# Experimental design

To test the effects of patch size on meta-ecosystem dynamics, we ran a four-week protist experiment (Altermatt et al., 2015). We run a full factorial design by crossing seven system types (three closed ecosystems, four meta-ecosystems) and two disturbance levels. The three closed ecosystems treatments were of different size (small = 7.5 ml, medium = 22.5 ml, large = 37.5 ml). Three meta-ecosystems treatments had patches of the same size but of different sizes among meta-ecosystems (small and small, medium and medium, large and large). A fourth meta-ecosystem treatment had patches of different sizes (small and large). The two disturbance levels resulted from the death of 5.25 ml (smaller perturbation) or 6.75 ml (higher perturbation) of the community. These were respectively 70% or 90% of the volume of the small patches. We had a five-fold replication. Therefore, we had 110 microcosms (30 closed ecosystems and 80 meta-ecosystem patches). See Figure M1.

Within a meta-ecosystem, the two patches exchanged exclusively resources. No organisms dispersed. The flow of resources among meta-ecosystem patches happened by turning part of the community into detritus which was then made flow to the other patch. Such detritus flow was volumetrically the same for both patches (e.g., 5.25 ml flowed from patch 1 to 2 and from patch 2 to 1). We also perturbed closed ecosystems to compare the patches of a meta-ecosystem with closed ecosystems. However, the detritus produced in closed ecosystems stayed inside the ecosystem and did not flow to another patch.

Each ecosystem was composed of six compartments: a detritus compartment (ground straw contained in the medium and organic material released by the wheat seeds), inorganic nutrients (included in the medium and released by the bacteria when decomposing the detritus), decomposers (the bacteria *Serratia fonticola*, *Bacillus subtilis*, and *Brevibacillus brevis*), producers (the alga *Euglena gracilis* and the water ciliate *Euplotes aediculatus*), primary consumers feeding on the decomposers (the ciliates *Colpidium sp.*, *Loxocephalus sp.*, *Paramecium aurelia*, *Paramecium caudatum*, *Spirostomum sp.*, *Spirostomum teres*, and *Tetrahymena cf. pyriformis*, and the rotifer *Cephalodella sp.*), and a secondary consumer feeding on the primary consumers (the predatory ciliate *Blepharisma sp.*). Throughout the paper, we refer to this community of an alga, a rotifer, and nine protists simply as protists.

Diagram

Description automatically generated

Figure M1. (A) S = small patch (7.5 ml), M = medium patch (22.5 ml), L = large patch (37.5 ml), arrows = resource flows, x5 = five replicates. (B) Initial composition of the ecosystem within each of the patches.

# 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), which constituted 5% of the total volume. On the day of the experiment assembly, a large, autoclaved bottle was inoculated with the eleven protist species. The same volume was inoculated for each protist species. 15% of the volume was added as the standard protist medium (0.46 g/L of Protozoa Pellet by Carolina). Throughout this paper, we refer to this as simply medium. 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.

# Disturbance and resource flow

During the experiment, six disturbance events occurred – one every four days (on days 5, 9, 13, 17, 21, and 25). In closed ecosystems, 5.25 ml (smaller perturbation) or 6.75 ml (larger perturbation) of the volume was boiled and then poured back into the original ecosystem. In meta-ecosystems, the part of the giving ecosystems was sampled, boiled, and poured into the receiving ecosystem. The boiling turned living biomass into detritus. This resource flow method mimics the detritus flow arising from the death of organisms from ecosystems' recurrent perturbation. The closed ecosystems were also perturbed to compare meta-ecosystem patches and closed ecosystems (otherwise, the differences we would see might arise from perturbations). As the volume exchanged between ecosystems was the same, 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 5 second video following a standardised video procedure (Pennekamp & Schtickzelle, 2013; Pennekamp, Schtickzelle, & Petchey, 2015). 0.175 ml of those 0.2 ml were 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 tried to reduce the evaporation from microwaving as much as possible. 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 rates of 2.43 ml (SD = 0.87), we boiled four tubes for one minute for the final three exchanges. Switching to this 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 metaecosystem 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.

# Bibliography

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