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The abundance and diversity of Enchytraeidae and Naididae (Oligochaeta) in Amazonian forest ecosystems at different stages of human impact

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We dedicate this contribution to Dr Rut Collado (1967–2015), University of A Coruña, Spain, who uncovered the diversity of naidid oligochaetes, mainly species of Pristina, at the sites sampled in this investigation.

Abstract

Enchytraeidae are known to be an important group of soil animals in temperate regions of the world but their diversity as well as their contribution to soil functions, esp. litter decomposition, in the humid tropics remain largely unexplored. Therefore, as part of the SHIFT ('Studies of Human Impact on Floodplains and Forests in the Tropics') project ENV 52, entitled 'Soil Fauna and Litter Decomposition', their species composition, abundance and biomass were determined in an experimental agroforestry area located about 20 km north of Manaus (Amazonia), Brazil, between 1997 and 2000, focusing on four plots with differing degree of human impact. In addition, individuals of the family Naididae were sampled at these plots as well. The aim of the project was to study the regeneration and anthropogenic usage of degraded forest areas, to mitigate the human impact on primary rain forests in Amazonia. Study sites were two polyculture tree plantations (POA, POC) and two plots of nearby secondary (growing since 1984, SEC) and undisturbed primary forest (FLO). Samples for both Enchytraeidae and Naididae were taken quarterly for two years and the worms were extracted by wet extraction (120 samples, each divided into litter and soil layer) per sampling date. The enchytraeids were identified in vivo, whereas naidids were initially only counted. Later on, and after fixation in EtOH, selected specimens were identified. Identification of the worms followed a site-specific key, based on information from the literature and own experience. The biomass of larger enchytraeids (i.e., the genus *Guaranidrilus*) was determined via weighing, while the biomass of smaller enchytraeids was estimated by using values previously determined for European species of similar size. In total, 18 enchytraeid and 14 naidid species were found. Most of the former belonged to the mainly neotropical genus *Guaranidrilu*s (5) and to the cosmopolitan genus *Hemienchytraeus* (5). Species of genera typical for temperate regions, such as *Achaeta* spp. (4) and *Enchytraeus* spp. (2) were also found. The abundance of enchytraeids found in the primary forest (1,000–10,000 Ind/m²) was comparable to those found at other tropical rain forest sites. The abundance in all four plots was similar, whereas the biomass was lower in POA and POC than in FLO and SEC. However, variability between replicates was high. No annual phenology pattern was observed, but dry conditions in 1997 had a negative influence on enchytraeids. The four plots were similar concerning species number and composition, but dominance patterns differed: the dominant genus in FLO was *Hemienchytraeus,* but *Guaranidrilus* on all other plots. Naidids seem to regularly occur in terrestrial samples of tropical rain forests; their species number was high in relation to their abundance but most species were new to science. The micro-annelid community indicated a clear distinction between forest (FLO, SEC) and plantation (POA, POC) sites.

Keywords Microdriles | Brazil | land use change | tropical forest soil | Clitellata | soil ecology

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1. Introduction

Based on experiences from temperate ecosystems it is assumed that important soil functions and services such as sustainable primary production or nutrient cycling (Adhikari & Hartemink 2016) depend mainly on the activities of soil organisms, such as microbes (e.g., Förster et al. 2009) and invertebrates, especially earthworms (Brown et al. 2018). However, in central Amazonia earthworms are rare and, despite their partly huge individual size (i.e., with a length of up to one meter), their impact on the soil system seems to be limited (Römbke et al. 1999). Little is known about the role of other soil organisms, especially the mesofauna, in providing these services in Amazonian soils (Lavelle et al. 1997). Thus, the work described in this contribution was aiming to increase our knowledge of two oligochaete groups in these soils, i.e., Enchytraeidae and Naididae.

Initially, the work focused only on enchytraeids as the representatives of mesofauna annelids. They are known to be an important group of invertebrate soil animals in temperate regions of the world but their contribution to soil functions, especially litter decomposition, in the humid tropics remains largely unexplored (Römbke 2007). However, during the project it became clear that another family of mesofauna-annelids is also diverse, frequent and abundant in these soils, namely the Naididae, usually considered as an aquatic group (Timm & Martin 2015). This family, including the former Tubificidae according to recent phylogenetic classifications (Erséus et al. 2008), was subsequently included in the investigations, i.e., by performing additional samplings at the project site (mainly in order to get more material for the description of the new species). In the following, enchytraeids and naidids will be referred to as ‘micro-annelids’ (in contrast to ‘mega-faunal’ oligochaetes, the earthworms).

As part of the SHIFT (‘Studies of Human Impact on Floodplains and Forests in the Tropics’) project ENV 52 ‘Soil Fauna and Litter Decomposition’, carried out from 1997 to 2004, the species composition, abundance, and biomass of these two groups were determined in two polyculture forestry plantations and in nearby secondary and primary forest (one plot each) in central Amazonia, Brazil (Höfer et al. 2001). The contribution of these faunal groups to litter decomposition was assessed as well. The two polyculture plots were designed as an alternative to the conventionally grown annual crops in the Amazon region. The general aim of the project was to study the regeneration and anthropogenic usage of already degraded areas, to mitigate the human impact on primary rain forest in Amazonia, with a special focus on the soil ecology of these areas. This contribution focuses on one basic aspect of the project: the diversity of Enchytraeidae and, to a lesser extent, Naididae. Preliminary results of these investigations have already been published (e.g., Römbke & Meller 1999). Here, we include abundance data and records of micro-annelids covering all sampling methods applied in the study: sampling of soil cores, exposing litter bags, and qualitative sampling.

2. Material and methods

2.1 Characteristics of the study sites

The study area of the SHIFT project ENV 52 was located at km 29 on the road Manaus-Itacoatiara (AM-010) adjacent to the research station of EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) – Amazonia Ocidental (Amazonas, Brazil) (Collado & Schmelz 2000a). The geographical coordinates are 02°53‘47‘‘S and 59°59‘45‘‘W. The area was flat, i.e. its highest elevations was 50–100 m above sea level. Mean annual precipitation during the study period was 2400 mm.

The area was divided into four study plots: an agroforestry plantation, divided into two adjacent study plots (POA, POC), a secondary forest (SEC), and a primary forest (FLO), all of them located close to each other. The FLO and SEC plots had a size of 40 m x 40 m, while the POA and POC plots had a size of 32 m x 48 m. In the latter two, several tree species of commercial use had been planted in rows, but the tolerated secondary vegetation (mainly *Vismia* spp., Guttiferae) still dominated the stand and especially the litter production (Beck et al. 1998; Höfer et al., 2001). The primary forest was an undisturbed (> 100 years) typical ‘terra firme’ (Portuguese for ‘firm land’) site, and comprised a mixed vegetation of hundreds of different plant species. None out of the approximately 40 tree species were dominant. An overview of the study site is given in **Figure 1.**

2.2 Climate

The climate of our study area was classified as an Af-type (A = mean temperature of all months > 18°C; f = monthly precipitation > 60 mm), according to Köppen (Schröder 2000). Monthly maximum temperature, average air and soil temperatures were highest in September 1997, and total annual rainfall in 1997 was 12–28 % lower than in the other study years (**Fig. 2**).

The year 1997 (when sampling started, see below) was classified as an ‘El Niño year’ (Wolter and Timlin 1998). Mean temperatures in the litter layer usually ranged from 25.6 to 27.7°C (min. 23.7°C, max. 34.7°C), and in the top soil (0–5 cm) from 25.7 to 26.1°C (min. 23.7°C, max. 27.7°C) (Martius et al. 2004a). Average moisture in the litter layer was 97%. The monthly litter temperatures were on average 2–4°C higher at POA and POC than at FLO and SEC. The highest average litter and soil temperatures as well as temperature maxima were recorded in POA and POC. The extent of canopy closure strongly determined litter temperatures at all sites (Martius et al. 2004b).

2.3 Soil properties

Soil properties were determined according to ISO guidelines (ISO 1992, 1994a,b). The soil type at all sites was Xanthic Ferrasol (sandy acid clay): 60% clay, 25% sand, 15% silt (FAO/UNESCO 1990). (**Table 1**). Soil pH was between 3.5 and 4.5 and the carbon and nitrogen content in the top soil layer (0–5 cm) showed a range of 2.5–4.5% and 0.20–0.31%, respectively. These values agree well with those of another study carried out at the same sites (Vohland and Schroth 1999). Water holding

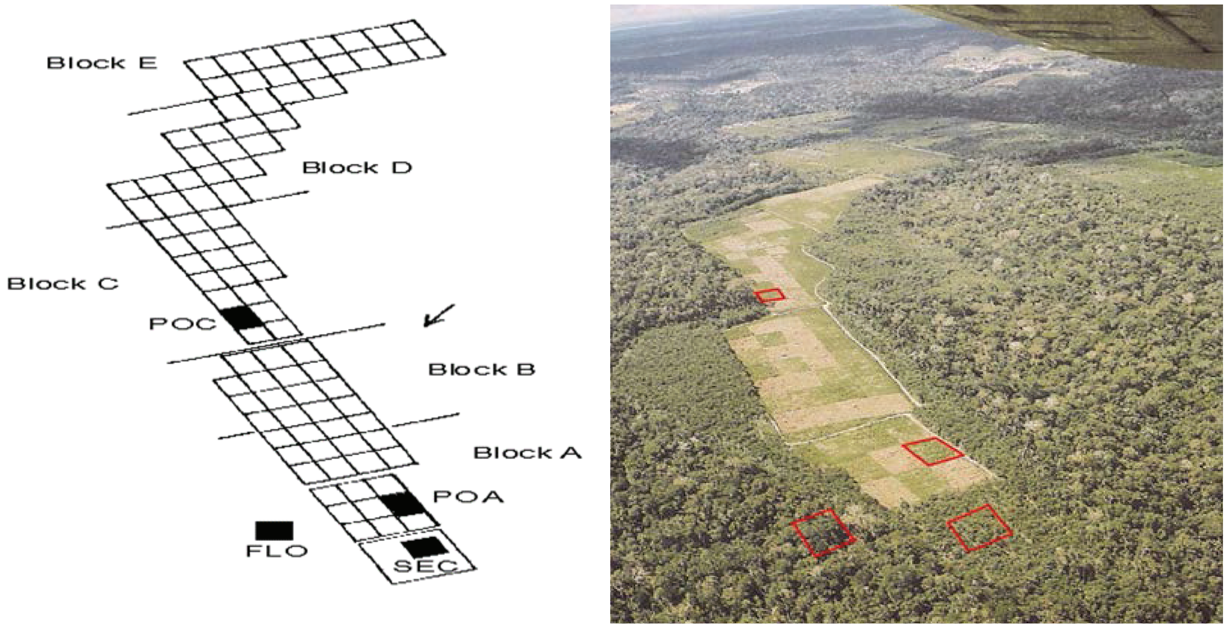

Figure 1. Design and aerial view of the field study at the EMBRAPA station (see text for details).

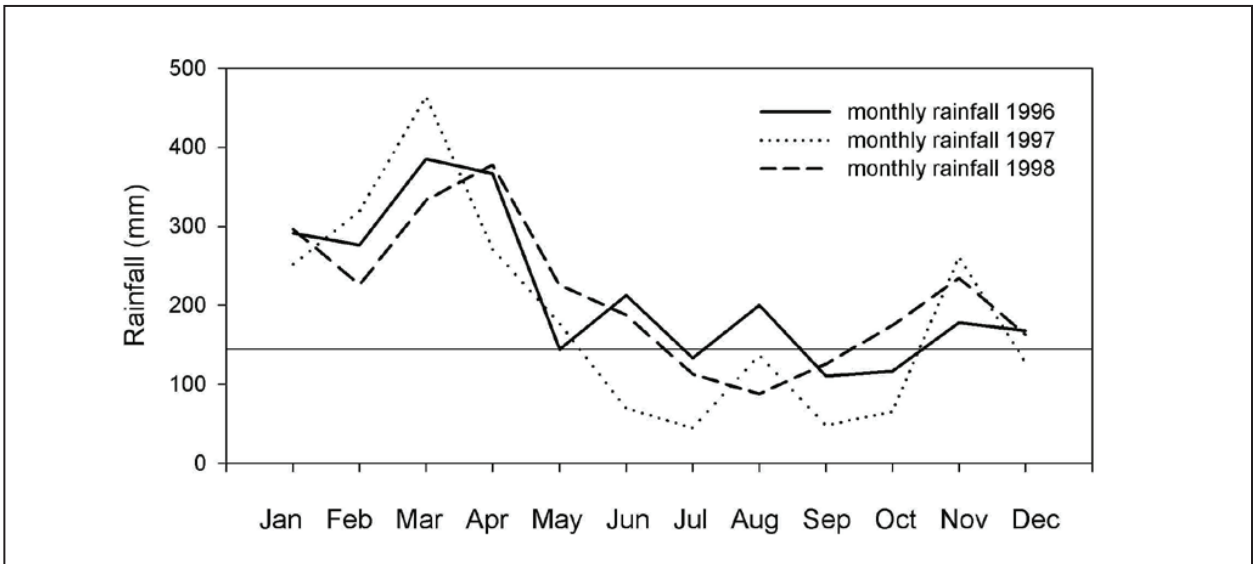

Figure 2. Monthly rainfall in each year: 1996–1999, measured at the weather station of Embrapa Amazonia Occidental, Manaus, Amazônia. Annual mean: 2392 mm (Martius et al. 2004a).

capacity was markedly higher at FLO than at SEC and POA (no data are available for POC).

2.4 Soil core sampling

Soil and litter samples (out of the same core) were taken every two to three months over a period of almost two years between July 1997 and March 1999, resulting in eight sampling dates. Samples were taken using a soil corer with a diameter of 6.4 cm (ISO 2006). In the field, each core was divided into two depth layers, the litter-layer including the root-mat and the uppermost mineral soil layer (0–5 cm depth). Each layer per core was stored separately. In total, 60 cores = 120 samples were taken per date: in POA and POC 10 cores each; in SEC and FLO 20 cores each. Micro-annelids were collected via the water flotation method (Römbke 1995; compliant with ISO 23611-3 (2007)) from the soil core samples. Extraction was done separately for the two layers when possible. In particular at the POA and POC plots, but, depending on the season, occasionally at the other sites as well, the litter layer was very thin or even absent. In addition to the general sampling programme, further soil core samplings were performed at the same sites but after the original sampling programme in order to get more material for the description of the new species, in particular of the naidids.

2.5 Litterbag sampling

In the same plots, organic matter decomposition was measured using the litter bag method (Swift et al. 1979), which in the meantime has been described in detail in an OECD guidance document (OECD 2006). Micro-annelids were collected from litterbags with three mesh sizes in order to assess their contribution to the process of litter decomposition (Höfer et al. 2001). Based on the results of a preliminary study (Beck et al. 1998), the following mesh-sizes were chosen: 10 mm gauze for the coarse mesh bags, 0.25 mm gauze as medium mesh and 20 µm for fine mesh bags. The experiment started in the summer of 1997 and the bags were exposed in the field in September 1997. At each of the six sampling dates between November 1997 and July 1998, usually two bags with the same mesh-size were taken from the FLO and SEC areas except at the first date (November 1997), when only one bag per mesh-size was retrieved. In POA and POC, one bag per mesh-size and plot was taken at each sampling date, respectively. In November 1997, POC was not sampled. A preliminary examination of some samples revealed an abundance relationship between enchytraeids and naidids of 1 : 1 in the coarse litterbags and 1 : 2 to 1 : 4 in the medium and fine mesh litterbags, respectively. All worms were stored in 70% EtOH immediately after determination.

2.6 Micro-annelid extraction

The micro-annelids were collected from the soil core samples and from the litter bags by same the standard wet-extraction-method described already for the soil cores (Römbke 1995; compliant with ISO 23611-3 (2007)). As a first step the fresh weight of all litter samples was determined. Extraction time was approximately 3 days at 22 ± 3°C (20–22°C is optimal). Subsequently the enchytraeids were immediately identified in vivo, whereas naidids were identified after fixation in EtOH.

2.7 Micro-annelid identification

Selected animals were stained and mounted individually on object slides. Identification of the micro-annelids was carried out to genus-level, following a site-specific key, prepared for the EMBRAPA site based on information from the literature and from the INPA (Instituto Nacional de Pesquisas da Amazônia) collection. Specimens were separated into morpho-species using light-microscopical characters. Consistent identification to species-level was not possible because at the time of the study almost

Table 1. Soil properties of the four study plots at Embrapa Amazônia Occidental. WHCmax: Maximum water-holding capacity.

Parameter	FLO	SEC	POA	POC
Vegetation	Primary forest	Secondary forest	Polyculture agroforestry plantations	
Site History	Extensively used for timber extraction	Burned primary forest; rubber trees cut and burned twice (1984; 1992)	Slashed and burned twice; timber trees planted (1993); re-forested with 8 different tree species (four on each plot)	
pH value ($CaCl_2$)	4.0 ± 0.2	4.0 ± 0.1	4.2 ± 0.1	4.0 ± 0.2
WHCmax (%)	86.4	79.7	76.8	n.d.
C content (%)	3.5–4.5	2.5–3.3	2.5–3.5	3.1–4.5
N content (%)	0.26–0.31	0.21–0.25	0.20–0.26	0.23–0.30

nothing was known about micro-annelid species diversity from Central Amazonia, with exception of few individual species (e.g., Righi 1978). Some of the enchytraeid species were recognized and described only later (e.g., Schmelz & Collado 2005; Schmelz & Römbke 2005), but most of them are still not described. In addition, several new naidid species were described after the end of the study (Collado & Schmelz 2000a,b; Collado & Schmelz 2001; 2002). Due to these taxonomic uncertainties, most of the ecological results presented here focus on the genus level.

2.8 Biomass measurement

This parameter was only determined for enchytraeids (no method and/or comparable data were available for the Naididae). The determination of the biomass of these small and quickly desiccated worms is very difficult. Therefore, the following procedure was performed: some of the larger individuals (especially adult worms of the genus *Guaranidrilus*) were weighed directly. The biomass of other enchytraeid species was estimated by using values previously determined for European species of similar size (Römbke & Kreysch 1988).

3. Results

3.1 Soil core and litterbag samples: Species number and composition

About thirty taxa of micro-annelid annelids were distinguished at the sampled sites (**Table 2**). Most of the enchytraeid species (in total 18) belonged to the neotropical genus *Guaranidrilus* (5 species) and to the tropical/

Table 2. List of taxa of Enchytraeidae and Naididae found at the four EMBRAPA plots.

Taxon	Comment
ENCHYTRAEIDAE	
Achaeta neotropica Černosvitov, 1937	sensu lato*, see Schmelz et al. (2011)
Achaeta spp.	3 species, possibly new
Enchytraeus buchholzi Vejdovský, 1878	species aggregate; small, sexually reproducing species of *Enchytraeus*
Enchytraeus bigeminus Nielsen & Christensen, 1963	sensu lato*; fragmenting species, genetically different from *E. bigeminus* sensu stricto (Collado et al. 2011: as *Enchytraeus* sp. 'Manaus')
Guaranidrilus spp.	5 species, unidentified, possibly new
Hemienchytraeus stephensoni Cognetti, 1927	'cf.', identification not fully confirmed
Hemienchytraeus solimoensis Righi, 1978	'cf.', identification not fully confirmed
Hemienchytraeus patriciae Schmelz & Römbke, 2005	new species described on material from this study
Hemienchytraeus siljae Schmelz & Römbke, 2005	new species described on material from this study
Hemienchytraeus tanjae Schmelz & Römbke, 2005	new species described on material from this study
Marionina sp.	1 species, unidentified, possibly new species
Enchytraeidae gen. sp.	1 species, unidentified, possibly new species
NAIDIDAE	
Bothrioneurum righii Collado & Schmelz, 2000b	new species described on material from this study
Pedonais crassifaucis Collado & Schmelz, 2000b	new species described on material from this study
Pristina jenkinae (Stephenson, 1937)	redescribed (Collado & Schmelz 2001)
Pristina marcusi Collado & Schmelz, 2001	new species described on material from this study
Pristina notopora Cernosvitov, 1937	redescribed (Collado & Schmelz 2001)
Pristina silvicola Collado & Schmelz, 2000a	new species described on material from this study
Pristina terrena Collado & Schmelz, 2000a	new species described on material from this study
Pristina trifida Collado & Schmelz 2002	new species described on material from this study
Pristina spp.	5 species, unidentified, possibly new species

* Taxonomic comments: *Achaeta neotropica* sensu lato: First described as *Achaeta becki* sp. nov. in Schmelz & Collado (2005), later tentatively synonymized with *A. neotropica*, a putative species complex (Schmelz et al. 2011). *Enchytraeus bigeminus* sensu lato: A fragmenting species of *Enchytraeus* with 2 chaetae per bundle, genetically distinct from other fragmenting species of *Enchytraeus*, including *E. bigeminus* sensu stricto (Collado et al. 2011). COI-barcodes available (Schmelz, unpublished). *Hemienchytraeus* cf. *stephensoni*, *H.* cf. *solimoensis*: Reference material unavailable.

subtropical, cosmopolitan genus *Hemienchytraeus* (5 species) (Schmelz et al., 2013) Species of genera known also from the Northern Hemisphere, such as *Achaeta* (4 species) and *Enchytraeus* (2 species) were also found. Some identifications remained tentative ('cf.'), and many species still await description. One species could not be assigned to an established genus so far.

Most of the naidid species belonged to *Pristina* (11 species, 6 of them identified; of these, 4 new to science, **Table 2**). Two further species belonged to *Bothrioneurum* and the newly erected genus *Pedonais* Collado & Schmelz, 2000b, respectively. Occurrence of this aquatic family in terrestrial habitats was not unprecedented, for example, *Dero multibranchiata* had previously been found in Amazonian inundation forests (Irmler, 1989), its high species diversity was nonetheless surprising.

Regarding the distribution of genera at the four study plots, most of the differences were not very pronounced, except in the case of specimens of the genus *Hemienchytraeus*: they were dominant at the FLO plot, whereas members of the genus *Guaranidrilus* were dominant on the other three plots

3.2 Soil core samples

Enchytraeid number and biomass:

Mean abundance and biomass of enchytraeids in the four plots (on average 4,300–6,200 Ind/m^2) are presented in **Tables 3 + 4** and in **Figure 3** (abundance only, sum of soil and litter samples). Average abundance as well as biomass values were lower at the two plantation areas than in the two forest plots, on average by about 20%. However, the variability at each date in 1997 and 1998 was so high that these differences are statistically not significant. In 1998 both values were higher in the wet season (December–April) than in the dry season (June–October). Lowest numbers were found in 1997, probably due to the extreme drought in late 1997. Biomass dynamics follow the abundance curve, with very few exceptions; thus, biomass curves are not presented here (but see **Table 5**). However, these values are based on few samplings, mainly in the wet season.

Juvenile/adult age ratio:

On average (i.e., all plots and sampling dates together), 71% of enchytraeids were juveniles and 29% adult – a

Table 3. Number +/- Standard-Deviation (SD) (Ind/m^2 * 1000) of Enchytraeidae from the four study plots and at different sampling dates. (SD: Standard Deviation).

Date	FLO		SEC		POA		POC	
	AB	±SD	AB	±SD	AB	±SD	AB	±SD
07/97	5.5	3.5	7.0	5.1	2.5	1.8	3.9	2.3
09/97	2.5	1.4	2.8	1.6	3.3	2.3	2.1	2.3
12/97	1.7	0.9	1.8	1.2	2.0	1.8	2.2	1.6
03/98	3.4	3.4	3.0	2.6	6.4	6.8	3.7	2.6
06/98	8.2	4.1	9.8	6.8	9.9	13.9	6.6	8.0
09/98	5.8	5.3	3.9	4.5	1.9	2.1	2.3	3.5
12/98	10.4	23.1	9.1	10.7	6.7	7.8	7.1	6.1
03/99	7.3	7.0	12.6	9.0	8.5	9.9	6.5	4.9
Mean	**5.6**	**3.0**	**6.3**	**3.9**	**5.2**	**3.2**	**4.3**	**2.1**

Table 4. Biomass (mg FW/m^2) of Enchytraeidae from the four study plots and at different sampling dates.

Date	FLO		SEC		POA		POC	
	BM		BM		BM		BM	
07/97	412		554		194		314	
09/97	153		234		250		158	
12/97	119		167		133		170	
03/98	309		290		566		314	
06/98	903		871		792		453	
09/98	669		301		146		303	
12/98	668		796		517		523	
03/99	875		1350		653		672	
Mean +/- SD	**514**	**+/- 289**	**570**	**+/- 383**	**406**	**+/- 240**	**363**	**+/- 165**

ratio which is in the normal range known from ecological studies in temperate regions (e.g., Römbke 1989, Beck et al. 2007). Nearly no differences of this ratio were found between the four plots (**Table 5**): 69 to 75 % of all enchytraeids were juvenile. This ratio was quite stable during the whole study period in all four plots. Due to the problematic taxonomic situation and the fact that often juveniles can only be determined to the genus level, no data on the juvenile/adult ratio for the individual species are presented here.

Litter / soil ratio:

Enchytraeid abundance and biomass were determined separately in the two layers (litter and soil); the litter to soil ratio (based on abundance) is given in **Figure 4** and **Table 5**. On average, the worms were more or less evenly distributed between the two layers at FLO and SEC, but at the two plantation sites the majority was found in the soil. During the dry season no worms were found at all in many litter samples. In general, in POA and POC the abundance in the litter layer was much lower than in the soil layer. The litter/soil ratio in FLO (41 : 59 %) and SEC (58 : 42 %) differed strongly between the first three sampling dates compared to the remaining study period: in the latter period the abundance in the litter was much higher than in the soil. These differences were probably caused by the moisture regime: in the dry period in 1997 there were about twice as many worms in the soil than in the litter layer while in the remaining time period more than twice as many enchytraeids were found in the litter except for the last sampling date. In short: if the moisture was sufficient, the worms preferred the litter layer. Only during periods of drought stress, they either retreated to deeper layers, or died. The same but even more pronounced behavior was true for the naidids.

Naidid number:

Naididae were found at very different numbers in the four sampling plots and at various sampling dates (**Figure 5**). At FLO, they were usually found in numbers

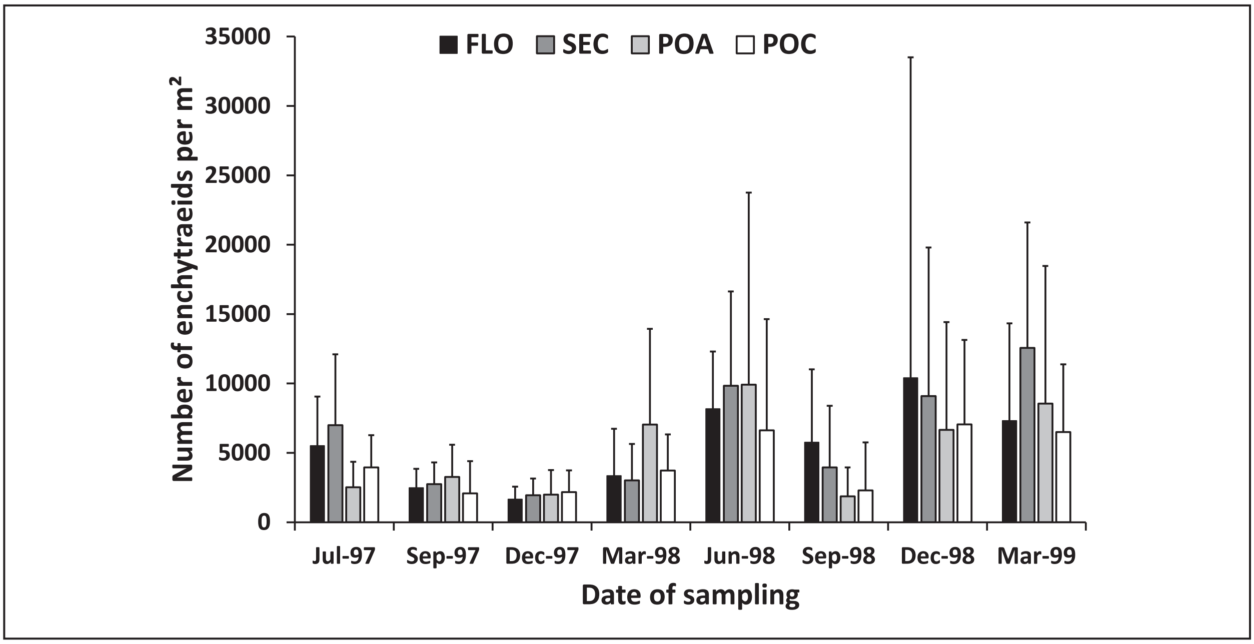

Figure 3. Enchytraeid abundance [Ind/m²] at the eight sampling dates (soil cores).

Table 5. Summary of data describing the enchytraeid biocoenosis at the four investigated plots.

Parameter	FLO	SEC	POA	POC
Abundance [Ind/m²]	5,600	6,300	5,200	4,300
Coefficient of variation	54 %	63 %	61 %	49 %
Biomass [mg FW/m²]	514	570	406	363
Coefficient of variation	56 %	67 %	59 %	45 %
Number of species	18	17	18	19
Juven./adult ratio [%]	69 : 31	71 : 29	74 : 26	75 : 25
Litter/soil ratio [%]	41 : 59	58 : 42	33 : 67	27 : 73

of up to 1000 Ind/m^2 in in the months March till June, but did only rarely occur in samples taken in the other months, in the soil cores. Peaks in abundance coincided with the rainy season in March and lasted for one to three months (no samples were taken in January). Basically, the same pattern was found at the other three plots as well, meaning that naidids were found most regularly and also in the highest numbers in the primary forest (FLO), while on the other three plots they occurred less regularly and in (slightly) lower numbers.

3.3 Litterbag samples

Enchytraeid numbers:

Numbers of enchytraeids counted in the litterbags are presented in Table 6. Enchytraeids were found at each sampling date. Two bags with the same mesh-width were taken from the FLO and SEC areas except at the first date (November 1997), where only one bag was used. In POA and POC always just one bag per area was taken, respectively (**Table 6**). Again, in November 1997 only POA was sampled. In February 1998, erroneously both bags per mesh-width were taken from POC whereas no bag was used from POA. However, due to the dry soil at these dates no worms were expected in the respective bags. By far the highest number of worms were found at the FLO plot while, with one exception (January 1998 at SEC), no or only very few individuals (1–6 per bag) were sampled at the other two sites. Thus, only at the FLO site the three mesh sizes could be compared regarding enchytraeid numbers. No clear difference between the three mesh sizes was found.

Naidid numbers:

Naidids were found at all sampling dates but in very different numbers in the four plots (**Table 7**). They occurred most regularly in the primary forest (FLO; maximum: 92 Ind/bag in July 1998, minimum: 3 Ind/bag in December 1997). In SEC, with one exception (46 Ind/bag in July 1998), always low to very low numbers were found. In both agroforestry plantation plots (POA, POC) no naidid worms were found even during the rainy season. This situation was probably caused by the lack of a continuous litter layer in the rows between the trees at both plantation sites, meaning that there were regularly times with dry, i.e., unfavourable, conditions at these sites. Similar but less drastic observations were also made for enchytraeids.

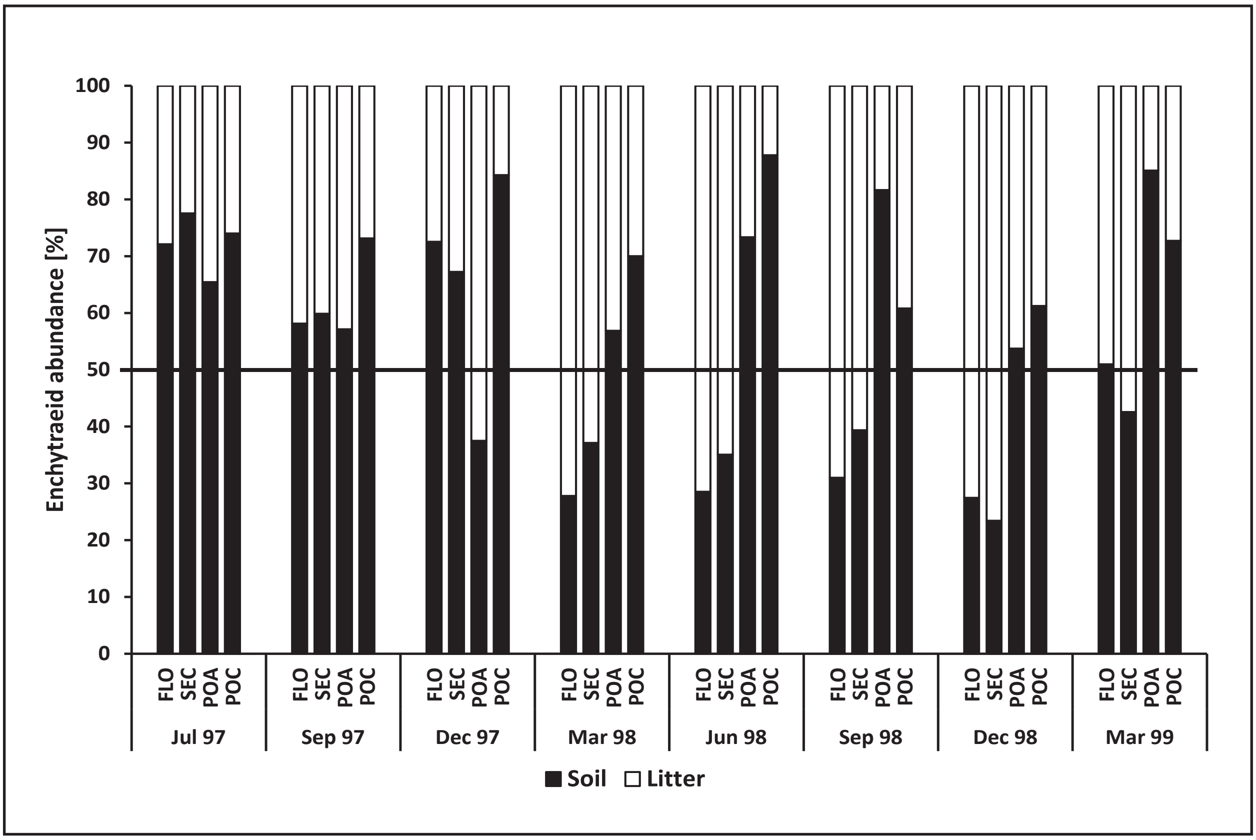

Figure 4. Litter/soil ratio of enchytraeid abundance at the four study sites at eight sampling dates.

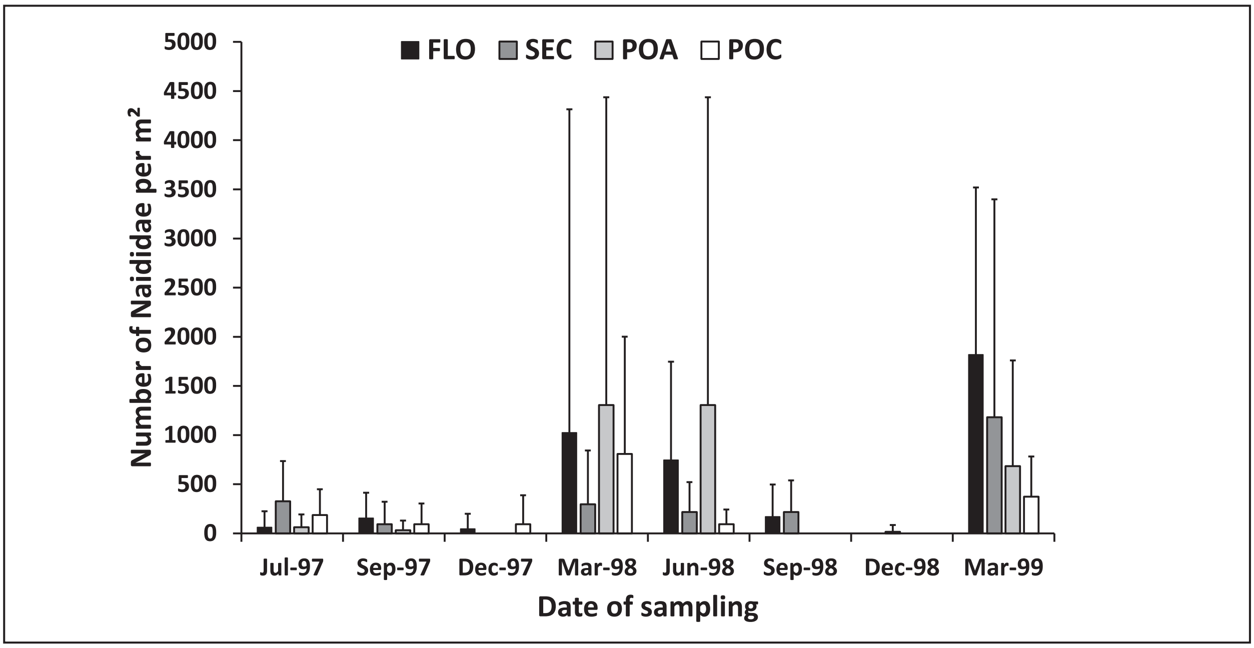

Figure 5. Naidid abundance [Ind/m^2] at the eight sampling dates (soil cores).

Table 6. Number of Enchytraeidae per litter bag (three mesh-widths) from the 4 study areas and 6 sampling dates (X = erroneously, no bag was taken from this plot in Feb. 1998).

	FLO			SEC			POA			POC		
Date:	Coar.	Med.	Fine	Coar.	Med.	Fine	Coar.	Med.	Fine	Coar.	Med.	Fine
11/97	25	55	155	5	0	0	0	0	0	-	-	-
12/97	5	4	0	0	0	0	0	0	0	1	1	0
01/98	24	112	9	37	38	1	0	0	0	0	2	1
02/98	25	3	49	0	0	0	X	X	X	1	0	0
04/98	144	64	15	25	0	0	1	0	0	1	0	5
07/98	93	192	268	0	0	0	0	0	0	4	0	6

Table 7. Number of Naididae per litter bag (three mesh-widths) from the 4 study areas and 6 sampling dates (D = sample destroyed).

	FLO			SEC			POA				POC		
Date:	Coar.	Med.	Fine	Coar.	Med.	Fine	Coar.	Med.	Fine		Coar.	Med.	Fine
11/97	15	27	0	D	D	0	-	-	-	-	-	-	-
12/97	3	0	0	D	D	0	-	-	-	-	-	-	-
01/98	14	51	5	D	D	0	-	-	-	-	-	-	-
02/98	17	0	16	D	D	0	-	-	-	-	-	-	-
04/98	80	0	15	16	0	0	-	-	-	-	-	-	-
07/98	56	92	32	18	46	8	-	-	-	-	-	-	-

4. Discussion

4.1 Literature records of enchytraeids and naidids in Central Amazonia

Prior to this study, very little was known about the species diversity of enchytraeids in Amazonian forest soils, and the few previous finds are poorly documented. For example, the type material of the only enchytraeid species described from the region of Manaus (an inundation forest (Varzea)), *Hemienchytraeus solimoensis* Righi, 1978, and deposited in the collection of the INPA museum (Manaus), was found to be destroyed (Römbke, pers. obs.).

No comparable data on naidids from terrestrial sites are known to us from the Manaus region. However, in inundation forests of the Manaus region the abundance of the naidid species *D. multibranchiata* is usually about 200–300 Ind/m^2 (Irmler, 1989), whereas in blackwater regions a maximum of up to 10,000 Ind/m^2 can be reached (Irmler 1975). The investigation of the naidids was not part of the original project but these organisms were found regularly, both in the soil core samples and in the litterbags. Not much was done with this material, but Augustsson showed, as part of her Ph.D. thesis, that they are quite common in the soils of the study area, especially in the primary forest (Augustsson 2001). In any case, it is highly likely that these micro-annelids are much more common in terrestrial habitats than it is known today. In fact, except for some isolated information, like the occurrence of the naidid species *Pristina jenkinae* (Coates & Stacey, 1994, as *Pristinella jenkinae*) in a Peruvian rain forest, nearly nothing is known about naidid species in tropical soils. The high diversity of a predominantly aquatic family at the study sites was therefore surprising. Interestingly all species belong to genera where asexual reproduction is common (Timm & Martin 2015). Collado & Schmelz (2000b) suggest this as a prerequisite for aquatic oligochaete taxa to colonize terrestrial habitats.

Under certain favorable conditions (e.g., in January and, partly, in April 1998) enchytraeid and naidid worms entered the SEC litterbags. Nearly no worms were found in POA and only very few in POC litterbags. Probably this situation was caused by the unfavorable abiotic conditions in the litterbags, which were exposed on the soil surface to very high temperatures and thus dry conditions, killing all worms or driving them out of the litter back into the soil.

The worms from the two annelid families differed strongly in size. The naidids, mostly represented by *Pristina* spp., were only up to 1 mm long, and juveniles (fragmenting specimens) often much smaller (length ca. 0.5 mm, diameter often down to 60 µm). This explains, at least partly, why they were so abundant, especially in the fine litterbags, leading to the situation that the overall number in all three mesh-widths of FLO litterbags was similar but in tendency even higher in the medium and fine mesh bags. However, an open question remains: How could have enchytraeids entered a fine-mesh litterbag? Adult worms, with a body diameter of 0.14 to 0.4 mm, were too big to pass a mesh size of 20 µm. Cocoons falling through the mesh can also be excluded, since their smallest width equals the body diameter of an adult worm (Westheide & Müller 1995). Freshly hatched juvenile specimens, often not wider than 0.06 mm, may be able to pass: due to the plasticity of these soft-bodied worms, enchytraeids are able to pass holes of less than 1/4 of their usual body diameter (Schmelz, pers. obs.). However, we suspect that tropical soil arthropods created small holes exceeding the size of the fine mesh, undetectable with the naked eye but large enough for enchytraeids to pass.

The contribution of micro-annelids to litter decomposition is evidently zero or very low at sites without or with very few specimens in the litter bags (Table 6). Due to the presence of micro-annelids even in bags with the finest mesh size, our data cannot be used to estimate the contribution of these worms to litter decomposition. Their contribution at sites without severe drought, however, deserves further study, since there are indications from studies in temperate regions that micro-annelids worms influence nutrient mobilization in flooded soils (Plum & Filser 2008).

In general, and considering field methods under tropical conditions, sampling and extraction of soil annelids are quite similar to ecological studies in temperate regions. In case that an air-conditioned laboratory is available within a short distance from the study site, almost no differences regarding the technical performance occur. But even if such a laboratory is not at hand, the investigation of micro-annelids is possible. In such a case, a temperature range of 15–25°C has to be secured during the transfer of the cooled samples to a laboratory. For quantitative studies, cooled samples should be stored not longer than one week; for qualitative studies, however, worms can be obtained even after two months of storage at room temperature (20 ºC, Schmelz & Collado, pers. obs.). Niva et al. (2010) give an introduction into the methodology of studying enchytraeids under tropical conditions. They recommend storage of samples not below 12ºC.

Influence of climate on enchytraeids:

Our results indicate that the numbers of enchytraeids depend on the climatically influenced soil properties, especially soil moisture (Petersen & Luxton 1982). Martius et al. (2004) showed that biomass of macrofauna (including earthworms) and arthropod mesofauna is correlated with the canopy closure as a proxy for different climate-related factors. Enchytraeids were not included in this study, but due to their life form it is very likely that they react at least as much as the other organism groups. Enchytraeids are very sensitive to drought in combination with increased temperatures (Holmstrup et al. 2012), a condition that occurred regularly in the litter layer of all study plots of the present study. Similarly, earthworms were found mostly in the wet season (with a peak in March) but due to their low absolute numbers this relationship is difficult to prove (Römbke et al. 1999).

Comparison of our results with other tropical soil ecological studies:

The abundance and biomass values found in the four investigated plots are very difficult to compare with literature values from the tropics since almost none exist so far. In **Table 8** ecological studies with enchytraeids performed in subtropical and tropical rainforests are listed. As far as can be said from this limited information (e.g., only in this study and at Pasoh (Malaysia) samples were taken in different seasons), enchytraeids seem to be relatively rare (i.e., less than 10,000 Ind/m^2) in tropical rain forests in comparison to moist temperate soils (Petersen & Luxton 1982). [In contrast, abundance can be very high at cool montane sites, see the review in Schmelz et al. (2013)]. In an intensive study performed in two regions with differently aged forests on two soil types in south-eastern Brazil (Paraná; Atlantic Rain Forest), using the same wet-extraction method, also less than 10,000 Ind/m^2 were found (Römbke et al. 2015). In that study, diversity was very high – 61 enchytraeid species could be discriminated. Actually, with the exception of this last study no other example was found in which the enchytraeid fauna would have been studied in such detail, i.e., during several years in different plots with various parallel investigations (e.g., on litter decomposition or the influence of canopy closure on soil fauna) and – last but not least - addressing the species level.

5. Conclusions and outlook

The most important results gained when investigating the micro-annelid worms of the four EMBRAPA plots can be summarized as follows, taking into consideration that the study described here is one of the few ecological investigations of micro-annelids in neotropical rain forests:

- slightly modified methods routinely used in temperate regions are also useful in the tropics;
- the number of enchytraeids in the primary forest was comparable to those few described from other rain forest sites; however, the data basis is very small;
- nothing is known about enchytraeid assemblages of tropical secondary forests or agro forestry sites; thus, a literature comparison with our data was not possible;
- enchytraeid abundance at all four plots was quite similar whereas the biomass was lower on POA and POC than in FLO and SEC;
- no clear annual population dynamics were observed for enchytraeids, but the dry conditions in 1997 had a negative influence on numbers and biomass; in contrast, naidids showed peaks of abundance in the rainy season;
- in general, the four plots were similar concerning species number (high in comparison to many forest sites in temperate regions) and composition, but the dominance spectrum was different (e.g., the dominant genus was *Hemienchytraeus* in FLO and *Guaranidrilus* in all other plots);
- the juvenile/adult ratio was very similar in all plots and at all sampling dates;
- the litter layer was significantly less inhabited in POA and POC than in FLO and SEC;
- nothing can be said so far concerning the ratio between endemic and peregrine species since the taxonomic assessment is not completed;
- a clear relationship between climatic parameters (positively: rainfall, humidity; negatively: soil and litter temperature and dryness) with enchytraeid biomass were found; some exceptions (especially in POA) cannot be fully explained;
- naidids were regularly found in terrestrial samples of a tropical rain forest for the first time; their species number (14) seems to be high (about two-thirds of them are known to science so far);
- based on the results of litterbag samples, micro-annelids (especially naidids) can reach high numbers under favorable conditions (especially in the permanently moist litter layer of FLO);
- the same distribution pattern was found when assessing the soil-core samples: naidids were quite abundant in FLO, rare in SEC and practically lacking in POA and POC.

Table 8. Ecological studies with enchytraeids in subtropical tropical rain forests (#: Species distinguished or not).

Study site	Method; Rep. #	Ind/m²	Species #	Reference
Not specified	Not specified	Up to 1000	No	Swift et al. 1979
Honshu, Japan	Nielsen; 7. Rep.	10.000	No	Kitazawa 1971
Sepilok, Malaysia	Nielsen, no Rep	1.000	No	Kitazawa 1971
Pasoh, Malaysia	O Connor, 20 Rep.	2000–23000	No	Chiba et al, 1976.,
Panguana, Peru	Wet extract., 10 Rep.	4000–5000	No	Römbke, unpubl.
Manaus, Brazil	Wet extract., 10 Rep.	1000–10000	Yes	This study
Cachoeira, Brazil	Wet extract., 10 Rep.	1600–2500	Yes	Römbke et al., 2015
Itaqui, Brazil	Wet extract., 10 Rep	3200	Yes	Römbke et al., 2015

Based on these results, the micro-annelid biocoenosis indicated a clear distinction of FLO (and partly SEC) in comparison to POA and POC. There are still some open questions:

- the correlation between micro-annelid biomass and litter stock and/or decomposition rates has to be determined;
- the role of naidids has to be assessed further (including laboratory tests on feeding habits and respiration (Augustsson, 2001));

Finally, these data could be used as an incentive to study the specific contribution of these organisms to the decomposition of organic matter at tropical moist forest sites. Since it is not to be expected that the direct influence of these small worms (taking their relatively low biomass into account) on decomposition is very high, further assessment should concentrate on their potential role as indicators, e.g., for anthropogenic effects.

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