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Suggested field procedures for collecting soil/litter arthropods in a tropical rainforest for long-term monitoring with DNA metabarcoding

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ABSTRACT

Suggested field procedures for collecting soil/litter arthropods in a tropical rainforest for long-term monitoring with DNA metabarcoding.

Standardized field protocols to monitor the soil fauna exist but they are not tailored to surveys in tropical rainforests and not designated to obtain samples that can be later processed with metabarcoding. In general, the soil fauna is extracted by Berlese-Tullgren apparatus, while the litter-dwelling and more active fauna is better surveyed with Winkler extractors. Here, we used Berlese-Tullgren which represent the choice method for Collembola but included in our samples both soil and litter.

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Standardized field protocols to monitor the soil fauna exist (Römbke et al. 2006) but they are not tailored to surveys in tropical rainforests and not designated to obtain samples that can be later processed with metabarcoding. In general, the soil fauna is extracted by Berlese-Tullgren apparatus (Southwood 1978, Andréet al. 2002, Bano and Roy 2016), while the litter-dwelling and more active fauna is better surveyed with Winkler extractors (Besuchet et al. 1987, Agosti et al. 2000). Here, we used Berlese-Tullgren which represent the choice method for Collembola (Karyanto et al. 2008) but included in our samples both soil and litter.

1. Pilot study and designating sample locations

1.1. Tropical rainforests can be rather heterogeneous in terms of forest age, composition and topography, including soil conditions. It is advisable to conduct a pilot study of the study area to estimate the different soil conditions and the number of soil samples that need to be obtained to be representative of the study area. For example, taking samples from a trail along a stream may lead to severely biased estimates of abundance and composition. An area of 50ha is appropriate for monitoring tropical trees (Losos and Leigh 2004), and smaller areas may be appropriate for most arthropods. Larger areas than 50ha may include a variety of habitats that may require high number of replicates to yield representative data. In our case the area of the ForestGEO permanent vegetation plot on Barro Colorado Island (Anderson-Teixeira et al. 2015) is relatively homogenous. We took 100 samples each including 2 liters of scooped litter and soil dispersed over an area of ca. 60ha with good results (e.g. species accumulation curves near saturation).

1.2. Depending on the local rainfall regime, the sampling scheme should also address faunal seasonality. On Barro Colorado Island, distinct dry and wet seasons occur, with implications for the soil fauna (Levings and Windsor 1982). To account for arthropod seasonality, we collected 50 samples during the dry season and 50 samples during the wet season. This appears sound to estimates annual population indices (see item 9).

1.3. A random sampling scheme is also advisable. For example, this could be an evenly spaced grid system where samples are selected at random. On BCI, it is only possible to walk in designated trails inside the ForestGEO plot, to reduce seedling trampling within the plot (Goldsmith et al. 2006). In this case, we choose a stratified random sampling method, where samples were selected at random from a limited number of locations available (i.e., locations on trails). In our case we first selected 10 sections of 500m trails within/near the plot, each with 50m landmarks. For one survey, we randomly chose five 50m-locations within one trail section (5 out of 10 locations possible). Then for each of the 5 chosen locations, we randomly selected one compass direction (45 deg. angles; 8 possible orientations). We went to the selected location, read the selected compass direction, walk 10m outside of the trail, and took the two paired samples there, each distant of 10cm (taxonomic and metabarcoding samples, see main text).

1.4. Avoid spatial autocorrelation between locations. This simplifies statistical analysis of data by preventing inflated degrees of freedom. For soil arthropods the minimal distance between samples can be short, as they typically do not move much around. In our case, the minimum distance between each paired sample was 50m. In these conditions spatial pseudoreplication was negligible (Basset et al. 2020).

1.5. Avoid taking samples near the nests of social insects (ants, termites), to avoid biased estimates of abundance/occurrence in samples.

2. Equipment

2.1. Berlese-Tullgren.We used a collapsible (ideal for transport) Berlese-Tullgren, adapted after the commercial Bioquip model #2832 (Bioquip 2321 Gladwick Street, Rancho Dominguez, CA 90220, USA; https://bioquipinc.com/catalog/collecting-equipment-supplies/traps/collapsible-berlese-funnel-trap/). The funnel is 31 cm in diameter and 91 cm in height when extended. The upper portion of the funnel section is made of muslin to allow evaporation of moisture. The funnel is smooth with a plastic funnel at the bottom. A 0.635cm mesh galvanized metal screen is secured to the lower ring. The user can place a smaller mesh screen on top of this screen if desired. Ground litter from which insects are to be collected is placed on the screen. A lamp base with socket, on/off knob, hanger loop, and cable are secured by a drawstring at the top of the funnel section. The unit operates on 120V AC electrical current, and a low-wattage light bulb (25W) is used to heat and dry out the material placed on the screen. The collector is a sterile plastic urine container (see item 2.3). A hole is drilled into the cap so that it can affixed to the plastic funnel. The container is filled with ethanol 95%.

2.2. Tools and small equipment needed, for 50 samples: garden spades to scoop litter and soil; brushes to clean tools and recipients; two spray bottles for bleach and distilled water; two plastic containers (20 liters each) for bleach and distilled water; a wooden frame of 20cm x 20cm (400 cm^2) to delineate coarsely the sample; a graduated plastic container of 2 liters for calibrating the sample; 50 plastic boxes about 20cm x 10cm x 10cm (or larger) to hold each sample. Ice boxes to accommodate all plastic boxes to avoid overheating samples when transporting them. Large trays to set up soil samples in Berlese-Tullgren.

2.3. Supplies needed.
Commercial bleach (for example in Panama: Clorox, Clorox de Centroamérica; hypoclorite of sodium 3.5%, hydroxide of sodium 0.3%).
Distilled water.
Face masks.
Latex gloves, sterile.

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Disposable sterile wipes, for example Kimwipes: https://www.sigmaaldrich.com/catalog/product/aldrich/z188956?lang=en&region=PA&gclid=CjwKCAjwjqT5BRAPEiwAJlBuBSYIJo7wwQirz17VKYXTnBw0b8PAP8J9Pt4kSeR6S8mQTm9_zFfe0BoCoTQQAvD_BwE
100+ vials to store samples, leak-proof. A cheap solution is to use 100ml sterile plastic urine containers, available in pharmacies and drugstores. Consider more vials so that the tap could be left in place in Berlese-Tullgren (item 2.1).
100 medium-sized transparent garbage bags.
Pre-print labels for samples
Parafilm tape to seal samples for transportation, for example: https://www.sigmaaldrich.com/catalog/product/sigma/p7543?lang=en&region=PA&gclid=CjwKCAjwjqT5BRAPEiwAJlBuBfFKckzvqNcHVWlV4de_CDUd3QiM9FJ1NxRtju5_RRjkUGEMUMNcgBoC6d8QAvD_BwE
Ropes to hang the Berlese-Tullgren apparatus.
Masking tape.
25W bulbs.
Ziplock bags

Note that most supplies should be cleaned with bleach and rinsed with distilled water before use.

3. Avoiding contamination

3.1. To avoid contamination between samples, use first face masks and latex gloves. Change gloves after the manipulation of each sample. Each time that a new sample is obtained, clean all recipients and tools with bleach in a spray bottle or with brushes, rinse with distilled water (to clean bleach residues) and then wipe the extra moisture with Kimwipes or equivalent.

4. Collecting samples in the field

4.1. Timing. To avoid soggy samples and a difficult extraction of the fauna, we do not collect samples if there was rain less than 6 hours before actual collection of samples.

4.2. We also avoid collecting in areas rather different than the main forest, such as large forest gaps.

4.3. Main procedure to obtain one sample. First, clean a garden spade with a small brush and then disinfect the brush and spade with spraying bleach and rinsing with distillated water. Second, use the spade to scoop a sample of 2,000cm^3 (=2,000ml) of soil and litter. To delineate coarsely the sample, use a 20cm x 20cm frame (400cm^2), and scoop inside the frame soil and litter up to a depth of ca. 5cm (so volume = 2,000cm^3). Spray with bleach and rinse with distilled water a calibrated 2,000ml (2 liters) plastic container, transfer the soil into the container with the spade and calibrate to 2000ml. Transfer the samples into a plastic box 20cm x 10cm x 10cm, which has been beforehand cleaned with bleach and rinsed with distilled water. Place the box and a large pre-printed label into a medium-sized transparent garbage bag, close it and put the bag into a rucksack or ice box.

4.4. If possible, record variables characterizing the sample: coordinates, forest cover, type of soil, topography, etc. If recorded into a forest vegetation plot, the coordinates may allow relating samples with the composition of vegetation nearby.

5. Transporting samples

5.1. Do not allow samples to heat under the sun light. Use ice boxes to transport samples to the place where they will be extracted. This will reduce overheating and concomitant arthropod mortality, which would otherwise bias extraction.

5.2. Ideally extract as quickly as possible samples with Berlese-Tullgren. In our case we were able to set samples in Berlese-Tullgren about 4-5 hours after collection.

6. Extracting the fauna

6.1. Berlese-Tullgren should be emplaced on ropes, ideally in a ventilated area with a roof and access to electricity. If AC current is not available, consider heating Berlese-Tullgren with heat packs (for example see https://bioquipinc.com/catalog/collecting-equipment-supplies/heat-pack/).

6.2. Wear face mask and latex gloves and change gloves after handling each sample. Each Berlese-Tullgren should be cleaned with brushes and bleach, rinsed with distilled water and allowed to dry. Likewise, clean and rinse each recipient or tool, before and after handling each sample.

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6.3. Samples should be gently set in Berlese, with all falling debris recuperated in large trays (which should be first cleaned with bleach and rinsed with distillated water) and then poured again in the samples until little debris fell back.

6.4. Screw a sterile urine container containing 3cm of ethanol 95% to the cap attached to the plastic funnel of the Berlese-Tullgren. Check that the container never gets dry during extraction. Write with an indelible pen the sample code on the outside container and additionally secure the container with masking tape.

6.5. Extract the samples for 72 hours. The first 24 hours without additional heat, the next 48 hours with heat provided by a 25W bulb.

7. Storage of samples

7.1. When samples are fully extracted (arthropod soup and debris), refill fresh ethanol 95%, screw a sterile plastic cap on the collector, rewrite or affix outside the collector the sample code, and emplace the collector into a ziplock bag with a second label in the bag.

7.2. Store samples in freezer at -20°C until they can be processed for metabarcoding. Ideally samples should be proceeds for metabarcoding within 2-3 months.

8. Shipping samples

8.1. In case samples need to be shipped to a laboratory for metabarcoding analyses, drain first much of the ethanol (wear face mask and latex gloves as usual) from the vial, screw the cap and close it firmly with parafilm tape, put it into a ziplock bag with label, and send all samples by courier mail to the laboratory. You may need to fill a IATA-180 form (exemption for scientific samples in ethanol) to ship the samples, as well as an export permit. Samples need to be refill with fresh ethanol 95% on arrival to the laboratory.

9. Long-term monitoring and annual indices

9.1. Once samples are sequenced, sequences may be uploaded in platform such as mBrave (Ratnasingham 2019) for user-friendly bioinformatic analyses and retrieval of summary data (Barcode Index Number, species identification, etc.). We then recommend using the occurrence of species in metabarcoding data as a surrogate for species' annual population indices (upper limit of 100 samples with species present in case 100 samples are processed each year).

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