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RESEARCH ARTICLE

Nest density and other observations on a population of *Aneuretus simoni* Emery, 1893 (Formicidae, Aneuretinae) and other ants in Indikada Mukalana Forest Reserve in Sri Lanka

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Abstract: The Sri Lankan Relict Ant, *Aneuretus simoni* Emery, survives in several wet zone and intermediate zone forests in Sri Lanka. Nests of this species and other ants were surveyed at 159 m and 291 m elevations in Indikada Mukalana Forest Reserve by laying 20 quadrats of 1 m × 1 m at two plots of each locality in December, 2015. The number of ant nests within each quadrat was recorded; then the frequency of nest occurrence out of 40 quadrats, percentage nest abundance and mean nest density of *A. simoni*, as well as associated ant fauna were calculated. Percentage frequency of worker ant occurrence was also investigated using pitfall traps. Eighteen genera and 21 species in Aneuretinae, Dolichoderinae, Formicinae, Myrmicinae and Ponerinae were recorded from the two methods. Nests of *A. simoni* were found only in the locality at 291 m altitude in the forest; 17.5 % of quadrats with an *A. simoni* colonies, 9.7% of nest abundance in relation to that of other ant species and 0.18 m^2 of mean nest density were observed. Nest density of *A. simoni* had the fourth rank among that of the other species. Frequency of occurrence of *A. simoni* workers in the pitfall traps at lower and upper elevations was 2% and 3%, respectively. An actualized map showing the current distribution of *A. simoni* is produced.

Key words: Pitfall trapping, quadrat method, nest survey, habitats of *Aneuretus simoni*, forest reserves, ant diversity.

Introduction

Twenty extant subfamilies of ants are currently recognized globally and the subfamily Aneuretinae has been included among the dolichoderomorphs in the formicoid clade (Ward

2007). The fossil genera, *Aneuretellus* Dlussky, 1988, *Mianeuretus* Carpenter, 1930, *Paraneuretus* Wheeler, 1915, *Protaneuretus* Wheeler, 1915 and the single extant genus, *Aneuretus* Emery, are included in the subfamily Aneuretinae (Bolton 2003). *Aneuretus simoni* Emery, 1893 ("Sri Lankan Relict Ant") is the sole extant species of the subfamily characterized with a long peduncle followed by a posterior petiolar node with dorsal and lateral swellings; it is endemic to Sri Lanka and was recently reported from several wet and intermediate zone forests in the island (Gunawardene *et al*. 2008; Dias *et al*. 2013; Karunarathna & Karunaratne 2013; Dias & Ruchirani 2014). Nest density of *A. simoni* was reported in Gilimale Forest Reserve in 1979 (Jayasuriya & Traniello 1985) and the species was reported from the forests that receive an annual rainfall of 2000 - 5000 mm and lie at the range of elevation, 57 m to above 450 m (Jayasuriya & Traniello 1985; Dias *et al.* 2013; Dias & Ruchirani 2014; Dias & Udayakantha 2016).

The "Indikada Mukalana" Forest Reserve (Fig. 1) is the second largest tropical lowland rainforest in Colombo District, Sri Lanka. It has 572 ha of extent and is situated in the Western Province of Sri Lanka. It contributes to the conservation of a number of plants and animals including many endemics (Chamikara 2011). Ants were surveyed for the first time in the Indikada Mukalana Forest Reserve and we here report on the discovery of *A. simoni* in the forest. Observations on nest density and related aspects of the species and other species in the ant community were made.

Material and methods

Description of sampling localities

The survey was conducted at Locality A in lower elevation, 159 m, and Locality B in upper elevation, 291 m, in Indikada Mukalana Forest Reserve in the Colombo District, Sri Lanka (Fig. 1). Locality A consisted of moist, clay mixed sandy floor and a taller canopy was formed by trees such as *Dipterocarpus zeylanicus* Thwaites, *Artocarpus nobilis* Thwaites, *Gyrinops walla* Gaertn. and *Pericopsis mooniana* Thwaites which shaded the area. Lower canopy was also present while the forest floor was covered with a leaf litter layer. Locality B had moist clay soil covered with a thin layer of leaf litter. Small to large pieces of decaying wood resulted from fallen trees were seen on the ground at the Locality B.

Preliminary survey for *A. simoni* workers

Honey baiting, breaking of decaying wood pieces and leaf litter sifting were conducted during daytime at Locality A in November 2015. Fifty pieces of gauze, each of 2 × 2 cm, with a drop of honey were placed throughout the area and were collected after an hour. Decaying logs and pieces of wood were broken and checked for *A. simoni* colonies and workers. Leaf litter was sifted and the ants fallen to the white tray were collected. Collected ants were preserved in labelled vials with 70% ethanol and checked for the presence of *A. simoni* workers under a low-power stereo-microscope in the field.

Ant sampling and identification

Ant nests at two 100 m^2 (10 m ×10 m) plots were surveyed at the both Locality A and Locality B that were approximately of 150 m apart in the study area in December, 2015. Rainy weather persisted before and during the sampling period. Table 1 presents the GPS coordinates of each plot surveyed at each locality. Twenty 1 m^2 quadrats were delimited by fixing four wooden pegs connected between them with a cord, at least 0.5 m away from each

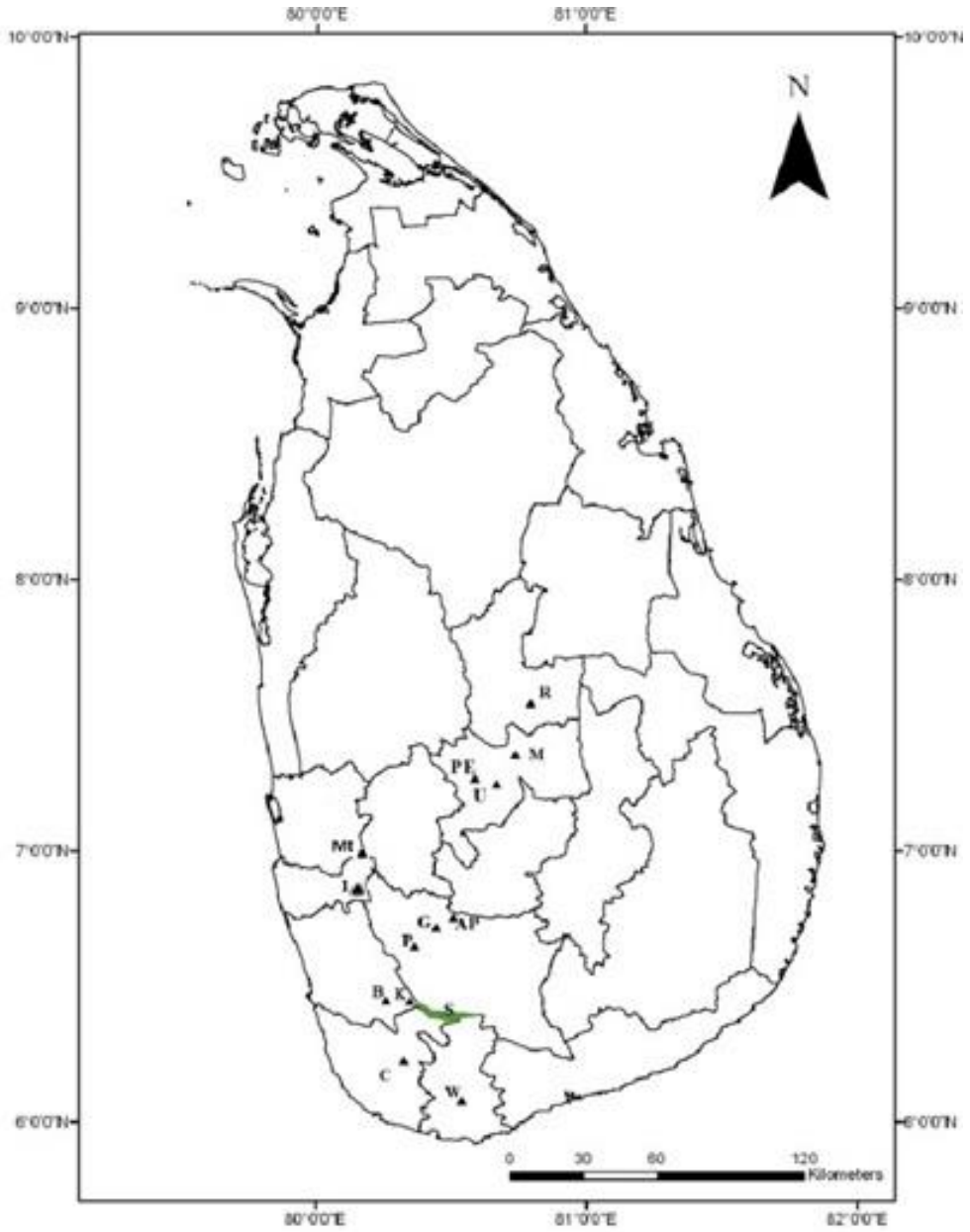

Figure 1. Map of Sri Lanka showing the location of Indikada Mukalana Forest Reserve (I) and other habitats of *A. simoni*. AP - Adam's Peak, B - Kalugala Proposed Forest Reserve, C - Kuluna Kanda Proposed Forest Reserve, G - Gillimale Forest Reserve, K - Kalugala Proposed Forest Reserve, M - Moragahakanda Forest, Mt - Meethirigala Forest Reserve, P - "Pompekelle", PE - Peradeniya, R - Rambukoluwa, S - Sinharaja Forest Reserve, U - Udawaththa Kele, W - Wilpita "Aranya Kele".

Table 1. GPS coordinates of each plot in Locality A and Locality B of Indikada Mukalana Forest Reserve.

Locality A (159 m)		**Locality B (291 m)**	
Plot 1	**Plot 2**	**Plot 1**	**Plot 2**
06°87'49.97" N, 80°16'36.90" E	06°87'49.97''N, 80°16'36.90'' E	06°87'27.47''N, 80°16'09.49''E	06°87'21.88''N, 80°16'09.06''E

other to cover each 100 m^2 plot marked at each elevation. Within each quadrat, nests of the ant species were surveyed by careful checking, breaking decaying pieces of wood, removing leaf litter and examining the soil. Three workers from each nest were preserved in the labelled vials with 70% ethanol. In addition, hundred honey-baited pitfall traps (diameter at the mouth = 7.5 cm and volume = 80 ml) (Dias & Perera 2011), were set at 4 m distance along each of the eight transects laid at each elevation outside each 100 m^2 plot marked for the ant nest survey. All pitfall traps were collected after 6 hours and collected ants were preserved as already indicated.

Worker ants were identified to the furthest taxonomic level using a low-power stereo-microscope with reference to Bingham (1903), Bolton (1994), Dias (2014), Schmidt & Shattuck (2014) and Sarnat *et al*. (2015). Antennae, mandibles, maxillary palps and legs of workers were dissected out when necessary and observed under a high-power microscope with a drop of glycerol. All voucher specimens are kept in the reference collection maintained at the Department of Zoology and Environmental Management, University of Kelaniya, Sri Lanka.

Measurement of environmental parameters

Environmental parameters were measured at three representative places in each plot within two localities and mean values were calculated. Air and soil temperature was measured using a digital thermometer. The depth of leaf litter was measured using a ruler. Three soil samples from each plot were collected into polythene bags; a known weight of soil from each sample was dried in an oven at 105°C until a steady dry weight was observed, and the soil moisture content was calculated according to Brower *et al*. (1998). Soil organic matter content was determined according to Sutherland (2006). Rainfall for the period of sampling recorded by the nearest available meteorological station at Labugama was obtained from the Department of Meteorology in Colombo.

Estimation of nest density, frequency of nest occurrence and percentage nest abundance of each ant species and the frequency of occurrence in pitfall traps

The nest density (ND = number of nests of the species per locality/ sum of the quadrat areas at the locality (40 m^2)), frequency of nest occurrence (FNO = number of quadrats with nests of the focal species/ total number of quadrats laid) and the relative nest abundance (NA% = number of nests of the focal species / total number of nests of all species) were calculated for each species. Significant differences, if any, among FNO values were tested using Chi-Square Homogeneity Test. Rank-abundance diagram was drawn for the mean nest density observed for each ant species. Percentage frequency of occurrence of workers of each species (FO% = Number of pitfall traps with the workers of a species; total number of pitfall traps fixed was 100) is also presented. One Way Analysis of Variance followed by Tukey's test (Minitab 14.0) was conducted to test significant differences among the log transformed mean nest density values. Mean values of soil temperature, depth of leaf litter, soil moisture content, soil organic matter content and rainfall were calculated.

Results

Preliminary observations, frequency of nest occurrence and percentage nest abundance

Workers of *A. simoni* were found in several samples collected by honey baiting in the preliminary survey. Twenty one ant species of 15 genera in 5 subfamilies, Aneuretinae,

Dolichoderinae, Formicinae, Myrmicinae and Ponerinae, were observed at the two localities of Indikada Mukalana Forest Reserve in December, 2015 (Table 2). Nests of *A. simoni* were encountered at Locality B only (Table 2). Frequency of nest occurrence (FNO) and percentage nest abundance (NA %) of ant fauna observed at Locality A and Locality B are presented in Table 2. The highest FNO and NA values were observed for *Odontomachus simillimus* and *Technomyrmex bicolor* whereas *Technomyrmex albipes* had the second highest FNO and NA at Locality A. However, FNO and NA values of the species, at the Locality A, were not significantly different (Chi-square Test, $p>0.05$). *Odontomachus simillimus* had the highest FNO and NA values at the Locality B while *Pheidole noda* had slightly lower FNO and NA values. Significant difference was not evident between FNO or NA values of the ant species observed at the Locality B (Chi-square Test, $p<0.05$).

Table 2. Relative frequency of occurrence (FO %), nest abundance (NA %), nest occurrence (FNO), relative nest abundance (NA %) and mean nest density per m^{-2} (MND) of each species observed by pitfall trapping and quadrat method in Indikada Mukalana Forest Reserve.

Subfamily	Ant genus/ species	Pitfall Trapping		Quadrat Method					
		Locality A FO%	Locality B FO%	Locality A			Locality B		
				NA %	FNO	MND m^{-2}	NA %	FNO	MND m^{-2}
Aneuretinae	*Aneuretus simoni* Emery, 1893	2	3	-	-	-	9.7	7/40	0.18
Dolichoderinae	*Technomyrmex albipes* Smith F., 1861	8	1	13.8	18/40	0.45	4.2	3/40	0.08
	Technomyrmex bicolor Forel, 1909	12	16	16.2	21/40	0.53	8.3	6/40	0.15
Formicinae	*Camponotus* sp.	-	1	-	-	-	-	-	-
	Nylanderia sp.	1	-	-	-	-	-	-	-
	Oecophylla smaragdina (Fabricius, 1775)	-	1	-	-	-	-	-	-
	Paratrechina longicornis (Latrielle, 1802)	-	3	-	-	-	2.8	2/40	0.05
	Polyrhachis (*Hemioptica*) *bugnioni* Forel, 1908	1	1	6.2	8/40	0.2	1.4	1/ 40	0.03
Myrmicinae	*Cardiocondyla nuda* Mayr, 1866	-	16	1.5	2/40	0.05	8.3	6/40	0.15
	Carebara sp.	2	2	-	-	-	-	-	-
	Myrmicaria brunnea Saunders, 1915	-	10	6.2	8/40	0.2	12.5	9/40	0.23
	Pheidole sp. 1	12	-	5.4	7/40	0.18	8.3	6/40	0.15
	Pheidole sp. 2	1	24	0.8	8/40	0.03	4.2	3/40	0.08
	Pheidole noda Smith F., 1902	16	22	10.8	14/40	0.35	-	-	-
	Solenopsis sp.	1	9	9.2	12/40	0.3	2.8	12/40	0.05
	Tetramorium bicarinatum (Nylander, 1846)	-	2	3.1	4/40	0.1	-	-	-
	Tetramorium smithi Mayr, 1879	-	-	0.8	1/40	0.03	-	-	-
	Tetramorium walshi (Forel, 1890)	-	3	1.5	2/40	0.05	-	-	-
Ponerinae	*Odontomachus simillimus* Smith F., 1858	19	19	16.2	21/40	0.53	19.4	14/40	0.35
	Mesoponera melanaria (Emery, 1893)	-	4	3.1	4/40	0.1	-	-	-
	Pseudoneoponera rufipes (Forel, 1911)	-	-	-	-	-	1.4	1/40	0.03

Mean nest density and the rank of *A. simoni*

Mean nest density (MND) of *O. simillimus*, *T. bicolor* and *T. albipes* was significantly higher (One Way ANOVA followed by Tukey's Test; $p < 0.05$) than that of other species observed at Locality A (Table 2). The ranked mean nest density (MND) of each ant species observed at Locality B is shown in Figure 2. The MND of *A. simoni* was significantly lower (One Way ANOVA followed by Tukey's Test; $p < 0.05$) than that of *O. simillimus* and had the fourth rank whereas that of *O. simillimus*, *P. noda* and *M. brunnea* had the rank of 1-3, respectively. Nests of *A. simoni* were observed only in decaying wood pieces seen within the quadrats laid at Locality B.

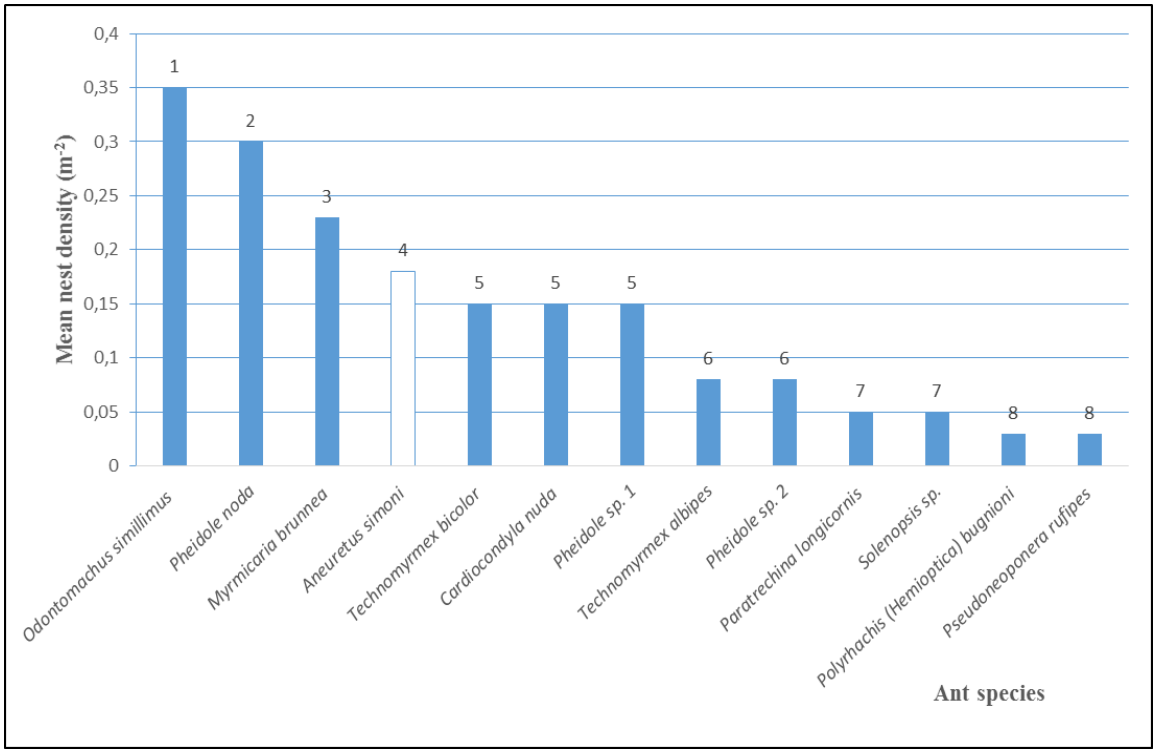

Figure 2. Ranked mean nest density of each ant species observed at Locality B of Indikada Mukalana Forest Reserve in December, 2015.

Table 3 shows that lower soil moisture content and more leaf litter were evident at the Locality B. Mean rainfall for November and December recorded at Labugama station was 392 mm.

Table 3. Mean environmental parameters observed at each elevation in Indikada Mukalana Forest Reserve in December, 2015.

Environmental parameter	Locality A	Locality B
Soil moisture content (%)	18.7 ± 0.60	13.3 ± 0.69
Soil organic matter content (%)	7.3 ± 0.07	8.2 ± 0.07
Soil temperature (ºC)	24.3 ± 0.06	25.3 ± 0.0
Air temperature (°C)	26 ± 0.0	27± 0.0
Depth of leaf litter (cm)	0.5 ± 0.50	4 ± 0.87

Frequency of worker occurrence in pitfall traps

Workers of *A. simoni* were observed in the pitfall traps fixed at both elevations (Table 2) and FO % of *A. simoni* was lower at each locality than those observed for several other species.

Discussion

The occurrence of *A. simoni* nests is reported for the first time in Indikada-Mukalana Forest Reserve in the Colombo District of western Sri Lanka. Nests of the species were found only at Locality B but pitfall trapping showed the presence of foraging workers in Locality A indicating the possibility for further nest surveying in this area. The nest density of *A. simoni* recorded at Locality B of the current Forest Reserve was lower than that recorded from Kuluna Kanda Proposed Forest Reserve (Dias & Ruchirani 2014), Kalugala Proposed Forest Reserve and Wilpita "Aranya Kele" (Dias & Ruchirani 2014) but higher than those observed at Kirikanda Forest (Dias *et al*. 2013) in 2010. The rank of *A. simoni* nest density at Locality B of Indikada-Mukalana Forest Reserve was higher than those observed for nine other ant species, in turn, indicated that it was a major component of the ant community of the locality although further nest sampling at the Forest Reserve should be conducted to confirm this observation. Heavy raining persisted for about a month prior to sampling period and also within the sampling period; surface run off had washed away leaf litter and decaying twigs and wood pieces in the Locality A. Also, the sand and silt had covered the forest floor in the sampling area due to the overflowing of the tributary running through the region (personal observation, Mr. Sudesh Udayakantha; Dias & Udayakantha 2016). Washing away of ideal substrates for nesting by the surface run-off may have led to the devoid of *A. simoni* nests at the Locality A. The range of elevation recorded recently for the species was 57 m (Meethirigala Forest Reserve) to 250 m ("Kirikanda" Forest) (Dias & Udayakantha 2016) and the current finding extends the upper elevation to 291 m in the wet zone of Sri Lanka.

Indikada-Mukalana Forest Reserve receives an average annual rainfall of about 2,000 – 3,000 mm (personal communication, Department of Meteorology, Colombo, Sri Lanka, 31 July, 2014) which is comparable with the range of annual rainfall of the previously recorded habitats, 2,000 – 5,000 mm in Gilimale Forest Reserve (Jayasuriya & Traniello 1985), 2,000-3,000 mm in Kirikanda forest (Dias *et al*. 2013) but lower than the annual rainfall, 4,000-5,000 mm in Kalugala proposed Forest Reserve (Dias & Ruchirani 2014). Soil temperature, air temperature and the litter depth recorded at the time of detection of *A. simoni* at the current Forest Reserve were comparable with those recorded in Gilimale Forest Reserve (Dias & Perera 2011), Sinharaja Forest Reserve (Perera *et al.* 2006) and Kirikanda Forest (Dias *et al.* 2013) but a lower soil moisture content than that of other habitats was observed at Indikada-Mukalana Forest Reserve in December, 2015.

Similar types of microhabitats to that reported in other habitats of *A. simoni* such as hollow cavities of decaying fallen twigs, leaf litter and bark of rotting logs (Wilson *et al*. 1956; Jayasuriya & Traniello 1985; Dias *et al*. 2001; Dias 2004; Perera & Dias 2004) were only observed at the current locality. There is an urgent need for the re-assessment of the local and global status of *A. simoni* (Dias 2014), after repeating the survey in the current Forest Reserve located in Colombo District and conducting a similar survey in a Forest Reserve in the remaining wet zone District, Kegalle, in Sri Lanka, in the future.

Aneuretus simoni is a resident species with a nest density of 0.18 m^{-2} at Locality B in December, 2015, at 291 m elevation of Indikada-Mukalana Forest Reserve in Colombo District that lie in the Western Province of Sri Lanka and it survives in several Forest

Reserves and disturbed forests in Southern, Western, Sabaragamuwa and Central Provinces of Sri Lanka; favourable environmental conditions are above 2,000 mm annual rainfall, 26 ºC – 29 ºC air temperature range, 24 ºC – 29 ºC soil temperature range, 13 % – 40 % soil moisture range, 4 cm or a higher litter depth and 8 % soil organic matter %.

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