams are concerning. To elucidate the effects of horse, cattle and sheep grazing, hot spring scenic development, and historic iron ore mine development on macroinvertebrate communities, we conducted a study in three glacier-fed streams of the Tianshan Mountains in northwest China in April 2021. Our results showed that the species richness and density significantly decreased due to grazing ($p < 0.05$). There were more taxa with resilience traits such as "small size at maturity" in the grazing stream. The EPT richness and density affected by hot spring scenic development significantly decreased compared to the undisturbed point ($p < 0.05$). There was a significant increase in taxa with resistance traits such as "bi-or-multivoltine" in the hot spring stream. The stream affected by historic mine development is currently in the self-recovery stage following the closure of the mine ten years ago. Additionally, the species richness, EPT richness, and density at the mining site were significantly higher than the source site ($p < 0.05$), reflecting that the habitat fragmentation caused by previous mining activities prevented the upward dispersal of macroinvertebrates. The taxa in the mining stream were also characterized by higher resistance traits such as "abundant occurrence in drift". These results were attributed to the impacts of human disturbance on habitat stability, habitat heterogeneity, water quality, and material cycling of stream ecosystems, indicating human disturbance on the efficiency of resource utilization and functional diversification. In addition, our results showed that functional indicators of macroinvertebrate communities are helpful for monitoring and evaluating habitat conditions.

Keywords: macroinvertebrate; grazing; hot spring; mine development; glacier-fed stream

Citation: Liu, Y.; Tian, Y.; Gao, Y.; Cui, D.; Zhang, W.; Jiao, Z.; Yao, F.; Zhang, Z.; Yang, H. The Impacts of Different Anthropogenic Disturbances on Macroinvertebrate Community Structure and Functional Traits of Glacier-Fed Streams in the Tianshan Mountains. *Water* **2022**, *14*, 1298. https://doi.org/10.3390/w14081298

Academic Editors: Peter Bitušík and Ladislav Hamerlik

Received: 3 February 2022
Accepted: 14 April 2022
Published: 15 April 2022

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1. Introduction

Glacier-fed streams are one of the environments most sensitive to global climate change [1–3]. They are usually characterized by harsh and fragile environmental conditions, such as low water temperature, channel stability, high turbidity, dissolved oxygen, and temporal variability in water flow [4]. These environmental conditions may reduce the local species diversity of glacier-fed streams, but some endemic taxa have been found to adapt to these extreme conditions [5–7]. Previous research has studied the macroinvertebrate community structure and their distribution in glacier-fed streams [3,8,9]. However, when anthropogenic disturbances occur in glacier-fed streams, they are less resilient and sometimes do not recover as well as other types of streams. Therefore, it is crucial to determine how biodiversity and functional traits of macroinvertebrates change in response to human activities in glacier-fed streams.

Due to human disturbances, global biodiversity is being lost at an unprecedented rate [10–12]. Aquatic ecosystems are particularly affected by human pressures, including habitat degradation, water abstraction, and pollution from industrial, agricultural, and urban sources [13–16]. Macroinvertebrates are one of the most diverse groups of aquatic organisms. Their feeding habits are extensive as an intermediate link of the food chain and the food web in aquatic ecosystems [17]. They feed on plankton, benthic algae, leaf litter, or fine particulate organic matter, and serve as prey for fish and other large aquatic organisms [18]. As the macroinvertebrate community structure is diverse, the types of biological indices are rich, the response to environmental changes is sensitive, and easy to collect and fix [19–21]. They are often used as bioindicators of water quality and habitat conditions [17], which give an advantage over traditional water quality assessments. Recently, the effects of anthropogenic disturbances on aquatic macroinvertebrate communities have been successfully assessed using trait-based methods [22]. By using a statistical analysis of macroinvertebrate functional traits, the information of individual shape, size at maturity, occurrence in drift, trophic habits, and so on could be obtained. The macroinvertebrates' functional traits reflect their survival strategies under the effects of natural selection pressure over time, which is a result of evolution [23]. Functional traits determine how species adapt to the environment since they exhibit requirements regarding food acquisition and type of trophic resources, physical habitat preferences, and water quality [24,25]. Functional traits can not only reveal the adaption of macroinvertebrates to the environment, but also identify the source of aquatic ecosystem instability [26]. Therefore, the functional traits of macroinvertebrates are also helpful for biological monitoring. Resistant traits can be considered as ability to withstand disturbance, while resilience traits can be thought of as the ability to recover from disturbance [27]. Based on these properties, it is more meaningful to explore how the macroinvertebrate functional traits respond to human disturbances.

Alpine mountains around the world are rich in natural resources such as glaciers, grasslands, hot springs, and mines. Natural grasslands constitute the main source of forage for livestock. The impacts of grazing on stream ecosystems include changes in nutrient load [28], changes in hydrology (e.g., flow regime, predictability, and water quantity) [29], and erosion processes that result in soil loss and sediment inputs to streams [30]. Livestock trampling the riverbed destroys habitats for benthic organisms [31]. Therefore, there is an urgent need to understand the impacts of grazing on macroinvertebrate diversity in glacier-fed streams.

Alpine springs are usually small in size but complex and species-rich. They are ecotones linking an aquifer to the uppermost section of a surface running water system [32], and can be considered as unique ecosystems that harbor different kinds of aquatic biota, including macroinvertebrates. Lamberti and Resh examined a natural thermal gradient formed by hot springs in Little Geysers Creek, California, USA. They found macroinvertebrates were existing at temperatures <45 °C [33]. Thermal springs and thermal pools are now generally exploited by humans. However, various anthropogenic activities around thermal spring areas such as swimming, bathing, cooking, and healing cause habitat loss and species reduction or extinction [34,35]. Thus, it is essential to study macroinvertebrates in streams that run through hot springs to document biodiversity changes.

Increasing global demand for minerals and metals creates opportunities for the economy and employment, yet exploitation of these resources may result in unexpected environmental impacts and consequences on biodiversity [36]. During placer mining, flora and fertile topsoil are removed, and the riverbed morphology is altered. Mining increases turbidity and sediment deposition [37], introduces nutrients, especially phosphorus [38], and heavy metals pollute the water [39], all of which culminate in the degradation of the habitat's environment quality [40]. Habitat fragmentation occurs when localized habitat loss gradually accumulates, resulting in the fragmentation of formerly patchy habitats into several isolated and discontinuous habitats [41]. Several studies have focused on the effects of mining processes on stream macroinvertebrates [42–45], yet there are only a few cases of stream habitat and macroinvertebrate status recovery after mining has ceased.

While investigating macroinvertebrate communities in glacier-fed streams in the Tianshan Mountains of Xinjiang, China, we identified three types of anthropogenic disturbances (horse, cattle and sheep grazing, hot spring scenic development, and historic iron ore mine development). Glaciers are an important source of streams in the Tianshan Mountains area, and glacier-fed streams are sensitive to environmental changes. However, the impacts of these disturbances on biodiversity in this unique cold system remain unclear. To elucidate how the three disturbances affect stream macroinvertebrate communities and functional traits, we investigated the macroinvertebrates in three disturbed streams. We hypothesized that macroinvertebrates have different response patterns to different anthropogenic disturbances. First, grazing and hot spring disturbances can lead to a decrease in macroinvertebrate diversity and density, while habitat fragmentation caused by the mine development, even in mines that have been closed for 10 years, still disrupts macroinvertebrate communities. Second, there will be more taxa with resilience traits in the grazing streams, and more taxa with resistance traits in hot springs and mine streams due to the environmental disturbance characteristics.

2. Materials and Methods

2.1. Study Area

The study area is located at 1500–2700 m in the western Tianshan Mountains, Xinjiang Province of China. This area belongs to the continental temperate climate zone. The mean annual water temperature is 10.4 °C, and the mean annual precipitation is 417.6 mm. The three surveyed streams are the source of the Gongnaisi River, one of the three tributaries of the Ili River. They all originate in the Tianshan Mountains and are influenced by the melting of glaciers and snow.

We established five sampling sites (A–E, that A is closest to the melting edge of the glacier, while E is the most downstream location in each catchment) along each surveyed stream to study the effects of different disturbances on macroinvertebrates in glacier-fed streams (Figure 1). The first stream (S1) is affected by grazing in the catchment, the second stream (S2) is affected by hot springs, and the third stream (S3) is affected by mining. The site S1-A and S1-B are undisturbed by grazing. The grazing sites are mostly concentrated in sites S1-C, S1-D and S1-E, and S1-E is next to a village. The S2 passes through Alxian Hot Spring Resort (site S2-B). The other sites of S2 are undisturbed by the hot spring, and there are no houses or towns on this stream. The S3 is disturbed by the development of a placer mine, while site S3-B is the location of the mine, which was suspended in 2012. The other sites of S3 are undisturbed by the mining, and there are no houses or towns on this stream. We use undisturbed upstream sites as a reference for more disturbed and more recovered downstream sites.

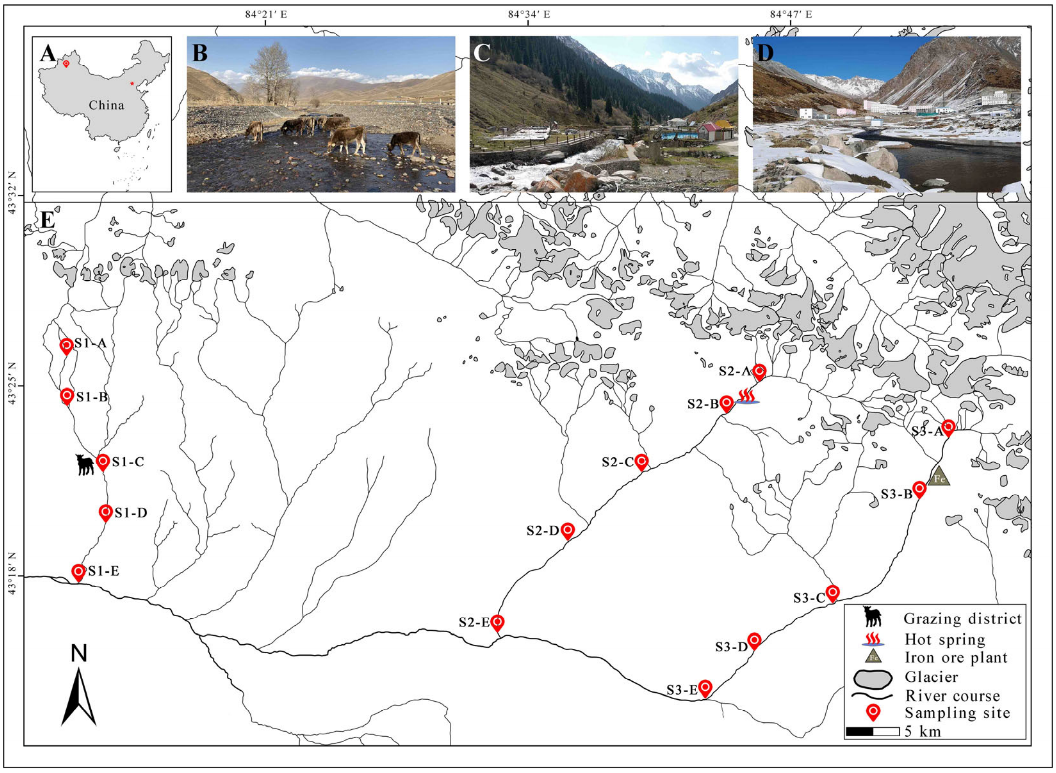

Figure 1. (**A**) Map showing the Ili River Basin located in the People's Republic of China; (**B**) The grazing stream (S1 C–E); (**C**) The hot spring stream (S2-B); (**D**) The mining stream (S3-B); (**E**) The locations of the sampling sites within the three streams of study. Glacier data sites from the second glacier inventory dataset of China [46].

2.2. Macroinvertebrate Collection and Functional Traits

Macroinvertebrates were sampled in April of 2021. We established five sampling sites along the surveyed streams. Each sampling site was sampled with a Surber net (30 × 30 cm, 500 μm mesh size) in three riffles and two pools. The specimens were filtered through a mesh size sieve and stored temporarily in plastic bags in the field. Then, the specimens were manually sorted from sediment on a white porcelain plate and preserved in 75% ethanol in the lab. In the laboratory, the macroinvertebrates were identified to the lowest possible taxonomic level (usually genus) under a stereoscopic microscope (Olympus SZX10). The Chironomidae and Oligochaetes were slide mounted for identification.

All macroinvertebrate taxa were assigned different functional traits in each site. These traits were compiled from database information published by Poff et al. [47] and Moretti et al. [48]. According to the habitat properties of the watershed, we selected six biological traits which included: the life history (voltinism), mobility (occurrence in drift), morphology (shape and size at maturity), and ecology (habit and trophic habit) traits. The six functional traits were coded in 21 trait modalities, and the code signifies the abbreviation of each trait (Table 1).

Table 1. The functional traits, trait state, and code of macroinvertebrates.

Functional Traits	Trait State	Code
Life history		
Voltinism	Semivoltine	Volt 1
	Univoltine	Volt 2
	Bi- or- multivoltine	Volt 3
Mobility		
Occurrence in drift	Rare	Drft 1
	Common	Drft 2
	Abundant	Drft 3
Morphology		
Shape	Streamlined	Shpe 1
	Not streamlined	Shpe 2
Size at maturity	Small (<9 mm)	Size 1
	Medium (9–16 mm)	Size 2
	Large (>16 mm)	Size 3
Ecology		
Habit	Burrower	Habi 1
	Climber	Habi 2
	Sprawler	Habi 3
	Clinger	Habi 4
	Swimmer	Habi 5
Trophic habit	Collector-gatherer	Trop 1
	Collector-filterer	Trop 2
	Herbivore	Trop 3
	Predator	Trop 4
	Shredder	Trop 5

Notes: The letter in each code refers to the trait, while the number refers to the trait state.

2.3. Measurements of Environmental Factors

Environmental data were collected at each site within each stream on the same day of sampling. We measured the altitude and substrate, alongside ten environmental factors related to both hydrological indicators: velocity and depth, and water quality indices: water temperature, dissolved oxygen, electrical conductivity, pH, turbidity, ammonia nitrogen, total nitrogen, and total phosphorus. We described the composition of the substrate within each Surber sample by visually estimating the percentages of sand (<2 mm), gravel (2–16 mm), cobbles (64–256 mm), and boulders (>256 mm) following the established protocol by Cummins [49]. The water depth was measured with a depth-sounding rod, and the velocity was measured by an LS300-A portable current analyzer at a value that was 0.6-fold the water depth. The temperature, dissolved oxygen, electrical conductivity, pH, and turbidity were measured using a portable water quality analyzer (Hanna, HI9829T, Italy). The water samples were refrigerated and transported to the laboratory for analysis. Total nitrogen, ammonia nitrogen, and total phosphorus concentrations were analyzed using an ultraviolet (UV) spectrophotometer.

2.4. Data Analysis

We used the species richness, Margalef diversity, EPT richness (Ephemeroptera, Plecoptera, and Trichoptera), and density to evaluate the macroinvertebrate communities in each site of three disturbed streams. We also used functional trait percentages to indicate the composition of functional traits at each sampling site. One-way analysis of variance (ANOVA) was used to analyze the differences in environmental factors, macroinvertebrate, and relative abundance of functional traits in each stream. Where significant

ANOVA results were obtained ($p < 0.05$), a least significant difference (LSD) multiple comparisons test was conducted. The above analyses were performed in IBMSPSS 25.0 and the figures were constructed in Origin 2020. The diversity was conducted by software PAST 4.01.

The constrained ordination method was used to analyze the relationship between macroinvertebrate community distribution, functional traits, and environmental factors by using CANOCO (Version 5.0). Detrended correspondence analysis (DCA) was conducted to test for linear relationships before redundancy analysis (RDA) was used to assess the relationships between the environmental factors and community distribution in the three streams. A Monte Carlo randomization test with 499 permutations was carried out to filter key environmental factors by using forward screening. All analyses were conducted with $\log_{10}(X + 1)$ transformed abundance data.

3. Results

3.1. Environmental Factors

We observed significant differences in electrical conductivity, ammonium nitrogen, and total nitrogen in three streams ($p < 0.05$, Table 2), with higher electrical conductivity observed in S3 and higher ammonium nitrogen and total nitrogen in S1. In addition, the temperature and total phosphorus in S1 was significantly higher than S2 and S3 ($p < 0.05$, Table 2). The turbidity in S2 was significantly lower than S1 and S3 ($p < 0.05$, Table 2). Additionally, all three streams were dominated by gravels and cobbles, but the sand concentration in S1 was significantly higher than S2 and S3 ($p < 0.05$, Table 2).

Table 2. A summary of the differences in altitude (m), velocity (m·s^{-1}), depth (m), water temperature (°C), dissolved oxygen (%), electrical conductivity (μS·cm^{-1}), pH, turbidity (FNU), ammonium nitrogen (mg·L^{-1}), total nitrogen (mg·L^{-1}), total phosphorus (mg·L^{-1}), and substrate between S1, S2, and S3 (mean ± SD). The significant differences between the three streams are indicated by small letters ($p < 0.05$). S1: Grazing stream; S2: Hot spring stream; S3: Mining stream.

Environment variables	S1	S2	S3
Altitude (m)	1788.59 ± 234.31a	2417.73 ± 141.75 b	2439.45 ± 292.71 b
Velocity (m·s^{-1})	0.56 ± 0.05	0.60 ± 0.16	0.52 ± 0.17
Depth (m)	0.32 ± 0.06	0.36 ± 0.09	0.35 ± 0.09
Water temperature (°C)	7.29 ± 0.70 a	4.58 ± 0.34 b	5.10 ± 0.76 b
Dissolved oxygen (%)	93.18 ± 1.64	95.38 ± 8.30	92.44 ± 6.89
Electrical conductivity (μS·cm^{-1})	242.28 ± 46.66 a	191.08 ± 40.52 b	243.52 ± 80.92 c
pH	8.22 ± 0.41	8.10 ± 0.26	8.11 ± 0.41
Turbidity (FNU)	39.99 ± 5.11a	30.52 ± 1.54 b	38.67 ± 0.74 a
Ammonium nitrogen (mg·L^{-1})	0.34 ± 0.04 a	0.25 ± 0.01 b	0.19 ± 0.01 c
Total nitrogen (mg·L^{-1})	0.93 ± 0.05 a	0.81 ± 0.01 b	0.72 ± 0.01 c
Total phosphorus (mg·L^{-1})	0.82 ± 0.08 a	0.64 ± 0.02 b	0.59 ± 0.01 b
Substrate:			
Sands (<2 mm)	30.0% ± 10.85 a	17.9% ± 3.64 b	21.6% ± 5.65 c
Gravels (2–64 mm)	33.1% ± 3.23	35.6% ± 3.99	36.5% ± 7.01
Cobbles (64–256 mm)	30.6% ± 5.94	37.7% ± 3.9	37.5% ± 7.38
Boulders (>256 mm)	7.4% ± 6.70	8.8% ± 5.13	4.4% ± 3.21

3.2. Macroinvertebrate Community Structure in Three Disturbed Streams

The macroinvertebrate community structure of three disturbed streams were reflected in the observed species richness, Margalef diversity, EPT richness, and density (Figure 2). There were 21 taxa identified in S1, and the most abundant taxa were *Baetis* sp., Orthocladiinae sp., and *Pericoma* sp. The species richness and density significantly declined from site S1-B to site S1-C, S1-D, and S1-E in S1 ($p < 0.05$). There were 40 taxa identified in S2, and the most abundant taxa were *Iron* sp., *Nemoura* sp., and *Baetis* sp. The EPT richness and density of site S2-A significantly declined to site S2-B in S2 ($p < 0.05$). There were 40 taxa identified in S3, and the most abundant taxa were also *Baetis* sp., *Nemoura* sp., and *Iron* sp. The species richness, EPT richness, and density of site S3-A significantly rose to site S3-B in S3 ($p < 0.05$). The macroinvertebrate taxa of the three streams are shown in Table A1.

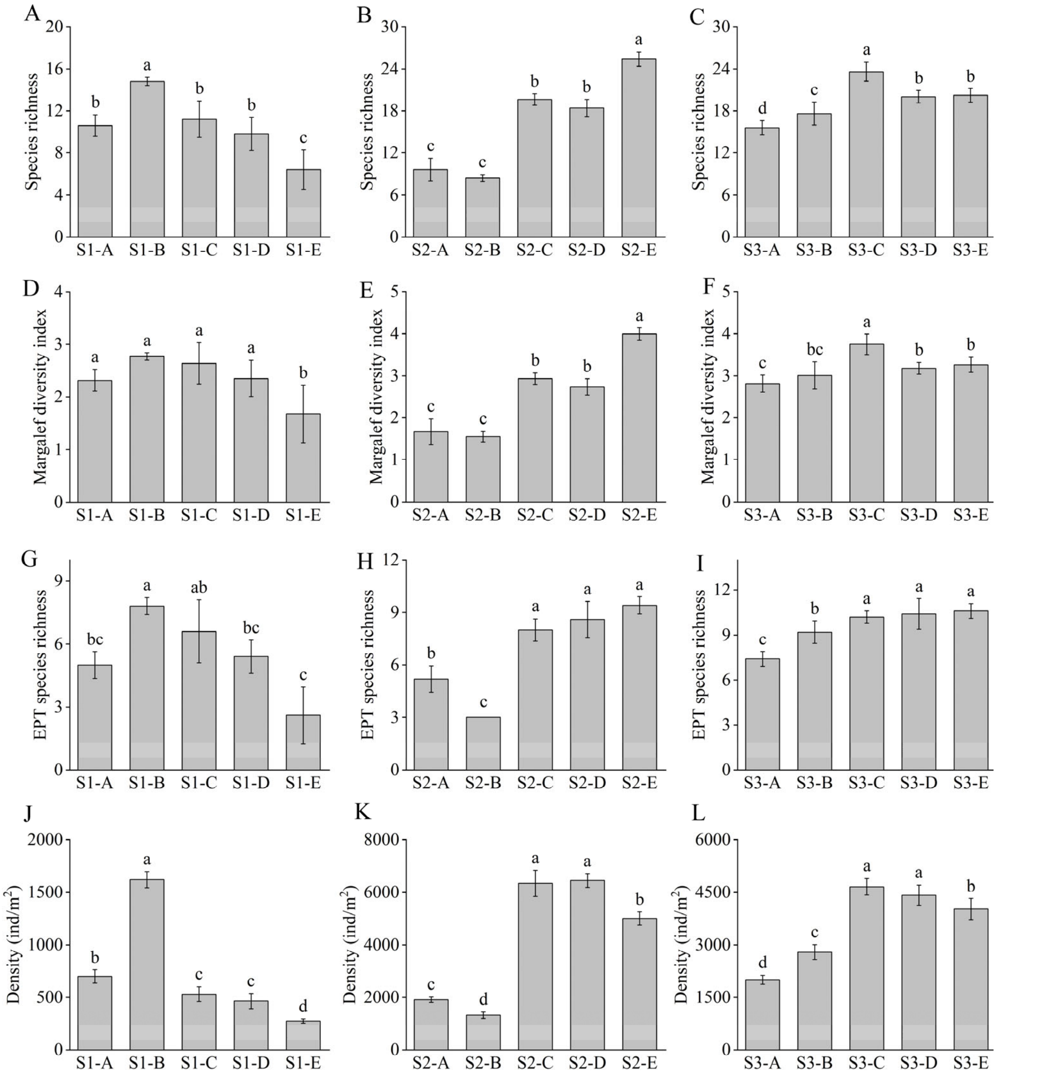

Figure 2. Differences in species richness (**A**–**C**); Margalef diversity (**D**–**F**); EPT richness (**G**–**I**), and density (**J**–**L**) in S1, S2, and S3 (mean ± SD). S1: Grazing stream; S2: Hot spring stream; S3: Mining stream. The significant differences among each site are indicated by small letters ($p < 0.05$).

3.3. Functional Traits of Macroinvertebrates

The spatial pattern of rank traits was compared and analyzed (Figure 3). The macroinvertebrate functional traits also varied between sampling sites in three streams. When considering life-history traits (Figure 3A), S1 was mainly characterized by the trait "univoltine". Additionally, the relative abundance of "univoltine" was significantly higher in sites C–E than sites A and B ($p < 0.05$). In S2, the relative abundance of "univoltine" in site B was significantly higher than site C–E ($p < 0.05$), while in S3, the relative abundance of "univoltine" was the highest in site B.

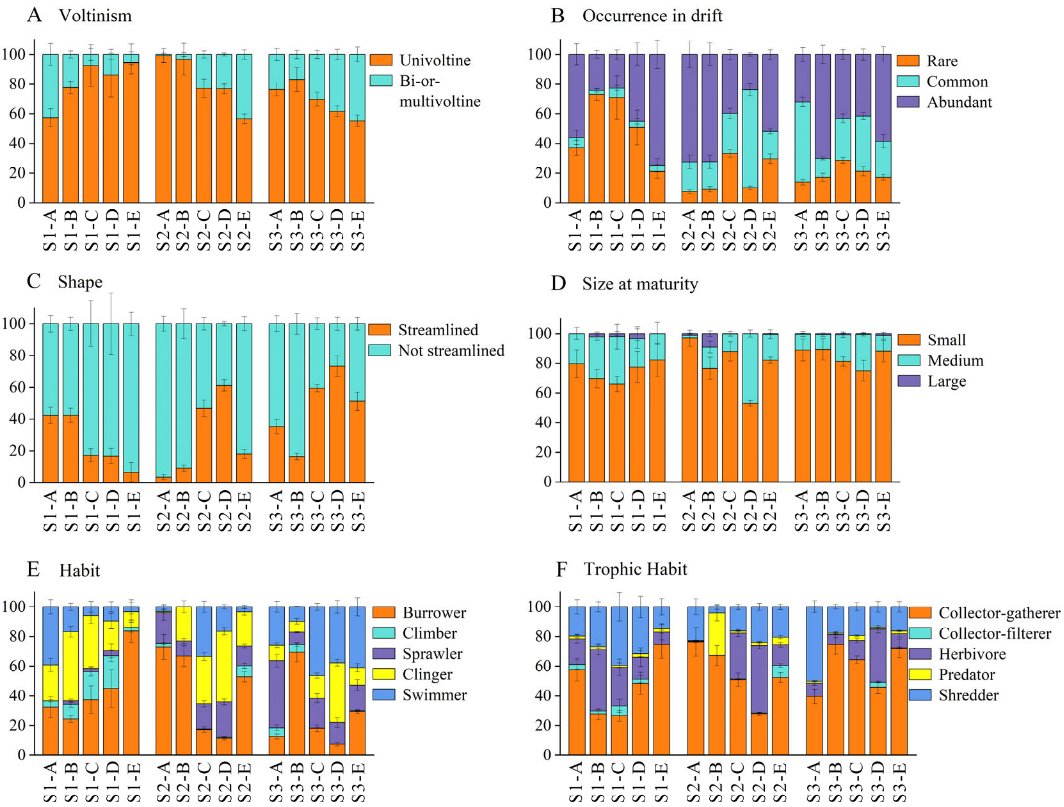

Figure 3. Average percentage (±SD) of a given trait category: voltinism (**A**); occurrence in drift (**B**); shape (**C**); size at maturity (**D**); habit (**E**), and trophic habit (**F**) of S1, S2, and S3. S1: Grazing stream; S2: Hot spring stream; S3: Mining stream.

For mobility traits (Figure 3B), the relative abundance of "abundant occurrence in drift" gradually rose in sites C–E of S1. However, the relative abundance of "rare occurrence in drift" significantly declined in sites C–E of S1 ($p < 0.05$). The relative abundance of "abundant occurrence in drift" in sites A and B were significantly higher than other sites of S2 ($p < 0.05$), while site D of S2 was mainly characterized by the trait "common occurrence in drift". The relative abundance of "abundant occurrence in drift" was significantly augmented in site B of S3 ($p < 0.05$).

For morphological traits (Figure 3C–D), sites C–E exhibited significantly higher abundances of taxa with the traits "not streamlined shape" than sites A and B of S1 ($p < 0.05$). Additionally, the S1 was mainly characterized by the trait "small size at maturity", especially for site E. The relative abundance of "streamlined shape" and "large size at maturity" gradually increased from site A to site B of S2. Additionally, site B of S3 was mainly characterized by the trait "not streamlined shape", while the S3 was mainly characterized by the trait "small size at maturity".

For ecology traits (Figure 3E–F), the relative abundances of "burrower" and "shredder" were significantly increased, while the relative abundances of "clinger" and "herbivore" were significantly decreased from site B to site C of S1 ($p < 0.05$). The relative abundances of "clinger" and "predator" were significantly increased, while the relative abundances of "sprawler" and "shredder" were significantly decreased from site A to site B of S2 ($p < 0.05$). In S3, the relative abundances of "burrower" and "collector-gatherer" were significantly increased, while the relative abundances of "swimmer" and "shredder" were significantly decreased from site A to site B ($p < 0.05$).

3.4. Relationships between Macroinvertebrate Communities and Functional Traits and Environmental Factors

The RDA tri-plot (Figure 4A) showed the relationship between the main environmental factors (forward selection) and macroinvertebrate communities. The first ordination RDA axis explained 20.53% of the variation, and the second ordination RDA axis explained 12.09% of the variation. The environmental factors that contributed most to the macroinvertebrate community were TN ($p = 0.002$) and DO ($p = 0.004$). The macroinvertebrate communities in sites C–E of S1 were mainly affected by total phosphorus, while the macroinvertebrate communities in sites C–E of S2 were mainly affected by dissolved oxygen. Different macroinvertebrates adapted to different sites. For example, *Drunella*, *Haxotoma*, and *Glossosoma* can adapt to high phosphorus concentrations (Figure 4A).

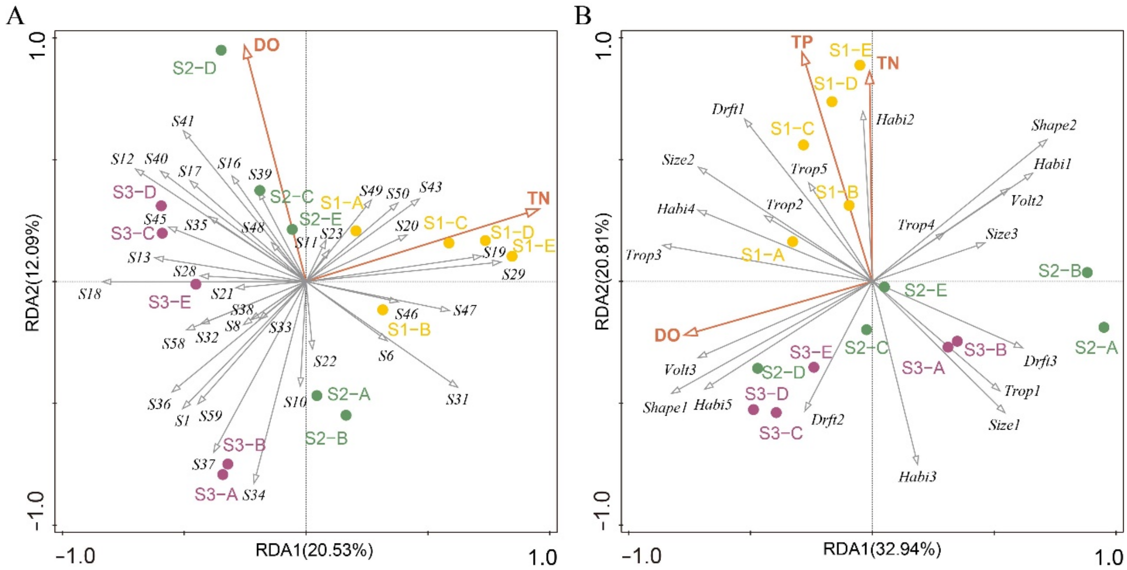

Figure 4. Redundancy analysis (RDA) ordination biplots of macroinvertebrate communities (**A**); functional traits (**B**) and main environmental factors for all sampling sites of three streams. S1: Grazing stream; S2: Hot spring stream; S3: Mining stream; DO: dissolved oxygen; TN: total nitrogen; TP: total phosphorus. Functional trait code refers to Table 1, and the species codes are as follows: S1, *Capnia* sp.; S6, *Isoperla* sp.; S8, *Nemoura* sp.; S10, *Perlodes* sp.; S11, *Suwallia* sp.; S12, *Epeorus* sp.; S13, *Cinygmula* sp.; S16, *Rhithrogena* sp.; S17, *Baetis* sp.; S18, *Ameletus* sp.; S19, *Drunella* sp.; S20, *Dicranota* sp.; S21, *Cheilotrichia* sp.; S22, *Cryptolabis* sp.; S23, *Antocha* sp.; S28, *Tipula* (*Sinotipula*) sp.; S29, *Hexotoma* sp.; S31, Orthocladiinae sp.1; S32, Orthocladiinae sp.2; S33, Orthocladiinae sp.3; S34, Orthocladiinae sp.4; S35, Tanytansini sp.; S36, Chironomini sp.; S37, *Boreoheptagyia* sp.; S38, Tanypodinae sp.; S39, *Simulium* sp.; S40, Empididae sp.1; S41, Empididae sp.2; S43, Psychodidae sp.; S45, *Blepharicera* sp.; S46, *Pseudostenophylax* sp.; S47, *Theliopsyche* sp.; S48, *Lepidostoma* sp.; S49, *Brachycentrus* sp.; S50, *Glossosoma* sp.; S58, Naididae sp.; S59, Hydrachnellae sp.

The RDA tri-plot (Figure 4B) showed the relationship between the main environmental factors (forward selection) and the macroinvertebrates' functional traits. The first ordination RDA axis explained 32.94% of the variation, and the second ordination RDA axis explained 20.81% of the variation. The environmental factors that contributed most to the macroinvertebrate functional traits were DO ($p = 0.004$), TP ($p = 0.002$), and TN ($p = 0.004$). The macroinvertebrate functional traits in S1 were mainly affected by total phosphorus and total nitrogen (Figure 4B).

4. Discussion

4.1. The Effects of Three Disturbances on Stream Environmental Factors

Human activities will affect the stream habitats and macroinvertebrate communities in our study. Higher levels of ammonia nitrogen, total nitrogen, and total phosphorus were identified in the grazing stream (S1) compared to the other two streams. These were likely caused by the manure from cattle, sheep, and horses [50]. Additionally, the S1-E sample site is next to a village, which promotes disturbances of domestic sewage. We observed some rocks covered by a thick mat of algae in sites C–E of the grazing stream. We speculated such growth is likely affected by the run-off of manure and sanitary sewage, which is corroborated by the higher values of nitrogen and phosphorous found in these sites. Moreover, livestock grazing activities can lead to increased streambank erosion [31]. This leads to a higher proportion of sediment content in the stream, which is consistent with the results for some polluted streams [51]. The increased sediment content also means that the available substrate becomes less stable, less complex, and coarse organic particulate matter for the fauna decreases [52]. As a consequence, the refuges for the biota in streams progressively disappear.

For hot springs, the water temperature has been recognized as an essential environmental factor that affects the community structure on a small [53] and large [54] spatial scale. There are 12 springs distributed in our study's hot spring scenic area, and the hot spring water temperature averages between 43 °C and 63 °C. In Sunbeam Hot Springs of Idaho, USA, spring surface water temperatures ranged from 52 to 58 °C [55]. However, in the Ethiopian Rift Valley area, the water temperature ranged from 38.5 to 90 °C in different thermal springs [56]. In our studied stream, the water temperature was higher at the sample sites (S2-B) affected by the hot springs; thus, the dissolved oxygen was lower. The RDA tri-plots also indicated that both structural and functional traits of macroinvertebrate communities at site S2-B were negatively correlated with dissolved oxygen (Figure 4). Additionally, the pH of our hot spring streams was between 7 and 8, which is consistent with the results in the Bernese Alps of Switzerland [57].

Mining disturbances typically increase sediment load and thus turbidity at the affected sample sites [58]. Drover's results also showed that community structural metrics representing taxonomic- and functional-group composition were often associated with indicators of specific conductance in central Appalachian coalfield streams [59]. Although the mine in our study had been nonfunctional for some years, the conductivity and turbidity in the streams remained high as the glacial snow meltwater flows through the mine site, because the damage to the river geomorphology (the stream bed was excavated) and mountains caused during mining has not been repaired.

4.2. The Effects of Three Disturbances on Macroinvertebrate Community Structure

The effects of grazing on macroinvertebrates were primarily feces, trampling, and disturbances caused by drinking and foraging. Some studies have reported higher species richness and diversity in forest streams relative to streams flowing through pastures [60,61]. Our results also showed the diversity and density decreased at sites C–E of the grazing stream when grazing intensity increased. However, there were some taxa sensitive to environmental changes such as EPT species in the grazing stream [62]. Our findings showed that EPT species were also present in the grazing stream, but they decreased with the presence of grazing disturbance. Some Diptera, such as Chironomidae, were more abundant in the grazing site (S1-C–E), mainly due to their characteristics that can tolerate degraded environments. The *Glossosoma* (Trichoptera) was associated with grazing sites; they have the functional trait "herbivore" and mainly feed on algae and organic particles [63]. Streams that flow through pastures are more likely to have forage flowing into them. Even so, they are usually considered intolerant to severe environmental degradation [64,65]. We investigated the presence of *Glossosoma* in grazing streams, providing further evidence that the grazing sites studied here, although disturbed, were not heavily degraded. Most macroinvertebrates found in hot springs and mine sites were present in grazing sites, but with lower densities (especially for sites C–E of the grazing stream). Some studies also indicated that macroinvertebrate fauna responded well to the low level of nutrient enrichment, in addition to other impairments caused by agriculture and pasture, such as physical habitat alterations [66].

Thermal spring ecosystems are extremely susceptible to anthropogenic activities because they are small and isolated, provide specific services, and are easily altered [32]. The French Pyrénéés [67] demonstrated a high α-and β-diversity of the organism for Alpine springs. In our study, the species richness and biodiversity of macroinvertebrates affected by the hot spring development at site B decreased compared to the undisturbed site (S2-A). The Ethiopian Rift Valley region's thermal springs had no sensitive taxa groups such as Ephemeroptera, Plecoptera, and Trichoptera (EPT species); instead, they were dominated by the dipterans and coleopteran groups [56]. EPT species also existed in the hot spring-influenced streams we studied and increased as the influence of the hot springs diminished. Water temperature is one of the factors that strongly affects the species composition in freshwater ecosystems [68]. The study performed in Ethiopia's highland thermal springs [69] also indicated that macroinvertebrate communities could exist in waters up to 52 °C. Most of the glacier-fed streams are characterized by low water temperatures, and this has led to the identification of endemic species that have adapted to the harsh environment of low temperatures. However, the presence of hot springs caused higher water temperatures at the sampling sites and an increase in the number of some species adapted to hot spring habitats, such as water mites, which was consistent with previous studies [70].

Mining disturbances affected macroinvertebrate community structure by disturbing stream turbidity, conductivity, etc. Further, Heishman and Mcluky [71] showed a strong positive correlation between very high electronic conductivity and the absence of an aquatic macroinvertebrate community. Stream communities could respond quickly to the restoration of physical characteristics and increased heterogeneity [72]. However, the study in the central Sierra Nevada of California showed that biological integrity in streams nearest the mine might be year-round to recover fully [73]. Dispersal is likely a primary colonization obstacle of macroinvertebrates in a restored stream besides water quality [74,75]. The mining stream in the Tianshan Mountains is currently in the self-recovery stage following the closure of the mine ten years ago. The species richness, diversity, and density in site B of the mine were larger than in site A owing to previous mining activities, which led to habitat fragmentation and the division of formerly patchy habitats into several isolated and discontinuous habitats [41]. This has prevented the upward dispersal of macroinvertebrates, leading to the accumulation of some macroinvertebrates at site B of the mine stream.

The site A of S3 had lower dissolved oxygen than the other four sampling sites since it is the source of the stream with lower velocity. The RDA results (Figure 4A) also showed that site S3-A was negatively correlated with dissolved oxygen. Some Trichoptera, such as Lepidostomatidae, have a larger proportion in this environment since they breathe with gills and can survive in lower oxygen environments. Lower oxygen availability should promote the presence of taxa with gills that help them increase their rates of oxygen uptake. Most mining streams have considered the effect of pH on macroinvertebrate communities because acidic conditions may result in toxicity. Most commonly, a decrease in pH will result in an increase in the availability of toxic free metal ions due to changes in metal morphology, mobility, and bioavailability [72]. The macroinvertebrate communities decreased gradually with increasing acidity to acid mine drainage in rivers of the High Andes (Bolivia), and pH was the best predictor of macroinvertebrate community richness [76]. However, our results indicated that the effect of pH on the macroinvertebrate of the mine streams was not significant, probably due to the dormant nature of the mine.

4.3. The Effects of Three Disturbances on Macroinvertebrate Functional Traits

The difference in the relative abundance of traits is the result of habitat filtering, whereby hierarchical traits with a higher relative abundance can be regarded as better adapted to the regional environment [77]. Species with the trait "univoltine" or "semivoltine" require habitats with long-term stability to grow and reproduce, whereas "bi- or multivoltine" species can maintain populations in constantly evolving habitats [78].

Indeed, the lower abundance of "semivoltine" species in the grazing stream site indicates the partial degradation of habitat stability. "Mobility" and "habit" traits are associated with dispersal capacity, resource availability, and competition [79]. The decrease in *Baetis* sp. abundance was the main contributor to the decrease in individuals with "abundant occurrence in drift" and "swimmer" traits in the grazing stream, indicating a decline in water quality and the destruction of food web complexity. Higher temperatures at the hot spring sampling sites increased the "burrower" relative abundance through an increase in Chironomidae. Springs in the Alps were characterized by a typical "burrower" biocoenosis [58]. The mining stream had higher turbidity and sediment content in the streams, and some burrowing organisms such as *Pseudostenophylax* (Trichoptera) adapted to this environment and increased significantly.

The "shape" traits reflect the ability of macroinvertebrates to avoid adverse habitats to a certain extent. The relative abundance of "not streamlined" in the hot spring site and mine site was significantly higher than other later sampling sites ($p < 0.05$), demonstrating that the two sites are more susceptible to hydrological disturbance. Our results showed that the relative abundance of "small size at maturity" taxa was the highest, and the three disturbances had little effect on this trait. This result supports the hypothesis that being small-bodies may offer resilience to environmental conditions. Clinging or attaching to the substrate may provide resistance to the high hydraulic stress experienced by invertebrates in glacier-fed streams [80,81]. Furthermore, small individuals usually have a shorter life cycle (*r*-selection) so that the community can recover faster after being disturbed [82]. Nutritional habits reflect material circulation and energy flow in the ecosystem where the communities are located. The relative abundance of collectors was the highest in hot springs and mine streams, indicating that the macroinvertebrates of the two glacier-fed streams mainly played the role of secondary producers in the water ecosystem [23]. A larger proportion of herbivores was present in grazing streams because some forage plants and litter leaves accumulated in the streams' channels, supplying sufficient food sources. Longitudinal distribution of macroinvertebrates in our studied streams partially fitted to prediction models for glacierized temperate systems, for example, Perlodidae, Taeniopterygidae, Baetidae, Simuliidae, and Empididae can be expected to occur above 4 °C [83].

5. Conclusions

We investigated the effects of grazing, hot spring development, and historic mine development on macroinvertebrate community structure and functional traits in glacier-fed streams in the Tianshan Mountains of China. The macroinvertebrate communities decreased due to grazing and hot springs, and the habitat fragmentation caused by the previous mining activities prevented the upward dispersal of macroinvertebrates in the mining stream. These findings reflected human disturbances in the Tianshan Mountains have affected the natural habitat conditions and macroinvertebrate communities. Taxa with resilience traits such as "small size at maturity" increased in the grazing stream, while taxa with resistance traits such as "bi- or multivoltine" and "abundant occurrence in drift" were identified in the hot spring stream and mining stream. Such results reflected the effects of human interference on resource-use efficiency and functional diversification in this region. Therefore, it is important to reduce human activity to conserve the stream ecosystems in the Tianshan Mountains. The results of our study are limited in some aspects due to the natural environment and geographical location, but the human disturbances in the environment and biodiversity changes must be treated with caution. Moreover, our results showed that functional indicators of macroinvertebrate communities are helpful for monitoring and evaluating habitat conditions.

Author Contributions: Yuwei Liu and Yilin Tian contributed equally to this work and should be considered co-first authors. Conceptualization, Y.L. and Y.T.; methodology, Y.L. and Y.T.; software, Y.L. and Y.T.; validation, Y.G.; formal analysis, Y.L., Y.T., and Y.G.; investigation, Y.L., Y.T., D.C.,

W.Z., Z.J., F.Y., and Z.Z.; resources, H.Y.; data curation, Y.G.; writing—original draft preparation, Y.L. and Y.T.; writing—review and editing, Y.L., Y.T., and Y.G.; visualization, H.Y.; supervision, H.Y.; project administration, H.Y.; funding acquisition, H.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (grant number 31860139; 32071587), the Scientific and Technological Aid Project of Science and Technology Department of Xinjiang Uygur Autonomous Region (grant number 2018E02095), and Program of Introducing Talents of Discipline to Universities (grant number B16011).

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A

Table A1. Macroinvertebrate taxa list of the grazing stream, hot spring stream, and mining stream in April.

Class	Order	Family	Subfamily/Genus	Grazing Stream	Hot Spring Stream	Mining Stream
Oligochaeta	Haplotaxida	Naididae sp.			+	+
Gastropoda	Basommatophora	Planorbidae	*Anis* sp.		+	
Malacostraca	Amphipoda	Gammaridae	*Gammarus* sp.			+
Insect	Ephemeroptera	Heptageniidae	*Iron* sp.	+	+	+
			Cinygmula sp.		+	+
			Heptagenia sp.			+
			Rhithrogena sp.	+	+	+
		Baetidae	*Baetis* sp.	+	+	+
		Ameletidae	*Ameletus* sp.		+	+
		Ephemerellidae	*Drunella* sp.	+		
	Plecoptera	Capniidae	*Capnia* sp.		+	+
		Taeniopterygidae	*Rhabdiopteryx* sp.			+
		Perlodidae	*Arcynopteryx* sp.			+
			Isoperla sp.	+	+	+
			Diura sp.			+
			Perlodes sp.		+	+
		Nemouridae	*Nemoura* sp.	+	+	+
		Chloroperlidae	*Suwallia* sp.	+	+	
	Trichoptera	Limnephilidae	*Pseudostenophylax* sp.	+	+	+
		Lepidostomatidae	*Theliopsyche* sp.	+	+	+
			Lepidostoma sp.		+	+
		Brachycentridae	*Brachycentrus* sp.	+	+	+
		Glossosomatidae	*Glossosoma* sp.	+	+	+
		Hydropsychidae	*Hydropsyche* sp.	+		
	Diptera	Tipulidae	*Dicranota* sp.	+	+	+
			Cheilotrichia sp.		+	+
			Cryptolabis sp.		+	
			Antocha sp.	+	+	+
			Tipula (*Arctotipula*) sp.		+	
			Tipula (*Sinotipula*) sp.		+	+
			Hexotoma sp.	+	+	+

			Pedica sp.		+	
		Chironomidae	Orthocladiinae sp.1	+	+	+
			Orthocladiinae sp.2	+	+	+
			Orthocladiinae sp.3	+	+	+
			Orthocladiinae sp.4		+	+
			Tanytansini sp.	+	+	+
			Chironomini sp.		+	+
			Tanypodinae sp.		+	+
		Diamesinae	*Boreoheptagyia* sp.		+	+
		Simuliidae	*Simulium* sp.	+	+	+
		Empididae sp.1			+	+
		Empididae sp.2			+	+
		Blephariceridae	*Blepharicera* sp.		+	+
		Ceratopogonidae sp.			+	
		Psychodidae	*Pericoma* sp.	+	+	+
		Dixdae	*Dixa* sp.			+
Arachnida	Acariformes	Hydrachnellae sp.			+	+

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