

# Trophic energy flows increase in more diverse communities of coastal food webs: Testing the vertical diversity hypothesis

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## Abstract

Biomass is a common, universal indicator of ecosystem productivity for exploring biodiversity–ecosystem functioning (BEF) relationships in all types of ecosystems. However, positive BEF is often missing in aquatic consumer communities with multi-trophic interactions. Here, we apply a new indicator, integrated trophic position (iTP), which is defined as the summed TPs of all consumers weighed by the relative biomass of each taxon, for multitrophic systems to test the vertical diversity hypothesis (VDH) that functional diversity can enhance trophic energy flows within a food web. Using a meta-community of coastal benthic macroinvertebrates, we demonstrate that iTP increases in more diverse communities, supporting the VDH. Comparing our results with previous findings that stream benthic macroinvertebrates exhibit a negative BEF in contrast to the VDH, we discuss a possible mechanism to explain this contrasting pattern. Finally, we use a size-based food web approach to deepen our mechanistic understanding of the observed BEF.

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## Abstract

Biomass is a common, universal indicator of ecosystem productivity for exploring biodiversity–ecosystem functioning (BEF) relationships in all types of ecosystems. However, positive BEF is often missing in aquatic consumer communities with multitrophic interactions. Here, we apply a new indicator, integrated trophic position (*i* TP), which is defined as the summed TPs of all consumers weighed by the relative biomass of each taxon, for multitrophic systems to test the vertical diversity hypothesis (VDH) that functional diversity can enhance trophic energy flows within a food web. Using a meta-community of coastal benthic macroinvertebrates, we demonstrate that *i* TP increases in more diverse communities, supporting the VDH. Comparing our results with previous findings that stream benthic macroinvertebrates exhibit a negative BEF in contrast to the VDH, we discuss a possible mechanism to explain this contrasting pattern. Finally, we use a size-based food web approach to deepen our mechanistic understanding of the observed BEF.

## INTRODUCTION

Since Tilman & Downing (1994)’s pioneering work, many studies have so far investigated biodiversity–ecosystem functioning (BEF) relationships and demonstrated that ecosystem productivity often increases with increasing biodiversity (Tilman et al., 2014). These results attract ecological and societal interests as biodiversity loss leads to a decline in ecosystem goods and services for humanity by decreasing ecosystem productivity (Balvanera et al., 2006; Harrison et al., 2014; Millennium Ecosystem Assessment, 2005), stimulating biodiversity conservation and restoration. In the assessment of BEF relationships, biomass is often used as a proxy for productivity due to difficulties in *in situ* measurements (e.g., Duffy et al., 2015; Gamfeldt et al., 2015; Scurlock et al., 2002). Despite unclear causal mechanisms, positive relationships between biodiversity and biomass are found in many observational studies in nature (Duffy et al., 2017). From a meta-analysis on non-experimental and experimental data, Waide et al. (1999) concluded that such a positive BEF is commonly found for terrestrial consumers, whereas the dominant pattern is unimodal or non-significant for aquatic consumers.

There are two possible explanations for the scarcity of positive BEF in aquatic consumer communities. One is the dominance of top-down effects on consumer biomass in aquatic food webs, i.e., the negative effect of predators on herbivore biomass is stronger in aquatic than in terrestrial systems (Shurin et al., 2002; Strong, 1992). Another is the difference in vertical diversity between these two systems, i.e., a greater number of trophic levels (TLs) in aquatic than in terrestrial food webs due to wider body size distribution and higher

metabolic efficiency in aquatic systems that permits more extended trophic links (Rossiter et al., 2017). Since the energy entering consumer communities becomes progressively attenuated with increasing TLs (Lindeman, 1942), we may underestimate ecosystem productivity if substituted with community biomass measurements in systems with multitrophic interactions.

Recently, multitrophic perspectives have provided new insights into empirical studies on BEF (Eisenhauer, 2017; Eisenhauer et al., 2019; Soliveres et al., 2016). In multitrophic systems, a comprehensive understanding of BEF relationships requires the measurement of energy flows but not community biomass in food webs because the former can be a common currency for ecosystem productivity across a variety of ecosystem types (Barnes et al., 2018; Hines et al., 2015). Supported by size-based food web models, the vertical diversity hypothesis (VDH) states that functional diversity across TLs can enhance energy flows within a community (Schneider et al., 2016; Wang & Brose, 2018). However, this hypothesis has been poorly tested empirically (Hines et al., 2015; Thompson et al., 2012a). Although experimental approaches are effective in testing the VDH, simplified experimental systems with small species pools and few TLs may lack the power needed to demonstrate that more energy travels to higher TLs in real food webs with high species richness and trophic network complexity. Barnes et al. (2016) employed the energy-based approach to explore BEF relationships in non-manipulative terrestrial food webs with multitrophic interactions, classifying consumers into given trophic groups based on ecological traits. However, realized consumer trophic positions (TPs) vary broadly depending on the ecological context, as is often the case for aquatic consumers (Nakazawa et al., 2010). Such flexibility in TPs can generate analytical uncertainty in energy-based BEF.

Stable isotope analysis is a powerful tool for accurately estimating consumer TPs (Chang et al., 2014; Jennings et al., 2002b; Post, 2002b). Using this technique, many ecologists measure food chain length (FCL) defined as top predator TPs (Post, 2002a). FCL is a convenient indicator for empirical studies but may not accurately capture the overall energy flow within a food web as not all energy flow distributions among trophic links are fully considered (Figure 1). To overcome this limitation, Ishikawa et al. (2017) recently proposed an alternative indicator to characterize food web topology, integrated trophic position (hereafter,  $i$  TP), which is defined as the summed TPs of all consumer species in a focal food web weighed by the relative biomass of each species (see Eq. 5 for details). The  $i$  TP indicates how many times consumer biomass undergoes metabolic turnover, on average, as a whole community. Moreover, it reflects dynamical changes in the biomass pyramid *via* trophic interactions, e.g., a decrease in prey biomass due to predation can be offset by increasing predator TP and predator biomass. Therefore, the  $i$  TP can provide more appropriate and quantitative estimates of trophic energy flows within a food web (Figure 1).

Here, we test the VDH, i.e., whether and how functional diversity can enhance overall trophic energy flow, by applying the  $i$  TP to complex real food webs. We focus on spatial variation in a meta-community of coastal benthic macroinvertebrates in the ancient Lake Biwa, which exhibits high biodiversity with endemism (Kawanabe et al., 2012; Timoshkin et al., 2011). Its huge lake basin has hundreds of tributary rivers, which greatly vary in land use and local human population sizes (Kohzu et al., 2009; Ohte et al., 2010). Thus, its coastal habitats have high spatial heterogeneity in nutrients and basal resource availability (Karube et al., 2010; Sakai et al., 2013). Because benthic macroinvertebrates are sedentary or less mobile, we compare  $i$  TP across a wide variety of local communities that share the species pool within a meta-community but greatly vary in species composition in response to local environments (Shibata et al., 2014). Since land use can be a strong driver that alters species composition, a between-community comparison provides an opportunity to explore ecosystem consequences of biodiversity change across anthropogenically induced environmental gradients at the landscape level (Barnes et al., 2014; Gossner et al., 2016; Thompson et al., 2012b). Comparing our results with previous findings that the  $i$  TP decreases in more diverse communities of stream benthic macroinvertebrates (Ishikawa et al., 2017), we discuss a possible mechanism to explain such a negative BEF in contrast to the VDH. To deepen our mechanistic understanding of observed patterns in biodiversity and  $i$  TP for the coastal meta-community, we finally employed a size-based food web approach, which enables us to link size-specific community properties, such as abundance, biomass, and TP, to ecosystem functioning based on the scaling theory (Cohen et al., 2003; Jennings & Mackinson, 2003; Jennings et al., 2002b).

## MATERIALS AND METHODS

### BIODIVERSITY MONITORING

We collected benthic macroinvertebrates in three replicates from each of the 33 shallow coastal habitats of Lake Biwa in winter (February 2006) using a 475- $\mu\text{m}$ -mesh Sarvar net with a  $30 \times 30$  cm quadrat (see Karube et al. 2010 for details). At each sampling site, we also collected basal resources, particulate organic matter (POM) and epilithic organic matter (EOM), in November 2005, February, May, and July 2006, and measured the quarterly dissolved oxygen (DO) concentration (mg/L) and water temperature (WT) of the coastal waters (see Sakai et al., 2013 for details). The average chlorophyll-*a* concentration for quarterly POM and EOM samples was used as an indicator for phytoplankton and benthic algal biomass, respectively, and the average DO and WT were used as indicators for coastal environments. Among these coastal sites, 29 were located near the mouth of tributary rivers, which greatly vary in catchment size and land use patterns, whereas four sites separate from the river mouth were considered control sites with the least fluvial impacts (see Sakai et al. 2013 for details). In this watershed, agricultural land use is regarded as the most critical driver of biodiversity loss in stream macroinvertebrate communities (Ko et al., 2021), which can also impact lake macroinvertebrate diversity *via* river inflows (Okano et al., 2018).

For three sub-samples at each site, macroinvertebrates were sorted, identified, and counted, after which their dry mass was measured for each taxon. The average biomass per unit area (mg/m<sup>2</sup>) was calculated from the average individual body mass (mg) multiplied by the average numerical abundance (individuals/m<sup>2</sup>) for three replicates of each taxon at each site. The Shannon–Wiener diversity index ( $H'$ ) was calculated as follows:

$$H' = \sum_{i=1}^S (p_i \times \ln p_i) \quad (1)$$

where  $S$  and  $p_i$  denote the total number of taxa (i.e., taxonomic richness) and the relative numerical abundance of each taxon in each of the local communities. Some small-sized animals, such as oligochaetes, chironomids, and midges, were difficult to quickly identify to a fine taxonomic resolution under fresh conditions for stable isotope analysis, and leeches (Hirudinea) were difficult to identify without anatomical observation. Since these animals were classified into higher taxonomic levels, which can be characterized by functional feeding groups,  $H'$  is regarded as an indicator of functional diversity when considering numerical abundance and equality.

For carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope analysis, each taxon at each site was provided individually or in bulk in one to three replicates, depending on individual mass (see Karube et al., 2010; Sakai et al., 2013 for methods of isotope sample treatment).

### FOOD WEB ANALYSIS

After the stable isotope analysis for macroinvertebrates and their two basal resources using mass spectrometers (CF/IRMS; Conflo II & Delta S, Finnigan MAT, Germany, analytical precision:  $\pm 0.1\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ; Conflo III and Delta plus XP, Thermo Fisher Scientific, USA, analytical precision:  $\pm 0.1\delta^{13}\text{C}$  and  $\pm 0.2$  estimated the TP of each taxon at each site based on the following stable isotope mixing model (Okuda et al., 2017; Okuda et al., 2020):

$$f_1 + f_2 = 1 \quad (2)$$

$$f_1 \delta^{13}\text{C}_1 + f_2 \delta^{13}\text{C}_2 + \Delta \delta^{13}\text{C}_{ef} \times (\text{TP}-1) = \delta^{13}\text{C}_{\text{taxon}} \quad (3)$$

$$f_1 \delta^{15}\text{N}_1 + f_2 \delta^{15}\text{N}_2 + \Delta \delta^{15}\text{N}_{ef} \times (\text{TP}-1) = \delta^{15}\text{N}_{\text{taxon}}, \quad (4)$$

where  $f_1$  and  $f_2$  denote the proportion of reliance of POM and EOM, which are regarded as basal resources of planktonic and benthic food webs at each coastal site, respectively.  $\delta R_1$ ,  $\delta R_2$ , and  $\delta R_{\text{taxon}}$  ( $R = {}^{13}\text{C}$  or  ${}^{15}\text{N}$ ) are carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope ratios of POM, EOM, and each macroinvertebrate taxon, respectively. The isotope values were averaged for each taxon at each site to consider individual variation and for time-series samples of POMs and EOMs at each site to consider temporal variation, excluding POM data

from February 2006 when temporary enrichment of its  $N^{15}$  occurred due to winter mixing-driven advection of profundal POM with a higher  $\delta^{15}N$  than primary consumers (Yoshimizu et al., 2008). Trophic enrichment factors,  $\Delta\delta^{13}C_{ef}$  and  $\Delta\delta^{15}N_{ef}$ , for macroinvertebrates were set to  $0.31\delta^{15}N$ , respectively, based on metadata for invertebrate body tissues (Caut et al., 2009).

Next, we calculated the  $i$  TP according to (Ishikawa et al., 2017). The  $i$  TP of a coastal macroinvertebrate community containing  $n$  taxa is expressed as follows:

$$i \text{ TP} = \sum_{i=1}^n (TP_i \times \frac{B_i}{B_T}) \quad (5)$$

where  $TP_i$  denotes the TP of taxon  $i$ , and  $B_i$  and  $B_T$  denote the biomass of taxon  $i$  and the total biomass of the local community, respectively. Hence,  $i$  TP is a calculation of the average number of times  $x$  that assimilated biomass is transferred along TEs in a focal food web (i.e.,  $i$  TP =  $x + 1$ ). In the present study,  $i$  TP does not include primary producers (i.e., TP = 1), such as planktonic and benthic algae, and coastal predatory vertebrates, such as fish and birds, whose biomasses are difficult to estimate at the same spatial scale as benthic macroinvertebrates. During the winter sampling period, since most fish predators were absent or inactive in coastal areas due to cold WT (mean  $\pm$  SD =  $4.93^\circ\text{C} \pm 1.09^\circ\text{C}$ , N = 33), we assume that top-down trophic cascading effects of fish predators were low in our focal communities.

Out of the 33 sites, 3 showed no macroinvertebrates, and 1 showed small biomass measurement of a few taxa that was insufficient for isotope analysis. For the other three muddy sites, since macroinvertebrate communities are dominated by hypoxia-tolerant deposit feeders (oligochaetes and chironomids) with  $\delta^{13}C$  and  $\delta^{15}N$  much lower than the two basal resources, the  $i$  TP could not be appropriately estimated, suggesting that these sites relied on methanotrophs in hypoxic sediment (Jones et al., 2008; Jones & Grey, 2011; Kiyashko et al., 2001). These communities were excluded from the  $i$  TP calculations. Biomass and abundance data were log-transformed for statistical analysis.

## RESULTS

A total of 27 benthic macroinvertebrate taxa were found in the coastal waters of Lake Biwa. Taxonomic richness varied from 0 to 13 ( $5.55 \pm 2.92$ , N = 33). In coastal communities, oligochaetes and chironomids were the most dominant taxa in numerical abundance ( $p_i = 0.45 \pm 0.28$  and  $0.24 \pm 0.25$ , respectively; Table S1), whereas grazing snails (*Semisulcospira* spp.) and a suspension-feeding bivalve (*Unio douglasiae biwae*) were the most dominant in biomass abundance ( $B_i/B_T = 0.17 \pm 0.22$  and  $0.11 \pm 0.25$ , respectively; Table S2).

Based on isotope data (Tables S3 and S4), TP was estimated for each taxon at each site (Table S5). Most grazer and deposit-feeder taxa were located around TP = 2 (Figure 2a). Among community members, carnivorous leeches and a freshwater prawn exhibited the highest TP ( $3.39 \pm 0.50$  and  $3.28 \pm 0.44$ , respectively), which are regarded as top predators in macroinvertebrate communities. In contrast, carnivorous insects, i.e., stoneflies and dragonflies, showed lower TPs than expected from their carnivorous habits, whereas grazing and suspension-feeding gastropods showed higher TPs than expected from their herbivorous habits, which are regarded as trophic omnivores. When all data were pooled for the whole coastal community, individual TPs significantly increased in body mass, but the slope of TP against the body mass was gentle ( $slope = 0.17$ ,  $P < 0.001$ , regression analysis; Figure 2a). Such a gentle slope could be ascribed to the omnipresence of grazing and suspension-feeding gastropods with large body masses and intermediate TPs. When these data were excluded from the regression analysis, the body mass-TP slope became steeper ( $slope = 0.30$ ,  $P = 0.009$ ). As observed in the general patterns of allometric scaling, body mass and numerical abundance demonstrated a clear negative correlation ( $P < 0.001$ ; Figure 2b). The numerical abundance-TP slope was also weakly negative ( $P = 0.028$ ; Figure 2c), but the biomass abundance-TP slope was flat ( $P = 0.14$ ; Figure 2d), as observed in another lake food web (Cohen et al., 2003).

Community biomass varied from 0.00 to  $6515.28 \text{ mg/m}^2$  ( $2309.70 \pm 2207.64 \text{ mg/m}^2$ , N = 33; Table S6). The basal resource availability defined by the phytoplankton and benthic algal biomasses impacted the biomass of each functional group. The benthic algal biomass showed bottom-up effects only on the chironomid biomass

( $r = 0.39$ ,  $P = 0.039$ ). Contrarily, the phytoplankton biomass had no bottom-up effects on any taxa but rather a negative effect on grazing insects and crustaceans ( $r = -0.46$ ,  $P = 0.01$ ). The phytoplankton biomass showed a strong negative correlation with DO in coastal waters ( $r = -0.54$ ,  $P < 0.001$ ), which was critical to the abundance of grazing insects and crustaceans ( $r = -0.59$ ,  $P < 0.001$ ) and prey taxonomic richness ( $r = -0.44$ ,  $P = 0.015$ ).

For BEF, the community biomass was significantly correlated with taxonomic richness ( $P < 0.001$ ,  $N = 30$ ; Figure 3a). This correlation, however, is partly due to the added species biomass to communities with low numerical abundance and thus low taxonomic richness, as shown by the community biomass almost reaching an asymptote at a value of 4 for taxonomic richness. Even when using the diversity index  $H'$  to consider numerical abundance and equality, the BEF relationship remained weak and varied greatly among communities with lower  $H'$  ( $P = 0.013$ ,  $N = 29$ ; Figure 3b). Using  $i$  TP, however, the positive BEF became stronger with a higher significance level ( $P = 0.002$ ,  $N = 26$ ; Figure 3c). In contrast, the variation in  $H'$  could not account for any change in FCL, which is defined as the maximum TP for each local community ( $P = 0.20$ ,  $N = 26$ ; Figure 3d).

The two top predator taxa more frequently appeared in local communities with higher prey richness (Wald = 4.17, odds ratio = 2.20,  $P = 0.041$ , logistic regression analysis) but not with higher biomass ( $P = 0.41$ ) or greater abundance ( $P = 0.57$ ) of prey taxa, except for predator-resistant gastropods. Although the  $i$  TP was positively correlated with the maximum TP ( $r = 0.42$ ,  $P = 0.03$ ,  $N = 26$ ), multiple regression analysis revealed that the within-lake variation in the  $i$  TP was best explained by the relative biomass abundance ( $B_i/B_T$ ) of deposit feeders, excluding deposit-feeding bivalves, with lower TPs and higher numerical abundance ( $t = -3.18$ , adjusted  $R^2 = 0.27$ ,  $P = 0.004$ ), rather than by top predators with the highest TP. Neither the  $i$  TP nor the maximum TP was affected by the basal resource availability.

## DISCUSSION

### VERTICAL VS HORIZONTAL DIVERSITY

Our study demonstrated that trophic energy flows defined by the  $i$  TP increase in more diverse communities of coastal benthic macroinvertebrates, supporting the VDH in real food webs with multitrophic interactions. When consumer biomass was used as an indicator of ecosystem productivity, in contrast, the positive BEF relationship was less clear. This may be due to the multitrophic nature of aquatic food webs showing strong top-down effects on prey biomass combined with Lindeman's (1942) law on consumer biomass progressively decreasing across TLs. FCL showed no significant biodiversity effects. Although the positive relationship between biodiversity and FCL has previously been reported in a comparative study, in which species richness and FCL scales were extended across a wide range of ecosystem sizes (Rossiter et al., 2017; Vander Zanden et al., 1999), empirical studies have often failed to highlight these relationships for individual ecosystems (Schriever & Lytle, 2020; Schriever & Williams, 2013). In open systems, whether predators with the highest TP are sampled for analysis is often random owing to their high mobility and low abundance, which may generate uncertainty when estimating the realized FCL. Therefore, to empirically test the VDH for multitrophic systems, the  $i$  TP can be a more reliable indicator for energy flow-based ecosystem functioning than FCL as it is more robust to the presence/absence of top predators.

In the multitrophic model testing the VDH, Wang & Brose (2018) demonstrated that trophic transfer indicated by nutrient flows increases when nutrient availability is high. In contrast, our observational data revealed that basal resource availability signified by algal biomass exerted no bottom-up effects on the  $i$  TP, as well as on consumer biomass, except for deposit-feeding chironomids. In aquatic ecosystems, high primary productivity is crucial to benthic macroinvertebrates as it causes organic matter loading and consequently oxygen depletion in benthic habitats (Paerl et al., 1998; Wolowicz et al., 2007). This is the reason why the abundance and richness of hypoxia-sensitive grazers decreased with increasing phytoplankton biomass in the present study. In general, productive conditions favor hypoxia-tolerant deposit feeders because algae-derived organic matters are embedded within detrital food webs in the sediment (Sokolowski et al., 2012). Combined, trophic energy flows may show a non-linear response to overall primary productivity in benthic food webs

(Vadeboncoeur et al., 2002).

Our within-lake comparison of meta-community also revealed that the  $i$  TP was affected by the relative biomass of deposit feeders rather than top predators. The dominance of deposit feeders implies that trophic energy is retained into a functional group with lower TPs, decreasing both  $H'$  and  $i$  TP. This is simply a phenomenological explanation for the observed correlation between  $H'$  and  $i$  TP; here, further research is needed to explain the reason why  $i$  TP is higher for more diverse communities. In our study, two carnivorous taxa with the highest TPs more frequently appeared in communities with higher prey taxonomic richness. Theoretical models demonstrated that prey–predator interactions become stable where predators have prey options, allowing for predator biomass to persist in the diverse community (Kondoh, 2003; Post et al., 2000). In laboratory experiments manipulating both prey and predator diversity, Gamfeldt et al. (2005) also demonstrated that prey richness can increase predator biomass *via* prey consumption by multiple predators. These studies provide circumstantial evidence that prey–predator diversity can enhance trophic energy flows to higher TLs. In mesocosms with tri-trophic interactions, Duffy et al. (2005) conducted prey diversity manipulation to explore how the presence of predators alters the prey diversity effects on biomass distribution. They found that prey diversity increased prey biomass only in the presence of predators but did not show topological changes in food webs throughout the experiment. Future experiments to manipulate functional diversity in multitrophic systems will further leverage the  $i$  TP to test the VDH.

More interestingly, Ishikawa et al. (2017) reported that the  $i$  TP decreases in more diverse communities of stream benthic macroinvertebrates in a tributary river of our study lake. To explain such a negative BEF relationship, Ishikawa et al. (2017) proposed alternative, but not mutually exclusive, hypotheses, i.e., variance in edibility and trophic omnivory hypotheses, although these hypotheses were not empirically tested. On the one hand, the former hypothesis assumes that the relative biomass of inedible prey increases with increasing prey diversity (Wilby & Orwin, 2013). This hypothesis is true if inedible preys have lower TPs. In our study communities, however, anti-predator armored gastropods, i.e., snails and bivalves, which were among the most dominant in the biomass abundance, had intermediate TPs, sometimes exceeding  $TP = 3$  (Table S5). Therefore, the dominance of these inedible preys does not always decrease the  $i$  TP in coastal macroinvertebrate communities, rejecting the variance in edibility hypothesis.

Conversely, the trophic omnivory hypothesis assumes that predators' omnivory decreases their TPs (Bruno & O'Connor, 2005), as reported for well-studied food webs where 12%–43% of consumers are omnivores (Williams & Martinez, 2004). In our study communities, the trophic omnivory hypothesis is applicable to carnivorous insects, which exhibited lower TPs than expected from their carnivorous habits (Table S5). In fact, predatory stoneflies can undergo a seasonal niche shift from carnivory to omnivory in winter, which results in lower TPs than carnivores (Miyasaka & Genkai-Kato, 2009). This type of trophic omnivory has a potential to decrease the  $i$  TP. Considering that their relative biomass is negligible ( $0.00 \pm 0.01$ ; Table S2), however, their trophic omnivory should only marginally affect the  $i$  TP in coastal macroinvertebrate communities. In conclusion, the trophic omnivory hypothesis can be rejected for our study communities.

When comparing coastal and stream benthic communities, the most critical difference is the diversity of basal resources and consumer functional feeding groups (FFGs), i.e., the former is characterized by the dominance of autochthonous algal products supporting deposit feeders and the latter by a combination of autochthonous algal and allochthonous terrestrial products supporting a variety of FFGs (Allan, 2008; Horne & Goldman, 1994). Within the study watershed, for instance, coastal communities harbored more abundant hypoxia-tolerant deposit feeders ( $p_i = 0.45 \pm 0.28$  for oligochaetes and  $0.24 \pm 0.25$  for chironomids; Table S1) than their stream counterparts ( $p_i = 0.09 \pm 0.15$  and  $0.20 \pm 0.18$ , respectively; Ko et al., 2021). In contrast, in stream communities, EPT taxa (i.e., Ephemeroptera, Plecoptera, and Trichoptera), which are considered the dominant FFGs, account for 45.7% of species richness (75/164; Ko et al., 2021), which is much greater than their coastal counterparts ( $25.9\% = 7/27$ ; Table S1). Even within a given EPT taxon, species diversity can enhance basal resource use *via* the interspecific complementarity effects (Cardinale et al., 2002). When compared with our coastal cases, the stream benthic food webs are characterized by the functional diversity of primary consumers.

Duffy et al. (2007) proposed a conceptual framework in which BEF relationships are altered by interactions between horizontal and vertical diversities in complex ways. Developing this framework, Kato et al. (2018) constructed a theoretical model to define complex food webs, in which biodiversity ( $H'$ ) can be broken down into three components, namely, horizontal diversity ( $D_H$ ), vertical diversity ( $D_V$ ), and range diversity ( $D_R$ ), defined as functional diversity within a TL, diversity of TLs, and degree of trophic omnivory, respectively, with a formula of  $H' = D_H + D_V - D_R$ . From a comparative analysis of three riverine systems, Kato et al. (2018) concluded that  $D_H$  can explain spatiotemporal variations in the diversity of stream macroinvertebrate communities. This may also hold true for stream communities in our study watershed (Ishikawa et al., 2014). Negative BEF can be observed through between-community comparisons if more diverse primary consumers ( $TP = 2$ ) increase their relative biomass, leading to a lower  $i$  TP. In contrast, for vertical diversity, the spatial variation in  $i$  TP is much greater for our coastal communities (range = 1.69) than the stream counterparts (0.40; Ishikawa et al. 2017). Therefore, it is likely that positive BEF in coastal communities can be explained by vertical diversity and negative BEF in stream communities by horizontal diversity of primary consumers, resulting in contrasting BEF patterns.

## SIZE-BASED FOOD WEBS AND ECOSYSTEM FUNCTIONING

Body size plays a significant role in aquatic food webs based on the general rule that larger predators eat smaller preys (Barnes et al., 2010; Cohen et al., 2003; France et al., 1998; Jennings et al., 2002a; Jonsson et al., 2005). Using the regression slope of TP against body mass in Figure 2a, we calculated the predator-prey body mass ratio (PPMR) using Eq.  $10^{(1/\text{slope})}$  (see Jennings et al. 2002). The PPMR was  $10^{5.21}$  for all pooled data and  $10^{3.03}$  when data on gastropods were excluded. These ratios were much higher than the literature average ( $10^{0.96 \pm 0.05\text{SE}}$ ) for lake invertebrates and comparable to lake ectothermic vertebrates ( $10^{4.15 \pm 0.18\text{SE}}$ ; Brose et al., 2006). Gastropods exhibited the most dominant biomass abundance in our study communities. They feed on small-sized preys, such as benthic microalgae and microplankton-derived POM, and reach their largest size among macroinvertebrates based on predator resistance due to their armored traits. This explains why PPMR is much higher for the whole community of coastal macroinvertebrates in Lake Biwa. Such predator-resistant taxa are often decoupled from the rest of the food web, as observed in a trophic cul-de-sac (Bishop et al., 2007), which makes the scaling allometry of size-based food webs non-linear (Jennings et al., 2002b).

Although our field research stresses the importance of unraveling BEF relationships in real food webs with multitrophic interactions, such a correlational approach limits our ability to fully understand the causal mechanisms that explain why more diverse communities show higher  $i$  TP. There are alternative but not mutually exclusive mechanisms for this. One possible mechanism is that the overall basal energy gained by consumers is greater in more diverse communities, and another is that the trophic transfer efficiency (TTE) is higher in more diverse communities.

Here, approximation allows us to test the former hypothesis. For simplicity, we assume that consumer biomass is trophically transferred at a given rate, irrespective of TPs, within a limited size range (0.03 mg to 1.33 g in dry weight) of benthic macroinvertebrate communities. Generally, TTE is defined as the production rate (biomass multiplied by the turnover rate) for two adjacent TLs (Lindeman, 1942). Based on a theoretical derivation of TTE for communities with a size-specific trophic structure (Andersen et al., 2009), Garcia-Comas et al. (2016) used the prey-predator biomass ratio as a proxy for TTE in aquatic food webs. Considering the small within-lake variations in coastal WT (range = 2.4°C–7.2°C), which is the primary factor affecting metabolic rate, using biomass measurements may be a valid approximation for TTE in our study communities. While setting the TTE at a given rate, we aimed to estimate consumer basal biomass ( $C_{BB}$ ), which is defined as a proxy for secondary production needed to support a whole consumer community, which was back-calculated to the primary consumer biomass using the following equation:

$$C_{BB} = \sum \{B_i \times (1/\beta)^{(TP_i - 2)}\} \quad (6)$$

where  $C_{BB}$  is the sum of the primary consumer biomass converted from  $B_i$ , the consumer biomass of taxon  $i$  with  $TP_i$ , and  $\beta$  the TTE. For  $\beta$ , we took the minimum (3.7%) and maximum (27.1%) TTE values



reported for benthic communities (Jennings et al., 2002b).

Our calculations revealed that  $C_{BB}$  significantly increases with increasing biodiversity within the possible TTE range (Figure 4). This means that more diverse communities rely on greater secondary production. Considering that primary productivity did not increase *i* TP, the underlying mechanism for this may be that an increase in resource use, but not resource availability, allows trophic transfer of more biomass to higher TLs *via* diverse trophic interactions. This suggests that positive BEF relationships may be more common in many multitrophic systems, where significant biodiversity effects on consumer biomass could not be detected when using *i* TP as an indicator for ecosystem productivity.

To test another hypothesis, we examined the relationship between TTE and *i* TP for metacommunities. We estimated the TTE for each local community from slope  $a$  of production against TPs for individual taxa (Box S2), according to the allometric scaling empirically formulated by Banse & Mosher (1980) for invertebrates. We estimated the TTE to be 9.77% for a local community, which is within reported literature values for benthic communities, but our estimates were unrealistic for many other communities due to insignificant slope  $a$  (Table S6). As aforementioned, a trophic cul-de-sac and allochthonous energy inputs from pelagic food webs may make allometric scaling non-linear and less significant in benthic macroinvertebrate communities, hindering us from testing the VDH under the assumption of size-based food webs.

Benthic macroinvertebrate communities form a part of lake food webs as intermediate consumers linking microalgae and vertebrate predators. The present study was conducted during the winter season when fish predators are absent or inactive in order to minimize their top-down effects on the size and trophic structure of localized food webs. During the productive season, however, coastal benthic food webs are linked with pelagic and adjacent coastal food webs *via* mobile predators, and eventually, the overall energy flows up to apex predators (e.g., a giant catfish with 3.75 of TP) of the whole lake food web (Okuda et al., 2020). In aquatic food webs, predators also increase their spatial movement scale as TPs increase with increasing body size (McCann et al., 2005; Tucker et al., 2014). Upscaling multitrophic consumer networks that link different ecosystem compartments to the whole ecosystem is a challenging and promising approach to gain better understanding of BEF relationships and achieve improved biodiversity management at the landscape level (Barnes et al., 2018; Eisenhauer et al., 2019; Gounand et al., 2018; Hines et al., 2015; Manning et al., 2019). Future studies should explore how highly compartmentalized and thus heterogeneous coastal food webs integrate mobile predators across spatiotemporal scales, which may allow for more accurate TTE estimates based on a rigid allometric relationship between body mass, biomass, and TP across the entire lake food web that encompasses size ranges from microscopic autotrophs to carnivorous megafauna.

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## Figure legends

**FIGURE 1** Comparison of the FCL, mean TP, and  $i$  TP between more diverse (A) and less diverse (B) communities. Triangles and squares indicate basal resources and circles consumers. The TP of each consumer can be quantitatively estimated *via* stable isotope analysis. Circle size with number and arrow thickness represent consumer biomass and trophic energy flows, respectively. The two communities have the same FCLs (4.0) and similar arithmetic mean of consumer TPs (i.e., 2.81 for community A and 2.80 for community B), whereas community A has higher  $i$  TP (2.62) than community B (2.42), indicating that more energy flowed up through the former food web. In dynamical aspects, a top predator population may be less stable in community B than A due to its smaller population size supporting less available energy, resulting in greater FCL fluctuation.

**FIGURE 2** Allometric relationships between body size and TP (a), between body size and numerical abundance (b), between numerical abundance and TP (c), and between biomass abundance and TP (d) in coastal benthic macroinvertebrate communities of Lake Biwa.

**FIGURE 3** BEF relationships in coastal benthic macroinvertebrate communities of Lake Biwa. Biomass as a universal measure of ecosystem productivity is plotted against taxonomic richness (a) or biodiversity index ( $H'$ ) (b). As indicators for trophic energy flows, the  $i$  TP (c) or maximum TP (d) is plotted against  $H'$ .

**FIGURE 4** Consumer basal biomass ( $C_{BB}$ ), back-calculated to the primary consumer biomass, against the biodiversity index ( $H'$ ) of macroinvertebrate communities with minimum ( $\beta = 3.7\%$ ) and maximum ( $\beta = 27.1\%$ ) TTE values.

## FIGURE 1

### Hosted file

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**FIGURE 2a**

**FIGURE 2b**

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**FIGURE 2c**

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**FIGURE 2d**

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**FIGURE 3a**

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**FIGURE 3b**

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**FIGURE 3c**

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**FIGURE 3d**

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**FIGURE 4**

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