The earthworm fauna of regenerating forests and anthropogenic habitats in the coastal region of Paraná

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Abstract – The aim of this study was to evaluate abundance, biomass and diversity of earthworms in the southern coast region of the Mata Atlântica biodiversity hotspot. A total of 51 study sites in pastures, banana monocultures, mixed agroforestry systems, secondary forests in succession and old-growth forests near the coast of Paraná, Brazil, were evaluated. Each site was sampled once. Species richness of the earthworms was generally low and varied little between sites. At all sites except for one, the peregrine species Pontoscolex corethrurus (Glossoscolecidae) strongly dominated. Three other peregrine species, Amynthas corticis, Amynthas gracilis (Megascolecidae) and Ocnerodrilus occidentalis (Ocnerodrilidae), were frequent in moist sites. No autochthonous species were found. Abundance and biomass of earthworms varied strongly within and between sites (0–338 individuals m\(^{-2}\), 0–96 g m\(^{-2}\) fresh weight). Pastures had significantly lower abundance than all other sites. The forest sites had similar earthworm abundance and biomass, with a tendency to be higher in younger succession stages. The coastal plain region has been strongly altered by human activities. Reasons for the lack of any autochthonous species and the dominance of one peregrine species require further investigation.

Index terms: Pontoscolex corethrurus, Atlantic Forest, biodiversity, Oligochaeta, peregrine species, secondary forest.

Introduction

Earthworms are a dominant group of soil animals in the humid and subhumid tropics (Fragoso et al., 1999). In recent years, research on this topic increased, particularly in Brazil (González, 2006; Brown & Fragoso, 2007), resulting in major compilations of earthworm diversity (Brown & Fragoso, 2007), assessments of earthworms role in agricultural systems and in secondary and primary forests, the identification of impacts of different land uses on earthworm populations and diversity, and their role as bioindicators. Although these questions have been partly addressed in several parts of Brazil (Lavelle & Lapied, 2003), knowledge on the earthworm fauna of the second largest forest biome in Brazil, the Atlantic Forest (Mata Atlântica) – worldwide recognized as a hotspot of biodiversity – is still scarce. Therefore, earthworms
The earthworm fauna of regenerating forests were sampled as important representatives of the soil fauna with an expected high functional importance, as part of the activities of the German-Brazilian project SOLOBIOMA. This project aims to assess the ecosystem quality of secondary forests, in the coastal region of Paraná state, and especially their potential for conservation of biodiversity and provision of ecosystem services.

The objectives of this study were to: assess the diversity and structure of earthworm assemblages, in the southern Mata Atlântica; evaluate how the earthworm fauna recovers during forest regeneration from pastures; and assess which site properties determine the abundance, biomass and diversity of the earthworm fauna.

**Materials and Methods**

The study sites were located within an area of about 30x80 km², along the coast of Paraná state, in the municipalities Antonina, Guaraqueçaba and Paranaguá. Most sites are situated within and adjacent to the “Área de Proteção Ambiental” Guaraqueçaba (Ferretti & Britez, 2006). Climate of this coastal region is mesothermic subtropical humid, corresponding to Köppen’s Cfa-type (Schröder, 2000; Strahler & Strahler, 2005). Mean annual temperature is above 18°C, and monthly precipitation is over 60 mm. Frost rarely occurs in areas from sea level to 700 m a.s.l. (Instituto Paranaense de Desenvolvimento Econômico e Social, 2001). Annual rainfall in the region varies between 2,000 and 3,000 mm (Roderjan & Kunyoshi, 1988) and is seasonal. Lower rainfall occurs from the autumn end to winter (April to August), and higher rainfall occurs during the warmer months of Brazilian summer (September to March) (Instituto Paranaense de Desenvolvimento Econômico e Social, 2001). Soils of the region are mainly Cambisols in the submontane range, and Podzols and Gleysols in the lowland range (Food and Agriculture Organization of the United Nations, 1998). These soil types are characterized by low pH, [3.5–4.3 (CaCl₂)], sometimes reaching 5.0, under pastures], C/N-ratios from 15–40 and organic matter contents of 2–5% in the upper 20 cm of soil.

One group of study sites (1–15 and 19–30, in Table 1) is situated within the “Reserva Natural do Rio Cachoeira” (herein called Cachoeira forest) and includes pastures, secondary forest stages naturally regenerating from pastures, and old-growth forests (>80 years old). The latter were never completely cut down and used as pastures. Pastures and secondary forests were found on the two most frequent soil types: well drained Cambisols (1–15) on slopes, and hydromorphic Gleysols (19–30) in the plain. For every stage on each soil type, three replicate sites were studied, and analyses of variance were done for the effects of stages and soil type. The second group of study sites (31–42), also comprising regenerating secondary forests and old-growth forests on Cambisols, is situated approximately 30 km to the east, within the “Reserva Natural do Itaqui” (herein called Itaqui forest). Likewise for all stages, three replicate sites were studied, allowing statistical analysis of the effect of stage and a comparison of the two areas, Cachoeira and Itaqui, based on Cambisol sites. These 39 sites within the submontane forest region represent the natural regeneration process, covering mosaic-like distributed patches of forests, which differ in age since abandonment of use, as buffalo pastures, from 3 to >80 years and, thus, represent a gradient of decreasing anthropogenic influence. These are the core sites of a large biodiversity study within the German-Brazilian project SOLOBIOMA. All sites are owned and administrated by the Brazilian NGO “Sociedade de Pesquisa em Vida Silvagem e Educação Ambiental”.

Within the Cachoeira forest area, three further sites (16–18) of three different regeneration stages on Cambisols were used for functional studies of litterfall, decomposition and nutrient cycling. The same accounts for three sites (43–45) of lowland forest on Podzols, situated in the municipality of Paranaguá, within the reserve “Floresta Estadual do Palmito” (530 ha, herein called Palmito forest). A detailed description of these sites, which represent the forest formations submontane and lowland Atlantic forest, is reported by Schmidt et al. (2008).

Six sites were located just outside the Cachoeira forest reserve, at a place called “Rio Pequeno”, about 20 km north of Antonina (Table 1), and represent currently cultivated sites with agroforestry plantations (46–51). Three were banana monocultures, and three mixed systems with bananas and palmito palms (*Euterpe edulis*), both very frequent smallholder systems in the region. Nearly all these sites stock on gleyic, groundwater-influenced soils.
Earthworms were sampled in a total of 51 sites, situated in two regions with different forest formations – lowland and submontane forest –, different land cover types – plantations, grassland and forest regeneration stages –, on three different soil types, Cambisols, Gleysols, Podzols, covering a gradient of anthropogenic influence, from agricultural sites and pastures to regenerating and old-growth forests.

At each site, five earthworm samples were taken on one occasion (Table 1), by using a combination of hand-sorting of soil, within a quadrate of 50x50 cm, to a depth of approximately 20 cm (International Organization for Standardization, 2007), followed by formol extraction (0.5% aqueous formol). Since almost no worms were caught after formol application, only hand-sorting was performed at the six agroforestry sites. All worms were stored in 70% ethanol before being counted, weighted and determined to the species level. Abundance, biomass and species composition were used as measurement endpoints. The statistical tests (ANOVA) for effects of soil type and for differences between stages were done with Statistica 8.0 (StatSoft, 2007).

Table 1. Location, site characteristics, of the 51 sampling sites and earthworm abundance and fresh weight (FW) biomass.

<table>
<thead>
<tr>
<th>Site a</th>
<th>Municipality</th>
<th>Location</th>
<th>Land use(1)</th>
<th>Soil type</th>
<th>Regeneration stage(2)</th>
<th>Age (2003)</th>
<th>Altitude m.a.s.l.</th>
<th>UTM coordinates (SAD99)</th>
<th>Species number</th>
<th>Abundance</th>
<th>Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Antonina</td>
<td>Cachoeira</td>
<td>Pasture</td>
<td>Cambisuel</td>
<td>0-0.2</td>
<td>2003</td>
<td>754354</td>
<td>350,025</td>
<td>3</td>
<td>114.8</td>
<td>24.3</td>
</tr>
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<td>2</td>
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<td>Cachoeira</td>
<td>Pasture</td>
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(1) Agro mono, agroforestry system with banana monoculture; agro mixed, agroforestry system with banana and palmito. (2) Herb, young herbaceous stage; arb, young arboreal stage; med, medium old arboreal stage; old, old-growth forest; ini, initial succession stage; adv, advanced succession stage. Stdev, standard deviation.

Results and Discussion

Earthworms belonging to three families (Glossoscolecidae, Megascolecidae, Ocnerodrilidae) were found. Species richness was generally low (1–4 species per site) and did not differ considerably between the sites or vegetation types. Altogether five species, all of them peregrine, were regularly found. In anthropogenic habitats in Brazil, like pastures, crop and agroforestry systems, as well as secondary forests, 2 to 4 species are usually found, whereas at primary forest sites, species richness is generally higher (on average 5, range: 2–10) (Brown & James, 2007). In all sites, except for banana/palmito plantations, Pontoscolex corethrurus (Müller, 1857) strongly dominated the samples, usually representing 90–99% of abundance (Figure 1). Very moist sites (e.g. close to small creeks) and also some agroforestry sites showed higher proportions of Amynthas spp. – A. corticis (Kinberg, 1867) and A. gracilis (Kinberg, 1867). In the agroforestry sites, Ocnerodrilus occidentalis (Eisen, 1878) or Dichogaster spp. were frequently found. At two banana/palmito sites, Amynthas spp. as well as O. occidentalis reached about 25% of abundance, respectively. One of these sites was located very close to a human settlement, while the other was adjacent to a small river.

![Abundance and biomass of earthworms per plot varied between 0 and 338 individuals m⁻² and 0 and 96 g fresh weight m⁻² (Table 1). Within the secondary forests, there was high variance of abundances (Figure 2). Biomass was almost always very similar to abundance, due to the fact that one species (P. corethrurus) dominated all samples and was almost always present with all development stages, from juvenile to adult. The only sites strongly differing from all other sites were the lowland forest sites in Palmito forest (43–45), with very low abundance (and biomass). In one site, no earthworm was found at all. This is readily explained by the very sandy (90 to 98% sand) and nutrient depleted soils in this site (Boeger et al., 2005). In these sandy soils, the upper horizons dry out quickly due to drainage and, in the lower horizons, the high ground water level creates anoxic situations, both of which are fatal for worms. The lack of available nutrients (organic matter), due to the relatively low input (litter fall) from the sparse plant cover, and very irregular (clumped) distribution of litter in the younger stages (Pinto & Marques, 2003) might negatively influence earthworm abundance (Lee, 1985).

In the three corresponding Cachoeira forest sites (16–18), on Cambisols, which were sampled at the same...
time, earthworm numbers (70–250 individuals m\(^{-2}\)) were distinctly higher and in the range of the other sites in Cachoeira (see Table 1). Especially biomass was surprisingly high (20–70 g m\(^{-2}\) fresh weight) (Schmidt et al., 2008).

Based on the replicated stages, several designs were analyzed using ANOVA. Comparison of pastures and secondary forests on Gleysols and Cambisols (sites 1–12, 19–30), in the Cachoeira reserve showed a significant effect of regeneration stage, caused by the lower abundance and biomass in the pastures and no effect of the soil type (Figure 3). Comparison of secondary forests and old-growth forests on Cambisols, in Cachoeira, with the same stages in Itaqui, showed a significant effect of stage, with decreasing abundances and biomass from the herbaceous to the old-growth forests (Figure 4). Differences between the two areas were significant for abundance, but not for biomass. In the old-growth forests of Itaqui, abundance and biomass were slightly higher than in the medium old forests. When looking at the whole succession, from pastures to older forests, on Cambisols in the Cachoeira reserve, the increase of earthworm biomass from pastures to the two younger succession stages was significant. In addition, earthworm abundance and biomass were significantly lower in the two older stages (medium, old-growth), in comparison with the two younger succession stages (Figure 5).

The plantations showed high abundances and very high biomass, compared with the forests. They were significantly higher in the mixed banana/palmito plantations than in the banana monocultures. Actually, the highest abundance of all sites (338 individuals m\(^{-2}\)) was found in a banana/palmito plantation (n=50). This site was the most closely located to a house and, also, the only one which was not dominated by *P. corethrurus*, but by the small peregrine species *O. occidentalis*.
The interpretation of these findings is facilitated by the fact that the earthworm assemblage was so strongly dominated by *P. corethrurus*, which is the best studied tropical earthworm species. Its high tolerance for a wide range of soil properties is confirmed by the absence of differences in abundance and biomass between sites on Cambisols and Gleysols. Low earthworm abundance and biomass in pastures can be explained by unfavourable conditions: the soil is compacted by buffalos, which impedes burrowing. The negative effect of soil compaction on the activities of *P. corethrurus* has been confirmed in laboratory tests (Zund et al., 1997). In addition, the daily temperature amplitude is higher than in shaded environments, causing low moisture values, at least in the uppermost soil layers. According to Lavelle et al. (1987), *P. corethrurus* can tolerate temperatures between 15 and 35°C, but reproduces only between 23 and 27°C. Significantly higher temperatures, in the 0–10 cm soil layer in pastures compared to forests, were cited as a possible reason for impacts on earthworm feeding, burrowing and reproductive activities in Puerto Rico (Liu & Zou, 2002).

The increase of *P. corethrurus* abundance in the two younger regeneration stages may partly be caused by a decrease of soil density and improved moisture levels caused by the emergence of shading higher vegetation. Both this increase of *P. corethrurus* and the decrease in earthworm abundance and biomass at the two oldest succession stages are probably caused by changes in the chemical composition of the residues and soil organic matter available as food. Originally, *P. corethrurus* has been classified as a mesohumic endogeic species, which means that it consumes intermediate quality organic matter and not fresh or slightly decomposed litter. The discrepancy between using such low energy food and its often high abundance and biomass has been explained by the presence of a highly efficient gut microflora, well adapted to this type of food as well as to root and fungal (including arbuscular mycorrhizal fungi) substrates (Lattaud et al., 1998). Based on studies with labelled sugarcane material, it was proposed that this species may derive much of its tissue C from rhizosphere sources, which due its energy-rich composition would better explain its astonishing success in many different soils (Spain et al., 1990). In our study sites, in the coastal rainforest region, *P. corethrurus* seems to find its ideal C-sources only in the younger succession stages and, in particular, in the agroforestry sites. Lapied & Lavelle (2003) also found higher numbers in banana plantations (361 individuals m⁻²), compared to different forest sites (50 – 250 individuals m⁻²), in Costa Rica.

Studies of earthworm populations along forest succession in the tropics, with a replicated design, are rare. A study in Puerto Rico using three replicates of pastures (about 60 years old) young secondary forests (25 – 40 years old) and “mature secondary” forests (>77 years old) (Sanchez-De Leon et al., 2003) revealed strong dominance of *P. corethrurus*, in the two early stages, and co-occurrence with five anecic endemic species in mature forest. Number and biomass of the endemic species, all together reaching about one-third of *P. corethrurus*, were positively correlated with the amount of litter mass. Abundance and biomass were highest in the pastures and decreased significantly along the succession. Similar findings, in a nearby sequence of sites, were reported by Zou & González (1997). While no causal explanation was given, a positive correlation was found between earthworm density and fine-root biomass, thus pointing to root exudates as the most important factor. Interestingly, no negative effects on soil bulk density were found at the pasture sites in Puerto Rico, in contrast to Amazonia (Barros et al., 2004). In the study, we also observed a decrease along the succession, although it must be remembered that our “old growth” forests were never cut and used as pastures. But, even so, they did not contain any native species.

Almost no data on abundance or biomass of earthworms were available from eastern Paraná state. Therefore, we compare the data from the coastal region of Paraná with abundance data from western and central Paraná, representing mainly highland areas (Table 2) (Brown & James, 2007), being aware of the differences in climate, geology, soils, human activities etc. It seems that abundance and biomass of earthworms, at the agroforestry sites, are higher than those at other sites in Paraná, while there are no differences for pastures and secondary forests. Abundance and biomass data from the old-growth forests were higher than the ones reported in the literature (Table 2). This might be due to the fact that in our sites *P. corethrurus* dominated, while other “old” forest sites had endemic species. But it should also be considered that the category “old-growth/native/primary forest” is not well defined and, in most cases, no site history is known or reported.
It is likely that the coastal region of Paraná was originally inhabited by endemic glossocolecid species, as this has been observed in other coastal regions with Atlantic Forest of Brazil (Brown & James, 2007). Using a relationship first proposed by Fragoso (2001) for Mexico, and later modified for the state of São Paulo (31 spp. per 100 thousand km$^2$), the Atlantic Forest, which originally covered approximately 1.3 million km$^2$ of Brazil, could have up to 400 species of earthworms. Currently, 144 species are known from this region (35 peregrine and 109 endemic) (Brown & James, 2007). For the state of Paraná, 55 species are known today (20 peregrine and 35 endemic), implying that there are probably many species not yet been discovered (Sautter et al., 2006). Most samples taken up to the present date have been taken in highland areas of western and central Paraná, with few samples from the lowland coastal rainforest (Brown & James, 2008).

The present study implies that the endemic species expected for the Atlantic Forest, in this region, have been replaced by the peregrine invasive species *P. corethrurus*, or by South-Eastern Asian *Amynthas* spp. (Blakemore, 2002) exotic species, since these were the main species found at the sampling sites, which represented different vegetation types, soil types and land use forms. Thus, it seems that even the old forests of the coastal plain region (which is the oldest colonization front of settlers in the state of Paraná, beginning in the 1600s), have been affected by anthropogenic activities, leading to the loss of native species and the invasion by peregrine and exotic ones.

Similar findings have also been made in other Latin American natural forests as in Costa Rica (Lapied & Lavelle, 2003; Brown et al., 2006). The coastal region of Brazil was strongly colonized for centuries and, consequently, *P. corethrurus* has been reported in almost every agriculturally used area of the Mata Atlântica for about 150 years (Müller, 1857; Brown & James, 2006), including near-natural, old-growth forests like our study sites. Today, *P. corethrurus* is probably the most widely distributed earthworm species in tropical areas, where it is often the dominant species, at least in anthropogenic disturbed soils (Fragoso et al., 1999). Due to its very wide ecological preferences and some morphological variability, it was proposed that this is a species complex, consisting of at least four species (Moreno, 2004) – a view, which has not been proven so far. This species has expanded over the Neotropics during the European expansion, and is still colonizing plantations and secondary forests throughout Brazil, for example in central Amazonia (Zínci et al., 2001). The species has already been associated with negative effects on soil structure due to its casting activities (Sparovek et al., 1999; Barros et al., 2004), on plant production (Brown et al., 1999), and on native earthworm communities (Lapied & Lavelle, 2003). However, it seems that in agricultural soils its net effect is mainly positive, while in native ecosystems the net effect of invasion may be negative (Brown & James, 2007). It is still not known to which extent *P. corethrurus* colonized “empty” soils after the native earthworm fauna was annihilated by human activities, or whether this species invaded pristine sites because it is simply more competitive than native species (González et al., 2006). The latter could be caused by the fact that the species is able to survive even in nutrient-poor soils (Lavelle et al., 1999). This explanation is supported by the results of a mesocosm study in Puerto Rico, which indicates that the recolonization of disturbed sites by native species *Estherella* spp. (epigeic and anecic) is possible, but only if peregrine species are not present (Huang et al., 2006). In this context, it is worthwhile to mention that at some sites a co-existence between *P. corethrurus* and native species is possible, simply by living at different layers (soil depths, litter) (Sanchez-De Leon et al., 2003). Based on field studies in Puerto Rico, Sanchez-De León et al. (2003) also pointed out that a recovery of native species is possible only if there are primary forest patches in close distance, in order to allow their recolonization, which, as far as we know today, was not the case of our study sites.

### Table 2. Comparison of literature data on earthworm abundance and biomass with present work data.

<table>
<thead>
<tr>
<th>Land use type</th>
<th>Abundance (individuals m$^{-2}$)</th>
<th>Biomass (g fresh weight m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present work data</td>
<td>Literature$^{(1)}$</td>
</tr>
<tr>
<td>Pastures</td>
<td>56–64</td>
<td>1–270</td>
</tr>
<tr>
<td>Secondary forest</td>
<td>54–187</td>
<td>0–392</td>
</tr>
<tr>
<td>Old-grown/native/primary forest</td>
<td>75–94</td>
<td>6–54</td>
</tr>
<tr>
<td>Agroforestry</td>
<td>126–209</td>
<td>0–32</td>
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</tbody>
</table>

$^{(1)}$Compiled in Brown & James (2007).

Much less is known about the invasive ocnerodrilids or megascolecids, the latter being often associated with human settlements. Based on laboratory experiments, Garcia & Fragoso (2003) concluded that *A. corticis* thrives best when feeding on a mixture of high and low quality organic material, while *P. corethrurus* is less demanding. This may partly explain why *Amynthas* spp. are usually less abundant. No data are known from eastern Paraná or from the coastal lowlands, but abundance values of 0 to 200 individuals m\(^{-2}\) of *Amynthas* spp. were reported from the highlands of Paraná (Brown & James, 2007). Especially at no-tillage treated crop sites, farmers acknowledge the occurrence of megascolecid worms (Peixoto & Marochi, 1996). The high ecological tolerance of these peregrine species, in terms of soil properties, vegetation types and climatic factors explains why they are so wide-spread in the secondary forests studied here.

The often very high abundance and, in particular, biomass (by far the biggest part of the whole soil invertebrate community) of the earthworms, at almost all sites (except for the forests with sandy Podzol soils), underlines their important role for the provision of ecological services (Schmidt et al., 2008). This is rather interesting, since for a long time, the contribution of earthworms to soil structure, organic matter decomposition and soil fertility was considered less important in the tropics than in temperate regions, as it was assumed that their biomass in tropical soils was lower than in temperate soils, and that the tropical earthworm community did not contain vertical burrowers (Beck, 1971). However, within the last two decades, the existing knowledge on earthworms, in different tropical ecosystems (pastures, secondary or primary forests) and, in particular, their role in decomposition processes increased considerably, mainly due to work in Latin America (Höfer et al., 2001; Liu & Zou, 2002). The latter authors pointed out that *P. corethrurus* can significantly increase litter decomposition indirectly by its casting activity at the soil surface: organic material is covered by the casts, which firstly enhances microbial activity by providing better moisture conditions and, secondly, may facilitate feeding by the worms. Despite this progress, the contribution of species like *P. corethrurus* to processes like organic matter decomposition are far from understood. Further research is necessary, in particular regarding the role which secondary forests as well as agroforestry sites can play for the long-term protection of earthworm biodiversity in Mata Atlântica.

The results of the present work raise new questions concerning the impacts of human activities on (soil organism) biodiversity in the coastal plain forests. How many species of native earthworms were formally present at these sites? Is there a threshold for a regeneration of native fauna? Why is one peregrine species so abundant and dominant? Will endemic species be kept to a few isolated spots, or is it possible to manage sites, in particular secondary forests, in a way that more diverse earthworm communities can thrive there?

**Conclusions**

1. The coastal Mata Atlântica of Paraná state, in Brazil, presents a depleted earthworm fauna in terms of species richness and diversity, while abundance and biomass values observed in this region reconfirm the importance of earthworms for the functioning of the ecosystems.

2. Endemic species expected for the Atlantic Forest have been replaced by the peregrine invasive species *Pontoscolex corethrurus*, or by South-Eastern Asian *Amynthas* spp. exotic species. This fact indicates that even the old forests of the Brazilian coastal plain region have been affected by anthropogenic activities.

3. Reasons for the overwhelming dominance of the peregrine species *P. corethrurus* and for the fact that not one native earthworm species was found in the 51 studied sites remain as a challenge for further research on the conservation and sustainable management of biodiversity, in lowland Atlantic rainforests of Brazil.

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