

Research article

Different components of biodiversity mediate two ecosystem functions derived from naturally assembled filter-feeder communities

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Positive biodiversity–ecosystem functioning (BEF) relationships observed in experiments can be challenging to identify in natural communities. Freshwater animal communities are disproportionately harmed by global change that results in accelerated species loss. Understanding how animal-mediated ecosystems functions may change as a result of global change can help determine whether biodiversity or species-specific conservation will be effective at maintaining function. Unionid mussels represent half of imperiled species in freshwater ecosystems globally and perform important ecological functions such as water filtration and nutrient recycling. We explored BEF relationships for 22 naturally assembled mussel aggregations spanning three river basins. We used the Price equation to partition the contributions of species richness, composition, and context dependent interactions to two functions of interests: spatially-explicit standing-stock biomass (indirect proxy for function) and species-specific nitrogen (N) excretion rates (direct measure of N recycling). Random and non-random species loss each reduced biomass and N recycling. Many rare species with low contributions to biomass contributed to standing-stock biomass in all basins. Widespread species had variable function across sites, such that context dependent effects (CDEs) outweighed richness effects on standing-stock biomass in two basins, and were similar to richness effects in the third. Richness effects outweighed CDEs for N recycling. Thus, many species contributed a low proportion to overall N-recycling; a product we attribute to the high evenness and functional effect trait diversity associated with these communities. The loss of low-functioning species reduced the function of persisting species. This novel result using observational data adds evidence that positive species interactions, such as interspecific facilitation, may be a mechanism by which biodiversity enhances ecosystem functions. Our work stresses the importance of evaluating species-specific contributions to functions in diverse systems, such as nutrient cycling when maintaining specific animal-mediated functions is a management goal because indirect proxies may not completely characterize BEF relationships.

Keywords: biodiversity-ecosystem functioning, biogeochemical cycling, complementarity, ecosystem services, evenness, freshwater mussels

Introduction

Biodiversity loss strongly reduces ecosystem functioning, particularly in experimental settings (Tilman et al. 1997, Cardinale et al. 2012). Declining species richness and or loss of certain species (species composition or identity effects), both affect ecosystem function (Symstad et al. 1998, Dangles and Malmqvist 2004). However, there is a lack of consensus about whether this applies at broad scales in natural ecosystems (Jiang et al. 2008, Cardinale et al. 2012, Jochum et al. 2020). Understanding of the role of biodiversity in maintaining natural ecosystem function may be important to conservation and management given high rates of biodiversity loss and the relevance of natural communities to food webs and biogeochemical cycles (Mora et al. 2011, Isbell et al. 2017), but consensus is lacking (Srivastava and Vellend 2005).

Conclusions drawn from experiments investigating biodiversity–ecosystem functioning (BEF) relationships may not match those from natural communities. Evenness tends to be high in BEF experiments because abundances or biomass is established equally among species, but these properties can be variable among natural communities. For instance, low dominance is characteristic of experimental communities (Dangles and Malmqvist 2004), whereas strong dominance patterns are common in natural communities (McGill et al. 2007). Therefore, abundance-mediated BEF relationships may occur in natural communities, despite the lack of experimental evidence (Winfree et al. 2015, Genung et al. 2020). Furthermore, randomly assembled communities across levels of species richness are typical of BEF experiments, whereas natural communities lose species non-randomly such that the most extinction-prone species are the first to be lost (Smith and Knapp 2003, Larsen et al. 2005, Suding et al. 2005). Extinction proneness may be related to local abundance of a species or specific traits that leave species vulnerable to natural or human-caused disturbances, such as when overharvest of commercially valuable species leads to extinction and loss of function (McIntyre et al. 2007). If extinction-prone species also contribute disproportionately to ecosystem function, function mediated by extinction-prone species should deteriorate rapidly with species loss. Alternatively, if species that contribute disproportionately to ecosystem function are lost last, function should diminish slowly. Both cases can be compared to the null expectation of linear loss of function with decreasing richness, which occurs under the random loss scenario (Schwartz et al. 2000). The order of species loss in natural ecosystems are still uncertain in most cases (Wardle et al. 2011) and whether such effects scale across space remains an important conservation issue. Because biomass or abundance can reasonably predict function for a suite of ecosystem functions and services (Smith and Knapp 2003, Dangles and Malmqvist 2004, Hooper et al. 2005, Kremen 2005), species rich communities comprised of a few species with relatively high abundance (i.e. dominant species) and many rare species might weaken richness effects because rare species often contribute little to overall function.

Drawing conclusions from experimental settings about how different biodiversity components influence ecosystem functions in natural settings can be challenging. Inconsistencies between experiments and natural ecosystems are often associated with covarying ecological contexts that act on biodiversity effects (Tilman et al. 2014). These factors are closely coupled in natural ecosystems but decoupled in experiments. Inferences from small-scale biodiversity experiments still offer valuable insight that may be fundamental to management or useful in predicting ecosystem functioning when novel combinations of species emerge in the future (Jochum et al. 2020). Data on natural systems is critical to bridging gaps between experimental and real-world studies of BEF relationships (Manning et al. 2019), but are often associational and the components of biodiversity can be strongly correlated (Cardinale et al. 2012, Duncan et al. 2015, De Laender et al. 2016, Winfree et al. 2018, Jochum et al. 2020). Analytical tools that can separate the effects of the different components of biodiversity (species richness, composition and abundance) of natural communities on ecosystem function may be useful to rigorously separate the causal role of the different components of biodiversity to functions.

The Price equation has made more rigorous BEF analyses of natural communities possible (Fox 2006, Fox and Harpole 2008, Fox and Kerr 2012). The Price equation is based on the change in ecosystem function between a baseline site, with greater ecosystem function and a lower functioning comparison site. The total difference in ecosystem function between the two sites is partitioned into the five terms of the Price equation. Briefly, the five additive components reflect: 1) species richness losses that are random with respect to the ecosystem function the species provides (RICH-L); 2) species richness gains that are random with respect to an ecosystem function the species provides (RICH-G); 3) species composition effects that capture any non-randomness with respect to function of the species that were lost (COMP-L); 4) species composition effects that capture any non-randomness with respect to function of the species that were gained (COMP-G); 5) and context dependent changes in function of species shared by both sites context dependent effect (CDE). The RICH-L is conceptually like the richness effect in BEF experiments that attempts to remove composition effects through combinatorial experiments and statistical approaches (Hector et al. 2002), but they measure different things (Fox 2006, Fox and Harpole 2008, Fox and Kerr 2012). Conventional statistical models estimate the functional form of the relationship between ecosystem function and species richness (e.g. linear, log-linear) while controlling for other sources of variation (Hector et al. 2002). The RICH-L measures the absolute functional reduction that would occur with a given level of species loss, assuming species were lost randomly with respect to their functional contribution and is necessarily linear (Fox 2006). The COMP-L is the compositional and/or identity effect and measures the change in function attributable to the fact that a species is lost non-randomly with respect to its function. As an example, a positive COMP-L indicates that lower functioning species were lost, and therefore the

ecosystem will have increased function over what it would be if species loss were random. The last term, CDE reflects change in function independent of changes in richness. A suite of ecological settings can contribute to this sum, including abiotic conditions and species interactions that differ between sites (Fox 2006). For example, a species shared by two sites should increase function at the site with favorable conditions. In this case, the function at comparison site relative to the baseline site $CDE > 0$. This term is called the context dependence effect because it captures variation in species' functional contribution between two sites representing different ecological settings. The five terms are additive and the sum of RICH-L+COMP-L reflects the total change in function tied to both random and non-random components of species loss. The two terms for species gain, RICH-G+COMP-G, are comparable to those for species loss, but measure species gain effects. Including both species gains and species losses is an advantage of this approach in a BEF context because species composition is rarely nested across sites in nature, but it is often nested in experiments (Raffaelli 2004, Zavaleta and Hulvey 2004). For a review of the mathematical derivation of the Price equation used herein, we refer readers to Fox 2006, Kerr and Godfrey-Smith (2009), Fox and Kerr (2012), the supplemental material of Winfree et al. (2015) and to our supplemental R code (www.r-project.org).

Here, we use the extended Price equation to partition datasets on species-specific biomass and nitrogen recycling rates associated with naturally assembled mussel (family: Unionidae) aggregations spanning an aquatic biodiversity hotspot in North America. Mussels are long-lived (4 to > 100 years), sedentary, filter-feeders that live buried in benthic habitats (Haag 2012). Mussels occur in spatially and temporally stable multispecies aggregations where their densities can exceed other areas of the stream by ~ 10 to 100-fold. Unfortunately, mussels represent half of imperiled species in freshwater ecosystems globally, with 74% of North American species considered imperiled and at least 35 species considered extinct (Lopes-Lima et al. 2018, Patterson et al. 2018, Böhm et al. 2020). Mussels provide important ecological functions that couple energy and nutrients in water column and benthic habitats through filtration, biodeposition of feces and pseudofeces, and nutrient mineralization (Vaughn 2018, Vaughn and Hoellein 2018, Atkinson et al. 2023). Integrating indirect predictors of ecosystem function such as biomass alongside more direct predictors related to species functional effect traits may be useful when the management goal is to preserve a particular function, such as nutrient cycling. Mussel biomass has been used as a reasonable proxy for mussel-mediated functions in field- and lab-based experimental studies (Atkinson and Vaughn 2015, Hopper et al. 2018, 2021, Nickerson et al. 2019, 2021). However, depending on the function of interest, species-specific functional effect traits may shed more light onto assemblage-level function (Spooner and Vaughn 2008, Vaughn et al. 2008). As such we took an approach that investigated both standing-stock biomass and an ecosystem function provided by mussels that can directly and indirectly influence nutrient limitation

status, food web support, and microbially-mediated biogeochemical cycling (Atkinson et al. 2013, Atkinson and Vaughn 2015, Nickerson et al. 2019, Atkinson and Forshay 2022). Contemporary mussel communities contend with habitat degradation and faunal-wide die-offs that are sometimes enigmatic (Haag 2019). Thus, the need to understand BEF relationships for this group is elevated by the level of imperilment combined with the suite of mussel-mediated ecosystem functions.

Using mussels as a model, we address two specific questions: 1) what is the relative importance of changes in species richness, species composition, and environmental context in explaining variation in ecosystem function and services (i.e. biomass and nitrogen recycling)? 2) Is the order in which species are lost random with respect to function? Whereas these natural communities are more complex than our study can capture, these data reflect field measurements of standing stock mussel biomass and nitrogen recycling rates.

Material and methods

Study region

North American rivers support the one of the most diverse collections of freshwater mussels, with the Mobile and Tennessee River faunas comprising ~ 60% of the diversity (Parmalee and Bogan 1998, Williams et al. 2008). To address BEF relationships for mussels, we intentionally selected rivers that would reflect a natural gradient of mussel species richness across this region. The Paint Rock River, Bear Creek and Duck River are direct tributaries to the Tennessee River while the Sipsey River and Buttahatchee Rivers meet the Tombigbee River in the western half of the Mobile Basin. Bogue Chitto Creek and the Cahaba River flow into the Alabama River prior to joining the eastern part of the Mobile basin in southwestern Alabama. Although some species occur in each system, each major basin supports distinct faunal groups. Therefore, we defined the regional species pool as the group of all sites combined for each basin (Alabama Basin, Tombigbee Basin and Tennessee Basin) separately.

Quantifying standing stock biomass

Biomass is a key proxy for a suite of ecosystem functions and services mussels perform, such as water filtration (Vaughn 2018, Vaughn and Hoellein 2018). We quantified mussel biomass across 22 river reaches throughout 2018–2020 (Kelley et al. 2022): four in the Cahaba and two in Bogue Chitto Creek (Alabama), five in the Sipsey River, two in the Buttahatchee River (Tombigbee), four in the Duck River, three in the Paint Rock River, two in Bear Creek (Tennessee). Briefly, we snorkeled areas with known aggregations of mussels to delineate their spatial extent. We excavated 0.25-m² quadrats to 15 cm deep, traversing the river's width every 2.5 m at four random transects every 20 m along the entire mussel aggregation for Bear Creek, Sipsey River, Buttahatchee

River, Bogue Chitto Creek and reach lengths varied from 40–150 m. The Cahaba, Duck, and Paint Rock Rivers were surveyed using a fixed number of quadrats (80–100 quadrats) within each ~ 50–100 m mussel aggregation. We identified live mussels to species, measured the longest shell axis (mm), and returned them to the stream. Length–mass regressions were used to estimate species-specific soft tissue dry mass (STDM (g); (Atkinson et al. 2020). Mussel biomass for an aggregation was the sum of the sampled species-specific areal biomass (g STDM m⁻²) and is employed here as a proxy for ecosystem functions and services provisioned by mussels.

Quantifying mussel-mediated nitrogen recycling

We used previously published NH₄⁺ (hereafter N) excretion rates scaled to each mussel assemblage to address our questions pertaining to mussel-mediated N recycling (Hopper et al. 2021, 2022). Briefly, we measured N excretion rates for 1281 individuals from 34 species from July to September 2019 and 2020 when water temperatures ranged from 24.0 to 31.1°C. Nitrogen excretion rates were measured for at least five individuals of species common in surveys. We gently scrubbed and rinsed biofilms from the shells of each individual before placing them into separate plastic containers with 150–500 ml of filtered stream water (GF/F; 0.7 µm pore size; Millipore) depending on body size for 60–130 min. We incubated control containers without animals simultaneously. We measured the total shell length of the individuals after incubation, re-filtered the water to partition biodeposits (i.e. egesta) from the soluble nutrients (i.e. excreta), took 50 ml samples and kept them refrigerated (~4°C) until analysis. We used a Seal AQ300 discrete analyzer (Seal Analytical) to analyze NH₄⁺ using the phenol method for filtered excretion samples. Per capita excretion rates of N for each species were calculated as the difference between containers with animals and the controls while correcting for water volume. We scaled per capita N excretion rates to the assemblage level to by combining species-specific excretion rates and biomass from the quantitative surveys to estimate areal N excretion rates for the sampled area (µmol N m⁻² h⁻¹).

Applying the Price equation partition to assemblages

We used the Price equation partition developed by Fox (2006) to explore biodiversity–ecosystem function relationships for the 22 naturally assembled mussel aggregations. In our study we use the Price equation to compare two ecosystem function metrics (standing stock biomass and assemblage level N recycling) between a baseline site with *s* species and a comparison site (multiple sites in this case) with *s'* species, with the goal of partitioning the differences between the two sites into ecologically relevant components characterizing different underlying mechanisms (e.g. loss of species richness from the baseline site). Species at the baseline site are analogous to individuals of an ancestral population in evolution, with the species at the comparison site analogous to a descendent

population (Kerr and Godfrey-Smith 2009, Fox and Kerr 2012). This framework is useful to our study because the approach compares observed sites to one another, instead of a null expectation, and therefore does not require data on species' functional contributions in monoculture as in most BEF experiments (Fox 2005).

We calculated the Price equation using mussel standing stock biomass (g STDM m⁻²), and areal N excretion rates (µmol m⁻² h⁻¹) for each species at a site. In addition to comparing the five terms separately, we compared CDE to the combined random and non-random component of species loss (RICH-L + COMP-L), species gain (RICH-G + COMP-G) and sum of all species-related effects, including both random and non-random losses, and both loss and gain of species (RICH-L + RICH-G + COMP-L + COMP-G). To make the relative strength of each of the terms comparable across basins that had different changes in standing stock biomass and areal N recycling, we present results for each site normalized by the strongest of terms for a given comparison to rescale values between -1 to 1. Analyses were conducted with R ver. 4.0.5 (www.r-project.org). In addition, as CDE may vary because of variation in the identity of species lost and gained between the baseline and comparison site, we evaluated the direction and strength of correlations between CDE and non-random species loss (COMP-L) along with CDE and species gain (COMP-G) effects.

We compared spatially explicit observed biomass and aggregate N-recycling estimates of the highest functioning site (baseline site) to each lower functioning site (comparison site) within each basin. The baseline site was not the same site for each function. The diversity gradient observed within each river is not strictly nested. Whereas sites within a basin are separated spatially, we make no assumptions regarding why sites contain subsets of species from the baseline site. Additionally, the choice to use the 'highest-functioning' site as the baseline constrains the sum of the terms to negative values but does not constrain the relative importance of the five terms, which is the primary concern here. Comparing low functioning sites to the highest functioning site makes our results more interpretable with respect to which components of biodiversity are most important in altering function across space.

Results

Quantitative surveys and mussel-mediated N recycling rates

We collected 4156 individuals belonging to 68 species during quantitative surveys. Species richness ranged from 4–12, 5–20 and 11–32 for the Alabama, Tombigbee and Tennessee basins, respectively. Standing stock biomass (g STDM m⁻²) ranged from 0.5–16.4, 0.9–11.4 and 1.9–40.2, for the Alabama, Tombigbee and Tennessee, respectively. Mussel-mediated N recycling rates (µmol N m⁻² h⁻¹) ranged from 1.08–6.40, 1.65–23.73 and 2.11–27.29 for the Alabama, Tombigbee and Tennessee basins, respectively.

Price equation terms: standing-stock biomass

The Price equation terms were qualitatively similar for all three river basins, with similar rank order of the five terms (Fig. 1). The random species loss effect (RICH-L) had a strong negative effect in each basin, suggesting that if species were randomly lost with respect to their function, standing-stock biomass would be strongly reduced. Each basin's random gain species effect (RICH-G) was positive but not as strong as the RICH-L effect was on standing-stock biomass. The species composition effects (COMP-L and COMP-G) in each basin contrasted their respective richness effects on biomass and had smaller effects. This means that most species contributing to richness have lower than average contributions to standing-stock biomass. The CDE term was negative for all basins but similar to RICH-L indicating that context dependent changes in the function of shared species at the baseline and comparison sites alter standing-stock biomass as much as species richness.

Additive combinations of the Price-equation terms: mussel biomass

The total effect of species loss on biomass, including both random and non-random components (RICH-L + COMP-L), was always negative but the strength of the effects varied among basins (Fig. 2). The net effect of species gains on biomass, including both random and non-random components (RICH-G + COMP-G), was near zero for each basin. The sum of all species-related effects on biomass, including both random and non-random losses and gains of species (RICH-L + RICH-G + COMP-L + COMP-G), was always negative because of the strong contribution from species loss effects. The effect of ecological context (CDE) on biomass was negative in all basins, but was particularly strong in the Alabama and Tombigbee, indicating that the biomass of species shared by sites in those systems is suppressed at comparison sites relative to the baseline site. This means there are typically more species at sites with more biomass and that changes in species

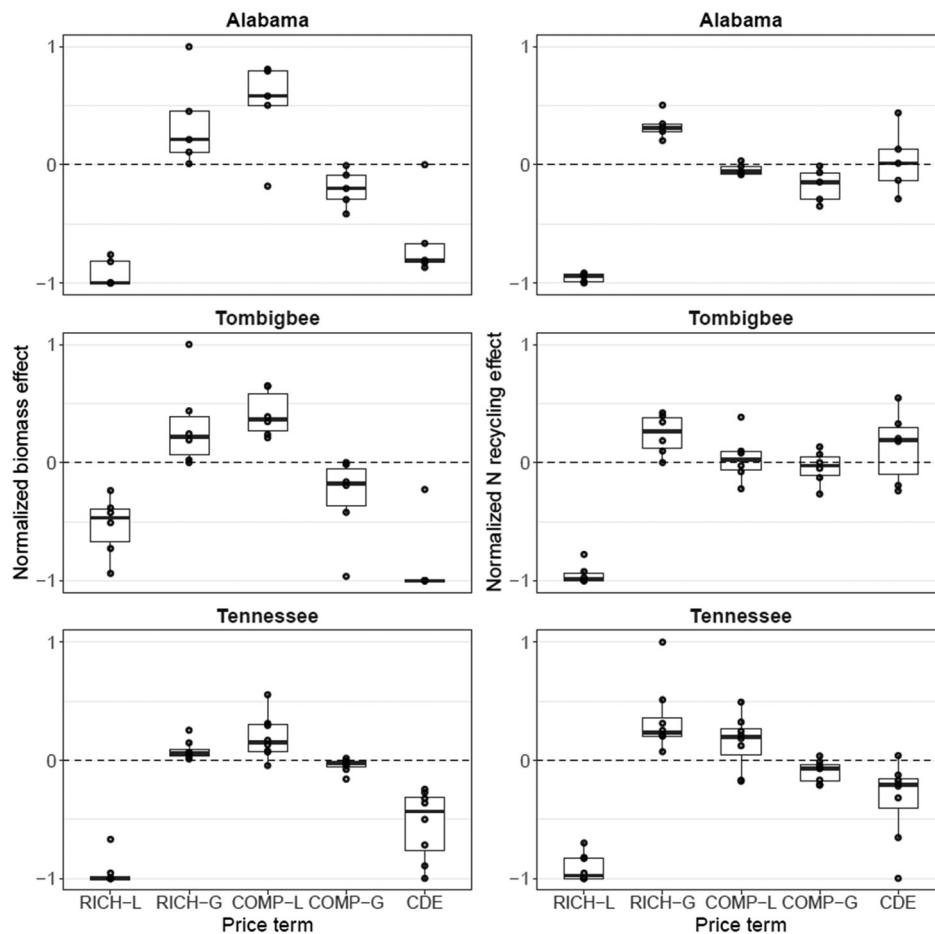


Figure 1. Relative importance of the five terms in explaining differences in standing stock biomass (left) and mussel-mediated nitrogen recycling (right) across sites. Y-axis for each graph is originally in units of soft-tissue dry mass (g m^{-2}) and areal excretion rates ($\mu\text{mol N m}^{-2} \text{h}^{-1}$) but has been normalized such that it represents the relative strength of the five effects. Boxes encompass the 25th–75% quartiles and whiskers extend to 1.5 times the interquartile range.

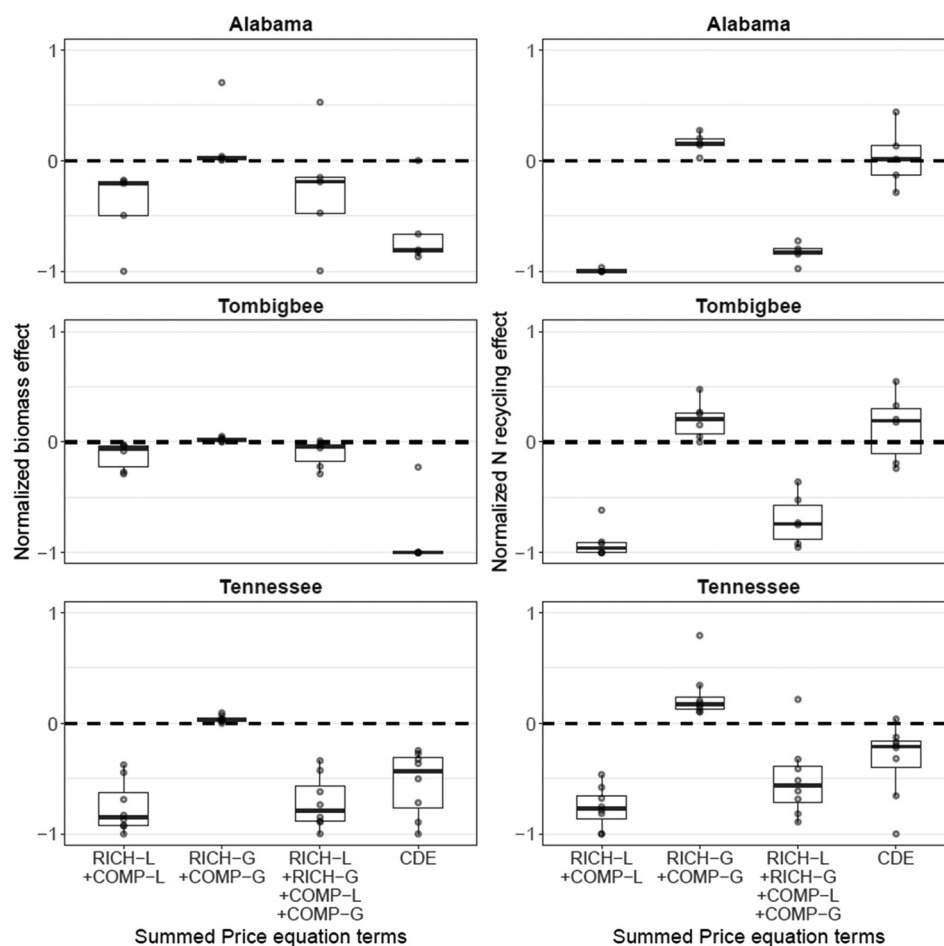


Figure 2. Comparing the original context dependence effect (CDE) with three combinations of Price equation terms representing effects of total changes in function due to species loss (RICH-L + COMP-L); total changes in function due to species gain (RICH-G + COMP-G); and total changes in richness and composition (RICH-L + COMP-L + RICH-G + COMP-G). Y-axis for each graph is originally in units of soft-tissue dry mass (g m^{-2}) and areal excretion rates ($\mu\text{mol N m}^{-2} \text{h}^{-1}$) but has been normalized such that it represents the relative strength of the five effects. Boxes encompass the 25th–75% quartiles and whiskers extend to 1.5 times the interquartile range.

functioning between sites due to environmental differences reduces biomass more than richness effects in the Alabama and Tombigbee basins. The CDE was still negative in the Tennessee Basin but was more similar to the species effects (Fig. 2). This means there are typically more species in sites with more biomass and that changes in species functioning between sites due to environmental context reduces biomass as much as richness effects.

Price equation terms: mussel-mediated nitrogen excretion

The Price equation terms for N-recycling were qualitatively similar for all three river basins, with similar rank order of the five terms (Fig. 1). The effect of random species loss (RICH-L) was the strongest and reduced mussel-mediated N recycling. The random gain species effect (RICH-G) in each basin weakly increased mussel-mediated N recycling. Both species composition effect (COMP-L and COMP-G) terms were nearly zero for all three basins. This suggests that species richness strongly influences mussel N-recycling in all three

systems and most species have below average contributions to N-recycling. Each basin's CDE term was near-zero or small, meaning either no species' N-recycling contributions are context dependent or that species' N-recycling contributions are context dependent in opposite ways (Fox 2006).

Additive combinations of the Price-equation terms: mussel-mediated nitrogen recycling

As for standing-stock biomass, the total effect of species loss on mussel-mediated N recycling, including both random and non-random components (RICH-L + COMP-L), was always negative (Fig. 2). The net effect of species gains toward N recycling by mussels, including both random and non-random components (RICH-G + COMP-G), was positive but relatively weak for each basin. The sum of all species-related effects on mussel-mediated N cycling, including both random and non-random losses and gains of species (RICH-L + RICH-G + COMP-L + COMP-G), was consistently negative because of the strong effects from both species loss terms. The CDE on N recycling in all basins was nearly zero and weaker

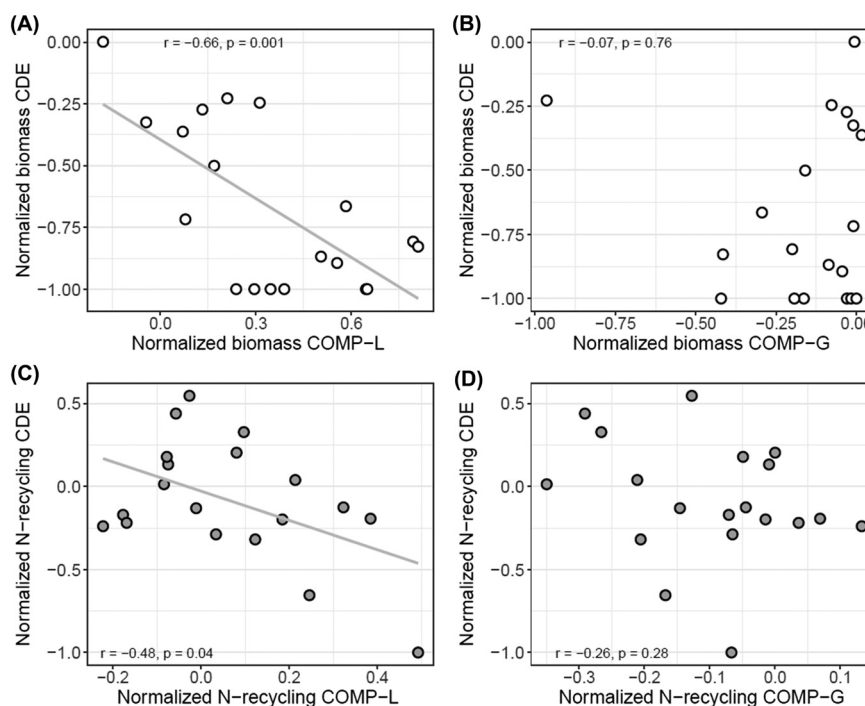


Figure 3. The CDE as a function of the COMP-L (A) and COMP-G (B) for mussel standing-stock biomass. The CDE as a function of the COMP-L (C) and COMP-G (D) for mussel mediated N-recycling. Significant correlations are indicated with lines. Correlation coefficients and p-values are shown for each.

than the combined species effects, indicating sites with greater N recycling by mussels had more species and species that turn over between sites have low functional contributions, while species shared between sites have similar function at both sites.

Species identity as a driver of context dependent effects on mussel ecosystem function

The CDE may vary between the baseline site and comparison sites because of the identity of lost and gained species. For example, gaining a highly competitive, high-biomass species might reduce the biomass of persisting species, while losing a highly competitive, high-biomass species would be expected to have the opposite effect (Fox and Harpole 2008). Our results contrast this expectation, in that there is no correlation with COMP-G for either standing-stock biomass or N-recycling, indicating that species gained from the baseline to comparison sites contribute below average functions compared to persisting species (Fig. 3). However, COMP-L was negatively correlated with CDE for both standing-stock biomass and N-recycling functions. This indicates that losing many low-functioning species from the baseline to the comparison sites suppresses the functioning of remaining species.

Discussion

Our results for naturally occurring freshwater mussel assemblages show some similarities and differences with small-scale BEF experiments and observations from other natural

systems. Similar to BEF experiments, we found that random loss of species and species identity both can have strong effects on ecosystem functions contributed by assemblages of mussels, although interpretation of these effects in experimental settings remains contentious (Grime 1997, Huston 1997, Wardle et al. 2000, Loreau et al. 2001). Our data reflect the distribution of species biomass in natural systems, which buffered the effects of random species loss on standing-stock biomass and N-recycling in different ways. Species loss was typically non-random with respect to each function, with many lower functioning species contributing to richness. This result is consistent with other Price equation studies using observational datasets and the typical skewed distribution of species abundances observed in natural systems. Natural variation in species distributions and N-recycling traits led to contrasting results for each mussel-mediated function. The total observed effect of species loss on function (RICH-L+COMP-L) was weak for standing stock-biomass in two systems (Alabama and Tombigbee) but was strong for N-recycling in all three systems. Another key result is that CDEs attributed to between site differences had stronger effects on standing-stock biomass than all species effects combined in the two systems with strong species effects, but often had minimal to no effects on N-recycling. Since BEF experiments standardize abundances of species in an assemblage, the likelihood that richness effects will be found increases because evenness maximizes complementarity effects that yield positive species richness-function relationships (Cardinale et al. 2005, Crowder et al. 2010). Yet, species abundances vary in natural communities and can be

a key factor in offsetting random species loss (Winfree et al. 2015, Genung et al. 2020). Thus, when deciding between biodiversity or function focused conservation plans, standing-stock biomass may provide a useful proxy for potential functions and services mediated by animal communities, but species-specific functional effect traits should be considered if maintaining particular functions is the management goal.

Sites with greater standing-stock biomass and N recycling by mussels were typically species-rich and most species that were lost or gained between the baseline and comparison sites had lower than average contributions to each function. Thus, the total effect of species loss (RICH-L+COMP-L) tends to weaken, particularly for standing stock biomass, but not for N-recycling. N-recycling by mussels in these systems was mediated by many species and CDEs were less important. The fact that many species additively contribute to ecosystem functions is a novel result of our study relative to other BEF studies in natural systems, likely due to the use of naturally occurring mussel assemblages. Studies of vertebrate and invertebrate communities from real-world terrestrial systems have shown a few common species contribute most to ecosystem functions (Kleijn et al. 2015, Winfree et al. 2015, Mateo-Tomás et al. 2017). In contrast to those systems, the potential for function to be dominated by a few species is low in mussel aggregations because a common characteristic of most North American mussel communities is high evenness, with a single species rarely composing > 50% of an assemblage (Haag 2012). Altogether these results highlight the value of prioritizing conservation of species-rich watersheds where more species additively contribute critical ecosystem functions and services rather than focusing on species-specific protection (Srivastava and Vellend 2005, Elkins et al. 2019).

CDE differed between the two functions of interest. Standing-stock biomass was strongly controlled by ecological differences that variably affected the performance of species shared by the baseline and comparison sites. On the other hand, mussel-mediated N-recycling reflected species' function that was not context dependent between the baseline and comparisons sites. However, the near zero CDE for N-recycling function could also arise because shared species have functional contributions that are context dependent but in opposite ways (Fox 2006). Such context dependent responses are challenging to interpret, but we attribute the results at least partially to the identity of the species present. The negative correlation between species identity effects (COMP-L) and CDE for both mussel-mediated functions demonstrates that the loss of low-functioning species does not increase the function of persisting species, but instead reduces their functions. The strength of this relationships was stronger for standing-stock biomass than for N-recycling. This novel result from our study adds evidence that positive species interactions, such as interspecific facilitation, may be a mechanism by which biodiversity enhances the functioning of ecosystems (Cardinale et al. 2012, Albertson et al. 2021). Although disentangling the underlying mechanisms of this result is beyond this study (Fox 2006, Fox and Harpole 2008), complementarity is hypothesized to play a strong part in maintaining the

density and diversity of mussel aggregations. More broadly, this result highlights the key role of environmental context, species occurrences, and abundance fluctuations, toward functions of animal communities and emphasizes the importance of studying BEF-relationships in natural systems.

While our data represent naturally assembled communities, assessing the biodiversity–ecosystem function relationships in nature is more complex than our approach can fully characterize. For example, this approach is unable to identify species interactions that can influence BEF relationships, such as spatial or temporal complementarity. Additionally, we know how many species are lost between baseline and comparison sites, but we do not know the causal factors leading to species loss between sites (Fox and Kerr 2012). However, species losses between these sites might be caused by the same mechanisms that drive declines at broader scales. For instance, enigmatic mussel declines progress over decades from lower stream reaches toward headwater reaches (Haag 2019). Diversity and densities increase upstream in some rivers in the Tennessee and Alabama Basins compared to downstream reaches (Kelley et al. 2022, Hopper et al. 2022), whereas this is not the case in the two rivers representing the Tombigbee, but more extensive surveys of these systems are warranted. Finally, the Price equation only assesses underlying causes of ecosystem function changes across space in our study but does not provide details about the number of species needed to attain a level of ecosystem function. The sites surveyed for this study represent some of the most diverse and intact mussel assemblages remaining in this region of North America but do not represent the full extent of historical mussel diversity (Haag 2012). Thus, it is impossible to know the extent to which mussel-mediated ecosystem functions and service have been altered. Although challenges remain, our work is a first attempt at assessing how changes in species abundance and richness of diverse communities of filter-feeding mussels impact freshwater ecosystem function and services.

Conclusion

We demonstrated that important changes to ecosystem function can occur both in response to and independently of changes in species richness using a rigorous analytical approach applied to freshwater mussel communities. Our study of biomass and N-recycling suggests that species richness does not have consistent effects across ecosystem functions within and among distinct systems. This result stresses the importance of evaluating species contributions to particular functions, such nutrient recycling, because functional effect traits can vary among species such that a few species contribute more to a particular function, while many species, or different combinations of species are needed to contribute a similar proportion to another. Overall, we provide information about how changes in community composition, rather than solely biodiversity loss, could be linked to freshwater ecosystem functioning in an important biodiversity hotspot.

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Author contributions

Garrett W. Hopper: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Irene Sánchez González:** Investigation (supporting); Methodology (supporting); Writing – review and editing (equal). **Jamie R. Bucholz:** Methodology (supporting); Writing – review and editing (supporting). **Jeffrey D. Lozier:** Funding acquisition (equal); Methodology (supporting); Supervision (equal); Writing – review and editing (supporting). **Carla L. Atkinson:** Funding acquisition (equal); Methodology (supporting); Supervision (equal); Writing – review and editing (supporting).

Data availability statement

Data and supporting code are available at Open Science Framework <https://osf.io/n3c5s/> (Hopper et al. 2023).

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