Air quality, the response of ecosystems

Design and construction of the Berlese-Tullgren trap for the LIFE MODERn (NEC) project

Leonardo Latilla, Bruno De Cinti, Serena Carloni, Flavia Sicuriello.

- CNR - Research Institute on Terrestrial Ecosystems, Strada Provinciale 35d, km. 0,700, Montelibretti - Rome (Italy)

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Introduction

The European Community, to safeguard human health and the planet, in response to the increasing levels of pollutant emissions into the atmosphere caused by anthropogenic activities, issued Directive 2016/2284 (NEC Directive), which establishes commitments for their reduction and the implementation of a monitoring system. The Life MODERn (NEC) Project was developed to address the need to monitor the effects of pollution reduction on ecosystems through a network of terrestrial and aquatic sites. The QBS-ar is one of the techniques employed in the monitoring activities. Was developed in recent years by an Italian team (Parisi, 2001; Parisi et al., 2005). The term "QBS" is the acronym of Soil Biological Quality (in Italian: Qualità Biologica del Suolo) and "ar" refers to the arthropod community. The index is based on soil invertebrates that belong to the phylum of arthropods with a size between 0.2 and 2 mm (mesofauna). Edaphic microarthropod communities are an important reservoir of biodiversity and play an essential role in several soil ecosystem functions; furthermore, they are often used as soil quality indicators (Menta et al., 2011).

QBS-ar index focuses on the presence of morphological characters that indicate the degree of adaptation to soil such as the reduction or loss of pigmentation, streamlined body form, reduced or loss of appendages (hairs, antennae, legs), reduction or loss of structure to fly, jump or run, reduced visual apparatus, reduced water-retention capacity and presence of specialized organs (i.e., post-antennal organs). Soil community is therefore composed by epi-edaphic, hemi-edaphic and eu-edaphic taxa progressively more closely related to the hypogeal environment and intolerant towards the disturbance.

The index is based on the concept that the higher is the number of microarthropod groups morphologically well adapted to this soil habitat and the higher is the soil quality (Joseph et al., 2016). So, this index combines the presence of microarthropods in the soil, intended as biodiversity and their capability to adapt to soil conditions intended as vulnerability (Menta et al., 2018)

Because of these characteristics QBS-ar index was considered to be a standard protocol for measuring soil fauna across Europe LTER sites ExpeEr Ecosystem Research Program (Experimentation in Ecosystem Research, proj. no. 262060, (http://www.expeeronline.eu/about-expeer/context.html, Firbank et al., 2017), and it is reported in the European Commission DG ENV, 2010. In addition, the index was inserted in the COMMON GUIDELINES FOR ANALYTICAL METHODS (Malusà et al., 2019; D'Avino et al., 2022).

Sampling method. Under stable conditions, such as in woodlands, soil can be sampled once a year at the same time, preferably in spring or autumn. Winter and summer should be avoided due to low temperatures and dryness, which can reduce the presence and activity of soil fauna. Samples for QBS-ar calculation must be collected when soil moisture ranges between 40% and 80% of field capacity (Parisi et al., 2005).

Adherence to the protocol during the extraction stage is particularly important for the quality assurance of the indicator. The procedure requires trained personnel for the four steps of the protocol: soil sampling, animal extraction, identification of groups, and scoring. By focusing on the presence of characteristics that indicate adaptation to soil environments, and not requiring complex taxonomic identification to the species level, QBS-ar analysis can also be performed by non-specialists (Galli et al., 2014) after a few days of training.

The QBS-ar protocol follows four phases (Parisi et al., 2005; Menta et al., 2008; Joseph et al., 2016; D'Avino et al., 2022):

1. **Soil sampling**. An appropriate sampling area of 10x10 m should be chosen within the study site, taking into account the high variability of soil communities and the potential edge effect. For each sampling unit, three cubic soil samples of 1 dm^3 (10x10x10 cm) should be taken from within the sampling area after removing the litter layer. Samples should be stored in plastic bags to protect them from thermal shock and physical impact, and must be placed in the trap as soon as possible, but no later than 48 hours.

2. **Microarthropod extraction**. Microarthropods are extracted from soil cores using a Berlese-Tullgren funnel (Berlese, 1905; Tullgren, 1918; Southwood, 1994), in which heat from a lamp causes the arthropods to migrate downward. The funnel setup consists of an incandescent lamp (40 watts) placed 30 cm above the soil sample, a sieve (2 mm mesh, 20 cm in diameter), a funnel (plastic or glass), and a container filled with a fixing liquid (2/3 alcohol and 1/3 glycerol). The duration of microarthropod extraction depends on soil moisture and should never be less than 5 days. Adherence to the protocol during the extraction stage is particularly important to ensure the quality of the indicator.

3. **Determination of Biological Forms and Assignment of the Ecological-Morphological Index (EMI).** The extracted specimens are observed using a stereomicroscope at low magnification (40X) and classified at the order or class level. For myriapods (Diplopoda, Chilopoda, Symphyla, Pauropoda), classification is done at the class level, while for insects, Chelicerata, and Crustacea, classification is performed at the order level. Within each higher taxon, the QBS method requires identifying the biological form (morphotype) that is most adapted to the soil. This form is assigned an Eco-Morphological Index (EMI) score proportionate to its level of adaptation. As a general rule, **eu-edaphic** forms (deep-dwelling) receive an EMI score of 20, **hemi-edaphic** forms (intermediate dwellers) are assigned a score proportionate to their degree of specialization, and **epi-edaphic** forms (surface dwellers) receive an EMI score of 1. Specimens belonging to each taxon are counted and separated into their respective biological forms, with scores ranging from 1 to 20 based on their soil adaptation.

4. **QBS-ar Index Computation.** The QBS-ar index is calculated by summing the EMI values obtained from the extracted sample. If two EMI values are assigned to the same taxon, the higher EMI value should be used for the QBS-ar computation. Field data will be initially recorded on paper forms, and then the survey teams will transcribe the data into an electronic spreadsheet. Specific software packages will be used to compute the indices, with a preference for open-source systems (e.g., R).

Berlese-Tullgren Trap

The QBS-ar technique, to be effectively applied, requires a process of extracting arthropods present in soil samples collected from the field.

This extraction can be carried out using the Berlese-Tullgren trap.

Fig. 1.

The equipment consists of a sieve placed above a funnel connected to a container that traps and preserves microarthropods in a mixture of alcohol and glycerol.

The extraction of organisms from a soil sample measuring one cubic decimeter is carried out by placing the sample on the metal mesh of the device and exposing it to a heat source, provided by a 40-watt incandescent lamp, for a duration of 5 days.

This process results in the progressive drying of the soil clump from top to bottom, driving the arthropods to move downward until they fall into the underlying container, where they are trapped in the fixative liquid.

Construction of the Berlese-Tullgren Trap

The traps were constructed using recycled materials, which allowed for a considerable reduction in costs.

List of Materials Used (Single Unit)

To build the sieve:

- Woven metal mesh with a square 2x2 mm grid;
- Self-tapping wood screws;
- Orange PVC pipe, 200 mm in diameter;
- Funnel, 220 mm in diameter;
- 250 ml plastic liquid container.

Fig. 2. The metal mesh used to create the sieve was hand-cut into a circular shape with a diameter of 220 mm using tin snips. It was then shaped and secured to the inner base of the orange PVC pipe with screws and glue. The mesh has 2x2 mm square openings, which effectively hold the soil clump above while allowing microorganisms to pass through, thus acting as an efficient filter. This type of mesh is sold by the meter at stores specializing in steel materials.

Fig. 3. The sieve was created by cutting a PVC pipe with a diameter of 200 mm to a height of 10 cm and securing the metal mesh (as shown in Fig. 1) to its base. The funnel below was made using the neck of an 18-liter PET bottle.To attach the sieve to the funnel, four vertical cuts were made along the neck of the bottle, and the two parts were joined with duct tape. Inside the mouth of the bottle, the perforated cap of the underlying container was glued (see Fig. 3), allowing it to be screwed onto the bottom of the funnel.

Fig. 4. The 250 ml container, filled with approximately 40 ml of an alcohol and glycerin-based solution, is screwed beneath the funnel using the threading of its perforated cap, which is glued inside the funnel (see Fig. 4).

Fig. 5. The perforated cap is glued to the inside of the funnel base.

To Create the Heat Source:

- 40-watt incandescent bulb;
- E27 lamp holder;
- Two-core rubber electrical cable;
- 16A male plug.

Fig. 6. The incandescent bulb was chosen because, in addition to emitting light, it generates heat essential for drying the soil samples, a feature not achievable with other types of bulbs. Due to energy-saving regulations, these bulbs are difficult to source, so they were purchased online from a non-European supplier.

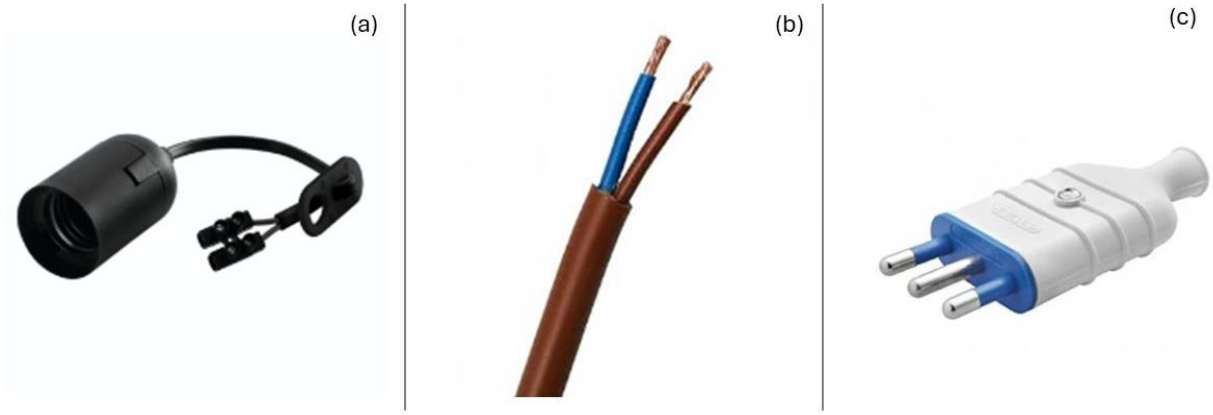

Fig. 7. (a) E27 lamp holder with cable,(b) 2.5 mm² two-core rubber electrical cable, (c) Power plug for connecting the power lines to the electrical panel.

Shelf for Simultaneous Extraction

Fig. 8. To allow the simultaneous extraction of multiple samples, a shelf with twenty-seven positions distributed over four tiers has been created. The structure was made using a metal shelf measuring 40x100x200, whose tiers were drilled with a column drill and a hole saw (hole diameter 8 cm) to support the traps. Each level accommodates 8 traps, except for the last one, which holds 3.

Each tier is served by an independent power line, to which the lamp holders are connected using clamps and insulated by a plastic channel, which functions as an adjustable support hanging above the traps. This allows a variable number of traps to be turned on depending on the number of samples to be extracted.

Conclusions

Practical experience and functionality tests have confirmed the reliability of the trap, achieving excellent results in the extraction of microarthropods. The shelf, designed to optimize space usage, allowed for the simultaneous handling of 27 samples in a compact area. It was constructed using easily available and recycled materials, ensuring a significant cost reduction. The total budget for the project was approximately 350 euros. The only flaw identified is its sensitivity to accidental impacts, which can cause soil to fall into the collection container located at the base of the trap. This results in an excessive amount of soil mixed with the extracted microarthropods, making the separation and analysis of the samples more complex. To mitigate this issue, the shelf was anchored to the wall, improving the overall stability of the system.

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