

Article

The Impacts of the Freezing–Thawing Process on Benthic Macroinvertebrate Communities in Riffles and Pools: A Case Study of China’s Glacier-Fed Stream

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Abstract: Glacier-fed streams are one of the environments most sensitive to global climate change. However, the effects of the freezing–thawing process on benthic macroinvertebrate communities in different habitats of glacier-fed streams are unclear. In this paper, we investigated benthic macroinvertebrates in riffles and pools of a glacier-fed stream in Xinjiang, China, during the pre-freezing period (November, 2018), freezing period (January 2019), and thawing period (April, 2019). Our results showed that the freezing–thawing process resulted in a decline in benthic macroinvertebrate species richness and diversity, both of which were attributed to the effects of the freezing–thawing process on habitat stability, water quality, and cycling of the stream ecosystems. During the whole freezing–thawing process, the indicator taxa of riffles were *Rhithrogena* sp. and *Baetis* sp., while the only indicator taxon of pools was *Chironomus* sp. The species richness, Margalef diversity, and EPT richness (Ephemeroptera, Plecoptera, and Trichoptera) of benthic macroinvertebrates in riffles were higher than those in pools, due to the higher habitat heterogeneity in the riffles. However, the density in riffles was significantly lower than that in pools during the freezing period ($p < 0.05$). Additionally, pools were dominated by taxa with higher resilience and resistance traits, such as “bi- or multi-voltine”, “abundant occurrence in drift”, and “small size at maturity”. This result indicated that pools provide a temporary refuge for benthic macroinvertebrates in the extreme environment of glacier-fed streams. The freezing–thawing process plays an essential role in the formation of the structure and function of the stream ecosystem. Our results can help us to further understand the winter ecological process of headwater streams, and provide a reference for stream biodiversity conservation in cold regions.

Keywords: glacier-fed stream; benthic macroinvertebrates; freezing–thawing process; riffles; pools



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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 environmental conditions, such as low water temperature and channel stability, and high turbidity and dissolved oxygen [4]. These characteristics are related to the freezing–thawing process [5], which directly causes changes in physical and chemical factors in the habitat. Harsh environmental conditions may reduce the local species diversity of glacier-fed streams; however, it has been found

that some endemic taxa have adapted to these extreme conditions [6–8]. The freezing–thawing process includes three periods: the pre-freezing period (PP), freezing period (FP), and thawing period (TP). Previous studies have mainly focused on the impact of floods generated by the thawing period on river aquatic organisms [9–12]. However, less attention has been paid to the impacts of the whole freezing–thawing process on aquatic organisms in rivers.

Benthic macroinvertebrates are one of the most diverse groups of aquatic organisms and are often used as bioindicators of water quality. They play an essential role in the circulation of matter and the energy flow of aquatic ecosystems [13]. Previous studies have mainly focused on the structure of benthic macroinvertebrate communities and their distribution in glacier-fed streams [2,14]; however, few studies have focused on how environmental change influences their functional traits [3,15]. Functional traits can reveal ecological and evolutionary differences among species [16,17] and can also reflect selectivity and adaptability among different habitats and benthic macroinvertebrates [18,19].

Sharp discontinuities often exist between channel geomorphic units, giving rise to the characteristic riffle–pool sequences [20]. There are differences in geomorphic morphology, hydraulic characteristics, and changes in deposition between riffles and pools [21]. This has led to significant differences in the benthic macroinvertebrate communities found in the two habitats [22,23]. The presence and distribution of benthic macroinvertebrates are closely related to river habitats. For example, the ratio of lentic to lotic habitat features strongly affects macroinvertebrate metrics in southern Europe [24]. Additionally, some disturbance processes can also affect the benthic macroinvertebrate communities in both lentic and lotic ecosystems [25]. Riffles and pools are the most common habitat structures in glacier-fed streams. Freezing has different effects on the two habitats. The study of these two critical habitats (riffles and pools) can reveal the distribution pattern and ecological influence of benthic macroinvertebrates in glacier-fed streams during the freezing–thawing process.

We investigated the community structure and functional traits of benthic macroinvertebrates in the headwater of a glacier-fed stream in Xinjiang, China. We mainly focused on the impacts of the freezing–thawing process on benthic macroinvertebrates in riffles and pools. We hypothesized that the freezing–thawing process causes a wide range of habitat changes, leading to significant changes in species composition, biodiversity, and functional properties during different winter periods. The fewest changes were predicted to occur during the freezing period. We also predicted that the diversity and density of benthic macroinvertebrate communities in riffles would be higher than that in pools in glacier-fed streams.

2. Materials and Methods

2.1. Study Area

The surveyed stream is located in the Banchan Nature Reserve of Tianshan Mountains, Xinjiang Province, China (N 43°17′ 51.229″–43°13′52.591″, E 84°51′45.844″–84°43′38.392″) (Figure 1). It is the source of the Gongnaisi River, one of the three tributaries of the Ili River, and is mainly affected by the melting of glaciers and snow. The study area is located in the hinterland of the western Tianshan Mountains, at an altitude of 2150–2511 m, which belongs to the continental temperate climate zone. In this area, winter lasts nearly five months, from November to April, and seasonal freezing–thawing characteristics are apparent. The annual average temperature is 8–9.2 °C, and the annual snowfall is 300–418 mm. We established five sampling sites along the surveyed stream (Figure 1A), to study the impact of the freezing–thawing process on the structural and functional characteristics of benthic macroinvertebrate communities in glacier-fed streams. Each sampling site was approximately 2.5 km apart.

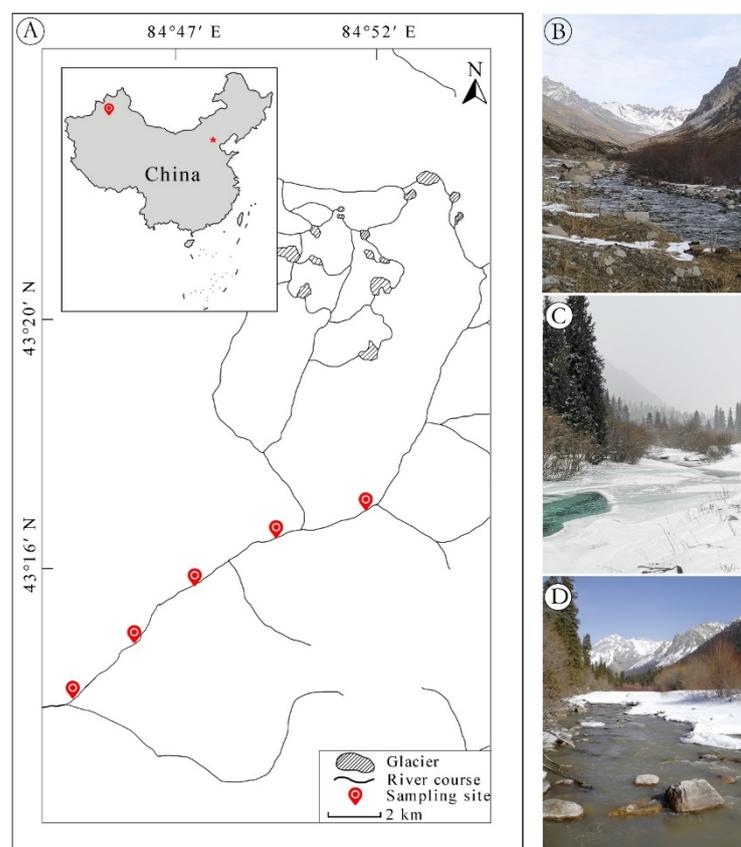


Figure 1. Locations of the Ili River Basin in the People’s Republic of China, and the distribution of the five sampling sites (A). The pre-freezing period (B). The freezing period (C). The thawing period (D). Glacier data site from the second glacier inventory dataset of China [26].

2.2. Benthic Macroinvertebrate Collection

Benthic macroinvertebrates were sampled across three periods in winter: the pre-freezing period (November in 2018), freezing period (January in 2019), and thawing period (April in 2019). The sample size was $n = 5$. Each site was sampled using a Surber net (30 × 30 cm, 500 μm mesh size) in a riffle–pool sequence. Three replicated samples in riffles and three replicated samples in pools were carried out in each site. The specimens were filtered through sieves with a 500 μm mesh size 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 laboratory, the benthic macroinvertebrates were identified to the lowest possible taxonomic level (usually genus) under a stereoscopic microscope (Olympus SZ × 10).

2.3. Environmental Characterization

We measured eight environmental factors, including velocity, depth, width, temperature, dissolved oxygen, electrical conductivity, pH, and substrate. Environmental factors were measured at five sampling sites (the sample size was $n = 5$), and three replicated measurements were made in each sampling site. The river width was measured with a meter scale, the water depth was measured with a depth-sounding rod, and the velocity was measured with a LS300-A portable current analyzer, at a value that was 0.6-fold the water depth. The temperature, dissolved oxygen, electrical conductivity, and pH were measured using a portable water quality analyzer (Hanna, HI9829T). According to Cummins [27],

the substrate was classified as boulders, gravel, sand, and silt. The average substrate score (MSUBSTD) was calculated [28] as follows (1) and (2):

$$\text{MSUBSTD} = \frac{-7.75 \times \text{BOLDCOBB} - 3.25 \times \text{PEBBGRAV} + 2 \times \text{SAND} + 8 \times \text{SILTCLAY}}{\text{TOTSUB}} \quad (1)$$

$$\text{TOTSUB} = \text{BOLDCOBB} + \text{PEBBGRAV} + \text{SAND} + \text{SILTCLAY} \quad (2)$$

BOLDCOBB, PEBBGRAV, SAND, and SILTCLAY indicate the percentage cover of bolder/cobble, pebble/gravel, sand, and silt/clay, respectively. A higher score indicates higher proportions of sand and silt, whereas a lower score indicates a higher proportion of large rocks and cobble.

2.4. Functional Traits

All benthic macroinvertebrate taxa were assigned to different functional traits. These traits were compiled from database information published by Poff et al. [18] and Moretti et al. [29]. According to the habitat properties of the watershed, we selected ten biological traits coded in 31 states, as follows: the life history (voltinism), mobility (occurrence in drift and swimming ability), morphology (attachment, shape, and size at maturity), and ecology (rheophily, thermal preference, habit, and trophic habit) (Table A1).

2.5. Data Analysis

The autocorrelation test results, regarding environmental and macroinvertebrate data via the Mantel test, were not statistically significant ($p > 0.05$). The benthic macroinvertebrate data were shown to have a normal distribution, after applying the normal distribution test. Therefore, we used related-samples t-tests to analyze the differences in environmental factors, species richness, biodiversity, EPT richness, density, and relative abundance of functional traits in riffles and pools. Additionally, repeated-measures ANOVA was used to compare the parameters mentioned above between different periods. Where significant ANOVA results were obtained ($p < 0.05$), least significant difference (LSD) multiple comparisons tests were conducted. The above analyses were performed in IBMSPSS 25.0, and the figures were constructed in Origin 2020. The software PAST 4.01 was used to calculate diversity.

We used indicator species analysis (ISA) to determine indicator taxa in riffles and pools. ISA uses the indicator value (IV), which combines information regarding relative abundance (RA) and relative frequency (RF) of the species, to reflect the intensity of the effect of the species on the environment [30,31]. The IV calculation formula is $IV = 100 \times RA \times RF$. RA refers to the ratio of the abundance of a particular species in a particular type of environment to the total species in all environmental types. RF refers to the frequency at which a species appears in a sample of a specific environment. A related-samples t-tests was used to determine the significant differences in the indicator species of riffles and pools in each period. Factors with $p < 0.05$ were considered significant. Canonical correlation analysis (CCA) was used to analyze the relationship between benthic macroinvertebrate community distribution and environmental factors, using CANOCO (version 5.0). A Monte-Carlo randomization test with 499 permutations was carried out to filter key environmental ($p < 0.05$) factors during the freezing–thawing period, using forward screening. All analyses were conducted with $\log_{10}(X + 1)$ transformed abundance data.

3. Results

3.1. Environmental Characteristics

We observed significant differences in velocity, depth, and MSUBSTD between riffles and pools during the freezing–thawing process ($p < 0.05$, Table 1), with higher values of depth and MSUBSTD observed in pools. The higher MSUBSTD value indicated that the substrate in riffles was mostly cobble and pebble, with limited sand, whereas there were high levels of sand and gravel in pools. The dissolved oxygen level in riffles was

significantly higher than that in pools during the pre-freezing period and thawing period ($p < 0.05$, Table 1). The electrical conductivity level in riffles was significantly higher than that in pools in the pre-freezing period ($p < 0.05$, Table 1). The temperature and pH in pools were lower than those in riffles during the thawing period ($p < 0.05$, Table 1).

Table 1. Summary of the differences in velocity ($\text{m}\cdot\text{s}^{-1}$), depth (m), width (m), temperature ($^{\circ}\text{C}$), dissolved oxygen ($\text{mg}\cdot\text{L}^{-1}$), electrical conductivity ($\text{ms}\cdot\text{cm}^{-1}$), pH, and MSUBSTD, between riffles and pools during the winter freezing–thawing process (mean \pm SD). In each period, significant differences between the two habitats are indicated by bold font ($p < 0.05$). Sample size ($n = 5$).

Parameters	Pre-Freezing Period		Freezing Period		Thawing Period	
	Riffles	Pools	Riffles	Pools	Riffles	Pools
Velocity ($\text{m}\cdot\text{s}^{-1}$)	0.35 \pm 0.10 A	0.20 \pm 0.05 a	0.20 \pm 0.01 B	0.18 \pm 0.00 a	0.50 \pm 0.03 C	0.30 \pm 0.04 b
Depth (m)	0.26 \pm 0.03 A	0.39 \pm 0.05 a	0.10 \pm 0.03 B	0.31 \pm 0.02 b	0.30 \pm 0.05 A	0.41 \pm 0.04 a
Width (m)	4.25 \pm 0.30 A	5.18 \pm 0.60 ab	4.19 \pm 0.28 A	5.01 \pm 0.53 a	5.48 \pm 0.25 B	5.71 \pm 0.16 b
Temperature ($^{\circ}\text{C}$)	1.74 \pm 0.04 A	1.75 \pm 0.04 a	0.35 \pm 0.11 B	0.36 \pm 0.12 b	4.77 \pm 0.09 C	4.27 \pm 0.05 c
Dissolved oxygen ($\text{mg}\cdot\text{L}^{-1}$)	9.25 \pm 0.03 A	9.01 \pm 0.09 a	8.66 \pm 0.12 B	8.62 \pm 0.10 b	9.34 \pm 0.03 C	9.14 \pm 0.04 c
Electrical conductivity ($\text{ms}\cdot\text{cm}^{-1}$)	0.15 \pm 0.05 A	0.12 \pm 0.04 a	0.23 \pm 0.02 B	0.22 \pm 0.01 b	0.29 \pm 0.03 C	0.26 \pm 0.02 c
pH	7.83 \pm 0.19 A	7.73 \pm 0.09 a	7.86 \pm 0.04 A	7.86 \pm 0.03 b	8.18 \pm 0.03 B	8.04 \pm 0.05 c
MSUBSTD	−4.14 \pm 0.66 A	1.26 \pm 0.88 a	−4.16 \pm 0.64 A	1.26 \pm 0.92 a	−4.16 \pm 0.64 A	1.26 \pm 0.92 a

3.2. Indicator Taxa in the Riffle–Pool Habitat

In total, 46 taxa were identified in the study area; of these taxa, 25 were identified in riffles and 23 were identified in pools (Table A2). There were 12 indicator taxa for riffles and six for pools during the pre-freezing period, 11 indicator taxa for riffles and 12 for pools during the freezing period, and 14 indicator taxa for riffles and nine for pools during the thawing period. During the whole freezing–thawing process, the indicator taxa were *Rhithrogena* sp. and *Baeits* sp. in riffles and *Chironomus* sp. in pools. There were two indicator taxa for different habitats at different periods. *Psychodidae* sp., which was an indicator species for riffles during the pre-freezing period and thawing period, indicated pools during the freezing period. *Theliopsyche* sp. was an indicator taxon for riffles during the pre-freezing period, but it was an indicator taxon for pools during the freezing period (Table 2).

Table 2. The indicator taxa in riffles and pools during the pre-freezing period, freezing period, and thawing period. * denotes a significant difference between riffles and pools ($p < 0.05$). Sample size ($n = 5$).

Taxa	Indicated Habitat		
	Pre-Freezing Period	Freezing Period	Thawing Period
Turbellaria			
Tricladida			
Planariidae			
<i>Planaria</i> sp.			Pools *
Oligochaeta			
Haplotaxida			
<i>Naididae</i> sp.			Pools *
Gastropoda			
Basommatophora			
Planorbidae			
<i>Anis</i> sp.		Pools *	
Malacostraca			
Amphipoda			
Gammaridae			
<i>Gammarus</i> sp.			Pools *

Table 2. Cont.

Taxa	Indicated Habitat		
	Pre-Freezing Period	Freezing Period	Thawing Period
Insect			
Ephemeroptera			
Heptageniidae			
<i>Rhithrogena</i> sp.	Riffles *	Riffles *	Riffles *
<i>Iron</i> sp.	Riffles *		Riffles *
<i>Heptagenia</i> sp.		Riffles *	
<i>Epeorus</i> sp.		Riffles *	
<i>Cinygmula</i> sp.			Riffles *
Baetidae			
<i>Baetis</i> sp.	Riffles *	Riffles *	Riffles *
Ameletidae			
<i>Ameletus</i> sp.		Riffles *	Riffles *
Plecoptera			
Perlodidae			
<i>Isoperla</i> sp.	Riffles *		
<i>Perlodes</i> sp.		Riffles *	
<i>Starsolus</i> sp.	Riffles *		
Nemouridae			
<i>Nemoura</i> sp.	Riffles *		Riffles *
<i>Amphinemura</i> sp.		Pools *	
Capniidae			
<i>Capnia</i> sp.		Riffles *	Riffles *
Chloroperlidae			
<i>Suwallia</i> sp.		Riffles *	
Coleoptera			
Haliplidae			
<i>Haliplus</i> sp.	—	—	—
Trichoptera			
Hydropsychidae			
<i>Homoplectra</i> sp.	—	—	—
Glossosomatidae			
<i>Glossosoma</i> sp.		Riffles *	
Lepidostomatidae			
<i>Theliopsycha</i> sp.	Riffles *	Pools *	
Brachycentridae			
<i>Brachycentrus</i> sp.	Riffles *		Riffles *
<i>Amiocentrus</i> sp.		Pools *	
Rhyacophilidae			
<i>Rhyacophila</i> sp.			Pools *
Limnephilidae			
<i>Desmona</i> sp.	—	—	—
<i>Pseudostenophylax</i> sp.	—	—	—
Diptera			
Tipulidae			
<i>Dicranota</i> sp.	Pools *	Pools *	
<i>Cheilotrichia</i> sp.		Pools *	
<i>Tipula (Arctotipula)</i> sp.	Pools *		Pools *
<i>Tipula (Sinotipula)</i> sp.		Pools *	
<i>Hexatoma</i> sp.			Riffles *
<i>Antocha</i> sp.	Riffles *		
Chironomidae			
Chironominae			
Tanytarsini sp.	Pools *		Pools *
<i>Chironomus</i> sp.	Pools *	Pools *	Pools *
Orthocladiinae sp.1		Pools *	Riffles *
Orthocladiinae sp.2	Pools *		Pools *
Orthocladiinae sp.3			Riffles *
Tanypodinae sp.	Pools *	Pools *	
Ceratopogonidae			
<i>Sphaeromias</i> sp.		Pools *	Pools *
Psychodidae sp.			
Riffles *	Riffles *		Riffles *
Blefariceridae sp.			
Riffles *	Riffles *		
Tabanidae sp.1			
Riffles *			Riffles *
Tabanidae sp.2			
Riffles *		Riffles *	
Blephariceridae			
<i>Blepharicera</i> sp.			Riffles *

Table 2. *Cont.*

Taxa	Indicated Habitat		
	Pre-Freezing Period	Freezing Period	Thawing Period
Arachnida			
Acariformes			
Hydrachnellae sp.		Riffles *	

3.3. Benthic Macroinvertebrate Community Structure in the Riffle–Pool Habitat

The differences in the community structure of benthic macroinvertebrates between riffles and pools were reflected in the observed species richness, Margalef diversity, EPT richness (Ephemeroptera, Plecoptera, and Trichoptera), and density (Figure 2). In general, the species richness and Margalef diversity values in riffles tended to be higher than those in pools, and they showed a significant difference during the freezing period and thawing period ($p < 0.05$, Figure 2A,B). EPT richness was significantly higher in riffles than in pools, during the three periods ($p < 0.05$, Figure 2C). The density in riffles showed a significantly higher value than in pools in the pre-freezing period, but it was significantly lower than in pools in the freezing period ($p < 0.05$, Figure 2D).

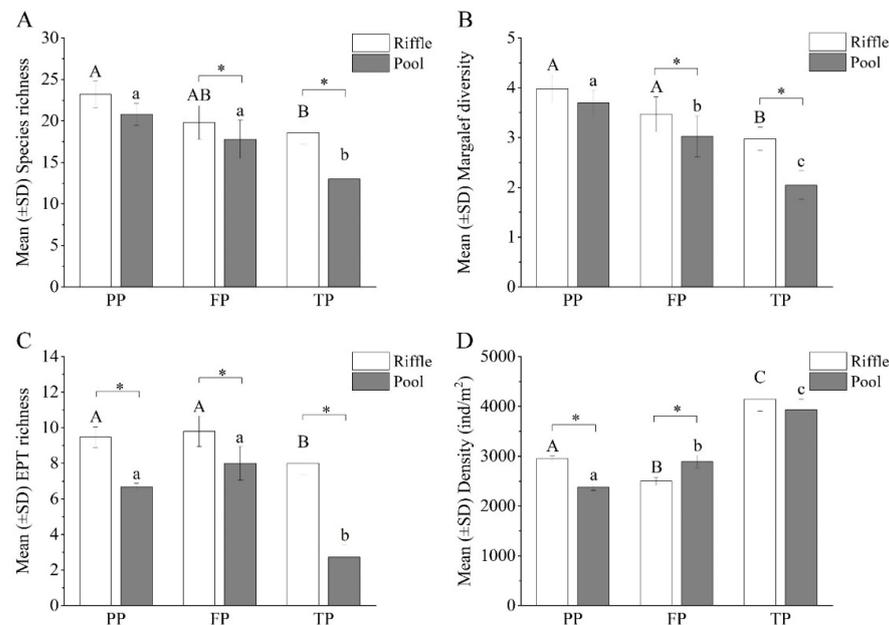


Figure 2. Differences in species richness (A), Margalef diversity (B), EPT richness (C), and density (D) between riffles and pools during the pre-freezing period (PP), freezing period (FP), and thawing period (TP) (mean ± SD). Different capital and small letters indicate significant differences among the three periods in riffles and pools, respectively. * denotes a significant difference between the two habitats ($p < 0.05$). Sample size ($n = 5$).

3.4. Functional Traits of Benthic Macroinvertebrates

The spatial pattern of rank traits was compared and analyzed (Figure 3). When considering life history traits, the relative abundance of “univoltine” taxa was significantly higher in riffles than in pools, whereas that of “bi- or multi-voltine” taxa showed the opposite result ($p < 0.05$; Figure 3A). For mobility traits, there were more taxa with “common occurrence in drift” and “rare occurrence in drift” in riffles than in pools. However, the relative abundance of “abundant occurrence in drift” was higher in pools than in riffles ($p < 0.05$; Figure 3B). The relative abundance of “strong swimming ability” and “weak swimming ability” was higher in riffles, while that of “none swimming ability” accounted for the largest proportion in pools ($p < 0.05$; Figure 3C). In terms of morphological traits, riffles exhibited significantly higher abundances of taxa with the traits “both attachment”, “streamlined shape”, and “medium

size at maturity”, whereas pools exhibited significantly higher abundances of taxa with the traits “none attachment”, “not streamlined shape” and “small size at maturity” ($p < 0.05$; Figure 3D–F). For ecology traits, pools were characterized by a significantly higher relative abundance of “depositional only” and “cool eurythermal” taxa than riffles ($p < 0.05$; Figure 3G,H). In terms of habit, “burrower” had an absolute advantage in pools during the three periods. However, the dominant taxa in riffles were “clinger” in the pre-freezing period and “swimmer” in the freezing period and thawing period ($p < 0.05$; Figure 3I). The relative abundance of “collector–gatherer” taxa was higher in pools than in riffles. In comparison, in riffles, the relative abundance of “herbivore” and “collector–filterer” taxa were higher in the pre-freezing period, the relative abundance of “shredder” and “predator” taxa were higher in the freezing period, and the relative abundance of “swimmer” and “herbivore” taxa were higher in the thawing period ($p < 0.05$; Figure 3J).

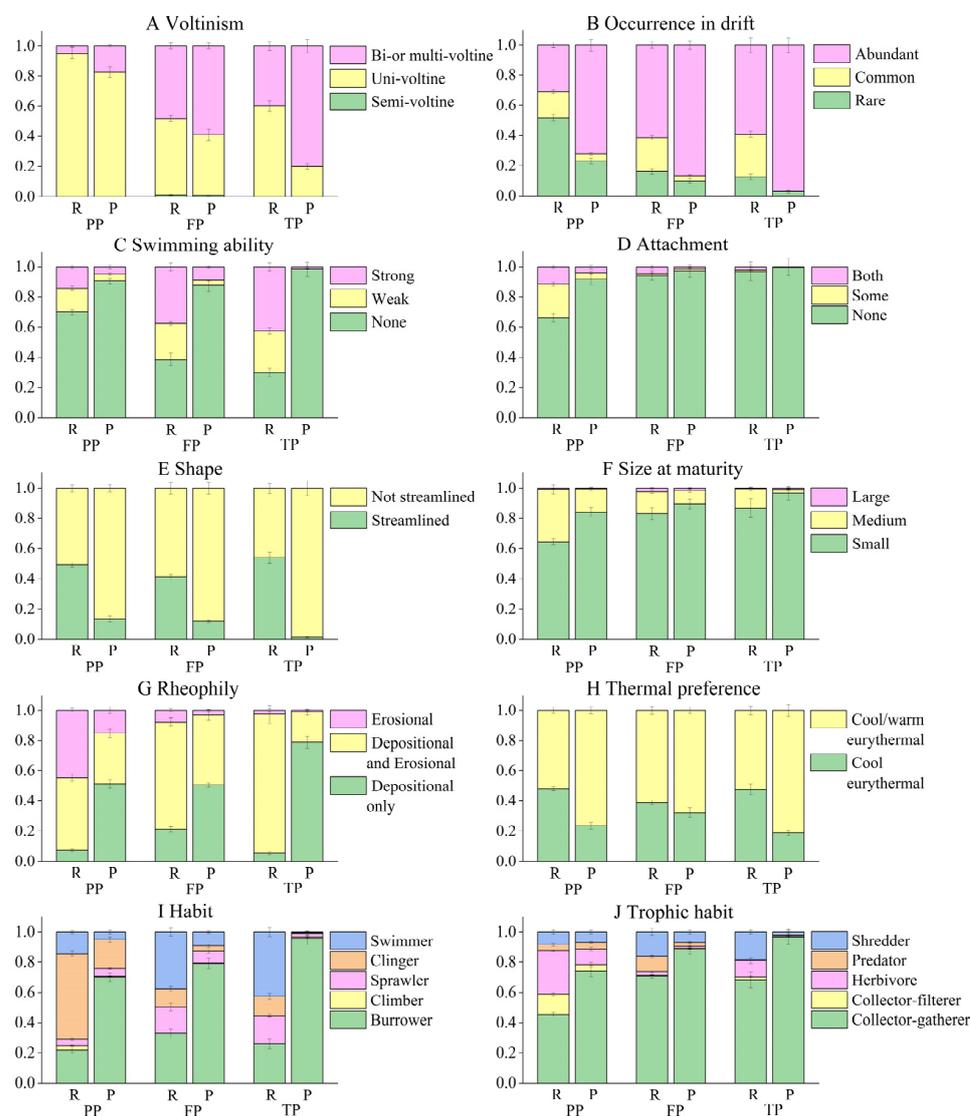


Figure 3. Average percentage (\pm SD) of a given trait category: voltinism (A), occurrence in drift (B), swimming ability (C), attachment (D), shape (E), size at maturity (F), rheophily (G), thermal preference (H), habit (I), and trophic habit (J) in riffles (R) and pools (P) during the pre-freezing period (PP), freezing period (FP), and thawing period (TP). Sample size ($n = 5$).

3.5. Relationships between Benthic Macroinvertebrate Communities and Environmental Factors

The relationships between benthic macroinvertebrate communities and environmental factors were shown in Figure 4. The first ordination CCA axis (Figure 4A) explained 33.18%

of the variation of benthic macroinvertebrate communities, and showed that velocity, dissolved oxygen, temperature, pH, electrical conductivity, and MSUBSTD were the critical environmental factors affecting the distribution of benthic macroinvertebrates during the freezing–thawing periods ($p < 0.05$). The first ordination CCA axis was mainly correlated with velocity and dissolved oxygen. This axis clearly distinguished the freezing period sampling sites from those of the pre-freezing period and thawing period, and the freezing period sampling sites had low velocity and dissolved oxygen. The second ordination axis, which was strongly associated with MSUBSTD, pH, and electrical conductivity reflected the amount of disturbance. All thawing period sampling sites characterized by higher levels of disturbance were positioned near the upper section of the ordination.

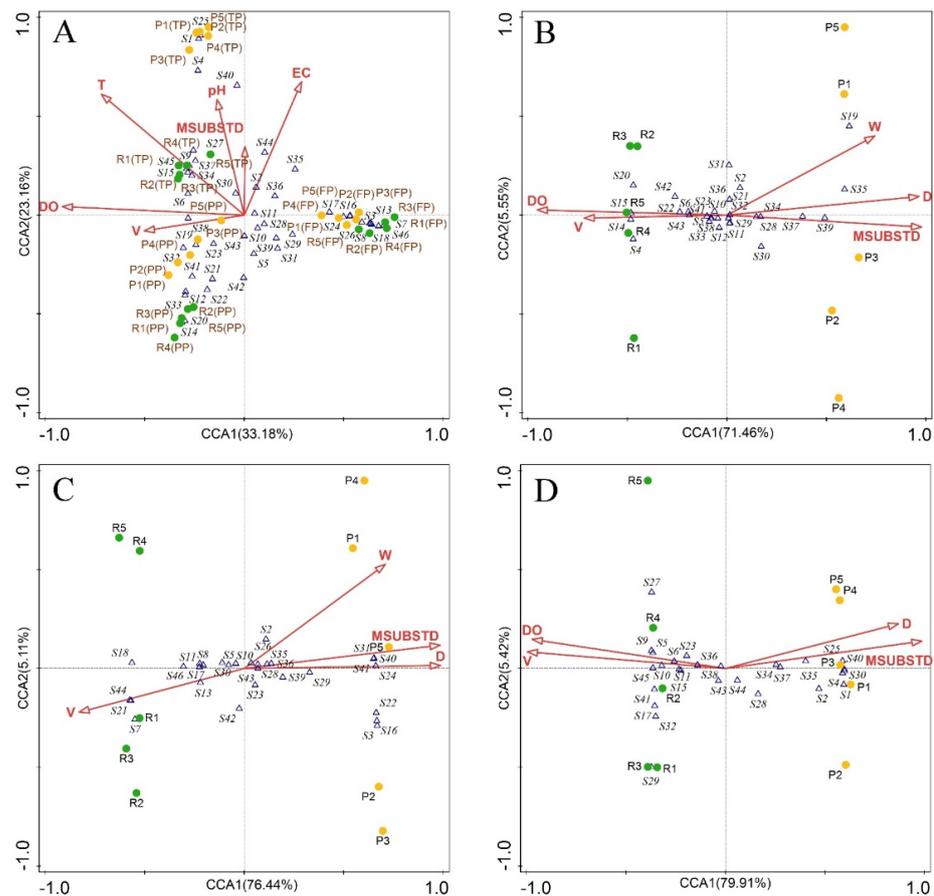


Figure 4. Canonical correlation analysis ordination bi-plots of benthic macroinvertebrates (blue triangle), sampling sites (riffles, green circles; pools, yellow circles), and forward-selected environmental factors (red arrows) for all periods (A), the pre-freezing period (B), freezing period (C), and thawing period (D). Sample size ($n = 5$). R, riffles; P, pools. PP, pre-freezing period; FP, freezing period; TP, thawing period. V, velocity; D, depth; W, width; T, temperature; DO, dissolved oxygen; EC, electrical conductivity; M, MSUBSTD. The species code are as follows: S1, *Planaria* sp.; S2, Naididae sp.; S3, *Anis* sp.; S4, *Gammarus* sp.; S5, *Rhithrogena* sp.; S6, *Iron* sp.; S7, *Heptagenia* sp.; S8, *Epeorus* sp.; S9, *Cinygmula* sp.; S10, *Baetis* sp.; S11, *Ameletus* sp.; S12, *Isoperla* sp.; S13, *Perlodes* sp.; S14, *Starsolus* sp.; S15, *Nemoura* sp.; S16, *Amphinemura* sp.; S17, *Capnia* sp.; S18, *Suwallia* sp.; S19, *Haliplus* sp.; S20, *Homoplectra* sp.; S21, *Glossosoma* sp.; S22, *Theliopsyche* sp.; S23, *Brachycentrus* sp.; S24, *Amiocentrus* sp.; S25, *Rhyacophila* sp.; S26, *Desmona* sp.; S27, *Pseudostenophylax* sp.; S28, *Dicranota* sp.; S29, *Cheilotrichia* sp.; S30, *Tipula* (*Arcotipula*) sp.; S31, *Tipula* (*Sinotipula*) sp.; S32, *Hexatoma* sp.; S33, *Antocha* sp.; S34, *Tanytarsini* sp.; S35, *Chironomus* sp.; S36, *Orthoclaadiinae* sp.1; S37, *Orthoclaadiinae* sp.2; S38, *Orthoclaadiinae* sp.3; S39, *Tanyptodinae* sp.; S40, *Sphaeromias* sp.; S41, *Psychodidae* sp.; S42, *Blefariceridae* sp.; S43, *Tabanidae* sp.1; S44, *Tabanidae* sp.2; S45, *Blepharicera* sp.; S46, *Hydrachnellae* sp.

The riffle and pool sites were separately distributed along with the positive and negative directions, respectively, of axis one in each period (Figure 4B–D). The first ordination CCA axis (Figure 4B) explained 71.46% of the variation of benthic macroinvertebrate communities and was mainly correlated with dissolved oxygen, velocity, temperature, MSUBSTD, depth, and width in the pre-freezing period ($p < 0.05$). Riffles were mainly affected by dissolved oxygen and velocity, while pools were mainly affected by MSUBSTD, depth, and width in this period. The first ordination CCA axis (Figure 4C) explained 76.44% of the variation of benthic macroinvertebrate communities and was mainly correlated with depth, MSUBSTD, velocity, and width in the freezing period ($p < 0.05$). Riffles were primarily influenced by velocity, but pools were primarily influenced by depth, width, and MSUBSTD in this period. The first ordination CCA axis (Figure 4D) explained 79.91% of the variation of benthic macroinvertebrate communities and was mainly correlated with velocity, MSUBSTD, dissolved oxygen, and depth in the thawing period ($p < 0.05$). Riffles were mostly impacted by velocity, and dissolved oxygen; however, pools were mostly impacted by depth in this period.

4. Discussion

4.1. Habitat Characteristics of Riffles and Pools during the Freezing–Thawing Process

The habitat environmental characteristics showed a decreasing trend, followed by an increasing trend during the freezing–thawing process. The ice surface prevented air exchange between the aquatic ecosystem and the atmosphere, resulting in the lowest dissolved oxygen and temperature being during the freezing period. Low precipitation also leads to lower dissolved oxygen values during the winter months [32]. Our results showed that the water temperature, velocity, dissolved oxygen, and pH significantly increased in the thawing period ($p < 0.05$). This is due to the fact that melting glacial ice contributes significantly to the harsh environmental key conditions (low water temperature, increased discharge dynamics, unstable substrate and riverbed, increased turbidity, and sediment load) in glacier-fed streams [33]. The CCA tri-plot (Figure 4A) indicated that the pH had the strongest correlation with the thawing period sampling sites. This is explained by the melting and leaching of glaciers and moraine. Glacial meltwater runoff is mainly affected by rock weathering, water–rock interaction is widespread, and the erosion and uplift of bedrock caused by glacial movement enhance the hydrolysis capacity of minerals [34]. Water temperature is an important environmental variable that determines the metabolic rate, spatial distribution, and population succession of aquatic organisms, especially for highlands [35]. Our results showed that the water temperature had a greater effect on benthic macroinvertebrate communities during the melting period.

The water quality conditions between riffles and pools differed slightly, due to the rapid flow and short water residence time in the river channel. Nevertheless, the difference in hydrological conditions in the two habitats was more prominent, consistent with Wang’s results [31]. The substrate of riffles was dominated by cobble and gravels, while the substrate of pools was dominated by silt and sand; previous studies found the same results [36]. Pools were slow enough to allow some sediment to be deposited, and this habitat was suitable for feeding on organic matter in the mud or for burrowing species to survive. However, heavy sediment deposition can negatively affect the inhabitants by reducing matrix porosity, reducing matrix heterogeneity, and enveloping organisms [37].

4.2. Insights on Indicator Taxa

Indicator taxa are sensitive and responsive to certain ecological disturbances and can provide information on the biological and environmental aspects of a watershed or ecosystem; thus, they can be used to monitor the environment [38]. By monitoring indicator taxa, we can gain a better understanding of the ecological situation of glacier-fed streams. In our study, the freezing–thawing process changed the indicator taxa in the riffles and pools of glacier-fed streams.

Diptera is often considered an indicator of glacier-fed streams [39–41]. Our results also showed that Diptera had the most species among the indicator taxa and showed strong habitat selectivity. Among the 18 genera of Diptera, there were seven indicator taxa of riffles and nine indicator taxa of pools. The body color of the Psychodidae in our results was primarily brown or black, and they were the indicator taxa in riffles during the pre-freezing and thawing period. This is consistent with Tanchet's findings, that brown or black genera of Psychodidae are distributed in flowing water habitats [42]. During the freezing period, the stream surface froze and there was more flowing water under pools than riffles. At this time, Psychodidae was the indicator of pools, and it seems that pools become their temporary refuge in the freezing period.

In addition to Diptera, EPT species (Ephemeroptera, Plecoptera, and Trichoptera) accounted for a large proportion of indicator taxa. Ephemeroptera was an indicator group in riffles of glacier-fed streams. Most of them have flat bodies (such as *Heptagenia*) with fixed suckers (such as *Rhithrogena*) or are small and fish-like (such as *Baetis*), which are adapted to a flowing water environment. Plecoptera originated in cold water rivers at high latitudes [43]. Our results showed that Plecoptera is an indicator taxa of both riffles and pools in glacier-fed streams. Additionally, the Nemouridae (Plecoptera) has a wide diversification, being distributed in running waters [44] and still water [42]. Although *Amphinemura* was an indicator of pools during the freezing period, which had the lowest temperature, another study showed that it can live in a high-flow and high-temperature habitat [2]. This is related to geographical location and natural conditions.

Food source is one of the critical criteria for the habitat selection of benthic macroinvertebrates. *Glossosoma* (Trichoptera) mainly feeds on diatoms and organic particles [45] that are primarily distributed on the surface of riffles due to the size, stability, and velocity of sediment particles [46]. Therefore, *Glossosoma* was the indicator taxon for riffles during the freezing period when environmental conditions were stable. *Brachycentrus* (Trichoptera) larvae like to gather on the surface of rocks, branches, or submerged plants facing the water [47]; thus, this was the indicator taxon for riffles during the pre-freezing period and thawing period when the water was flowing. *Theliopsycha* (Trichoptera) indicated riffles in the pre-freezing period, but indicated pools in the freezing period. This means that it moves to a more suitable location as the environment changes. It can swim between riffles and pools, choosing a habitat to settle in, according to its environment.

Generally, during the pre-freezing period in glacier-fed streams, the indicator taxa of riffles were Ephemeroptera, Plecoptera, Diptera, and Trichoptera. These taxa have a flat body (Ephemeroptera), wide and flat hocks (Plecoptera), a small tail with rows of hooks (Diptera), or gravel nests (Trichoptera). These structures give them a strong attachment to stone. The indicator taxa of pools were Diptera (Tipulidae and Chironomidae), which were both burrowers. The dissolved oxygen level was lowest during the freezing period. Lower oxygen availability should promote the presence of taxa with gills that help them increase their rates of oxygen uptake. Therefore, the dominant groups at this time were *Amphinemura*, *Theliopsycha*, and *Amiocentrus*, which breathe through gills. In addition to Diptera, the indicator taxa of pools were Trichoptera and Basommatophora. The indicator taxa of riffles were Ephemeroptera, Plecoptera, Diptera, and Trichoptera in the thawing period, but the prevalence of some Diptera species increased. This increase may have been because glacier and snow meltwater washed them out of pools into riffles. Meanwhile, the indicator taxa of pools increased during the thawing period, including *Rhyacophila*, *Gammarus*, Naididae, and *Planaria*. This is because they bred in large numbers at this time.

4.3. Benthic Macroinvertebrate Community Structure in Riffles and Pools during the Freezing–Thawing Process

Our research confirmed the benthic macroinvertebrate community structure in a glacier-fed stream in the Tianshan mountains in China, during the snow-covered period. The seasonal glacial melt led to high flow, high turbidity, the destruction of food sources, and changes in the original habitat conditions. This glacial melt strongly influenced the

dynamics of the benthic macroinvertebrate communities. Similar results have been reported in Europe and South America [2,48,49].

During the freezing–thawing process, the species richness and diversity of benthic macroinvertebrates in riffles and pools showed a decreasing trend. Our results showed that these values were significantly higher in riffles than in pools in the thawing period ($p < 0.05$). CCA analysis (Figure 4D) also showed that the influence of “melting glacier and snow” on riffles was more significant than on pools. This allows riffles to provide varied environmental conditions for aquatic organisms, due to their increasing flow, velocity, and oxygen cycle. Therefore, riffles were more suitable for different kinds of benthic macroinvertebrates than pools. Previous studies only obtained higher EPT taxa abundance in riffles than in pools [23], and did not measure these during the freezing–thawing process. The EPT taxa in both habitats were the most abundant in the freezing period. The taxa usually possess the functional trait “large size at maturity.” Benthic macroinvertebrates with a large body size are more likely to maintain their regulatory function in a heterogeneous environment, and, thus, have advantages in cold habitats [50].

The densities of the benthic macroinvertebrate communities during the pre-freezing period and thawing period were consistent with most research, which showed that riffles had significantly higher densities than pools [51,52]. However, the results obtained in the freezing period contradicted this, and these findings were also different from our second hypothesis regarding density. This contradiction may be due to the low temperature in the freezing period, leading to the higher stability of pools than riffles. As organic debris was easy to deposit in pools during the pre-freezing period, the food source was sufficient in pools, and their overwintering environment was more suitable for benthic macroinvertebrate survival; thus, there were more aquatic organisms in pools.

4.4. Benthic Macroinvertebrate Functional Traits in Riffles and Pools during the Freezing–Thawing Process

Functional traits affect the species coexistence pattern of the entire community by impacting the arrangement of species along the environmental gradient, interspecific competition, and resource allocation within the community [53]. The difference in the relative abundance of traits is the result of habitat filtering; that is, hierarchical traits with a higher relative abundance can be regarded as better adapted to the regional environment [54]. Given the imminent threat of glacier retreat to aquatic communities in these catchments [10] due to global warming, more information is needed to clarify the functional consequences of species loss and community changes [55,56].

In our study, the higher relative abundance of “uni-voltine” and “bi- or multi-voltine” taxa indicated that there were apparent seasonal rhythms and hydrology conditions at the source of the Tianshan Mountain glacier-fed stream [57,58]. Furthermore, the “bi- or multi-voltine” taxa had a higher relative abundance in pools, and these taxa can maintain populations in constantly changing habitats [59]. The “swimming ability”, “attachment”, and “shape” traits reflect the ability of benthic macroinvertebrates to avoid adverse habitats to a certain extent. The relative abundance of “none swimming ability”, “none attachment”, and “not streamlined” taxa in pools were significantly higher than those in riffles ($p < 0.05$), demonstrating that riffle taxa are more susceptible to hydrological disturbance. In contrast, pools provide a crucial mobile refuge for benthic macroinvertebrates, including rheophilic taxa [60]. The relative abundance of “large size at maturity” taxa during the freezing period was greater than that during the other two periods in glacier-fed streams, which is consistent with the results observed in forest streams [61]. However, the overall proportion of “large size at maturity” was deficient in riffles and pools during the freezing–thawing process. Our results showed that the relative abundance of “small size at maturity” taxa was the highest, and in pools the relative abundance was significantly larger than that in riffles ($p < 0.05$). This result supports the hypothesis that being small may offer resilience to environmental conditions. Clinging or attaching to a substrate may provide resistance to the high hydraulic stress experienced by invertebrates in glacier-fed streams [62,63].

Furthermore, small individuals usually have a shorter life cycle (*r*-selection), so the community can recover faster after being disturbed [64]. These results also show that pools in the glacier-fed streams are more suitable than riffles for benthic macroinvertebrates to deal with extreme environments.

In terms of rheophily, the relative abundance of “erosional” benthic macroinvertebrates in riffles was higher than that in pools, indicating the high heterogeneity of the riffle habitat. Owing to greater depths and slower currents, organic matter and sediments tend to accumulate in pools. The winter freezing–thawing process was mainly based on “cool/warm eurythermal” thermal preference. As the snow continued to melt, there was an increase in “cool/warm eurythermal” benthic macroinvertebrates in pools, reflecting slower temperature changes in pools. Concordant with the results of streams in the Smith River Basin (northern California) and the Sierra Nevada (California), we found that the abundances of clinger taxa dominated in riffles, whereas burrowers dominated in pools [23,65]. Differences in habitats are related to the type of substrate present. Riffles mainly consist of boulders, which provide living conditions suitable for “clinger” benthic macroinvertebrates. Meanwhile, pools mainly consist of gravels, which is suitable for supporting burrowing benthic macroinvertebrates. As such, the trait “clinger” was the dominant group in riffles in the pre-freezing period in our study. At this time, the river is not frozen, and the water in riffles is more fluid. Sessile benthic macroinvertebrates usually attach to the substrate surface of the riverbed. They generally have abdominal suckers, powerful tarsal claws, a fixed nest, and a flat dorsal surface and abdomen to avoid or withstand hydraulic impact. In the thawing period, the trait “swimmer” had an advantage in riffles, owing to glacier and snow melting, because taxa with this trait can control the direction and speed of their motion.

Nutritional habits reflect circulation of matter and energy flow in the ecosystem where communities are located. Changes in the composition of functional feeding groups indicated that the winter freezing–thawing process affected the structure of the benthic macroinvertebrate food web [66]. The relative abundance of collectors in riffles and pools was the highest, indicating that the benthic macroinvertebrates in the Tianshan Mountains glacier-fed stream mainly played the role of secondary producers in the water ecosystem [67]. We found that filterers were more common in riffles and gatherers were more common in pools, which was in agreement with the results obtained regarding Arizona streams [68]. Although leaves and wood chips are deposited in pools, they are also intercepted and accumulated by gravel or pebbles in riffles. The relative abundance of herbivores and shredders in riffles was more significant than in pools ($p < 0.05$). Conversely, some stonefly taxa classified as shredders consume a large amount of algae; thus, their abundances may not depend on allochthonous detritus alone [69]. Overall, there were more taxa with resilience and resistance traits in pools than in riffles, and the same results were shown in other disturbed streams [70].

5. Conclusions

We investigated the effects of the freezing–thawing process on benthic macroinvertebrates based on the analysis of their community structure and functional traits in riffles and pools of a glacier-fed stream in Xinjiang, China. The freezing–thawing process resulted in declines in benthic macroinvertebrate species richness and diversity, but the functional traits adapted to adverse habitat increased. These findings indicated that the freezing–thawing process affected the natural habitat conditions and diversification of the benthic macroinvertebrate communities, suggesting that freezing and thawing play an essential role in forming the structure and function of the stream ecosystem. The levels of species richness, EPT richness, and biodiversity in riffles were higher than those in pools of the glacier-fed stream, but the species density was lower in riffles than in pools in the freezing period. The groups of benthic macroinvertebrates with resilience and resistance were greater in number in pools than in riffles. The disturbance of meltwater during the thawing period is the critical factor that affects the survival of benthic macroinvertebrates.

Riffles are more disturbed by meltwater and, despite increasing habitat heterogeneity, pools provide a temporary refuge for benthic macroinvertebrates. The results of our study are limited in some respects, due to the natural environment and geographical location, and conclusions about changes in biodiversity in changing habitats must be made cautiously. The study of benthic macroinvertebrates can allow us to better evaluate the ecological process and habitat status of glacier-fed streams. Furthermore, our results can also be used as an indicator to assess the impact of glacier and snow line retreat on river ecosystems in the future.

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

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

Appendix A

Table A1. The functional traits and trait state of benthic macroinvertebrates.

Trait	Trait State	Trait	Trait State
Life history Voltinism	Semi-voltine	Ecology Rheophily	Depositional only
	Uni-voltine		Depositional and erosional
	Bi- or multi-voltine		Erosional
Mobility		Thermal preference	Cool eurythermal
Occurrence in drift	Rare		Cool/warm eurythermal
	Common Abundant		Warm eurythermal
Swimming ability	None	Habit	Burrower
	Weak		Climber
	Strong		Sprawler
Morphology Attachment			Clinger
	None	Trophic habit	Swimmer
	Some		Collector-gatherer
Both	Collector-filterer		
Shape	Streamlined		Herbivore
	Not streamlined		Predator
Size at maturity	Small (<9 mm)		Shredder
	Medium (9–16 mm)		
	Large (>16 mm)		

Table A2. Density compositions of benthic macroinvertebrates (individuals/m², *n* = 5).

Code	Taxa	Pre-Freezing Period				Freezing Period				Thawing Period			
		Riffle		Pool		Riffle		Pool		Riffle		Pool	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	mean	SD	Mean	SD
S1	<i>Planaria</i> sp.	0	0	0	0	0	0	0	0	0	0	6	4
S2	<i>Naididae</i> sp.	10	2	11	7	12	5	16	7	2	2	27	15
S3	<i>Anis</i> sp.	0	0	0	0	0	0	8	9	0	0	0	0
S4	<i>Gammarus</i> sp.	2	2	0	0	0	0	0	0	0	0	34	19
S5	<i>Rhithrogena</i> sp.	384	27	87	17	127	34	47	4	24	6	0	0
S6	<i>Iron</i> sp.	24	10	4	1	0	0	0	0	366	60	7	4
S7	<i>Heptagenia</i> sp.	0	0	0	0	10	6	0	0	0	0	0	0
S8	<i>Epeorus</i> sp.	0	0	0	0	23	5	7	1	0	0	0	0
S9	<i>Cinygmula</i> sp.	0	0	0	0	0	0	0	0	41	23	0	0
S10	<i>Baetis</i> sp.	387	25	84	15	656	32	232	20	1422	103	4	9
S11	<i>Ameletus</i> sp.	40	8	33	13	185	22	23	8	339	57	8	4
S12	<i>Isoperla</i> sp.	27	4	16	6	0	0	0	0	0	0	0	0
S13	<i>Perlodes</i> sp.	0	0	0	0	22	2	7	6	0	0	0	0
S14	<i>Starsolus</i> sp.	12	8	0	0	0	0	0	0	0	0	0	0
S15	<i>Nemoura</i> sp.	13	2	0	0	0	0	0	0	693	27	13	9
S16	<i>Amphinemura</i> sp.	0	0	0	0	0	0	7	7	0	0	0	0
S17	<i>Capnia</i> sp.	0	0	0	0	333	44	32	10	34	22	0	0
S18	<i>Suxvallia</i> sp.	0	0	0	0	79	13	0	0	0	0	0	0
S19	<i>Haliplus</i> sp.	0	0	2	2	0	0	0	0	0	0	0	0
S20	<i>Homoplectra</i> sp.	5	6	0	0	0	0	0	0	0	0	0	0
S21	<i>Glossosoma</i> sp.	220	15	143	25	17	10	0	0	0	0	0	0
S22	<i>Theliopsycha</i> sp.	82	14	14	5	0	0	7	4	0	0	0	0
S23	<i>Brachycentrus</i> sp.	345	23	96	25	12	5	12	4	74	24	7	6
S24	<i>Amiocentrus</i> sp.	0	0	0	0	0	0	16	7	0	0	0	0
S25	<i>Rhyacophila</i> sp.	0	0	0	0	0	0	0	0	0	0	4	0
S26	<i>Desmona</i> sp.	0	0	0	0	27	8	25	7	0	0	0	0
S27	<i>Pseudostenophylax</i> sp.	0	0	0	0	0	0	0	0	15	15	0	0
S28	<i>Dicranota</i> sp.	35	4	74	9	59	9	70	13	12	8	15	9
S29	<i>Cheilotrichia</i> sp.	9	4	7	3	11	5	58	8	7	9	0	0
S30	<i>Tipula (Arctotipula)</i> sp.	8	3	18	12	7	3	4	0	0	0	19	11
S31	<i>Tipula (Sinotipula)</i> sp.	5	2	5	7	0	0	19	3	0	0	0	0
S32	<i>Hexatoma</i> sp.	21	11	16	7	0	0	0	0	22	14	0	0
S33	<i>Antocha</i> sp.	73	25	25	8	0	0	0	0	0	0	0	0
S34	Tanytarsini sp.	216	24	1198	64	0	0	0	0	206	47	2526	139
S35	<i>Chironomus</i> sp.	0	0	16	6	519	51	1454	39	22	15	550	61
S36	Orthocladiinae sp.1	26	7	21	9	246	35	776	72	295	31	59	8
S37	Orthocladiinae sp.2	12	1	219	23	0	0	0	0	72	21	603	66
S38	Orthocladiinae sp.3	257	22	90	17	0	0	0	0	438	66	24	12
S39	Tanypodinae sp.	3	4	77	10	17	7	37	8	0	0	0	0
S40	<i>Sphaeromias</i> sp.	0	0	0	0	0	0	4	0	0	0	16	14
S41	Psychodidae sp.	593	34	94	23	0	0	4	0	17	8	0	0
S42	Blefariceridae sp.	61	9	8	7	10	6	8	9	0	0	0	0
S43	Tabanidae sp.1	79	18	13	4	20	5	23	8	17	4	7	5
S44	Tabanidae sp.2	0	0	0	0	8	9	0	0	7	8	4	1
S45	<i>Blepharicera</i> sp.	0	0	0	0	0	0	0	0	24	11	0	0
S46	Hydrachnellae sp.	0	0	0	0	99	23	10	3	0	0	0	0

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