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Efficiency of sampling methods for capturing soil-dwelling ants in three landscapes in southern Cameroon

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Abstract

Soil-dwelling ants are the most diverse and abundant groups of animals in most terrestrial ecosystems. However, reliable techniques for assessing the abundance and diversity of ant communities with respect to land management remain poorly studied. The present study aimed to evaluate the efficiency of three trapping procedures (Pitfall trapping, quadrat sampling and baiting) in three habitats (urban, upland and littoral). A total of 169,934 ant workers belonging to 7 sub-families, 51 genera and 198 species were recorded in the three habitats. High species richness and abundance were recorded in upland habitats, compared to urban and littoral habitats. Similarly, contribution diversity approach based on species richness and Simpson's index was high in upland habitats and low in littoral and urban habitats. Quadrat sampling was more efficient and reliable technique (138 species) than pitfall trapping (133 species) and baiting (126 species). Ant pitfall trapping was less efficient in urban and littoral habitats, but significantly effective in upland habitats. All techniques recorded more predominantly epigaeically foraging species than hypogaeically species. Ant community composition significantly varied among habitats, but no between sampling methods. These results highlight the influence of habitat traits on ant trapping success and indicate that sampling techniques used seem to be more suitable to sample a specific stratum, particularly the epigaeic ant fauna. Additional methods are therefore needed to increase the likelihood of recording hidden ant fauna and obtaining an adequate impression of the local ant fauna.

Keywords sampling techniques | ants | epigaeic | hypogaeic | land management | Cameroon

1. Introduction

Arthropods are the most diverse and abundant soil-dwelling organisms and represent more than 75 % of the earth's terrestrial biodiversity. They are found in nearly all terrestrial habitats, performing at many levels in an ecosystem and consequently play important roles in maintaining ecosystem stability (Wilson 1987, McIntyre et al. 2001, McKinney 2008). Many arthropod groups (e.g. ants) have been considered as potential indicator taxa due to their high diversity and ecological importance (Williams 1993, Andersen & Sparling 1997, Longcore 2003).

Ants are one of the most ubiquitous, widespread and abundant groups of soil-dwelling arthropod species in most terrestrial ecosystems (Hölldobler & Wilson 1990). They can nest in different soil microhabitats including the soil surface and deeper soil layers. Epigaeic ant species are closely related to the soil surface resources, especially in the litter, in tropical ecosystems (Yanoviak & Kaspari 2000) while hypogaeic or subterranean ant species appear to be more related to physical characteristics of their microhabitat, such as soil density (Schmidt et al. 2013). These insects play critical roles in every terrestrial ecosystem, such as recycling nutrients, dispersing seeds,

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and engaging in mutualistic associations with other organisms (Beattie 1985, Schultz & McGlynn 2000). Ants are also sensitive to environmental changes and particularly appropriate for biodiversity assessment programs (Agosti & Alonso 2000, Hoffmann & Andersen 2003). Compared to other arthropods moving frequently from their habitats in search of food or nesting sites, ants possess stationary and perennial nests that can be marked and revisited. Therefore, they can be more reliably sampled and monitored (Majer 1983).

Different sampling techniques have been developed to quantify ant species or assemblages in many habitats. These techniques are divided into two broad classes: passive sampling techniques including pitfall trapping, baiting, and quadrat sampling, while active sampling methods are represented by direct sampling, colony counts and intensive sampling (Greenslade 1973, Marsh 1984, Andersen 1997, Agosti & Alonso 2000, Bestelmeyer et al. 2000, Fisher 2002, 2004, Longino 2000, Laeger & Schultz 2005, Longino et al. 2019, Delabie et al. 2021). It is recognized that the sampling success of each method depends mainly on nest density, ground vegetation cover, researchers' choice, specific-species traits, habitat in question, and the objective of the study (Romero & Jaffe 1989, Andersen & Sparling 1997, Bestelmeyer et al. 2000, Schlick-Steiner et al. 2006). Numerous studies reported pitfall trapping technique as the most commonly used in ant fauna surveys (Andersen 1991, Vorster et al. 1992, Majer 1997, Lindsey & Skinner 2001, LeBreton et al. 2003, Jiménez-Carmona et al. 2020, Hacala et al. 2021). Although that method enables continuous sampling over a prolonged period (day and night) and can better estimate abundance and species composition of epigaeic ant fauna, it provides an inadequate sample of ant fauna (Olson 1991, Majer 1997, Agosti et al. 2000, Wang et al. 2001).Thus, pitfall trapping is used simultaneously with others techniques to collect the hidden ant fauna, hypogaeic or subterranean ants in tropical ecosystems.

Many studies have compared the performance of association of pitfall trapping with nest counting (Romero & Jaffe 1989, Schlick-Steiner et al. 2006), and pitfall trapping with bait trapping (Hacala et al. 2021) in ant fauna sampling. However, few studies have directly compared association of pitfall trapping with dig sampling and quadrat sampling (Lindsey & Skinner 2001) or pitfall trapping with bait trapping and quadrat sampling (Fotso et al. 2015). The present study aimed to evaluate the strengths and the weaknesses of pitfall, quadrat and bait trapping of recording epigaeic and/or hypogaeic ant fauna in three different habitats. We hypothesise pitfall trapping is suitable for epigaeic ant sampling while quadrat sampling and bait trapping are adequate for hypogaeic ant recording.

2. Material and methods

Study sites

Investigations were carried out in southern Cameroon rainforest (Figure 1). The southern part of this country extends from 2° 20' 43" N, 9° 59' 28" E to 7° 20' 33", N 13° 34' 58" E. The vegetation is dominated by the dense rainforests divided into two predominant types: lowland evergreen and lowland semi-deciduous forest. The topography of this area extends from coastline to hill regions with elevations of up to 2 000 m a.s.l. The southern Cameroon experiences an equatorial climate of the Guinea subtype characterized by four distinct seasons: two wet seasons and two dry seasons. Rainfall is high especially along the coast. Rainfall averages 1500–2000 mm per year and the mean annual temperature is about 25°C. Ants were sampled during rainy and dry seasons in three different habitats as followed:

(1) *Urban areas.* Ant species assemblages were sampled in 2007 and 2017 in two Yaoundé districts located at the metropolis' southeast, including Biyem assi (3°51'10 N, 11°28'10E) and Mendong (3°51'10 N, 11°27'35 E). The vegetation of Yaoundé formerly belonged to the semi-deciduous forest type, but is currently dominated by human settlements and building areas covering more than 90% of available area with few spaces for natural forest (Nkwewoh et al. 2017). The smallest patches of remnant forest are found only on hill summits.

(2) *Upland forests.* Ants were sampled from September 2018 to April 2019 in two low altitudes mounts in Cameroon: Mount Eloundem (3°49′ N, 11°26′ E, 1 156 m above sea level [asl]) and Mount Kala (3°50′ N, 11°21′ E, 1 159 m asl). The sampling sites were selected at an elevation of 800 m, 900 m, 1000 m, and 1100 m a.s.l. which represent formerly the typical pattern of vegetation of this mountain (Achoundong 1996). The landscape is characterized by the steep and rugged rock slopes and it is dominated by seven most important plant families: Leguminosae, Clusiaceae, Myristicaceae, Burseraceae, Sterculiaceae, Annonaceae and Rubiaceae (Achoundong 1996, Madiapevo et al. 2017). The percentage of area covered by vegetation is approximately 80%, consisting of cloud forests, rotten woods, plant and leaf litter cover at high altitudes, while the remaining area (less than 20%) located at low altitudes is threatened by logging and subsistence farming with neither leaf litter nor vegetation coverage.

(3) *Littoral evergreen forests.* Ant sampling was conducted at the Campo Ma'an National Park (CMNP) (2°52'N, 10°54'E), with an area that covers about 776202 ha. Ants were collected from June 2015 to June 2016 in two sites located at the southern periphery of the CMNP and separated by the Ntem River: a nearly primary forest

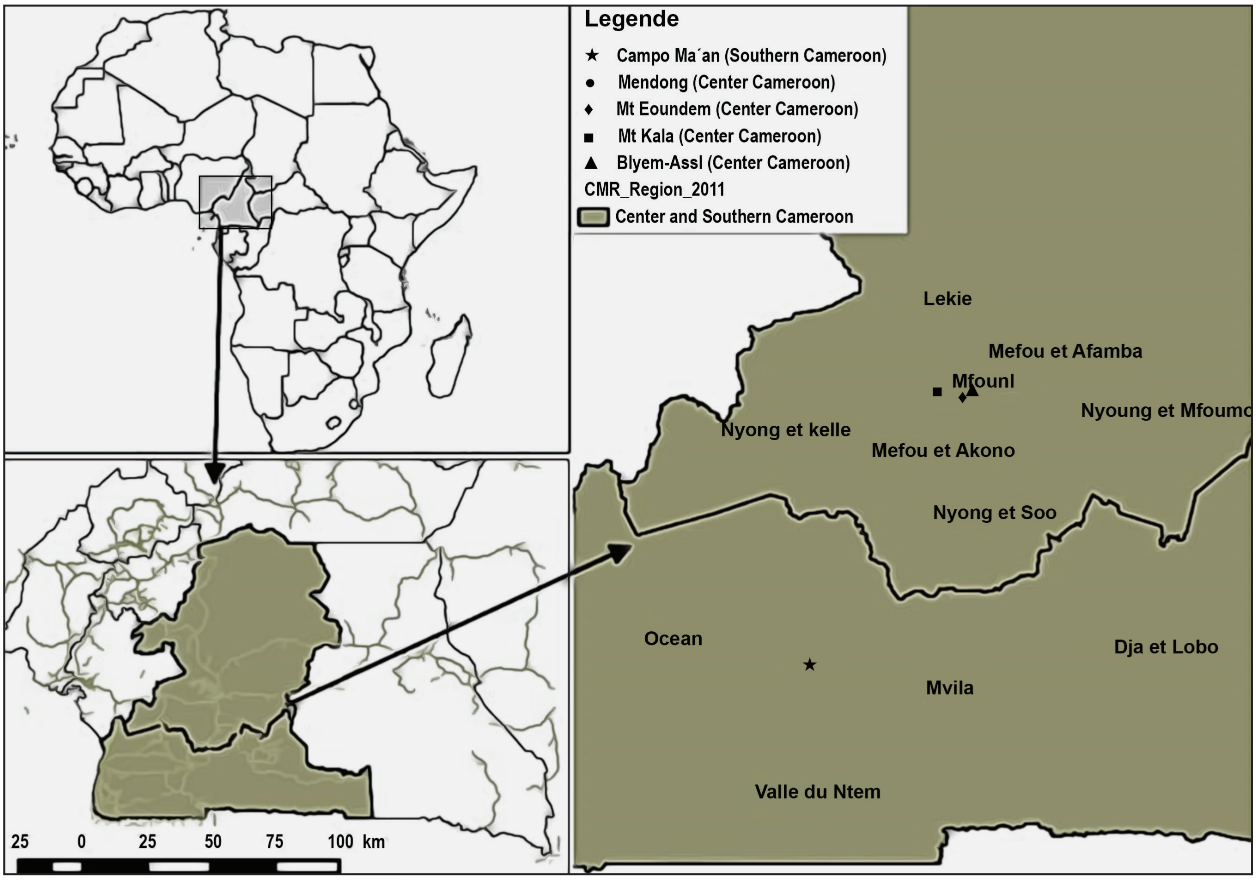

Figure 1. Map showing the study sites.

site (PF) located in the protection zone (Dipikar Island) and a secondary forest site (SF) situated in one of the five logging concessions surrounding the park 'UFA 09025'. The vegetation of the site forms part of the Atlantic Biafran forest and Lowland evergreen forest of the Congo Basin and Equatorial Guinea, rich in Caesalpinioideae with *Calpocalyx heitzii* and *Sacoglottis gabonensis* (Letouzey 1985*,* Tchouto et al. 2009). This is a regenerated forest consisting of 60% closed canopy vegetation and less plant and leaf litter coverage than upland areas.

Sampling of ant species composition structure

Ant richness and abundance were monitored by pitfall trapping, quadrat sampling and baiting to ensure as complete a sampling effort as possible (Di Castri et al. 1992). Two sites were selected at each habitat. Three transect lines representing the three sampling methods were placed at each site. Each transect line was 110 m long and spaced 10 m apart. Sampling points were delimited on every 10 m alongside that line. Four sampling events were conducted at two-month intervals during the study period. Traps were set between 8h00 and 11h00 a.m. in residential backyard, gardens, and lawns in urban areas whereas they were placed in herbaceous plants and in leaf litter in upland and littoral habitats.

Pitfall trapping – Traps were set along the first transect. They were constituted of plastic cups (diameter 20 mm, 150 mm length) inserted into sunken plastic pipes. A quarter of the cup (about 100 ml) was filled with water (98%) and soap (2%). Prior to the commencement of trapping, the pitfall traps were left for one week to reduce 'digging-in' effects (Greenslade 1973).

Quadrat sampling – Ten quadrats measuring 1m^2 each were set along the second transect. Two consecutive quadrats were 10 m apart and 10 m from the nearest pitfall trap. Ants were searched for in rotten logs and stumps, under stones, bark, layers of leaf litter and directly in the soil. Ants were sampled by using an aspirator and collected by two trained entomologists during 15 minutes of active searching.

Bating – A mixture of honey 30%, tuna 50% and soya oil 20% were used as bait and were placed along the third transect on the ground on a square plastic (20 cm x 20 cm). Ants were checked every 5 minutes during 30 minutes of observation.

At the laboratory of Zoology of the University of Yaoundé I, ants were removed from the traps and placed in 70% ethanol for later identification. Ant voucher specimens were identified to species level using keys from Bolton (1994) and a new taxonomic revision for genera (Borowiec 2016, Fisher & Bolton 2016), and the web sites ant sub-Saharan African (Taylor 2010), Antweb.org and AntCat.org for species. Species that could not be named were designated as a morphospecies with labels. Ant specimens were mounted on card board triangles and then kept in a reference collection at the laboratory.

Statistical analyses

To evaluate sampling success, two relevant non-parametric richness estimators, Abundance-based coverage estimator-ACE and Chao 1, were used for each habitat and sampling method, and species accumulation curves were generated. Plots of cumulative species per sample curves were generated in which species accumulation was plotted as a function of the number of samples taken (Colwell 2005). Curves reaching a plateau at values of maximum sampling effort indicate that the number of sampled species likely represents the actual number of species within each habitat or method (Soberon & Llorente 1993). We also performed non-parametric one-way analyses of ranked similarities (ANOSIM, 999 randomisations) to test whether the pairwise similarities of the ant assemblages between habitats and sampling methods were similar. To evaluate species diversity, we used a contribution diversity approach based on unit distinctiveness, for species richness (γ_{ST}) and Simpson's indices (Lu et al. 2007):

$$\alpha_{ST\ or\ DT} = \frac{1}{n}\sum_{i}^{S} ni\ ,\ \beta_{ST\ or DT} = 1 - \sum_{i} P_i^2 \text{ and}$$

$$\gamma_{ST\ or\ DT} = \alpha_{ST\ or\ DT} + \beta_{ST\ or\ DT}$$

Where Pi is the relative abundance of the ith species among n units in the region, ni is the number of units where the ith species occurs in the region, $\alpha_{ST\ \mathrm{or}\ DT}$ is the contribution of the tth unit to within-unit diversity, $\alpha_{\mathrm{ST\ or\ DT}}$ is the distinctiveness or the contribution of the tth unit to among-unit diversity.

To evaluate the distribution of species diversity within and among units, a differentiation coefficient based on species richness and Simpson's index contribution diversity approach was defined by:

$$D_{ST\ or\ DT} = \beta_{ST}/\gamma_{ST}\ or\ \beta_{DT}/\gamma_{DT}$$

A value of D > 0.5 means that most diversity is distributed among units (habitat) or D < 0.5 means that most diversity is distributed within units.

All analyses were performed using package Vegan with package R (4.0.3) software.

3. Results

Ant community

A total of 169,934 ant workers from 198 species, seven sub-families and 51 genera were recorded in the three habitats (Table 1). *Tetramorium* was the most species-rich genus with 27 species, followed by *Camponotus* and *Cataulacus* (13 species each). The most speciose and abundant subfamily was Myrmicinae (100 species; 50.51%), followed by Ponerinae (38 species; 19.19%) and Formicinae (32 species; 16.16%) (Table 2).

Species richness estimator and sampling success

Values of species richness for the three habitats as function of sampling methods are presented in Table 4. Overall, sample efficiency varied between 80 and 99% depending on the species richness estimator. The highest estimated richness was observed with Ace estimator while the lowest estimated richness was obtained with Chao 1. Species accumulation curves using baiting as sampling methods reached a horizontal asymptote when approximately 10.000 samples were collected in upland and urban areas respectively or 1000 samples in littoral areas (Figure 2). In contrary, additional sampling efforts and more samples would be required to reach a real asymptote plateau using quadrat sampling and pitfall sampling in upland and urban areas respectively. Likewise, the sampling efforts are needed to see the curves flatten out in littoral areas using pitfall as sampling method.

Abundance and uniqueness

The most abundant species over all three habitats were: *Wasmannia auropunctata* Roger, 1863 (35.98%), *Dorylus (Anomma) nigricans* Illiger, 1802 (29.45%), *Crematogaster (Sphaerocrema) concava* Emery, 1899 (7.46%), *Pheidole megacephala* (Fabricius, 1793) (4.20%) and *Tetramorium aculeatum* Mayr, 1866 (4.09%). The upland habitats yielded the highest species richness and abundance (91759 individuals of 166 species), followed by urban habitats (70230 individuals of 64 species) and littoral habitats (8003 individuals of 38 species). In upland habitats, the most abundant species were: *Dorylus*

Table 1. List of ant species and species-traits as a function of the three sampling methods used in upland, littoral and urban habitats. Values in the table represent occurrence of each species. **Nb**: Epi = epigaeic ants and Hyp = Hypogaeic ants.

subfamily/species	Stratum	Bait	Pitfall	Quadrat
Dolichoderinae				
Axinidris bidens Shattuck, 1991	Epi	18	17	20
Tapinoma sp.1	Epi	24	18	20
Tapinoma sp.2	Epi	5	9	10
Tapinoma sp.3	Epi	10	14	13
Tapinoma specie T3	Epi	1		2
Technomyrmex albipes (Smith, 1861)	Epi	4	5	10
Technomyrmex sp.1	Epi			3
Technomyrmex sp.2	Epi			3
Technomyrmex sp.3	Epi			1
Technomyrmex sp.4	Epi	6	1	
Dorylinae				
Aenictus decolor Mayr, 1876	Epi			1
Aenictus sp.	Epi	1		
Aenictus weissi Santschi, 1910	Epi		3	
Dorylus (Anomma) nigricans Illiger, 1802	Hyp	13	84	16
Dorylus (Dorylus) braunsi Emery, 1895	Hyp	4	8	1
Parasyscia sp.1	Hyp			1
Parasyscia foreli (Santschi, 1914)	Hyp	2	5	1
Parasyscia nitidulus (Brown, 1975)	Hyp	2		
Parasyscia nkomoensis (Forel, 1907)	Hyp		1	
Parasyscia sp.2	Hyp	1	1	5
Parasyscia sp.3	Hyp	1		
Parasyscia sudanensis (Weber, 1942)	Hyp	2	2	
Formicinae				
Anoplolepis tenella Santschi, 1911	Hyp	15	126	82
Camponotus (Myrmacrhaphe) sp.	Hyp		7	1
Camponotus (Myrmopelta) sp.1	Hyp	2	2	19
Camponotus (Myrmopelta) sp.2	Hyp	8	6	33
Camponotus (Myrmopelta) sp.3	Hyp	2	2	22
Camponotus (Myrmopelta) sp.4	Hyp		1	1
Camponotus (Myrmosericus) flavomarginatus Mayr, 1862	Hyp/Epi		12	14
Camponotus (Myrmotrema) foraminosus Forel, 1876	Hyp		7	3
Camponotus (Myrmotrema) sp. 1	Hyp	2	10	5
Camponotus (Myrmotrema) sp. 2	Hyp		1	1
Camponotus (Tanaemyrmex) acvapimensis Mayr, 1862	Hyp/Epi		5	2
Camponotus (Tanaemyrmex) brutus Forel, 1886	Hyp/Epi	2	87	6
Camponotus (Tanaemyrmex) maculatus Fabricius, 1782	Hyp/Epi	2	158	14
Camponotus (Tanaemyrmex) pompeius Forel, 1886	Hyp		5	2
Lepisiota sp.1	Hyp	2	11	19
Lepisiota sp.2	Hyp	2	1	15
Lepisiota sp.3	Hyp		1	2
Oecophylla longinoda Latreille, 1802	Hyp/Epi	7		22
Paratrechina sp.1	Epi	9	3	9
Paratrechina sp.2	Epi			1
Petalomyrmex sp.	Hyp		1	
Polyrachis ayousi Taylor, 2005	Epi		2	1
Polyrachis concave André, 1889	Epi			1
Polyrachis decemdentata André, 1889	Hyp/Epi		1	18
Polyrachis laboriosa Smith F., 1858	Hyp/Epi			3
Polyrachis militaris Fabricius, 1782	Hyp/Epi	1	13	13

Table 1. Continued.

subfamily/species	Stratum	Bait	Pitfall	Quadrat
Polyrachis phidias Forel, 1910	Epi		1	2
Polyrachis rufipalpis Santschi, 1910	Epi			1
Polyrachis sp.1	Epi	1	5	9
Polyrachis sp.2	Epi		3	3
Polyrachis sp.3	Epi		1	
Polyrachis weissi Santschi, 1910	Epi			1
Myrmicinae				
Atopomyrmex mocquerysi André, 1889	Epi		3	14
Baracidris sp.	Epi	1		
Calyptomyrmex nummuliticus Santschi, 1914	Epi	7	1	
Calyptomyrmex sp.	Epi	2		
Cardiocondyla wassmanni Santschi, 1926	Hyp/Epi		2	
Cataulacus kohli Mayrs, 1895	Epi			1
Cataulacus mocquerysi André, 1889	Epi			2
Cataulacus sp.1	Epi	1		10
Cataulacus sp.2	Epi			2
Cataulacus sp.3	Epi		3	14
Cataulatus centrurus Bolton, 1982	Epi		7	6
Cataulatus egenus Santschi, 1911	Epi		8	8
Cataulatus guineensis Smith F., 1853	Epi		1	7
Cataulatus kohli Mayr, 1895	Epi			3
Cataulatus lujae Forel, 1911	Epi			1
Cataulatus taylori Bolton, 1982	Epi			5
Crematogaster (Atopogyne) clariventris Mayr, 1895	Epi	1		4
Crematogaster (Atopogyne) depressa Latreille, 1802	Epi	18	17	15
Crematogaster (Atopogyne) sp. 1	Epi		1	
Crematogaster (Atopogyne) sp. 2	Epi			2
Crematogaster (Crematogaster) sp.1	Epi	4	7	21
Crematogaster (Crematogaster) sp.2	Epi		1	
Crematogaster (Crematogaster) sp.3	Epi	4	2	1
Crematogaster (Crematogaster) striatula Emery, 1892	Hyp/Epi	6		17
Crematogaster (Orthocrema) pulchella Bernard, 1953	Epi	2	16	4
Crematogaster (Sphaerocrema) concave Emery, 1899	Epi	88	112	82
Crematogaster (Sphaerocrema) gabonensis Emery, 1899	Epi		1	
Crematogaster (Sphaerocrema) sp.1	Epi	1		1
Crematogaster (Sphaerocrema) sp.2	Epi		2	5
Decamorium sp.	Hyp		1	
Discroapis sp.	Hyp		1	
Melissotarsus weissi Santschi, 1910	Hyp		5	
Meranoplus sp.	Hyp	1	1	4
Microdaceton tibialis Weber, 1952	Hyp	2	1	
Monomorium bicolor Emery, 1877	Epi	3		3
Monomorium sp.1	Epi		4	1
Monomorium sp.2	Epi	3		2
Monomorium sp.3	Epi	1		2
Monomorium sp.4	Epi	3	1	2
Myrmicaria opaciventris Emery, 1893	Hyp	64	31	74
Oligomyrmex diabolus Santschi, 1913	Hyp	18	3	
Oligomyrmex sp.1	Hyp	7	2	
Oligomyrmex sp.2	Hyp	2	1	
Oligomyrmex sp.3	Epi	9	4	1

subfamily/species	Stratum	Bait	Pitfall	Quadrat
Pheidole impressifrons Wasmann, 1905	Epi	2		
Pheidole megacephala (Fabricius, 1793)	Hyp/Epi	37	57	31
Pheidole pulchella Santschi, 1910	Epi	12	33	7
Pheidole sp.2	Epi	4	15	1
Pheidole sp.3	Epi	26	26	21
Pheidole sp.4	Epi	9	7	6
Pheidole sp.5	Epi		1	
Pheidole sp.6	Epi	2		
Pheidole sp.7	Epi		4	
Pheidole speculifera Emery, 1877	Hyp/Epi	19	57	42
Pristomyrmex orbiceps Santschi, 1914	Hyp	12	3	1
P*ristomyrmex* sp.	Hyp	5		1
Rhoptromyrmex sp.	Hyp	2	9	8
Serrastruma (Epitritus) sp.	Hyp	5		
Serrastruma (Glamyromyrmex) sistrura Bolton, 1983	Hyp/Epi	5		
Serrastruma (Glamyromyrmex) sp.	Hyp	1		
Serrastruma (Serrastruma) dotaja Bolton, 1983	Hyp	1		
Serrastruma (Serrastruma) sp.1	Hyp	27	6	1
Serrastruma (Serrastruma) sp.2	Hyp	3	4	1
Serrastruma (Serrastruma) sp.3	Hyp	3	1	
Serrastruma (Smithistruma) sp.1	Hyp	3		
Serrastruma (Smithistruma) sp.2	Hyp	1		
Solenopsis sp.	Epi	2		
Strumigenys sp.1	Epi	1		
Strumigenys sp.2	Epi		1	
Strumigenys sp.3	Epi	17	2	
Strumigenys sp.4	Epi	30	7	
Strumigenys sp.5	Hyp	2		
Tetramorium aculeatum Mayr, 1866	Hyp/Epi	53	72	98
Tetramorium angulinode Santschi. 1910	Epi	3		
Tetramorium ataxium Bolton,1980	Epi	22	18	2
Tetramorium boltoni Hiter Garcia. Fischer et Peters, 2010	Epi		1	
Tetramorium brevispinosum Stitz, 1910	Epi	7	20	29
Tetramorium coloreum Mayr, 1901	Epi	13	28	21
Tetramorium dogieli Karavaiev, 1931	Epi		2	1
Tetramorium guineense Bernard, 1953	Epi	30	67	33
Tetramorium lucayanum Wheeler. W.M., 1905	Epi		1	
Tetramorium minisculum Santschi, 1914	Epi	5	5	3
Tetramorium philippwagneri Hita Garcia, Fisher & Peters, 2010	Epi	3		
Tetramorium rugosum Taylor, 2007	Epi		1	
Tetramorium sericeiventre Emery. 1877	Epi	2		2
Tetramorium sp.1	Epi	8	5	1
Tetramorium sp.10	Epi		1	
Tetramorium sp.11	Epi			4
Tetramorium sp.2	Hyp/Epi	7	6	
Tetramorium sp.3	Epi	3	3	7
Tetramorium sp.4	Epi	4	4	2
Tetramorium sp.5	Hyp		1	3
Tetramorium sp.6	Epi		3	1
Tetramorium sp.7	Epi	1	1	2
Tetramorium sp.8	Epi	10	7	1
Tetramorium sp.9	Epi	6	4	
Tetramorium speculifera Emery, 1877	Epi		4	3

Table 1. Continued.

subfamily/species	Stratum	Bait	Pitfall	Quadrat
Tetramorium versiculum Bolton, 1980	Epi		2	
Tetramorium zonacaciae Weber, 1943	Epi	22	17	9
Wasmannia auropunctata Roger, 1863	Epi	60	60	60
Ponerinae				
Anochetus bequarti Forel, 1913	Epi	6		1
Anochetus sp.1	Epi	1	3	8
Anochetus sp.2	Epi	8	5	8
Anochetus sp.3	Epi		1	1
Anochetus sp.4	Epi	1	1	2
Anochetus sp.5	Epi		2	
Anochetus sp.6	Epi	4	1	1
Hypoponera cognata (Santschi, 1912)	Hyp	3		5
Hypoponera rothkirchi Wasmann, 1953	Hyp	2		
Hypoponera sp.1	Hyp	27	2	2
Hypoponera sp.2	Hyp	2	1	4
Hypoponera sp.3	Hyp	7		
Hypoponera sp.4	Hyp	1		
Hypoponera sp.5	Hyp	6		1
Hypoponera sp.6	Hyp	3		
Leptogenys sp.1	Epi	3	10	1
Leptogenys sp.2	Epi		6	
Leptogenys sp.3	Epi		1	
Leptogenys sp.4	Epi	1		1
Loboponera basalis Bolton et Brown, 2002	Epi	2		
Odontomachus assiniensis Emery, 1892	Epi	23	49	48
Odontomachus troglodytes Santschi, 1914	Epi	4	9	4
Palthothyreus (Bothroponera) sp.1	Epi		7	5
Palthothyreus (Xiphopelta) sp.1	Epi	7	6	2
Palthothyreus (Bothroponera) sp.2	Epi	8	5	1
Palthothyreus (Bothroponera) sp.3	Epi	7	8	15
Palthothyreus (Brachyponera) sp.	Epi			3
Palthothyreus (Trachymesopus) sp. 1	Hyp/Epi	1	7	4
Palthothyreus (Trachymesopus) sp. 2	Epi	9	27	21
Palthothyreus (Xiphopelta) sp.2	Epi	1		
Palthothyreus (Xiphopelta) sp.3	Epi		2	1
Palthothyreus (Xiphopelta) sp.4	Epi	2		1
Palthothyreus tarsatus Fabricius, 1798	Hyp	30	123	40
Phrynoponera sp.	Hyp	1	8	6
Platythyrea conradti (Emery, 1899)	Hyp			2
Platythyrea occidentale André, 1890	Hyp		1	1
Plectroctena cristata Emery, 1899	Hyp		3	2
Plectroctena sp.	Hyp	1		2
Proceratiinae				
Discothyrea sp.	Hyp	2		
Probolomyrmex filiformis Mayr, 1901	Epi	4	2	
Probolomyrmex sp.	Epi	2		
Pseudomyrmecinae				
Tetraponera aethiops Smith, 1877	Hyp/Epi			1
Tetraponera anthracina Santschi,1910	Hyp		1	21
Tetraponera ledouxi Terron, 1969	Hyp/Epi			1
Total		**1067**	**1736**	**1364**

Table 2. distribution of genera and species in three habitat types in southern Cameroon.

	Genus	%	Species	%
Dolichoderinae	4	7,84	10	5,05
Dorylinae	3	5,88	12	6,06
Formicinae	8	15,69	32	16,16
Myrmicinae	24	47,06	100	50,51
Ponerinae	9	17,65	38	19,19
Proceratiinae	2	3,92	3	1,52
Pseudomyrmecinae	1	1,96	3	1,52
Total	**51**	**100**	**198**	**100**

(*Anomma*) *nigricans* (54.01 %) and *Crematogaster (Sphaerocrema) concava* (13.92 %). In contrast, *Wasmannia auropunctata* (87.07 %) and *Tetramorium aculeatum* (6.58 %) were the ant species encountered most frequently in urban habitats.. In littoral habitats, *Pheidole megacephala* (67.89 %) and *Monomorium* sp.2 (6.01 %) were the most represented ant species among these communities.

Eight species were unique to the littoral habitats (*Tetramoruim species* T3, *Technomyrmex* sp.3, *Camponotus (Myrmopelta*) sp.2, *Cataulacus mocquerysi*, *Crematogaster (Atopogyne)* sp.2, *Solenopsis* sp., *Tetramoruim speculifera* and *Paltothyreus (Xiphopelta)* sp.4, whilst 134 and 18 species were unique to upland and urban habitats, respectively.

Sampling method efficiency

In all habitats, quadrat sampling was the more efficient and reliable technique (137 species) than either pitfall (133 species) or baiting (126 species) (Table 3). More specifically, quadrat sampling caught more species than pitfall and bait trapping in littoral habitats as well as urban habitats. In contrast, pitfall trapping recorded more species than quadrat sampling and bait trapping in upland habitats. The number of unique species was greater in bait trapping (26 species) than pitfall and quadrat (22 and 23 species, respectively). The most represented rare species caught by bait trapping belonged to cryptic species, such as *Parasyscia* spp., *Serrastruma* spp., *Hypoponera* spp., *Discothyrea* sp., *Probolomyrmex* sp. and *Strumigenys* sp.

Ant stratum

Of the 198 species recorded using the combination of the three sampling techniques, 119 species (60 % of the total number of the species) represented predominantly

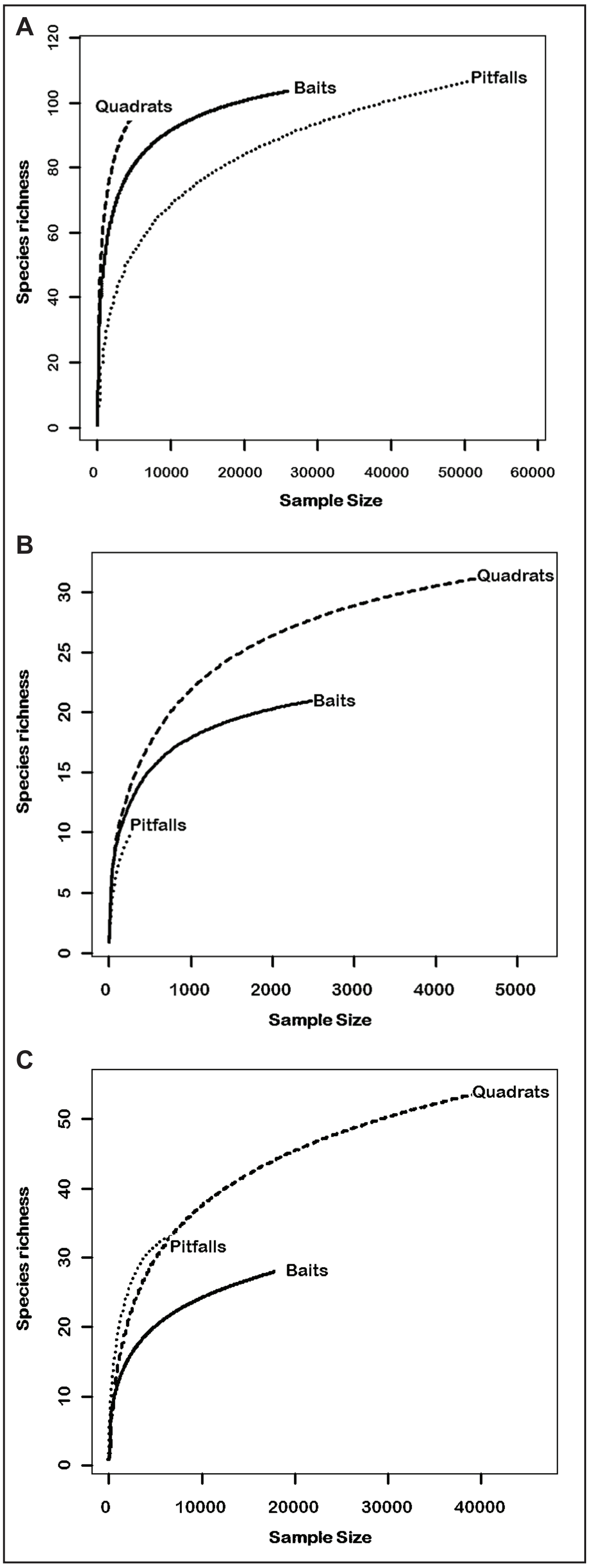

Figure 2. Rarefaction curves of ant species collected in upland (**A**), littoral (**B**) and urban sites (**C**), Cameroon. The species accumulation curves in each graph plot the number of ant species observed as a function of sample size.

Table 3. Number of genera, species, workers, unique species and traits of ants as a function of sampling methods.

Techniques	Genera	Species	workers	Unique species	Stratum (%)		
					Hyp.	Epi.	Epi/ Hyp
Quadrat	37	137	65038	23	32.49	35.84	31.67
Pitfall	45	133	56648	22	23.24	64.84	11.92
Bait	44	126	48258	26	38.12	42.26	19.62

Legend: Hyp – Hypogaeic ants, Epi – Epigaeic ants, Epi/ Hyp – Epigaeic and Hypogaeic ants

Table 4. Observed species richness (Sobs) and expected number of species (Chao & and Ace estimators)as calculated with two species richness estimators. The sampling success given as proportion (%) of sampled species to the theoretical species richness is given in brackets.

	Littoral			Upland			Urban		
	Bait	Pitfall	Quadrat	Bait	Pitfall	Quadrat	Bait	Pitfall	Quadrat
Sobs	21	10	32	102	116	97	28	33	55
Chao1	22 (95)	11 (91)	34 (94)	103 (99)	137 (85)	116 (83)	35 (80)	35 (94)	60 (92)
Ace	23 (96)	13 (91)	36 (89)	105 (97)	139 (84)	111 (87)	35 (80)	35 (94)	63 (87)

Table 5. Species diversities estimated by species richness and Simpson's index based contribution diversity approach in three habitat types, Cameroon (bold values in the table indicate the average within unit diversity α and the average amount of diversity β not found in a single, randomly-chosen unit).

Species richness – based contribution diversity approach			
	α_{ST}	β_{ST}	$\gamma_{ST}=\alpha_{ST}+\beta_{ST}$
Upland x Bait	34.000	22.166	56.166
- x Pitfall	38.666	22.500	61.166
- x Quadrat	32.333	15.333	47.666
	35.000	**19.000**	**54.000**
Littoral x Bait	7.000	4.833	11.8333
- x Pitfall	3.333	3.000	6.333
- x Quadrat	10.666	10.166	20.833
	7.000	**6.000**	**13.000**
Urban x Bait	9.333	2.500	11.833
- x Pitfall	11.000	4.833	15.833
- x Quadrat	18.333	18.000	36.333
	12.889	**8.444**	**21.333**
Simpson's index based contribution diversity approach			
	α_{PT}	β_{PT}	$\gamma_{PT}=\alpha_{PT}+\beta_{PT}$
Upland x Bait	0.825	0.091	0.917
- x Pitfall	0.353	0.308	0.662
- x Quadrat	0.807	-0.0005	0.806
	0.661	**0.132**	**0.793**
Littoral x Bait	0.574	0.124	0.698
- x Pitfall	0.552	0.298	0.850
- x Quadrat	0.450	0.206	0.657
	0.525	**0.209**	**0.734**
Urban x Bait	0.416	-0.070	0.346
- x Pitfall	0.136	0.062	0.199
- x Quadrat	0.166	0.044	0.211
	0.239	**0.012**	**0.251**

epigaeically foraging species. Another 61 species (30.80 %) foraged hypogaeically, while 18 species (9.10 %) were hypogaeically as well as epigaeically. Pitfall trapping caught the most epigaeic ant species (64.84 %) and least hypogaeic ants (23.24 %). Similar trends were observed with quadrat sampling (35.84 % and 32.49 %, respectively) and bait trapping (42.26 % and 38.12 %) (Table 3).

Difference in ant community

There was no significant difference in the ant community compositions between sampling methods (ANOSIM, R = 0.0028, P = 0.103) suggesting that ant assemblages recorded by the different methods are similar (Figure 3A). However, upland, urban and littoral habitats had distinct ant community composition at all sample times (ANOSIM, R = 0. 203, p = 0.002) (Figure 3B).

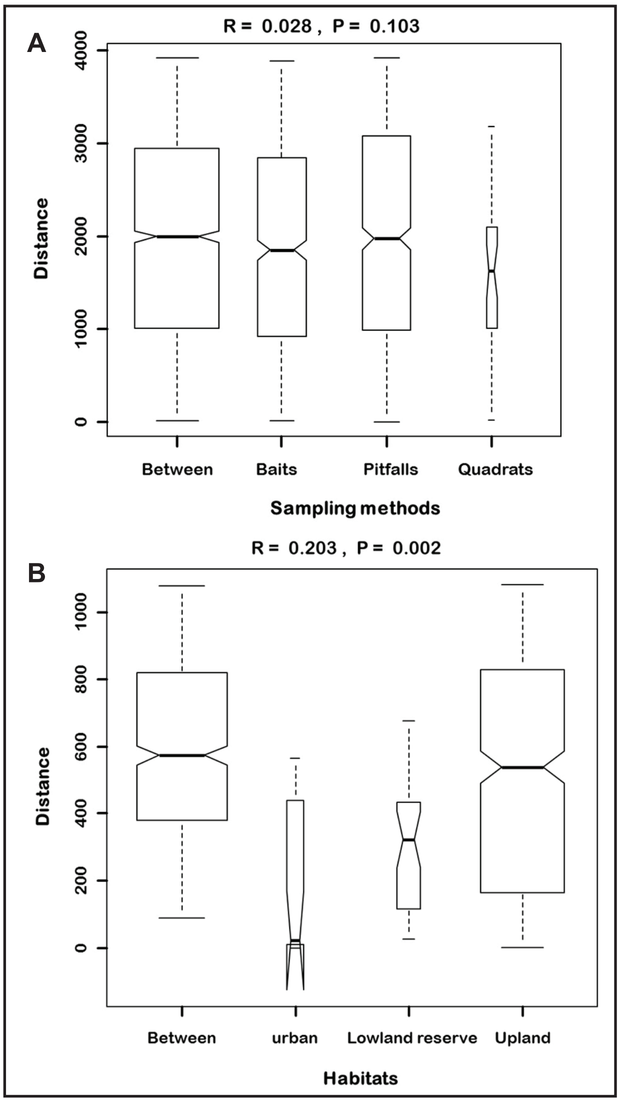

Figure 3. Difference in ant community compositions between sampling methods (**A**) and habitat (**B**) using ANOSIM (999 randomisations).

Contribution diversity approach

According to species richness based on the contribution diversity approach, γ_{ST} diversity was 54, means of α and β diversity were 35 and 19, respectively indicating higher contribution to diversity in upland habitats. In littoral site, diversity was 13 while 21.33 was obtained in Urban habitats (Table 5). The Simpson's indices were γ_{DT} = 0.79, γ_{DT} = 0.73 and γ_{DT} = 0.251 in upland, littoral and urban habitats, respectively. The differentiation coefficient were D_{ST} = 0.364 of the upland community, D_{ST} = 0.462 and D_{ST} = 0.396 of the littoral and urban ant communities, respectively indicating that most diversity was partitioned within habitats. Similarly, the differentiation index was also inferior to 0.5 in upland (D_{DT} = 0.167), in littoral (D_{DT} = 0.285), and in Urban habitats (D_{DT} = 0.049), based on Simpson's index (Figure 4). In contrast, the differentiation index between the three sampling methods was 0.601 in bait, 0.602 in pitfall and 0.556 in quadrat indicating that most diversity was partitioned among methods (figure 5).

4. Discussion

Ant richness and contribution to diversity

Our study shows that a combination of the three sampling methods was responsible for recording 198 ant species among the three habitats. The observed species richness was below 237 ant species recorded in locally vegetation types (mixed-crop fields, short –fallows, and regenerated forests) in southern Cameroon using the same sampling methods (Fotso et al. 2015). This difference in species richness between the two studies may be due to habitat composition and species traits. In fact, numerous studies showed that specific microclimate features such as tree density, tree height, circumference at breast height, density of the herbaceous and shrubby vegetation, weight and heterogeneity of the leaf litter, and canopy cover may affect ant richness more closely than other characteristics (Queiroz et al. 2013). Moreover, a positive correlation between vegetation structure and the diversity of ants is frequently reported (Room 1975).

Species diversity in the present study was evaluated by a contribution diversity approach based on unit distinctiveness, for species richness (γ_{ST}) and Simpson's

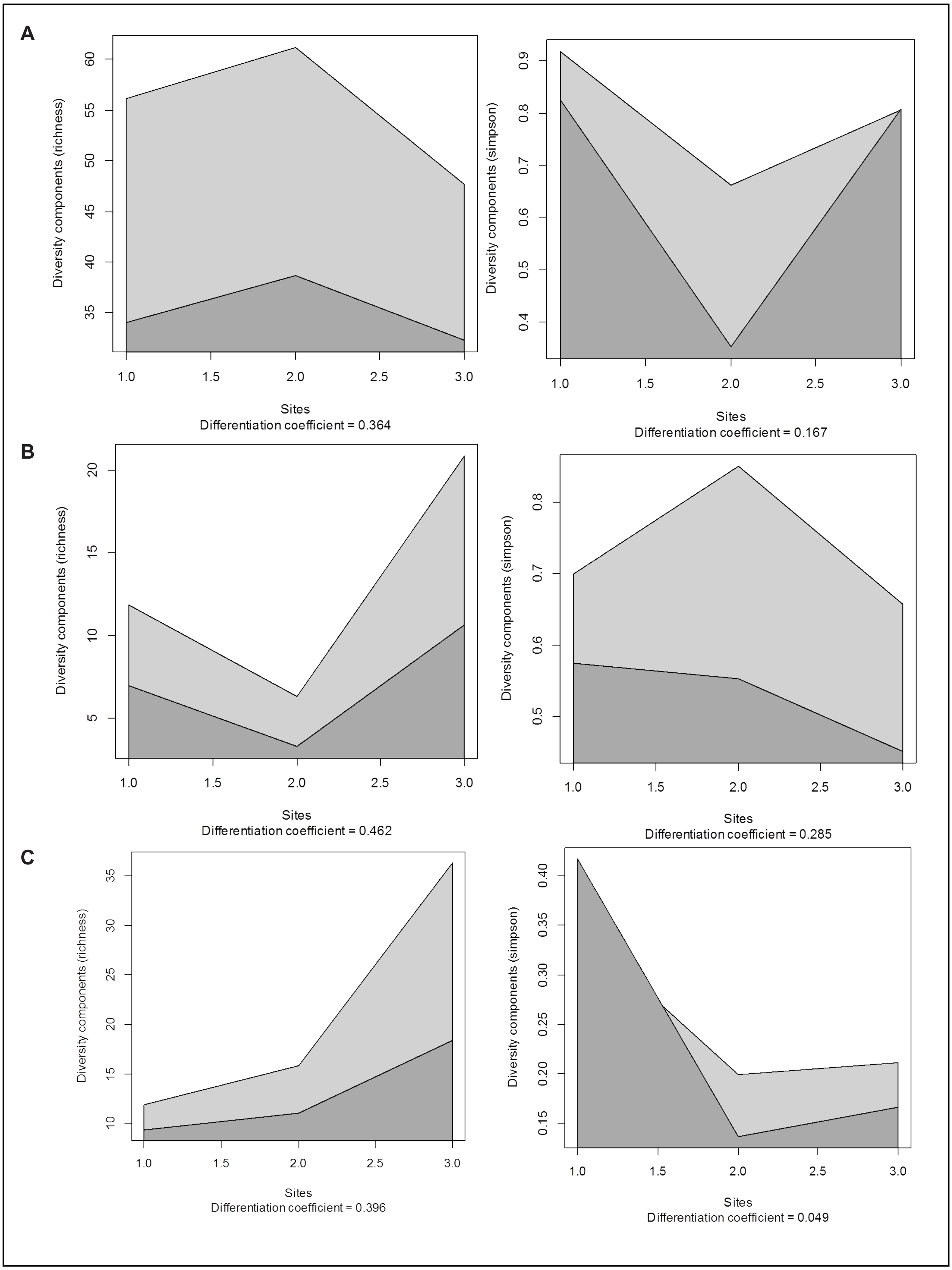

Figure 4. Differentiation coefficient based on species richness (left) and simpson's index (right) in three habitats: (**A**) Upland, (**B**) Littoral, (**C**) Urban.

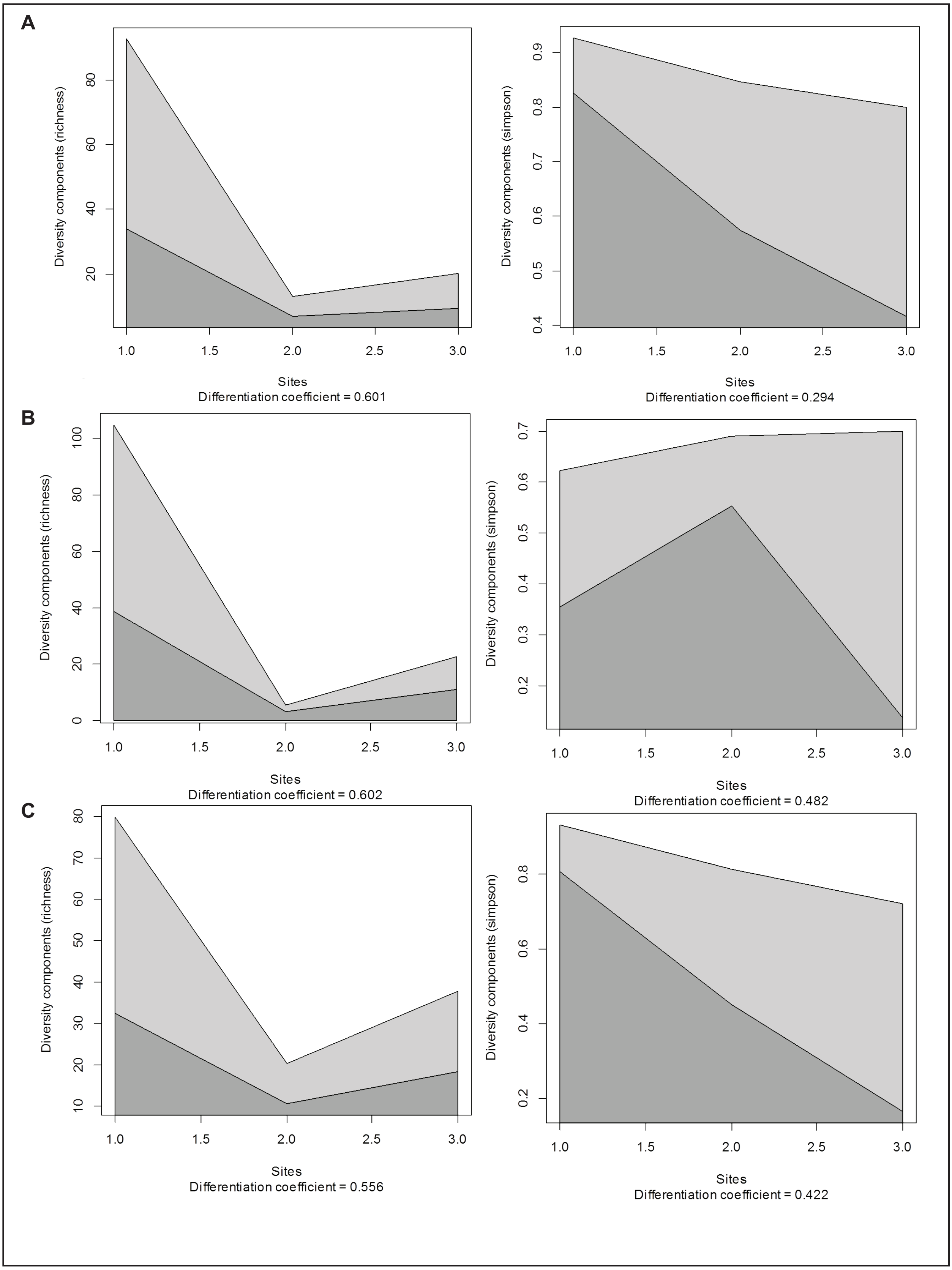

Figure 5: Differentiation coefficient based on species richness (left) and Simpson's index (right) in three sampling methods (**A**) Bait, (**B**) Pitfall, (**C**) Quadrat.

index (γ_{DT}), both taking into account **α** and **β** diversities. Our results demonstrate highest contribution to diversity in upland and lowest in urban and littoral habitats. Many studies usually considered these indices separately, which may lead to biased results. The contribution diversity approach can evaluate the contribution of each unit to the average **α** diversity or to the **β** diversity of the region, provides an objective foundation for determining conservation priorities, and can evaluate unit distinctiveness based on the distinctiveness of all species in the unit, not only of the endemic species (Lu et al. 2007, Gavish et al. 2019).

Habitat differences

Our results also showed that eight species were unique to the littoral habitats whilst 134 and 18 species were unique to upland and urban habitats respectively. In addition, a non-parametric one-way analysis of ranked similarities (ANOSIM) revealed a significant difference in the ant community composition between the three habitats. These results suggest that each habitat harbours different ant assemblages. Numerous studies have highlighted that ant assemblages are strongly influenced by a number of habitat variables including: geology, soil type, soil moisture, physiognomy, vegetation cover, plant and leaf litter covers (Koen & Breytenbach 1988). Upland habitats yielded the highest diversity (166 species) compared to urban (64 species) and littoral habitats (38 species). Highland vegetation was characterized by human modified-areas without canopy cover, low or no leaf litter at lower altitudes, and it is floristically dominated by several plant families such as Meliaceae, Sterculiaceae, Euphobiaceae, and Olacaceae (Achoundong 1996). At highest elevations, the landscape was characterized by hydro-mesophilic and submontane forest with closed canopy, high leaf litter. These elevations were dominated mainly by seven plant families: Leguminosae, Clusiaceae, Myristicaceae, Burseraceae, Sterculiaceae, Annonaceae and Rubiaceae (Madiapevo et al. 2017). Furthermore, elevational gradients in upland areas yield a greater variety of microclimates over relatively short distances when compared to latitudinal gradients. These features could explain the high species diversity observed in this study in upland areas and why so many biodiversity hotspots are located in these habitats (Myers et al. 2000, Kollmair et al. 2005).

Conversely, urban and littoral habitats had the lowest diversity after upland habitats. As over 80% of available area of urban habitat is covered by pavement and buildings, less than 20%, remains as vegetated area (Nkwewoh et al. 2017). Likewise, littoral evergreen forest is a regenerate forest formerly threatened by logging and poaching, and has received protected status from Cameroon's government. As a result, ant assemblages found in these habitats include mixture of native ant species and disturbance specialists. This could explain the numerical and behavioural dominance of two invasive species *W. auropunctata* and *Pheidole megacephala* in urban and littoral habitats, respectively. These ant species are recognized as tramp species (Lowe et al. 2000) and exhibit specific traits (fast resource discovering, rapid recruitment of nestmates, and unicoloniality) that facilitate their spread in many regions around the world through human-mediated dispersal (Hölldobler & Wilson 1990, Passera 1994, McGlynn 1999).

Efficiency of sampling methods

The sampling success in different habitats varied between 80 and 99%. This suggest that the number of sampled species likely represents the actual number of species even if an asymptote plateau was not reached for some methods used at each habitat. In all three habitats, few samples were sufficient in baiting to reach a horizontal asymptote while others techniques would require more samples to see their curves flatten out. In practice, sampling more individuals until no new species are found and the species accumulation curve reaches an asymptote is routinely impossible. The main reason is that the number of individuals that must be sampled to reach an asymptote can often be prohibitively large (Chao et al. 2009). Although, baiting curves were the first to reach an asymptote plateau compared to others curves, it caught the fewest species during the study period. Each method seems to be specific and may record different ant foraging patterns and speeds (Bestelmeyer et al. 2000). Because activity of different ant varied with microclimate, daily and seasonally, baiting performed at different times of the day (and night) or year in the same area may attract foragers of different strata faster than others sampling methods.

Our results demonstrate that quadrat sampling was more efficient than pitfall and baiting sampling. These findings are consistent with recent studies carried out along a gradient of increasing vegetation disturbance in southern Cameroon (Fotso et al. 2015). The data recorded in quadrat sampling include richness and composition, relative abundance, frequency of occurrence in sets of quadrats, and time and duration of activity (Agosti et al. 2000). Quadrat can be used to examine hourly and daily patterns, whereas pitfall trapping sums activity over time (Bestelmeyer et al. 2000). Therefore, to obtain an adequate impression of the local ant faunae, many researchers indicated the importance of using a variety of sampling methods (Lindsey & Skinner 2000, Fisher 2002, 2004).

Specific-species traits

All sampling methods used in the present study caught 60% of epigaeically foraging species, 30.80% of hypogaeically foraging species and 9.10% of ant species either hypogaeic or epigaeic species. As a result, it appears that additional methods are needed to increase the likelihood of recording rare and hypogaeic species. With this regard, leaf litter sifting (Winkler) and dig sampling may be added and employed to ensure a more complete survey of ant fauna. Both winkler and dig sampling are especially appropriate for use in forest and woodland habitats, where many ant species inhabit the top soil and litter layer (Bestelmeyer et al. 2000). Moreover, litter sampling and dig sampling sampled the abundant and diversified leaf litter fauna including hypogaeic species, which is severely undersampled using other methods owing to the ants' cryptic habits and small foraging ranges (Greenslade & Greenslade 1971, Majer 1996).

5. Conclusion

This study showed the efficiency of three sampling for capturing soil-dwelling ants in three landscapes in tropical rainforest. Quadrat sampling was more efficient than pitfall and bait trapping. Quadrat sampling and bait trapping seem to be an optimal method in urban and littoral habitats, whereas pitfall trapping is probably relevant for the closed habitats like upland forests. Although these three methods are the most widely used methods in surveys of ant fauna in tropical ecosystems, they seem to be inadequate of recording cryptic species and hypogaeically foraging species. Further studies are therefore needed in the same habitats to evaluate a combination of these methods with those relevant to sample hidden ant fauna like winkler extraction and dig sampling.

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