

## Recommendations for assessing earthworm populations in Brazilian ecosystems

**Abstract** – Earthworms are often related to fertile soils and are also frequently used as environmental quality indicators. However, to optimize their use as bioindicators, earthworm populations must be evaluated together with the environmental and anthropogenic variables regulating their communities. This review sought to identify the earthworm-sampling, soil chemical and physical, and environmental and anthropogenic attributes evaluated in 124 published studies that quantified earthworm abundance (>7,300 samples) in 765 sites with different types of climate, soils, land use, and management systems in Brazil. Soil chemical and physical attributes (except pH) were less reported ( $\leq 50\%$  of studies) than other environmental variables such as sampling date, altitude, temperature, precipitation, climate and soil type, and land use (>50% of studies). Earthworms were rarely identified (24%) and few studies (31%) measured their biomass, although most provided adequate information on sampling protocol. Based on their importance in regulating earthworm populations, a set of variables is proposed to be evaluated when studying earthworm communities and other macrofauna groups. This should help guide future studies on earthworms in Brazil and other countries, optimize data collection and replicability, allow comparisons between different studies, and promote the use of earthworms as soil quality bioindicators.

**Index terms:** bioindicators, macrofauna, Oligochaeta, soil quality.

### Recomendações para avaliação de populações de minhocas em ecossistemas brasileiros

**Resumo** – As minhocas são frequentemente relacionadas a solos férteis e, também, bastante usadas como indicadores da qualidade ambiental. No entanto, para otimizar seu uso como bioindicadores, as populações de minhocas devem ser avaliadas juntamente com as variáveis ambientais e antropogênicas que regulam as suas comunidades. Esta revisão buscou identificar os atributos relacionados à amostragem de minhocas, físicos e químicos dos solos, e ambientais e antropogênicos avaliados em 124 estudos publicados que quantificaram a abundância de minhocas (>7.300 amostras) em 765 locais com diferentes tipos de clima, solos, uso da terra e sistemas de manejo no Brasil. Os atributos químicos e físicos do solo (exceto pH) foram menos relatados ( $\leq 50\%$  dos estudos) do que outras variáveis ambientais, como data de coleta, altitude, temperatura, precipitação, tipo de solo e de clima, e uso do solo (>50% dos estudos). As minhocas foram raramente identificadas (24%) e poucos estudos (31%) mediram sua biomassa, embora a maioria tenha fornecido informações adequadas sobre o protocolo de amostragem. Com base na sua importância para a regulação das populações de minhocas, propõe-se um conjunto de variáveis que devem ser avaliadas no estudo de comunidades de minhocas e outros grupos da macrofauna do solo. Isso deve ajudar a guiar futuros estudos sobre minhocas no Brasil e em outros países,

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#### Received

August 28, 2018

#### Accepted

March 31, 2020

#### How to cite

NADOLNY, H.; SANTOS, A.; DEMETRIO, W.; FERREIRA, T.; MAIA, L. dos S.; CONRADO, A.C.; BARTZ, M.; GARRASTAZU, M.; SILVA, E. da; LAVELLE, P.; BARETTA, D.; PASINI, A.; VEZZANI, F.; SOUSA, J.P.; CUNHA, L.; MATHIEU, J.; RÖMBKE, J.; BROWN, G. Recommendations for assessing earthworm populations in Brazilian ecosystems. **Pesquisa Agropecuária Brasileira**, v.55, e01006, 2020. DOI: <https://doi.org/10.1590/S1678-3921.pab2020.v55.01006>.

otimizar a coleta e a replicabilidade de dados, permitir comparações entre diferentes estudos e promover o uso de minhocas como bioindicadores da qualidade do solo.

**Termos para indexação:** bioindicadores, macrofauna, Oligochaeta, qualidade do solo.

## Introduction

Earthworms are among the most well-known soil animals, being ecosystem engineers (Jones et al., 1994; Lavelle et al., 1997) that actively contribute to many ecosystem services, including carbon sequestration and gaseous exchanges, plant production, and erosion control, as well as soil genesis, decomposition, and nutrient cycling (Stockdale & Watson, 2012; Jouquet et al., 2014; Brown et al., 2015). Most farmers and gardeners are quick to recognize the value of earthworms for soil fertility (Brown et al., 2003; Lima & Brussaard, 2010) and tend to associate the presence of a high number of earthworms with more fertile soils.

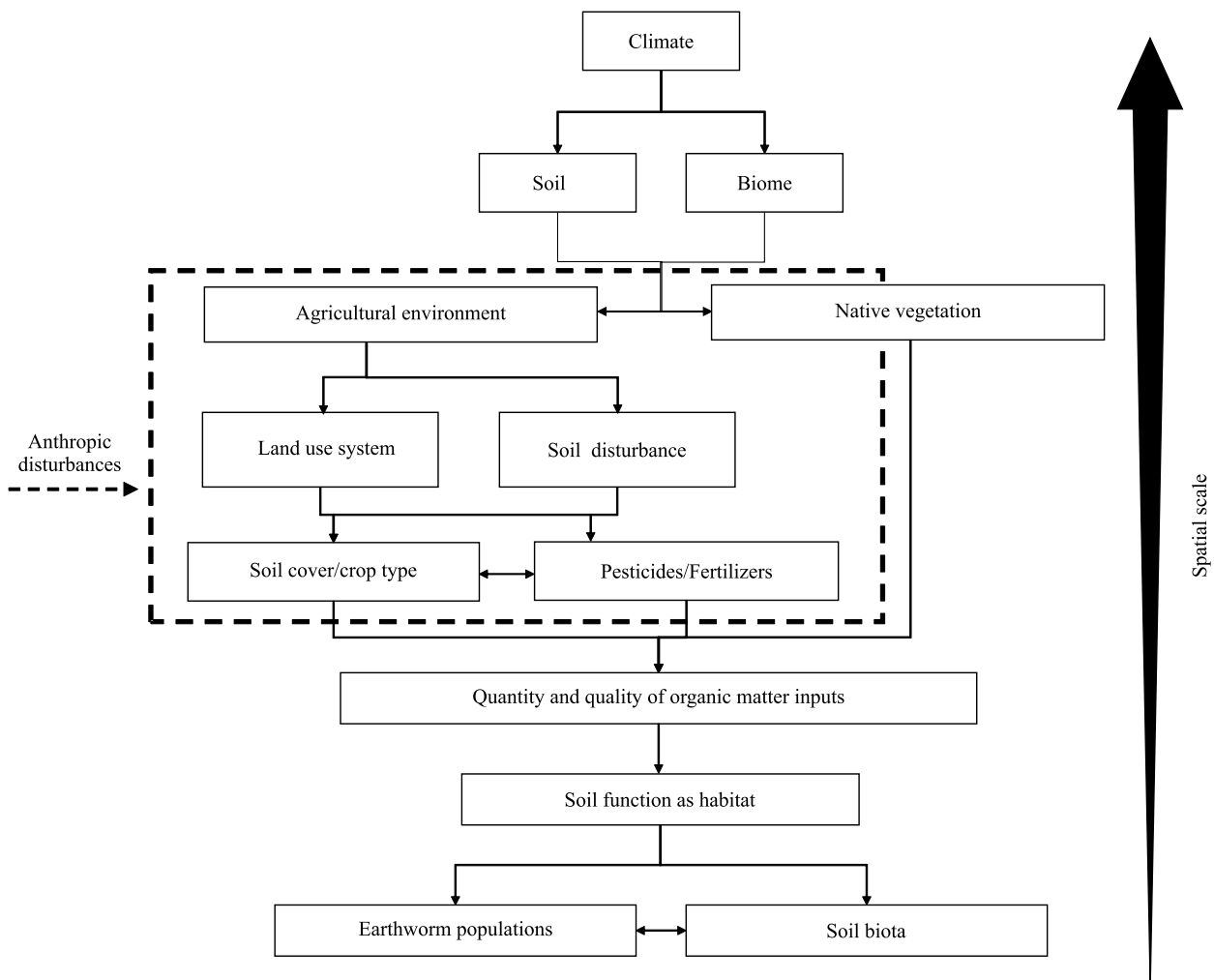
The community and abundance of earthworms at a given location are controlled by several biotic and abiotic factors, which act at different spatial scales (Figure 1) and include: climatic conditions, such as climate type, especially precipitation and temperature; soil properties, mainly its type and chemical and physical conditions, among which stand out pH, organic matter, moisture, and texture; vegetation, indicating the type of ecosystem, primarily plant cover; and history of the site, particularly human activities but also geological processes (Reynolds & Jordan, 1975; Brown & Domínguez, 2010). At the largest spatial scale, climate is the most important hierarchical factor (Lavelle et al., 1993; Phillips et al., 2019), because it generally regulates the biome and type of ecosystem (vegetation), also influencing the formation of soil layers (Blume et al., 2016). At lower spatial scales, that is, at regional and local levels, other important determinants of earthworm communities are: human disturbance, such as soil management; type of crop or forest plantation; and inputs and cultural practices, including tillage and pesticide and fertilizer use (Curry, 2004). All these directly or indirectly affect many of the soil characteristics that are important for earthworms, like organic matter content, pH, and nutrients, as well as plant productivity and cover that influence litter quality and quantity and soil temperature (Curry, 2004). At the lowest spatial scale, i.e., within a soil profile of a particular site, it is mainly the soil physical and chemical characteristics that affect the soil as a habitat for earthworms and also the interactions (e.g., predation, parasitism, and mutualism) with other organisms (e.g. other soil biota) that can affect earthworm populations (Brown & Domínguez, 2010).

Because of their usefulness as environmental and, particularly, as soil quality indicators, earthworm communities have been regularly studied in European countries (Fründ et al., 2011; Pulleman et al., 2012; Bünemann et al., 2018). Unfortunately, so far, there are few

nation-wide monitoring programs in place, but both public and scientific interest in the state of the soil organism community, mainly of earthworms, are growing notably in France, the Netherlands, and Germany (Jeffery et al., 2010; Cluzeau et al., 2012; Römbke et al., 2016). In Brazil, the use of earthworm communities as bioindicators has been explored in several publications (Nunes et al., 2007; Uzêda et al., 2007; Andréa, 2010; Bartz et al., 2010; Fernandes et al., 2010; Lima & Brussaard, 2010; Marichal et al., 2010; Rousseau et al., 2010); however, up to now, only one earthworm-based soil quality classification has been proposed, considering the density of these invertebrates in areas under no-tillage in the western region of the state of Paraná, Southern Brazil (Bartz et al., 2013). Based on earthworm abundance, these

authors classified soil quality under no-tillage in four classes: poor, with  $< 25$  individuals per square meter; moderate, with  $\geq 25$ –100 individuals per square meter; good, with  $> 100$ –200 individuals per square meter; and excellent, with  $> 200$  individuals per square meter. Earthworm abundance in farms was positively related to the sum of bases, but negatively associated with soil organic matter contents. Clearly, there is still much to be done both in Brazil and even worldwide concerning the use of earthworms for the indication of soil quality and monitoring purposes, especially considering the relative ease and low cost of sampling and the value given by land managers to earthworms.

Due to the large variety of factors that can influence earthworm communities in soils, the use of these organisms as bioindicators requires the sampling of



**Figure 1.** Hierarchical model of the factors that determine earthworm communities in Brazilian ecosystems.

several environmental data and soil attributes that are important for the soil to function and to work as a habitat for the development and activity of earthworm populations (Römbke et al., 2005). Furthermore, in order to be able to compare the effects of different ecosystems, types of vegetation, and land use management systems on earthworm populations, the collection of data – on the environment, earthworm communities, and soil – must be standardized in each study and between studies, e.g., according to the International Organization for Standardization (ISO, 2018). Standardization proposals have been made before (Römbke et al., 2006; Römbke, 2007) and are available in ISO (2018), but their level of adoption is quite variable and often requires local adaptations (Silva et al., 2019).

Therefore, the aim of this review was to assess the environmental and soil variables considered in the studies that quantitatively sampled earthworms in Brazil, and, based on this literature survey, to propose a set of variables that should be evaluated when studying earthworm populations. This should help guide future studies on earthworms in Brazil and other countries, optimize data collection, allow comparisons between different studies, and promote the use of earthworm communities as soil quality bioindicators in Brazilian ecosystems.

### **Characterization of studies on earthworm populations in Brazil**

For this review, studies on earthworm populations in Brazilian ecosystems published from 1976 to 2017 were considered, being obtained from searchable online databases such as Web of Science, Scielo, Lattes-CNPq Platform, Biblioteca Digital de Teses e Dissertações, Google Scholar, and the Alice repository of Empresa Brasileira de Pesquisa Agropecuária (Embrapa). For an exhaustive review and to determine which soil, environmental, management, earthworm, and sampling-related factors were evaluated, non-indexed journals, book chapters, and conference proceedings on soil science, zoology, ecology, agroecology, and conservation agriculture were also included.

Over 150 studies on earthworm populations or soil macrofauna in general, including earthworms, were found. Different methods were used to sample earthworms and to make them rise to the soil surface

(Peixoto & Marochi, 1996; Römbke et al., 1999; Ressetti, 2006; Ressetti et al., 2008; Steffen et al., 2013), including chemical solutions, such as diluted formaldehyde, mustard or onion extracts, or their main chemical components, e.g., Allyl isothiocyanate (AITC) (Zaborski, 2003; Pelosi et al., 2009). Although, in some locations, some species of earthworms – particularly epigeics, epi-endogeics, or anecics – may be better sampled by chemical extraction or by combining both hand-sorting and chemical extraction (Römbke et al., 1999; Römbke, 2007), only hand-sorting studies were selected, because this was the most common method used and would allow a more thorough comparison between studies. Studies were excluded when they did not present data on earthworm density per sample site, but rather as a means per land use system or type of soil management in several sites, making data recovery from individual sites impossible (Mathieu et al., 2009; Marichal et al., 2010; Pimentel et al., 2011a; Baretta et al., 2013; Vasconcellos et al., 2013; Rousseau et al., 2014; Santos et al., 2016).

This resulted in the evaluation of a total of 124 published studies that are listed in Table 1, which includes the source and location (municipality and state in Brazil) of the study, biome, land use systems sampled, number of sites, and type of measurements performed (earthworm density and/or biomass and associated soil data). Overall, only about 40% of all studies were journal articles and a large proportion (~60%) were material produced outside the traditional commercial or academic publishing and distribution channels, including 36 theses and dissertations and 44 conference proceedings papers.

The data on soil, environmental, and earthworm sampling variables, as well as on the management practices adopted at each sampling site, obtained from the 124 publications are available for download at Dryad, an online open-access repository (Nadolny et al., 2020). This dataset provides information on the number of publications containing each environmental, earthworm, and soil physical and chemical variable, besides the corresponding number of points/sampling sites and their proportion. The data covers over 7.300 earthworm samples, from a wide range of soils, vegetation types, and management systems in Brazil. In the following sections, these studies and their data were reviewed according to the geographical spread of the samples, climate and vegetation-related variables,

**Table 1.** Location of the experiment, land use systems evaluated, number of sampled sites, and type of data identified in the 124 published studies on earthworm populations in Brazilian ecosystems, used to build the database available at Nadolny et al. (2020).

Study	Biome	Municipality – State	Land use system <sup>(1)</sup>	Number <sup>(2)</sup>	Data <sup>(3)</sup>	References
1	Pampa	Pelotas, Morro Redondo – RS	Crop	4	a, b, s	Schiavon (2012)
2	Pampa	Pelotas – RS	Native vegetation, forestry plantation, crop	5	a	Schiavon et al. (2009)
3	Pampa	Pelotas, São Lourenço do Sul – RS	IPS	4	a	Hipólito et al. (2015)
4	Pampa	Santa Maria – RS	Crop	5	a	Giracca et al. (2005)
5	Pampa	Santa Maria – RS	Crop	5	a	Campos et al. (1997)
6	Atlantic Forest	Teutônia – RS	Crop, native vegetation	4	a, s	Krabbe et al. (1993)
7	Atlantic For-est	Campos Novos – SC	Crop	6	a, s	Alves (2007)
8	Atlantic For-est	Canoinhas – SC	Crop	4	a, b, s	Freitas (2007)
9	Atlantic For-est	Chapecó – SC	Crop, pasture, native vegetation	7	a, s	Baretta et al. (2003)
10	Atlantic Forest	Florianópolis – SC	Native vegetation, forestry plantation	5	a	Gois et al. (2007)
11	Atlantic Forest	Lages – SC	Native vegetation, forestry plantation	3	a, s	Pompeo et al. (2016)
12	Atlantic For-est	Orleans – SC	Native vegetation, pasture, crop	4	a, s	Alberton et al. (2010); Oliveira Filho (2009)
13	Atlantic For-est	Antonina – PR	Native vegetation, pasture, crop, IPS	6	a, b	Maschio et al. (2010)
14	Atlantic For-est	Antonina, Guaraqueçaba, Paranaguá – PR	Pasture, native vegetation, crop, IPS	51	a, b, s	Römbke et al. (2009)
15	Atlantic For-est	Arapongas, Londrina, Rolândia – PR	Native vegetation, pasture, crop	5	a, b, s	Bartz et al. (2014)
16	Atlantic Forest	Barra do Turvo – SP, Adrianópolis – PR	IPS, native vegetation	6	a, b, s	Brown et al. (2009)
17	Atlantic Forest	Bela Vista do Paraíso – PR	Crop, native vegetation, pasture	12	a, b	Benito (2002); Brown et al. (2003)
18	Atlantic Forest	Bituruna – PR	Crop, native vegetation	3	a	Bianchi et al. (2007)
19	Atlantic Forest	Cafeara, Campo Mourão, Cornélio Procópio, Jataizinho, Londrina, Sertanópolis, São Jerônimo da Serra – PR	Crop, pasture, native vegetation	29	a, b	Brown et al. (2003, 2004, 2008)
20	Atlantic Forest	Londrina – PR	Crop	6	a, b	Brown et al. (2001, 2004)
21	Atlantic Forest	Rolândia – PR	Crop, IPS, pasture, native vegetation	4	a, s	Benito et al. (2008); Brown et al. (2003)
22	Atlantic Forest	Campina Grande do Sul – PR	Native vegetation	8	a, b, s	Cardoso et al. (2014)
23	Atlantic Forest	Castro – PR	Crop, native vegetation	4	a, b, s	Tanck (1996); Tanck et al. (2000)
24	Atlantic Forest	Cianorte – PR	IPS, pasture	2	a, b	Jardevski (2005)
25	Atlantic Forest	Clevelândia – PR	Crop	2	a, s	Trogello et al. (2008)
26	Atlantic Forest	Colombo – PR	Forestry plantation, native vegetation	15	a, b, s	Lima (2011); Santos et al. (2016); Silva (2010)

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Study	Biome	Municipality – State	Land use system <sup>(1)</sup>	Number <sup>(2)</sup>	Data <sup>(3)</sup>	Reference
27	Atlantic Forest	Colombo – PR	Forestry plantation	5	a, b, s	Maschio (2012); Maschio et al. (2014)
28	Atlantic Forest	Colorado – PR	Crop, native vegetation	5	a, s	Pasqualin (2009); Pasqualin et al. (2012)
29	Atlantic Forest	General Carneiro – PR	Crop, native vegetation	6	a, b	Mafra et al. (2002)
30	Atlantic Forest	Jaguapitã – PR	Crop, pasture, native vegetation	11	a, b, s	Nunes (2006); Nunes et al. (2007)
31	Atlantic Forest	Jardim Olinda – PR	IPS	4	a, s	Franchini et al. (2009)
32	Atlantic Forest	Londrina – PR	Crop, native vegetation, pasture	6	a, b, s	Fernandes (2009)
33	Atlantic Forest	Londrina – PR	Crop, native vegetation	6	a, b, s	Bartz et al. (2009a)
34	Atlantic Forest	Londrina – PR	Crop, native vegetation, pasture	4	a, b	Azevedo et al. (2010); Brown et al. (2008)
35	Atlantic Forest	Londrina, Rolândia – PR	Crop	7	a, s	Derpsch et al. (1986); Kemper & Derpsch (1980/1981, 1981)
36	Atlantic Forest	Miraselva – PR	Pasture, native vegetation	4	a	Benito (2005)
37	Atlantic Forest	Paranaguá – PR	Native vegetation, IPS	2	a, s	Santos et al. (2015)
38	Atlantic Forest	Pinhais – PR	Pasture	3	a, s	Klenk (2010)
39	Atlantic Forest	Ponta Grossa – PR	Native vegetation	2	a, b, s	Ferreira (2015)
40	Atlantic Forest	Ponta Grossa – PR	IPS, forestry plantation, crop, pasture	5	a, b, s	Zagatto (2014)
41	Atlantic Forest	Ponta Grossa – PR	Crop	4	a	Voss (1986)
42	Atlantic Forest	Nova Aurora, Cafelândia, Araçongas, Cascavel, Palotina – PR	Crop	8	a, b, s	Lima et al. (2008/2009); Brown et al. (2008)
43	Atlantic Forest	Mercedes, Marechal Cândido Rondon, Itaipulândia, Entre Rios do Oeste, Santa Helena, Toledo – PR	Crop	40	a, b, s	Gorte (2016)
44	Atlantic Forest	Mercedes, Marechal Cândido Rondon, Itaipulândia, Entre Rios do Oeste, Santa Helena, Toledo – PR	Crop, native vegetation, forestry plantation	29	a, s	Bartz et al. (2010, 2013)
45	Atlantic Forest	Campos do Jordão – SP	Native vegetation, forestry plantation, IPS	4	a, b, s	Baretta (2007); Baretta et al. (2007)
46	Atlantic Forest	Campos do Jordão – SP	Native vegetation, forestry plantation	3	a, s	Merlim (2005)
47	Atlantic Forest	São Roque – SP	Crop, native vegetation	3	a, s	Uzêda et al. (2007)
48	Atlantic Forest	Ubatuba – SP	Crop, pasture	5	a, s	Marchiori (2008)
49	Atlantic Forest	Duque de Caxias – RJ	Native vegetation	2	a, s	Buch et al. (2015)
50	Atlantic Forest	Itaboraí – RJ	Native vegetation	6	a	Ferreira et al. (2012)
51	Atlantic Forest	Barra do Piraí – RJ	Forestry plantation	4	a, s	Bianchi (2009); Correia et al. (2003)
52	Atlantic Forest	Paty do Alferes, Valença – RJ	Native vegetation, crop	12	a, s	Pimentel (2005); Pimentel et al. (2002, 2011a)
53	Atlantic Forest	Queluz – RJ	Native vegetation, pasture	4	a, s	Menezes (2008); Menezes et al. (2009)
54	Atlantic Forest	Seropédica – RJ	IPS, pasture	4	a	Dias et al. (2006b)

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Study	Biome	Municipality – State	Land use system <sup>(1)</sup>	Number <sup>(2)</sup>	Data <sup>(3)</sup>	Reference
55	Atlantic Forest	Seropédica – RJ	IPS, pasture	6	a	Dias et al. (2007); Silva et al. (2015a)
56	Atlantic Forest	Seropédica – RJ	Crop	3	a, s	Merlim et al. (2005)
57	Atlantic Forest	Seropédica – RJ	Crop	5	a, b	Rodrigues et al. (2004)
58	Atlantic Forest	Seropédica – RJ	Crop	5	a	Cordeiro et al. (2004)
59	Atlantic Forest	Nova Friburgo, Seropédica – RJ	Crop	7	a	Aquino et al. (2005)
60	Atlantic Forest	Linhares – ES	Crop	4	a, s	Benazzi (2011)
61	Atlantic Forest	Alagoa, Bocaina de Minas – MG	IPS, pasture, native vegetation	6	a, b, s	Camargo (2016); Camargo et al. (2015)
62	Atlantic Forest	Araponga – MG	Crop, native vegetation	8	a, s	Souza (2010)
63	Atlantic Forest	Campos Gerais – MG	Crop, native vegetation, forestry plantation	3	a	Marques & Silva (2011)
64	Atlantic Forest	Governador Valadares – MG	Native vegetation, pasture	2	a	Vicente et al. (2010)
65	Atlantic Forest	Ouro Fino – MG	Native vegetation, crop	3	a	Silva et al. (2014)
66	Atlantic Forest	Pedralva – MG	Crop	3	a, s	Madeira et al. (2011); Oliveira (2012)
67	Atlantic Forest	Fátima do Sul – MS	Crop, native vegetation	5	a, s	Silva et al. (2007)
68	Atlantic Forest	Fátima do Sul – MS	Crop, native vegetation	3	a, s	Silva et al. (2001)
69	Atlantic Forest	Fátima do Sul – MS	Crop, native vegetation	3	a, s	Otsubo et al. (2002)
70	Atlantic Forest	Dourados – MS	Crop, pasture, native vegetation, IPS	5	a, s	Silva et al. (2006)
71	Atlantic Forest	Dourados – MS	Crop, pasture, native vegetation, IPS	9	a, s	Portilho et al. (2011)
72	Atlantic Forest	Itaporã – MS	Crop	2	a	Pezarico et al. (2006)
73	Atlantic Forest	Selvíria – MS	Native vegetation, forestry plantation, IPS	6	a, s	Marchini et al. (2011)
74	Atlantic Forest	Cruz das Almas – BA	Crop, Native vegetation, IPS, Forest-ry plantation	5	a	Pereira et al. (2012)
75	Atlantic Forest	João Pessoa, Areia – PB	IPS, native vegetation	3	a, b, s	Guerra & Silva (1994)
76	Pantanal	Aquidauana – MS	Native vegetation, crop	4	a, s	Brito et al. (2016)
77	Pantanal	Corumbá – MS	Native vegetation	3	a	Dias et al. (2006a)
78	Cerrado	Brasília – DF	Native vegetation, pasture, IPS, crop	14	a, s	Marchão et al. (2009)
79	Cerrado	Brasília – DF	Native vegetation	5	a, b, s	Dias et al. (1997)
80	Cerrado	Brasília – DF	Native vegetation	2	a, s	Bento (2009); Corrêa & Bento (2010)
81	Cerrado	Correntina – BA	Crop, pasture, native vegetation	6	a, s	Marchão et al. (2008a, 2008b)
82	Cerrado	Formosa – DF, Santo Antônio do Descoberto – GO	Pasture	4	a, s	Vendrame (2008); Vendrame et al. (2009)
83	Cerrado	Jataí – GO	Native vegetation, crop	7	a, s	Blanchart et al. (2007)
84	Cerrado	Planaltina – GO	Native vegetation, pasture	5	a, b	Benito et al. (2000)
85	Cerrado	Santo Antônio de Goiás – GO	Crop, IPS	8	a, s	Santos et al. (2008)
86	Cerrado	Maracaju – MS	Native vegetation, IPS	4	a, s	Batista (2011); Batista et al. (2014)

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Study	Biome	Municipality – State	Land use system <sup>(1)</sup>	Number <sup>(2)</sup>	Data <sup>(3)</sup>	Reference
87	Cerrado	Maracaju – MS	Crop, pasture, IPS, forestry plantation, native vegetation	6	a, s	Lourente et al. (2007)
88	Cerrado	Miranda – MS, Rio Brilhante – MS	Crop	2	a	Barrigossi et al. (2009)
89	Cerrado	São Carlos – SP	Pasture, native vegetation	3	a, s	Brigante (2000)
90	Cerrado	São José do Rio Preto – SP	Native vegetation	1	a	Caballero (1976)
91	Cerrado	Taciba – SP	Crop, pasture	3	a, b	Brown & James (2007)
92	Cerrado, Atlantic Forest	Valparaíso, Ipaussu – SP, Jataí – GO	Crop, pasture, native vegetation	9	a, s	Franco et al. (2016)
93	Cerrado	São Sebastião do Paraíso – MG	Crop	2	a, b, s	Aquino et al. (2000); Ricci et al. (1999)
94	Cerrado	Uberlândia – MG	Crop, pasture, native vegetation	5	a, b, s	Brossard et al. (2012); Pasini et al. (2003)
95	Cerrado	Uruçui – PI	Crop, native vegetation	3	a	Santos et al. (2013)
96	Amazon	Vila Bela da Santíssima Trindade – MT	IPS	2	a, s	Mendes et al. (2010)
97	Amazon	Alcântara – MA	Native vegetation	8	a	Triana (2014)
98	Amazon	São Luís – MA	IPS	6	a, s	Moura et al. (2015)
99	Amazon	Igarapé-Açu – PA	Pasture, IPS, native vegetation	6	a	Rousseau et al. (2010)
100	Amazon	Itupiranga – PA	Pasture, forestry plantation, IPS	16	a, s	Laossi et al. (2008); Velásquez et al. (2012)
101	Amazon	São Félix do Xingu – PA	Native vegetation, pasture, IPS	5	a, s	Braga (2015)
102	Amazon	Benjamin Constant – AM	Native vegetation, pasture	3	a, b, s	Alves (2010)
103	Amazon	Manaus – AM	Native vegetation, forestry plantation	6	a, s	Bandeira & Harada (1998); Harada & Bandeira (1994)
104	Amazon	Manaus – AM	Native vegetation	4	a	Araújo & Luizão (2011)
105	Amazon	Manaus – AM	Crop, pasture	4	a, b, s	Pontes (2009)
106	Amazon	Manaus – AM	Native vegetation	6	a, b	Lins-Teixeira et al. (2007)
107	Amazon	Presidente Figueiredo – AM	Native vegetation, forestry plantation	4	a, s	Viana (2012)
108	Amazon	Rio Preto da Eva – AM	IPS	10	a, b	Tarrá (2003); Tarrá et al. (2012)
109	Amazon	Rio Preto da Eva – AM	IPS, native vegetation	5	a, b, s	Barros et al. (2003)
110	Amazon	Rio Preto da Eva – AM, Itapiranga – AM	Native vegetation, pasture, IPS	3	a, b, s	Barros et al. (1996)
111	Amazon	Bonfim – RR	Native vegetation	3	a, b	Guerra (1994a)
112	Amazon	Cantá – RR	IPS, native vegetation, pasture	6	a	Costa et al. (2004)
113	Amazon	Macapá – AP	Crop	4	a, b, s	Silva (2009)
114	Amazon	Ji-Paraná – RO	IPS	1	a	Pequeno et al. (2005)
115	Amazon	Nova Califórnia – RO	IPS, native vegetation	4	a, s	Luizão et al. (2002)

## Continuation...



## Continuation...

Study	Biome	Municipality – State	Land use system <sup>(1)</sup>	Number <sup>(2)</sup>	Data <sup>(3)</sup>	Reference
116	Amazon	Ouro Preto do Oeste – RO	IPS	3	a	Silva et al. (2005)
117	Amazon	Rio Branco – AC	Pasture, native vegetation	2	a, b, s	Guerra (1994b)
118	Amazon	Theobroma, Porto Velho, Ji-Paraná – RO, Acrelândia – AC	Native vegetation, crop, IPS, pasture	15	a, b, s	Barros et al. (2002)
119	Amazon, Cerrado, Atlantic Forest	Itapuã do Oeste – RO, Uruará – PA, Botucatu – SP, São Paulo – SP	Native vegetation, pasture, IPS	11	a	Catanozi (2010)
120	Caatinga	Crato – CE	Native vegetation	5	a, s	Araújo (2010)
121	Caatinga	Esperantina – PI	IPS, native vegetation, crop	5	a, s	Lima (2008); Lima et al. (2010)
122	Caatinga	Bom Jesus – PI	Native vegetation, crop, pasture	6	a, s	Santos et al. (2017)
123	Caatinga	Juazeiro – BA	Crop	1	a, s	Pimentel et al. (2011b)
124	Caatinga	Tauá – CE	Crop	9	a, s	Lima et al. (2007)
	6 biomes <sup>(4)</sup>	135 municipalities <sup>(5)</sup>	Number by category <sup>(6)</sup>			Number by category <sup>(7)</sup>
Total	Atlantic Forest (73), Pantanal (2), Cerrado (21), Amazon (24), Caatinga (4), and Pampa (5)	DF, ES, MT, AP (1); AC, MA, PB, RR, CE (2); BA, PI (3); PA (4); AM, GO (5); RO, SC (6); RS (5); RJ (8); MG, MS (9); SP (11); PR (47)	Crop (70/322) Native vegetation (82/214) IPS (32/101) Pasture (40/87) Forestry plantation (17/41)	765 sites	a (124) b (42) s (83)	Papers in journal (49) Book chapters (7) Theses and dissertations (36) Abstracts/conference papers/others (42)

<sup>(1)</sup>IPS, integrated production system, which includes different species. <sup>(2)</sup>Number of sampling sites. <sup>(3)</sup>a, abundance; b, biomass; and s, soil attributes, even if partial, chemical, and/or physical. <sup>(4)</sup>The number between parentheses represents the total number of studies that were carried out in each biome. <sup>(5)</sup>The number between parentheses represents the total number of studies that were carried out in each municipality in different Brazilian states. <sup>(6)</sup>The numbers between parentheses represent the total number of studies/number of cases in the database. <sup>(7)</sup>The sum of all studies in each category is greater than 124, because data from some studies were published in more than one format (e.g., thesis, paper, and other). Brazilian states: RS, Rio Grande do Sul; SC, Santa Catarina; PR, Paraná; SP, São Paulo; RJ, Rio de Janeiro; ES, Espírito Santo; MG, Minas Gerais; MS, Mato Grosso do Sul; BA, Bahia; PB, Paraíba; DF, Distrito Federal; GO, Goiânia; PI, Piauí; MT, Mato Grosso; MA, Maranhão; PA, Pará; AM, Amazonas; RR, Roraima; AP, Amapá; RO, Rondônia; AC, Acre; and CE, Ceará.

management practices adopted at the sites, and various soil and earthworm sampling-related variables.

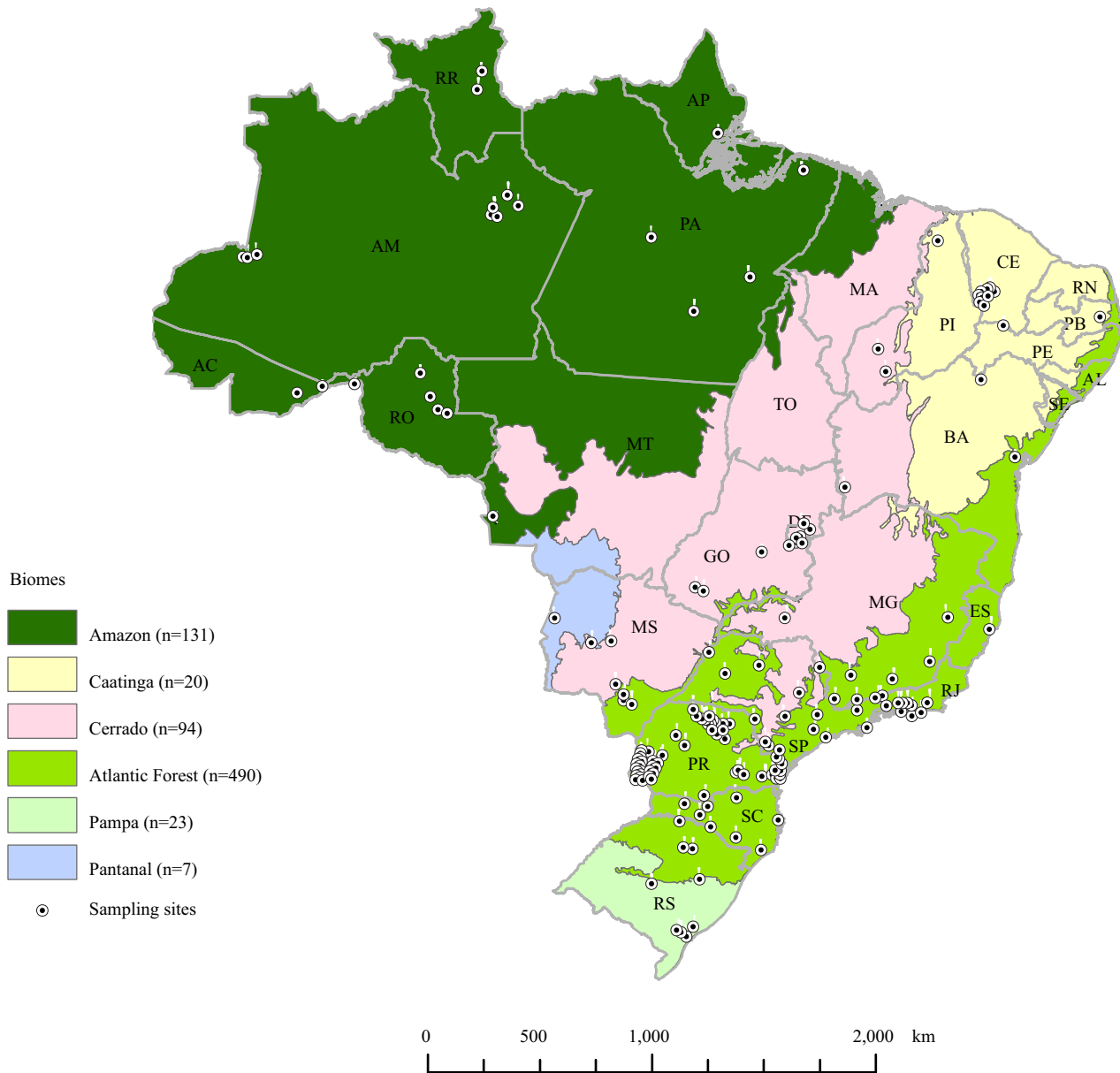
### Geographic representation of the studies

The 124 evaluated publications showed earthworm abundance for 765 sites throughout Brazil (Figure 2), the majority located in the Atlantic Forest biome (64% of the total), with a much smaller proportion in the Amazon (17%), Cerrado (12%), Pampa (3%), Caatinga (3%), and Pantanal (1%). As most Brazilian researchers work in the Atlantic Forest, considered a biodiversity hot spot (Myers et al., 2000) with 144 known earthworm species (Brown & James, 2007), it is not surprising that most samples were collected from this biome (Table 1), specifically from sites ranging from the state of Rio Grande do Sul (seven

studies in seven municipalities), in Southern Brazil, to the state of Paraíba in the Northeast (only one study in two municipalities) (Guerra & Silva, 1994). Paraná was the best assessed state, comprising 38% of all studies, which were performed in 47 municipalities. Of the three states exclusively in the Atlantic Forest biome, i.e., Espírito Santo, Rio de Janeiro, and Santa Catarina, the former requires much more attention because there is scant information on earthworms from this state (Brown & James, 2007), with only one known study in one municipality until now (Figure 2). The states from Northeastern Brazil were generally little represented in the publications. In fact, several states in this region – Pernambuco, Rio Grande do Norte, Sergipe, and Alagoas – have no quantitative data on earthworm populations. Another state with

notoriously few records on earthworms (Brown & James, 2007) and with no quantitative sampling in Tocantins. Clearly, sampling efforts in these states are urgently needed in order to reduce the knowledge gap on earthworm ecology and distribution in the country.

Only four studies examined the Caatinga and two the Pantanal (Table 1), revealing the lack of earthworm research in these biomes that occupy approximately 10 and 1.8% of Brazil's surface area, respectively (IBGE, 2019). Although only three species of earthworms



**Figure 2.** Geographic distribution and number of earthworm sampling sites in each Brazilian biome. Brazilian states: RO, R ndonia; AC, Acre; AM, Amazonas; RR, Roraima; PA, Par ; AP, Amap ; MA, Maranh ; PI, Piau ; CE, Cear ; RN, Rio Grande do Norte; PB, Para ba; PE, Pernambuco; AL, Alagoas; SE, Sergipe; BA, Bahia; ES, Esp rito Santo; RJ, Rio de Janeiro; SP, S o Paulo; PR, Paran ; SC, Santa Catarina; RS, Rio Grande do Sul; MS, Mato Grosso do Sul; MT, Mato Grosso; TO, Tocantins; GO, Goi nia; DF, Distrito Federal; and MG, Minas Gerais.

from the Caatinga are known (Brown & James, 2007), there are records of large and very active earthworms in this biome (Cordero, 1943; Almeida et al., 2009), and clayey soils and/or those with a higher potential to maintain moisture can harbor earthworm population densities of over 100 individuals per square meter (Araújo et al., 2010; Lima et al., 2010). In the Caatinga, earthworms are subjected to a higher seasonal variation and to a lower precipitation than in other locations in the country, which causes them to undergo diapause or prolonged inactivity, generally at greater soil depths (Silva et al., 2015b). Therefore, future sampling in this biome should seek to better understand the climatic and edaphic limitations to earthworm populations, also taking into account the time of year for sampling, prioritizing the rainy season, when the soil is moister and the earthworms are active and closer to the surface. In two sites in the Caatinga, earthworms were found only in samples collected in the rainy season (Araújo et al., 2010; Lima et al., 2010).

In the Pantanal, where climate seasonality is also important, the yearly flooding of vast areas may cause difficulties, both for the sampling of earthworms and of their activity in the soil. Currently only 18 earthworm species from this biome are known, and some of them are well adapted to living in flooded soils (Carter & Beadle, 1931; Brown & James, 2007). The data of the two studies carried out in this biome (Table 1 and Figure 2) showed that the earthworm populations there had a high density in native vegetation (Dias et al., 2006a), but a very low abundance under cassava (*Manihot esculenta* Crantz) crops (Brito et al., 2016). Unfortunately, the earthworm species were not identified, but it is known that flooded areas, including rice (*Oryza sativa* L.) plantations (Barrigossi et al., 2009; Bartz et al., 2009b), can harbor a significant numbers of native species, especially of the Ocnerothrilidae and Almididae families (Brown & James, 2007). Therefore, future sampling in this biome should consider the particularities of each region and its earthworm species, adapting the methodology to the local conditions.

In the Pampa biome, which occupies 2% of Brazil's territory, only five studies evaluated earthworm populations (Table 1) in the regions of Santa Maria and Pelotas, in the state of Rio Grande do Sul (Figure 2). From this biome, 36 earthworm species are known, but 70% of them are exotic, i.e., non-native species,

originally from other countries (Brown & James, 2007). The predominant Cfa climate in this biome has no defined dry season (Alvares et al., 2013), which allows earthworms to be active all year, even during winter, at very low ambient temperatures of  $-4^{\circ}\text{C}$  (Santos et al., 2019), since the soil does not freeze.

In the Cerrado biome, another biodiversity hotspot (Myers et al., 2000) that covers 24% of Brazil (IBGE, 2019), 20 studies assessed earthworm populations. Although termites and ants predominate in this biome (Benito et al., 2004; Marchão et al., 2009), earthworm populations can have a considerable abundance and biomass in certain locations, mainly in pastures or integrated production systems (Brigante, 2000; Brown & James, 2007; Marchão et al., 2009), but also in coffee (*Coffea arabica* L.) plantations (Ricci et al., 1999) and under no-tillage agriculture (Blanchart et al., 2007). However, native Cerrado vegetation tends to have very few earthworms, especially in Central Brazil (Benito et al., 2008; Dias et al., 1997). Sampling time in this biome is especially important, because soils tend to be very dry and hard in winter, making it difficult to collect earthworms, which undergo diapause or aestivation (Abe & Buck, 1985; Silva et al., 2015b), normally in deeper layers.

In the Amazon, earthworm populations were reported in 24 publications (19% of the total). However, greater efforts are needed to better understand earthworm populations in this biome and the possible impacts of human activities, considering the region's size, the large variation in vegetation, soils, and natural and human-altered environments in this biome, and the high diversity of earthworm species in the Amazon Basin (Lavelle & Lapied, 2003). In addition, the presence of earthworms is high in this biome: no earthworms (0 individual per square meter) were reported only in five cases (3.5%), in dense Ombrophylous forest (Silva et al., 2005; Catanozi, 2010; Viana, 2012), and in corn (*Zea mays* L.) (Moura et al., 2015) and coffee (Silva et al., 2005) plantations.

Clearly, sampling efforts in the different Brazilian biomes have been highly variable (Figure 2). Furthermore, the specificities of each biome in terms of soils, vegetation, climate, and earthworm species present must be taken into account in order to optimize sampling schemes. Moreover, the data presented here can be used to target or prioritize future sampling sites and regions, in order to improve the understanding of

earthworm communities in different ecosystems and their role in soil processes.

### **Climate and vegetation-related variables**

Only three variables were considered by all authors: municipality, state, and soil cover/main vegetation type; other attributes that are easily available or that can be easily determined were often not included. The absence of these data, particularly sampling date (provided in 93% of the studies), sampling season (in 37%), geographical coordinates (in 65%), or the name of the specific location (in 74%), complicates data interpretation and comparison. Since climatic and vegetation-related variables are key determinants of earthworm biodiversity and activity in soils, their adequate reporting is crucial to correctly identify the context in which the data on earthworm communities was collected and to interpret the obtained results (Lavelle et al., 1993; Brown & Domínguez, 2010; Phillips et al., 2019).

A total of 13 climate and vegetation-related variables were identified and selected from the evaluated studies (Table 2). Climate is among the environmental attributes considered most important for earthworms (Phillips et al., 2019), regulating annual average temperature and precipitation and, consequently, driving soil moisture (Lavelle & Spain, 2001; Blume et al., 2016; Rutgers et al., 2016).

Altitude influences climate, especially temperature, and the type of vegetation, which affect soil and litter quantity and quality at the collection sites; therefore, it can have an important impact on earthworm abundance and species composition (Cardoso et al., 2014). Climate-related variables are also fundamental to determine the best assessing season (dry or rainy) and if the weather, particularly rainfall regime and potential soil moisture, is favorable for sampling earthworm activity and abundance (Satchell, 1967; Lavelle, 1983; Kale & Karmegam, 2010). Due to the high seasonal variability in earthworm abundance (Nadolny, 2017), especially in seasonally dry climates, the earthworm population should be assessed preferably towards the end of the rainy season, when most of the individuals have reached the adult stage (Lavelle, 1983), facilitating their identification (Richard et al., 2010).

Of the 12 climatic types described in Brazil (Alvares et al., 2013), only 9 were represented in the publications

(Nadolny et al., 2020). Climate at the sampling site – the main factor affecting earthworms in most population models (Phillips et al., 2019; Lavelle et al., 1993) – was omitted in 46% of the studies, although approximately 60% of them included information on precipitation and temperature. Even though climate data can be derived from the collection site using GPS coordinates and climate-specific databases, information on particular climate conditions during or just prior to sampling (e.g., large rainfall events) are crucial to better understand the results obtained for the earthworm community (Satchell, 1967).

Less than half (42%) of the studies described the biome and only 44% described the native vegetation of the sampling site (Table 2). Although this information can be easily obtained from other sources, more detail on the conditions of the vegetation are always a fundamental factor for assessing earthworm communities, since well-preserved or primary vegetation are more likely to have native species than secondary forests or highly disturbed sites (Brown & James, 2007). In addition, the age of regeneration of the vegetation can also have a key impact on earthworm abundance (Rousseau et al., 2014). Therefore, knowledge on the vegetation at the experimental sites is especially important because it allows determining the amount, type, and quality of the organic matter inputs made available both above- and belowground for earthworm consumption (Curry, 2004).

### **Management-related variables**

Eight management-related variables were identified and selected from the evaluated studies (Table 2). Human disturbance and soil management practices have major impacts on earthworm communities in Brazil (Brown & James, 2007; Nadolny, 2017) and were addressed in the majority of the studies included in the database (Nadolny et al., 2020). However, reporting of management practices and land use history in the publications was highly variable. Current and former land use are important variables in determining earthworm populations, and information on both should be provided in earthworm sampling schemes. In this regard, the number of years in current land use was provided in 58% of the publications, i.e., for 410 sites, but only 28% of the authors indicated previous land use.

Agricultural cropping was the most frequently assessed land use system (70 studies), representing 322 sites, i.e., 52% of all data (Table 1). Approximately half (49%) of the studies also described the soil management systems and crop type at sampling time (Table 2). Information on the type of soil tillage is essential because conventional tillage can negatively affect earthworm populations, whereas no-tillage systems usually have positive effects (Briones & Schmidt, 2017; Brown et al., 2003). Information on crop type and management is also important because these influence the amount and type of crop residues and their C:N ratio, which determine the food value for earthworms. Low C:N materials tend to decompose faster and have a higher N content, one of the main

components of earthworms (11% N in dry matter), essential for their growth and reproduction (Huerta et al., 2005, 2007).

Integrated production systems – such as agroforestry, integrated crop-livestock (agropastoral), integrated crop-livestock-forestry (agrosilvopastoral), and livestock-forestry (silvopastoral) systems – were reported in 32 studies and represented 26% of the sampled sites (Table 1). Pastures and integrated production systems, like agropastoral and agrosilvopastoral systems, tend to be good for earthworms (Lourente et al., 2007; Franchini et al., 2009; Marchão et al., 2009; Batista et al., 2014) and generally have higher earthworm population densities than annual crops, particularly in the tropics (Decaëns

**Table 2.** Data on the overall environmental (except soil) and management-related variables collected in the literature review, number of studies (percentage of total in parentheses) with the information available, and number of samples included in the database (N° db)<sup>(1)</sup>.

Variable	Description	N° of studies (%)	N° db
Climate and vegetation-related			
Sampling date	Month/year of sampling	115 (93)	722
Sampling season	Specification of wet or dry season	46 (37)	758
Location	Name of sampling site (such as farm and experimental field)	92 (74)	587
Municipality	Name of municipality	124 (100)	765
State	Acronym of the Brazilian state	124 (100)	765
Geographical coordinates	Coordinates of the collection site, preferably in degrees, minutes, and seconds, or transformed to decimals	80 (65)	765
Altitude	Altitude (meters) above sea level	66 (53)	765
Annual mean precipitation	Annual mean precipitation in millimeters	76 (61)	765
Annual mean temperature	Annual mean temperature in degree Celsius	73 (59)	765
Köppen's climate	Climate type according to Köppen's classification	66 (53)	765
Biome	Brazilian biome where sampling was done	52 (42)	765
Soil cover/vegetation type	Soil cover or type of vegetation	124 (100)	765
Type of native vegetation	Type of local native vegetation according to IBGE (2012)	54 (68) <sup>(2)</sup>	208
Management-related			
Crop type	Name of crop at or just before sampling date	65 (98) <sup>(2)</sup>	284
Soil management	Soil disturbance (conventional seeding, minimum tillage, no-tillage, permanent crop)	60 (48)	279
Current land use in years	Time in years of the soil in its current use	72 (58)	410
Previous land use	Previous use of the soil in the area	35 (28)	134
Pesticide use	Pesticide use (yes or no)	7 (6)	20
Pesticide type	Insecticides, fungicides, and herbicides	2 (2)	7
Fertilizers	Fertilizers applied (inorganic, organic, or none)	65 (52)	215
Fertilizer type	Name of used fertilizers	16 (13)	41

<sup>(1)</sup>The database is available at Nadolny et al. (2020) and includes the 124 publications evaluated. <sup>(2)</sup>Proportion calculated in relation to the total number of studies that sampled native vegetation or crops.



et al., 2004; Nunes et al., 2007). Agroforestry systems are also beneficial to earthworm populations (Luizão et al., 2002; Brown et al., 2006b, 2009; Römcke et al., 2009; Tarrá et al., 2012; Tapia-Coral et al., 2015), not only due to the protection offered by the greater vegetation cover (trees), which affects soil temperature and humidity, but also due to the diversification in the sources of the organic matter added to the soil.

Forestry plantations represented only 16% of the sampling sites and were addressed in five studies, mostly (77%) performed in sites located in Southern and Southeastern Brazil. Considering the area of 10 million ha that these plantations occupy in the country (IBGE, 2015) and their economic importance, greater sampling efforts are needed regarding forestry systems. Tree species and management affect both surface litter and underground organic matter quality, affecting the role of these systems as earthworm habitats. Higher Ca contents in the litter of some tree species can positively affect soil pH over time, influencing earthworm populations (Reich et al., 2005). The forestry species most common in Brazil are *Eucalyptus* and *Pinus*, both of which provide litter with low nutritional quality for earthworms (Bernhard-Reversat et al., 2001); however, large populations can still be found in these plantations (Maschio et al., 2014; Silva et al., 2019), where they may be providing important environmental services, like organic matter decomposition, nutrient cycling, and soil aggregation, a topic deserving further attention (Silva et al., 2019).

Besides soil disturbance, the use of pesticides and fertilizers at the sampling site(s) must also be known. However, only 5% of the studies recorded pesticide use in the sampling sites and just 2% revealed the type of product used. Moreover, only 13% of the studies mentioned fertilization, including chemical, organic, or mineral fertilizers, and just 10% detailed which types were applied. These management practices can deeply influence earthworm communities, since several pesticides cause reductions in the fecundity and changes in the feeding behavior and mortality of earthworms (Pelosi et al., 2014). However, little is known about the effects of most pesticides used in Brazilian agriculture on the earthworms actually present in the soils of the country (Sisinno et al., 2019). Some studies have reported that the application of fertilizers may increase earthworm densities because it also tends to increase plant production

(Edwards & Lofty, 1982; Misra & Tripathy, 1988), generally increasing the input of organic matter (food for earthworms) into the soil. For the same reasons, the application of manure-based organic fertilizers also tends to be beneficial to earthworm populations (Tiwari, 1993; Curry, 2004). Conversely, the prolonged use of large quantities of inorganic N-based fertilizers can cause soil acidification, which, if not corrected, may decrease earthworm abundance (Ma et al., 1990).

Therefore, soil and crop management practices have major impacts on the soil as a habitat for earthworms, with intense positive or negative effects (Curry, 2004), depending on the specific practice or on the combination of practices adopted. For this reason, standardizing the obtained data will provide important insights into possible mechanisms of population regulation in individual studies and future reviews on these topics.

### Soil-related variables

In Brazil, the majority of the earthworm species belong to the endogeic ecological category, living in and feeding within the soil matrix, rarely coming out onto soil surface (Lavelle, 1988b; Brown & James, 2007). Therefore, it is expected that soil type and physical and chemical attributes will affect their activity and populations in Brazilian ecosystems.

A total of 17 soil-related variables were selected from the publications and are listed in Table 3. Several studies in Brazil have highlighted the complex relationships between earthworm populations and some soil characteristics, particularly pH, organic matter, Ca, Mg, P, Al, texture and bulk density (Brown et al., 2003; Silva, 2010; Lima, 2011; Baretta et al., 2013; Bartz et al., 2013). However, these relationships are difficult to establish, because earthworms inhabit a multivariate habitat, where several factors act and interact simultaneously, affecting the soil's potential as a habitat for the development and activity of earthworm populations.

The soil type of the sampling site was given in 80% of the studies. This factor is generally considered key for earthworm species, whose preferences vary in terms of soil texture, pH, or organic matter content, all largely determined by the type of soil, whose properties also strongly influence the bioavailability of chemical stressors such as metals or pesticides (Ortega-Calvo



et al., 2015; Romero-Freire et al., 2015; Marchand et al., 2017). This shows that the classification of soils can provide useful information on their physical and chemical properties, water regime, depth and nutrient content, important to determine earthworm populations at a given site (Curry, 2004). However, little is known about the preference of earthworm species for soil types in Brazil – only one study on this topic has been performed so far, in the Cerrado region of northwestern São Paulo (Caballero, 1973, 1976). Furthermore, unfortunately, the soil maps available for most regions of Brazil are still at geographic scales too gross for the adequate estimation of the soil types at a collection site, indicating that more precise and, preferably, primary data on soil types should be provided.

Soil pH was informed in 56% of the evaluated studies (Table 3), being the most mentioned of all soil attributes. Most Brazilian soils are naturally acid (Motta & Melo, 2009), so the earthworms living in them are generally adapted to acidic conditions. However,

there is little information on the pH preferences of Brazilian earthworm species (Steffen, 2012), which may also vary depending on soil type. Some species of earthworms of the Lumbricidae family, which is typical of cooler regions with a temperate climate, show a clear preference for particular soil pH intervals (Satchell, 1967; Graefe & Beylich, 2003), but only a few lumbricids actually inhabit Brazilian soils and those that do are all exotic and occur almost exclusively in the Southern region of the country (Brown et al., 2006a). The common invasive species *Pontoscolex corethrurus*, from the Rhinodrilidae family, the most widespread earthworm in Brazil (Brown et al., 2006a), inhabits soils with pH ranging from 4.5 to 6.2 (Knapper & Porto, 1979; Steffen, 2012), while *Amyntas* spp. (*Amyntas gracilis* and *Amyntas corticis*) of the Megascolecidae family, also widely distributed in the country (Brown et al., 2006a), live in soils with higher pH, ranging from 4.8 to 7.2 (Knapper & Porto, 1979; Steffen, 2012). Soil pH also determines the availability of a number of other soil elements important for plant

**Table 3.** Soil-related variables, including soil type and chemical and physical parameters, identified in the literature review, number of studies (percentage of total in parentheses) with this information available, and number of samples included in the database (N° db)<sup>(1)</sup>.

Variable <sup>(2)</sup>	Description	N° of studies (%)	N° db
Soil type	Soil classification according to the Brazilian classification system (Santos et al., 2018) or to the Food and Agricultural Organization (FAO, 2015)	99 (80)	765
pH	Soil pH in CaCl <sub>2</sub> , KCl, or H <sub>2</sub> O (pH in water transformed to CaCl <sub>2</sub> )	70 (56)	438
H+Al	Soil potential acidity (cmol <sub>c</sub> dm <sup>-3</sup> )	39 (31)	277
K	Available potassium (cmol <sub>c</sub> dm <sup>-3</sup> )	62 (50)	386
Ca	Available calcium (cmol <sub>c</sub> dm <sup>-3</sup> )	59 (48)	360
Mg	Available magnesium (cmol <sub>c</sub> dm <sup>-3</sup> )	59 (48)	360
P	Total phosphorus by Mehlich (mg dm <sup>-3</sup> )	58 (47)	361
C	Total carbon by combustion or Walkley-Black (g dm <sup>-3</sup> )	44 (35)	305
Sum of bases	Sum of bases (cmol <sub>c</sub> dm <sup>-3</sup> )	41 (33)	386
CEC	Soil CEC (cmol <sub>c</sub> dm <sup>-3</sup> )	42 (34)	320
Base saturation	Base saturation (%)	36 (29)	324
N	Total nitrogen by Kjeldahl or combustion (g dm <sup>-3</sup> )	15 (12)	104
C:N	Soil carbon:nitrogen ratio	11 (9)	62
Sand	Total sand content (g kg <sup>-1</sup> )	42 (35)	295
Clay	Clay content (g kg <sup>-1</sup> )	48 (39)	319
Silt	Silt content (g kg <sup>-1</sup> )	44 (35)	295
Textural class	Textural class according to IBGE (2007)	55 (44)	423

<sup>(1)</sup>Database available at Nadolny et al. (2020). <sup>(2)</sup>CEC, cation exchange capacity.

and animal life, especially bases, cation exchange capacity, and Al and P contents. In Brazil, liming is one of the most common agricultural and forestry practices to increase soil pH, and can have profound effects on plant production and earthworm populations (Lavelle et al., 1995a). In many cases, liming is performed with calcium carbonate, increasing Ca availability in the soils.

Even though Ca is one of the most important elements to determine in a soil chemical analysis due to its direct relationship to pH, its contents were reported in less than half (48%) of the studies. The Ca content can be close to 1% dry mass in earthworms tissues (Paoletti et al., 2003), and this element plays a vital role in earthworm metabolism, being used to produce  $\text{CaCO}_3$  in the calciferous glands, reduce  $\text{CO}_2$  levels in the body, and regulate the pH of the gut (Pierce, 1972; Versteegh et al., 2014). In fact, the physiological activities involving Ca in earthworms are indicative of a mechanism of C sequestration in soils (Briones et al., 2008).

Data on the K and Mg cations in the soil were provided in approximately half (50 and 48%, respectively) of the studies; however, soil potential acidity (H+Al), cation exchange capacity, base saturation, and sum of bases were rarely provided (29–34%). These factors are generally well related to soil fertility levels (Ribeiro et al., 1999), and, therefore, commonly determined in soil quality assessments, especially in agricultural fields (Raij, 1987). However, the relationships between earthworm populations and the potential acidity, cation exchange capacity, base saturation, and K and Mg contents of the soils are not well known, particularly in Brazilian ecosystems.

Although soil P is generally tightly bound and is one of the most limiting elements in Brazilian soils (Malavolta, 2006), it was reported in only 47% of the studies. Therefore, there is still no clear relationship between P levels in the soil and earthworm populations, even though higher earthworm abundance has been associated with higher soil P contents (Bartz et al., 2013). It has also been shown that soil P is important for the metabolism of earthworms, whose tissues contain about 0.5% P (Paoletti et al., 2003), and a unique phosphagen, called phospholombricine (Wilson et al., 1992).

Despite being well known to positively affect soil quality (Sparling et al., 2008) and fauna populations

and activity (Lavelle et al., 2001), soil organic matter content is not always included in routine soil analyses in Brazil (Raij, 1987). Hence, only 34% of the studies provided soil C values (Table 3). Of these, some showed positive relationships between earthworm abundance and soil organic matter content (e.g., Brown et al., 2004), considered the main energy source for the earthworm metabolism (Martin et al., 1992). Endogeic earthworm species, the most common in Brazil, must ingest large amounts of soil due to their generally low C content and have also developed a mutualistic digestion system with bacteria in their gut to help them increase the assimilation of the ingested organic materials (Lavelle et al., 1995b).

Only 12% of the studies provided soil N values, probably because total N estimates are not included in routine soil analyses in Brazilian laboratories (Raij, 1987). Earthworm tissues have high proportions of N, in general about 70% protein in dry matter (Paoletti et al., 2003), even though the abundance of this element in the soil is low. Clearly, this is an essential element for earthworm populations in the soils, especially regarding their growth and reproduction; however, relationships between soil N and earthworms have not been frequently established (Huerta et al., 2005, 2007). Although the soil C:N ratio was evaluated in only 9% of the studies, being the least reported soil variable, it may still be important for earthworm communities, since it was identified as one of the factors governing the distribution of earthworm species in German soils (Römbke et al., 2012).

Soil texture, particularly clay content, is a key factor for earthworm activity because it generally influences both soil C content and water retention in different pore sizes (Feller & Beare, 1997; Costa et al., 2013). The preference of many lumbricid earthworm species for specific soil textural classes was studied by Guild (1948), who found that medium-textured soils appeared to be more favorable than sandy or clayey ones. More recently, Vendrame et al. (2009) observed that some earthworm species in the Cerrado preferred soils with high levels of kaolinite; however, very little information is available on the soil textural and mineralogical preferences of Brazilian species (Steffen, 2012). Soil textural classes were provided in 44% of the studies, and the specific contents of clay, sand, and silt were given in 35–39% of them. Stony or very coarse sandy soils may restrict earthworm populations (Guild, 1948),

mainly because of their low water retention capacity, but also of the presence of sand quartz crystals that may damage the earthworm's epidermis (Curry, 2004). At the other end of the textural triangle, heavy clay soils have a higher tendency to compaction, which can hinder earthworm activity, reducing soil displacement and ingestion (Klok et al., 2007). Furthermore, clayey soils often have lower water infiltration rates, which can negatively affect water availability in the soil profile due to a higher runoff, reducing earthworm activity (Edwards & Bohlen, 1996). Under more intense rainfall, these soils could become flooded, particularly in flatlands, complicating gas exchanges and earthworm respiration. However, most earthworms can survive short periods of anaerobiosis in case their burrows are flooded after heavy rains (Lee, 1985).

Soil moisture is an extremely important factor for the survival of earthworms (Lee, 1985; Edwards, 2004) – first, because their bodies are formed by > 80% H<sub>2</sub>O (Caballero, 1979; Ayres & Guerra, 1981) and, second, because they breathe through their skin, which needs to be kept continuously moist. However, this attribute was reported in only 17% of the evaluated publications. The ideal available water content for earthworms differs from one species to another and depends on the respective soil properties, especially texture and the amount of organic matter (Edwards & Bohlen 1996). Therefore, gravimetric soil moisture measurements must be related to soil texture and compared with overall water holding capacity. Lavelle et al. (1987), for instance, found that the ideal soil moisture values for the optimum development of *P. corethrurus* were all well above the field capacity of 35% H<sub>2</sub>O in a Mexican clayey loam soil, with 31% clay and 40% sand. Hydrophilic species, such as most representatives of the Ocerodrilidae, Sparganophilidae, Almididae, and Criodrilidae families (Righi, 1997), as well as some members of the Glossoscolecidae, Rhinodrilidae, and Acanthodrilidae families (Ayres & Guerra, 1981; Gavrilov, 1981; Barrion & Litsinger, 1997; Bartz et al., 2012), live in saturated soils with a low oxygen pressure. For instance, 33 of the 40 species from Central Amazon were found only next to water bodies, while 10% were collected in upland sites, being presumably more resistant to soil moisture variations (Ayres & Guerra, 1981). Unfortunately, very little is known regarding the soil moisture preferences of most Brazilian earthworm species, which is an indicative

that this is another important topic for basic biology research on earthworms.

### Sampling and earthworm-related attributes

The most widely used method of assessing earthworm abundance in tropical countries is hand-sorting, following the tropical soil biology and fertility (TSBF) method (Anderson & Ingram, 1993), combined with formalin expulsion of large earthworms (Römbke et al., 2006). The TSBF method (ISO, 2018), originally devised by Lavelle (1988a), recommends digging and hand-sorting five to ten holes of 25x25 cm width and 30 cm depth – 15 cm in ISO (2018) – in each area/plot/treatment/site to be studied (Anderson & Ingram, 1993). Although the method has some limitations, especially when collecting smaller individuals and cocoons (Lavelle et al., 1981; Jiménez et al., 2006), it has been widely used in the tropics and subtropics, with a reasonable success rate (Rossi et al., 2006). All publications selected for this review applied this method or adaptations of it (Nadolny et al., 2020), which included digging shallower (10 cm) or deeper (40 cm) holes, increasing the size of the holes (40x40 cm), and increasing (n = 36 holes) or decreasing (n = 3) sample frequency number.

Most studies (73%) used standard sizes of 25x25 cm or smaller for the sampling holes (Nadolny et al., 2020). The main limitation to smaller holes is that larger-sized earthworms are frequently cut and, therefore, not adequately sampled. To avoid this, when adult earthworms larger than 12.5 cm, i.e., half the width, are present, wider holes of 30x30 or 40x40 cm should be dug to reduce earthworm amputation. If there are only small earthworms with mean lengths of 5–10 cm, such as the size of *P. corethrurus*, then the standard hole of 25x25 cm is suitable. If anecic or large-bodied earthworms – which form casts and open burrows on the soil surface and usually respond to chemical extractants – are present at the site, then sampling should follow the ISO (2018) norm. This consists of hand-sorting soil from holes 50x50 cm wide and 20 cm deep, and posteriorly applying 5 to 10 L of the chemical extractant AITC, at the concentration of 0.1 g L<sup>-1</sup>; if giant earthworms, greater than 50 cm in length, are present, then the sampling area should be expanded to 4 m<sup>2</sup> and 80 L AITC should be applied (Römbke, 2007; ISO, 2018). AITC replaced formalin

as the recommended chemical extractant in the latest ISO norm (ISO, 2018), due the possible carcinogenic properties of formalin. However, only one study has tested the efficiency of AITC in Brazil (Ressetti et al., 2008), which was higher than formalin in cropping systems, but lower in pasture and native forest. Therefore, further research is needed to evaluate the adoption of AITC as the recommended chemical extractant in the country.

There is little published information on the ideal size of soil monoliths for quantitative earthworm sampling. Two Brazilian studies (Baretta et al., 2007; Cardoso et al., 2014) found that 40x40 or 50x50-cm wide holes were better than the standard TSBF-sized holes of 25x25 cm for collecting worms, probably because the experimental sites had large or fast-moving species. Caballero (1976) tested many hole sizes and proposed that the 50x50 or 60x60-cm dimensions were the best for her research conditions, that included large earthworm species. However, due to the size of these holes, more effort and time was needed to dig and hand-sort the soil from them, making it a procedure difficult or impossible to adopt in smaller plots (experimental fields) and when human resources are limited.

Most studies (98%) reported sampling depth (Table 4), which was 15 cm or less in 18% of them. The ideal sample depth should be chosen based on: the characteristics of the earthworm community, such as the presence or not of larger species that build deep galleries; soil structure, including the depth of specific layers; anthropogenic influence, like ploughing depth; and sampling season or climatic conditions at and/or just before the sampling date. During the rainy season, most earthworms tend to be concentrated in the 0–10-cm layer (Lavelle & Kohlmann, 1984), but, in the dry

season, they often migrate to greater depths and/or coil up into a ball, staying in a state of quiescence or even diapause (Abe & Buck, 1985; Drumond et al., 2013), when they are often more difficult to collect and/or in a lower abundance. However, even in the rainy season, particularly when it coincides with warmer temperatures, earthworms often move below 10-cm depth, especially at the hottest time of the day, in order to escape excessive heat and the lower soil moisture in the upper layer (Lavelle, 1983, 1988b). Therefore, it is important to choose the adequate monolith size and sample depth, based on previous observations in-situ, which will reveal the possible occurrence of larger earthworm species and the depth of their activity, as well as where larger holes should be dug (Caballero, 1976; Römbke et al., 2005; Cardoso et al., 2014).

Earthworm biomass is rarely reported in soil fauna studies in Brazil (Brown & James, 2007). It was only measured in 31% of the publications (314 sites), likely due to the additional effort – especially time – needed for this task (Bignell et al., 2008). Still, biomass measurements must be standardized for use in ecological investigations and comparisons between studies. Active earthworms normally have a variable proportion of soil in their intestine, generally 10–20% fresh weight, which can affect the precision of the fresh earthworm biomass measurements (University of Minnesota, 2020). For this reason, some researchers allow earthworms to void their guts before sacrificing and weighing them (Lee, 1985); however, this procedure is not feasible when collecting earthworms in the field, where they are often amputated and/or injured, which can affect the survival of the collected individuals. The best way to measure biomass in order to allow comparisons between studies is to determine

**Table 4.** Variables associated with earthworm sampling identified in the literature review, number of studies (percentage of total in parentheses) with this information available, and number of samples included in the database (N<sup>o</sup> db)<sup>(1)</sup>.

Variable	Description	N <sup>o</sup> of studies (%)	N <sup>o</sup> db
Size of dug holes	Dimensions of hole side x side or diameter if cylindrical (cm)	122 (98)	758
Number of holes	Number of samples per site	118 (95)	748
Depth	Sample depth (cm), with a minimum of 10 cm	121 (98)	755
Density	Mean number of earthworm individuals (m <sup>-2</sup> ) found at the site	124 (100)	765
Biomass	Mean earthworm biomass (g m <sup>-2</sup> ) at the site (fresh weight in preservative liquid, including intestinal contents)	38 (31)	314
Species identification	Species identified (yes or no)	30 (24)	266

<sup>(1)</sup>Database available at Nadolny et al. (2020).



ash-free dry weights with a freeze-drier (Brown et al., 1998). However, once this procedure is done, it is no longer possible to identify individuals, but it is possible to make some projections of gut weight for a subsample of the species, making a correction for the others (Martin, 1986). Therefore, a practical way to present biomass data is to specify how weighing was done; normally fresh weight, including intestinal content, is determined in alcohol at concentrations of 70% or greater or in formaldehyde from 4 to 10% (Baker & Lee, 1993). If there is time or it is possible, an adjustment can be made to obtain the corrected biomass (without gut contents) using data from the species. Material preserved in formaldehyde is easier to weigh and less dehydrated than that in ethanol, since the water content of the formaldehyde solution is higher (generally > 90%) than that of ethanol (generally < 30%) (Baker & Lee, 1993; ISO, 2018). However, for molecular studies using DNA, ethanol (> 90%) is recommended.

The ISO (2015) proposal is regularly used in ecotoxicological standard field tests. According to Dunger & Fiedler (1997), earthworms seem to lose about 10 to 20% of their mass during fixation, but, since this is about the same as the mass of the gut content, compensation is not necessary. The measured fresh mass can be converted to dry mass by multiplying its value by a factor of 0.15 (Petersen & Luxton, 1982), which was determined using mineral soil dwellers (endogeic species) from European grassland sites; therefore, this factor may vary considerably depending on the ecological category, i.e., it is smaller in epigeic species than in endogeic ones. Hence, adaptations may be needed in local studies, as well as further work to obtain more precise estimates for the species in-situ.

After sampling, species are normally identified in the laboratory, since most of the common tropical and subtropical earthworms require dissection for an adequate identification (Righi, 1997). For this reason, in Brazil, earthworm identification is not an easy task, having been performed in only 24% of the studies (Table 4). The proper identification of species improves the assessments of their interactions with the soil environment, and the estimation of their potential effects on soil properties and processes, which are closely linked to their ecological category/functional group (Brown et al., 2000). Therefore, in as far as is possible, the collected species should be identified, in

order to evaluate the impacts of land use on biodiversity and to estimate possible impacts of species on soil and associated ecosystem services (Podgaiski et al., 2011).

### **Recommendations for standardization**

Studies relating earthworms to environmental and soil factors must consider the multifactorial nature of soil-animal-plant relationships, and evaluate a minimum set of variables that are important determinants of earthworm populations in terrestrial ecosystems. A list of these variables and a brief explanation of the reasons for their recommendation is given in Table 5, aiming to increase the number of publications that simultaneously provide data on a wide range of soil and environmental variables. A good, detailed description of the sampling site, climate, and vegetation and soil type, including previous and current land uses and soil management, could improve the understanding of the relationships between environmental factors and earthworm abundance and diversity at a particular site.

Soil variables, evaluated in less than 50% of the studies, are not so difficult or costly to determine, and can be easily incorporated into the project budget of studies involving earthworm and/or soil fauna sampling. Furthermore, it is not difficult to obtain a soil sample for routine soil analysis, which would be enough to adequately describe the soil chemical and physical environment of a site as an earthworm habitat. Considering the data evaluated in this review, it is recommended that future studies evaluating earthworm populations measure, from at least a composite soil sample: pH; moisture; Ca, Mg, K, P, C, and N contents by combustion; CEC; and soil texture, including percentage of sand, silt, and clay. However, if time and resources are not an issue, then samples could be taken from around or within each earthworm monolith (Swift & Bignell, 2001). Furthermore, those variables should be measured using standard methods, in order to enhance comparisons between studies. Slightly similar lists were proposed by ISO (2018) and Swift & Bignell (2001) for soil biodiversity studies worldwide.

The data on earthworm abundance and biomass measured at each site and/or individual treatment type should be either presented in a table in the publication, or as an appendix or supplementary table

to the paper. The data shown in the figures as means per set of treatments or as a part of larger “groups” of soil animals (e.g., detritivores or engineers) should be individualized for earthworms (and other macrofauna groups) per treatment and provided in supplementary tables, so that they can be used in future comparative studies (Nadolny, 2017). A preliminary, rapid sampling

at a site is essential before undertaking intensive sampling, to reveal the size of the earthworms present and the depth of their activities and also to determine the intensity and appropriate soil volume for sampling.

Although earthworm species-level data provide essential information on niches and the relationships of species presence and abundance with soil, vegetation

**Table 5.** List of standard environmental, soil, and earthworm attributes to be provided/measured in studies on earthworm populations.

Attribute	Standard list of variables	Reason for data inclusion
Environmental	Sampling date/season and Köppen’s climate	Allows estimation of related weather conditions, particularly temperature, rainfall, and soil moisture
	Location/geographical coordinates	Allows mapping of sampling sites and relation to other environmental variables such as soil type and vegetation
	Annual mean precipitation	Affects mainly soil moisture contents
	Soil cover/vegetation type and type of native vegetation	Affects soil cover (protection) and the inputs of organic resources (food quantity and quality)
	Crop type	Affects soil cover, soil and crop management, and the inputs of organic resources (food quantity and quality)
	Soil management	Soil preparation (various forms of tillage) impacts soil structure; for example, a higher intensity is prejudicial to earthworms, whereas no-tillage tends to be beneficial
	Past and current land use	Time in current land use is a measure of the intensity of positive or negative potential impacts on earthworms; previous land use determines possible legacy effects on earthworm populations
	Pesticide use/type	Various pesticide formulations and active ingredients may have negative impacts on earthworms
	Fertilizer use/type	Fertilization increases primary production and the inputs of organic materials and mineral resources to the soil; long-term intensive fertilization with inorganic N acidifies soil, impacting earthworm populations
	Soil	Soil type
pH		Earthworm species have variable tolerance to pH, which is also related to the available mineral and organic resources
Soil moisture, K, Ca, Mg, and P		Important for earthworm metabolism
C and N		Related to food resource availability (quantity and quality), important for earthworm metabolism
Cation exchange capacity		Related to plant productivity potential and ability to provide food resources
Sand, clay, silt, and textural class		Affects the physical environment of the earthworm; related to food resource availability and soil hydrology
Earthworm sampling	Size of holes	Affects sampling efficiency; larger holes are needed when large earthworm species are present
	Number of holes	Related to sampling effort; a minimum number is needed to adequately sample earthworm communities
	Depth of holes	Affects sampling efficiency; deeper holes are needed in the dry season and when deep-burrowing earthworms are present
	Density	Main parameter related to earthworm presence in soils
	Biomass	Important to determine potential impacts of earthworms on soil physical properties
	Species identification	Allows determining biodiversity and facilitates the estimation of potential impacts on soil functioning



and management variables, total earthworm biomass and density are easier to determine and were considered by Doube & Schmidt (1997) as more stable (less variable) than data on species in studies using earthworms as environmental bioindicators. However, given the large number of native and endemic species present in Brazilian soils (Brown et al., 2013), it is important to obtain data on these species and any preferences they might have for particular soil and environmental conditions, in order to enhance conservation and management efforts. The identification of earthworm species requires expertise, and, in many cases, proper identification is only possible by a taxonomist, although there are a few specialists in Brazil and elsewhere who can also identify Brazilian earthworm species. When the identification of the material is not possible, care should be taken to preserve the collected species correctly, and contact should be made with an appropriate specialist or institution that can receive and store the material (e.g., a museum collection) to be identified later (if possible), reducing the risk of loss or damage by improper conservation.

The above suggestions for data collection are valid not only for earthworm sampling, but also for overall soil macrofauna population studies. This should facilitate the standardization of the information provided in publications and comparisons between/among them. Considering the growing scientific body in soil zoology and overall interest in the potential use of earthworms and other soil animals as soil and environmental quality indicators (Bünemann et al., 2018), the use of standard methods of analysis and data collection are essential to optimize research efforts, allow a wider use of the data and derived publications, and increase the life-span and usefulness of studies on earthworm populations in Brazilian ecosystems and worldwide.

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