Ecosystem size mediates the effects of resource flows on biodiversity and ecosystem function at different scales

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**Abstract**

Ecosystem size and resource flows are key factors driving biodiversity and ecosystem function. However, the question of whether and how these drivers interact has been largely overlooked. Here, we investigated how ecosystem size asymmetry affects biodiversity and function of two-patch meta-ecosystems connected through flows of non-living resources. We conducted a microcosm experiment, mimicking spatial resource flows between ecosystems of different sizes yet otherwise identical properties or between ecosystems of the same size. Meta-ecosystems with asymmetric ecosystem sizes displayed higher α- diversity but lower β-diversity and ecosystem function (total biomass) than their unconnected counterparts. At the same time, such an effect was not found for meta-ecosystems of identical patch sizes. Our work demonstrates how the size of ecosystems, interconnected via resource flows, can modulate cross-ecosystem dynamics, having implications for biodiversity and function across scales.

Keywords: homogenisation, differentiation, detritus, nutrients, scaling, subsidies, allochthonous input, disturbance, species-area relationship, landscape

**Introduction**

Ecosystem size is a key factor driving biodiversity. Ecologists have long known that larger ecosystems harbour more biodiversity than smaller ecosystems (species-area relationship; MacArthur & Wilson, 1963). The concept dates back to the late 1700s during the second Pacific voyage of James Cook. There, naturalists Johann Reinhold Forster and Georg Forster noted that 'Islands only produce a greater or less number of species, as their circumference is more or less extensive' (Forster, 1778), which has been empirically and experimentally corroborated many times since (e.g., Fukami, 2004; Losos & Ricklefs, 2009; Wilson, 1961). The various reasons why larger ecosystems harbour more biodiversity remains an ongoing area of research (Losos & Ricklefs, 2009),(Losos & Ricklefs, 2009), and the individual roles of different processes (e.g., speciation, dispersion) contributing to this pattern are still debated (e.g., Valente et al., 2020). The main explanation for this phenomenon has been that species go extinct at lower rates in larger ecosystems (MacArthur & Wilson, 1963, 1967), as they have more habitat types (Kallimanis et al., 2008; Williams, 1943), more niche diversity (e.g., Ren et al., 2022), and experience less ecological drift (e.g., Gilbert & Levine, 2017), with extensive theoretical, comparative, and experimental extensions (e.g., Hanski & Ovaskainen, 2000; Luo et al., 2022; Wang & Altermatt, 2019). Furthermore, ecosystem size can also influence ecosystem function (LeCraw et al., 2017a; Yang et al., 2021). For example, larger ecosystems can be more productive because their community composition allows them to use resources more efficiently (complementarity effects) (Delong & Gibert, 2019).

Furthermore, irrespective of their size, ecosystems are rarely isolated in space. Spatial flows and subsidies of non-living resources among ecosystems (e.g., leaf litter, carcasses, inorganic nutrients; herein, "resource flows"; see Gounand et al., 2018 for a review) are—next to ecosystem size—a key abiotic factor affecting biodiversity and ecosystem function. For example, salmon carcasses transported from rivers to land by wolves and bears bring abundant nutrients, which can decrease riparian plant biodiversity as they promote the dominance of some species (Hocking & Reynolds, 2011). Subsidies from marine algal wrack can either increase plant biodiversity on sand dunes (Del Vecchio et al., 2017) or decrease plant biodiversity in rainforests on tiny islands (Obrist et al., 2022). As another example, aquatic insects can increase the function (production) of riparian birds which feed on them (Nakano & Murakami, 2001). Supporting such empirical evidence, meta-ecosystem theory predicts that resource flows can affect biodiversity by modifying species interactions and persistence (Gravel, Mouquet, et al., 2010; Marleau et al., 2014; Peller et al., 2022). For example, resource flows can delay competitive exclusion by increasing locally available resources (Gounand et al., 2017) or instead prevent the local establishment of dispersing species by increasing the abundance of the resident competitors (Gravel, Mouquet, et al., 2010). Furthermore, resource flows should increase meta-ecosystem production if they transport resources from ecosystems good at producing biomass to ecosystems good at transforming non-living resources into consumers (Harvey et al., 2023). However, despite widespread recognition that ecosystem size and resource flows can, individually, affect biodiversity and ecosystem function, their interactive effect has largely been overlooked.

These two drivers–ecosystem size and resource flows–likely interact since ecosystem size influences the amount and the effect of resource flows. For example, the size of a body of water regulates the amount of resources it exports: the larger a lake or river, the more insects emerge from it per metre of reach (Gratton & Vander Zanden, 2009). Whereas, the size of the receiving watershed would determine the effects of aquatic resource import: for instance, the larger a watershed, the more diluted its fertilisation from salmon carcasses (Hocking & Reimchen, 2009). Also, larger islands that receive algal wrack and carrions from the ocean experience a more diluted positive effect on their secondary production (Polis & Hurd, 1995). As resource flows can influence biodiversity and ecosystem function, and ecosystem size can influence resource flows, the hypothesis that ecosystem size can influence biodiversity and ecosystem function through resource flows emerges naturally as a general concept.

Here, we tested if and how the size of interconnected ecosystems mediates the influence of resource flows on biodiversity and ecosystem function using a protist microcosm experiment (Altermatt et al., 2015; Benton et al., 2007; Cadotte & Fukami, 2005). We constructed two-patch meta-ecosystems connected by resource flows between ecosystems (for clarity we use “patch” as a synonym for “ecosystem”). We manipulated (i) the relative size of the two patches within the meta-ecosystem (symmetric vs asymmetric sizes) while keeping the total size of the meta-ecosystem constant and (ii) the connection between the two ecosystems (connected vs unconnected). Our results showed that ecosystem size asymmetry significantly influences biodiversity and ecosystem function through resource flows. Specifically, we observed resource flows increasing α-diversity and a decreasing β-diversity and ecosystem function (total biomass) in asymmetric meta-ecosystems when comparing their connected to their unconnected treatment. Contrastingly, resource flows did not affect α-diversity, β-diversity, or ecosystem function in symmetric meta-ecosystems, as shown by comparing symmetric connected and unconnected meta-ecosystems.

**Materials and methods**

*Experimental design*

We studied how asymmetry in ecosystem size mediates the effect of resource flows on biodiversity and ecosystem function in meta-ecosystems via a microcosm experiment involving an aquatic protist community (Altermatt et al., 2015). Specifically, we compared two-patch meta-ecosystems with either symmetric or asymmetric sizes (yet identical total meta-ecosystem size), and either connected by non-living resource flows or unconnected (see Fig. 1). All replicates started with identical initial communities and we evaluated the resource flow effect by comparing connected meta-ecosystems with pairs of unconnected ecosystems of the same size and symmetry properties (controls, referred to as unconnected meta-ecosystems). Meta-ecosystems were of identical total size (volume: 45 ml), with symmetric meta-ecosystems composed of two identical sized patches (each 22.5 ml), and asymmetric meta-ecosystems composed of a 7.5 ml and a 37.5 ml patch, respectively. We call symmetric meta-ecosystems MMMM and asymmetric meta-ecosystems SLLs, with S, M, and L referring to Small (7.5 ml), Medium (22.5 ml), and Large (37.5 ml) ecosystems, respectively, and subscripts referring to the size of the connected ecosystem. We call the respective unconnected controls of the resource effect (unconnected meta-ecosystems) MM and SL, respectively (without subscripts).

To understand the effects of the size of the connected ecosystem, we also established two control meta-ecosystems connecting two small ecosystems (SSSS) and two large ecosystems (LLLL), respectively (see Fig. 1) and compared local ecosystems that were connected to either small or large ecosystems. Specifically, we compared SL with SS and LS with LL. All the above combinations were replicated five-fold.

*Experimental setup*

Our initial communities consisted of eight heterotrophic ciliates (*Blepharisma* sp., *Colpidium* sp., *Loxocephalus* sp., *Paramecium aurelia*, *Paramecium caudatum*, *Spirostomum* sp., *Spirostomum teres*, and *Tetrahymena cf. pyriformis*), two mixotrophic ciliates able to photosynthesise (*Euglena gracilis* and *Euplotes aediculatus*), and one rotifer (*Cephalodella* sp.), subsequently all referred to as “protists”. We cultured protists in pre-autoclaved bottles with standard protist medium (0.46 g of Protozoa Pellet by Carolina per L of water) and a bacterial mix (*Serratia fonticola*, *Bacillus subtilis*, and *Brevibacillus brevis*) serving as food for protists and constituting 5 % of the total culture volume. See Altermatt et al. (2015) for further details and protocols.

At the start of the experiment (day zero), we established a master mix of the protist community consisting of all 11 species mixed at 1/11 of their carrying capacity and volumetrically supplemented with 15 % standard protist medium. The experiment was conducted in 50 ml centrifuge tubes, with each tube representing an ecosystem. We pipetted 7.5 ml, 22.5 ml, and 37.5 ml of the master mix to constitute the small, medium, and large ecosystems, respectively. The replicates were randomised in position and kept in an incubator at 20 °C with constant lighting for the remainder of the experiment.

*Disturbances and* *non-living* *resource flows*

Because it is logistically difficult to separate living species from non-living resources in aquatic microcosms, spatial flows were associated with disturbances, whose mortality effect was controlled in the unconnected ecosystems Jacquet et al., 2020). Such temporal synchrony of resource flows and disturbances also commonly reflects natural systems in which disturbance and subsequent flows or resources are coinciding (e.g., forest fire, landslide, etc.). Specifically, every four days (starting on day five), we boiled a fixed volume of the community of each ecosystem for 30 seconds to kill every organism, thereby turning all organisms into non-living resources (i.e., local disturbance). After boiling the sampled volumes in a microwave, we let them cool down to room temperature and then poured them into the connected recipient ecosystems, creating bidirectional resource flows. We investigated whether resource flow level affects our results by considering two levels of disturbances, either boiling 5.25 ml of each ecosystem (low disturbance) or boiling 6.75 ml (high disturbance), which represented 70 % and 90 % of a small ecosystem, 23.3 % and 30 % of a medium ecosystem, and 14 % and 18 % of a large ecosystem, respectively in the low and high disturbance treatments. In the unconnected controls, the same volume was disturbed but poured back to the originating ecosystem to control for the mortality associated with cross-ecosystem resource flows (‘resource retention’ in Fig. 1). We here focus on the results of the high disturbance level yet give the results for the low one in Appendix S4. Resource flow effects on biodiversity were mediated by patch size at both low and high disturbance, but effects on ecosystem function only at high disturbance. Throughout the experiment, the total volume of the ecosystems was held constant by compensating evaporation loss with deionised water (see Appendix S1).

*Sampling*

To determine the abundance, species identity, biomass, and traits of protists in each ecosystem, we took videos of 0.2 ml samples from each ecosystem every four days, starting at day zero. While the first two time points (days 0 and 4) took place before the first disturbance, all other time points were always taken three days after the disturbance to leave communities to recover as much as possible from disturbances. We took a 5 s-video of each sample at 1.6x magnification, using a Hamamatsu Orca Flash 4.0 (Herrsching am Ammersee, Germany) camera. During the last two time points (days 24 and 28), we took two samples per ecosystem to reduce the sampling error and increase the chances of detecting individuals at low densities (each metric was averaged across the two samples). We also took videos of all protist monocultures to construct a training dataset of each species’ traits for species identification. We took sufficient videos of each monoculture to capture at least 100 individuals of each species.

*Quantifying biomass and biodiversity*

We used the R-package BEMOVI to identify and characterise protist species in the communities (Altermatt et al., 2015; Pennekamp et al., 2015; Pennekamp & Schtickzelle, 2013). We first extracted moving particles' traits (e.g., speed, shape, size) in the videos. We then used these traits to filter out particles that were not protists and obtain an average abundance of protist individuals per volume. We also measured protist biomass as our focal ecosystem function. We calculated the total area of protists (as area per volume medium) and subsequently used this “bioarea” as a proxy of biomass (hereafter referred to as “biomass”), which is a fair assumption given the roundish shape of protists (see also previous work using the same approximation; e.g., Jacquet et al., 2020; Pennekamp et al., 2018). We then identified protist species using a support vector machine model (Cortes et al., 1995; r-package “e1071”: Dimitriadou et al., 2006), employing traits extracted from species monocultures as predictor variables. Last, we calculated local biodiversity (α-diversity) using the Shannon Index. At the meta-ecosystem scale, we calculated biodiversity through (i) mean local biodiversity (mean α-diversity) as the Shannon Index averaged across ecosystems, (ii) among-community biodiversity (β-diversity) as the Bray-Curtis index, and (iii) total biodiversity (γ-diversity) as the total number of species persisting at the meta-ecosystem level.

*Statistical analysis*

To understand the effects of resource flows on biodiversity over time in symmetric and asymmetric meta-ecosystems, we performed statistical analysis using mixed-effect models with the 'lme4' package in R (Bates et al., 2015). The analysis excluded the initial two time points preceding the disturbances (grey zones in all figures), as their inclusion would interfere with our understanding of the impact of disturbances and resource flows.

At the meta-ecosystem level, to examine resource flow effects, we compared SLLS to SL and MMMM to MM. SL and MM were virtual meta-ecosystems created from unconnected ecosystems, that is, pairing two ecosystems to calculate the biodiversity and total biomass (yet without having these ecosystems connected by flows of resources). We constructed these virtual control meta-ecosystems by creating all possible pairs (without replacement) of unconnected ecosystems (25 SL pairs and 10 MM pairs). To test the influence of the resource flow connection on a response variable (α-, β-, γ-diversity, total biomass), we examined the effects of the resource flow connection and its interaction with time by comparing a full model (including the predictor variable interacting with time as a fixed effect) to a null model (including only time as a fixed effect) using ANOVA. Significant results indicated that the predictor variable interacted with time to affect the response variable. Similarly, we assessed the influence of the predictor variable alone (without its interaction with time) by comparing with ANOVA a reduced model (including the predictor variable and time as fixed effects) with the null model. Significant results indicated that the predictor variable affected the response variable without interacting with time. In the full, reduced, and null models, random effects considered the impact of replicates on the correlated intercept and the slope of the relationship between the response variable and time. We performed multiple comparisons iteratively when comparing (i) asymmetric connected to asymmetric unconnected meta-ecosystems and (ii) symmetric connected to symmetric unconnected meta-ecosystems, resulting in a distribution of p-values. Each iteration involved unconnected meta-ecosystems with differently paired ecosystems (without resampling). The presented p-values are the means of their respective distributions. See Table S1 for the p values associated with meta-ecosystem models.

At the local level, we investigated whether the size of the connected ecosystem influenced resource flow effects–comparing SL to SS and LS to LL–and whether resource flows had an effect when happening between ecosystems of the same size–comparing SS to S, MM to M, and LL to L. To test the influence of the resource flow connection or connected ecosystem size on a response variable (Shannon Index, biomass), we made the same model comparisons as at the meta-ecosystem level (but without iterations). See Table S2 for the p-values associated with ecosystem models.

**Results**

At the meta-ecosystem level, resource flows increased mean α-diversity, decreased β-diversity, and decreased total biomass in asymmetric meta-ecosystems (SLLS; Fig. 2: purple solid vs. dashed lines) but not in symmetric meta-ecosystems (MMMM; Fig. 2: green solid vs. dashed lines): SLLS had a higher mean α-diversity (p = 0.019), lower β-diversity (p = 0.012), and lower total biomass (p = 0.003) compared to SL (purple lines in Fig. 2a, 2b, and 2d, respectively). MMMM had the same mean α- and β-diversity and total biomass (green lines in Fig. 2a, 2b, and 2d, respectively, p > 0.1) as MM. Resource flows did not influence γ-diversity in either asymmetric or symmetric meta-ecosystems. That is, SLLS had the same γ-diversity as SL (purple lines in Fig. 2c, p > 0.1), and MMMM had the same γ-diversity as MM (green lines in Fig. 2c, p > 0.1).

At the local level, small ecosystems that were connected to large ecosystems (SL vs. S) had higher biodiversity (Shannon Index) (solid vs. dotted brown lines in Fig. 3a, p = 0.002) and biomass (solid vs. dotted brown lines in Fig. 3b, p = 0.019, connection interacted with time) than when unconnected. This effect on biodiversity can be broken down into two components. First, the size of the connected ecosystem, as being connected to large ecosystems led to greater biodiversity (SL vs. SS; solid vs. dashed brown lines in Fig. 3a, p = 0.013). Second, the presence or absence of the connection, as small ecosystems when connected to other small ecosystems were more diverse (dashed vs. dotted brown lines in Fig. 3a, p = 0.012) than when unconnected (SS vs. S). We observed a similar but weak (marginally not significant) trend for biomass. That is, when a small ecosystem was connected to a large ecosystem, it led to greater biomass (solid vs. dashed brown lines in Fig. 3b, p = 0.060) than when connected to small ecosystems (SL vs. SS) and when a small patch was connected to another small patch sustained greater biomass (dashed vs. dotted brown lines in Fig. 3b p = 0.071) than when unconnected (SS vs. S).

Also at the local level, large ecosystems that were connected to small ecosystems were similar in their biodiversity (solid vs. dotted blue lines in Fig. 3a, p > 0.1) but had lower biomass (solid vs. dotted blue lines in Fig. 3b, p = 0.001) relative to when they were unconnected (LS vs. L). For large ecosystems, the connection to small ecosystems decreased their biomass (solid vs. dashed blue lines in Fig. 3b, p = 0.036) (LS vs LL). This effect was mediated by the size of the connected ecosystem, as when large ecosystems were connected to other large ecosystems, the effect was not observed (dashed vs. dotted blue lines in Fig. 3b, p > 0.1) (LL vs L).

Finally, in medium ecosystems we observed a weak, yet marginally non-significant, trend of resource flows increasing biodiversity (Fig. S1, p = 0.081) and biomass (Fig. S1, p = 0.062) in connected ecosystems relative to those that were unconnected (MM vs. M).

**Discussion**

We experimentally demonstrate in a proof-of-concept study that ecosystem size asymmetry can mediate the effects of bidirectional resource flows on biodiversity and ecosystem function. Meta-ecosystems with asymmetric patch sizes (SLLS) had more similar communities (lower β-diversity) and lower function (lower total biomass) but maintained higher biodiversity across the two local patches (higher mean α-diversity) than asymmetric yet unconnected meta-ecosystems (SL). The fact that these effects were not observed in meta-ecosystems with symmetric ecosystem sizes indicates a mediating role of ecosystem patch size. These results could be explained by the connection of a small ecosystem to a larger ecosystem resulting in an increase in biodiversity and biomass in the small ecosystem (SL had greater biodiversity and biomass than SS and S) while leaving the biodiversity of the large ecosystem unchanged (biodiversity was similar between Ls, LL, and L) and decreasing the biomass of the large ecosystem (LS had lower biomass than LL, and L). As small and large ecosystems were identical aside from their size (resource concentration, community composition, etc.), the effects of the connection can be attributed to ecosystem size. Ultimately, our findings suggest that considering the size of interconnected ecosystems can help us understand how bidirectional resource flows shape biodiversity and function.

Notably, we found resources flowing between ecosystems of different sizes impacted both α- and β-diversity by increasing the biodiversity of the smaller patch within the meta-ecosystem. Furthermore, they decreased total meta-ecosystem biomass by decreasing the biomass of large patches, which was not compensated by the congruent increase of biomass in small patches. We suggest three mutually non-exclusive mechanisms by which small patches may have gained biodiversity and biomass while large patches lost biomass compared to the unconnected control patches.

First, resource quantity: small ecosystems may have had a net import of resources. Although the volume exchanged between ecosystems was identical, larger ecosystems had a greater dominance of photosynthetic species than small ecosystems (Fig. S9), which might have increased carbon availability more in large versus small ecosystems. Consequently, small ecosystems may have imported a greater quantity of sequestered carbon from large ecosystems relative to what they exported, creating an emergent source-sink dynamics of resources (sensu Gravel, Guichard, et al., 2010 and Loreau et al., 2013).This net import of resources could have allowed small ecosystems to sustain more biodiversity as more resources allow more individuals to persist, promoting a greater abundance of rare species and preventing their extinction (species energy theory, see Wright, 1983). The net import of C-rich necromass to small patches and net export from large patches could have increased basal resources for bacteria–primary resources of protists–in small patches at the detriment of large patches, which cascaded up on biomass production: the decrease in meta-ecosystem biomass caused by a larger decrease in biomass in large patches than the increase in biomass in small patches could be explained by a net movement of resources to the small patch, which could have had lower recycling rates. Indeed, higher recycling rates in larger patches are reasonable to expect, as they can be found in nature (Donghao et al., 2021; LeCraw et al., 2017; Yang et al., 2021). Furthermore, large patches in our experiment had higher biodiversity, which can be positively related to recycling rates as well (van der Plas, 2019). In natural ecosystems, we would expect differences in ecosystem sizes to lead to differences in the quantity of resources exchanged as well, potentially through different mechanisms. The trophic island biogeography theory (Gravel et al., 2011; Holt, 2009), for instance, predicts variation in the ratio between autotrophs and consumers between ecosystems of different sizes. Gravel et al. (2011) supported this prediction by parameterising a trophic metacommunity model using 50 pelagic food webs (Havens, 1992) and showing that larger ecosystems contained more consumers relative to autotrophs. The explanation for this result is that in larger ecosystems consumers are more likely to find one of their prey and, therefore, establish.

Second, resource quality: small ecosystems may have had a net import of detritus (protist detritus) of better/different quality. Protists can be rich in PUFAs (Boëchat et al., 2007; Martin-Creuzburg et al., 2005, 2006), while most bacteria are deficient in PUFAs (Okuyama et al., 2007; Ratledge, 2001). If the detritus of protists was of higher quality as a resource for the local community compared to other resource forms (e.g., bacterial detritus, inorganic nutrients), it would have sustained a higher growth of individuals and, therefore, higher biodiversity in the small ecosystem. Consequently, the movement of resources of higher quality to the small ecosystem and of lower quality to the large ecosystem would have increased the function of small ecosystems and decreased the function of the large ecosystem, as a meta-ecosystem model showed that good quality subsidies should increase the function of the receiving ecosystem and bad quality subsidies should decrease it (Osakpolor et al., 2023). We would also expect this mechanism, where size differences between connected ecosystems create differences in the quality of resources exchanged and cascade to influence biodiversity and function, to occur in natural ecosystems, potentially though different mechanisms. For example, ecosystems of different sizes can have different biomass distributions across trophic levels (Petermann et al., 2015), with often higher maximal trophic levels in larger ecosystems (Guo et al., 2023; Post et al., 2000; Ward & McCann, 2017) Moreover, different trophic levels might produce detritus of different qualities as consumers often have higher nitrogen content than producers (Elser et al., 2000). As a consequence, the relative quantities of biomass in trophic levels determine the overall quality of the resources exchanged with other ecosystems, which depends on ecosystem size.

Third, resource heterogeneity: small ecosystems might have imported resources that were more heterogeneous than their own. As there was greater protist biodiversity in large than in small ecosystems, the corresponding exported detritus might have been more diverse with respect to carbon compounds and biomolecules, potentially creating more niches for protists to coexist in small ecosystems (resource diversity hypothesis, Lawton, 1983). The positive correlation between detritus heterogeneity and consumer feeding on it has been observed in nature (Moore & William Hunt, 1988; Yodzis, 1988). Moving resource heterogeneity more to the small patches could have decreased the productivity of the meta-ecosystem by moving them to an ecosystem with slower recycling. We expect that also in nature differences in ecosystem size would cause differences in resource heterogeneity and, therefore, cause resource flows to influence biodiversity and ecosystem function.Larger ecosystems generally have higher biodiversity within trophic levels (horizontal diversity, MacArthur & Wilson, 1963, 1967) and higher number of trophic levels (vertical diversity or maximum food chain length, Guo et al., 2023; Post et al., 2000; Ward & McCann, 2017). Such higher biodiversity should translate into a change in their biomass composition (e.g., biodiversity can be related to stoichiometry, Striebel et al., 2009) and higher resource heterogeneity, which would constitute more heterogeneous resources that would determine the effects of resource flow on biodiversity and function.

Our study highlights that the size of the donor ecosystem, where resource flows originate, can shape the effect of resource flows on a recipient ecosystem’s biodiversity. In particular, in our experiment, biodiversity increased in ecosystem with small patch size when connected to ecosystems of large patch size. The subsidised island biogeography theory (Anderson & Wait, 2001) states that resources flowing into an ecosystem can influence its biodiversity, making its biodiversity deviate from what we would expect from species-area relationships, especially in small ecosystems. There is some comparative evidence by field studies, for instance, with resource flows increasing the biodiversity of bird species more in smaller than in larger islands (Obrist et al., 2020). Here, we give a formal experimental corroboration that resource exchanged between differently sized ecosystems indeed has direct effects on biodiversity and ecosystem functions (e.g., biomass) and is directly modulated by the differential patch size. In particular, we highlight and discuss how the quantity, quality and heterogeneity of resource flows may be mediated by the size of the exporter ecosystem through a variety of mechanisms that would modulate the effect on the diversity and functioning of the recipient ecosystem.

Decades of research on spatial subsidies have documented that donor ecosystems commonly vary in size. For example, islands which export nitrogen to coral reefs (Lorrain et al., 2017), kelp forests which exchange non-living resources with their adjacent intertidal zone (Tallis, 2009), or forests that export leaf litter to streams (Larsen et al., 2016). Moreover, evidence from natural systems supports our finding that the size of donor ecosystems can influence the biodiversity and function of recipient ecosystems. Such evidence is found in lakes and rivers embedded in terrestrial watersheds of different sizes. Notably, studies found that larger watersheds can (i) increase lake primary production, as they export more phosphorus (Knoll et al., 2003), (ii) sustain fewer lake consumers that rely on sediments, as they export lower quantities of sediments (lower water flow, gentler slopes, and increased sedimentation in terrestrial ecosystems) (Babler et al., 2011), and (iii) sustain longer river food chains, as they have more water flow, hence less hydrological variation and therefore a more stable environment (Sabo et al., 2010). This, in conjunction with our findings, suggests that subsidised island biogeography (Anderson & Wait, 2001) would gain in integrating how the size of the connected ecosystems mediates the effects of resources on the shape of species-area relationships and possibly changes this relationship. According to our results, we expect, for example, that the biodiversity of macroinvertebrates in a lake might be higher than expected by their area only (according to subsidised island biogeography) when the lake is connected to a larger rather than a small forest.

In conclusion, our experiment provides experimental evidence that asymmetry in ecosystem size can indirectly affect biodiversity and function in meta-ecosystems through its effects on a ubiquitous connection among ecosystems–spatial flows of resources. Consequently, this implies a need to consider how ecosystem size changes resource flow between ecosystems when aiming to generally understand the drivers of biodiversity and ecosystem function in spatially structured systems.

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**Figure legends**

Figure 1. Experimental design. SL = small ecosystem connected to large ecosystem, LS = large ecosystem connected to small ecosystem, SS = small ecosystem connected to small ecosystem, MM = medium ecosystem connected to medium ecosystem, LL = large ecosystem connected to large ecosystem, S = small unconnected ecosystem, M = medium unconnected ecosystem, L = large unconnected ecosystem. Red portion of the ecosystem with a lighting bolt = disturbed portion of the community turned into non-living resources and reciprocally exchanged between ecosystems (in connected ecosystems) or put back into the ecosystem (in unconnected ecosystems). Treatments shown in this figure were crossed with two levels of disturbance, low and high, and each treatment combination was replicated five times. This resulted in 120 microcosms paired in 60 meta-ecosystems.

Figure 2. Time series of biodiversity and biomass at a meta-ecosystem scale. (a) mean α-diversity (Shannon), (b) β-diversity (Bray-Curtis), (c) γ-diversity (species richness), and (d) total biomass (bioarea in mm2) of asymmetric and symmetric (purple vs green) and connected and unconnected (solid versus dotted lines) meta-ecosystems. For connected meta-ecosystems, dots represent means across replicates. For unconnected meta-ecosystems, dots represent the mean of all possible combinations of unconnected ecosystems assembled as virtual pairs of ecosystems with the respective ecosystem size structure. Error bars represent 95 % confidence intervals; vertical grey lines represent disturbance events followed by resource flows. Points were minimally jittered along the x-axis to make the figure clear. The area in grey indicates time points not considered for analysis, as meta-ecosystems were sampled before the first disturbance and resource flow.

Figure 3. Time series of biodiversity and biomass at a local scale. (a) biodiversity (Shannon index) and (b) biomass (bioarea per volume in mm2/ml) of small and large ecosystems (red vs blue lines) connected to an ecosystem of the same size, connected to an ecosystem of different size, or unconnected (solid vs dashed vs dotted lines). Dots represent means across replicates; error bars represent 95 % confidence intervals; vertical grey lines represent disturbance events followed by resource flows. All systems were sampled on the same day. Points were minimally jittered along the x-axis to make the figure clear. The area in grey indicates time points not considered for analysis, as ecosystems were sampled before the first disturbance and resource flow.

**Figures**

A diagram of a meta-ecosystems

Description automatically generated

Figure 1.

A graph of different colored lines

Description automatically generated

Figure 2.

A graph of different colored lines

Description automatically generated with medium confidence

Figure 3.

**Appendix S1 Experimental design: volume balance**

During the experiment, we kept the volume of ecosystems constant by replenishing the inevitable losses of volume that occurred through sampling and evaporation. Three days before each sampling day, we added the protist medium equal to the same volume to be sampled (0.2 ml) to avoid a decrease in total volume. Secondly, we counteracted evaporation losses by replenishing the volumes that evaporated with autoclaved deionised water. Right before the first two disturbances, we added 1.0 ml of deionised water to all tubes right before each disturbance. However, before the third exchange event, we observed slightly higher than anticipated evaporation rates, and the ecosystems were, on average, 1.17 ml (SD = 0.37) smaller than their initial volumes. Therefore, before the third exchange and after each subsequent exchange, we refilled the ecosystems with water until they reached their initial volume.

**Appendix S2 Mixotrophs-heterotrophs ratio analysis**

At the local level, to examine the effects of ecosystem size, we investigated whether the size of unconnected ecosystems influenced the ratio between mixotrophic and heterotrophic individuals (mixotrophs-heterotrophs ratio) by comparing S, M, and L. Mixotrophic individuals belonged to the species *Euglena gracilis* and *Euplotes aediculatus*. Heterotrophic individuals belonged to the other nine species in the community. To test the influence of ecosystem size on this ratio, we examined the effects of ecosystem size and its interaction with time by comparing a full model (including ecosystem size interacting with time as a fixed effect) to a null model (including only time as a fixed effect) using ANOVA. Significant results indicated that ecosystem size interacted with time to affect the mixotrophs-heterotrophs ratio. Similarly, we assessed the influence of ecosystem size alone (without its interaction with time) by comparing with ANOVA a reduced model (including ecosystem size and time as fixed effects) with the null model. Significant results indicated that ecosystem size affected the response variable without interacting with time. In the full, reduced, and null models, random effects considered the impact of replicates on the correlated intercept and the slope of the relationship between the mixotrophs-heterotrophs ratio and time.

**Appendix S3 Results: high disturbance**

Larger ecosystems were denser with mixotrophic individuals, as attested by the mixotrophs-heterotrophs ratio increasing with ecosystem size in unconnected ecosystems (Fig. S2, p = 0.026, ecosystem size interacted with time) (S vs M vs L).

A screenshot of a graph

Description automatically generated

Figure S1. Time series of biodiversity and biomass at a local scale. (a) biodiversity (Shannon index) and (b) biomass (bioarea per volume in mm2/ml) of medium ecosystems connected to another medium ecosystem or unconnected (dashed vs dotted lines). Dots represent means across replicates; error bars represent 95 % confidence intervals; vertical grey lines represent disturbance events followed by resource flows. All systems were sampled on the same day. Points were minimally jittered along the x-axis to make the figure clear. The area in grey indicates time points not considered for analysis, as meta-ecosystems were sampled before the first disturbance and resource flow.

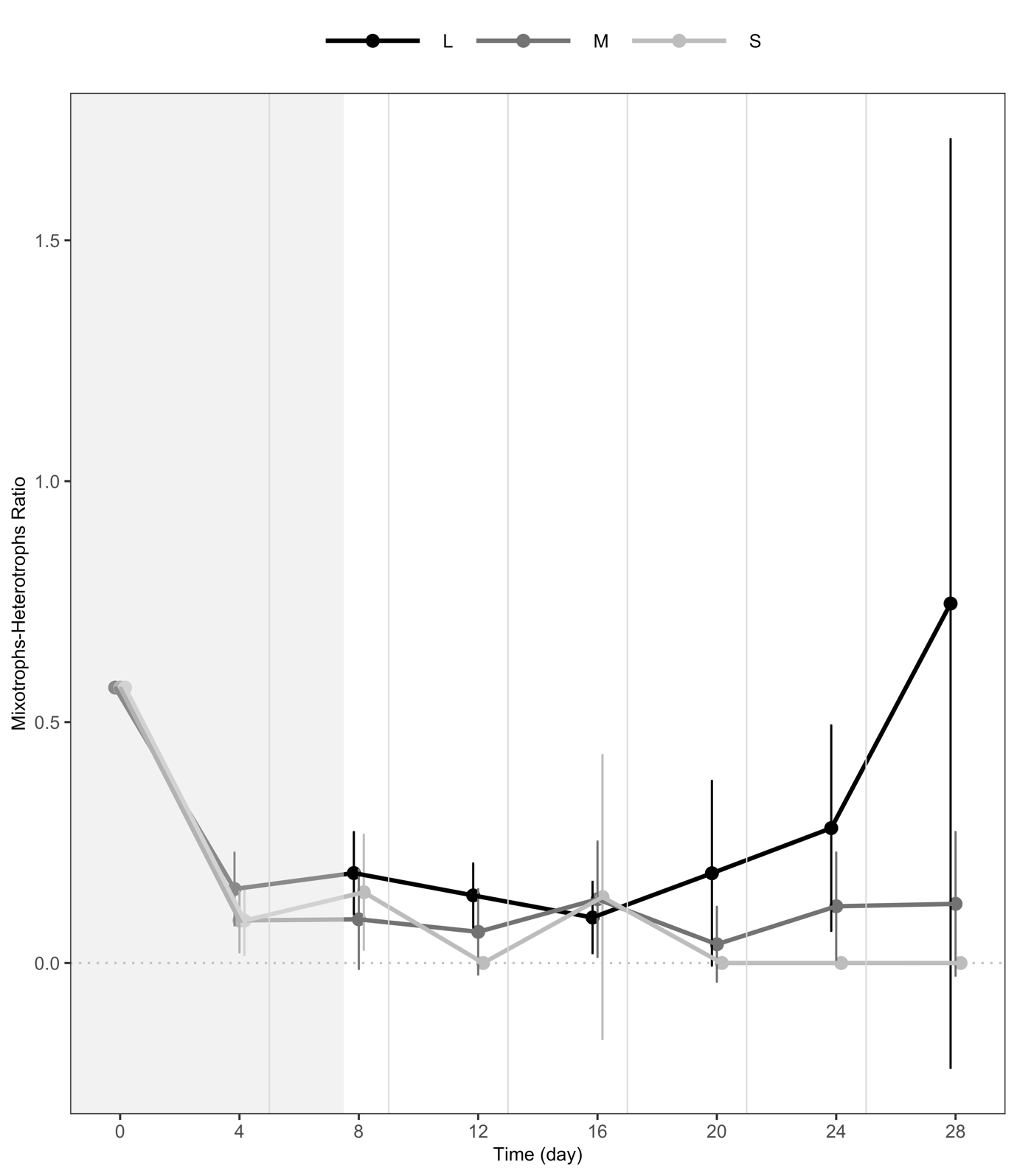


Figure S2. Time series of the mixotrophs-heterotrophs ratio at a local scale in small, medium, and large (light grey vs dark grey vs black lines) unconnected ecosystems. Dots represent means across replicates; error bars represent 95 % confidence intervals; vertical grey lines represent disturbance events followed by resource flows. All systems were sampled on the same day. Points were minimally jittered along the x-axis to make the figure clear. The area in grey indicates time points not considered for analysis, as meta-ecosystems were sampled before the first disturbance and resource flow.

**Appendix S4 Results: low disturbance**

At the meta-ecosystem level, resource flows increased mean α-diversity and decreased β-diversity in meta-ecosystems with asymmetric patch sizes (SLLS; Fig. S3 purple solid versus dashed lines) and increased total biomass in identical (symmetric) patch sizes (MMMM; Fig. S3 green solid versus dashed lines). SLLS had a higher mean α-diversity (p = 0.002, connection interacted with time), lower β-diversity (p = 0.007, connection interacted with time), same γ-diversity (p > 0.1), and same total biomass (p > 0.1) compared to SL (purple lines in Fig. S3a, S3b, S3c, S3d respectively). MMMM had the same mean α-, β-, and γ-diversity (green lines in Fig. S3a, S3b, and S3c, respectively, p > 0.1), but higher total biomass (green lines in Fig. S3d, p = 0.026), compared to MM.

At the local level, small ecosystems that were connected to large ecosystems had higher biodiversity (Shannon Index) (solid vs dotted brown lines in Fig. S4a, p = 0.001, connection interacted with time) than when unconnected (SL vs S). This effect on biodiversity was due to the size of the connected ecosystem, as being connected to large ecosystems led to greater biodiversity (solid vs dashed brown lines in Fig. S4a, p < 0.001, connection interacted with time) (SL vs SS). The presence or absence of the connection alone had no effect, as small ecosystems when connected to other small ecosystems were as diverse (dashed vs dotted brown lines in Fig. S4a, p > 0.1) than when unconnected (SS vs S).

Furthermore, small ecosystems connected to large ecosystems had higher biomass (solid vs dotted brown lines in Fig. S4b, p = 0.004) than unconnected (SL vs S). This effect on biomass was also due to the size of the connected ecosystem, as being connected to large ecosystems led to greater biomass (solid vs dashed brown lines in Fig. S4b, p < 0.001) (SL vs SS). The presence or absence of the connection alone had no effect, as small ecosystems, when connected to other small ecosystems, had as much biomass (dashed vs dotted brown lines in Fig. S4a, p > 0.1) as when unconnected (SS vs S).

Also at the local level, large ecosystems that were connected to small ecosystems had less biodiversity (solid vs dotted blue lines in Fig. S4a, p = 0.048, connection interacted with time) than when unconnected (LS vs L). This might have been due to the presence of the connection itself and not the size of the connected ecosystem, as large ecosystems had the same biodiversity when connected to different patch sizes (solid vs dashed blue lines in Fig. S4b, p > 0.1) (LS vs LL) but there was weak evidence of being connected to another large ecosystem decreased biodiversity (dashed vs dotted blue lines in Fig. S4b, p = 0.064) (LL vs L). We detected weak evidence for large ecosystems connected to small ecosystems to have less biomass (solid vs dotted blue lines in Fig. S4b, p = 0.064) than unconnected (LS vs L). However, this was not significant. We found no effect of the size of the connected ecosystem on biomass (solid vs dashed blue lines in Fig. S4, p > 0.1) (LS vs LL) and only weak evidence for the same size effect (dashed vs dotted blue lines in Fig. S4, p = 0.071, connection interacted with time) (LL vs L).

In medium ecosystems, we observed no effect of connection on biodiversity (dashed vs dotted lines in Fig. S5a, p > 0.1) in connected ecosystems compared to if they were unconnected (MM vs M). However, we observed increased biomass when ecosystems were connected (dashed vs dotted lines in Fig. S5b, p = 0.014, connection interacted with time) (MM vs M).

Finally, larger ecosystems were not denser with mixotrophic individuals, as attested by ecosystem size not affecting the mixotrophs-heterotrophs ratio in unconnected ecosystems (Fig. S6, p > 0.1) (S vs M vs L).

A graph of different colored lines

Description automatically generated

Figure S3. Time series of biodiversity and biomass at a meta-ecosystem scale. (a) mean α-diversity (Shannon), (b) β-diversity (Bray-Curtis), (c) γ-diversity (species richness), and (d) total biomass (bioarea in mm2) of asymmetric and symmetric (purple vs green) and connected and unconnected (solid versus dotted lines) meta-ecosystems. For connected meta-ecosystems, dots represent means across replicates. For unconnected meta-ecosystems, dots represent the mean of all possible combinations of unconnected ecosystems assembled as virtual pairs of ecosystems with the respective ecosystem size structure. Error bars represent 95 % confidence intervals; vertical grey lines represent disturbance events followed by resource flows. Points were minimally jittered along the x-axis to make the figure clear. The area in grey indicates time points not considered for analysis, as meta-ecosystems were sampled before the first disturbance and resource flow.

A graph of different sizes and colors

Description automatically generated with medium confidence

Figure S4. Time series of biodiversity and biomass at a local scale. (a) biodiversity (Shannon index) and (b) biomass (bioarea per volume in mm2/ml) of small and large ecosystems (red vs blue lines) connected to an ecosystem of the same size, connected to an ecosystem of different size, or unconnected (solid vs dashed vs dotted lines). Dots represent means across replicates; error bars represent 95 % confidence intervals; vertical grey lines represent disturbance events followed by resource flows. All systems were sampled on the same day. Points were minimally jittered along the x-axis to make the figure clear. The area in grey indicates time points not considered for analysis, as ecosystems were sampled before the first disturbance and resource flow.

A graph of a number of red dots

Description automatically generated with medium confidence

Figure S5. Time series of biodiversity and biomass at a local scale. (a) biodiversity (Shannon index) and (b) biomass (bioarea per volume in mm2/ml) of medium ecosystems connected to another medium ecosystem or unconnected (dashed vs dotted lines). Dots represent means across replicates; error bars represent 95 % confidence intervals; vertical grey lines represent disturbance events followed by resource flows. All systems were sampled on the same day. Points were minimally jittered along the x-axis to make the figure clear. The area in grey indicates time points not considered for analysis, as meta-ecosystems were sampled before the first disturbance and resource flow.

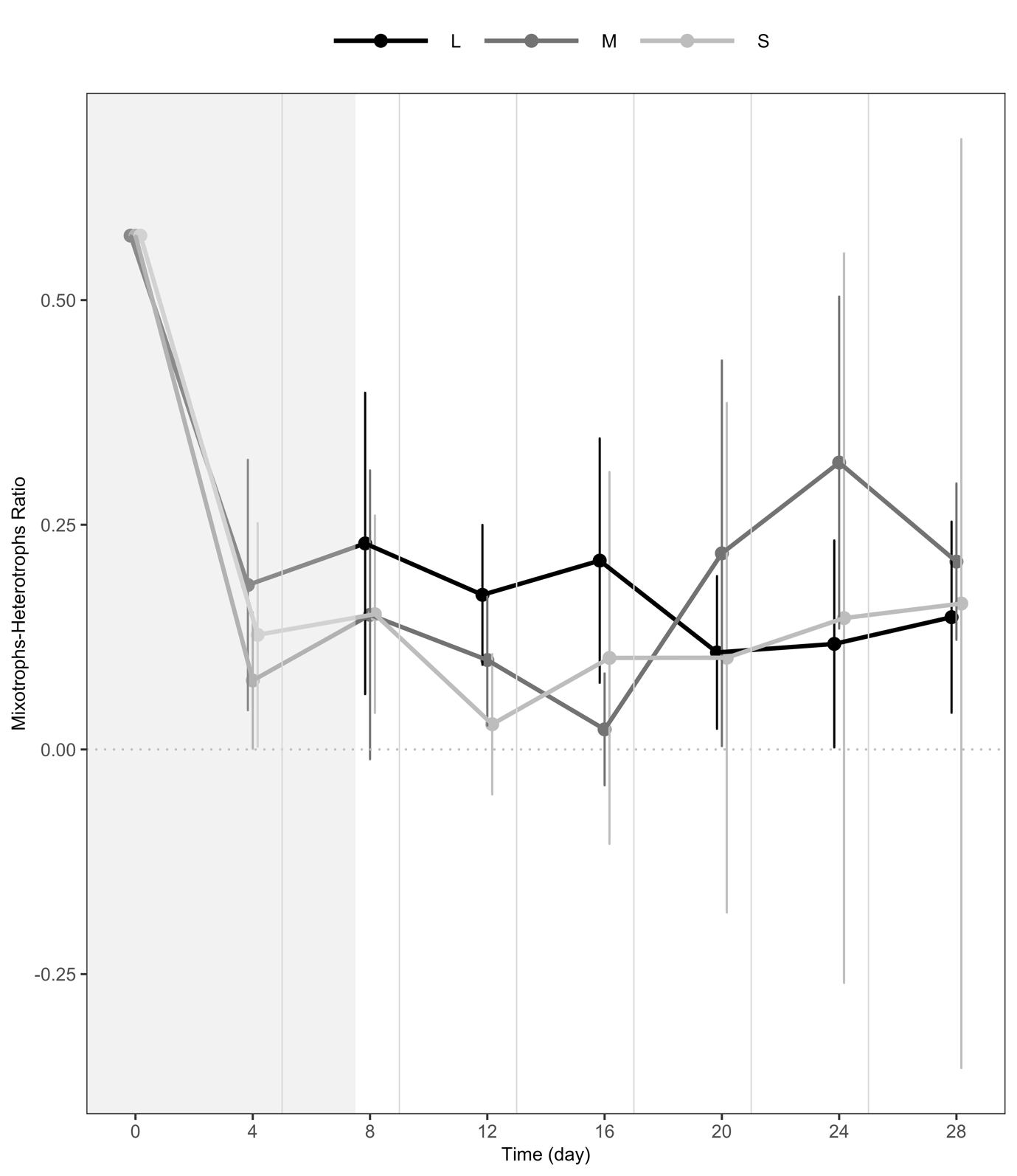


Figure S6. Time series of the mixotrophs-heterotrophs ratio at a local scale in small, medium, and large (light grey vs dark grey vs black lines) unconnected ecosystems. Dots represent means across replicates; error bars represent 95 % confidence intervals; vertical grey lines represent disturbance events followed by resource flows. All systems were sampled on the same day. Points were minimally jittered along the x-axis to make the figure clear. The area in grey indicates time points not considered for analysis, as meta-ecosystems were sampled before the first disturbance and resource flow.

**Appendix S5 p values of the comparisons**

Table S1. P values of the meta-ecosystems mixed effect models. The full and null models are compared to the null models. The effects that passed a test with a significance level below p = 0.05 are in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Response variable** | **Comparison** | **Full model**  **p (high disturbance)** | **Reduced model p (high disturbance)** | **Full model  p (low disturbance)** | **Reduced model p (low disturbance)** |
| Mean  α-Diversity (Shannon) | SLLS vs SL | .061 | **.019** | **.002** | .483 |
| MMMM vs MM | .225 | .115 | .591 | .788 |
| β-Diversity (Bray-Curtis) | SLLS vs SL | **.027** | **.012** | **.007** | .075 |
| MMMM vs MM | .469 | .333 | .308 | .236 |
| γ-Diversity (Species Richness) | SLLS vs SL | .449 | .318 | .432 | .737 |
| MMMM vs MM | .576 | .620 | .282 | .242 |
| Total Biomass (mm2) | SLLS vs SL | **.011** | **.003** | .105 | .264 |
| MMMM vs MM | .167 | .356 | .078 | **0.026** |

Table S2. P values of the ecosystems mixed effect models. Both the full model and the reduced model are compared to the null model. The effects that passed a test with a significance level below p = 0.05 are in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Response variable** | **Comparison** | **Full model**  **p (high disturbance)** | **Reduced model p (high disturbance)** | **Full model  p (low disturbance)** | **Reduced model p (low disturbance)** |
| Biodiversity (Shannon) | SL vs S | **.009** | **.002** | **.001** | **.031** |
| SL vs SS | **.045** | **.013** | **< .001** | **.004** |
| Ss vs S | **.040** | **.012** | .907 | .767 |
| MM vs M | .193 | .081 | .578 | .817 |
| LS vs L | .220 | .109 | **.048** | .330 |
| LS vs LL | .271 | .134 | .431 | .932 |
| LL vs L | .887 | .682 | .064 | .068 |
| Biomass (mm2/ml) | SL vs S | **.019** | **.032** | **.012** | **.004** |
| SL vs SS | .060 | .060 | **.001** | **< .001** |
| Ss vs S | .142 | .071 | .778 | .805 |
| MM vs M | .062 | .231 | **.014** | **.003** |
| LS vs L | **.003** | **.001** | .064 | .148 |
| LS vs LL | .085 | **.036** | .685 | .397 |
| LL vs L | .396 | .185 | .071 | .130 |
| Mixotrophs-Heterotrophs Ratio | S vs M vs L | **.026** | .109 | .161 | .291 |