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Influence of leaf litter moisture on the efficiency of the Winkler method for extracting ants

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Abstract

The Winkler extraction is one of the two fundamental sampling techniques of the standardized "Ants of the Leaf Litter" protocol, which aims to allow qualitative and quantitative comparisons of ant (Hymenoptera: Formicidae) assemblages. To achieve this objective, it is essential that the standard 48–hour extraction provides a reliable picture of the assemblages under study. Here, we tested to what extent the efficiency of the ant extraction is affected by the initial moisture content of the leaf litter sample. In an Ecuadorian mountain rainforest, the leaf litter present under rainfall–excluded and rainfall–allowed plots was collected, its moisture content measured, and its ant fauna extracted with a mini–Winkler apparatus for a 48–hour and a 96–hour period. The efficiency of the Winkler method to extract ant individuals over a 48–hour period decreased with the moisture content of the leaf litter sample. However, doubling the extraction time did not improve the estimations of the ant species richness, composition, and relative abundance. Although the moisture content of the leaf litter slightly affected the ant sampling, our results indicated that a 48–hour Winkler extraction, as recommended by the "Ants of the Leaf Litter" protocol, is sufficient to allow reliable comparisons of ant assemblages.

Keywords: ants of leaf litter protocol, Ecuador, Formicidae, mountain rainforest, rainfall exclusion, rapid biodiversity assessment, sampling method evaluation, Winkler extraction time
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Introduction

The Winkler extraction is a rapid, simple, cost–effective, and repeatable method to collect ants (Hymenoptera: Formicidae) of the leaf litter (Bestelmeyer et al. 2000; Delabie et al. 2000). This method, along with pitfall traps, constitute the fundamental sampling techniques of the standardized "Ants of the Leaf Litter" (A.L.L.) protocol (Agosti and Alonso 2000). The latter was developed to allow qualitative and quantitative comparisons of ant assemblages at different localities or over time, for use in biological evaluation and conservation, assemblage monitoring, and description of diversity patterns. In order to carry out reliable comparisons, the Winkler extraction duration should ideally be sufficient to collect all the ants present in the sample, or at least to provide a correct picture of the assemblage structure. The standard Winkler extraction of the A.L.L. protocol lasts 48 hours, but a survey of the literature shows that a large variety of extraction durations have been used, often without a justification (the extraction time ranged from 0 to 10 days or was not given; a 48–hour extraction was used in less than 50 % of the 73 studies surveyed. Supplementary details are provided in the Appendix). This diversity makes inter–study comparisons potentially challenging, especially because a very long time is often necessary to obtain a complete extraction of the ant fauna (Krell et al. 2005; Sakchoowong et al. 2007). For instance, up to 15 days were necessary to extract all ants present in leaf litter samples from temperate forests of England (Krell et al. 2005). In addition, because the Winkler method is partly based on the passive dessication of the leaf litter (Bestelmeyer et al. 2000; Krell et al. 2005), the completeness of ant extraction might be affected by the moisture content of the sample, with wetter samples requiring longer extraction times than drier ones. If it is the case, using the Winkler method to compare the ant assemblage structure among seasons, between moist and dry habitats, or even before and after a rain might be irrelevant. In this study, the leaf litter moisture of a mountain rainforest was experimentally manipulated to test the following hypotheses. First, the completeness of a 48–hour Winkler extraction, as recommended by the A.L.L. protocol, is not affected by the initial moisture content of the leaf litter sample. This result would be obtained if the ant extraction relies mainly on the disturbance of the leaf litter rather than on its passive dessication. Second, a 48–hour extraction is sufficient to obtain a reliable picture of the ant assemblage, whatever the initial moisture content of the leaf litter sample. To test this hypothesis, we compared the composition and the species relative abundance of the ant assemblages after a 48–hour and a 96–hour Winkler extraction for both dry and moist leaf litter samples.

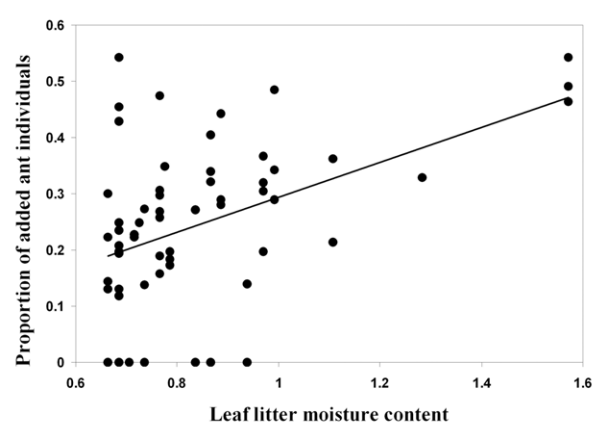

Figure 1. Relationship between the moisture content of the leaf litter sample and the proportion of ant individuals added by the second Winkler extraction. Only data from rainfall–excluded plots were analyzed (n = 60). Data from rainfall–allowed plots were not included because the moisture content of almost all these samples was maximal (Table 1). Both axes are arcsin square root transformed. The best–fitting equation of the regression analysis was: asinsqrt (Proportion of added individual) = −0.017 + 0.311 × asinsqrt (Leaf litter moisture); adjusted r^2 = 0.189, p < 0.01. High quality figures are available online.

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Materials and Methods

The study was carried out at 2000 m a.s.l. at the "Reserva Biologíca San Francisco" situated within the Eastern Cordillera of the Ecuadorian Andes, in the province of Zamora-Chinchipe (3° 58' S, 79° 5' W). Vegetation corresponds to an evergreen upper montane forest (Homeier et al. 2008). Mean annual precipitation is 2100 mm (Bendix et al. 2008). Mean temperature in the leaf litter during the experiment was 16 °C (min-max: 12.7-21.5 °C). Atmospheric relative humidity at 1.5 m above ground ranged from 91 to 95% during sampling.

In November 2009, six 3 × 3 m plots, spaced 2 to 20 m apart, were randomly assigned to either rainfall–excluded (n = 3) or rainfall–allowed (n = 3) plots. Rainfall exclusion was achieved by installing transparent plastic sheets at 1-1.2 m above ground. At the top side of the plot, a supplementary sheet was buried to a depth of 30 cm to keep running water from going inside. The three other sides were left open to limit any greenhouse effect. A mesh replaced the sheets at rainfall–allowed plots to exclude falling leaves but to allow rainfall inputs (Figure 2).

In May 2010, ants were collected using the Winkler method (Bestelmeyer et al. 2000). One plot of each treatment was always sampled during a single day. Sampling was carried out at least a day after significant rainfall to limit the risk of arthropods (especially small ones) sticking to the wet litter, and thus not being effectively extracted (Fisher 1996). The leaf litter present inside a 0.5 m² or a 0.25 m² quadrat (n = 16 and 4/plot, respectively) was collected and sifted (Figure 3 shows details of the quadrat disposition within each plot). The moisture content (using a Protometer Mini moisture meter, www.romus.org), volume, and weight of the sifted leaf litter were measured and its fauna was extracted with a mini–Winkler apparatus (Fisher 1996, 1998). All the extractions operated in the same room. After a 48–hour extraction, the collecting container was replaced by a new one and a second extraction was performed over a 48–hour period. No additional search for remaining ants was made

Figure 2. Rainfall–excluded (A, B) and rainfall–allowed (C, D) plots. High quality figures are available online.

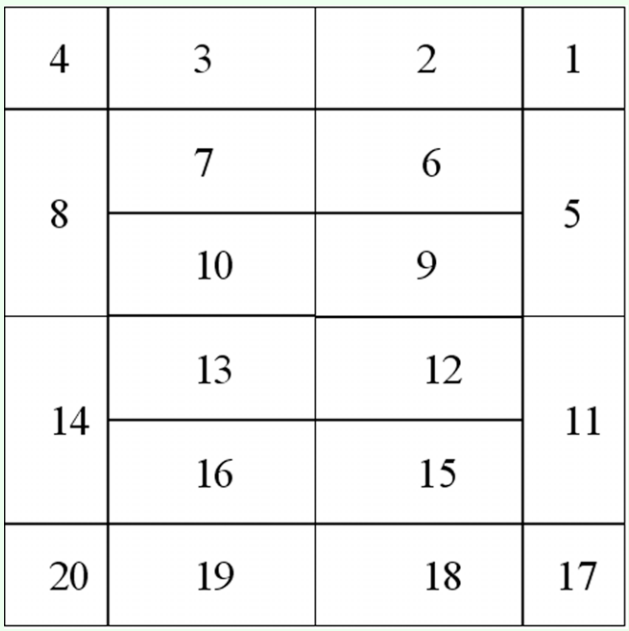

Figure 3. Quadrat disposition within each plot. Each plot was a 3 × 3 m square. The disposition of the quadrats was chosen in order to obtain a 50 cm border around a 4 m² area. The fact that quadrats are of two different sizes (0.5 and 0.25 m²) is a consequence of this design. High quality figures are available online.

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after the second extraction, since such a procedure may be highly time–consuming (Ivanov et al. 2010). Rather, the efficiency of the first 48–hour extraction was estimated by calculating the proportion of individuals and species collected after the first extraction relative to the total number of individuals and species present after a 96–hour extraction.

Analyses of similarity (ANOSIM) was used to test for differences in the composition of ant assemblages between treatments and between the first and second Winkler extractions. The ANOSIM test is a non–parametric permutation procedure applied to similarity matrices. It produces a global R–statistic, which represents an absolute measure of distance between groups. When the R–value is close to 1, groups are highly distinct; whereas when the R–value is close to 0, groups are strongly similar (Clarke and Gorley 2006). Abundance data were fourth–root transformed prior to analyses to reduce the weight of common species. Similarity matrices were built using Bray–Curtis similarity measures. Tests were performed with the PRIMER v.6.1.6. software (PRIMER-E Ltd., www.primer-e.com). Other analyses were carried out using the SigmatStat v.2.03 software (Systat Software Inc., www.systat.com).

Our protocol was designed to answer to two independent, although complementary, questions. The first one is methodological and aims to evaluate the impact of leaf litter moisture on the efficiency of the Winkler method for extracting ants. The second is ecological and is about understanding the impact of an extended drought *per se* on ant assemblages. Here, we focused on the first question; differences between ant assemblages from rainfall–excluded and rainfall–allowed plots will be discussed in detail elsewhere (Delsinne et al. in prep.). Voucher specimens were deposited at the Royal Belgian Institute of Natural Sciences, Brussels, Belgium and at the "Universidad Técnica Particular de Loja", Loja, Ecuador.

Results and Discussion

The leaf litter samples from rainfall–excluded plots were on average 43% drier than samples from rainfall–allowed plots (Table 1). In total, 5649 ant specimens and 28 species were collected (Tables 1 and 2). Doubling the Winkler extraction time allowed the collection of 7.8 and 23.5% of supplementary individuals for rainfall–excluded and rainfall–allowed samples, respectively (Table 1; Mann–Whitney Rank Sum Test, $p < 0.01$). For samples collected under rainfall–excluded plots ($n = 60$), the proportion of added individuals increased significantly with increasing moisture content of the leaf litter sample; both variables were arcsin square root transformed prior to linear regression analysis ($p < 0.01$; Figure 1). Adding either the volume or the weight (both $\log_{10}$ transformed) of the leaf litter into the model did not significantly improve the ability of the equation to predict the proportion of added individuals (arcsin square root transformed) (stepwise regression). Interestingly, when the three very wet samples (moisture content = 100 %; asinsqrt (100) = 1.57; Figure 1) were excluded from the analysis, the significance of the trend disappeared; the best–fitting equation of the regression analysis became: asinsqrt (Proportion of added individual) = 0.0316 + 0.248 × asinsqrt (Leaf litter moisture); adjusted $r^2 = 0.042$, $p = 0.068$). These results indicated that the moisture content of the leaf litter sample significantly affected the efficiency of the Winkler method to extract ant individuals, at least when the moisture content was very high. The wetter the leaf

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litter, the longer the extraction should ideally last in order to collect all the specimens present within the sample. More data are needed to accurately estimate (1) the moisture content, above which it would be useful to extend the Winkler extraction, and (2) the duration of the extraction necessary to achieve similar extraction efficiencies.

Fortunately, the standard 48–hour extraction was sufficient to provide a reliable estimation of the composition and species richness of the ant assemblage, even when based on very wet samples. Indeed, there were no significant differences in the composition of the ant assemblage between Winkler extraction times (R = −0.333; p = 1 for both treatments; anosim tests). Moreover, the proportion of species added was not significantly different between samples from the two treatments (Table 1; Mann–Whitney Rank Sum Test, p = 0.395). Only three and eight samples, containing between one and six species after the first extraction, had one supplementary species documented after the second extraction for rainfall–excluded (n = 60) and rainfall–allowed plots (n = 60), respectively. At the treatment level, all the species collected after a 96–hour extraction were already documented after the first 48–hour extraction. Because ants are social insects, it is generally recommended to work with occurrence rather than abundance data (Longino 2000). Our results suggested this also limits biases caused by the leaf litter moisture.

The ant species rank–abundance curves based on 48–hour and 96–hour extracted samples were very similar for both treatments (Spearman Rank Order Correlations; for rainfall–excluded plots: n = 24 species; r = 0.990, $p < 0.01$; for rainfall–allowed plots: n = 20 species; r = 0.984; $p < 0.01$). Thus, doubling the extraction time did not substantively change the shape of the species relative abundance curve obtained after a standard 48–hour extraction.

There were no significant differences in the composition of the ant assemblage between treatments (R = 0.296; p = 0.2, anosim test based on the 48–hour Winkler extraction). At the species level, changes in relative abundance between rainfall–excluded and rainfall–allowed plots (Table 2) may be caused, for instance, by specific differences in drought tolerance. Nevertheless, it is possible that some individuals stuck to the wet litter of rainfall–allowed samples and were lost during the sifting process. This is suspected to be especially true for small ants, such as *Brachymyrmex* and *Solenopsis* species, since they are more prone to stick to wet litter. As a result, at least part of the differences in species relative abundance between treatments may be caused by the sampling procedure itself.

The few studies that have investigated the Winkler extraction efficiency for different periods of time demonstrated that a large proportion of both ant specimens and species were rapidly extracted from the samples (Ward 1987; Beshaw and Bolton 1994; Krell et al. 2005; Delsinne et al. 2008; Ivanov et al. 2010). For instance, a 48–hour extraction of samples from the Brazilian Atlantic rainforest allowed documentation of 85 and 95% of ant individuals and species, respectively (J.H.C. Delabie pers. comm.). Moreover, based on the analysis of 110 tropical and temperate assemblages collected with Winkler samples but with an extraction period varying from 10 to 72 hours (mean ± SD: 32.3 ± 21.1 hours; median: 24 hours), Ward (2000) found that the extraction period had no significant effect on several measures of diversity such as species richness. Relatively short extraction

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times seem therefore justified when focusing on ants. Because the moisture content of the leaf litter only slightly decreased during the Winkler extraction (e.g., Sakchoowong et al. 2007; Delsinne pers. obs.), it is probable that the ant fauna migration out of the leaf litter relies mainly on the disturbance of the habitat rather than on its passive dessication.

In conclusion, a 48–hour Winkler extraction duration, as proposed for the A.L.L. protocol (Agosti and Alonso 2000), allows researchers to carry out reliable comparisons of leaf litter ant assemblages. Absolute abundance may be slightly underestimated when the moisture content of the leaf litter sample is high (e.g., ≥ 80%), but the assemblage structure (i.e., species richness, composition, and relative abundance) is correctly documented.

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References

Agosti D, Mohamed M, Arthur CYC. 1994. Has the diversity of tropical ant fauna been underestimated? An indication from leaf litter studies in a West Malaysian lowland rain forest. *Tropical Biodiversity* 2: 270-275.

Agosti D, Alonso LE. 2000. The A.L.L. protocol. A standard protocol for the collection of ground–dwelling ants. In: Agosti D, Majer JD, Alonso LE, Schultz TR, Editors. *Ants: Standard Methods for Measuring and Monitoring Biodiversity*. pp. 204-206. Smithsonian Institution Press.

Armbrecht I, Rivera L, Perfecto I. 2005. Reduced diversity and complexity in the leaf–litter ant assemblage of Colombian coffee plantations. *Conservation Biology* 19: 897-907.

Belshaw R, Bolton B. 1993. The effect of forest disturbance on the leaf litter ant fauna in Ghana. *Biodiversity and Conservation* 2: 656-666.

Belshaw R, Bolton B. 1994. A survey of the leaf litter ant fauna in Ghana, West Africa (Hymenoptera: Formicidae). *Journal of Hymenoptera Research* 3: 5-16.

Bendix J, Rollenbeck R, Richter M, Fabian P, Emck P. 2008. Climate. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R, Editors. *Gradients in a Tropical Mountain Ecosystem of Ecuador, Ecological Studies* 198: 63-73.

Bestelmeyer BT, Agosti D, Alonso LE, Brandão RF, Brown Jr WL, Delabie JHC, Silvestre R. 2000. Field techniques for the study of ground–dwelling ants: An overview, description, and evaluation. In: Agosti D, Majer JD, Alonso LE, Schultz TR, Editors. *Ants: Standard Methods for Measuring and Monitoring Biodiversity*. pp. 122-144. Smithsonian Institution Press.

Bihn JH, Verhaagh M, Brändle M, Brandl R. 2008. Do secondary forests act as refuges for old growth forest animals? Recovery of ant

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diversity in the Atlantic Forest of Brazil. *Biological Conservation* 141: 733-743.

Braga DL, Louzada JNC, Zanetti R, Delabie J. 2010. Avaliação Rápida da Diversidade de Formigas em Sistemas de Uso do Solo no Sul da Bahia. *Neotropical Entomology* 39: 464-469.

Brandão CRF, Silva RR. 2008. Synecology of *Wasmannia auropunctata*, an invasive ant species (Hymenoptera: Formicidae), in continuous and fragmented areas in the Brazilian Atlantic Forest. In: Paine TD, Editor. *Invasive Forest Insects, Introduced Forest Trees, and Altered Ecosystems*. pp. 141-151. Springer Science and Business Media B.V.

Brühl CA, Eltz T, Linsenmair KE. 2003. Size does matter – effects of tropical rainforest fragmentation on the leaf litter ant community in Sabah, Malaysia. *Biodiversity and Conservation* 12: 1371-1389.

Brühl CA, Mohamed M, Linsenmair KE. 1999. Altitudinal distribution of leaf litter ants along a transect in primary forests on Mount Kinabalu, Sabah, Malaysia. *Journal of Tropical Ecology* 15: 265-277.

Calcaterra LA, Cuezzo F, Cabrera SM, Briano JA. 2010. Ground ant diversity (Hymenoptera: Formicidae) in the Iberá Nature Reserve, the largest wetland of Argentina. *Annals of the Entomological Society of America* 103: 71-83.

Clarke KR, Gorley RN. 2006. *PRIMER v.6: User Manual/Tutorial*. PRIMER-E.

Davidson DW, Lessard J-P, Bernau CR, Cook SC. 2007. The tropical ant mosaic in a primary Bornean rain forest. *Biotropica* 39: 468-475.

Delabie JHC, Fisher BL, Majer JD, Wright IW. 2000. Sampling effort and choice of methods. In: Agosti D, Majer JD, Alonso LE, Schultz TR, Editors. *Ants: Standard Methods for Measuring and Monitoring Biodiversity*. pp. 145-154. Smithsonian Institution Press.

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

Donoso DA, Johnston MK, Kaspari M. 2010. Trees as templates for tropical litter arthropod diversity. *Oecologia* 164: 201-211.

Donoso DA, Ramón G. 2009. Composition of a high diversity leaf litter ant community (Hymenoptera: Formicidae) from an Ecuadorian pre–montane rainforest. *Annales de la Société Entomologique de France (Nouvelle Série)* 45: 487-499.

dos Santos Oliveira Jr A, Santos Melo J, Santos Sampaio L, Neves Alves M, Silva Santos MC, Freire Silva R, Biggi de Souza AL. 2009. Eficiencia de métodos amostrais para a colecta de formigas epigéica em uma floresta tropical no parque Estadual da Serra do Conduru, URUC¸ UCA - BAHIA. *Anais do IX Congresso de Ecologia do Brasil, São Lourenço* - MG. 1-3.

Ellison AM, Record S, Arguello A, Gotelli NJ. 2007. Rapid inventory of the ant assemblage in a temperate hardwood forest: species composition and assessment of sampling methods. *Environmental Entomology* 36: 766-775.

Ellwood MDF, Jones DT, Foster WA. 2002. Canopy ferns in lowland dipterocarp forest support a prolific abundance of ants, termites,

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and other invertebrates. *Biotropica* 34: 575-583.

Fayle TM, Turner EC, Snaddon JL, Khen Chey V, Chung AYC, Eggleton P, Foster WA. 2010. Oil palm expansion into rain forest greatly reduces ant biodiversity in canopy, epiphytes and leaf–litter. *Basic and Applied Ecology* 11: 337-345.

Fisher BL. 1996. Ant diversity patterns along an elevational gradient in the Réserve Naturelle Intégrale d'Andringitra, Madagascar. *Fieldiana Zoology* 85: 93-108.

Fisher BL. 1998. Ant diversity patterns along an elevational gradient in the Réserve Spéciale d'Anjanaharibe-Sud and on the western Masoala Peninsula, Madagascar. *Fieldiana Zoology* 90: 39-67.

Fisher BL, Robertson HG. 2002. Comparison and Origin of Forest and Grassland Ant Assemblages in the High Plateau of Madagascar (Hymenoptera: Formicidae). *Biotropica* 34: 155-167.

Fisher BL. 1999. Improving inventory efficiency: A case study of leaf–litter ant diversity in Madagascar. *Ecological Applications* 9: 714-731.

Groc S, Orivel J, Dejean A, Martin J-M, Etienne M-P, Corbara B, Delabie JHC. 2009. Baseline study of the leaf–litter ant fauna in a French Guianese forest. *Insect Conservation and Diversity* 2: 183-193.

Groc S. 2006. Diversité de la myrmécofaune des Causses aveyronnais - Comparaison de différentes méthodes d'échantillonnage. *Mémoire de DESUPS. Université Paul Sabatier*.

Guerrero RJ, Sarmiento CE. 2010. Distribución altitudinal de hormigas (Hymenoptera, Formicidae) en la vertiente noroccidental de la Sierra Nevada de Santa Marta (Colombia). *Acta Zoológica Mexicana* 26: 279-302.

Gunawardene NR, Majer JD, Edirisinghe JP. 2010. Investigating residual effects of selective logging on ant species assemblages in Sinharaja Forest Reserve, Sri Lanka. *Forest Ecology and Management* 259: 555-562.

Hites NL, Mourão MAN, Araújo FO, Melo MVC, de Biseau JC, Quinet Y. 2005. Diversity of the ground–dwelling ant fauna (Hymenoptera: Formicidae) of a moist, montane forest of the semi–arid Brazilian "Nordeste". *Revista de Biología Tropical* 53: 165-173.

Homeier J, Werner FA, Gradstein SR, Breckle S-W, Richter M. 2008. Potential vegetation and floristic composition of Andean forests in South Ecuador, with a focus on the RBSF. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R, Editors. *Gradients in a Tropical Mountain Ecosystem of Ecuador, Ecological Studies* 198*:* 87-100.

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

King JA, Ansersen AN, Cutter AD. 1998. Ants as bioindicators of habitat disturbance: validation of the functional group model for Australia's humid tropics. *Biodiversity and Conservation* 7: 1627-1638.

Kolo Y. 2006. A rapid assessment of the ants of the Boké Region, Guinea. In: *RAP Bulletin*

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of Biological Assessment: A Rapid Biological Assessment of Boké Préfecture, Northwestern Guinea. pp. 120-126. Conservation International.

Kone M, Konate S, Yeo K, Kouassi P, Linsenmair KE. 2010. Diversity and abundance of terrestrial ants along a gradient of land use intensification in a transitional forest–savannah zone of Côte d'Ivoire. *Journal of Applied Biosciences* 29: 1809-1827.

Krell F-T, Chung AYC, DeBoise E, Eggleton P, Giusti A, Inward K, Krell-Westerwalbesloh S. 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.

Lapolla JS, Suman T, Sosa-Calvo J, Schultz T. 2007. Leaf litter ant diversity in Guyana. *Biodiversity and Conservation* 16: 491-510.

Leponce M, Theunis L, Delabie JHC, Roisin Y. 2004. Scale dependence of diversity measures in a leaf–litter ant assemblage. *Ecography* 27: 253-267.

Lessard J-P, Dunn RR, Sanders N J. 2009. Temperature–mediated coexistence in temperate forest ant communities. *Insectes Sociaux* 56: 149-156.

Lessard J-P, Sackett TE, Reynolds WN, Fowler DA, Sanders NJ. 2011. Determinants of the detrital arthropod community structure: the effects of temperature and resources along an environmental gradient. *Oikos* 120: 333-343.

Longino JT. 2000. What to do with the data? In: Agosti D, Majer JD, Alonso LE, Schultz TR, Editors. *Ants: Standard Methods for Measuring and Monitoring Biodiversity*. pp. 186-203. Smithsonian Institution Press.

Longino JT, Coddington J, Colwell RK. 2002. The ant fauna of a tropical rain forest: estimating species richness three different ways. *Ecology* 83: 689-702.

Lopes CT, Vasconcelos HL. 2008. Evaluation of three methods for sampling ground–dwelling ants in the Brazilian Cerrado. *Neotropical Entomology* 37: 399-405.

Majer JD, Delabie JHC. 1994. Comparison of the ant communities of annually inundated and terra firme forests at Trombetas in the Brazilian Amazon. *Insectes Sociaux* 41: 343-359.

Majer JD. 1996. Ant recolonization of rehabilitated bauxite mines at Trombetas, Para, Brazil. *Journal of Tropical Ecology* 12: 257-273.

Malsch AKF, Fiala B, Maschwitz U, Mohamed M, Nais J, Linsenmair KE. 2008. An analysis of declining ant species richness with increasing elevation at Mount Kinabalu, Sabah, Borneo. *Asian Myrmecology* 2: 33-49.

Martelli MG, Ward MM, Fraser AM. 2004. Ant diversity sampling on the Southern Cumberland Plateau: A comparison of litter sifting and pitfall trapping. *Southeastern Naturalist* 3: 113-126.

Mezger D, Pfeiffer M. 2011. Partitioning the impact of abiotic factors and spatial patterns on species richness and community structure of ground ant assemblages in four Bornean rainforests. *Ecography* 34: 39-48.

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Mikheyev AS, Tchingnoumba L, Henderson A, Alonso A. 2008. Effect of propagule pressure on the establishment and spread of the little fire ant *Wasmannia auropunctata* in a Gabonese oilfield. *Diversity and Distributions* 14: 301-306.

Morrison LW. 1998. A review of Bahamian ant (Hymenoptera: Formicidae) biogeography. *Journal of Biogeography* 25: 561-571.

Nadkarni NM, Longino JT. 1990. Invertebrates in canopy and ground organic matter in a Neotropical montane forest, Costa Rica. *Biotropica* 22: 286-289.

Neville PJ, O'Dowd DJ, Yen AL. 2008. Issues and implications for research on disturbed oceanic islands illustrated through an ant survey of the Cocos (Keeling) Islands. *Journal of Insect Conservation* 12: 313-323.

Olson DM. 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.

Olson DM. 1994. The distribution of leaf litter invertebrates along a Neotropical altitudinal gradient. *Journal of Tropical Ecology* 10: 129-150.

Parr CL, Chown SL. 2001. Inventory and bioindicator sampling: Testing pitfall and Winkler methods with ants in a South African savanna. *Journal of Insect Conservation* 5: 27-36.

Philpott SM, Bichier P, Rice RA, Greenberg R. 2008. Biodiversity conservation, yield, and alternative products in coffee agroecosystems in Sumatra, Indonesia. *Biodiversity and Conservation* 17: 1805-1820.

Ratsirarson H, Robertson HG, Picker MD, van Noort S. 2002. Indigenous forests versus exotic eucalypt and pine plantations: a comparison of leaf–litter invertebrate communities. *African Entomology* 10: 93-99.

Robertson HG. 2002. Comparison of leaf litter ant communities in woodlands, lowland forests and montane forests of north–eastern Tanzania. *Biodiversity and Conservation* 11: 1637-1652.

Rowles AD, O'Dowd DJ. 2009. Impacts of the invasive Argentine ant on native ants and other invertebrates in coastal scrub in south–eastern Australia. *Austral Ecology* 34: 239-248.

Ryder Wilkie KT, Mertl AL, Traniello JFA. 2007. Biodiversity below ground: probing the subterranean ant fauna of Amazonia. *Naturwissenschaften* 94: 725-731.

Sabu TK, Shiju RT. 2010. Efficacy of pitfall trapping, Winkler and Berlese extraction methods for measuring ground–dwelling arthropods in moist–deciduous forests in the Western Ghats. *Journal of Insect Science* 10: 98. Available online, insectscience.org/10.98

Sabu TK, Vineesh, PJ, Vinod, KV. 2008. Diversity of forest litter–inhabiting ants along elevations in the Wayanad Region of the Western Ghats. *Journal of Insect Science* 8: 69. Available online, insectscience.org/8.69

Sagata K, Mack AL, Wright DD, Lester PJ. 2010. The influence of nest availability on the abundance and diversity of twig–dwelling ants in a Papua New Guinea forest. *Insectes Sociaux* 57: 333-341.

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Sakchoowong W, Nomura S, Ogata K, Chanpaisaeng J. 2007. Comparison of extraction efficiency between Winkler and Tullgren extractors for tropical leal litter macroarthropods. *Thai Journal of Agricultural Science* 40: 97-105.

Sanders NJ, Lessard J-P, Fitzpatrick MC, Dunn RR. 2007. Temperature, but not productivity or geometry, predicts elevational diversity gradients in ants across spatial grains. *Global Ecology and Biogeography* 16: 640-649.

Silva RR, Machado Feitosa RS, Eberhardt F. 2007. Reduced ant diversity along a habitat regeneration gradient in the southern Brazilian Atlantic Forest. *Forest Ecology and Management* 240: 61-69.

Soares SM, Schoereder JH, Desouza OG. 2001. Processes involved in species saturation of ground–dwelling ant communities (Hymenoptera, Formicidae). *Austral Ecology* 26: 187-192.

Sobrinho TG, Schoereder JH. 2007. Edge and shape effects on ant (Hymenoptera: Formicidae) species richness and composition in forest fragments. *Biodiversity and Conservation* 16:1459-1470.

Solomon SE, Mikheyev AS. 2005. The ant (Hymenoptera: Formicidae) fauna of Cocos Island, Costa Rica. *The Florida Entomologist* 88: 415-423.

Souza JLP, Moura CAR, Harada AY, Franklin E. 2007. Diversidade de espécies dos gêneros de *Crematogaster*, *Gnamptogenys* e *Pachycondyla* (Hymenoptera: Formicidae) e complementaridade dos métodos de coleta durante a estação seca numa estação ecológica no estado do Pará, Brasil. *Acta Amazonica* 37: 649-656.

Spiesman BJ, Cumming GS. 2008. Communities in context: the influences of multiscale environmental variation on local ant community structure. *Landscape Ecology* 23: 313-325.

Tista M, Fiedler K. 2011. How to evaluate and reduce sampling effort for ants. *Journal of Insect Conservation*. 15: 547-559.

Vargas AB, Queiroz JM, Mayhé-Nunes AJ, Souza G, Ramos EF. 2009. Teste da regra de equivalência energética para formigas de serapilheira: efeitos de diferentes métodos de estimativa de abundância em floresta ombrófila. *Neotropical Entomology* 38: 867-870.

Vasconcelos HL, Vilhena JMS, Caliri GJA. 2000. Responses of ants to selective logging of a central Amazonian forest. *Journal of Applied Ecology* 37: 508-514.

Vasconcelos HL, Vilhena JMS, Facure KG, Albernaz ALKM. 2010. Patterns of ant species diversity and turnover across 2000 km of Amazonian floodplain forest. *Journal of Biogeography* 37: 432-440.

Vasconcelos HL, Vilhena JMS, Magnusson WE, Albernaz ALKM. 2006. Long–term effects of forest fragmentation on Amazonian ant communities. *Journal of Biogeography* 33: 1348-1356.

Ward D, Beggs J. 2007. Coexistence, habitat patterns and the assembly of ant communities in the Yasawa islands, Fiji. *Acta Oecologica* 32: 215-223.

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Ward PS. 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 PS. 2000. Broad–scale patterns of diversity in leaf litter ant communities. In: Agosti D, Majer JD, Alonso LE, Schultz TR, Editors. *Ants: Standard Methods for Measuring and Monitoring Biodiversity*. pp. 99-121. Smithsonian Institution Press.

Watt AD, Stork NE, Bolton B. 2002. The diversity and abundance of ants in relation to forest disturbance and plantation establishment in southern Cameroon. *Journal of Applied Ecology* 39: 18-30.

Zelikova TJ, Dunn RR, Sanders NJ. 2008. Variation in seed dispersal along an elevational gradient in Great Smoky Mountains National Park. *Acta Oecologica* 34: 155-162.

Table 1. Leaf litter sample properties and efficiency of the ant fauna extraction for relatively dry and wet Winkler samples from rainfall–excluded and rainfall–allowed plots, respectively. Data are medians, interquartiles between parentheses, total values in bold.

	Winkler samples from rainfall-allowed plots (n = 60)	Winkler samples from rainfall-excluded plots (n = 60)
Leaf litter humidity (%)	100 (100;100)	48 (40; 62.5)
Leaf litter weight (g)	632 (484.5; 776)	490.5 (339.5; 670.5)
Leaf litter volume (L)	1.4 (1; 1.8)	2.5 (1.7; 3.2)
Number of species collected after a 48-hour extraction	4.0 (3.0; 4.0)	5.0 (5.0; 6.0)
Total number of species collected after a 48-hour extraction	**20**	**24**
Number of species collected after a 96-hour extraction	4.0 (3.0; 4.0)	5.0 (5.0; 6.0)
Total number of species collected after a 96-hour extraction	**20**	**24**
Proportion of species added by the second extraction (%)	0 (0; 0)	0 (0; 0)
Proportion of species added by the second extraction (based on total values) (%)	**0**	**0**
Number of individuals collected after a 48-hour extraction	18.5 (11; 29.5)	50.5 (29.0; 76.5)
Total number of individuals collected after a 48-hour extraction	**1295**	**3756**
Number of individuals collected after a 96-hour extraction	22.0 (14.0; 36.5)	53.0 (31.5; 83.5)
Total number of individuals collected after a 96-hour extraction	**1599**	**4050**
Proportion of individuals added by the second extraction (%)	19.1 (9.1; 30.3)	3.5 (1; 6)
Proportion of individuals added by the second extraction (based on total values) (%)	**23.5**	**7.8**

Table 2. The 28 morphospecies collected and their relative abundance (%) for Winkler samples from rainfall–allowed and rainfall–excluded plots. Data from the 48–hour and the 96–hour extractions were computed separately.

	Rainfall-allowed		Rainfall-excluded	
	48-hour extraction	96-hour extraction	48-hour extraction	96-hour extraction
Hypoponera sp.A	59.61	61.66	21.92	21.74
Solenopsis sp.A	13.75	13.7	10.47	10.51
Solenopsis sp.B	10.12	9.57	22.42	21.56
Brachymyrmex sp.A	4.86	4.44	21.33	22.53
Strumigenys sp.A	3.55	3.38	15.74	15.5
Pheidole sp.A	3.01	2.56	1.01	0.96
Pheidole sp.B	2.01	1.81	1.46	1.38
Pheidole sp.C	0.85	0.75	1.07	1.01
Pheidole sp.D	0.93	0.75	0.11	0.1
Pheidole sp.E	0.31	0.38	0	0
Nylanderia sp.A	0.15	0.25	0.48	0.45
Camponotus sp.A	0.08	0.13	0	0
Pachycondyla sp.A	0.15	0.13	0.91	0.84
Pheidole sp.F	0.15	0.13	0	0
Stigmatomma sp.A	0.08	0.06	0.05	0.05
Linepithema sp.A	0.08	0.06	0.08	0.07
Megalomyrmex sp.A	0.08	0.06	1.01	1.36
Pachycondyla sp.B	0.08	0.06	0.03	0.02
Simopelta sp.A	0.08	0.06	0	0
Solenopsis sp.C	0.08	0.06	0.67	0.69
Leptanilloides sp.A	0	0	0.93	0.89
Pachycondyla sp.C	0	0	0.08	0.07
Pheidole sp.G	0	0	0.08	0.07
Typhlomyrmex sp.A	0	0	0.03	0.05
Pheidole sp.H	0	0	0.05	0.05
Pachycondyla sp.D	0	0	0.03	0.02
Pheidole sp.I	0	0	0.03	0.02
Solenopsis sp.D	0	0	0.03	0.02

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Appendix. Winkler extraction duration used in published ant surveys. In December 2010, keywords such as "ants + Winkler", "Formicidae + Winkler" and "A.L.L. protocol" were used to search studies dealing with ant diversity, ecology and biogeography on Web of Science and Google Scholar. Ant taxonomical studies were not included because their aim is not to compare ant assemblages in a standardized way. Where the same data set was used in several papers, only one was listed. The study locality is given in order to show that extraction time was rarely selected according to where the sampling was carried out.

Extraction time	Study locality	Reference
Not provided, extracted until sample "thoroughly dry"	Kuala Belalong Field Studies Centre, Brunei Darussalam, Borneo	Davidson et al. 2007
Not provided but referred to Ward (1987): 72h?	Bahamas	Morrison 1998
Not provided but referred to the A.L.L. protocol (Agosti and Alonso 2000): 48h?	Mount Kinabalu, Sabah, Borneo	Malsch et al. 2008
Not provided but referred to the A.L.L. protocol (Agosti and Alonso 2000): 48h?	Borneo	Mezger and Pfeiffer 2011
Not provided but referred to the A.L.L. protocol (Agosti and Alonso 2000): 48h?	Tiputini Biodiversity Station, Ecuador	Ryder Wilkie et al. 2007
Not provided but referred to the A.L.L. protocol (Agosti and Alonso 2000): 48h?	Florida, USA	Spiesman and Cumming 2008
Not provided but referred to Olson (1991): 48h?	Viçosa, south-east Brazil	Soares et al. 2001
Not provided	Ulu Gombak, Selangor, West Malaysia	Agosti et al. 1994
Not provided	Nouragues Research Station, French Guiana, France	Groc et al. 2009
Not provided	Trombetas, Pará, Brazil	Majer 1996
Not provided	Cocos (Keeling) Islands, Australia	Neville et al. 2008
Not provided	Mornington Peninsula National Park, Australia	Rowles and O'Dowd 2009
Hand-sorted during 15 min	Yasawa islands, Fiji	Ward and Beggs 2007
Hand-sorted	Cornwall, NY, USA	Ellison et al. 2007
Hand-sorted	Wayanad region of Western Ghats, India	Sabu et al. 2008
10 days	Cape Peninsula National Park, South Africa	Ratsirarson et al. 2002
1 week	Danum Valley Conservation Area in Sabah, Borneo	Ellwood et al. 2002
At least 6 days + remaining ants were hand-sorted	Mount Kinabalu, Sabah, Borneo	Brühl et al. 1999
5 days	Western Ghats, India	Sabu and Shiju 2010
4-5 days	Papua New Guinea	Sagata et al. 2010
At least 3 days + remaining ants were hand-sorted	Lower Austria	Tista and Fiedler 2010
2-4 days	North-eastern Tanzania	Robertson 2002
72h + remaining ants were hand-sorted	Danum Valley Conservation Area in Sabah, Borneo	Fayle et al. 2010
72h + remaining ants were hand-sorted	Cleveland area of northeastern Ohio, USA	Ivanov et al. 2010
72h	Ghana	Belshaw and Bolton 1993, 1994
72h	Rio Cachoeira Nature Reserve, Parana, Brazil	Bihn et al. 2008
72h	Bahia, Brazil	Braga et al. 2010
72h	DanumValley Conservation Area, Sabah,Borneo	Brühl et al. 2003
72h	Sierra Nevada de Santa Marta, Colombia	Guerrero and Sarmiento 2010
72h	Atherton Tablelands in north-eastern Queensland, Australia	King et al. 1998
72h	Great Smoky Mountains National Park, Tennessee, USA	Lessard et al. 2011
72h	La Selva Biological Station, Heredia Province, Costa-Rica	Longino et al. 2002
72h	Cumberland Plateau in southern Tennessee, USA	Martelli et al. 2004
72h	Monteverde Cloud Forest Reserve, Costa-Rica	Nadkarni and Longino 1990
72h	Viçosa, southeastern Brazil	Sobrinho and Schoereder 2007
72h	Lower Sacramento Valley, USA	Ward 1987
48h + "subsequently picked through by hand for an hour"	The Caribbean slope of the Cordillera Central in western Panama	Olson 1994
48h the litter being removed, shaken and returned after the first 24h	Sinharaja Forest Reserve, Sri Lanka	Gunawardene et al. 2010
48h	Andes on the northwest side of Colombia	Armbrecht et al. 2005
48h	Atlantic Forest, Brazil	Brandão and Silva 2008
48h	Iberá Nature Reserve, Argentina	Calcaterra et al. 2010
48h	Otongachi forest, Ecuador	Donoso and Ramón 2009.
48h	Barro Colorado Island, Panama	Donoso et al. 2010
48h	Parque Estadual da Serra do Conduru, Bahia, Brasil	dos Santos Oliviera et al. 2009
48h	Madagascar	Fisher 1999
48h	Province of Fianarantsoa, Madagascar	Fisher and Robertson 2002
48h	Montane forest of the semi-arid Brazilian "Nordeste"	Hites et al. 2005
48h	Boké Region, Guinea	Kolo 2006
48h	Lamto Reserve, Côte d'Ivoire	Kone et al. 2010
48h	Guyana	LaPolla et al. 2007
48h	Southern Appalachians, USA	Lessard et al. 2009
48h	Reserva Ecológica do Panga, Minas Gerais, Brazil	Lopes and Vasconcelos 2008
48h	Lowland rain forest, Gabon	Mikheyev et al. 2008
48h	La Selva Biological Station, Heredia Province, Costa Rica	Olson 1991
48h	Kruger National Park, South Africa	Parr and Chown 2001
48h	Lampung province of Sumatra, Indonesia	Philpott et al. 2008
48h	Great Smoky Mountains National Park, USA	Sanders et al. 2007
48h	Parque das Nascentes, Santa Catarina state, South Brazil	Silva et al. 2007
48h	Floresta Nacional de Caxiuanã, Pará, Brazil	Souza et al. 2007
48h	Reserva Biológica do Tinguá, Brazil	Vargas et al. 2009
48h	Pará, Brazil	Vasconcelos et al. 2006
48h	Amazon River, Brazil	Vasconcelos et al. 2010
48h	Cameroon	Watt et al. 2002
48h	Great Smoky Mountains National Park, USA	Zelikova et al. 2008
"Approximately 48h"	Cocos Island, Costa Rica	Solomon and Mikheyev 2005
24h	Brazil	Delabie et al. 2000
24h	Gran Chaco, Argentina and Paraguay	Delsinne et al. 2008
24h	Grands Causses, France	Groc 2006
24 h	Rio Pilcomayo National Park, Northern Argentina	Leponce et al. 2004
24 h	Near Trombetas, Brazilian Amazonia	Majer and Delabie 1994
24h	North of Manaus, Brazil	Vasconcelos et al. 2000
3h up to 7 weeks + hand-sorted	England, Hants, New Forest / Malaysia, Sabah, Borneo	Krell et al. 2005
3h up to 7 days	Eastern Thailand	Sakchoowong et al. 2007

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