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The use of inert pads to study the Collembola of suspended soils

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Abstract

A simple, cheap and non-destructive technique is introduced for the collection of micro-arthropods in sensitive habitats, focussing on suspended soils (accumulations of humus and organic detritus in trees, elevated above soil level). These habitats contain distinctive communities of soil fauna, but in most European forests suspended soil volumes are so small and slow to regenerate that systematic collections can destroy much of the suspended soil resource in a woodland, requiring years to recover. Here I calibrate a non-destructive collection technique based on inserting a pad of inert material with a wide pore space, allowing the material to be colonised then removing the pad for Tullgren extraction. Standard domestic scouring pads are ideal for this purpose and extract quickly in high gradient extractors. This method has the potential to become a standard non-destructive collection technique for suspended soils as well as in other sensitive habitats such as caves. It is also a suitable platform for a wide variety of small-scale projects involving experimental manipulations.

Keywords Arboreal arthropods | vertical migration | synthetic habitat

1. Introduction

Although most organic detritus within woods ends up as humus in the upper layers of the soil, a small amount can accumulate in natural traps such as branch bases or wounds. These habitats, variously called ‘arboreal soils’ (Nadkarni 1994), ‘canopy litter’ (Yoshida & Hijii 2006), ‘suspended litter’, ‘suspended soils’ and ‘crown humus’ (Lindo & Winchester 2006) are often stabilised by epiphytes (mosses, ferns or bromeliads) to create a long-lasting humus habitat well above ground level, as high as 50 m, containing many sorts of arthropods (Palacios-Vargas & Castaño-Menesesa 2002, Lindo & Winchester 2006), as well as a diverse array of invertebrata including oligochaetes (Affeld et al. 2009, Krombheim et al. 1999). Karasawa & Hijii (2006) found evidence of habitat stratification within individual *Asplenium* ferns, acting as islands of suspended soils. Nadkarni (1994) stated that ‘The arboreal soil and litter is one of the least studied components of humid tropical forest communities’. These suspended soils contain arthropod communities that differ qualitatively from what is found in leaf litter at soil level (Yanoviak et al. 2004, Rodgers & Kitching 1998, 2011) and are not collected by standard fogging techniques (as they do not fall after death; Yanoviak et al. 2003). Although suspended organic material is routinely found in tropical moist forests, the volume of suspended soil in UK woodlands is often minimal. O’Reilly et al. (1969) reported 1580 kg ha^{-1} of moss epiphytes in a particularly rich North Wales forest, but this standing crop value seems large and there seem to be few published records of the standing crop of suspended humus, in moss mats or elsewhere in the UK. The habitat is effectively absent from many young woods, and requires either old trees with decayed holes or old woodland with epiphyte-covered bark. During one (unpublished) sampling exercise in White Wood Devon, one post-doctoral student removed much of the safely accessible suspended litter in the wood, finding one rare collembolan species (*Uzelia setifera* Absolon, 1901), but still collecting too few Collembola for any meaningful statistical analyses. In many sites the most

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common form of suspended litter will probably be old birds' nests, which are known to contain a diverse range of non-parasitic arthropods (Hicks 1953).

The suspended soil community of Collembola is derived from, but qualitatively distinct from the 'bark surface' or epiphytic Collembola, which are ubiquitous (but often overlooked due to size) in vacuum / fogging collections. These are dominated by Entomobryids [in the UK *Entomobrya albocincta* (Templeton, 1835)*, Entomobrya nivalis* (Linnaeus, 1758)*, Entomobrya intermedia* Brook, 1884 and *Orchesella cincta* (Linnaeus, 1758)] (Hopkin 1997, 2007, Prinzing 2001, Shaw & Ozanne 2006), though many other Collembola may be found here (Bowden et al. 1976), including species of Sminthuridae (*Allacma fusca* Linnaeus, 1758), plus the podurid genus *Xenylla* (e.g. Yoshida & Hijii 2006). Hicks (1953) found birds' nests to contain Collembola only of the Entomobryid genera *Entomobrya* and *Seira*.

These two Collembola communities require different collection methods. Surface active animals may be collected by beating, pyrethrum knock-down or vacuum collection. Beating and chemical knock-down are ineffective for animals living within litter or deep in bark crevices. One extreme solution has been to employ professional climbers to ascend trees and saw off branches, which are then soaked in KOH to expel arthropods deep in bark crevices (Bolger, pers. comm.). By contrast, to collect animals from suspended humus, the material is normally collected and bagged by hand for immediate placement in a Tullgren extractor (Rodgers & Kitching 2011). Karasawa & Hijii (2006) cut down (hence killed) all the 37 *Asplenium* ferns studied in their project.

As an alternative, here I introduce the idea of temporarily introducing inert pads into the suspended soil habitat, as artificial habitats for mesofauna. After allowing for colonisation, the pads are returned from the field to the laboratory for extraction of mesofauna in a standard high-gradient extractor. Domestic scouring pads prove to be ideal for this function. The work described here sets out a basic introduction to the method and seeks to answer three questions: (1) does the material of the pads affect their colonisation? (This has implications for the choice of materials used by future researchers.) (2) Are the densities of animals comparable to densities in the surrounding matrix? (3) Is the Collembola community of pads representative of the local fauna surveyed by alternative methods? These questions were focussed on Collembola in one specific habitat, that of suspended soils in woodlands, where slow accretion rates generally preclude bulk removal of soil.

2. Methods

Standard domestic scouring pads (chemical free) were used, purchased in bulk either as plastic or steel loops. The volume occupied by these was slightly different; 150 cc (plastic) vs. 200 cc (steel). Two small experiments are described here, as part of the validation of the use of these inert pads to collect soil fauna from sensitive habitats. Where data were irremediably non-Gaussian, inferential analyses used non-parametric statistical techniques (Siegel 1956) run on SPSS 19.

Table 1. Summary data on field sites.

site	species	Days	site	Coordinates	H, m	tree hole?	total spp
1	hazel	93	Bookham common	51°14'51"N 0°16'0"W	0.5	0	14
2	hazel	93	Bookham common	51°14'52"N 0°16'0"W	0.1	0	11
3	beech	93	Bookham common	51°14'52"N 0°16'0"W	2.0	1	8
4	ash	133	Inholms, nr Dorking	51°12'54"N 0°18'56"W	1.5	1	8
5	ash	133	Inholms, nr Dorking	51°12'54"N 0°18'55"W	1.5	1	9
6	oak	117	Inholms, nr Dorking	51°12'44"N 0°19'12"W	0.5	1	3
7	willow	133	Inholms, nr Dorking	51°12'55"N 0°19'35"W	0.2	0	12
8	gutter	97	Inholms, nr Dorking	51°12'55"N 0°18'52"W	2.0	0	5
9	alder	131	Holmwood common	51°12'15"N 0°18'46"W	0.8	0	7
10	oak	107	Holmwood common	51°12'58"N 0°19'24"W	1.9	0	6
11	hazel	137	Inholms, nr Dorking	51°12'39"N 0°19'19"W	0.2	0	8
12	ash		Froebel college woods	51°27'25"N 0°14'46"W	1.0	0	12

Days = days of exposure in the field, **H** = Elevation, metres.
'Gutter' is not a tree species but part of a roof drainage system, where leaf litter had accumulated.

The aim of experiment 1 was to compare Collembola collections between steel and plastic pads. Paired plastic and steel pads (N = 24 pairs) were placed or suspended in a variety of arboreal settings between 0.5 and 2.0 m elevation, left for between two and four weeks, then all pads were bagged separately and their fauna collected in a Tullgren funnel.

The aim of experiment 2 was to establish the accuracy with which inert pads give a representative picture of the 'true' soil faunal community in suspended soil systems, compared to Tullgren extraction of the bulk soil or surface vacuum extraction. Inert pads (steel) were placed in a range of suspended soil situations (N = 12) in February 2012, and re-collected June 2012 along with a sample of the actual suspended soil and a surface vacuum sample. Details are listed in Table 1. The pads and suspended soil samples were bagged separately and returned to the lab for extraction in a standard Tullgren apparatus. For each location, the 'correct' total species list was defined as the species collected by all 3 methods pooled together. Comparisons were made between species balance sampled by different collection methods by the Jaccard index; densities (animals per unit volume) were compared between pads and suspended soil by correlation. The balance between bark-surface forms and suspended soil forms within inert pads was estimated from the predicted outputs from a regression model where the dependent variable was a habitat code (1 = surface vacuum, 3 = suspended soil), while 'the pad community' was coded as missing. The independent variables were presence/absence data (since densities in soil and bark communities could not be compared directly). Initially all species found > 3 times were entered into the regression; this was used to select species whose occurrence correlated with collection type (using the threshold of $p < 0.1$), and the regression was re-run using only these species to estimate the 'collection type' associated with the community within the steel pads.

3. Results

The pads were all colonised by a range of soil mesofauna including acari, coleoptera, pseudoscorpiones, diploda and chilopoda along with aranae, opiliones and

Table 2. A summary of the number of collections of each species Collembola during experiment 2 (N = the number of collections, maximum = 36).

species	N	species	N	species	N
Allacma fusca (Linnaeus, 1758)	1	*Hypogastrura* spp	12		
	3	*Orchesella cincta* (Linnaeus, 1758)	27		
Desoria tigrina Nicolet, 1842	3	*Isotomiella minor* (Schäffer, 1896)			
	1	*Orchesella villosa* (Geoffroy, 1764)	1		
Deuterosminthurus pallipes (Bourlet, 1843)	1	*Lepidocyrtus cyaneus* Tullberg, 1871			
	16	*Parisotoma notabilis* (Schäffer, 1896)	15		
Dicrytoma fusca (Lubbock, 1873)	1	*Friesea* spp	3	juvenile isotomids	2
Dicyrtomina minuta (O. Fabricius, 1783)	2	*Isotoma viridis* Bourlet, 1839		*Sminthurinus trinotatus* Axelson, 1905	3
Entomobrya albocincta (Templeton, 1835)	14	*Isotomiella minor* (Schäffer, 1896)		*Sphaeridia pumilis* (Krausbauer, 1898)	5
Entomobrya intermedia Brook, 1884	4	*Lepidocyrtus cyaneus* Tullberg, 1871	3	*Tomocerus minor* (Lubbock, 1862)	6
Entomobrya nicoleti (Lubbock, 1867)	5	*Lepidocyrtus curvicollis* Bourlet, 1839	1	*Tomocerus vulgaris* (Tullberg, 1871).	6
Entomobrya nivalis (Linneaus, 1758)	3	*Lepidocyrtus lanuginosus* (Gmelin, 1788)	16	*Tomocerus longicornis* (Müller, 1776)	8
Folsomia quadrioculata (Tullberg, 1871)	6	*Megalothorax minimus* Willem, 1900	18	*Vertagopus arboreus* (Linnaeus, 1758)	1
Friesea spp	3	*Monobella grassei* (Denis, 1923)	1		
Heteromurus major (Moniez, 1889)	1	*Neanura muscorum* (Templeton, 1835)	3		

psocoptera (data not presented). Extraction in a standard Tullgren funnel (40W bulb) was visibly underway within an hour and completed within 24 hours, several times faster than soil cores.

Experiment 1: The density of animals per unit volume in plastic and paired steel pads is shown in Fig. 1 (N = 24 pairs). Differences were not significant for total animal density, species richness or for any individual species (all $p > 0.05$) by the Wilcoxon signed rank test.

Experiment 2: The number of Collembola species and density per unit volume are summarised in Table 2. There were no significant trends relating to effects of height (Spearman's correlation coefficient), tree hole (Mann Whitney U test) or tree species (Kruskal-Wallis test with 3 df) on Collembola density or species richness. Jaccard similarities between vacuum, pad-collected and soil Collembola communities are shown in Fig. 2. This shows the highest similarities were between the suspended soil community and that collected in inert pads (though the differences between the similarities for each site was $p > 0.05$ by anova). No collection method was clearly superior in terms of % species recovered, with each method recording 50–60 % of the total species present in/on the suspended soil (Fig. 3).

After removal of one outlier (a suspended soil whose density of *Hypogastrura* exceeded 1600 animals litre^{-1}, eight times higher than any other), densities of animals litre^{-1} were correlated between pads and soil ($r = 0.57$, $p < 0.05$ after log-transformation), though slightly lower in the pads (Fig. 4). The zero-intercept line has $b = 0.80$, hence a 20 % underestimate in true density.

When presence/absence data were regressed onto collection method, with 1 = vacuum, 3 = soil and pads left as missing, only four species were significant at $p < 0.1$; *Folsomia quadrioculata* (Tullberg, 1871) ($p < 0.05$) and *Tomocerus minor* (Lubbock, 1862) ($p < 0.1$) were soil-associated, while *Orchesella cincta* ($p < 0.05$) and *Entomobrya albocinta* ($p < 0.05$) were the predictors of the bark community. Rerunning the regression on these species alone suggested that the community of Collembola within the pads was approximately intermediate between the bark and soil communities (Fig. 5), shown as predicted habitat codes being around 1.8, where 1 = bark and 3 = suspended soil).

4. Discussion

The work described above was aimed at establishing the general validity of using inert porous pads as a surrogate for the local Collembola community and offers answers to the three questions listed in the Introduction section. Inert

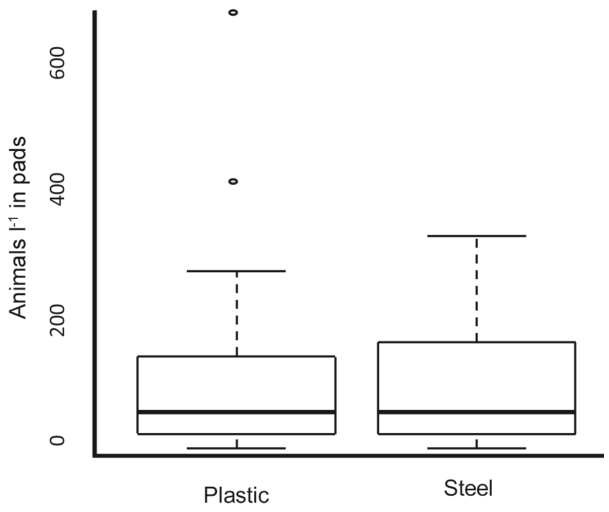

Figure 1. Collembola densities in paired plastic vs. steel pads. The difference is not statistically significant.

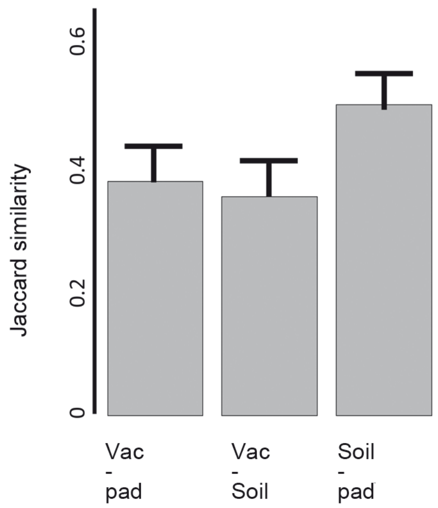

Figure 2. Jaccard similarities between 3 collection methods, based on 12 suspended soil habitats; bars show 1 se, $p > 0.05$ by 1-way anova.

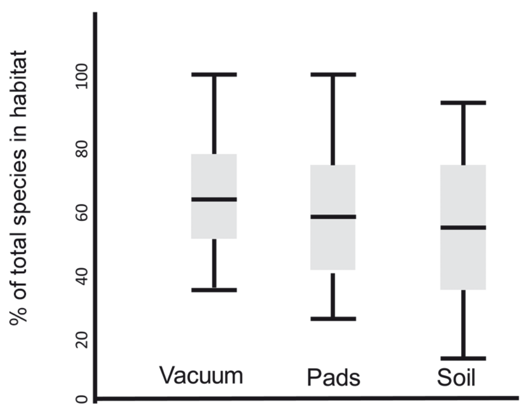

Figure 3. The proportion of total Collembola species collected in each habitat, as a function of sampling technique.

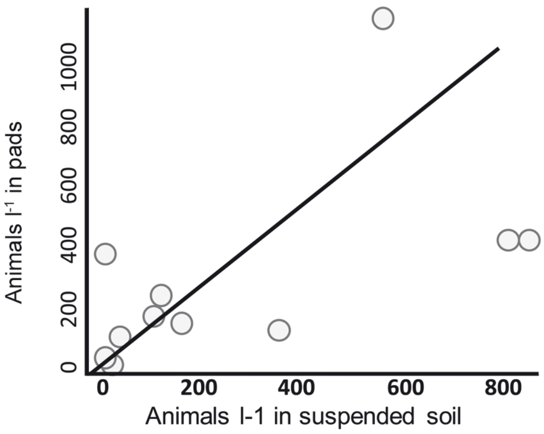

Figure 4. A comparison of faunal density in pads vs. density in matched suspended soil. Y = 0.6X + 135, r = 0.57, $p < 0.05$; zero intercept line b = 0.80 (shown).

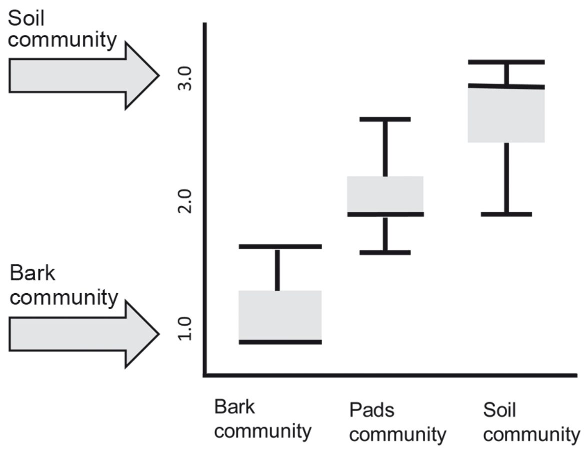

Figure 5. Predicted Collembola community scores of steel pads based on a regression model contrasting 'bark' (= 1) against 'soil' communities (= 3).

pads have been used as experimental substrata in aquatic research (e.g. King et al. 1990), but this appears to be the first analysis of data collected from them as an arboreal pseudo-habitat. Coarse inert pads attached epiphytically were readily colonised by a range soil invertebrates, apparently irrespective of its material composition. They accumulate densities of Collembola that agree with and correlate to naturally occurring values, and the species collected describe what is present locally. Accordingly, these simple pads have the potential to become a standard collecting tool. Their use could be for population studies of Collembola (or other mesofauna) in habitats where bulk collection of material is impractical (caves, tree bark) or unethical (small, slow-forming systems such as Antarctic moss tussocks). In such cases the temporary introduction of inert pads followed by their removal to a Tullgren extractor allows researchers good estimates of the local soil faunal community without causing any habitat damage, beyond removal of the animals. These could lead to a variety of small research projects, notably on use of attractant baits / pheromones to maximise capture.

5. Acknowledgements

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