Soil Fauna and Litter Decomposition in Primary and Secondary Forests and a Mixed Culture System in Amazonia

Final Report 1996-1999

Project leadership:

Prof.Dr. Ludwig Beck (Staatliches Museum für Naturkunde, Karlsruhe, D)
Dr. Luadir Gasparotto (Embrapa Amazônia Ocidental, Manaus, BR)

Project coordination:

Dr. H. Höfer [4], Dr. C. Martius [1;6], Dr. W. Hanagarth [1], MSc. M. Garcia [2], Dr. E. Franklin [3], Dr. J. Römbke [5]

Further scientific collaborators:

Dr. B. Förster [5], Dr. T. Gasnier [7], Dipl. Biol. C. Hanne [6], Dr. F. Luizão [3], Dr. R. Luizão [3], MSc. L. Medeiros [3], Dr. W. Morais [3], MSc. R. Ott [3]

[1] Staatliches Museum für Naturkunde Karlsruhe (SMNK)
[2] Embrapa Amazônia Ocidental, Manaus
[3] Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus
[4] II. Institute of Zoology, University of Göttingen
[5] ECT Oekotoxikologie GmbH, Flörsheim
[6] Center for Development Research (ZEF), Bonn
[7] Universidade do Amazonas, Manaus

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Earthworms

Jörg Römbke[1] & Marcos Garcia[2]

[1] ECT Oekotoxikologie GmbH, Flörsheim, Germany
[2] *Embrapa Amazônia Ocidental*, Manaus, Brazil

1. Introduction

Earthworms are known to be the most important group of soil animals in temperate regions of the world but their contribution to ecosystem soil functions, especially litter decomposition, in the humid tropics remains largely unexplored. Therefore, the species composition, abundance and biomass of these organisms were determined in two plots of a polyculture forestry plantation and in plots of nearby secondary and primary forest as part of the SHIFT ("Studies on Human Impact on Floodplains and Forests in the Tropics") project since 1997. The aim of the project is to study the regeneration and better use of already degraded plots, to diminish the human impact on primary rain forest in Amazonia (Beck et al. 1998). The basic hypothesis of all investigations within this project is that soil fauna (especially ecosystem engineers like earthworms; Lavelle et al. 1997) and micro-organisms are extremely important for the maintenance of "healthy" (functional) nutrient cycles (Fragoso & Lavelle 1995). In particular the following questions were investigated:

- number of species and species composition of the earthworm biocoenosis (including the ratio between native and peregrine species)
- number and biomass of the earthworms of the four study plots
- juvenile/adult age ratio of the earthworms
- the correlation between various climatic data and earthworm biomass.

Since some general results of the earthworm investigations have been published already (Römbke et al. 1999), this chapter will concentrate on the species level.

1. Study sites and methods

Study sites

Study sites were four plots of three different forest systems - one plot of 40 x 40 m in a primary forest (FLO), one plot of 40 x 40 m in a nearby secondary forest (growing since 1984, SEC) and two plots of 32 x 48 m large polycultures (POA, POC), where 4 different tree species of commercial use have been planted in rows. In the polyculture plots the tolerated secondary vegetation (mainly *Vismia* spp., Guttiferae) still dominated the stand and especially the litter production (Beck et al. 1998; Höfer et al. 1999).

Earthworm sampling, determination and biomass measurement

In contrast to recommendations in the literature (Anderson & Ingram 1993), hand-sorting was not successful for collecting the earthworms for two reasons: The individual size (approx. 2 cm to 110 cm in length) and the behaviour of the various species differ greatly. Secondly, the worms are very inhomogeneously distributed. A preliminary study confirmed that formalin extraction in plots of 4 m² is an easy method to sample earthworms qualitatively and quantitatively. Therefore, two replicates of 4 m² each were taken per plot (one in POA and POC, respectively) every three months (in total: 8 sampling dates). Per plot 80 L of 0.5 % aqueous formalin solution were added within 30 – 60 min. After appearance of the worms at the soil surface (the litter layer was removed carefully by hand before) they were collected by hand or by using forceps. Unfortunately, the use of mustard, used successfully in Europe as a less toxic alternative to formalin (Gunn 1992) was not efficient.

It should be noted that some earthworms have been found when using other methods (e.g. extraction from macro-sonda samples or in Berlese funnels). Since these worms were always very small (and often juvenile), not all of them are determined yet and their (very low) biomass has not been measured. However, since these additional samples were taken close from the plots where the formalin samples were taken, the numbers for both methods are given. Some small differences between numbers in various tables are due to the fact that in some cases (especially when presenting data on species composition) worms were not included, which could not be determined – mainly due to bad preservation,.

Immediately after sampling all earthworms were fixed in 70% ethanol. The animals must be transferred for 1 or 2 weeks to 4% formalin (one to several hours after the fixation in EtOH). Afterwards the worms were stored unlimited in 70% EtOH. Identification of the animals follows a site-specific key, prepared for the EMBRAPA site based on information from the literature and the INPA collection. No review on earthworm taxonomy from the Manaus region is available, despite the fact that several individual species were described (e.g. Righi et al. 1976). Currently some of the species are reviewed taxonomically (Zicsi et al. 2000). The determined species are mainly stored in the collection of EMBRAPA-CPAA (Manaus).

Determination of biomass was performed using the fixed material. The animals were washed in water (5 min) and weighed. Afterwards the worms were stored again in 70% EtOH. Due to the change of weight during preservation and the soil content in the gut the measurements had been corrected by published factors to get the dry weight of the animals. According to literature (e.g. Lee 1985, Dunger & Fiedler 1997), preserved worms seem to loose about 10 - 27 % of weight. This is approximately similar to the weight of the gut content. Therefore, no compensation would be necessary. According to Bouche (cited in Petersen & Luxton 1982) the conversion factor from fresh weight, full gut to dry weight, empty gut is 10. Statistical calculations, partly done using Excel, are not finished yet.

3. Results and Discussion

Species number and composition

Considering the differences like vegetation cover and anthropogenic influence in the four plots, the number of species seems to be slightly higher (approximately 9; Table 1) than the normal range reported from rainforests (6.5 ± 1.3; Fragoso & Lavelle 1992). All of them belong to the mainly neotropical family Glossoscolecidae. The most conspicuous (since up to 110 cm long) belong to the genus *Rhinodrilus*. Besides some species widely distributed in Amazonia (*A. amazonius*, *U. brasiliensis*; Righi 1990), at least two of them seem to be endemic to the Manaus region (*R. contortus*, *R. priollii*). Both species were found for the first time since they have been described scientifically in 1938 and 1967, respectively. Very rarely and only in Berlese samples two small species were found (*P. vandersleeni, C. righhii*; Zicsi et al. 2000). The peregrine species (i.e. circumtropical) *Pontoscolex corethrurus* was found on all plots except FLO. Another small peregrine species, *Dichogaster bolaui* (Octochaetidae), which is often found at anthropogenic sites in Brazil, occurred in another plantation plot nearby. Information on the range of environmental conditions, their distribution in different land-use systems and main biological properties (e.g. reproduction potential) for the two peregrine species have been recently compiled by Lavelle et al. (1999).

Table 1: List of earthworm species (family Glossoscolecidae) found at the four Embrapa plots

Genus	Species	Author
Andiorrhinus	*amazonicus*	Michaelsen, 1918
Andiorrhinus	*venezuelanus tarumanis*	Righi et al. 1976
Cirodrilus	*righii*	Zicsi et al., 2000
Pontoscolex	*corethrurus*	(Müller, 1857)
Pontoscolex	*vandersleeni*	Michaelsen, 1933
Rhinodrilus	*contortus*	Cernosvitov, 1938
Rhinodrilus	*priollii*	Righi, 1967
Urobenus (Rhinodrilus)	*brasiliensis*	(Benham, 1887)
Tuiba	*dianaea*	Righi et al. 1976

This list is not complete since taxonomic work on some of the earthworms found in additional samples (both on the four study plots as well as nearby) is not finished yet.

Earthworm number and biomass

The abundance of earthworms in the four plots is low in comparison to many other tropical lowland rain forest sites (Table 2), even when taking the worms from macro-sonda samples into consideration (only three worms were found in macroarea samples during the whole study period). Concerning the mean abundance, no differences between the four plots are indicated – despite the fact that no worms were found at all on POA at two sampling times (September 1997 and 1998; Fig. 1). In various other polyculture plots nearby higher mean earthworm numbers were found (approximately by a factor of 3 to 4; Araujo, pers. comm.). However, these values are based on less sampling dates, mainly in the wet season. Very differentiated results were got by Vohland & Schroth (1999), also in various agroforestry plots at the EMBRAPA site: The number of earthworms differed approximately between 0 and 10 worms per square meter. A direct comparison between their data and the results

presented here is not possible since these authors took samples only once (in the wet season) and plotted them as number per kg of litter.
Since most of the species living on the investigation plots are very large, the amount of biomass found at one point of time is very high (up to 35 g FW m^{-2}). Considering the results from FLO, the results are within the normal range reported from tropical rain forests (Fragoso & Lavelle 1992). The literature values shown in Table 2 for sites comparable to SEC, POA and POC are based on just two studies on plantations and secondary forests (Gilot et al. 1995; Gonzalez et al. 1996). The high numbers found in these studies are caused by the occurrence of peregrine species like *P. corethrurus* which can reach incredibly high densities but which is rare on our plots; probably because the whole study area was cleared relatively recently. It can be expected that the number and biomass of *P. corethrurus* will increase drastically in the near future since this worm is widely distributed in older pastures and plantations of the Manaus region (Barros, pers. comm.). Many of the studies on tropical earthworms conducted so far have used very few sampling dates. For example, data from a *Seringueira* plantation in Acre (Brazil) or a secondary forest in Sabana (Puerto Rico) were not included in Table 2 since samples were taken only once (Guerra 1988; Zou & Gonzalez 1997).

Table 2: Abundance [ind m^{-2}] and biomass [g FW m^{-2}] of earthworms in the four study plots (mean values of eight sampling dates and standard deviations are given) and data from the literature (Fragoso & Lavelle 1992, Gilot et al. 1995; Gonzalez et al. 1996)

Parameter	FLO	SEC	POA	POC
Abundance				
This study				
Formalin extraction	2.8 ± 2.3	1.6 ± 1.4	1.1 ± 0.8	2.5 ± 2.8
Macro-sonda	13.4 ± 10.4	10.1 ± 8.4	10.9 ± 7.8	12.0 ± 17.4
Literature	68 ± 32	171 - 189	91 - 150	
Biomass				
This study	15.6 ± 10.4	2.6 ± 2.1	4.0 ± 8.2	9.6 ± 11.9
Literature	12.9 ± 6.2	52 - 61	29 – 59	

The average number of earthworms in the four plots seems to be more or less the same, showing a maximum in the rainy season (March 1998 and March 1999; Fig. 1). However, due to the very low number, these data are highly variable. On average, the earthworm biomass is highest in the primary forest and lowest in the secondary forest, whereas the plantation plots are somewhere in between (POC resembles more FLO and POA more SEC; Fig. 2). No annual cycle is obvious for this parameter. The extreme climatic conditions, partly induced by the open tree rows, might be responsible for the variability in the plantation plots which is clearly higher than in the two forests. In addition it is notable that only on POA no or nearly no earthworms were found in the dry season (September to December). The data gained so far have not been compared statistically due to their high variability in time, but it seems that the difference between the FLO and the SEC plot (and maybe the plantation plots too) is not accidental.

When looking at the individual species, the composition at the four sites is quite similar (Table 3). For example, the percentage of *A. amazonius* oder *Rh. priollii* differs only between 25 to 33 % or 5 to 12 %, respectively. Only in few cases one species is completely missing (e.g. *Rh. contortus* on POA) or clearly more or less abundant than on the other plots (e.g. *U. brasiliensis* on POC). Two issues should be highlighted:

- concerning their species composition FLO and SEC seem to be more similar than the two polyculture plots;
- the percentage of the only peregrine species (*P. corethrurus*) is increasing steadily from FLO (0 %) to SEC (2 %), POC (5 %) and POA (12 %).

In general, the species composition indicates a closer relationship between the two forest sites in comparison to the two polyculture sites.

Table 3: Percentage of the most important earthworm species at the four study plots at the EMBRAPA site based on individual numbers (for details see Table 4 in the Annex)

Species	FLO	SEC	POA	POC
A. amazonicus	11 %	10 %	12 %	22 %
P. corethrurus	0 %	2 %	12 %	5 %
Rh. contortus	31 %	17 %	0 %	14 %
Rh. priollii	6 %	5 %	12 %	5 %
U. brasiliensis	20 %	34 %	33 %	10 %
T. dianaea	25 %	28 %	30 %	33 %
Rest	7 %	6 %	1 %	11 %

When investigating the species composition based on biomass values (Table 5 in the Annex; Fig. 3 - 6), the differences between the four plots are even smaller: The large *Rhinodrilus* species (especially *Rh. priollii*) are responsible for 88 (SEC), 92 (POC), 94 (POA) and 97 (FLO) % of the total biomass, respectively. Within this genus it seems that *Rh. priollii* is more adapted to the polyculture situation since on FLO and SEC it shows a percentage similar to that of *Rh. contortus*, whereas on POC and, especially, on POA it is highly dominant. This leads to an extremely steep dominance rank curve on POA. No other species reaches more than 5 %. Only on POA *P. corethrurus* had a measurable biomass (1 %).

Juvenile/adult age ratio

If all earthworms are assessed together, the juvenile to subadult to adult ratio is 72 to 13 to 15 % based on numbers and 28 to 14 to 58 % based on biomass, indicating the much higher weight of the adult worms (Fig. 7 and 8). Despite the fact that biomass is the ecologically more important parameter, for reasons of convenience (comparability with literature data) only results based on numbers are presented here. However, the average numbers are quite different when the various species (not distinguished between study plots) are compared individually (Table 6). These numbers reflect probably different life strategies and maybe methodological problems (e.g. the relatively small juveniles of *P. corethrurus* might be underestimated since they are more abundant in the macro-sonda samples than in the formalin samples presented here).

Table 6: Percentage of the three age stages for the most important earthworm species found at the four EMBRAPA plots so far

Species	Juvenil	Subadult	Adult
A. amazonicus	66 %	8 %	26 %
P. corethrurus	40 %	0 %	60 %
Rh. contortus	72 %	14 %	14 %
Rh. priollii	52 %	4 %	44 %
U. brasiliensis	44 %	1 %	55 %
T. dianaea	91 %	4 %	5 %

Concerning the age ratio at the four study plots (Table 7; Fig. 9 - 12) it seems that the juvenile number (including subadults) is higher on FLO and POC compared to POA and SEC. The same tendency was already found concerning biomass. Except of FLO (where the total biomass of juveniles seems to be higher in the wet season (November – April)) no correlation between number and biomass of age classes and climatic variables is visible.

Correlation between climatic data and earthworm biomass
Based on an idea of C. Martius, the biomass of the all earthworms on the four study plots was correlated with rainfall, humidity, soil temperature and litter temperature 3, 5, 10 and 30 days before the actual sampling dates (Table 8 in the Annex). Nearly no correlation was found: Only the rainfall immediately (i.e. three days) before sampling on POA and soil temperature at any time (increasing coefficient from 3 to 30 days) again on POA seems to be positively correlated with earthworm biomass. This result is explainable due to the relative homogenous climatic conditions on the other plots (especially FLO) and, more important, due to the long life-cycle, large body size and high mobility of these organisms.

4. Conclusions and outlook
The most important results gained so far when investigating the earthworms of the four EMBRAPA plots can be summarised as follows (cf. Table 8):

- the earthworm biocoenosis of the primary forest (FLO) is comparable to those described from other (neotropical) rain forest sites
- some of the species seem to be confined to the region of Manaus, whether others are widely distributed in Amazonia
- the number at all four sites is quite low whereas the biomass is in the expected range
- the variability is high, especially on the two polyculture sites (POA, POC)
- in general, the four sites are not completely different concerning their earthworm biocoenosis (e.g. the species number is comparable):
    - the biomass on FLO (and partly POC) is higher than on POA and SEC
    - the same tendency is visible concerning the juvenile/adult ratio;
    - the species composition is more similar on FLO / SEC than on POA / POC
- the amount of peregrine species (*P. corethrurus*) is 0 % on FLO and 12 % on POA with POC and SEC in between
- a correlation between climatic parameters (litter temperature and, partly, rainfall) and earthworm biomass was only found (as expectable) on POA.

Table 8: Summary of the most important data describing the earthworm biocoenosis at the EMBRAPA site (* = data including the values from macro-sonda samples)

Parameter	FLO	SEC	POA	POC
Abundance [Ind/m^2] *	2.8 (16.2)	1.6 (11.7)	1.1 (12.0)	2.5 (14.5)
Variance	80 %	85 %	81 %	115 %
Biomass [FW/m^2]	15.6	2.6	4.0	9.6
Variance	68 %	79 %	205 %	122 %
Number of species	7	8	5	8
Juvenile/adult ratio [%]	81 : 19	64 : 36	67 : 33	77 : 23
Endemic/peregrine species ratio [%]	100 : 0	98 : 2	88 : 12	95 : 5

The reasons for the observed distribution pattern in time and space are not yet clear. However, the high biomass of earthworms, which can be as high as that of all other soil invertebrates together, indicates that they play a key role in ecological soil functions at the EMBRAPA sites. Similar conclusions have already been drawn by Römbke & Verhaagh (1992) and Fragoso & Lavelle (1995) who emphasised their important (direct or indirect) influence on the decomposition process. In contrary, the low biomass values (partly together with the changed species composition) indicate that this role might be impeded on SEC and, especially, POA plots. The intermediate situation at POC is probably due to two facts:

- this plot is separated from the primary forest only by a unpaved road;
- due to the closed BLÄTTERDACH the abiotic conditions are more favourable.

There are still some open questions which have to be assessed when all data are availabie:

- the taxonomic investigation has to be completed
- the various species have to be classified according to their ecological role
- the correlation between earthworm biomass and litter stock and/or decomposition rates should be clarified in detail
- in order to clarify the role of earthworms their respiration has been measured but the data is still under investigation

Finally, these data will be used to model the specific contribution of these organisms to the decomposition of the organic matter, and on the importance of these processes for the nutrient supply to the plants.

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6. Annex

See following pages

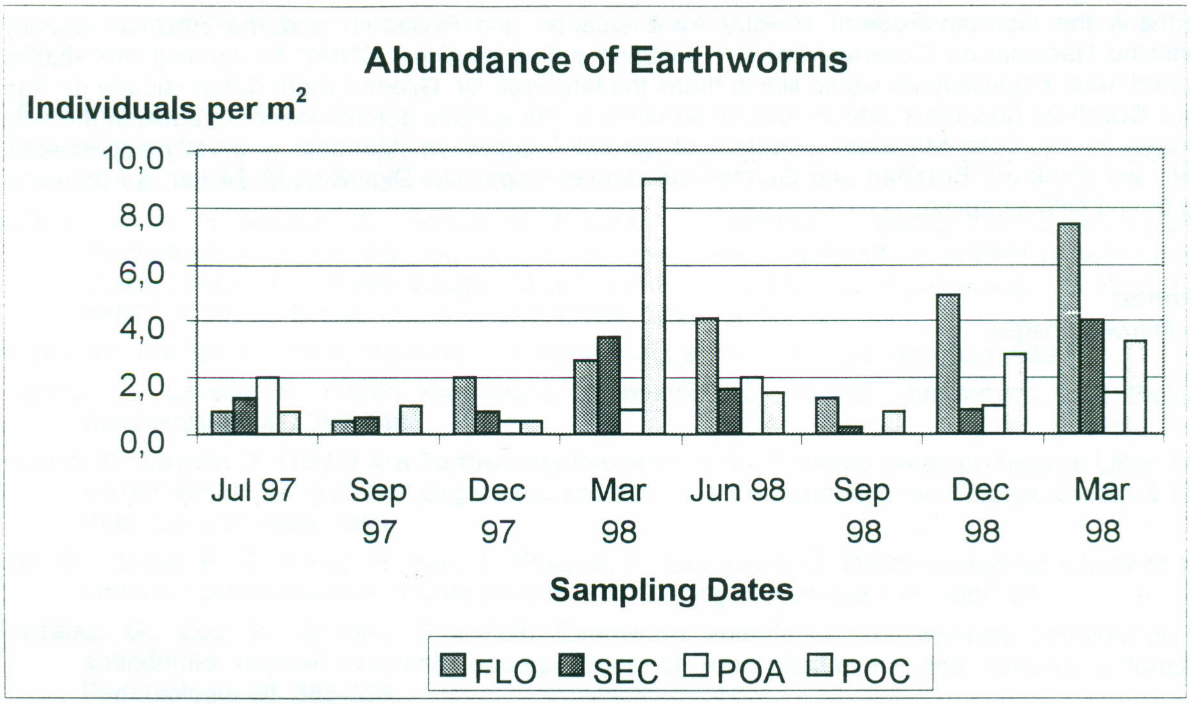

Fig. 1: Abundance [ind/m^2] of earthworms in the four study plots (FLO, SEC, POA, POC) at the eight sampling dates

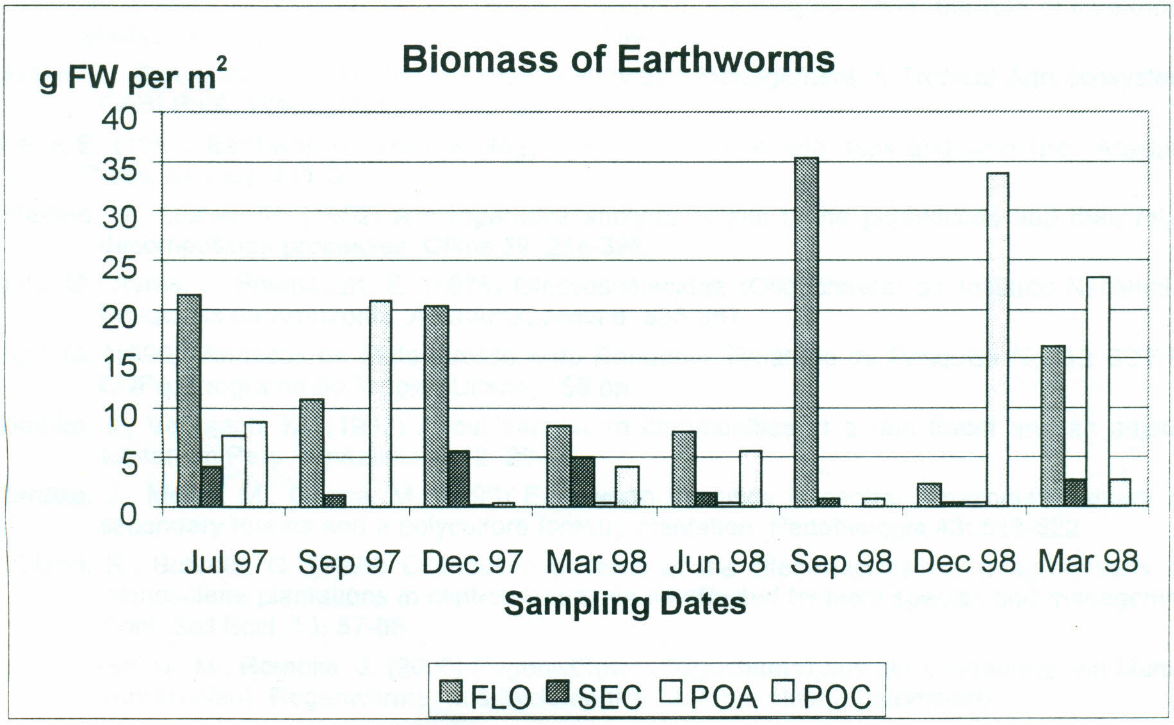

Fig. 2: Biomass [g fresh weight /m^2] of earthworms in the three study plots (FLO, SEC, POA, POC) at the eight sampling dates

Table 4: Percentage of the earthworm species based on numbers on the four study plots

FLO	Individuals	Individuals/m²	%
R. contortus	50.00	6.25	31%
T. dianaea	41.00	5.13	25%
U. brasiliensis	32.00	4.00	20%
A. amazonius	17.00	2.13	11%
R. priollii	10.00	1.25	6%
Andiorrhinus sp.	7.00	0.88	4%
Rhinodrilus sp.	4.00	0.50	2%
P. corethrurus	0.00	0.00	0%
Total	161.00	20.13	100%

POA	Individuals	Individuals/m²	%
U. brasiliensis	11.00	2.75	33%
T. dianaea	10.00	2.50	30%
A. amazonius	4.00	1.00	12%
P. corethrurus	4.00	1.00	12%
R. priollii	4.00	1.00	12%
R. contortus	0.00	0.00	0%
Rhinodrilus sp.	0.00	0.00	0%
Andiorrhinus sp.	0.00	0.00	0%
Total	33.00	8.25	100%

POC	Individuals	Individuals/m²	%
T. dianaea	24.00	6.00	33%
A. amazonius	16.00	4.00	22%
R. contortus	10.00	2.50	14%
U. brasiliensis	7.00	1.75	10%
Andiorrhinus sp.	5.00	1.25	7%
P. corethrurus	4.00	1.00	5%
R. priollii	4.00	1.00	5%
Rhinodrilus sp.	3.00	0.75	4%
Total	73.00	18.25	100%

SEC	Individuals	Individuals/m²	%
U. brasiliensis	34.00	4.25	34%
T. dianaea	28.00	3.50	28%
R. contortus	17.00	2.13	17%
A. amazonius	10.00	1.25	10%
R. priollii	5.00	0.63	5%
P. corethrurus	2.00	0.25	2%
Rhinodrilus sp.	2.00	0.25	2%
Andiorrhinus sp.	1.00	0.13	1%
Total	99.00	12.38	100%

Table 5: Percentage of the earthworm species based on biomass on the four study plots

FLO	FW	FW/m²	%
R. contortus	237,68	59,42	48%
R. priollii	207,29	51,82	42%
Rhinodrilus sp.	33,98	8,49	7%
T. dianaea	8,03	2,01	2%
U. brasiliensis	4,71	1,18	1%
A. amazonius	1,32	0,33	0%
Andiorrhinus sp.	0,12	0,03	0%
P. corethrurus	0,00	0,00	0%
Total	493,13	123,28	100%

POA	FW	FW/m²	%
R. priollii	119,57	29,89	94%
U. brasiliensis	3,11	0,78	2%
T. dianaea	2,70	0,68	2%
P. corethrurus	0,82	0,20	1%
A. amazonius	0,74	0,18	1%
R. contortus	0,00	0,00	0%
Rhinodrilus sp.	0,00	0,00	0%
Andiorrhinus sp.	0,00	0,00	0%
Total	126,93	31,73	100%

POC	FW	FW/m²	%
R. priollii	191,44	47,86	62%
Rhinodrilus sp.	47,15	11,79	15%
R. contortus	47,06	11,77	15%
T. dianaea	12,09	3,02	4%
U. brasiliensis	6,39	1,60	2%
A. amazonius	2,74	0,69	1%
P. corethrurus	1,19	0,30	0%
Andiorrhinus sp.	0,04	0,01	0%
Total	308,10	77,02	100%

SEC	FW	FW/m²	%
R. contortus	32,18	8,04	39%
R. priollii	25,17	6,29	30%
Rhinodrilus sp.	15,95	3,99	19%
T. dianaea	4,08	1,02	5%
U. brasiliensis	4,03	1,01	5%
A. amazonius	1,07	0,27	1%
P. corethrurus	0,09	0,02	0%
Andiorrhinus sp.	0,00	0,00	0%
Total	82,57	20,64	100%

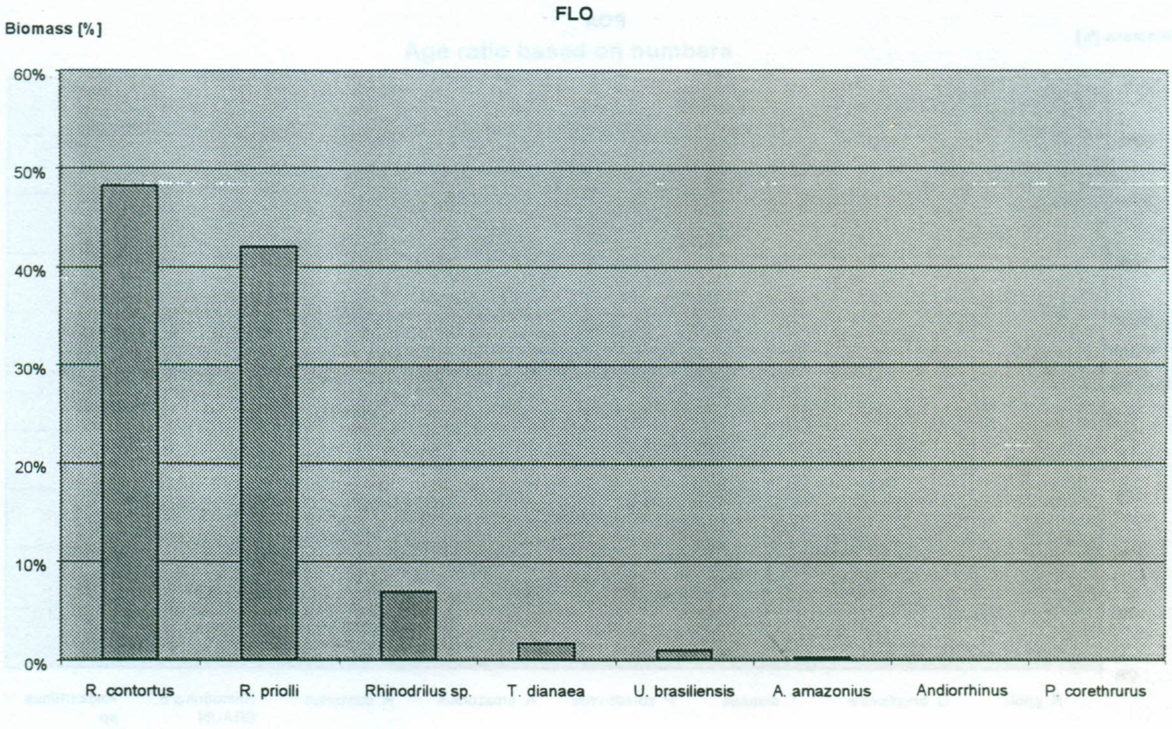

Fig. 3: Percentage of the earthworm species at the FLO study plot based on biomass data

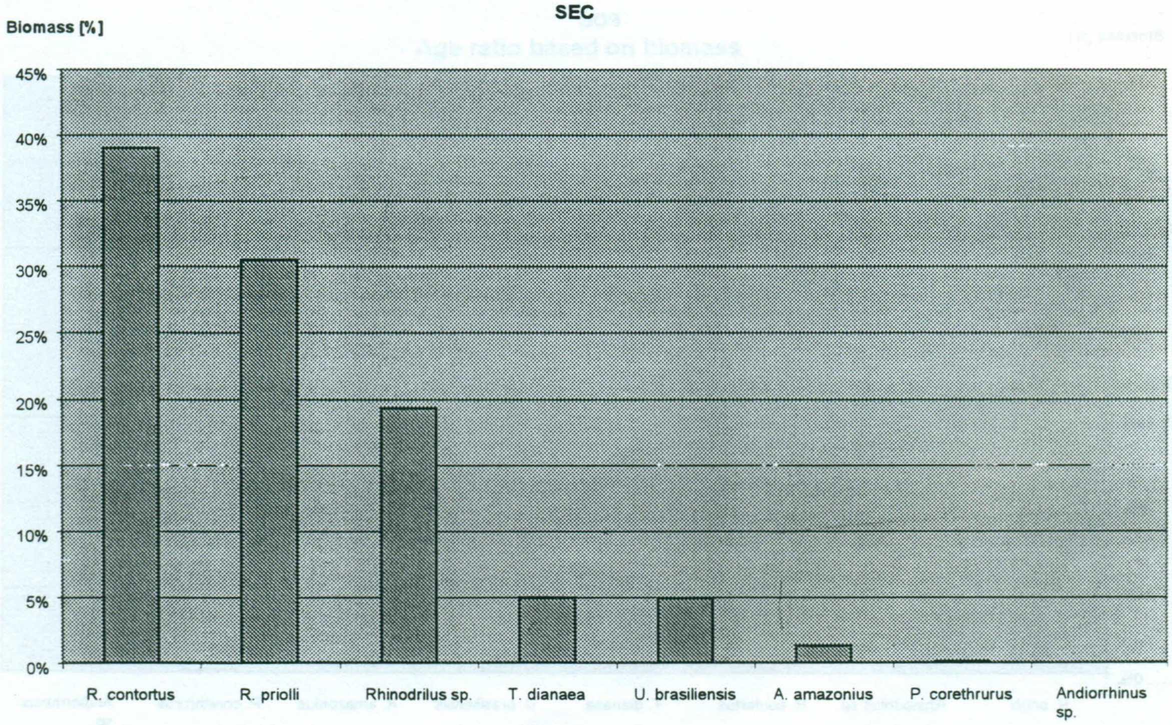

Fig. 4: Percentage of earthworm species at the SEC study plot based on biomass data

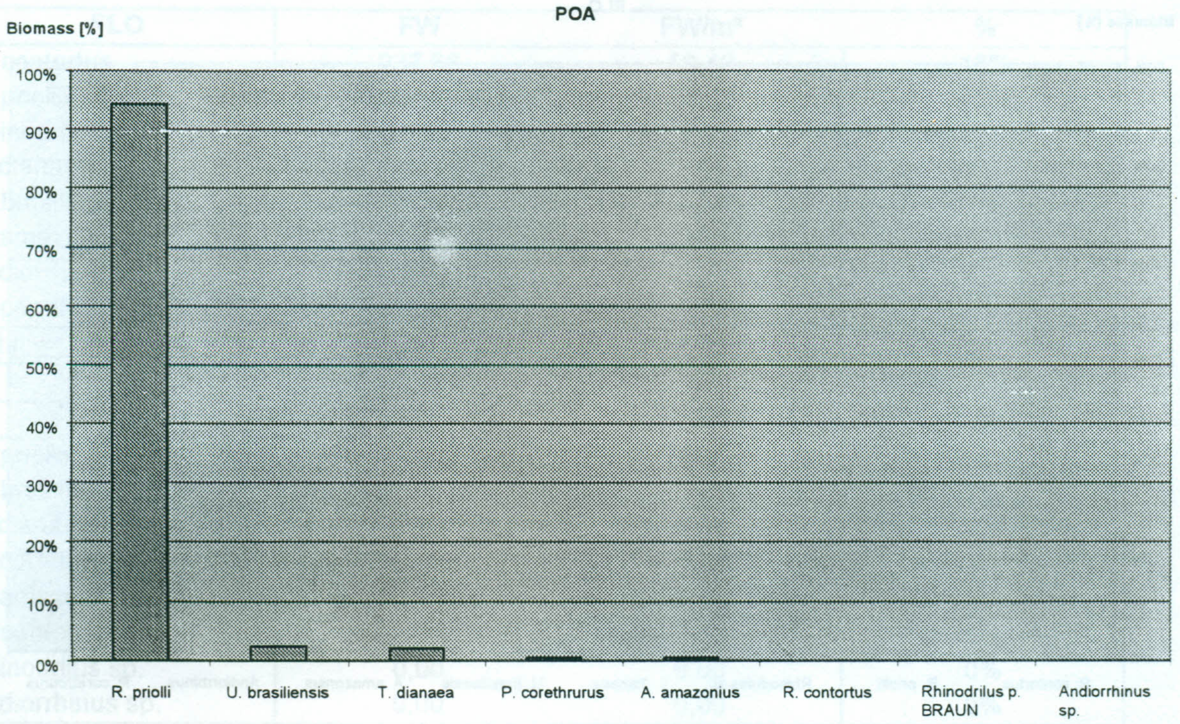

Fig. 5: Percentage of earthworm species at the POA study plot based on biomass data

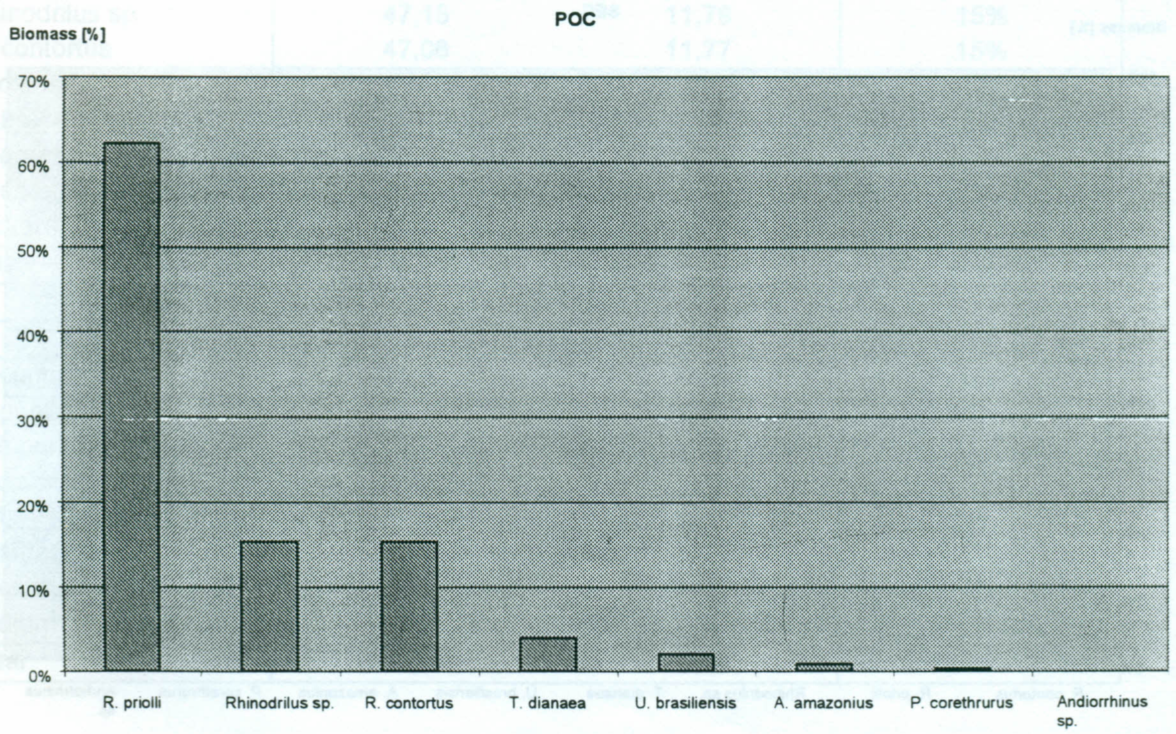

Fig. 6: Percentage of earthworm species at the POC study plot based on biomass data

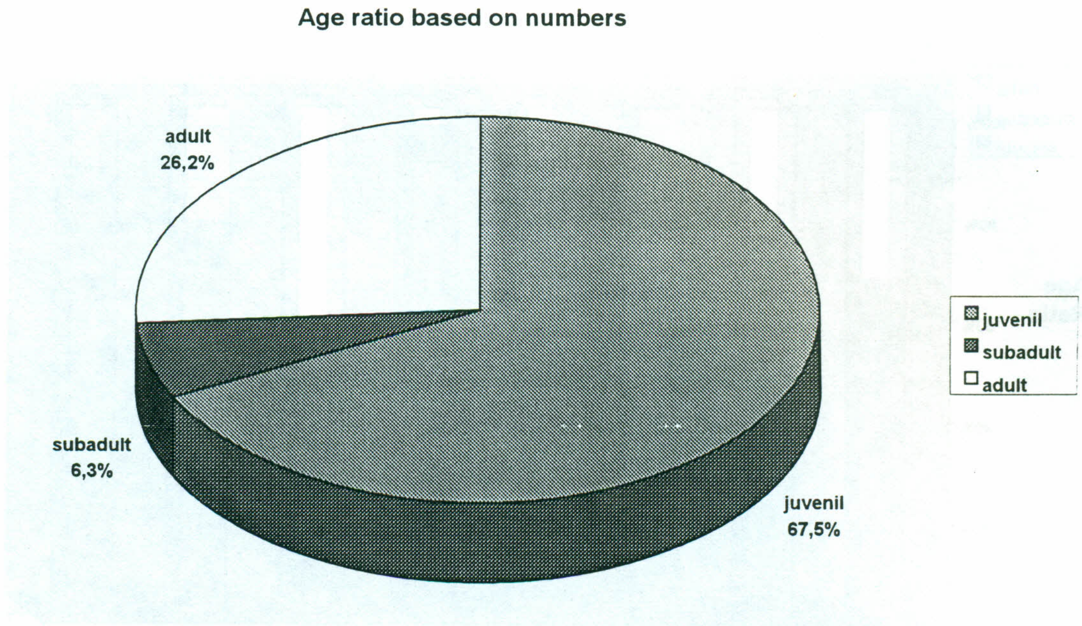

Fig. 7: Age ratio of all earthworms in the four study plots (FLO, SEC, POA, POC) at the eight sampling dates based on numbers

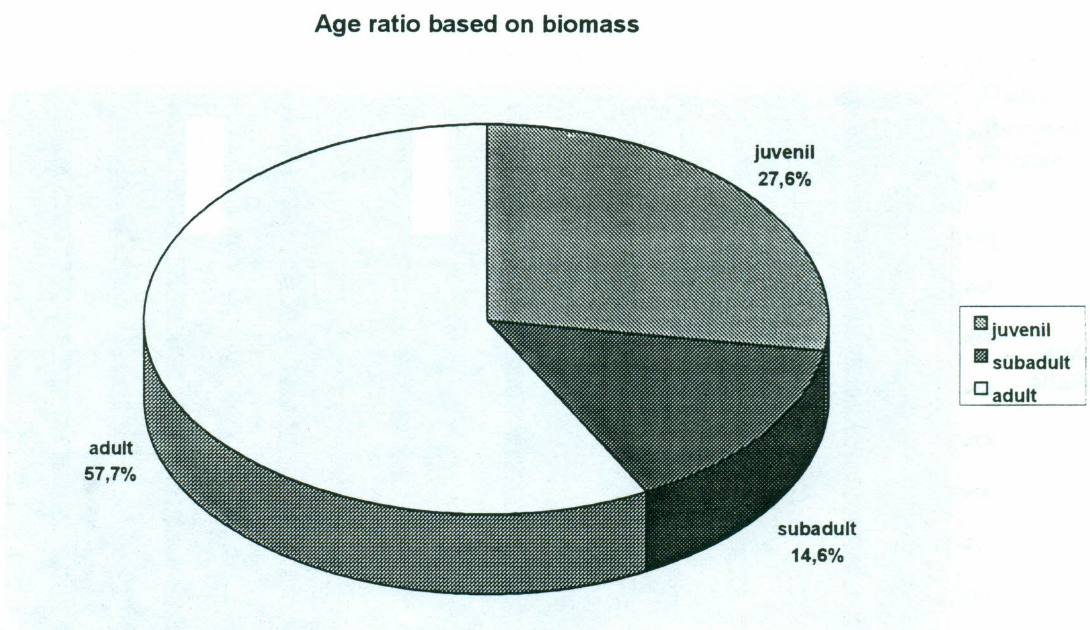

Fig. 8: Age ratio of all earthworms in the four study plots (FLO, SEC, POA, POC) at the eight sampling dates based on biomass

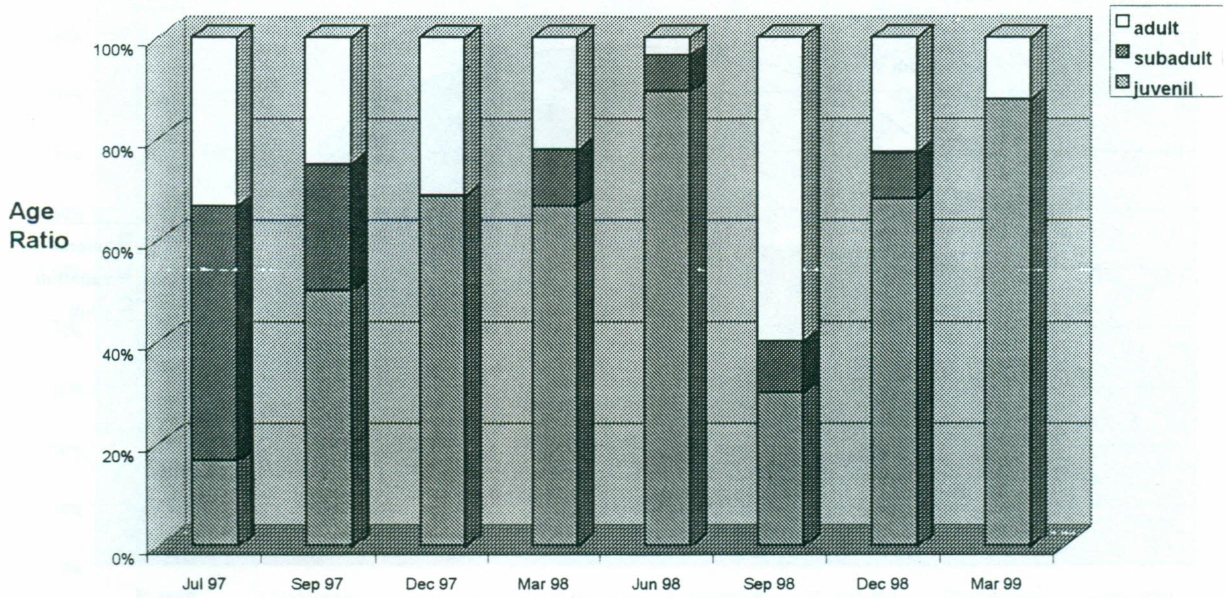

Fig. 9: Age ratio of all earthworms in plot FLO at the eight sampling dates based on numbers

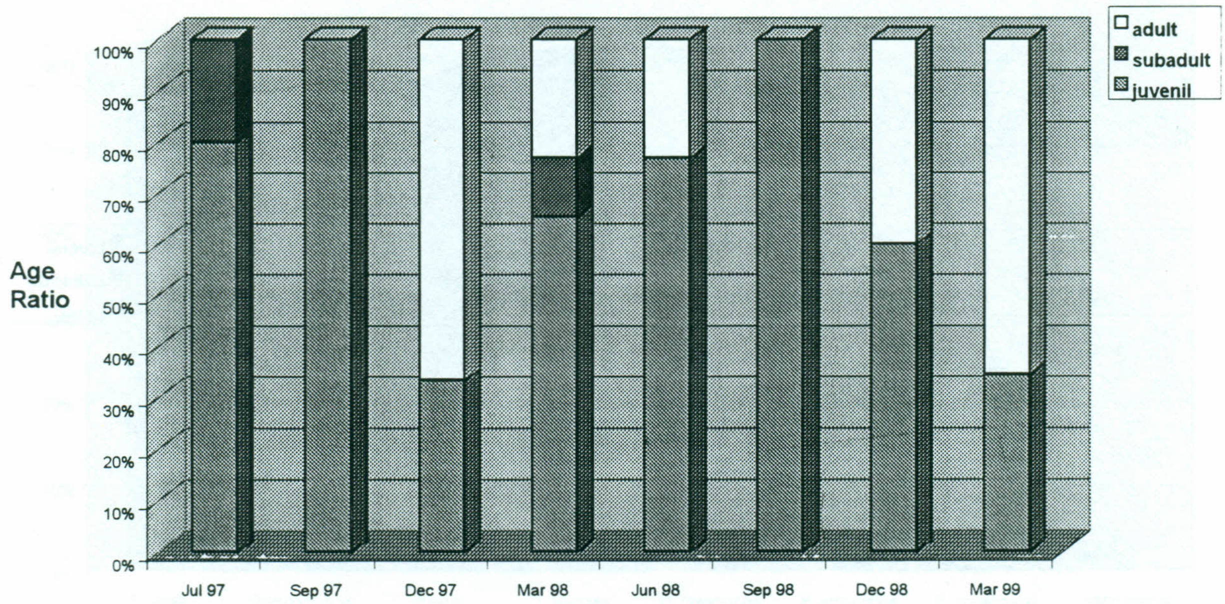

Fig. 10: Age ratio of earthworms in plot SEC at the eight sampling dates based on numbers

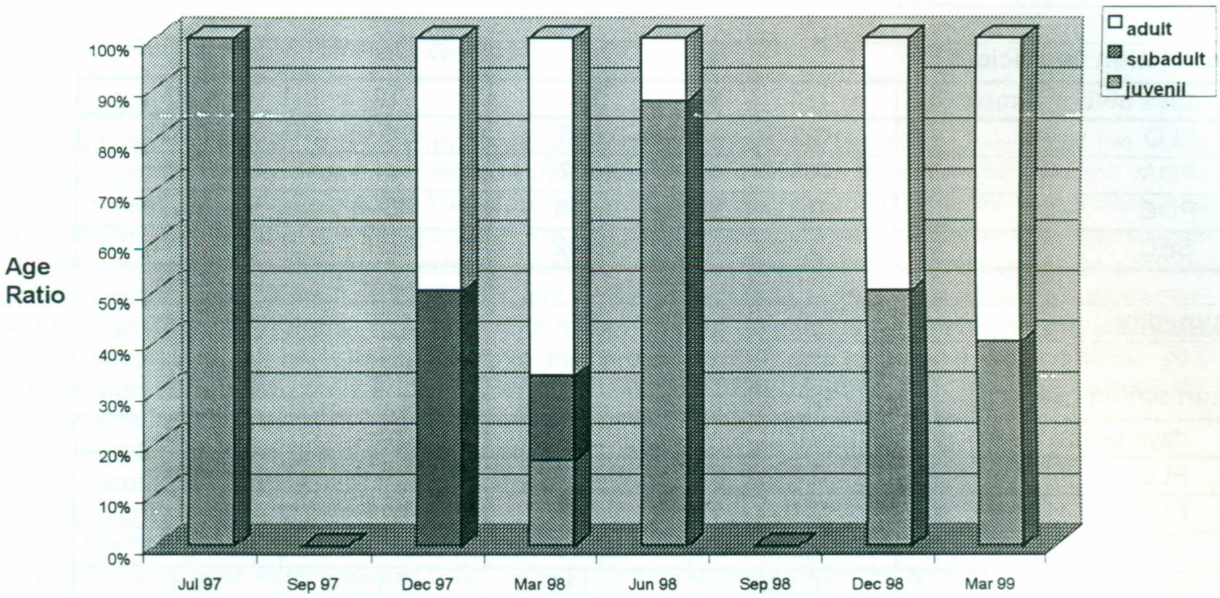

Fig. 11: Age ratio of earthworms in plot POA at the eight sampling dates based on numbers

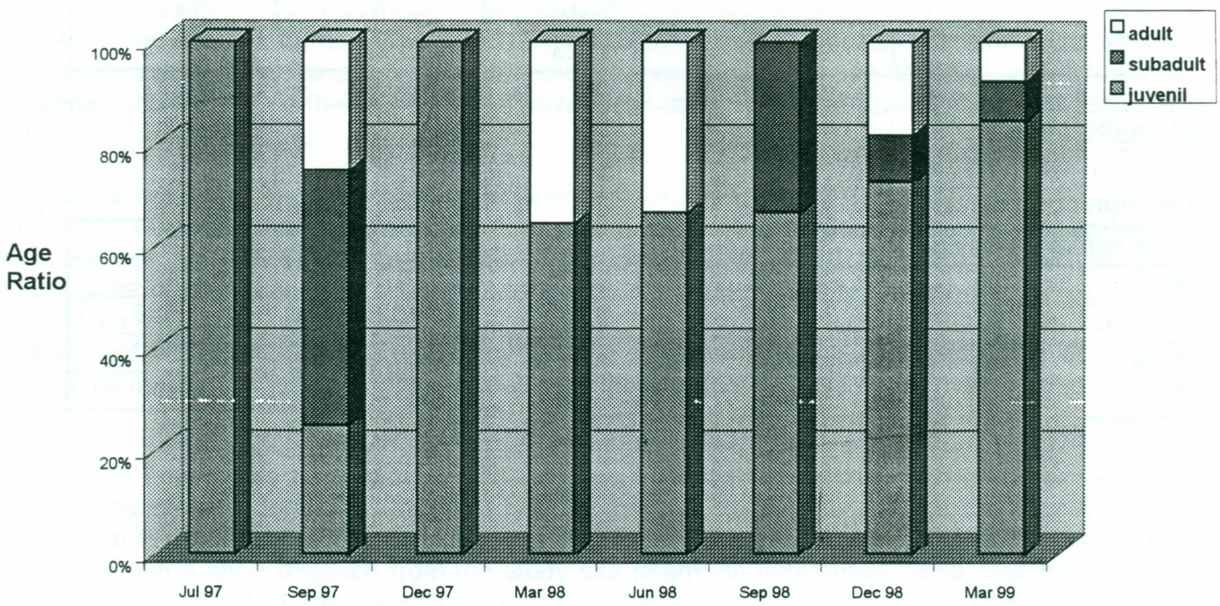

Fig. 12: Age ratio of earthworms in plot POC at the eight sampling dates based on numbers

Table 8: Correlation factors between various climatic parameters and earthworm biomass at the four study plots.

rainfall				
correlation coefficient				
days before sampling	3	5	10	30
FLO	0,221	0,006	-0,348	-0,318
POA	**0,722**	0,622	0,454	0,510
POC	-0,294	-0,053	0,302	-0,079
SEC	-0,361	-0,462	-0,271	0,119

humidity				
correlation coefficient				
days before sampling	3	5	10	30
FLO	0,293	0,502	0,504	0,499
POA	0,109	0,460	0,513	0,422
POC	0,616	0,616	0,621	0,394
SEC	-0,350	-0,135	-0,156	-0,419

soil temperature				
correlation coefficient				
days before sampling	3	5	10	30
FLO	0,036	0,131	0,189	-0,058
POA	-0,687	**-0,774**	**-0,872**	**-0,888**
POC	0,257	0,046	-0,041	0,438
SEC	0,310	0,223	0,282	0,396

litter temperature				
correlation coefficient				
days before sampling	3	5	10	30
FLO	-0,172	-0,076	0,140	-0,158
POA	-0,483	-0,493	-0,521	-0,638
POC	0,245	0,146	-0,146	-0,130
SEC	0,288	0,192	0,263	0,489