



Article Additive Effects of Sediment and Nutrient on Leaf Litter Decomposition and Macroinvertebrates in Hyporheic Zone

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Abstract: Despite the fact that leaf decomposition constitutes an important function in rivers, how multiple environmental stressors simultaneously affect it remains largely unknown. This study investigated the interactive effects of fine sediments (particle size: <2 mm; experimentally manipulated) and a specific nutrient (i.e., nitrate) on subsurface (hyporheic) leaf litter decomposition rate and macroinvertebrates in a gravel-bed river and its tributary in eastern Hokkaido, Japan. The experiment was conducted by measuring leaf litter decomposition of dried *Alnus japonica* leaves (3 ± 0.05 g) in benthic and hyporheic zones with and without sediment treatments at four sites that had a gradient of nitrate concentration. The decomposition rate was comparable between the two zones but was slowed down by sediment addition in the hyporheic zone. The functional responses were highly predictable for the individual stressors. Detritivore invertebrates were the main driving component of decomposition as well as detritivore feeding. Overall, the negative effect of fine sediment could be offset in the presence of nitrate while considering gross functional responses. We demonstrated the additive effects of fine sediment and nitrate on leaf litter decomposition in the hyporheic zone.

Keywords: detritivores; ecosystem function; gravel-bed river; manipulation; multiple stressors

1. Introduction

Rivers are threatened worldwide by human activities coupled with the alterations in physical structures and ecosystem functions [1]. Land use activities such as intensive agricultural practices and/or urbanization have been immensely accelerating the degree of alteration in freshwater ecosystems via a myriad of stressors. Two common concerning stressors are excessive loads of nutrients originating from the usage of synthetic fertilizers or wastewater and fine sediments (organic and inorganic particles <2 mm) through surface erosion in contributing areas [2–4]. In rivers, the resulting impairment of water quality often leads to structural changes in stream assemblages [5–9] and their functions [10–12]. Although those stressors often co-occur to affect ecosystems simultaneously as multiple stressors [13], there is still a relatively low understanding of how multiple stressors affect the functional properties of river ecosystems beyond their structural properties, such as a community structure [14].

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 [15,16]. 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 [17,18]. The



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). processing of leaf litter (decomposition) in rivers is driven by leaching, microbial conditioning, invertebrate shredding, mechanical fragmentation by water flow, and sediment transport [19]. 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 [20–23]. 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 [24–26], because a part of organic matter can be buried beneath the streambed during flooding or sediment movement at higher water velocity [27–30]. 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 [31–34] or examined overall effects of co-occurring multiple stressors from catchments [35,36]. Some also tested the interactive effects of multiple stressors on community structures and microbial functioning [37–42], 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 [23]. 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 [5,43] and indirectly by imposing limitations in the resources (e.g., dissolved oxygen, nutrients) and habitat for microbial and invertebrate assemblages [44,45]. In the hyporheic zone, increased sediment deposition can clog interstices, thereby lowering hydrological exchanges [38,46]. This in turn could create an oxygen-limiting condition that negatively affects the habitat of hyporheic invertebrate decomposers [44]. The reported effects of nutrients on leaf litter decomposition have been context-dependent and mixed [47,48]. Additional nutrients can increase ecosystem productivity under oligotrophic conditions by enhancing microbial and algal production as a subsidy (see also [9]). Stimulated microbial activities under high nutrients may promote organic matter decomposition [49]. 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 [8,50,51]. Relatively few studies have examined the responses of hyporheic processes to nutrient pollution [35,52,53], 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, with the effects being additive (response is equal to the sum of the effects of both stressors), synergistic (response is greater than the sum of the individual stressors), or antagonistic (response is less than the sum of the individual stressors) [54]. 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 [6,55–57]. The consequences of possible multiple stressors in hyporheic processes have also been reported in some studies [35,41], but only a few were designed to disentangle the interactions of environmental factors. To fill this knowledge gap, we 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. 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. We 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. We 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 [9,53], was predicted to become a stressor under high sediment conditions (synergistic effects).

2. Materials and Methods

2.1. Site Description and Sampling Design

The study was conducted from July 2019 to November 2019 in four sites in the main channel of the Satsunai River (catchment area 725 km²; channel length 82 km) and one spring-fed tributary. Leaf litterfall of trees including Japanese Alder (*Alnus japonica* Steud.) commonly takes place in the region between August and December [58–60], and thus our experiment setup was reasonably representative of natural leaf litter decomposition. The river originates from Mt. Satsunai (42°41′ N, 142°47′ E; 1895 m a.s.l.) and flows into the Tokachi River, in eastern Hokkaido, Japan (Figure 1). Three sites were located in the main channel and one site was located in a small tributary collecting water from spring-fed sources in the upstream area which originates from a groundwater reservoir in the alluvial fan of the Satsunai River. Site A1 was located downstream of the confluence with the Totsutabetsu River, which is as large as the Satsunai River. All sites in the main channel were characterized by typical gravel-bar river landscapes with riparian areas, gravel bars, and braided channels. One site in the tributary was characterized by a stable channel without well-developed gravel bars because of infrequent sediment movements associated with the stable flow of spring-fed water sources.



Figure 1. Location of the Tokachi watershed in Hokkaido, Japan where the study area is indicated by a dotted box (**a**) and four study sites (gray-filled circles) in the Satsunai River (**b**). A red-filled point indicates the confluence with a tributary affected by a wastewater treatment plant (WWTP). An arrow denotes the flow direction of the river.

A wastewater treatment plant (WWTP) for sewage discharges dissolved and particulate solids enriching water through a small tributary (flow rate = approximately 0.8 m³/s) (Figure 1). This point source, in addition to other diffuse sources (i.e., agricultural activities), forms a longitudinal gradient along the surface water in terms of nutrients (i.e., nitrate) from upstream to downstream (detailed information can be found in [53,61]). Thus, we utilized this gradient of nitrate to examine the effect of nutrient levels. The regional climate is characterized by lower air temperature and precipitation during winter and higher temperature and precipitation during summer. The annual precipitation was 1181.3 (\pm 204.2) mm (from 1999 to 2018) (Kami-satsunai station, Japan Meteorological Agency). The mean (\pm standard deviation) daily flow rate from 1999 to 2016 was 11.1 (\pm 17.6) m³/s (Kami-satsunai station, Ministry of Land, Infrastructure, Transport and Tourism (MLIT)) in the main channel, while the tributary had smaller flow rates recorded between 0.9 and 1.5 m³/s from 2013 to 2019 (unpublished data, J.N. Negishi).

The litter bag method [62] 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; [26,36,63]) and set in the river on 8th and 9th of July 2019 (Figure S1). 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 (Figure S1; see [53] for details about the traps). 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 litter bags were installed in each of the six pits. The distance between pits along the channel (Figure S2) 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; see 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, we installed a total of 24 litter bags in the benthic zone with six bags at each site. One slow-current depositional area (e.g., backwater area) was chosen within a 50 m distance from the pits and six bags were tethered to a metal lever driven into the riverbed (Figure S2).

Fine sediment addition treatment was conducted by depositing 40 kg of commercially available fine sediments (particle size: <2 mm) on top of two colonization traps in three pits (1st, 3rd, and 5th pits from the upstream end of the pit row) at each site (Figures S2 and S3). The other three pits were left without sediment additions 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. Both treatment and control pits were buried back with the original deposited sediments within one hour from the pit excavation. Nylon ropes attached to the colonization traps were left visible on the riverbed to easily locate the traps during sample collection.

2.2. 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 we predicted relatively faster loss of organic matter in the surface zone. 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 [9,28,53]. On each collection occasion, we collected a total of four litter bags (two in each treatment and control pit) 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 S1). Bags in the hyporheic zone were collected through two steps. First, a colonization trap was retrieved as described in [53] and cut open immediately after retrieval. The litter bags in the colonization traps were quickly taken out and placed in a zip-lock bag and the remaining contents of the colonization traps were saved for other studies. 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.

2.3. 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 S2). Hyporheic water was collected using a handheld bilge pump from the PVC wells twice per site (two PVC wells) as described in [53]. 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 (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 \pm standard deviations: 9.95 \pm 1.07 mg/L) with pH being almost neutral (6.98 \pm 0.37) all the time. We considered DO and pH unimportant as limiting factors and thus they were excluded from further analyses.

2.4. 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 μ m 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., [64,65]). We focused on Lepidostomatidae, Nemouridae, Leptophlebidae, and Tipulidae and treated them as detritivores in the following analyses based on [53], where these taxa have been identified as being dependent on allochthonous carbon sources. Thus, we 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 μ m (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).

2.5. 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). We defined the sampling season 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* (Figure S4). 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, habitattreatment 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 matteras the number of individuals per gram of AFDM and were log_{10} transformed. Scores of two NMDS axes were compared among habitattreatment 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 [66], and the effects of temperature were statistically removed. For each litter bag, we calculated a habitat-treatment-type specific two-week mean water temperature and found that there was a significant effect of water temperature on leaf litter decomposition rate (k) (Figure S5). Therefore, we developed a GLM 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 S2). To supplement the missing nitrate data for each litter bag, we developed a linear relationship between nitrate and EC for the surface water and derived missing nitrate values based on this relationship (Figure S6). We believe that this approach was acceptable because our previous study also found a highly significant relationship between nitrate and EC [61] 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, R Core Team [67]). Data in GL(M)Ms were log₁₀ 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.

3. Results

Nitrate concentrations were consistently higher in some sites regardless of habitattreatment types, as shown by significant effects of site with insignificant interaction effects (Table 1). The concentration tended to be higher in sites downstream of the WWTP and was the highest in Site L1 (Figure 2). 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 1) and was highest in A1 and lowest in site S1 (Figure 2). The amount of fine sediment showed significant effects of interaction, indicating that the extent of differences among habitat-treatment types varied across sites (Table 1c). 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 (Figure 2). 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.

Table 1. Results of GLMMs testing the effects of site (Site: four sampling sites) 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 models 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)	Explanatory Variables	logLik	AIC	<i>p</i> -Value
	Full model			
	Site (S), Type (T), S \times 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	
	Т	-85.06	178.13	
(b)	Explanatory Variables	logLik	AIC	<i>p</i> -Value
	Full model			
	Site (S), Type (T), S \times 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	
	Т	105.04	-198.1	
(c)	Explanatory Variables	logLik	AIC	<i>p</i> -Value
	Full model			
	Site (S), Type (T), $S \times T$	-97.47	224.9	<0.05
	1st Reduced model			
	S, T	-105.81	229.6	



Figure 2. 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.

The leaf litter decomposition rate differed among habitat-treatment types (Table 2). 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 (Figure 3a). 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 (Figure S4).

Table 2. Results of GLMMs testing the effects of habitat-treatment type (Type: benthic zone, control hyporheic zone, and treatment hyporheic zone) on \log_{10} -transformed leaf litter decomposition rate (*k*) (a), and the effects of \log_{10} -transformed standardized abundance of detritivores on \log_{10} -transformed leaf litter decomposition rate (b). Full models were compared to null models using log-likelihood tests. Bold letters for *p*-value denote statistical significance.

(a)	Explanatory Variables	logLik	AIC	<i>p</i> -Value
	Full model Type Null model	-4.25	18.5	<0.05
	N.A.	-8.20	22.4	
(b)	Explanatory Variables	logLik	AIC	<i>p</i> -Value
	Full model Detritivore Null model	-18.99	46.0	<0.01
	N.A.	-23.63	53.3	

A total of 16 macroinvertebrate taxa (hereafter called either order or family) and 3076 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 S3). 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 S3). The community structure was clustered according to habitat-treatment-type groups in the NMDS plot with significant differences between the benthic and 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



(Figure 3c; Table 2), indicating that habitat-treatment-type specific *k* variation was partially related to the activities of detritivores.

Figure 3. 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.

Residual *k* was not affected by the interaction between nitrate and sediment, showing both independent significant effects (Table 3). The residual *k* was negatively affected by sediment and positively affected by nitrate (Figure 4). 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 3). 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, $r^2 = 0.88$).

Table 3. 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 leaf litter decomposition rate (b). Full models and reduced models were each compared using log-likelihood tests; when full models 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)	Explanatory Variables	logLik	AIC	<i>p</i> -Value
	Full model			
	Nitrate (N), sediment (S), N $ imes$ 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			
	Ν	-16.99	46	
	S	-19.28	50.6	
(b)	Explanatory Variables	logLik	AIC	<i>p-</i> 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	



Figure 4. 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**).

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 our 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 particular 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.

Our results did not support previous reports that leaf litter decomposition rate is lower in the hyporheic zones compared with surface water zones [28,36]. 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 [28,36] 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 [28] and sandy to coarse gravel in [36]. 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 [53]. 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) [36,68,69]. In our study, no Gammaridae including a species *Pseudocrangonyx yezonis* [70], which is known to inhabit the study site [61], 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 detri-

tus 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 [9,53]) 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 habitattreatment-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. 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 [68,71,72].

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 [73,74]. Second, the stimulated activities of the microbial community enhance the palatability of leaves that could accelerate the macroinvertebratederived decomposition and thus mediate the carbon flow [66,75,76]. 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 our results. 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 [53]. 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.

5. Conclusions

Our 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. Future studies on the systems that cover a wider range of stressor gradients including the range that shows the stress-subsidy responses of invertebrates and functions will help

in developing more generalized predictive models of hyporheic functions in response to multiple environmental stressors.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/ 10.3390/w13101340/s1: Figure S1: Photos showing a litter bag and prepared colonization traps; Figure S2: Diagram showing the sampling design of each study site in a longitudinal section of a river; Figure S3: Photos showing how the experimental treatment of sediment addition was conducted; Figure S4: Temporal changes of remaining leaf litter mass (fraction) after the installation (Day 0) in relation to habitat-treatment groups and sites; Figure S5: The relationship between two-week mean water temperature and leaf litter decomposition rate (*k*); Figure S6: The relationship between electrical conductivity and nitrate in the surface water (benthic) zone; Table S1: Summary of litter bag collection schedule in the surface water (benthic) and hyporheic zones; Table S2: Summary of water-sampling dates in 2019; Table S3: Summary of all invertebrates caught in litter bags.

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