Ecosystem size reverses the effect of the spatial coupling between autotrophic and heterotrophic ecosystems

Emanuele Giacomuzzo[1], Tianna Peller[1], Isabelle Gounand[2], and Florian Altermatt[1]

[1]University of Zurich
[2]IEES

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Abstract

The flow of non-living resources between autotrophic and heterotrophic ecosystems can impact their ecosystem function. However, ecosystem size is similarly known to influence ecological properties and it is uncertain how the size of coupled ecosystems mediates the effect of resource flows. Here, we used a protist experiment to examine how differences in relative ecosystem sizes mediated the effect of non-living resource flows on the functioning of two-patch, autotrophic-heterotrophic meta-ecosystems. We found that when the autotrophic patch was larger, resource flows between the patches led to an increase in the total biomass of the meta-ecosystem. Conversely, when the heterotrophic patch was larger, resource flows decreased the total biomass of the meta-ecosystem. Patch size also played a critical role in determining the effects of resource flows on biomass at the ecosystem level. Our findings reveal that differences in relative ecosystem size can significantly influence cross-ecosystem dynamics and their implications for ecosystem function.

Introduction

One of the features that most universally affects ecological systems is ecosystem (patch) size. Ever since the foundational work by MacArthur & Wilson (1967), it has been known that ecosystem size affects diversity, abundance, and extinction risks. This finding has broad implications from theoretical ecology (Luo et al., 2022) to conservation biology (Riva et al., 2024). A second feature that universally affects ecological systems is their spatial connection through the movement of non-living materials, creating spatial coupling or even spatial feedbacks (Gravel et al., 2010; Loreau et al., 2003; Peller et al., 2024), whose effects can depend on the type of ecosystems connected (Gounand et al., 2017; Osakpolor et al., 2023; Pichon et al., 2023), as ecosystem type can determine the type and quality of resources flowing (Gounand, Harvey, et al., 2018; Gounand, Little, et al., 2018; Pichon et al., 2023; Sitters et al., 2015). However, despite recognising the ecological significance of these two features, we do not know how the size of ecosystems of different types influences the effects of resource flows on their function.

Worldwide, different and heterogeneous ecosystems are connected through flows of resources (Gounand, Little, et al., 2018), forming meta-ecosystems (Gounand, Harvey, et al., 2018; Harvey et al., 2023; Leroux et al., 2017; Loreau et al., 2003; Osakpolor et al., 2023). For example, a meta-ecosystem could be composed of a stream and a forest (Harvey et al., 2023; Leroux et al., 2017; Osakpolor et al., 2023), where leaf litter falls from the forest into the stream (Cereghetti & Altermatt, 2023; Naiman & Link, 1997), fish carcasses are brought from the stream to the forest by bears fishing (Helfield & Naiman, 2002), and emergent aquatic insects end their life cycle in the forest (Scharnweber et al., 2014). In a meta-ecosystem, ecosystems can sustain their consumers mainly through their resources (autotrophic ecosystems) or through resources coming from other ecosystems (heterotrophic ecosystems). For example, a forest can be net autotrophic because it has enough plant production to sustain the growth and reproduction of terrestrial insects. In contrast, a stream can be net heterotrophic because it does not produce enough algae to sustain insects–such as stoneflies–that, therefore, rely on the input of leaves from the terrestrial ecosystem. The flow of resources between autotrophic and heterotrophic ecosystems is widespread in nature (Gounand, Little, et al., 2018). For example, deep benthic systems (heterotrophic) are coupled to pelagic systems (autotrophic) (Griffiths et al., 2017; Schindler & Scheuerell, 2002), small headwater streams (heterotrophic) are coupled to riparian forests (autotrophic) (Carter et al., 2024; Gounand, Little, et al., 2018), and coral reefs (heterotrophic) are coupled to pelagic ecosystems (Morais et al., 2021; Morais & Bellwood, 2019).

Resources flowing between autotrophic and heterotrophic ecosystems can positively or negatively affect the overall meta-ecosystem production (Allen et al., 2024; Gounand et al., 2017; Pichon et al., 2023). Resource flows are predicted to increase the production of the two ecosystems if the production of the two systems is limited by the compounds that the other provides (Pichon et al., 2023). By contrast, resource flows would likely decrease production if the ecosystems provided each other with unnecessary compounds, making limiting resources less dense and, therefore, less available (Pichon et al., 2023). For example, if a stream connected to a forest receives leaf litter rich in carbon, this flow of resources will increase stream production if the stream is carbon-limited and decrease it if it is nitrogen-limited (Pichon et al., 2023). Also, if a stream is connected to a terrestrial ecosystem from which it receives too many resources such as hippo faeces, these resources cannot be absorbed by algae as quickly as received, and subsequently provoke hypoxia, reducing fish function (Dutton et al., 2018). As the connection among autotrophic and heterotrophic ecosystems via resource flows is widespread in nature, understanding how their connection influences their production will deepen our understanding of a ubiquitous ecological process, ultimately enhancing our ability to manage landscapes effectively.

However, how the size of the autotrophic and heterotrophic ecosystems shape the effects of this spatial coupling has been overlooked. The size of an ecosystem can alter its permeability to resource flows and, therefore, how they affect its production. This interaction between ecosystem size and resource flows was shown, for example, by field studies on unidirectional flows of resources to islands, where smaller islands get proportionally more resources coming from the ocean than larger ones, increasing the density of spiders and insects in islands in Baja California (Mexico) (Polis & Hurd, 1995) and birds in islands in British Columbia (Canada) (Obrist et al., 2020). Furthermore, resources flowing bidirectionally between ecosystems of different sizes can change the overall biomass in these ecosystems, as shown by a protist experiment (Giacomuzzo et al., 2024). In light of the influence of ecosystem size on meta-ecosystem total biomass and the fact that ecosystems have different sizes in nature, we expect that including ecosystem size should be important in understanding how resource flows between autotrophic and heterotrophic ecosystems change the total biomass of meta-ecosystems.

Here, we studied how relative ecosystem size mediates the effects of spatial couplings on meta-ecosystem biomass using a protist experiment. Such microcosm experiments are a well-known approach to study general questions in ecology and allow a high level of control and a close conceptual analogy to mathematical models, yet still contain realistic ecological dynamics (Altermatt et al., 2015; Benton et al., 2007). We studied two-patch meta-ecosystems composed of one autotrophic and one heterotrophic patch with only non-living resources flowing between the two ecosystems ('patch' and 'ecosystem' are used as synonyms). We investigated the effects of resource flows in three meta-ecosystems with the same total volume but different relative patch sizes: one in which the autotrophic patch was larger, one in which the heterotrophic patch was larger, and one in which both patches were of the same size. We investigated the effects of resource flows by comparing the same three meta-ecosystems (varying in patch size of heterotrophic and autotrophic systems) and manipulating if patches were coupled or not (i.e., connected or unconnected by resource flows).

Materials and method

Experimental design

We studied how relative ecosystem size mediates the effect of resource flows between autotrophic and heterotrophic patches in experimental meta-ecosystems using aquatic protist microcosms (Altermatt et al., 2015). We compared meta-ecosystems containing two patches: one autotrophic and one heterotrophic patch. The total size of each of these three meta-ecosystem types was the same (i.e., the total volume of both patches per meta-ecosystem totalled always 45 mL), but they had different relative patch sizes per meta-ecosystem. The first meta-ecosystem type had a large heterotrophic and a small autotrophic patch, referred to as heterotrophic-dominated. The second meta-ecosystem type had a large autotrophic and a small heterotrophic patch, referred to as autotrophic-dominated. The third meta-ecosystem type had two medium-sized patches, referred to as equally-dominated. The volumes of the small, medium, and large patches were 7.5, 22.5, and 37.5 mL, respectively. The three meta-ecosystem types were either connected by non-living resource flows or unconnected (see Fig. 1). Equally-dominated, heterotrophic-dominated, and autotrophic-dominated connected meta-ecosystems are referred to asMA_{MH}–MH_{MA} ,SA_{LH}–LH_{SA} , andLA_{SH}–SH_{LA} , respectively (dashes indicating the two connected patches). S , M , and L refer to Small (7.5 ml), Medium (22.5 ml), and Large (37.5 ml) patches, respectively. A and H refer to autotrophic and heterotrophic patches, respectively. When looking at individual patches, subscripts refer to the size and trophic type of the connected patch (e.g., MA_{MH} indicating that it is a medium-sized autotrophic patch connected to a medium-sized heterotrophic patch). Respective unconnected controls of the resource flow effect are$MA|MH$, $SA|LH$, and $LA|SH$ (without subscripts; vertical line indicating that they were unconnected). All meta-ecosystems started with identical initial communities and were replicated five-fold.

Experimental setup

All meta-ecosystems consisted of a heterotrophic and an autotrophic patch (ecosystem), respectively. The heterotrophic ecosystems consisted of a community of nine, mostly heterotrophic ciliates (*Blepharisma* sp., *Colpidium* sp., *Euplotes aediculatus* , *Loxocephalus* sp., *Paramecium aurelia* ,*Paramecium caudatum* , *Spirostomum* sp., *Spirostomum teres* , and *Tetrahymena cf. pyriformis)* , and one rotifer (*Cephalodella* sp.). The autotrophic ecosystem consisted of a photosynthesising mixotrophic flagellate (*Euglena gracilis*). We subsequently refer to all these organisms as "protists". Before the experiment, the protists were individually cultured in pre-autoclaved bottles with protist medium (0.46 g of Protozoa Pellet by Carolina per L of water) and bacteria (*Serratia fonticola* , *Bacillus subtilis* , and *Brevibacillus brevis*) acting as food for protists and constituting 5 % of the total volume of the culture. See Altermatt et al. (2015) for further details and protocols.

At the beginning of the experiment (day zero), we prepared two large bottles either containing *E. gracilis* or the mix of the 10 heterotrophic protists (the latter at 1/10 of their carrying capacity). From these large bottles, we prepared 60 cell flasks (40 mL volume) with different culture volumes—20 flasks of small ecosystem size (7.5 mL), medium ecosystem size (22.5 mL), and large patch size (37.5 mL) (3 sizes x 2 trophic types x 2 connection treatments x 5 replicates = 60 flasks). Subsequently, the position of the cultures was randomised within an incubator, maintained at a constant temperature of 20 °C with continuous illumination.

Disturbances and non-living resource flows

Spatial flows were associated with disturbances, during which living biomass was turned into detritus and used for the resource flows. More specifically, every seven days (starting on day seven), we heated a fixed volume of the community of each patch to 100 °C in a laboratory oven to kill all organisms, thereby turning them into non-living resources (i.e., local disturbance). Heating was done such that any loss of water through evaporation was minimised, and replenished, respectively. Resource flows were carried out at a fixed volume of 5.25 ml per patch (this represented 70 % of a small patch, 23.3 % of a medium patch, and 14 % of a large patch). After cooling down, this detritus part of patches that belonged to a connected meta-ecosystem was transferred between the connected patches, while in the unconnected controls the same volume was poured back to the patch where it originated to control for the perturbation/mortality associated with cross-ecosystem resource flows ('resource retention' in Fig. 1), yet without having an actual cross-ecosystem flow. The loss of water through evaporation was replenished to keep the volume of cultures consistent by adding 1 ml of sterile protist medium to each patch after the third disturbance event.

Sampling

To assess the abundance, species identity, biomass, and traits of protists in each patch, we recorded videos of

0.2 ml samples from each patch every seven days, starting from day five. The only exception was on day zero, when we filmed the autotrophic and heterotrophic in the two source mixtures as a proxy for the individual autotrophic and heterotrophic patches, respectively. While the first two time points (days 0 and 5) took place before the first disturbance, all other observations were always recorded five days after the disturbance to allow communities to recover from disturbances. Each video was 5 s long and recorded using a Hamamatsu Orca Flash 4.0 (Herrsching am Ammersee, Germany) camera at 1.6x magnification.

Quantifying biomass, median body size, and biodiversity

With these videos, we identified and characterised protist species in the communities using the R-package BEMOVI (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 and used such traits to exclude particles that were not protists to obtain an average abundance of protist individuals per volume. We used protist biomass as the focal ecosystem function. Specifically, we measured the total area of protists (as area per volume medium) and used this "bioarea" as a proxy of biomass (hereafter referred to as "biomass")–a fair assumption given that protists have a roundish shape (see also other work using the same proxy; e.g., Jacquet et al., 2020; Pennekamp et al., 2018). The area of protists gave us a metric of body size, from which we calculated the median body size of individuals in each patch at each time point (the median gives an average metric robust to different types of distributions). We then used a support vector machine model to identify protist species (Cortes et al., 1995; r-package "e1071": Dimitriadou et al., 2006), using traits in species monocultures as predictor variables. Finally, we calculated patch biodiversity using the Shannon Index to account for relative abundances.

Statistical analysis

To understand how resource flows affected function and biodiversity in meta-ecosystems, we performed statistical analysis using mixed-effect models with the 'glmmTMB' (for Tweedie distributions, Mollie et al., 2017) and 'lme4' (for normal distributions, Bates et al., 2015) packages in R. The analysis did not include the initial two time points before the disturbances (grey zones in all figures), as they are not related to the understanding of the effects of disturbances and resource flows.

At the meta-ecosystem level, to examine resource flow effects, we compared connected and unconnected meta-ecosystems for each meta-ecosystem type (autotrophic-dominated, heterotrophic-dominated, and equally-dominated). To test the influence of relative patch size and the resource flow connection on total biomass, we examined the effects of the resource flow connection and its interaction with relative patch size in a mixed effect model where random effects were time and baseline (response variable at the time point before the first disturbance) using analysis of deviance. As we set the distribution of biomass as a Tweedie distribution, which considers distribution to be bound to positive numbers (biomass cannot be negative) and to have a spike at zero (when both patches' biomass crashes), we performed an analysis of deviance, which can handle Tweedie assumptions (non-normality, zero inflation, and overdispersion). Next, we performed a post hoc Tukey test with the package 'emmeans' (Lenth, 2022) in R to see in which meta-ecosystems resource flows affected total biomass by comparing connected to unconnected scenarios for each meta-ecosystem type. Finally, also using a post hoc Tukey test, we compared the biomass among meta-ecosystems types, when they are connected and when they are unconnected.

At the local level, we investigated whether patch size and its interaction with the connection via resource flows influenced response variables (biomass density, Shannon Index, median body size) by constructing two identical models: one for heterotrophic patches (i.e.,SH_{LA} , SH , MH_{MA} ,MH , LH_{SA} , and LH) and one for autotrophic patches (i.e., SA_{LH} , SA ,MA_{MH} , MA , LA_{SH} , andLA). In each of these two models, we examined the effects of the interaction between resource flow connection and patch size in a mixed effect model where random effects were time and baseline (response variable at the time point before the first disturbance) using analysis of deviance as above. Next, to understand the effects of this interaction, as above, we performed an analysis of deviance for biomass density and median body size (we set distribution as Tweedie) and an analysis of variance (ANOVA) for Shannon Index (we set distributions as normal). Next, as also above, using a Tukey test we saw in which patches the connection via resource flows had an effect (comparing in heterotrophic patches SH_{LA} to SH , MH_{MA} to MH , and LH_{SA} to LH and in autotrophic patches SA_{LH} to SA , MA_{MH} to MA , and LA_{SH} to LA), and whether size had an effect in unconnected patches (comparing in heterotrophic patches SH to MH and SH to LH and in autotrophic patches SA to MA , and SA to LA).

Results

At the meta-ecosystem level, relative patch size and resource flows interacted to influence the total biomass of the meta-ecosystems, as revealed by the analysis of deviance of the mixed effect model (Fig. 2, Chisq = 21.21, Df = 2, $p < 0.001$). Resource flows increased the total biomass in autotrophic-dominated meta-ecosystems (turquoise solid vs. dashed lines in Fig. 2, LA_{SH}–SH_{LA} sustained on average 0.22 mm^2 more biomass than $LA|SH$, SE = 0.08, z ratio = 2.72, p = 0.006) and decreased the total biomass in heterotrophic-dominated meta-ecosystems (yellow solid vs. dashed lines in Fig. 2, LH_{SA}–SA_{LH} sustained on average 0.39 mm^2 less biomass than $LH|SA$, SE = 0.11, z ratio = -3.71, $p < 0.001$). Resource flows did not affect the total biomass in equally-dominated meta-ecosystems (grey solid vs. dashed lines in Fig. 2, SE = 0.09, z ratio = -0.29, p = 0.769). Furthermore, relative patch size made total biomass differ among connected meta-ecosystems. Autotrophic-dominated connected meta-ecosystems had more total biomass than equally-dominated meta-ecosystems (turquoise vs grey solid lines in Fig. 2, LA_{SH}–SH_{LA} sustained on average 0.48 mm^2 more biomass than MH_{MA}–MA_{MH} , SE = 0.08, z ratio = 5.67, $p < 0.001$) and equally-dominated meta-ecosystems had more total biomass than heterotrophic-dominated meta-ecosystems (grey vs. yellow solid lines in Fig. 2, MH_{MA}–MA_{MH} sustained on average 0.62 mm^2 more biomass than LH_{SA}–SA_{LH} , SE = 0.10, z ratio = -6.06, $p < 0.001$).

Relative patch size similarly influenced the total biomass among unconnected meta-ecosystems, with total biomass decreasing from autotrophic-dominated meta-ecosystems to equally-dominated meta-ecosystems and heterotrophic-dominated meta-ecosystems (Fig. 2, dashed lines; comparing turquoise vs. grey, $LA|SH$ sustained on average 0.23 mm^2 more biomass than $MH|MA$, SE = 0.09, z ratio = 2.67, p = 0.008; comparing grey vs. yellow, $MH|MA$ sustained on average 0.26 mm^2 more biomass than $LH|SA$, SE = 0.94, z ratio = -2.74, p = 0.006).

At the local level, in autotrophic patches, relative patch size and connection via resource flows interacted to influence biomass density (Fig. 3, Chisq = 6.44, Df = 2, p = 0.040). The connection increased the biomass density of large and small but not medium autotrophic local patches (solid vs. dashed green lines in Fig. 3; in top panel, LA_{SH} sustained on average 0.26 mm^2/ml more biomass density than LA , SE = 0.12, z ratio = 2.16, p = 0.031; in bottom panel, SA_{LH} sustained on average 0.48 mm^2/ml more biomass density than SA , SE = 0.20, z ratio = 2.37, p = 0.018; in middle panel, biomass did not differ between MA_{MH} and MA , SE = 0.13, z ratio = - 0.57, p = 0.568). Furthermore, patch size influenced local autotrophic biomass density, as in unconnected autotrophic patches the biomass density significantly increased from small to medium and to large patches (green dotted lines in Fig. 3; in top vs. bottom panels, LA sustained on average 1.53 mm^2/ml more biomass density than SA , SE = 0.18, z ratio = -8.46, $p < 0.001$; in middle vs. bottom panels, MA sustained on average 1.49 mm^2/ml more biomass density than SA , SE = 0.18, z ratio = 8.254, $p < 0.001$).

Also at the local level, in heterotrophic patches, patch size and connection via resource flows interacted to influence biomass density (Fig. 3, Chisq = 15.002, Df = 2, p = 0.001). The connection decreased the biomass density of small and large but not medium heterotrophic local patches (solid vs. dashed blue lines in Fig. 3; in top panel, SH_{LA} sustained on average 0.76 mm^2/ml less biomass density than SH , SE = 0.18, z ratio = -4.19, $p < 0.001$; in bottom panel, LH_{SA} sustained on average 0.50 mm^2/ml less biomass density than LH , SE = 0.15, z ratio = -3.292, p = 0.001; in middle panel, biomass density in MH_{MA} and MH are not significantly different, SE = 0.15, z ratio = 0.61, p = 0.540). Furthermore, patch size influenced biomass density. Indeed, biomass density increased from small to large patches in unconnected heterotrophic patches, although not between small and medium patches (dotted blue lines in Fig. 3; in top vs. bottom panels, LH sustained on average 0.50 mm^2/ml more biomass density than SH , SE = 0.15, z ratio = 3.33, p = 0.001; in middle vs. bottom panels, biomass density in MH and SH are not significantly different, SE = 0.16, z ratio

= 1.23, p = 0.217).

At the local level, patch size and connection via resource flows also interacted to influence the median body size in both heterotrophic and autotrophic ecosystems (see Appendix S1) and the biodiversity of heterotrophic ecosystems (see Appendix S2). Detailed results of these analyses can be found in the respective appendices.

Discussion

We experimentally demonstrated that relative ecosystem size can mediate the impact of resource flows on the function of autotrophic-heterotrophic meta-ecosystems. Positive effects of resource flow exchanges on meta-ecosystem total biomass were observed when the larger patch was autotrophic, while the effects were negative when the larger patch was heterotrophic. These findings were explained by the coupling via resource flows between autotrophic and heterotrophic patches increasing the local biomass density of autotrophic patches while decreasing it in heterotrophic patches. Overall, our findings suggest that resource flows between autotrophic and heterotrophic ecosystems of different sizes can affect ecosystem functions at larger scales and that the trophic balance of the larger ecosystem (net autotrophic vs. heterotrophic) can determine the direction of the meta-scale effect.

The negative impact of resource flows on heterotrophic ecosystem biomass contrasts with previous results. We expected the carbon-enriched resources from autotrophs to have a bottom-up positive effect on heterotrophic protist biomass via a boost of the bacterial populations they prey on (Gounand et al., 2017). We see at least two mutually non-exclusive explanations for this counterintuitive result: stoichiometric mismatch or oxygen limitation. A stoichiometric mismatch would occur if imported resources dilute the limiting nutrients compared to local resources (Pichon et al., 2023). This would mean that our heterotrophic ecosystems were not carbon-limited, unlike in Gounand et al. (2017). A difference with this previous experiment is the higher diversity of our heterotrophic protist community (up to 10 protist species compared to one or two), which could have caused more efficient recycling (Delong & Gibert, 2019) and made the heterotrophic patch less responsive to carbon-rich resources coming from the autotrophic patch in the equally-dominated meta-ecosystems. Alternatively, resource flows from autotrophic patches may have caused hypoxia through increased bacterial growth. This might have negatively affected protist biomass in small heterotrophic patches due to high relative carbon import and in large ones due to pre-existing oxygen limitation from a low area-volume ratio. Similar hypoxia from resource flows is widespread in nature, for instance, when massive amounts of hippo faeces and urine enter confined areas in streams (Dutton et al., 2018) or when fertilisers leach into lakes or estuaries (e.g., Rabalais & Turner, 2019; Steffen et al., 2012).

In contrast, the positive effect of resources coming from heterotrophic ecosystems on the local production of autotrophic ecosystems has consistently been observed in experiments (e.g., Gounand et al., 2017; Harvey et al., 2016), as well as in empirical studies (Montagano et al., 2019). This positive effect likely comes from relaxing the nutrient limitation in autotroph species, which in our control settings might just emerge from the differences in the stoichiometry of the abiotic resources between ecosystem types resulting from carbon fixation and direct use of inorganic nutrients in autotrophic ecosystems. In this context, heterotrophic ecosystems export resources that are much richer in nutrients relative to carbon, which should boost autotrophic ecosystem production.

Our study suggests that resource flows between ecosystems of different sizes can exacerbate the differences in production between meta-ecosystems that were already present in their unconnected counterparts. Autotrophic patches in our experiment sustained higher biomass density than heterotrophic patches, leading to a decrease in meta-ecosystem total biomass from autotrophic-dominated to equally-dominated, and heterotrophic-dominated unconnected meta-ecosystems (without resource flows connecting ecosystems). The total biomass of a meta-ecosystem was, therefore, influenced by how much of the size of the meta-ecosystem was allocated to the two types of patches. Adding resource flows between patches allowed autotrophic-dominated meta-ecosystems to become even more productive and heterotrophic-dominated meta-ecosystems to become even less productive. This suggests that disparities in function among ecosystems within a landscape may arise not only from their intrinsic properties but also from differences in their size, which influence resource flows and their effects. Further, our findings suggest that ongoing changes to resource flows connecting ecosystems around the world (e.g., Elser & Bennett, 2011; Peller & Altermatt, 2024; Smith et al., 2025) can significantly impact ecosystem function across scales.

The results of this experiment also show that relative ecosystem size influences the effects of resource flows and that it matters if the heterotrophic or autotrophic patch is dominating in size, even when resource flow quantities are equal and bi-directional. Unidirectional resource flow effects on the recipient ecosystem have been shown in field surveys and experiments to depend on the size of the ecosystem they enter. For example, marine subsidies (e.g., seaweed detritus and seabird fish scraps) increase secondary production the most in smaller islands (Polis & Hurd, 1995, 1996), nitrogen brought by salmon gets incorporated into plants and invertebrates the most in smaller river watersheds (Hocking & Reimchen, 2009), and ocean seaweed provided to the shores of islands increases lizards densities only on small islands (Wright et al., 2020). Many ecosystems, however, are reciprocally coupled, with resources flowing in both directions between ecosystems (Gounand et al., 2020; Gounand, Little, et al., 2018). A recent protist experiment manipulating the relative size of connected patches showed that the connection through flows of resources could decrease the total biomass of the meta-ecosystem (Giacomuzzo et al., 2024). While this work explored the interaction between resource flows and ecosystem size, it did not address the common case where the connected ecosystems differ in type or trophic status. Our findings support the results of Giacomuzzo et al., (2024), but significantly extend our knowledge by showing that the trophic status of the ecosystem dominating the meta-ecosystem's size, can reverse the impact of resource flows on meta-ecosystem function. Global change is altering the size of ecosystems, and our findings suggest the potential for resource flows to mediate the effects on ecosystem function.

An important factor to consider when interpreting these results in relation to natural systems is the role of ecotones or the interfaces between connected ecosystems. In our microcosm experiment, ecosystems are relatively well-mixed and thus, resource flows can influence all areas of the ecosystem. However, in many natural systems, the extent of the ecotone, rather than the total size of the ecosystem, determines the extent of coupling between ecosystems (Muehlbauer et al., 2014). For instance, not all parts of a forest receive resource flows from an adjacent river; rather, the sections at the interface (ecotone) are predominantly involved (Harvey et al., 2023; Muehlbauer et al., 2014). Consequently, it can be that in some landscapes the size of ecosystems affected by resource exchanges is determined by the size of their ecotones, while in others it is more affected by the spatial structure/layout. Such finding is also consistent with continental-scale observations that landscape diversity, that is, the diversity of different ecosystems, per se can result in positive ecosystem function effects (Mayor et al., 2025; Oehri et al., 2020), which has been directly postulated through resource couplings and other cross-ecosystem dynamics. In the context of our study, this suggests that the influence of size may not be solely determined by the total area of autotrophic and heterotrophic patches, but by the spatial extent of their ecotone, where resource exchanges are most pronounced. Notably, ecotones are recognized as relatively dynamic areas that naturally change in size across time (Smith & Goetz, 2021). Therefore, our results can potentially explain why resource flows have different effects at different times (Anderson et al., 2008; Mulholland et al., 2006; Valett et al., 2008).

In conclusion, our experiment provides experimental evidence that the relative size of coupled autotrophic and heterotrophic ecosystems can indirectly affect ecosystem function at local and meta-ecosystem scales. Autotrophic and heterotrophic ecosystems are ubiquitously connected by spatial flows of resources, yet these ecosystems commonly differ in size—a feature that has generally been overlooked in studies of spatial flows. Our findings underscore the importance of considering how ecosystem size and type, as well as resource flow dynamics, interact to drive ecosystem function across scales. By acknowledging the role of ecosystem size and resource flows, we can better address the complexities of ecosystem function in the context of ongoing global change.

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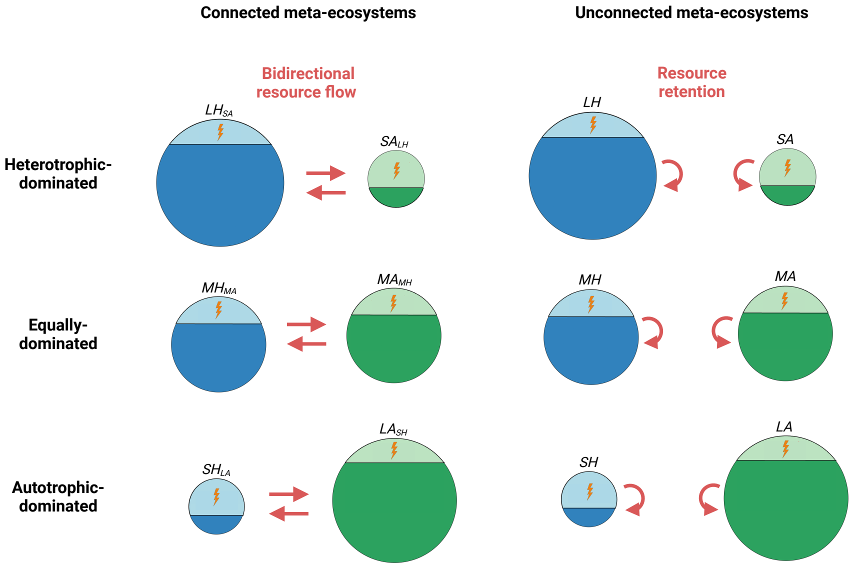
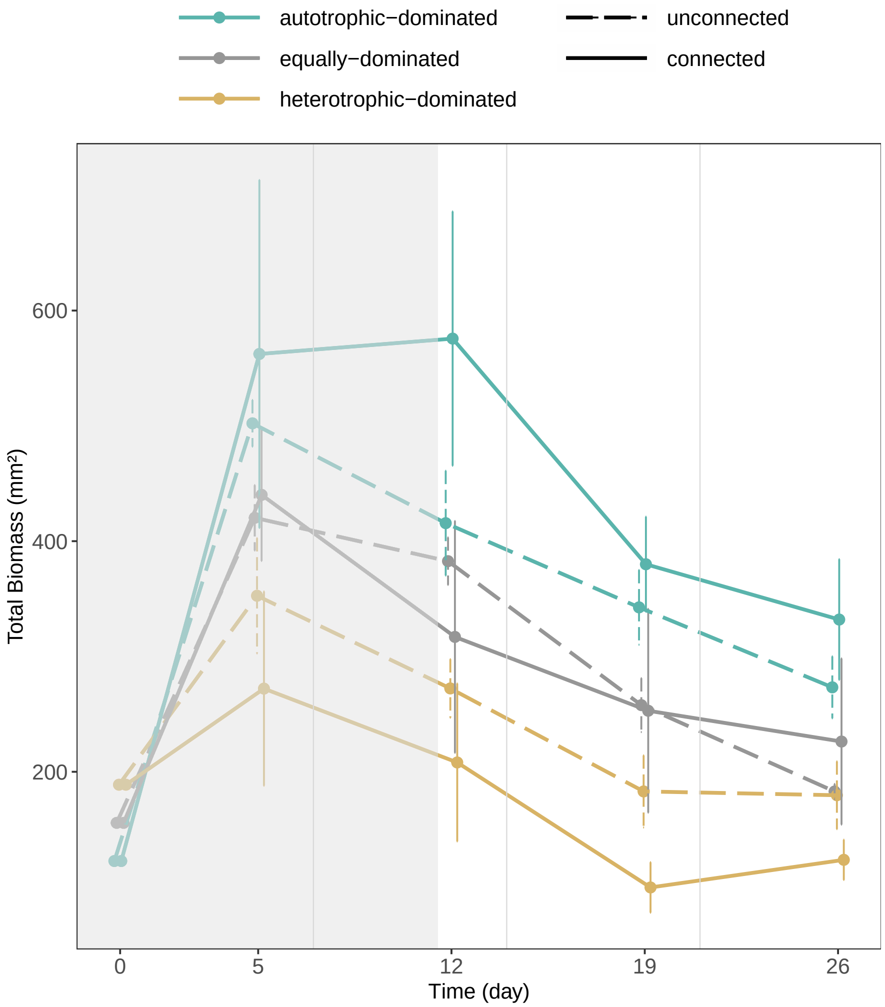
Figure legends

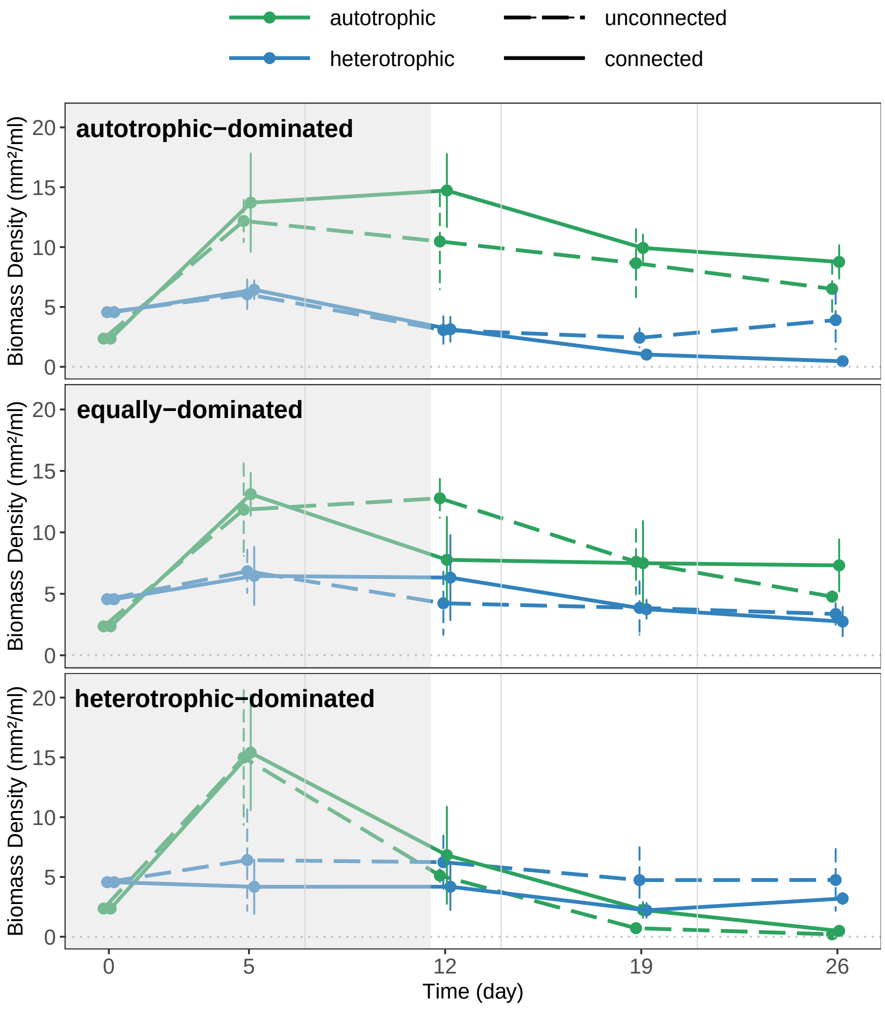
Figure 1: Experimental design. We manipulated relative patch size to study its effect on the spatial coupling between autotrophic and heterotrophic ecosystems in experimental protist two-patch meta-ecosystems. Capital letters describe patch size and type: S = small patch, M = medium patch, L = large patch, A = autotrophic patch, H = heterotrophic patch. The subscript indicates the size and trophic type of the connected patch. The disturbed part of the respective system is indicated with a lightning bolt. This part of the patch was heated to turn living biomass into detritus and then used for bi-directional resource flow or retained, respectively, in the connected vs. unconnected meta-ecosystems. Each treatment was replicated five times, resulting in 60 patches.

Figure 2: Time series of meta-ecosystem protist total biomass. For connected meta-ecosystems, dots indicate means across replicates. For unconnected meta-ecosystems, dots indicate the mean of possible combinations of unconnected ecosystems, assembled as unconnected meta-ecosystems. Error bars indicate 95 % confidence intervals; vertical grey lines indicate disturbance events that were followed by resource flows. Points are slightly jittered along the x-axis to improve the clarity of the figure. The area in grey encompasses the time points that were not considered for analysis, as meta-ecosystems at these time points were sampled before the first disturbance and resource flow event.

Figure 3: Time series of local level protist biomass density and effects of the connection via resource flows on local patches in autotrophic-, equally-, and heterotrophic-dominated connected meta-ecosystems, respectively. Dots represent means across replicates. Error bars indicate 95 % confidence intervals; vertical grey lines indicate disturbance events which were followed by resource flows. Points are slightly jittered along the x-axis to improve the clarity of the figure. The area in grey encompasses the time points that were not considered for analysis, as patches at these time points were sampled before the first disturbance and resource flow.


Connected meta-ecosystems
Unconnected meta-ecosystems
Bidirectional resource flow
Resource retention
Heterotrophic-dominated
Equally-dominated
Autotrophic-dominated
LH_{SA}
SA_{LH}
LH
SA
MH_{MA}
MA_{MH}
MH
MA
SH_{LA}
LA_{SH}
SH
LA


autotrophic−dominated
equally−dominated
heterotrophic−dominated
unconnected
connected
Total Biomass (mm²)
600
400
200
0
5
12
19
26
Time (day)


autotrophic
heterotrophic
unconnected
connected
autotrophic−dominated
equally−dominated
heterotrophic−dominated
Biomass Density (mm²/ml)
0
5
10
15
20
0
5
12
19
26
Time (day)