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Below-ground pitfall traps for standardised monitoring of soil mesofauna: Design and comparison to Berlese/Tullgren funnels

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ABSTRACT

Sampling of soil mesofauna has been traditionally carried out with Berlese/Tullgren extractions, a century old technique. However, sampling methods involving the extractions of soil are becoming increasingly difficult to implement and standardise due to the lack of commercially available equipment. Moreover, they are poorly suited to repeated sampling in the same locations and underestimate more mobile taxa.

Below-ground (hypogean) pitfall trapping is a promising new technique that up to now was only attempted with bulky custom-manufactured tools. In the present work we test a cheap and easily deployable setup made using standard pipe fittings.

The new design was compared across different environments with Berlese/Tullgren extractions in order to ascertain whether they produce similar species lists and detect the same environment-induced changes in communities. The two trap types were found to yield structurally different assemblages, with the new design producing significantly higher abundance and diversity of springtails and larger taxa. Beta-diversity profiles resulted however perfectly comparable, characterising the same pattern of dissimilarities. In addition, a new method is proposed to use the two sampling types in combination to estimate the dispersal of soil organisms.

Below-ground pitfall traps have the potential to complement Berlese extractions for reliable and standardised monitoring of soil arthropods, thanks to their effectiveness, low cost and ease of operation.

1. Introduction

The important role of soil fauna in ecosystems is widely acknowledged in scientific literature (Creamer et al., 2022). Earthworms often form parts of dedicated sampling schemes addressed by farmers (Ebitu et al., 2021) and microbial communities have become much less expensive to investigate and describe (Oliverio et al., 2018). However, the rest of below-ground communities are usually given little attention. This is partly due to inherent difficulties in sampling communities living within the soil profile in an efficient, scalable and reliable manner.

The traditionally accepted standard for sampling soil mesofauna is a protocol commonly named Berlese/Tullgren extraction. It was first developed by Antonio Berlese as a way to flush and channel invertebrates in a collected soil core through a funnel by heating the surface or the sides of the core with a gas-fuelled flame (Berlese, 1905). The mechanism exploits the behaviour of many soil invertebrates when faced with increasing temperature and decreasing moisture gradients, which they escape by moving to zones of lower temperature a higher humidity. The system was streamlined by Hugo Albert Tullgren, who replaced the gas flame with an incandescent light bulb lit above the soil surface (Tullgren, 1918). The resulting equipment, the Berlese/Tullgren funnel, has been a standard of soil ecological investigations for many years, with setups offered by several commercial manufacturers. In recent times, the bulkiness and the high energy requirements of high-throughput Berlese Tullgren setups have led to their discontinuation in many research institutes. Commercial implementations have ceased to be widely available, and even spare parts for the maintenance of existing setups, such as incandescence light bulbs, have become increasingly hard to source, and expensive. Standardised layouts have been replaced by improvised implementations, which have led to a

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reduction in replicability potential. This situation is conductive to the search for alternative systems. Centrifugal flotation is a substitute with a long tradition (Hale, 1964) and shows recovery rates of invertebrates higher than the Berlese/Tullgren extractors, but it produces large amounts of contaminated slurry and is generally impracticable for large numbers of samples. Moreover, both Berlese/Tullgren extraction and centrifugal flotation present a bias in that they are active methods, requiring collection of a soil core from the field. Organisms capable of fast movement across the soil profile, such as many Entomobryomorpha springtails and mite families like Eupodiidae and Rhagiididae in addition to edaphic beetles and other arthropods, are very likely to escape detection or at least their numbers be severely underestimated.

This is the reason why standard, small-volume, Berlese/Tullgren extraction is not considered an appropriate method for sampling larger size classes. These are traditionally approached with methods that have in common the extraction of large monoliths, followed by a variety of techniques including manual sorting, different refinements of the Moczarski passive separator or custom large-volume Berlese/Tullgren setups (Bremner, 2012; Holdhaus, 1910). The extraction of monoliths is still likely to introduce bias against the most mobile elements of soil fauna and the above-mentioned methods either require costly specialised setups or a substantial amount of manpower and time, both obstacles to high-throughput applications.

Pitfall trapping, which does not require removal of the matrix where invertebrates live, has been the technique of choice for sampling invertebrates moving on the soil surface for a long time (Woodcock, 2005). In its most basic implementation, it consists just of a vessel containing a preservative and inserted in the soil so that its upper edge is flush with the soil surface. The method has also been successfully adapted for target vertebrate species, chiefly reptiles and amphibians (Weddeling et al., 2004). However, pitfall traps with surface openings are not suitable to collect endogean fauna.

The first design of pitfall traps modified to sample invertebrates moving not on the surface but within the soil was produced by Owen (1995). Other complex designs appeared later specifically targeting invertebrates moving across leaf litter at different depths (Kaplin et al., 2017; Ruiz-Lupión et al., 2019; Schmidt and Solar, 2010; Wagner et al., 2003) or fauna in highly specific matrices like scree (Růžička and Klimeš, 2005) or mesovoid shallow substratum (López and Oromí, 2010). The original design, boosted by its conceptual simplicity, enjoyed wider application as perfected by Mark G. Telfer (Sims et al., 2019b) and was proficiently used to characterise a wide variety of target soil clades (Sims et al., 2016), not limited to the traditional targets of Berlese/-Tullgren extraction. It was compared to the golden standard of Berlese/Tullgren extraction and with epigean pitfall traps with very good results (Sims et al., 2020; Sims et al., 2019a).

The “Owen design” of hypogean pitfall trap still has some limitations that can be addressed, while building on the success of the original prototype. The original traps are heavy and bulky (11 cm in diameter, 50 cm in depth with a combined sampling port dimension of 500 cm^2), making transport and deployment in the field of more than a handful a significant logistical challenge. Moreover, the wide diameter of their structure results in specialised equipment being required to dig a clean circular hole to deploy them. In cases where this is not available, deploying the traps requires substantial amounts of soil backfilling, with extensive disturbance of the surrounding soil profile, requiring long settling periods before meaningful sampling could begin. Additionally, the manufacture of the original design was assembled using extensively modified or customised components, making traps dependent on the availability of specialized materials.

A new design of hypogean pitfall trap, addressing all of these issues, with an external diameter equal to one of the most common auger sizes (4 cm) and realised with readily available and lightweight hydraulic fittings, was tested in a pilot study against the original version, and it showed very promisingly similar rarefaction curves and beta-diversity distributions (Fioratti Junod et al., 2021). The total catch size per trap was reduced compared to the old version, but when abundances were normalized for sampling port size, the new design was found to be significantly more efficient.

In addition to this potential benefits, if the new pitfall trap design is found to provide reliable estimates of soil biodiversity, and if its catch can be postulated to be a measure of activity in the same way as Berlese/Tullgren funnels are postulated to provide an accurate measure of absolute density, the opportunity to mechanistically estimate the dispersal capability of different clades of soil invertebrates is opened by the combined use of two techniques, potentially superseding indirect, impractical or time consuming methods like mark and recapture, experimental manipulation and modelling (Auclerc et al., 2009; Mathieu et al., 2018; Pequeno et al., 2021).

Here we present a detailed description of the new trap design and provide a comparison of their sampling efficiency with Berlese/Tullgren extractions across a range of environments. The focus will be on the mesofaunal clades of springtails and mites, for which the new design is proposed as a standardised method of monitoring. However, the catch of the proposed pitfall traps includes other soil arthropod classes in higher numbers compared to Berlese/Tullgen extracions. These clades will be taken into account for the purpose of replicable whole-community environmental profiling and fingerprinting.

The objective of the present work is describing structural differences in diversity and abundance in the catches of the two sampling types, which may orient the practitioner towards the more suitable technique, as well as a comparison of the community-level profiles of environmental gradients the two methods generate.

The results will be used to recommend the ideal use of pitfall traps, highlighting opportunities and drawbacks of its use compared to Berlese/Tullgren extractions, and suggesting a new method to estimate dispersal in soil arthropods.

2. Methods

2.1. Design and operation

2.1.1. Pitfall trap design

The development process for the new pitfall trap design took into account several requirements including manufacturing costs and materials, deployment, operation and downstream processing of samples.

The main requirement was for the trap to be light, inexpensive and easy to manufacture and deploy. Readily available components were therefore favoured, with the choice falling on standard rain-waste-vent 40 mm drainage pipes and paired fittings (Fig. 1). Acrylonitrile butadiene styrene (ABS) is a cheap material, stress-resistant and impervious to substantial deformation while being very easy to work without the need for specialised tools. A pipe-cutter blade is needed to cut the external case at the required length and create the lateral openings (windows, or sampling ports). The trap in the present study had two 2.5 per 20 cm windows cut on opposite sides, for a total of 100 cm^2 area, but these specifications are easily adapted to sample deeper in the soil profile if required, or to reduce or extend their sampling depth. A pipe coupler was inserted at the top and a pipe-end lid with an O-ring for good sealing, completing the setup by isolating the system from rainwater. A standard plastic 50 mm conical centrifuge tube was found to be the ideal collection vessel due to its dimensions, wide availability, low cost and screw-on cap facilitating safe sample transport and storage, as well as being standard laboratory equipment for centrifuging and lysis.

No readily available fitting was available to create a tight fit between the inner trap-body and the centrifuge tube. This required the design of the only custom-made component, a polylactic acid (PLA) connector that was manufactured inexpensively and can be made by almost any commercial or entry-level 3-d printer (Fig. 2). The design was based on an inner threaded surface that can be screwed onto the standard 50 mL centrifuge tubes, and an external smooth surface providing a tight but sliding fit to the inner wall of the trap body. The upper edge of the

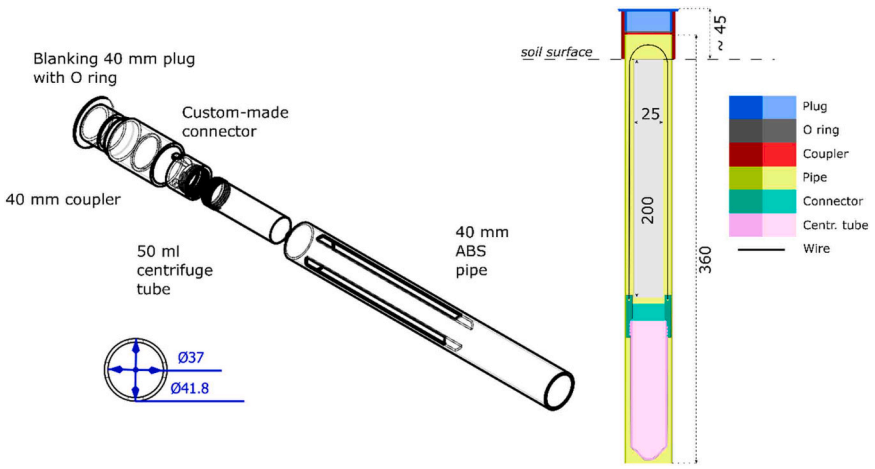

Fig. 1. Exploded view of the components, horizontal cross-sectional dimensioning of the case and operational vertical cross sectional view showing the trap in its deployed state. Openings on the side of the case can be adapted to sampling requirements: the images show two 2.5 × 20 cm ports as tested in the present study. All dimensions in the figure are expressed in millimetres.

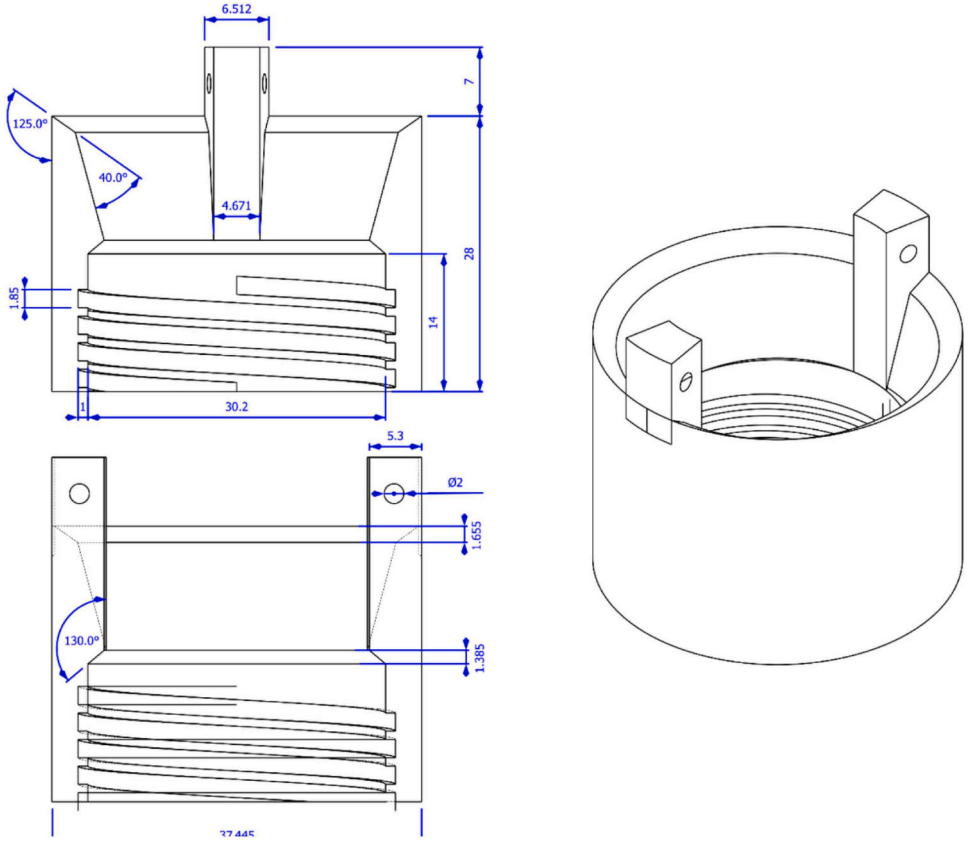

Fig. 2. View and dimensioning of the 3d printed connector. The printing material for this trial was PLA, which provided a good fit to the centrifuge tube threading and the inner walls of the external case. All measures are expressed in millimetres.

connector was chamfered a 45°, for easier collection of specimens into the tube below, and on opposite sides two protruding ribs were included, with a hole cut through them to enable a length of thin metal wire loop to be included facilitating the removal and replacement of the sampling tube using a wire hook. The overall cost per trap was dependent on the equipment supplier, but should not exceed 10 USD, including the 3d printed connectors, based on retail prices and a batch of 20 traps. The weight of the setup was also very low, at roughly 100 g per trap, including the empty collection tube.

2.1.2. *Field operation*

For deployment, it was envisaged that the traps could be inserted into the soil with tools readily available to any fieldworker, without the need for soil backfilling and minimising disturbance to the surrounding soil. A circular-section small-bore Dutch helical auger (40 mm) was used. This removed a plug of soil producing a hole of the correct size to accommodate the trap, causing negligible disturbance of the soil profile. An appropriately sized auger allowed the trap to be inserted smoothly into the soil while maintaining tight contact between the soil and the sampling port openings. No backfilling was required, and therefore the

trap could be immediately operational without any need for a settling period. The required depth of the hole is limited to bottom depth of the sampling range increased by the length of the collection tube, which in the case of the recommended 50 mL conical centrifuge tube is around 12 cm. This means that for standard topsoil sampling, the hole does not need to extend beyond 36 cm, minimising deployment issues due to stony or highly compacted deeper soil layers.

Operationally, the requirement was for a trap capable of performing equally well for point sampling and for extended monitoring periods. For point sampling strategies, requiring rapid turnover times among locations, the design offers quick deployment and retrieval of the external structure from the soil, each necessitating not more than a couple of minutes. For extended monitoring, the trap can be left in place for months or longer with only the collection tube regularly collected and replaced. The sampling tube replacement takes less than a minute. A weekly interval between tube replacements was found to be optimal, allowing the collection of a good number of specimens while not allowing evaporation to significantly reduce the level of collection liquid (pure ethanol).

While deployed, the setup can withstand many stresses, short of being driven over by traffic. Therefore, active tramlines in arable fields are not suitable for deployment, but the presence of traps was compatible with all major agricultural operations (not involving soil cultivation or drilling) like spraying, harvesting and cutting above 5 cm.

For subsequent storage and analysis, collected tubes can be sealed with screw-on lids and easily transported and stored, even without refrigeration, provided fresh ethanol is added. Tubes can then be handled by transferring the contents to a Petri dish for sorting and morphological identification of specimens. Alternatively, lysis and the first steps of purification for DNA extraction can take place directly in the original collection tube. Normally, only a small amount of soil enters the collection tube if the trap is properly deployed, but the presence of large burrowing beetles can dislodge larger quantities, requiring an additional sorting and cleaning step before identification or extraction of genetic material. Short of loose unstructured sand, which would be challenging to work with even in traditional Berlese/Tullgren setups, soil intrusion is minimal and easily managed.

2.2. Experimental comparison

2.2.1. Trial setup

The site chosen for testing the traps in a comparison with Berlese/Tullgren extractions was the Wendling Beck Exemplar Project (British OS reference TF 97000 15000, 52.6968 N, 0.9138 E), a mixed area of seminatural and agricultural land spanning different properties under a coordinated management plan. The area is located north of Dereham, Norfolk, United Kingdom (Fig. 3), and the underlying soil shows substantial variability at the sub-hectare scale, with a general west east gradient within the study area between freely draining slightly acid sandy soils of the Newport series and slightly acid loamy and clayey soils with impeded drainage of the Burlingham series (Cranfield University, 2018). Land under five different types of land use was selected for the trial. These included: an active wheat (*Triticum aestivum*) field; a field formerly under wheat in its first year of conversion to herbal fallow; an active blackcurrant (*Ribes nigrum*) field; a former blackcurrant field in its first year after conversion to herbal fallow; a minimally improved

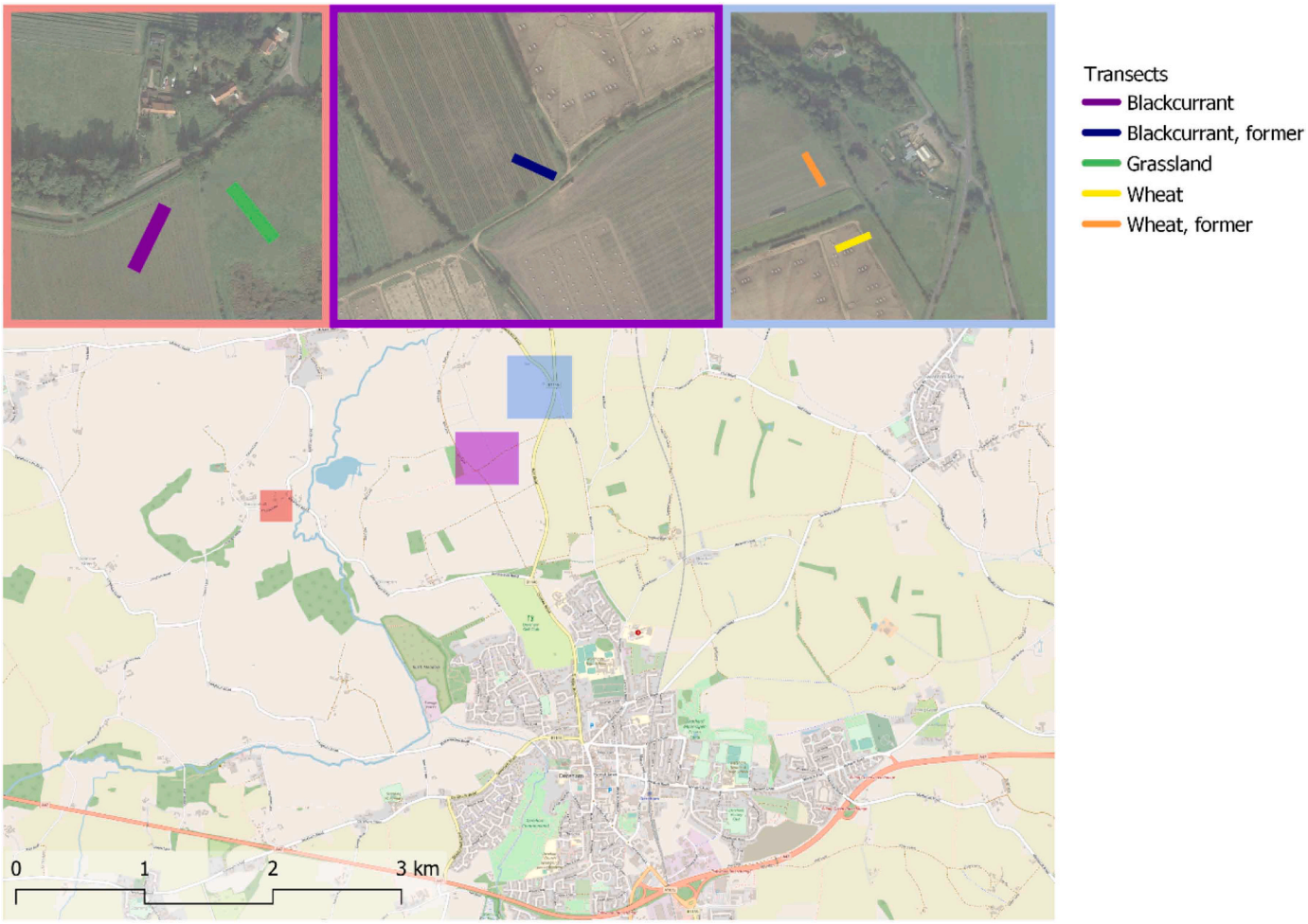

Fig. 3. Location of the five transects on which pitfall traps were deployed and soil cores were collected at 10 m intervals. (© OpenStreetMap contributors).

managed grassland. At each site a 40 m transect was identified with sampling points located every 10 m. For each of the 5 sampling points, a pitfall trap was deployed and left in place for a single week, after which the tube was collected, sealed and stored for further processing. At the time of collection, a 5 cm diameter soil core sample was taken for Berlese/Tullgren extraction. Soil cores extended to a depth of 20 cm and were taken from an area within 50 cm of the pitfall trap. Collection dates were the 9th of June 2021 for the wheat and former wheat transects, the 16th of June 2021 for the blackcurrant and grassland transects and 23rd of June 2021 for the former blackcurrant transect.

2.2.2. Sample processing

The soil cores were stored in sealed plastic bags and each was loaded into a Berlese/Tullgren extractor within two hours from collection. The extractor consisted of a wooden frame encasing 15 cm diameter funnels. The entrance to the funnel tube contained a 1 cm nylon mesh screen upon which the soil core was laid. The heat source was a 46 W incandescence light bulb, located centrally at 15 cm above each funnel, which was kept lit for the duration of the four-day extraction period. A vessel filled with pure ethanol was placed at the bottom of the funnel to collect specimens.

All catches, those generated by pitfall traps and those collected with Berlese/Tullgren extractions were then processed in the same way. The collection vessel was emptied into a Petri dish, together with the eluate of a further rinse to dislodge specimens from the sides of the container. Once the sample had settled all invertebrates were located and individually identified under a stereomicroscope. Contrasting backgrounds of black or white ceramic were used to pick all specimens, and invertebrates requiring detailed observation were transferred on glass slides under a brightfield microscope. Springtails were identified to species (Hopkin, 2007). Mites were assigned to one of four main clades, namely Astigmatina, Prostigmata. Mesostigmata and Oribatida (Shepherd and Crotty, 2018). Adult beetles were identified to family whereas larvae were kept separate (Unwin, 1984), other insects were identified to order and other invertebrates, namely Annellida, Araneae, Chilopoda, Diplopoda, Isopoda, Mollusca, Opiliones, to higher ranks.

2.2.3. Data analysis

The abundance matrix was separated by main component groups identified at a similar taxonomic resolution, namely springtails, mites, beetles and all other clades.

Abundance, species richness and Shannon's diversity index were computed for each of the groups. For diversity, a linear model was fitted having the Shannon's index as a response variable and environment, position in the transect and sampling mode as explanatory variables. For abundance and species richness, considering the integer count nature of the data, a Poisson regression was fitted instead, with a log link function and the same structure of predictors. For each fitted model, statistical summaries for the magnitude and significance of the sampling mode predictor were generated, as well as estimated marginal means obtained with the R package emmeans (Lenth, 2022).

A community-level approach was also adopted to ascertain whether the two sampling types allow to detect the same patterns of dissimilarity across environments (Legendre et al., 2005; Whitaker, 1972). The whole catches of the two types of sampling were used to graphically represent structural variation among assemblages in different environments for each sampling method using biaxial non-metric multidimensional scaling (Kenkel and Orlóci, 1986), with dimensional scores computed using the metaMDS function of the vegan R library (Oksanen, 2018; Oksanen et al., 2008). Dissimilarity matrices based on the Bray-Curtis algorithms were computed with the vegdist function of the same package, and the results were fed to a permutational multivariate analysis of variance model having environment as an explanatory variable.

The same dataset was processed to generate rarefied species curves, aggregated for environment and sampling method, computed using the rarefy function of the eponymous R package (Bacaro et al., 2021).

3. Results

3.1. Springtails

Seven species of springtails (*Lepidocyrtus lignorum*, *Orchesella villosa*, *Pseudosinella alba*, *Pseudosinella immaculata*, *Sminthurinus aureus*, *Sminthurinus niger*, *Tomocerus longicornis*) were exclusively detected in pitfall traps, two (*Folsomia candida* and *Metaphorura affinis*) were recorded only in Berlese/Tullgren extractions and 11 were common to both datasets. Poduromorpha were relatively more abundant in Berlese/Tullgren catches, whereas Entomobryomorpha and Symphypleona significantly more abundant in pitfall trap communities. Overall, pitfall trap catches were characterised by a higher modelled Shannon's diversity (Fig. 4, Table 1). Mean modelled springtail abundance for pitfall traps was also significantly higher than Berlese/Tullgren extractions. The same trend can be observed for species richness, with Berlese/Tullgren extraction recording a modelled mean of 2.14 species less than pitfall traps.

3.2. Mites

All four clades represented in the current analysis were recorded with both trapping methods. However, mite assemblages diverged substantially between traps, with Berlese/Tullgren extraction recovering higher relative numbers of Oribatida and Astigmatina and pitfall traps yielding more Mesostigmata and Prostigmata specimens (Fig. 5). None of the three summary parameters under analysis (Shannon's diversity index, abundance and clade richness) showed modelled significant differences attributable to sampling method, with Berlese/Tullgren extractions showing marginally higher diversity and mean clade number and pitfall traps recovering more specimens (Table 1).

It is envisaged that larger studies will confirm the observed trends in diversity with identification to lower taxonomic rank.

3.3. Beetles and other clades

All beetle families recovered in Berlese/Tullgren extractions were also found in pitfall trap catches, whereas four families (Coccinellidae, Nitidulidae, Ptilidae, Scotilidae) were not represented among specimens recovered in Berlese/Tullgren catches. Although small numbers of Carabid and Staphylinid beetles were recovered in Berlese/Tullgren extractions, there is a striking difference with pitfall trap catches, where they make up respectively more than 4.2% and 1.3% of the total community (Table 1). The Shannon's diversity index was significantly higher in pitfall traps than in Berlese/Tullgren extractions, as was the size of beetle catch. The modelled mean number of coleopteran families recovered in pitfall samples was higher by 2.62 units compared to Berlese/Tullgren extractions.

All other clades represented in Berlese/Tullgren extractions were also recovered by pitfall traps, whereas no molluscs, harvestmen, woodlice, centipedes, and no representatives of the insect orders Dermaptera and Lepidoptera were recorded by Berlese/Tullgren extractions. The mean modelled diversity for other clades was significantly higher than in Berlese/Tullgren extractions, like the average number of other clades in pitfall trap samples and the number of specimens belonging to these clades.

Care should be taken in the interpretation of the results in this section. It must be noted that the core size used for the Berlese/Tullgren extractions is substantially smaller than what would be used for targeted ground beetle surveys. Extraction and sieving of large monoliths (or potentially the use of larger bore hypogean pitfall traps) cannot be replaced by the proposed design for targeted macrofauna description. Nevertheless, cores of comparable dimensions to those tested for Berlese/Tullgren extractions are often used to take extract arthropods other than springtails and mites for the purpose of environmental profiling of whole below-ground communities (Cifuentes-Croquevielle et al., 2020; Manu et al., 2022; Ostandie et al., 2021; Salmon, 2018; Ustinova et al.,

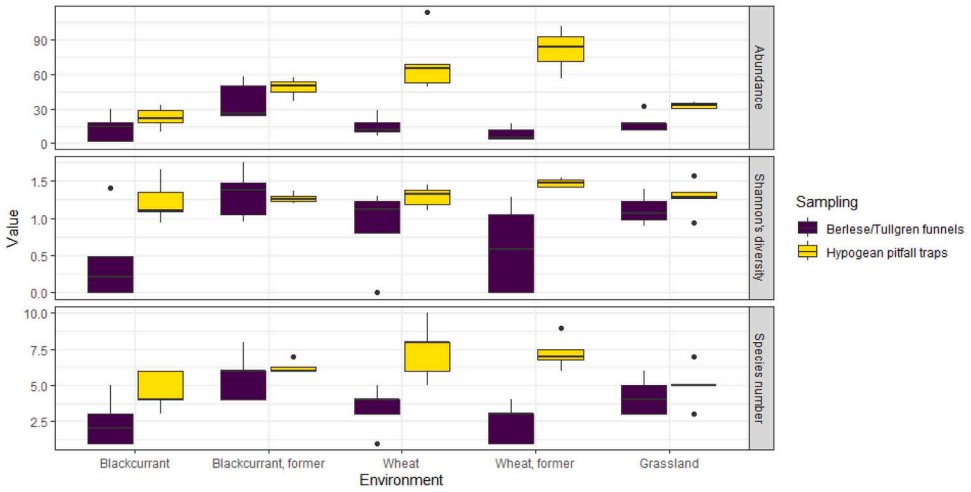

Fig. 4. Boxplots with interquantile ranges for springtail abundance, Shannon's Diversity Index and species richness.

2021).

3.4. Rarefaction curves and beta diversity profiles

Ordination is traditionally used to visually represent the structural proximity or divergence between communities pertaining to different environments or treatments. The distance and overlap between distributions is indicative of their degree of similarity. By applying the same ordination pipeline to datasets stemming from the two sampling techniques, a remarkably similar profile is obtained (Fig. 6). Non-metric multidimensional scaling representation shows a striking similarity in terms of relative distances among group centroids for each environment, as well as for average spread. Similar trends in how different environments shape the below-ground assemblages were also shown by applying a permutational analysis of variance to the Bray-Curtis dissimilarity matrix calculated for samples from each trap. This analysis shows analogous results in terms of variance explained by the type of environment (R^2 0.60 for Berlese/Tullgren extractions, R^2 0.71 for pitfall traps) and the associated explanatory-variable specific p value (0.001 for both sampling types).

Rarefaction curves based on random resampling at different sizes also show flattening occurring at lower catch sizes for all environments in the Berlese/Tullgren extractions compared to the pitfall traps, showing the overall better performance of the latter in covering soil invertebrate diversity (Fig. 7), the phenomenon being largely driven by springtails and beetles.

4. Discussion

4.1. General observations

Pitfall traps have been shown to provide significantly higher springtail catches, and comparably-sized mite catches when tested against Berlese/Tullgren extractions, with these two groups being the usual targets of mesofaunal surveys. In addition, pitfall traps provide much more varied assemblages, including good representation of groups like rove and ground beetles, woodlice and centipedes, that have substantial potential as environmental indicators (Gerlach et al., 2013; Koivula, 2011; Méndez-Rojas et al., 2021; Paoletti and Hassall, 1999) and otherwise require specialised extractors or time-consuming manual sorting (Pfiffner and Luka, 2000). All major groups of soil invertebrates were collected in larger numbers by the pitfall traps. For springtails, beetles and non-target taxa, diversity and species/clade richness were also significantly higher than their Berlese/Tullgren counterparts. The use of pitfall traps seems therefore particularly promising for surveys targeting holistically different size ranges of soil fauna. A single sampling method is able to provide significant amounts of biotic data spanning from microarthropods to ground beetles, that would require a combination of destructive extraction and hand sorting, each more time and resource consuming than the pitfalls.

4.2. Sources of bias

Additionally, discrepancies in the community assemblages sampled with the two methods, are suggestive that pitfall traps can help reduce bias introduced by sampling of soil cores followed by Berlese/Tullgren extractions. Firstly, many of the more mobile invertebrates are likely to escape the portion of soil where the corer is slowly lowered and rotated for extracting an intact soil core sample. This may lead to a substantial underestimation of clades like carabid beetles (albeit usually sampled with larger cores in targeted studies), Entomobryomorpha springtails and Prostigmata mites, known for their rapid movement across the soil profile (Sabu et al., 2011). Substantially higher abundance of these clades in pitfall trap catches (3.5, 32.1 and 3.6 more individuals per trap respectively) strongly supports this hypothesis. Additionally, the heat and light necessary for the Berlese/Tullgren extraction can potentially wake from dormancy invertebrates that are otherwise inactive, masking seasonal effects in recovered samples (Belozerov, 2009; Block and Zettel, 2003). Third, the principle of the Berlese/Tullgren extraction are the avoidance of light, high temperature and dry conditions by soil invertebrates, but it was possible that some groups of organisms, again likely to be the more mobile ones, might show an opposite reaction and escape the channelling through the funnel. At the same time, slower moving organisms may be desiccated in situ and die, so not leave the soil for collection in the ethanol.

On the other hand, some relevant below-ground clades particularly relevant for soil biological studies, like Oribatid mites and Poduromorph springtails, were sampled with higher relative frequency by Berlese/Tullgren extractions. It is matter of hemiedaphic and euedaphic species, characterised by slow movement and generally reduced exploratory behaviour (Chauvat et al., 2007; Lehmitz et al., 2012). It is envisaged that studies focusing on these clades may adopt a pitfall trap residence time longer than one week, to obtain richer and more representative samples.

4.3. Cross-comparability

A significant obstacle for new sampling techniques if they are to be widely accepted, even if they prove to be substantially more efficient than the accepted standard, is the complexity of comparing data

Table 1
Modelled means and standard errors for the different groups of invertebrates, as derived from a model including environment, sampling type and position within the transect as explanatory variables (with an underlying Poisson distribution for abundance and diversity). Effect size and p values reported in the rightmost two columns refer to the sampling type variable.

		Blackcurrant				Blackcurrant, former				Wheat				Wheat, former				Grassland				Effect size	p
		Berlese		Pitfall		Berlese		Pitfall		Berlese		Pitfall		Berlese		Pitfall		Berlese		Pitfall			
		μ	SE	μ	SE	μ	SE	μ	SE	μ	SE	μ	SE	μ	SE	μ	SE	μ	SE	μ	SE		
Springtails	*Diversity*	0.62	0.14	1.03	0.14	1.11	0.14	1.52	0.15	0.88	0.14	1.29	0.14	0.72	0.14	1.12	0.15	0.99	0.14	1.40	0.14	0.41	* *
	Abundance	8.23	0.81	22.13	2.01	23.29	1.60	62.66	4.04	22.39	1.45	60.24	3.15	16.74	1.91	45.04	5.02	13.60	1.03	36.59	2.39	*0.99*	* **
	Richness	2.52	0.50	4.12	0.77	4.50	0.75	7.35	1.23	3.95	0.65	6.46	0.97	2.86	0.71	4.68	1.16	3.38	0.59	5.53	0.89	*0.49*	* **
Mites	*Diversity*	0.53	0.14	0.36	0.14	0.87	0.15	0.70	0.16	0.57	0.14	0.41	0.14	0.94	0.15	0.77	0.16	0.83	0.14	0.66	0.14	-0.17	ns
	Abundance	2.86	0.71	3.07	0.76	7.57	0.98	8.12	1.10	15.86	1.45	17.02	1.53	10.18	1.25	10.92	1.40	9.74	1.10	10.46	1.17	*0.44*	ns
	Richness	1.52	0.46	1.34	0.41	2.92	0.64	2.56	0.62	2.10	0.51	1.85	0.46	3.12	0.67	2.74	0.64	2.64	0.58	2.32	0.52	*-0.13*	ns
Beetles	*Diversity*	0.08	0.13	0.98	0.13	-0.09	0.13	0.82	0.14	0.23	0.13	1.13	0.13	0.10	0.13	1.00	0.14	-0.19	0.13	0.72	0.13	0.91	* **
	Abundance	0.54	0.16	5.46	1.02	0.66	0.22	6.76	1.88	1.21	0.31	12.29	1.54	1.08	0.32	10.93	2.64	0.23	0.09	2.37	0.72	*2.32*	* **
	Richness	0.58	0.20	3.37	0.78	0.49	0.19	2.85	0.99	0.72	0.24	4.23	0.87	0.77	0.26	4.51	1.29	0.29	0.12	1.71	0.57	*1.77*	* **
Others	*Diversity*	0.00	0.13	0.88	0.13	-0.07	0.13	0.82	0.14	0.22	0.13	1.11	0.13	0.28	0.13	1.17	0.14	0.33	0.13	1.22	0.13	0.88	* **
	Abundance	0.29	0.08	4.08	0.88	3.36	0.62	47.15	5.43	0.89	0.21	12.43	1.70	0.63	0.18	8.83	2.21	0.77	0.18	10.82	1.46	*2.64*	* **
	Richness	0.49	0.17	2.37	0.65	0.88	0.28	4.22	1.19	0.83	0.24	3.96	0.85	0.91	0.29	4.38	1.24	0.88	0.26	4.22	0.91	*1.57*	* **

collected with the new method to that of the previously published data. The remarkable similarity of beta diversity profiles across the environments sampled with the two systems is a clear indication that meaningful comparison of environmental and ecological patterns was not compromised, allowing a solid link with the published literature.

The observed differences in alpha diversity profile, on the other hand, should not be seen as a reason to reject comparability. The inadequacy of alpha diversity indices in below-ground communities for detecting and describing environmental change is well-established in literature (Giller, 1996; Heneghan et al., 1998; Rusek, 1998; Tian et al., 2018).

4.4. Arthropod dispersal coefficient

Berlese/Tullgren extractions and pitfall trapping have so far been presented as exclusive methods in the present work. However, their coupling in surveys might help shed light on a parameter difficult to assess as the movement of invertebrates in the soil profile. The application of traditional mark and recapture techniques to estimate mobility and dispersal patterns is made complex by the small size of target groups. In soil, some success is documented in earthworms (Mathieu et al., 2018), but for smaller clades attempts have usually been limited to modelling-based inference (Pequeno et al., 2021) or substantial experimental manipulation (Auclerc et al., 2009).

The combined use of the two sampling methods allows for a more mechanistic and less intrusive estimate of dispersal capabilities. Provided the sampling depth of the pitfall traps and the soil cores for Berlese/Tullgren extractions is the same and moving from the assumptions of accurate density determination from Berlese/Tullgren extractions and of activity determination for pitfall traps, Eq. (1) is proposed as a method to calculate a coefficient of dispersal (D), calculated as the horizontal speed of a taxon within the soil profile in a unit of time.

$$D = \frac{C_p}{\frac{C_b}{V_b} * A_p * t} \tag{1}$$

C_p represents the catch of the target taxon in the pitfall traps, C_b the catch of the same taxon in Berlese/Tullgren extractions, V_b the volume of the soil core used for extractions, A_p the area of sampling ports on pitfall traps and t the time they are deployed. The catch of pitfall traps is assumed to be directly proportional to the sampling port area, to the duration of the deployment, the density of the target taxon in the soil (as measured by Berlese/Tullgren extractions) and the speed of horizontal movement, which is the proposed metric. The method is conceptually similar to techniques widely used above ground, for instance with flight interceptor traps (Byers, 2012), which also involve calculations based on the transit of invertebrates through a diaphragm per unit of time. Application to large datasets will be needed to show the potential of this metric to accurately describe dispersal of soil clades with well-defined error margins.

4.5. Operational opportunities and drawbacks

The use of pitfall does not require significant amounts of work or costly and cumbersome equipment following sample collection from the field. Moreover, the possibility of keeping the external pipe structure in place while replacing the collection tube allows to sample consistently the same exact spot across time, without the risk of microenvironmental spatial variability issues. The advantages of the pitfall trapping method are manifold and substantial, but their possible drawbacks can be an important issue in some environmental settings and must also be carefully considered. The main weakness of the pitfall system was the necessity to keep the traps in place for an extended period of time. This can lead to tampering, removal or damage to traps by both humans and animals, and indeed one of the traps deployed for the present trial was removed and not recovered. On the contrary, the physical habitat

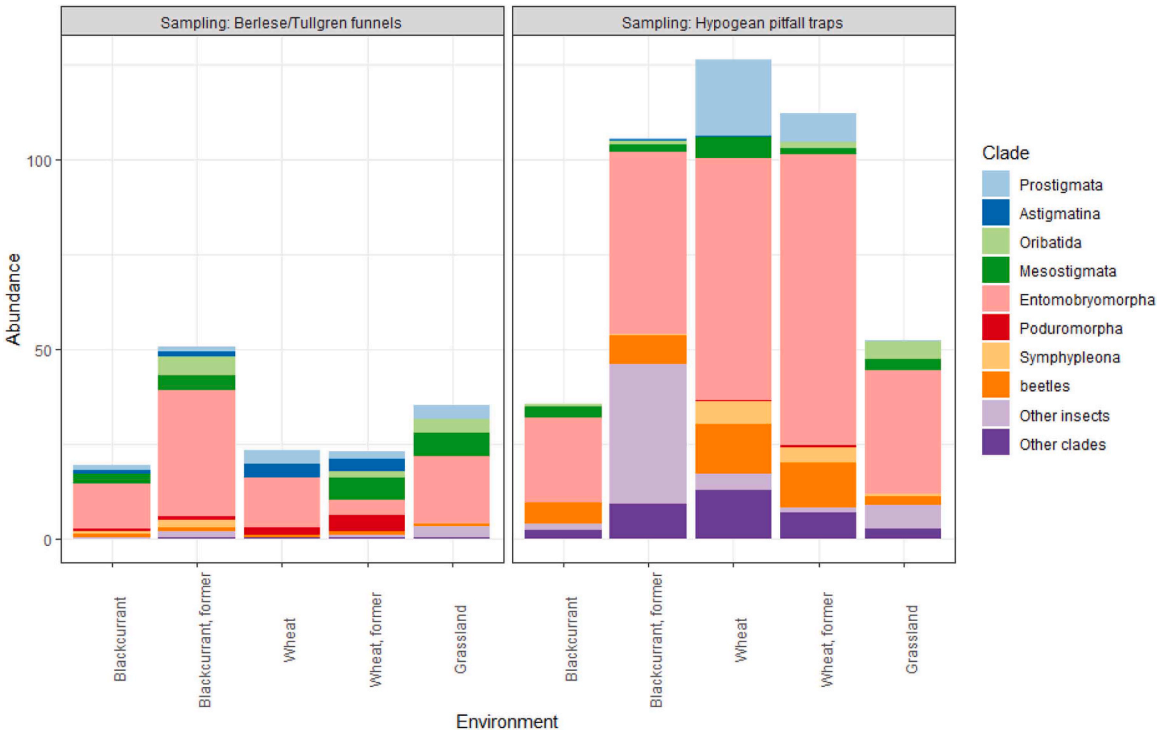

Fig. 5. Community breakup for environment and sampling mode. Values are means per individual trap/extraction.

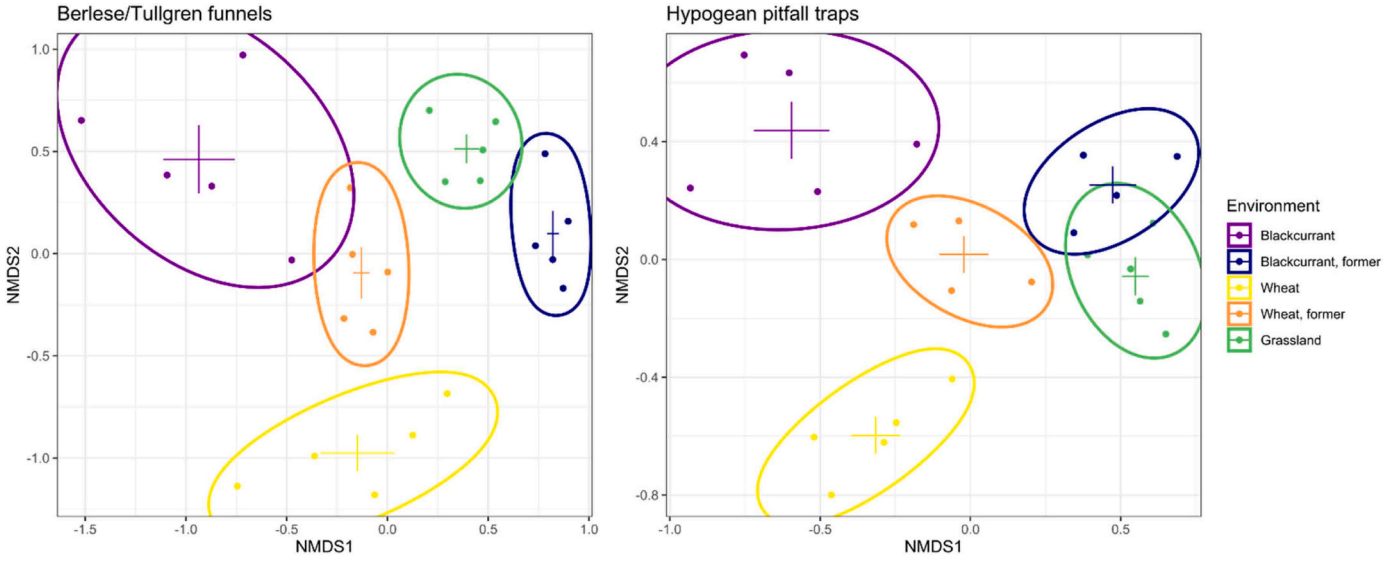

Fig. 6. Non-metric multidimensional scaling of communities recovered with each sampling mode. Centroids and concentration ellipses refer to single environments. Stress was 0.17 for the Berlese/Tullgren ordination and 0.13 for the pitfall trap ordination.

represented by traps can prove attractive to some non-target species. The data from one of the traps was discarded because it was occupied by an active ant colony of *Formica fusca*. Pitfall traps of the proposed design, with sampling ports located beneath the surface, are unlikely to allow access to amphibians, but the presence of vulnerable non-target species should be assessed before deployment, as customary with surface pitfall traps. Finally, while pitfall traps have proved robust enough to withstand significant amounts of rainfall without negative consequences for their operability, persistent waterlogging above the sampling depth can substantially compromise the quality of recovered samples, but this problem is also likely to affect alternative methods.

5. Conclusions

The number, breadth and diversity of invertebrates sampled with the proposed design of pitfall traps was found to be overall superior to conventional low-volume Berlese/Tullgren extractions. As for the traditional mesofaunal targets of Berlese/Tullgren extractions, pitfall traps yielded more diverse and abundant springtail communities and comparable diversity and abundance for mites, although for the latter group additional studies with higher taxonomic resolution are required. The two methods are however not equivalent in terms of sampled communities. In studies whose objective is the production of exhaustive lists of species, the two techniques are better considered as

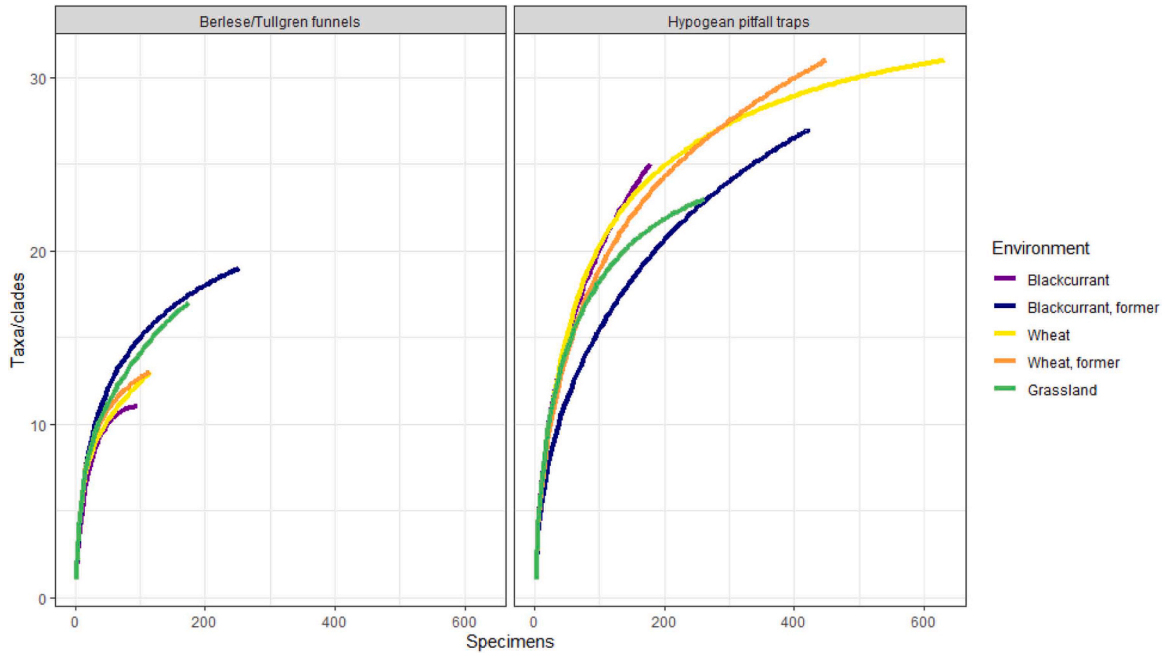

Fig. 7. Rarefaction curves for each sampling mode and environment.

complementary.

In addition to springtail and mites, pitfall trap catches sample beyond the size-range offered by traditional small-volume Berlese/Tullgren implementations, extending with good variety and numbers to relevant clades, like edaphic beetles, centipedes or isopods. For targeted studies of soil microarthropods and the production of exhaustive inventories, large extractions involving monoliths will still be the technique of choice. However, when the objective is environmental profiling of whole-communities for beta-diversity fingerprinting, the proposed design can offer, with low-cost and easy operability, a more holistic view of soil arthropod assemblages than small-volume Berlese/Tullgren extraction.

The structural differences in the species list obtained with the two methods do not affect the comparability of community-level profiles in response to environmental gradients. The two beta-diversity profiles show substantial similarity, with a promising degree of comparability for datasets collected with the two sampling types.

The introduction of hypogean pitfall traps in the toolbox of a soil biologist results in manifold advantages. For chronologically extended monitoring studies, pitfall traps have an obvious competitive edge over the current standard. For studies targeting springtails and soil mites, pitfall traps can provide – depending on the objectives of the research - either an effective alternative or an ideal complement to Berlese/Tullgren extractions, with the additional possibilities in the latter case, of refining estimates for dispersal capabilities for single species or clades. Finally, for resource-limited research contexts, the low cost and ease of deployment of pitfall traps could just make soil biotic sampling affordable and the broad characterisation of arthropod communities for environmental profiling economically viable.

CRediT authorship contribution statement

M. Fioratti Junod and I. Sims conceived the ideas and sampling principles; M. Fioratti Junod collected and analysed the data, designed and carried out the field test and produced the prototype; M. Fioratti Junod and A. J. Miller led the writing of the manuscript. B. Reid identified the sampling area. A. J. Miller, B. J. Reid and I. Sims provided supervision. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Anthony J Miller reports financial support was provided by Biotechnology and Biological Sciences Research Council. Ian Sims reports a relationship with Syngenta International AG that includes: employment. Nothing else to declare.

Data availability

Data will be made available on request.

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Data archive

Raw data from the field test will be made available as supplemental materials on Dryad.

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