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## CALL FOR COLLABORATION

**Global monitoring of soil animal communities using a common methodology**

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## Abstract

Here we introduce the Soil BON Foodweb Team, a cross-continental collaborative network that aims to monitor soil animal communities and food webs using consistent methodology at a global scale. Soil animals support vital soil processes via soil structure modification, consumption of dead organic matter, and interactions with microbial and plant communities. Soil animal effects on ecosystem functions have been demonstrated by correlative analyses as well as in laboratory and field experiments, but these studies typically focus on selected animal groups or species at one or few sites with limited variation in environmental conditions. The lack of comprehensive harmonised large-scale soil animal community data including microfauna, mesofauna, and macrofauna, in conjunction with related soil functions, microbial communities, and vegetation, limits our understanding of biological interactions in soil systems and how these interactions affect ecosystem functioning. To provide such data, the Soil BON Foodweb Team invites researchers worldwide to use a common methodology to address six long-term goals: (1) to collect globally representative harmonised data on soil micro-, meso-, and macrofauna communities, (2) to describe key environmental drivers of soil animal communities and food webs, (3) to assess the efficiency of conservation approaches for the protection of soil animal communities, (4) to describe soil food webs and their association with soil functioning globally, (5) to establish a global research network for soil biodiversity monitoring and collaborative projects in related topics, (6) to reinforce local collaboration networks and expertise and support capacity building for soil animal research around the world. In this paper, we describe the vision of the global research network and the common sampling protocol to assess soil animal communities and advocate for the use of standard methodologies across observational and experimental soil animal studies. We will use this protocol to conduct soil animal assessments and reconstruct soil food webs at sites associated with the global soil biodiversity monitoring network, Soil BON, allowing us to assess linkages among soil biodiversity, vegetation, soil physico-chemical properties, climate, and ecosystem functions. In the present paper, we call for researchers especially from countries and ecoregions that remain underrepresented in the majority of soil biodiversity assessments to join us. Together we will be able to provide science-based evidence to support soil biodiversity conservation and functioning of terrestrial ecosystems.

**Keywords** Biogeography | ecosystem functioning | macroecology | soil fauna | soil biodiversity

## Introduction

Soil animals are an essential component of virtually all terrestrial ecosystems (Petersen & Luxton 1982, Fierer et al. 2009). They support ecosystem functions by directly contributing to decomposition and nutrient cycling, and indirectly through engineering activities, as well as by influencing microbial communities and plant growth (Hassall et al. 2006, Lavelle et al. 2006, Briones 2014, Handa et al. 2014). In exclusion experiments, the presence of soil animals can enhance aboveground plant productivity by up to 70%, depending on the vegetation type (Sackett et al. 2010, van Groenigen et al. 2014, Trap

et al. 2016), or facilitate litter decomposition by up to 50%, depending on climatic conditions (García-Palacios et al. 2013). These effects largely emerge from biological interactions within and among functional groups of soil animals, microbes, and plants (Bonkowski et al. 2009, Coulibaly et al. 2019, Potapov 2021). Local variations in animal communities may sum up to considerable effects on ecosystem processes at the biosphere level (Handa et al. 2014, Seibold et al. 2021). There have been several calls to include explicitly soil animal effects in global biogeochemical (Filser et al. 2016, Soong & Nielsen 2016, Deckmyn et al. 2020) and soil erosion models (Orgiazzi & Panagos 2018), but the required large-scale

comprehensive community data to validate these animal-based models are lacking.

The most comprehensive overview on the contribution of soil animal communities to ecosystem functioning comes from the International Biological Programme and dates back to the 1980s (Petersen & Luxton 1982, Huhta 2007). However, the observational data compiled at that time was not linked to soil properties and functions in a spatially-explicit way, which limited their use for biogeochemical modelling and for a broader ecosystem level understanding. Several recent studies have collected global spatially-explicit data and extrapolated global distributions of earthworms (Phillips et al. 2019) and nematodes (van den Hoogen et al. 2019), while syntheses on springtails (#GlobalCollembola) (Potapov et al. 2020a, Potapov et al. 2022) and soil macrofauna (GlobalSoilMacrofauna) (Lavelle et al. *under review*) are in progress. These studies showed that the distribution of the local diversity of soil animals differs strongly from that of aboveground organisms (Cameron et al. 2019, Phillips et al. 2019) due to their contrasting responses to environmental drivers (Bardgett & van der Putten 2014).

To date, global assessments of soil micro-, meso- and macrofauna have been done independently by different expert communities and using different methodologies. This limits our understanding of relationships among key functional groups of soil organisms and prevents us from global upscaling of soil communities and food webs they form. A comprehensive community-level assessment will make it possible to reconstruct soil food webs and infer energy fluxes to describe biotic controls, trophic functioning, and stability patterns of soil biodiversity. Food webs is a particularly powerful approach to link soil biodiversity to both ecosystem functioning and environmental drivers (de Vries et al. 2013, Potapov 2021). Existing global extrapolations of soil biodiversity remain poorly linked to soil functions with only 0.3% of soil ecological studies simultaneously assessing soil biodiversity and functions (Guerra et al. 2020). This important knowledge gap makes a robust quantification of soil animal contribution to biosphere functioning impossible and hampers projections of soil functioning under future global change scenarios (Guerra et al. 2021b). Furthermore, gaps in knowledge of soil animal distribution and diversity, especially of tropical soil animal communities, severely limits our understanding of human impact on soil animals and the design of appropriate conservation strategies (Eisenhauer et al. 2019, Geisen et al. 2019, Guerra et al. 2021a). These knowledge gaps call for a comprehensive soil animal biodiversity assessment at a global scale using a common methodology (Geisen et al. 2019, White et al. 2020, Eisenhauer et al. 2021), which is achievable only through a major joint effort.

## A standardised soil animal monitoring at the global scale

The Soil Biodiversity Observation Network (Soil BON) was launched in 2018 as a part of The Group on Earth Observations Biodiversity Observation Network (GEO BON), a United Nations initiative that aims to monitor Earth's biodiversity (Scholes et al. 2008, Guerra et al. 2021c). Soil BON is a collaborative network supported by the Global Soil Biodiversity Initiative (GSBI) that focuses on soil biodiversity (Guerra et al. 2021c) and at present includes research teams across 80+ countries. Local teams will collect soil samples at approximately 1,000 sites across all continents, except Antarctica, for the first time in 2022. Additional sampling is planned every three years to establish long-term global-scale monitoring of soil biodiversity. The resulting soil samples will be shipped to a central hub (German Centre for Integrative Biodiversity Research, iDiv, Leipzig, Germany) to perform measurements of soil properties (water holding capacity, stoichiometry of nutrients, pH, root characteristics), microbiome (eDNA sequencing of prokaryotes, fungi, protists), and functions (soil respiration, substrate-induced respiration, microbial biomass, enzymatic activity, litter decomposition, soil aggregate stability). The sampling also includes nematodes from topsoil. However, this approach is not well-suited to assess in full soil animal communities due to the large amount of material that needs to be transported (kilograms of soil per site), and the high mortality of soil animals (particularly macrofauna) in long-term stored and shipped soil samples. Hence, it is important that soil animals are collected, extracted, and assessed immediately after sampling with limited disturbance to samples.

Here, we introduce the Soil BON Foodweb Team (SBF Team), an extension of Soil BON that focuses on the assessment of soil animal communities (Fig. 1a). We aim at producing new harmonised global data on densities and biomasses of all major taxonomic groups of soil invertebrates across micro-, meso-, and macrofauna, and thus expand the scope of the Soil BON initiative by linking soil biodiversity to ecosystem level processes through a soil food web perspective. This effort widens the core Soil BON network by involving local research communities of soil zoologists and taxonomists and applying additional sampling approaches. Instead of having a central hub, we will coordinate local researchers and facilities to form a complementary global network aiming at six main long-term goals:

1. To deliver *open, comprehensive, and globally representative methods and harmonised datasets* on soil micro-, meso-, and macrofauna in conjunction with soil functions.

2. To explore effects of *climate, land use, and other environmental variables* on soil animal communities, soil food web structure, resistance, and resilience.
3. To assess the efficiency of current *nature conservation* approaches in protecting soil animal communities.
4. To relate soil animal communities and food webs with *soil functioning* across climates, soils, and land use types.
5. To establish a *global soil fauna expert network* for soil biodiversity monitoring and other collaborative projects.
6. To reinforce local collaboration networks and *local expertise* in soil animal ecology and taxonomy.

Our initial objective is to sample at least 240 out of the planned ~1,000 Soil BON sites in 2022. Soil animal data will be linked to the core Soil BON network data on climate, land use, soil parameters, microorganisms, and functions via common sampling sites and similar sampling periods. Therefore, *this is the first initiative linking quantitative soil animal data across the size spectrum to a range of soil functions worldwide*. The *Global coordination team* includes the *Central team* which is responsible for data acquisition and storage, the co-Chairs of Soil BON, and the *National coordinators* who communicate with the local survey networks (Fig. 1c). The extended network includes *Field teams* and *Local hubs*, i.e. research teams that have expertise in soil animal ecology and perform soil sampling and animal extraction. All these roles are not mutually exclusive (e.g. a person can be involved in the *Global coordination team*, but also form a *Local hub* and a *Field team*). To date, research teams from 25 countries have volunteered to join the Team, partly covering also less explored regions such as Africa, South America, Asia, and Russia. This paper is a call for soil animal ecologists from around the world to join the SBF Team. Priority will be given to research teams from undersampled countries and ecoregions; at present, these include mainly countries in Africa, Asia, South America, and the Middle East (Fig. 1b). To participate as a *Field team*, expertise in soil zoology, basic equipment for the field work (Supplementary protocol S1), and possibility to collect soil animals from several sampling sites with standardised methods is needed. To establish a *Local hub*, a laboratory equipped with wet and/or dry extractors is needed. This equipment can be also built with little monetary costs and guidelines will be provided by the Team participants (Edwards 1991, Niva et al. 2015, Cesarz et al. 2019). *National coordinators* will help establish, support, and coordinate local collaboration networks of *Field teams* and *Local hubs*. The current list of *National coordinators*, sampling video, and extraction

guidelines will be available from the web page of the SBF Team ([www.soilbonfoodweb.org](http://www.soilbonfoodweb.org)). All material and data contributors and national coordinators are invited to join collaborative publications, workshops, and add-on projects of the SBF Team and synthesis publications of the Soil BON consortium including their data. Below, we describe our sampling protocol, data acquisition and storage strategies.

## A common methodology

### Target variables

Our initiative and methodologies have been designed to be as *comprehensive* as possible and cover all key functional groups of soil micro-, meso- and macrofauna, to be *globally representative*, with significant coverage across continents, different countries, and environmental conditions, and to be *inclusive* for research groups and countries with limited facilities (Maestre & Eisenhauer 2019). This is a bold and challenging task that demands low-cost and labour-efficient approaches. To ensure feasibility, at the initial stage we will focus on biomass of taxonomic and functional groups, rather than at species level. The latter implies a need for taxonomic expertise in all major soil invertebrate groups which is limiting particularly in tropical regions, where a large proportion of the individuals collected are singletons, and where many species are unknown/undescribed (Rossi et al. 2006, Barnes et al. 2014, Potapov et al. 2020b, Demetrio et al. 2021). Nevertheless, building taxonomic expert capacities locally is one of the long-term goals of the SBF Team (see our aims above). Our methodologies are designed to represent well all major functional groups of soil animals while being feasible for implementation across the globe. Additional methodologies that improve representation of specific functional groups (e.g. very large or spatially-aggregated macrofauna) are considered for add-on projects in the future.

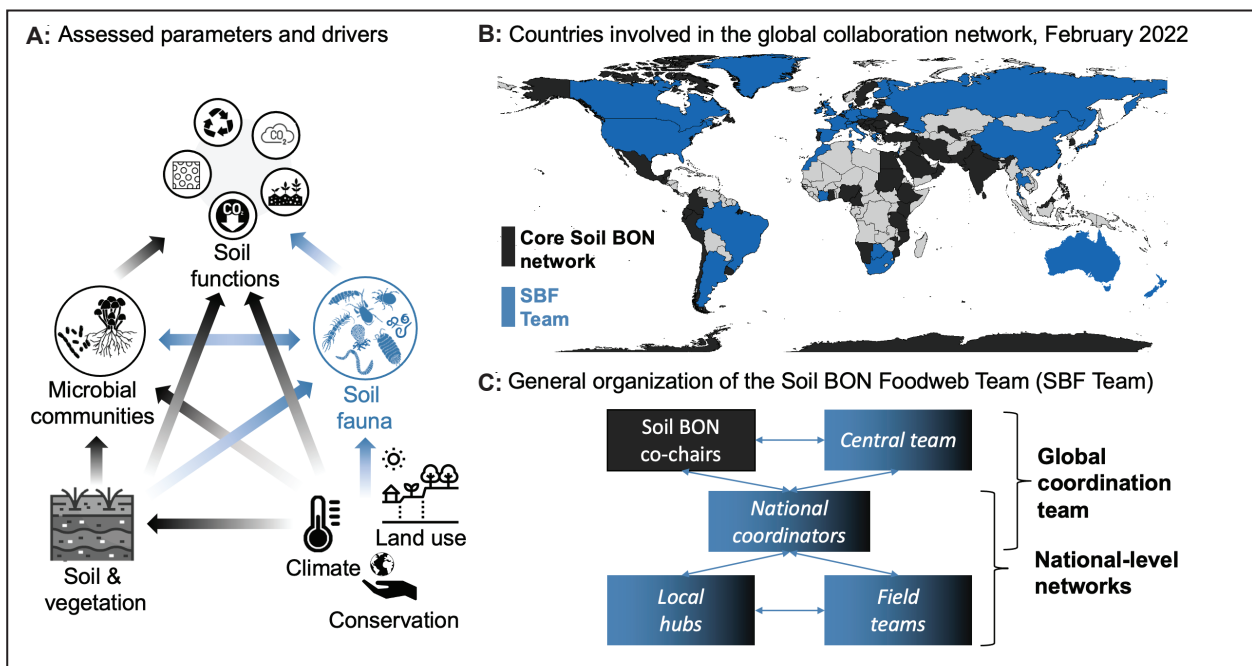
### Site selection, sampling size and time

Site selection is linked to the Soil BON core design, where soil parameters, functions, and microbial communities are assessed. Therefore, sampling sites for animal assessment are chosen from a pre-selected set of sites in communication with researchers involved in the core Soil BON network. The core design includes sites outside and inside areas that have a 'protection' status (e.g. nature conservation reserves) in each country to

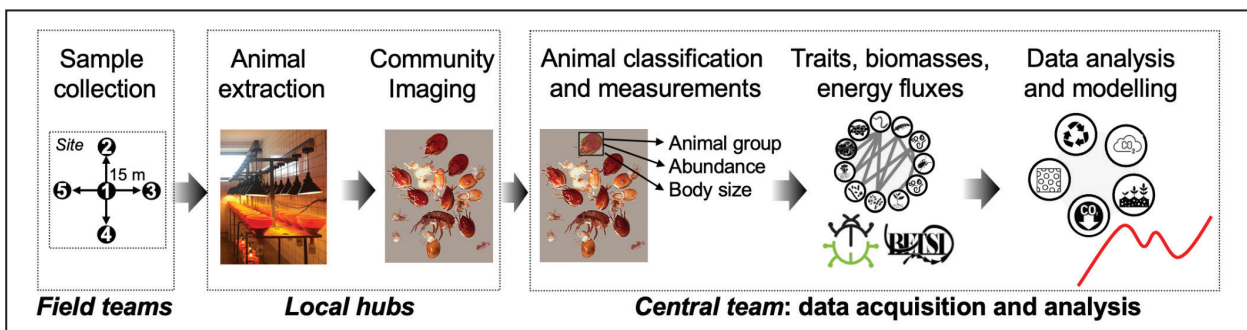


evaluate the effect of present conservation strategies on soil biota (Guerra et al. 2021a). The sampling of soil animals should be done at least on two sites by each *Field team* (e.g. one site inside and one outside of a protected area within the same geographical region and habitat type), but ideally cover more ecosystems and geographic areas. The final site selection is designed by *National coordinators* to identify priority ecosystems that cover wide environmental gradients (Guerra et al. 2020). At the initial stage, we target to sample at least 240 sites (120 pairs) globally, which will be sufficient for general analyses (Delgado-Baquerizo et al. 2020). For all sites we collect information on the sampling date, person,

location, vegetation characteristics, and history. The sampling is done at the peak vegetation biomass season according to the Soil BON approach (Guerra et al. 2021c). A 3-month window will be provided for each site and these time frames will vary across sites. At each site, soil animals are assessed at five sampling points (within a 30 x 30 m area) to account for within-site variation as the recommended minimum for large soil animals, whose spatial distribution is commonly heterogeneous (Fig. 2) (Rossi et al. 2006, Ruiz et al. 2011). All samples will be processed separately, making it possible to assess local beta-diversity. Animal sorting will be done outside sampling sites to minimise disturbance.



**Figure 1. The Soil BON Foodweb Team concept.** Effects of climate, land use, and conservation on soil functions are mediated by soil properties, microbes, and animals, the latter are assessed by the SBF Team (A). At present, research groups from 80+ countries are officially involved in Soil BON (black), the *Global coordination team* of the SBF Team (blue) covers all major continents (B). The *Global coordination team* includes the SBF *Central team*, Soil BON co-chairs, and SBF *National coordinators*. National-level networks are coordinated by *National coordinators* and may also include teams involved in the core Soil BON network, showed by black-blue gradient (C).



**Figure 2. Workflow of the global soil animal assessment.** Soil animals are collected by local *Field teams* at the Soil BON sites. Animals are extracted and photographed in mixed samples at *Local hubs*. Abundances and body sizes of taxonomic groups are estimated by the *Central team* using manual image annotations and a trained image analysis algorithm (Schneider et al. 2021). Biomasses and energy fluxes are estimated using allometric regressions and food-web reconstruction approaches (Ehnes et al. 2011, Sohlström et al. 2018, Jochum et al. 2021, Potapov 2021) and used in statistical analyses and modelling.

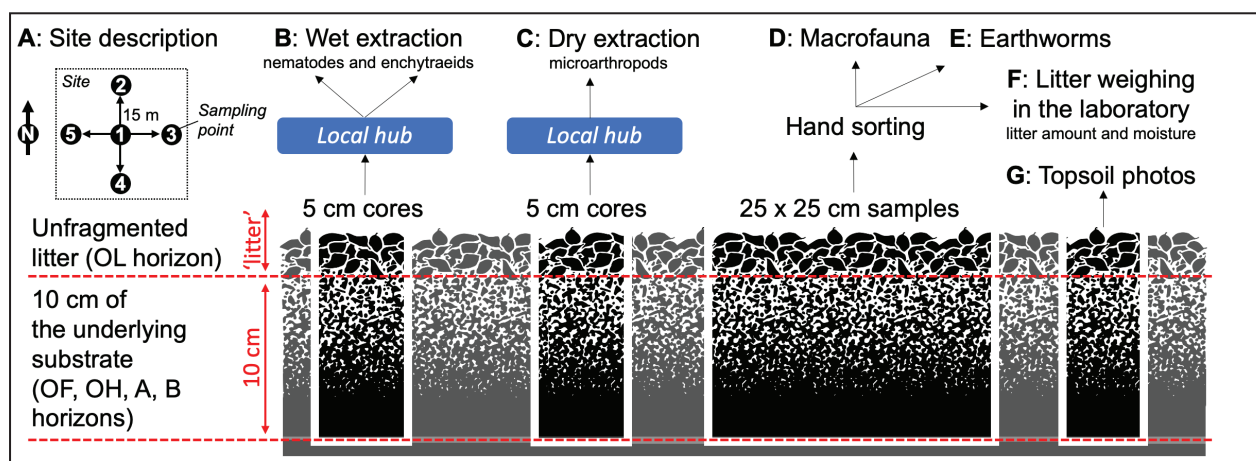
## Field sampling methods

**Selected methods.** We will use a combination of sampling methods to comprehensively represent soil animal communities, including micro-, meso-, and macrofauna (Fig. 3; Geisen et al. 2019, White et al. 2020). Microfauna and enchytraeids will be collected using wet extractions (Baermann funnels; Niva et al. 2015, Cesarz et al. 2019), microarthropods using dry extraction (e.g. Berlese-like; Edwards 1991), and macrofauna using hand-sorting (Anderson & Ingram 1993, Bignell 2009; details are given below). We have chosen these sampling methods as the most commonly used for the corresponding animal size groups and are straightforward enough to be used even by researchers with basic experience in soil animal ecology. These methods provide area-based estimations of biomass and density which is necessary for data comparability and calculations of energy fluxes and functional impacts of soil animals (Jochum & Eisenhauer 2022). For the latter reason, pitfall traps are not included in the main methods, but suggested as an auxiliary method (Supplementary protocol S1). We will also take pictures of topsoil profiles and sampling sites to collect environmental data (Fig. 3, Supplementary protocol S1).

**Sample areas.** We follow a common 19.6 cm<sup>2</sup> sample area for microarthropods, i.e. soil corer of 5 cm diameter (also used in the core Soil BON network sampling). We use the same sample area for microfauna and enchytraeids, but the sample is divided in two equal parts at *Local hubs* (see below). A 625 cm<sup>2</sup> sample area is used for macrofauna (soil monoliths 25 x 25 cm). Although a larger sample area would better represent

large-sized actively-moving animals and social insects, we have to limit collection efforts to make the sampling of many sites feasible and thus make the initiative more inclusive for research teams and globally representative.

**Sample depth.** We sample the entire fresh litter layer (OL horizon, unfragmented litter) together with the first 10 cm of the underlying substrate ('soil', here referring to OF, OH, A, and sometimes top B horizons). Animals from litter and soil layers are collected and processed together to maximise efficiency and avoid data mismatch due to ambiguities in the definition of 'litter' (Fig. 4). Our assessment will focus on animals and processes in top soil and will miss deep-living soil animals (e.g., some endogeic earthworms; Lavelle 1988, Jiménez & Decaëns 2000), especially in soils with well-developed organic horizons (Andre et al. 2002, Rossi et al. 2006, Potapov et al. 2017). We also acknowledge that other special soil habitats such as suspended soils, subsoils and subterranean soils will not be included in this effort (see Fig. 1 in White et al. 2020). This would allow the application of a single standard protocol and increase the number of sites to make the analysis globally representative. Furthermore, we plan to address the issue of undersampling of deep-living soil fauna by (1) performing sampling of deeper soil layers as an add-on project (Supplementary protocol S1), and (2) modelling the vertical distributions of different animal groups depending on the soil type based on existing data (van den Hoogen et al. 2019, Phillips et al. 2019, Potapov et al. 2022, Lavelle et al. *under review*). The uniform sampling depth is used for all samples to make the energy flux calculations comparable across size classes. To correctly measure the sampling depth, we strongly



**Figure 3. Sampling concept and animal extraction methods.** A combination of wet extraction, dry extraction, and hand-sorting is used to collect soil micro-, meso-, and macrofauna. Five samples of each type are taken per *site*. Sample depth (litter and 10 cm of the underlying soil) has been chosen to ensure comparability of data on animal communities with data on microorganisms, soil parameters, and functions measured in the first 10 cm of soil by the core Soil BON network. Note that we define 'litter' to include the OL horizon only, i.e. unfragmented leaves (Zanella et al. 2018a, Zanella et al. 2018b). Extractions of microarthropods, nematodes and enchytraeids are done at *Local hubs*.

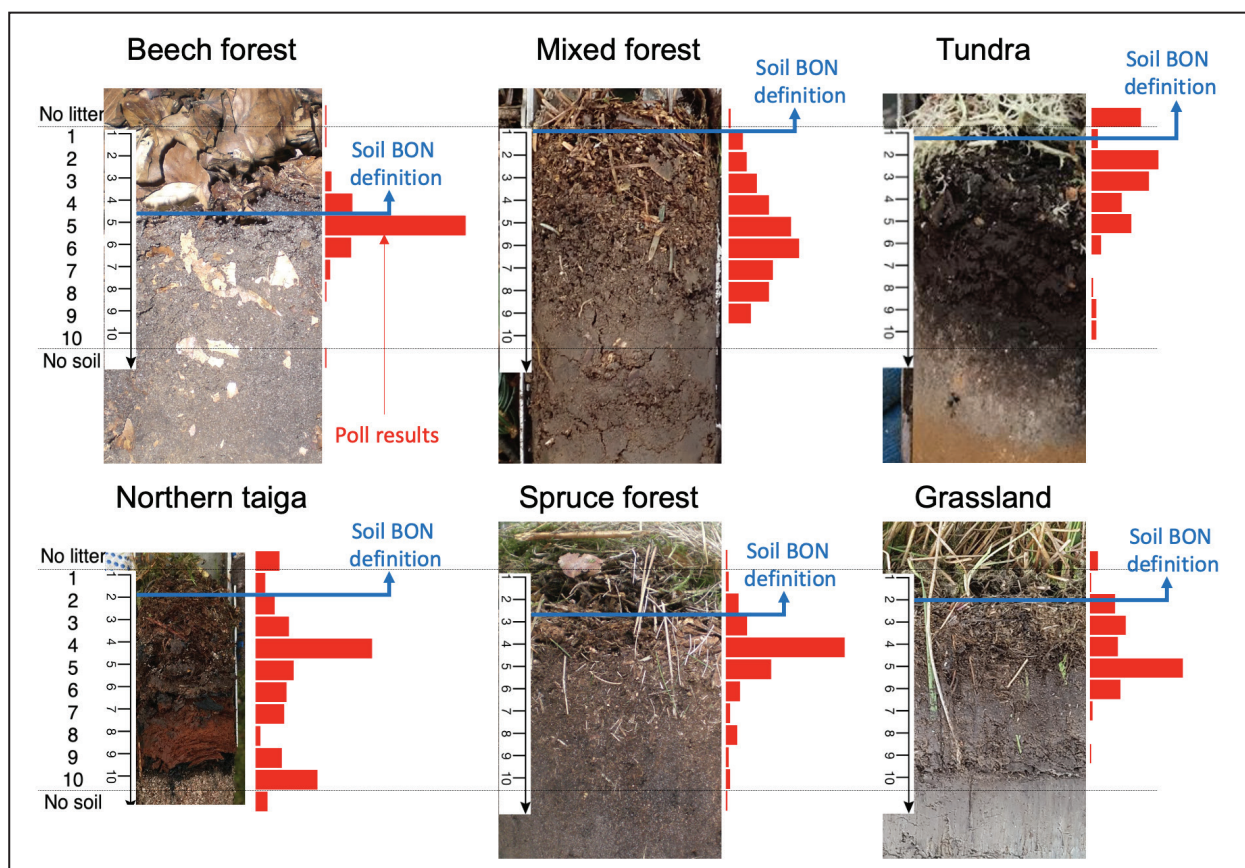
encourage soil zoologists to become familiar with the recent HUMUSICA publications to define the diagnostic horizons (Zanella et al. 2018a, Zanella et al. 2018b).

**Macrofauna collection.** Large invertebrates (>3 mm in body length) including earthworms are picked up by hand from litter and soil, generally following the Tropical Soil Biology and Fertility method (TSBF; Anderson & Ingram 1993). We excavate soil monoliths 25 x 25 cm to a 10 cm depth and animals are hand sorted either in the field or brought to a laboratory. All social insects (ants, termites) regardless their size and all other animals belonging to the size class between 3 and 30 mm and not belonging to taxa of microarthropods (mites, pseudoscorpions, symphylans, diplurans, pauropods etc.) (Gongalsky 2021) are collected and stored in 96% alcohol. Hand-sorting of macrofauna is preferred over dry extraction, because it has better compatibility with other existing global-scale initiatives (Lavelle et al. *under revision*), is conventionally used to collect earthworms, and catches less mobile animals like gastropods and insect larvae

(Anderson & Ingram 1993). Besides, large amounts of material do not have to be transported to the laboratory, which is hard for remote sites and may be disruptive.

### Animal extraction: Local hubs

Animal extractions are done at *Local hubs* to improve consistency of extractions across sites and make possible participation of research teams without extraction equipment. We distinguished *Wet extraction hubs* that are equipped with Baermann funnels or other wet extraction equipment and *Dry extraction hubs* that are equipped with dry Berlese, Tullgren, or high-gradient extractors. These two types of hubs could (but do not have to) be at different locations and coordinated by different research teams. Each hub is expected to do extractions and animal imaging from 4-10 sites in the sampling year (i.e., 20-50 soil samples). *Local hubs* can receive central support from the SBF Team (e.g. imaging equipment),



**Figure 4. Variation in the definition of ‘litter’ by soil ecologists and the SBF Team solution.** Results of a non-representative poll with the aim of finding the ‘litter-soil threshold’ on topsoil images are presented as red histograms. Responses were acquired anonymously from 170 soil ecologists after disseminating the poll through email contacts and Twitter, and represent an overview of opinions mostly from Europe (66% of respondents) and North and South America (27%). The poll was mostly completed by soil zoologists and functional ecologists (39% each). The SBF Team defines ‘litter’ as *green vegetation remains, mosses, lichens and unfragmented dead leaves/wood with only little decomposition damage* (100% organic matter, OL horizon, marked with blue lines and arrows; Zanella et al. 2018a, Zanella et al. 2018b). Photograph credits: Daniil I. Korobushkin, Melanie M. Pollierer, Johanness Lux, Pierre Ganault.



their coordinators are closely engaged in the planning of sampling and add-on projects together with *National coordinators*, and have priority to lead regional-scale synthesis studies. The global assessment in the framework of the SBF Team can be combined with ongoing regional and local-scale projects or monitoring activities as long as the sites are registered in Soil BON and the common sampling protocol is used. *Local hubs* are also responsible for organising mid-term (at least 5 years) storage of samples, but are *not* expected to do field sampling (unless research teams form both a *Local hub* and a *Field team*), animal sorting and identification (the latter is done by the *Central team* from the images at iDiv, Leipzig). A collaboration with museums for long-term sample storage is encouraged. We also will provide detailed manuals and protocols of the extraction equipment and process on our web page ([www.soilbonfoodweb.org](http://www.soilbonfoodweb.org)).

**Nematode and enchytraeid extraction.** These two groups are extracted separately using wet extractors. For both groups, we recommend Baermann funnels as the most commonly used and more accessible method in comparison to e.g. Oostenbrink elutriator (De Goede & Verschoor 2000). Nematodes together with other microfauna are extracted through milk filters to produce clean samples. We will generally follow a recent protocol for large-scale nematode assessments to improve extraction efficiency (Cesarz et al. 2019). Formalin will be used as a storage agent to allow for potential future identifications. Enchytraeids are extracted separately through a 0.5 mm mesh (ISO 23611-3 2019) and stored in ~96% ethanol to make the material suitable for potential genetic analyses.

**Microarthropod extraction.** To extract microarthropods, we recommend light bulb-equipped

Tullgren/Berlese funnels (Tullgren 1917, Edwards 1991) or Macfadyen/Kempson high-gradient extractors (Macfadyen 1961, Kempson et al. 1963). These different extractor types provide a fair representation of community composition and biomass of microarthropods and, despite in some cases favouring different groups, yield comparable results (Macfadyen 1961, Edwards 1991, Andre et al. 2002). The extraction is done through a 2 mm mesh to exclude large macrofauna, and ~96% ethanol is used as the collection and storage solution to make the material suitable for potential genetic analyses. Since each laboratory is equipped with slightly different extractors, we will provide detailed protocols on both wet and dry extraction procedures to participants and openly distribute in via [www.soilbonfoodweb.org](http://www.soilbonfoodweb.org).

## Animal identification

Soil communities will be characterised at each sampling site based on the list of taxonomic and functional groups with data on their abundance, individual body masses, and total biomasses (the tentative list of target groups is given in Table 1). On the one hand, a taxonomic grouping should be detailed enough to make functional inferences as well as a food web reconstruction possible (Brussaard 1998, Briones 2014, Buchkowski & Lindo 2021, Potapov 2021). On the other hand, the grouping should be generic enough to include all major taxa and regions and easy enough to allow the sorting by a general soil ecologist from a mixed community image, i.e. under a low magnification microscope. In most cases, we follow a taxonomic classification, because it allows for unambiguous

**Table 1. The list of animal groups are counted and measured.** Both taxonomic and functional groups are used for sorting. Individual groups are divided by comma. ‘Parent’ groups that include several target groups are given in *italics* and their ‘children’ are separated with full stop from other groups.

Method	Target animal groups
Wet extraction of nematodes and other microfauna	<i>Nematoda</i> : Plant feeders, Bacterivores, Fungivores, Omnivores, Predators. Rotifera, Tardigrada.
Wet extraction of enchytraeids	Enchytraeidae
Dry extraction of microarthropods	Collembola, Protura, Oribatida, Mesostigmata, Astigmata, Prostigmata, Pseudoscorpiones, Symphyla, Pauropoda. <i>Diplura</i> : Campodeoidea, Japygoidea and Projapygoidea. Also <i>small-sized</i> Araneae, Thysanoptera, Sternorrhyncha, other insects.
Hand sorting	Earthworms. <i>Gastropoda</i> : snails, slugs. <i>Orthoptera</i> : Ensifera, Caelifera. Cockroaches (Blattidae and Corydiidae), Isoptera, Formicidae. <i>Hemiptera</i> : Auchenorrhyncha, Sternorrhyncha, Heteroptera herbivores, Heteroptera predators. <i>Coleoptera</i> : Carabidae, Cantharidae, Elateridae, Staphylinidae, Curculionidae, Scarabaeidae, Tenebrionidae, Silphidae, other beetle families. Psocoptera, Lepidoptera, Diptera, Dermaptera, Thysanoptera. Collembola (only large epigeic species), other insect groups. <i>Chilopoda</i> : Geophilomorpha, Lithobiomorpha and Scolopendromorpha. Diplopoda. Isopoda, other crustaceans. Araneae, Opiliones, Amblypygi, Uropygi, Solifugae, Scorpiones.

grouping of animals and because functional roles and trophic niches of soil animals are in general related to their taxonomic position (Cardoso et al. 2011, Potapov et al. 2019). In several functionally diverse groups, such as mites, flies, ants, and termites, a broad taxonomic resolution may lead to information loss (Frouz 1999, Eggleton & Tayasu 2001, Schneider et al. 2004, King 2016), but more detailed identification is constrained by the absence of appropriate taxonomic expertise across many regions. We are planning to approach these limitations in the follow-up molecular and taxonomic projects by establishing thematic collaborative networks. For instance, nematodes are planned to be sorted to trophic groups (Table 1) in collaboration with experts. In perspective, linking the global assessment of soil biodiversity with networks of taxonomic experts and organisation of taxonomic training programmes would allow for a great progress in the understanding of global soil biodiversity.

### Animal identification and measurement

Animal sorting and identification is probably the most difficult and laborious part of soil animal assessments. Rapidly developing metabarcoding and metagenomic approaches are often seen as an alternative to visual identification (Oliverio et al. 2018, Liu et al. 2020), however, they have strong limitations in the light of our aims: (1) large-scale assessments using these approaches are expensive and require complex local facilities, thus are not inclusive for research teams/regions with limited resources, (2) mass-sequencing methods are non-quantitative due to extraction and amplification biases (Dopheide et al. 2019, Pereira-da-Conceicao et al. 2021), (3) due to a limited trait data availability, it is not possible to extract body sizes for all soil invertebrate taxa based on genetic sequences and thus reliably calculate energy fluxes, (4) barcode libraries are absent for vast majority of soil invertebrate species to match phylogenetic positions with traits such as body size.

As an alternative high-throughput approach, we adopt image analyses of mixed invertebrate community samples (Schneider et al. 2021). Image analysis is based on visual animal identification and allows for direct body size estimations which can be used to estimate biomasses and energy fluxes. To acquire high resolution photographs, we will use a recently tested imaging pipeline for soil communities using a flatbed scanner (Potapov et al. *unpublished data*). This approach lowers the costs of imaging equipment to max 350 USD, thus being inclusive for teams with limited resources (Fig. 5). The imaging is done at *Local hubs* and all images are stored on central servers at iDiv, Leipzig.

### Data acquisition

High-resolution pictures will be processed by the *Central team* using manual image annotations to develop a computer-vision pipeline based on deep learning algorithms (RCNN) (Schneider et al. 2021, Sys et al. *under review*). Preliminary tests on a community of 10 springtail and two mite species showed a detection rate of 77% with identification accuracy of 90% at the species level. We expect improvement of the two metrics with further developments of the training library and at a coarser taxonomic resolution. Manual annotations will be used at the initial stages and then deep learning algorithms will be applied to streamline the identification and measurement processes. With this approach, all individuals of micro-, meso-, and macrofauna will be identified to the group level (Table 1) and body dimensions will be measured. Individual body sizes will be converted to body masses and total community biomass using allometric equations (Andrassy 1956, Petersen 1975, Newton & Proctor 2013, Sohlström et al. 2018). With development of a training image library, scanning and image analysis are expected to speed up group-level identification and body size estimations of soil invertebrates and considerably reduce workload of *Local hubs*. Our methodology can be applied by most soil ecology laboratories across the globe and will produce reliable and comparable data on density, biomass, and size distributions of all key functional groups of soil micro-, meso- and macrofauna found in the topsoil. All images and collected data will be centrally stored and accessible for the data providers who will have priority of analysing and publishing these data. Collected data and developed machine learning algorithms will be made openly available through public online platforms upon publication for the use of the research community.

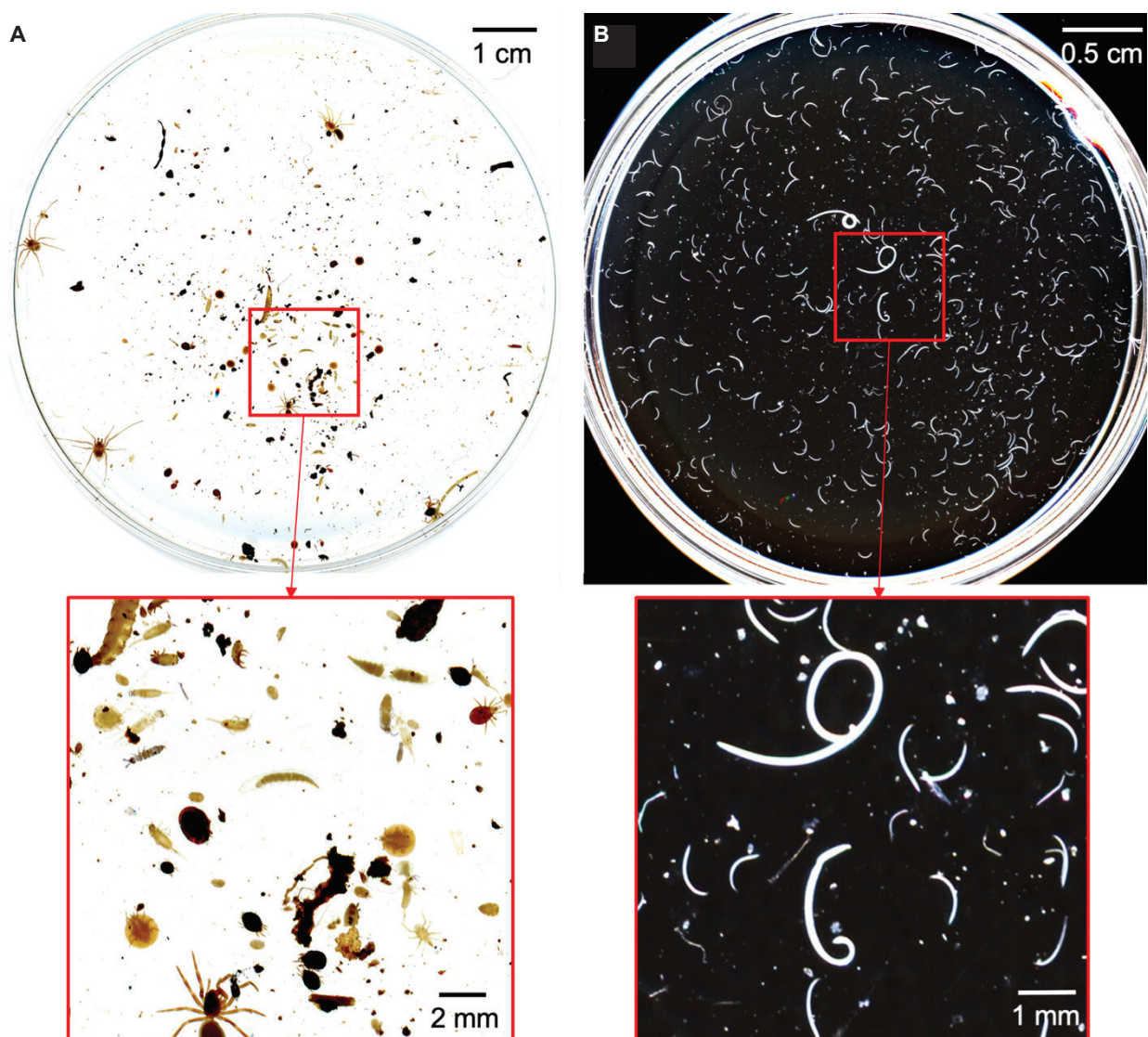
### Future prospects

Data collected by the SBF Team will be linked to the data on soil, vegetation, climatic, and microbial parameters generated by ongoing work at respective Soil BON sites, and relationships between soil animals and ecosystem functioning and their responses to environmental factors and human activities will be assessed. We will also compare soil animal communities inside and outside of the protected areas worldwide to evaluate the effect of current conservation practices on soil animal communities. To analyse the animal data, we will use different approaches, such as path analysis (Eisenhauer et al. 2015), geospatial modelling (van den Hoogen et al.

2021), food-web reconstruction and modelling (de Vries et al. 2013, Potapov 2021), and energy flux approaches (Barnes et al. 2018, Jochum et al. 2021). We also plan to link collected animal data with multiple functional traits to assess global variation in the functional diversity of soil animal communities (Pey et al. 2014, Brousseau et al. 2018) and work on integration of our results in suggested animal-based biogeochemical models (Chertov et al. 2017, Deckmyn et al. 2020, Flores et al. 2021). Finally, any SBF Team participant can propose further ideas of how to use the collected data to the *Global coordination team* and test his/her/their hypotheses.

The developed protocol can be used beyond the SBF Team in any other compatible observational or experimental study that aims to accumulate standard and

comprehensive data on soil animal communities across environmental and biotic gradients. To ensure the long-term data safety, compatibility, and accessibility, common databases on community images and animal counts will be established. We intend to upload collected data to Edaphobase (Burkhardt et al. 2014) which will make it publicly available directly and through the linked Global Biodiversity Information Facility (GBIF; Heberling et al. 2021). To ensure the safety of collected materials, all animals will be stored in ~96% ethanol in the freezer for at least five years after the sampling, and this period is likely to be prolonged. These storage conditions will allow us to run potential add-on projects on biodiversity-related questions, such as metabarcoding of soil animal communities and taxonomic identification of selected



**Figure 5. Images of mixed communities of soil arthropods (A) and nematodes (B) made with a flatbed scanner.** The images have been taken from a Petri dish with 96% ethanol with a 4800 dpi resolution using Epson Perfection V600 (Seiko Epson Corporation, Japan). Close-up frames show that even small mites and nematodes can be detected on the images.



animal groups. Established collaborative networks at the global and regional scales will serve as the coordination basis for such add-on projects, facilitating soil animal ecology and providing science-based evidence to policymakers to support soil biodiversity conservation and functioning of the terrestrial biosphere.

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## Supplementary materials

Protocol S1 describing field sampling; as supplementary file to this article on [www.soil-organisms.org](http://www.soil-organisms.org).

## References

- Anderson, J. M. & J. S. I. Ingram (1993): *Tropical Soil Biology and Fertility: A Handbook of Methods*. – C.A.B. International, Wallingford, Oxfordshire, England.
- Andrassy, I. (1956): Die rauminhalts- und gewichtsbestimmung der fadenwürmer (Nematoden). – *Acta Zoologica Hungarica* **2**: 1–5.
- André, H. M., X. Ducarme & P. Lebrun (2002): Soil biodiversity: myth, reality or conning? – *Oikos* **96**: 3–24.
- Bardgett, R. D., & W. H. van der Putten (2014): Belowground biodiversity and ecosystem functioning. – *Nature* **515**: 505–511.
- Barnes, A. D., M. Jochum, J. S. Lefcheck, N. Eisenhauer, C. Scherber, M. I. O’Connor, P. de Ruiter & U. Brose (2018): Energy Flux: The Link between Multitrophic Biodiversity and Ecosystem Functioning. – *Trends in Ecology & Evolution* **33**: 186–197.
- Barnes, A. D., M. Jochum, S. Mumme, N. F. Haneda, A. Farajallah, T. H. Widarto & U. Brose (2014): Consequences of tropical land use for multitrophic biodiversity and ecosystem functioning. – *Nature Communications* **5**: 5351.
- Bignell, D. E. (2009): Towards a universal sampling protocol for soil biotas in the humid tropics. – *Pesquisa Agropecuaria Brasileira* **44**: 825–834.
- Bonkowski, M., C. Villenave & B. Griffiths (2009): Rhizosphere fauna: the functional and structural diversity of intimate interactions of soil fauna with plant roots. – *Plant and Soil* **321**: 213–233.
- Briones, M. J. I. (2014): Soil fauna and soil functions: a jigsaw puzzle. – *Frontiers in Environmental Science* **2** [<https://doi.org/10.3389/fenvs.2014.00007>].
- Brousseau, P.-M., D. Gravel & I. T. Handa (2018): On the development of a predictive functional trait approach for studying terrestrial arthropods. – *The Journal of Animal Ecology* **87**: 1209–1220.
- Brussaard, L. (1998): Soil fauna, guilds, functional groups and ecosystem processes. – *Applied Soil Ecology: A Section of Agriculture, Ecosystems & Environment* **9**: 123–135.
- Buchkowski, R. W., & Z. Lindo (2021): Stoichiometric and structural uncertainty in soil food web models. – *Functional Ecology* **35**(1): 288–300.
- Burkhardt, U., D. J. Russell, P. Decker, M. Döhler, H. Höfer, S. Lesch, S. Rick, J. Römbke, C. Trog, J. Vorwald & et al. (2014): The Edaphobase project of GBIF-Germany—A new online soil-zoological data warehouse. – *Applied Soil Ecology* **83**: 3–12.
- Cameron, E. K., I. S. Martins, P. Lavelle, J. Mathieu, L. Tedersoo, M. Bahram, F. Gottschall, C. A. Guerra, J. Hines, G. Patoine & et al. (2019): Global mismatches in aboveground and belowground biodiversity. – *Conservation Biology* **33**: 1187–1192.
- Cardoso, P., S. Pekár, R. Jocqué & J. A. Coddington (2011): Global patterns of guild composition and functional diversity of spiders. – *PloS One* **6**: e21710.
- Cesarz, S., A. E. Schulz, R. Beugnon & N. Eisenhauer (2019): Testing soil nematode extraction efficiency using different variations of the Baermann-funnel method. – *Soil Organisms* **91**: 61–72.
- Chertov, O., A. Komarov, C. Shaw, S. Bykhovets, P. Frolov, V. Shanin, P. Grabarnik, I. Pripulina, E. Zubkova & M. Shashkov (2017): Romul\_Hum—A model of soil organic matter formation coupling with soil biota activity. II. Parameterisation of the soil food web biota activity. – *Ecological Modelling* **345**: 125–139.
- Coulibaly, S. F. M., B. R. Winck, M. Akpa-Vinceslas, L. Mignot, M. Legras, F. Forey & M. Chauvat (2019): Functional assemblages of Collembola determine soil microbial communities and associated functions. – *Frontiers*



- of Environmental Science 7 [https://doi.org/10.3389/fenvs.2019.00052].
- Deckmyn, G., O. Flores, M. Mayer, X. Domene, A. Schnepf, K. Kuka, K. Van Looy, D. P. Rasse, M. J. I. Briones, S. Barot & et al. (2020): KEYLINK: towards a more integrative soil representation for inclusion in ecosystem scale models. I. review and model concept. – *PeerJ* **8**: e9750.
- De Goede, R. G. M. & B. Verschoor (2000): The nematode extraction efficiency of the Oostenbrink elutriator-cottonwool filter method with special reference to nematode body size and life strategy. – *Nematology* **2**: 325–342.
- de Vries, F. T., E. Thebault, M. Liiri, K. Birkhofer, M. A. Tsiafouli, L. Bjornlund, H. B. Jorgensen, M. V. Brady, S. Christensen, P. C. de Ruiter & et al. (2013): Soil food web properties explain ecosystem services across European land use systems. – *Proceedings of the National Academy of Sciences of the United States of America* **110**: 14296–14301.
- Delgado-Baquerizo, M., P. B. Reich, C. Trivedi, D. J. Eldridge, S. Abades, F. D. Alfaro, F. Bastida, A. A. Berhe, N. A. Cutler, A. Gallardo & et al. (2020): Multiple elements of soil biodiversity drive ecosystem functions across biomes. – *Nature Ecology & Evolution* **4**: 210–220.
- Demetrio, W. C., A. C. Conrado, A. N. S. Acioli, A. C. Ferreira, M. L. C. Bartz, S. W. James, E. da Silva, L. S. Maia, G. C. Martins, R. S. Macedo & et al. (2021): A “Dirty” Footprint: Macroinvertebrate diversity in Amazonian Anthropogenic Soils. – *Global Change Biology* **27**: 4575–4591.
- Dopheide, A., D. Xie, T. R. Buckley, A. J. Drummond & R. D. Newcomb (2019): Impacts of DNA extraction and PCR on DNA metabarcoding estimates of soil biodiversity. – *Methods in Ecology and Evolution* **10**: 120–133.
- Edwards, C. A. (1991): The assessment of populations of soil-inhabiting invertebrates. – *Agriculture, Ecosystems & Environment* **34**: 145–176.
- Eggleton, P. & I. Tayasu (2001): Feeding groups, lifestyles and the global ecology of termites. – *Ecological Research* **16**: 941–960.
- Ehnes, R. B., B. C. Rall & U. Brose (2011): Phylogenetic grouping, curvature and metabolic scaling in terrestrial invertebrates. – *Ecology Letters* **14**: 993–1000.
- Eisenhauer, N., Bonn, A., & A Guerra, C. (2019): Recognizing the quiet extinction of invertebrates. — *Nature Communications* **10**: 50.
- Eisenhauer, N., M. A. Bowker, J. B. Grace & J. R. Powell (2015): From patterns to causal understanding: Structural equation modeling (SEM) in soil ecology. – *Pedobiologia* **58**: 65–72.
- Eisenhauer, N., F. Buscot, A. Heintz-Buschart, S. D. Jurburg, K. Küsel, J. Sikorski, H.-J. Vogel & C. A. Guerra (2021): The multidimensionality of soil macroecology. – *Global Ecology and Biogeography* **30**: 4–10.
- Fierer, N., M. S. Strickland, D. Liptzin, M. A. Bradford & C. C. Cleveland (2009): Global patterns in belowground communities. – *Ecology Letters* **12**: 1238–1249.
- Filser, J., J. H. Faber, A. V. Tiunov, L. Brussaard, J. Frouz, G. De Deyn, A. V. Uvarov, M. P. Berg, P. Lavelle, M. Loreau & et al. (2016): Soil fauna: key to new carbon models. – *Soil* **2**: 565–582
- Flores, O., G. Deckmyn, J. Curiel Yuste, M. Javaux, A. Uvarov, S. van der Linde, B. De Vos, H. Vereecken, J. Jiménez, O. Vinduskova & A. Schnepf (2021): KEYLINK: towards a more integrative soil representation for inclusion in ecosystem scale models-II: model description, implementation and testing. – *PeerJ* **9**: e10707.
- Frouz, J. (1999): Use of soil dwelling Diptera (Insecta, Diptera) as bioindicators: a review of ecological requirements and response to disturbance. – *Agriculture, Ecosystems & Environment* **74**: 167–186.
- García-Palacios, P., F. T. Maestre, J. Kattge & D. H. Wall (2013): Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. – *Ecology Letters* **16**: 1045–1053.
- Geisen, S., M. J. I. Briones, H. Gan, V. M. Behan-Pelletier, V.-P. Friman, G. A. de Groot, S. E. Hannula, Z. Lindo, L. Philippot, A. V. Tiunov & D. H. Wall (2019): A methodological framework to embrace soil biodiversity. – *Soil Biology & Biochemistry* **136**: 107536.
- Geisen, S., D. H. Wall & W. H. van der Putten (2019): Challenges and Opportunities for Soil Biodiversity in the Anthropocene. – *Current Biology* **29**: R1036–R1044.
- Gongalsky, K. B. (2021): Soil macrofauna: Study problems and perspectives. – *Soil Biology & Biochemistry* **159**: 108281.
- Guerra, C. A., R. D. Bardgett, L. Caon, T. W. Crowther, M. Delgado-Baquerizo, L. Montanarella, L. M. Navarro, A. Orgiazzi, B. K. Singh, L. Tedersoo & et al. (2021a): Tracking, targeting, and conserving soil biodiversity. – *Science* **371**: 239–241.
- Guerra, C. A., M. Delgado-Baquerizo, E. Duarte, O. Marigliano, C. Gørgen, F. T. Maestre & N. Eisenhauer (2021b): Global projections of the soil microbiome in the Anthropocene. – *Global Ecology and Biogeography* **30**: 987–999.
- Guerra, C. A., A. Heintz-Buschart, J. Sikorski, A. Chatzinotas, N. Guerrero-Ramírez, S. Cesarz, L. Beaumelle, M. C. Rillig, F. T. Maestre, M. Delgado-Baquerizo, F. Buscot & et al. (2020): Blind spots in global soil biodiversity and ecosystem function research. – *Nature Communications* **11**: 3870.
- Guerra, C. A., D. H. Wall & N. Eisenhauer (2021c): Unearthing soil ecological observations. – *Soil Organisms* **93**: 79–81.
- Handa, I. T., R. Aerts, F. Berendse, M. P. Berg, A. Bruder, O. Butenschoen, E. Chauvet, M. O. Gessner, J. Jabiol, M. Makkonen & et al. (2014): Consequences of biodiversity loss for litter decomposition across biomes. – *Nature* **509**: 218–221.
- Hassall, M., S. Adl, M. Berg, B. Griffiths & S. Scheu (2006): Soil fauna–microbe interactions: towards a conceptual framework for research. – *European Journal of Soil Biology* **42**: 54–60.

- Heberling, J. M., J. T. Miller, D. Noesgaard, S. Weingart & D. Schigel (2021): Data integration enables global biodiversity synthesis. – *Proceedings of the National Academy of Sciences of the United States of America* **118**: e2018093118.
- Huhta, V. (2007): The role of soil fauna in ecosystems: A historical review. – *Pedobiologia* **50**: 489–495.
- ISO 23611-3 (2019): Soil quality - sampling of soil invertebrates. Part 3, Sampling and extraction of enchytraeids.
- Jiménez, J. J. & T. Decaëns (2000): ‘Vertical Distribution of Earthworms in Grassland Soils of the Colombian Llanos’. – *Biology and Fertility of Soils* **32**: 463–73.
- Jochum, M., A. D. Barnes, U. Brose, B. Gauzens, M. Sünemann, Amyntas & N. Eisenhauer (2021): For flux’s sake: General considerations for energy-flux calculations in ecological communities. – *Ecology and Evolution* **11**: 12948–12969.
- Jochum, M. & N. Eisenhauer (2022): Out of the dark: Using energy flux to connect above- and belowground communities and ecosystem functioning. – *European Journal of Soil Science* **73**: e13154.
- Kempson, D., M. Lloyd & R. Ghelardi (1963): A new extractor for woodland litter. – *Pedobiologia* **3**: 1–21.
- King, J. R. (2016): Where do eusocial insects fit into soil food webs? – *Soil Biology & Biochemistry* **102**: 55–62.
- Lavelle, P., T. Decaëns, M. Aubert, S. Barot, M. F. Blouin, F. Bureau, P. Margerie, P. Mora & J.-P. Rossi (2006): Soil invertebrates and ecosystem services. – *European Journal of Soil Biology* **42**: 3–15.
- Lavelle, P., L. Mathieu, A. Spain, G. Brown, C. Fragoso, C. E. Lapiéd, A. De Aquino, I. Barois, E. Barrios, M. E. Barros & et al. Soil macroinvertebrate communities: a worldwide assessment. – *Under review* in *Global Ecology and Biogeography*.
- Liu, M., L. J. Clarke, S. C. Baker, G. J. Jordan & C. P. Burridge (2020): A practical guide to DNA metabarcoding for entomological ecologists. – *Ecological Entomology* **45**: 373–385.
- Macfadyen, A. (1961): Improved funnel-type extractors for soil arthropods. – *The Journal of Animal Ecology* **30**(1): 171–184.
- Maestre, F. T. & N. Eisenhauer (2019): Recommendations for establishing global collaborative networks in soil ecology. – *Soil Organisms* **91**: 73–85.
- Moreira, F. M. S., E. Jeroen Huising & D. E. Bignell (2012): *A Handbook of Tropical Soil Biology: Sampling and Characterization of Below-ground Biodiversity*. Routledge, Milton Park, Abingdon, Oxfordshire.
- Newton, J. S., & H. Proctor (2013): A fresh look at weight-estimation models for soil mites (Acari). – *International Journal of Acarology* **39**: 72–85.
- Niva, C. C., R. M. Cezar, P. M. Fonseca, M. R. G. Zagatto, E. M. Oliveira, E. F. Bush, L. A. Clasen & G. G. Brown (2015): Enchytraeid abundance in Araucaria Mixed Forest determined by cold and hot wet extraction. – *Brazilian Journal of Biology* **75**: 169–175.
- Oliverio, A. M., H. Gan, K. Wickings & N. Fierer (2018): A DNA metabarcoding approach to characterize soil arthropod communities. – *Soil Biology & Biochemistry* **125**: 37–43.
- Orgiazzi, A. & P. Panagos (2018): Soil biodiversity and soil erosion: It is time to get married. – *Global Ecology and Biogeography* **27**: 1155–1167.
- Pereira-da-Conceicao, L., V. Elbrecht, A. Hall, A. Briscoe, H. Barber-James & B. Price (2021): Metabarcoding unsorted kick-samples facilitates macroinvertebrate-based biomonitoring with increased taxonomic resolution, while outperforming environmental DNA. – *Environmental DNA* **3**: 353–371.
- Petersen, H. (1975): Estimation of dry weight, fresh weight, and calorific content of various Collembolan species. – *Pedobiologia* **15**: 222–243.
- Petersen, H. & M. Luxton (1982): A comparative analysis of soil fauna populations and their role in decomposition processes. – *Oikos* **39**: 288–388.
- Pey, B., J. Nahmani, A. Auclerc, Y. Capowiez, D. Cluzeau, J. Cortet, T. Decaëns, L. Deharveng, F. Dubs, S. Joimel & et al. (2014): Current use of and future needs for soil invertebrate functional traits in community ecology. – *Basic and Applied Ecology* **15**: 194–206.
- Phillips, H. R. P., C. A. Guerra, M. L. C. Bartz, M. J. I. Briones, G. Brown, T. W. Crowther, O. Ferlian, K. B. Gongalsky, J. van den Hoogen, J. Krebs & et al. (2019): Global distribution of earthworm diversity. – *Science* **366**: 480–485.
- Potapov, A. (2021): Multifunctionality of belowground food webs: resource, size and spatial energy channels. – *bioRxiv* [<https://doi.org/10.1101/2021.06.06.447267>].
- Potapov, A., B. Bellini, S. Chown, L. Deharveng, F. Janssens, L. Kováč, N. Kuznetsova, J.-F. Ponge, M. Potapov, P. Querner & et al. (2020a): Towards a global synthesis of Collembola knowledge – challenges and potential solutions. – *Soil Organisms* **92**: 161–188.
- Potapov, A. M., N. Dupérré, M. Jochum, K. Dreczko, B. Klärner, A. D. Barnes, V. Krashevskaya, K. Rembold, H. Kreft, U. Brose & et al. (2020b): Functional losses in ground spider communities due to habitat structure degradation under tropical land-use change. – *Ecology* **101**: e02957.
- Potapov, A. M., A. A. Goncharov, E. E. Semenina, A. Y. Korotkevich, S. M. Tsurikov, O. L. Rozanova, A. E. Anichkin, A. G. Zuev, E. S. Samoylova, I. I. Semenyuk & et al. (2017): Arthropods in the subsoil: Abundance and vertical distribution as related to soil organic matter, microbial biomass and plant roots. – *European Journal of Soil Biology* **82**: 88–97.
- Potapov, A. M., C. A. Guerra, J. van den Hoogen, A. Babenko, B. C. Bellini, M. P. Berg, S. L. Chown, L., Deharveng, L. Kovac, N. A. Kuznetsova & et al. (2022): Globally invariant metabolism but density-diversity mismatch in springtails. – *bioRxiv* [<https://doi.org/10.1101/2022.01.07.475345>].

- Potapov, A. M., S. Scheu & A. V. Tiunov (2019): Trophic consistency of supraspecific taxa in below-ground invertebrate communities: Comparison across lineages and taxonomic ranks. – *Functional Ecology* **33**: 1172–1183.
- Rossi, J.-P., J. Mathieu, M. Cooper & M. Grimaldi (2006): Soil macrofaunal biodiversity in Amazonian pastures: Matching sampling with patterns. – *Soil Biology & Biochemistry* **38**: 2178–2187.
- Ruiz, N., J. Mathieu, L. Célini, C. Rollard, G. Hommay, E. Iorio & P. Lavelle (2011): IBQS: A synthetic index of soil quality based on soil macro-invertebrate communities. – *Soil Biology & Biochemistry* **43**: 2032–2045.
- Sackett, T. E., A. T. Classen & N. J. Sanders (2010): Linking soil food web structure to above- and belowground ecosystem processes: a meta-analysis. – *Oikos* **119**: 1984–1992.
- Schneider, K., S. Migge, R. A. Norton, S. Scheu, R. Langel, A. Reineking & M. Maraun (2004): Trophic niche differentiation in soil microarthropods (Oribatida, Acari): evidence from stable isotope ratios ( $^{15}\text{N}/^{14}\text{N}$ ). – *Soil Biology & Biochemistry* **36**: 1769–1774.
- Schneider, S., G. W. Taylor, S. C. Kremer, P. Burgess, J. McGroarty, K. Mitsui, A. Zhuang, J. R. deWaard & J. M. Fryxell (2021): Bulk arthropod abundance, biomass and diversity estimation using deep learning for computer vision. – *Methods in Ecology and Evolution* [https://doi.org/10.1111/2041-210X.13769].
- Scholes, R. J., G. M. Mace, W. Turner, G. N. Geller, N. Jurgens, A. Larigauderie, D. Muchoney, B. A. Walther & H. A. Mooney (2008): Toward a global biodiversity observing system. – *Science* **321**: 1044–1045.
- Seibold, S., W. Rammer, T. Hothorn, R. Seidl, M. D. Ulyshen, J. Lorz, M. W. Cadotte, D. B. Lindenmayer, Y. P. Adhikari, R. Aragón & et al. (2021): The contribution of insects to global forest deadwood decomposition. – *Nature* **597**: 77–81.
- Sohlström, E. H., L. Marian, A. D. Barnes, N. F. Haneda, S. Scheu, B. C. Rall, U. Brose & M. Jochum (2018): Applying generalized allometric regressions to predict live body mass of tropical and temperate arthropods. – *Ecology and Evolution* **8**: 12737–12749.
- Soong, J. L. & U. N. Nielsen (2016): The role of microarthropods in emerging models of soil organic matter. – *Soil Biology & Biochemistry* **102**: 37–39.
- Sys, S., S. Weißbach, L. Jakob, S. Gerber & C. Schneider: CollembolAI, a macrophotography and computer-vision workflow to digitize and characterize samples of soil invertebrates communities preserved in fluid. – *Under review*. – In: *Methods in Ecology & Evolution*.
- Trap, J., M. Bonkowski, C. Plassard, C. Villenave & E. Blanchart (2016): Ecological importance of soil bacterivores for ecosystem functions. – *Plant and Soil* **398**: 1–24.
- Tullgren, A. (1917): En enkel apparat for automatiskt vittjande av sallgoods. – *Entomologisk Tidskrift* **38**: 97–100.
- van den Hoogen, J., S. Geisen, D. Routh, H. Ferris, W. Traunspurger, D. A. Wardle, R. G. M. de Goede, B. J. Adams, W. Ahmad, W. S. Andriuzzi & et al. (2019): Soil nematode abundance and functional group composition at a global scale. – *Nature* **572**: 194–198.
- van den Hoogen, J., N. Robmann, D. Routh, T. Lauber, N. van Tiel, O. Danylo & T. W. Crowther (2021): A geospatial mapping pipeline for ecologists. – *bioRxiv* [https://doi.org/10.1101/2021.07.07.451145].
- van Groenigen, J. W., I. M. Lubbers, H. M. J. Vos, G. G. Brown, G. B. De Deyn & K. J. van Groenigen (2014): Earthworms increase plant production: a meta-analysis. – *Scientific Reports* **4**: 6365.
- White, H. J., L. León-Sánchez, V. J. Burton, E. K. Cameron, T. Caruso, L. Cunha, T. Dirilgen, S. D. Jurburg, R. Kelly, D. Kumaresan & et al. (2020): Methods and approaches to advance soil macroecology. – *Global Ecology and Biogeography* **29**: 1674–1690.
- Zanella, A., J.-F. Ponge, J.-M. Gobat, J. Juilleret, M. Blouin, M. Aubert, O. Chertov & J. L. Rubio (2018a): Humusica 1, article 1: Essential bases – Vocabulary. – *Applied Soil Ecology* **122**: 10–21.
- Zanella, A., J.-F. Ponge, B. Jabiol, G. Sartori, E. Kolb, J.-M. Gobat, R.-C. L. Bayon, M. Aubert, R. D. Waal, B. Van Delft, A. Vacca & et al. (2018b): Humusica 1, article 4: Terrestrial humus systems and forms — Specific terms and diagnostic horizons. – *Applied Soil Ecology* **122**: 56–74.