Distribution and diversity of earthworm (Annelida, Clitellata) populations across four land use types in northern Cameroon

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Abstract: Earthworms are present in all types of soil. Changes in land use influence populations of earthworms. However, information on the dynamics of earthworm populations in northern Cameroon is not available. This paper highlights the research carried out on the abundance, biomass, and diversity of earthworm populations throughout four types of land use in northern Cameroon. Earthworms were sampled throughout the rainy season in three regions, across pastures, woody savannahs, grass savannahs, and fields, using formaldehyde and hand sorting. Earthworms of the woody savannah and pasture have a high mean density (63 and 45 individuals/m², respectively) and biomass (25.2 and 22 g/m², respectively). Region rather than land use had a significant effect (P = 0.031) on earthworm diversity. The increase in earthworm abundance in woody savannah and pasture is certainly due to the modification of the soil microclimate and litter by ligneous plants, and to the input of cow dung in pastures, both of which improve the quality and quantity of the organic matter brought to the soil. This research provides insight into the patterns of earthworm populations in northern Cameroon and the need to implement adequate use and management strategies for the maintenance of these soil organisms that provide major ecosystem services.

Key words: Annelida, Clitellata, earthworm sampling, density, biomass, diversity, land use types, northern Cameroon

1. Introduction
Earthworms are soil invertebrates and taxonomically belong to the phylum Annelida, class Clitellata (Pechenik, 2014). They are mainly found in soils and organic matter accumulations in terrestrial ecosystems, with a few species adapted to aquatic conditions (Tomlin, 2001; Raja and Karmegam, 2009; Siddaraju et al., 2013). Earthworms play a prominent role in regulating soil processes and have been recognized as the most important soil ecosystem engineers (Lavelle, 1988; Santra and Bhowmik, 2001). About 3700 earthworm species have been described to date (Decaëns et al., 2013), mostly from tropical environments. Cameroon has a wide latitudinal range and a very complex topography, which results in a contrasting climate that ranges from equatorial in the southern regions to tropical in the northern regions. It has been divided into 3 large geoclimatic units and 5 vast agroecological zones for the implementation of agricultural planning and policies. The northern part of Cameroon belongs to 2 agroecological zones and 2 geoclimatic units (Suchel, 1988; Ngachie, 1992) and is adequate for assessing regional earthworm populations.

Changes in land use pattern directly affect the composition and population structure of earthworm species in different agroclimatic regions (Behera et al., 1999). Processes of land-use changes are increasing in the northern region of Cameroon. The dominant pattern is the transition from the native vegetation (savannahs, gallery forests) to agriculture through firewood exploitation and burning. Cattle production and traditional burn-and-till agriculture are the main land uses in converted areas (MINEF, 1996). Moreover, the low fertility of soils in the region leads to the frequent abandonment of fields to pastures, fallows, and habitable lands. Consequently, the landscape that results from these anthropogenic activities is a mosaic mostly composed of agricultural lands, pastures, and grass, shrub, and woody savannahs. Even if the effects of land use changes on earthworm populations in tropical areas have been studied elsewhere (Decaëns et al., 1994; Fragoso et al., 1999), little information is known about earthworm population dynamics in Cameroon, especially in the Guinean and Sahelian zones in northern Cameroon where rapid soil degradation occurs. In this context, savannahs play a key role in maintaining soil
and vegetation (Seiny-Boukar et al., 1992), but their contribution to earthworm population dynamics has been overlooked.

Considering the significant contribution of earthworms to soil health and the direct and indirect ecosystem services they provide (Keith and Robinson, 2012; Blouin et al., 2013), the aim of the present study was to assess earthworm population dynamics under four land use types (fields, pastures, grass, and woody savannahs) of Adamawa, the north region, and the far-north region of Cameroon. The two key objectives were to examine the effect of land use and region on the abundance and biomass of earthworms and the effect of region and land use on the diversity of earthworms. We predicted that the abundance and diversity of earthworms would decrease from Adamawa to far-north Cameroon.

2. Materials and methods

2.1. The study area

An extensive survey was conducted in 2015 and 2016 in Adamawa and the north and far-north regions of Cameroon. These parts of the country occupy approximately 33% of the national area. Adamawa and the far-north are respectively the most southern and northern regions of the study area and represent a 600-km transect from south to north (Figure 1). These regions fall into 2 agroecological zones (Ngachie, 1992), namely the Sudano-Sahelian zone or zone I, and the Guinea-savannah zone or zone II.

The north and far-north regions (NR and FR) cover the first agroecological zone, with a surface area of about 100,000 km² (Figures 2 and 3). It is made up of low-altitude plains, many of which are flooded during the rainy season, which lasts for less than 6 months. The mean annual temperature is 28 °C (Table 1). The main vegetation is Sudano-Sahelian savannah and flooded prairie. Cattle production is the most important agricultural activity. The major food crops are millets and maize. Cattle production is less important than in the other two regions (Ngachie, 1992).

The Adamawa region (AR) belongs to the second agroecological zone and covers a surface area of about 62,000 km² (Figure 4). Its climate is of Sudano-Guinean highland type with an annual rainfall of 1600 to 1800 mm distributed over 7 months (Table 1). The mean annual temperature is 23 °C (Pamo and Yonkeu, 1986). The main vegetation is Sudano-Guinean savannah. The main food crops are millets, peanut, and maize (Ngachie, 1992).

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Table 1 provides more information about the soil types and altitudes of these regions (FAO-UNESCO, 1977).

2.2. Land use classification

The field reconnaissance was done by a group comprising an ecologist, a botanist, and a specific guide for each locality.

The study sites were chosen to reflect a range of landscapes encountered in the three regions. We investigated four land use types, namely woody savannah (WS), grass savannah (GS), pasture area (PA), and agricultural land (AL).

2.3. Earthworm sampling

At each of the sampling sites, earthworms were collected using a combination of chemical and physical extraction methods (Baretta et al., 2007). Earthworms were sampled using three randomly selected metal quadrats of 1 × 1 m in each plot in order to get an equilateral triangle pattern of 20 m in size. Before sampling, the plot was cleared and when necessary the litter layer was removed, and any earthworms found in the organic matter were collected. Specimens were extracted using a formaldehyde solution at a concentration of approximately 50 mL of formol for 10,000 mL of water. Two successive applications of 5000 mL of formaldehyde solution were slowly poured within the quadrat in 10-min intervals. Emerging earthworms were collected and fixed in 60% ethanol. Afterwards, a soil pit (25 cm wide × 25 cm wide × 20 cm deep) was dug at the center of the quadrat. All excavated soils were placed on a tarp and then crumbled and hand-sorted for detection of individual earthworms. Specimens collected from the three quadrats were pooled into one sample.

2.4. Earthworm screening and determination of density and biomass

After 24 h, the earthworms were rinsed with water and transferred to 95% ethanol for preservation (Huang et al., 2007). Preserved earthworms were separated into two age classes based on clitellum development: juveniles (lacking clitellum) and adults (clitellate). Specimens that were damaged during the sampling process were counted when head portions were present and were included in abundance analyses. Specimens without heads were included in biomass analyses only (Smith et al., 2008). For biomass estimation, each specimen was patted dry using a paper towel to remove excess skin moisture and weighed (Smith et al., 2008) to the nearest 0.001 g. The diversity of earthworms per sample was estimated by counting the number of distinct morphotypes per sample according to Ljungström (1970) and Sims and Gerard (1985). Clitellate individuals were examined by noting morphological parameters such as the length of the body, the skin coloration, the diameter of the body, the clitellum position, the number of total body segments, and the type of prostomium. The morphotypes were used as an indicator of species diversity (Gift, 2009).

2.5. Statistical analysis

Descriptive statistics such as means ± standard deviation and percentages were calculated. The data were tested for normality using the Shapiro–Wilk test. Normally
distributed data were processed using a parametric multiple test (one-way ANOVA, followed by Tukey’s test). Nonparametric data were analyzed using the Kruskal–Wallis test. The effects of the factors land use type (WS, GS, PA, AL) and region (AR, NR, FR) were studied by 2-way analysis of variance (ANOVA) at significance level $P < 0.05$ using R (version 2.13.0, 2011, R cmdr package).

3. Results
3.1. Abundance
A total of 3201 earthworm individuals were sampled. Some specimens were too damaged to be examined. Earthworms sampled in the north region accounted for 47% of the total number of earthworms sampled; the Adamawa region and the far-north region represented 30% and 22% of the total,
respectively. Throughout the regions, woody savannah represented 36% of the total abundance of earthworms, pasture area 30%, grass savannah 20%, and finally agricultural land 14%.

Statistical analyses revealed that land use type had a significant effect on earthworm abundance (number of individual earthworms per m²) (Table 2). Overall abundance of total earthworm and age class group varied between the regions; in the Adamawa region, more earthworms were found in pasture than in any other land use type (P < 0.05). In terms of age classes, in Adamawa, the total number of juveniles was statistically higher in pasture areas than in any other land use type (P < 0.05). Adults showed no preference between woody savannah and pasture area (Table 2). In the north region, earthworms were more abundant in woody savannah than other land use types (P < 0.05). In terms of age classes, the total numbers of adults and juveniles seemed more abundant in woody savannah, but statistical analyses revealed that they were as abundant in grass savannah and pasture area, respectively (Table 2). The far-north region had a higher abundance of earthworms in woody savannah than in other land use types (P < 0.05). In terms of age classes, adults seemed more abundant in woody savannah, but statistical analyses revealed that they were as abundant in grass savannah and pasture area. Juveniles were statistically more abundant in woody savannah than in any other land use type (P < 0.05). In general, the effects of land use type and region on earthworm abundance were significant for all adult, juvenile, and total earthworms (P < 0.001, P < 0.001, and P < 0.001, respectively). Likewise, a significant interaction (land use type × region) at P < 0.001 was observed for all adult, juvenile, and total earthworms (Table 2).

Earthworm abundance viewed in terms of age classes varied little between the regions. Overall, juveniles were
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statistically more abundant than adults (P < 0.05) and represented more than 80% of the total earthworm samples in each region (Figure 5).

When considering the differences in age classes between land use types, grass savannah had a higher proportion of adult earthworms than other land use types (P < 0.05) (Figure 6). The order of land use preference for adult earthworms was as follows: GS > PA = AL = WS, indicating that the numbers in PA, AL, and WS were the same statistically (P > 0.05).

3.2. Biomass
Land use type had a significant effect on the biomass of adult and juvenile earthworms (Table 3). In the Adamawa and far-north regions, more earthworm biomass was found in pasture area than in other land use types (P < 0.05). In the north region, statistical analyses showed higher biomasses in woody savannah, pasture, and grass savannah (P < 0.05). The effect of land use type on earthworm biomass was significant for all adult and juvenile groups and total earthworms (P < 0.001, P < 0.001, and P < 0.001, respectively). Moreover, the region had a significant effect on earthworm biomass for all adult and juvenile groups and total earthworms (P < 0.05, P < 0.001, and P < 0.001, respectively). A significant interaction between land use type and region was observed for all adult and juvenile groups and total earthworms (P < 0.001, P < 0.001, and P < 0.001, respectively) (Table 3).

3.3. Diversity
In terms of diversity, morphotype distribution was similar everywhere except in the agricultural lands of the north region, where fewer morphotypes were found (P < 0.05). The effect of land use type on earthworm diversity was not significant (P = 0.1608). The region had a significant influence on earthworm diversity (P < 0.05). However, no significant interactions were observed between the land use type and region for earthworm diversity (P = 0.1608) (Table 4).

4. Discussion
Earthworms have been sampled and studied since Darwin (Darwin, 1881). Until recently, no standardized earthworm sampling method was approved. Many techniques have been used in studies and have resulted in a high variation in earthworm population assessment (Edwards, 1991; Fründ and Jordan, 2003). Fründ and Jordan (2003) found that more than 50% of endogeic earthworms stay in the soil
even after application of formaldehyde solution. Physical extraction is laborious and time-consuming, but accurate for sampling endogeic and epigeic species (Edwards, 1991; Edwards and Bohlen, 1996). Hence, to ensure the most effective sampling for estimating populations, the combination of chemical and physical extraction is preferred (Edwards, 1991).

In the present study, juvenile earthworms were more abundant (about 85%) than adults. This was similar to reports from other studies (Callaham and Hendrix, 1997; Sackett, 2012). In the forest systems of Quebec, Whalen (2004) found the range of juveniles to be between 70% to 95%.

Table 1. Soil orders, precipitation, temperature, and altitude by region in northern Cameroon (FAO-UNESCO, 1977; Pamo and Yonkeu, 1986).

<table>
<thead>
<tr>
<th>Region</th>
<th>Soil order</th>
<th>Average annual precipitation (mm)</th>
<th>Average annual temperature (°C)</th>
<th>Altitude (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adamawa</td>
<td>Nitosols and ferralsols</td>
<td>1497</td>
<td>22</td>
<td>1104</td>
</tr>
<tr>
<td>North</td>
<td>Luvisols and fluvisols</td>
<td>997</td>
<td>28</td>
<td>244</td>
</tr>
<tr>
<td>Far north</td>
<td>Vertisols, regosols, and fluvisols</td>
<td>790</td>
<td>28</td>
<td>422</td>
</tr>
</tbody>
</table>

Our findings indicated that the mean overall density and biomass of earthworms were 41 ind/m² and 20.6 g/m², respectively. The average high values were from woody savannah (63 ind/m² and 22 g/m²) and pasture (45 ind/m² and 25.2 g/m²). The mean lowest values were from grass savannah (36 ind/m² and 25.4 g/m²) and agricultural land (21 ind/m² and 12.1 g/m²). These values found in the savannah were low compared to those of Decaëns et al. (1994), who found a higher value of earthworm density and a much lower value of biomass in the Oriental Llanos savannah of Colombia (157 ind/m² and 4.8 g/m²). In the central savannah of Lamto in Côte d’Ivoire, Lavelle (1983) observed density and biomass ranges of 188–400 ind/m² and 17–48.6 g/m², respectively.
When compared to the study by Decaëns et al. (1994) conducted in Colombia, the current results in pasture lands were similar for density (45 ind/m² vs. 32–192 ind/m²), but higher for biomass (25.2 g/m² vs. 4.5–13.8 g/m²). Moreover, other studies have reported high densities and biomasses, such as 474–573 ind/m² and 78–116.4 g/m² in traditional pasture and 546–740 ind/m² and 103.2–153 g/m² in improved pasture in Peru (Lavelle and Pashanasi, 1989). Senapati (1980) recorded density and biomass ranges of 17.4–800 ind/m² and 30.2–56 g/m² in improved pasture of India.

In our study, earthworm density range in agricultural land was relatively higher than those of Decaëns et al. (1994) in Colombia (18–27 ind/m² and 0.5–2.3 g/m²) and Lavelle and Pashanasi (1989) in Peru (14 ind/m² and 1.5 g/m²). The differences between the current results and those mentioned above may be due to the difference in sampling period and methods, regional climate, and other abiotic factors such as topography. It would be difficult to compare studies carried out on density and biomass due to differences in factors such as methods of sampling, data analyses, and assemblages of species (Lavelle, 1983; Decaëns et al., 1994).

In contrast to earthworm density and biomass, diversity of community composition appeared to be less reflective of the differences in land use types and regions. Although earthworm samples were not identified to species level, the examined morphotypes showed an overall average of 3.5 species among the three regions.

General trends in abundance followed NR > AR > FR for region and WS > PA > GS > AL for land use type. Biomass followed NR > FR > AR for region and PA > WS.
Figure 5. Percentage of age class group composition based on region. AR = Adamawa region, NR = North region, FR = Far-north region.

Figure 6. Percentage of age class group composition based on land use types. WS = Woody Savannah, GS = Grass Savannah, PA = Pasture Area, AL = Agricultural Land.
The diversity followed AR > NR > FR for region and PA = WS = GS ≥ AL for land use.

The relatively high abundance and biomass of earthworms observed across pastures may certainly be due to grazing. The increased inputs of cow dung to the soil probably explain increased earthworm populations. Decaëns et al. (1994) and Fragoso et al. (1999) argued that intensive pastures are suitable for the activity of soil fauna, but in some cases, they may decrease diversity. In woody savannah, the high inputs of organic matter in quality and quantity from the litter may help maintain earthworm populations (Decaëns et al., 1994). Additionally, the positive effect of ligneous plants on ensuring a suitable soil microclimate and wet conditions, as well as protecting the soil from intense sunlight, could provide optimal conditions for earthworm population growth. Earthworms are highly sensitive to light. The ultraviolet light intensity of sunlight is lethal to earthworms, but depending on the degree of pigmentation of some species, they can withstand limited sunlight exposure (Edwards and Bohlen, 1996).

Furthermore, the low abundance in grass savannah may be due to the frequent burning of savannahs in northern Cameroon. This seems to limit the population growth and expansion of a large part of soil macrofauna (Decaëns et al., 1994). The conversion of natural ecosystems into agroecosystems generally has very dramatic impacts on the communities of soil macroinvertebrates (Dangerfield, 1990). They are sensitive to agricultural practices (such as tillage) that modify the disturbance frame and availability of trophic resources. This situation has been mostly described in tropical environments where communities are highly modified when forests or savannahs are converted into

<table>
<thead>
<tr>
<th>Land use types</th>
<th>Average earthworm biomass, g/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Adamawa</td>
<td></td>
</tr>
<tr>
<td>Woody savannah</td>
<td>9.4 (4.9) b</td>
</tr>
<tr>
<td>Grass savannah</td>
<td>10.1 (5.8) b</td>
</tr>
<tr>
<td>Pasture area</td>
<td>21.7 (11.3) a</td>
</tr>
<tr>
<td>Agricultural land</td>
<td>5.8 (4.0) c</td>
</tr>
<tr>
<td>North</td>
<td></td>
</tr>
<tr>
<td>Woody savannah</td>
<td>43.9 (14.0) a</td>
</tr>
<tr>
<td>Grass savannah</td>
<td>36.3 (14.6) a</td>
</tr>
<tr>
<td>Pasture area</td>
<td>38.9 (11.3) a</td>
</tr>
<tr>
<td>Agricultural land</td>
<td>14.5 (6.4) b</td>
</tr>
<tr>
<td>Far north</td>
<td></td>
</tr>
<tr>
<td>Woody savannah</td>
<td>14 (8) b</td>
</tr>
<tr>
<td>Grass savannah</td>
<td>14.3 (4.8) b</td>
</tr>
<tr>
<td>Pasture area</td>
<td>34.8 (12.2) a</td>
</tr>
<tr>
<td>Agricultural land</td>
<td>10 (4.9) c</td>
</tr>
</tbody>
</table>

ANOVA †

<table>
<thead>
<tr>
<th>Land use type (LU)</th>
<th>&lt;0.0001</th>
<th>&lt;0.0001</th>
<th>&lt;0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region (RE)</td>
<td>&lt;0.0001</td>
<td>0.0008</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LU × RE</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
agroecosystems (Dangerfield, 1990; Gilot, 1995). These changes can occur at the taxonomical (e.g., substitution of native species by exotic species), ecological (e.g., increase or decrease in number of species, abundance of ecological groups), or quantitative (e.g., the biomass or the density of populations are modified) (Dangerfield, 1990; Gilot, 1995) levels. Annual crops are generally detrimental for both the abundance and the diversity of the communities (Fargoso et al., 1999). Finally, most of the farmers investigated use chemical fertilizers and pesticides to control pests and weeds and to increase agricultural production. It is therefore possible that earthworm populations would be reduced by some of these chemicals and pesticides (Card et al., 2002).

In conclusion, this observational study is the first one carried out in northern Cameroon. It showed the distribution trends of earthworms from Adamawa to the far-north region of Cameroon. The results showed that land use type and region, as well as their interaction, significantly affected the abundance and biomass of earthworms, while only region had a significant effect on the diversity of earthworms. At the land-use scale, grass savannah and agricultural land showed low abundance and biomass of earthworms. These results highlight the importance of implementing adequate policies for land use and management for the maintenance of these soil organisms, which provide major ecosystem services.

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