

Composite sampling enhances the confidence of soil microarthropod abundance and species richness estimates

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Summary

The quantification of abundance and species richness of soil microarthropods is most often severely hindered by extraordinary data variability, highly skewed frequency distributions, many extreme and zero counts, and small sample sizes. We developed a composite sampling technique to enhance the confidence of abundance and species richness estimates. Many soil cores ($n \geq 100$) are sampled, animals extracted, the extracts pooled, mixed, and subsamples (aliquots) taken. Compared to the standard (separate sampling units), no microarthropods were lost or mechanically damaged during the compositing procedure. The confidence of abundance estimates was substantially greater in the composite than in the standard, although not for taxa of low abundance ($< \approx 10^3$ ind.m⁻²). Moreover, compositing was the superior technique in estimating species richness. The number of sampling units needed to recover a certain number of species with the composite was 70 % of the standard method. We conclude that composite sampling is a promising alternative to the standard technique and may help to increase the generally low confidence of microarthropod field data. Finally, potential limitations of composite plans are discussed: a great number of field cores from an unbiased sampling plan have to be composited; comparisons between composites of unequal size should be avoided; all information on the variation among field cores is lost by compositing; parallel measurements of fauna and other variables in the same cores are not possible.

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Introduction

Sample data of field collections of soil microarthropods typically exhibit extraordinary variability, highly skewed frequency distributions far from normality, and many extreme and zero counts (Bruckner unpubl. data; Ekschmitt 1998). Sample size (the number of cores) is usually low, rarely exceeding 10 to 20 (Bruckner unpubl. data). This may have serious consequences for data analysis and for biological conclusions drawn from a study: The confidence of abundance estimates is generally very low; species of low frequency are likely to be missed by the sampling design and the number of species may be underestimated accordingly; and, when comparing treatments or sites, a high probability of type II errors (i.e. failing to detect existing differences between treatments or sites) has to be accepted (Ekschmitt 1998).

Increasing sample size seems to be a straightforward remedy to the situation. However, the confidence of estimates and the number of sampling units are related by non-linear power functions (e.g. Karandinos 1976), and the benefits of added precision by intensified sampling are rapidly outweighed by increasing costs. Thus, "more samples" is not an adequate way to improve estimates of soil microarthropods: The expenses to achieve even modest levels of data quality are prohibitive (Bruckner unpubl. data).

A notable feature of soil microarthropod studies is that sampling costs are substantially lower than processing costs. While a number of cores can usually be collected within a few hours per site, the extraction, identification and counting of animals usually take months. For studies of this kind, composite sampling techniques are known to be time-efficient alternatives to separate measurements (Boswell et al. 1996; Garner et al. 1988). When forming a composite, a great number of sampling units is taken from the population, pooled, mixed, and a small number of subsamples (or aliquots) is then taken and analyzed. Data variability of the subsamples is usually minimized and the number of units to be processed is much smaller compared with standard designs. Study costs can be reduced or, if the time budget is fixed, the confidence of estimated means can be increased.

Composites are frequently used in soil science and in censuses of plant-parasitic soil nematodes (Carter & Lowe 1986; McSorley & Walter 1991; Petersen & Calvin 1986; Schouten 1995). When sampling nematodes, soil is collected, mixed, and the worms then extracted from subsamples. It is assumed that the physical action of mixing has no influence on the viability of the worms (but see Schouten 1995). This procedure is certainly not suitable for microarthropods, since the grinding action of soil particles would injure or kill a considerable portion of the fauna. This would depress the extraction efficiency and lead to underestimation of abundance and species numbers.

In this paper, we propose a composite sampling technique for soil microarthropods that was developed to improve the poor estimates of abundance and species richness. Our principal idea was to mix the sampling units in liquid medium *after* extraction to avoid damaging fragile specimens, instead of mixing soil material of the cores *before*

extraction. We report on two studies conducted to test the quantitative performance of the technique and compare it to the standard sampling method. For more analyse and tentative results of the first study see Bruckner & Barth (1997).

Materials and Methods

The first site was a Norway spruce (*Picea abies*) forest monoculture near Gumpenstein, Styria, Austria (47°29'N, 14°7'E, 750m above sea level), situated on a 15° inclined, west-facing slope. The stand was approximately 40 years old and contained no undergrowth. The soil was dystric Cambisol over quaternary sediments (silty sand), the humus form was humimor (Green et al. 1993) with distinct L (depth ≈ 2 cm), F (≈ 2 cm), and H (≈ 6 cm) layers. The F layer was densely rooted. For recent studies of soil biota at the site, see Bauer et al. (1994), Bruckner et al. (1995, 1999), Kandeler et al. (1994), Vedder et al. (1996), and Zechmeister-Boltenstern et al. (1998). A total of 130 soil cores were taken randomly within a 100×40m plot with a corer of the bulb planter type (Ø 7 cm, depth 12 cm) on October 28, 1993. Cores were extracted in a Berlese-Tullgren apparatus into picric acid.

The second site was a moist, nutrient-rich meadow in Sankt Koloman, Salzburg, Austria (47°38'N, 13°14'E, 1005m above sea level). Inclination of the site increased from 5° to 22° towards the southwest. The soil was chromic Cambisol over moraine deposits of variable depth. The meadow is mown at least once a year. The site is part of the permanent soil monitoring programme of Salzburg (Juritsch 1996; Bruckner & Bauer 1997). A total of 130 soil cores were taken randomly within a 70×85 m plot with a bulb planter (Ø 6 cm, depth 6 cm) on June 19, 1996. Cores were extracted in a modified Macfadyen apparatus into 50% ethylene glycol.

From the extracts (animals and debris floating in the collecting fluid) of the 130 soil cores 30 were randomly selected, and microarthropods determined and counted, forming the *standard* or *separate sample*. The remaining 100 extracts were combined and transferred into glycerol for one week to increase the specific gravity of the particles. Then, extracts were decanted into a self-made, rectangular PVC vat. To simplify subsequent calculations, the ground surface of the vat was 100 times the surface of the soil corers (we used two different corers in this study and thus constructed two vats of different dimensions. We later found 70×50×15 cm convenient for laboratory practice). The liquid level in the vat was raised to 5cm by adding approximately 20 litres of 80% denatured ethanol, which caused the glycerol-soaked animals and the debris to sink. The sediment particles were homogenized by gently agitating and stirring with a spoon for about 10 minutes. Thirty aliquots were taken randomly from the vat by pressing a plastic tube (of the same diameter as the corers) to the sediment and sucking it up with a modified wash bottle (pipe shortened). The animals and the debris had to sink completely to ensure that they could be sucked up completely. No extra ethanol was added to compensate for the removed volume because the sediment should not be agitated once the sucking procedure started. Microarthropods were determined and counted in the aliquots and form the *composite sample*.

In the forest sample, only taxa identifiable with a binocular microscope at 40× magnification were censused, i.e. total number of Collembola and adult Oribatida, the oribatid species *Hermannia gibba*, *Liacarus coracinus*, *Tectocepheus velatus* and *Phthiracarus spp.*, and the collembolan *Isotomiella minor*. In the meadow sample, the Collembola were determined and counted at species level.

A numeric simulation was run to assess the influence of sample size on the confidence of abundance estimates. There was no frequency distribution that fit the sample data of all taxa. However, lognormal models were found to be the best general descriptors of the standard data, and normal models of the composite (details not shown, Barth & Scheibengraf unpubl. data).

Table 1. Sample statistics of soil microarthropods of the forest and meadow site. stand: standard sample, comp: composite sample. The species of the forest sample are oribatids (except the collembolan *Isotomiella minor*). The species of the meadow sample are Collembola. Zero counts are sampling units containing no specimens of a taxon

taxa	total number of specimens		[ind·m ⁻²]		coeff. variation [%]		skewness		number of zero counts	
	stand	comp	stand	comp	stand	comp	stand	comp	stand	comp
<i>Folsomia quadrioculata</i>	458	588	5399	6932	147.60	26.62	1.79	1.48	6	0
<i>Isotomurus palustris</i>	941	665	11094	7840	105.23	25.38	1.56	0.85	1	0
<i>Isotomiella minor</i>	267	343	3148	4044	144.81	31.30	1.91	0.35	5	0
<i>Isotoma notabilis</i>	284	222	3348	2617	240.16	41.33	3.73	0.96	7	0
<i>Mesophorura tenuisensillata</i>	30	38	354	448	159.74	87.80	1.74	0.56	18	9
<i>Metaphorura affinis</i>	13	8	153	94	301.11	168.65	3.60	1.11	25	22
<i>Stenaphorura denisi</i>	11	17	130	200	316.12	151.47	3.48	2.41	26	17
<i>Ceratophysella denticulata</i>	21	19	248	224	273.51	140.52	4.28	1.45	22	17
meadow sample <i>Protaphorura armata</i>	10	21	118	248	240.71	150.77	2.31	1.60	25	18
<i>Hymenaphorura cf. sibirica</i>	9	7	106	83	278.90	216.03	3.53	2.15	25	24
<i>Brachystomella parvula</i>	50	43	589	507	154.65	85.32	1.50	0.53	17	8
<i>Friesea mirabilis</i>	0	3	0	35	0.00	305.10	–	2.81	30	27
<i>Folsomia litsteri</i>	0	3	0	35	0.00	305.10	–	2.81	30	27
<i>Lepidocyrtus cyaneus</i>	41	33	483	389	145.69	105.02	1.615	0.80	15	12
<i>Pseudosinella sp.</i>	1	0	12	0	548.35	0.00	5.48	–	29	30
<i>Heteromurus nitidus</i>	14	20	165	236	230.19	120.34	3.04	1.13	23	15
<i>Bourletiella cf. hortensis</i>	115	179	1356	2110	83.09	41.63	0.92	0.00	3	0
<i>Dicyrtoma fusca</i>	11	8	130	94	208.59	168.65	1.72	1.11	24	22
total Collembola	2276	2217	26832	26137	92.54	16.61	2.39	-0.04	0	0
total Oribatida	3107	3400	26911	29449	118.85	27.63	2.14	0.60	0	0

	total Collembola	4708	5186	40778	44919	121.70	10.61	2.28	0.12	0	0
forest	<i>Hermannia gibba</i>	64	89	554	771	145.52	83.27	2.16	1.11	12	1
sample	<i>Liacarus coracinus</i>	265	220	2295	1906	136.66	59.42	2.58	1.04	3	0
	<i>Phthiracarus</i> spp.	142	241	1230	2087	214.45	41.67	4.28	0.62	9	0
	<i>Tectocepheus velatus</i>	259	173	2243	1498	243.79	57.19	2.92	0.52	11	0
	<i>Isotomiella minor</i>	1984	1862	17187	16128	167.71	17.34	2.37	-0.04	0	0

For the simulation, we used these frequency distributions to generate 10,000 random data points for selected taxa of low and high abundance. These data sets were sampled with sizes of 5, 10, ... 30 units, and arithmetic means calculated for each sample. The number of means falling within a given interval around the (standard or composite) sample mean were counted and used as a measure of sample precision. Barker & Campbell (1981) and Ferris (1984/85) regarded an interval of $\pm 5\%$ as acceptable for the assessment of plant-pathogenic nematodes in agricultural fields. Unlike nematodes, soil microarthropods are rarely of economic importance, so we arbitrarily set the range to $\pm 10\%$.

We used the meadow Collembola data to assess the power of the methods to estimate species richness, i.e. the number of species at a site, and adopted Pielou's pooled quadrat method (Magurran 1988) to relate species richness to sample size. The number of species in a randomly selected sampling unit was noted, then the records of new species in a second unit added, then a third unit added, until all 30 units had been accounted for. One hundred permutations of this process were run for each method, and the means calculated for 1, 2, ... 30 sampling units.

Statistical analyses and simulations were performed with Microsoft Excel 97 SR-1 and the Statgraphics 3.0 package.

Results

It took about 16 hours to sample 130 cores in the forest study, and 40 hours of laboratory manipulations including the compositing procedure (hours per operator). Thus, if we assume a sample of 100 cores per composite, the extra effort for composite sampling is 4 to 5 days at maximum. In our opinion the time for laboratory work can be much reduced by experienced personnel who process the sample as a matter of routine. Even 4 to 5 extra days, however, seem to be acceptable against the background of the many months that are usually spent for extraction, identification and counting.

We checked for physical damage of the microarthropods in the composites (detached setae, crushed bodies, etc.) that may have occurred due to the manipulations or to the different osmotic properties of ethanol and glycerol. Microscopical examination of all meadow collembolan and randomly selected forest oribatid specimens revealed no damage. The animals that went through the compositing procedure were as readily identifiable as the specimens in the standard.

The total number of specimens of each taxon in all 30 cores/aliquots were approximately the same for both methods (Tab. 1). There were no indications of a systematically smaller or larger recovery by either method. A 2×2 contingency table did not indicate a significant difference between the methods for total numbers of meadow Collembola (two-sided Chi-squared test, smaller or larger recovery of a method classed as - and +, $\chi^2 = 0.125$, $P > 0.40$).

In contrast to the abundance estimates, data variability (expressed as coefficient of variation, CV %) was substantially smaller in the composite than in the standard, that is, the confidence of abundance estimates was greatly enhanced by compositing. The frequency distributions of abundance data were generally highly skewed in the separate sample and more symmetrical in the composites. Except for very rare species, the number of zero counts was generally high in the standard and much reduced in the composite (Tab. 1).

However, as was expected from the predictions of Taylor's power law and related variance-mean relationships (e.g. Kuno 1991), the ability of the two methods to reduce data variability was not the same for all densities. In order to assess the depen-

density of variability on abundance, we pooled the data of the forest and the meadow study, and plotted CV% against \bar{x} for the whole range of densities (Fig. 1). There was a hyperbolic relationship between abundance and data variability for both methods. Variability was relatively low for taxa of high abundance, but increased sharply when density fell below a certain value (inflection point of the hyperbola). The CV% of taxa below this abundance was high or very high and the confidence of the estimates consequently poor. A multiplicative power regression model gave the best fit to the data of 12 models tested:

$$\text{standard: } \bar{x} = 2.04 * 10^{11} * CV \%^{-3.71} \quad (r = -0.74, P < 0.01)$$

$$\text{composite: } \bar{x} = 1.61 * 10^7 * CV \%^{-2.28} \quad (r = -0.98, P < 0.01)$$

The inflection points of both models lay at 3,500 to 4,000 indiv.m⁻². Below this value, precision decreased rapidly and a meaningful estimation of a taxon's abundance was hardly possible, irrespective of the method used. However, the composite method often performed better even for taxa of low abundance (Fig. 1).

For the simulated data, the composite was superior to the standard method for all abundances tested (Figs. 2a, b). At a given sample size, a greater number of composite than of separate means fell within the interval of $\pm 10\%$ around the sample mean. Commonly used sample sizes (10 to 20 units) were sufficient to achieve acceptable levels of precision ($\geq 80\%$ of means within the specified range) in the composite for taxa of high abundance ($> \approx 8,000$ ind.m⁻²) (Fig. 2a). This was not true for low-density taxa. For abundances below $\approx 1,000$ ind.m⁻², estimates were poor with both methods, with the number of means falling $\pm 10\%$ of the sample mean always less than 50% (Fig. 2b).

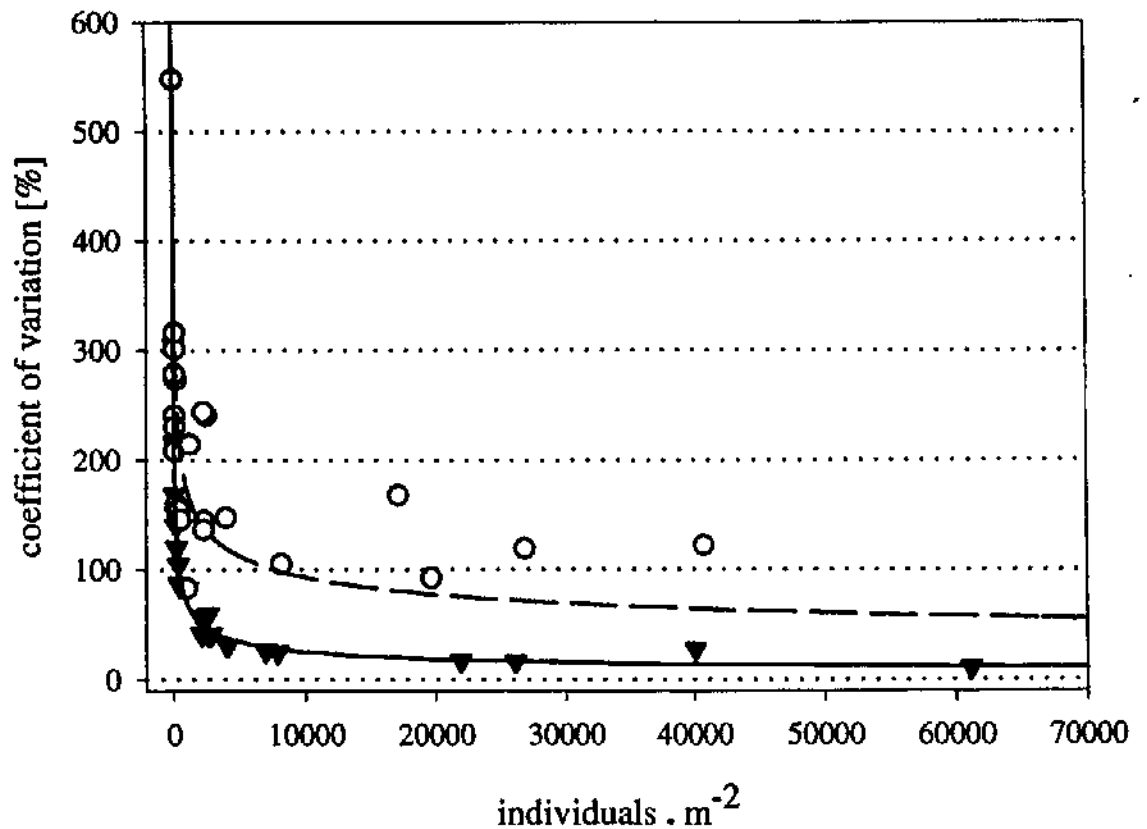


Fig. 1. Regression of abundance on variability of pooled data of forest and meadow microarthropod taxa. Solid triangles and line indicate data points and a multiplicative power model of the composite, open circles and dashed line the standard.

The number of species in the standard and composite of the meadow site was 16 and 17, respectively. The sampling effort to obtain a certain percentage of species was consistently higher for the standard than for the composite (Tab. 2). It took 28 of 30 sampling units to obtain 100 % of species with the standard method, and 20 with the composite (averages of 100 permutations of the "pooled quadrates" procedure). The absolute difference between the methods rapidly became smaller with decreasing probability of including a species in the sample, but the number of composite units was consistently about 70 % of the separate (Tab. 2). If we had decided for a recovery probability of 95 % in the meadow study, a relatively small sampling effort would have been sufficient using either method (13 standard and 9 composite units, on average).

Discussion

The advantages of compositing are obvious in our two data sets. At a given sample size, the composite provided abundance and species richness estimates of higher confidence than the standard, but did not appreciably increase the total costs of data collection. If, on the other hand, a certain level of data confidence is fixed at the onset of a study, the number of sampling units can be reduced by compositing. Thus, the proposed technique is a promising alternative to the standard method, particularly for monitoring, conservation, or other applied research, where confident data have to be gathered from a small number of sites at low cost. Whenever the research objectives are "good means and species lists", the use of composites is strongly recommended.

However, the benefits of compositing do not apply for taxa of low abundance. As a rough guide, we suggest that the extra effort of compositing does not pay for taxa with less than $\approx 10^3$ ind.m⁻² (note that this figure holds true only for standard corers of 5–7 cm diameter). In approximate agreement with this value, a recent analysis of a great number of soil fauna data sets by Ekschmitt (1998) revealed that data variability is nearly constant for abundances >10 specimens/core, but rapidly increases above this value. Poor abundance estimates and low detection probabilities are a general feature of rare species (Karandinos 1976) and can be improved only to a limited extent by alternative sampling techniques (Morrison et al. 1995).

Table 2. Number of sampling units necessary to obtain a percentage of species richness with the standard and the composite method. Values are averages of 100 permutations of a meadow Collembola sample (n=30)

number of species [%]	number of sampling units	
	standard	composite
100	28	20
99	22	15
97	16	11
95	13	9
90	9	6
80	5	3
70	3	2

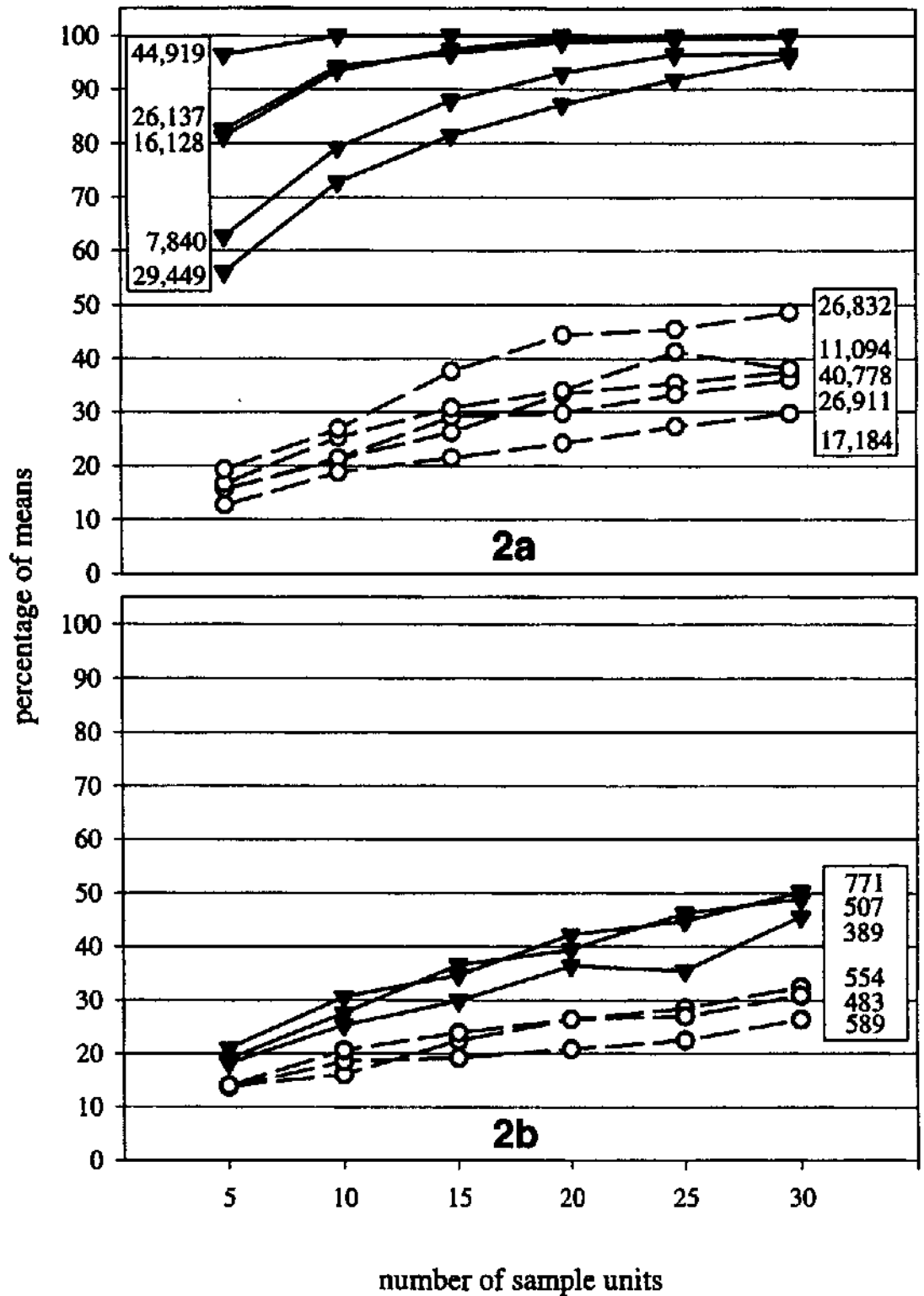


Fig. 2. Simulated random sampling of taxa of high (2a) and low (2b) density. The percentage of arithmetic means falling within $\pm 10\%$ of the (standard or composite) sample mean (ordinate) was used to assess the influence of sample size (abscissa) on the confidence of abundance estimates. Solid triangles and lines indicate taxa of the composite, open circles and dashed lines taxa of the standard. The numbers beside the lines are densities (ind.m^{-2})

The proposed composite method is not a universal remedy for all sampling problems (see Garner et al. 1988, for a general account of potential limitations). Above all, the subsample has to represent the (unknown) field population. If this condition is not satisfied, means and species numbers will not be proper estimates of their respective field values. To ensure adequate representation, a large sample from an unbiased sampling plan (e.g. true random sampling or systematic sampling) has to be composited. Due to limited experience with the new technique, we cannot give even vague figures of what a "large sample" is. As a precaution, we advise not to use less than 100 cores in richly structured habitats such as forest soils. It should be possible for most laboratories to extract a sample of this size in two or three successive runs. Storage of cores for up to several weeks does not significantly reduce the extraction efficiency of microarthropods if the material is kept cool (Edwards & Fletcher 1971; Leinaas 1978).

Comparisons between composites of unequal size should be avoided, e.g. between subsamples of 100 and 200 units. As long as the field population is not fully represented in the composite, the species number may be especially sensitive to sample size.

Provided the field population is adequately represented in the composite, the subsample means are proper estimates of field means. An important disadvantage of composite techniques is that all information on the variation among field sampling units and data confidence is lost by compositing. Thus, unconfident means cannot be distinguished from "good" ones.

Composites are inappropriate whenever estimates of within-sample variance are needed, e.g. for ANOVA. In principle, composites may be formed for every replicate of a treatment and the variance within and between treatments calculated. In practice, the number of composites in a study will be limited by the capacity of extractors. As a compromise, cores from all replicates of each treatment may be combined and composite means compared. However, there is no test statistic for significance of differences between treatments.

Composite sampling should be excluded in investigations which require single-core analyses of species spectrum or abundance along with soil moisture, nutrient content, and microbial biomass. In such cases, correlations between faunal characteristics and additional variables are searched for, but composite designs can only accomplish the comparison of means.

The collection of reliable field data on soil microarthropods is a difficult task, and the development of sampling strategies to cope with these difficulties is decades overdue. There are long-known as well as recently developed sampling methods with potential applicability in soil zoology (e.g. binomial sampling: Binns & Nyrop 1992; Legg & Lockwood 1995; stratified random sampling: Abrahamsen 1969, Blackith 1974; randomization and bootstrapping: Pitt & Kreutzweiser 1998). We look enviously at related disciplines with similar problems, where sampling optimization is current practice and much effort is directed towards tailoring strategies for specific demands (Been & Schomaker 1996; Clay et al. 1997; Fons et al. 1997, among many others). We hope that this paper will stimulate soil zoologists to conduct research in this direction.

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