SOIL ORGANISMS **94** (3) · December 2022 pp. 163–169

Effects of soil core handling, transport and storage on numbers and body sizes of edaphic predatory mites (Gamasina)

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Received 14 November 2022 | **Accepted** 28 November 2022
Published online at www.soil-organisms.de 1 December 2022 | **Printed version** 15 December 2022
DOI 10.25674/so94iss3id304

Abstract

In recent years, a number of collaborative projects began to collect consistent data on soil animal communities with the aim to understand, model, and map edaphic biodiversity, and to support environmental planning and decision making. Especially when operating on an international scale, it is vital for these programs to develop standardized protocols for properly sampling and processing soil cores. While guidelines for sampling, extracting, identifying and enumerating animals from soils have been published, the influence of transport and storage conditions on the recovery of animals has received very little attention. In this paper, the effects of improper treatment of cores on the extraction efficiency of predatory mites (Gamasina) from a temperate deciduous forest soil are investigated. Neither prolonged storage, shaking, compression or any of two combination treatments (compression + prolonged storage; shaking + prolonged storage) exerted a significant influence on the total abundance or the body size distributions of the mites. In contrast, both warming over 25°C and overfilling the sample containers of the Tullgren extractor significantly and drastically reduced the recovery of the animals, irrespective of body size. In conclusion, while the total (group) abundance of gamasid mites seems to be rather insensitive against improper sample treatment, the temperature of cores during transport and storage and a suitable volume of material in the extractor containers need to be adressed when planning the logistics of large scale sampling campaigns. Further studies on this topic are encouraged that also include other animal groups, other climate zones, and preferrably work on the level of species.

Keywords Biodiversity | extraction | sampling | soil fauna | soil microarthropods

1. Introduction

Before the background of the ongoing biodiversity crisis, scientists and research institutions are beginning to join forces for collating consistent data on edaphic animal communities. Due to their global extent, involvement of many researchers, and coverage of a wide range of environmental conditions, the Soil Observation Network (Soil BON, Guerra et al. 2021a, b) the Soil BON Foodweb Team (SBF Team, Potapov et al. 2022), and the MACROFAUNA database and its projected development (Mathieu et al. 2022) are especially ambitious in this respect. But also older, nation-wide inventories and monitorings (overview in Pulleman et al. 2012, Food and Agriculture Organization 2020) show the growing need to better understand, model, and map soil animal biodiversity and functions, and to provide the scientific basis for environmental planning and decision making.

Large scale soil biodiversity programs have to address a multitude of challenges that originate from the collaboration of research teams from many countries (Maestre & Eisenhauer 2019). A rather technical, yet decisive, aspect is to have standardized protocols for properly collecting, transporting, and processing

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ISSN 1864-6417 (print) · **ISSN** 2509-9523 (online)
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soils from selected study sites. With regard to edaphic animals, the rich (and sometimes inconsistent) literature on extraction techniques alone demonstrates that the quality of the collected data will heavily depend on these practical details (e.g. André et al. 2002).

For example, the SBF Team has recently published instructions on where, when and how to sample, how to extract edaphic animals, and how to identify and enumerate individuals (Potapov et al. 2022, https://soilbonfoodweb.org/protocols-and-manuals/). These guidelines are an excellent example of successfully combining the required detail for a standardized approach with the practicalities of field work. However, a particular step in the process of processing soil cores in large scale studies has yet not found attention: It is the phase between the sampling and the extraction of animals, that is, the phase of soil core transport and storage.

Since data are especially scarce in undersampled 'blind spots' of biodiversity research, we may assume that global biodiversity projects will prioritize regions where careful and quick sample treatment may not always be possible, for example tropical biomes, montane grassland, and arid areas (Guerra et al. 2020). Remote places like these may often be accessible only by foot and soil cores have to withstand a long and bumpy transport in rucksacks. Sampling tours may go over days, thus cores may have to be stored for prolonged periods. Without proper cooling and not in darkness, animals can be killed during the transport (or, vice versa, quiescent development stages become active and bias the catch). Field sampling may be conducted by less qualified personnel to save travel cost and make experts free for identification and counting. Finally, in order to keep pace with a tight project schedule, laboratory staff may be tempted to charge extraction apparatuses more than appropriate, which can trap animals inside the cores.

While there is at least some information available on the effects of sample treatment on soil microbial activity and community structure (e.g. Aragão et al. 2020, Brandt et al. 2014, Rubin et al. 2013), we know very little about transport and storage effects on edaphic animals: Lawrence et al. (2005) reported that the numbers of a plant pathogenic nematode recovered from soil dropped gradually within 1080 days of storage. Presenting preliminary data of an apparently never fully published study, Edwards & Fletcher (1971) showed little change of the abundances of meso- and macrofauna groups after storage at 5°C for 28 days, while numbers de- or increased more significantly at 20°C after 56 days. No appreciable effect of storage at 4°C for 12 days on the recovery of oribatid and trombidiform mites (except *Nanorchestes sp.*) were detected by Murphy (1962), however on a very small sample size. Lakly & Crossley (2000) found that the abundances of edaphic mites decreased linearly when cores where stored in a refrigerator - after only 8 days of storage, numbers were approximately halved.

Even otherwise elaborate textbooks provide, at best, only a few vague sentences on the subject. For instance, Wolfram Dunger noted that 'avoidable mistakes [that decrease the extraction efficiency of animals] may be caused by [...] poor transport conditions, wrong and overly long storage [...] but empirical data are hardly existent', Dunger & Fiedler 1997, p. 440, translation from German original). Empirical and consistent data are therefore urgently needed.

In this paper, and using gamasid mites as a test group, the sensitivity of edaphic microarthropods to improper sample treatment was estimated, as is likely to occur in large scale biodiversity programs. Soil material was collected in the field and improper transport and storage were simulated under laboratory conditions to enable controlled and repeatable experimentation. Specifically, prolonged storage, shaking, compression, warming, and charging of extractor containers were simulated separately and in combination, and their effects on total numbers and body sizes of the mites assessed.

2. Materials and methods

2.1 Sampling site

Experimental soil was collected in the 'Schottenwald' (16°16' E, 48°14' N, 300 m above sea level) in the forested western outskirts of the city of Vienna, Austria. The locality is in a transition zone between the cool humid alpine and the semiarid climate of the pannonian plain (Kilian et al. 1993). It was a mature European beech (*Fagus sylvatica*) and hornbeam (*Carpinus betulus*) forest with an even-aged, single canopy layer (6.1 stems * 100 m^{-2}, tree height approximately 25 m). The stand was strongly (20°) inclined to the south. The soil was a haplic planosol (Pseudogley, Nestroy et al. 2000) with mull humus, moderately moist and fertile, and moderately to pronouncedly acidic (indicator analysis of vascular plants, Ellenberg et al. 1992). Ground vegetation was classified as *Galio odorati-Fagetum* Sougnez et Thill 1969 (Wallnöfer et al. 1993).

2.2 Field sampling and experimental treatments

Gamasids were sampled on July 4, 2005, on a 400 m^2 plot within the study site on a regular grid. Cores were

taken with a 57 x 57 mm quadratic steel tube (Bruckner 1998) to a depth of 100 mm including the litter layer, put individually in plastic freezer bags, and transported to the laboratory on trays. Each core was handled with uttermost care and any compression, shaking, and exposure to sunlight avoided. Each core arrived in a cooling room (temperature 6 ± 0.5°C) no longer than 30 minutes after sampling.

The cores were randomly assigned to one of seven experiments. The experimental levels and the controls consisted of 16 replicate cores each; thus, in total, 368 cores were processed (7 experiments, 2 to 4 levels, 16 replicates). The cores remained inside their freezer bags during the experimental procedures, and following the manipulations, were slightly crumbled and extracted in a modified Tullgren apparatus (diameter of sample containers: 20 cm, 70 W light bulbs) into 10% sodium benzoate solution for three days.

(1) To test the influence of prolonged storage on the number of Gamasids extracted, soil cores were stored for one, three, and six months at 6°C inside their freezer bags. In order to avoid any disturbance or compression during that time, the samples were arranged in a single layer (side by side) and not moved. Potential evaporative water loss through the plastic during storage was measured gravimetrically, and found insignificant. A set of cores extracted immediately after field sampling served as a control.

(2) To assess the effects of heat during sample transport, cores were subjected to warming at constant 25, 35, and 45°C for five hours in a Heraeus VT 5042 EK climatic cabinet. Preliminary tests showed that this time was sufficient to bring the whole volume of the cores to the desired temperatures. A set of cores remained in the 6°C cooling room during that time and served as a control.

(3) Effects of shaking were tested by treating cores for three hours on a GFL 3006 orbital shaker at 100, 200, and 300 strokes minute^{-1}. Before this treatment, air was sucked out of the freezer bags to avoid any cushioning effects. 16 control cores were not agitated and extracted together with the treated ones.

(4) To estimate the effects of compression, cores were individually weighed down with the one-, three-, and tenfold average core mass (300, 900, and 3000 g, respectively) for ten hours, using plastic bags filled with gravel of 6 mm grain. A control level was not burdened and extracted together with the treated ones.

(5) Effects of extractor filling were assessed by charging Tullgren containers with one, two, and three cores, which resulted in approximately 15, 30, and 45 mm soil layer thickness, respectively. There was no true control treatment in this experiment, but note that we usually charge the sample containers with one core. The number of emerging mites were divided by 2 for the two-core treatment and by 3 for the three-core treatment, respectively, to make them comparable to the one-core treatment.

Two combi treatments simulated the combined effects of several influences, as may be more realistically encountered in field work: (6) In the combi1 experiment, the effects of compression and prolonged storage were combined by keeping cores in a cool box on top of each other for ten days at room temperature. (7) The combi2 experiment subjected cores to three hours of shaking at 200 strokes minute^{-1}, followed by a 5 days period of storage under the same conditions as in combi1. 16 cores from the cooling room each served as controls for the two combi treatments.

2.3 Measurements

Adult gamasid mites were counted in the Tullgren extracts under a binocular microscope at 16- to 40-fold magnification. The body length of each specimen was determined with reference to an ocular graticule on an ordinal scale, choosing class limits that divided the expected size range into four equal parts. Due to very few counts, the two largest size classes were combined and only three classes used for data analysis (< 560, 560–920, > 920 µm).

2.4 Data analysis

To test for significant differences of abundance among the experimental levels, negative binomial generalized linear models with log links were used (procedure glm.nb() in R package MASS 7.3.57, Venables & Ripley 2002). Various checks (e.g. check_model() in performance 0.9.1, Lüdecke et al. 2021) showed that the models were adequate for the data. Only the results of the temperature experiment exhibited significant zero-inflation (check_zeroinflation() in performance 0.9.1), and these data were tested using a hurdle regression with negative binomial counts (log link) in the count part, and with binomial counts (logit link) in the zero part of the model (hurdle() in pscl 1.5.5, Zeileis et al. 2008).

Before analyzing the body size results, the 45°C treatment was excluded from the temperature data, since there was only one surviving individual in a single core. Due to very uneven counts, there was significant zero-inflation in the size data of all experiments. To account for this and test for significant differences among treatment levels, 'treatment' was fit as being nested in 'size class' for the count part of the model, 'size class'

was used for the zero part, and the same model structure was used as described above for the abundance data of the temperature experiment.

All analyses were run and figures produced in R 4.1.2 under macOS High Sierra 10.13.6 (R Core Team 2021).

3. Results

The abundance of predatory mites recovered from the samples was not significantly influenced by storage time, shaking, compression, or any of the two combination treatments (Fig. 1, Tab. S1-2). Charging the Tullgren containers with two or three soil cores significantly decreased the number of emerging mites to 32% on average (median), as compared to one core (the control). While there was no significant difference between the control and the 25°C treatment, warming at 35°C significantly reduced median abundances to about 25% of the control, and at 45°C, only a single individual was found (Fig. 1, Tab. S1-2).

The storage time, shaking, compression, or any of the two combination treatments did not exert a significant influence on the body size distribution of the mites (Fig. 2, Tab. S1-2). In contrast, charging two or three soil cores resulted in significantly different body size distributions as compared to one core. This difference could be attributed to diverging counts in all size classes, were numbers were reduced between 15 and 50% of the control on average. Warming cores to 25°C did not affect the body size distribution significantly, while heating to 35°C diminished the counts in the small and medium size class to 14–17% of the control on average, but not in the large size class (Fig. 2, Tab. S1-2).

4. Discussion

Overall, the gamasid mites in this study turned out to be surprisingly robust against improper sample treatment. Neither prolonged storage, shaking, compression or any of the two combination treatments exerted a significant influence on total (group) abundance or the body size distributions of the animals. Among these treatments, it is especially the high resistance against long storage that makes the mites highly attractive for large scale biodiversity programs.

In contrast, the depth of the soil volume in the Tullgren containers did have a significant effect on the recovery of mites, irrespective of size class. Compared to their body size, microarthropods have to travel a long distance through the sample material during extraction, to eventually fall into the collection cups. Overcharging the containers made this distance longer on average, probably decreasing the steepness of the temperature/moisture gradient in the containers, and resulted in a reduced recovery. This finding is especially relevant for large scale biodiversity projects, as they need to process many cores in short time and may hence be inclined to use bigger cores, or place several cores per container, to process samples more efficiently.

The importance of the depth of material on microarthropod recovery is occasionally emphasized in the older literature (e.g. Edwards & Fletcher 1971), and specifically the usage of a shallow depth is recommended (e.g. < 2.5 cm, Murphy 1962), but without providing sufficient empirical data. I assume that there is an optimal depth to charge the containers with, which can be seen as a tradeoff between the minimized animal loss in little material and the increased sample throughput in more material. The optimal depth may depend on many parameters that influence the temperature/moisture gradient in the sample volume, like the speed of the extraction process (commonly 3–4 days,) and the diameter of the sample containers (commonly 10–15 cm, Edwards & Fletcher 1971), and is possibly hard to standardize over the various variants of dynamic extraction apparatuses. However, future large scale programs should acknowledge that that the depth of material in the sample containers may affect extraction efficiency very considerably, and that this parameter needs to be optimized early in the design phase.

The other treatment that significantly influenced extraction efficiency was the temperature of storage. There was no difference between the control (6°C) and room conditions (25°C), but storage at 35°C and especially 45°C drastically reduced the catch. This was to be expected: In testing the effects of forest fire to microarthropods kept inside moist soil cores from the field, Malmström (2008) reported that the numbers of many species declined significantly after a 12-hours exposure to 30°C or higher. Notably, the gamasid species in her experiment were slightly less tolerant towards high temperatures than the Protura, Collembola and Oribatida (Malmström 2008).

It will be logistically feasible to keep soil cores cool between sampling site and laboratory (e.g. in an insulated bag) if the amount of collected material is small and the transport does not take more than several hours. This is the conventional situation for soil zoologists, who wisely tend to select their sampling sites close to accessible places. Large scale biodiversity programs, in contrast, will need to put a focus on remote and so far undersampled regions of the globe (for instance, see Fig. 1 in Mathieu et al. 2022) where long travelling distances

have to be taken into account. Here, and especially in warmer climates, continuously cooling the cores using external energy (e.g. in a battery-powered container) is highly recommendable before extraction. 20 to 25°C appears to be a reasonable upper temperature level to observe, at least in temperate regions.

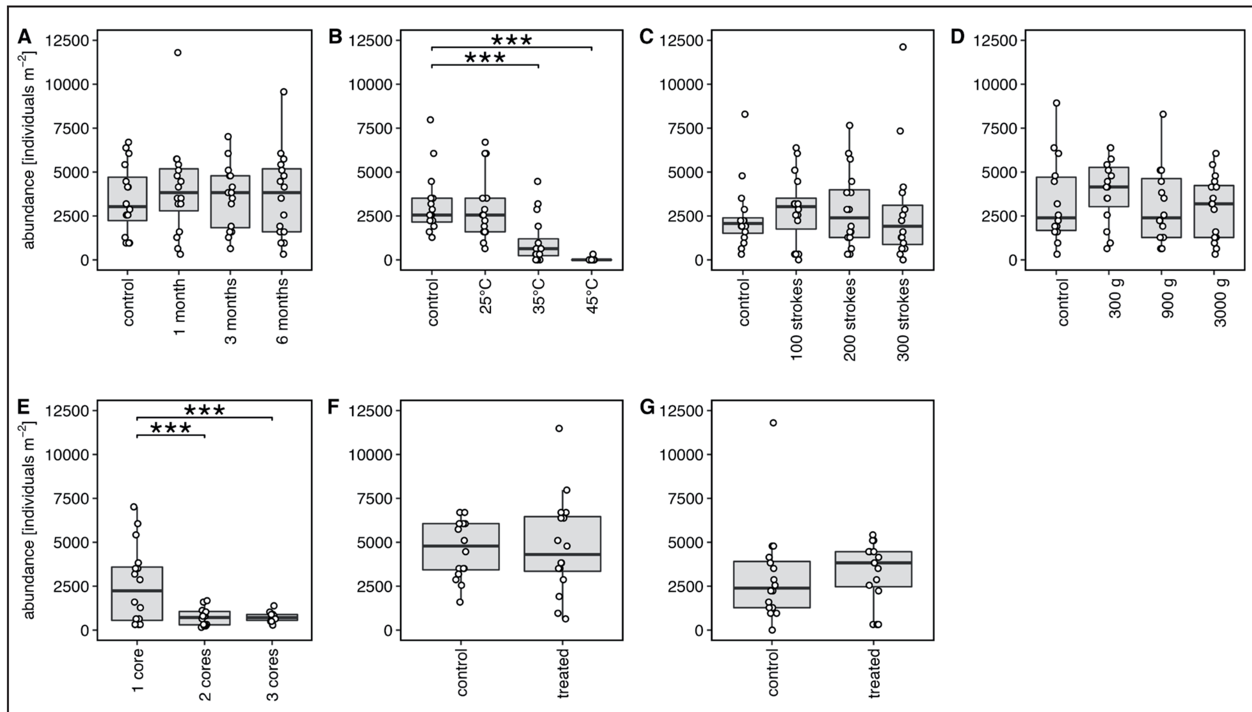

Figure 1. Effects of improper core treatment on the total abundance of predatory mites (Gamasida) extracted from soil. (**A**) storage duration, (**B**) storage temperature, (**C**) shaking intensity, (**D**) sample compression, (**E**) filling of sample containers in extraction apparatus, (**F**) combination of compression and prolonged storage, and (**G**) combination of shaking and prolonged storage.

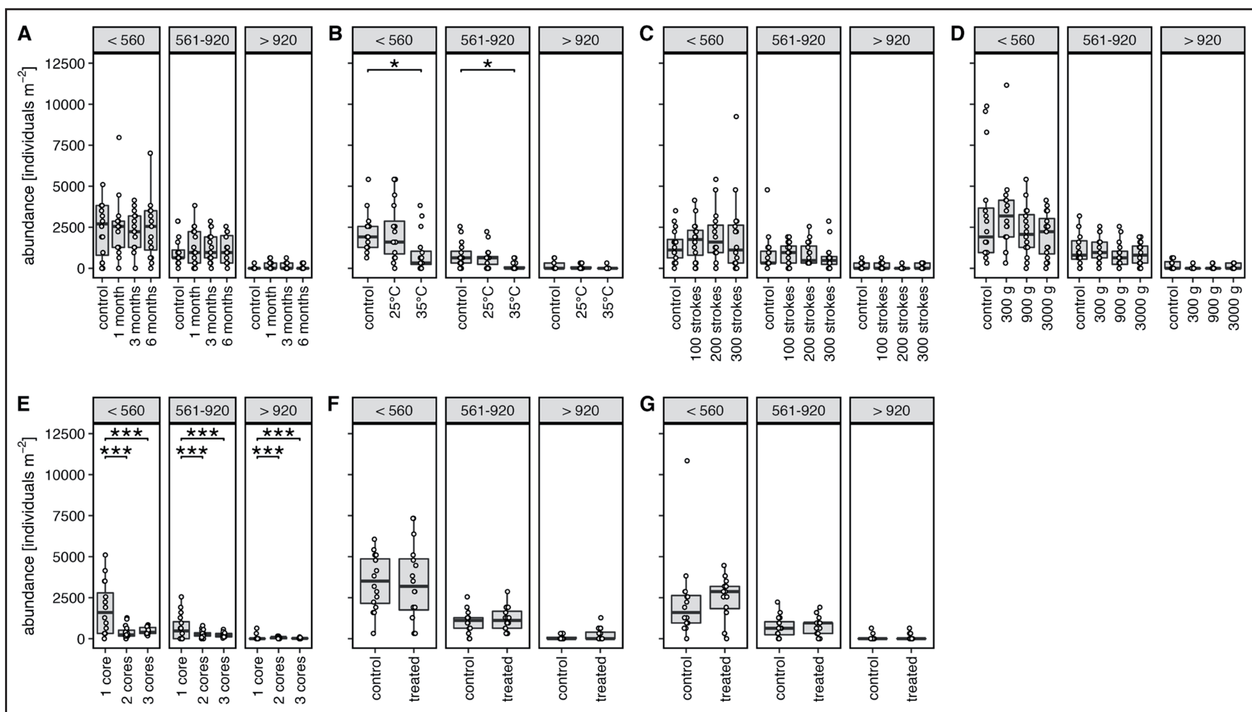

Figure 2. Effects of improper core treatment on the body size distribution of predatory mites (Gamasida) extracted from soil samples. Numbers in panel heads are body sizes in micrometers [µm]. See Fig. 1 for plot labels.

This study is a starting point for further research to understand potential effects of transport and storage conditions on soil microarthropods. As a caveat, please note that its taxonomic resolution is low, as only the total abundance of gamasid mites was considered. For simplicity, body size was measured just on an ordinal scale with only three classes. Future studies may find more sensitive reactions to the handling of samples, at least for certain species. However, as they are, the results are valuable for programs that aim at characterizing edaphic faunas not at the species, but at the group level (e.g. Potapov et al. 2022). Further research on the topic is needed for more ambitious endeavors. They should consider more animal groups (e.g. nematodes, collembolans, oribatid mites), ideally analyze at the level of species, and, taking into account the global scale of recent biodiversity programs, also include other climate zones.

In conclusion, this study suggests that the total abundance of gamasid mite assemblages is rather insensitive against improper sample treatment, as I suspect to often occur in large scale soil biodiversity programs. Collected cores can be shaken, compressed, and stored for longer periods without the danger of biasing the catch. Overfilling of extractor canisters and, especially, warming cores above room temperature during and after transport turned out to drastically decrease extraction efficiency. Thus, these two factors need to be adressed when designing the logistics of sampling campaigns.

5. Acknowledgements

Tamara Čoja, Thomas Müllner, Thomas Peham and Michael Reichmann assisted sampling in the field. Michael Reichmann and Tamara Čoja processed the mite material after extraction. Hanno Richter (Inst. Botany) provided the cooling room for sample storage. Stefan Jeitler of the Forstverwaltung Stift Schotten permitted access to the study site. The comments of two anonymous reviewers helped improving the text considerably.

6. References

André, H. M., X. Ducarme & P. Lebrun (2002): Soil biodiversity: myth, reality or conning? – Oikos **96**: 3–24.

Aragão, O. O. S., S. M. Oliveira-Longatti, A. A. Souza, E. C. Jesus, M. N. Merlo, E. P. Oliveira & F. M. S. Moreira (2020): The Effectiveness of a Microbiological Attribute as a Soil Quality Indicator Depends on the Storage Time of the Sample. – Journal of Soil Science and Plant Nutrition **20**: 2525–2535.

Brandt, F. B., B. Breidenbach, K. Brenzinger & R. Conrad (2014): Impact of short-term storage temperature on determination of microbial community composition and abundance in aerated forestsoil and anoxic pond sediment samples. – Systematic and Applied Microbiology **37**: 570–577.

Bruckner A. (1998): Augers may bias field samples of soil mesofauna. – Pedobiologia **42**: 309–315.

Dunger, W. & H. J. Fiedler (1997): Methoden der Bodenbiologie. 2nd ed. Gustav Fischer, Jena: 539 pp.

Edwards C. A. & K. E. Fletcher (1971): A comparison of extraction methods for terrestrial arthropods. – In: J. Phillipson (ed.): Methods of Study in Quantitative Soil Ecology: Population, production, and energy flow. – IBP Handbook **18**, Blackwell Scientific Publishers, Oxford: 150–185.

Ellenberg, H., H. E. Weber, R. Düll, V. Wirth, W. Werner & D. Paulißen (1992): Zeigerwerte von Pflanzen in Mitteleuropa. – Scripta Geobotanica **18**: 1–258.

Food and Agriculture Organization (2020): State of knowledge of soil biodiversity – Status, challenges and potentialities. – Report 2020, Rome: 583 pp.

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 & et al. (2020): Blind spots in global soil biodiversity and ecosystem function research. – Nature Communications **11**: 3870.

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. A monitoring and indicator system can inform policy. – Science **371**: 239–241.

Guerra, C. A., D. H. Wall & N. Eisenhauer (2021b): Unearthing soil ecological observations. – Soil Organisms **93**: 79–81.

Kilian, W., F. Müller & F. Starlinger (1993): Die forstlichen Wuchsgebiete Österreichs. Eine Naturraumgliederung nach waldökologischen Gesichtspunkten. – Berichte der forstlichen Bundesversuchsanstalt Wien **82**: 1–60.

Lakly, M. B. & D. A. Crossley (2000): Tullgren extraction of soil mites (Acarina): Effect of refrigeration time on extraction efficiency. – Experimental and Applied Acarology **24**: 135–140.

Lawrence, K. S., G. W. Lawrence & E. van Santan (2005): Effect of controlled cold storage on recovery of *Rotylenchulus reniformis* from naturally infested soil. – Journal of Nematology **37**: 272–275.

Lüdecke, D., M. S. Ben-Shachar, I. Patil, P. Waggoner & D. Makowski (2021): performance: An R Package for Assessment, Comparison and Testing of Statistical Models. – Journal of Open Source Software **6**: 1–8.

Maestre, F. T. & N. Eisenhauer (2019): Recommendations for establishing global collaborative networks in Soil Ecology. – Soil Organisms **91**: 73–85.

Mathieu, J., A. C. Antunes, S. Barot, A. E. Bonato Asato, M. L. C. Bartz, G. G. Brown, I. Calderon-Sanou, T. Decaëns, S. J. Fonte, P. Ganault & et al. (2022): sOilFauna - a global synthesis effort on the drivers of soil macrofauna communities and functioning. – Soil Organisms **94**: 111–126.

Murphy P. W. (1962): Extraction methods for soil animals. I. Dynamic methods with particular reference to funnel processes. – In: Murphy, P. W. (ed): Progress in Soil Zoology. – Butterworths, London: 75–114.

Nestroy O., O. H. Danneberg, M. Englisch, A. Geßl, H. Hager, E. Herzberger, W. Kilian, P. Nelhiebel, E. Pecina, A. Pehamberger & et al. (2000): Systematische Gliederung der Böden Österreichs (Österreichische Bodensystematik 2000). – Mitteilungen der Österreichischen Bodenkundlichen Gesellschaft **60**: 1–124.

Potapov, A. M., X. Sun, A. D. Barnes, M. J. I. Briones, G. G. Brown, E. K. Cameron, C.-H. Chang, J. Cortet, N. Eisenhauer, A. L. C. Franco & et al. (2022): Global monitoring of soil animal communities using a common methodology. – Soil Organisms **94**: 55–68.

Pulleman, M., R. Creamer, U. Hamer, J. Helder, C. Pelosi, G. Pérès & M. Rutgers (2012): Soil biodiversity, biological indicators and soil ecosystem services - an overview of European approaches. – Current Opinion in Environmental Sustainability **4**: 529–538.

R Core Team (2021). R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria.

Rubin, B. E. R., S. M. Gibbons, S. Kennedy, J. Hampton-Marcell, S. Owens & J. A. Gilbert (2013): Investigating the Impact of Storage Conditions on Microbial Community Composition in Soil Samples. – PLOS ONE 8: e70460.

Stone D., P. Blomkvist, N. Bohse Hendriksen, M. Bonkowski, H.Bracht Jørgensen, F. Carvalho, M. B. Dunbar, C. Gardi, S. Geisen, R. Griffiths & et al. (2016): A method of establishing a transect for biodiversity and ecosystem function monitoring across Europe. – Applied Soil Ecology **97**: 3–11.

Venables, W. N. & B. D. Ripley (2002): Modern Applied Statistics with S. 4th ed. Springer, New York: 495 pp.

Wallnöfer S., L. Mucina & V. Grass (1993): Querco-Fagetea – In: Mucina L., G. Grabherr & S. Wallnöfer (eds): Die Pflanzengesellschaften Österreichs. Teil III: Wälder und Gebüsche. – Gustav Fischer, Jena: 85–236.

Zeileis, A., C. Kleiber & S. Jackman (2008): Regression Models for count data in R. – Journal of Statistical Software **27**: 1–25.