# Experimental design

The effects of patch size on local and regional meta-ecosystem properties were tested using a protist microcosm experiment (Altermatt et al., 2015). Each meta-ecosystem was composed of two cultures subjected to a disturbance regime. During each disturbance event, part of the community was turned into detritus. This detritus then flowed between cultures bidirectionally, connecting them through resource flow. Within a meta-ecosystem, only resources were exchanged. No organisms dispersed.

Our focal meta-ecosystem was composed of a small patch (7.5 ml) and a large patch (37.5 ml). To study the effects that such size difference had on regional properties, we compared this meta-ecosystem to a meta-ecosystem of the same total volume (45 ml) but that – instead of having a small and large patch – had two medium-sized patches (22.5 ml). For the study of local patch properties, we compared the small patch to other small patches that – instead of being connected to a large patch – were connected to another small patch or were isolated. Furthermore, we studied the effects that patch size had by comparing isolated patches of different size (small, medium, and large) and meta-ecosystems with patches of different size (meta-ecosystems with small, medium, and large patches). We here call the meta-ecosystems using the size of their patches (e.g., meta-ecosystems with a small and a large patch are referred to as small-large meta-ecosystems).

All meta-ecosystems and isolated patch treatments were subjected to disturbance intensities, either low or high. This resulted in a full factorial design in which we varied (1) the size of meta-ecosystems and isolated patches and (2) disturbance intensity. Each treatment was replicated five times, resulting in 110 microcosms (30 isolated patches and 80 meta-ecosystem patches). See Figure M1.

# Experimental setup

Eight days before assembling the experiment, we grew protist densities to carrying capacity in autoclaved bottles with medium, two wheat seeds, and a bacterial mix containing *Serratia fonticola*, *Bacillus subtilis*, and *Brevibacillus brevis* (see Altermatt et al., 2015 for protocols). The medium was a standard protist medium (0.46 g/L of Protozoa Pellet by Carolina). The bacterial mix constituted 5% of the total culture volume. On the day we assembled the experiment, a large, autoclaved bottle was inoculated with the eleven species of the protist community. The same volume was inoculated for each protist species. 15% of the final total volume of this large bottle was composed of. This large bottle's volume was pipetted into sterile 50 ml centrifuge tubes (SPL life sciences skirted conical centrifuge tubes). We pipetted 7.5 ml into the small patches, 22.5 ml into the medium patches, and 37.5 ml into the large patches. Cultures were then randomised on four foam boards. The cultures were kept in an incubator at 20 °C and under constant lighting. What we here refer to as protists is a community of nine water ciliates (*Euplotes aediculatus*, *Colpidium sp.*, *Loxocephalus sp.*, *Paramecium aurelia*, *Paramecium caudatum*, *Spirostomum sp.*, *Spirostomum teres*, *Tetrahymena cf. pyriformis,* and *Blepharisma sp.*), one alga (*Euglena gracilis*), and one rotifer (*Cephalodella sp.*).

# Disturbance and resource flow

Six disturbances occurred during experiment – one every four days, starting from the fifth day (on days 5, 9, 13, 17, 21, and 25). During a disturbance event, culture subsamples (5.25 ml for low disturbance and 6.75 ml for high disturbance) were boiled using a microwave, turning the community into detritus. These subsamples corresponded to 70% and 90% of the volume of the small patches for the low and high disturbance, respectively. In isolated patches, the boiled subsample was poured back into the original patch. In meta-ecosystems, it was poured into the connected patch. This resource flow method mimics the detritus flow arising from the death of organisms from patch recurrent disturbance. As the volume exchanged between patches was the same (e.g., 5.25 ml flowed from patch 1 to 2 and 5.25 ml from patch 2 to 1), the patch volume remained the same across time.

# Sampling

We tracked changes in community dynamics across time throughout the whole experiment. Sampling took place eight times – once every four days (on days 0, 4, 8, 12, 16, 20, 24, and 28). Each time we sampled, we took 0.2 ml samples per microcosm. We recorded a five second video following a standardised video procedure (Pennekamp & Schtickzelle, 2013; Pennekamp, Schtickzelle, & Petchey, 2015). Each sample was placed under a dissecting microscope connected to a camera, which recorded the culture for 5 seconds. Using the R-package BEMOVI (Pennekamp et al. 2015), we used an image processing software (ImageJ) to extract the number of moving organisms along with their traits (e.g., speed, shape, size). These traits were then used to filter out background movement noise (e.g., medium particles) and identify species in mixed cultures.

# Volume balance

Throughout the experiment, we monitored and compensated for variation in evaporation from microwaving across microcosms. For the first three exchange events, we boiled 15 tubes in a rack at 800 W for three minutes using a microwave (Sharp R-202). However, because we noticed high evaporation volumes of 2.43 ml (SD = 0.87), we boiled four tubes for one minute for the final three exchanges. This switch in boiling protocol produced a mean evaporation rate of 1.25 ml (SD = 0.37).

The evaporated water was replenished with autoclaved deionised water. Before the two exchange events, 1 ml of water was added to all tubes. However, before the third exchange event, we noticed that the evaporation rates were higher than expected. Cultures were a mean 1.17 ml (SD = 0.37) smaller than their initial volumes. Therefore, before the third exchange and after every following exchange, we replenished the cultures with water until their initial volume. During the first exchange event, we microwaved most tubes with other full tubes, except for the last five tubes, which were microwaved with ten empty tubes. Placing empty instead of full tubes made them evaporate more than the others. These tubes were all part of the high disturbance small-large meta-ecosystem treatment. To make up for this, we added 3.15 ml of water right before the second resource exchange (as we calculated that this was the difference in evaporated volume). We microwaved all tubes with other full tubes in the following exchange events.

Furthermore, we added medium to the cultures during each exchange event to make up for the volume sampled at each time point (0.2 ml). The addition of medium, however, did not happen at the sixth exchange, as it was right before the last time point. The sampling of 0.2 ml of culture at the last time point would not have mattered as it was the last day of the experiment.

# Analyses

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# References

Altermatt, F., Fronhofer, E. A., Garnier, A., Giometto, A., Hammes, F., Klecka, J., … Petchey, O. L. (2015). Big answers from small worlds: A user’s guide for protist microcosms as a model system in ecology and evolution. *Methods in Ecology and Evolution*, *6*(2), 218–231. https://doi.org/10.1111/2041-210X.12312

Pennekamp, F., & Schtickzelle, N. (2013). Implementing image analysis in laboratory-based experimental systems for ecology and evolution: A hands-on guide. *Methods in Ecology and Evolution*, *4*(5), 483–492. https://doi.org/10.1111/2041-210X.12036

Pennekamp, F., Schtickzelle, N., & Petchey, O. L. (2015). BEMOVI, software for extracting behavior and morphology from videos, illustrated with analyses of microbes. *Ecology and Evolution*, *5*(13), 2584–2595. https://doi.org/10.1002/ece3.1529