MICROcosm: a protocol for a global microcosm experiment

Coordinators:

Gustavo Romero, Universidade Estadual de Campinas (UNICAMP), Brazil
Martin Gossner, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Switzerland

Core team:
Gustavo Romero
Martin Gossner
Akihiro Nakamura
Jana Petermann
Pavel Kratina

1. Motivation

Forest ecosystems have been threatened by global anthropogenic stressors, such as climate change and habitat degradation, with consequent loss in biodiversity and ecosystem function worldwide. Understanding the mechanisms that underlie the relationships between global changes, biodiversity and related processes is often hindered by the complexity of forest ecosystems. Replicated microcosm experiments provide suitable model systems to study such impacts because they are simple enough to allow studying the whole system, but sufficiently complex to derive results that can be applied to larger ecosystems. Impacts of forest mangement are being successfully accessed using freshwater microcosms, such as water filled tree holes, tank bromeliads and artificial tree holes (Ngai et al. 2008, Gossner et al. 2016, Petermann et al. 2016, 2020). These microcosms are frequently inhabited by insects, crustaceans, nematodes, aquatic mites, protists, bacteria and fungi (Kitching 2000, Srivastava et al. 2004), which comprise an important community in forest ecosystems, affecting key ecosystem functions and services (Ladino et al. 2019). Natural microcosms can hold up to 50,000 L of rain water per hectare in tropical forests (Ladino et al. 2019) and several litres of rain-water in temperate beech forests (Gossner et. al. 2016), thus acting as water source for many forest organisms and additionally provide important food resources for terrestrial organisms (Ladino et al. 2019). Beside their importance for ecosystem functions in natural ecosystems, forest degradation can favour the development of mosquito-transmitted diseases in such ecosystems (Becker 2003).

2. Aims

We propose a global protocol to assess human impacts on forest ecosystems using experimental microcosms. The main question we aim to address is:

How do current and future climatic conditions, forest habitat degradation and management, influence biodiversity and ecosystem functioning in water filled microcosms worldwide?

This general question can be answered by applying a multifaceted approach of diversity, including taxonomic, functional and phylogenetic diversity at different temporal and spatial scales. For example, are taxonomic, functional and phylogenetic diversity affected by the same drivers? How do global drivers affect different diversity levels (alpha, beta, gamma)? For the phylogenetic approach, we will construct a barcoding database, upon submission of specific proposals to ask for funding for such molecular analyses. We will focus on invertebrates and vertebrates in the first step. In the future we plan to ask funds for metagenomic analyses, which would help answering similar questions on multifaceted approach of diversity also for micro-organisms.

This project will focus on an important ecosystem function - detritus mass loss (decomposition of organic material), which can vary across latitudes and degrees of forest integrity (natural, managed in different degrees). It will also evaluate changes in several limnological variables (water pH, dissolved CO_2 and O_2, turbidity, CDOM, chlorophyll-a concentrations, ammonium and nitrate concentrations, temperature along the experiment using data loggers). These variables will help understanding the ecosystem functioning (e.g., Antiqueira et al. 2018, Lewington-Pearce et al. 2020).

3. General protocol

In general, we aim to cover a large range of different dry and wet forest sites around the globe covering tropical and temperate rain forests as well as broad-leaved and conifer forests of different tree diversity and composition in temperate and boreal forests.

3.1. The study area

For each site, choose two forest locations: a natural forest and an adjacent forest influenced by degradation or management. Provide detailed descriptions of the type of degradation or management, including (i) forest inside a biological reserve (protected) vs. adjacent unprotected forest, (ii) native, unmanaged forest vs. managed forest (see Petermann et al. 2020), (iii) native, protected forest vs. forests close to cities, and/or with access to human exploration (e.g., wood extraction, palm tree heart extraction etc.), or even more extreme situations, such as (iv) native protected forests vs. monoculture forests (coniferous, eucalyptus, palm trees).
These paired forests should be at least 500 m apart to minimize data dependency.

3.2. The microcosm

The artificial aquatic microcosm should consist of 1L transparent plastic (polypropylene) jar of ca. 12 cm diameter (top) x 13 cm height, holding 800 ml of rain water. To prepare the microcosm, wrap the jar from the bottom until the level of 800 ml with a commercial black insulating tape. Make two parallel holes to the height of 800 ml (red arrows below) to (i) keep the water volume constant at 800 ml and (ii) fix the jar to the tree (see *Experimental set up* below). Insert a stick (from a hardware store, preferably untreated) into the microcosm for easy access to egg laying insects.

3.3. Experimental set up

Clean well the microcosms with fresh water. For each forest type (natural or managed/degraded), please, attach 10 microcosms on tree trunks, at 1.5m height from the ground. The total number of microcosms per site is 20 (10 in preserved and 10 in degraded forest). The microcosms could be fixed on tree trunks (random position: north, south, east or west) with help of rustless, resistant wire, or rope. Within each forest type, please set up these microcosms at least 30 m apart each other to minimize data dependency. Avoid installing the microcosms near the forest edges; long exposure to the sun can overheat the system. Keep them in areas shaded by the forest. Fill up all the 20 microcosms with 800 ml of rainwater collected in open areas of the study site (you may want to use several clean bowls or buckets to collect the rain water). Please filter the water using a commercial fine mesh sieve to remove eggs etc. from collected water.

Insert the "detritus" (i.e., cotton strips) within the microcosms (see below). Please, keep the microcosms completely open, i.e., with no physical barrier for organism colonization, during the whole experiment.

Record the position of each microcosm in the tree (north, south, east or west), as well as its geographic position, latitude, longitude (in decimals), and elevation. Record the tree taxon supporting each microcosm, and its diameter at breast height (dbh). Also record the canopy openness around the microcosm (four categories, estimated by sight: < 25% cover, 25-50% cover, 51-75% cover and >75% cover).

3.4. Experimental time span and season
The experimental duration is 90 days.
The experiment should be performed during the suitable season for invertebrate development (warm, rainy or summer season). For example, for temperate regions, one could start the experiment in spring (April) and finish in summer time (July). In tropical regions one could start the experiment early in the rainy season (e.g., November for southeast Brazil) and finish in the mid of rainy season.

3.5. Detritus preparation and measurements
Natural leaf litter chemistry could affect local patterns of species colonization, community structure and development, and thus the rates of detritus loss. Because of that, researchers have been widely using cotton strips as a standardized alternative to leaf litter (Tiegs et al. 2007, 2013, 2019, Imberger et al. 2010).
Here we adopt the use of 100% unbleached calico cotton strips inside bags of different mesh sizes for quantifying detritus decomposition by micro-organisms alone (fine mesh bag) and micro-organisms plus detritivores (coarse mesh bag) (see LeCraw et al. 2017).
For each microcosm, insert two detritus bags, one containing 2 2x4 cm cotton strips into a ~2 mm nylon or polyester mesh bag (coarse), and other 2 cotton strips of the same size into a ~100 µm nylon (or polyester) mesh bag (fine). In addition, insert two extra 2 4x2 cm cotton strips freely (without bags) to control for bag (nylon) tissue, thus totalizing 6 cotton strips per microcosm (see the scheme below).

Standardized cotton strips, from the same source tissue, will be provided by the core team (sent via regular mail). So please, contact the core team (gqromero@gmail.com), and we will send the strips to you. Then you insert the cotton strips inside the bags you prepared. The bag tissues can be easily found in commercial shops, e.g.:

~2 mm mesh (white tulle) can be easily found in commercial shops, e.g.:
https://www.amazon.co.uk/White-bridal-tulle-fabric-300cm/dp/B00MB4HXJ4/ref=sr_1_3?dchild=1&keywords=tulle&qid=1586897689&s=kitchen&sr=1-3

~100 µm mesh (white organza) can be easily found in commercial shops, e.g.:
https://www.amazon.co.uk/Premium-Crystal-Organza-Costume-Decorations/dp/B07VQKY38F/ref=sr_1_8?dchild=1&keywords=organza&qid=1586895846&s=kitchen&sr=1-8

The bags can be fully sealed using aquarium silicone or chemically inert hot glue. Prior to inserting the cotton strips into the bags (this includes also the two extra strips), please, wash them using 70% ethanol, rinse in clean water (preferring deionized water), and dry at 60°C for 72 hours before weighting. Please, use a precise scale/balance of at least 4 decimals. Avoid cotton strips to absorb atmospheric water before weighing (use exicator or store the strips into plastic

bags with silica gel or measure immediately after taking it out of the drying oven).
At the end of the experiment, remove the bags from the microcosms, open them to carefully collect the cotton strips and follow the same procedure described above (thoroughly washing, rinsing, and weighting). Detritus mass loss will be the difference of biomass of the beginning and end of the experiment. After weighting, keep the cotton strips well dry (exicator or into plastic bags with silica gel) for later tensile strength analysis, which will be conducted in specialized laboratory; address to ship the cotton strips will be provided in due time.

Scheme by Gustavo Migliorini

3.6. Limnological parameters
At the beginning (1 day after experiment set up), and at end of the experiment, measure water pH in each microcosm (middle of the container).
The following measurements are not mandatory, but very useful for our ecosystem functioning monitoring: in the end of the experiment, please, measure dissolved O_2 (this parameter should be the first to be measured), turbidity, chromophoric dissolved organic matter (CDOM), dissolved CO_2, ammonium, nitrate, and in vivo chlorophyll-*a*. These limnological measurements can be assessed using multi-parameter probes (e.g., CyberScan 600, Eutech) and portable fluorometer (e.g., Aquafluor Handheld Fluorometer, Turner Designs). If possible, use water-proofed ibottons to measure water temperature over time; please schedule the ibuttons to measure at 1-h intervals. If you are unable to include one ibutton per microcosm because of financial restrictions, please include as many as possible (at least in five microcosms within each forest type). Please measure remaining water volume at the end of the experiment.
At the end of the experiment, record the detritus dry mass (separated by fine <0.5mm and coarse >0.5mm detritus using a sieve), recovered with help of a filter paper; put the detritus in paper bags and dry them at 60°C for 24h. Then, weigh the detritus using a precise scale/balance of at least 4 decimals.

3.7. Material for metagenomic analyses
In the end of the experiment, sample water plus fine detritus particle in the bottom of the microcosm. With help of a pipette, mix the water to gently detach the biofilm from the microcosm wall. Then, sample water and fine detritus by pipetting 10 ml solution, and store in sterilized 15 ml tubes. The samples should be frozen (~ -20^{o}C) immediately after collecting.
If insects or other organisms were collected in this procedure, please, separate them in another vial, keeping only water and detritus in such samples for metagenomics. Metagenomic analyses will be done pending approval of funding from future proposals.

3.8. Macroinvertebrate surveys and measurements
In the end of the experiment, after taking data on limnological parameters, collecting the litter bags, and material for metagenomics (sections 3.5, 3.6 and 3.7 above), pour the entire contents of the microcosm on a white tray to sort and collect aquatic invertebrates visible to the naked eye in trays. Please, ensure that all macroinvertebrates were sampled. If you find invertebrates in detritus bags, please, remove them from the bags and pool them together with the material collected in the vial. Measure the volume of the recovered water. Then, wash the microcosms with clean water upon a white tray to collect those invertebrates that were stuck on the microcosm walls.
Fix the invertebrates using 99% ethanol for future quantifications and identifications (e.g., traits and barcoding analyses). For the purpose of this immediate study, identify each specimen to the lowest taxonomic level, and then classify its feeding guild (e.g., predators, shredders, scrapers, filter feeders etc.) using specific literature (e.g., Merritt & Cummins 1996). Measure their length (head to end of body, excluding legs and antennae) for later estimates of biomass with help of allometric equations. Also, record if they present morphological defence against predation, e.g., case in Trichoptera ("yes" or "no"), the type of life cycle (simple or complex), and the habitat they occur (pelagic or benthic). Please, store the fixed material in 99% ethanol and in freezer at *ca* -20˚C for molecular analyses (phylogenetic diversity).

4. Optional treatments
Authors are invited to apply additional treatments (with additional microcosms) to the basic protocol above, including (i) influence of vertical distribution along the tree canopy and/or (ii) influence of allochthonous detritus on ecosystem functioning.
(i) *vertical distribution*: for each microcosm inserted at 1.5m height, please insert another one at 15 m height, in the same tree, to allow for a paired comparison. The set up of these additional microcosms, including all the measures, should follow the same procedure described in the general protocol above.
(ii) *allochthonous detritus*: to control the influence of fallen detritus (e.g., dead leaves) on the ecosystem functioning (physico-chemical properties), set up extra sets of 20 microcosms (10 per each forest type, as above) following the general protocol above. Set up the microcosms at least 30 m apart each other, and at least 30 m apart other microcosms from the standard protocol (above) to

minimize data dependency. For this new set of microcosms, insert a roof upon their tops to impede detritus entrance (see the figure below). Leave a distance of ca. 8-10 cm between the roof and the top of the jar for the insects to access the microcosm. All the measures should follow the general protocol.


5. Authorship, data submission and deadline

Among other criteria to be further defined, the order of authorship will be related to the amount of databases (sites) provided.

The database should be organized according to the spreadsheets accompanying this protocol. Please, submit your spreadsheets to Gustavo Romero (gqromero@gmail.com) and Martin Gossner (Martin.gossner@wsl.ch). If you have conducted the experiment in more than one site, please, prepare one spreadsheet per site.

Please, register your participation in the following Google docs:
https://docs.google.com/spreadsheets/d/1swmLyUDqE-jkQTdBdyjnJjCB1sTWxTJvwDUsBATjdzI/edit?usp=sharing

Deadline to send the spreadsheets: December 2022

Any doubt, please, contact the coordinators.

6. Literature cited

Antiqueira, P. A. P., O. L. Petchey, and G. Q. Romero. 2018a. Warming and top predator loss drive ecosystem multifunctionality. Ecology Letters 21:72–82.

Becker, N., 2003. Mosquitoes and their control. Kluwer Academic / Plenum Publishers.

Bernabé, T. N., P. M. de Omena, V. P. dos Santos, V. M. de Siqueira, V. M. de Oliveira, and G. Q. Romero. 2018. Warming weakens facilitative interactions between decomposers and detritivores, and modifies freshwater ecosystem functioning. Global Change Biology 24:3170–3186.

Gossner, M.M. et al. 2016. Effects of management on aquatic tree‐hole communities in temperate forests are mediated by detritus amount and water chemistry. Journal of Animal Ecology 85: 213-226.

Imberger, S. J., R. M. Thompson, and M. R. Grace. 2010. Searching for effective indicators of ecosystem function in urban streams: Assessing cellulose decomposition potential. Freshwater Biology 55:2089–2106.

Kitching, R.L. 2000. Food webs and container habitats: The Natural history and ecology of phytotelmata. Cambridge University Press, Cambridge.

Ladino, G., Ospina-Bautista, F., Varón, J.E., Jerabkova, L. Kratina, P. 2019. Ecosystem services provided by bromeliad plants: A systematic review. Ecology and Evolution.
https://doi.org/10.1002/ece3.5296

LeCraw, R. M., G. Q. Romero, and D. S. Srivastava. 2017. Geographic shifts in the effects of habitat size on trophic structure and decomposition. Ecography 40:1445–1454.

Lewington-Pearce, L., Parker, B., Narwani, A., Nielsen, M.J. and Kratina P. 2020. Diversity and temperature indirectly reduce CO2 concentrations in experimental freshwater communities. Oecologia 192: 515–527.

Merritt R.W. & Cummins K.W. 1996. An Introduction to the Aquatic Insects of North America. Kendall Hunt Publishing Company, Dubuque.

Ngai, J. T., K. R. Kirby, B. Gilbert, B. M. Starzomski, A. J. D. Pelletier, and D. S. Srivastava. 2008. The impact of land-use change on larval insect communities: Testing the role of habitat elements in conservation. Ecoscience 15:160-168.

Petermann, J. S., A. Rohland, N. Sichardt, P. Lade, B. Guidetti, W. W. Weisser, and M. Gossner. 2016. Forest management intensity affects aquatic communities in artificial tree holes. PloS ONE 11:e0155549.

Petermann, J. S., A. L. Roberts, C. Hemmerling et al. 2020. Direct and indirect effects of forest management on tree-hole inhabiting aqua- tic organisms and their functional traits. Science of the Total Environment, doi.org/10.1016/j.scitotenv.2019.135418

Srivastava, D. S., J. Kolasa, J. Bengtsson, A. Gonzalez, S. P. Lawler, T. E. Miller, P. Munguia, T. Romanuk, D. C. Schneider, and M. K. Trzcinski. 2004. Are natural microcosms useful model systems for ecology? Trends in Ecology and Evolution 19:379–384.

Tiegs, S. D., S. D. Langhans, K. Tockner, and M. O. Gessner. 2007. Cotton strips as a leaf surrogate to measure decomposition in river floodplain habitats. Journal of the North American Benthological Society 26:70–77.

Tiegs, S.D., J.E. Clapcott, N.A. Griffiths and A.J. Boulton. 2013. A standardized cotton-strip assay for measuring organic-matter decomposition in streams. Ecological Indicators 32:131-139.

Tiegs, S.D., D. M. Costello, M. W. Isken, G. Woodward, P. B. McIntyre, M. O. Gessner, E. Chauvet, N. A. Griffiths, A. S. Flecker, et al. 2019. Global patterns and drivers of ecosystem functioning in rivers and riparian zones. Science Advances 5, eaav0486.