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Shuffling Leaf Litter Samples Produces More Accurate and Precise Snapshots of Terrestrial Arthropod Community Composition

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ABSTRACT Understanding the accuracy of sampling techniques is critical to accurate interpretation of local and global ecological patterns. Over the past 20 yr, Winkler leaf litter extractors have become one of the most common techniques used to collect terrestrial arthropods. Although the original description of this technique recommends the use of disturbance during extraction to increase extraction efficiency, most published studies do not follow this recommendation. Here we test experimentally how disturbing, or "shuffling" leaf litter during extraction affects collection efficiency for four groups of arthropods: ants, micro-Hymenoptera, beetles, and spiders. Our results show that extraction efficiency for abundance and richness of ants is increased with disturbance. Disturbance also improves extraction efficiency for beetle abundance, but does not affect micro-Hymenoptera and spider abundance or richness. Significantly, our results also demonstrate that shuffling litter can greatly reduce the variability of extraction efficiency between different sites. Because of this greater efficiency, we recommend use of the shuffling technique, when sampling leaf litter via Winkler extractors to maximize the collection of ants and beetles, especially for studies that compare abundance and richness across multiple sites.

RÉSUMÉ La compréhension de l'efficacité des méthodes d'échantillonnage est cruciale pour une interprétation exacte des patrons écologiques locaux et globaux. Au cours des 20 dernières années, l'extracteur de type Winkler est devenu l'une des techniques les plus communément utilisées pour collecter les arthropodes présents dans la litière du sol. Alors que la description originale de cette technique recommande la perturbation du substrat pendant la phase de séchage pour augmenter l'efficacité d'extraction des arthropodes, la plupart des études réalisées ne suivent pas cette recommandation. Ici, nous testons expérimentalement comment la perturbation de la litière pendant l'extraction affecte l'efficacité de cette technique sur quatre groupes d'arthropodes : les fourmis, les micro-Hyménoptères, les Coléoptères, et les araignées. Nos résultats démontrent que la perturbation du substrat augmente l'efficacité d'extraction sur l'abondance et la richesse des fourmis et sur l'abondance des coléoptères. L'abondance et la richesse des micro-Hyménoptères et des araignées ne sont pas affectés. Nos résultats démontrent également que la variabilité dans l'abondance des fourmis observée entre les différents sites est fortement réduite. De par son efficacité accrue, nous recommandons l'utilisation de la technique utilisant la perturbation régulière du substrat, ici appelée ≪ de secouage ≫, pendant l'utilisation d'extracteurs de type Winkler pour maximiser la capture de fourmis et de Coléoptères, et ce spécialement pour les études portant sur des comparaisons d'abondance ou de richesse entre plusieurs sites.

KEY WORDS Winkler extractor, Formicidae, Coleoptera, Arachnida, Micro-Hymenoptera

Knowing the inherent biases of any data collection method is essential for accurate inferences about ecology, and as sampling is a heuristic for measuring species composition and abundance, knowledge of the efficiency and biases of sampling methods is vital. Many statistical techniques take sampling efficiency and, to a lesser extent, biases into account when calculating species diversity (e.g., EstimateS, Shannon or Simpson's diversity index), but unfortunately less attention is paid to quantifying the efficiency and variability of the methods themselves. This paper focuses on the extraction efficiency of a method for sampling ground dwelling arthropods, and suggests a modification in sampling technique that both increases sampling efficiency and reduces variability.

Leaf litter and soil arthropods represent an important focus in many ecological studies because they are easy to sample, ecologically relevant (Engelmann 1961), and important in ecosystem functioning (De

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Deyn et al. 2003). Although multiple techniques can be used to collect soil arthropods, Winkler extractors are among the most widely employed methods for sampling arthropods of the leaf litter and soil (Krell et al. 2005) and have proven to be one of the most successful sampling techniques for collecting soil arthropods over a diverse range of habitats (Besuchet et al. 1987, Olson 1991, Fisher 1999, Delabie et al. 2000, Martelli et al. 2004, Groc et al. 2007, Lopes and Vasconcelos 2008, Ivanov and Keiper 2009; but see the cases of xeric habitats: Parr and Chown 2001, Delsinne et al. 2008).

Part of the appeal of Winkler extractors is that they can be used at remote field sites, and unlike Berlese or Tullgren extractors, are lightweight, easily collapsed, and require no electricity. In the Winkler method, sifted leaf litter hangs suspended in a mesh bag enclosed in a cloth sack (Bestelmeyer et al. 2000). As the substrate dries, mobile arthropods in the sample fall into a hanging ethanol-filled collection cup. A critical aspect of Winkler extractor efficiency is the duration of time that the litter is suspended. In studies focusing on ant diversity and abundance, extraction time periods vary, with some authors preferring a 48-h interval (Olson 1991; Fisher 1998, 1999; Delabie et al. 1999; Parr and Chown 2001; Lopes and Vasconcelos 2008; Donoso and Ramón 2009; Ryder Wilkie et al. 2010) and others a 72-h interval (Ward 1987, Nadkarni and Longino 1990, Belshaw and Bolton 1994, Martelli et al. 2004, Krell et al. 2005, Ivanov and Keiper 2009). Thanks to several recent studies, users of this method are aware that these limited time periods do not ensure extraction of all individuals, or even all species from litter samples. In a study comparing Winkler extractor efficiency through time to extract all resident arthropods, Krell et al. (2005) demonstrated that no <15 d were necessary to extract all ant individuals. Clearly, the extraction method is not exhaustive in the time frame commonly used, but the efficiency of the method and whether it can be improved have not yet been addressed. In the first description of the Winkler method, Besuchet and collaborators (1987) argued for the importance of shuffling samples to maximize extraction of arthropods from sifted leaf litter. Most studies using Winkler extractor technique no longer use this practice. To our knowledge, no empirical evaluation has been conducted to test the importance of shuffling on the efficiency of arthropod extraction.

Ants are among the best-studied arthropods, and are common focal taxa in diverse fields of study including biodiversity studies, community ecology, conservation, or biological invasions (Lach et al. 2010). For this reason, the precision with which ant richness and abundance is measured becomes crucial when comparing different sites. Recently, several authors have used ants to address large-scale biogeographical questions (Dunn et al. 2007, 2009; Groc et al. 2010; Weiser et al. 2010), and further global studies are expected to take advantage of large multi-author data sets. In this context, the use of standardized sampling methodologies and incorporating measures of sampling efficiency and variability are crucial for making comparisons across space, time, and habitats. For these reasons, we place specific emphasis on ants. In addition, we also consider three other groups of arthropods commonly found in leaf litter: (non ant) micro-Hymenoptera, beetles (Coleoptera), and spiders (Arachnida).

In this study we compare the efficiency and variability of the Winkler technique as it is currently applied (hereafter referred to as "traditional") and with the inclusion of repeated shuffling during the drying process (hereafter referred to as "shuffle") (Besuchet et al. 1987). We specifically ask if the proportion of individuals extracted relative to the total number present in any sample is higher when the shuffle technique is used than when using the traditional method. Similarly, we ask if the same pattern is observed with species diversity for each group of arthropods considered. Finally, we assess the variability in efficiency observed among plots within a site with the shuffle method relative to the traditional method. We specifically aim to answer the questions: does the traditional method introduce variability where it does not actually exist, and can using the shuffle technique reduce this variability? This final aspect is especially important in studies that compare species abundance across multiple sites to characterize the importance of a given factor (e.g., presence or absence of invasive species).

Materials and Methods

Leaf litter was collected at four different forested sites in North and South Carolina between July and September 2010 (Table 1). At each site, 7–10.5 liters sifted litter was sampled, in total. All the sifted litter collected at a single site was thoroughly mixed before being divided into 1.75-liter allotments and hung, uncompressed, in individual extractor bags (mini-Winklers). All samples were suspended in a row within 6 hr of collection under identical ambient conditions, with treatments applied to alternating extractor bags. The two treatments, "traditional" and "shuffle" differed in the absence or presence of disturbance. Half of all samples were subjected to standard Winkler protocol and left to dry undisturbed. The remaining samples were "shuffled" 12, 36, and 60 h after the initial placement of the mini-Winklers by removing the sifted litter from the extractor bag, pouring the contents into a large bucket, and mixing the litter before replacing it in the mesh bag and returning it to the mini-Winkler sack. The entire "shuffle" operation for each mini-Winkler took ≈5 min. The extracted arthropods that had fallen into a cup filled with 4 cm of 95% alcohol were collected at 12, 36, 60, and 84 h after installation for both treatments. Collection of the sample cup preceded the "shuffle", and fresh alcohol cups were installed after the litter was replaced. After 84 h, the extractors were taken down and the leaf litter from each was sorted manually to collect any remaining arthropods in the drying litter.

Richness and abundance of ants extracted was recorded for each individual extractor during 0–12 h,

Table 1. Locations, dates, sampling effort, and efficiency for each site as a result of the traditional treatment (T) or the shuffle treatment (S)

Site name	Coordinates	Date	No. of mini-Winklers	Ants	Beetles	Spiders	Micro-Hymenoptera
Raven Rock State Park, NC (T)	35.482N, −78.917W	18/07/10	2	87.1	77.5	68.8	100
Raven Rock State Park, NC (S)	35.482N, −78.917W	18/07/10	2	96.8	83.3	100	92.3
Eno River State Park, NC (T)	36.044N, −79.002W	25/07/10	3	91.1	69.4	74.2	85.9
Eno River State Park, NC (S)	36.044N, −79.002W	25/07/10	3	99.1	79.4	88.0	100
Centennial Forest, Raleigh, NC (T)	35.762N, −78.685 W	6/09/10	2	99.8	100	76.2	100
Centennial Forest, Raleigh, NC (S)	35.762N, −78.685 W	6/09/10	2	99.8	100	96.4	100
Myrtle Beach State Park, SC (T)	33.655N, −78.929W	28/09/10	2	81.2	68.9	100	100
Myrtle Beach State Park, SC (S)	33.655N, −78.929W	28/09/10	2	96.5	98.4	100	100

Percentage of total individuals extracted after 84 h for each treatment for each site are presented for ants, beetles, spiders, and micro-Hymenoptera.

12–36 h, 36–60 h, and 60–84 h as well as for the ants remaining in the leaf litter at 84 h. In addition to ants, the species richness of micro-Hymenoptera was recorded, as was the abundance of beetles and spiders. Vouchers were deposited in Rob Dunn laboratory's collection and in the NC State University Insect Museum.

Variability of the Collected Abundance Between Treatments. Variability in abundance of species collected among samples within sites was compared for the shuffle and traditional treatments for ants. We considered the proportion of the abundance of ants collected after 84 h for each replicate minus the average proportion found within its specific treatment. Data were log transformed to obtain normal distribution and a *t*-test was used to compare the variability in collected abundance between treatments.

Statistical Analysis. Data were ArcSine square-root transformed to normalize values expressed as percentage. A matched pairs *t*-tests for the different periods tested the effect of the two treatments (shuffle and traditional) in the extraction efficiency of the abundance of ants, beetles, spiders, and micro-Hymenoptera, and separately, species richness for ants and micro-Hymenoptera across the different time periods (12, 36, 60, and 84 h). Pairs of mini-Winkler bags were established on the base of their site origin. If a specific group of arthropod studied was not collected during the extraction process, then we excluded the specific pair of data from the final analysis. Analyses were performed with the statistical software JMP (SAS Institute 2009).

Results

In total, 7,505 ants, representing 34 species were collected (Supp. Table S1 [online only]). The number of ants collected from each 1.75-liter allotment of sifted litter, ranged from 99 to 995 individuals, and from 9 to 14 species. In total, 114 micro-Hymenoptera individuals were collected, representing 46 species (Supp. Table 2 [online only]). In total, 191 individual spiders and 579 individual beetles also were collected. A number of regionally noteworthy species were collected, including the first record of the genus *Discothyrea* (*D. testacea*) in South Carolina.

Ant Abundance. The shuffle technique proved approximately 10% more efficient than the traditional method in extracting ants from sifted leaf litter, with a capture efficiency of 97.7% (range: 93.9–99.6%; 95% CL = 94–101.4%) of individual ants over an 84-h extraction (Fig. 1). Over the same period, the traditional technique averaged 87.1% (range: 77.8–96.4%; 95% CL = 83.4–90.8%) of individual ants. No statistically significant differences were observed between techniques during the first 36 h (encompassing the effects of one shuffle). However, differences emerged after 60 h (after two shuffles; Df = 12; $t_{ratio} = 3.97$; $P = 0.0019$) as well as for 84 h (after three shuffles; Df = 12; $t_{ratio} = 7.88$; $P < 0.0001$). Block effects between the different sites were not significant ($P = 0.35$).

Ant Species Richness. We observed no effects of shuffle treatments on the proportion of species sampled (Fig. 2). After 84 h no differences between the traditional and shuffle treatment were observed (Df = 12; $t_{ratio} = 2.11$; $P = 0.057$). Overall, shuffle treatment extractions yielded 98.9% of species present (95% $CL_{shuffle}$ = 94.9–103%), whereas the traditional treatment yielded 95.6% of species present (95% $CL_{traditional}$ = 91.6–99.6%).

Variability of the Abundance Collected Between Treatments. The shuffle treatment produced lower variance than the traditional treatment ($t_{1-16} = -2.79$, $P = 0.013$) in ant abundance among sites. The variability observed in the shuffle treatment (mean = 1.56%; min = 0.2; max = 4.3) was almost four times lower than the variability observed in the traditional treatment (mean = 5.84%; min = 0.7; max = 12.2), indicating that the traditional protocol for using Winkler extractors can artificially introduce variability into measures across sites.

Spider and Beetle Abundance. The percentage of individual spiders collected was not significantly different between treatments up to 36 h, however, at 84 h, the shuffle treatment was slightly more efficient than the traditional treatment, though this result is nonsignificant statistically ($t_{1-15} = 2.06$, $P = 0.079$; 95% $CL_{shuffle}$ = 81.3–108%; 95% $CL_{traditional}$ = 63.3–90%) (Fig. 1). The percentage of individual beetles collected was not significantly different between treatments for the first three periods considered. After 84 h, the percentage of beetle abundance collected was

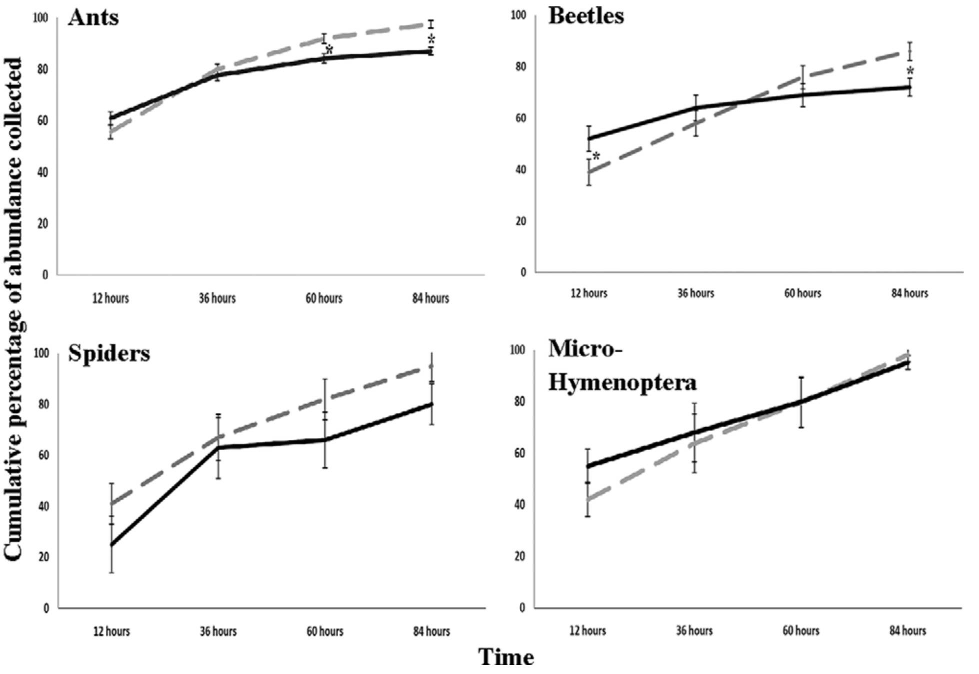

Fig. 1. Cumulative abundance of individuals collected through time with the traditional Winkler technique (black line) and the shuffled technique (broken gray line) for the four groups of arthropods studied.

superior in the shuffle treatment than in the traditional treatment (t_{1-14} = 2.74, P = 0.033; 95% $CL_{shuffle}$ = 81.9–96.1%; 95% $CL_{traditional}$ = 70.7–84.9%) (Fig. 1).

Micro-Hymenoptera Richness and Abundance. After 84 h both techniques extracted a high percentage of all individuals present, with 98.3% and 95.0% collected by the shuffle and traditional techniques, respectively (95% $CL_{shuffle}$ = 92.1–104.5%; 95% $CL_{traditional}$ = 89.9–101.5%). Similarly, almost all the species present in our initial collection (after 84 h) were extracted, respectively 98 and 97.8% for the shuffle and the traditional technique (95% $CL_{shuffle}$ = 92.6–103%; 95% $CL_{traditional}$ = 92.8–103.2%). No difference in the extraction efficiency of the micro-Hymenoptera abundance or richness has been observed between treatments for the different period considered (Fig. 1).

Discussion

Efficient sampling methods are crucial to conducting successful ecological studies. Leaf litter arthropods are abundant and diverse, and their use in ecology relies on appropriate collecting methods that accurately represent this abundance and diversity. In our study we demonstrate that a simple modification of the Winkler extraction method can significantly increase the reliability of results collected for two of the most abundant and ecologically important groups of terrestrial arthropods, the ants and beetles (Fig. 3). The

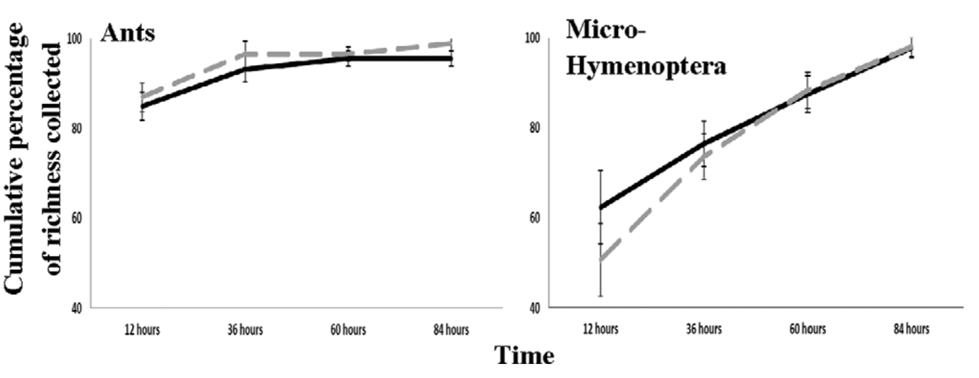

Fig. 2. Cumulative richness of species collected through time with the traditional Winkler technique (black line) and the shuffled technique (broken gray line) for ants and micro-Hymenoptera.

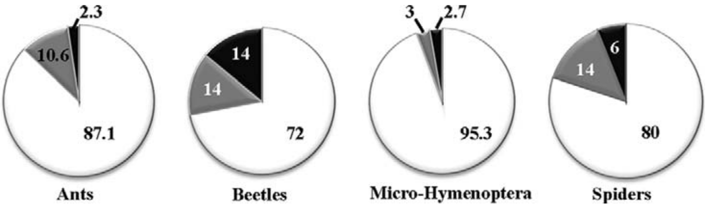

Fig. 3. Percentage of abundance extracted after 84 h for the different arthropod groups tested. The percentage of abundance collected by the traditional and shuffle treatments are represented in white, the percentage of abundance collected only by the shuffle treatment is in gray, the percentage of abundance not collected by any of the two methods appear in black.

shuffle technique offers three important advantages over the traditional method: 1) abundance of individual ants and beetles extracted with the shuffle technique is higher, 2) species richness extracted tends to be higher when the shuffle technique is used, and 3) variability in abundances between different collection sites is reduced by a factor of four when the shuffle technique is used instead of the traditional method.

The results presented here concur with previous findings about the relatively high efficiency of the traditional Winkler method in both temperate and tropical ecosystems, and suggest that these results also can be applied to many other ecosystems. Our extraction of 87.1% of ant abundance with the traditional technique is comparable to previous studies in other temperate ecosystems—85% of ants in California (Ward 1987), 84% of ants in Ohio (Ivanov et al. 2010), and 92% of ants in England (Krell et al. 2005) were extracted after 3 d. Similarly, in tropical ecosystems, 86% of individuals were extracted after 3 d in Ghana (Belshaw and Bolton 1994), and 85% in Brazil after only 2 d (Delabie et al. 1999). Regarding species richness, our result also echoed previous findings in temperate environments. Using the traditional technique, 96% of the species initially present on our samples were extracted after 3 d, quite similar to 98% in California (Ward 1987), and 99% in Ohio (Ivanov et al. 2010).

Besides, it is interesting to note that despite the efficiency of the traditional method in temperate regions, species extraction is considerably less efficient in tropical ecosystems where ant communities are more diverse and where samples are often wetter, as shown by the results in Ghana, with only 88% efficiency after 3 d (Belshaw and Bolton 1994), but interestingly, not for Brazil with 95% efficiency after only 2 d (Delabie et al. 1999). These results suggest that our results and the shuffle technique could be extrapolated to other regions. A test of this method in tropical systems would be useful; nevertheless, in the absence of such tests, all available data point toward improved efficiency with the shuffle technique in tropical areas, where disturbance should encourage greater movement of individuals and species out of the sampled substrate and into collection cups.

As more ecological studies increasingly amass large data sets to compare different sites across time and space, it is crucial that data reflect the actual community sampled as accurately as possible. This is especially vital in a context where these data are being used to measure the effects of invasive species (Morrison 2002, Guénard and Dunn 2010); to quantify disturbance (Majer et al. 1997, Brühl et al. 2003, Ivanov and Keiper 2010); or to understand patterns of diversity across microhabitats (Longino and Nadkarni 1990), ecosystems (Chung and Mohamed 1996, Pacheco et al. 2009) or regions (Groc et al. 2010, Weiser et al. 2010); and ultimately where these data will be used to predict future outcomes.

In addressing the question "Does the standard method introduce variability where it does not actually exist?", our results suggest that the stochasticity observed among the individual replicates of the traditional treatment was relatively high. As such, it is of great interest to reduce this variability because of a sampling effect to accurately measure biological response. The shuffle technique provides an interesting solution to this problem by sharply reducing variability between replicates. More generally, at a community level, the shuffle technique increases the accuracy of a Winkler sample's "snapshot" of community composition by closing the gap between the number of species or individuals observed in a sample and the total actually present. The extraction efficiency achieved for ants in this study, 98% extraction efficiency of abundance and 99% of diversity, gives great confidence that the technique accurately reflects the community.

The appeal of Winkler extractors stems from their efficiency in extracting arthropods from the leaf litter, convenience in the field, and wide adoption, inviting cross-study comparisons (Ward 2000). The downside of this technique, however, is the fact that exhaustive extraction of all arthropods sampled from sifter litter requires an extraordinarily long extraction time (Krell et al. 2005). Intervals exceeding several days are not usually feasible given the constraints of field work, therefore, we focused on improving the extraction efficiency over the time periods most often cited in the literature: 48–72 h (Ward 1987; Olson 1991; Belshaw and Bolton 1994; Fisher 1998, 1999; Delabie et al. 1999; Parr and Chown 2001; Martelli et al. 2004; Krell et al. 2005; Lopes and Vasconcelos 2008; Donoso and Ramón 2009; Ivanov and Keiper 2009; Ryder Wilkie et al. 2010). Here too, the shuffle method provides a better alternative between time and efficiency than what is observed with the traditional Winkler method for ants and beetles. However, for studies focusing exclusively on micro-Hymenoptera or spiders we do not necessarily recommend the application of a shuffling treatment, although it would not decrease the efficiency of the process.

Our results from micro-Hymenoptera did not demonstrate any difference between techniques; both show high efficiency and collect most individuals even after 3 d. Our results contrast with those found by Krell et al. (2005) where non-ant hymenoptera were among one of the slowest groups to be extracted. We collected 98.3% and 95.3%, respectively, of the micro-Hymenoptera (non-ants) with the shuffle and the traditional technique after 3 d, whereas only around 25% of the total micro-Hymenoptera was collected by Krell et al. (2005) after the same period. This difference partly may be explained by the fact that our results reflect mostly the micro-Hymenoptera present as adults in our initial sampling, whereas the much longer Krell study allowed the emergence of new adults during the 7-wk extraction period.

Efficiency of extraction and time needed to collect arthropods also depend on the initial conditions of the leaf litter. One of our sites (Centennial forest) was collected during an intense period of drought and the leaf litter collected initially was much drier than other samples. Despite the fact that arthropods were still abundant, they tended to exit the leaf litter more quickly than in other samples. This observation, though anecdotal, suggest that desiccation could be an important factor forcing arthropods out of the leaf litter, although Besuchet et al. (1987) suggested that desiccation is only secondary to disturbance as an extraction factor.

In conclusion, we suggest that the shuffle technique be adopted in studies seeking to maximize the extraction of ants and beetles during short periods of time. In the case of ants, not only is abundance increased with the shuffle technique, but variability in abundance observed between different replicates also is strongly reduced. The lowered variability has important consequences for studies that compare different sites at local scales, or studies that seek to understand difference or similarities between communities around the world (Dunn et al. 2007, 2009; Weiser et al. 2010). Finally it is important to keep in mind that Winkler techniques should be used in association with other sampling methods as they offer important complementary information about species composition (Agosti and Alonso 2000, Gotelli et al. 2011).

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References Cited

Agosti, D., and L. E. Alonso. 2000. The ALL protocol: a standard protocol for the collection of ground-dwelling ants, pp: 204–206. *In* D. Agosti, J. D. Majer, L. E. Alonso, and T. R. Schultz (eds.), Ants: standard methods for measuring and monitoring biodiversity. Smithsonian Institution Press, Washington, DC.

Belshaw, R., and B. Bolton. 1994. A survey of the leaf litter ant fauna in Ghana, West Africa (Hymenoptera: Formicidae). J. Hymenopt. Res. 3: 5–16.

Bestelmeyer, B. T., D. Agosti, L. E. Alonso, C.R.F. Brandao, W. L. Brown, Jr., J.H.C. Delabie, and R. Silvestre. 2000. Field techniques for the study of ground-dwelling ants: an overview, description, and evaluation, pp. 122–144. *In* D. Agosti, J. D. Majer, L. E. Alonso, and T. R. Schultz (eds.), Ants: standard methods for measuring and monitoring biodiversity. Smithsonian Institution Press, Washington, DC.

Besuchet, C., D. H. Burckhardt, and I. Lobl. 1987. The "Winkler/Moczarski" eclector as an efficient extractor for fungus and litter Coleoptera. Coleopt. Bull. 41: 392–394.

Brühl, C. A., T. Eltz, and K. E. Linsenmair. 2003. Size does matter – effects of tropical rainforest fragmentation on the leaf litter ant community in Sabah, Malaysia. Biodivers. Conserv. 12: 1371–1389.

Chung, A.Y.C., and M. Mohamed. 1996. A comparative study of the ant fauna in primary and secondary forest in Sabah, Malaysia, pp. 357–366. *In* D. S. Edwards, W. E. Booth, and S. C. Choy (eds.), Tropical rainforest research. Current Issues. Kluwer Academic Publishers, Dodrecht, The Netherlands.

De Deyn, G. B., C. E. Raaijmakers, H. R. Zoomer, M. P. Berg, P. C. de Ruiter, H. A. Verhoef, T. M. Bezemer, and W. H. van der Putten. 2003. Soil invertebrate fauna enhances grassland succession and diversity. Nature 422: 711–713.

Delabie, J.H.C., I. C. do Nascimento, and C.S.F. dos Mariano. 1999. Importance de l'agriculture cacaoyère pour le maintien de la biodiversité: étude comparée de la myrmécofaune de différents milieux du sud-est de Bahia, Brésil (Hymenoptera : Formicidae), pp. 23–30. *In* Proceedings, the 12th International Cocoa Research Conference, 18–22 November 1996, Bahia, Brazil. Cocoa Producers Alliance, Lagos, Nigeria.

Delabie, J.H.C., B. L. Fisher, J. D. Majer, and I. W. Wright. 2000. Sampling effort and choice of methods, pp. 145–154. *In* D. Agosti, J. D. Majer, L. E. Alonso, and T. R. Schultz (eds.), Ants: standard methods for measuring and monitoring biodiversity. Smithsonian Institution Press, Washington, DC.

Delsinne, T., M. Leponce, L. Theunis, Y. Braet, and Y. Roisin. 2008. Rainfall influences ant sampling in dry forests. Biotropica 40: 590–596.

Donoso, D., and G. Ramón. 2009. Composition of a high diversity leaf litter ant community (Hymenoptera: Formicidae) from an Ecuadorian pre-montane rainforest. Ann. Soc. Entomol. Fr. 45: 487–499.

Dunn, R. R., N. J. Sanders, M. C. Fitzpatrick, E. Laurent, J. P. Lessard, D. Agosti, A. Andersen, C. Brühl, X. Cerdà, A. Ellison, et al. 2007. Global ant biodiversity and biogeography: a new database and its possibilities. Myrmecol. News 10: 77–83.

Dunn, R. R., D. Agosti, A. Andersen, C. Brühl, X. Cerdà, A. Ellison, B. Fisher, M. C. Fitzpatrick, H. Gibb, N. Gotelli, et al. 2009. Climatic drivers of hemispheric Asymmetry in global patterns of ant species richness. Ecol. Lett. 12: 324–333.

Engelmann, M. D. 1961. The role of soil arthropods in the energetics of an old field community. Ecol. Monogr. 31: 221–238.

Fisher, B. L. 1998. Ant diversity patterns along an elevational gradient in the Reserve Speciale d'Anjanaharibe-Sud and on the western Masoala Peninsula, Madagascar. Fieldiana Zool. 90: 39–67.

Fisher, B. L. 1999. Improving inventory efficiency: a case study of leaf-litter ant diversity in Madagascar. Ecol. Appl. 9: 714–731.

Gotelli, N. J., A. M. Ellison, R. R. Dunn, and N. J. Sanders. 2011. Counting ants (Hymenoptera: Formicidae): biodiversity sampling and statistical analysis for myrmecologists. Myrmecol. News 15: 13–19.

Groc, S., J.H.C. Delabie, R. Cereghino, J. Orivel, F. Jaladeau, J. Grangier, C.S.F. Mariano, and A. Dejean. 2007. Ant species diversity in the 'Grands Causses' (Aveyron, France): in search of sampling methods adapted to temperate climates. C. R. Biologies 330: 913–922.

Groc, S., J.H.C. Delabie, J. T. Longino, J. Orivel, J. D. Majer, H. L. Vasconcelos, and A. Dejean. 2010. A new method based on taxonomic sufficiency to simplify studies on Neotropical ant assemblages. Biol. Conserv. 143: 2832–2839.

Guénard, B., and R. R. Dunn. 2010. A new (old), invasive ant in the hardwood forests of eastern North America and its potentially widespread impacts. PLoS ONE 5: e11614. doi:10.1371/journal.pone.0011614.

Ivanov, K., and J. Keiper. 2009. Effectiveness and biases of Winkler litter extraction and pitfall trapping for collecting ground-dwelling ants in northern temperate forests. Environ. Entomol. 38: 1724–1736.

Ivanov, K., and J. Keiper. 2010. Ant (Hymenoptera: Formicidae) diversity and community composition along sharp urban forest edges. Biodivers. Conserv. doi:10.1007/s10531-010-9937-3.

Ivanov, K., J. Milligan, and J. Keiper. 2010. Efficiency of the Winkler method for extracting ants (Hymenoptera: Formicidae), from temperate-forest litter. Myrmecol. News 13: 73–79.

Krell, F. T., A.Y.C. Chung, E. DeBoise, P. Eggleton, A. Giusti, K. Inward, and S. Krell-Westerwalbesloh. 2005. Quantitative extraction of macro-invertebrates from temperate and tropical leaf litter and soil: efficiency and time-dependent taxonomic biases of the Winkler extraction. Pedobiologia 49: 175–186.

Lach, L., C. L. Parr, and K. L. Abbott. 2010. Ant ecology. Oxford University Press, New York.

Longino, J. T., and N. M. Nadkarni. 1990. A comparison of ground and canopy leaf litter ants (Hymenoptera: Formicidae) in a neotropical montane forest. Psyche 97: 81–93.

Lopes, C. T., and H. L. Vasconcelos. 2008. Evaluation of three methods for sampling ground-dwelling ants in the Brazilian Cerrado. Neotrop. Entomol. 37: 399–405.

Majer, J. D., J.H.C. Delabie, and N. L. McKenzie. 1997. Ant litter fauna of forest, forest edges and adjacent grassland in the Atlantic rain forest region of Bahia, Brazil. Insectes Soc. 44: 255–266.

Martelli, M. G., M. M. Ward, and A. M. Fraser. 2004. Ant diversity sampling on the southern Cumberland Plateau: a comparison of litter sifting and pitfall trapping. Southeast. Nat. 3: 113–126.

Morrison, L. W. 2002. Long-term impacts of an arthropod-community invasion by the imported fire ant, *Solenopsis invicta.* Ecology 83: 2337–2345.

Nadkarni, N. M., and J. T. Longino. 1990. Invertebrates in canopy and ground organic matter in a Neotropical montane forest, Costa Rica. Biotropica 22: 286–289.

Olson, D. M. 1991. A comparison of the efficacy of litter sifting and pitfall traps for sampling leaf litter ants (Hymenoptera, Formicidae) in a tropical wet forest, Costa Rica. Biotropica 23: 166–172.

Pacheco, R., R. R. Silva, M. S. De C. Morini, and C.R.F. Brandão. 2009. A comparison of the leaf-litter ant fauna in a secondary Atlantic forest with an adjacent pine plantation in southeastern Brazil. Neotrop. Entomol. 38: 55–65.

Parr, C. L., and S. L. Chown. 2001. Inventory and bioindicator sampling: testing pitfall and Winkler methods with ants in a South African savanna. J. Insect Conserv. 5: 27–36.

Ryder Wilkie, K. T., A. L. Mertl, and J.F.A. Traniello. 2010. Species diversity and distribution patterns of the ants of Amazonian Ecuador. PLoS ONE 5: e13146. doi:10.1371/journal.pone. 0013146.

SAS Institute. 2009. JMP – statistical discovery software. SAS Institute, Cary, NC.

Ward, P. S. 1987. Distribution of the introduced Argentine ant (*Iridomyrmex humilis*) in natural habitats of the lower Sacramento Valley and its effects on the indigenous ant fauna. Hilgardia 55: 1–16.

Ward, P. S. 2000. Broad-scale patterns of diversity in leaf litter ant communities, pp. 99–121. *In* D. Agosti, J. D. Majer, L. E. Alonso, and T. R. Schultz (eds.), Ants: standard methods for measuring and monitoring biodiversity. Smithsonian Institution Press, Washington, DC.

Weiser, M. D., N. J. Sanders, D. Agosti, A. Ellison, A. Andersen, B. Fisher, H. Gibb, N. Gotelli, A. Gove, K. Gross, et al. 2010. Canopy and litter ant assemblages share similar climate-species density relationships. Biol. Lett. doi:10.1098/rsbl. 2010.0151.

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