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Earthworm richness in land-use systems in Santa Catarina, Brazil

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ABSTRACT

Brazil is a megadiverse country from which around 10% of all species are known. However, many areas in Brazil have not been adequately studied, particularly for soil animals. This includes the state of Santa Catarina, where only 18 of the approximately 300 known Brazilian earthworm species occur, and where very little is known of the impacts of land use management on earthworm populations (density and diversity). Therefore, the aim of this study was to evaluate earthworm species richness in five different land-use systems (LUS) with increasing anthropogenic impact: native forest (NF), eucalyptus plantation (EP), pasture (PA), integrated crop-livestock (ICL) and no-tillage cropping (NT), in six counties, three each from the Western and Plateau regions of the state. Nine monoliths of 25 cm × 25 cm × 20 cm depth were sampled at each site and worms hand sorted. Qualitative samples were also taken by digging at least 20 holes per site to improve the likelihood of collecting rare species. Samples were taken in the Winter and Summer months of 2011–2012 (July–August 2011, December 2011–January 2012). Contrary to expectations, species richness was higher in LUS with higher (ICL and NT = 15 and 17 spp.) vs. lower anthropogenic impacts (EP, PA, NF = 9–10 spp.), mainly due to the presence of exotic species in the cropping systems. Native species predominated in PA and NF, although natives were also found in highly disturbed ecosystems (NT, ICL) and in the West region all worms collected in NT were native. In total 24 species were identified in all LUS, with 19 native species, including several that were new to science. Several species were collected exclusively in each region, and overall qualitative samples yielded 24 species while quantitative samples only 16. Therefore, qualitative sampling appears to be more effective in determining earthworm species richness at regional levels, although quantitative samples are a useful addition when LUS are compared within a region, and if abundance and diversity index calculations are needed.

1. Introduction

Brazil is a megadiverse country that holds approximately 10% of all described species, although only about 10% of the estimated 1.8 million species have been described from the country (Lewinsohn and Prado, 2005). Of the 1013 known earthworm species in Latin America, Brazil hosts 336 (Brown et al., 2013), but large areas of the country still remain poorly known both in terms of biodiversity and the presence of species in different land use systems (LUS). One of these areas is the Araucaria forest region in Southern Brazil, particularly in the state of Santa Catarina (SC) (Brown and James, 2007). Until recently, very little work had been done on earthworms in SC, and only 18 species (of which only eight were native) were recorded for that state by Brown and James (2007), mainly due to the low number of sites sampled.

The state of SC occupies an area of around 95.7 thousand km^2, and is home to approximately 6.5 million persons (IBGE, 2010). Despite its small size (1% of Brazil's landmass), it contributes significantly to national economy and has the 6th largest gross domestic product in the country, being also an important agricultural

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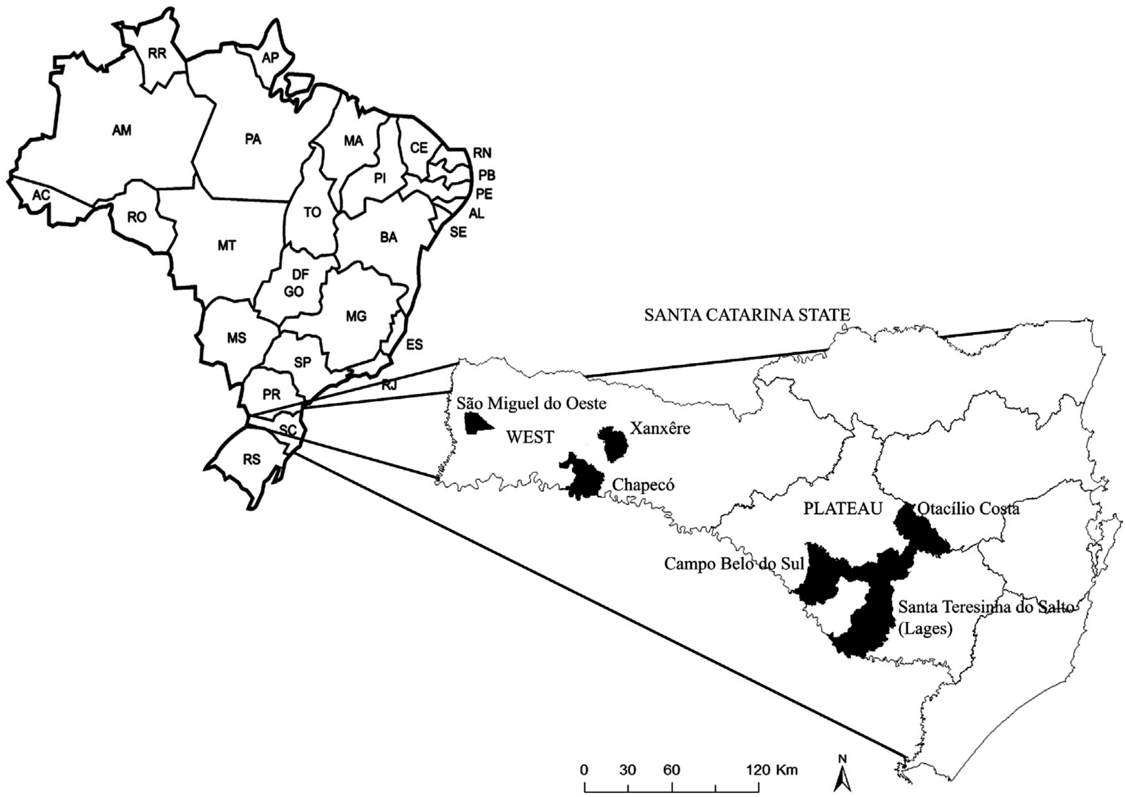

Fig. 1. Map showing the counties and regions in Santa Catarina State, Brazil, where earthworm sampling was performed.

producer, mainly of crops such as apples, onions, tobacco, rice, wheat, banana, garlic, maize and beans (CEPA, 2012). As in most of Brazil, the first European colonizers in SC arrived along the coast, making safe harbor in cities like São Francisco do Sul (established 1553), and then later made their way inland. The Plateau region (Fig. 1, "Plateau") was largely occupied in the 18th century by migrants from the state of São Paulo, who moved to SC to establish sawmills and cattle production. In the last 20–30 years old pasture lands have been progressively converted to annual cropping, mainly soybean and maize (Dufloth et al., 2005). The western most part of SC (Fig. 1, "West") was extensively colonized only recently (20th century) by immigrants of European origin coming from Rio Grande do Sul, who first established sawmills and then family farms with annual crops and animals (Waibel, 1955). Until 40 years ago, all cultivation practices were performed with conventional tillage (moldboard or soil inversion plows; ICEPA, 1999), but since then no-tillage has spread rapidly and most farmers in SC now employ this tillage practice (Veiga and Trombetta, 1997; Denardin et al., 2008) that generally promotes the maintenance and recovery of earthworm populations (Brown et al., 2003).

It is well known that land use intensification, mainly by agriculture, affects the composition of soil fauna and earthworm communities at various levels (Baretta et al., 2003, 2006; Decaëns, 2010; Postma-Blaauw et al., 2012). Intensification is generally accompanied by greater soil perturbation, greater proportion of annual cropping and use of external inputs such as fertilizers, insecticides, fungicides and herbicides that can affect the ability of soils to provide ecosystem services such as primary production, nutrient cycling, biological pest control and conservation of biodiversity (Giller et al., 1997). Among the organisms affected by intensification are the earthworms (Fragoso et al., 1997), considered important for soil function and the delivery of ecosystem services (Blouin et al., 2013). In most cases, intensification negatively affects earthworm communities (biomass, density, species richness; Decaëns and Jiménez, 2002; Decaëns et al., 2003; Feijoo et al., 2007; Smith et al., 2008; Ponge et al., 2013), but in some cases it may increase biomass and density and have no effect on species richness (Tondoh et al., 2007, 2011). Reasons for these differences may be related to the natural and anthropogenic history of the site, the extent of difference between the natural and disturbed ecosystems (e.g., native savanna vs. forest converted to pasture or crops), the biology of the species encountered, and the presence/absence of exotic or invasive species (Decaëns et al., 2004; Tondoh et al., 2011).

The whole of the state of Santa Catarina is within the Atlantic Rainforest biome, one of the 25 world hotspots of biodiversity, highly endangered and fragmented and in need of preservation (Myers et al., 2000). About 40% of the state was also originally covered by Araucaria forests, of which <1% remain intact (Alarcon et al., 2011). As very little is known of the soil biota in that state, and of the impact of land use practices on their populations, a large 3-year project (2010–2013) was undertaken by a consortium of universities and research institutes, to evaluate soil biodiversity along a land use intensification gradient (www.biotasc.com). In the present study, biodiversity of earthworms in SC was assessed comparing different regions (West and Plateau) and different land use systems. The efficiency of two different sampling schemes (qualitative and quantitative) for estimating species richness was tested, and earthworms communities were evaluated in order to assess their sensitivity to land use changes (Paoletti, 1999).

2. Materials and methods

2.1. Sites

The study was carried out in two regions (West and Plateau) of Santa Catarina (SC) state, Brazil. Study sites were randomly selected in both regions, with three counties per region: São Miguel do Oeste, Chapecó and Xanxerê in the West, and Lages (Santa

Table 1
Selected environmental and management characteristics of the sampling sites and LUS in each county and region (West, Plateau) of Santa Catarina.

Region	County	Parameter	Native forest (FN)	*Eucalyptus* plantation (EP)	Pasture (PA)	Integrated crop-livestock (ICL)	No-tillage (NT)
West	Xanxerê	Altitude (m)	714	709	723	714	746
		UTM (J22 zone)	355588.13 7031160.6	354841.11 7036628.9	353941.05 355588.13	7031160.6 354841.11	7036628.9 7030597.3
		Size (ha)	1	6	4.2	1.9	6.2
		Age (yr)	–	4	12	8	18
		Vegetation and management	Transition of mixed ombrophilous forest and semidecidual stational forest. Secondary forest.	Native grassland previously.	Introduced pasture (*Axonopus affinis*). Manure input.	Minimun tillage with crop rotation (soybean, corn, oat, ryegrass). Use of herbicides, insecticides and fungicides.	No-tillage (soybean, corn, wheat). Use of herbicides, insecticides and fungicides.
		Soil texture	Loam	Sandy clay loam	Sandy clay loam	Clay loam	Loam
		Organic matter (%)	4.8	4.1	5.1	3.9	3.9
	São Miguel do Oeste	Altitude (m)	648	659	660	641	642
		UTM (J22 zone)	247891.07 7040008.5	247846.98 7040639.5	247970.74 247891.07	7040008.5 247846.98	7040639.5 7039329.9
		Size (ha)	10.8	2.6	1.9	1.8	3.2
		Age (yr)	>50	7	50	18	4
		Vegetation and management	Transition of mixed ombrophilous forest and semidecidual stational forest. Secondary forest. Entrance of people.	Native grassland previously.	Introduced pasture mixed with native. Accidentally burned in 2007.	No-tillage with crop rotation (soybean, corn, oat, ryegrass). Use of herbicides, insecticides and fungicides.	No-tillage with crop rotation (soybean, corn, oat, ryegrass). Use of herbicides. Last two yr. without insecticides.
		Soil texture	Loam	Clay loam	Clay loam	Clay loam	Clay loam
		Organic matter (%)	5.2	4.0	4.8	3.6	3.6
	Chapecó	Altitude (m)	623	653	642	593	679
		UTM (J22 zone)	331686.98 7008603.1	332063.29 7009274.9	336900.37 331686.98	7008603.1 332063.29	7009274.9 7010937.3
		Size (ha)	7.6	3.5	5.4	5.1	2.2
		Age (yr)	>50	15	50	10	10
		Vegetation and management	Transition of mixed ombrophilous forest and semidecidual stational forest. Secondary forest. Entrance of people.	Manure input. Accidentally burned in 2006.	Native grass.	No-tillage with crop rotation (soybean, corn, oat, ryegrass). Use of herbicides, insecticides and fungicides.	No-tillage (soybean, corn, wheat). Use of herbicides, insecticides and fungicides.
		Soil texture	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam
		Organic matter (%)	4.8	4.1	4.6	4.6	4.6
Plateau	Campo Belo do Sul	Altitude (m)	1016	989	1004	947	923
		UTM (J22 zone)	6915778.9 534356.22	6915002.2 534167.46	6917279.1 6915778.9	534356.22 6915002.2	534167.46 6917279.1
		Size (ha)	5	1	6.9	4	1.1
		Age (yr)	-	20	12	25	11
		Vegetation and management	Mixed ombrophilous forest. Secondary forest. Entrance of people and cattle.	Native grassland previously. Entrance of people and cattle.	Native grass. Controled burning every 2 years.	No-tillage with crop rotation (soybean, corn, wheat, oat). Use of herbicides, insecticides and fungicides.	No-tillage (soybean, corn, wheat and fallow). Use of herbicides, insecticides and fungicides.
		Soil texture	Clay	Clay	Clay	Clay	Clay

Table 1 (*Continued*)

Region	County	Parameter	Native forest (FN)	*Eucalyptus* plantation (EP)	Pasture (PA)	Integrated crop-livestock (ICL)	No-tillage (NT)
		Organic matter (%)	6.2	4.1	4.9	4.3	4.0
		Altitude (m)	895	852	858	873	883
	Santa Teresinha do Salto	UTM (J22 zone)	6924955.1 539289.77	6925346.4 539402.33	6925122.7 6924955.1	539289.77 6925346.4	539402.33 6925122.7
		Size (ha)	3.5	2.8	7.6	14	4
		Age (yr)	-	7	15	10	7
		Vegetation and management	Mixed ombrophilous forest. Secondary forest. Entrance of cattle.	Entrance of cattle.	Native grass.	No-tillage with crop rotation (soybean, corn, oat, ryegrass). Use of herbicides, insecticides (physiological) and fungicides.	No-tillage (soybean, corn, wheat). Use of herbicides, insecticides (physiological) and fungicides.
		Soil texture	Clay	Clay	Clay	Clay	Clay
		Organic matter (%)	6.0	4.5	5.2	4.3	4.1
		Altitude (m)	919	855	900	902	879
	Otacílio Costa	UTM (J22 zone)	6947179.5 603859.81	6951380.3 612712.11	6944460.5 6947179.5	603859.81 6951380.3	612712.11 6944460.5
		Size (ha)	3	1.5	6.3	11.3	5.5
		Age (yr)	-	21	-	12	10
		Vegetation and management	Mixed ombrophilous forest. Secondary forest. Entrance of cattle.	-	Native grass. Annual controlled burning.	No-tillage with crop rotation (soybean, corn, oat, ryegrass). Use of herbicides, insecticides and fungicides.	No-tillage (soybean, corn and fallow). Use of herbicides, insecticides and fungicides.
		Soil texture	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Loam
		Organic matter (%)	6.4	4.7	5.7	5.6	5.3

Teresinha do Salto district), Campo Belo do Sul and Otacílio Costa in the Plateau (Fig. 1). Each county was considered a true replicate and five different LUS were selected per county, representing a gradient of increasing land-use intensity: native forest fragments (NF), eucalyptus plantations (EP), pastures (PA), crop-livestock integrated systems (ICL) and no-tillage crops (NT).

Selected characteristics of each sample site are presented in Table 1. Climate in both regions is temperate humid with warm (Cfa) or cool (Cfa) summer (Köppen classification) in the West and Plateau region, respectively, and with mean temperatures 18–22 °C (West) and 14–18 °C (Plateau). Both regions show no marked dry season and rain falls throughout the year. Soils of the sites in Western SC are classified generally as clayey and loamy red Latossols, while in the Plateau region they are clayey and loamy brown Nitossols or Cambissols (Brazilian classification; Embrapa, 2009).

The NF fragments were generally small (1.2–10.8 ha) and consisted of secondary Araucaria forests (mixed ombrophilous forest, mainly in the Plateau) or a transition to semi-deciduous seasonal forest (West region), where a part of the forest trees lose their leaves in the winter season. In several forests either humans or cattle regularly entered, and paths were frequently observed. The EP ranged from 1 to 6 ha in size and 4–21 yr of age and in several locations had previously been native grassland. The PA ranged from 1.9 to 7.6 ha in size in size and 12–50 yr old; in the Plateau they were native grass pastures while in the West they were native, introduced or mixed grass pastures. The NT systems involved minimum soil disturbance, permanent soil cover and crop rotation (at least 4 crops in three years), according to the standards of the Brazilian NT federation (FEBRAPDP, 2013). NT fields ranged from 1.1 to 6.2 ha in size and 4–18 yr since adoption of no-tillage. The ICL fields ranged from 1.8 to 14 ha in size and 8–25 yr of age. ICL is an agricultural management system with annual cropping (planted using NT) in summer and cover crops (oat, wheat, grass) in winter used for livestock grazing. Both ICL and NT where considered more intensified systems, due the frequent use of external inputs such as herbicides, fungicides and insecticides. We qualitatively ranked these five LUS along a land-use intensity gradient as follows: NF < EP < PA < ICL < NT.

Due to the lack of information on local earthworm assemblages, we reduced the sampling areas to approximately 1 ha in order to avoid confounding factors that may influence earthworm distribution within each system, such as slope, differential solar exposure and variation in soil characteristics.

2.2. Earthworm sampling

In each site, earthworms were sampled following two complementary approaches: qualitative and quantitative sampling.

Quantitative sampling was carried out on a square grid with 9 points 30 m away from each other (Fig. 2). Each grid was centered in a 1 ha plot so that sampling points were at least 20 m away from the plot boundaries. At each point earthworms were sampled using the TSBF (Tropical Soil Biology and Fertility) method (Anderson and Ingram, 1993), i.e., hand sorting of a 25 cm × 25 cm × 20 cm deep soil monolith. Other soil fauna was sampled as well; results will be presented elsewhere.

The qualitative sampling consisted in the digging of at least 20 randomly selected holes in each site. The sampling effort was adapted to the level of intensification of the LUS, with a smaller number of holes in the more intensely managed systems. The number of holes ranged from 45 to 50 in NF, 35 to 40 in EP and PA and 20 to 25 in ICL and NT. Usually, the holes were dug randomly in the 1 ha area of the quantitative (TSBF) samplings (Fig. 2), but in some cases, especially in NF, selected microhabitats (in bromeliads, in and under decaying trunks, under stones, and in wet areas) were also sampled. The size of the holes was usually of 20 cm × 20 cm

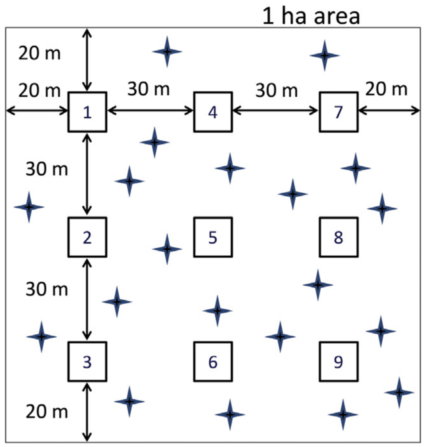

Fig. 2. Example of a sampling scheme in a land use system, using a 30 m × 30 m grid for the quantitative (TSBF) method (squares) and random digging of ≥20 holes for the qualitative method (stars) within the 1 ha sampling area.

to 20–30 cm depth, but in sites where big species were found (i.e., "minhocuçus" with >20 cm body length) larger holes (usually 50 cm × 50 cm) were dug. The time used for this sampling was usually the same as that needed to perform the quantitative sampling, reaching up to 90 min per plot for NF and 40–60 min for the other land use systems. The reason for the differences in sampling effort in each LUS were based on previous experience of the authors, and the fact that NF usually have much lower earthworm abundance and mainly native species (Brown and James, 2007), requiring higher sampling efforts to describe the communities present. On the other hand, LUS such as NT, PA, ICL and EP usually have higher abundances and exotic species (Bartz et al., 2013, in press; Maschio et al., in press), requiring lower efforts to describe the communities.

Sampling was carried out in both winter (July–August 2011) and summer (December 2011–January 2012) so each plot was sampled twice with the same sampling design. Earthworms were fixed and preserved in 92.8% ethanol, counted and identified at family, genus or species level using keys and original descriptions found in Michaelsen (1900), Righi (1990, 1995) and Blakemore (2002).

2.3. Data analysis

Since seasonal effects were not of interest in the present study, winter and summer data were summed for both quantitative and qualitative samples. For calculation of rarefaction curves and species richness, data from both quantitative and qualitative samples were treated separately and also combined for each region to assess the overall efficiency of the sampling effort in each LUS. Mean species richness per LUS was calculated using the data from each county as true replicates within each region (West and Plateau). Mean species richness per sample was calculated using the number of samples for each sampling method, i.e., nine for the quantitative (TSBF) and n = variable for the qualitative samples (see above). Mean species richness per LUS and mean species richness per sample were submitted to an ANOVA, followed by Fischer's Protected LSD test at P = 0.05 confidence level (Statsoft Inc., 2001).

Shannon and *Pielou* indices were calculated for the quantitative data only, using standard formulas (Odum, 1983). Rarefaction curves were computed with EcoSim 7.7.1 software (Gotelli and Entsminger, 2012). We used the number of collected specimens as the measure of sampling effort in order to allow relevant analyses of datasets that combined both quantitative and qualitative samples. This was done for: (a) data from both regions (totals of West + Plateau) for each sampling method separately (quantitative vs. qualitative) and combined (total worms collected using both methods), to assess the effectiveness of sampling methods in capturing the earthworm species present; (b) total earthworm abundance data (total of both sampling methods) for each region separately (West vs. Plateau) and combined (total of both regions) to assess the proportion of species richness collected in each region and each LUS. Additionally, we calculated rarefied richness (RAR) and ACE estimated richness (Gotelli and Colwell, 2001; Oksanen et al., 2008) using the Vegan module in the R software (Oksanen et al., 2008; R Development Core Team, 2004).

A multivariate cluster analysis was performed with the TSBF data by the complete linkage (furthest neighbor) method (Everitt, 1993; Jongman, 1995) and by calculating Euclidean distances (Frakes and Yates, 2000) with Statistica v.6.0 (Statsoft Inc., 2001).

3. Results

3.1. Earthworm species richness in LUS

Overall 2227 individuals were collected and identified, of which 615 were from the Western and 1612 from the Plateau region. These worms belonged to 24 species from six families (Table 2). More earthworms were collected in the winter (1389 individuals; 385 in West, 1004 in Plateau) than in summer (838 individuals; 230 in West, 608 in Plateau). In the West, 14 species from six families were found, of which nine were native. In the Plateau, 19 species from five families were found, of which thirteen were native.

Most widespread was an unidentified species of Ocnerodrilidae (Table 2, "sp. 1"), found in all but one site (EP in West) and *Urobenus brasiliensis*, found in 8 of the 10 sampling sites (Table 2). The most abundant species were *Octalasion tyrtaeum* (442 indiv.), *U. brasiliensis* (218 indiv.) and *Fimoscolex* sp. 2 (165 indiv.). Several species were rare, found in low numbers (down to one specimen) or at only one or two sites (Table 2). Nine species (five native and four exotic) were common to both regions. Five species (four native and one exotic) were found exclusively in the West while ten (eight native and two exotic) were exclusive to the Plateau region. The unnamed species of the native genera *Glossoscolex* and *Fimoscolex* are new to science, whereas the unnamed species of Ocnerodrilidae and *Metaphire* require more careful taxonomic consideration and genetic evaluation to adequately assess species status.

Considering both regions together, richness among the LUS followed the order: ICL > NT > PA = NF > EP. However, two main groups of systems were identified, according to species richness: NF, PA and EP, with similar richness (9–10 spp.), and NT and ICL (15 and 17 spp.), with higher species richness than in the other systems (Table 3). In the West region, higher numbers of individuals were collected in systems with higher anthropogenic impact (PA, NT and ICL), and species richness was higher in ICL (11 spp.) and PA (8 spp.), when considering both sampling methods (Table 2). In the Plateau higher number of individuals were again collected in the systems with highest human interference (ILC and NT; Table 1), and the highest number of species was observed in NT (13 spp.), followed by ICL and NF with the same number of species overall (9 spp.) (Table 2).

Mean species richness per LUS was highest in ICL in both regions, using the TSBF method (Table 3). Using the qualitative method in the Plateau, mean richness was higher in NT than in ICL, PA and EP, and combining both methods, richness was higher in ILC and NT than in PA. Mean species richness per sample (no. species collected in each hole) followed similar trends (Table 3).

Shannon indices also showed a trend of lower to higher diversity from less impacted to the more intensively managed LUS in the West region. However, in the Plateau region, this trend was not seen: EP had lowest diversity followed by PA, ICL, while NT and NF had the highest diversity index. Pielou evenness indices were

Table 2
Earthworm species (number of individuals collected) in the natural forest (NF), eucalyptus plantation (EP), pasture (PA), integrated crop-livestock (ICL) and no-tillage (NT) using quantitative (TSBF) and qualitative sampling (sums of two sampling seasons) in the West and Plateau regions of Santa Catarina state, Brazil.

Family *Genus & species*	Origin[a]	WEST										PLATEAU									
		TSBF[b]					Qualitative[b]					TSBF[b]					Qualitative[b]				
		NF	EP	PA	ICL	NT	NF	EP	PA	ICL	NT	NF	EP	PA	ICL	NT	NF	EP	PA	ICL	NT
Glossoscolecidae																					
Glossoscolex sp.1	n											1	11	15	1	2		40			
Glossoscolex sp.2	n											2					1				
Glossoscolex sp.3	n			16	5									2			7		27		
Glossoscolex sp.4	n			1														3	25		28
Glossoscolex sp.5	n							2	19	6											
Glossoscolex sp.6	n																2				
Fimoscolex sp.1	n													3	1				35		
Fimoscolex sp.2	n																				5
Fimoscolex sp.3	n	1	1	72	2	3	1	4	55	4	22										
Fimoscolex sp.4	n		1					4													
Glossoscolecidae juveniles			2	5	1	2				1			4	5	1	20	3	1			2
Rhinodrilidae																					
Urobenus brasiliensis	n	16	5		15	17	14	30		45	46	4	1			1	23				
Andiorrhinus duseni	n																13	18			
Ocnerodrilidae																					
Ocnerodrilidae sp.1	n	1		8	4	5			5	9		8	2	6	38		1	2	3	24	1
Ocnerodrilidae sp.2	n			5	14	1					1	3		1	6	10			2		
Ocnerodrilidae sp.3	n														5	2				1	13
Ocnerodrilidae sp.4	n																			46	
Ocnerodrilidae sp.5	n									9	4										
Megascolecidae																					
Amynthas gracilis	e				4					12					2	14	1				8
Amynthas corticis	e									6					16	13				35	26
Metaphire californica	e									3						3					21
Metaphire sp.1	e																				1
Megascolecidae juveniles					6					10			1		35	65				11	77
Lumbricidae																					
Octolasion tyrtaeum	e														130	5				287	20
Bimastus parvus	e				3				2	3											1
Acanthodrilidae																					
Dichogaster gracilis	e		1		3				24												
NI Juveniles[c]		1	10	4	5	5		2	12	4	11	7	6	6	221	102	2		2	9	4
TOTAL		19	20	111	62	33	15	42	117	112	84	25	25	38	456	237	53	64	94	413	207

[a] n: native and e: exotic.
[b] Numbers of individuals not transformed per unit area (i.e., not m^{-2}).
[c] NI: not identified juveniles independent of the family.

Table 3
Earthworm species parameters in the natural forest (NF), eucalyptus plantation (EP), pasture (PA), integrated crop-livestock (ICL) and no-tillage (NT) using TSBF, qualitative samplings and total data for the West and Plateau regions of Santa Catarina state, Brazil.

Region	Method	LUS	Species richness per LUS	Mean species richness per LUS[a]	Mean species richness per sample[a]	No native	No exotic	Proportion native spp. per LUS	Proportion native spp. per sampling method	Total species richness (native + exotic)	Mean species richness	Shannon index (H')	Pielou index (J)
		NF	3	1.7a	0.19aB	3	0	100				0.05a	0.18a
		EP	4	2.0a	0.22a	3	1	75				0.27a	0.78b
	TSBF	PA	5	2.7ab	0.30abB	4	0	100	88	10 (7 + 3)	2.3	0.24a	0.39ab
		ICL	8	3.7b	0.41bB	5	3	63				0.30b	0.64b
		NT	4	1.7a	0.19a	4	0	100				0.14a	0.23a
		NF	2	1.5	0.03A	2	0	100					
		EP	4	2.3	0.07	4	0	100					
West	Qualitative	PA	5	1.7	0.05A	3	2	60	83	12 (7 + 5)	2.0		
		ICL	9	2.8	0.08A	5	4	56					
		NT	4	1.8	0.08	4	0	100					
		NF	3	1.5	0.11a	3	0	100					
		EP	5	2.2	0.14a	4	1	80					
	Total	PA	8	2.2	0.17ab	6	2	75	87	14 (9 + 5)			
		ICL	11	2.8	0.24b	6	5	58					
		NT	5	1.8	0.13a	5	0	100					
		NF	5	2.3a	0.16a	5	0	100				0.30bc	0.61bc
		EP	4	1.7a	0.12a	4	0	100				0.05a	0.11a
	TSBF	PA	5	1.5a	0.10aB	5	0	100	83	12 (8 + 4)	2.5	0.08ab	0.26ab
		ICL	8	4.3b	0.29bB	5	3	63				0.15ab	0.40abc
		NT	8	2.7abA	0.23abB	4	4	50				0.49c	0.70c
		NF	7	3ab	0.07a	6	1	86					
		EP	4	1.7a	0.05a	4	0	100					
Plateau	Qualitative	PA	5	1.7a	0.05aA	5	0	100	79	19 (13 + 6)	2.6		
		ICL	5	2.3a	0.09bA	3	2	60					
		NT	10	4.3bB	0.17cA	5	5	50					
		NF	9	2.3abc	0.16ab	8	1	88					
		EP	5	1.7ab	0.19a	5	0	100					
	Total	PA	6	1.3a	0.15a	6	0	100	82	19 (13 + 6)			
		ICL	9	4.3bc	0.29c	6	3	67					
		NT	13	5.7c	0.23bc	7	6	54					

[a] Different lower case letters behind numbers mean significant ($P < 0.05$) differences between LUS within a particular sampling method (TSBF, qualitative, or total = the combination of both), while different upper case letters mean significant differences between sampling methods (TSBF vs. qualitative) in a particular LUS.

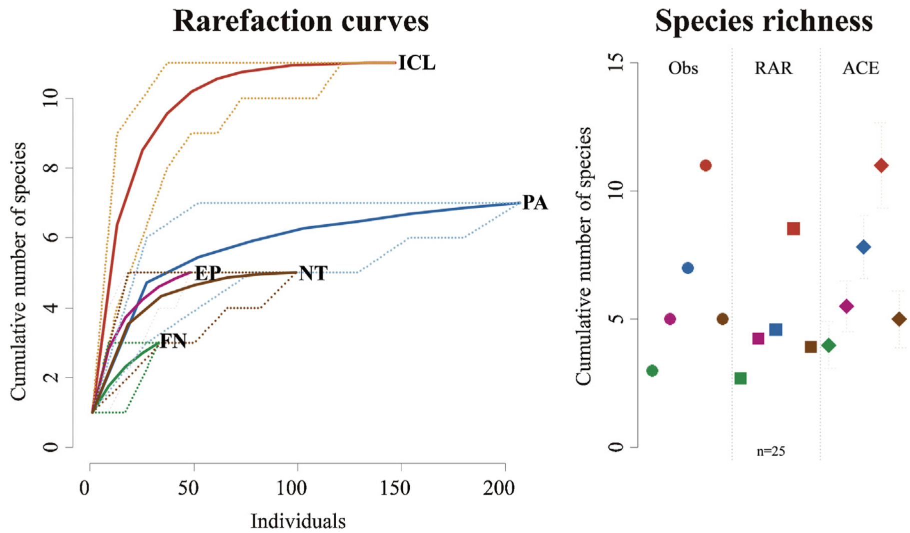

Fig. 3. Rarefaction curves, RAR and ACE specific richness of earthworms in each LUS, irrespective of sampling method (total of qualitative and quantitative samples) in the West region of Santa Catarina State (West), Brazil. (LUS order in "Species richness": FN, EP, PA, ICL and NT).

highest in EP (0.78) and ICL (0.64) in the West region, while in the Plateau the higher values were observed for NT (0.70), followed by NF (0.61).

The high species richness values observed in agricultural sites were due to the presence of exotic species additional to the native species. Considering both regions together, native species accounted for 90%, 89% and 80% of species richness in NF, EP and PA systems, respectively, while in the cropping systems, native species accounted for 65% and 60% total richness in ICL and NT, respectively (Table 2). In the West region, assemblages of NF and NT were exclusively composed of native species, while in PA and ICL they represented 75% and 58% (Table 2). The Plateau assemblages were composed of 100% native species in EP and PA, 88% in NF and only 67% and 54% in ICL and NT, respectively (Table 2).

For most sites in the West the rarefaction curves reached an asymptote, indicating that in these sites, the sampling effort was adequate to describe the species richness present (Fig. 3). This is despite the difference in sampling efforts made in each LUS. Observed (Obs) and estimated (ACE) species richness showed few differences. The rarefaction curve for the Plateau region showed that PA had already reached a plateau, while EP and ICL were asymptotic in terms of species richness, indicating that the number of samples was sufficient or nearly adequate to describe the earthworm community in these systems (Fig. 4). On the other hand, the Obs vs. ACE richness were very different for NT, indicating that more species will probably still be found with further sampling in this system. The number of individuals collected in NF was very low and inadequate to describe the true species richness in this system.

The dendrograms obtained by cluster analysis of the TSBF data are shown in Fig. 5, where numbers on the vertical axis represent the "Euclidean distance" rescaled from 0 to 120. For the in West there is one cluster with 4 LUS, and PA is outside, in Plateau there is one cluster with 3 LUS, and NT and ICL are outside. Similarities in community composition do not reflect the hypthothesized gradient of land use intensity.

3.2. Sampling efficiency for earthworm richness

In both regions, the total species richness collected with quantitative method was lower than that obtained using qualitative

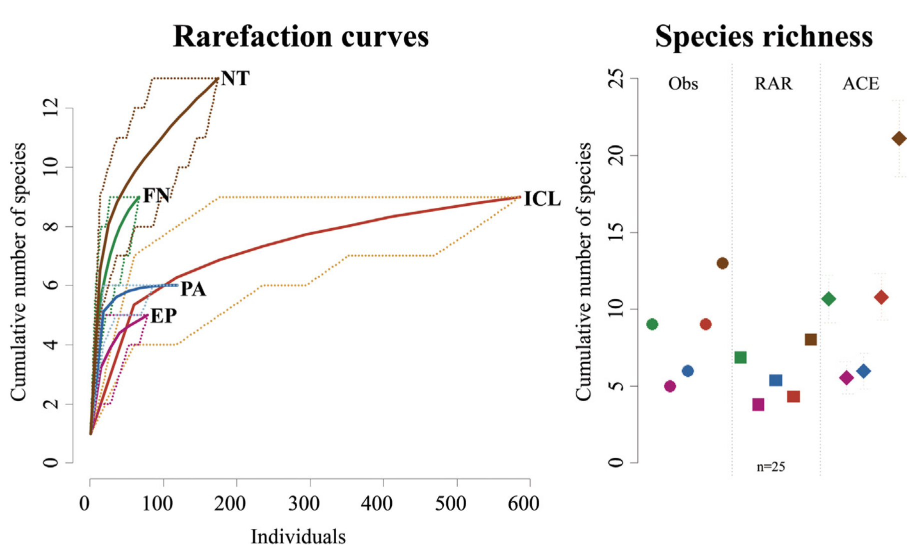

Fig. 4. Rarefaction curves, RAR and ACE specific richness of earthworms in each LUS, irrespective of sampling method (total of qualitative and quantitative samples) in the Plateau region of Santa Catarina State (Plateau), Brazil. (LUS order in "Species richness": FN, EP, PA, ICL and NT).

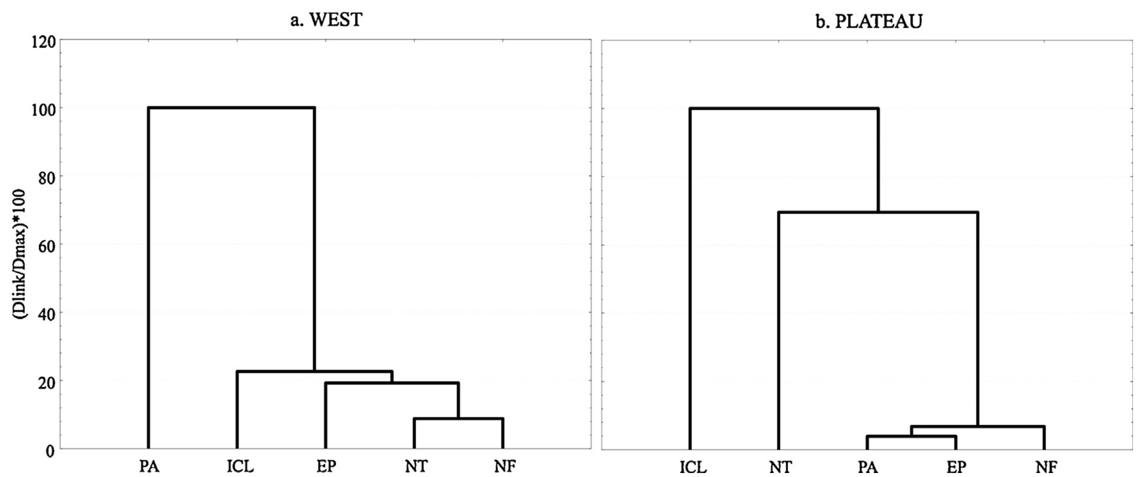

Fig. 5. Dendrograms obtained by cluster analysis discriminating by "Euclidean Distance" the five different land-use systems (natural forest, NF; eucalyptus plantation, EP; pasture, PA; no-till, NT; and integrated crop-livestock, ICL), independent of the sampling method used (total number of individuals collected in each LUS), in the West (a) and Plateau (b) regions of Santa Catarina State, Brazil.

sampling. In the West and Plateau 9 and 13 species were collected with TSBF monoliths, while 13 and 19 species were collected by digging >20 random holes and niche searching, respectively. Nevertheless, in the West, three species were exclusively found using the TSBF method and four with the qualitative method. In the Plateau, qualitative sampling yielded all species found with the TSBF methods, and seven more. Considering the methods independently of region, 16 species were found using the TSBF method while 24 were found in the qualitative samples.

Rarefactions curves performed using all data available (sum of LUS and regions) for the quantitative and qualitative methods separately and combined (sum of both methods), showed that the qualitative method was more efficient at sampling the earthworm species than the TSBF method (Fig. 6). In fact, even if a greater sampling effort using TSBF is made, rarefaction curves predict that this method would never reach the same level of biodiversity obtained from the qualitative sampling. Combining both methods led to little difference in observed and estimated species richness, indicating that little is gained by combining both methods when performing biodiversity studies at a higher geographic level (two regions).

4. Discussion

4.1. Earthworm species richness in LUS

In total, twenty-four earthworm species were found overall, increasing the number of known species in the state of Santa Catarina from 18 (Brown and James, 2007) to 39. Only three of the 24 species found had been previously reported from SC: *U. brasiliensis*, *Amynthas gracilis* and *Metaphire californica* (Brown and James, 2007). Of the 21 new species records 16 are native (at least 10 of them new, undescribed species) and 5 exotic species (Table 3).

Forest systems are often reported to have greater numbers of earthworm species (particularly of native species) than cultivated agricultural land (Fragoso et al., 1997, 1999; Feijoo et al., 2007) due their higher complexity and greater number of niches that allow for persistence of species with variable ecologies (Lee, 1985; Edwards and Bohlen, 1996). However in the present study, higher earthworm species richness and abundance were observed in the more impacted sites (ICL and NT), as compared with the less disturbed ecosystems such as EP and NF. Furthermore, both native vegetation systems (NF and PA) had lower overall species richness than ICL and NT. The difference in richness among LUS appeared to be due to the presence of several exotic species in the more intensively managed systems, especially in ICL (both regions) and NT in the Plateau. The presence of these exotics was expected, as disturbed ecosystems Brazil are frequently colonized by these widespread earthworms (Brown et al., 2006). Additionally, the somewhat degraded nature and small size of the forest fragments available in the region for sampling, particularly in the West region may also have been a limiting factor in the available species pool, resulting in smaller richness collected.

Surprising, however, was the observation of 100% native species present in the three NT fields sampled in the Western region, despite the frequent use of insecticides, fungicides and especially herbicides (glyphosate) for pest control. Previous surveys, performed in NT agroecosystems the neighboring Paraná State had reported mainly exotic or invasive earthworms in NT agroecosystems, particularly of the genera *Dichogaster* and *Pontoscolex*, although some native species of the genera *Andiorrhinus*, *Belladrilus*, *Glossoscolex* and *Fimoscolex* were also found at low densities (Sautter et al., 2007; Brown et al., 2008; Bartz, 2011; Bartz et al., 2013). The most probable reasons for the presence of native species in the intensified agricultural LUS of both regions is the relatively recent adoption of cultivated agriculture in both regions and the use of soil conservation practices (NT), which reduce soil disturbance and allows for accumulation of organic matter in the topsoil (Derpsch et al., 1991; Derpsch and Florentín, 2000; Landers, 2001; Pieri. et al., 2002; Franchini et al., 2004; Casão Junior et al., 2006), i.e., food for the earthworms.

Native secondary forests and pastures established after forest clearing after forest clearing allow the survival of native species, as in both regions 100% of worms collected in NF and PA using TSBF were native. However, exotic species appeared in qualitative samples of both systems (PA in West and NF in Plateau), indicating they are also susceptible to invasion or colonization by exotics. As observed in other studies in Paraná (e.g., Brown et al., 2008; Nunes et al., 2006, 2007; Bartz, 2011), earthworm abundance tended to be much higher in PA than in NF. Nevertheless, contrary to these previous studies that found mostly exotics (*Dichogaster* spp. and *Pontoscolex corethrurus*) in high abundance in pastures, native species predominated in numbers in the native pastures of both regions. The few exotics found were *Dichogaster gracilis*, collected under manure spread on the surface of one of the pastures in the West (Chapecó). Reasons for the dominance of natives in these pastures may be related to the land use history in the region,

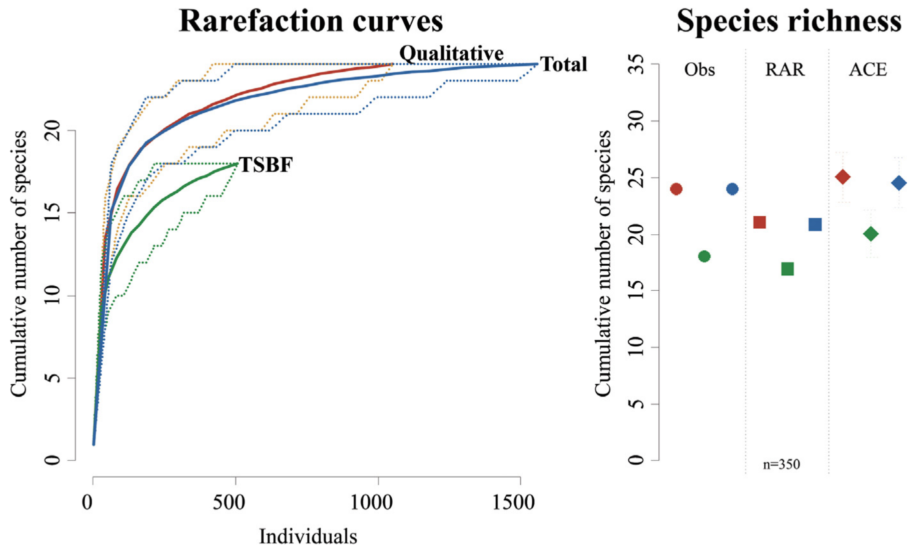

Fig. 6. Rarefaction curves, RAR and ACE specific richness of earthworms irrespective of LUS (all LUS combined), performed using quantitative (TSBF) and qualitative sampling, considering together both regions (West and Plateau) of Santa Catarina State, Brazil. (Methods order in "Species richness": Qualitative, TSBF and Total).

particularly its more recent colonization (mainly 19th and 20th century in Plateau and West, respectively), and the widespread use of native pastures in both regions after deforestation.

Most of the genera found in the present study had been recorded previously in other surveys performed in Paraná, although many of the species we collected were new to science, and must still be described. *Glossoscolex* and *Fimoscolex* are native genera with wide occurrence in S and SE Brazil, and many new species of these genera have been collected by the authors in natural and agricultural ecosystems over the last 10 years (Bartz et al., 2012). The species collected in the present study were mostly small to medium-sized (<12 cm long) unpigmented endogeics, although large (>20 cm) pigmented species with endo-anecic habit were also found in EP and NT. Unfortunately almost nothing is known of their biology and ecology, although we can presume that the endogeics and particularly the large species will be important for soil functioning in their habitats, creating large galleries (observed in the field), that aid in soil aeration and root penetration in compacted soils.

The earthworms of the genera *Dichogaster* and the Ocnerodrilidae species found in the present study were all small (3–5 cm long) and usually reddish, with epi-endogeic habit. They inhabited the soil surface (between soil and straw), but were sometimes found at 10–15 cm depths in drier conditions. The Ocnerodrilidae were especially abundant especially in ICL, and similar species in the genus *Belladrilus* have been found in pastures and NT systems in North and Western Paraná (Sautter et al., 2007; Bartz, 2011; Bartz et al., 2013). In fact, this is the first time that earthworms sampled quantitatively were identified to species level in ICL in Brazil. Exotic megascolecids and lumbricids were found in high numbers especially in NT and ICL of the Plateau region. High abundance of megascolecids had been previously reported in various natural and disturbed ecosystems in Southern Brazil, where the cooler climate appears to be more conducive to their presence in these sites (Brown et al., 2006; Brown and James, 2007). However, the lumbricid *O. tyrtaeum* was known only from one location previously (Porto Alegre, RS; Knäpper, 1976), and *Bimastus parvus* from only four locations (Brown and James, 2007). The high abundance of these exotic worms (particularly *O. tyrtaeum* in ICL), especially in the Plateau was probably due to the cooler climate and high levels of soil organic matter (OM) in the topmost 10 cm of soil in these systems (>5% OM; Bartz et al., unpublished results), where they tend to concentrate their activities. The higher number of individuals collected in ICL overall and compared with NT, may also be due to the presence of livestock in this system.

One of the notable results was the absence of *P. corethrurus* in samples of both regions. This species is widely adapted to a range of environmental conditions and was originally described from specimens collected by Fritz Müller in Eastern Santa Catarina. Although competition with native species has not been adequately proven for *P. corethrurus* (Huang et al., 2006; Marichal et al., 2010) we cannot completely discard the possibility that its absence in both regions may be benefitting the presence and prevalence of native species in the more impacted LUS, where it is generally dominant in other sites of Brazil (Brown et al., 2006).

Another important result was the frequent occurrence of *U. brasiliensis*, a widespread native Brazilian species (Brown and James, 2007), in annual cropping systems (NT and ICL). This species was considered an indicator of well-preserved ecosystems (native forest) and/or sites with dense surface layer of organic matter (Sautter et al., 2007), and had only recently been reported in agricultural fields in SC (Bartz et al., 2011). Its absence in cultivated systems had been attributed to soil disturbance and/or the disappearance of the dense litter layer that represented the necessary habitat for this species (Brown et al., 2003). The presence of this species in NT, ICL and EP, especially in the West, and the finding of adults as well as cocoons (Bartz et al., 2011) indicate that these systems, like NF, provide adequate conditions for its survival, and that *U. brasiliensis* is able to tolerate low levels of disturbance. The presence of this epi-endogeic species in high numbers in NT and ICL is probably important for soil function because its feeding (i.e., surface litter), burrowing and casting habits assist in the incorporation of residue and aeration of the topsoil (0–10 cm; Bartz et al., 2011).

4.2. Sampling efficiency for earthworm richness estimation

The TSBF method has been widely used throughout the tropics for quantitative estimates of soil macrofauna and earthworm populations (e.g., Lavelle et al., 1994; Brown et al., 2001; Pauli et al., 2011), although the ISO (2006) methodology recommends the use of larger monoliths, to account for the presence of larger earthworm species in the samples (Römbke et al., 2006). Furthermore, while studying the effect of land-use on earthworms in semi-decidous forest of Ivory Coast, Tondoh et al. (2007) suggested that the results obtained with TSBF sampling were sufficient to assess both abundance and species richness of earthworm communities at the landscape level. On the other hand, Baretta et al. (2007) found that the TSBF method was adequate to collect smaller individuals species of *Glossoscolex*, but inadequate to collect the surface-active *A. corticis* and *U. brasiliensis* in Araucaria forests and plantations in São Paulo State, Brazil. In the present study, both soil macrofauna and earthworms were being evaluated, so the TSBF method was adopted. To "compensate" for potential under-representation of large or surface active species or those from particular niches, qualitative sampling was performed as well. No significant differences were found in species richness per LUS comparing both methods, but mean species richness was higher on one occasion (NT in Plateau) using qualitative sampling. However, mean species richness per sample was generally higher using TSBF than qualitative samples (and significantly so for several LUS), meaning that the chance of recovering more species with fewer samples is higher with the quantitative vs. qualitative method.

Overall, many more species (24 spp.) were collected using the qualitative vs. the quantitative method ($n = 17$ spp.) considering both regions. Although rarefaction curves showed little difference between the use of only qualitative method vs. the combination of both methods (Fig. 6), we found that two *Glossoscolex* species were only collected using the TSBF method in the West region. On the other hand, seven species overall were found using only the qualitative method (nine if considering regions separately). Many fewer individuals of several megascolecid species (including *Amynthas corticis*) were collected with the TSBF method compared with the qualitative method, confirming that the latter method is more efficient for collecting members of this family in the ecosystems sampled.

Although sampling efforts were different for qualitative sampling, the sampling scheme used (combination of both quantitative and qualitative methods) appeared to be adequate, especially for PA and ICL (in both regions), likely related to the higher number of identified individuals in these LUS. Considering each region separately, the scheme described better earthworm richness in the West region (Fig. 3) vs. the Plateau (Fig. 4), despite the lower number of individuals collected overall in the West. Comparing LUS, the scheme was not effective for describing richness in NF and EP in both regions, despite the higher sampling effort in these systems. For NT in the West, sampling effort was sufficient, but in the Plateau rarefaction curves showed that a greater sampling effort would likely increase species richness recovered.

From the present study, it appears that the qualitative method is much more effective in determining species richness of LUS within a region, although quantitative methods are needed for a more detailed description of the earthworm community, since it provides data on species richness per unit area and also allows for calculations of diversity indices that can be used to compare land use impacts (of LUS).

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