Efficiency of the Winkler method for extracting ants (Hymenoptera: Formicidae) from temperate-forest litter

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Abstract

Winkler litter extraction is a commonly used collecting technique for sampling arthropods from leaf litter in forested areas. We evaluated the efficiency of the Winkler method for extracting ants from temperate-forest litter for a typical extraction period of 72 h. The Winkler extraction was followed by hand-sorting sufficient to assure that no ants remained in the dry litter. We collected 7777 ants, from 31 species, of which 6511 were extracted with Winkler extractors during 72 h and an additional 1266 ants collected afterwards by hand sorting. Three days were sufficient to recover representatives of all ant species from the collected samples, with an average species extraction efficiency of 99%. The lack of significant differences in the number of observed and estimated species between the materials extracted after 72 h and the totals (including hand-sorted material) suggests that three days of extraction are sufficient to obtain an unbiased estimate of the local ant species richness. Winkler extraction also was efficient in terms of the number of individuals extracted with an average of 91% of the individuals, present in the collected samples, extracted during the 72 h period. As a result, an extraction time of 72 h allowed for valid and unbiased estimates of the structure and the composition of the local ant community. Thus, the 72 h Winkler litter extraction is a rapid and efficient way of collecting ants in temperate forest areas.

Key words: Ohio, sampling method, Winkler extraction time, species richness.

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Introduction

Winkler litter extraction is among the preferred ant-collecting techniques used by myrmecologists in forested areas, as it is efficient both in the number of individuals and the number of species collected. In closed canopy habitats with plentiful leaf litter, Winkler extraction has proven superior to other commonly applied collecting methods, such as pitfall trapping, Berlese extraction and baiting (OLSON 1991, FISHER 1998, 1999, DELABIE & al. 2000, KALIF & MOUTINHO 2000, MARTELLI & al. 2004, GROC & al. 2007, LOPES & VASCONCELOS 2008). In addition to capturing the dominant and the more common elements of the local ant communities, Winkler extraction also collects many smaller and cryptic ants inhabiting the leaf litter and the top soil layers, which are not readily sampled by other techniques (OLSON 1991, BESTELMEYER & al. 2000, FELLNER & al. 2009). Winkler extraction has been a preferred method not only for its effectiveness but also for its low methodological and technical requirements, and ease of use (KRELL & al. 2005). This technique also requires relatively inexpensive equipment, and does not require electricity often unavailable in remote areas. In addition, the data collected by Winkler extraction can be used to assess community structure and composition both qualitatively and quantitatively, as it provides information on species richness, relative abundance and frequency of occurrence (FISHER 1999).

The Winkler method is a passive technique designed to measure the abundance and composition of litter inhabiting ants. A discrete amount (usually 1 m^2) of leaf litter and top soil is scraped from the surface and sifted in the field through a wire mesh to exclude larger fragments, such as twigs and leaves, and to reduce the volume of the collected material. The material then is loaded in flat mesh bags suspended inside a framed outer cloth bag, which is tied shut. In response to the disturbance of the litter inside the mesh bags, and as a result of their generalized movements, the ants leave the substratum, and are collected and preserved in an alcohol-filled container placed at the bottom of the apparatus (FISHER 1998, BESTELMEYER & al. 2000).

Ideally, the time period that each Winkler extractor is allowed to process the collected samples should be sufficient to extract all ants in the collected litter. This is, however, rarely feasible as it can drastically increase the length, and therefore the costs of a study. This is especially true for rapid inventorying protocols in which time is of utmost importance. A compromise therefore should be made between the length of the chosen extraction time and the completeness of extraction. A variety of extraction times have been used by different researchers, ranging from a single day (DELABIE & al. 2000, LEPONCE & al. 2004) to over six days (BRÜHL & al. 1999), with 2 days (OLSON 1991, FISHER

1998, DELABIE & al. 1999, FISHER 1999, BESTELMEYER & al. 2000, KALIF & MOUTINHO 2000, PARR & CHOWN 2001, LESSARD & al. 2007, LOPES & VASCONCELOS 2008) and 3 days (WARD 1987, BELSHAW & BOLTON 1994, LONGINO & al. 2002, MARTELLI & al. 2004) being the most commonly used extraction times. The effect of longer extraction times of up to 7 weeks has also been explored (KRELL & al. 2005).

To our knowledge only a few published studies have explored the effect of extraction-time duration on the quality of the collected data. KRELL & al. (2005) found ants to be the invertebrate group most rapidly extracted from litter samples. Even so, complete extraction of all ants present in the collected samples was not achieved until day fifteen. Nevertheless, a large proportion of the individuals (over 92%), had left the substratum by 72 h, and the subsequent extraction time added only a small portion of individuals and almost no species, to the total (KRELL & al. 2005). Similarly high extraction efficiencies have been reported from Brazil after 48 h (DELABIE & al. 1999), California after 72 h (WARD 1987), and the Afrotropics after 72 h of extraction (BELSHAW & BOLTON 1994). Relatively short extraction times therefore seem warranted when focusing on ants. Finding optimal extraction times is critical for designing rapid, complete and cost-effective inventories. Estimation of Winkler-extraction efficiency is also important for validating the reliability of the extracted materials for representing the "true" structure and composition of the studied community.

Based on published extraction efficiencies we chose a commonly applied extraction time of 72 h to evaluate the efficiency of the Winkler method for extracting ground-dwelling ants from temperate-forest litter. We address two main questions. First, are there taxonomic biases in the resulting data caused by the relatively short extraction time? Second, is the chosen extraction time sufficient to provide an unbiased picture of the composition and the structure of the studied community?

Materials and methods

We sampled nine forest fragments during the summer months (late May – late August) of 2005 and 2006 in the Cleveland Metropolitan area of northeast Ohio, USA. We conducted the study in temperate mixed mesophytic forest types dominated by combinations of red and white oak (*Quercus rubra* L. and *Q. alba* L.), sugar and red maple (*Acer saccharum* MARSHALL and *A. rubrum* L.), American beech (*Fagus grandifolia* EHRH.), tulip tree (*Liriodendron tulipifera* L.), and hickories (*Carya* spp.) (for a complete list of sites, fragment size and vegetation characteristics, see IVANOV & KEIPER in press). The climate of the area is temperate continental with annual precipitation of 1011 mm in 2005, and 1032 mm in 2006 (NOAA 2009). The elevation of all sampling locations ranged between 180 and 370 m a.s.l.

We collected ten 1 m^2 samples of leaf litter and top soil from each of the nine forest fragments, for a total of 90 samples. Sampling locations were chosen at random from a large dataset of randomly pre-selected sampling points. Sampling was conducted at least a day after a heavy rain to assure reliable extraction as insects are less effectively extracted from wet litter (FISHER 1998).

We used a 1 m × 1 m plastic quadrat with movable joints to enclose each sampling point, collected the leaf litter inside by hand and scraped the top layer (2 - 3 cm) of loose soil using a trowel. We sifted the collected material through a sifter with a mesh opening of approximately 10 mm to exclude larger fragments, such as leaves, twigs and stones. We placed the sifted material in nylon sample bags and transported them to the laboratory. We loaded the sifted litter into flattened mesh bags, with an opening of 4 mm, over a plastic tray to capture any falling litter, which was then returned to the mesh bags. We used an empty container suspended at the bottom of each Winkler sack to collect any material falling out of the mesh bags during their placement in the extractor. This procedure reduces the amount of litter fragments in the final samples and thus facilitates subsequent sorting. At the onset of the extraction we replaced the empty container with one containing 95% ethanol. The Winkler extractors were left to operate at room temperature (~ 23ºC) for 72 h. At the end of the extraction time, we rinsed the content of each collecting container into a labeled vial with 95% ethanol. The dry litter inside each extractor then was emptied onto a plastic tray and hand sorted by two researchers until all remaining ants were collected (on average 40 - 45 min).

Winkler extractors, sample bags and mesh bags were supplied by Marizete Pereira dos Santos, Rua do Ciqueiral, 60 – Conquista, CEP: 45650-140, Ilhéus, Bahia, Brazil.

We sorted, counted and identified all individuals to species using available taxonomic keys (SMITH 1957, COOVERT 2005, FRANCOEUR 2007). We also consulted the ongoing work of A. Francoeur, who is currently revising the North American members of the genus *Myrmica*, to account for the presence of a yet undescribed species in our samples. We sent challenging specimens to experts. Vouchers are deposited at the Cleveland Museum of Natural History, Department of Invertebrate Zoology, and the remaining materials are in the first author's collection. Nomenclature follows BOLTON & al. (2007).

We calculated individual extraction efficiencies for each sample as the proportion of all individual ants extracted after 72 h (hereafter referred to as extracted samples), relative to the total number of individuals (extracted plus hand-sorted materials; hereafter referred to as totals) present in that sample without regard to species. Similarly, we calculated genus and species extraction efficiencies for each sample by taking the proportion of the genera (species) extracted after 72 h to the total number of genera (species) without regard to number of individuals. The extraction efficiencies reported here are given in percentages and represent averages across samples for each type of extraction efficiency (e.g., individual, genus and species), unless otherwise specified. We calculated species-specific extraction efficiencies for each species by taking the number of individual workers of a particular species, extracted after 72 h, expressed as a percentage of the total number of individuals of that species. The reported values are averages across all samples in which a particular species was found.

We used sample-based rarefaction to calculate and compare the observed species richness (rarefaction curves were scaled to number of occurrences) and species density (curves were scaled to number of accumulated samples), separately for the samples obtained after 72 h of extraction and for the totals (GOTELLI & COLWELL 2001). We created all sample-based rarefaction curves using the analytical method of COLWELL & al. (2004), implemented in Es-

Tab. 1: List of the collected ant species with corresponding abundances and species-specific extraction-efficiency values. Species are arranged in decreasing order of total abundances recorded. Abbreviations are as follows: n-Occ (number of occurences); n-Ind-T (total number of individuals); n-Ind-extr (number of individuals extracted, 72 h); n-Ind-hand (number of individuals hand sorted); aver-EE (average sample extraction efficiency in %); SD (standard deviation).

Species	n-Occ	n-Ind-T	n-Ind-extr	n-Ind-hand	aver-EE	SD
Aphaenogaster picea (W.M. WHEELER, 1908)	59	2252	1423	829	87.5	18.9
Lasius alienus (FOERSTER, 1850)	32	1132	1068	64	99.1	2.8
Myrmecina americana EMERY, 1895	67	1066	986	80	93.6	11.2
Myrmica punctiventris ROGER, 1863	70	938	827	111	94.7	10.6
Ponera pennsylvanica BUCKLEY, 1866	28	508	393	115	89.0	23.5
Tapinoma sessile (SAY, 1836)	3	442	424	18	71.8	25.4
Stenamma impar FOREL, 1901	45	351	350	1	99.9	0.5
Temnothorax curvispinosus (MAYR, 1866)	43	219	215	4	98.5	5.5
Myrmica sp.	8	185	177	8	94.4	10.3
Lasius umbratus (NYLANDER, 1846)	4	123	119	4	97.8	3.3
Stenamma schmitti W.M. WHEELER, 1903	21	92	91	1	95.2	21.8
Stenamma brevicorne (MAYR, 1886)	15	76	71	5	90.6	16.0
Amblyopone pallipes (HALDEMAN, 1844)	30	74	67	7	93.8	15.1
Lasius nearcticus W.M. WHEELER, 1906	4	61	61	0	100.0	0.0
Camponotus pennsylvanicus (DE GEER, 1773)	28	51	50	1	100.0	18.9
Brachymyrmex depilis EMERY, 1893	3	36	31	5	88.9	10.2
Myrmica emeryana FOREL	2	34	33	1	97.9	2.9
Formica subsericea SAY, 1836	6	33	33	0	100.0	0.0
Aphaenogaster rudis J. ENZMANN, 1947	4	22	21	1	93.8	12.5
Temnothorax longispinosus (ROGER, 1863)	6	21	20	1	98.3	4.1
Prenolepis impairs (SAY, 1836)	5	21	20	1	98.5	3.4
Myrmica pinetorum W.M. WHEELER, 1905	1	9	4	5	44.4	–
Myrmica semiparasitica FRANCOEUR, 2007	3	8	6	2	77.8	38.5
Formica glacialis W.M. WHEELER, 1908	3	7	6	1	66.7	57.7
Camponotus nearcticus EMERY, 1893	3	5	4	1	88.9	19.3
Formica neogagates EMERY, 1893	3	4	4	0	100.0	0.0
Camponotus subbarbatus EMERY, 1893	3	3	3	0	100.0	0.0
Crematogaster cerasi (FITCH, 1854)	1	1	1	0	100.0	–
Temnothorax schaumii (ROGER, 1863)	1	1	1	0	100.0	–
Protomognathus americanus (EMERY, 1895)	1	1	1	0	100.0	–
Camponotus chromaiodes BOLTON, 1995	1	1	1	0	100.0	–

timateS 8.0 (COLWELL 2006). To estimate the asymptotic species richness in the samples extracted after 72 h and the totals, we calculated the non-parametric Chao2 estimator using 100 randomizations of sample accumulation order (COLWELL 2006). We used the Chao-Jaccard abundance-based similarity index (CHAO & al. 2005), available in EstimateS 8.0, to assess the degree of compositional similarity between the extracted materials and the totals. Prior to the analysis we pooled all extracted samples into a single large sample, and we did the same with the totals. We followed this procedure because we were interested in the composition of the studied community as a whole and not in the compositional variation of individual samples. Lastly, we constructed and visually compared rank-abundance curves based on our extracted samples and the totals, in order to assess any differences in the estimated structure of the local community caused by the chosen extraction time. The obvious lack of independence between the two variables precluded formal statistical comparison of the shape of the rank-abundance distributions. We used Spearman Rank Correlation to assess the association between the two rank-abundance distributions (SPSS 16.0, SPSS Inc, Chicago, IL).

Results

We collected 7777 ant workers representing 31 species from 15 genera (Tab. 1). These numbers include 6511 individuals obtained from the collected litter after 72 h, in the Winkler extractors, and an additional 1266 individuals hand sorted from the remaining dry litter. Nearly all (89 of 90) of the collected samples contained at least one ant.

We calculated the average genus extraction efficiency at 99.8% ± 2.1 standard deviations (SD). All genera in nearly all samples (88 of 89) were extracted after 72 h, with single workers of two genera remaining in a single sample at the end of the extraction period. All species in 86 of the 89 samples were extracted, and only 3 samples contained species not extracted after 72 h. We found only
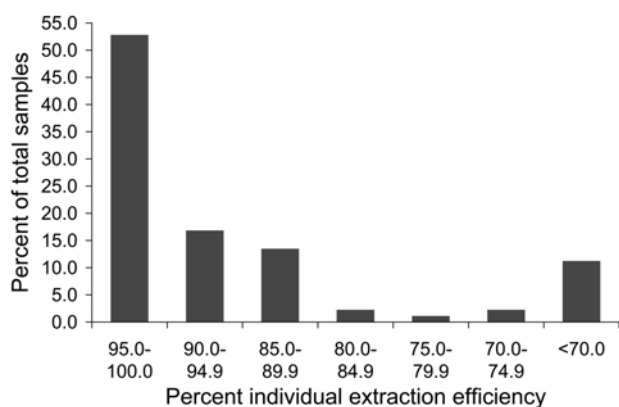
Fig. 1: Frequency distribution of the estimated extraction efficiencies based on individuals presented as proportion of total samples.

Tab. 2: Extraction time (ET), individual extraction efficiencies (IEE) and species extraction efficiencies (SEE) based on published studies.

Study	ET (h)	IEE (%)	SEE (%)
DELABIE & al. (1999)	48	85	95
WARD (1987)	72	85	98
BELSHAW & BOLTON (1994)	72	86	88
KRELL & al. (2005)	72	92	–
This study	72	91	99

single *Camponotus pennsylvanicus* (DE GEER, 1773) and single *Formica glacialis* W.M. WHEELER, 1908 workers remaining in two samples, and single individuals of two species (*Stenamma schmitti* W.M. WHEELER, 1903 and *Ponera pennsylvanica* BUCKLEY, 1866) remaining in a third sample. All of the species mentioned above, however, were present in the materials extracted from other samples. Thus, all 31 species found in the study area occurred in the samples extracted after 3 days, and no species that were absent from the extracted materials emerged during hand sorting. We calculated the average species extraction efficiency per sample at 99.5% ± 3.0 SD.

We calculated the average individual extraction efficiency at 90.7% ± 12.7 SD. All individuals present were extracted after 72 h in nearly a third (~ 33%) of the samples. Ten of all collected samples showed an individual extraction efficiency of less than 70%, with only two of these samples (2.2% of total) showing an extraction efficiency of < 50% (Fig. 1). All ten samples that showed lower individual extraction efficiency (< 70%) contained at least 50 *Aphaenogaster picea* (W.M. WHEELER, 1908) workers, with the highest total number of workers in a single sample being 478. We calculated the average species-specific extraction efficiency for *A. picea* in samples containing over 50 workers at approximately 62%, and that for samples containing < 50 workers at 96%. *Aphaenogaster picea* was the only species with high numbers of individuals remaining in the substratum after 3 days of extraction, and a large proportion (0.65) of the 1266 individual workers remaining in the dry litter after the 72 h extraction time belonged to this species.

Average species-specific extraction efficiencies ranged from a low of 44.4% (*Myrmica pinetorum* W.M. WHEELER, 1905, occurring in a single sample) to a high of 100% (9 species; Tab. 1). Twenty-seven of the 31 species collected were extracted with an average efficiency of over 85%, and only two with an efficiency of < 70% (Tab. 1).

The sample-based rarefaction curves (not included) scaled to number of samples, and to number of individuals, were nearly identical, with almost completely overlapping 95% confidence intervals. The species density and species richness estimates of the studied community therefore did not differ significantly when based on samples extracted after 72 h or on totals. Our estimates of the expected number of species when based on materials extracted after 72 h

(34.3 species) or on totals (35.9 species) also were nearly identical and did not differ significantly from each other as evidenced by the broad overlap of the 95% confidence intervals.

The compositional similarity between the extracted samples and the totals was estimated at 1.0 (i.e., 100%), showing no differences in the composition of the studied community when estimated based on samples extracted after 72 h or when estimated based on totals.

The rank-abundance curves based on extracted samples and on totals were very similar (Spearman's rho = 0.996), with shifts in species ranks occurring only in three positions along the distribution (Fig. 2). These shifts included species increase / decrease in rank of no more than two positions (i.e., forward or backward shift in rank by one or two places). Moreover, only one of these shifts concerned the abundance rank of the 15 most common species in our study, with the remaining two shifts occurring towards the tail end of the rank-abundance distribution. Our data thus provide no evidence of a substantive difference between the rank-abundances based on Winkler-extracted materials and on totals.

Discussion

Our data document a high efficiency of Winkler litter extraction, with a large proportion of all individuals and representatives of all species extracted from the collected samples after 72 h. The chosen time duration therefore is appropriate for extracting ants from temperate-forest litter. The 72 h duration did not cause taxonomic bias or distortion in the collected data, as all species found during hand sorting of the dry litter also were present in the extracted materials. Our estimates of both the observed and the expected species richness were not affected by the length of the extraction time, which thus is sufficient for a valid representation of the number of species in the study area. The complete overlap between the extracted samples and the totals in terms of species composition shows that the composition of the local community indeed was accurately represented by the materials extracted after 72 h. The high similarity in the rank-abundance curves between the extracted samples and the totals shows that the three-day period was sufficient to recover the abundance rank of the majority of the species, and therefore allows for an accurate estimation of the structure of the studied community.

The high average individual extraction efficiency shows that the majority of the individuals left the substratum after three days. Only about 11% of the 89 samples containing ants showed an individual extraction efficiency of < 70% (Fig. 1). All of these samples contained large numbers
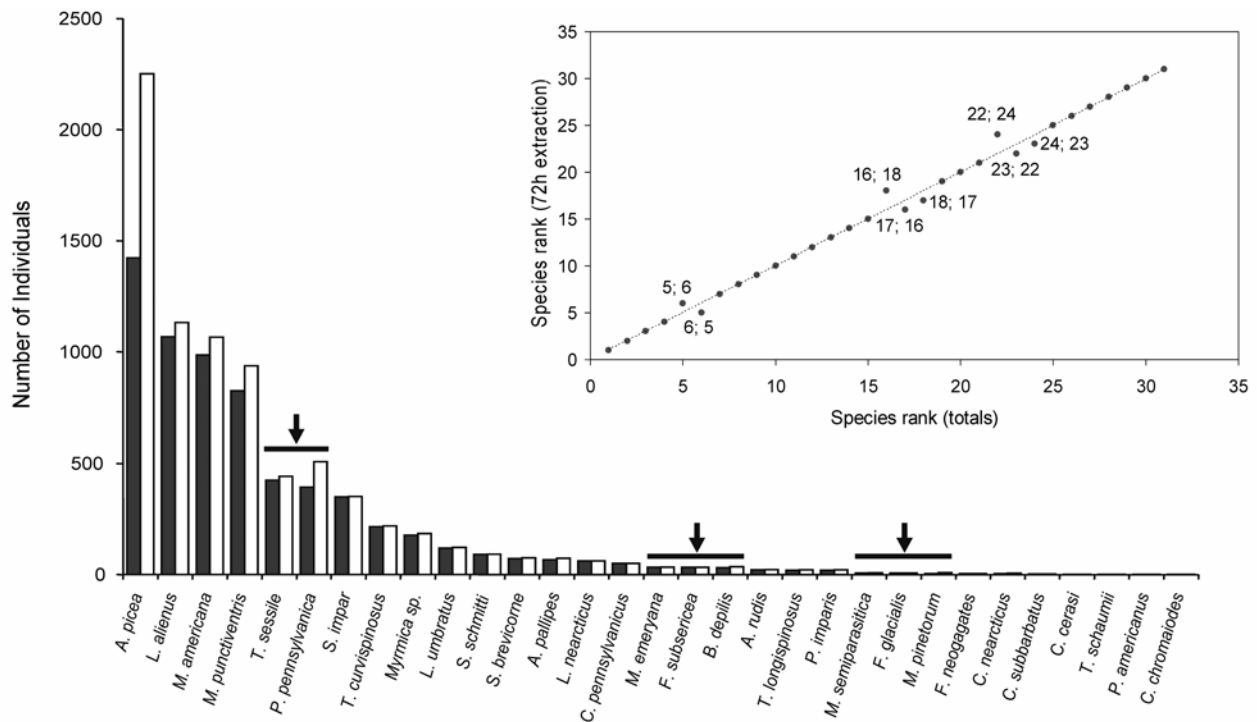
Fig. 2: Rank-abundance curves based on samples extracted after 72 h (dark bars) and on totals (extracted plus hand-sorted material; open bars). Species are arranged by their abundance in the extracted samples. Black arrows and horizontal lines pinpoint shifts in species rank. Inset graph shows shifts in species rank between Winkler-extracted materials and totals. Points lying on the dotted line show no shift in species rank, those above the line represent forward shift in rank after hand sorting, and those below represent drop in rank. The magnitude of the shift is represented by the distance from the line.

of *A. picea*, with many of the workers still present in the dry litter inside the mesh bags after 72 h. On the occasions when un-extracted *A. picea* workers were present, we found those settled down in the center of the litter. Thus, when large colony fragments of that species are present in the collected samples, the individual workers seem to cluster together in the mesh bags (K. Ivanov, unpubl.), and accordingly are extracted with a lower efficiency. This fact should be taken in consideration by researchers employing Winkler extraction in areas where this or similar, numerically dominant, species occur. The high numbers of un-extracted *A. picea* workers, however, did not lead to changes in abundance rank, due to the species' high numerical dominance in the collected samples. Breaking up nesting aggregations, such as the ones described above, and thus improving extraction efficiency, can be achieved through removing, remixing, and reloading the litter after a day of extraction. This procedure however, as pointed out by KRELL & al. (2005) may be hard to standardize and may lead to loss of mobile arthropods during the remixing and reloading process.

Four species showed an average species-specific extraction efficiency of < 80% (Tab. 1). These species, however, either were represented in too few samples or by too few individuals, or both, to judge if they indeed show a tendency towards extraction with lower efficiency, or if the observed results were caused by chance.

Our calculated individual and species extraction efficiencies are very similar to those reported from other studies (Tab. 2). The Winkler method shows striking consistency in extraction efficiency, especially considering the broad range of vegetation types and geographic areas represented in these studies.

Our hand sorting of the dry litter took approximately 1.5 person hours per sample to process. Thus, for all 89 samples containing ants, an additional 15 days (assuming 10 h work day) were required to remove all remaining ants from the collected samples. This increase in sample-processing time seems impractical considering that the materials collected after hand sorting did not lead to changes in the estimates of the community species richness, composition, and structure. A more practical, and time effective, approach, therefore, would be focusing one's efforts on collecting a larger number of litter samples from a study area, rather than spending time searching for remaining ants in the dry litter after extraction.

Based on our findings, we agree with KRELL & al. (2005) that shorter extraction times are efficient and practical when focusing on ants. Due to time constraints, longer extraction periods (up to 1 week), or the addition of hand sorting through the dry litter seem unwarranted, especially viewed in the light of only a small gain in the number of species and / or individuals collected after 72 h of extraction. In conclusion, the 72 h Winkler litter extraction is an efficient, rapid collecting technique that allows for valid representation of the structure and composition of the local ant communities inhabiting the leaf litter and the top soil layers in temperate forest areas. However, given the differences in litter moisture and composition, species tolerances, and ambient drying temperatures the efficiency of the Winkler-extraction duration will almost certainly vary across studies and / or geographic areas. We recommend that researchers use approaches similar to the ones presented in this paper to determine optimal extraction times and establish extraction periods most appropriate to their studies and research areas.

Zusammenfassung

Die Winkler-Extraktion ist eine häufig angewandte Methode zur Beprobung von Arthropoden der Laubstreu bewaldeter Gebiete. Wir haben die Effizienz der Winkler-Methode für das Extrahieren von Ameisen aus der Laubstreu gemäßigter Wälder bei der häufig gewählten Extraktionsdauer von 72 Stunden evaluiert. Anschließend an die Winkler-Extraktion wurden händisch jegliche noch in der trockenen Streu verbliebenen Ameisen aussortiert. Wir sammelten 7777 Ameisen von 31 Arten, von denen 6511 mit den Winkler-Extraktoren während der 72 Stunden und 1266 durch händisches Sortieren erfasst wurden. Drei Tage erwiesen sich als ausreichend, um Individuen aller Ameisenarten in der beprobten Laubstreu zu erfassen, mit einer durchschnittlichen Extraktionseffizienz von Arten von 99 %. Das Fehlen von signifikanten Unterschieden bei der beobachteten und der geschätzten Zahl von Arten zwischen den nach 72 Stunden extrahierten und den insgesamt erfassten Ameisen (inklusive jener mit der Hand aussortierten) legt nahe, dass drei Tage Extraktion für eine unverzerrte Abschätzung des lokalen Ameisenarteninventars ausreichen. Die Winkler-Extraktion war auch hinsichtlich der Zahl der extrahierten Individuen effizient: Durchschnittlich 91 % der in den Proben vorhandenen Individuen wurden während der 72 Stunden extrahiert. Insgesamt hat also eine Extraktionszeit von 72 Stunden eine stichhaltige und unverzerrte Abschätzung der Struktur und Zusammensetzung der lokalen Ameisengemeinschaft ermöglicht. Die Winkler-Extraktion mit einer Extraktionsdauer von 72 Stunden ist eine rasche und effiziente Methode zum Besammeln der Ameisen temperater Wälder.

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