Structural and functional assessments of hyporheic macroinvertebrates across multiple environmental gradients in rivers of Hokkaido, Japan

（北海道河川における複数環境傾度に沿った河床飽和間隙水域無脊椎動物の構造および機能評価）

Md. Khorshed Alam

Ph.D. Dissertation

2023

Division of Environmental Science Development

Graduate School of Environmental Science

Hokkaido University, Japan

Abstract

Knowledge of organisms' diversity pattern and their ecological drivers is central to ecology, which may vary at local to spatial scales. Rivers are composed of benthic habitat and a vertically saturated area below the riverbed which can be a habitat for diverse macroinvertebrate communities known as hyporheic habitat. In rivers, understanding the contribution of macroinvertebrates in ecosystem structure and function is largely limited for hyporheic habitat across multiple environmental gradients. This study tested the hyporheic habitat-type contribution to total diversity of macroinvertebrates and demonstrated how macroinvertebrate's structural and functional responses vary across multiple environmental gradients in the hyporheic zone. The first part of the research was conducted in 15 river reaches within eight river segments consisting of five rivers in Hokkaido, Japan (Toyohira, Horonai, Sorachi, Tokachi, and Satsunai River). The latter parts were conducted only in the Satsunai River.

The study first demonstrated the importance of habitat contribution from the hyporheic zone to the total diversity of macroinvertebrates and the factors that influence the contribution. Hyporheic and benthic samples were collected from August to November 2020 by installing colonization traps at 30 cm depth and a Surber sampler respectively. The macroinvertebrates were separated among segments, river reaches, and between benthic and hyporheic habitats. Habitat contribution (up to 25.9%) was most clearly detected at the reach scale, suggesting that habitat contribution should be recognized more at relatively small local scales. The higher alpha diversity of the benthic habitat and together with the dominance of turnover between habitats, suggested the presence of unique species in hyporheic habitat. The variable contribution pattern across reaches did not vary under variable environmental gradients. The number of hyporheic species predicted the contribution pattern across reaches and responded to environmental variables independently with the negative effect of fine sediment amount and positive effects of nitrate concentration, dissolved oxygen, and riverbed median particle size. Thus, the number of hyporheic species and their variable responses to the local environmental variables interactively cause varying contribution patterns across reaches.

The study then examined how fine sediment (particle size: <2 mm) influences the macroinvertebrates in hyporheic zone under different levels of nutrient pollution through experimental sediment addition in sites with different nutrient levels. The field survey and sampling were conducted in three sites in the Satsunai River and one of its tributaries in 2019. The amount of fine sediments varied between habitat treatment types, indicating that sediment addition effectively manipulated the hyporheic zone. The responses of hyporheic macroinvertebrates were predictable for the independent stressors. Higher nitrate increased the taxonomic richness and relative abundance. The interaction between two stressors was not apparent. A community structure would have dominated under the higher nutrient levels that might offset any adverse effects of fine sediment. Thus, the effect of fine sediment was not appeared as strong adverse effects.

The study finally examined the effects of fine sediments and nutrients on hyporheic leaf litter decomposition rate and macroinvertebrates. The field survey and sampling were conducted in three sites in the Satsunai River and one of its tributaries in 2019. The field experiment was conducted by measuring the leaf litter decomposition of dried Alnus japonica leaves in benthic and hyporheic zones with and without sediment treatments at four sites with a gradient of nitrate concentration. The decomposition rate was comparable between the two zones but slowed down by sediment addition in the hyporheic zone. The leaf litter decomposition rate was highly predictable for the individual stressors. The detritivore invertebrate community was the main driving component of decomposition in the decreased leaf litter decomposition rate under higher sediment levels. Higher nitrate levels increased the leaf litter decomposition rate by stimulating microbe-driven decomposition and detritivore feeding. The adverse effects of fine sediment could be offset in the presence of nitrate and represented the additive effects of fine sediment and nitrate on leaf litter decomposition in the hyporheic zone.

The findings estimated habitat contribution to the total diversity of macroinvertebrates and how environmental stressors affect the structure and function of macroinvertebrates in the hyporheic zone. The finding showed that hyporheic habitat could add unique species to river macroinvertebrate diversity in particular when examined at smaller reach scale. Moreover, hyporheic macroinvertebrates' structural and

functional properties were influenced by the fine sediment and nutrients in the hyporheic zone, in which functional responses were adversely affected by the fine sediment with a decrease in detritivore abundance. Conservation of hyporheic species is important as they could provide essential roles (e.g., role in the food web) in hyporheic habitat. Detritivore invertebrates might not be unique species in the hyporheic zone, but their role is crucial for providing hyporheic functions. Thus, effective conservation measures can vary based on observed community structure including hyporheic species and taxa with more important functions, and multiple environmental gradients.

Table of contents

Chapter 1

General introduction

1.1 Background

Freshwater ecosystems are essential for human life as they provide economic, scientific, educational, aesthetic, and cultural value (Dudgeon et al., 2006). The river is one of the most critically essential freshwater components in providing resources for human life and well-being. Resources, including water and biota (e.g., fish) living in the river, have direct importance for human survival that can be maintained by interacting with a complex food web. For example, fish depend on invertebrates, and invertebrates depend on other autochthonous and allochthonous resources from instream production and terrestrial zone, respectively (Marczak and Richardson, 2007). This is how life on earth is maintained across aquatic and terrestrial compartments and invertebrates play crucial roles in maintaining the integrity of temperate river ecosystems.

Despite the aforementioned importance, freshwater ecosystems are among the most endangered ecosystems and have been altered by human activities' direct and indirect interventions (Sala et al., 2000). As a result, the habitat for freshwater biota, including invertebrates, becomes increasingly degraded, thereby indirectly affecting the human resource supply and increasing the rate of biodiversity loss (Dudgeon et al., 2006; Strayer and Dudgeon, 2010). Thus, it is urgent to investigate the diversity and ecology of freshwater invertebrates to understand how they are spatially structured and distributed, their roles in the environment and how they can be affected by environmental stressors. Such knowledge will contribute to realizing sustainable conservation and management of freshwater systems.

Ecosystem structure and function in rivers

Ecosystem structures are the physical features of the ecosystem and organisms that inhabit it (Cummins, 1974; Schowalter, 2011). It includes the networks of interaction between biotic and abiotic components of the system. The biotic components include the biological community, e.g., species number, biomass, life form, life history, and spatial distribution of species. The species diversity of producers, consumers, and decomposers is among the critical characteristics of the ecosystem structures (Schowalter, 2011). On the other hand, functions are the role of different structural components for their natural

survival in the system (Schowalter, 2011). Functions are mainly physical and biogeochemical processes in the ecosystem through the exchange of matter and energy as a joint activity of the biological communities (Tilman et al., 2014). Together, ecosystem structure and function constitute the integrity of ecosystems.

The river can be viewed as a continuum of changes in ecosystem structure and function from upstream to downstream reaches (Vannote, 1980). The river ecosystem structure varies regarding channel characteristics, water characteristics, diversity, and distribution of biological communities (Schowalter, 2011). Ecosystem functions in rivers generally refer to organic matter decomposition, metabolism, and primary and secondary productions (Lindeman, 1942; Sandin and Solimini, 2009; Schowalter, 2011), in which application of leaf litter breakdown has been established as a proxy of decomposition function (Gessner and Chauvet, 2002). The structure and functioning of rivers are complex, and often one cannot be inferred from the other (Cardinale et al., 2013). In addition to being an important biotic structural component of rivers, invertebrates are vital to the function of rivers because their secondary production connects primary production to other consumers as food resources and affects organic matter processing in the process of production (Wallace and Webster, 1996; Covich et al., 1999; Huryn and Wallace, 2000). Also, they are highly diverse, sensitive to environmental changes, and ubiquitous occurrences (Rosi-Marshall and Wallace, 2002, Negishi et al., 2019a). As a whole, the diversity and distribution of aquatic macroinvertebrates (invertebrate fauna with a body size > 500 μm; Hauer and Resh, 2017) and their functional role in the river ecosystem have been considered a key to assessing the ecosystem health of a river (Wallace and Webster, 1996; Fierro et al., 2017; Wu et al., 2020).

Multiple stressors in freshwater ecosystems

The diversity and/or abundance of the biological community in rivers is decreasing at an alarming rate, not entirely due to single causes but rather due to multiple causes (Schinegger et al., 2016; Feld et al., 2016; Gatti, 2016). Such causes include the over-extraction of resources, river regulation, water abstraction, and wastes and effluents released into the rivers because of agricultural, domestic, and industrial activities

(Dudgeon et al., 2006; Feld et al., 2016). The pressures from human actions generally alter more than one environmental factor (e.g., urbanization affects runoff quantity, water quality, thermal regimes, and habitat availability). The response of the biological community usually reflects the multivariate effects from variable sources because environmental variables from variable sources often coincide (Strayer and Dudgeon, 2010).

This biodiversity loss and abundance change can directly affect ecosystem function. For example, the loss of species with an important functional role can cause a reduction in ecosystem functions (Cardinale et al., 2013). The relationship between ecosystem functioning and biodiversity loss depends on several factors, including community structure, interactions among species, sequence of species loss, species traits, and environmental context (Statzner and Moss, 2004; Vaughn, 2010; Schmera et al., 2017). The number of studies examining the multiple stressors effect on aquatic biota is increasing (Statzner and Bêche, 2010; Wagenhoff et al., 2012; Sabater et al., 2016). However, the understanding is far from clear, and existing studies report contrasting results (Wagenhoff et al., 2011; Piggott et al., 2015). This might be partially because the co-occurring environmental stressors and co-occurring traits in species can potentially mask the predicted cause-effect relationships (Piggott et al., 2015).

The hyporheic zone and hyporheos

Rivers are dynamic ecosystems on earth and can be conceptualized as a four-dimensional framework. It includes longitudinal, lateral, vertical, and temporal dimensions (Ward, 1989). These dimensions of rivers have been studied extensively except for the vertical three-dimensional space in the riverbed, called the hyporheic zone (Fig. 1.1; Boulton et al., 2010). This zone works as a physical filter, where groundwater and surface water mix through the hydrological exchange of water, potentially maintaining longitudinal and lateral connectivity through the sub-surface corridor depending on the sediment structures of the riverbed which typically ranges in the centimeter vertically (Stanford and Ward, 1993). It is typically the zone that contains all flow paths that begin and end at the sediment-water interface and where a certain percentage of surface water (e.g., 10%) is

present. The exact boundary and depth differ in space and time based on sediment grain size, hydrological exchange patterns, and sediment stability (Wondzell and Gooseff, 2013). The depth of hyporheic zone was generally reported to be 30 cm vertically for gravel-bed stream, based on the hydrological mixing and detectable oxygen. The declination in light and current velocity could define the upper boundary of benthic and hyporheic zone (Willaiams and Hynes, 1974). It is a dynamic ecotone and vital structural component of rivers for several reasons. First, hydrological, and bio-geological processes in the zone drive essential ecosystem functions such as productivity, nutrient cycling, leaf litter decomposition, and carbon cycling (Lefebvre et al., 2004; Birgand et al., 2007). Second, the zone provides habitat for a diverse group of invertebrates, known as hyporheos, in addition to a relatively well-known and studied benthic habitat (ecological zone located at the bottom of any freshwater body including substrate soft sedimentary or rocky bottoms, such as a river, lake, or pond; Fig. 1.1; Williams and Hynes, 1974; Ward, 1989; Boulton et al., 2010). It is important to increase our understanding of the structure and functional characteristics of the hyporheic zone, in contrast with the other major entity of vertical spatial dimension, i.e., the benthic zone.

Spatial analyses of species diversity

Spatially explicit species diversity analyses have been extensively conducted in various types of ecosystems and biota, including rivers (Wiens et al., 1993; García-Roger et al., 2003; Diekotter et al., 2008; Bonada et al., 2008; Guénard et al., 2016; Altermatt et al., 2020). In short, this approach helps quantitatively evaluate and identify relatively more important habitats compared to the others in attempts to conserve biodiversity efficiently and effectively (Isaak et al., 2012; Guénard et al., 2016). Specifically, the diversity in community structure varies at local and spatial scales representing dissimilarities (β) in composition that link local alpha (α) to total diversity (γ) (Legendre et al., 2005; Fig. 1.2a, b). In river ecosystems, the diversity of macroinvertebrates and their functional traits generate different patterns at multiple spatial scales because different mechanisms could be operated at each scale (Levin 1992; Allan and Johnson, 1997; Bonada et al., 2008; García-Roger et al., 2013). This spatial analytical approach to appreciate diversity structure has been extensively applied to benthic macroinvertebrates (Underwood and

Chapman, 1996; Collier and Clements, 2011) However, understanding of the diversity structure contribution of the invertebrates in the hyporheic habitat is still limited. By inhabiting the diverse hyporheic fauna, this zone can contribute to the total biodiversity of the river ecosystem.

1.2 Gaps of past studies

The environmental variables at higher spatial scales (i.e., ecoregion) are key determinants of the environmental characteristics observed at lower scales (i.e., reach and habitat) that have a direct effect on the macroinvertebrate structure and function in the freshwater systems (Malmqvist, 2002; Allan, 2004a). Thus, changes in community structure and functional traits of macroinvertebrates at variable spatial scales have been used to assess the level of alterations in aquatic ecosystems, factors limiting these alterations, and at which scale they occur (Allan et al., 1997; Allan, 2004a; Sandin and Johnson, 2003). Many studies have investigated the diversity of macroinvertebrates and have been concentrated on the benthic community and their contribution to regional diversity with very few reported on hyporheic alpha diversity indices (Lencioni and Spitale, 2015; Stubbington et al., 2016; Araújo et al., 2016; Stubbington et al., 2019). However, none attempted to examine the contribution of the hyporheic zone to the regional diversity of macroinvertebrates and the factors that influence the contribution.

Due to the increasing effects of stressors on freshwater ecosystems, the necessity to understand how macroinvertebrate communities are being affected structurally and functionally is also increasing (Cox and Rutherford, 2000; Verdonschot and Nijboer, 2004; Larsen et al., 2009). The effects of multiple environmental stressors on the benthic macroinvertebrate structure and functions were examined extensively in rivers (Dangles et al., 2004; Niyogi et al., 2013; Graebar et al., 2017; Cornejo et al., 2019). On the other hand, a few studies attempted to examine the interaction between environmental stressors and their effects on the hyporheic macroinvertebrate community structure (Wagenhoff et al., 2012; Matthaei et al., 2010; Piggott et al., 2015; Beermann et al., 2018). The studies concerning the effects of stressors on the hyporheic function were limited to the single stressor context (Stubbington et al., 2011; Nogaro et al., 2013).

1.3 Objectives

This study assessed structural and functional characteristics of hyporheic macroinvertebrates across multiple environmental gradients in rivers of Hokkaido, Japan. By fulfilling specific objectives below, this study aimed to fill the knowledge gaps mentioned above.

The specific objectives were:

- To examine the importance of habitat contribution from the hyporheic zone to the total diversity of macroinvertebrates and the factors that influence the contribution pattern of the hyporheic habitat

- To examine the effects of fine sediments on hyporheic macroinvertebrates under different levels of nutrient pollution

- To examine the effects of fine sediment on leaf litter decomposition in the hyporheic zone under different levels of nutrient pollution

1.4 Structure of the report

The following chapters of this research thesis are as follows-

Chapter 2: This chapter quantified the extent of contribution by the hyporheic habitat to the total diversity of macroinvertebrates and the factors that influence these contributions to fulfill the first specific objective. The specific questions were: How important is the hyporheic habitat was in contributing to the total diversity, and what factors influenced the habitat contribution? It showed that the hyporheic habitat played an important role in contributing to the total diversity of macroinvertebrates.

Chapter 3: This chapter quantified the effects of multiple stressors on hyporheic macroinvertebrates to fulfill the second specific objective. The specific questions in this chapter were: How does sediment and nutrient affect hyporheic macroinvertebrate community structure, and were the effects interactive or independent from each other? It

showed that the hyporheic macroinvertebrate taxonomic richness was affected by nutrient and fine sediment independently.

Chapter 4: This chapter quantified the effects of multiple stressors on the hyporheic leaf litter decomposition to fulfill the third specific objective. The specific questions in this chapter were: How does sediment and nutrient affect hyporheic litter decomposition, and were the effects interactive or independent from each other? It demonstrated that the leaf litter decomposition was highly predictable for individual stressors.

Chapter 5: This is the concluding chapter. It describes the general discussion on the major findings and implications of the study.

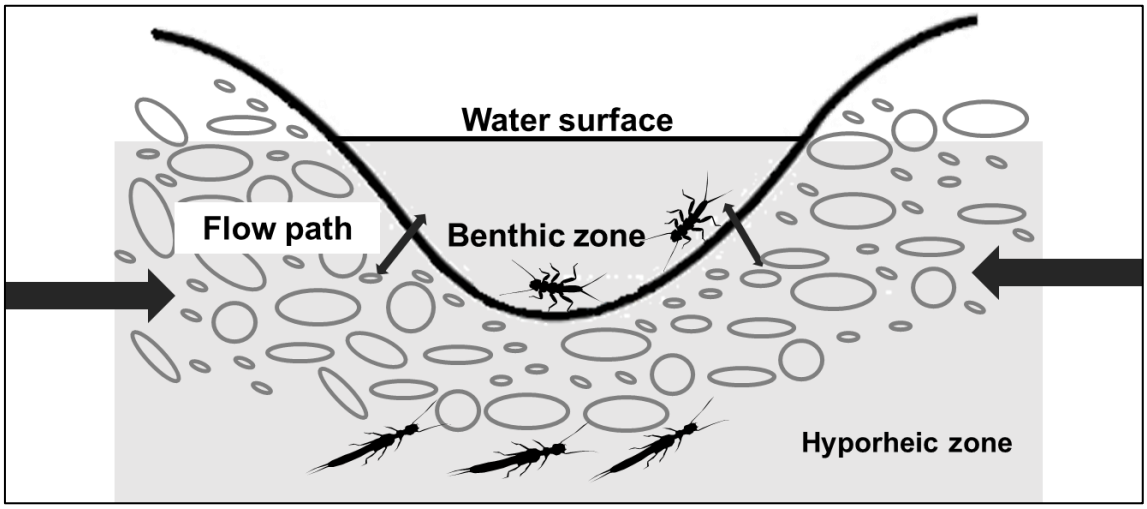


Fig. 1.1 A diagram of river cross section showing benthic zone and saturated underground area (i.e., where surface water and ground water mix) called hyporheic zone.

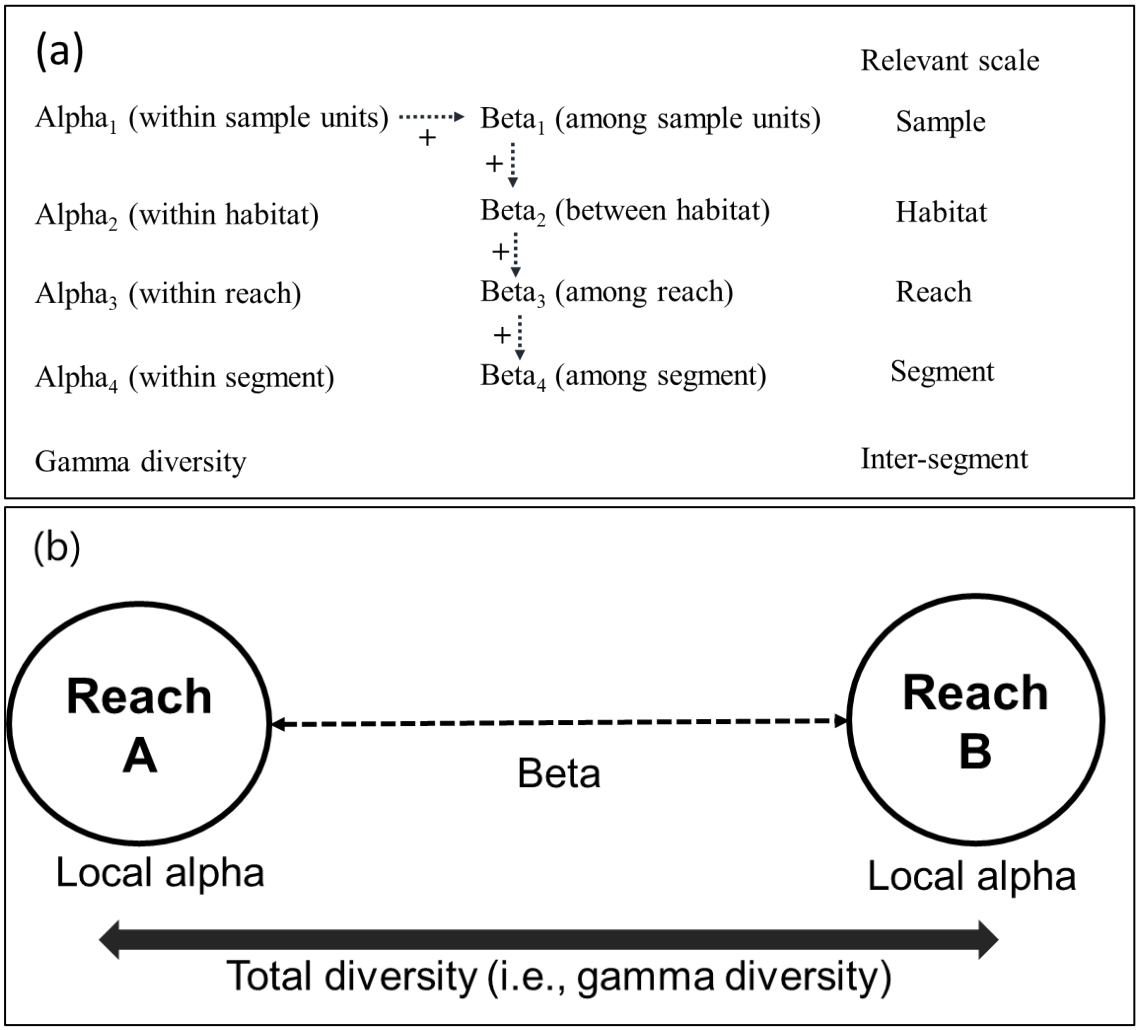


Fig. 1.2 A diagram showing the definition of diversity decomposition across multiple spatial scales where lower sample units are nested within higher units (a) and definition of alpha, beta, and gamma diversity considering two river reach (i.e., Reach A and Reach B) as an example where the number of species at each reach is alpha diversity, dissimilarity between two reach is beta diversity and total number of species in two river reach is gamma diversity (b).

Chapter 2

Habitat contribution of the hyporheic zone to the total diversity of macroinvertebrates in rivers

2.1 Introduction

Effective biodiversity conservation benefits from spatially explicit identifications of areas that comprise a high proportion of total biodiversity (i.e., gamma diversity). It is well known that the chosen measurement scale to study an ecological community can be considered a window of assessing an ecosystem (Levin, 1992). This principle also applies to biodiversity structure and the appropriate scale at which the interested diversity patterns in space and time can be observed should be determined to understand an ecosystem (Chave, 2013; Miyazawa et al., 2020). Consequently, significant challenges in conservation ecology are understanding, determining, and predicting the biodiversity pattern in space, how it is organized at multiple scales, and the mechanisms behind these arrangements (Richardson and Whittaker, 2010; Suurkuukka et al., 2012: Loreau et al., 2001; Solan et al., 2004; Diekotter et al., 2008, 2013). Rivers requires the same consideration because they possess complex hierarchical organizations in which the multi-scaled species diversity patterns and the processes that regulate them could vary from local to regional processes (Mykrä et al., 2007; Chase and Knight, 2013; Garzon–Lopez et al., 2014).

The arrangement of diversity and ecological processes that shape the diversity pattern can be addressed through alpha (α), beta (β), and gamma (γ) diversity patterns which Whittaker first proposed as a multiplicative approach (Whittaker, 1960) as $\gamma = \alpha \times \beta$. This diversity decomposition framework was extended by additive partitioning of diversity ($\gamma = \alpha + \beta$), which can describe more precisely the components found within (α) and among samples (β) (Lande, 1996; Gering et al., 2003; Crist et al., 2003; Matsuda et al., 2015). Beta-diversity can be further partitioned into its nestedness and turnover components, where turnover refers to the replacement of species from one site (or habitat type) to another, and nestedness refers to the taxa in species-poor sites that represent a subset of species-rich sites (Baselga 2010, Baselga and Orme 2012; Baselga and Leprieur, 2015; Legendre 2014). These structural assessment methods could help to detect the effect of spatial scales in structuring the diversity patterns (Rahbek et al., 2005; Hernández-Hernández et al., 2017).

The hyporheic zone often harbors diverse invertebrate taxa known as hyporheos (Boulton et al., 1998, Williams et al., 2010). They are characterized by different ecological requirements having differences in spending their life span in the zone compared to those found in benthic zone. For example, some of them spend part of their life (temporary dwellers) or entire larval life-cycle stage in the zone called as amphibites whereas some are obligated to live in the zone called stygobites (Boulton, 2007). This group of taxa composes the hyporheic habitat as a highly biologically diverse zone (Marmonier et al., 2020; Hutchins et al., 2020). Thus, if species unique to this zone is present, the hyporheic habitat can contribute to the total biodiversity. However, understanding of how hyporheic habitat add to the total diversity of macroinvertebrates and how their contribution vary across the observation scales are largely limited.

The additive partitioning has the potential to reveal how hyporheic habitat can contribute to the total biodiversity as a beta diversity component at multiple observation scales. Many studies addressed the species diversity patterns over different spatial scales in benthic habitats (Underwood and Chapman, 1996; Collier and Clements, 2011). Significant knowledge gaps remain in spatial scale species diversity patterns and distribution in the hyporheic habitat (Stubbington et al., 2019). Some studies addressed the alpha (Xu et al., 2012; Descloux et al., 2013; Stubbington et al., 2014; Mathers et al., 2014; Lencioni and Spitale, 2015; Stubbington et al., 2016; Araújo et al., 2017; Mathers et al., 2017; Durkota et al., 2019; Hutchins et al., 2020; Duan et al., 2021), and very few addressed the beta diversity patterns of the hyporheic community (Stubbington et al., 2014, 2019). It remains also unknown as to how hyporheic invertebrates mediate total diversity via nestedness and/or turnover processes in relation to invertebrate diversity characteristics in benthic habitat.

This chapter examined the importance of habitat contribution from the hyporheic zone to the total diversity of macroinvertebrates and the factors that influence the contribution of the hyporheic habitat. The specific objectives were to (i) examine the variabilities in macroinvertebrate community structure between habitat types (hyporheic and benthic zones), (ii) determine the contribution of the habitat-type scale as a beta component to the total biodiversity at larger spatial scales and its mechanisms (turnover

and/or, nestedness), and (iii) examine factors that control the variable patterns of habitat contribution. The hypothesis was that the hyporheic macroinvertebrates would contribute to the total diversity. It was also hypothesized that the contribution from the hyporheic habitat would vary under varying environmental gradients across river reaches. It was predicted that hyporheic zone would increase the total diversity by harboring unique taxa available in the hyporheic zone but absent in the benthic zone, and that the environmental variables, such as riverbed sediment median particle size, nutrient levels, or dissolved oxygen (DO) would positively correlate with the habitat contribution. On the other hand, hyporheic fine sediment would negatively affect the habitat contribution. These predictions were based on the adverse effects of fine sediments and positive effects of other environmental variables on the macroinvertebrates in the hyporheic habitat (Fowler and Death, 2001; Fowler and Scarsbrook, 2002; Descloux et al., 2013; Jones et al., 2015; Dunscombe et al., 2018; Negishi et al., 2019b).

2.2 Materials and methods

Study area

Field surveys, experiments, and sampling were conducted from August to November 2020 at fifteen river reaches of eight river segments, namely, Satsunai L7, L1, S1, and A1 (catchment area, 725 km^2; channel length, 82 km), Tokachi (catchment area, of 9010 km^2; channel length, 156 km), Sorachi (catchment area, 2618 km^2; channel length, 195 km), Horonai (catchment area, 9.4 km^2; channel length, 3.8 km), and Toyohira River (catchment area, 859 km^2; channel length, 72.5 km) in Hokkaido, Japan (Fig. 2.1; Table 2.1). One river segment was chosen in each river system and each segment was divided into two reaches except for the Satsunai River. The reaches varied in distance from each other across the segments (Table 2.1). Each of the reach was subdivided into at least three sub-reaches and were approximately 10 m distant from each other. Therefore, each river reach was at least 20 m long. For Satsunai River, four segments were chosen which were subdivided into six reaches (L7_up, L7_down, L1_up, L1_down, and A1_up and A1_down) were in the main channel, and two (S1_up and S1_down) were in a spring-fed tributary originating from a groundwater reservoir in the alluvial fan of the Satsunai River

(Fig. 2.1; Table 2.1). Each of the reach was subdivided into at least two sub reaches and were 10 m distant from each other. The segment A1 was located at the Totsutabetsu River's confluence with Satsunai River, a tributary of Tokachi River, which is the same size as the Satsunai River. A detailed description of the characteristics of the river reaches can be found in Negishi et al. (2019b). Due to floods during the colonization period, the downstream reach of A1 (A1_down) was lost, and thus, the segment A1 had A1_up only.

The river reaches were characterized by typical well-developed gravel bar landscapes with gravel beds (except for the Horonai River), riparian vegetation, and braided channel. The gravel bars were interconnected with riparian forests on one side, the channel on the other, or the channel/riparian forests on both sides, except for the Toyohira River. Toyohira River's reaches are typically gravel bars, riparian vegetation with encroachments from human settlements like embankments or bridges, and braided channels. The channels are connected either with riparian forests on one side or embankments on the other side and gravel bars on one side, or embankments on the other side of the channel. In the Horonai River, the bedrock is very coarse pumice, so the basin drains very well (Shibata et al., 1998). Relatively stable discharges and higher baseflow indicate that the contribution of deeper groundwater to runoff is greater than subsurface runoff through the surface unsaturated soil horizon (Shibata et al., 2001). However, the reaches are characterized by stable channels without well-developed gravel bars and with riparian vegetation on both sides of the channel.

Collection of hyporheic macroinvertebrates

Hyporheic macroinvertebrates were collected with colonization traps in the glide habitat of the riverbed. Colonization traps were made from available riverbed sediments (4-22.4 mm and 22.4-64 mm in a 3:1 ratio). The sediments were collected in situ from the riverbed sediments, washed well, and tightly enclosed a volume of 3800 cm^3 sediment in a 4 mm × 3 mm nylon net with an area of 50 cm × 50 cm. Further details describing the quantitative colonization trap can be found in Negishi et al. (2019b) and Alam et al. (2020). Eighty traps were prepared. Pits were excavated to a depth of 30 cm using either an excavator or by hand. A total of 12 traps were placed in each river (6 at each reach),

with two traps placed in each pit (at least 5 cm apart) except for Satsunai River. In the Satsunai River, a total of 32 traps were set, four traps at each river reach. At each reach, one to two T-shaped hyporheic wells (PVC pipe with an inner diameter of 25 mm; 8 mm holes at two ends of the pipe) were installed perpendicular to the colonization traps (Fig. 2.2). Traps were manually excavated and retrieved using a hand-held D-frame net (375 µm mesh) after 60 to 90 days since the installation. Traps were retrieved after carefully removing the sediment above the trap to ensure that benthic macroinvertebrates and detritus at the surface did not contaminate the hyporheic samples. Trap processing was completed by washing and sieving the trap material with a 500-µm stainless steel sieve within 30 minutes of collection and preserved with 75% ethanol for later processing. The remaining sediments were sieved using a stack of stainless-steel sieves (22.4 mm, 2 mm, and 500 µm). Sediments from 2 mm to 500 µm were collected and considered to represent fine sediment amount in the hyporheic habitat.

Collection of benthic macroinvertebrates

Benthic macroinvertebrates were collected on the same occasion and immediately before retrieving the colonization traps. Benthic samples were collected by inserting a Surber sampler (0.0625 m^2, 324-µm mesh) into the riverbed to a depth of ~7 cm for a total sample volume of 4375 cm^3 and ensured that benthic samples matched hyporheic assemblages by collecting sediment above the hyporheic traps in each pit (1 sample per pit). In pits that dried up, benthic samples were collected from the nearest wetted channel associated with each pit. Samples were collected immediately downstream from each trap so that sampling would not contaminate the hyporheic samples (Fig. 2.2).

Measurement of environmental conditions

The temperature of the hyporheic water was measured continuously at 30 min intervals using data loggers (HOBO pendant logger, Onset Co., MA, USA); at each river reach, one to two temperature loggers were inserted into the bottom of hyporheic wells. Water samples were collected within one month of installation and immediately before the removal of hyporheic traps. Samples were collected in acid-washed polyethylene bottles to determine nitrate concentrations in surface and hyporheic water. On both occasions, it

was not possible to collect hyporheic water samples from some sub-reaches due to drying of the riverbed (i.e., Tokachi and Sorachi River segments) and clogging of the well (i.e., reach of Satsunai L7 segment), but it was possible to collect samples at each reach because replicates were available (Tables 2.2, 2.3, and 2.4). Hyporheic water was collected using a manually operated suction hose, and surface water was collected simultaneously as the hyporheic water samples. Temperature, dissolved oxygen, electrical conductivity (EC), and pH were measured for the hyporheic and surface water using probes (HACH-HQ40d, Hach Co., Loveland, CO, USA; WM -32EP, TOA-DKK Co., Tokyo, Japan) immediately after the water was collected for the nitrate measurements. At each reach, one to two sediment samples were collected from the riverbed to characterize the sediment of each river. Samples were collected by manually excavating the riverbed and placing a plastic bucket at 30 cm depth from the bed to obtain a heterogeneous lamination of riverbed materials. After collection, samples were placed in a transparent plastic bag and returned to the lab for further analysis.

Laboratory analyses

After reaching the laboratory, water samples were filtered using a glass fiber filter with a pore size of 0.5 µm (GC-50, Advantec Co., Tokyo, Japan) and kept refrigerated until measurements nitrate (NO_3^-) concentrations by ion chromatography (IA-300, TOA-DKK Co., Tokyo, Japan), within a week. Macroinvertebrates were separated from organic material and inorganic particles and sorted from species to family level (Kawai and Tanida, 2018; Merritt, and Cummins, 2019). The retained residual was oven-dried at 60 °C for at least a week and was combusted at 500 °C for 4 hours (FO310, Yamato Scientific Co., Tokyo, Japan) to determine the ash-free dry mass (AFDM) and the weight of inorganic matter. The weight of this inorganic matter and the weight of sediment retained in the field from the colonization trap were combined to obtain the fine sediment weight from each trap. The riverbed sediments were air dried for at least 15-30 days. Then, each sample was sieved through a stack of stainless sieves (22.4 mm, 16 mm, 8 mm, 4 mm, 2 mm, 1 mm, 500 µm, and 100 µm), and gravels larger than 63 mm were measured separately. The sediments retained on each sieve were weighed using a weighing scale (A & D, FP -6000). Sediments were then classified according to the Wentworth sediment

grading scale (Wentworth, 1922), and the fine sediment fraction obtained from this measurement was used for further analysis. D_{50} was measured as a proxy for sediment size class of riverbed sediment using GRADISTAT (Blott and Pye, 2001).

Statistical analyses

The differences of macroinvertebrates across the river reaches, river segments, and between habitat type (i.e., benthic and hyporheic) were investigated using a non-metric multidimensional scaling (NMDS) in two dimensions with Bray-Curtis spacing and one hundred maximum iterations. Invertebrate abundance data were used with $\log_{10}(x+1)$ transformation. The values of the two NMDS axes were compared to test for differences between habitat types, river reaches, and segments using permutational variance analysis (PERMANOVA with 999 permutations). In this procedure, stress value was checked, and the included taxa was sequentially reduced to the level where stress reached <0.20 to obtain more reliable community characterizations.

The additive diversity partitioning (Crist et al., 2002; Bo et al., 2020) was adopted to determine the contribution of hyporheic invertebrates to total diversity at larger spatial scales. Hierarchically nested spatial scales with five levels were established for diversity structure data: sample (each Surber or hyporheic trap community data as the smallest observation unit), habitat-type (multiple sample community data pooled for each of hyporheic and benthic zones), reach (multiple community data pooled for each reach), segment (multiple reach community data pooled for each segment), and inter-segment scales (multiple segment data pooled together) in an increasing order of spatial extent (Fig.2.3A). Although Satsunai River had several segments within a same river, they were treated as independent segments because they were distant to each other, and the segments were highly different in terms of environmental conditions. In the first partition analysis at the inter-segment scale, total diversity (total taxonomic richness observed in all the pooled samples; Surber and colonization trap samples) was partitioned into four hierarchical levels, where α was the diversity within the samples, $\beta 1$ was the diversity among samples, $\beta 2$ was the diversity between habitat types (or habitat-type beta), $\beta 3$ was the diversity among river reaches, and $\beta 4$ was the diversity among the river segments (Fig.

2.3B). Then, sequentially, data size was reduced; for example, in the segment-scale partitioning, total diversity for a particular segment was partitioned into three levels (α, β1, β2, and β3), and the same analysis was conducted for each of other segments. The statistical significance of each β was tested using a null model based on hypothetical random observations of data across all the scales. Specifically, individual-based randomization was repeated to the null model 999 times, generating a distribution of expected values. The statistical significance was determined using the proportion of every component's null value (the expected value) relative to the observed value and based on whether they were greater or less than the expected value.

To investigate the community diversity dissimilarities at habitat-type scale and unravel the mechanisms behind the patterns of community, incidence-based dissimilarities were calculated using Sørensen dissimilarity (Baselga, 2010). The overall Sørensen dissimilarities were ($\beta_{sør}$) decomposed into its additive components, species turnover (βsim), and nestedness (β_{nes}), where turnover denotes the replacement of some species by others between benthic and hyporheic habitats, and nestedness refers to the differences in richness between nested subsets. This analysis detected the pairwise dissimilarities between habitats (e.g., hyporheic-hyporheic, benthic-benthic, and benthic-hyporheic); however, it was considered only the beta pairs between benthic and hyporheic habitats. The beta dissimilarity was calculated based on the taxonomic matrix using the following equation.

$$\beta_{S\phi r} = \frac{b + c}{2a + b + c} = \beta_{sim} + \beta_{nes}$$

$$\beta_{sim} = \frac{\min(b, c)}{a + \min(b, c)}$$

$$\beta_{nes} = \frac{\max(b, c) - \min(b, c)}{2a + \min(b, c) + \max(b, c)} \times \frac{a}{a + \min(b, c)}$$

Where a, b, and c represent the species common in both habitats (benthic and hyporheic), species that occur in the benthic habitat, not in the hyporheic, and species that occur in the hyporheic habitat, not in the benthic habitat, respectively.

To determine the driving component of beta diversity, the relative contribution of the turnover to the overall beta diversity was assessed using the following equation.

$$P_{turn} = \frac{turnover}{overall\ dissimilarity}$$

Then, turnover contribution to beta diversity were compared across reaches by constructing generalized linear mixed models (GLMMs) considering turnover contribution to beta as a response variable and sampling reach as the main factor (Gaussian error distribution). After that, the habitat contribution was determined by multiplying the reach-scale habitat-type beta from additive diversity partitioning and P_{turn} by using the following equation.

$$Habitat\ contribution\ to\ regional\ diversity\ = \text{habitat} - \text{type beta} \times\ P_{turn}$$

The habitat contribution was then compared across river reaches by constructing GLMMs considering habitat contribution as a response variable and river reach as the main factor (Gaussian error distribution). At each reach, the species richness and Shannon-Wiener diversity index of the two habitats were compared to examine whether the turnover pattern was due to the presence of unique taxa in the hyporheic habitat. To examine whether taxonomic richness and Shannon-Wiener diversity index differ across reaches and between habitats, GLMMs were constructed using taxa richness and Shannon-Wiener diversity index as the response variable, habitat and reach identity and their interaction as main factors, and sample identity as random variables (Poisson and Gaussian error distribution, respectively).

The habitat affinity of macroinvertebrates was determined based on the incidence-based matrix by comparing the invertebrates in hyporheic colonization trap samples at the respective river reach to those from the benthic Surber sample, and the relative proportion (%) of species found in the hyporheic zone was determined. All the combinations between samples from two zones were prepared with presence and absence data of each taxon, which provided multiple measurements of incidence probability of either 1 (present) or 0 (absent). The overall species-specific proportional mean of these

multiple incidence measurements for each taxon at each river reach was determined and considered an index of habitat affinity of the macroinvertebrates. The species with a proportional mean value greater than "0.5" were considered unique taxa with a relatively high habitat affinity to the hyporheic habitat as "hyporheic species". A generalized linear model (GLM) was constructed to examine whether the changes in hyporheic habitat contribution were dependent upon the changes in the number of hyporheic species across reaches by considering hyporheic habitat contribution as a response variable and the number of hyporheic species as explanatory variable (Gaussian error distribution). This analysis was conducted at the reach scale; total number of "hyporheic species" observed in multiple calculations were summed up and used. Several GLMs were constructed to examine the effects of environmental variables on hyporheic habitat contribution by including habitat contribution as response variables and environmental variables as explanatory variables (Gaussian error distribution). These analyses were conducted at the reach scale; both response and explanatory variables were averaged for each and used. Lastly, the relationships between the richness of hyporheic species and environmental variables were tested by developing GLMMs with sample identity as a random factor (Poisson error distribution). These analyses were conducted at the sample scale; the richness was measured for each hyporheic trap. When multiple explanatory variables have correlations with each other, the effects of the explanatory variables on the response variables can be distorted due to collinearities with redundant effects. For this reason, environmental variables without strong correlations (Pearson's correlation analyses; coefficient > 0.7; $p < 0.05$) among them (i.e., explanatory variables) to be included in GLM(M)s to avoid multicollinearity (Graham et al., 2003; Dormann et al., 2013).

Species diversity is well known to be affected by sampling efforts and/or individual numbers (Colwell et al., 2004). Whether different methods used to collect macroinvertebrates between hyporheic and benthic zones caused flawed data representation and interpretation was examined by drawing relationships between taxonomic richness and the number of sampled individuals. Both sampling area (or volume, and thus individual density) and sampled individuals per sample (colonization traps or Surber sampler) differed and thus the individual number was chosen as explanatory variable that can affect taxonomic richness (Moreno and Halffter, 2001). At

the segment scale, all the samples were pooled for each habitat type, and sample-based taxonomic richness was obtained based on randomized resampling of individuals and calculation of alpha diversity of each sample; individual abundance for each sample was ranged at the interval of 5 starting from 5 individuals until the individual number reached 50% of total individual number in pooled sample.

All statistical analyses (significance threshold = 0.05) were performed using R (version 4.2.0, R Core Team 2022). The packages "glmmADMB", "vegan", and "ADONIS" were used for GLMMs, NMDS, and PERMANOVA, respectively. Multiple comparisons between groups in GLMMs were performed using the package "multcomp". The vegan package (Oksanen et al., 2018) was for additive diversity partitioning and betapart with the beta.pair command in R (Baselga and Orme, 2012) was used to determine beta partitioning.

2.3 Results

A total of 23,815 individuals (14,556 in benthic habitat and 9,259 in hyporheic habitat) were collected among the 68 macroinvertebrate taxa. Macroinvertebrate communities were separated largely among segments in the NMDS plot (Stress = 0.17; Fig. 2.4). Both the community categorizations based on reaches and habitat types overlapped between groups and were less separated in the plot. The results of the NMDS ordination and PERMANOVA showed significant differences in the taxonomic composition of the communities at all three spatial scales, but the community composition variation was largely explained by segment scale categorization as shown in much high r-squared value, which reflected the pattern shown in the biplot (Table 2.5).

A non-random pattern was observed in spatial distributions of taxa across the hierarchical levels. At the inter-segment scale, habitat-type beta (β2) was 12.9% and was insignificant ($p>0.05$) (Fig. 2.5A; Table 2.6). β1, β3, and β4 components accounted for 14.2%, 8.77%, and 39.9%, respectively, of the total taxon richness. β3 and β4 were significantly higher than expected values ($Prop_{exp>obs} < 0.001$), whereas β1 was significantly lower than expected. Alpha (within samples) diversity contributed 24.18% and the percentage of explained variance was significantly lower than expected (Fig.

2.5A; Table 2.6). The second partition at segment scale showed that the contribution of habitat-type beta ranged from 17.8% to 31.9% of the total diversity across river segments. Most observed value was higher than predicted significantly except for Satsunai_L7 and Sorachi river segments (Fig. 2.5B). The highest values of beta diversity contribution were detected among sample units, and the lowest was between river reaches in each segment. At the reach scale, the contribution of the β2 (habitat-type beta) component ranged from 17.5% to 46.8% and was significantly ($p<0.05$) higher than predicted values in 13 out of 15 reaches. Habitat-type beta contribution was highest at Sorachi_up and lowest at Satsunai L1_up reach (Fig. 2.5C; Table 2.7). The α (within sample units) component contributed 27.5 to 65.7% to the total diversity, highest at the Satsunai L1_up reach and lowest at the Tok_up reach. The diversity within sample units was significantly lower than the predicted values in most reaches. The β1 (among sample units) component accounted for the range from 13.4 to 40.6% of the total diversity, where Hor_down reach was the highest (Fig. 2.5C; Table 2.7). In almost all the reaches, the diversity among sample units was either significantly or not significantly lower than predicted except for Satsunai L7_down. Overall, the habitat contribution was well detected at the reach scale, and the following analysis focused on the reach scale.

The mean values of total beta diversity ranged from 0.28 to 0.84 across the reaches, and turnover ranged from 0.18 to 0.54 across the reaches (Table 2.8). The turnover varied across reaches and was highest at Satsunai S1_down reach. On the other hand, the turnover contribution to overall beta diversity varies across river reaches (Table 2.9A) that ranged from 0.20 to 0.91 and was highest at Satsunai_L7_up and lowest at the Tokachi_up reach (Fig. 2.6A; Table 2.8). The hyporheic habitat contribution differed across river reaches (Fig. 2.6A; Table 2.9B), with a higher contribution at the Satsunal_S1 reach and lowest at Sorachi_down reach. Hyporheic habitat contributed up to 25.9% to the total diversity and averaged 15.4% across reaches (Fig. 2.6B; Table 2.8). Species richness was highest at the Satsunai L1_down reach and lowest at the Tokachi_up reach (Fig. 2.7). Species richness and Shannon diversity in the benthic habitat were higher than hyporheic between habitat across the river reaches (Fig. 2.7A, B; Table 2.10).

A total of 44 taxa showed habitat preference in the hyporheic habitat across river reaches. Among the reaches, a maximum of 16 taxa at Satsunai S1_up and a minimum of

2 taxa at Sorachi_down and Tokachi_up reaches showed habitat affinity to the hyporheic zone, with other reaches being intermediate (Fig. 2.8A). *Plectrocnemia* sp. (Order: Trichoptera) was relatively more often found (eight reaches), while some species appeared only once across the reaches (13 taxa at nine reaches). The species richness of hyporheic species differed across sites with a higher number in Satsunai river reaches (Fig. 2.8A). The number of species in the hyporheic habitat significantly predicted the habitat contribution from the hyporheic zone to the total diversity of macroinvertebrates (Fig. 2.8B, Table 2.11). The contribution of hyporheic habitat did not systematically vary under varying environmental gradients. None of the environmental variables influenced the contribution (Table 2.12). The number of hyporheic species significantly decreased with the increasing amount of fine sediment in the colonization traps (Table 2.13). On the other hand, number of hyporheic species was increased with D_{50} of the riverbed sediment, nitrate, and dissolved oxygen (Table 2.13).

Sample-based taxonomic richness estimated for randomized resampling of individuals pooled for each habitat type in eight segments demonstrated that taxonomic richness increased with an increase of collected individual abundance (Fig 2.9). No relationships appeared to have reached to plateaus with the abundance range examined. In all cases, hyporheic taxonomic richness was higher or as high as when compared with the relationship for benthic invertebrates.

2.4 Discussion

This study examined whether hyporheic habitat contributes to the total diversity of macroinvertebrates and the factors controlling the pattern of contribution in the rivers in Hokkaido, Japan. The findings showed that the hyporheic habitat had a significant contribution mediated by the turnover with the presence of unique species being present in hyporheic zone, which supported the initial prediction. However, the prediction that the contribution is affected by some environmental conditions was not supported. Numerous previous studies have reported the differences in terms of invertebrate community composition between two zones also by identifying species unique to the hyporheic zone (De Walt and Stewart, 1995; Negishi et al., 2019b; Marmonier et al., 2020; Negishi et al., 2022) but did not quantify how important they are in spatial diversity

structure. Overall, this study reports a new understanding of the importance of the hyporheic habitat to the total diversity of macroinvertebrates and the knowledge of taxonomic responses of hyporheic species across environmental gradients in the hyporheic zone.

The hyporheic habitat contributed to the total diversity on an average of about 15%. Although sampling efforts differed, the taxonomic richness and thus, hyporheic contribution was unlikely overestimated, because higher or comparable levels of diversity relative to the benthic zone was expected to be observed in the hyporheic zone if similar hypothetical levels of sampling efforts (individual number) were considered; the macroinvertebrate abundance in the hyporheic samples was lower or comparable relative to that in the benthic zone in this study (unpublished data) and thus the possibility of underestimation needs to be cautioned instead. Although total taxonomic diversity was underestimated because richness-abundance curves did not reach to plateaus, this should not systematically bias the findings' interpretation. Few studies have examined both subsurface and benthic zones simultaneously in the perspective of community structure and diversity structure. Lencioni and Spitale (2014) and Gray et al. (2006) both studied gravel-bed river systems at the spatial scale close to the reach to segment scales in this study (i.e., two rivers only apart from each other by 20 m in European Alps and one several sections in New Zealand) and found that groundwater or hyporheic communities are unique in terms of species diversity. The former study specifically reported that about 20% of community structure variations in two rivers could be explained by the presence of benthic and hyporheic communities while the latter did not quantify the differences. It should be noted that our estimated contribution was somehow similar to their estimated importance to faunal organizations but in a slightly different sense.

The magnitude of hyporheic contribution was scale dependent in this study. For example, variation among river segments and reaches were the components that accounted for the most significant amount of explained variances in the taxonomic richness, indicating that the river reaches and segments were the spatial scales that mainly contributed to the total richness of macroinvertebrates. One of the plausible explanations for this is that species pool increased substantially when multiple river systems were

included in the analyses, and this increase overwhelmed species pool increase in the hyporheic zone. This was also supported by the community structure differences most pronounced across river segments on the NMDS plot, which suggests the availability of unique niches across river reaches and segments (Tornwall et al., 2015; Bo et al., 2016; Piano et al., 2019; Bo et al., 2020). The environmental heterogeneity could influence the composition of macroinvertebrates due to the variability among studied reaches and segments observed in the study. The river segments and reaches contributed more than other smaller spatial scales supporting the previous findings (Ligeiro et al., 2010; Bo et al., 2020). The river segments and reaches are situated in different watersheds, which explains a large spatial extent to which macroinvertebrate community diversity and composition could explain a large proportion of variances (Gray and Harding, 2009; Ligeiro et al., 2010; Bo et al., 2020). The river segments in the study region are dissimilar in terms of physical structure, geomorphology, and landscape structures (Shibata et al., 1998; Negishi et al., 2019b), thus could create heterogeneity. Among them, the Horonai Rver segment and Satsunai_S1 are spring-water dominated, whereas other segments are typical gravel-bed rivers with surface-runoff being dominant. This study thus reported quantitative and scale-dependent importance of hyporheic zone in macroinvertebrate diversity for the first time.

Beta diversity has been a point of interest among all the diversity components because they address the dissimilarities in community composition across spatial scales (Anderson et al., 2011). The prediction that the beta diversity would be mediated by turnover was supported suggesting that presence of unique species between benthic and hyporheic habitats. The dominance of the turnover component in the beta diversity patterns suggested the deterministic niche-related processes between habitats (Nunes et al., 2016; Guzman and Alcocer, 2022). This reflects the replacement of taxa and the presence of unique taxa between the habitats. The presence of unique taxa created a distinctive community structure between habitats. The variability in turnover contribution to overall beta dissimilarities suggested the variable species replacement between habitats across reaches (Datry, 2012; Stubbington et al., 2019). Previous studies reported similar findings that habitat heterogeneity (e.g., lentic vs. lotic) accompanies the turnover contribution to the total dissimilarity (Datry, 2012; Lencioni and Spitale, 2014; Nunes et

al., 2016; Guzman and Alcocer, 2022). The present findings were consistent with a pioneering study by Stubbington et al. (2019) who reported that beta dissimilarity in benthic and hyporheic habitats differed across the different catchments, in which dissimilarity in the hyporheic habitat was triggered mainly by turnover. Furthermore, the hyporheic alpha diversity index lower than that of benthic zone indicated that the hyporheic community comprised a well nested subset of benthic community. This was caused by fewer but unique species present in the hyporheic habitat, which supported the study prediction. Overall, habitat contribution to total diversity was largely driven by turnover with the presence of unique species in the hyporheic habitat (Baselga and Orme, 2012; Lencioni and Spitale, 2015).

Macroinvertebrate community structure in both zones differed spatially in particular across river segments with high variation of hyporheic contribution to the total diversity. Segments were highly variable in terms of environmental conditions, which presumably caused variable invertebrate responses in a complex way. The contribution from the hyporheic habitat did not systematically vary under varying environmental gradients across river reaches. Instead, contrasting evidence was found for the responses of hyporheic species to the environmental variables. The independent negative effect of fine sediment amount on the hyporheic species richness indicated the stress condition originating from the gradient of fine sediments limiting the habitat requirement for the hyporheic species in the zone (Navel et al., 2010; Beerman et al., 2018; Collins et al., 2018; Zhang et al., 2018). The fine sediment deposition is one of the significant contributors to the impairment of hyporheic habitat worldwide (Wagenhoff et al., 2012; Mathers et al., 2014). Deposited fine sediment in the surface layer can be introduced to the hyporheic zone by hydrological exchange, and the presence of a higher amount can cause the colmation in the hyporheic zone (Huettel et al., 1996; Olsen and Townsend, 2003; Ren and Packman, 2007; Simpson and Meixner, 2012). The increasing amount can negatively influence the particle size distribution of the streambed, the amount of fine sediments in the hyporheic zone, thermal characteristics, etc. (Evans and Petts, 1997; Boulton et al., 1998; Bo et al., 2006).

The findings suggest that increased levels of nitrate, dissolved oxygen, and median particle size provided favorable habitat for hyporheic invertebrates (Mathers et al., 2014; Collins et al., 2018; Negishi et al., 2019b; Zhang et al., 2020; Hutchins et al., 2020; Bo et al., 2020; Duan et al., 2021). These were consistent with previously reported positive effects of nutrients and dissolved oxygen on hyporheic taxonomic richness (Zhang et al., 2018; Hutchins et al., 2020; Mathers et al., 2014; Collins et al., 2018; Negishi et al., 2019b). On the other hand, riverbed particle size could be an essential physical filter for structuring macroinvertebrate communities (Pardo and Armitage, 1997; Robson and Chester, 1999; Buss et al., 2004; Costa and Melo, 2008). The habitats are often characterized by the physicochemical variables determining the macroinvertebrate community structure. The near-bed conditions often are a strong predictor in determining the filter effects of species-specific traits (Beisel et al., 1998; Lamouroux et al., 2004; Sagnes et al., 2008). The substrate size and stability frequently control the distribution of food availability in the streams (Minshall, 1988; Fenoglio et al., 2005; Doretto et al., 2016, 2017). Thus, fine sediment amounts in the hyporheic zone, nutrients, DO, and riverbed sediment particles (D_{50}) characterized the hyporheic habitat and influenced the hyporheic species richness.

2.5 Conclusion

The findings extended the critically important understanding of hyporheic habitat as a contributor to the total diversity of the macroinvertebrates and the factors that affect the contribution based on simultaneous measurement of the benthic and hyporheic metacommunities. The contribution was significantly detected at the smaller reach scale, which suggests focusing on the conservation needs of hyporheic biodiversity at smaller scales. The beta diversity pattern between habitats also extended the understanding of how hyporheic habitats can act as a vital community by interacting and forming a metacommunity and critically contributing to the macroinvertebrates' total biodiversity by adding unique species. The underlying mechanisms of the variable pattern of the hyporheic habitat contribution provided a unique understanding of how the richness of unique species, their variable responses to the environmental variabilities, and their contribution to the spatial turnover can be the key to changing diversity contribution. In

the context of freshwater resource management and conservation, knowledge of the importance of hyporheic habitat as a significant contributor to the total diversity of macroinvertebrates in rivers would signify the conservation priority of the habitat. Also, it provided information on the suitable scale at which the hyporheic habitat should be assessed, which could be crucial guidelines to set up the conservation importance of natural habitats. This would help the river manager to set up the conservation priority based on the effects of environmental variables on hyporheic macroinvertebrates. High contributions in Satsunai River calls an attention for hyporheic conservation and further research on the mechanical driver of the findings.

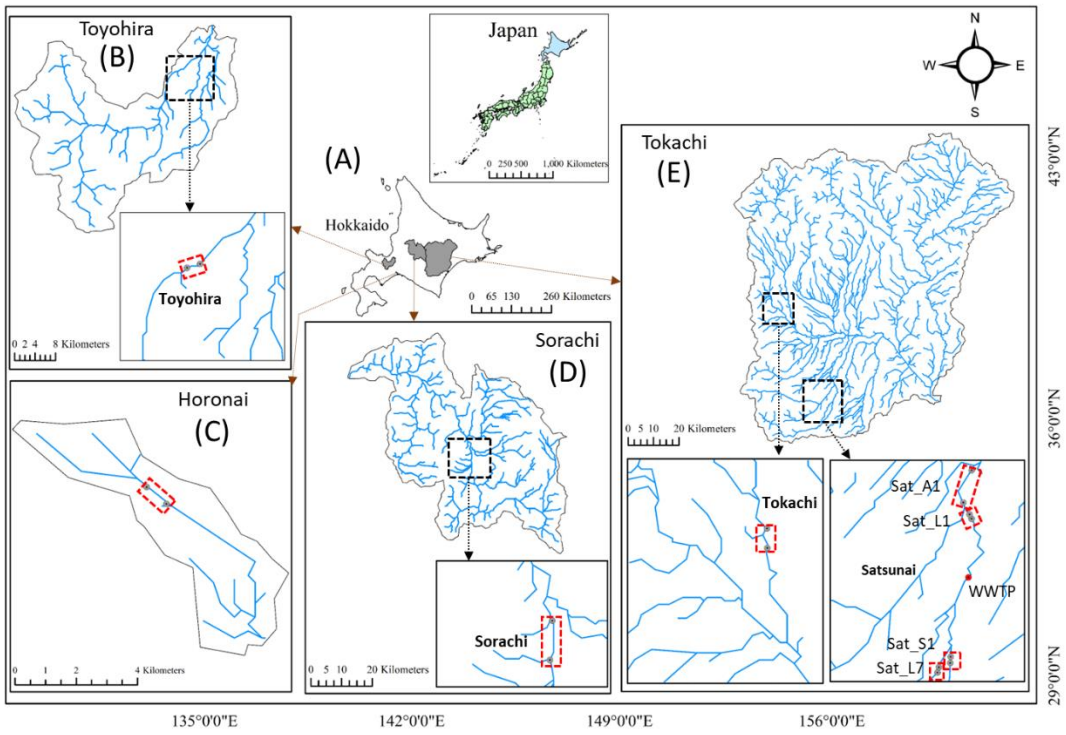


Fig. 2.1 Location of the Hokkaido prefecture in Japan, where studied catchments are indicated by gray shaded area (A). River segments are shown in four panels: Toyohira (B), Horonai (C), Sorachi (D), Tokachi (Tokachi and Satsunai river in Tokachi catchment) (E). In each panel, the area enclosed with dotted black lines were enlarged when necessary; river reaches are shown by red dotted box (two reaches in each box shape and gray-filled point denotes each river reach). A red-filled point in Satsunai River channel denotes the confluence with a tributary affected by a wastewater treatment plant (WWTP).

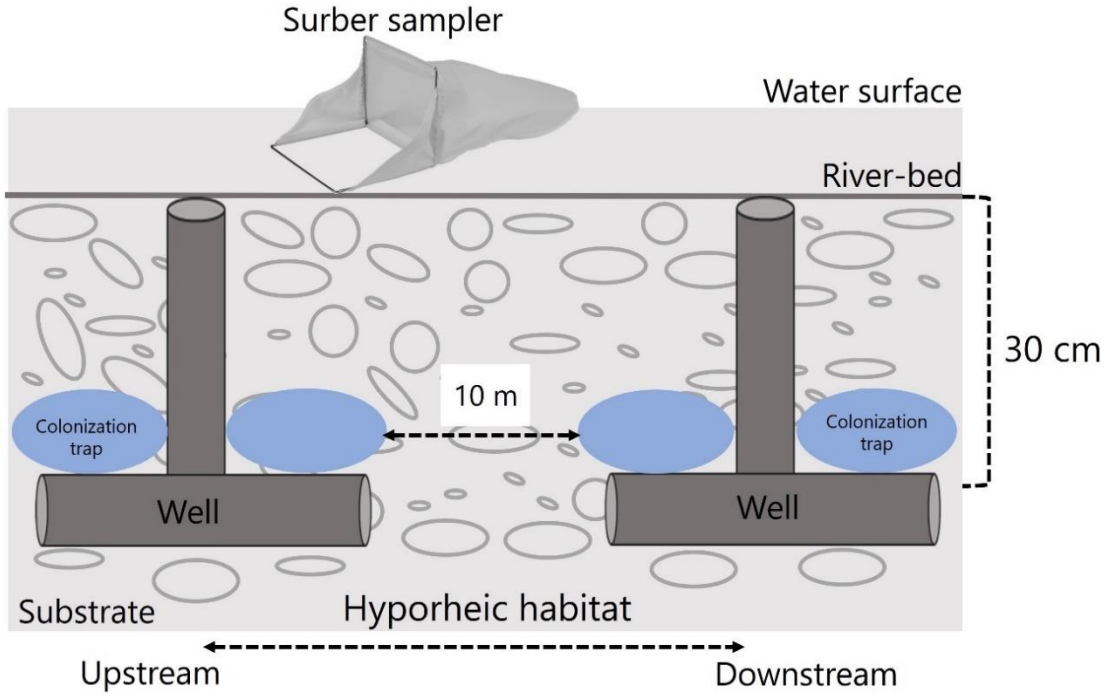
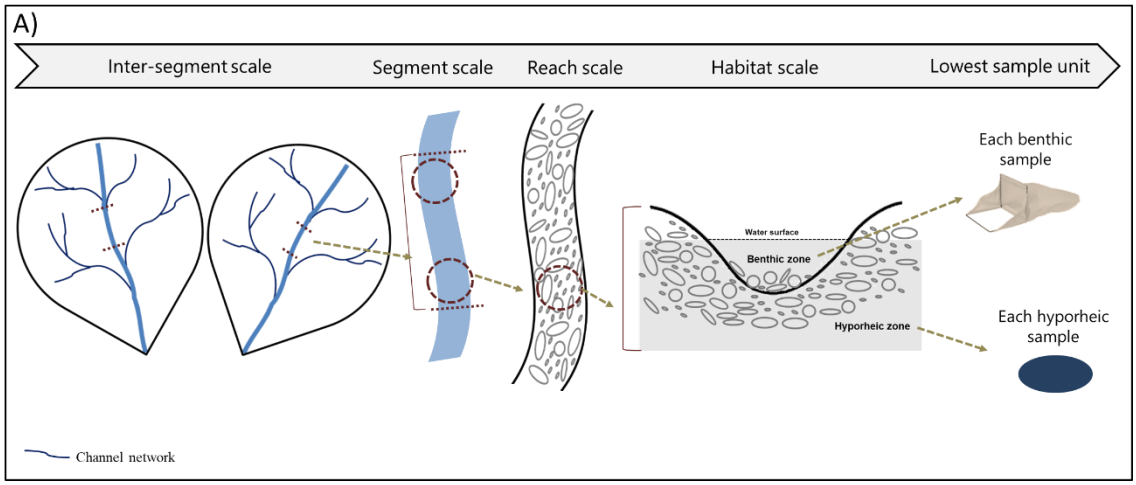


Fig. 2.2 Diagram showing the experimental design of each river reach detailing longitudinal locations of where the hyporheic and benthic macroinvertebrate samples were collected using colonization traps and Surber sampler respectively. "T-shaped" hyporheic well were used in the hyporheic zone to collect hyporheic water samples.



B)

	Relevant scale
$Alpha_1$ (within sample units) + $Beta_1$ (among sample units)	Sample
$Alpha_2$ (within habitat) + $Beta_2$ (between habitat)	Habitat
$Alpha_3$ (within reach) + $Beta_3$ (among reach)	Reach
$Alpha_4$ (within segment) + $Beta_4$ (among segment)	Segment
Total diversity	Inter-segment

Total diversity (inter-segment) = $Alpha_1+Beta_1+Beta_2+Beta_3+Beta_4$
Total diversity (segment) = $Alpha_1+Beta_1+Beta_2+Beta_3$
Total diversity (reach) = $Alpha_1+Beta_1+Beta_2$

Fig. 2.3 A diagram on the conceptual study design of the study showing how the scales were determined from small (i.e., sample unit) to large scale (i.e., inter-segment). Across the scales, the maroon color dotted line at catchment scale showed the segment at catchment scale, the dotted maroon color circle denoted the river reach at segment scale and at reach scale dotted maroon color circle denoted habitat at each reach (A) and diversity decomposition of the components across spatial scales. The total diversity and components were shown at different scales (B).

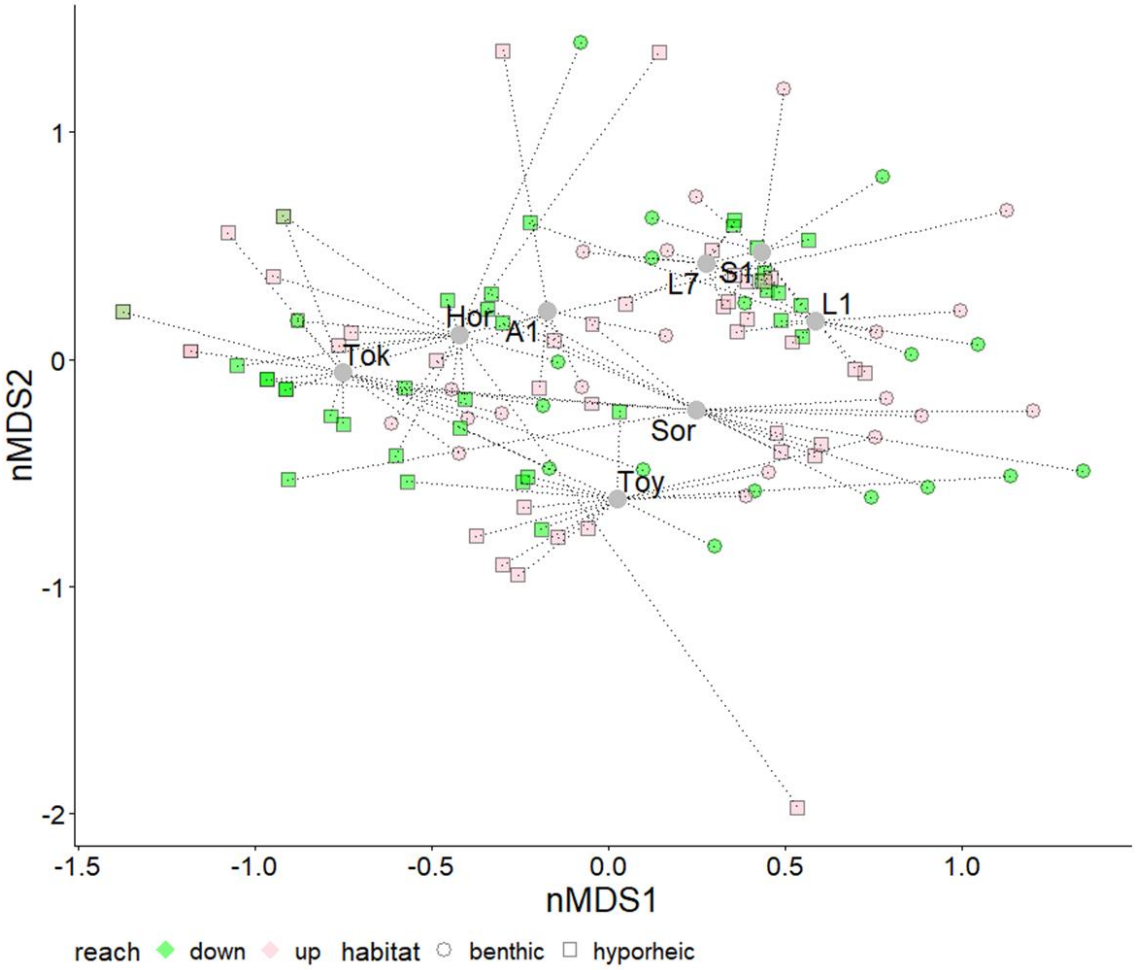


Fig. 2.4 A biplot showing macroinvertebrate community structure using the results of NMDS with log10-transformed abundance of taxa that comprised >5% of total catch. Three spatial scales were indicated: Segment-scale clustering was denoted by connecting each data point with their centroids (gray dots), which were accompanied with segment abbreviations; reach-scale and habitat-type scale clustering were indicated using different colors and symbols. Segment abbreviations were: Tok for Tokachi River segment; Hor for Horonai River segment; Toy for Toyohira River segment; Sor for Sorachi River segement; A1, L7, L1, and S1 for Satsunai River segment.

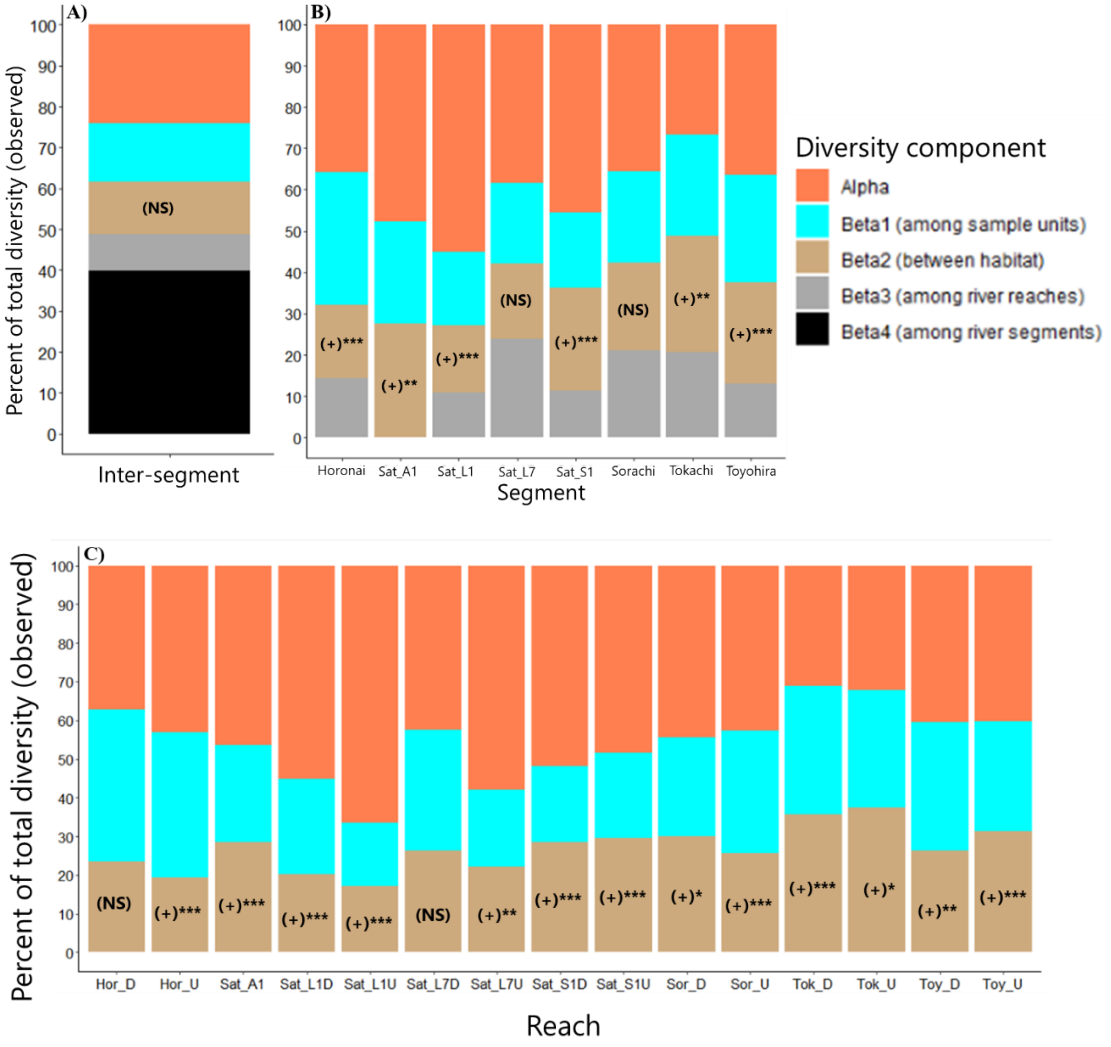


Fig. 2.5 Bar plots showing the additive partition of species richness at inter-segment scale (A), segment scale (B) and, reach scale (C). Values are expressed as the percent of total diversity of macroinvertebrates explained by each hierarchical level. The observed partitions were compared to expected values to evaluate the significance threshold. Statistical significance ("+/-*": observed value is significantly higher or lower than expected in randomization and "NS": not significant) was shown only habitat-type beta diversity.

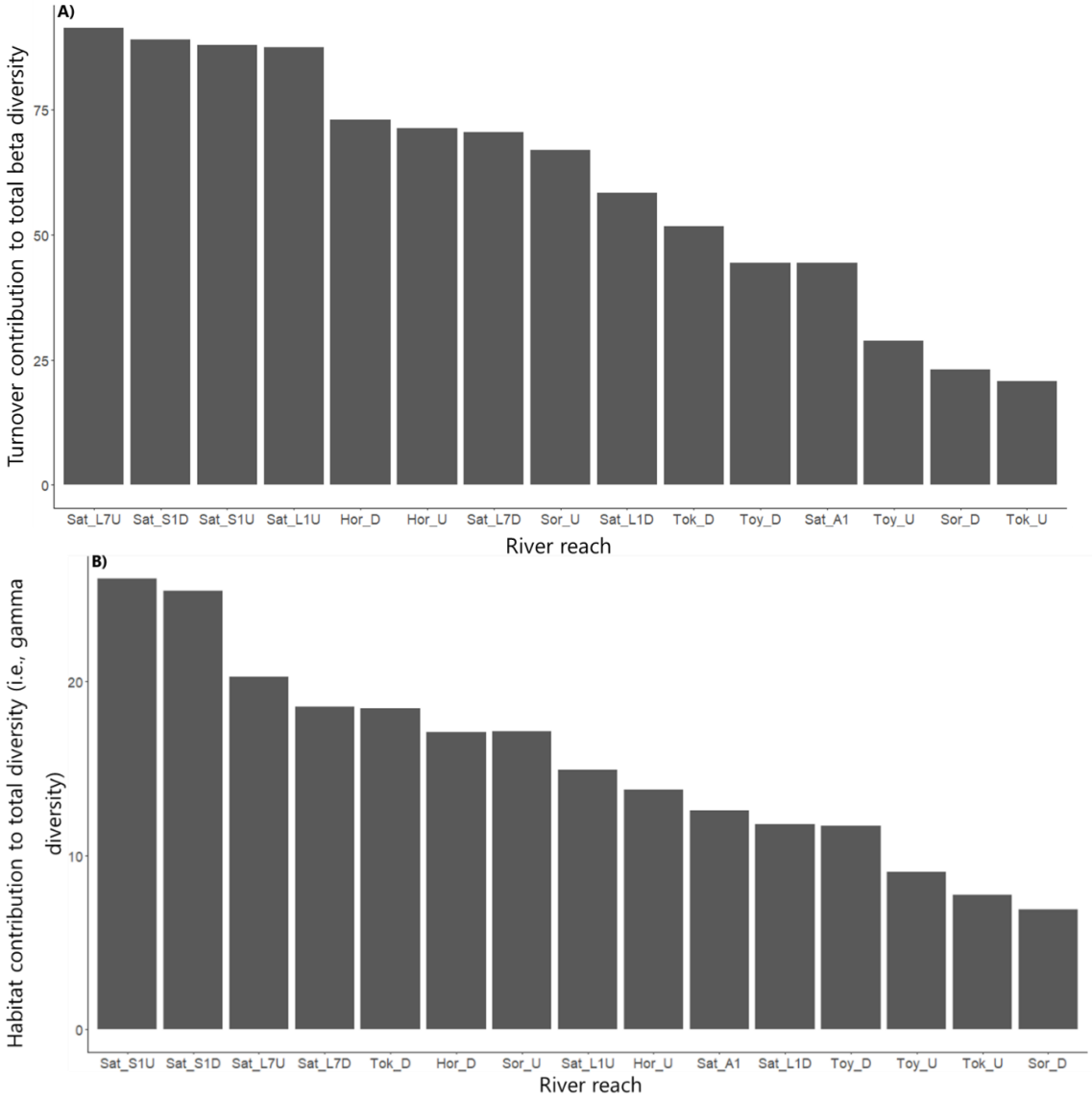


Fig. 2.6 Bar plots showing the turnover contribution to the beta diversity that was determined based on the pairwise analysis between benthic and hyporheic macroinvertebrates (A) and habitat contribution at each river reach that was calculated by multiplying habitat-type beta contribution to total diversity with turnover contribution to beta diversity (B).

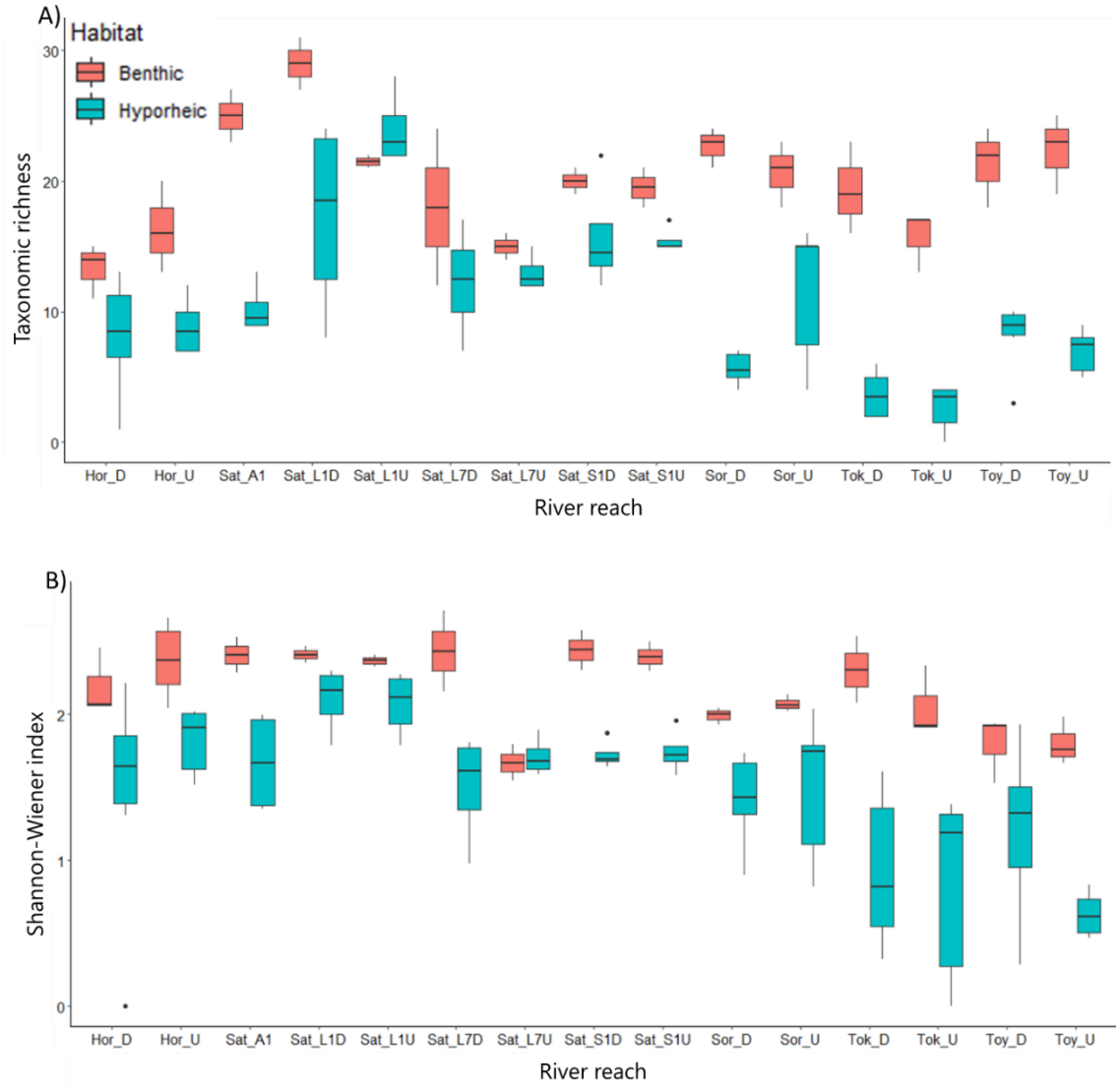


Fig. 2.7 Boxplots showing comparison of the alpha diversity components between habitats across reaches as species richness (A) and Shannon-Wiener diversity index (B).

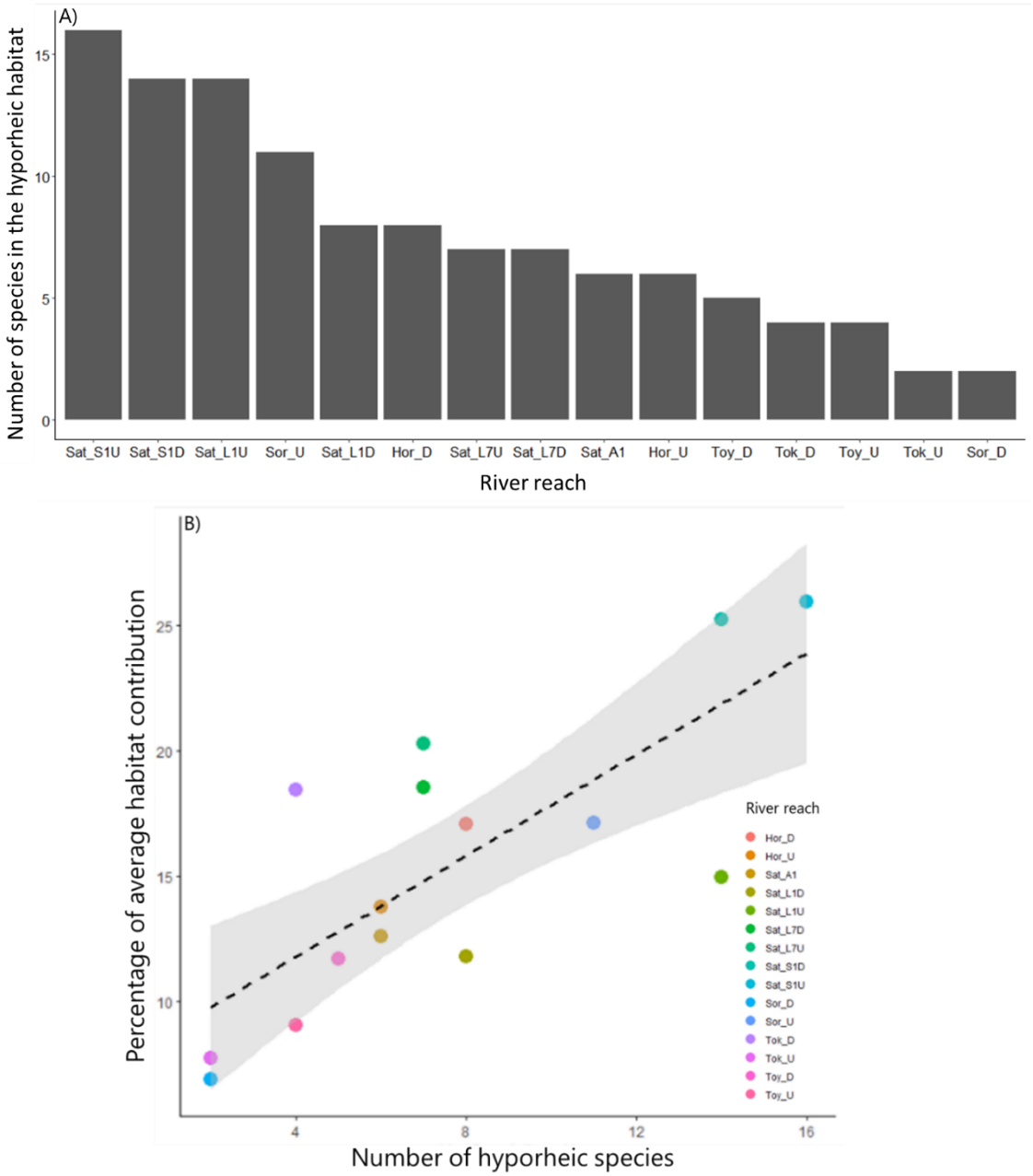


Fig. 2.8 Bar plot showing the number of hyporheic species across the river reaches (hyporheic taxa were those disproportionately higher in the hyporheic habitat as well as showed high habitat affinity) (A) and a biplot showing the relationship between number of hyporheic species and habitat contribution to the total diversity of macroinvertebrates (B). The dotted line and gray zones in (B) denote a statistically significant regression line developed from a generalized linear model and a 95% confidence interval of the mean.

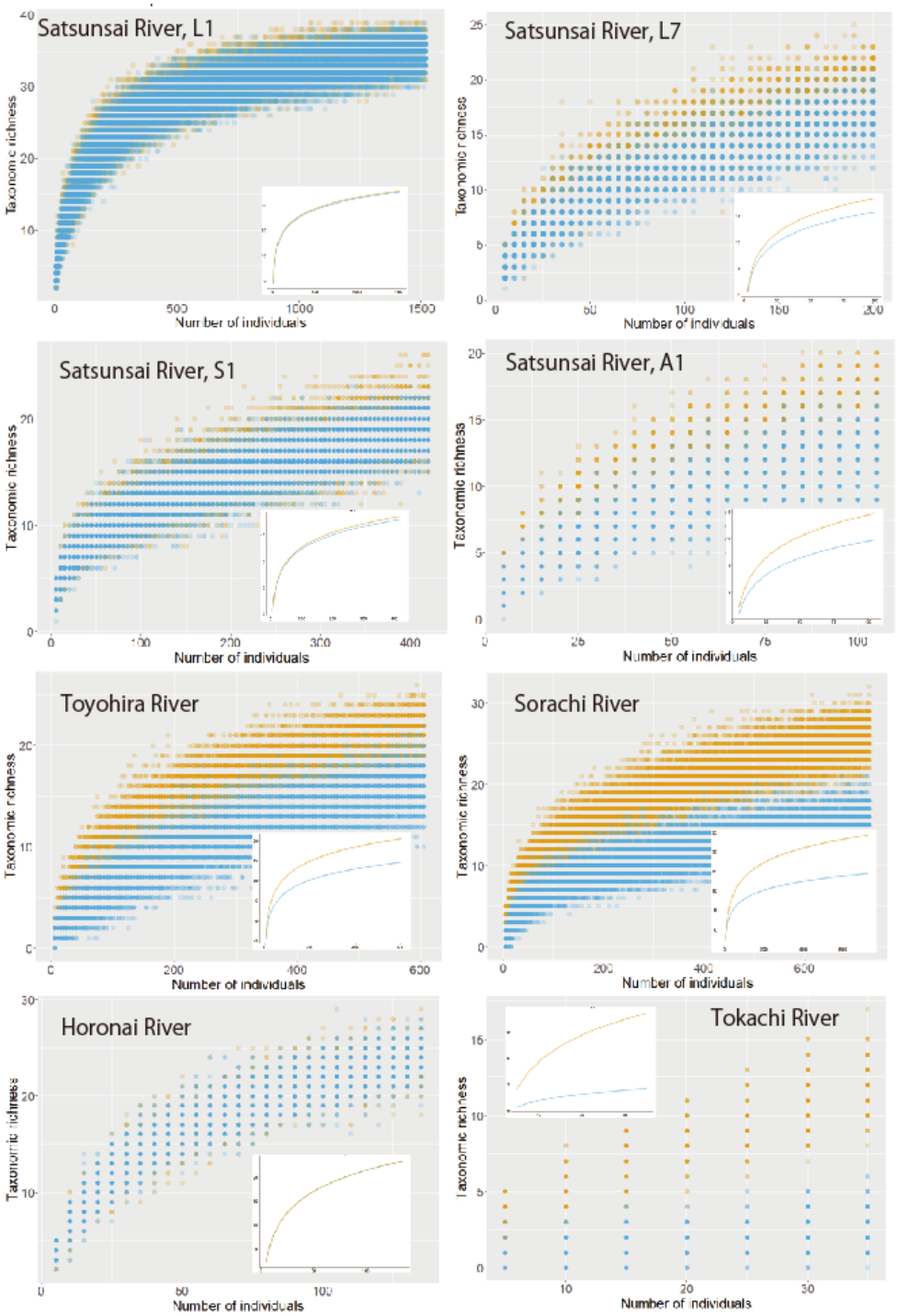


Fig. 2.9 Sample-based taxonomic richness estimated for randomized resampling of individuals pooled for each habitat type in eight segments. Blue and brown color represent benthic and hyporheic data, respectively. The inset represented statistically significant logarithmic curves obtained for each relationship.

Table 2.1 A table showing the general characteristics of the studied segments.

River segment	Segment length (km)	Water depth (cm)	Flow velocity (cm/s)
Satsunai_A1	2.86	20.16	37.38
Satsunai_L1	0.20	21	42.82
Satsunai_L7	0.14	34.25	74.28
Satsunai_S1	0.10	20.5	65.53
Tokachi	1.31	17.83	26.47
Sorachi	2.59	9.87	22.57
Horonai	0.85	13	48.38
Toyohira	0.90	7.43	12.75

Table 2.2 A summary of sampling dates of hyporheic and benthic samples in 2020. Numbers in bracketed superscripts indicate replicated samples measured at each reach.

River	River reach	Installation date (hyporheic)	Collection date (hyporheic and benthic)
		2020	2020
Satsunai	Sat_L1_down	8/4$^{(4)}$	10/28$^{(4,2)}$
	Sat_L1_up	8/4$^{(4)}$	10/28$^{(4,2)}$
	Sat_L7_down	8/4$^{(4)}$	10/29$^{(4,2)}$
	Sat_L7_up	8/4$^{(4)}$	10/29$^{(4,2)}$
	Sat_A1	8/3$^{(4)}$	10/28$^{(4,2)}$
	Sat_S1_down	8/4$^{(4)}$	10/28$^{(4,2)}$
	Sat_S1_up	8/4$^{(4)}$	10/29$^{(4,2)}$
Tokachi	Tok_down	8/17$^{(6)}$	11/12$^{(6,3)}$
	Tok_up	8/17$^{(6)}$	11/12$^{(6,3)}$
Sorachi	Sor_down	8/10$^{(6)}$	10/13$^{(6,3)}$
	Sor_up	8/10$^{(6)}$	10/13$^{(6,3)}$
Horonai	Hor_down	8/17$^{(6)}$	10/13$^{(6,3)}$
	Hor_up	8/17$^{(6)}$	10/13$^{(6,3)}$
Toyohira	Toy_down	8/6$^{(6)}$	10/16$^{(6,3)}$
	Toy_up	8/6$^{(6)}$	10/16$^{(6,3)}$

Table 2.3 A summary of sampling dates of water samples in 2020 for the measurements of electrical conductivity, pH, dissolved oxygen, and temperature (probe) in the surface-water (benthic) zone and in the hyporheic zone. Numbers in bracketed superscripts indicate replicated samples measured at each reach; no superscripts indicate one sample.

River	River reach	Dates (hyporheic)	Dates (benthic)	N
Satsunai	Sat_L1_down	9/14, 10/28	9/14, 10/28	Benthic$^{(2)}$, Hyporheic$^{(2)}$
	Sat_L1_up	9/14, 9/15, 10/28	9/14, 10/28	Benthic$^{(2)}$, Hyporheic$^{(3)}$
	Sat_L7_down	10/29	9/15, 10/29	Benthic$^{(2)}$, Hyporheic
	Sat_L7_up	9/15	9/15, 10/29	Benthic$^{(2)}$, Hyporheic
	Sat_A1	9/14	9/14, 10/28	Benthic, Hyporheic
	Sat_S1_down	9/14, 10/28	9/14, 10/28	Benthic$^{(2)}$, Hyporheic$^{(2)}$
	Sat_S1_up	9/14, 10/29	9/14, 10/29	Benthic$^{(2)}$, Hyporheic$^{(2)}$
Tokachi	Tok_down	9/15, 11/12$^{(2)}$	9/15, 11/12	Benthic$^{(2)}$, Hyporheic$^{(3)}$
	Tok_up	9/15$^{(2)}$, 11/12$^{(2)}$	9/15, 11/12	Benthic$^{(2)}$, Hyporheic$^{(4)}$
Sorachi	Sor_down	10/13	9/15, 10/13	Benthic$^{(2)}$, Hyporheic
	Sor_up	9/15	9/15, 10/13	Benthic$^{(2)}$, Hyporheic
Horonai	Hor_down	9/16$^{(2)}$, 10/13$^{(2)}$	9/16, 10/13	Benthic$^{(2)}$, Hyporheic$^{(4)}$
	Hor_up	9/16$^{(2)}$, 10/13$^{(2)}$	9/16, 10/13	Benthic$^{(2)}$, Hyporheic$^{(4)}$
Toyohira	Toy_down	9/16$^{(2)}$, 10/16$^{(2)}$	9/16, 10/16	Benthic$^{(2)}$, Hyporheic$^{(2)}$
	Toy_up	9/16$^{(2)}$, 10/16$^{(2)}$	9/16, 10/16	Benthic$^{(2)}$, Hyporheic$^{(2)}$

Table 2.4 A summary of sampling dates of water samples in 2020 for the measurements of nitrate in the surface-water (benthic) zone and in the hyporheic zone. Numbers in bracketed superscripts indicate replicated samples measured at each reach; no superscripts indicate one sample.

River	River reach	Dates (hyporheic)	Dates (benthic)	N
Satsunai	Sat_L1_down	9/14, 10/28	9/14, 10/28	Benthic$^{(2)}$, Hyporheic$^{(2)}$
	Sat_L1_up	9/14, 10/28	9/14, 9/15, 10/28	Benthic$^{(3)}$, Hyporheic$^{(2)}$
	Sat_L7_down	10/29	9/15, 10/28	Benthic$^{(2)}$, Hyporheic
	Sat_L7_up	9/15	9/15, 10/29	Benthic$^{(2)}$, Hyporheic
	Sat_A1	9/14	10/28	Benthic, Hyporheic
	Sat_S1_down	9/14, 10/28	9/14, 10/28	Benthic$^{(2)}$, Hyporheic$^{(2)}$
	Sat_S1_up	9/14, 10/29	9/14, 10/29	Benthic$^{(2)}$, Hyporheic$^{(2)}$
Tokachi	Tok_down	9/15, 11/12$^{(2)}$	9/15, 11/12	Benthic$^{(2)}$, Hyporheic$^{(3)}$
	Tok_up	9/15$^{(2)}$, 11/12$^{(2)}$	9/15, 11/12	Benthic$^{(2)}$, Hyporheic$^{(4)}$
Sorachi	Sor_down	9/15	9/15, 10/13	Benthic$^{(2)}$, Hyporheic
	Sor_up	10/11	9/15, 10/13	Benthic$^{(2)}$, Hyporheic
Horonai	Hor_down	9/16$^{(2)}$, 10/13$^{(2)}$	9/16, 10/13	Benthic$^{(2)}$, Hyporheic$^{(4)}$
	Hor_up	9/16$^{(2)}$, 10/13$^{(2)}$	9/16, 10/13	Benthic$^{(2)}$, Hyporheic$^{(4)}$
Toyohira	Toy_down	9/16$^{(2)}$, 10/16$^{(2)}$	9/16, 10/16	Benthic$^{(2)}$, Hyporheic$^{(2)}$
	Toy_up	9/16$^{(2)}$, 10/16$^{(2)}$	9/16, 10/16	Benthic$^{(2)}$, Hyporheic$^{(2)}$

Table 2.5 Results of multivariate analysis of variance using PERMANOVA on Bray-Curtis matrix and log10-transformed abundance of taxa that comprised >5% of total catch. Variation sources of community composition was partitioned into three spatial scales, i.e., segment, reach and habitat types (benthic vs. hyporheic).

	df	SS	R squared	F	Probability
Segment	7	11.4071	0.46178	15.5749	0.001
Reach	1	0.2583	0.01046	2.4685	0.035
Habitat type	1	2.26	0.09149	21.6005	0.001
Residual	103	10.7768	0.43627		
Total	112	24.7021	1		

"*p<0.05; **p<0. 01; ***p<0.001**"**

Table 2.6 Results of additive diversity partitioning at inter-segment scale: diversity component = components of diversity, description = each component of diversity, observed = percentage variance of observed richness, expected = percentage variance of predicted richness, significance = significance threshold between observed and predicted variance.

Diversity component	Description	Observed (%)	Expected (%)	Significance
Alpha	Within sample units	24.17	44.28	(-)***
Beta 1	Among sample units	14.20	23.05	(-)***
Beta 2	Between habitats	12.91	11.91	(+)NS
Beta 3	Among river reaches	8.77	6.77	(+)**
Beta 4	Among river segments	39.93	13.95	(+)***

(+/-)*: observed value is significantly higher or lower than expected in randomization

"NS": not significant; *p<0.05; **p<0. 01; ***p<0.001.

Table 2.7 Results of additive diversity partitioning at reach scale: diversity component = components of diversity, description = each component of diversity, observed = percentage variance of observed richness, expected = percentage variance of predicted richness, significance = significance threshold between observed and predicted variance (obs. = observed value; exp. =predicted value).

River reach	Within sample units (obs.)	Within sample units (exp.)	Among sample units (obs.)	Among sample units (exp.)	Between habitats (obs.)	Between habitats (exp.)
Sat_L1_down	55.15 (-)***	63.36	24.60 (-)^{NS}	26.18	20.23 (+)***	10.45
Sat_L1_up	66.54 (-)***	74.04	16.35 (-)^{NS}	20.04	17.10 (+)***	5.90
Sat_L7_down	42.54 (-)***	50.52	31.13 (+)^{NS}	27.40	26.31 (+)^{NS}	22.06
Sat_L7_up	58.02 (-)***	65.03	19.75 (-)^{NS}	24.30	22.22 (+)**	10.66
Sat_A1	46.39 (-)***	52.18	25.22 (-)**	31.76	28.37 (+)***	16.04
Sat_S1_down	51.80 (-)***	69.00	19.81 (+)^{NS}	19.00	28.37 (+)***	11.99
Sat_S1_up	48.29 (-)***	63.35	22.22 (+)^{NS}	20.15	29.48 (+)***	16.49
Tok_up	32.14 (+)^{NS}	30.75	30.35 (-)**	39.50	37.5 (+)*	29.73
Tok_down	31.11 (-)***	35.97	33.17 (-)^{NS}	38.21	35.71 (+)***	25.81
Sor_up	42.73 (-)***	52.67	31.62 (-)^{NS}	34.55	25.64 (+)***	12.76
Sor_down	44.44 (-)^{NS}	46.79	25.55 (-)*	30.80	30 (+)*	22.39
Hor_up	43.00 (-)***	52.51	37.63 (-)^{NS}	37.86	19.35 (+)***	9.62
Hor_down	37.15 (-)^{NS}	39.15	39.40 (-)^{NS}	40.51	23.43 (+)^{NS}	20.32
Toy_up	40.31 (-)***	51.81	28.25 (-)**	33.85	31.42 (+)***	14.33
Toy_down	40.43 (-)***	46.76	33.17 (+)^{NS}	37.17	26.38 (+)**	16.05

(+/-)*: observed value is significantly higher or lower than expected in randomization; "^{NS}": not significant; *p<0.05; **p<0. 01; ***p<0.001.

Table 2.8 Table showing the turnover as a beta diversity component, turnover contribution to beta dissimilarity, and habitat contribution to the total diversity. Pairwise beta diversity was determined considering the pairs between macroinvertebrates in benthic and hyporheic habitats.

River reach	Turnover	Contribution of turnover to beta diversity	Average habitat contribution
Sat_L1_down	0.2379	0.5834	0.1180
Sat_L1_up	0.2887	0.8737	0.1494
Sat_L7_down	0.3555	0.7050	0.1855
Sat_L7_up	0.4494	0.9120	0.2026
Sat_A1	0.2630	0.4433	0.12582
Sat_S1_down	0.5347	0.8889	0.25225
Sat_S1_up	0.4759	0.8787	0.25911
Tok_up	0.1499	0.2067	0.07751
Tok_down	0.4351	0.5165	0.18449
Sor_up	0.3567	0.6682	0.17133
Sor_down	0.1633	0.2303	0.06910
Hor_up	0.4202	0.7125	0.13790
Hor_down	0.4384	0.7290	0.17088
Toy_up	0.1819	0.2885	0.09067
Toy_down	0.2702	0.4441	0.11720

Note: Habitat contribution at each river reach: turnover contribution to beta × percentage of habitat-type contribution to total diversity

Table 2.9 Results of GLMMs testing the effects of river reach on the contribution of turnover to total beta diversity (A) and hyporheic habitat contribution to total diversity (B). Full models were compared to null models using log-likelihood tests. Bold letters for p-value denote statistical significance.

Explanatory variables	logLik	AIC	p-value
A) Contribution of turnover to beta diversity			
River reach	50.30	-68.6	**<0.001**
Null model	-45.30	94.6	
B) Habitat contribution			
River reach	302.62	-573.3	**<0.001**
Null model	233.21	-462.4	

"*p<0.05; **p<0. 01; ***p<0.001"

Table 2.10 Results of GLMMs testing the effects of river reach, habitat (benthic and hyporheic), and their interactions on the taxonomic richness (A) and Shannon-Wiener diversity index (B). Full model and reduced model was compared using log-likelihood tests. Bold letters for p-value denote statistical significance.

(A)	**logLik**	**AIC**	**p-value**
Full model			
Site (S), Habitat (H), S×H	-288.39	640.8	**<0.001**
1st Reduced model			
S, H	-329.85	695.7	

(B)	**logLik**	**AIC**	**p-value**
Full model			
Site (S), Habitat (H), S×H	-36.78	135.6	**<0.05**
1st Reduced model			
S, H	-49.80	133.6	

"*p<0.05; **p<0. 01; ***p<0.001"

Table 2.11 Generalized linear model (GLM) testing the effect of number of hyporheic species on the habitat contribution. Bold letters for p-value denote statistical significance.

Explanatory variables	Estimate	AIC	p-value
Number of hyporheic species	0.460314	-59.528	**<0.001**

"*p<0.05; **p<0. 01; ***p<0.001"

Table 2.12 Results of GLMMs testing the effects of hyporheic environmental variables on the hyporheic habitat contribution. Full models were compared to null models using log-likelihood ratio tests.

Explanatory variables	Estimate	logLik	AIC	p-value
Temperature (probe)	-0.00763	22.7513	-39.5	0.2286
Temperature (logger)	-0.00154	22.0885	-38.2	0.809
Fine sediment amount in hyporheic trap	$2.73e^{-05}$	22.0832	-38.2	0.8272
D_{50} of the riverbed sediment	$3.25e^{-07}$	22.1173	-38.2	0.73
Dissolved oxygen	0.00817	22.7071	-39.4	0.24
Ash Free Dry Mass	0.1034	23.3207	-40.6	0.097
Nitrate concentration	-0.00279	22.2958	-38.6	0.49
Null model		22.059	-40.1	

"*p<0.05; **p<0. 01; ***p<0.001"

Table 2.13 Results of GLMMs testing the effects of hyporheic environmental variables on the hyporheic species richness. Full models were compared to null models using log-likelihood tests. Bold letters for p-value denote statistical significance.

Explanatory variables	Estimate	logLik	AIC	p-value
Temperature (probe)	-0.0878	-143.614	295.2	0.091
Temperature (logger)	0.0239	-144.849	297.7	0.54
Fine sediment amount in hyporheic trap	-0.001715	-142.712	293.4	**0.05**
D_{50} of the riverbed sediment	$1.74e^{-05}$	-140.741	289.5	**0.05**
Dissolved oxygen	0.1855	-137.564	283.1	**0.001**
Ash Free Dry Mass	1.376	-140.044	288.1	**0.001**
Nitrate	0.0540	-142.563	293.1	**0.05**
Null model		-145.033	296.1	

"*p<0.05; **p<0. 01; ***p<0.001"

Chapter 3

Effects of multiple stressors on macroinvertebrate community in the hyporheic zone

3.1 Introduction

The rapid shift in water use is associated with the physical and biological alteration of freshwater ecosystems (Vörösmarty et al., 2010). The ongoing urbanization and agricultural intensification have led to the degradation of lotic habitats worldwide by providing the nutrients and fine sediment (organic and inorganic particles <2 mm) inputs (Allan, 2004a; Ormerod et al., 2010; Tockner et al., 2010; Schinegger et al., 2012, 2016; Hering et al., 2015; Jackson et al., 2016). It causes adversely stress condition to stream community as water travel from the headwater areas through the channels altered by the effluents (Allan et al., 1997; Nelson and Roline, 2003; Wenger et al., 2009). The two most ubiquitous stressors originating mainly from different point or diffuse sources are deposited fine sediments (Jones et al., 2012) and elevated nutrient concentration (Jarvie et al., 2010; Nhiwatiwa et al., 2017; Gonzales-Inca et al., 2018; Zhang et al., 2018). Those have been recognized as the main stressors for the freshwater systems and causing the alteration of water quality in rivers (Nuttall and Bielby, 1973; Newcombe and MacDonald, 1991; Wood and Armitage, 1997). In natural system, stressors can co-occur and affect the biological community in an interactive way as multiple stressors and it is challenging to deal with multiple stressors originating from land use activities for the conservation of freshwater biodiversity (Crain et al., 2008; Piggott et al., 2015). There is evidence of biological responses originating from multiple stressors in marine and rivers systems (Crain et al., 2008; Darling and Côté, 2008; Ban et al., 2014; Côté et al., 2016; Jackson et al., 2016). Thus, it is important to know how multiple stressors affect structural properties of ecosystems, although knowledge on the framework of mechanistic understanding of multiple stressors is limited (Nõges et al., 2016; Orr et al., 2020).

Macroinvertebrates are commonly used as biomonitoring tools due to their different tolerance levels to environmental changes, which can provide information on any disturbances in aquatic systems (Larsen et al., 2009). The response of aquatic communities may vary under different gradients of environmental variables, such as water quality, nutrients, temperature, or sediment clogging (Cox and Rutherford, 2000, Larsen et al., 2009). In a particular habitat and at a given time, a similar set of traits (e.g., life history strategies) of the macroinvertebrates indicate the highest fitness to individuals

(Towsend and Hildrew, 1994). Any exception from these traits can be considered as the responses caused by the changes in their habitat's physical or chemical characteristics. These changes can be reflected in the community structure of the macroinvertebrates, and such a relationship is the basis of assessing the effects of environmental changes on the biological community in the freshwater systems (Verdonschot and Nijboer, 2004). A similar set of stressors can play a differential role in contrasting habitats, such as benthic and hyporheic habitats (Folt et al., 1999; Jooste, 2000). The responses of macroinvertebrates are well documented for the different environmental variables in the benthic zone (Beerman et al., 2018; Davis et al., 2018; Cornejo et al., 2019; Castro-Català et al., 2020), but understanding is limited for the hyporheic zone (Nelson and Roline, 2003; Pacioglu and Moldovan, 2016).

The macroinvertebrates often show sensitivity to rivers' fine sediments load and nutrient pollution (Piggott et al., 2015). Increased sediment deposition could lead to the streambed and interstitial clogging characterized by low hydrological exchange due to reduced porosity (Soulsby et al., 2001; Nogaro et al., 2010). This could create an oxygen-limiting condition that could negatively affect macroinvertebrates (Lefebvre et al., 2004). It can limit the access of macroinvertebrates within the sediments and thus could decrease the abundance of sensitive taxa in the coarse sediments (Dole-Olivier et al. 1997; Hakenkamp and Morin 2000; Boulton 2007). On the other hand, the effect of nutrients often produces subsidy-stress gradients in the invertebrate community (Wagenhoff et al., 2012). The effect of those stressors on benthic macroinvertebrates has been reported extensively (Wagenhoff et al., 2012; Beerman et al., 2018; Davis et al., 2018); however, a substantial gap remains for the hyporheic macroinvertebrates. The individual effect of those stressors on the hyporheic macroinvertebrates assemblages has been investigated by few researchers (Pacioglu and Moldovan, 2016). Some studies attempted to examine the effects in the multiple stressor context (interactive and/or, combined; Nelson and Roline, 2003; Piscart et al., 2011), but knowledge is limited for nutrient and fine sediment context. Understanding the combined and/or, interactive effects of stressors on macroinvertebrates and underlying mechanisms are essential to unveil the structural properties under multiple stressor context in the hyporheic zone.

The interactions among stressors are complex and could be additive (response is equal to the sum of the effects of both stressors), synergistic (response is greater than the sum of the individual stressors) and/or, antagonistic (response is less than the sum of the individual stressors; Folt et al., 1999; Ormerod et al., 2010). Understanding the response and sensitivity of hyporheic macroinvertebrate to the multiple stressors would provide the important information for the river conservation and management (Hering et al., 2015). This chapter examined the effects of fine sediment on macroinvertebrates in the hyporheic zone under different levels of nutrient pollution through experimental sediment addition in sites with different nutrient levels. The findings of the chapter 2 suggested that hyporheic habitat contribution is important to the macroinvertebrate diversity and among all the studied river, Satsunai River reaches contributed most. Also, the taxonomic richness of hyporheic species in the Satsunai river reaches was higher. So, the reaches of the Satsunai River segments were chosen as a model system where environmental stressors on diverse hyporheic macroinvertebrate community can be addressed. The multiple stressors were chosen based on the consistent negative and positive effects of fine sediments and nutrients on the taxonomic richness of species in hyporheic zone respectively. The objective was to test the effects of fine sediments on the macroinvertebrate community structure in the hyporheic zone under different levels of nutrients. It was predicted that the higher nutrient would offset the adverse effects of sediment (e.g., increase in abundance and taxa richness). This was because nutrient as a subsidy in the study sites, Satsunai River (Negishi et al., 2019a, b) and was predicted to offset the negative effect of fine sediment.

3.2 Materials and Methods

Study area

The field survey and experiment were conducted from July 2019 to November 2019 in the main channel of Satsunai river (catchment area 725 km^2; channel length 82 km) and one of its spring-fed tributaries. The river originates from Mt. Satsunai (42° 41′ N, 142° 47′ E; 1,895 m above sea level) to the Tokachi river, in eastern Hokkaido, Japan (Fig. 3.1). Three sites were located in the main channel and one site was in a small tributary

which collects water from the spring-fed sources. Site A1 was located downstream of the confluence with the Totsutabetsu River, which is as large as the Satsunai River. All sites were characterized by typical gravel bed river landscapes with riparian areas, gravel bars (with an exception for spring-fed tributary) and braided channels. The bars were interconnected with riparian forest in one side and river channel in the other side or, channel in both sides or, riparian forest in both sides.

A wastewater treatment plant for sewages discharges dissolved and particulate solids enriched water through a small tributary (flow rate = approximately 0.8 m^3 /sec) (Fig. 3.1). The point source in addition with other diffuse sources (i.e., agricultural activities) formed a longitudinal gradient in surface water in terms of nutrients (i.e., nitrate) from upstream to downstream (detailed information can be found in Negishi et al. 2019a, b). Thus, this gradient of nutrient was utilized to examine the effect of nutrient on the hyporheic macroinvertebrates. Two sites were chosen in the upstream and downstream of the tributary confluence from where the sewage treated water enters in the main channel of Satsunai River. The regional climate is characterized by lower air temperature and precipitation in the winter and higher temperature and precipitation in the summer. The annual precipitation was 1181.3 (±204.2) mm (1999 to 2018; Kami-satsunai station, Japan Meteorological Agency), and the mean (±standard deviation) daily flow rate from 1999 to 2016 was 11.1 (±17.6) m^3 /sec (Kami-satsunai station, Ministry of Land, Infrastructure, Transport and Tourism (MLIT), while the tributary had smaller flow rates recorded between 0.9 and 1.5 m^3/s from 2013 to 2019 (unpublished data, J.N. Negishi).

Installation of colonization traps

Hyporheic macroinvertebrates were collected with colonization traps in the riverbed which were installed together with the leaf litter bags inside as a part another parallel study on the hyporheic zone (see, methodology part in chapter 4). Colonization traps were made using the available riverbed sediments (4-22.4 mm and 22.4-64 mm in a 3:1 ratio; Fig. 3.2). Sediment collected from the available riverbed materials were rinsed well, and enclosed in a 4 mm × 3 mm blue nylon net with an area of 50 cm × 50 cm. Further details

describing the quantitative colonization trap can be found in (Negishi et al., 2019b; Alam et al., 2020). In total, sixty colonization traps were prepared. Six pits were excavated in a glide habitat with approximately 15 cm surface water depth and <50 cm/s current velocity at each site. Colonization traps were set at a depth of 30 cm from the riverbed with a pair in each pit having a distance of 5 cm from each other. As a result, two colonization traps were installed in each of the six pits. The distance between pits along the channel (Fig. 3.3) was approximately 5 m. The first and fourth pits from the upstream side were chosen to set a Polyvinyl chloride (PVC) pipe for later water sample collections (one with sediment treatment and the other in a control pit; next paragraph for the treatment). The lower end of the pipe was perforated for 10 cm and set at a depth of 50 cm. The top end was enclosed with PVC caps except during sampling occasions. In addition, six pits were excavated in L1 and A1 (three pits each) as a blank control to examine the influence of presence of leaf litter bag and sediment treatment in the colonization traps and how the community differs with the general colonization pattern.

Fine sediment treatment

Fine sediment treatment was conducted at each site to examine the effect of sediment on hyporheic macro-invertebrates. At each pit after excavation of 30 cm, two traps were set up with 5 cm distant from each other and covered up with at least 40 kg commercially available fine sediments (particle size: < 2 mm) on top of two colonization traps in three pits (1^{st}, 3^{rd}, and 5^{th} pits from the upstream end of the pit row) at each site and other three pits were kept without sediment addition as control. Sediment was introduced to the bottom of the pit and traps through a 10 cm diameter PVC pipe to minimize the loss of sediment. Sediment was introduced in a way that fine sediment could evenly be distributed in treatment and control pits and were buried back with the original deposited sediments within one hour from the pit excavation (Fig. 3.4). Total twenty-four traps were treated and other twenty-four remained untouched. White nylon string ropes were attached from the traps up to the riverbed during incubation so that, traps could be recognized later during collection.

Collection of colonization traps

The colonization traps were manually excavated and retrieved using a hand-held D-frame net (375 µm mesh) between August and November 2019. On each collection occasion, a total of four colonization traps (two in each treatment and control pit) were collected by excavating one control pit and one treatment pit in the hyporheic zone. And at site L1 and A1 one control, one treatment, and one blank control pit. Due to the flow conditions and unexpected sediment deposition, however, some sample collections were delayed causing a minor inconsistency in the sampling design (Table 3.1). The pits with PVC wells were collected on the last occasion to allow water quality measurement at the end of the experiment. Traps were retrieved after carefully removing the sediment above the trap to ensure that benthic macroinvertebrates and detritus at the surface did not contaminate the hyporheic samples. It was done in two steps. First, the colonization traps were cut, opened and the leaf traps which were inside the colonization traps were quickly taken out and placed in a zip lock and saved for chapter 4. The leaf traps were carefully removed and treated in a way that there were no effects of having them inside the colonization traps. Second, processing of the colonization trap was completed by washing and sieving the trap material with a 500-µm stainless steel sieve within 30 minutes of collection and preserved with 75% ethanol for further processing. The remaining sediments were sieved using a stack of stainless-steel sieves (22.4 mm, 2 mm, and 500 µm). Sediments from 2 mm to 500 µm were collected and stored for further analysis.

Measurement of environmental conditions

The water temperature was continuously measured at 15- or 30-min intervals using data loggers (HOBO pendant logger, Onset Co., MA, USA) buried together with the colonization traps with one for each treatment and control pit in the hyporheic zone (four loggers: two control and two treatment pits). Two-week means of temperature were calculated for each habitat in each site as measurement replicates. Water samples were collected in acid-washed polyethylene bottles to determine nutrient concentrations. Hyporheic water was collected using a handheld bilge pump from the PVC wells installed at 50 cm within the traps (Negishi et al., 2019b; S4). The flow conditions were sometimes unsuitable for the sampling and one PVC pipe with sediment treatment at L1 was damaged to the point of malfunction before the first sampling occasion, resulting in a

slightly modified schedule (Table 3.2). Dissolved oxygen, electrical conductivity (EC), and pH were also measured for surface water using probes (HACH-HQ40d, Hach Co., Loveland, CO, USA; WM-32EP, TOA-DKK Co., Tokyo, Japan) immediately after the water was collected for nitrate measurements. Dissolved oxygen was always at a saturated level (means ± standard deviations: 9.95 ± 1.07 mg/L) with pH being almost neutral (6.98 ± 0.37) all the time. We considered DO and pH unimportant as limiting factors and thus they were excluded from further analyses. All samples were stored in an ice chest and transported to the laboratory.

Laboratory analysis

After reaching to the laboratory, water samples were filtered using a glass fiber filter with a pore size of 0.5 µm (GC-50, Advantec Co., Tokyo, Japan) and kept refrigerated until further analysis within seven days. Nitrate (NO_3^-) concentrations were measured by ion chromatography (IA-300, TOA-DKK Co., Tokyo, Japan). Macroinvertebrates were separated from organic material and inorganic particles and sorted down to family level according to available resources (Kawai and Tanida, 2018; Webstar and Benfield, 2018). The macroinvertebrates were divided into detritivore and non-detritivore. For that, the Lepidostomatidae, Nemouridae, Leptophlebidae, and Tipulidae were focused as detritivores according to Negishi et al., 2019b because these taxa have been identified as being largely dependent on allochthonous carbon sources. The retained mixture was oven-dried at 60 °C for at least seven days and was combusted at 500 °C for 4 hours (FO310, Yamato Scientific Co., Tokyo, Japan) to determine the ash-free dry mass (AFDM) and the weight of inorganic matter. The weight of this inorganic matter and the weight of sediment retained in the field from the colonization trap were combined to obtain the fine sediment weight from each trap.

Statistical analysis

To test whether the intended environmental gradient was found between habitat treatment and across sites in relation to sediment treatment, nutrient, and water temperature, the data were examined using generalized linear mixed models (GLMMs). It was examined if amount of fine sediment differed between habitat treatment types (i.e., control and

treatment) and across sites by developing a generalized linear mixed model (GLMM) using amount of fine sediment in colonization traps as a response variable, habitat treatment type, site, and their interactions as main factors, and sampling season as a random factor (Gaussian error distribution). It was tested if nitrate concentration and temperature varied across sites and between habitat treatment types by developing generalized linear mixed models (GLMMs) using nitrate concentration as main factor, site, habitat treatment type and their interactions as main factors and sampling season as a random factor (Gaussian error distribution). The sampling season was defined to account for the possible correlation of data in a similar seasonal context (periods before 8 September, between 9 September and 12 October, and between 13 October and 11 November were considered as different seasons).

Additionally, at least one colonization trap sediment was collected from each of the control and treatment traps from each site to examine that how much fine sediment was retained in the colonization trap. The fine sediment ratio was then determined from the total weight of colonization trap sediment.

It was examined if the community structure of hyporheic invertebrate assemblages differed between habitat treatment type and among sites using a non-metric multidimensional scaling (NMDS) in two dimensions with Bray-Curtis spacing and one hundred maximum iterations (‘vegan package’). Invertebrate abundance data were used with $\log_{10}(x+1)$ transformation. The scores of the two NMDS axes were compared to test for differences between habitat treatment types and sites using permutational analysis of variance (PERMANOVA with 999 permutations). The alpha diversity indices, such as, taxonomic richness, Shannon-Wiener diversity, total abundance, detritivore abundance, non-detritivore abundance, and EPT abundance were determined. The alpha diversity indices were then compared between habitat treatment types by developing generalized linear mixed models (GLMMs), using alpha diversity indices as response variables and habitat treatment types as main factors, and sampling occasion, pit id nested within site as random factors (Poisson error distribution for taxonomic richness, Negative binomial error distribution for total abundance, detritivore, non-detritivore abundance, and EPT abundance and Gaussian error distribution for Shannon-Wiener diversity index).

To examine the type and extent of the interactions of nitrate and sediment, a GLMM was developed using hyporheic macroinvertebrates alpha diversity indices as response variables and nitrate, fine sediment, and their interaction as main factors, with pit identity nested within site, and sampling seasonality as a random factor (Poisson error distribution for taxonomic richness, Negative binomial error distribution for taxa abundance, EPT, detritivore and non-detritivore abundance, and Gaussian error distribution for Shannon-Wiener diversity index)

All statistical analyses were performed using R (version 3.5.2). Data in GLMMs were log10 transformed to improve normality among groups when necessary. The effects of the main factors in GLMMs were examined by sequentially comparing models with reduced models without the effect of the factor of interest, using likelihood-ratio tests. The "glmmADMB", "vegan", and "ADONIS2" packages were used for GLMMs, NMDS, and PERMANOVA, respectively. Multiple comparisons among groups in GLMMs were carried out with the "multcomp" package. The statistical significance level was set at $p = 0.05$.

3.3 Results

Nitrate concentration differed among sites but did not differ between habitat treatment types which reflected in insignificant effects of habitat treatment types and interaction terms. Values of nitrate concentration were higher in the sites located at the downstream to the wastewater treatment plant (Fig. 3.5A; Table 3.3a). Site L1 had the highest concentration, while site A1 was the intermediate in concentration between L1 and S1 sites (Fig. 3.5A). In site S1 nitrate concentration was lowest (Fig. 3.5A). The amount of fine sediment in the hyporheic colonization traps was significantly different among sites and between habitat treatment types (Fig. 3.5B, Table 3.3b). The amount of sediment was higher in all the treatment traps compared to that of control traps. The site A1 had the highest level of fine sediments, while L1 had the lowest levels across the sites (Fig. 3.5B; Table 3.3b). The continuous measurement of water temperature differed across sites but was not different between habitat treatments. Temperature was highest at A1 site and lowest at S1 site (Fig. 3.5C, Table 3.3c).

A total of 36 macroinvertebrates taxa (either order or family) and 6,189 individuals were caught in the hyporheic colonization traps. In which 23 family were identified as EPT, and 2,460 individuals were found from those families which was 39.75% of the total abundance (Table 3.4). Total 1,351 individuals from detritivore individuals were found among four taxa which was 21.83% of total abundance. Lepidostomatidae taxon was most abundant and covered 57.29% of the detritivore abundance. Leptophlebidae, Tipulidae, and Nemouridae taxa covered 33.75, 6.66, and 2.29% of the total detritivore abundance. On the other hand, Chironomidae taxa covered 46.71 and 59.76% of total abundance and non-detritivore abundance respectively (Table 3.4). Some of taxa responded under habitat treatments, such as, Chironomidae and Tipulidae taxa increased under higher sediment treatments. On the other hand, Nemouridae decreased with increasing fine sediments (Table 3.4).

The macroinvertebrates were clustered according to habitat treatment type and sites in NMDS. The macroinvertebrates were not separated according to the habitat treatments, however, were different across the sites (stress = 0.21; Fig. 3.6A, B). In addition, comparison of blank control hyporheic community with treatment and control groups did not show differences (Stress = 0.20; Fig. 3.7) which indicated that the leaf traps did not have any influence on colonization trap community. Taxa richness did not vary between habitat treatments (Fig. 3.8A, Table 3.5a). Shannon-Wiener diversity index did not vary between habitat treatments (Fig. 3.8B, Table 3.5b). At L7 site Shannon index was lowest among sites. Total abundance and EPT abundance did not vary between habitat treatment types (Fig 3.8C, D, Table 3.5c, d). The detritivore and non-detritivore abundance also did not vary between habitat treatments (Fig. 3.8E, F, Table 3.5e, f).

The macroinvertebrates alpha diversity indices did not show any response to the interaction of nitrate and fine sediments (Table 3.6). The taxonomic richness was positively affected by nitrate concentration (Table 3.6a). Nitrate concentration had positive effects on Shanon-Wiener diversity index without significant effects of fine sediments (Table 3.6b). Neither nitrate nor fine sediments had significant effects on EPT, detritivore, or non-detritivore abundance (Table 3.6c-f).

3.4 Discussion

The hyporheic macroinvertebrate responses to the multiple stressors have been reported in past (Connolly and Pearson, 2007; Larsen et al., 2009 which is critically important to understand their counteracting responses in the stressed conditions However, none of them reported in the context of nutrient and fine sediment simultaneously. It was examined if the multiple stressors (nutrient and fine sediment) can interact with each other and combinedly influence the macroinvertebrate community within the riverbed. The results showed that fine sediment did not have significant influence on overall alpha diversity of hyporheic macroinvertebrate community, however, nitrate concentration affected the number of taxa and relative abundance positively. The macroinvertebrate's alpha diversity indices were not significantly influenced by fine sediments, which did not support the hypothesis. But taxa level response was found between habitat treatment types. Also, the prediction that effects of fine sediment would be offset at higher nutrient levels was not supported. This study provided an improved and extended understanding on mechanisms behind the macroinvertebrate responses under the gradients of nutrient and fine sediment in hyporheic zone.

The gradual longitudinal increases in the nutrient gradient along river catchment dominated by anthropogenic activities (e.g., agricultural activities, urban land uses) are often observed (Carey and Migliaccio, 2009; Morrissey et al., 2013). The effects of the point and other diffuse sources were observed in the spatial differences in the surface water quality that was consistent with the earlier reports in this experimental channel (Alam et al., 2020; Negishi et al., 2019b). Thus, spatial nutrient gradient existed in the hyporheic habitat that worked as a natural surrogate of testing the effects of higher nutrient levels. The higher nutrient enrichment in the downstream of the point source leads to a significant change in the macro-invertebrate with an increase in the abundances (Negishi et. al., 2019b). Negishi et al. (2019b) showed that, the response of hyporheic macro-invertebrates was numerically positive to the water pollution originated from point or other diffuse sources. The fine sediment amount was found to be higher in the treatment hyporheic traps that indicated that sediment treatment was effective to manipulate the subsurface zone. The fine sediments were also variable across sites which indicated the

background fine sediment gradient was different across sites. The changes in nitrate concentration across sites and fine sediments between habitat treatments indicated that, the detectable gradient of nutrient and fine sediment was existed in the study stretch.

Hyporheic macroinvertebrate assemblages were variable among sampling sites for the taxonomic richness. Macroinvertebrate assemblages in the higher fine sediment deposited area were generally dominated by Chironomidae and Oligochaeta due to their intermediate body size, flexibility, and adapted to burrowing (Descloux et al., 2013; Korbel, 2019). The assemblage structure from EPT dominated structure to the Chironomidae and Oligochaeta dominated structure could be in the streams or reaches with heavy fine sediment deposition (Buendia et al., 2013; Larsen and Ormerod, 2014). Such shift was not observed in this study for EPT taxa, although, Chironomidae and Oligochaeta abundances increased in the habitat treatments (Table 3.4). Previous field manipulation experiment also did not find marked effect on macroinvertebrate community compositions as exception from the general trends (Larsen and Ormerod, 2014) and effects might be consistent with seasons (Duan et al., 2021). Thus, the effects of fine sediment on macroinvertebrates in hyporheic zone could be chronic and thus the net effect could differ due to the differences in taxa level sensitivities, amount and source of fine sediment, sedimentary structure of the study reaches, differences in hydrological exchange and uncontainable natural influences (Descloux et al., 2013).

The critical factors that could contribute to the hyporheic macroinvertebrate are living space, oxygen, and food availability (Strayer et al., 1997; Boulton et al., 2010). These influential factors largely depend on the sediment size of the substratum, where gravel-bed-dominated streams provide a richer habitat than sand and silt beds (Duan et al., 2009; Xu et al., 2012). In this study, macroinvertebrate taxonomic richness and relative abundance did not significantly decrease with sediment treatment and were almost similar to those of controls. It suggests that sediment treatment increased the relative amount of fine sediment, but the macroinvertebrate community wasn't influenced largely in terms of alpha diversity in the hyporheic zone. The effects of sediment treatment could not resist the macroinvertebrates from colonizing across the sites. However, some macroinvertebrate taxa substantially reduced in the treatment traps, such

as, the family Hydropsychidae and Rhyacophylidae. The fine sediment accumulation in streams has reportedly impacted these taxa. Turley et al. (2016) reported that family-level sensitivity of macroinvertebrates to fine sediments where those two taxa showed a higher level of sensitivity supported the study's findings. Among the detritivores, the Tipulidae increased substantially compared to other taxa (Matthaei et al., 2006; Buendia et al., 2013; Larsen and Ormerod, 2014). However, Nemouridae decreased substantially with fine sediment. The alpha diversity indices did not vary between sediment treatments due to the different taxa-level sensitivity to the fine sediments. It could be due to some taxa colonized regardless of any effect of fine sediment due to their physiological and morphological adaptations (Zuellig, Kondratieff, and Rhodes, 2002). On the other hand, some taxa responded negatively to the fine sediments, which counterbalanced the effects of fine sediment.

Alpha diversity indices did not respond to the sediment treatments. This inconsistency from the usual pattern (Descloux et al., 2013; Jones et al., 2015; Davis et al., 2018; Mathers et al., 2021) could be explained by the sedimentary characteristics of the Satsunai river, which is dominated by cobble with higher hydrological connectivity between surface and sub-surface zone (Negishi et al., 2019b). The average median particle size (D_{50}) of 30 cm cores of study reaches were ranged from 27.19 to 40.39 mm (D_{50} of L1, A1, L7 and S1 are 40.39, 26.71, 44.88, 27.17 mm respectively; Alam, M.K., unpublished data) which indicated the median particle size could be an important factor regardless of sediment treatment in this study (Ligeiro et al., 2010). The fine sediment ratio was in the range of 10% in most the previous studies which generally created impairment on the macroinvertebrate community. However, the fine sediment ratio in the colonization traps in the study were above the ranges (Alam M.K., unpublished data) found in the previous reports (Cormier et al., 2008). Although the sediment at 30 cm of the riverbed was manipulated in the study pits, however, higher hydrological exchange between hyporheic and benthic zone might support the hyporheic community due to the relatively large median particle size (Buss et al., 2004; Costa and Melo, 2008; Ligeiro et al., 2010).

As expected, nutrient enrichment generally acted as a subsidy, increasing the number of taxa and relative abundance of macroinvertebrates. It indicated that the nitrate concentration in the hyporheic zone was at the range providing subsidy effects (Negishi et al., 2019b). The results supported the prediction that the increase of macroinvertebrate taxa richness and Shannon-Wiener diversity index with nitrate concentration was consistent (Negishi et al., 2019a, b). The water quality was not different between control and treatment traps which suggests water quality does not have influence on the hyporheic macroinvertebrates under different levels of treatments. The prediction that the stressors would influence the macroinvertebrates in a way that adverse effect of fine sediments would be offset under higher nitrate levels was not supported. The absence of significant interaction term between two factors indicated that no interactive effects was present. In this study, the community responses of macroinvertebrates could be biased by the nutrient enrichment which could lead the increase in primary productivity as well as positive effects on macroinvertebrate structure.

3.5 Conclusion

Overall, the study revealed that the macroinvertebrate taxonomic richness and Shannon-Wiener index were influenced by nitrate concentration independently. In these study sites, a notable exception to the previous literatures was that effects of fine sediments would not always be adverse but could be neutral as net effect or could have taxa level effects on the hyporheic macroinvertebrates. The positive effect of nutrient was existed while no effect on alpha diversity indices of fine sediment which is new addition of understanding of stress-response relationships in the hyporheic zone. The interaction effects of nutrients and fine sediments were not apparent in the study reaches, so the effect of one variable was not dependent with another. This study would add the improved information on how field manipulation of fine sediments could affect the macroinvertebrates in the hyporheic zone under different nutrient gradients, which has been lacking and difficult through field observations and sampling (Jones et al., 2012) often limited in providing cause-effect relationship. The hyporheic zone is very important since the biodiversity in the hyporheic zone plays important roles and shows variable responses to the environmental variables. A simultaneous quantitative measurement including benthic zone, with the functional

responses could tell us further insight about the structural properties under multiple stressor effects in the hyporheic zone.

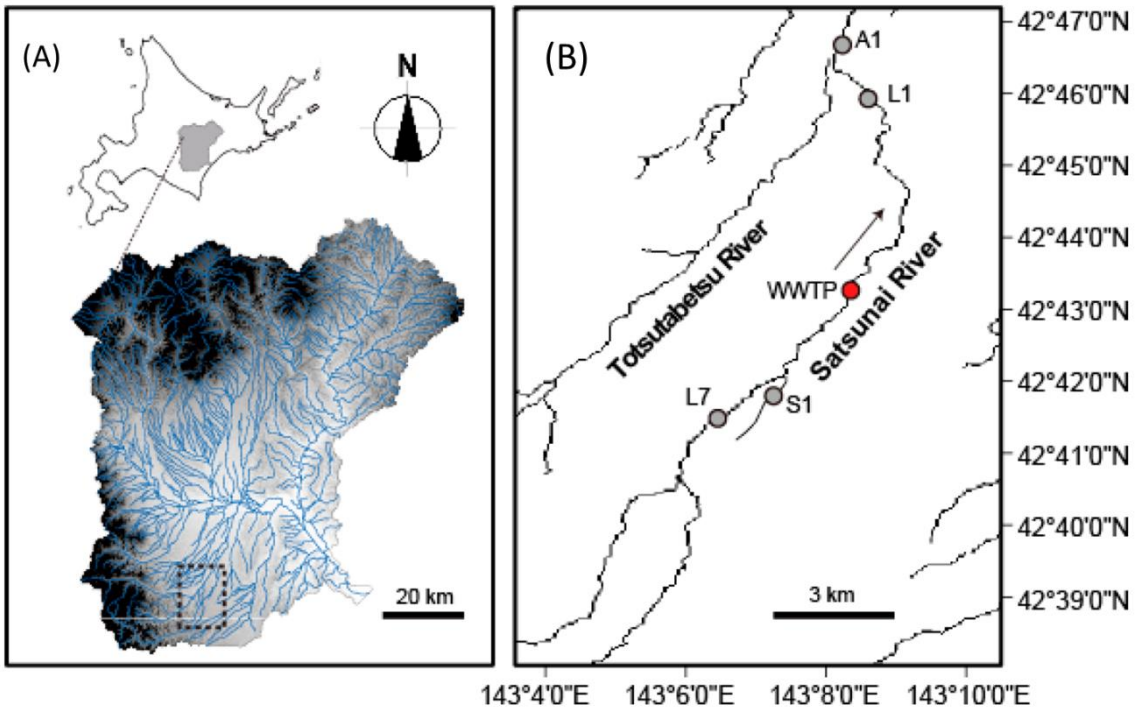


Fig. 3.1 Location of the Tokachi watershed in Hokkaido, Japan where the study area was indicated by a dotted box (A) and four study sites (gray filled circles) in the Satsunai river (B). A red filled point indicates the conflux with a tributary affected by a wastewater treatment plant (WWTP). An arrow denotes flow direction of the river.

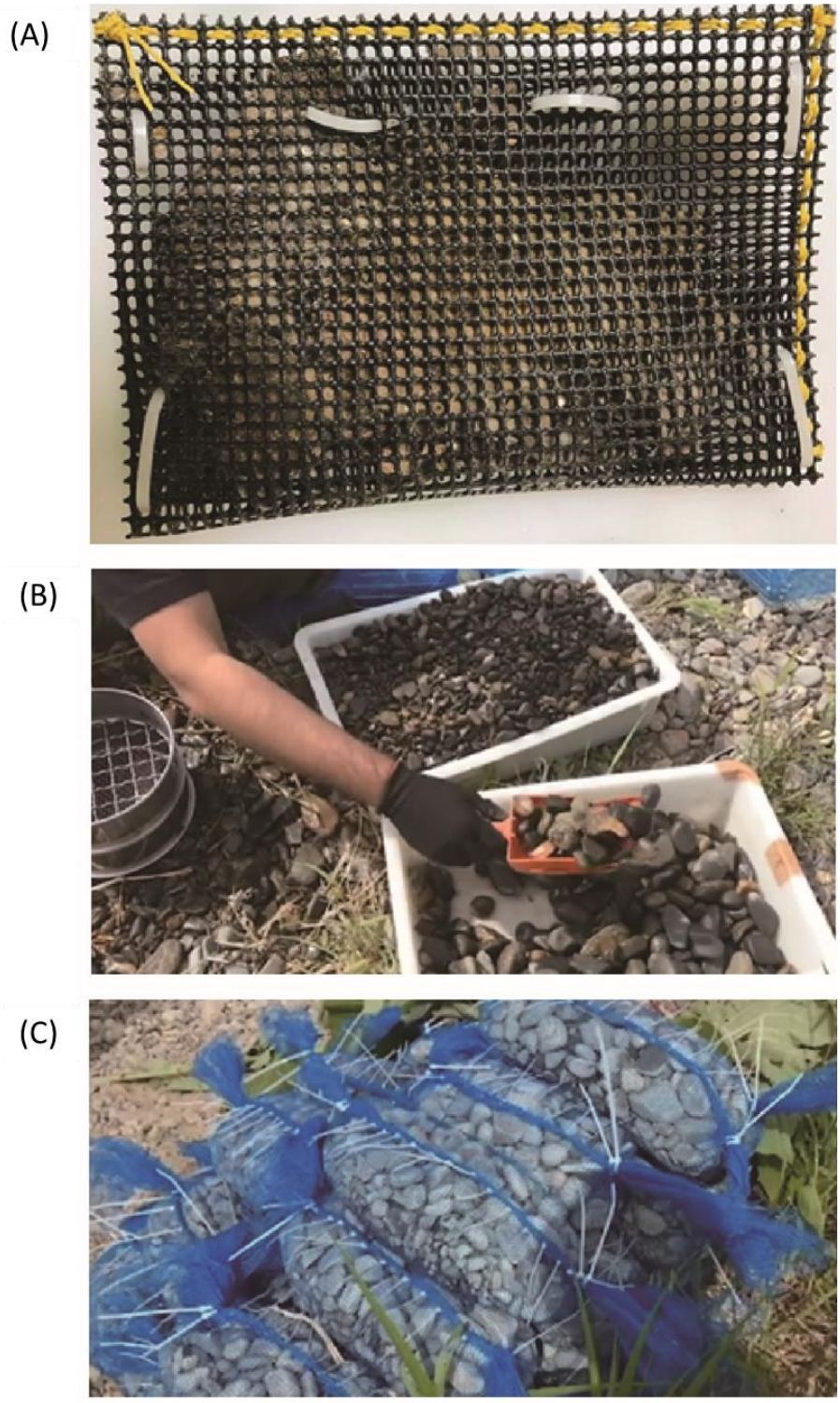


Fig. 3.2 Photos showing a litter bag that contains dried leaves of Alnus japonica (A), mixed sediment used for colonization traps (B), and prepared colonization traps before the installation (C).

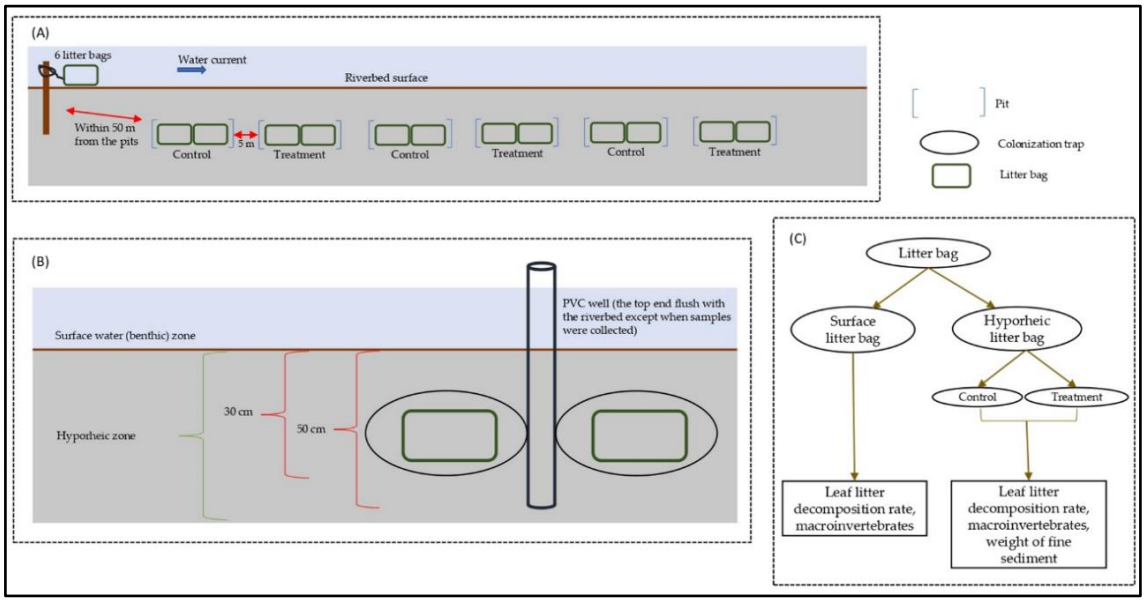


Fig. 3.3 Diagram showing the sampling design of each site detailing the longitudinal locations of litter bags in hyporheic control and treatment pits (two litter bags in one pit) in relation to litter bags in surface water (benthic) zone (A), a magnified view of a pit (control and/or, treatment) with hyporheic well for collecting water sample (B), and simplified steps of sample collection from litter bags (C). The treatment pits were manipulated by adding fine sediment on top of litter bags.

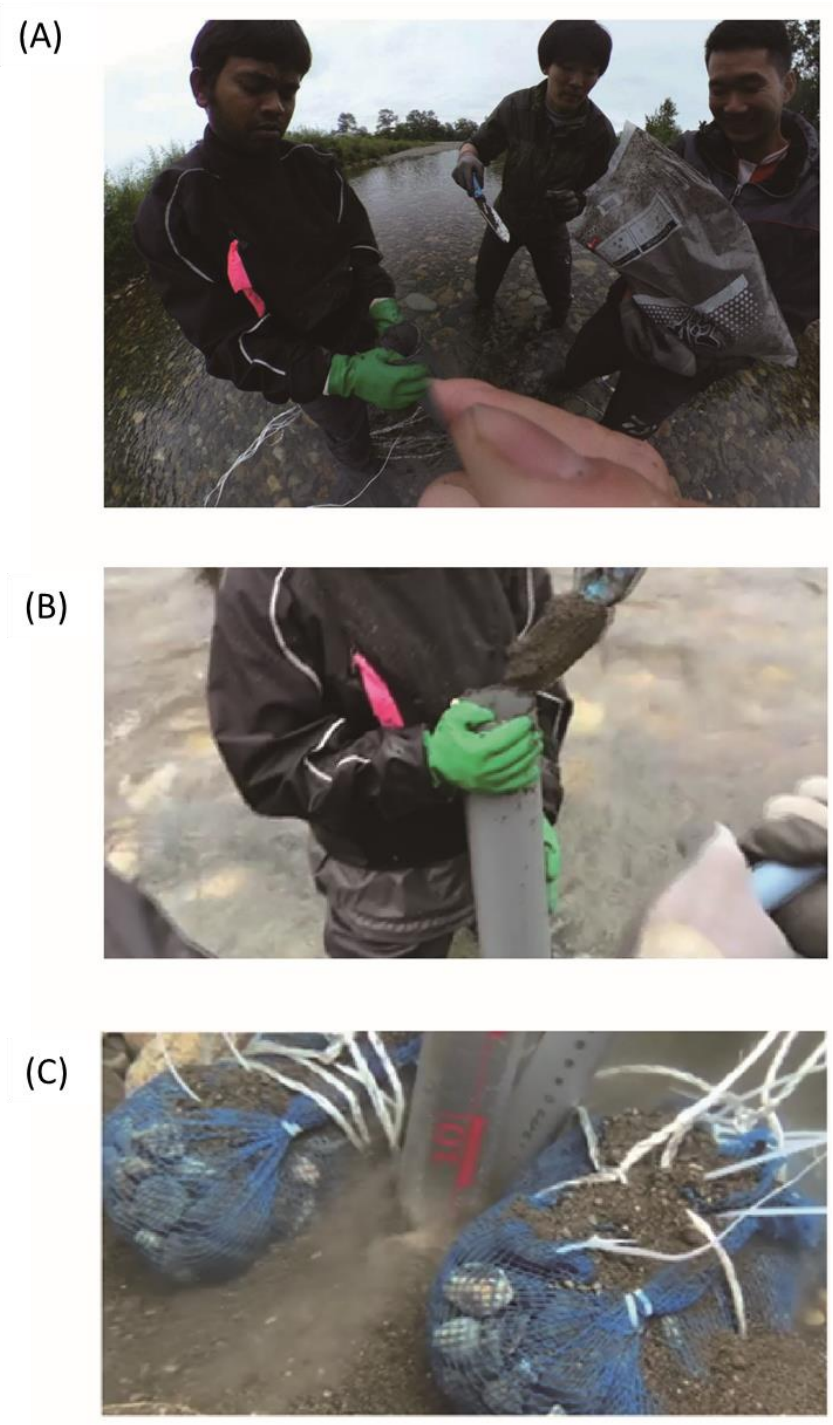


Fig. 3.4 Photos showing how experimental treatment was conducted with a group of people and a bag commercially available fine sediment in a bag (A), how fine sediment was introduced into a PVC pipe which directly delivered sediment to the top of the traps in water (B), and the introduced sediment deposited on the top of traps in water (C).

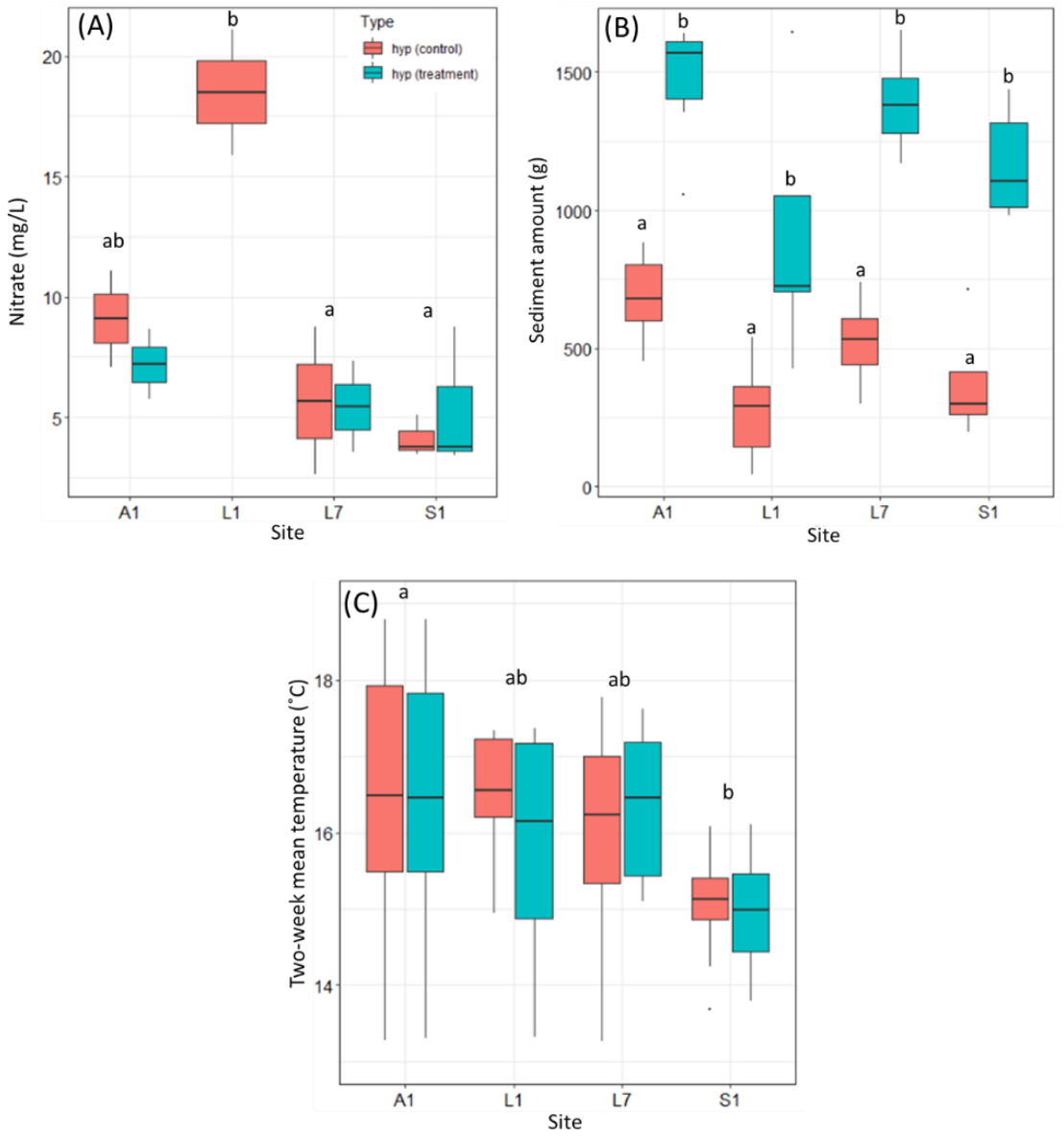


Fig. 3.5 Comparisons of nitrate (A), fine sediment (B), and water temperature (C) in relation to sites and habitat-treatment types. Alphabetical letters denote results of multiple comparisons based on GLMM; those with same letters were statistically undistinguishable. Multiple comparisons were performed among sites for nitrate and temperature and among habitat-type groups within each site for fine sediment.

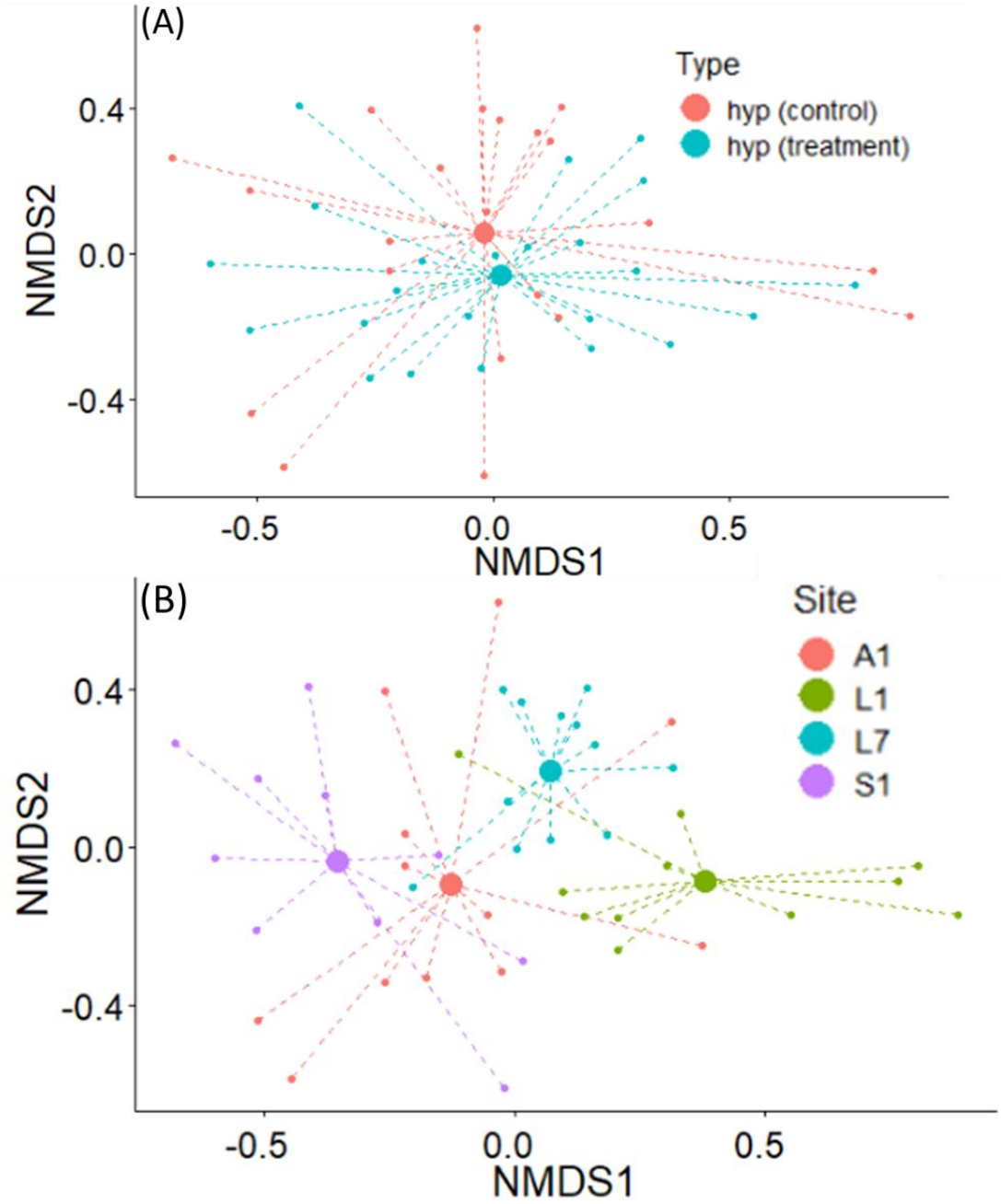


Fig. 3.6 Biplots showing macroinvertebrate community structure using the results of NMDS (Stress=0.21) with log10-transformed abundance of taxa between habitat treatments (A), and among sites (B). Large circles represent centroids of data points either for habitat treatment types or for sites.

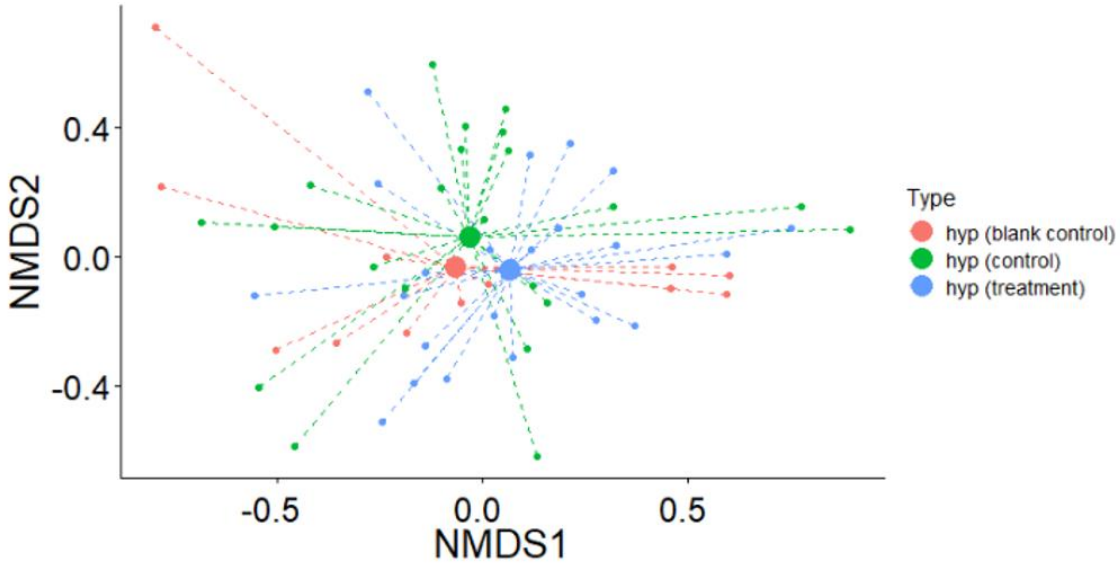
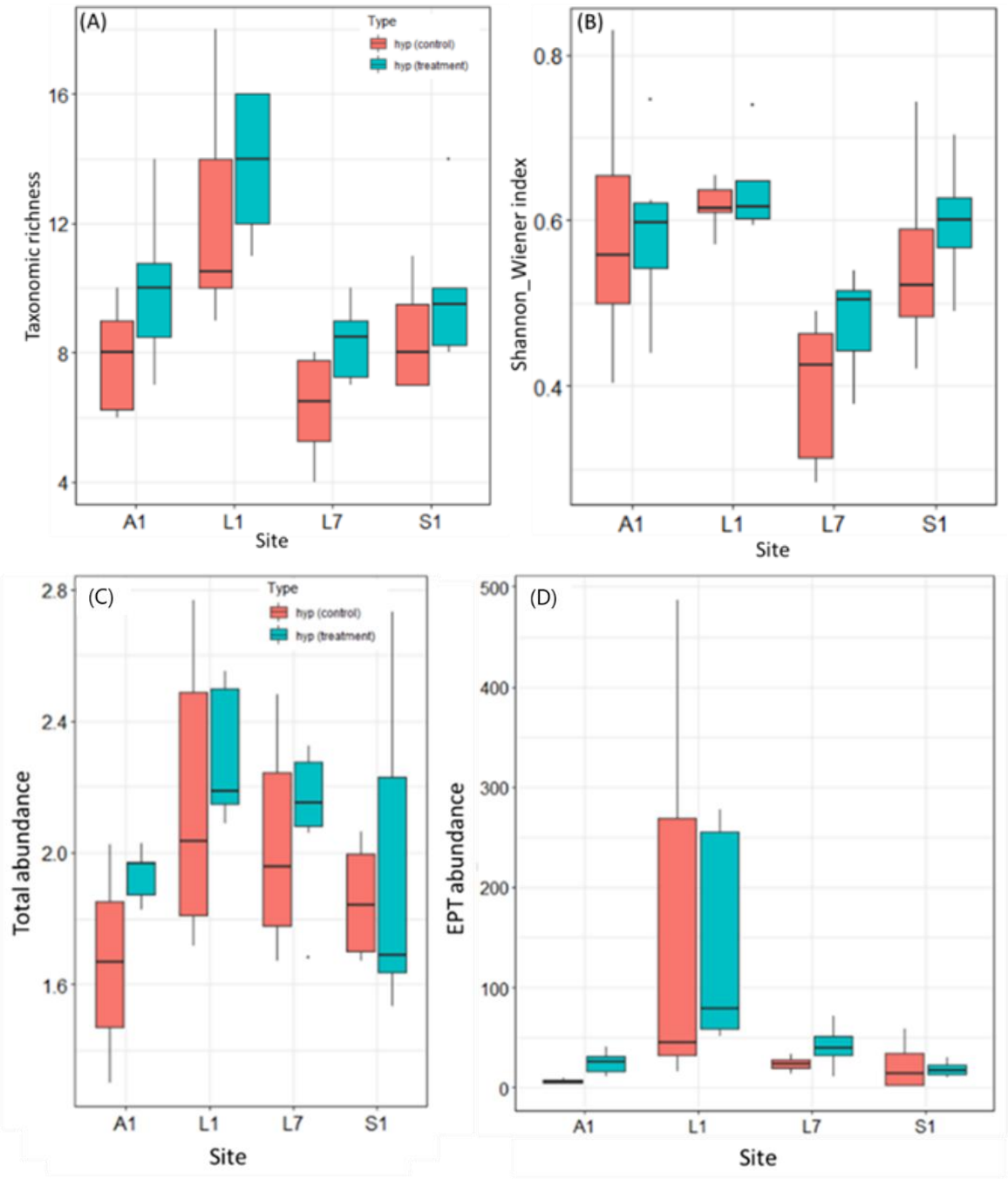


Fig. 3.7 A biplots showing macroinvertebrate community structure using the results of NMDS (Stress=0.20) with log10-transformed abundance of taxa among the habitat treatments and blank control community in the hyporheic zone. Large circles represent centroids of data points for habitat treatment types including blank control.


(A)
Type
hyp (control)
hyp (treatment)
Taxonomic richness
16
12
8
4
A1
L1
L7
S1
Site
(B)
Shannon_Wiener index
0.8
0.6
0.4
A1
L1
L7
S1
Site
(C)
Type
hyp (control)
hyp (treatment)
Total abundance
2.8
2.4
2.0
1.6
A1
L1
L7
S1
Site
(D)
EPT abundance
500
400
300
200
100
0
A1
L1
L7
S1
Site

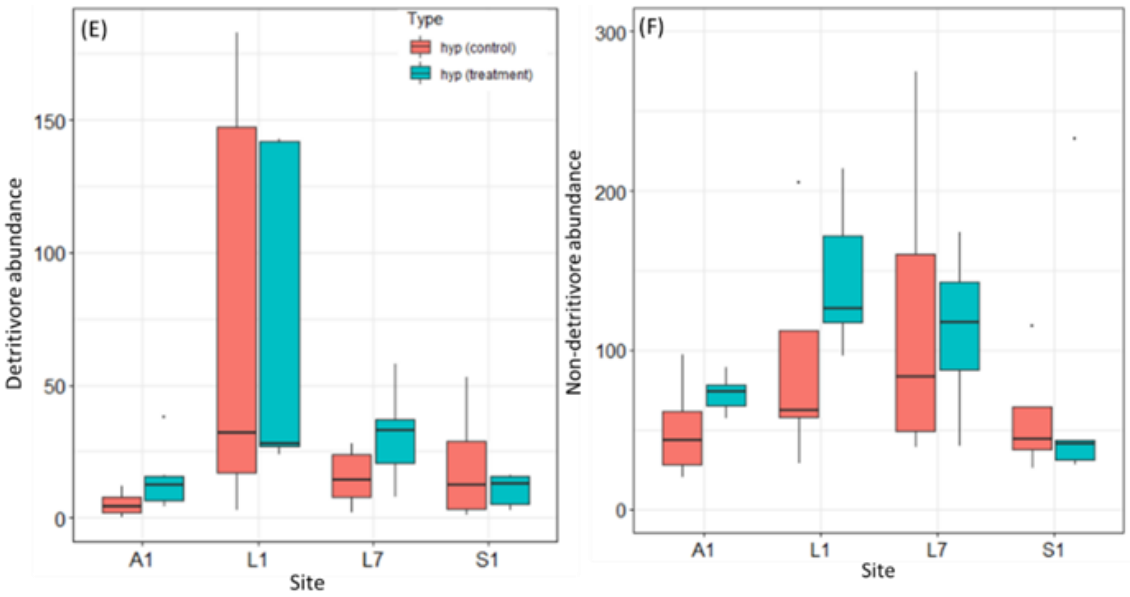


Fig. 3.8 Boxplots showing comparisons of taxonomic richness (A), Shannon-Wiener diversity index (B), and total abundance (C), EPT abundance (D), detritivore abundance (E), and non-detritivore abundance (F) in relation to habitat-treatment types.

Table 3.1 A summary of sampling dates of water in 2019 for the measurements of electrical conductivity, pH, dissolved oxygen in the surface-water (benthic) zone (a), nitrate in the surface-water zone (b), and nitrate in the hyporheic zone (c). Numbers in bracketed superscripts indicate replicated samples measured at each site; no superscripts indicate one sample. N denotes sample sizes.

(a)

Site	**Habitat type**	**Dates**	**N**
A1	Surface	9/7$^{(3)}$, 10/12$^{(3)}$, 10/24$^{(3)}$	9
L1	Surface	9/7$^{(3)}$, 10/12, 10/25$^{(3)}$	7
S1	Surface	9/7$^{(3)}$, 10/10$^{(3)}$, 10/26$^{(3)}$	9
L7	Surface	9/8$^{(3)}$, 10/12$^{(3)}$, 11/8$^{(3)}$	9

(b)

Site	Habitat type	Dates	N
A1	Surface	9/7, 10/12, 10/24	3
L1	Surface	9/7, 10/12, 10/25	3
S1	Surface	9/8, 10/10, 10/26	3

(c)

Site	Habitat type	Treatment type	Dates	N
A1	Hyporheic	Control	9/7, 10/24	2
		Treatment	9/7, 10/24	2
L1	Hyporheic	Control	9/7, 10/25	2
		Treatment	Sample loss due to washout of the well	0
S1	Hyporheic	Control	9/8, 10/10, 10/26	3
		Treatment	9/8, 10/10, 10/26	3
L7	Hyporheic	Control	9/7, 10/26	2
		Treatment	9/7, 10/26	2

Table 3.2 A summary of colonization trap collection schedule in hyporheic zone. Numbers in bracketed superscripts denote the number of traps retrieved from the hyporheic zone. N denotes sample sizes.

Site	Habitat type	Installation date	Collection date	N
		2019	2019	
A1	Hyporheic	7/8	$9/7^{(4)}$, $10/24^{(8)}$	12
L1	Hyporheic	7/8	$9/7^{(4)}$, $10/25^{(8)}$	12
S1	Hyporheic	7/9	$9/8^{(4)}$, $10/10^{(4)}$, $10/26^{(4)}$	12
L7	Hyporheic	7/9	$9/7^{(4)}$, $10/26^{(2)}$, $11/08^{(6)}$	12

Table 3.3 Results of GLMMs testing the effects of site (Site) and habitat-treatment type (Type: control hyporheic zone, and treatment hyporheic zone) and their interactions on log_{10}-transformed nitrate concentration (a), the effects of site and habitat-treatment type and their interactions on log_{10}-transformed fine sediment amount (b), and the effects of site and habitat-treatment type and their interactions on two-week mean water temperature (c). Full models and reduced models were each compared using log-likelihood tests; when Full model were insignificant, 1st reduced models were compared to 2nd reduced models sequentially. Superscripts of p-values indicate the variables removed from the model to test with those from reduced models by one level. Bold letters for p-value denote statistical significance (*p<0.05; **p<0. 01; ***p<0.001).

(a)	**logLik**	**AIC**	**p-value**
Full model			
Site (S), Type (T), S×T	6.16	1.7	0.68
1st Reduced model			
S, T	5.78	-1.6	**<0.01**^{S}, 0.89^{T}
2nd Reduced model			
S	5.77	-3.5	
T	3.18	-0.4	

(b)	**logLik**	**AIC**	**p-value**
Full model			
Site (S), Type (T), S×T	-23.61	67.2	0.32
1st Reduced model			
S, T	-25.35	64.7	**<0.001**^{S}, **<0.001**^{T}
2nd Reduced model			
S	-36.31	80.6	
T	-46.98	106	

(c)	**logLik**	**AIC**	**p-value**
Full model			
Site (S), Type (T), S×T	-184.93	389.9	0.53
1st Reduced model			
S, T	-186.03	386.1	**0.05**^{S}, 0.17^{T}
2nd Reduced model			
S	-186.95	385.9	
T	-189.88	387.8	

Table 3.4 A summary of all the invertebrate taxa caught in colonization traps for two types of habitat-treatment group. NA indicates that identification was conducted only at the order level.

Invertebrate taxa		**Hyporheic (control)**		**Hyporheic (treatment)**	
Order	Family	Mean±SD	Abundance	Mean±SD	Abundance
Ephemeroptera	Philopotamidae	0.40±1.11	9	0.72±1.13	16
Ephemeroptera	Baetidae	0.22±0.66	5	0.72±1.57	16
Ephemeroptera	Ephemeridae	0.95±2.36	21	0.68±1.22	15
Ephemeroptera	Ephemerellidae	10.45±33.25	230	4.22±12.16	93
Ephemeroptera	Leptophlebiidae*	10±23.39	220	10.68±22.01	236
Ephemeroptera	Heptageniidae	4.05±7.29	89	7.63±15.17	168
Ephemeroptera	Caenidae	0.13±0.45	3	0.18±0.64	4
Ephemeroptera	Ameletidae	0.04±0.20	1	0	0
Trichoptera	Lepidostomatidae*	17.13±25.49	377	17.86±17.57	397
Trichoptera	Brachycentridae	0	0	0.22±0.73	5
Trichoptera	Glossosomatidae	0.04±0.20	1	0	0
Trichoptera	Hydropsychidae	3.86±10.87	85	1.22±2.44	27
Trichoptera	Stenopsychidae	0.77±2.08	17	0.68±2.11	15
Trichoptera	Rhyacophylidae	3.5±12.26	77	0.77±2.17	18
Trichoptera	Polycentropodidae	0.13±0.45	3	0	0
Trichoptera	Leptoceridae	0	0	0.04±0.20	1
Trichoptera	Apataniidae	0.04±0.20	1	0	0
Plecoptera	Perlidae	0.27±0.74	6	0.31±0.55	7
Plecoptera	Perlodidae	0.36±1.18	8	0.04±0.20	1
Plecoptera	Leuctridae	0.04±0.20	1	0.63±1.33	15
Plecoptera	Capniidae	0.04±0.20	1	0.36±1.18	8
Plecoptera	Nemouridae*	1.18±3.32	26	0.22±1.04	5
Plecoptera	Chloroperlidae	2.27±1.65	50	8±7.50	182
Diptera	Chironomidae	57.31±60.58	1261	73.54±99.08	1630
Diptera	Ceratopogonidae	1.59±2.88	35	2.63±3.99	62
Diptera	Tipulidae*	1.13±2.05	25	2.95±3.90	65
Diptera	Simuliidae	0.36±1.46	8	0.5±2.29	11
Diptera	Athericidae	0.09±0.28	2	0.36±0.82	8
Diptera	Tabanidae	0.09±0.41	2	0	0
Diptera	Dolichopodidae	0.40±1.49	9	0.31±0.92	7
Diptera	Empididae	0.13±0.45	3	0	0
Crustacean	Amphipoda	0.59±1.40	13	1.90±2.93	55
Crustacean	Isopoda	0.36±0.82	8	0.13±0.45	3
Oligochaeta	NA	5.90±8.46	130	13.13±14.39	315
Coleoptera	Elmidae	0.86±1.89	19	2.59±6.15	57
Coleoptera	Hydrophilidae	0	0	0	1
Total			2746		3443

"*" denotes the detritivores in the study.

Table 3.5 Results of GLMMs testing the effect habitat-treatment type (Type: control hyporheic zone, and treatment hyporheic zone) on macroinvertebrate taxa richness (a), log_{10}-transformed Shannon-Wiener diver index of macroinvertebrates (b), on log_{10}-transformed EPT abundance (c), log_{10}-transformed total abundance (d) log_{10}-transformed detritivore abundance (e), and log_{10}-transformed non-detritivore abundance (f). Full models were compared with null model using log-likelihood tests. Bold letters for p-value denote statistical significance (*p<0.05; **p<0. 01; ***p<0.001).

(a)	**logLik**	**AIC**	**p-value**
Full model			
Type	-110.55	227.1	0.078
Null model			
	-112.10	228.2	
(b)	**logLik**	**AIC**	**p-value**
Full model			
Type	33.990	-60	0.2829
Null model			
	33.414	-60.8	
(c)	**logLik**	**AIC**	**p-value**
Full model			
Type	-77.653	163.3	0.2418
Null model			
	-78.338	162.7	
(d)	**logLik**	**AIC**	**p-value**
Full model	-38.36	84.7	0.76
Type			
Null model	-38.40	82.8	
(e)	**logLik**	**AIC**	**p-value**
Full model			
Type	-197.51	403	0.8993
Null model			
	-197.52	401	
(f)	**logLik**	**AIC**	**p-value**
Full model			
Type	-251.38	510.8	0.3178
Null model			
	-251.88	509.8	

Table 3.6 Results of GLMMs testing the effects of nitrate and fine sediment and their interactions on macroinvertebrate taxa richness (a), log_{10}-transformed Shannon-Wiener diversity index (b), and log_{10}-transformed total abundance (c), log_{10}-transformed EPT abundance (d), log_{10}-transformed detritivore abundance (e), and non-detritivore abundance (f). Full models and reduced models were each compared using log-likelihood tests; when Full model were insignificant, 1st reduced models were compared to 2nd reduced models sequentially. Superscripts of p-values indicate the variables removed from the model to test with those from reduced models by one level. Bold letters for p-value denote statistical significance (*p<0.05; **p<0. 01; ***p<0.001).

(a)	**logLik**	**AIC**	**p-value**
Full model			
Nitrate (N), Fine sediment (S), N×S	-104.09	220.2	0.69
1st Reduced model			
N, S	-104.17	218.3	0.21^{S}, $\mathbf{<0.001^{N}}$
2nd Reduced model			
N	-104.93	217.9	
S	-112.07	232.2	
(b)	**logLik**	**AIC**	**p-value**
Full model			
Nitrate (N), Fine sediment (S), N×S	37.224	-60.4	0.7997
1st Reduced model			
N, S	37.191	-62.4	0.15^{S}, $\mathbf{0.01^{N}}$
2nd Reduced model			
N	36.17	-62.4	
S	33.99	-58	
(c)	**logLik**	**AIC**	**p-value**
Full model			
Nitrate (N), Fine sediment (S), N×S	-59.68	133.4	1
1st Reduced model			
N, S	-59.68	131.4	0.70^{S}, 0.75^{N}
2nd Reduced model			
N	-59.75	129.5	
S	-59.85	127.7	
(d)	**logLik**	**AIC**	**p-value**
Full model			
Nitrate (N), Fine sediment (S), N×S	-52.92	119.9	0.81
1st Reduced model			
N, S	-52.95	117.9	0.32^{S}, 0.07^{N}
2nd Reduced model			
N	-53.44	116.9	
S	-54.56	119.1	

(e)	logLik	AIC	p-value
Full model			
Nitrate (N), Fine sediment (S), N×S	-50.61	115.2	0.91
1st Reduced model			
N, S	-50.62	113.2	$< 0.40^{S}$, $< 0.13^{N}$
2nd Reduced model			
N	-50.96	111.9	
S	-51.74	113.5	

(f)	logLik	AIC	p-value
Full model			
Nitrate (N), Fine sediment (S), N×S	-248.3	510.6	0.49
1st Reduced model			
N, S	-248.53	509.1	0.11^{S}, 0.08^{N}
2nd Reduced model			
N	-249.74	509.5	
S	-250.00	510	

Chapter 4

Additive effects of sediment and nutrient on leaf litter decomposition and macroinvertebrates in hyporheic zone

4.1 Introduction

Coarse particulate organic matter originating from the riparian forests (i.e., leaf litter) is among the major sources of carbon and nutrients that fuel food webs in rivers (Doucett et al., 2007; Hayden et al., 2016). Continuous water and nutrient availability in rivers can stimulate the inland aquatic organic matter decomposition process by having a higher metabolic rate compared to the terrestrial zone, which contributes significantly to the global carbon cycle (Battin et al., 2009; Tranvick et al., 2009). The processing of leaf litter (decomposition) in rivers is driven by leaching, microbial conditioning, invertebrate shredding, mechanical fragmentation by water flow, and sediment transport (Gessner et al., 1999). Because leaf litter decomposition is affected by biological, physical, and chemical processes the decomposition rate can be used as a key indicator of ecosystem functioning in streams and rivers (Young et al., 2008; Gulis and Suberkropp, 2003; Dangles et al., 2004; Niyogi et al., 2013). This function is maintained both in the surface water zone including the benthic zone and the subsurface hyporheic zone, where groundwater and surface water mix (Boulton et al., 2010; Corson-Rikert et al., 2016; Burrows et al., 2017), because a part of organic matter can be buried beneath the streambed during flooding or sediment movement at higher water velocity (Metzler and Smock, 1990; Rulík et al., 2001; Cornut et al., 2010; Pinay et al., 2009). Previous studies on the effects of stressors (e.g., sedimentation, temperature changes, nutrient pollution, or drying events) on hyporheic processes have been conducted in a single-stressor context (Bärlocher et al., 2008; Feris et al., 2009; Stubbington et al., 2011; Nogaro et al., 2013) or examined overall effects of co-occurring multiple stressors (see introduction section in chapter 3) from catchments (Piscart et al., 2011; Sánchez-Morales et al., 2018). Some also tested the interactive effects of multiple stressors on community structures and microbial functioning (Boulton et al., 1998; Nelson and Roline, 2003; Nogaro et al., 2010; Harvey et al., 2012; Solagaistua et al., 2016; Mori et al., 2019), but none have examined the responses of leaf litter decomposition.

The leaf litter decomposition rate can represent the sensitivity of river ecosystem function to sediment and nutrient pollution (Niyogi et al., 2013). Fine sediments often lead to a reduction of decomposition rate in the benthic zone directly through

physiological damage to decomposers through the burial of individuals and suffocation of gills (Jones et al., 2012; Conroy et al., 2018) and indirectly by imposing limitations in the resources (e.g., dissolved oxygen, nutrients) and habitat for microbial and invertebrate assemblages (Lefebvre et al., 2004; Lowell et al., 2009). In the hyporheic zone, increased sediment deposition can clog interstices, thereby lowering hydrological exchanges (Soulsby et al., 2001; Nogaro et al., 2010). This in turn could create an oxygen-limiting condition that negatively affects the habitat of hyporheic invertebrate decomposers (Lefebvre et al., 2004). The reported effects of nutrients on leaf litter decomposition have been context-dependent and mixed (Niyogi et al., 2007; Aristi et al., 2015). Additional nutrients can increase ecosystem productivity under oligotrophic conditions by enhancing microbial and algal production as a subsidy (see also Negishi et al., 2019a). Stimulated microbial activities under high nutrients may promote organic matter decomposition (Menéndez et al., 2003). Beyond a threshold level, however, eutrophic conditions and consumption of oxygen can cause the structural degradation of a habitat with anoxic conditions and even directly impose toxic effects (Graeber et al., 2017; Zhang et al., 2018; Cook et al., 2018). Relatively few studies have examined the responses of hyporheic processes to nutrient pollution (Atashgahi et al., 2015; Sánchez-Morales et al., 2018; Negishi et al., 2019b), and none have rigorously examined possible interactions between the effects of sediment and nutrients.

Ecosystem responses to multiple stressors (e.g., nutrients, fine sediment) are complex because of different types of interactions (Folt et al., 1999). Understanding the type and thresholds of the occurrence of the interaction effects provides important insights for the management of a river environment. In particular, understanding how decomposition processes respond to stressors provides insights on how nutrient cycling and community structures or aquatic organisms may also respond. Recent research found that the adverse effects of fine sediment escalated with higher nutrient concentration in the benthic zone (Matthaei et al., 2010; Wagenhoff et al., 2011; Piggott et al., 2015; Beermann et al., 2018). The consequences of possible multiple stressors in hyporheic processes have also been reported in some studies (Sánchez-Morales et al., 2018; Mori et al., 2019), but only a few were designed to disentangle the interactions of environmental factors. To fill this knowledge gap, this chapter tested the effects of fine sediment on leaf

litter decomposition in the hyporheic zone under different levels of nutrient pollution through experimental sediment addition in sites with different nutrient levels was tested. The objectives and hypotheses were developed based on the key previous observations and evidence. Firstly, the hyporheic habitat was very important by accommodating unique taxa and those taxa responded to environmental variables (chapter 2). Secondly, the fine sediment and nutrient showed gradients in the sediment addition experiment in Satsunai river where the effects of those stressors were found on the taxonomic richness of the hyporheic macroinvertebrates (chapter 3). The first objective was to test how the macroinvertebrate community structure and leaf litter decomposition rates would differ between high sediment and low sediment loading in the hyporheic zone. It was predicted that sediment would significantly reduce the leaf litter decomposition rate by impoverishing the invertebrate community. For comparison baseline data, the benthic zone was also monitored. The second objective was to test whether the leaf litter decomposition rate and the community structure in the hyporheic zone differ by sediment addition under different nutrient levels. It was predicted that the adverse effects of sediment (e.g., decrease in leaf litter decomposition rate) would increase under a high nutrient condition. This was because nutrient as a subsidy, which was the case in the study site, Satsunai River (Negishi et al., 2019a, b), was predicted to become a stressor under high sediment conditions (synergistic effects).

4.2 Materials and Methods

Site description and sampling design

The study was conducted from July 2019 to November 2019 by using the similar study sites as chapter 3 (Fig. 3.1). Leaf litterfall of trees including Japanese Alder (*Alnus japonica* Steud.) commonly takes place in the region between August and December (Kanda, 1996; Xu and Shibata, 2007; Nakamura et al., 2010), and thus the experiment setup was reasonably representative of natural leaf litter decomposition.

The litter bag method (Boulton and Boon, 1991) was used to determine the leaf litter decomposition rate. Dried leaves of *Alnus japonica* were weighed up to 3 (±0.05) g, enclosed within mesh bags (mesh size: 3.9 × 3.9 mm; Piscart et al., 2011; Cornut et al.,

2012; Burrows et al., 2017; Chapter 3; Fig. 3.2). This mesh size allowed macroinvertebrates to enter the traps and feed on leaves. A total of 48 litter bags in the hyporheic zone, with 12 bags at each site, were installed. To prevent the physical breakdown of the leaves and the loss of colonized invertebrates and fine sediment in retrieval processes, the litter bags were installed in the hyporheic zone by enclosing them inside the colonization traps (Fig. S1; see Negishi et al., 2019b for details about the traps). Details of study setup was described in chapter 3 (Fig. 3.3). Similar method was used for the fine sediment addition treatment (Chapter 3; Fig. 3.4)

Collection of litter bags

The litter bags were retrieved between August and November 2019. The collection timing and duration were slightly different between zones because relatively faster loss of organic matter in the surface zone was predicted. This was an effort to avoid losing all the mass by the end of the experiment; a prediction based on the observation of more abundant macroinvertebrates in the benthic zone as well as data from previous reports (Rulík et al., 2001; Negishi et al., 2019a, b). On each collection occasion, a total of four litter bags (two in each treatment and control pit) was collected by excavating one control pit and one treatment pit in the hyporheic zone. The pits with PVC wells were collected on the last occasion to allow water quality measurement at the end of the experiment. For the benthic zone, two litter bags were collected on each sampling occasion. Due to the flow conditions and unexpected sediment deposition, however, some sample collections were delayed causing a minor inconsistency in the sampling design (Table 4.1). Bags in the hyporheic zone were collected through two steps. First, a colonization trap was retrieved as described in (Negishi et al., 2019b) and cut open immediately after retrieval. The litter bags in the colonization traps were quickly taken out and placed in a zip-lock bag. The bags in the benthic zone were removed from the attached ropes and placed immediately in zip-lock bags. After collection, the litter bag samples were transported to the laboratory in an ice chest for further processing.

Water physicochemical measurements

The water temperature was continuously measured at 15- or 30-min intervals using data loggers (HOBO pendant logger, Onset Co., MA, USA) buried together with the colonization traps with one for each treatment and control pit in the hyporheic zone (four loggers; two control and two treatment pits) and one logger in the benthic zone at each site (tethered to a water collection PVC pipe). Two-week means of temperature were calculated for each habitat in each site as measurement replicates. On each occasion of hyporheic sampling, water samples were collected in acid-washed polyethylene bottles to determine nitrate concentrations both in surface and hyporheic water. The flow conditions were sometimes unsuitable for the sampling and one PVC pipe with sediment treatment at L1 was damaged to the point of malfunction before the first sampling occasion, resulting in a slightly modified schedule (Table 4.2). Hyporheic water was collected using a handheld bilge pump from the PVC wells twice per site (two PVC wells) as described in (Negishi et al., 2019b). Surface water was collected at the same time once per site. Dissolved oxygen, electrical conductivity (EC), and pH were also measured for surface water using probes (HACH-HQ40d, Hach Co., Loveland, CO, USA; WM -32EP, TOA-DKK Co., Tokyo, Japan) immediately after the water was collected for nitrate measurements. Dissolved oxygen was always at a saturated level (means ± standard deviations: 9.95 ± 1.07 mg/L) with pH being almost neutral (6.98 ± 0.37) all the time. The DO and pH were considered unimportant as limiting factors and thus they were excluded from further analyses.

Laboratory Analyses

Upon arrival at the laboratory, within 1–2 days, the litter bag samples were washed to recover *A. japonica* leaves. These leaves were oven dried at 60 °C for at least 7 days and were combusted at 500 °C for 4 h (FO310, Yamato Scientific Co., Tokyo, Japan) to obtain ash free dry mass (AFDM) as well as the weight of inorganic matter.

The remaining matter was sieved through a 500 µm sieve and the retained mixture of inorganic particles (found on top and/or entered into the traps), macroinvertebrates, and other exogenous organic matter were processed further. The macroinvertebrates were separated and sorted down to the family level based on existing resources (e.g., Kawai

and Tanida, 2018; Merritt and Cummins, 2019). The Lepidostomatidae, Nemouridae, Leptophlebidae, and Tipulidae were focused and treated them as detritivores in the following analyses based on (Negishi et al., 2019b), where these taxa have been identified as being dependent on allochthonous carbon sources. Thus, it was predicted that they are main contributors to invertebrate-driven leaf litter decomposition. The remaining matter was processed the same as for *A. japonica* leaves to obtain the weight of inorganic material. This weight was added to the weight of the inorganic matter obtained from the *A. japonica* AFDM measurements to represent the total fine sediment in the litter bags.

Upon reaching the laboratory, the water samples were filtered using a glass fiber filter that had a pore size of 0.5 µm (GC-50, Advantec Co., Tokyo, Japan) and were refrigerated until further analyses within a week. The nitrate concentrations were measured using ion chromatography (IA-300, TOA-DKK Co., Tokyo, Japan).

Statistical Analyses

In order to test if the intended environmental gradient was found and how the environment differed between the hyporheic and benthic zones in relation to the sediment treatment, nitrate, water temperature, and fine sediment, the data were examined using generalized linear (mixed) models (GL(M)Ms). A GLM was developed using nitrate concentration as a response variable and site and habitat-treatment type (surface, control, and treatment) as main factors (Gaussian error distribution). Furthermore, generalized linear mixed models (GLMMs) were developed using water temperature and fine sediment in litter bags as response variables and pit identity nested within site, habitat-treatment type, and their interaction as main factors, with sampling season as a random factor (Gaussian error distribution). The sampling season was defined to account for the possible correlation of data in a similar seasonal context (periods before 8 September, between 9 September and 12 October, and between 13 October and 11 November were considered as different seasons).

The leaf litter decomposition rate (k) was obtained using the exponential decay model and compared among habitat-treatment types:

$$W_t = W_i \times e^{-k \times t}$$

where W_t is the leaf AFDM remaining at time t (days since the installation) and W_i is the leaf AFDM at the initial time. The decay model was fitted for each site for each habitat-treatment type (a total of 12 models) using nonlinear least squares regression to obtain habitat-treatment specific *k* (Fig. 4.1). The initial AFDM (W_i) was assumed to be consistently 89% of initial dry mass (3 g) based on a separately established relationship between dry mass and AFDM (unpublished data, J.N.N.). The *k* was compared among habitat-treatment types by developing a GLMM with *k* as a response variable, habitat-treatment type as a main factor, and site as a random factor (Gaussian error distribution).

The community structures of the litter bag invertebrates were summarized using nonmetric multidimensional scaling (NMDS) based on Bray–Curtis distance and 100 maximum iterations. Invertebrate abundance data were standardized by dividing them with the remaining organic matter as the number of individuals per gram of AFDM and were $\log_{10}$ transformed. Scores of two NMDS axes were compared among habitat-treatment types using permutational multivariate analyses of variance (PERMANOVA). To examine the effects of invertebrates on leaf litter decomposition rate, habitat-treatment-type specific *k* was regressed against the standardized abundance of detritivores using a GLMM, with site as a random factor (Gaussian error distribution).

To test whether the effects of sediment treatment varied under different nutrient levels, leaf litter decomposition rate (*k*) was obtained for each litter bag based on the remaining AFDM and duration of time until respective retrievals. Temperature is a crucial factor that affects leaf decomposition rates in rivers (Webster and Benfield, 1986), and the effects of temperature were statistically removed. For each litter bag, a habitat-treatment-type specific two-week mean water temperature was calculated and found that there was a significant effect of water temperature on leaf litter decomposition rate (*k*) (Fig. 4.2). Therefore, a GLM was developed to regress *k* against water temperature and used the residual of the model (residual *k*) in the following analyses.

To determine the type and extent of the interactions of nitrate and sediment, a GLMM was developed using residual *k* as a response variable and nitrate, fine sediment, and their interaction as main factors, with pit identity nested within site sampling seasonality as a random factor (Gaussian error distribution). For the nitrate in the same treatment type of the same site, the same data were adapted for multiple litter bags. However, because the PVC well in L1 was lost and water collection was sometimes conducted only for surface water, nitrate data were missing for some occasions in the hyporheic zone (Table 4.2). To supplement the missing nitrate data for each litter bag, a linear relationship between nitrate and EC was developed for the surface water and derived missing nitrate values based on this relationship (Fig. 4.3). It was believed that this approach was acceptable because the previous study also found a highly significant relationship between nitrate and EC (Alam et al., 2020) and no difference was found among habitat-treatment types (see the Results section). When multiple nitrate values were available for the same litter bag until the retrieval, the mean value was used in the analyses. A significant interaction of main factors in this model was interpreted as supporting evidence of complex interactive effects of nutrients and sediment. Lastly, a standardized abundance of detritivores was added to this model as a covariate and the model was rerun to examine whether any of the significant effects of the main factors were related to invertebrate activities. Any effects negated by the invertebrate covariate were interpreted as being under strong control of invertebrate activities, whereas the persistent significant effects were interpreted as being under the control of microbes.

All statistical analyses were performed using R (version 3.5.2). Data in GL(M)Ms were $\log_{10}$ transformed to improve normality among groups when necessary. The normality assumption was checked by Shapiro–Wilk tests and was confirmed in all cases. The effects of the main factors in GL(M)Ms were examined by sequentially comparing models with reduced models without the effect of the factor of interest, using likelihood-ratio tests. The "glmmADMB", "nls", "vegan", and "ADONIS" packages were used for GLMMs, nonlinear least square regression, NMDS, and PERMANOVA, respectively. Multiple comparisons among groups in GLMMs were carried out with the "multcomp" package. The statistical significance level was set at $p = 0.05$ with Bonferroni corrections in group comparisons.

4.3 Results

Nitrate concentrations were consistently higher in some sites regardless of habitat-treatment types, as shown by significant effects of site with insignificant interaction effects (Table 4.3). The concentration tended to be higher in sites downstream of the WWTP and was the highest in Site L1 (Fig. 4.4). Site A1, downstream of the confluence and with a large tributary, had intermediate concentrations, between Sites L1 and L7, with the spring-fed tributary site S1 having the lowest. Water temperature also differed between sites (Table 4.3) and was highest in A1 and lowest in site S1 (Fig. 4.4). The amount of fine sediment showed significant effects of interaction, indicating that the extent of differences among habitat-treatment types varied across sites (Table 4.3c). The sediment was at the highest level in litter bags treated with sediment across all the sites, and benthic litter bags contained the lowest amount of sediment (Fig. 4.4). The sediment amount in L1 and L7 treatment bags was not statistically different from the control bags, but the medians and upper quartiles in the treatment bags were higher than those in the control bags.

The leaf litter decomposition rate differed among habitat-treatment types (Table 4.4). The rate in the benthic zone did not differ from that in the control hyporheic zone, but the rate in the treatment hyporheic zone was significantly lower than that in the benthic zone (Fig. 4.5a). The rate in the treatment hyporheic zone was insignificant compared with the rate in the control hyporheic zone largely because the rate in the Site L1 remained high even with the sediment treatment (Fig. 4.1).

A total of 16 macroinvertebrate taxa (hereafter called either order or family) and 3,076 individuals were found in the leaf litter bags of the benthic and hyporheic zones. Detritivore macroinvertebrate taxa (e.g., Lepidostomatidae, Nemouridae, Leptophlebiidae, and Tipulidae) were present in the benthic and hyporheic zones where all taxa except Chironomidae were numerically abundant in the hyporheic zone compared to that of the benthic zone (Table 4.4). Detritivore taxa Tipulidae were found to be relatively lower in number in the treatment hyporheic zone compared to the benthic and control hyporheic zones (Table 4.4). The community structure was clustered according to

habitat-treatment-type groups in the NMDS plot with significant differences between the benthic and hyporheic treatment bags (stress = 0.16, Fig. 4.5b, and Table 4.4). Community in the control hyporheic zone was intermediate and was not distinguishable from the two other groups. The leaf litter decomposition rates had a positive relationship with the abundance of detritivores (Fig. 4.5c; Table 4.5), indicating that habitat-treatment-type specific *k* variation was partially related to the activities of detritivores.

Residual *k* was not affected by the interaction between nitrate and sediment, showing both independent significant effects (Table 4.6). The residual *k* was negatively affected by sediment and positively affected by nitrate (Fig. 4.6). When the abundance of detritivores was added as a covariate to the model of residual *k* with nitrate and fine sediment, the effect of sediment became insignificant with significant effects of the abundance of detritivores (Table 4.6). The positive effects of nitrate on the residual *k* remained significant. An additional examination of the relationship between the abundance of detritivores and nitrate level showed a highly positive relationship (GLM with the abundance of detritivores as a response variable and nitrate as an explanatory variable: $p < 0.001$, $r^2 = 0.88$).

4.4 Discussion

The results showed that both fine sediment and a specific nutrient (i.e., nitrate) mediate leaf litter decomposition within the riverbed. Contrary to the predictions, there was no evidence that two potential stressors synergistically or antagonistically interact with each other when affecting leaf litter decomposition in a multiple stressor context. Independent effects of sediment and nutrients were consistently observed and thus the two stressors had additive effects. Because the two effects were directionally opposite to each other, the additive effects appeared as an offset of one by the other. Invertebrate detritivores played an important role in these functional responses of the hyporheic zone, in response to fine sediment. These findings add to the existing pioneer studies on the effects of sediment and nutrients on hyporheic processes in a multiple stressor context.

The results did not support previous reports that leaf litter decomposition rate is lower in the hyporheic zones compared with surface water zones (Rulík et al., 2001;

Piscart et al., 2011). The estimated leaf litter decomposition rate in the control hyporheic zone was similar to that in the benthic zone. This discrepancy can be explained by the different environmental contexts of the riverbed in these studies. Two previous studies (Rulík et al., 2001; Piscart et al., 2011) both reported conspicuous differences between the surface water zone and the hyporheic zone in terms of dissolved oxygen with lower values in the latter, suggesting that hydrological exchanges with the surface water zone might have been relatively low compared with those in the Satsunai River. It is probable that this might be related to the sediment characteristics in the riverbed, which was sandy in (Rulík et al., 2001) and sandy to coarse gravel in (Piscart et al., 2011). Riverbed materials in the Satsunai River are dominated by cobbles with high hydrological connectivity between the benthic and hyporheic zones with little differences in water quality (Negishi et al., 2019b). Such sediment differences might have caused direct and indirect effects on the leaf litter decomposition rates. When treated with the sediment, the leaf litter decomposition rate was reduced, providing clear support to this idea. However, water quality differences were not apparent between the control and treatment bags and thus water quality was not the main limiting factor of leaf litter decomposition rate in the hyporheic zone.

Variations in the leaf litter decomposition rates among habitat-treatment-type groups were driven by the gradient of the abundance of detritivores. In the hyporheic zone, frequently reported shredders are amphipods (family: Gammaridae) (Navel et al., 2010; Piscart et al., 2011; Vander Vorste et al., 2017). In this study, no Gammaridae including a species *Pseudocrangonyx yezonis* (Akatsuka and Komai, 1922), which is known to inhabit the study site (Alam et al., 2020), were captured. Alam et al. (2020) showed that *P. yezonis* is a top predator in the hyporheic food web. Therefore, a low direct affinity of *P. yezonis* with detritus resources was probably the reason that no individuals were caught. Instead, relatively abundant aquatic insect detritivores such as Lepidostomatidae were found in both the benthic and hyporheic zones. These detritivores were also found in the surface water zone (see also (Negishi et al., 2019a, b)) and thus were considered as a temporal dweller of the hyporheic zone. Relatively high hydrological exchanges between the benthic and hyporheic zones associated presumably with high interstitial spaces might have allowed surface dwellers' access to the hyporheic zone. The leaf litter decomposition

rate variation among three habitat-treatment-type groups was explained by the abundance of these detritivores, indicating that the insect detritivores contributed to leaf litter decomposition in both the benthic and hyporheic zones. The negative effects of sediment addition on the leaf litter decomposition rate were largely caused by the negative effects of sediment on the abundance of these detritivores and their function. This explanation was supported by the disappearance of the sediment effects by the inclusion of detritivore abundance as a covariate in the model. The specific population process would have occurred at control pits and were absent in the treatments. The immigration of detritivores was a possible process that occurred when food resources were available in the hyporheic zone, which was limited in treatment pits having limited access to the food resources by detritivores. It is probable that the community structure and distribution of invertebrates and the feeding activities of invertebrate detritivores were more limited by finer materials due to reduced access to their food resources and interstitial habitat (Navel et al., 2010; Stubbington et al., 2011; Xu et al., 2012).

The effect of nitrate on the leaf litter decomposition rate of organic matter was independent of the effects of fine sediment as indicated by the absence of statistical interactions between the two factors. The nitrate effect was consistently positive by promoting faster loss of leaf materials. There are two partially related mechanisms that were possibly responsible for this relationship. First, nutrients stimulate microbial activity and thus promote the microbial decomposition of organic matter (Grattan and Suberkropp, 2001; Menendez et al., 2003). Second, the stimulated activities of the microbial community enhance the palatability of leaves that could accelerate the macroinvertebrate-derived decomposition and thus mediate the carbon flow (Webster and Benfield, 1986; Gulis et al., 2006; Graham et al., 2016). The inclusion of the detritivore abundance as a covariate did not offset the positive nutrient effects on the leaf litter decomposition rate and there was a strong relationship between the abundance of detritivores and nitrate levels, thus providing support to the second explanation of results of this study. Negishi et al. (2019a,b) have reported that invertebrate community, including detritivores in the present study, was more abundant in the nutrient-polluted lower section of the Satsunai River with clear isotope evidence of trophic assimilation of synthetic nitrogen, suggesting overall that nitrate acted as a subsidy in the hyporheic zone though strong coupling of the

processes in the two zones (Negishi et al., 2019b). The estimates of the contribution of microbial activities requires the measurements of leaf litter decomposition rates using a fine mesh as well as other functional properties such as the carbon-to-nitrogen ratio.

4.5 Conclusion

The findings demonstrated that detritivore community and leaf litter decomposition rates differed between the benthic and hyporheic zones and hyporheic leaf litter decomposition was influenced by two stressors independently in a highly predictable manner. In the Satsunai River, it is projected that excessive loads of fine sediment may result in a predictable impact on leaf litter decomposition rate in the hyporheic zone with the same degree of reduction regardless of background nitrate levels. From the viewpoint of overall functional response, the negative effects of sediment stress can be offset by the counteracting effects of the nutrient pollution. Intriguingly, the mechanisms that cause the respective responses differed between the types of stressors. These findings provide two important implications to the predictions of how the hyporheic ecosystem may respond to sediment and nutrient stressors. First, ecosystem functioning in terms of leaf litter decomposition rates is resistant to stressors because of counteracting responses. Second, the changes of ecosystem structure (community structure of macroinvertebrates and microbes) due to stressors may be substantial despite the functional changes appearing to be small. In other words, it is suggested that the functional resilience is maintained by the functional redundancy of detritivore invertebrates and microbes in the decomposition processes.

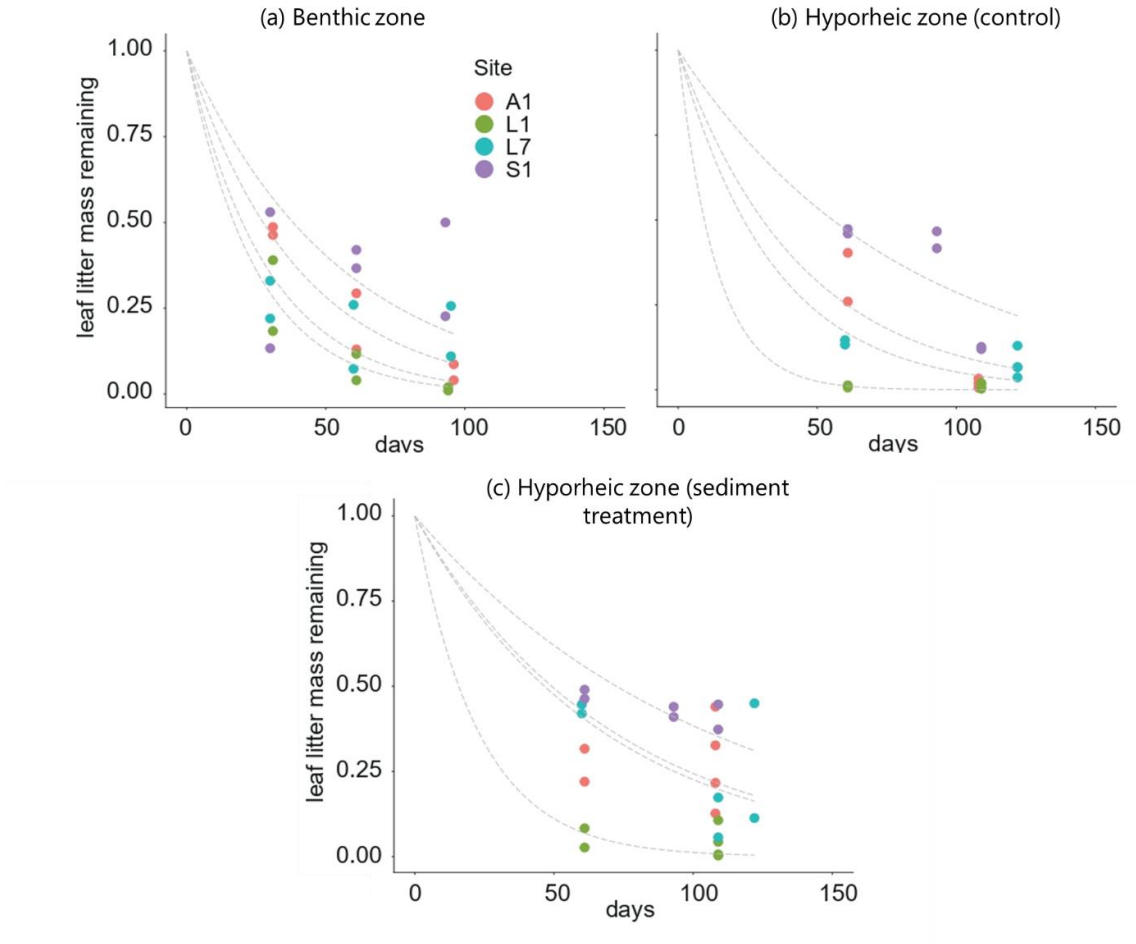


Fig. 4.1 Temporal changes of remaining leaf litter mass (fraction) after the installation (day 0) in relation to habitat-treatment groups, benthic (a), hyporheic control (b), hyporheic sediment treatment (c) in respective sites. Dotted lines represent regressions based on nonlinear least square regression models.

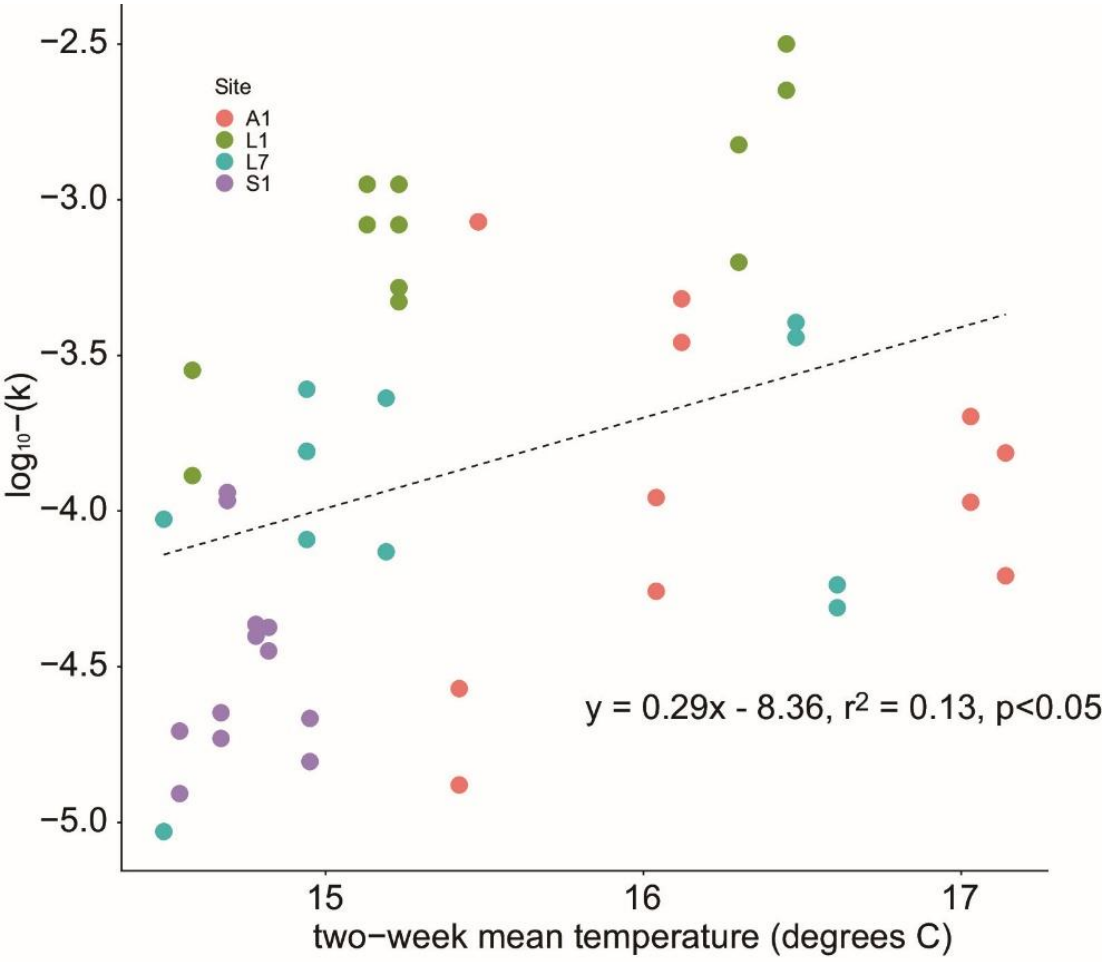


Fig. 4.2 A relationship between two-week mean water temperature and leaf litter decomposition rate k (in a logarithmic scale). Water temperature for each data point was obtained by taking averages of all two-week temperature data between the installation and retrieval occasions. For the same treatment type in the same site, the same temperature data was adapted for multiple litter bags. A dotted line denotes a regression line.

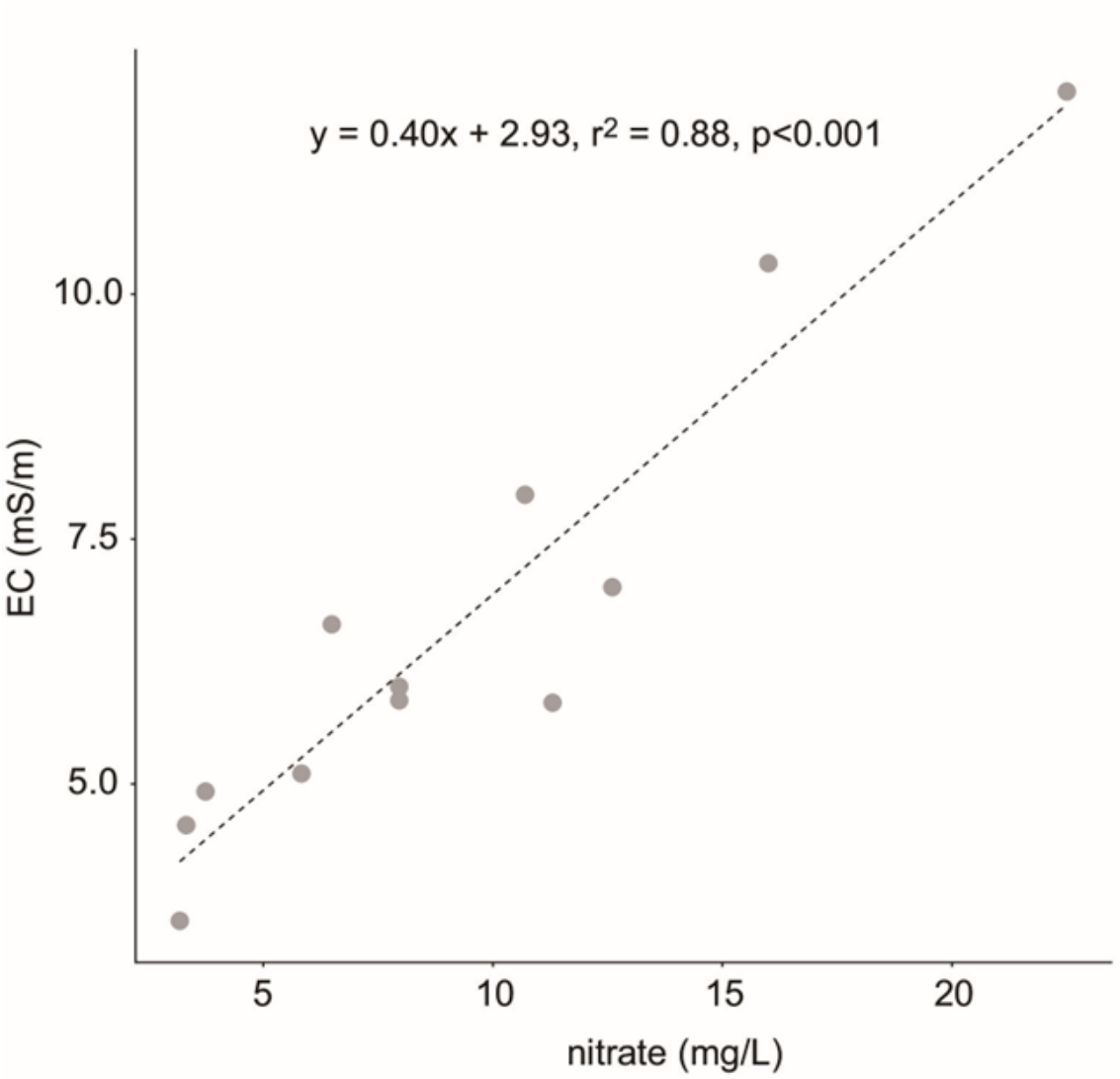


Fig. 4.3 A relationship between electrical conductivity and nitrate in the surface-water (benthic) zone. A dotted line denotes a regression line.

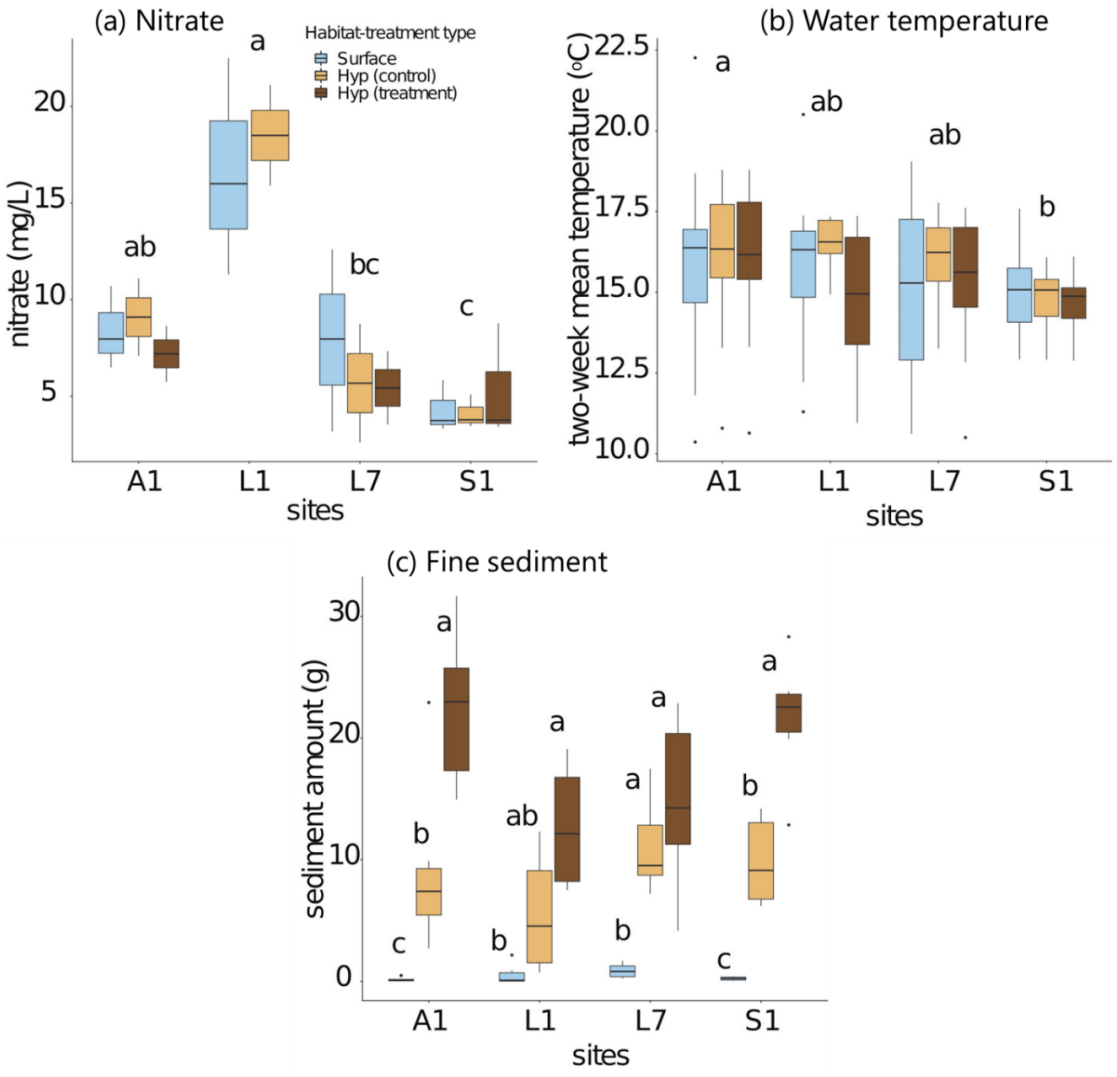


Fig. 4.4 Comparisons of nitrate (a), water temperature (b), and fine sediment (c) in relation to habitat-treatment types. Alphabetical letters denote results of multiple comparisons based on GLMM; those with the same letters were statistically undistinguishable. Multiple comparisons were performed among sites for nitrate and temperature and among habitat-type groups within each site for fine sediment.

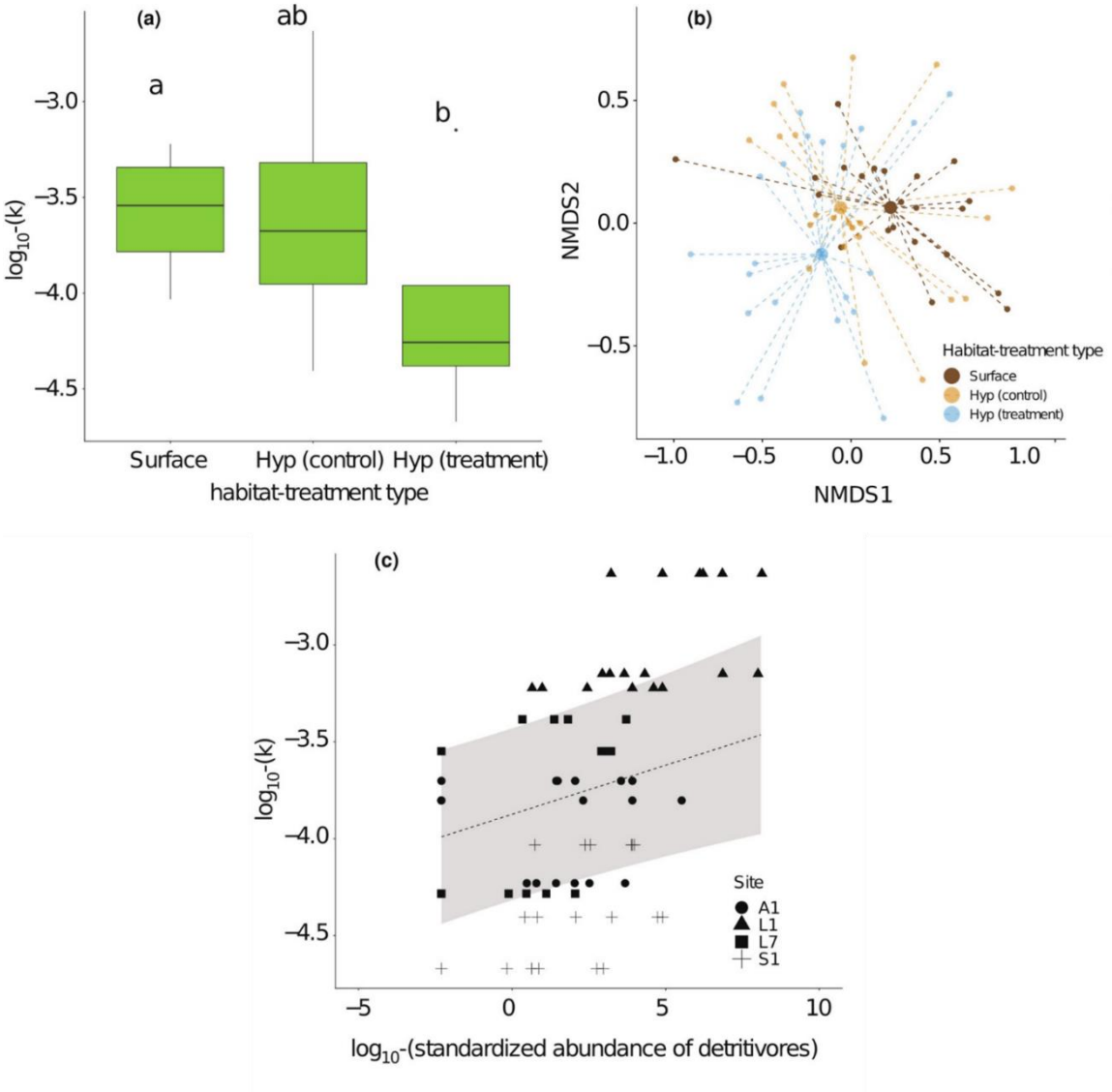


Fig. 4.5 Comparisons of $\log_{10}$-transformed leaf litter decomposition rate (*k*) in relation to the habitat-treatment types (a), biplots as a result of NMDS (b), and a relationship between the $\log_{10}$-transformed abundance of detritivores and *k* (c). In (a), alphabetical letters denote results of multiple comparisons based on GLMM; those with the same letters were statistically undistinguishable. Multiple comparisons were performed among sites. In (b), large circles represent centroids of data points for each habitat-treatment group. In (c), a regression line based on a GLMM and 95% confidence intervals are shown.

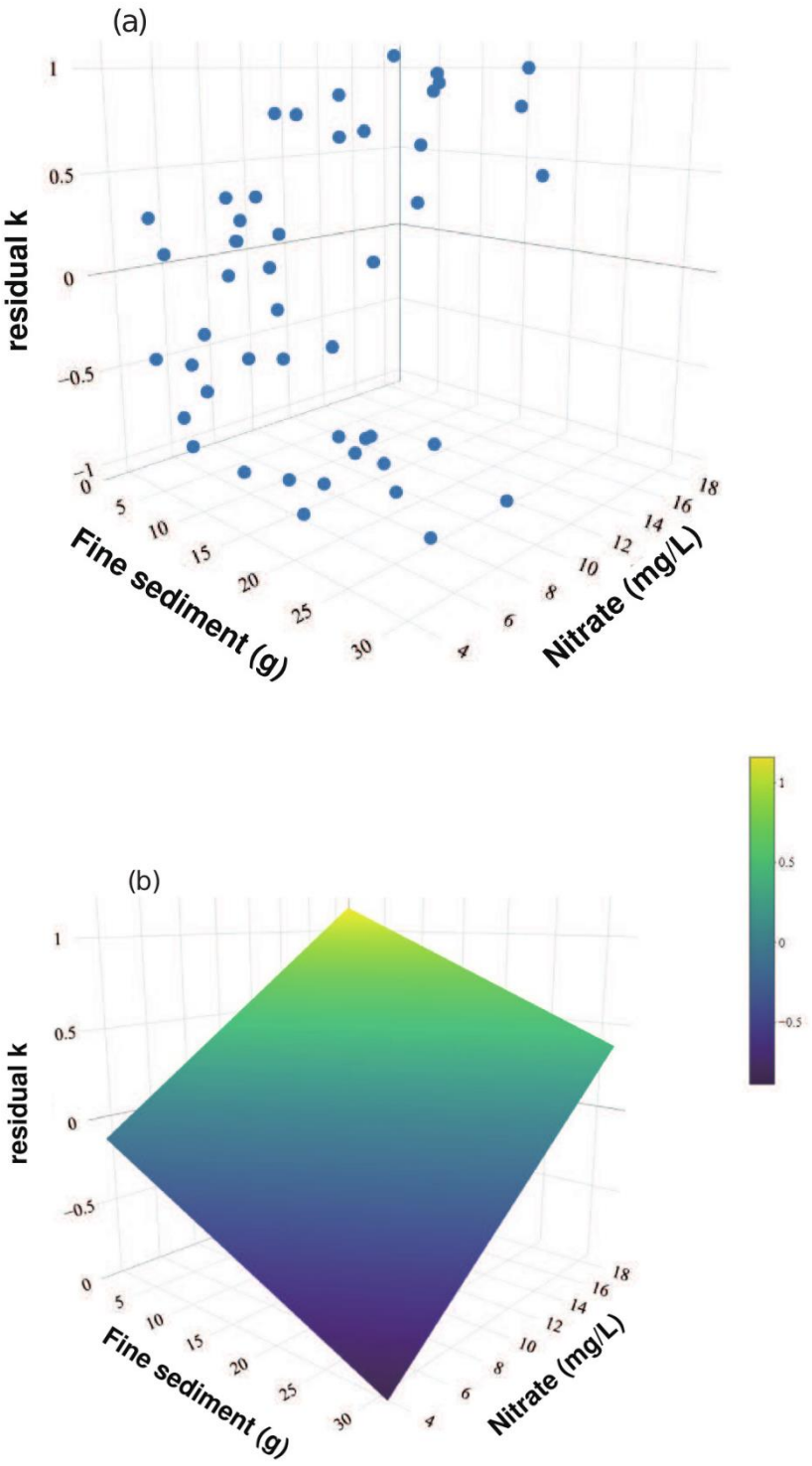


Fig. 4.6 A three-dimensional plot for raw residual *k* in relation to nitrate and fine sediment (a) and a projected surface model based on the results of GLMM (b).

Table 4.1 A summary of litter bag collection schedule in the surface-water (benthic) and hyporheic zones. Numbers in bracketed superscripts denote the number of bags retrieved for the surface-water zone and the number of pits excavated for retrieving traps for the hyporheic zone.

Site	Habitat type	Installation date	Collection date
		2019	2019
A1	Surface	7/8	8/8$^{(2)}$, 9/7$^{(2)}$, 10/12$^{(2)}$
	Hyporheic		9/7$^{(2)}$, 10/24$^{(4)}$
L1	Surface	7/8	8/8$^{(2)}$, 9/7$^{(2)}$, 10/10$^{(2)}$
	Hyporheic		9/7$^{(2)}$, 10/25$^{(4)}$
S1	Surface	7/9	8/8$^{(2)}$, 9/8$^{(2)}$, 10/10$^{(2)}$
	Hyporheic		9/8$^{(2)}$, 10/10$^{(2)}$, 10/26$^{(2)}$
L7	Surface	7/9	8/8$^{(2)}$, 9/7$^{(2)}$, 10/12$^{(2)}$
	Hyporheic		9/7$^{(2)}$, 10/26$^{(1)}$, 11/08$^{(3)}$

Table 4.2 A summary of sampling dates of water in 2019 for the measurements of electrical conductivity, pH, dissolved oxygen in the surface-water (benthic) zone (a), nitrate in the surface-water zone (b), and nitrate in the hyporheic zone (c). Numbers in bracketed superscripts indicate replicated samples measured at each site; no superscripts indicate one sample. N denotes sample sizes.

(a)

Site	Habitat type	Dates	N
A1	Surface	9/7$^{(3)}$, 10/12$^{(3)}$, 10/24$^{(3)}$	9
L1	Surface	9/7$^{(3)}$, 10/12, 10/25$^{(3)}$	7
S1	Surface	9/7$^{(3)}$, 10/10$^{(3)}$, 10/26$^{(3)}$	9
L7	Surface	9/8$^{(3)}$, 10/12$^{(3)}$, 11/8$^{(3)}$	9

(b)

Site	Habitat type	Dates	N
A1	Surface	9/7, 10/12, 10/24	3
L1	Surface	9/7, 10/12, 10/25	3
S1	Surface	9/8, 10/10, 10/26	3
L7	Surface	9/7, 10/12, 10/26	3

(c)

Site	Habitat type	Treatment type	Dates	N
A1	Hyporheic	Control	9/7, 10/24	2
		Treatment	9/7, 10/24	2
L1	Hyporheic	Control	9/7, 10/25	2
		Treatment	Sample loss due to washout of the well	0
S1	Hyporheic	Control	9/8, 10/10, 10/26	3
		Treatment	9/8, 10/10, 10/26	3
L7	Hyporheic	Control	9/7, 10/26	2
		Treatment	9/7, 10/26	2

Table 4.3 Results of GLMMs testing the effects of site (Site) and habitat-treatment type (Type: benthic zone, control hyporheic zone, and treatment hyporheic zone) and their interactions on log_{10}-transformed nitrate concentration (a), the effects of site and habitat-treatment type and their interactions on log_{10}-transformed two-week mean water temperature (b), and the effects of site and habitat-treatment type and their interactions on log_{10}-transformed fine sediment amount (c). Full models and reduced models were each compared using log-likelihood tests; when Full model were insignificant, 1st reduced models were compared to 2nd reduced models sequentially. Superscripts of p-values indicate the variables removed from the model to test with those from reduced models by one level. Bold letters for p-value denote statistical significance.

(a)	**logLik**	**AIC**	**p-value**
Full model			
Site (S), Type (T), S×T	-66.65	157.31	0.74
1st Reduced model			
S, T	-67.99	149.99	**<0.001**^{S}, 0.93^{T}
2nd Reduced model			
S	-68.06	146.11	
T	-85.06	178.13	

(b)	**logLik**	**AIC**	**p-value**
Full model			
Site (S), Type (T), S×T	109.68	-189.4	0.98
1st Reduced model			
S, T	109.06	-200.1	**<0.05**^{S}, 0.13^{T}
2nd Reduced model			
S	107.07	-200.1	
T	105.04	-198.1	

(c)	**logLik**	**AIC**	**p-value**
Full model			
Site (S), Type (T), S×T	-97.47	224.9	**<0.05**
1st Reduced model			
S, T	-105.81	229.6	

Table 4.4 A summary of all the invertebrate taxa caught in litter bags for three types of habitat-treatment group. NA indicates that identification was conducted only at the order level.

		Surface		Hyporheic (control)		Hyporheic (treatment)	
Order	**Family**	**Mean ± SD**	**Total**	**Mean ± SD**	**Total**	**Mean ± SD**	**Total**
Ephemeroptera	Ephemerellidae	4.54 ± 6.29	109	1.63 ± 5.55	39	1.21 ± 4.12	29
Ephemeroptera	Ephemeridae	0.58 ± 1.53	14	0.75 ± 2.44	18	0.00 ± 0.00	0
Ephemeroptera	Heptageniidae	0.17 ± 0.48	4	0.13 ± 0.45	3	0.08 ± 0.28	2
Ephemeroptera	Leptophlebidae	1.58 ± 2.21	38	1.79 ± 3.82	43	0.92 ± 2.60	22
Plecoptera	Chloroperlidae	0.00 ± 0.00	0	0.08 ± 0.28	2	0.50 ± 0.98	12
Plecoptera	Nemouridae	0.54 ± 1.22	13	0.75 ± 1.89	18	0.13 ± 0.34	3
Plecoptera	Perlodidae	1.96 ± 3.07	47	0.17 ±0.64	4	0.04 ± 0.20	1
Trichoptera	Hydropsychidae	23.96 ± 50.24	575	0.21 ± 0.66	5	0.04 ± 0.20	1
Trichoptera	Lepidostomatidae	7.17 ± 12.08	172	9.42 ± 12.39	226	5.96 ± 7.46	143
Trichoptera	Rhyacophylidae	0.17 ± 0.48	4	0.00 ± 0.00	0	0.00 ± 0.00	0
Trichoptera	Stenopsychidae	0.71 ± 2.01	17	0.21 ± 0.83	5	0.04 ± 0.20	1
Diptera	Chironomidae	26.17 ± 19.86	628	15.29 ± 12.01	367	13.67 ± 10.45	328
Diptera	Tipulidae	0.17 ± 0.48	4	0.25 ± 0.61	6	0.17 ± 0.38	4
Oligochaeta	NA	1.21 ± 2.73	29	1.50 ± 3.04	36	2.21 ± 2.70	53
Coleoptera	Elmidae	1.63 ± 3.59	39	0.38 ± 1.13	9	0.04 ± 0.20	1
Odonata	NA	0.08 ± 0.41	2	0.00 ± 0.00	0	0.00 ± 0.00	0

Table 4.5 Results of GLMMs testing the effects of habitat-treatment type (Type: benthic zone, control hyporheic zone, and treatment hyporheic zone) on log_{10}-transformed processing rate (*k*) (a), and the effects of log_{10}-transformed standardized abundance of detritivores on log_{10}-transformed processing rate (b). Full models were compared to null models using log-likelihood tests. Bold letters for p-value denote statistical significance.

(a)	**logLik**	**AIC**	**p-value**
Full model			
Type	-4.25	18.5	**<0.05**
Null model			
N.A.	-8.20	22.4	

(b)	**logLik**	**AIC**	**p-value**
Full model			
Detritivore	-18.99	46.0	**<0.01**
Null model			
N.A.	-23.63	53.3	

Table 4.6 Results of GLMMs testing the effects of nitrate, sediment and their interactions on residual *k* (a), and the effects of nitrate, sediment, and $\log_{10}$-transformed standardized abundance of detritivores on residual *k* (b). the effects of $\log_{10}$-transformed standardized abundance of detritivores on $\log_{10}$-transformed processing rate (b). Full models and reduced models were each compared using log-likelihood tests; when Full model were insignificant, 1st reduced models were compared to 2nd reduced models sequentially. Superscripts of p-values indicate the variables removed from the model to test with those from reduced models by one level. Bold letters for p-value denote statistical significance.

(a)	**logLik**	**AIC**	**p-value**
Full model			
Nitrate (N), sediment (S), N×S	-11.43	38.9	0.44
1st Reduced model			
N, S	-11.73	37.5	**<0.001^{N}**, **<0.01^{S}**
2nd Reduced model			
N	-16.99	46	
S	-19.28	50.6	

(b)	**logLik**	**AIC**	**p-value**
Full model			
Nitrate (N), sediment (S), detritivores (D)	9.0	-2.1	**<0.01^{N}**, 0.09^{S}, **<0.001^{D}**
1st Reduced model			
N, S	-5.61	25.2	
S, D	4.35	5.3	
D, N	7.58	-1.2	

Chapter 5

General discussion

5.1 Summary of the study

The research that examined the importance of the critical contribution of structural and functional properties of hyporheic macroinvertebrates across multiple environmental gradients is limited (Niyogi et al., 2007; Descloux et al. 2013; Jones et al. 2015; Aristi et al., 2015; Sánchez-Morales et al., 2018; Mori et al., 2019), in particular when compared with a wealth of studies on benthic habitats. The current research effort was made to fill the remaining knowledge gaps, demonstrating how vital the hyporheic habitat is in terms of macroinvertebrate species diversity, and how common anthropogenic environmental stressors affect macroinvertebrate communities and their functions. The study was conducted in eight river segments (e.g., Toyohira, Horonai, Sorachi, Tokachi, and four segments in Satsunai River). Based on the findings in Chapter 2, the multiple stressor effects were thoroughly tested in four river segments in Satsunai River. This particular river system was chosen because the river was among the best preserved hyporheic environments with diverse hyporheic invertebrates. The river was idealistic because a nutrient gradient exists between the river reaches (Negishi et al., 2019a, b), which allowed the field testing of two stressor influences on river ecosystem structure and function.

The first part of the study investigated the contribution of hyporheic habitat to the total diversity of the macroinvertebrates and the factors that influence the contribution (Chapter 2). It revealed that the hyporheic habitat could contribute to the total macroinvertebrate diversity. The habitat-type beta contribution in the diversity decomposition was insignificant at inter-segment scales but significant at the smaller reach scale. The hyporheic habitat contributed up to 25.9% of the total macroinvertebrate diversity, influenced by the number of hyporheic species. The fine sediment, nitrate concentrations, dissolved oxygen contents, and the riverbed median particle size in the hyporheic habitat significantly influenced the taxonomic richness of these hyporheic species.

The second part of the study examined whether hyporheic macroinvertebrates could be influenced by the multiple stressors (Chapter 3). The findings showed that the nitrate and fine sediment did not act as stressors to the taxonomic richness of the

hyporheic macroinvertebrates. Sediment did not have significant effects on hyporheic community, which was inconsistent with predictions. In contrast, nitrate affected positively on community as predicted. A positive increase in the Shannon-Wiener diversity index with hyporheic nitrate concentration indicated that the number and relative abundance of the macroinvertebrates could be subsidized by the nitrate, which was found in the previous studies in the same river segments (Negishi et al., 2019a, b; Alam et al., 2020).

The final part of this research tested the effects of fine sediment under the variable levels of nutrient pollution on the hyporheic leaf litter decomposition as a function (Chapter 4). The leaf litter decomposition showed different patterns of fine sediments compared to the previous chapter in the hyporheic zone. It revealed the comparable decomposition rate between the surface and hyporheic zone but decreased under the fine sediment treatments. The stressors' effects were independent, where higher nitrate concentration accelerated the leaf litter decomposition rate and fine sediment decreased the decomposition rate with a significant adverse effect on the hyporheic detritivore community. The detritivore community appeared to be the driving factor of the decomposition rate under the sediment treatment in the hyporheic zone.

5.2 Importance of hyporheic macroinvertebrates in ecosystem structure and function

It is well known that the benthic macroinvertebrates play crucial roles in the ecosystem structure and functions of the river ecosystems (Wallace and Webster, 1996; Collier and Clements, 2011; In˜iguez-Armijos et al., 2017; Adner, 2017). This study provided several new evidence that the hyporheic macroinvertebrates could play roles in ecosystem structure and functions in several additional ways. First, its importance as a source of macroinvertebrate taxonomic diversity was quantified for the first time. Second, the number of hyporheic species could add to the structural properties of the ecosystems (Noy-Meir, 1979; Sandin and Solimini, 2009; Adner, 2017). They usually comprise a unique community structure (Chapters 2 and 3), which could add species to macroinvertebrates' diversity. Third, the functional role in the hyporheic zone is crucial

for the river ecosystems (Tilman et al., 2014). The hyporheic detritivores were the key group to determine the functions in the hyporheic zone in the current study (Chapter 4) which influences the carbon flow in the hyporheic zone and the whole river ecosystem (Gulis et al., 2006; Graham et al., 2016). It was one of the few reports where the leaf litter decomposition rate was tested across the multiple stressors gradient in the hyporheic zone (Piscart et al., 2011).

5.3 Importance of hyporheic species conservation

The hyporheic zone is as essential as a benthic zone in terms of macroinvertebrate diversity (Williams and Hynes, 1974; Boulton et al., 1998). The hyporheic zone's biodiversity is essential and often comprises unique taxa. This study found several unique species that made up a unique community structure in hyporheic habitat. The diversity of macroinvertebrates is a vital component of the river ecosystem. Protecting and conserving this habitat is very important because this habitat could make an essential contribution by adding unique species. Taxa proportionately dominated in the hyporheic zone could provide many important roles in the hyporheic habitat (Alam et al., 2020). The hyporheic specialist species could play an important role in the food web. Alam et al. (2020) showed that Amphipoda and *Alloperla ishikariana* were the top predator and secondary consumers in the hyporheic food web with an essential trophic link with each other. The hyporheic species could provide an important role by contributing to the food web. Consequently, hyporheic macroinvertebrates could provide crucial information on water pollution in the hyporheic habitat by showing the sensitivities to the stressors or by the assimilation (Alam et al., 2020). Thus, hyporheic specialist species could be used as indicators of water pollution in the hyporheic habitat. Besides, some macroinvertebrate taxa could provide essential functions, such as leaf litter decompositions, and those might not be hyporheic species but could be temporal dwellers in the zone (Alam et al., 2021). In such cases, conserving hyporheic species would not ensure the leaf litter decompositions in the hyporheic zone. Although there is no evidence of playing a direct role in the decomposition process in the hyporheic zone, their conservation is essential due to the crucial roles in maintaining carbon flow in the hyporheic food web and

important roles as indicators of water pollutions by providing information of hyporheic food web.

5.4 Factors influencing the structure and function of hyporheic macroinvertebrates

Multiple environmental stressors have reportedly affected the hyporheic zone; however, very few research tested the effects on the hyporheic macroinvertebrates and their functions (Descloux et al. 2013; Stubbington et al., 2015; Jones et al. 2015; Mori et al., 2019). The habitat contribution from the hyporheic zone may depend on the physical and chemical characteristics of the habitat. The determination of the hyporheic species is the prior task to know how importantly the hyporheic habitat is in providing contribution. The number of species in the hyporheic habitat and their interaction with the environmental variables could determine how hyporheic habitat could variably contribute to the total diversity of macroinvertebrates at the reach scale (Chapter 2).

The community structure and function of macroinvertebrates could depend on the environmental gradients in the hyporheic zone (Descloux et al. 2013; Jones et al. 2015; Mori et al., 2019). The hyporheic macroinvertebrate community wouldn't have any response to the fine sediment but positive response to nutrient enrichment (Chapter 3). On the other hand, fine sediment and nutrients affected the hyporheic leaf litter decomposition in a negative and positive directions, respectively (Chapter 4). The abundance of detritivores was decreased with a higher amount of fine sediment and were regarded as a key to limiting the function in the hyporheic zone (Chapter 4). These suggested that worldwide recognized adverse effects of fine sediment (Descloux et al., 2013; Jones et al., 2015; Mathers et al., 2021) could affect the community structure or function or both (Young et al., 2008). River geomorphology could determine the structure and function in two ways. First, increasing the amount of fine sediment could reduce the number of hyporheic species and thus reduce the habitat contribution from the hyporheic zone. Second, increasing fine sediment could reduce the ecosystem function in the hyporheic zone. The loss of specific taxa richness or abundance by fine sediments with specific functional roles could be reflected directly in a decrease in function without direct evidence in the community structure of macroinvertebrates (Chapter 4; Sandin and

Solimini, 2009). The fine sediment could resist the macroinvertebrates detritivores to access to the organic matter (Navel et al., 2010; Stubbington et al., 2011).

5.5 Discrepancy of macroinvertebrate responses to the fine sediment

This study reports the different responses of hyporheic macroinvertebrates to fine sediment accumulation in the hyporheic zone. Hyporheic species responded negatively to the fine sediments across the river reaches (Chapter 2). On the other hand, the macroinvertebrate community did not respond to fine sediments in the hyporheic zone (Chapter 3). The following could explain the different responses of macroinvertebrates to fine sediment. First, the hyporheic species were determined based on the proportionally higher occurrence of taxa in the hyporheic habitat compared with benthic (Chapter 2), which was different for the macroinvertebrate community in Satsunai River only (Chapter 3). In later cases, the different sensitivities of macroinvertebrate taxa counterbalanced the effects of fine sediments. Also, nitrate concentration in the hyporheic zone consistently subsidized the macroinvertebrates community regardless of any effect of fine sediments. Second, the natural gradients of fine sediments across multiple river reaches were considered, while the experimental sediment addition was employed in the Satsunai River (Chapter 3). So, in the Satsunai River, fine sediment might have limited effects based on the different sensitivities of taxa.

5.6 Implications in water resource management

The Ministry of Land, Infrastructure, and Transport in Japan, started supporting the local Government in 1990 for managing rivers not only for flood management but also maintaining, improving, and protecting natural environments (Yoshimura et al., 2005). Rivers' natural dynamics are important for a successful restoration (Ward et al., 2001). The Government has maintained integrated water management by incorporating the environment, flood management, and water utilization since 1997. The conservation of the river environment signifies the critical interaction between the biological community and environmental factors. In this transition, it is necessary to know structural and functional assessments of the macroinvertebrates under variable environmental gradients. Since the effects of different environmental stressors on river biological communities and

their function are important to evaluate river health, it is critical information for freshwater resource management. This zone should be taken care of and considered with great importance in the river management planning. This study suggested that the conservation of hyporheic habitat should be emphasized in the freshwater resource management at local and global scale with priority.

This study clearly suggested that the value of hyporheic diversity contribution should be recognized at smaller reach scales (Chapter 2). Within the studied segments, the Satsunai River accounted for the maximum diversity contribution and appeared to the most important among the rivers. The habitat size in the surface and sub-surface zone depends on the amount and type of sediment depositions (Wondzell and Gooseff, 2013). It constructs the spaces within interstices where the macroinvertebrates could be distributed (Wondzell and Gooseff, 2013). The higher amount of deposited sediments and sedimentary characteristics (e.g., cobble) could increase the habitat size in the Satsunai river. The rich hyporheic zone with well mixed hydrological connectivity supported the higher diversity contribution from the hyporheic habitat in the river segments (Negishi et al., 2019b). So, Satsunai river segments should be protected with great care by implementing the following steps. First, the knowledge of macroinvertebrate diversity, distributions, and their contribution to the ecosystem structure and functions should be incorporated into a database as future research directives. Second, the influence of environmental stressors on the macroinvertebrate structure and functions in the hyporheic habitat. Third, assessing whether ongoing river management activities such as, channelization and river regulation by dam etc. could influence the community structure and function. Fourth, setting up the priority of where conservation strategy should be employed. Fifth, based on the magnitude of the stressors in hyporheic habitat, the activities that are responsible for generating stressors need to be limited. Sixth, the hyporheic zone of the Satsunai River could be included in the regular river monitoring activities. The hyporheic habitat of the Satsunai River should be included as an integral part of integrated water resource management so that the hyporheic habitat and the stressors acting in the zone can be monitored regularly, like the benthic zone and riparian zone. Thus, a sustainable and solid management of integrated water resources by conserving the river's environmental health would be possible.

5.7 Limitations of the study

Despite the novelty and importance of the findings, there were some limitations that could be addressed in future studies. In Chapter 2, the study was conducted in the summer and autumn seasons, where the temporal replicates were missing. The temporal replicates could provide information on the community dynamics because some taxa in the hyporheic habitat might inhabit the hyporheic zone? temporally in other seasons. Different diversity structure pattern may appear in different seasonal contexts.

The second part of the study was limited to the hyporheic zone without considering the benthic zone. It tested the effects of multiple stressors on the hyporheic macroinvertebrates by artificial addition of fine sediment under different level of nutrients (Chapter 3). Future research might include the benthic community that would allow comparing background benthic and hyporheic macroinvertebrate communities. The functional responses of the hyporheic zone were one of the unique findings in the current study (Chapter 4; Alam et al., 2021). This study was able to detect the important role of detritivore macroinvertebrates in driving the leaf litter decomposition in the hyporheic zone. The future study might include microbial decomposition in the hyporheic habitat. It could add a comprehensive understanding of the role of microbial community and their responses to leaf litter decomposition in the hyporheic zone. Although the findings would not be changed from what was found in the present study because leaf litter traps included microbial decomposition with macroinvertebrates in this study, more knowledge on mechanistic processes can be understood. Overall, future studies in the multiple river systems with various comprehensive ranges of stressors gradients and more spatial and temporal replicates would help in developing more generalized predictive models of hyporheic structure and functions across multiple environmental gradients.

Acknowledgments

Pursuing this doctoral program has been really a life-changing experience for me and would not have been possible without the great help, support, and guidance that I received from many people.

First, I would like to give my heartiest gratitude to my supervisor Dr. Junjiro N. Negishi for granting me as his student and for his support, guidance, help, encouragement, patience, and kindness. I am also thankful for all the opportunities you have given me during this journey that increased my knowledge and experience. Your kind suggestions helped me a lot to develop my research all the way. Thank you, Sensei, for supporting me during all the hard field work and the continuous guidance for educating me in various ways. Without your uninterrupted support and constant feedback this PhD would not have been achievable.

I really appreciate all the faculty members of Hokkaido University who contributed and helped me during my research. I thank Dr. Kazuhiro Toyoda to allow me to use the laboratory facilities. I would like to say a very special thanks to my wife who has been with me every single minute during this entire period as my support, encouragement, and power and without whom it would not have been possible. Heartfelt thanks to my parents for those I am here today and thank you for your infinite support from my early days till now. I thank my siblings, friends, and all well-wishers for their honest prayer for me.

I am grateful to all my lab mates and friends. I want to mention Mr. ATM Mirza Tanvir Rahman and BM Refat Faisal especially. I am indebted for their supports physically and mentally in the whole tenure of my staying in Japan. I am also thankful to other lab members- Ms. Gao Yiyang, Ms. Janine Rodulfo Tolod, Mr. Pongpet Pongsivapai, Mr. Shohei Yamashita, Mr. Tomohiro Nakagawa, Mr. Hokuto Izumi, Ms. Wu Junyi, Mr. Mo Zhenwei, and others for their tremendous support during numerous field tours. I also want to mention Uno Hiromi for her advice and encouragements. I am thankful to the Obihiro Regional Office of Hokkaido Development Bureau, MLIT for their field assistance.

References

Adner, R., 2017. Ecosystem as structure: An actionable construct for strategy. J. manag. 43, 39-58.

Akatsuka, K., Komai, T., 1922. Pseudocrangonyx, a new genus of subterranean amphipods from Japan. Annot. Zool. Jpn. 10, 119–126.

Alam, M.K. Negishi, J.N., Pongsivapai, P., Yamashita, S., Nakagawa, T.,2021. Additive effects of sediment and nutrient on leaf litter decomposition and macroinvertebrates in hyporheic zone. Water 13, 1340.

Alam, M.K., Negishi, J.N., Rahman, M.A.T.M.T., Tolod, J.R., 2020. Stable isotope ratios of emergent adult aquatic insects can be used as indicators of water pollution in the hyporheic food web. Ecol. Indic. 118, 106738.

Allan, J. D., Erickson, D. L., Fay, J., 1997. The influence of catchment land use on stream integrity across multiple spatial scales. Freshw. Biol. 37, 149–161.

Allan, J.D. and Johnson, L., 1997. Catchment-scale analysis of aquatic ecosystems. Freshw. Biol. 37, 107-111.

Allan, J.D., 2004a. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annu. Rev. Ecol. Evol. Syst. 35, 257-284.

Altermatt, F., Little, C. J., Mächler, E., Wang, S., Zhang, X., Blackman, R. C., 2020. Uncovering the complete biodiversity structure in spatial networks: the example of riverine systems. Oikos 129, 607-618.

Anderson, M.J., Crist, T.O., Chase, J.M., Vellend, M., Inouye, B.D., Freestone, A.L., Sanders, N.J., Cornell, H. V., Comita, L.S., Davies, K.F., Harrison, S.P., Kraft, N.J.B., Stegen, J.C., Swenson, N.G., 2011. Navigating the multiple meanings of β diversity: A roadmap for the practicing ecologist. Ecol. Lett. 14, 19–28.

Araújo, C.B., Gomes, A.S., Boudebs, G., 2016. Techniques for nonlinear optical characterization of materials: a review. Rep. Prog. Phys. 79, 036401.

Araújo, T.Q., Checon, H.H., Garraffoni, A.R.S., 2017. Alpha and beta diversity of freshwater meiofauna at different spatial scales in a Neotropical lotic system. Mar. Freshw. Res. 68, 538–548.

Aristi, I., von Schiller, D., Arroita, M., Barceló, D., Ponsatí, L., García-Galán, M.J., 2015. Mixed effects of effluents from a wastewater treatment plant on river ecosystem metabolism: Subsidy or stress? Freshw. Biol. 60, 1398-1410

Atashgahi, S., Aydin, R., Dimitrov, M.R., Sipkema, D., Hamonts, K., Lahti, L., Maphosa, F., Kruse, T., Saccenti, E., Springael, D. et al., 2015. Impact of a wastewater treatment plant on microbial community composition and function in a hyporheic zone of a eutrophic river. Sci. Rep. 5, 17284.

Ban, S.S., Pressey, R.L., Graham, N.A.J., 2014. Assessing interactions of multiple stressors when data are limited: A Bayesian belief network applied to coral reefs. Glob Environ. Change. 27, 64-72.

Bärlocher, F., Seena, S., Wilson, K.P., Williams, D., 2008. Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes. Freshw. Biol. 53, 368–379.

Baselga, A., 2010. Partitioning the turnover and nestedness components of beta diversity. Glob. Ecol. Biogeogr. 19, 134–143.

Baselga, A., Leprieur, F., 2015. Comparing methods to separate components of beta diversity. Methods Ecol. Evol. 6, 1069–1079.

Baselga, A., Orme, C.D.L., 2012. Betapart: An R package for the study of beta diversity. Methods Ecol. Evol. 3, 808–812.

Battin, T.J., Luyssaert, S., Kaplan, L.A., Aufdenkampe, A.K., Richter, A., Tranvik, L.J., 2009. The boundless carbon cycle. Nat. Geosci. 2, 598–600.

Beermann, A.J., Elbrecht, V., Karnatz, S., Ma, L., Matthaei, C.D., Piggott, J.J., Leese, F., 2018. Multiple-stressor effects on stream macroinvertebrate communities: A mesocosm experiment manipulating salinity, fine sediment and flow velocity. Sci. Total Environ. 610-611, 961-971.

Beisel, J.-N., Usseglio-Polatera, P., Thomas, S., Moreteau, J.-C., 1998. Stream community structure in relation to spatial variation: the influence of mesohabitat characteristics, Hydrobiologia 389, 73-88.

Birgand, F., Skaggs, R.W., Chescheir, G.M., Gilliam, J.W., 2007. Nitrogen removal in streams of agricultural catchments—a literature review. Crit. Rev. Environ. Sci. Technol. 37, 381-487.

Blott, S.J., Pye, K., 2001. Gradistat: A grain size distribution and statistics package for the analysis of unconsolidated sediments. Earth Surf. Process. Landforms 26, 1237–1248.

Bo, T., Cucco, M., Fenoglio, S., Malacarne, G., 2006. Colonisation patterns and vertical movements of stream invertebrates in the interstitial zone: a case study in the Apennines, NW Italy. Hydrobiologia, 568, 67-78.

Bo, T., Doretto, A., Levrino, M., Fenoglio, S., 2020. Contribution of beta diversity in shaping stream macroinvertebrate communities among hydro-ecoregions. Aquat. Ecol. 54, 957–971.

Bo, T., Piano, E., Doretto, A., Bona, F., Fenoglio, S., 2016. Microhabitat preference of sympatric Hydraena Kugelann, 1794 species (Coleoptera: Hydraenidae) in a low-order forest stream. Aquat. Insects 37, 287–292.

Bonada, N., Rieradevall, M., Dallas, H., Davis, J., Day, J., Figueroa, R., Resh, V. H., Prat, N., 2008. Multi-scale assessment of macroinvertebrate richness and composition in Mediterranean-climate rivers. Freshw. Biol. 53, 772–788.

Boulton, A.J., 2007. Hyporheic rehabilitation in rivers: restoring vertical connectivity. Freshw. Biol. 52, 632-650.

Boulton, A.J., Boon, P.I., 1991. A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf?. Mar. Freshw. Res. 42, 1-43.

Boulton, A.J., Datry, T., Kasahara, T., Mutz, M., Stanford, J.A., 2010. Ecology and management of the hyporheic zone: stream–groundwater interactions of running waters and their floodplains. J. N. Am. Benthol. Soc. 29, 26-40.

Boulton, A.J., Findlay, S., Marmonier, P., Stanley, E.H. Valett, H.M., 1998. The functional significance of the hyporheic zone in streams and rivers. Annu. Rev. Ecol. Evol. Syst. 29, 59-81.

Buendia, C., Gibbins, C.N., Vericat, D., Batalla, R.J., Douglas, A., 2013. Detecting the structural and functional impacts of fine sediment on stream invertebrates. Ecol. Indic. 25, 184-196.

Burrows, R.M., Rutlidge, H., Bond, N.R., Eberhard, S.M., Auhl, A., Andersen, M.S., Valdez, D.G., Kennard, M.J., 2017. High rates of organic carbon processing in the hyporheic zone of intermittent streams. Sci. Rep. 7, 13198.

Buss, D.F., Baptista, D.F., Nessimian, J.L., Egler, M., 2004. Substrate specificity, environmental degradation and disturbance structuring macroinvertebrate assemblages in neotropical streams. Hydrobiologia 518, 179–188.

Cardinale, B.J., Gross, K., Fritschie, K., Flombaum, P., Fox, J.W., Rixen, C., Van Ruijven, J., Reich, P.B., Scherer-Lorenzen, M., Wilsey, B.J., 2013. Biodiversity simultaneously enhances the production and stability of community biomass, but the effects are independent. Ecology 94, 1697-1707.

Carey R.O., Migliaccio K.W., 2009. Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: a review. Environ. Manage. 44, 205-217.

Castro-Català, N., Dolédec, S., Kalogianni, E., Skoulikidis, N.T., Paunovic, M., Vasiljević, B., Sabater, S., Tornés, E., Muñoz, I., 2020. Unravelling the effects of multiple stressors on diatom and macroinvertebrate communities in European river basins using structural and functional approaches. Sci. Total Environ. *742*, 140543.

Chase, J.M., Knight, T.M., 2013. Scale-dependent effect sizes of ecological drivers on biodiversity: Why standardised sampling is not enough. Ecol. Lett. 16, 17–26.

Chave, J., 2013. The problem of pattern and scale in ecology: What have we learned in 20 years? Ecol. Lett. 16, 4–16.

Collier, K.J., Clements, B.L., 2011. Influences of catchment and corridor imperviousness on urban stream macroinvertebrate communities at multiple spatial scales. Hydrobiologia, 664, 35-50.

Collins, A.L., Newell Price, J.P., Zhang, Y., Gooday, R., Naden, P.S., Skirvin, D., 2018. Assessing the potential impacts of a revised set of on-farm nutrient and sediment 'basic' control measures for reducing agricultural diffuse pollution across England. Sci. Total Environ. 621, 1499–1511.

Colwell, R. K., Mao, C. X., Chang, J., 2004. Interpolating, extrapolating, and comparing incidence-based species accumulation curves. Ecology, 85, 2717-2727.

Connolly, N.M., Pearson, R.G., 2007. The effect of fine sedimentation on tropical stream macroinvertebrate assemblages: a comparison using flow-through artificial stream channels and recirculating mesocosms. Hydrobiologia, 592, 423-438.

Conroy, E., Turner, J.N., Rymszewicz, A., Bruen, M., O'Sullivan, J.J., Lawler, D.M., Stafford, S., Kelly-Quinn, M., 2018. Further insights into the responses of macroinvertebrate species to burial by sediment. Hydrobiol. 805, 399–411.

Cook, S.C., Housley, L., Back, J.A., King, R.S., 2018. Freshwater eutrophication drives sharp reductions in temporal beta diversity. Ecol. 99, 47–56.

Cormier, S.M., Paul, J.F., Spehar, R.L., Shaw-Allen, P., Berry, W.J., Suter, G.W., 2008. Using field data and weight of evidence to develop water quality criteria. Integr. Environ. Assess. Manag. 4, 490-504.

Cornejo, A., Tonin, A. M., Checa, B., Tuñon, A. R., Pérez, D., Coronado, E., González, S., Ríos, T., Macchi, P., Correa-Araneda, F., Boyero, L., 2019. Effects of multiple stressors associated with agriculture on stream macroinvertebrate communities in a tropical catchment. PLoS ONE 14, e0220528.

Cornut, J., Clivot, H., Chauvet, E., Elger, A., Pagnout, C., Guérold, F., 2012. Effect of acidification on leaf litter decomposition in benthic and hyporheic zones of woodland streams. Wat. Res, 46, 6430-6444.

Cornut, J., Elger, A., Lambrigot, D., Marmonier, P., Chauvet, E., 2010. Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams. Freshw. Biol. 55, 2541–2556.

Corson-Rikert, H.A., Wondzell, S.M., Haggerty, R., Santelmann, M.V., 2016. Carbon dynamics in the hyporheic zone of a headwater mountain stream in the Cascade Mountains, Oregon. Water Resour. Res. 52, 7556–7576.

Costa, S.S., Melo, A.S., 2008. Beta diversity in stream macroinvertebrate assemblages: among-site and among-microhabitat components. Hydrobiologia 598, 131–138.

Côté, I.M., Darling, E.S., Brown, C.J., 2016. Interactions among ecosystem stressors and their importance in conservation. Proc. R. Soc. B. 283, 20152592.

Covich, A. P., Palmer, M. A., Crowl, T. A., 1999. The role of benthic invertebrate species in freshwater ecosystems: zoobenthic species influence energy flows and nutrient cycling Small invertebrates are functionally important in many terrestrial and aquatic ecosystems. BioScience, 49, 119-127.

Cox, T. J., Rutherford, J. C., 2000. Thermal tolerances of two stream invertebrates exposed to diurnally varying temperature. N. Z. J. Mar. Freshw. Res. 34, 203–208.

Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecol. Lett. 11, 1304-1315.

Crist, T.O., Veech, J.A., Gering, J.C., Summerville, K.S., 2003. Partitioning species diversity across landscapes and regions: A hierarchical analysis of α, β, and γ diversity. Am. Nat. 162, 734–743.

Cummins, K.W., 1974. Structure and Function of Stream Ecosystems. BioScience, 24, 631-641.

Dangles, O., Gessner, M. O., Guérold, F., Chauvet, E., 2004. Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. J. Appl. Ecol. 41, 365-378.

Darling, E.S., Côté, I.M., 2008. Quantifying the evidence for ecological synergies. Ecol. Lett. 11, 1278-1286.

Datry, T., 2012. Benthic and hyporheic invertebrate assemblages along a flow intermittence gradient: effects of duration of dry events. Freshw. Biol. 57, 563–574.

Davis, S.J., Mellander, P.E., Kelly, A.M., Matthaei, C.D., Piggott, J.J., Kelly-Quinn, M., 2018. Multiple-stressor effects of sediment, phosphorus and nitrogen on stream macroinvertebrate communities. *Sci. Total Environ. 637*, 577-587.

Descloux, S., Datry, T., Marmonier, P., 2013. Benthic and hyporheic invertebrate assemblages along a gradient of increasing streambed colmation by fine sediment. Aquat. Sci. 75, 493-507.

DeWalt, R.E., Stewart, K.W., 1995. Life histories of stoneflies (Plecoptera) in the Rio Conejos of southern Colorado. Great Basin Nat. 55, 1-18.

Diekötter, T., Billeter, R., Crist, T. O., 2008. Effects of landscape connectivity on the spatial distribution of insect diversity in agricultural mosaic landscapes. Basic Appl. Ecol. 9, 298–307.

Diekötter, T., Crist, T.O., 2013. Quantifying habitat-specific contributions to insect diversity in agricultural mosaic landscapes. Insect. Conserv. Divers. 6, 607–618.

Dole-Olivier, M.J., Marmonier, P., Beffy, J.L., 1997. Response of invertebrates to lotic disturbance: is the hyporheic zone a patchy refugium?. Freshw. Biol. 37, 257-276.

Doretto, A., Bona, F., Falasco, E., Piano, E., Tizzani, P., Fenoglio, S., 2016. Fine sedimentation affects CPOM availability and shredder abundance in Alpine streams. J. Freshw. Ecol. 31, 299-302.

Doretto, A., Bona, F., Piano, E., Zanin, I., Eandi, A.C., Fenoglio, S., 2017. Trophic availability buffers the detrimental effects of clogging in an alpine stream. Sci. Total Environ. 592, 503–511.

Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J.R.G., Gruber, B., Lafourcade, B., Leitão, P.J., Münkemüller, T., Mcclean, C., Osborne, P.E., Reineking, B., Schröder, B., Skidmore, A.K., Zurell, D., Lautenbach, S., 2013. Collinearity: A review of methods to deal with it and a simulation study evaluating their performance. Ecography 36, 27–46.

Doucett, R.R., Marks, J.C., Blinn, D.W., Caron, M., Hungate, B.A., 2007. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. Ecol. 88, 1587–1592.

Duan, N., Song, J., Mao, H., Sun, H., Huang, P., 2021. Environmental context mediates the interaction between fine sediment and hyporheic macroinvertebrate. Ecohydrology 15, e2392.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81, 163-182.

Dunscombe, M., Robertson, A., Peralta-Maraver, I., Shaw, P., 2018. Community structure and functioning below the streambed across contrasting geologies. Sci. Total Environ. 630, 1028–1035.

Durkota, J.M., Wood, P.J., Johns, T., Thompson, J.R., Flower, R.J., 2019. Distribution of macroinvertebrate communities across surface and groundwater habitats in response to hydrological variability. Fundam. Appl. Limnol. 193, 79-92.

Evans, E.C., Petts, G.E., 1997. Comportement des températures hyporhéiques dans des rapides. Hydrol. Sci. J. 42, 199–213.

Feld, C.K., Birk, S., Eme, D., Gerisch, M., Hering, D., Kernan, M., Maileht, K., Mischke, U., Ott, I., Pletterbauer, F., Poikane, S., 2016. Disentangling the effects of land use and geo-climatic factors on diversity in European freshwater ecosystems. Ecol. Indic. 60, 71-83.

Fenoglio, S., Bo, T., Agosta, P., Malacarne, G., 2005. Temporal and spatial patterns of coarse particulate organic matter and macroinvertebrate distribution in a low-order apennine stream. J. Freshw. Ecol. 20, 539–547.

Feris, K.P., Ramsey, P.W., Gibbons, S.M., Frazer, C., Rillig, M.C., Moore, J.N., Gannon, J.E., Holben, W.E., 2009. Hyporheic microbial community development is a sensitive indicator of metal contamination. Environ. Sci. Technol. 43, 6158–6163.

Fierro, P., Bertrán, C., Tapia, J., Hauenstein, E., Peña-Cortés, F., Vergara, C., Cerna, C., Vargas-Chacoff, L., 2017. Effects of local land-use on riparian vegetation, water quality, and the functional organization of macroinvertebrate assemblages. Sci. Total Environ. 609, 724–734.

Folt, C.L., Chen, C.Y., Moore, M.V., Burnaford, J., 1999. Synergism and antagonism among multiple stressors. Limnol.oceanogr. 44, 864-877.

Fowler, R.T., Death, R.G., 2001. The effect of environmental stability on hyporheic community structure, Hydrobiologia 445, 85-95.

Fowler, R.T., Scarsbrook, M.R., 2002. Influence of hydrologic exchange patterns on water chemistry and hyporheic invertebrate communities in three gravel-bed rivers. *N. Z. J. Mar. Freshw. Res. 36*, 471-482.

Fraser, B.G., Williams, D.D., 1998. Seasonal boundary dynamics of a groundwater/surface-water ecotone. Ecology 79, 2019-2031.

García-Roger, E.M., Sánchez-Montoya, M.D.M., Cid, N., Erba, S., Karaouzas, I., Verkaik, I., Rieradevall, M., Gómez, R., Suárez, M.L. *et al*., 2013. Spatial scale effects on taxonomic and biological trait diversity of aquatic macroinvertebrates in Mediterranean streams. Fundam. Appl. Limnol. 183. 89-105.

Garzon-Lopez, C.X., Jansen, P.A., Bohlman, S.A., Ordonez, A., Olff, H., 2014. Effects of sampling scale on patterns of habitat association in tropical trees. *J. Veg. Sci. 25*, 349-362.

Gatti, R.C., 2016. Freshwater biodiversity: a review of local and global threats. Int. J. Environ. Stud, 73, 887–904.

Gering, J.C., Crist, T.O., Veech, J.A., 2003. Additive Partitioning of Species Diversity across Multiple Spatial Scales: Implications for Regional Conservation of Biodiversity. Conserv. Biol. 17, 488-499.

Gessner, M. O., Chauvet, E., 2002. A case for using litter breakdown to assess functional stream integrity. Ecol. Appl. 12, 498–510.

Gessner, M.O., Chauvet, E., Dobson, M. A., 1999. perspective on leaf litter breakdown in streams. Oikos85, 377–384.

Gonzales-Inca, C., Valkama, P., Lill, J.O., Slotte, J., Hietaharju, E., Uusitalo, R., 2018. Spatial modeling of sediment transfer and identification of sediment sources during snowmelt in an agricultural watershed in boreal climate. Sci. Total Environ. 612, 303-312.

Graeber, D., Jensen, T. M., Rasmussen, J. J., Riis, T., Wiberg-Larsen, P., Baattrup-Pedersen, A., 2017. Multiple stress response of lowland stream benthic macroinvertebrates depends on habitat type. Sci. Total Environ. 599–600, 1517–1523.

Graham E.B., Knelman J.E., Schindlbacher A., Siciliano S., Breulmann M., Yannarell A., Beman J.M., Abell G., Philippot L., Prosser J., Foulquier A., Yuste J.C., Glanville H.C., Jones D.L., Angel R., Salminen J., Newton R.J., Bürgmann H., Ingram L.J., Hamer U., Siljanen H.M.P., Peltoniemi K., Potthast K., Bañeras L., Hartmann M., Banerjee S., Yu R-Q, Nogaro G., Richter A., Koranda M., Castle S.C., Goberna M., Song B., Chatterjee A., Nunes O.C., Lopes A.R., Cao Y., Kaisermann A., Hallin S., Strickland M.S., Garcia-Pausas J., Barba J., Kang H., Isobe K., Papaspyrou S., Pastorelli R., Lagomarsino A., Lindström E.S., Basiliko N., Nemergut D.R., 2016. Microbes as Engines of Ecosystem Function: When Does Community Structure Enhance Predictions of Ecosystem Processes? Front. Microbiol. 7, 214.

Graham, M.H., 2003. Confronting multicollinearity in ecological multiple regression. Ecology 84, 2809-2815.

Grant, E.H.C., Lowe, W.H., Fagan, W.F., 2007. Living in the branches: population dynamics and ecological processes in dendritic networks. Ecol. Lett. 10, 165-175.

Grattan, R.M., Suberkropp, K., 2001. Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. J. N. Am. Benthol. Soc. 20, 33–43.

Gray, D., Harding, J. S., 2009. Braided river benthic diversity at multiple spatial scales: a hierarchical analysis of β diversity in complex floodplain systems. J. North Am. Benthol. Soc. 28, 537-551.

Gray, D., Scarsbrook, M. R., Harding, J. S., 2006. Spatial biodiversity patterns in a large New Zealand braided river. N. Z. J. Mar. Freshwater Res. 40, 631-642.

Guénard, G., Lanthier, G., Harvey-Lavoie, S., Macnaughton, C. J., Senay, C., Lapointe, M., Legendre, P., Boisclair, D., 2016. A spatially-explicit assessment of the fish population response to flow management in a heterogeneous landscape. Ecosphere 7, e01252.

Gulis, V., Ferreira, V., Graca, M.A.S., 2006. Stimulation of leaf litter decomposition and associated fungi and invertebrates by moderate eutrophication: implications for stream assessment. Freshw. Biol. 51, 1655–1669.

Gulis, V., Suberkropp, K., 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshw. Biol. 48, 123–134.

Guzmán, D., Alcocer, J., 2022. Turnover Drives High Benthic Macroinvertebrates' Beta Diversity in a Tropical Karstic Lake District. Diversity 14, 259.

Hakenkamp, C.C., Morin, A., 2000. The importance of meiofauna to lotic ecosystem functioning. Freshw. Biol. 44, 165-175.

Harvey, J.W., Drummond, J.D., Martin, R.L., McPhillips, L.E., Packman, A.I., Jerolmack, D.J., Stonedahl, S.H., Aubeneau, A.F., Sawyer, A.H., Larsen, L.G. et al., 2012. Hydrogeomorphology of the hyporheic zone: Stream solute and fine particle interactions with a dynamic streambed. J. Geophys. Res. 117, G00N11.

Hauer, F. R., Resh, V. H., 2017. Macroinvertebrates. In Methods in Stream Ecology: Third Edition 1, 297–319. Elsevier Inc.

Hayden, B., McWilliam-Hughes, S.M., Cunjak, R.A., 2016. Evidence for limited trophic transfer of allochthonous energy in temperate river food webs. Freshw. Sci. 35, 544–558.

Hering, D., Carvalho, L., Argillier, C., Beklioglu, M., Borja, A., Cardoso, A.C., Duel, H., Ferreira, T., Globevnik, L., Hanganu, J., Hellsten, S. *et al*., 2015. Managing aquatic ecosystems and water resources under multiple stress—An introduction to the MARS project. Sci. Total Environ. 503-504, 10-21.

Hernández-Hernández, R., Borges, P.A., Gabriel, R., Rigal, F., Ah-Peng, C. and González-Mancebo, J.M., 2017. Scaling α-and β-diversity: bryophytes along an elevational gradient on a subtropical oceanic Island (La Palma, Canary Islands). *J. Veg. Sci. 28,* 1209-1219.

Huettel, A., Ziebis, W., Forster, S., 1996. Flow-induced uptake of particulate matter in permeable sediments, Limnol. Oceanogr. 41, 309-322.

Huryn, A. D., Wallace, J. B., 2000. Life history and production of stream insects. Annual Rev. Entom. 45, 83–110.

Hutchins, B.T., Swink, A.P., Diaz, P.H., Schwartz, B.F., 2020. Environmental influences on invertebrate diversity and community composition in the hyporheic zone ecotone in Texas, USA: contrasts between co-occurring epigean taxa and stygobionts. Hydrobiologia 847, 3967–3982.

In˜iguez-Armijos, C., Hampel, C., Breuer, L., 2017. Land-use effects on structural and functional composition of benthic and leaf-associated macroinvertebrates in four Andean streams. Aquat. Ecol. 52, 77–92.

Isaak, D. J., Wollrab, S., Horan, D., Chandler, G., 2012. Climate change effects on stream and river temperatures across the northwest U.S. from 1980-2009 and implications for salmonid fishes. Clim. Change 113, 499–524.

Jackson, M.C., Woodford, D.J., Weyl, O.L., 2016. Linking key environmental stressors with the delivery of provisioning ecosystem services in the freshwaters of southern Africa. Geo: Geography and Environment, 3, e00026.

Jarvie, H.P., Withers, P.J.A., Bowes, M.J., Palmer-Felgate, E.J., Harper, D.M., Wasiak, K., Wasiak, P., Hodgkinson, R.A., Bates, A., Stoate, C. *et al* 2010. Streamwater phosphorus and nitrogen across a gradient in rural–agricultural land use intensity. Agric. Ecosyst. Environ. 135, 238-252.

Jones, I., Growns, I., Arnold, A., McCall, S., Bowes, M., 2015. The effects of increased flow and fine sediment on hyporheic invertebrates and nutrients in stream mesocosms. Freshw. Biol. 60, 813-826.

Jones, J.I., Murphy, J.F., Collins, A.L., Sear, D.A., Naden, P.S., Armitage, P.D., 2012. The impact of fine sediment on macro-invertebrates. River Res. Appl. 28, 1055–1071.

Jooste, S., 2000. A model to estimate the total ecological risk in the management of water resources subject to multiple stressors. Water SA. 26, 159-166.

Kanda, F., 1996. Survival curves and longevity of the leaves of Alnus japonica var. arguta in Kushiro Marsh. Vegetatio, 124, 61-66.

Kawai, S., Tanida, K., 2018. Aquatic Insects of Japan: Manual with Keys and Illustrations, 2nd ed.; Tokai University Press: Kanagawa, Japan; p. 1730, (In Japanese). ISBN 978-448-601-774-5.

Korbel, K.L., Stephenson, S. and Hose, G.C., 2019. Sediment size influences habitat selection and use by groundwater macrofauna and meiofauna. Aquat. Sci. 81, 39.

Lamouroux, N., Dolédec, S., Gayraud, S., 2004. Biological traits of stream macroinvertebrate communities: Effects of microhabitat, reach, and basin filters. J. N. Am. Benthol. Soc. 23, 449–466.

Lande, R., 1996. Statistics and partitioning of species diversity, and similarity among multiple communities. Oikos 76, 5-13.

Larsen, S., Ormerod, S.J., 2014. Anthropogenic modification disrupts species co-occurrence in stream invertebrates. Glob. Chang. Biol. 20, 51-60.

Larsen, S., Vaughan, I. P., Ormerod, S. J., 2009. Scale-dependent effects of fine sediments on temperate headwater invertebrates. Freshw. Biol. 54, 203–219.

LeFebvre, S., Marmonier, P., Pinay, G., 2004. Stream regulation and nitrogen dynamics in sediment interstices: comparison of natural and straightened sectors of a third-order stream. River Res. Applic. 20, 499-512.

Legendre, P., 2014. Interpreting the replacement and richness difference components of beta diversity. Glob. Ecol. Biogeogr. 23, 1324–1334.

Legendre, P., Borcard, D., Peres-Neto, P.R., 2005. Analyzing beta diversity: partitioning the spatial variation of community composition data. Ecol. Monogr. 75, 435-450.

Lencioni, V., Spitale, D., 2015. Diversity and distribution of benthic and hyporheic fauna in different stream types on an alpine glacial floodplain. Hydrobiologia 751, 73-87.

Levin, S.A., 1992. The problem of pattern and scale in ecology: the Robert H. MacArthur award lecture. Ecology, 73, 1943-1967.

Ligeiro, R., Melo, A.S., Callisto, M., 2010. Spatial scale and the diversity of macroinvertebrates in a Neotropical catchment. Freshw. Biol. 55, 424–435.

Lindeman, R.L., 1942. The trophic-dynamic aspect of ecology. Ecology 23, 399-417.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D., 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. Science, 294, 804-808.

Lowell, J.L., Gordon, N., Engstrom, D., Stanford, J.A., Holben, W.E., Gannon, J.E., 2009. Habitat heterogeneity and associated microbial community structure in a small-scale floodplain hyporheic flow path. Microb. Ecol. 58, 611–620.

Malmqvist, B., 2002. Aquatic invertebrates in riverine landscapes. Freshw. Biol. 47, 679–694.

Marczak, L.B., Richardson, J.S., 2007. Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. J. Anim. Ecol. 76, 687-694.

Marmonier, P., Creuzé des Châtelliers, M., Dole-Olivier, M.J., Radakovitch, O., Mayer, A., Chapuis, H., Graillot, D., Re-Bahuaud, J., Johannet, A., Cadilhac, L., 2020. Are surface water characteristics efficient to locate hyporheic biodiversity hotspots? Sci. Total Environ. 738. 139930.

Mathers, K.L., Hill, M.J., Wood, P.J., 2017. Benthic and hyporheic macroinvertebrate distribution within the heads and tails of riffles during baseflow conditions. Hydrobiologia 794, 17–30.

Mathers, K.L., Kowarik, C., Rachelly, C., Robinson, C.T., Weber, C., 2021. The effects of sediment traps on instream habitat and macroinvertebrates of mountain streams. J. Environ. Manag. 295, 113066.

Mathers, K.L., Millett, J., Robertson, A.L., Stubbington, R., Wood, P.J., 2014. Faunal response to benthic and hyporheic sedimentation varies with direction of vertical hydrological exchange. Freshwat. Biol. 59, 2278-2289.

Matsuda, J.T., Martens, K., Higuti, J., 2015. Diversity of ostracod communities (Crustacea, Ostracoda) across hierarchical spatial levels in a tropical floodplain. Hydrobiologia 762, 113–126.

Matthaei, C.D., Piggott, J.J., Townsend, C.R., 2010. Multiple stressors in agricultural streams: interactions among sediment addition, nutrient enrichment and water abstraction. J. Appl. Ecol. 47, 639-649.

Matthaei, C.D., Weller, F., Kelly, D.W., Townsend, C.R., 2006. Impacts of fine sediment addition to tussock, pasture, dairy and deer farming streams in New Zealand. Freshw. Biol. 51, 2154-2172.

Menéndez, M., Descals, E., Riera, T., Moya, O., 2011. Leaf litter breakdown in Mediterranean streams: Effect of dissolved inorganic nutrients. Hydrobiol. 669, 143–155.

Merritt, R.W., Cummins, K.W., Berg, M.B., 2019. An Introduction to the Aquatic Insects of North America, 5th ed.; Kendal Hunt Publishing Company: Dubuque, IA, USA; p. 1498. ISBN 978-152-496-854-0.

Metzler, G.M., Smock, L.A., 1990. Storage and dynamics of subsurface detritus in a sand-bottomed stream. Can. J. Fisheries Aquat. Sci. 47, 588–594.

Minshall, G.W., 1988. Stream ecosystem theory: a global perspective. J. N. Am. Benthol. Soc. 7, 263-288.

Miyazawa, E., Montilla, L.M., Agudo-Adriani, E.A., Ascanio, A., Mariño-Briceño, G., Croquer, A., 2020. On the importance of spatial scales on beta diversity of coral assemblages: A study from Venezuelan coral reefs. PeerJ 8, p.e9082.

Moreno, C. E., Halffter, G., 2001. On the measure of sampling effort used in species accumulation curves. J. Appl. Ecol. 487-490.

Mori, N., Brancelj, A., 2011. Spatial and temporal variability of hyporheic invertebrate community within a stream reach of the River Bača (W Slovenia). Nat. Slov. 13, 25-38.

Mori, N., Debeljak, B., Škerjanec, M., Simčič, T., Kanduč, T., Brancelj, A., 2019. Modelling the effects of multiple stressors on respiration and microbial biomass in the hyporheic zone using decision trees. Water Res. 149, e20.

Morrissey, C.A., Boldt, A., Mapstone, A., Newton, J., Ormerod, S.J., 2013. Stable isotopes as indicators of wastewater effects on the macroinvertebrates of urban rivers. Hydrobiologia 700, 231-244.

Mykrä, H., Heino, J., Muotka, T., 2007. Scale-related patterns in the spatial and environmental components of stream macroinvertebrate assemblage variation. Global Ecol. Biogeogr. 16, 149–159.

Nakamura, M., Muller, O., Tayanagi, S., Nakaji, T., Hiura, T., 2010. Experimental branch warming alters tall tree leaf phenology and acorn production. Agric. For. Meteor. 150, 1026-1029.

Navel, S., Mermillod-Blondin, F., Montuelle, B., Chauvet, E., Simon, L., Piscart, C., Marmonier, P., 2010. Interactions between fauna and sediment control the breakdown of plant matter in river sediments. Freshw. Biol. 55, 753–766.

Negishi, J.N., Alam, M.K., Rahman, M.A.T.M.T., Kawanishi, R., Uno, H., Yoshinari, G., Tojo, K., 2022. Three years in the dark: life history and trophic traits of the hyporheic stonefly, *Alloperla ishikariana* Kohno, 1953 (Plecoptera, Chloroperlidae). Hydrobiologia 849, 4203-4219.

Negishi, J.N., Hibino, A., Miura, K., Kawanishi, R., Watanabe, N., Toyoda, K., 2019b. Coupled benthic-hyporheic responses of macroinvertebrates to surface water pollution in a gravel-bed river. Freshw. Sci. 38, 591–604.

Negishi, J.N., Terui, A., Nessa, B., Miura, K., Oiso, T., Sumitomo, K., Kyuka, T., Yonemoto, M., Nakamura, F., 2019a. High resilience of aquatic community to a 100-year flood in a gravel-bed river. Landsc. Ecol. Eng. 15, 143-154.

Nelson, S.M., Roline, R.A., 2003. Effects of multiple stressors on hyporheic invertebrates in a lotic system. Ecol. Indic. 3, 65-79.

Newcombe, C.P., MacDonald, D.D., 1991. Effects of suspended sediments on aquatic ecosystems. North Am. J. Fish. Manage. 11, 72-82.

Nhiwatiwa, T., Dalu, T., Brendonck, L., 2017. Impact of irrigation based sugarcane cultivation on the Chiredzi and Runde Rivers quality, Zimbabwe. Sci. Total Environ. 587, 316-325.

Niyogi, D. K., Harding, J. S., Simon, K. S., 2013. Organic matter breakdown as a measure of stream health in New Zealand streams affected by acid mine drainage. Ecol. Indic. 24, 510–517.

Niyogi, D.K., Koren, M., Arbuckle, C.J., Townsend, C.R., 2007. Stream communities along a catchment land-use gradient: Subsidy-stress responses to pastoral development. Environ. Manage. 39, 213–225.

Nogaro, G., Datry, T., Mermillod-Blondin, F., Descloux, S., Montuelle, B., 2010. Influence of streambed sediment clogging on microbial processes in the hyporheic zone. Freshw. Biol. 55, 1288-1302.

Nogaro, G., Datry, T., Mermillod-Blondin, F., Foulquier, A., Montuelle, B., 2013. Influence of hyporheic zone characteristics on the structure and activity of microbial assemblages. Freshw. Biol. 58, 2567–2583.

Nõges, P., Argillier, C., Borja, Á., Garmendia, J.M., Hanganu, J., Kodeš, V., Pletterbauer, F., Sagouis, A., Birk, S., 2016. Quantified biotic and abiotic responses to multiple stress in freshwater, marine and ground waters. *Sci. Total Environ. 540*, 43-52.

Noy-Meir, I., 1979. Structure and function of desert ecosystems. Isr. J. Bot. 28, 1-19.

Nunes, C.A., Braga, R.F., Figueira, J.E.C., de Siqueira Neves, F., Fernandes, G.W., 2016. Dung beetles along a tropical altitudinal gradient: Environmental filtering on taxonomic and functional diversity. PLoS One 11, e0157442.

Nuttall, P.M., Bielby, G.H., 1973. The effect of china-clay wastes on stream invertebrates. Environ. Pollut. 5, 77-86.

Oksanen, J. Vegan: an introduction to ordination. *R Project*, https://cran.r-project.org/web/packages/vegan/vignettes/intro-vegan.pdf (2018).

Olsen, D.A., Townsend, C.R., 2003. Hyporheic community composition in a gravel-bed stream: influence of vertical hydrological exchange, sediment structure and physicochemistry. *Freshw. Biol. 48*, 1363-1378.

Ormerod, S.J., Dobson, M., Hildrew, A.G., Townsend, C., 2010. Multiple stressors in freshwater ecosystems. Freshw. Biol. 55, 1-4.

Orr, J.A., Vinebrooke, R.D., Jackson, M.C., Kroeker, K.J., Kordas, R.L., Mantyka-Pringle, C., Van den Brink, P.J., De Laender, F., Stoks, R., Holmstrup, M., Matthaei, C.D., 2020. Towards a unified study of multiple stressors: divisions and common goals across research disciplines. *Proc. Royal Soc. B. 287*, 20200421.

Pacioglu, O., Moldovan, O.T., 2016. Response of invertebrates from the hyporheic zone of chalk rivers to eutrophication and land use. Environ. Sci. Pollut. Res. 23, 4729–4740.

Pardo, I., Armitage, P.D., 1997. Species assemblages as descriptors of mesohabitats, Hydrobiologia 344, 111–128.

Piano, E., Doretto, A., Falasco, E., Gruppuso, L., Fenoglio, S., Bona, F., 2019. The role of recurrent dewatering events in shaping ecological niches of scrapers in intermittent Alpine streams. Hydrobiologia 841, 177-189.

Piggott, J.J., Townsend, C.R., Matthaei, C.D., 2015. Climate warming and agricultural stressors interact to determine stream macroinvertebrate community dynamics. Glob. Change Biol. 21, 1887-1906.

Pinay, G., O'Keefe, T.C., Edwards, R.T., Naiman, R.J., 2009. Nitrate removal in the hyporheic zone of a salmon river in Alaska. River Res. Appl. 25, 367–375.

Piscart, C., Navel, S., Maazouzi, C., Montuelle, B., Cornut, J., Mermillod-Blondin, F., Des Châtelliers, M.C., Simon, L., Marmonier, P., 2011. Leaf litter recycling in benthic and hyporheic layers in agricultural streams with different types of land use. Sci. Total Environ. 409, 4373-4380.

Rahbek, C., 2005. The role of spatial scale and the perception of large-scale species-richness patterns. Ecol. Lett. 8, 224–239.

Ren, J., Packman, A.I., 2007. Changes in fine sediment size distributions due to interactions with streambed sediments. Sediment Geol. 202, 529–537.

Richardson, D.M., Whittaker, R.J., 2010. Conservation biogeography - foundations, concepts and challenges. Diversity Distrib. 16, 313–320.

Robson, B.J., Chester, E.T., 1999. Spatial patterns of invertebrate species richness in a river: The relationship between riffles and microhabitats. Austral. J. Ecol. 24, 599–607.

Rosi-Marshall, E.J., Wallace, J.B., 2002. Invertebrate food webs along a stream resource gradient. Freshw. Biol. 47, 129-141.

Rulík, M., Zavřelová, P., Duchoslav, M., 2001. Decomposition of two different POM types in surface water and within hyporheic sediments of a small lowland stream (Sitka, Czech Republic). Int. Rev. Hydrobiol. 86, 487–500.

Sabater, S., Barceló, D., De Castro-Català, N., Ginebreda, A., Kuzmanovic, M., Petrovic, M., Picó, Y., Ponsatí, L., Tornés, E., Muñoz, I., 2016. Shared effects of organic microcontaminants and environmental stressors on biofilms and invertebrates in impaired rivers. Environ. Pollut. 210, 303-314.

Sagnes, P., Mérigoux, S., Péru, N., 2008. Hydraulic habitat use with respect to body size of aquatic insect larvae: Case of six species from a French Mediterranean type stream. Limnologica 38, 23–33.

Sala, O.E., Stuart Chapin, F.I.I.I., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., 2000. Global biodiversity scenarios for the year 2100. Science 287, 1770-1774.

Sánchez-Morales, M., Sabater, F., Muñoz, I., 2018. Effects of urban wastewater on hyporheic habitat and invertebrates in Mediterranean streams. Sci. Tot. Environ. 642, 937-945.

Sandin, L., Johnson, R. K., 2003. Local, landscape and regional factors structuring benthic macroinvertebrate assemblages in Swedish streams. Landsc. Ecol. 19, 501–514.

Sandin, L., Solimini, A.G., 2009. Freshwater ecosystem structure–function relationships: from theory to application. Freshw. Biol. 54, 2017-2024.

Schinegger, R., Palt, M., Segurado, P., Schmutz, S., 2016. Untangling the effects of multiple human stressors and their impacts on fish assemblages in European running waters. Sci.Total Environ. 573, 1079-1088.

Schinegger, R., Trautwein, C., Melcher, A., Schmutz, S., 2012. Multiple human pressures and their spatial patterns in e uropean running waters. Water Environ. J. 26, 261-273.

Schmera, D., Heino, J., Podani, J., Erős, T., Dolédec, S., 2017. Functional diversity: a review of methodology and current knowledge in freshwater macroinvertebrate research. Hydrobiologia 787, 27-44.

Schowalter, T. D., 2011. Ecosystem Structure and Function. Insect Ecology, 327–358. Elsevier.

Shibata, H., Kirikae, M., Tanaka, Y., Sakuma, T., Hatano, R., 1998. Proton budgets of forest ecosystems on volcanogenous regosols in Hokkaido, northern Japan. Water, Air, Soil Pollut. 105, 63-72.

Shibata, H., Mitsuhashi, H., Miyake, Y., Nakano, S., 2001. Dissolved and particulate carbon dynamics in a cool-temperate forested basin in Northern Japan. Hydrol. Process. 15, 1817–1828.

Simpson, S.C., Meixner, T., 2012. Modeling effects of floods on streambed hydraulic conductivity and groundwater-surface water interactions. *Water Resour. Res. 48*, W02515.

Solagaistua, L., Arroita, M., Aristi, I., Larrañaga, A., Elosegi, A., 2016. Changes in discharge affect more surface than subsurface breakdown of organic matter in a mountain stream. Mar. Freshw. Res. 2016, 1826–1834.

Solan, M., Cardinale, B.J., Downing, A.L., Engelhardt, K.A., Ruesink, J.L., Srivastava, D.S., 2004. Extinction and ecosystem function in the marine benthos. Science, 306,1177-1180.

Soulsby, C., Youngson, A.F., Moir, H.J., Malcolm, I.A., 2001. Fine sediment influence on salmonid spawning habitat in a lowland agricultural stream: a preliminary assessment. Sci. Total Environ. 265, 295-307.

Stanford, J.A., Ward, J.V., 1993. An ecosystem perspective of alluvial rivers: connectivity and the hyporheic corridor. J. N. Am. Benthol. Soc. 12, 48-60.

Statzner, B., Beche, L.A., 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? Freshw. Biol. 55, 80-119.

Statzner, B., Moss, B., 2004. Linking ecological function, biodiversity and habitat: a mini-review focusing on older ecological literature. Basic Appl. Ecol.5, 97-106.

Strayer, D.L., Dudgeon, D., 2010. Freshwater biodiversity conservation: recent progress and future challenges. J. N. Am. Benthol. Soc. 29, 344-358.

Strayer, D.L., May, S.E., Nielsen, P., Wollheim, W., Hausam, S., 1997. Oxygen, organic matter, and sediment granulometry as controls on hyporheic animal communities. Archiv. Hydrobiol. 140, 131-144.

Stubbington, R., Boulton, A.J., Little, S., Wood, P.J., 2014. Changes in invertebrate assemblage composition in benthic and hyporheic zones during a severe supraseasonal drought. Freshw. Sci. 34, 344–354.

Stubbington, R., Boulton, A.J., Little, S., Wood, P.J., 2015. Changes in invertebrate assemblage composition in benthic and hyporheic zones during a severe supraseasonal drought. Freshw. Sci. 34, 344–354.

Stubbington, R., Gunn, J., Little, S., Worrall, T.P., Wood, P.J., 2016. Macroinvertebrate seedbank composition in relation to antecedent duration of drying and multiple wet-dry cycles in a temporary stream. Freshw. Biol. 61, 1293-1307.

Stubbington, R., Sarremejane, R., Datry, T., 2019. Alpha and beta diversity of connected benthic–subsurface invertebrate communities respond to drying in dynamic river ecosystems. Ecography, 42, 2060–2073.

Stubbington, R., Wood, P.J., Reid, I., 2011 Spatial variability in the hyporheic zone refugium of temporary streams. Aquat. Sci. 73, 499–511.

Suurkuukka, H., Meissner, K.K., Muotka, T., 2012. Species turnover in lake littorals: Spatial and temporal variation of benthic macroinvertebrate diversity and community composition. Diversity Distrib. 18, 931–941.

Tank, J.L., Rosi-Marshall, E.J., Griffiths, N.A., Entrekin, S.A., Stephen, M.L., 2010. A review of allochthonous organic matter dynamics and metabolism in streams. *J. N. Am. Benthol. Soc. 29*, 118-146.

Tilman, D., Isbell, F., Cowles, J.M., 2014. Biodiversity and ecosystem functioning. Annu. Rev. Ecol. Evol. Syst. 45, 471-493.

Tockner, K., Pusch, M., Borchardt, D., Lorang, M.S., 2010. Multiple stressors in coupled river–floodplain ecosystems. Freshw. Biol. 55, 135-151.

Tornwall, B., Sokol, E., Skelton, J., Brown, B.L., 2015. Trends in stream biodiversity research since the river continuum concept. Diversity 7, 16-35.

Townsend, C.R., Hildrew, A.G., 1994. Species traits in relation to a habitat templet for river systems. Freshw. Biol. 31, 265-275.

Tranvik, L.J., Downing, J.A., Cotner, J.B., Loiselle, S.A., Striegl, R.G., Ballatore, T.J., Dillon, P., Finlay, K., Fortino, K., Knoll, L.B. et al., 2009. Lakes and reservoirs as regulators of carbon cycling and climate. Limnol. Oceanogr. 54, 2298–2314.

Underwood, A.J., Chapman, M.G., 1996. Scales of spatial patterns of distribution of intertidal invertebrates. Oecologia 107, 212-224.

Vander Vorste, R., Mermillod-Blondin, F., Hervant, F., Mons, R. and Datry, T., 2017. Gammarus pulex (Crustacea: Amphipoda) avoids increasing water temperature and intraspecific competition through vertical migration into the hyporheic zone: A mesocosm experiment. Aquat. Sci. 79, 45–55.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., Cushing, C.E., 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37, 130-137.

Vaughn, C.C., 2010. Biodiversity losses and ecosystem function in freshwaters: emerging conclusions and research directions. Bioscience 60, 25-35.

Veech, J.A., Summerville, K.S., Crist, T.O, Gering J.C. 2002. The additive partitioning of species diversity: recent revival of an old idea. OIKOS 99, 3–9

Verdonschot P.F.M., Nijboer R.C., 2004. Testing the European stream typology of the Water Framework Directive for macroinvertebrates. Hydrobiologia, 516, 35– 54.

Vinebrooke, R.D., Cottingham, K.L., Norberg, J., Scheffer, M., Dodson, S.I., Maberly, S.C., Sommer, U., 2004. Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos 104, 451-457.

Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. *nature. 467*, 555-561.

Wagenhoff, A., Townsend, C. R., Phillips, N., Matthaei, C. D., 2011. Subsidy-stress and multiple-stressor effects along gradients of deposited fine sediment and dissolved nutrients in a regional set of streams and rivers. Freshw. Biol. 56, 1916–1936.

Wagenhoff, A., Townsend, C.R., Matthaei, C.D., 2012. Macroinvertebrate responses along broad stressor gradients of deposited fine sediment and dissolved nutrients: a stream mesocosm experiment. J. Appl. Ecol. 49, 892-902.

Wallace, J. B., Webster, J. R., 1996. The role of macroinvertebrates in stream ecosystem function. Annu. Rev. Entomol. 41, 115-139.

Ward, J.V., 1989. The four-dimensional nature of lotic ecosystems. J. N. Am. Benthol. Soc. 8, 2-8.

Ward, J.V., Tockner, K., Uehlinger, U., Malard, F., 2001. Understanding natural patterns and processes in river corridors as the basis for effective river restoration. Regul. Rivers: Res. Mgmt. 17, 311-323.

Webster, J.R., Benfield, E.F., 1986. Vascular plant breakdown in freshwater ecosystems. Ann. Rev. Ecol. Syst. 17, 567–594.

Wenger, S.J., Roy, A.H., Jackson, C.R., Bernhardt, E.S., Carter, T.L., Filoso, S., Gibson, C.A., Hession, W.C., Kaushal, S.S., Martí, E., Meyer, J.L. et al., 2009. Twenty-six key research questions in urban stream ecology: an assessment of the state of the science. J. North Am. Benthol. Soc. 28, 1080-1098.

Wentworth, C.K., 1922. A scale of grade and class terms for clastic sediments.The Journal of Geology 30, 377-392.

Whittaker, R.H., 1960. Vegetation of the Siskiyou mountains, Oregon and California. Ecol. Monogr. 30, 279-338.

Wiens, J. A., Stenseth, N. C., Horne, B. V., Ims, R. A., 1993. Ecological Mechanisms and Landscape Ecology. Oikos, 66, 369-380.

Williams, D.D., Febria, C.M., Wong, J.C., 2010. Ecotonal and other properties of the hyporheic zone. Fundam. Appl. Limnol., Arch. Hydrobiol. 176, 349-364.

Williams, D.D., Hynes H.B.N., 1974. The occurrence of benthos deep in the substratum of a stream. Freshw. Biol. 4, 233-256.

Wondzell, S. M. and Gooseff, M. N., 2013. Geomorphic controls on hyporheic exchange across scales: watersheds to particles. – In: Shroder, J. F. (ed.), Treatise on geomorphology. Academic Press, 203–218.

Wood, P.J., Armitage, P.D., 1997. Biological effects of fine sediment in the lotic environment. Environ. Manage. 21, 203-217.

Wu, J., Mao, R., Li, M., Xia, J., Song, J., Cheng, D., Sun, H., 2020. Assessment of aquatic ecological health based on determination of biological community variability of fish and macroinvertebrates in the Weihe River Basin, China. J. Environ. Manag. 267, 110651.

Xu, M.-Z., Wang, Z.-Y., Pan, B.-Z., Zhao, N., 2012. Distribution and species composition of macroinvertebrates in the hyporheic zone of bed sediment. Int. J. Sediment Res. 27, 129-140.

Xu, X.N., Shibata, H., 2007. Landscape patterns of overstory litterfall and related nutrient fluxes in a cool-temperate forest watershed in northern Hokkaido, Japan. J. For. Res. 18, 249-254.

Yoshimura, C., Omura, T., Furumai, H., Tockner, K., 2005. Present state of rivers and streams in Japan. River Res. Applic. 21, 93-112.

Young, R.G., Matthaei, C.D., Townsend, C.R., 2008. Organic matter breakdown and ecosystem metabolism: Functional indicators for assessing river ecosystem health. J. N. Am. Benthol. Soc. 27, 605–625.

Zhang, Y., Cheng, L., Tolonen, K.E., Yin, H., Gao, J., Zhang, Z., Li, K., Cai, Y., 2018. Substrate degradation and nutrient enrichment structuring macroinvertebrate assemblages in agriculturally dominated Lake Chaohu Basins, China. Sci. Total Environ. 627, 57–66.

Zuellig, R.E., Kondratieff, B.C., Rhodes, H.A., 2002. Benthos recovery after an episodic sediment release into a Colorado Rocky Mountain River. West. N. Am. Nat. 59-72.