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Earthworm functions
VIII. — Population estimation techniques[1]

BY

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Synopsis: The paper deals with a review of earthworm sampling techniques. A comparison of three methods over three years (weekly sampled) provides an assessment of biases versus species, season, stages of development, activity level.
Key words: sampling, coring, gathering, faunal analysis, earthworm, activity, season.

INTRODUCTION

Earthworms play a major role in terrestrial ecosystems. They constitute the largest fraction of animal biomass in most soils and are important in the decomposition of organic matter. An accurate assessment of the populations of earthworms is a prerequisite for a quantitative estimation of their ecological rôle. In other words, knowledge of the population structure, and its changes with time, is necessary to estimate demographic patterns such as rate of birth, death, growth, etc. These population parameters provide the

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necessary foundation for using physiological parameters (e.g., respiration data measured under field conditions) in an ecological context without the danger of serious bias.

To adequately estimate and compare population parameters it is necessary to define: a) the sampling procedure used ; b) the capture and sorting techniques used to isolate earthworms from other material in the soil; c) the measurement of individual earthworms (i.e., stage, weight, etc.), and d) the mathematical treatment of the data. Although all these steps may vary with the aims of a particular study, the greatest difficulty is encountered with the capture and sorting techniques. The purpose of this paper is to compare the merits and faults of various methods of sampling earthworm populations.

Three general approaches have been used in attempting to estimate the number of earthworms living in the soil: 1. physical methods (direct capture of earthworms by taking soil cores); 2. ethological methods (physical or chemical methods used to expell earthworms from the soil); and 3. indicator methods (indirect measures of estimating earthworm numbers). The only type of indicator method used has been pitfall traps (BOYD, 1957). Since the success of pitfall trapping depends on unknown activity factors and species specific behavior, this method captures a very small fraction of the earthworm community (BOUCHÉ, 1976 *a*). The results are nearly impossible to use as an adequate representation of population levels. Earthworm casts are sometimes used to estimate population densities, but because of their ephemeral nature they are unreliable indicators. Capture-recapture methods have been used for migration and survival studies (MAZAUD and BOUCHÉ, 1980), but this method disturbs the natural populations (communities) by introducing additional labelled earthworms. Therefore, this paper will consider only physical and ethological methods of sampling earthworm populations.

I. — DATA COLLECTION

A) Review of sampling methods.

The literature comparing sampling techniques is scattered and requires, at least, an understanding of English, French and German. Because biases may result from an incomplete review of available information, we have attempted to compile citations of the available techniques (Tab. I) and compare the results (Tab. II; abbreviations from BOUCHÉ, 1975). Expellents such as mercury chloride (EATON and CHANDLER, 1942), heating the soil (KEMSON *et al.*, 1963), copper sulfate or ammonia (BOUCHÉ, 1978), which have been used alone, and thus can not be compared, are not included. We will not discuss problems related to random sampling, size of sampling units (see WILCKE, 1955; ZICSI, 1957; VAILLAUD, 1963), temporo-spatial distribution of samples, biases which depend on the structure of the population, or the accuracy and purpose of a particular study.

All techniques reviewed use at least two of the three following steps:

1. Coring: removal of soil samples from the field.

2. Extraction: separation of earthworms from the soil, by soil fractioning, by the use of expellents, etc.

3. Collecting: manual removal of earthworms from soil samples or the soil surface.

Before analyzing the efficiency of any method, it is necessary to recognize the inherent limitations of the procedure (for details see BOUCHÉ, 1969 *a*, *b*). Coring is nearly

TAB. I

Techniques used for quantitative estimates of earthworm populations

First Author	Name	Abbreviation	Coring	Extraction	Collecting
HENSEN, 1877.......	direct hand sorting	bm	yes	no	yes
WALTON, 1933.......	electricity expellent......	el	no	yes	yes
?, 1930-35...........	mowrah meal expellent	mm	no	yes	yes
DAWSON *et al.*, 1938..	K-permanganate expellent	pp	no	yes	yes
GUILD, 1951.........	vibration expellent	vi	no	yes	yes
RAW, 1959..........	formaline expellent	fo	no	yes	yes
RAW, 1960..........	washing/sieving/flotation	bf	yes	yes	yes
KEMPSON *et al.*, 1963.	wet heating/expellent	bh	yes	yes	yes
BOUCHÉ, 1969........	washing/sieving/automatic	bl	yes	yes	yes

always limited because the deeper soil horizons are not sampled. Earthworms will usually colonize the first 1 or 2 meters of the soil but may be found as deep as 6 meters below the surface. Coring is sometimes impractical because of disturbance to the study site, presence of rocky soils, interference of tree roots, etc. Separation of earthworms from the soil samples can be done by physical methods (washing, sieving, elutriation, density separation in liquids, etc.) or extraction can also be performed by the movement of earthworms themselves, using their behavior under the action of various expellents. It is obvious that ethologic methods are an ineffective means of sampling lethargic earthworms or cocoons.

NELSON and SATCHELL (1962) have compared the efficiency of manual separation of earthworms from the soil by introducing a known number of animals into the soil sample, and then subjecting the sample to two successive hand sortings. This work was done with earthworms previously selected by hand sorting and not on an actual population. AXELSSON *et al.* (1971) made comparisons of two successive hand sortings of the same soil sample. In neither case was an accurate estimate of the population available. These comparisons are incapable of estimating the efficiency of hand sorting.

Collection is affected by many factors, the most important being the ability of the collector, the surrounding medium (it is easier to separate worms from a water washed sample), the behavior of earthworms, and the conspicuousness of the earthworms (sizes may range from 0.6 to 1200 mm, and color may be more or less similar to the soil). Table II shows the results of different comparisons, most of which are based on a few sample-units collected on the same date. The relative efficiency of any method varies greatly with seasons (BOUCHÉ, 1969, 1975). Comparisons are rarely conducted at the species level for both biomass and number estimates, and do not take into account the quantity of cocoons present. The efficiency of direct hand sorting, made on samples previously soaked by a solution of formalin, K permanganate, etc., is also affected, especially when the soils is muddy.

A general comparison of the data available for the various techniques gives the following classifications of their efficiencies: [vi $<$ mm = pp = el $<$ fo $<$ bm $<$ bf $<$ bl] (symbols defined in Tab. I). Ethological methods are poorer than physical ones. For this reason, most studies have combined coring with hand collecting (PEREL, 1958, VAN RHEE and NATHANS, 1961, ZICSI, 1962, GRAFF, 1964, ZAJONC, 1970, LAVELLE 1971, etc.). Fre-

TAB. II

Comparisons of available sampling methods

Techniques are ethologic (ch : heating, el : electricity, fo : formaline, mm : mowrah meal, pp : potassium permanganate, vi : vibration), physic (bl : digging-washing on sieve, bm : hand sorting). Sample

Authors		Techn.	Sample				
			SU Da	N Da	Surf.	Vol.	Deepth
DAWSON *et al.*,	1938	mm	5 to 6	3	84	—	—
		pp	30 to 40	3	280	—	—
EVANS et GUILD,	1947	pp	10	1	5.3	—	—
		bm after pp	10	1	5.3	?	?
GUILD,	1951	vi	?	?	?	?	?
		pp	?	?	?	?	?
SATCHELL,	1955	el, pp and bm		complex experiment, no obvious conclusions			
SVENDSE,	1955	pp	10	1 (?)	10	—	—
		bm	10	1 (?)	1.41	0.28	20
RAW,	1959	pp	10	1 (?)	3.7	—	—
		fo	10	1 (?)	3.7	—	—
		bm	16	1 (?)	0.6	0.12	20
		pp	10	1	3.7	—	—
		fo	10	1	3.7	—	—
		bm	8	1	0.3	0.6	20
		fo 1	7	1	3.0	—	—
		fo 2	7	1	3.0	—	—
		pp	7	1	3.0	—	—
RAW,	1960	bm	9	?	?	?	?
		bl after bm	9	?	?	—	—
		bm	?	?	?	?	?
		bl after bm	?	?	?	—	—
		bm	?	?	?	?	?
		bl after bm	?	?	?	—	—
BOUCHÉ,	1969a	fo	20	3	30.0	—	—
		bl_{20} after fo	20	3	1.875	0.375	20
		bl_{60}	20	3	7.5	3.00	60
BOUCHÉ,	19691, b	fo	20	9	90.0	—	—
		bl_{20} after fo	10	9	5.625	1.125	20
SATCHELL,	1969	ch	?	?	?	?	10
ABRAHAMSEN,	1972	ch	?	?	?	?	?
		bm after ch	?	?	?	?	?
DASHS and PATRA,	1972	pp 1	10	1	0.625	—	—
		bm after pp 1	10	1	0.625	0.125	20
		pp 2	10	1	0.625	—	—
		bm after pp 2	10	1	0.625	0.125	20
		fo	10	1	0.625	—	—
		bm after fo	10	1	0.625	0.125	20
		bm	10	1	0.625	0.125	20
BOUCHÉ,	1975a	fo	4	58	116.0	—	—
		bl_{20} after fo	4	58	23.2	4.64	20
		bl_{60}	4	58	23.2	13.92	60
BOUCHÉ,		fo	4	109	218.0	—	—
		bl_{20} after fo	4	109	43.6	10.325	20
		bl_{60}	4	109	43.6	29.850	60

are constituted by a number of sample-unit per day (SU Da) repeatedly made, a number of day (N Da) giving a total surface in m^{-2} (Surf) or volume in m^{-3} (Vol) treated; deepth of coring is given in cm. Total number of animals was analyzed at species (Sp) or not (No) level, taking cocoons (Coc) and biomasses (Bm) in account or not. Number of catched individuals given in column « Number catched » is total or, if a cross is, the result of the last technique applied. Relative efficiency (Ef % N) of a first technique versus a second one is given in percentage of numbers.

Number catched	Anal.	BM	Coc.	Site	Ef % N	Author's conclusions
4 088	No	+	—	grass	mm/pp = 78.07	mm ≃ pp
9 827	No	+	—			
1 090	Sp	—	—	pasture	pp/bm+pp = 97.00	pp ≃ ph (difficult because watering)
+34	Sp	—	—			
?	No	—	—	pasture	—	vi < pp
?	No	—	—			
						pp ≃ el
644	Sp	—	—	pasture	pp/bm = 14.4	pp < < bm
639	Sp	—	—			
325	Sp	+	—	arable	pp/bm = 422.1	
597	Sp	+	—	light		
77	Sp	+	—	soil	pp/fo = 54.4	fo > pp
839	Sp	+	—	grass orchard	pp/bm = 30.00	deepth of bm
1 651	Sp	+	—	light	fo/bm = 59.00	too small
2 800	Sp	+	—	soil		
850	Sp	+	—	lawn		fo 1 ≠ fo 2
403	Sp	+	—	heavy		(two formaline concentration)
294	Sp	+	—	soil		
256	Sp	+	—	pasture, wet	bm/bl = 52.00	
+234	Sp	+	—	shallow soil		
?	?	+	—	arable orchard	bm/bl = 89.00	bl > bm
?	?	+	—	light soil		
?	?	+	—	grass orchard	bm/bl = 59.00	
?	?	+	—	light soil		
1 662	Sp	+	—	grassland	fo/bl_{60} = 14.06	fo < fo bl_{20}
+513	Sp	+	—	silt soil		
2 663	Sp	+	—		$fo+bl_{20}/bl_{60}$ = 83.5	fo bl < < bl_{60}
—	Sp	+	—	grassland	fo/fo+bl = 26.7	
—	Sp	+	—	silt soil		
?	Sp	+	—	Deschampsia flexuosa	?	fo < ch > bm for Bimastos eiseni
575	No	—	—	coniferous forest	ch/ch+bm = 95.00	ch < < bm
+25	No					
370	No	—	—	?	pp 1/pp 1+bm = 20.1	
+1 470	No	—	—	?		
410	No	—	—	?	pp 2/pp 2+bm = 24.5	pp > fo
+1 260	No	—	—	?		bm > pp
240	No	—	—	?	fo/fo+bm = 17.4	
+1 140	No	—	—	?		
1 680	No	—	—	?		
—	Sp	+	+	grassland	fo/bl_{60} = 39.4	fo < fo, bl_{20} < bl_{60}
—	Sp	+	+	grassland	fo, bl_{20}/bl_{60} = 74.6	possible corrections
—	Sp	+	+	grassland		
25 705	Sp	+	+	grassland	fo/bl_{60} = 16.23	fo<< fo, bl_{20} < bl_{60} and fo, bl_{20} >
+17 298	Sp	+	+	grassland	fo, bl_{20}/bl_{60} = 82.31	bl_{60} sometimes
24 898	Sp	+	+	grassland		

quently this is all done in the field, but sometimes the collecting is done with a soil sieving, washing, and extraction process (RAW, 1960, GERARD, 1967, LAVELLE, 1978). This latter method is laborious and impracticable where manpower is limited.

B) Sampling methods.

For the above reasons we developed, in 1965, an automated washing-sieving procedure (BOUCHÉ and BEUGNOT, 1972). Separation of earthworms by this method is nearly 100 % effective, because hand sorting from a mixture of stones, roots, leaves, earthworms and other microfauna is done in the laboratory under good working conditions. The result is that good information on soil standing crops is provided (BOUCHÉ, 1972 a). However, this technique has some limitations common to all coring and collecting methods; e.g., some small cocoons and larvae can be confused with stones, roots, and other debris, and sampling is still limited to 60 cm depth. Attempts to improve this process by using both elutriation and flotation have had little success (BOUCHÉ and BEUGNOT, 1972).

Because most cocoons lie in the 0-20 cm horizon (see also GÉRARD, 1967) it was thought that perhaps formalin expelled only a small fraction of the fauna from the surface layers. The remaining fauna could have moved into the upper soil layers but, because of light and/or other adverse surface conditions, they may have failed to emerge. This hypothesis was verified and gives a basis for combining the advantages of the formalin application with digging-washing-sieving to 20 cm (BOUCHÉ, 1969). This combined method will be called the ethophysical technique (the name describes the order of operations — ethological and then physical). The ethological technique using formalin as an expellent was recommended for and used by International Biological Program (SATCHELL, 1970). Thus, we compare three combinations of physical and ethological sampling methods:

1. Physical: soil collection to 60 cm depth with automated washing and sieving of the sample.

2. Ethological: formalin application technique.

3. Ethophysical: combined formalin application followed by soil sampling to 20 cm depth.

Details of the total procedure are given in the following section with abbreviations after BOUCHÉ, 1975 (i.e., bl_{60} for physical collection to 60 cm, fo for formalin sampling, and fo-bl_{20} for formalin followed by soil sampling to 20 cm).

C) Methods.

The study was conducted from November 28, 1966 to May 26, 1970. The study site, Cîteaux (BOUCHÉ, 1975), has been a permanent grassland since at least 1840. Soil samples were taken with hand spades according to the dimensions indicated in Table III. Sample-units were distributed in time and space following a grid which will be described in a later publication. It is only necessary to know that sample-units for the three techniques were taken close together on the same day, with 2 repetitions of each technique taken in the same plot, and two plots sampled on the same date (Tab. III). The same plot was never sampled twice, and adjacent plots were never closer than 5 meters.

Thus, for each date five sets of samples were taken: 1. formalin application (fo); 2. soil removal to 20 cm after formalin application (fo-bl_{20}); 3. soil removal to 20 cm (bl_{20}); 4. soil removal from 20 to 40 cm (bl_{20-40}); 5. and soil removal from 40 to 60 cm (bl_{40}-bl_{60}). A total of 94 samples were taken with a total surface area of 338.3 m^2 (fo = 86.5 %, including fo-bl_{20} = 13.30 %, and bl_{60} = 13.46 %). The total volume of soil sampled was = 40.18 m^3 with 25.7 % for fo-bl_{20} method.

The bl samples were treated by the automatic washing and sieving method of BOUCHÉ and BEUGNOT (1972). The earthworms, cocoons and hatched cocoons of each sample unit were carefully examined and individually characterized by weight, taxon, stage, substage

TAB. III

Surface and volume of sample per date. N.D. = number of dates of sampling; Loc = number of location sampled each date; Sudm² = surface of a sample unit in dm²; Sudm³, volume of a sample unit in dm³; Nloc = number of sample unit per location; (1) fo-bl$_{20}$ method use fo-method unit sample + one bl$_{20}$ sample here quoted; (2) bl$_{20}$ was always divided in 3 soil layers (0-20, 20-40, 20-60 cm depth); (3) the surface of the sample unit 0-20 cm layer was 2 times that of sample unit 20-40 and 40-60

Date	N.D.	Loc	fo-method		fo-bl$_{20}$ method (1)			bl$_{60}$ method (2)			
			Sudm²	Nloc	Sudm²	Sudm³	Nloc		Sudm²	Sudm²	Nloc
28/11/66 to 14/2/67......	3	10	50	2	6.25	12.5	1	(3)	25	50	1
									12.5	50	1
									(25)	100	1
6/04/67 to 5/03/67.......	8	10	50	2	6.25	12.5	1		0	0	0
8/04/67 to 22/04/67......	3	2	50	2	6.25	12.5	2		6.25	37.5	2
29/04/67 to 23/11/67.....	110	2	50	2	10.00	20.00	2		10.00	60.0	2

and state. Weight was established as fresh weight, full gut (ppho) or weight after fixation in a 4 % solution of formalin with full gut (ppfo; codes from BOUCHÉ, 1975 b). Taxa (i.e., species, subspecies or varieties) follow BOUCHÉ (1972 b) with a few modifications (BOUCHÉ, 1976 b). All earthworms were preserved in a 4 % solution of formalin, weighed, and then the weights were corrected and expressed as « alive with gut content » (pphc).

The stages accounted for are cocoons, larvae, subadults, and adults. More precise substages were recorded, but are not presented here. The state of the earthworms indicates whether it is in diapause, regenerating, or recently wounded. To avoid any confusion, we will later discuss earthworms numbers for all stages, as well as postembryos for larvae, subadults, and adults. Individuals that were amputated by the sampling process were also identified if they had a head, but they were not individually weighed. For each sample-unit, postembryos were grouped together and weighed. A total of 68305 individuals were collected, 37.6 % by the fo-method, 24,3 % by bl$_{20}$ after formalin application, and 37.0 % by bl$_{60}$ method. 2261 pieces of postembryos were also weighed.

Table IV shows the number of animals identified by taxon (0 sum). Some taxa are created here for convenience, i.e., *Nicodrilus immature (N. immatures)* for cocoons and larvae of the two anecic species *N. longus* and *N. nocturnus*. Some adults were initially confused under the label *Allolobophora terrestris* (Sav.) (BOUCHÉ, 1969 a), but are separated here in an *N. anecic* group. *Allolobophora rosea* and *A. icterica* larvae were grouped under the label *A. larvae*. Larvae of *Lumbricus* could not be separated, but only one species occurs in Cîteaux, so all larvae with a weight ppfo $<$ 300 mg were attributed to *L. castaneus*. The total number (0 sum) also includes any hatched cocoons that were observed.

When a taxon is represented in the total sample by less than 400 individuals (less than 0.6 % of the community) this species is no longer listed (TA, Tabl. IV). These rare species total only 1.1 % of the individuals captured. Non-identifiable individuals (4.3 %) were later prorated to identifiable taxa on the basis of numbers present (see section F).

TAB. IV

Animal catched in Cîteaux. Observed (O Sum) taken in account (T.A.) for subsequent estimates as sum of all daily estimates per square meter (Sum²) including cocoons. Reducer numbers (from only 113 dates) analyzed later (RC Sum²) includes full cocoons (C Sum²) and hatched cocoons (H sum²). Wmg: width of weight classes in mg

Full Name	Short Name	O Sum	T.A.	Sum²	RC Sum²	C Sum²	H Sum²	Wmg
Nicodrilus caliginosus caliginosus (Sav.) var *paratypicus* B.	*N. caliginosus*	2 093	+	3 501.7	3 448.2	1 919.0	57.5	60
Nicodrilus nocturnus (Evans) var. *cistercianus* B	*N. nocturnus*	2 988	+	3 894.8	3 874.0	0.0	0.0	240
Nicodrilus longus longus (Ude).	*N. longus*	4 020	+	6 252.9	6 210.0	0.0	0.0	300
Nicodrilus cocoons and larvae of anecic species..........	An. (immatures)	27 216	+	43 418.5	40 865.3	20 031.7	2 735.5	240
« *Allolobophora* » *rosea rosea* (Sav.)...................	*A. rosea*	3 561	+	6 583.5	6 374.5	3 276.0	117.5	47
Allolobophora icterica icterica (Sav.)...................	*A. icterica*	4 785	+	8 385.4	7 877.0	966.5	180.0	94
« *Allolobophora larvae* ».......	*A. larvae*	7 969	+	8 174.3	7 111.7	0.0	0.0	23
Allolobophora chlorotica chlorotica (Sav.) *typica* (= green)..	*A. chlorotica* T.	19		—	—	—	—	
Allolobophora chlorotica chlorotica (Sav.) *albinica*.........	*A. chlorotica* A.	316		—	—	—	—	
Lumbricus castaneus (Sav.) *typicus*.....................	*L. castaneus*	11 317	+	18 905.2	18 329.9	13 372.1	207.5	35
Lumbricus terrestris L. (em. Sims) = *L. herculeus* (Sav.)..	*L. terrestris*	399		—	—	—	—	
Lumbricus larvae...........	*L. larvae*	746	+	—	—	—	—	
Dendrobaena mammalis (Sav.).	*D. mammalis*	41		—	—	—	—	
Dendrobaena octaedra (Sav.)...	*D. octaedra*	1		—	—	—	—	
Haplotaxis gordioides (Hartm.).	*H. gordioides*	2		—	—	—	—	
Non-identifiable............		2 832	+	—	—	—	—	
TOTAL...............		68 305						

D) Data management.

The data were treated in the following manner:

1. For each data set, the original information for the different sample-units were added to constitute a single prelevat (a prelevat is the sum of the sample units for a given date and defines the space dimensions from which the population estimates will be derived (see BOUCHÉ, 1975 c). This procedure was carried out for all analysis versus time and vertical distribution, movement, etc. For this kind of analysis the sample-units are simply sub-prelavats.

2. Means per m² were established for each taxon taken into account by set, stage, and date. Sums of all dates and taxon are given in Table III (Sum²).

3. Because of the variations in sampling procedure and the initial confusion of the two anecic *Nicodrilus*, we have choosen to compare techniques only from April 29, 1968

to November 23, 1970. Preliminary comparisons have been made for some previous samples (BOUCHÉ, 1969 a, 1969 b). The consideration of only 113 sampling dates (one per week for more than two years) gives a new daily square-meter estimate for all sets (RC sum^2), including cocoons (C sum^2) and hatched cocoons (H sum^2, Tab. IV).

4. Earthworms were classified into 12 weight classes. Class 0 for worms cut during sampling (not weighed), Class 1 to 10 for equal weight intervals, and Class 11 for the largest specimens. Limits for the weight classes were chosen so that 1 to 2 % of the individuals fell into class 11 (Tabl. IV).

5. The daily values were prorated on the following basis:

a) Individuals identified by taxon but not identifiable by stage were assigned to known stages according to the proportion of that stage present for that day, set and taxon.

b) Individuals that were amputated and not weighed were assigned to weight classes according to the proportion of that class present for that day, stage, taxon and set. Because this calculation was made for each taxon, set, date, and stage, rounding errors

TAB. V

Animal catched in Cîteaux, by taxon, without cocoons, in sum of the daily, per square meter, estimates (R Sum^2), after proration (P Sum^2). Effect of proration in percent (DP %). Percentage of correction applied to Set 1 (PC 1), Set 2 (PC 2), Set 3 (PC 3), Set 4 (PC 4), Set 5 (PC 5) and resulting percentage on fo-bl$_{20}$ (PC T1) and bl$_{60}$ (PC T2) Results in sum of daily, per square meter estimates (PC Sum^2), increase versus R Sum^2 (C %) and difference with 4.33 % (D %)

Short Name	R Sum^2	P Sum^2 DP %	PC 1	PC 2	PC 3	PC 4	PC 5	PCT 1	PCT 2	PC Sum^2	C %	D %
N. caliginosus...	1 471.7	1 400.2 — 4.86	4.40	10.78	11.42	20.24	7.87	7.29	12.11	1 532.52	4.13	0.20
N. nocturnus....	3 874.0	3 540.0 — 8.62	4.38	19.28	15.66	18.16	13.84	11.12	16.11	4 027.25	3.96	0.37
N. longus.......	6 210.0	5 787.3 — 6.81	4.39	13.37	11.46	15.09	16.73	9.97	12.94	6 460.02	4.03	0.30
An. (immatures).	18 098.1	18 764.1 + 3.68	4.24	3.41	0.87	2.57	-5.27	1.52	0.18	18 910.81	4.49	0.16
A. rosea........	2 981.0	2 936.5 — 1.49	4.35	6.28	5.88	6.30	6.07	5.74	5.93	3 108.13	4.26	0.07
A. icterica......	6 730.5	6 615.4 — 1.71	4.35	6.09	6.04	7.29	6.82	5.75	6.31	7 016.98	4.26	0.07
A. larvae.......	7 111.7	6 908.7 — 2.85	4.41	7.77	7.23	9.99	10.08	6.95	7.53	7 410.68	4.20	0.13
L. castaneus....	4 750.3	4 850.0 + 1.05	4.06	0.53	1.99	0.20	-1.86	2.97	1.87	4 750.3	4.42	0.09

introduced an unexpected bias. Some individuals which were amputated during sampling were « lost » because no whole animals were recorded for that day and stage; hence, they could not be prorated. Table V shows the total number of animals (except cocoons which were not affected by this problem) by taxon before (R sum^2) and after (P sum^2). The data were prorated (sums of dates estimated by m^2, all sets), and the difference R sum^2 — P sum^2 expressed as a percent of sum^2 (DP %).

E) **Coring effect.**

Earthworms were often amputated by the spade during sampling and, during dry periods when the soil was hard, they were sometimes broken. Because the heads of amputated earthworms were counted, it is possible to calculate a percentage of cut postembryos versus all postembryos. Table VI gives these percentages by taxon, set, and sampling method.

All taxa show an increasing percentage of amputated individuals with depth of sample because the top layer was cut on only 5 sides, while the deeper layers were cut on 6 sides. Differences in the number of amputated individuals between the bl_{40} and bl_{60} data sets, respectively, are due to the small numbers of individuals, especially in the bl_{60} data set, and a concentration of animals at the top of the 40-60 cm soil layer. The formalin technique resulted in fewer amputated individuals. Nevertheless, animals are often broken when taken too quickly from their burrows: This is particularly noticeable for small and fragile larvae of *Nicodrilus*.

TAB. VI

Cutting effect of coring. Percentage of truncate postembryos in Set 1 (T 1), Set 2 (T 2), et 3 (T 3), Set 4 (T 4), Set 5 (T 5), and in fo bl_{20} (TT 1) on Bl_{60} method (TT 2)

Sgort Name	T1	T2	T3	T4	T5	TT1	TT2
N. *caliginosus*	0.92	18.65	20.75	37.50	10.42	14.85	22.26
N. nocturnus	0.16	37.67	32.37	35.13	24.58	18.90	32.42
N. longus	0.26	36.59	30.02	39.57	42.52	23.57	34.18
An. (immatures)	0.84	35.56	35.28	51.01	61.07	25.77	40.09
A. rosea	0.25	21.53	19.20	21.36	19.44	15.91	19.46
A. icterica	0.17	19.64	21.13	32.52	26.22	15.88	23.60
A. larvae	0.62	23.35	23.41	38.01	32.85	18.25	25.15
L. castaneus	3.09	36.58	29.93	36.78	49.54	14.55	30.73
Total	1.00	30.21	29.76	41.81	44.38	21.02	32.86

The number of amputated individuals of *Lumbricus castaneus* is always rather high, probably because of natural predation and poor regeneration by this epigeous species. Generally speaking, the larger the individual species, the greater the frequency of amputation. The bl_{60} gave the highest amputation rate (32.9 %). Ethophysical sampling reduces this figure to 21.0 %, because its fo-component (1 % of the cut worms).

F) **Correction factors.**

During the data correction phase, two numerical biases may be introduced which can cause an underestimation of earthworms numbers. Unidentified postembryos (4.33 % of total) are not accounted for and the proration procedure may produce significant roundoff errors. The former problem is corrected by assuming that unidentified postembryos belong proportionally to each species. To correct for roundoff errors we calculated for each taxon, set or technique the following factor:

$PC = DRP \times W / P \times S + 4.33$,

where P is the prorated number for the set and taxon under consideration; S is the observed number of amputated individuals (all sets) of a given taxon; W is the observed number of amputated individuals for a given set and taxon; DRP = sum^2 — P sum^2; and PC is the calculated percentage correction necessary for each set and taxon.

Table IV shows the different PC estimates which, applied on the different P values, gave a PC sum^2 (all sets added for a taxon, sum of days estimated per m^2) which increase the originial R sum² of a C % of P sum², of nearly 4.33 % (D % = 4.33 — C %). Table V shows PC sum², C % and D %. D % measures the remaining bias which is, at most, equal to 0.37 % of our estimates.

II. — RESULTS

A) Conventions.

This section is devoted to giving the relative efficiency of each method. All methods tend to underestimate the real population levels because it is easy to loose individuals, but they are never created during sampling. Therefore, the highest estimate is also the best estimate of the real earthworm community. However, seasonal activity, local conditions and demography all vary with each species, so the efficiency of a given technique will vary with each species and season. Environmental parameters such as temperature and humidity influence earthworm behavior the most. Earthworms need good soil humidity which is generally found at Cîteaux in the fall, winter and early spring. During late spring and summer the evapotranspiration is severe creating significant drying of the upper soil layers. During the wet periods temperature seems to be principal factor controlling earthworm mobility.

For these reasons we will limit our presentation to a comparison of the three sampling techniques with the data summarized by species and week or season (seasons were obtained by dividing the year into 9 equal parts starting from the Middle of August).

TAB. VII

Mean estimates of main components of the Cîteaux earthworms community. Both number and biomass are equal for « earthworms » and postembryos with formaline method (no cocoon catched)

	Number ($N.m^2$)					Biomass ($g.m^2$)				
	Earthworms		Postembryos			Earthworms		Postembryos		
	fo, bl_{20}	bl_{60}	fo	fo, bl_{20}	bl_{60}	fo, bl_{20}	bl_{60}	fo	fo, bl_{20}	bl_{60}
N. nocturnus......	16.47	19.66	8.43	16.47	19.66	21.30	24.77	11.92	21.30	24.77
N. longus.........	25.32	32.56	9.07	25.32	32.56	39.88	49.72	15.44	39.88	49.72
N. anecic.........	147.03	174.71	22.41	77.81	94.05	43.52	47.68	14.84	40.18	43.77
Total anecics ..	188.82	226.92	39.91	119.60	146.26	104.70	122.18	42.20	101.36	118.28
A. rosea..........	26.99	28.12	3.31	12.14	15.12	2.67	3.10	0.81	2.54	2.99
A. icterica.........	37.31	49.90	6.10	33.68	44.87	19.30	26.79	3.38	19.21	26.65
A. endogés........	29.51	35.77	6.36	29.51	35.77	4.05	4.95	0.90	4.05	4.95
Total endoges..	93.81	113.78	15.77	75.62	95.76	26.03	34.84	5.08	25.81	34.58
N. caliginosus.....	16.88	12.88	3.86	7.33	6.15	1.65	1.40	0.77	1.53	1.32
L. castaneus.......	60.08	83.32	11.39	16.53	27.87	3.13	4.67	1.94	2.88	4.38
Total community (except scarces species)..............	359.59	436.89	70.93	219.08	276.04	135.51	163.09	49.09	131.58	158.58

The relative efficiency of each method can be judged by the number (N) and biomass (B) estimated. Most previous studies did not take into account cocoons, and, so in order to make a comparisons with these studies, we will refer only to the postembryo data. In nearly all cases the physical method (bl_{60}) gave the best results for both biomass and number with or without cocoons (Tab. VII). For this reason the relative efficiency, in percent, of other methods is compared to the physical method.

B) **General trends.**

The mean number of individual earthworms per square meter for all species (rare species compose 1.1 % of the total and are not considered here, see section C) over the two years sampling period was 436.9. The mean biomass was 163.0 a m^{-2} (fresh weight with gut content). Cocoons accounted for 36.7 % of the total number and 2.8 % of the biomass.

The ethological method gives only 16.2 % of earthworms and 25.7 % of postembryos in numbers and respectively 30.7 % and 31.5 % in biomass. The ethophysical method provides an improved estimate of 82.6 % and 79.4 % in numbers and 83.1 % and 83.0 % in biomass. While cocoon samples are never obtained by the formalin method, the ethophysical procedure gives 88.4 % (number) and 87.1 % (biomass) of the cocoons estimated by the physical method. The reason for this improvement by the ethophysical method is that most cocoons occur in the top 20 cm of the soil and are, therefore, sampled by both the ethophysical and physical methods. The difference between the two methods are obviously due to the cocoons which lay deeper than 20 cm and were only sampled by the physical method.

Interpreting the results by species or ecological types (Tab. VII) yields the following: with cocoons included in the sample the ethological method gives less than 18 % of the total number observed and without considering cocoons gives only 43 %. *N. caliginosus* is an exception to this general rule giving 30 % and 62.8 %, with and without cocoons, respectively. Except for the *L. castaneus* postembryos, the ethophysical method always gives at least 72 % with or without cocoons. Obviously, the ethological method is not suitable for earthworm sampling, while the ethophysical gives better, though not completely satisfactory results.

Seasons and species-specific behavior influenced these results. The ethophysical method does not consume as much time and labor as the physical method and the ethological component produces information valuable for estimating earthworm mobility.

We now consider seasonal differences for each species, or ecological type, particularly the exceptional results for *L. castaneus* and *N. caliginosus.*

C) **The Anecics.**

At Cîteaux grassland site, as in most European soils (Bouché, 1975 *d*) with mull humus, anecics are functionally dominant (biomass = 74.9 %). These animals are large and dig deep into the soil. They produce casts on the surface where they feed on the litter. During the wet season these animals consume nearly all the dead plants on the top of the soil. Because of their large size, their dominance in total numbers (51.9 %) is not as obvious.

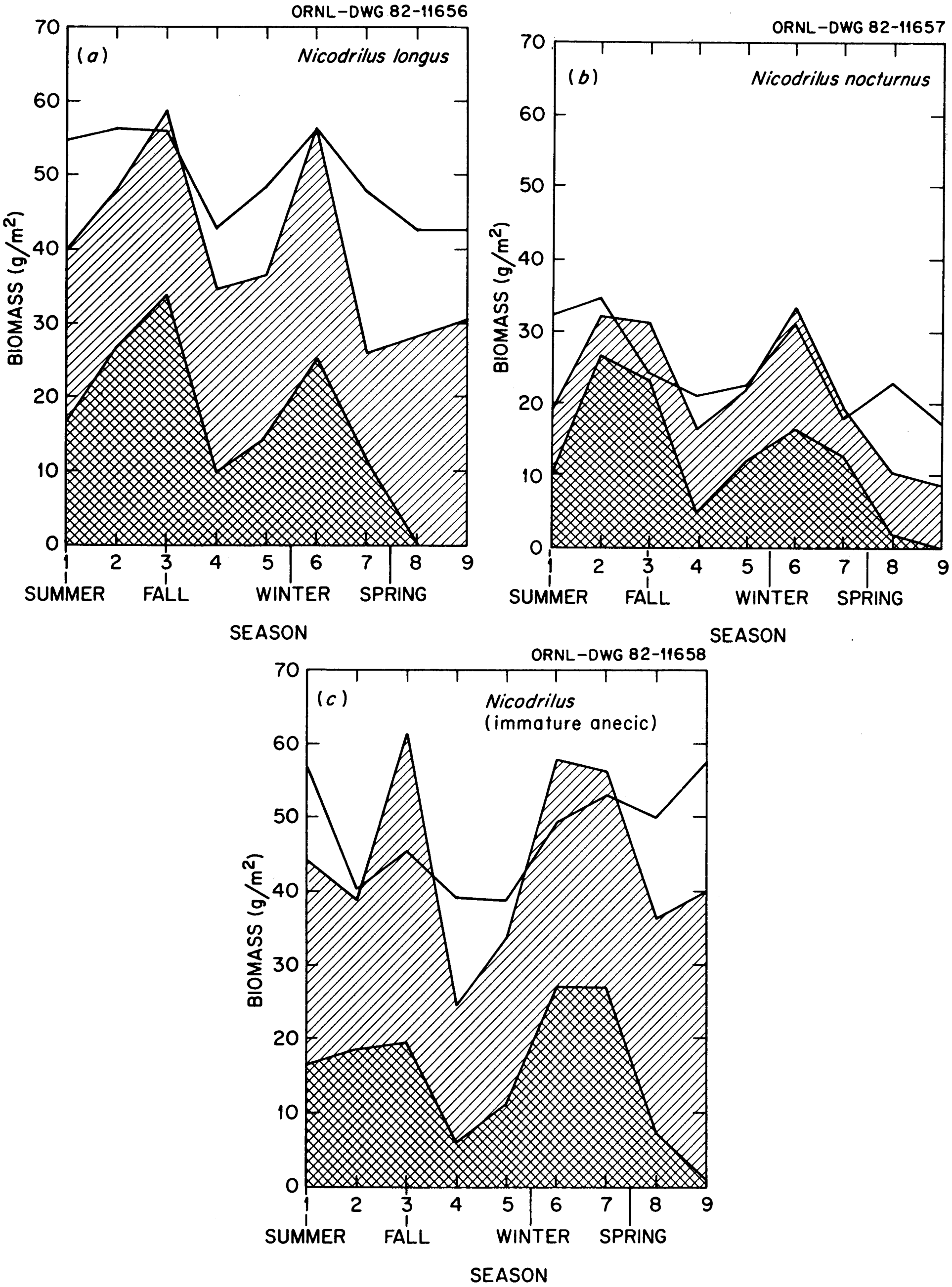


FIG. 1. — Comparison of three methods for estimating biomass (g m^{-2}) of earthworms: ethological (low line), ethophysical (middle line) and physical method (top line). a. *Nicodrilus longus* adults and and subadults; b. *Nicodrilus nocturnus* adults and subadults; c. Immature anecic *Nicodrilus*.

At the Cîteaux site two anecics species, *Nicodrilus longus longus* and *Nicodrilus nocturnus* variety *cistercianus* are allopatric. These species are morphologically distinguishable only in the adult stage. For this reason their larvae and cocoons are grouped under the label *N. anecics* (immatures). The ethological method is inefficient for sampling these earthworms (N = 17.6 %; B = 35.5 %) and for postembryos (N = 27.9 %; B = 35.7 %) while ethophysical results are, on the average, rather close to the best estimates obtained by the physical method (for earthworms; N = 83.2 %; B = 85.7 %; for postembryos N = 81.8 % and B = 85.7 %). The mean values for each group indicate a relative discrepancy between species for ethologic method, where *N. nocturnus* adults are rather well sampled versus *N. longus* (N = 42.9 % and 27.9 % and B = 44.1 % and 31.1 % respectively). This discrepancy is less for ethophysic method (N = 83.8 % and 80.2 %; B = 86.0 % and 80.2 %, respectively).

These results have been compared for the ethophysical and physical method for those two species by season (Fig. 1 a and 1 b). The physical method remains the most efficient for *N. longus*, while during the Fall and Spring the physic method underestimates the *N. nocturnus* population in comparison with ethophysical method. This result is also obvious for non-identified anecics (a mix of both species, Fig. 1 c). These differences are clear for estimated biomass levels and can not be attributed to a random effect of sampling (for each value there were between 44 and 72 sampling units). For earthworm numbers those inversions (where the ethophysical method is more efficient than the physical method), give the same shapes (Fig. 2 a, 2 b, and 2 c), but not for immature postembryos (Fig. 2 d).

Comparison among stages reveals that during the fall and spring seasons the underestimation of adult numbers explains the underestimation of *N. nocturnus* numbers (Fig. 3 a and 3 b). Total estimates for *N. longus* were similar in the Fall and Spring for the two methods, but absolute numbers (Fig. 4 a and 4 b) show an underestimation compensated for by an overestimation of subadults. Non identified anecics (Fig. 5 a and 5 b) are also underestimated by the physical method for the larger stages (adults and subadults) during Fall and Spring (seasons 3 and 6).

The Fall and Spring are also the periods when the ethological method gives the highest results (Fig. 1 and 2). Conversely, the dry periods (seasons 8 and 9) or cold periods (season 4) none or few earthworms were gathered by formalin application. During periods when the earthworm activity is low, the ethophysical method is relatively inefficient for all stages (except for *N. nocturnus* subadults in winter). During dry periods, adults of both species enter a true diapause and regress to a subadult stage (no sexual activity). During this diapause, even when the soil is wet, anecic species remain immobile and no earthworms are gathered by ethological methods.

Conclusions: The ethological method is unsuitable for population estimates of anecics, but gives a direct measure of activity influenced by soil humidity, and temperature and internal factors regulating diapause. The physical method is the best year around; nevertheless this technique underestimates the larger animals (mostly adults and subadults) which are below 60 cm. A fraction of these larger animals migrates upwards under formalin application. This forced movement is only efficient when activity levels are

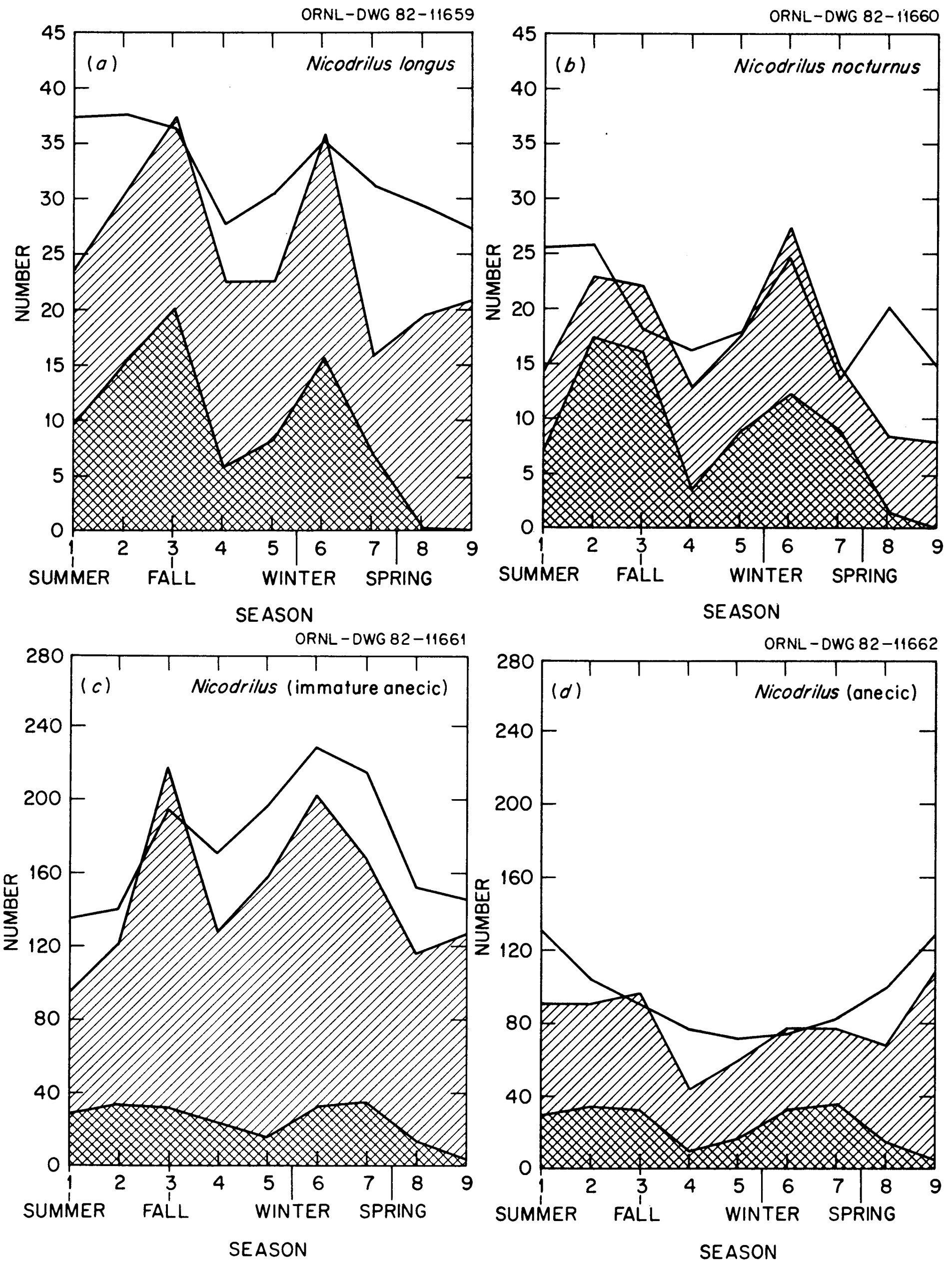
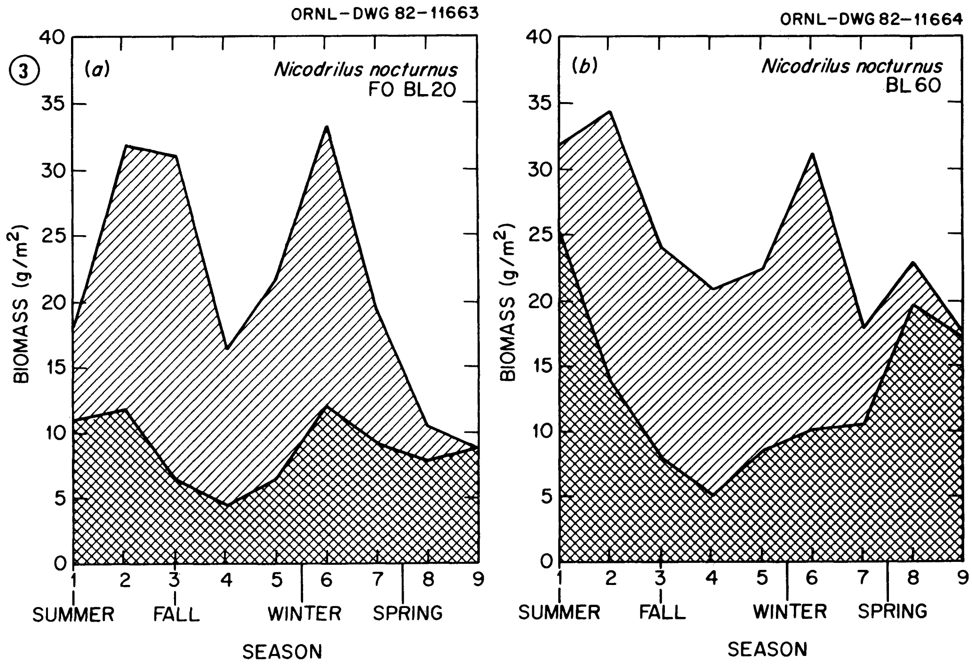


FIG. 2. — Comparison of three methods for estimating numbers per m^2 of earthworms: ethological (low line), ethophysical (middle line) and physical method (top line). a. *Nicodrilus longus* adults and subadults; b. *Nicodrilus nocturnus* adults and subadults; c. Cocoons and larvae of immature anecic *Nicodrilus;* d. Larvae of anecic *Nicodrilus.*



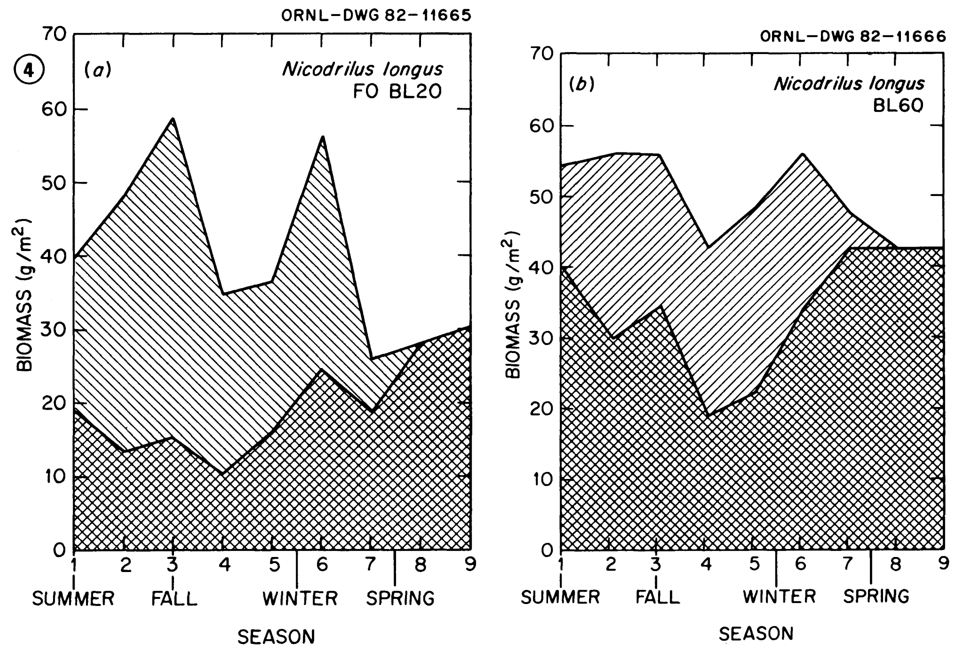


FIG. 3. — General seasonal trends of biomass (g m^{-2}) evolution of *Nicodrilus nocturnus* subadults (cross hatched pattern) and adults (striped pattern). a. The ethophysical method (FO-BL 20) ; b. The physical method (BL 60).

FIG. 4. — Cumulative seasonal trends of biomass (g m^{-2}) evolution of *Nicodrilus longus* subadults (crosshatched pattern) and adults (striped pattern). a. The ethophysical method; b. The physical method.

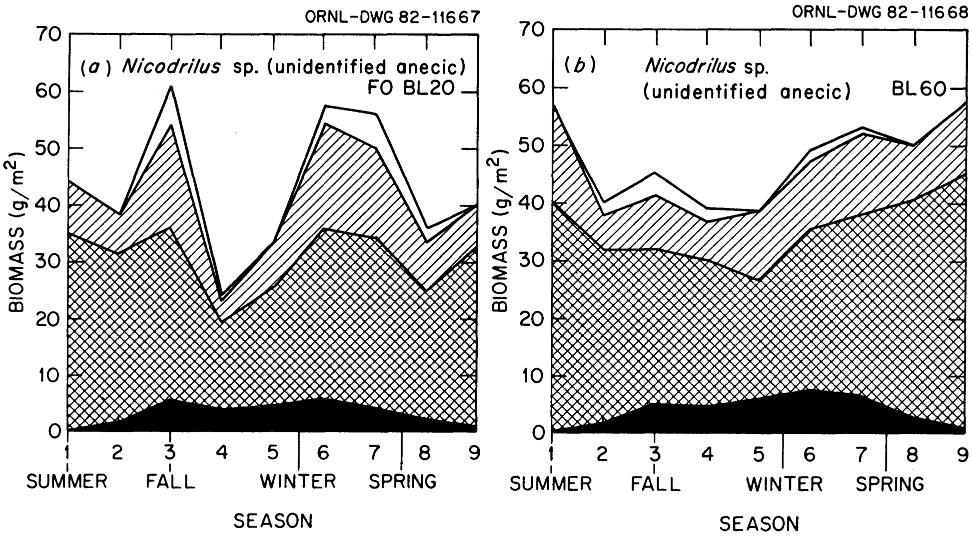


FIG. 5. — Cumulative seasonal trends of biomass (g m^{-2}) evolution of *Nicodrilus* sp. anecic cocoons (black striped pattern), larvae (crosshatched pattern), subadults (striped pattern) and adults (clear pattern). a. The ethophysical method; b. The physical method.

high. There is a slight underestimation of cocoons by the ethophysical method (Tab. VII); 14.2 % of ootheque were not sampled by this method because they are found below 20 cm.

D) The Endogés.

As in most soils, the absolute quantity (biomass and numbers) of endogeous earthworms increases when anecics increase, but their relative quantities decrease (BOUCHÉ, 1975 *d*). In Cîteaux grassland there are four endogés species: *Allolobophora icterica, A. rosea, Nicodrilus caliginosus* and *A. chlorotica* (green and white « forms »). The last species is very rare and not considered here. *N. caliginosus* is not a typical endogé, but has characteristics of anecics and epigés species and, from a methological point of view, it is also peculiar. For these reasons we report *N. caliginosus* separately.

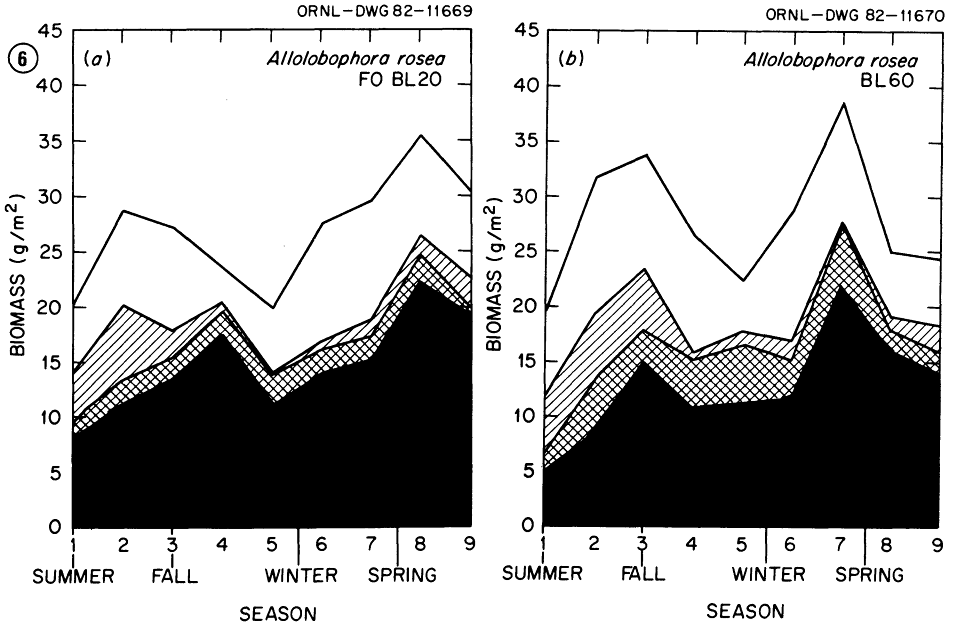
Allolobophora icterica is the most typical endogé in Cîteaux (as this group was redefined in BOUCHÉ, 1977), being primarily geophagic and living deep in the soil. *A. rosea* lives near the soil surface and has a diet partly composed of dead roots. The two species can be identified at the ootheque, subadult stages, while larvae are very similar and cannot be distinguished after formaline preservation. Nevertheless, larvae can be ascribed to *A. icterica* when their size is larger than *A. rosea* adults. For the Cîteaux data set we chose a weight limit of 282 mg (corrected fresh weight) for *A. rosea* adults and used this criteria for making *a posteriori* identification of *A. icterica* larvae. The remaining endogés larvae were called *Allolobophora* larvae, or simply *A.* larvae.

Accordingly, the endogés were divided into three groups: *A. rosea* (ootheques, subadults, adults), *A. icterica* (ootheque, larvae > 282 mg, subadults and adults) and *A.* larvae (larvae < 282 mg). The *Allolobophora* species represent 26.0 % in numbers and 21.4 % in biomass of the community. The ethological method is inefficient both for earthworms (N = 13.9 % ; B = 14.6 %) and postembryos (N = 6.5 %; B = 14.7 %), while the ethophysical results are, on the average, rather close to the best (physical) method for earthworms (N = 82.5 %; B = 74.7 %) and postembryos (N = 79.0 %; B = 74.6 %). The difference between species reflects the fact that *A. icterica* lives deeper in the soil (often below 20 cm) while *A. rosea* lives near the surface and is captured nearly as well by ethophysical (core to 20 cm deep) as by the physical method (core of 60 cm deep). For earthworms *A. rosea* N = 96.0 %; B = 86.2 % and *A. icterica* N = 74.8 %; B = 72.1 %, and for postembryos *A. rosea* N = 80.31 %; B = 85.7 % and *A. icterica* N = 80.3 %; B = 85.1 %. Notice that *A. rosea* earthworms, including the cocoons, are sampled with the greatest efficiency. This fact is due to a better sampling efficiency of cocoons by the ethophysical than the physical method (14.9 m^{-2} versus 13.0 m^{-2}, respectively). This difference can be accounted for by a failure of the automatic washing, sieving, and hand sorting procedure to separate the very small cocoons from the stones and plants. The physical method produces soil samples with entire plants while the ethophysical method contains only roots (the top parts of the plants were removed before formalin application). Conversely, estimation of *A. icterica* cocoons is better by the physical than the ethophysical method (5.0 m^{-2} versus 3.6 m^{-2}) because these cocoons are larger and lay below 20 cm where coring is not deep enough to sample all cocoons present.

Comparison between ethophysical and physical methods for each season reveals (Fig. 6 a and 6 b) an underestimation by the physical method for all life stages during June, July and August. This underestimation is also observed for *A. larvae* (Fig. 7 a) but not for *A. icterica* (Fig. 7 b) in June. During June *A. icterica* migrates downward and aestivates.

The failure of the physical method in comparison with the ethophysical method can not be explained by migration alone, because soil sampling of the ethophysical method is more limited than the physical method. The seasonal occurrence of plant parts in the samples also does not explain this effect. The explanation seems to be in the actual size of the soil core taken by the different methods. During the dryest periods (1 and 9) the soil hardens and sampling by spade is difficult with more earthworms cut and broken (see section on coring effects). The human factor also introduces a bias during dry periods, with the horizontal dimensions of the sample are approximately 3 cm less, resulting in an 18 % reduction of the sample volume. (Manual sampling by spade in a harder soil leads to this unintentional reduction). In the ethophysical method, where the soil is first softened by the formalin solution, the tendency to reduce the size of the soil core is absent. The results of *N. caliginosus* confirm this bias in soil coring (see below).

The ethological method always gives a smaller estimate of the populations in comparison with the physical method (average/year/square meter, earthworms: N = 13.9 %, B = 14.6 %; postembryos: N = 16.5 % and B = 14.7 %). The general weakness of the formalin method is indicated by the mean values per square meter (see Tab. VII). The best proportion is



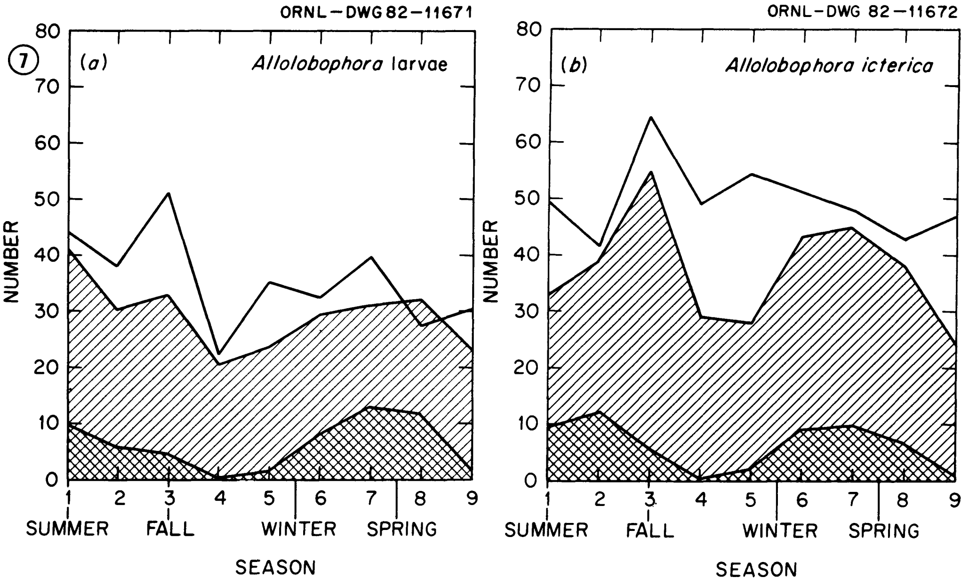
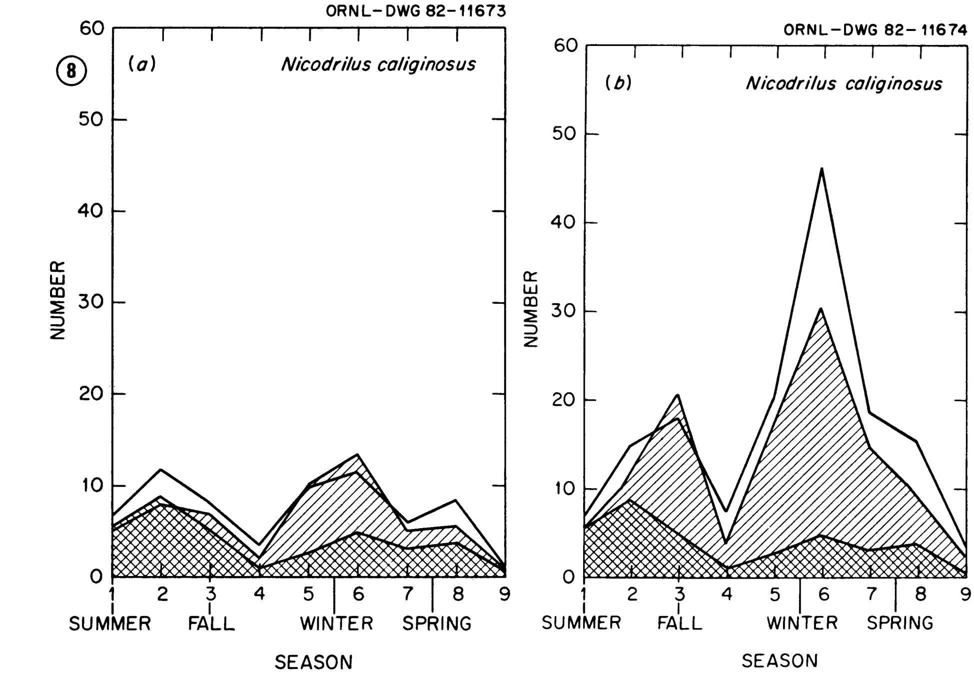


FIG. 6. — Cumulative seasonal trends of biomass (g m^{-2}) evolution of *Allolobophora rosea* for cocoons black pattern), larvae (crosshatched pattern), subadults (striped pattern), and adults (clear pattern) as determined by: a. The physical method; b. The ethophysical method.

FIG. 7. — Comparison of three sampling methods for estimating numbers per m^2 of *Allolobophora*: the ethological (low line), ethophysical (middle line) and physical (top line) methods. a. Non-identifiable larvae; b. *Allolobophora icterica*.



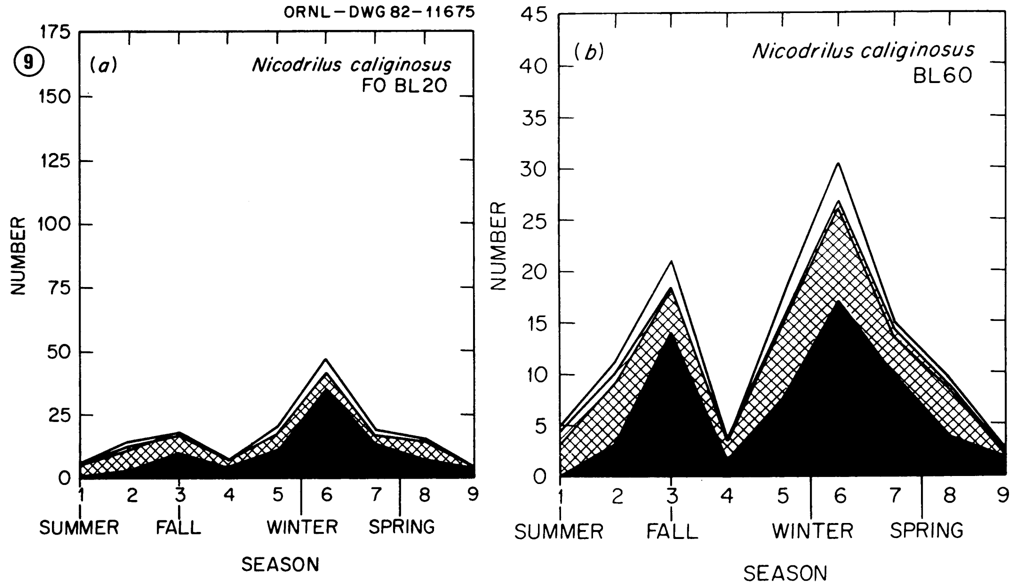


FIG. 8. — Comparison of three sampling methods for estimating numbers per m²: the ethological (cross hatched pattern), ethophysical (clear pattern) and physical (striped pattern) methods. a. *Nicodrilus caliginosus* - without cocoons; b. *Nicodrilus caliginosus* all stages.

FIG. 9. — General seasonal trends of the numbers per m² of *Nicodrilus caliginosus* cocoons (black pattern), larvae (cross hatched pattern), subadults (striped pattern), and adults (clear pattern). a. The ethophysical method; b. The physical method.

given by the biomass of *A. rosea* postembryos (N = 27.1 %). The yearly activity of the endoge's is bimodal and this method reflects this pattern. Activity is at a minimum in the winter (season 4), and is diminished in the summer. Inspection of the original data for the summer samples indicates a high variability depending on soil moisture. Lethargy of these two endoge's species is regulated by soil moisture and not by a specific season (compare Fig. 1 with anecic, having a true diapause).

E) ***Nicodrilus caliginosus.***

All life stages of *N. caliginosus* are distinct and clearly recognizable. The morphological characteristics of this species causes it to live in the top soil layers, with a tendency to both anecic and epigeous habits. Unfortunately the frequency of this species in the community is low (N = 2.95 %; B = 0,86 % by the physical method) and the variance between samples tends to be high. Mean annual estimates of the technical efficiency of each method indicates, in comparison with the physical method, a relatively high efficiency of the formalin (ethological) method (N = 30.0 %; B = 55.1 % for earthworms, and N = 62.8 %; B = 58.3 % for postembryos), and a better efficiency of the ethophysical than the physical method (N = 131.1 %; B = 117.7 % for earthworms, and N = 119.3 %; B = 115.9 % for postembryos). Thus, the physical method underestimates earthworm populations. The effect of this underestimation is reflected in the irregularities of most seasonal estimates (Fig. 8). Both cocoons and postembryos are underestimated, but not during the same seasons (Fig. 9).

F) ***Lumbricus castaneus*: the Epigeous Species.**

Lumbricus castaneus is a pure epigeous species of the Cîteaux grassland. Because it lives in cow dung, its distribution is patchy and not easily estimated. Fortunately, the number of individuals is high (83.32 m^{-2}) representing 19.1 % of the community, but because the individuals are small it comprises only 2.9 % of the biomass (estimates by the physical method). As usual, the ethological method gives a low efficiency for postembryos, but a good indication of earthworm mobility (Fig. 9 b). The ethophysical method is also partly inefficient: N = 72.1 %; B = 67.0 % for earthworms and N = 59.3 % and B = 65.6 % for postembryos. Underestimation also occurs for cocoons (N = 84.7 %). Cocoons are 78.6 % of the total number estimated by the physical method. In summer this species is almost always in the ootheque stage (the « resting » form: Bouché, 1977). Only 1.95 % of cocoons are found below 20 cm in June and July, while the ethophysical method estimates an N of only 75.8 % of the physical method. These underestimations could be explained by the removal of cow dung, and cocoons contained in the dung, during site preparation before formalin application. While migration below 20 cm occurs, most of the underevaluation by the ethophysical method for postembryos (Fig. 10) seems to be due to the removal of cow dung. Numerous postembryos of *L. castaneus* could be seen on the soil living in dung or moving into tuffs of grass. The underestimation of cocoons in the Spring by the physical method, in comparison with the ethophysical method, seems to be due to difficulties in handsorting those cocoons from the abundant grass fragments after washing and sieving.

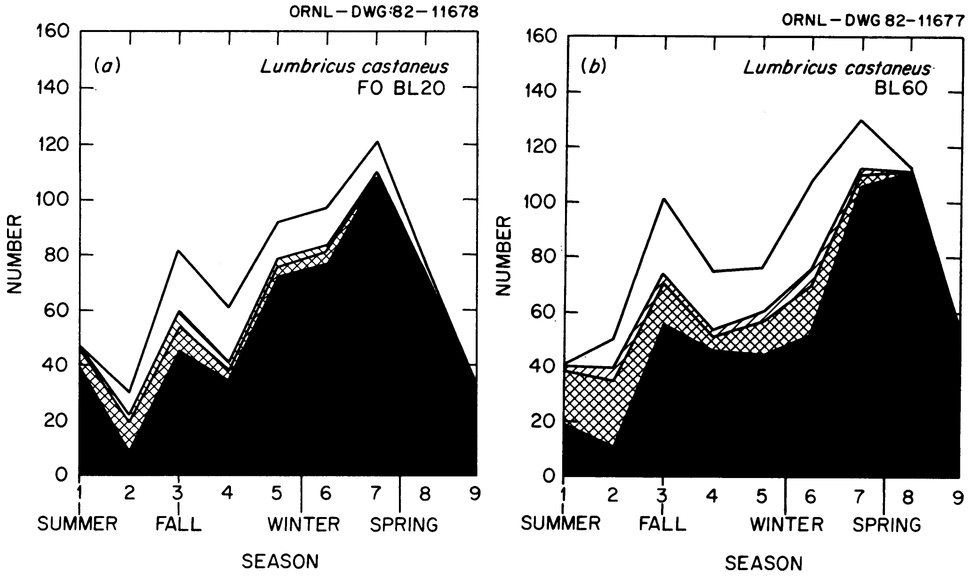


FIG. 10. — Cumulative seasonal trends of evolutions of numbers per m² of *Lumbricus castaneus* cocoons (black pattern), larvae (cross hatched pattern), subadults (striped pattern) and adults (clear pattern). a. The ethophysical method; b. The physical method.

G) **Other species.**

The Cîteaux soil community includes three species at low population densities. Because there is very little data to compare technical efficiencies, we present only the general results which can be made directly from the raw data. The same system has been used for each technique. Only samples from the 101 last dates (202 m^2 of formalin sampling and 32.32 m^{-3} of washed soil) are reported because sampling is most reliable during this period. As usual we report numbers as percent of the physical method.

Lumbricus terrestris L. (e.g., *L. herculeus* Savigny) is a very important species in wet temperate climates. In the Cîteaux site this species was found only in areas where the water table was high enough to ensure survival during the dry summer period. This species needs more than a year to reach reproductive maturity, but is unable to resist drought either by quiescence or diapause. Moisture, at least in the deeper horizons, is an absolute necessity for surviving dry seasons (BOUCHÉ and BEUGNOT, 1978). Because we did not know how to distinguish larvae smaller than 300 mg, we attributed all the smallest larvage to *L. castaneus*. Consequently, *L. terrestris* is underestimated by all methods. The results for the ethological method are N = 64.8 % for earthworms and N = 156.8 % for postembreyos. For the ethophysical method the results are N = 147.1 % for earthworms and N = 172.6 % for postembryos. For this species the physical method is not the best one, because most individuals living below 60 cm are not captured by it. Calculated by the physical method, 57.9 % of the postembryos were living below 20 cm (between

20 and 60 cm deep). It is possible that individuals of this fast moving species take refuge in the deeper horizons during the coring procedure. Conversely, this species is not strongly susceptible to light as most earthworms and lives frequently as an epigeous species (its ecological type is intermediate between anecic and epige).

Nevertheless, the total number of cocoons was 51.62 % in the ethophysical method, and probably many small larvae were missed by the formalin method. (In our results all adults were taken during formalin extraction, but only 84.7 % of the larger larvae. Smaller larvae are probably ommitted as *L. castaneus* postembryos, see Tab. VII). For the biomass estimate, it seems that the formalin method could be used in favorable conditions while the ethophysical is far better in all situations, including estimation of numbers and demographic parameters.

Allolobophora chlorotica chlorotica Savigny, with two varieties (an albinic form and a green form) are also scarce in the Cîteaux community. Both forms live mostly in the top (0-20 cm) layer (only 23.1 % of the postembryos and 8.1 % of the earthworms were caught below 20 cm and 8.1 % of the earthworms were caught below 20 cm by the physical method). One-third of the postembryos belong to the green form. The efficiency (N) of the ethological method was 6.7 % for earthworms and 20.0 % for postembryos, while the ethophysical method gives, respectively, 91.6 % and 82.0 %. The results of the ethophysical method and the physical method are nearly equal (density = 1.95 m^{-2} and 2.13 m^{-2}, respectively, with the proportion of cocoons, 69.8 % and 66.28 %, respectively, also similar).

Dendrobaena mammalis Savigny was very scarce, all cocoons and many larvae were probably confused with *L. castaneus.* Estimates of the number of identified individuals (44) prevents any conclusions.

III. — **CONCLUSIONS**

A comparison of data available from the literature and from the Cîteaux site leads to the following conclusions:

1. The ethological methods are inaccurate for estimating numbers and biomass of earthworms, except for the formalin method for *L. terrestris.*

2. The ethological (formalin) method reflects differences of behavior between species: the closer the species lives to the top of the soil, the larger the numbers obtained in each sample. The mean annual efficiency of this method declines from epigeous, to anecic, to endogeous species.

3. The performance of the formalin method is quite variable between species because of differences in the activity of postembryo invidivuals. Earthworm movement is dependant of external factors, such as soil humidity and temperature, and internal factors, particularly the endocrine control of diapause.

4. The physical methods are laborious, and, as a result are limited in volume and depth of soil which can be sampled.

5. The physical methods cut the postembryos and, therefore, requires careful handling of the samples and data derived from them.

6. Direct hand sorting both on the site (formalin) and on the soil in the laboratory is only efficient for estimating the biomass of medium to large sizes of postembryos. Cocoons and small juveniles are always underestimated.

7. Washing-sieving of the soil samples increases the reliability of estimates of total numbers. Plant parts, stones and other debris which remain can confuse the hand sorting of individuals from the soil samples.

8. The physical method, with automated washing-sieving is, at present, the only way to obtain an accurate estimate of the number, demography and biomass in all groups, excepting large *Lumbricus.*

9. The physical method is, nevertheless, inefficient for very deep burrowers (e.g., large anecics and *L. terrestris*).

10. A combination of physical and ethological methods, i.e., the ethophysical method, is a good alternative for sampling the deeper burrowers and estimating their numbers.

11. The ethophysical method is nearly always as efficient, and sometimes more efficient, than the physical method during favorable periods. This method could be the best one during some periods of the year.

12. The ethological portion of the ethophysical procedure allows a check on the periods when the ethophysical method is most efficient.

The ethological method, while it always underestimates actual numbers, can give comparable results in some situations because it underestimates by a similar proportion when samples are all taken at the same time and from soil of the same soil moisture succion (pF) and temperature. The physical method, with direct hand sorting, can, for some species, give a good estimate of biomass (within 60 to 80 % of true values). The ethophysical method could give good estimates during favorable climatic periods for the whole community. The study of the entire community during the whole year requires the best means for coring, for the treatment of samples, for identification of species and stages and, last but not least, for handling the data.

In the Cîteaux study the physical method had some serious limitations: The depth was only 60 cm, the removal of the soil caused frequent cutting of postembryos, and the volume of the soil removed was inaccurate. Some epigeous earthworms were eliminated by clearing the soil before sampling. Automated washing-sieving was very efficient even though a small percent of the larvae or cocoons are still missed. Exact identification is still a problem. Very similar species could not be distinguished except at the adult stage. Correction of the raw data is tedious and without the aid of a computer is nearly impossible. In short, a complete study of an earthworm community can not be made by simple methods.

While earthworms are the dominant fraction of animal biomass in most paleoarctic ecosystems, means are needed to study them quantitatively. This study indicates that current estimates of numbers range from 10 to 60 % of the actual total and from 20 to 80 % of the biomass of earthworm communities.

Summary

A review of available techniques for sampling populations of earthworms was made and the primary methods experimentaly compared over a three year period. The accuracy of these techniques varies with: species; season; stage of earthworm development; activity level; physical nature of the soil, particulary soil temperature and humidity; method of handling the soil sample; and the correction of the data and final computation of numbers. Most estimates of community dynamics are biased and, though the choice of a technique will depend on the objectives of the study, it is important to realize the extent of the inherent biases. This study provides, for the first time, a controlled experimental comparison which accurately assesses this biases.

Résumé

Fonction des Lombriciens. VIII. Techniques d'estimation des populations.

L'article traite de la comparaison des diverses méthodes de prélèvements des lombriciens ; il comporte une étude bibliographique et une comparaison, sur trois ans, des principales méthodes. La précision des techniques varie avec les espèces, stades de développement et niveau d'activité des lombriciens, avec les saisons, les conditions physiques du sol (particulièrement humidité et température), les méthodes de manipulation des prises de sol et des données numériques pour corrections et calculs. La plupart des estimations de peuplements lombriciens sont biaisées et, quoique le choix de la technique depende des buts de l'étude, il est important d'apprécier l'importance des biais. L'étude fournit, pour la première fois sur des bases expérimentales importantes, une mesure de ces biais.

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