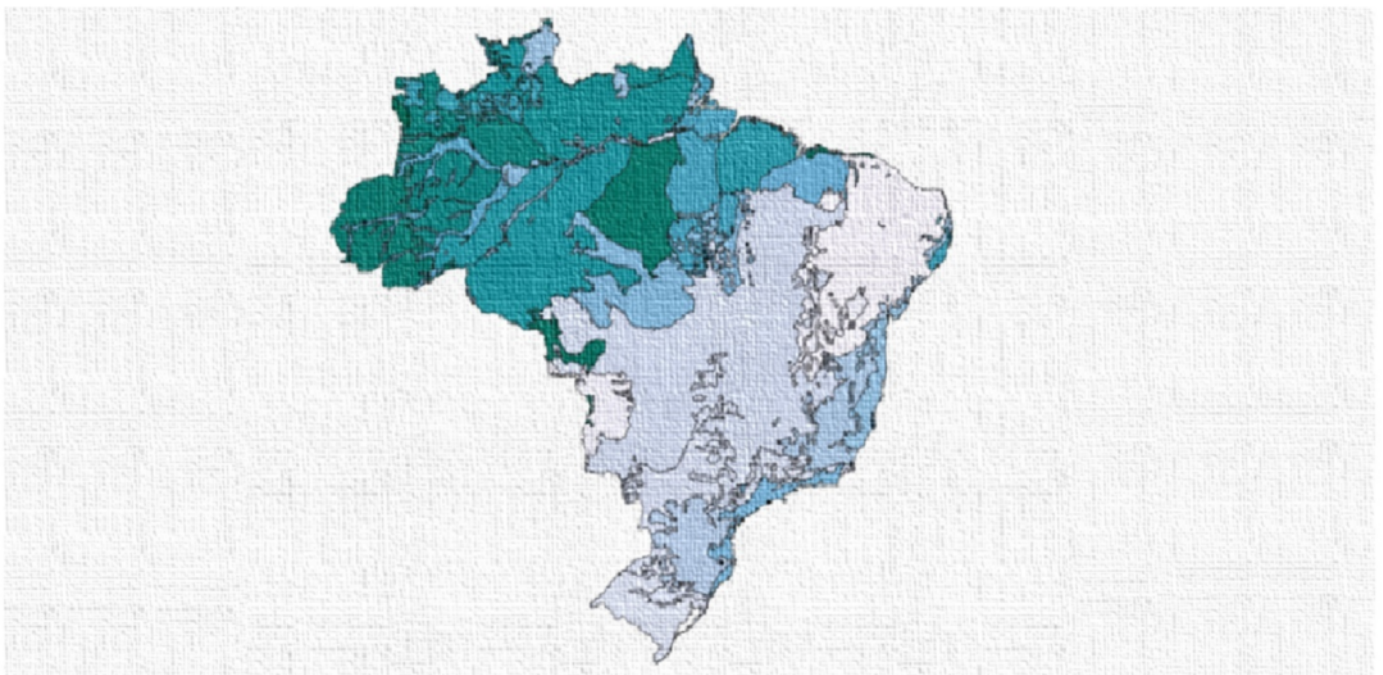


Plant species richness as indicator of terrestrial ecosystem damages:

Lessons from the Brazilian case



Natalia Crespo Mendes
PhD Thesis
February 2018

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Division for Quantitative Sustainability Assessment
Department of Management Engineering
Technical University of Denmark

Preface

This PhD thesis presents the outcome of the PhD project “Development of a Life Cycle Impact Assessment methodology for Brazil”. The research work was carried out at the Division for Quantitative Sustainability Assessment of the Department of Management Engineering at the Technical University of Denmark from December 2013 to December 2017.

The PhD project was funded by the CAPES Foundation, Ministry of Education of Brazil (process number 9365/13-3) and supervised by Professor Michael Zwicky Hauschild, as main supervisor, Associate Professor Alexis Laurent and by Professor Aldo Roberto Ometto, University of Sao Paulo – Brazil.

The thesis is a synopsis of three scientific articles of original research covering major findings of the project. The article manuscripts, of which one has been published and two accepted at the time of the thesis completion, are included as appendices. The articles are referred herein by adding roman numerals from I to III to the citation – e.g. **Article I**: Crespo-Mendes et al. (2014), and listed below:

Article I: Crespo-Mendes, N., Laurent, A., Ometto, A. R., & Hauschild, M. Z. (2014). Necessidade de uma metodologia de Avaliação de Impacto do Ciclo de Vida especialmente diferenciada para o Brasil. IV Congresso Brasileiro em Gestão do Ciclo de Vida, São Paulo, Brazil – Published, manuscript in post-print version.

Article II: Crespo-Mendes, N., Laurent, A., Bruun, H. H., & Hauschild, M. Z. Relationships between plant species richness and soil pH at the level of biome and ecoregion in Brazil. *Ecological Indicators* – Accepted (2018), manuscript in pre-print version.

Article III: Crespo-Mendes, N., Laurent, A., & Hauschild, M. Z. Effect factors of terrestrial acidification in Brazil for use in Life Cycle Impact Assessment. *The International Journal of Life Cycle Assessment* – Accepted (2018), manuscript in pre-print version.

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I would also like to thank the CAPES Foundation, Ministry of Education of Brazil, which funded this PhD project, the co-authors of the articles for their collaboration and all of those who I have contacted throughout this journey to help me put ideas into practice. I am particularly indebted to Sebastian Borchert from DTU Computing Center (DCC) and Flavia Pinto from the Spatial Ecology and Conservation Lab (LEEC), UNESP, Brazil. I would like to thank them for sharing their time and expertise, without even knowing me beforehand. People like them make this world a better place.

As everything in life is more pleasant when you have friends, in this case would not be different. I would like to thank all my friends for being present, regardless of the geographical distance. With special mention to: Daniella P. Galloro, who brilliantly always rescues good humor in my day, no matter how bad the situation seems to be. Ana Laura R. Pavan who helps me to keep the enthusiasm in moments of fatigue. Maria Rita R. Almeida for the moments of reflection on life. I guess all of them have already mastered the subject of this thesis, thanks for listening to me when I needed to organize my ideas. Carolina S. Conceição, Daniela Pigosso and Mariú A. Moro also deserve my gratitude for being my family in Denmark.

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A todos, muito obrigada!

Summary

Besides the intrinsic values of terrestrial ecosystems as regulators of the biogeochemical cycles of the Earth, ecosystem services such as food provision, climate regulation and air purification also generate direct benefits for human well-being. Preserving terrestrial ecosystems is, therefore, an international concern that has aroused the interest of scientists, economists and policy makers. To quantify potential damage to terrestrial ecosystems, Life Cycle Impact Assessment (LCIA) methodologies characterize impacts caused by anthropogenic activities through the use of indicators based on biodiversity loss. As primary producers, plants are the basis of the food chain and the foundation of most of the ecosystems, which justify their use as a biodiversity indicator at damage level for various impact categories such as climate change, photochemical ozone formation, terrestrial acidification, terrestrial ecotoxicity, land use and water consumption. In this context, this PhD thesis focuses on the use of plant species richness to assess the damage of terrestrial ecosystems.

Due to its extensive territorial area and great variations in terms of population density, anthropogenic activities and environmental characteristics throughout the country, Brazil is chosen as the study area of this thesis. Additionally, among the impact categories identified as concerns in the Brazilian context, terrestrial acidification is used as an example to carry out a detailed assessment of impacts on Brazilian flora. Thus, the effects of changes in soil hydrogen ion concentration on terrestrial plant species are evaluated based on the species richness distributions as a function of soil pH.

Data availability is a methodological challenge that is overcome by the development of a georeferenced botanical inventory containing 29712 terrestrial plants species, spatially differentiated at country, biome and ecoregion levels, with identification of range-restricted species (species only occurring in one ecoregion of Brazil). Based on this data set statistically strongly significant lognormal distributions are found, confirming the strong correlation between Brazilian plant species richness and soil pH. Similarly to the Potentially Not Occurring Fraction (PNOF) of species that is used for effects factor (EF) calculations in current LCIA models for terrestrial acidification, a new metric Potentially Extinct Fraction (PXF) of species is proposed and integrated into the effect factor calculations to enable the complementary assessment of unique species of a region, supporting a differentiated damage modelling indicating both the total loss of species and the potential for extinction of species.

More consistent spatially differentiated effect factors are provided while maintaining compatibility with existing models. Spatial differentiation proves meaningful when it is

possible to combine fine spatial scales (e.g. ecoregions) and highly representative data. In addition, area-weighted effect factors (EF_{aw}) are proposed to assess effects on the entire species richness curves and the contribution of each pH value in terms of the land area it represents, which has not been done in previous models. Resulting effect factors suggest that an increase in soil acidity may not necessarily be associated with a decrease in species richness if pH approaches the optimum pH (in which the species richness is at its maximum) from the alkaline side of the curve. Overall, this thesis questions the appropriateness of the metric that is currently used for impact characterization and damage modelling in LCIA and highlights the limitations of using species richness as the only indicator to assess terrestrial ecosystem damages caused by terrestrial acidification.

Additional research may include the expansion of the methodological elements presented in this thesis to other regions of the world and thus make feasible their integration into characterization factors for terrestrial acidification as well as for other impact categories. Furthermore, the data provided in the botanical inventory can support other ecologically related research areas enabling, for example, studies of the interactions between ecosystem changes and potential loss of species helping identify potential patterns of biodiversity loss to support conservation policies.

Dansk sammenfatning

Udover landjordsøkosystemers funktion som regulatorer af Jordens biogeokemiske cyklus, tjener de både som primærkilde for føde, klimaregulation, rensning af luften, og de er derfor direkte afgørende for menneskers trivsel. Bevaring af landjordsøkosystemer er derfor et emne af international vigtighed som har vakt interesse fra forskere, økonomer og politikere.

Skader på landjordsøkosystemer fra menneskeskabte aktiviteter kvantificeres i livscyklusvurdering af miljøpåvirkninger (LCIA) igennem indikatorer der udtrykker tab af biodiversitet. Som primærproducenter danner planter basis for fødekæden og fundamentet for de fleste økosystemer. Denne rolle retfærdiggør deres brug som indikator for biodiversitet ved endpoint vurdering af klimaforandringer, ozondannelse, forsurening af jord, økotoksicitet, samt arealanvendelse og vandforbrug. Denne PhD afhandling vil på denne baggrund fokusere på brugen af plantediversitet til at vurdere skaden til terrestriske økosystemer.

På grund af dets omfattende areal såvel som den store variation i både befolkningstæthed, menneskelig aktivitet samt forskelligartede miljømæssige forhold i landet, vil Brasilien blive brugt som fokusområde for denne afhandling. Forsuring af jord vil blive brugt som eksempel og vurderet i en detaljeret analyse af dens indflydelse på den Brasilianske flora. Indflydelsen af hydrogen-ion koncentrationen i jorden på diversiteten af plantelivet vil derfor blive evalueret.

Tilgængeligheden af data er en udfordring, som vil blive adresseret ved udviklingen af en georefereret botanisk liste indeholdende 29712 landplanter, differentieret på land, biom og økoregionsniveau med identifikation af område-begrænsede arter (arter, som kun forefindes i én økoregion i Brasilien). Baseret på disse data, er statistisk stærkt signifikante log-normalfordelinger blevet identificeret, demonstrerende en stærk korrelation imellem plantediversiteten og jordbundens pH værdi. I lighed med Potentielt-ikke-forekommende-fraktioner (PNOF) af arter, som bliver brugt ved udregning af effektfaktorer (EF) i nuværende LCIA modeller for forsurening, er den i denne tese foreslåede metrik, Potentielt udryddede fraktion (PXF) af arter, blevet anvendt i udregning af effektfaktorer for at muliggøre en komplementær beskrivelse af både det totale artstabilitet såvel som potentialet for arts-udryddelse. Mere konsistente stedligt differentierede effektfaktorer, som samtidig bibeholder kompatibiliteten med eksisterende modeller er introduceret. Stedlig differentiering viser sig meningsfuld, når det er muligt at understøtte fin-detaljerede geografiske skalaer (som f.eks. øko-regioner) med stærkt repræsentative data. Derudover foreslås udregning af EF baseret på arealvægtning (EF_{aw}) for at tage hensyn til effekten på hele artsrigdomskurven med

repræsentation af hver enkelt jord-pH værdi proportionalt med det areal, den repræsenterer, hvilket ikke tidligere er gjort. De resulterende effektfaktorer antyder, at et fald i pH værdien ikke nødvendigvis er associeret med faldende artsrighed, hvis ændringen i jordens pH værdi resulterer i en pH tættere på den optimale værdi for artsrigheden.

Sammenfattende indikerer denne afhandling, at den nuværende metrik til repræsentering af miljøskade ikke nødvendigvis er repræsentativ for den faktiske effekt og understreger, problemerne forbundet med udelukkende at anvende artsrighed som indikator for forureningsforårsagede skader på jordbundsmiljøer.

Yderligere forskning kan for eksempel inkludere brug af de metoder, der er udviklet og præsenteret i nærværende afhandling til andre regioner og herigennem muliggøre en karakterisering af en mere fyldestgørende karakterisering af forurening af jorden såvel som andre miljøpåvirkninger af jordøkosystemer. Ydermere kan de data, som er præsenteret i den botaniske fortegnelse anvendes til at understøtte studier af sammenhængen mellem økosystemforandringer og tab af artsdiversitet for at hjælpe til at identificere mønstre i tab af biodiversitet og hermed bidrage til optimering af rationelle politiske beslutninger for beskyttelse af miljøet.

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1. Introduction

1.1. Preserving terrestrial ecosystems

Biodiversity loss and consequent damage to ecosystems are global concerns that have been addressed by scientists, economists and policy makers. Besides emphasizing the importance of biodiversity and ecosystem functioning as regulators of Earth's biochemical cycles, international studies have been published to highlight the costs of biodiversity loss and ecosystems damages, estimating monetary values for ecosystem services, and provide recommendations for policy makers (Costanza et al., 1997; Loreau et al., 2001; MA, 2005; TEEB, 2010; Kubiszewski et al., 2017).

Preservation strategies focused on terrestrial ecosystems gained recognition over the last decades, which is reflected by the 2030 Agenda for Sustainable Development (United Nations, 2017). Several ecosystem and biodiversity-related targets are addressed among the Sustainable Development Goals (SDG), specifically in the Goal 15 - Life on Land.

In addition to the intrinsic values, biophysical structures and processes of terrestrial ecosystems have functions that provide fundamental services (also referred to as natural capital), generating benefits to people (Haines-Young and Potschin, 2010). As one of the major drivers of ecosystem changes, biodiversity loss can accelerate the damage in important processes such as productivity and decomposition (Hooper et al., 2012). Additionally, food provision, climate regulation, air purification and pollination are examples of services provided by the ecosystem that contribute to human well-being (TEEB, 2010).

While biophysical trade-offs and qualitative analyses are used by ecologists to measure the benefits provided by ecosystems, monetary units are used by economists to estimate the value of ecosystems and natural capital (Costanza et al., 2011). Costanza et al. (1997), for example, considered the economic value of 17 ecosystem services for 16 biomes to estimate the value for the entire biosphere, which corresponds to an average of US\$33 trillion per year.

To help prioritize global natural conservation efforts, the distribution of biodiversity across the Earth and the location of threatened species' populations have been identified by different approaches, most of them placed within the irreplaceability/vulnerability framework of systematic conservation planning (Brooks et al., 2006). However, even within countries and regions of high global conservation priority, biodiversity and threatened species are not evenly distributed, and conservation targets and priorities at finer resolutions are needed to improve the conservation planning and resource allocation (Brooks et al., 2006).

Complementarily to the degree of threat, species richness and endemism have been used as biodiversity indicators to manage biodiversity conservation plans for different regions in the world (Kier and Barthlott, 2001; Crisp et al., 2001; Pärtel et al., 2004; Klink and Machado, 2005; Kier et al., 2009; Ribeiro et al., 2009; Forzza et al., 2012). Different taxonomic groups (e.g. mammals, birds, amphibians, reptiles and plants) are used to assess impacts on biodiversity and ecosystems (Thomas et al., 2004; Kier et al., 2009). Although each group has its own importance in the environment, plants are the basis of the food chain (i.e. primary producers) and the foundation of most of the ecosystems, thus being indispensable for the maintenance of other life forms, such as animals, bacteria and fungi (Kapustka and Reporter, 1997). In addition, plants have been used as indicators of damage to terrestrial ecosystems caused by anthropogenic activities, for example, to assess the effects of sulfur and nitrogen pollutants emissions (Lee, 1998; Ruthewford, 1984), ionizing radiation (Woodwell, 1962), ozone exposure (van Goethem et al., 2013a), increased solar radiation associated with ozone depletion (Caldwell et al., 1998) and the effects of climate change (Convey and Smith, 2005). This thesis therefore focuses on the terrestrial plant species richness to assess damage to terrestrial ecosystems.

1.2. Plant species richness as biodiversity indicator in Life Cycle Impact Assessment (LCIA)

Life Cycle Assessment (LCA) is an environmental analysis tool used to quantify potential impacts of products or services throughout its life cycle, from the extraction of raw materials to the end-of-life (ISO 14044, 2006). From an LCA perspective, the emissions and resource consumption caused by anthropogenic activities are assigned to impact categories (e.g. climate change, acidification and land use) and translated into potential environmental impacts through the use of Life Cycle Impact Assessment (LCIA) methodologies (Hauschild and Huijbregts, 2015).

Historically LCIA methodologies have been primarily developed by European, North American and Japanese research institutions (EC, 2010). This has resulted in methodologies which have a strong bias towards the environmental issues that are of the highest concern in these regions – emission related impacts like climate change, ozone depletion, acidification, eutrophication, photochemical oxidants and environmental and human toxicity. In addition, the methods developed to characterize these impacts are typically based on environmental models

developed for these regions of the world and the parameter settings of the models thus represent European, North American or Japanese conditions. Efforts to bring spatial differentiation into LCIA methodologies with a global coverage have led to the development of LC-Impact (2017), Impact World+ (2017) and ReCiPe 2016 (Huijbregts et al., 2017), which are currently the most advanced LCIA methodologies in that regard. This thesis considers the characterization models adopted by these LCIA methodologies as a basis for the investigation on terrestrial ecosystem impact modelling.

In LCIA, environmental interferences resulting from biodiversity loss are addressed, at a damage level, in the area of protection (AoP) “ecosystem quality” (Woods et al., 2017). Damages related to ecosystem services – the instrumental values for humans – are still under development as an additional AoP, being rarely operationalized in current LCIA models (Verones et al., 2017b) and, therefore, are not part of the scope of this thesis.

Climate change, photochemical ozone formation, terrestrial acidification, terrestrial ecotoxicity, land use and water consumption are environmental problems that cause damage to terrestrial ecosystems. When addressed as impact categories related to the AoP “ecosystem quality”, it is recommended by the international LCA community (UNEP-SETAC Life Cycle Initiative) to use the “Potentially Disappeared Fraction of species (PDF)” as a damage level metric that relates the stress factor for each category (e.g. pollutant emissions) with its effects caused on biodiversity in terms of potential species loss (Verones et al., 2017b). In addition, the development of disaggregated impact indicators is recommended, i.e. damages expressed in PDF separately for the different compartments (e.g. terrestrial, freshwater or marine) and taxonomic groups (e.g. plants or animals) (Verones et al., 2017b). The compartments and taxonomic groups are chosen according to the environmental impact pathway of each impact category. As part of the terrestrial ecosystem damage modelling, plants have been used to indicate the effects – in terms of species loss – due to greenhouse gas (GHG) emissions and consequent temperature increase (climate change, De Schryver et al. (2009)), ozone exposure (photochemical ozone formation, Van Goethem et al. (2013b)), changes on H⁺ concentration in the soil due to the emission of acidifying substances (terrestrial acidification, Roy et al. (2014)), cumulative land use (land use, Chaudhary et al. (2015)) and limited water share of net primary productivity (water consumption, Verones et al. (2017a)). Rather than indicating the local effect of plant species loss, the most recently developed characterization models for land use (Chaudhary et al., 2015) and water consumption (Verones et al., 2017a) also adopt a vulnerability score to indicate the global effects of endemic species loss, i.e. the risk of

extinction. Species vulnerability is, however, a concept that still needs to be further developed and applied to other impact categories.

1.3. Spatially differentiated models

In the LCIA context, spatially differentiated models are meaningful for non-global impact categories (e.g. eutrophication, acidification and ecotoxicity), for which environmental impacts are assessed regionally depending on the location of the emission (Potting and Hauschild, 2006). EDIP 2003 was the first LCIA methodology to present spatial differentiation for all non-global impact categories, providing site-dependent characterization factors and normalization references for different regions of Europe (Hauschild and Potting, 2005). The development of spatially differentiated characterization models has spread to other continents and spatial differentiation has been directed from the continental scale to the country scale. Characterization factors of different impact categories have been developed for countries with a large territorial extension, such as Argentina (Civit et al., 2014), Canada (Saad et al., 2011), United States (Shah and Ries, 2009) and Sweden - one of the largest countries in Europe (Finnveden and Nilsson, 2005). According to these studies, atmospheric emissions, vegetation, climate, territorial area, population density and seasonal variations are some examples of regional parameters that can influence the results in an impact assessment. The significant difference in results, reported in studies comparing site-dependent factors with generic factors, reinforces the need to develop methodologies that represent regional environmental specificities (Finnveden and Nilsson, 2005; Shah and Ries, 2009; Saad et al., 2011; Civit et al., 2014; **Article I**: Crespo-Mendes et al., 2014; Souza et al., 2015; Verones et al., 2017b). The most recently developed LCIA methodologies represent progress in that regard. Different spatial levels have been adopted for calculations such as biomes, ecoregions, watersheds and wetlands, and country-average characterization factors are available in a global coverage (Roy et al., 2014; Van Zelm et al., 2016; Verones et al., 2017a; Huijbregts et al., 2017). Hereupon, exploring the spatial variability of impacts can support the choice of the most appropriate spatial level for characterization models and thereby increase their environmental relevance.

1.4. Relevance of Brazil

Brazil is chosen as the study area of this thesis, since:

- (i) The high number of species recorded in Brazil – more than 30000 species of plants (Forzza et al., 2012) – makes it one of the most biodiverse countries in the world. These high numbers are also reflected in terms of threatened species (SiBBr, 2017). Thus, the tropical and highly diverse Brazilian flora can be further explored to benefit research on the use of plant species richness as an indicator for damage to ecosystems.
- (ii) It has an extensive territorial area (8515759 km²), with great variations in terms of population density distribution and environmental conditions throughout the country (IBGE, 2017a,b). As a country with continental size, Brazil can be a good proxy for South American studies. In addition, being represented by different biomes and ecoregions enables the assessment of the influence of spatial granularity in LCIA models.
- (iii) Anthropogenic activities in Brazil, which vary considerably across the country, cover different sectors (e.g. agriculture, livestock, consumer goods industry and transport system) and they are therefore associated with various types of environmental impacts, such as human toxicity, acidification and climate change (Dalgaard et al., 2008; Malhi et al., 2008; Ometto et al., 2009; Alho, 2011; Filho and Horridge, 2014; Almeida et al., 2016; Horn et al., 2016; Araújo et al., 2017; **Article I**: Crespo-Mendes et al., 2014). Among the impact categories identified as concerns in the Brazilian context, terrestrial acidification is used as an example to carry out a detailed assessment of impacts on Brazilian flora.

Thus, it is expected that the lessons acquired through the Brazilian context can support the development of ecosystem damage assessment globally in future research.

1.5. Limitations and research gaps of current indicators

As pointed out in **Article II**: Crespo-Mendes et al. (2018), there is a lack of studies exploring the relationships between plant species richness and soil pH – which is the basis for assessing the effects of terrestrial acidification in LCIA – on continents other than North America and Europe. The study by Azevedo et al. (2013) is an exception, in which the relationships between

changes in plant species richness and soil pH variations are explored from the occurrences of 2409 plant species, attributed to 13 terrestrial biomes in the world. However, there are more than 300000 plant species distributed around the world (Kreft and Jetz, 2007; Kier et al., 2009), which emphasizes the need for more comprehensive datasets to support the investigation of this type of relationships for other regions such as in the southern hemisphere continents.

The low data representativeness is reflected by the LCIA factors that represent the effects of terrestrial acidification on plants (called effect factors, EF), which as a consequence are the dominant source of uncertainties in the characterization factors (Roy et al., 2014; Van Zelm et al., 2015). In addition, data availability is also a limitation with respect to spatial differentiation since low data availability may compromise analyses at finer spatial scales (Azevedo et al., 2013).

Furthermore, there is a lack of LCIA models for terrestrial acidification that focus on the differences between effects on all plant species and effects on critical species, e.g. range-restricted species (**Article III**: Crespo-Mendes et al., 2018). In addition to contributing to the conservation of species diversity, the complementary assessment of richness of both total species and critical species may reveal patterns of unique species to the environment and indicate potential permanent damage to ecosystems, i.e. potential risk of extinction.

1.6. Research goals

This thesis aims to investigate the bias in Brazilian ecosystem impact modelling in LCA resulting from ignoring Brazilian conditions and contribute to developing ecosystem damage assessment in support of a Life Cycle Impact Assessment methodology that is fully compliant with regional Brazilian conditions.

The impact category acidification is chosen as example and a detailed assessment of acidifying effects on the Brazilian flora is performed.

The proposed methodology is in accordance with existing LCIA methodologies covering the rest of the world to ensure compatibility between the results for Brazilian ecosystems and the impacts modelled for other regions, exposed to emissions from the product life cycle.

To guide the research on the above considerations specific research goals are defined:

1. Provide a methodological framework for the development of georeferenced inventories that can be applied in research on different taxonomic groups, for different regions of the world.

2. Create a quality assured and harmonized database of georeferenced terrestrial plant species occurrence to link to existing soil pH mapping for Brazil.
3. Investigate the relationships between terrestrial plant species richness and soil pH in a large tropical flora, at the level of country, biome and ecoregion.
4. Provide consistent spatially differentiated effect factors for terrestrial acidification, based on the use of a comprehensive botanic inventory of Brazil.
5. Analyze the variability of the species richness and the effect factor distributions across ecoregions and biomes of Brazil to assess the influence of spatial granularity and the benefits of using data at ecoregion, biome or country levels.
6. Assess potential differences in impact indicators based on species which are unique to single ecoregions, termed “range-restricted species” in the study, and those which have extended ranges of occurrence.
7. Discuss the appropriateness of the metrics used in LCIA to address biodiversity loss caused by terrestrial acidification.

1.7. How to read this thesis

The thesis is structured as follows. Initially, aspects that justify the need for a Life Cycle Impact Assessment methodology spatially differentiated for Brazil are presented in Chapter 2. This chapter is supported by **Article I**: Crespo-Mendes et al. (2014). Chapter 3 proposes a methodological framework for the development of georeferenced biodiversity inventories to support research on environmental impact assessment, ecology and related areas. This chapter is supported by **Article II**: Crespo-Mendes et al. (2018). Chapter 4 explores the relationship between the terrestrial plant species richness and soil pH, summarizing the main findings of **Article II**: Crespo-Mendes et al. (2018). Chapter 5 assesses the effects of terrestrial acidification on plant species richness in LCIA and provides different sets of spatially differentiated effect factors for Brazil. This Chapter is supported mainly by **Article III**: Crespo-Mendes et al. (2018). Chapter 6 presents a discussion based on the main findings of previous chapters, highlighting the influence of data availability, spatial granularity and appropriateness of metrics to address biodiversity loss in LCIA studies. This chapter is supported by **Article II**: Crespo-Mendes et al. (2018) and **Article III**: Crespo-Mendes et al. (2018). Finally, conclusions and outlook are presented in Chapter 7 and a list of major findings is subsequently provided in Chapter 8. All the scientific articles are attached as appendices.

2. Anthropogenic activities and environmental impact assessment in Brazil

2.1. Geographic data

Brazil is the fifth largest country in the world, both by geographical area and by population. Its area of 8515759 km² represents approximately 47% of the total area of countries that comprise the South America, making it the largest country in such a group (IBGE, 2017a). With an estimated population of 207660929 inhabitants in 2017 (IBGE, 2017b), the population distribution in Brazil is unequal. It has a high population density in the Southeast Region (94 hab/km²), followed by the South Regions (51 hab/km²) and the Northeast Region (37 hab/km²). Although the Central-West Region and North Region together represent 64% of the Brazilian territory, these are the least populated areas with 10 hab/km² and 5 hab/km², respectively (IBGE, 2017a,b).

Besides the geopolitical division in 27 Federative Units (26 states and one federal district) grouped in five main regions (North, Northeast, Central-West, Southeast and South Regions), the extensive geographical area of Brazil covers different biomes and ecoregions. The division into biomes considers aspects such as macroclimate, phytophysiognomy, soil and altitude to group terrestrial ecosystems with similar characteristics among them (Clements, 1949). While ecoregions are smaller units that constitute the biomes and represent natural communities with the same environmental conditions and inter-species interactions (Olson et al., 2001). Figure 2.1 illustrates the biomes and ecoregions delineated by Olson et al. (2001) a widely-applied classification that addresses regions around the world.

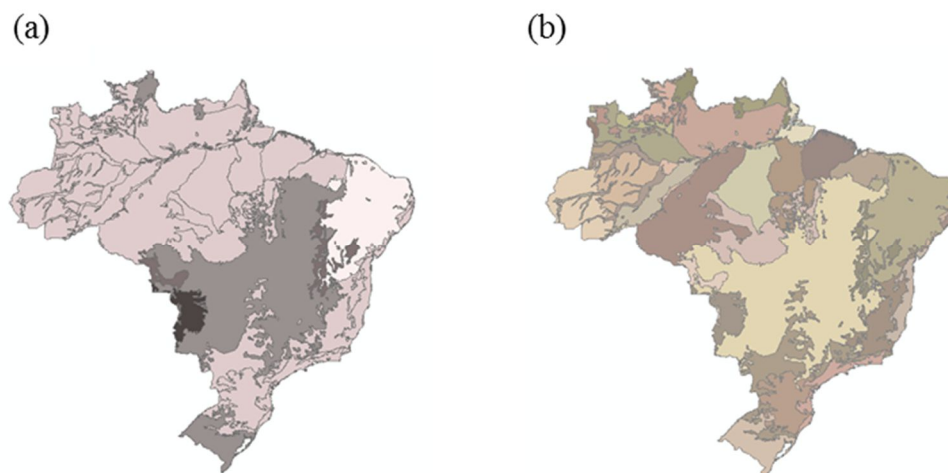


Figure 2.1. (a) Biomes (6) and (b) ecoregions (47) of Brazil, delineated by Olson et al. (2001).

2.2. Anthropogenic activities and LCIA in Brazil

The variation in population density and the richness of typical natural resources from each biome affect how anthropogenic activities are developed across the country. In **Article I**: Crespo-Mendes et al. (2014) the anthropogenic activities that are potentially causing environmental impacts in Brazil are identified through the literature review of scientific articles and reports published by national ministries and institutions (MME, 2009; MAPA, 2011a,b; Mendes and Ometto, 2012). These activities are then associated with the LCIA impact categories to which they contribute. In addition, the need for spatial differentiation for each impact category addressed in the study is analyzed. The main findings are presented in Table 2.1.

Table 2.1. LCIA impact categories and the anthropogenic activities that are potentially causing environmental impacts in Brazil.

Impact category	Anthropogenic activities	Literature references	Need for spatial differentiation
Climate change	Energy production (fossil fuels) Road transportation Agriculture Livestock	Silva et al. (2010) Dalgaard et al. (2008) Ometto et al. (2009) Solomon (2010) Alho (2011)	No
Ozone depletion	Chemical industry		No
Terrestrial acidification	Road transportation Agriculture Energy production (fossil fuels)	Dalgaard et al. (2008) Coltro et al. (2009) Ometto et al. (2009)	Yes
Eutrophication	Agriculture Household waste	Coltro et al. (2009) Ometto et al. (2009)	Yes
Photochemical ozone formation	Road transportation		Yes
Ecotoxicity	Mining Household waste Chemical industry		Yes
Human toxicity	Mining Chemical industry Road transportation		Yes
Land use	Agriculture Livestock Energy production (fossil fuels) Mining Metallurgical industry	Fearnside (2001) Solomon (2010) Alho (2011)	Yes

Water use	Agriculture Mining Chemical industry Household waste Clothing industry	Coltro et al. (2006) Coltro et al. (2009) Ometto et al. (2009) Solomon (2010)	Yes
Resources depletion	Energy production (fossil fuels) Mining Metallurgical, automotive, chemical, ceramics, airplanes, clothing and electronics industries		Yes

Besides agriculture and livestock, Brazil still has a varied industrial park of consumer goods and technology. Metallurgical, automotive, chemical, ceramics, airplanes, clothing and electronics industries are examples of anthropogenic activities highly performed in Brazil (**Article I**: Crespo-Mendes et al., 2014; IBGE, 2017a). All these activities are potential causes of impacts to the environment (see Table 2.1) and the specificities of each region make it difficult to properly assess environmental impacts using site-generic characterization factors. In this context, the application of spatially differentiated LCIA methodologies, fully compliant with regional Brazilian conditions, could better capture the differences of each region in Brazil. In addition, **Article I**: Crespo-Mendes et al. (2014) emphasizes the need for the development of normalization references – expressed in Brazilian citizens' equivalents – considering Brazilian national production and the importance of consistency between the results of national processes and those occurring outside the country, since the life cycle rarely occurs exclusively within Brazil.

Among the impact categories identified in the literature and presented in Table 2.1 as concerns in the Brazilian context, terrestrial acidification is used as an example to carry out a detailed assessment of impacts on Brazilian flora. Besides being an impact category that enables the analysis of both local and global effects, it also enables to explore how the relationship between plant species and abiotic factors, in this case soil pH, can benefit the assessment of damage to terrestrial ecosystems.

2.3. Assessing terrestrial acidification in Brazil

Terrestrial acidification is defined as the decrease in soil pH due to the accumulation of hydrogen and potentially free aluminum ions in the soil and the leaching of cation bases such

as calcium, magnesium, potassium and sodium. Acidity negatively affects soil fertility and compromises the production capacity of most agricultural soils (FAO and ITPS, 2015). Soil acidity is a major concern in soils with low capacity to buffer the decrease in pH and soils which already have low pH, such as acidic soils in highly weathered tropical areas (Harter, 2007; Johnson et al., 1982). Figure 2.2 shows the soil pH distribution around the world and from the pH values attributed to Brazil and the anthropogenic activities carried out in that area, terrestrial acidification is shown as a relevant issue for the Brazilian context. Among the impacts caused to terrestrial ecosystems are damage to agricultural areas, to food production and loss of biodiversity.

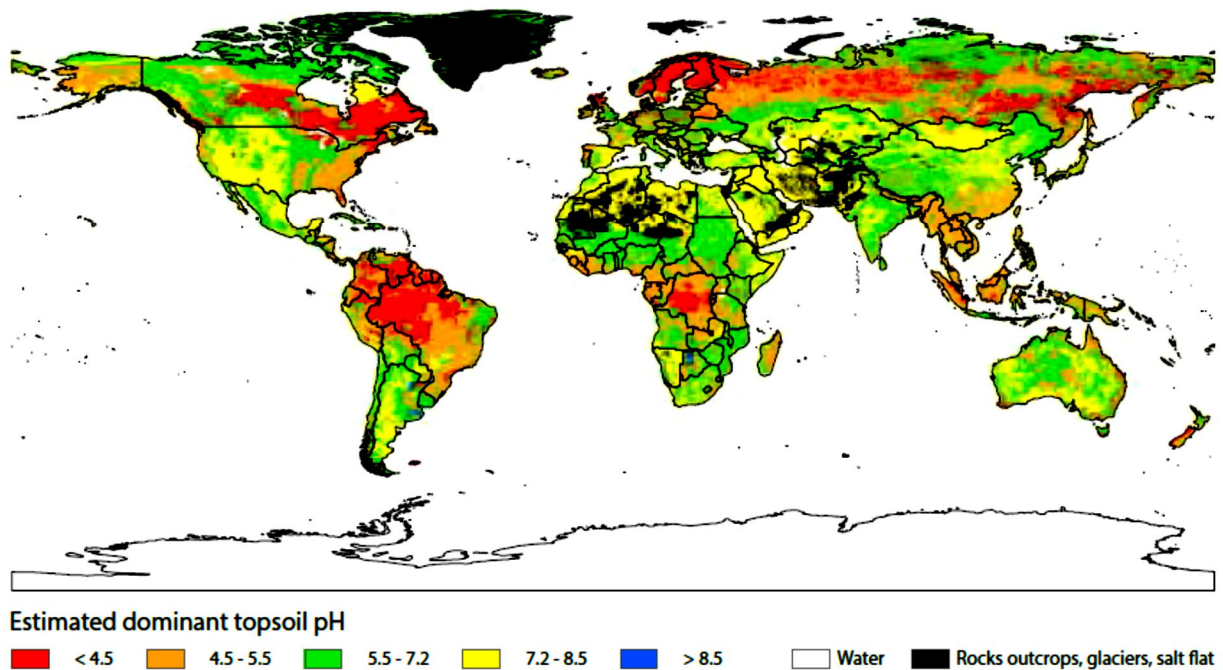


Figure 2.2. World map of soil surface pH.
Source: FAO/IIASA/ISRIC/ISS-CAS/JRC, 2009.

2.3.1. State of the art in terrestrial acidification impact assessment

The LCIA impact category “terrestrial acidification” addresses the potential environmental problems caused by emissions and depositions of acidifying substances, such as oxides of nitrogen (NO_x), sulfur dioxide (SO₂) and ammonia (NH₃). Impact characterization at the midpoint level is usually performed through the use of characterization factors (CF) composed of an atmospheric fate factor (FF) and an exposure factor (XF). FF represents the source-

receptor relationship, i.e. it considers the location of pollutants emissions and depositions in addition to environmental conditions and processes involved in atmospheric transport. XF addresses the properties and the sensitivity of the receiving soil to the impact characterization. The inclusion of an effect factor (EF) in this combination allows the endpoint characterization, i.e. the impact assessment at a damage level. Equation 2.1 shows the general framework of a characterization factor (Udo de Haes et al., 2002).

$$CF = FF \times XF \times EF \quad (\text{Equation 2.1})$$

The EF represents the damages of anthropogenic interferences on ecosystems and in most of the LCIA methods for terrestrial acidification it is expressed through the occurrences of plant species (**Article III**: Crespo-Mendes et al., 2018).

The characterization model developed by Roy et al. (2014) represents the state-of-the-art model for terrestrial acidification and it is adopted as a reference by the three most recently developed LCIA methodologies: LC-Impact (2017), Impact World+ (2017) and ReCiPe 2016 (Huijbregts et al., 2017). In that model, FF, XF and EF were combined to provide spatially differentiated CFs at a global scale. Table 2.2 presents an overview of the factors that compose a characterization model, according to Roy et al. (2014).

Table 2.2. Overview of factors that compose a characterization model for terrestrial acidification. Effect factor (EF) is highlighted in grey, as it is the main focus of the thesis.

Factor	Description	Unit	References
Atmospheric fate factor (FF)	Relationship between source and receptor location, considering the climatic conditions and deposition mechanisms.	$[k_{eq} \times \text{kg}_{emitted}^{-1}]$	Roy et al. (2012a) Roy et al. (2014)
Soil sensitivity factor (XF)	Changes in soil properties due to depositions of acidifying substances, with H ⁺ concentration as a soil indicator.	$[\text{mol H}^+ \times \text{L}^{-1} \times \text{m}^2 \times k_{eq}^{-1} \times \text{yr}]$	Roy et al. (2012b) Roy et al. (2014)
Effect factor (EF)	Relationship between plant species richness and the H ⁺ concentration in the soil, considering pH as indicator of soil acidity	$[\text{PNOF} \times (\text{mol H}^+ \times \text{L}^{-1})^{-1}]$	Azevedo et al. (2013) Roy et al. (2014)
Characterization factor (CF)	Change in relative loss of terrestrial plants due to an emission change of acidifying substances (NO _x , NH ₃ and SO ₂) CF = FF x XF x EF	$[\text{PNOF} \times \text{m}^2 \times \text{yr} \times \text{kg}_{emitted}^{-1}]$	Roy et al. (2014)

In this context, considering the limitations and research gaps presented in Subchapter 1.5, the effect factors are the focus of this thesis. From the botanical inventory presented in Chapter 3, the relationships between plant species and soil pH are investigated in Chapter 4. Thus, the calculations and findings on the effect factors are described and discussed in Chapters 5 and 6, respectively.

3. Development of comprehensive georeferenced biodiversity inventories

3.1. Methodological framework

In addition to providing information on the composition of biodiversity, georeferenced inventories enable the definition of species distribution in certain regions. Moreover, when combined with data on physicochemical properties, for example, they may contribute to studies on species-typical behavior in different environments.

The proposed framework for the development of georeferenced inventories can be applied to species from different taxonomic groups and different regions of the world. The procedures described in this subchapter are divided into five steps and illustrated in Figure 3.1.

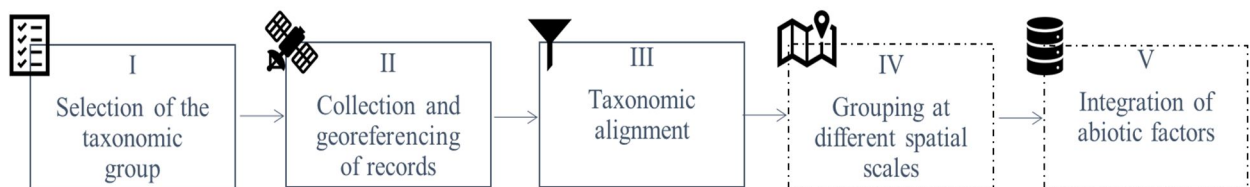


Figure 3.1. Conceptual framework for the development of georeferenced inventories. Dotted boxes indicate optional steps.

I. Selection of the taxonomic group:

The selection of a taxonomic group is done consistently with the scope of the study and with the taxonomic rank.

II. Collection and georeferencing of records:

Collected records must provide information on the location of species occurrences that can be aligned to a known geographic coordinate system (e.g. latitude and longitude). Data are processed by using Geographic Information Systems (GIS). The geographic coordinate system defined in this step is adopted as a reference during the following steps. This enables a consistent analysis of the records with other geographic data, reducing interferences and distortions.

III. Taxonomic alignment:

A taxonomic alignment is performed to ensure that only accepted names are listed in the inventory. It thus avoids the propagation of taxonomic errors and the inflated species count due to, e.g., the use of synonymous or misspelled names.

Taxonomic databases are used to support the alignment and querying a taxon name can result in different classifications (e.g. accepted, valid, synonymy, illegitimate, invalid and rejected names). Therefore, it is important to verify the definition of each taxonomic status according to the selected database before using the taxon name uncritically. A taxon name can be validly published but if it violates some other nomenclatural rule it will not be classified as accepted by the taxonomic sources.

During the verification of names, priority is given to the lowest taxon of interest. For example, if the study focuses on the species, the alignment considers both the genus and species names together and does not allow partial correspondences with the genus (which is a higher taxon) name only. If a match to the entire name cannot be found, that record is discarded.

IV. Grouping at different spatial scales (optional):

Data grouping at different spatial scales is accomplished through the use of maps delineated according to the chosen classification (e.g. biomes and hydrographic basins). This optional step may be used to evaluate the influence of spatial granularity through analysis of species richness variability.

V. Integration of abiotic factors (optional):

The integration of physicochemical aspects of the environment is achieved by editing the data on maps, processed in geographic information systems. The relationships between species richness and abiotic factors such as temperature and pH can contribute to the analysis of species interactions and ecosystem specificities.

3.2. Operationalization and data sources for Brazil

Following the steps described in Subchapter 3.1 a comprehensive and georeferenced botanical inventory for Brazil was built to support the research goals of this thesis. It is the main dataset for investigations on how plant species richness correlates with soil pH (Chapter 4) and may be used as indicator to evaluate the damage to terrestrial ecosystems in Life Cycle Impact Assessment (LCIA) (Chapter 5).

The inventory, which is based on the occurrence of plants in Brazil, presents a harmonized list of species names and it is spatially differentiated into biomes and ecoregions.

Procedures for the development of the inventory are presented below and summarized in Table 3.1. More details are found in **Article II**: Crespo-Mendes et al. (2018).

Table 3.1. Settings used to build the georeferenced botanical inventory for Brazil.

Step	Description / Sources
I. Selection of the taxonomic group	Species belonging to the kingdom <i>Plantae</i>
II. Collection and georeferencing of records	Records downloaded from Global Biodiversity Information Facility (GBIF.org, 2017) and processed using the software ArcGIS 10.3.1
III. Taxonomic alignment	Genus and species names evaluated together according to the classification provided by the Taxonomic Name Resolution Service v4.0 (Boyle et al., 2013)
IV. Grouping at different spatial scales	Biomes and ecoregions delineated by Olson et al. (2001)
V. Integration of abiotic factors	Soil pH data accessed through SoilGrids1km (Hengl et al., 2014)

I. Selection of the taxonomic group:

This study focuses on terrestrial plant species, therefore, kingdom *Plantae* is the selected taxonomic group.

II. Collection and georeferencing of records:

Georeferenced records of plant species were downloaded from Global Biodiversity Information Facility (GBIF.org, 2017) and processed using the software ArcGIS 10.3.1 (2017).

GBIF is an online platform that provides more than 37000 datasets of global biodiversity, published by over 1125 international institutions (GBIF.org, 2017). Besides being associated with the Brazilian biodiversity information system SiBBR (Sistema de Informação sobre a Biodiversidade Brasileira, 2017), GBIF gathers 1853 datasets with biodiversity data about Brazil (GBIF.org, 2017) and it is believed to be the data source with the largest number of digitized records freely accessible until the present moment.

Due to its global coverage in terms of participating countries and organizations, and species diversity, GBIF is a data source that can be widely used in biodiversity studies around the world.

III. Taxonomic alignment:

The Taxonomic Name Resolution Service v4.0 (TNRS, Boyle et al., 2013) was used to support the correction and harmonization of plant names. The configurations set and the list of taxonomic data sources used as reference in this step are presented in **Article II: Crespo-Mendes et al. (2018)**. TNRS is a specific tool for plant species and is therefore an option to extend this type of study to terrestrial plants in other parts of the world or to plants with other habitats than terrestrial (e.g. aquatic plants).

Genus and species names were evaluated together and according to the classification provided by the TNRS only names classified as 'accepted' and 'synonym' are valid names under the botanical code (Boyle et al., 2013). Based on this classification all 'accepted names' were maintained in the list, the 'synonyms' were replaced by their respective accepted names and names classified as 'illegitimate', 'invalid' or 'no opinion' were deleted (**Article II: Crespo-Mendes et al., 2018**).

IV. Grouping at different spatial scales (optional):

The biomes and ecoregions delineated by Olson et al. (2001) are the additional spatial scales adopted for this inventory. This is a widely-applied classification since it covers both biomes and ecoregions worldwide. It was thus selected to ensure compatibility with global data sets.

V. Integration of abiotic factors (optional):

Soil pH data was integrated to the inventory. The pH data stored in a raster format were collected through SoilGrids1km (Hengl et al., 2014) and processed using ArcGIS v.10.3.1. The pH values were extracted per 1km² grids cells within Brazil and matched with the georeferenced species occurrences. A soil pH value was thereby assigned to each species occurrence according to the grid in which the species is located. More details of how soil pH maps were generated are found in **Article II: Crespo-Mendes et al. (2018)**.

3.3. Data quality and uncertainties

Since this is the data set that supports the research goals of this PhD thesis and could directly influence the results presented in the following chapters, potential sources of uncertainties related to the development of this inventory are further discussed in **Article II**: Crespo-Mendes et al. (2018) and **Article III**: Crespo-Mendes et al. (2018). Among them are inaccurate georeferencing of plant occurrences, errors in taxonomic identification, classification and/or selection of plant species with terrestrial habitat and estimates of soil pH values. Due to the large number of species occurrence data recorded for the different biomes and ecoregions of Brazil, the first three sources of uncertainty are likely to be negligible (**Article II**: Crespo-Mendes et al., 2018). In addition, considering that this study uses the mean predicted pH values and the prediction interval used to propagate the uncertainties presents the same variation for all regions, it not expected that the uncertainties related to the statistical modeling of soil pH distribution influence the species richness curves (**Article III**: Crespo-Mendes et al., 2018).

3.4. Other possible applications

The georeferenced botanical inventory built on the basis of the Brazilian flora can support other studies in related areas such as ecology and environmental impact assessment.

In the same way that soil pH data were integrated to the inventory to explore its correlation with plant species richness, other environmental factors such as precipitation, soil organic carbon and nutrient deposition could be integrated to enable a more comprehensive assessment of possible patterns among the ecosystems. Relationships between species richness and levels of nitrogen or organic carbon in the soil, for instance, could be used as inputs to assess impacts related to eutrophication and land use, respectively.

3.5. Botanical inventory for Brazil

The framework developed in Subchapter 3.1 and operationalized in Subchapter 3.2 to botanical data in Brazil resulted in an inventory of terrestrial plant species in Brazil comprising 891313 occurrences of plants, representing 29712 different species of which 8242 (28%) are identified as range-restricted species (**Article II**: Crespo-Mendes et al., 2018). In this thesis, range-

restricted species is defined in a Brazilian perspective and correspond at ecoregion or biome level to species that are found to occur in only one of Brazil's ecoregions. Figure 3.2 presents the distribution of species and range-restricted species at the biome and ecoregion levels. Detailed information on the number of species, range-restricted species, area and number of collection spots per ecoregion, biome and for the whole country are available in **Article II: Crespo-Mendes et al. (2018)**.

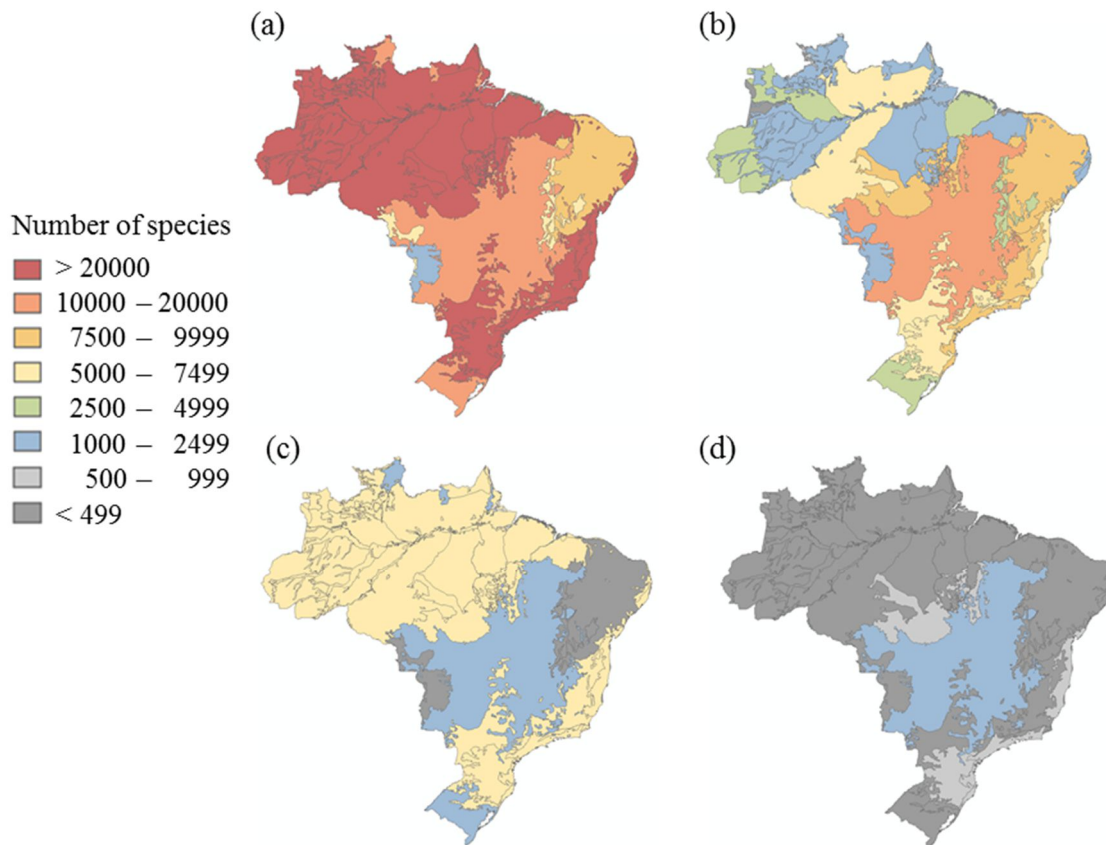


Figure 3.2. Terrestrial plant species in Brazil: (a) Total species per biome (six biomes), (b) Total species per ecoregion (47 ecoregions), (c) Range-restricted species per biome (six biomes) and (d) Range-restricted species per ecoregion (47 ecoregions). Retrieved from **Article II: Crespo-Mendes et al. (2018)**.

Considering that (i) many of the range-restricted species will be truly endemic to the ecoregion, i.e. they will not occur outside Brazil (**Article II: Crespo-Mendes et al., 2018**), (ii) endemism has already been combined with species richness as indicators (Kier and Barthlott, 2001; Kier et al., 2009; Crisp et al., 2001) and (iii) there is a weak relationship between species richness and range-restricted species richness (**Article II: Crespo-Mendes et al., 2018**), this thesis proposes the use of range-restricted species richness as a potential complementary indicator of

biodiversity. The implications of using species richness and range-restricted species richness to assess damage to terrestrial ecosystems are presented in the following chapters.

The complete list of Brazilian plant species used in this thesis are found in **Article II**: Crespo-Mendes et al. (2018) and in the digital media attached to the printed version of the thesis.

4. Plant species richness and soil pH

4.1. Correlation between species richness and soil pH

The relationships between plant species richness and soil pH are investigated through regression analyses. The species richness distributions, frequently obtained by using parametric fitting models (e.g. logistic and lognormal distribution models) (Guisan et al., 2002; Longino et al., 2002; Volkov et al., 2003; McGill et al., 2007; Azevedo et al., 2013; Colwell and Coddington, 1994), are determined as functions of the soil pH. Each species is assigned a pH range defined by the lowest and highest pH values at which it has been recorded. All the data are extracted from the botanical inventory presented in Chapter 3 and considering the high number of occurrences recorded, this pH range is assumed as being representative of species occurrences, even though a species has not been recorded at some of the intermediate pH values inside the interval or that the species may also exist unregistered at some value outside of this range (**Article II**: Crespo-Mendes et al., 2018). The analysis is performed at the ecoregion, biome and country level, for all species and for range-restricted species, separately. Lognormal distributions (see Equation 4.1) are selected as the preferred approach for this thesis since they show a slightly better fit (higher R^2) to the data than logistic distributions (**Article II**: Crespo-Mendes et al., 2018).

$$SR_{ij} = \frac{a}{C} \exp \left[-0.5 \left(\frac{\ln(C/x_0)}{b} \right)^2 \right] \quad (\text{Equation 4.1})$$

where SR_{ij} is the predicted value of species richness present at pH i in biome or ecoregion j , C is the soil concentration of H^+ (mol.L^{-1}) relative to the SR_{ij} and a , b and x_0 are regression parameters derived from the lognormal distribution model.

Data on species richness distributions are reported in **Article II**: Crespo-Mendes et al. (2018). Figure 4.1 shows the distribution of terrestrial plant species richness as a function of soil pH at whole country level and Table 4.1 presents corresponding statistical data for the whole country, biomes and ecoregions of Brazil. Results demonstrate statistically significant correlations – regardless of which spatial scale is used – for both total species richness distributions ($R^2 = 0.999$ at country level, R^2 above 0.955 for the six biomes and R^2 ranging 0.830-1.000 for 40

out of 45 ecoregions) and range-restricted species distributions ($R^2 = 0.982$ at country level, R^2 ranging 0.855-0.995 for five out six biomes and R^2 ranging 0.700-0.995 for 32 out of 41 ecoregions), the latter presenting slightly weaker correlations. Ecoregions with poor correlations (R^2 lower than 0.500 – see Table 4.1) have relatively small data sets, demonstrating the potential influence of unrepresentative data on the regression analysis and thereby on the species richness distributions (see discussion in **Article II**: Crespo-Mendes et al., 2018). The influence of data availability is discussed further in the next chapters.

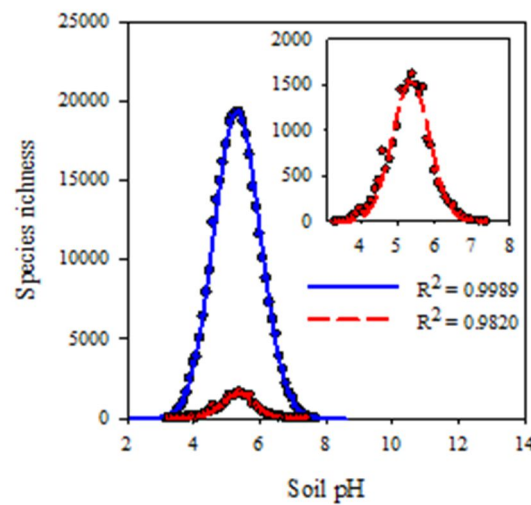


Figure 4.1. Distribution of terrestrial plant species richness (i.e. number of species in a given region) as a function of soil pH in Brazil, at the country level: the entire list of species (line, in blue) and only range-restricted species (dashes, in red). Insert shows distribution for range-restricted species in higher resolution. Dots represent the collected data. Data on distribution curves at biome and ecoregion levels are available in **Article II**: Crespo-Mendes et al. (2018). Corresponding statistical data are reported in Table 4.1. Retrieved from **Article II**: Crespo-Mendes et al. (2018).

Table 4.1. Relationships between plant species richness and soil pH: lognormal distribution model. Retrieved from **Article II:** Crespo-Mendes et al. (2018).

	R ²	Soil pH range	Optimum pH	Number of species at the optimum pH	R ² (range-restricted species)	Soil pH range (range-restricted species)	Optimum pH (range-restricted species)	Number of range-restricted species at the optimum pH
Brazil	0.997	2.9 - 9.6	5.2	19321	0.976	3.7 - 7.7	5.3	1535
Biome: Tropical and subtropical moist broadleaf forests								
Biome: Tropical and subtropical moist broadleaf forests	0.998	2.4 - 7.9	5.1	16399	0.971	3.2 - 7.0	5.1	927
Ecoregion:								
Alto Paraná Atlantic forests	0.984	4.1 - 6.9	5.5	3937	0.922	5.6 - 5.8	5.7	287
Araucaria moist forests	0.993	3.8 - 6.9	5.4	3619	0.978	4.2 - 6.5	5.3	180
Atlantic Coast restingas	0.935	3.0 - 7.5	5.2	339	0.699	5.2 - 5.3	5.2	4
Bahia coastal forests	0.994	3.3 - 7.0	5.1	4610	0.986	3.8 - 6.4	5.1	258
Bahia interior forests	0.994	3.8 - 7.4	5.6	4731	0.947	4.6 - 6.8	5.7	65
Caatinga Enclaves moist forests	0.927	4.4 - 5.9	5.2	333	0.798	4.9 - 5.5	5.1	3
Caqueta moist forests	---	---	---	---	---	---	---	---
Guianan Highlands moist forests	0.843	4.2 - 4.7	4.5	323	0.929	4.4 - 4.6	4.5	10
Guianan moist forests	0.787	3.1 - 4.8	3.9	542	0.932	3.9 - 4.1	4.0	59
Guianan piedmont and lowland moist forests	0.890	3.2 - 6.2	4.7	645	0.794	4.5 - 5.6	5.0	47
Gurupa varzeá	0.289	4.1 - 6.1	5.0	8	---	---	---	---
Iquitos varzeá	0.936	4.0 - 6.4	5.2	826	0.692	4.3 - 6.0	5.1	12
Japurá-Solimoes-Negro moist forests	0.937	2.4 - 7.2	4.8	1251	0.754	3.3 - 5.0	4.1	21
Juruá-Purus moist forests	0.856	3.1 - 6.2	4.7	459	0.709	3.7 - 5.7	4.5	5

Madeira-Tapajós moist forests	0.938	3.3 - 6.4	4.9	2784	0.759	4.0 - 5.5	4.8	62
Marajó varzeá	0.901	2.9 - 6.3	4.6	503	0.653	4.0 - 4.8	4.3	3
Maranhão Babaçu forests	0.986	3.7 - 6.7	5.2	846	0.863	4.5 - 6.0	5.3	10
Mato Grosso seasonal forests	0.859	3.4 - 6.5	5.0	3251	0.325	3.7 - 5.9	4.8	122
Monte Alegre varzeá	0.898	3.4 - 6.4	4.9	582	0.807	4.6 - 4.8	4.7	7
Negro-Branco moist forests	0.919	2.4 - 6.9	4.6	470	0.726	3.0 - 5.9	4.1	6
Northeastern Brazil restingas	0.498	4.3 - 7.2	5.7	50	---	---	---	---
Pantepui	0.539	3.6 - 5.7	4.6	15	0.204	4.3 - 5.5	4.4	1
Pernambuco coastal forests	0.974	3.9 - 6.1	5.0	816	0.313	4.5 - 5.7	4.8	2
Pernambuco interior forests	0.950	3.8 - 6.7	5.3	1190	0.694	4.9 - 5.5	5.2	4
Purus-Madeira moist forests	0.935	3.7 - 5.5	4.6	837	0.779	4.2 - 4.9	4.6	13
Purus varzeá	0.947	3.1 - 6.5	4.8	794	0.803	4.4 - 5.4	4.9	13
Rio Negro campinarana	0.830	2.7 - 7.1	4.9	328	0.367	3.1 - 6.4	4.5	6
Serra do Mar coastal forests	0.989	3.4 - 7.3	5.3	4719	0.952	3.8 - 6.8	5.3	155
Solimões-Japurá moist forests	0.561	3.2 - 5.0	4.1	101	---	---	---	---
Southwest Amazon moist forests	0.980	3.6 - 6.5	5.1	2031	0.961	4.0 - 6.1	5.0	112
Tapajós-Xingu moist forests	0.900	3.4 - 6.2	4.8	692	0.867	4.4 - 4.9	4.7	15
Tocantins/Pindare moist forests	0.967	3.2 - 5.9	4.6	1265	0.747	3.8 - 5.5	4.5	7
Uatuma-Trombetas moist forests	0.922	2.9 - 6.0	4.5	2662	0.889	3.8 - 4.9	4.4	136
Xingu-Tocantins-Araguaia moist forests	0.969	3.4 - 6.1	4.8	870	0.853	4.6 - 5.2	4.9	16

Biome: Tropical and subtropical dry

broadleaf forests	0.991	4.1 - 7.7	5.9	2.879	0.855	5.0 - 7.4	6.1	17
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Ecoregion:

Atlantic dry forests	0.978	4.3 - 7.4	5.8	2157	0.969	5.1 - 6.8	6.0	11
Chiquitano dry forests	0.849	3.6 - 8.3	6.0	648	0.783	6.0 - 7.0	6.5	16

Biome: Tropical and subtropical grasslands, savannas and shrublands	0.996	3.5 - 7.6	5.5	9764	0.990	4.3 - 6.7	5.5	823
Ecoregion:								
Campos Rupestres montane savanna	0.967	3.7 - 7.7	5.7	2358	0.741	4.4 - 6.9	5.6	26
Cerrado	0.995	3.7 - 7.3	5.5	7920	0.995	4.5 - 6.6	5.5	696
Dry Chaco	---	---	---	---	---	---	---	---
Guianan savanna	0.963	3.1 - 7.0	5.1	669	0.608	3.9 - 6.3	4.9	4
Humid Chaco	0.964	6.2 - 6.8	6.5	81	0.972	6.2 - 6.6	6.4	4
Uruguayan savanna	0.960	4.0 - 7.1	5.5	1519	0.913	4.3 - 6.9	5.6	112
Biome: Flooded grasslands and savannas	0.955	4.4 - 7.8	6.1	1038	0.859	5.4 - 7.1	6.2	13
Ecoregion:								
Pantanal	0.953	4.4 - 7.8	6.1	1035	0.853	5.4 - 7.1	6.2	13
Southern Cone Mesopotamian savanna	0.879	6.7 - 7.1	6.9	26	---	---	---	---
Biome: Deserts and xeric shrublands	0.998	3.7 - 8.3	6.0	4835	0.995	4.8 - 7.4	6.1	128
Ecoregion:								
Caatinga	0.998	3.7 - 8.3	6.0	4835	0.995	4.8 - 7.4	6.1	128
Biome: Mangroves	0.977	3.5 - 7.0	5.2	1184	0.583	5.3 - 5.9	5.6	8
Ecoregion:								
Amazon-Orinoco-Southern Caribbean mangroves	0.861	2.7 - 7.4	5.0	314	0.898	5.4 - 5.9	5.6	4
Southern Atlantic mangroves	0.979	3.8 - 6.7	5.3	963	0.439	4.3 - 6.1	5.0	3

4.2. Optimum pH (pH_{opt})

From the species richness distributions described in Subchapter 4.1 and from the information presented in Table 4.1 an optimum pH can be observed for each region, i.e., the pH value with the highest number of species richness within that region. Regardless of the spatial resolution chosen and disregarding other environmental factors that may co-vary with soil pH, for all species richness curves significantly correlated with soil pH, decreasing or increasing pH from optimum pH may be associated with a reduction in the number of species that occur (**Article II**: Crespo-Mendes et al., 2018).

In addition, **Article II**: Crespo-Mendes et al. (2018) shows different patterns across ecoregions within a given biome in terms of optimum pH and species richness distributions, leading to the conclusion that variations in species richness distribution and optimum pH values observed at the ecoregion level may no longer be observed at the biome level. Figure 4.2 illustrates the distribution of the optimum pH across biomes and ecoregions in Brazil. These findings corroborate the importance of spatial differentiation in environmental assessments, which is discussed further in Chapter 6.

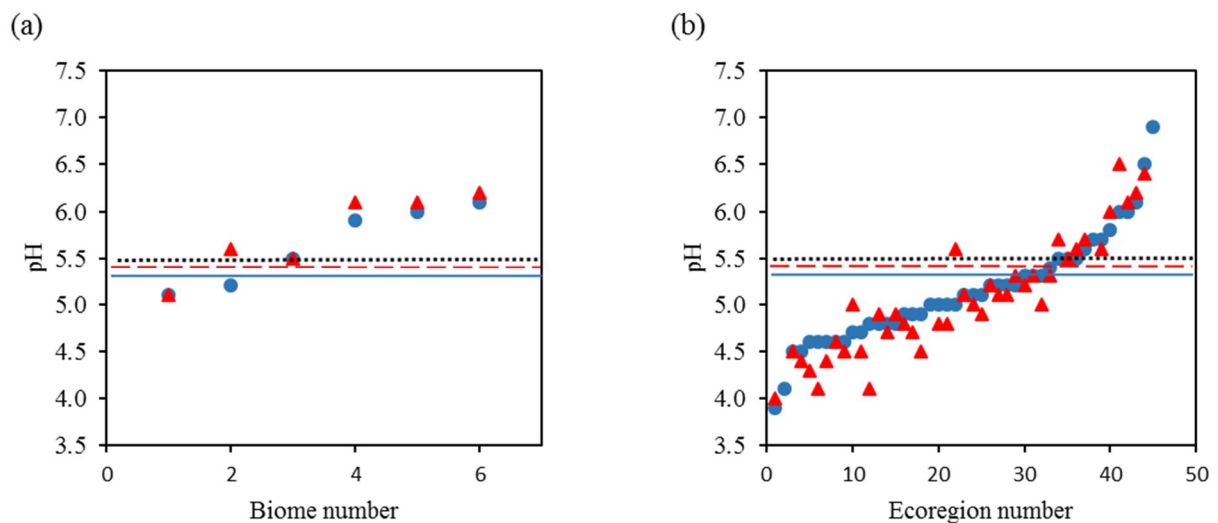


Figure 4.2. Optimum pH distribution at (a) biome level (total of 6 biomes) and (b) ecoregion level (total of 45 ecoregions). Circles (in blue) represent optimum pH for total species; Triangles (in red) represent optimum pH for range-restricted species; Thick line (in blue) represents optimum pH for total species in Brazil; and dashed line (in red) represents optimum pH for range-restricted species in Brazil. Dotted line (in black) represents a boundary between acidic and non-acidic soils, with $pH=5.5$ (Pärtel, 2002; Pärtel et al., 2004). Retrieved from **Article II**: Crespo-Mendes et al. (2018).

Considering a boundary between acidic and non-acidic soils at pH=5.5 (Pärtel, 2002; Pärtel et al., 2004), the optimum pH for the species richness distributions at the country level is acidic soil (< pH 5.5) for both total species and range-restricted species. At ecoregion level, the optimum pH tends to be the same or slightly lower for range-restricted species than for total species. In addition, ecoregions have their optimum pH nicely distributed around the national optimum while biomes tend to have their optimum pH above the national optimum. This shows a possible influence of the largest biome of Brazil ‘Tropical and subtropical moist broadleaf forests’ (with an area of 5213434 km² and 34 ecoregions) dominating the national average optimum pH while the other biomes and their respective ecoregions have less influence.

5. Effects of terrestrial acidification on plant species richness

5.1. Spatially differentiated effect factors

Effect factors (EF) proposed in this thesis were developed based on the state-of-the-art models for characterization of impacts related to terrestrial acidification. It thus ensures compatibility between the results for Brazilian ecosystems and the impacts modeled for other regions exposed to emissions from the product life cycle. Calculation procedures are described in detail in **Article III**: Crespo-Mendes et al. (2018) and summarized by the following three elements:

- I. Species richness distributions (Equation 4.1 – see Chapter 4)
- II. Potentially Not Occurring Fractions of species (Equation 5.1)
- III. Effect factor calculations (Equation 5.2)

Effect factors are meant to represent the effect on the indicator (species occurrence) from a change in soil pH. They are based on the species richness distributions as a function of the soil pH variation, i.e. changes in soil hydrogen ion concentration, and they are defined by the slope of the species richness distribution curve at the relevant soil pH value as described in the following. The species richness distributions presented in Chapter 4 are translated into modeled Potentially Not Occurring Fractions (PNOF) of species (Azevedo et al., 2013), which is a zero-to-one measure representing the presence or absence of species (Equation 5.1).

$$PNOF_{ij} = 1 - \frac{SR_{ij}}{SR_{\max j}} \quad (\text{Equation 5.1})$$

where the SR_{ij} is the predicted value of species richness present at pH i in biome or ecoregion j (see Subchapter 4.1) and $SR_{\max j}$ is the highest species richness occurring at any pH value in biome or ecoregion j .

Based on the PNOF as a function of the soil hydrogen ion concentration, effect factors are calculated as described by Equation 5.2.

$$EF_{ij} = \frac{dPNOF_{ij}}{dC} = \frac{(1 - PNOF_{ij})}{C} \left[1 + \frac{\ln(C/x_0)}{b^2} \right] \quad (\text{Equation 5.2})$$

where C is the soil concentration of H^+ (mol.L^{-1}) relative to the SR_{ij} and a , b and x_0 are regression parameters derived from the lognormal distribution model. Defined in this way, the effect factor represents the change in the fraction of species that could be present in the region but are not, as a function of a change in pH.

Different settings used to enable the evaluation of the calculated effect factors are presented and discussed in the following subchapters. Table 5.1 summarizes these settings through three scenarios. A first scenario reproduces the settings of previous studies and compares the influence of the data sets used for the effect factor calculations (Subchapter 5.2). A second scenario includes the methodological approaches proposed in this thesis that consider the bell shape of the species richness distribution curves (Subchapter 5.3). The third scenario presents a complementary metric on the range-restricted species, which may represent potential permanent damages to ecosystems (Subchapter 5.4). All calculated effect factors are presented in **Article III**: Crespo-Mendes et al. (2018).

Table 5.1. Settings for effect factors calculations. Adapted from **Article III**: Crespo-Mendes et al. (2018).

	Approach		Curve side	Regression	Spatial scale	Data set	Data source
Scenario 1	PNOF = 0.5	pH min.	Acid	Logistic	Biome	All species	Crespo-Mendes et al. (2018)
	PNOF = 0.5	pH min.	Acid	Logistic	Biome	All species	Azevedo et al. (2013)
	PNOF = 0.5	pH range	Acid	Logistic	Biome	All species	Crespo-Mendes et al. (2018)
	PNOF = 0.5	pH range	Acid	Logistic	Biome	All species	Azevedo et al. (2013)
Scenario 2	PNOF = 0.5	pH range	Acid / Alkaline	Lognormal	Biome / Ecoregion	All species	Crespo-Mendes et al. (2018)
	Area-weighted	pH range	Acid / Alkaline/ Entire curve	Lognormal	Biome / Ecoregion	All species	Crespo-Mendes et al. (2018)
Scenario 3	Area-weighted	pH range	Entire curve	Lognormal	Biome / Ecoregion	Range-restricted species	Crespo-Mendes et al. (2018)

5.2. Comparison with existing effect factors

A first scenario considers the settings presented in previous studies: Roy et al. (2014) and Azevedo et al. (2013). Both adopt logistic species richness distributions at the biome level based on the data reported by Azevedo et al. (2013). The main difference between the two studies is the way in which the species richness curve is determined as a function of soil pH values. Roy et al. (2014) adopt the lowest pH value where the species is recorded (identified in

this thesis as “pH min” approach) to count species, while Azevedo et al. (2013) consider the range between the lowest and the highest pH value at which the species is recorded (identified in this thesis as “pH range” approach).

Effect factors are then calculated based on the pH value – in terms of hydrogen ion concentrations in the soil – where 50% of plant species do not occur (PNOF = 0.5), which is the approach used by current models of terrestrial acidification (see discussion in **Article III**: Crespo-Mendes et al., 2018). Reproducing the calculation procedures adopted in previous studies allows analysis of the influence that the applied data sets have on the resulting effect factors, since the factors presented in this thesis are based on the data reported in **Article II**: Crespo-Mendes et al. (2018).

Table 5.2 highlights the much higher representation of actually occurring species offered by the data set reported in **Article II**: Crespo-Mendes et al. (2018) when comparing the number of species reported per biome between the studies. The number of species compared to the dataset in Azevedo et al. (2013) increase across all biomes with factors of 21-131.

Table 5.2. Overview of plant species richness data addressed in each study. Retrieved from **Article III**: Crespo-Mendes et al. (2018).

Biome	Roy et al. (2014) and Azevedo et al. (2013) (data extracted from Azevedo et al. (2013))		This study (data extracted from Article II: Crespo-Mendes et al. (2018))	
	Number of species	Number of species at the optimum pH	Number of species	Number of species at the optimum pH
Tropical and subtropical moist broadleaf forests	533	358	25774	16399
Tropical and subtropical dry broadleaf forests	139	65	5656	2879
Tropical and subtropical grasslands, savannas and shrublands	131	107	16172	9764
Flooded grasslands and savannas	18	18	1965	1038
Deserts and xeric shrublands	350	293	7505	4835
Mangroves	25	25	3268	1184

^a Data extracted from Azevedo et al. (2013) represent the number of species per biomes distributed throughout the world, whereas data extracted from **Article II**: Crespo-Mendes et al. (2018) represent the number of species per biomes within Brazil.

Calculated effect factors are presented in Table 5.3 and in **Article III**: Crespo-Mendes et al. (2018), where the differences are illustrated by the ratios between the effect factors: This

study/Roy et al. (2014): ranging 0.06-75.13; This study/Azevedo et al. (2013): ranging 0.07-9.27. These high differences result from the use of a much more comprehensive data set (Table 5.2). In this chapter it is therefore demonstrated that besides compromising the species richness distribution curves (as verified in Chapter 4) the strongly reduced representativeness of the applied data sets might also lead to unrepresentative values of effect factors (see also Subchapter 6.1).

Table 5.3. Effect factors at PNOF=0.5 [PNOF.(mol H⁺. L⁻¹)⁻¹]: Comparison with previous approaches at the biome level. Retrieved from **Article III**: Crespo-Mendes et al. (2018).

Biome	(pH min)			(pH range)		
	This study	Roy et al. (2014)	This study/ Roy et al., (2014)	This study	Azevedo et al. (2013)	This study/ Azevedo et al. (2013)
	[PNOF.(mol H ⁺ . L ⁻¹) ⁻¹]	[PNOF.(mol H ⁺ . L ⁻¹) ⁻¹]		[PNOF.(mol H ⁺ . L ⁻¹) ⁻¹]	[PNOF.(mol H ⁺ . L ⁻¹) ⁻¹]	
Tropical and subtropical moist broadleaf forests	1.43E+04	2.00E+03	7.16	1.12E+04	2.14E+03	5.23
Tropical and subtropical dry broadleaf forests	1.61E+05	2.14E+03	75.13	1.72E+05	-	-
Tropical and subtropical grasslands, savannas and shrublands	6.62E+04	8.33E+04	0.80	6.25E+04	2.41E+04	2.60
Flooded grasslands and savannas	2.37E+05	2.45E+06	0.10	2.56E+05	6.72E+04	3.80
Deserts and xeric shrublands	1.39E+05	2.31E+06	0.06	1.18E+05	1.74E+06	0.07
Mangroves	3.61E+04	5.03E+03	7.18	2.11E+04	2.28E+03	9.27

5.3. Area-weighted effect factors

A second set of settings is based on the species richness distributions determined in Chapter 4, i.e. lognormal distributions at the country, biome and ecoregion levels, based on the pH range approach and data reported by **Article II**: Crespo-Mendes et al. (2018). This scenario highlights two main analyses that have not yet been attempted in any previous studies found by the author:

- (i) Analysis of the entire curve, adopting the optimum pH as a boundary between acid (pH < pH_{opt}) and alkaline (pH > pH_{opt}) sides. As shown in Subchapter 4.2, optimum pH refers to the pH value at which the highest species richness occurs within a region. This scenario thus enables the assessment of potential effects on species

richness by increasing soil pH towards the optimum pH from the alkaline side of the curve.

- (ii) Calculation of effect factors weighted by the area of land that each pH unit represents in the studied region (area-weighted effect factors, EF_{aw} – see Equation 5.3). The reported values represent the average effect factors of each pH unit for the acid side and alkaline side, separately, and for the whole curve. These factors reflect the contribution of each pH value in terms of land area within the studied region.

$$EF_{aw} = \sum \frac{EF_{ij} \cdot A_{ij}}{A_{totj}} \quad (\text{Equation 5.3})$$

where EF_{ij} and A_{ij} are, respectively, the EF and the area (km^2) for each pH unit i in biome or ecoregion j , and A_{totj} is the total area (km^2) of biome or ecoregion j .

Table 5.4 shows the proposed effect factors per biome and for total Brazil. The effect factors based on $\text{PNOF}=0.5$ are also provided for comparisons between the different approaches. All calculated factors, including effect factors at ecoregion level, are available in **Article III**: Crespo-Mendes et al. (2018).

Table 5.4. Effect factors [$\text{PNOF} \cdot (\text{mol H}^+ \cdot \text{L}^{-1})^{-1}$] for terrestrial acidification per biome and for total Brazil. Retrieved from **Article III**: Crespo-Mendes et al. (2018).

Biome	EF [$\text{PNOF} \cdot (\text{mol H}^+ \cdot \text{L}^{-1})^{-1}$]		EF _{aw} [$\text{PNOF} \cdot (\text{mol H}^+ \cdot \text{L}^{-1})^{-1}$]			EF _{aw-rr} [$\text{PNOF} \cdot (\text{mol H}^+ \cdot \text{L}^{-1})^{-1}$]
	(PNOF=0.5)		(area-weighted)			(range-restricted)
	Acid side	Alkaline side	Acid side	Alkaline side	Entire curve	Entire curve
Tropical and subtropical moist broadleaf forests	1.12E+04	-3.05E+05	9.06E+03	-3.04E+04	-2.13E+04	-2.61E+04
Tropical and subtropical dry broadleaf forests	1.32E+05	-1.48E+06	7.14E+04	-3.64E+05	-2.93E+05	-1.07E+05
Tropical and subtropical grasslands, savannas and shrublands	5.29E+04	-6.84E+05	3.20E+04	-1.42E+05	-1.10E+05	-1.23E+05
Flooded grasslands and savannas	2.18E+05	-2.41E+06	1.22E+05	-6.19E+05	-4.98E+05	-5.03E+05
Deserts and xeric shrublands	1.11E+05	-2.17E+06	4.61E+04	-7.74E+05	-7.28E+05	-7.62E+05
Mangroves	2.71E+04	-3.25E+05	8.58E+03	-9.79E+04	-8.94E+04	1.88E+03
Brazil	1.14E+04	-5.04E+05	8.11E+03	-1.02E+05	-9.37E+04	-8.89E+04

Decreasing soil pH may not necessarily be associated with damages to the environment expressed as loss of species richness, since adding acidity to a soil on the alkaline side of the curve will be associated with an increase in species richness as the pH approaches the optimum pH – which is shown by the negative values for effect factors in Table 5.4 and in **Article III**: Crespo-Mendes et al. (2018).

The key point of this subchapter refers to the methodological choices for the effect factor calculations. In the current models for terrestrial acidification, hydrogen ions that cause soil acidification are treated in the same way as the toxic agents addressed in ecotoxicological effect models (Pennington et al., 2004; Larsen and Hauschild, 2007). Marginal effect factors based on the concentration of hydrogen ion in which PNOF = 0.5 are calculated exclusively for the acid side of the species richness curve and does not consider its bell shape nor the areas with soil pH higher than the optimum pH, thus ignoring that deposition of acidity in a region may just as well occur to soils with pH value above the optimum pH as to soils with pH below the optimum pH. By focusing on the entire curve, adding acidity may thus be associated with either higher or lower species diversity and it is the probability of both occurring that is intended to be represented by the proposed area-weighted average effect factor approach in this thesis (see also Subchapter 6.3).

5.4. Potentially Extinct Fraction (PXF) of species

Proposed as a potential complementary indicator of biodiversity (see Subchapter 3.4), the range-restricted species richness is integrated into the effect factors through the use of the Potentially Extinct Fraction (PXF) of species. The concept and approach is the same as for all species but just concentrated on the population of range-restricted species, i.e. species only occurring in one ecoregion in Brazil. Range-restricted species richness distributions presented in Chapter 4 are translated into modeled PXF following the procedure adopted for the PNOF calculation (see Subchapter 5.1). PXF is therefore a zero-to-one measure representing the presence or absence of range-restricted species, where $PXF=0$ corresponds to the optimum pH for range-restricted species (pH_{opt-rr}) and indicates that the highest number of range-restricted species occur. Figure 5.1 shows two distinct patterns observed in **Article III**: Crespo-Mendes et al. (2018) when the PNOF and PXF curves and their respective optimum pH ($pH_{opt-tot}$ and pH_{opt-rr}) are compared.

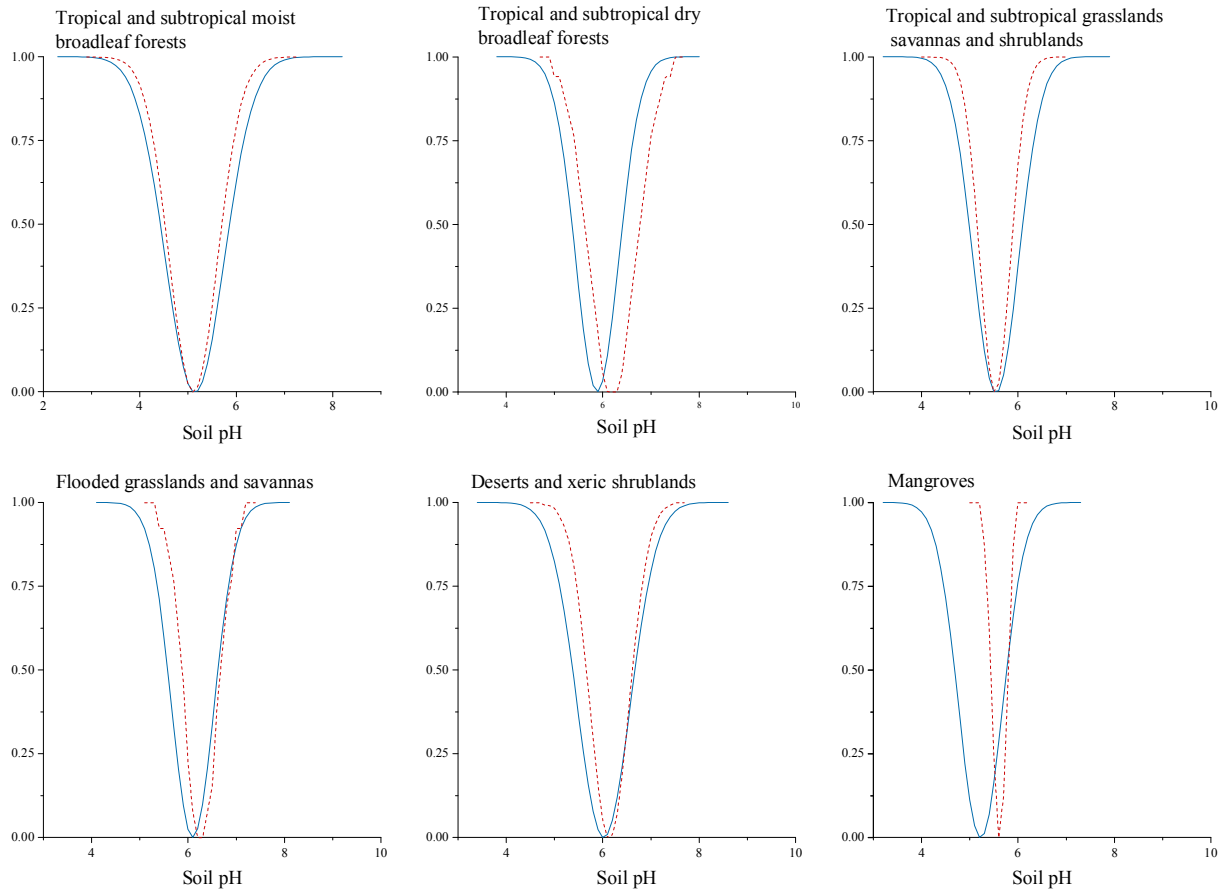


Figure 5.1. Potentially not occurring fractions (PNOF) of species (thick curve in blue) and Potentially Extinct Fraction (PXF) of species (dotted curve in red) per biome (6) in Brazil. Retrieved from **Article III:** Crespo-Mendes et al. (2018).

- (i) The PXF curve is narrower than the PNOF curve while the same optimum pH value is observed for all species and for range-restricted species ($\text{pH}_{\text{opt-tot}} = \text{pH}_{\text{opt-rr}}$): a decrease of range-restricted species associated with the acidity deposited in the soil is mirrored by a decrease of total species in the same region.
- (ii) The PXF curve is displaced relative to the PNOF curve ($\text{pH}_{\text{opt-tot}} \neq \text{pH}_{\text{opt-rr}}$): a decrease of range-restricted species associated with acidity deposited in the soil is not necessarily mirrored by a decrease of total species in the same region. This behavior is observed for some biomes and most ecoregions.

Since PNOF is thus often not a good proxy for PXF and the conservation of the largest number of species in a region does not guarantee the conservation of the largest number of range-restricted species (**Article III:** Crespo-Mendes et al., 2018), the PNOF is replaced by the PXF

in a third scenario, and area-weighted effect factors are calculated for the entire curve, at country, biome and ecoregion levels. Table 5.4 shows the area-weighted effect factors based on the range-restricted species richness curves (EF_{aw-rr}) at country and biome levels. Effect factors at ecoregion level are available in **Article III**: Crespo-Mendes et al. (2018).

PNOF and PXF are metrics applied for species preservation with different objectives: while the use of PNOF focuses on conserving the largest number of species in a region, the use of PXF focuses on conserving unique species of a region, preventing permanent damage to the ecosystem. The complementary use of these two metrics is therefore recommended to assess the effects of terrestrial acidification on biodiversity.

5.5. Outcome

LCIA effect factors to assess impacts related to terrestrial acidification are provided for six biomes and 45 ecoregions in Brazil. Different scenarios are presented in this chapter to highlight (i) the relevance of using a more comprehensive data set to determine the species richness distributions and consequently the effect factors (scenario 1, Subchapter 5.2); (ii) the influence of methodological choices for calculating the effect factors (scenario 2, Subchapter 5.3); and (iii) the need to include a complementary metric for critical species, since the loss of these species represents potential permanent damage to ecosystems (scenario 3, Subchapter 5.4).

Area-weighted effect factors, which consider the complete distribution of species richness, and the new metric Potentially Extinct Fraction (PXF) of species, which focuses on critical species to the ecosystems, are recommended for the integration within LCIA methodologies for terrestrial acidification. Additionally, more consistent spatially-differentiated effect factors are provided based on existing LCIA model approaches.

All calculated effect factors are available in **Article III**: Crespo-Mendes et al. (2018) at the country, biome and ecoregion level. For the ecoregions where effect factors could not be calculated (data not shown for their regression model (**Article II**: Crespo-Mendes et al., 2018)), it is recommended as default to use the effect factors of the biome to which they belong.

6. Implications for LCIA methodologies

6.1. Influence of data availability

The influence of data availability is demonstrated over Chapters 4 and 5. For species richness distributions (Chapter 4) data availability might significantly influence regression analyses when (i) the species occurrences of a certain region are defined from a small set of data points resulting in curves with several peaks, since the number of records may not be representative (e.g. for delimiting the pH range to be used for acidification; see Subchapter 5.2), and (ii) a region cannot be described by the regression model due to lack of data (**Article II**: Crespo-Mendes et al., 2018). This influence propagates to the effects factors (Chapter 5), since their calculations rely on the species richness distributions curves, thus yielding potentially-representative effect factors.

Providing comprehensive and representative inventories is a step forward to minimize influences in that regard. Furthermore, based on the botanical inventory presented in this thesis and considering the non-balanced geographic distribution of data collection in Brazil (**Article II**: Crespo-Mendes et al., 2018), it is possible to identify areas that need to be better explored in terms of data collection and recording. Benefits of high data availability are also demonstrated with respect to the spatial differentiation of the models, since it enables to capture variations in smaller spatial scales (see discussion in Subchapter 6.2).

6.2. Influence of spatial granularity

Spatial differentiation is meaningful when it is possible to combine fine spatial resolutions and highly representative data. Biomes and ecoregions are selected as the spatial scales for this study since they represent a set of terrestrial ecosystems delimited geographically according to environmental similarities and the data used in this thesis are representative for most of these regions in Brazil. According to **Article II**: Crespo-Mendes et al. (2018), identifying species richness using fine GIS grid cell resolutions, for example, could bring benefits such as eliminating interference related to species recorded at the boundaries. However, it would increase the uncertainties related to data availability, besides representing an arbitrary choice for the subsequent aggregation of data.

The different patterns across ecoregions within a given biome in terms of optimum pH and species richness distributions presented in Subchapter 4.2 highlight that the variations observed at the ecoregion level may no longer be observed at the biome level. These variations are reflected by the range of ecoregions' effect factors within the biome in which they belong (Figure 6.1 illustrates that point).

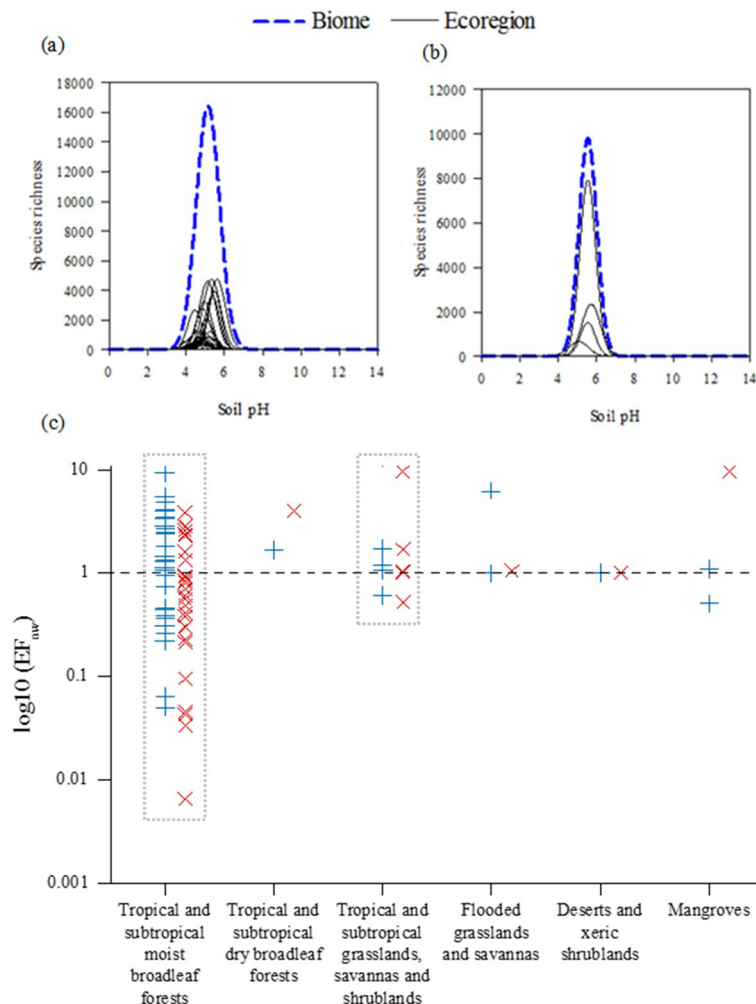


Figure 6.1. Grouping of ecoregions within each biome: (a) Species richness distribution for Tropical and subtropical moist broadleaf forests (incl. 34 ecoregions), (b) Species richness distribution for Tropical and subtropical grasslands, savannas and shrublands (incl. six ecoregions) and (c) Area-weighted effect factors (EF_{aw}), with emphasis (dotted boxes) on the regions of (a) and (b). The EF_{aw} for ecoregions are normalized against the EF_{aw} of the biome to which they belong, for all species [$PNOF \cdot (mol\ H^+ \cdot L^{-1})^{-1}$] (cross (+), in blue) and for range-restricted species [$PNOF \cdot (mol\ H^+ \cdot L^{-1})^{-1}$] (cross (x), in red). Dotted line represent the EF_{aw} at biome level. Adapted from **Article II**: Crespo-Mendes et al. (2018) and **Article III**: Crespo-Mendes et al. (2018).

It is important to note that the level of spatial differentiation of a characterization model also relies on the other elements that compose it. It needs to match the spatial resolution that is relevant for the inventory data, i.e. the typical size of the deposition area for emission from a source in the product system. Thus, considering the spatial resolution of the other factors that compose the characterization model (especially the atmospheric fate factor since it considers the location of emissions and depositions causing the impacts) and the dimensions of the Brazilian ecoregion areas, a recommendation for terrestrial acidification models is that the effect factors at ecoregion level should be preferred (see discussion in **Article III**: Crespo-Mendes et al., 2018). Using these factors can reflect variabilities that may be masked at biome level.

Although no consistent pattern is found in the distribution of effect factors of ecoregions within the biome to which they belong, the biome effect factors represent the area-weighted average of the underlying ecoregion effect factors quite well (see discussion in **Article III**: Crespo-Mendes et al., 2018). Biomes effect factors are then recommended as default when the ecoregion effect factors are not available.

6.3. Metrics for assessment of terrestrial ecosystem damage

The metrics currently used to assess environmental impacts to terrestrial ecosystems at a damage level (endpoint) are the key points highlighted in this thesis to be discussed within the LCA community.

Previous studies have adopted a similar approach to what is used for ecotoxicological impact models, treating acidity as a toxicant and focusing exclusively on the acid side of species richness and PNOF curves (Azevedo et al., 2013; Roy et al., 2014). In this thesis, the approach has relied on the assessment of the entire species richness curve given that acidic compounds may just as well deposit in soils on the alkaline side of the optimum pH as on soils on the acidic side. Thus, hydrogen ions that cause soil acidification are no longer treated as the toxic agents addressed in LCIA ecotoxicological models (Pennington et al., 2004; Larsen and Hauschild, 2007). Ecosystem behavior is thereby analyzed in terms of species loss from a broader perspective, considering the bell shape found for the species richness curves. The proposed approach gains relevance as the decrease in soil pH in Brazil can be associated with a decrease or increase in species richness, depending on the pH value of the analyzed soil in relation to the optimum pH of the region. This is reflected by the obtained negative effect factors and

suggests that soil acidification would not necessarily cause damage to the ecosystem, pointing to the inadequacy of biodiversity as a (sole) indicator of ecosystem quality (see discussion in **Article III**: Crespo-Mendes et al., 2018).

Despite being the most used indicator in current LCIA models, effects on species richness alone should be interpreted with caution. As shown in Subchapter 5.4 preserving the largest number of species guarantees high diversity but does not guarantee the preservation of the highest number of unique species to a region. In a country like Brazil, with high diversity of species and also high number of range-restricted species (considered in this thesis as a good proxy for endemic species), the loss of species besides representing local damages in diversity can also lead to permanent global damages, i.e. extinction of species. Range-restricted species richness is therefore a relevant complementary metric that contributes to the assessment of species vulnerability and could be integrated into LCIA characterization models.

Furthermore, neither the species richness and the associated PNOF curves nor the range-restricted species richness and its PXF curves consider the specificities and benefits of each species in the environment, i.e. the functional diversity. Complementary indicators linking species loss to key ecosystem functions have already been proposed for impacts on land use (Maia De Souza et al., 2013; Woods et al., 2017). However, further investigations are needed for its operationalization in LCIA models and species richness is still the most feasible indicator for assessing the effects on biodiversity and consequent damage to ecosystems.

6.4. Further research

LCIA methodologies are constantly being updated to better represent the environmental pathways of each impact category. For terrestrial acidification models, additional research is needed with regard to the causality between the occurrence or non-occurrence of species in the environment and changes in soil pH. The strong correlation observed between the two variables does not eliminate the possibility that other environmental factors also influence the conditions of species occurrence (**Article III**: Crespo-Mendes et al., 2018).

In addition, the implementation of the provided effects factors in characterization factors relies on the expansion of this work to regions other than Brazil. Global coverage and the combination of effect factors with fate factors and exposure factors enable emissions and impacts across borders to be effectively evaluated.

Besides possible adjustments to the definition of range-restricted species (e.g. identifying species present only in the transition areas between ecoregions, which are currently not counted as range-restricted according to the proposed definition), the comparison with lists of endemics and threatened species could also bring insights to the species conservation planning, since these indicators prioritize unique species and the risks of extinction, respectively (**Article II**: Crespo-Mendes et al., 2018).

In addition to the methodological elements for the integration of the PXF curves in the LCIA models (for terrestrial acidification and also for other impact categories), this thesis provides data that can support the development of vulnerability scores (VS) for terrestrial acidification, which is an approach that has been used in models for impacts related to land use (Chaudhary et al., 2015) and water consumption (Verones et al., 2017a).

7. Conclusions and outlook

Obtaining a comprehensive and representative dataset was the first methodological challenge overcome to reach the main outcomes of this thesis. A georeferenced inventory of terrestrial plant species is provided for Brazil and using these data the relationships between terrestrial plant species richness and soil pH was investigated in a large Tropical flora, at different geographical scales. Statistically-significant lognormal distributions were found for ecoregions, biomes and for entire Brazil. Similar distribution patterns were observed when limiting the study scope to range-restricted species (species only occurring in one ecoregion). Despite the strong correlation between plant species richness and soil pH for both scenarios (total species and range-restricted species), range-restricted species richness is poorly correlated with total species richness across all ecoregions in Brazil and, owing to its different focus, it is hence proposed as a complementary indicator of biodiversity. The metric of Potentially Extinct Fraction of species is therefore proposed as a new metric to consider species vulnerability through impacts on range-restricted species in LCIA models.

Based on the plant species richness distributions the Potentially Not Occurring Fraction of species and the Potentially Extinct Fraction of species were integrated into effect factors for terrestrial acidification. These factors, provided for the whole country, 6 biomes and 45 ecoregions of Brazil, represent the effects of changes in soil hydrogen ion concentration on terrestrial ecosystems in terms of species loss. Variations in the species richness distribution that are captured by effect factors at ecoregion level may not be observed at coarser resolutions. Hence the calculated effect factors at ecoregion level are recommended for integration into existing LCIA methodologies.

Along with the differentiation between the set of target species (total and range-restricted species) and spatial scales (ecoregion, biome and country levels), the area-weighted effect factors constitute another outcome of this thesis. These factors, which consider the complete species richness curves and the contribution of each pH value in terms of land area, bring significant contribution to improve currently models for terrestrial acidification in LCIA. As positive and negative values were found for the area-weighted effect factors, adding acidity to the soil could therefore be associated with an increase in species richness as pH approaches the optimum pH (in which the species richness is at its maximum) from the alkaline side of the curve, which questions the appropriateness of the metrics used for this impact category, since it implies that acidification of Brazilian ecoregions or biomes as a rule is associated with

increased species richness. Together, these outcomes successfully cover the research goals defined.

Overall, this thesis highlights the limitations of using species richness as the only indicator to assess terrestrial ecosystem damages caused by terrestrial acidification. Furthermore, the substantial data provided in this work may be useful for improving approaches used for other LCIA impact categories such as land use and water consumption, in addition to benefiting other ecological-related research, such as the evaluation of interactions between species and ecosystems and the analysis of potential patterns of biodiversity loss to support conservation policies.

8. Major achievements

- A. Geo-referenced inventory of 29712 terrestrial plants species was developed for Brazil.
- B. Relationships of species richness vs. soil pH were studied at three spatial scales: country, biome and ecoregion levels.
- C. Statistically-significant lognormal distributions with optimum pH were found, confirming a strong correlation between Brazilian plant species richness and soil pH.
- D. Effect factors (EF) were calculated for six biomes and 45 ecoregions in Brazil. These factors represent the effects of changes in soil hydrogen ion concentration on plant species richness. Different settings were used to enable the evaluation of the calculated EF for biomes and ecoregions and the comparison with existing factors.
- E. Area-weighted effect factors enable the assessment of potential effects on species richness by decreasing pH towards the optimum pH from the alkaline side of the curve, which is an extra element to existing models. They also reflect the contribution of each pH value in terms of land area within the studied region.
- F. Increasing or decreasing soil pH from the optimum pH value is correlated with loss of species diversity.
- G. Weak plant occurrence databases with low representativeness of the plants in a biome or ecoregion compromise the species richness distribution curves, which is reflected by the derived effect factors.
- H. Different patterns in terms of optimum pH and species richness distributions are observed across biomes and ecoregions.
- I. Different species richness metrics are compared for their use in biodiversity assessment. A weak correlation between species richness and range-restricted species richness (in Brazil) was found.
- J. Major contributions to the current state-of-the-art of LCIA indicators for terrestrial acidification reside in (i) providing effect factors (EF) for Brazil based on a uniquely comprehensive database, (ii) assessing effects of changes in soil hydrogen ion concentration along the entire curve of species richness distribution, identifying flaws in the existing effect factor calculations and questioning the validity of the use of species richness as an indicator of ecosystem damage, and (iii) introducing the Potentially Extinct Fraction (PXF) concept, which prioritizes the conservation of unique species from each region.

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Appendices

Article I: Crespo-Mendes, N., Laurent, A., Ometto, A. R., & Hauschild, M. Z. (2014). Necessidade de uma metodologia de Avaliação de Impacto do Ciclo de Vida espacialmente diferenciada para o Brasil. *IV Congresso Brasileiro em Gestão do Ciclo de Vida*, São Paulo, Brazil. Published, manuscript in post-print version.

Article II: Crespo-Mendes, N., Laurent, A., Bruun, H. H., & Hauschild, M. Z. Relationships between plant species richness and soil pH at the level of biome and ecoregion in Brazil. *Ecological Indicators*. Accepted (2018), manuscript in pre-print version.

Article III: Crespo-Mendes, N., Laurent, A., & Hauschild, M. Z. Effect factors of terrestrial acidification in Brazil for use in Life Cycle Impact Assessment. *The International Journal of Life Cycle Assessment*. Accepted (2018), manuscript in pre-print version.

List of attended conferences

The results of this PhD project were disseminated through participation in the following international conferences:

- Mendes, N. C., Laurent, A., Hauschild, M. Z. Effect factors for terrestrial acidification in Brazil. In: SETAC Europe 26th Annual Meeting, 2016, Nantes. Proceedings of the SETAC Europe 26th Annual Meeting, 2016.
- Mendes, N. C., Ometto, A. R., Laurent, A., Hauschild, M. Z. Fate factors for airborne contributions to acidification, eutrophication and photochemical ozone formation in Brazil. In: SETAC Europe 25th Annual Meeting, 2015, Barcelona. Proceedings of the SETAC Europe 25th Annual Meeting, 2015.
- Mendes, N. C., Laurent, A., Ometto, A. R., Hauschild, M. Z. Necessidade de uma metodologia de Avaliação de Impacto do Ciclo de Vida espacialmente diferenciada para o Brasil. In: IV Congresso Brasileiro sobre Gestão do Ciclo de Vida, 2014, São Bernardo do Campo. Anais do IV Congresso Brasileiro sobre Gestão pelo Ciclo de Vida, 2014.

Article I

Necessidade de uma metodologia de Avaliação de Impacto do Ciclo de Vida espacialmente diferenciada para o Brasil

Crespo-Mendes, N., Laurent, A., Ometto, A. R., & Hauschild, M. Z.

IV Congresso Brasileiro em Gestão do Ciclo de Vida

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IV CONGRESSO BRASILEIRO SOBRE GESTÃO PELO CICLO DE VIDA

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Necessidade de uma metodologia de Avaliação de Impacto do Ciclo de Vida espacialmente diferenciada para o Brasil

N. C. MENDES^{1*}, A. LAURENT¹, A. R. OMETTO² e M. Z. HAUSCHILD¹

¹ MAN-QSA – Technical University of Denmark

² SEP-EESC – Universidade de São Paulo

* Produktionstorvet, Building 424, 2800 Kongens Lyngby, Denmark

A seleção das categorias de impacto que serão abordadas em um estudo é a etapa inicial da Avaliação de Impacto do Ciclo de Vida (AICV). Assim, o principal objetivo deste trabalho é identificar as atividades antrópicas que são potencialmente causadoras dos impactos ambientais no Brasil e indicar quais são as categorias de impacto relevantes no contexto brasileiro. O procedimento metodológico utilizado foi a revisão bibliográfica das principais atividades antrópicas e seus potenciais impactos ambientais baseada em documentos publicados por órgãos nacionais e artigos científicos da área. Realizou-se também um levantamento bibliográfico dos estudos sobre desenvolvimento de fatores de caracterização espacialmente diferenciados a fim de identificar parâmetros que podem influenciar nos resultados da AICV. Os resultados obtidos permitiram verificar que todas as categorias de impacto podem ser relevantes para o contexto brasileiro. Foi possível, ainda, identificar parâmetros relevantes na aplicação de fatores de caracterização e como os impactos ambientais podem variar entre regiões, de acordo com os diferentes tipos de solo e clima, por exemplo. Desse modo, conclui-se que existe a necessidade do desenvolvimento de metodologias espacialmente diferenciadas que representem as características regionais do Brasil e forneçam resultados compatíveis com os dos modelos de caracterização usados nas outras regiões do mundo.

1. Introdução

A seleção das categorias de impacto, indicadores de categoria e modelos de caracterização é o primeiro elemento obrigatório da fase de Avaliação de Impacto do Ciclo de Vida. As categorias de impactos selecionadas devem ser consistentes com o objetivo e escopo da Avaliação do Ciclo de Vida, além de refletir as questões ambientais relacionadas ao sistema em estudo [1].

Neste contexto, este trabalho foi desenvolvido a fim de identificar como as atividades antropogênicas estão relacionadas com os impactos ambientais em uma determinada região. O principal objetivo deste estudo é identificar as atividades antrópicas que são potencialmente causadoras dos impactos ambientais no Brasil e, desse modo, indicar quais são as categorias de impacto que correspondem às preocupações ambientais no país.

Assim, com base nas categorias de impacto consideradas relevantes para o Brasil, pretende-se verificar se há a necessidade de desenvolver uma nova metodologia de AICV para melhor avaliá-las.

2. Métodos

De acordo com o objetivo deste trabalho os procedimentos metodológicos utilizados podem ser divididos em três etapas principais, sendo elas:



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2.1 Mapeamento das atividades antrópicas no Brasil

Realizou-se um levantamento bibliográfico dos produtos brasileiros de maior destaque frente ao crescimento econômico do país a fim de identificar as principais atividades antrópicas do Brasil. Para essa etapa foram consultados documentos publicados pelos Ministérios do Brasil, por outros órgãos nacionais e artigos científicos.

2.2 Identificação de categorias de impactos relevantes para o Brasil

As atividades antrópicas identificadas na etapa 2.1 foram relacionadas com as categorias de impacto comumente avaliadas em estudos de ACV, de acordo com os potenciais impactos que cada atividade pode causar. Em seguida, analisou-se a necessidade da diferenciação espacial para cada categoria de impacto abordada neste estudo.

Assim, os resultados obtidos, que utilizaram como base artigos científicos internacionais de estudos de ACV, foram sintetizados em uma tabela com os seguintes itens:

- Categorias de impacto
- Atividades antrópicas
- Exemplos da literatura
- Necessidade de diferenciação espacial

A partir dessa classificação foi possível analisar quais são as categorias de impacto relevantes para o Brasil.

Nessa etapa, o procedimento ideal seria quantificar a magnitude das atividades e estimar a magnitude dos impactos que cada atividade normalmente causa. Em seguida, ao relacionar os dois itens e usar referências de normalização globais, seria possível determinar quais impactos são os mais relevantes para serem abordados. No entanto, tal procedimento aparece como uma limitação do escopo atual do trabalho, sendo, assim, uma perspectiva futura para aperfeiçoamento do estudo.

2.3 Discussão

A discussão final foi estruturada sobre os resultados obtidos nas etapas anteriores 2.1 e 2.2. Adicionalmente, foi realizado um levantamento bibliográfico referente ao desenvolvimento de fatores de caracterização dependentes do local para diversas categorias de impacto. A seleção, não exaustiva, de estudos que abordam esse tema serviu para identificar parâmetros que podem influenciar nos resultados da ACV e também como suporte para a discussão no âmbito da necessidade da diferenciação espacial durante a fase de caracterização da AICV.

3. Resultados

O Brasil é considerado uma referência no agronegócio, sendo um dos principais produtores e fornecedores mundiais de alimentos. Os principais produtos produzidos para consumo interno e exportação são: café, açúcar, algodão, soja, milho, arroz, feijão, suco de laranja, carnes bovina e de frango. Adicionalmente, o Brasil lidera a agricultura de energia e o mercado de biocombustíveis, com destaque para o etanol extraído de cana de açúcar e o biodiesel produzido a partir de óleos vegetais ou gorduras animais [2].

Biocombustíveis e energia hidrelétrica são exemplos de fontes renováveis de energia que conferem um diferencial positivo a matriz energética brasileira. No entanto, o Brasil também é



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um dos maiores consumidores mundiais de petróleo e o inclui, juntamente com o gás natural, como uma fonte não renovável de energia [2].

Além da agricultura e pecuária, o Brasil conta ainda com um variado parque industrial de bens de consumo e tecnologia de ponta. Nesse contexto, as principais atividades antrópicas estão relacionadas às indústrias metalúrgicas, automobilísticas, químicas, cerâmicas, de aviões, vestuário e eletroeletrônicos [3]. Devem ser consideradas também as atividades antrópicas relacionadas à mineração, ao sistema de transporte brasileiro, que é predominantemente rodoviário, e à disposição de resíduos domiciliares.

Todas essas atividades são potencialmente causadoras de impactos ao meio ambiente. A tabela 01 apresenta as atividades antrópicas listadas anteriormente relacionando-as com as categorias de impacto comumente avaliadas em estudos de ACV, com base nos potenciais impactos que cada atividade pode causar.

Tabela 01: Relação entre as categorias de impacto da ACV e as atividades antrópicas do Brasil.

Categoria de impacto	Atividades antrópicas	Exemplos da literatura	Necessidade de diferenciação espacial
Mudanças climáticas	Produção de energia (combustíveis fósseis) Transporte rodoviário Agricultura Pecuária	[4, 5, 9, 10] [11]	Não
Depleção de ozônio	Indústria química		Não
Acidificação	Agricultura Transporte rodoviário Produção de energia (combustíveis fósseis)	[5, 8, 9]	Sim
Eutrofização	Agricultura Resíduos domiciliares	[8, 9]	Sim
Formação de ozônio fotoquímico	Transporte rodoviário		Sim
Ecotoxicidade	Mineração Resíduos domiciliares Indústria química		Sim
Toxicidade humana	Mineração Indústria química Transporte rodoviário		Sim



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Categoria de impacto	Atividades antrópicas	Exemplos da literatura	Necessidade de diferenciação espacial
Uso da terra	Agricultura Pecuária Produção de energia (hidrelétrica) Mineração Metalurgia	[6, 10] [11]	Sim
Uso da água	Agricultura Mineração Indústria química Resíduos domiciliares Indústria de vestuário	[7, 8, 9, 10]	Sim
Depleção de recursos	Produção de energia (combustíveis fósseis) Mineração Metalurgia Indústria automobilística, química, cerâmica, de aviões, vestuário e eletroeletrônicos		Sim

É possível verificar que as atividades antrópicas realizadas no Brasil estão relacionadas a diversos tipos de impactos ao meio ambiente, assim, todas as categorias de impacto da Tabela 01 podem ser consideradas relevantes no contexto nacional.

As categorias de impacto mudanças climáticas e depleção de ozônio são consideradas categorias globais por definição e dispõem de modelos de caracterização internacionalmente aceitos. Para as demais categorias, não globais, o ideal seria a aplicação de modelos de caracterização desenvolvidos para avaliação de impactos ambientais regionais.

O método EDIP 2003 foi um dos primeiros a apresentar diferenciação espacial para todas as categorias de impacto não globais, disponibilizando fatores de caracterização dependentes do local e referências de normalização para diferentes regiões da Europa [12].

O desenvolvimento de fatores de caracterização espacialmente diferenciados também se estendeu a outros continentes e a partir dos estudos publicados verifica-se que a diferenciação espacial tem se direcionado da escala continental para a escala nacional. Fatores de caracterização de diferentes categorias de impacto estão sendo desenvolvidos para a aplicação em países de grande extensão territorial como Argentina, Canadá e Estados Unidos, ou ainda a Suécia, que é um dos maiores países do continente europeu. A diferença significativa de resultados relatada nos estudos que comparam os fatores dependentes do local com fatores genéricos reforça a necessidade de se desenvolver metodologias que representem as características ambientais de cada região. Emissões atmosféricas, vegetação, clima, área territorial, densidade populacional e variações sazonais são alguns exemplos de parâmetros



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regionais que podem influenciar nos resultados obtidos em uma avaliação de impactos [13, 14, 15 e 16].

Nesse contexto, sendo o Brasil um país com extensa área territorial e grande diversidade ambiental, identifica-se a necessidade de desenvolvimento de novas metodologias para a caracterização de impactos que representem as características regionais do país. Além do desenvolvimento de referências de normalização, expressas em equivalentes de cidadão brasileiro, considerando a produção nacional do Brasil. Para isso, ressalta-se a importância da seleção de critérios para a divisão do território nacional, considerando as variações de clima, relevo, solo, fauna, flora e atividades humanas distintas de cada região brasileira.

4. Conclusões

A partir do estudo realizado conclui-se que todas as categorias de impacto comumente abordadas nos estudos de ACV podem ser consideradas relevantes para o Brasil. São elas: mudanças climáticas, depleção de ozônio, acidificação, eutrofização, formação de ozônio fotoquímico, ecotoxicidade e toxicidade humana, uso da terra, uso da água e depleção de recursos. Os resultados de uma avaliação de impacto podem variar de acordo com cada região e isso está relacionado com as diferentes características do meio ambiente. Assim, devido a sua grande extensão e biodiversidade, existe a necessidade de se desenvolver metodologias espacialmente diferenciadas que representem as características das diferentes regiões do Brasil. Deve-se destacar também que essas metodologias devem fornecer resultados consistentes com os modelos de caracterização usados em outras regiões do mundo, para que exista compatibilidade entre os resultados dos processos nacionais e os que ocorrem fora do país, já que raramente o ciclo de vida ocorre exclusivamente dentro do Brasil.

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Article II

Relationships between plant species richness and soil pH at the level of biome and ecoregion in Brazil

Crespo-Mendes, N., Laurent, A., Bruun, H. H., & Hauschild, M. Z.

Ecological Indicators

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Relationships between plant species richness and soil pH at the level of biome and ecoregion in Brazil

Natalia Crespo Mendes^{1*}, Alexis Laurent¹, Hans Henrik Bruun² and Michael Zwicky Hauschild¹

¹ Division for Quantitative Sustainability Assessment (QSA), Department of Management Engineering, Technical University of Denmark (DTU), 2800 Kgs. Lyngby, Denmark

² Department of Biology, University of Copenhagen (KU), 2100 Copenhagen Ø, Denmark

*To whom correspondence should be addressed; e-mail: cm.natalia@gmail.com

Abstract

Soil pH has been used to indicate how changes in soil acidity can influence species loss. The correlation between soil pH and plant species richness has mainly been studied in North America and Europe, while there is a lack of studies exploring Tropical floras. Here, our aim was therefore to investigate the relationships between terrestrial plant species richness and soil pH for the large Brazilian flora, with spatial differentiation into biomes and ecoregions. Data of plant species occurrences and soil pH in Brazil were compiled from public databases into a geo-referenced inventory of 29712 terrestrial plants species with a harmonized nomenclature. Based on the pH range, over which each species had been observed, the species richness for each unit of soil pH was determined and plotted as a function of pH for the 6 biomes and 47 ecoregions of Brazil. Lognormal distributions were found for entire Brazil ($R^2 = 0.999$), the six biomes ($R^2 > 0.955$) and for 40 out of 45 ecoregions, for which a sufficient number of observations was available (R^2 of 0.830-1.000). Similar distribution patterns were observed when limiting the study scope to range-restricted species, i.e. species only occurring in a single ecoregion in Brazil. Species richness is an indicator of plant biodiversity and we recommend a combined use of species richness for all species and for range-restricted species to address the overall status of the terrestrial plant ecosystem as well as the potential loss of unique species within it, including endemic species. We additionally propose that the developed inventory and the observed sensitivity distributions serve as basis for life cycle impact assessment of terrestrial acidification.

Keywords: Brazilian flora, Biodiversity conservation, Range-restricted species, Soil acidity, Ecoregion, Biome.

1. INTRODUCTION

Biodiversity loss is a worldwide concern and the central point of studies defining global conservation priorities (Myers et al., 2000; Orme et al., 2005; Brooks et al., 2006). Habitat loss and habitat change due to anthropogenic pressures are the prime drivers of biodiversity loss. To identify patterns of species loss in support of conservation policies, biodiversity indicator results can be analyzed together with physicochemical properties of the environment.

With regard to plants, the influences of soil acidity and availability of soil nutrients on plant species richness have received considerable attention in the scientific literature. The majority of studies have addressed North American and European regions (Gough et al., 2000; Roem and Berendse, 2000; Pärtel, 2002; Crawley et al., 2005; Duprè et al., 2010; Stevens et al., 2010), with few exceptions such as the study by Azevedo et al. (2013) focusing on different world biomes. Most studies found a correlation between soil pH and plant species richness, but they also indicated other possible drivers of change in the species richness, such as precipitation (Gentry, 1988), latitude (Duprè et al., 2010) and nitrogen deposition, which often accompanies airborne acidification (Duprè et al., 2010; Stevens et al., 2010). In this context, analyses considering physicochemical properties and biodiversity indicators together, such as soil pH and species richness respectively, contribute to identify specificities within ecosystems. In Pärtel (2002), for example, analyses considering where the pools of species are suited, whether for low or high pH soil, may relate to evolutionary history on local-scale diversity patterns. Additionally, Azevedo et al. (2013) use the relationships between species richness and soil pH to assess the potential effects that acidifying substances might cause in terrestrial ecosystems.

Furthermore, analyzing the relationships between species richness and soil pH within regions with common climate, vegetation, geology, etc. may help capture regional differences that have not been identified in studies covering more extensive territorial divisions. Ecoregions, which are biogeographic units containing a distinct assemblage of natural communities sharing a large proportion of species, dynamics, and environmental conditions (Olson et al., 2001), appear as a good choice for this type of investigation. Moreover, besides the concern with the loss of wide-ranging species, knowing what unique – or range-restricted - species of each ecoregion are can bring the

advantage of an analysis focused on the preservation of species that could potentially be extinct, preventing the loss of biodiversity.

Brazil is the country in the world hosting the largest floristic diversity, with more than 30000 species of higher plants recorded (Forzza et al., 2012; Brazil Flora G, 2015). However, there is a lack of studies exploring the relationships between Tropical plant species richness and soil pH. The Brazilian Flora Checklist (Brazil Flora G, 2015) provides information on the distribution of species into biomes and estimates the proportion of endemic species to Brazil (Zappi et al., 2015; Costa and Peralta, 2015; Prado et al., 2015). Nevertheless, information on species richness and range size of species, as well as the relationships between species richness and soil pH are not provided at the finer resolution of ecoregions. The most comprehensive study known to the authors is a global study that observed occurrences of 2409 plant species categorized in 13 terrestrial biomes across the world (Azevedo et al., 2013). The modest number of species covered at global scale, compared to the more than 30000 plant species reported for Brazil alone (Forzza et al., 2012; Brazil Flora G, 2015), renders the representativeness of this and other less ambitious studies questionable, pointing to the relevance of a more comprehensive analysis.

Rather than further analyze patterns of plant diversity in Brazil and the effects of human interactions on the ecosystems, in this study, we aim to 1) investigate the relationships between terrestrial plant species richness and soil pH in Brazil at the level of country, biome and ecoregion, using a large tropical flora, and 2) assess potential differences in these relationships between species, which are unique to single ecoregions, termed “range-restricted species” in the study, and those, which have extended ranges of occurrence.

2. METHODOLOGY

A large methodological challenge is to obtain sufficiently comprehensive and representative data to analyze the relationships between species richness and soil pH. To overcome this challenge, instead of retrieving data through a literature review, which is the approach used in previous studies (Pärtel, 2002; Azevedo et al., 2013), we have compiled information from separate databases of plant species occurrences and soil pH in Brazil, and analyzed the information using the Geographic Information System (GIS) software ArcGIS 10.3.1 (<https://www.arcgis.com>) and statistical tools. Details of the applied methodology are given in the following sections.

2.1. Occurrences of plant species in Brazil

An occurrence of a plant species refers to a recorded observation of the plant species at a specific location. Only records with information on the genus and species names of the plant, and the latitude and longitude of the observation site were used. The species richness of a region (ecoregion or biome) was defined as the number of different species observed in the region. The generated inventory presents an overview of the occurrence of species over the years and all occurrence data are treated the same, irrespective of the year of observation. Thus, ecological successions are not differentiated in this study, whether they are caused by natural forces or human interactions, such as forest fires or agricultural settlement. Such causality would however be worth investigating in further research work.

2.1.1. Data sources for species occurrence

Data for the Brazilian inventory were extracted from the Global Biodiversity Information Facility (GBIF, 2015). GBIF is integrated with the Brazilian platform (Sistema de Informação sobre a Biodiversidade Brasileira - SiBBr) and data quality is ensured by more than 90 endorsed data publishers for Brazil along with additional checks performed by GBIF (GBIF secretariat, 2017). However, the virtue of GBIF data is their exuberance, rather than the accuracy of the individual data entry (see Section 2.4). Collecting data from the GBIF database is believed to ensure that the greatest number of digitized records publicly available is considered in the present study. More details on the GBIF database are provided in Electronic Supplementary Material 1 (ESM-1), Supporting Methods A.

2.1.2. Development of terrestrial plant species inventory

In the extraction of species occurrence data, only records belonging to the kingdom *Plantae* were considered. ArcGIS 10.3.1 was used for processing the extracted data into a georeferenced inventory, and the World Geodetic System 1984 (WGS84) was adopted as the geographic coordinate system. The map of Brazil provided by the Brazilian Institute of Geography and Statistics (IBGE), which is responsible for statistical, geographic, cartographic, geodetic and environmental information in Brazil, was used as a reference for defining the Brazilian territory (IBGE, 2015).

To only use accepted names and avoid double counting of species, a taxonomic alignment was performed and supported by the use of the Taxonomic Name Resolution Service v4.0 (TNRS; Boyle et al., 2013). TNRS is an online application for automated standardization of plant scientific names

with reference to existing high-quality taxonomy sources (Boyle et al., 2013). The following taxonomic data sources were used in this study: Missouri Botanical Garden's Tropicos database (i.e. Tropicos, 2015), The Global Compositae Checklist (GCC; Flann, 2015), The Plant List (TPL, 2015) and The International Legume Database and Information Service (ILDIS, 2015). The list of species names was submitted to the TNRS and the configurations set for the analysis are presented in Supporting Methods. Inconsistent records were flagged from the run through TNRS tool and adjustments were performed – see details in the Supporting Methods B (ESM-1). The species habitat inventory list provided by the Brazilian Plant Checklist (Brazil Flora G, 2015) was also integrated into the inventory, enabling exclusion of non-terrestrial plant species.

2.1.3. Spatial resolution

Species in the resulting inventory of terrestrial plants in Brazil were grouped at different spatial resolutions. Several approaches exist for classification of biomes (Olson et al., 2001; Hoekstra et al., 2005; IBGE, 2004; Ellis and Ramankutty, 2008) and ecoregions in Brazil (Dinerstein et al., 1995; Olson and Dinerstein, 1998; Olson et al., 2001). The current study uses the widely-applied classification delineated by Olson et al. (2001) because it covers both biomes and ecoregions for the entire world and thus provides compatibility with global data sets that may be developed in the future. According to this classification the terrestrial world is subdivided into 14 biomes and 867 ecoregions to better reflect the distribution of the Earth's natural communities and species. The three spatial scales adopted in this study therefore are the ecoregion level (47 ecoregions), the biome level (six biomes) and the whole-Brazil level.

2.1.4. Range-restricted species

In this study, we adopted a Brazilian perspective and defined range-restricted species at the level of ecoregions, i.e. species only occurring in one of Brazil's ecoregions. Species that are range-restricted from this definition may still occur in other locations outside Brazil, but given the size of the country and the high species richness, this classification still provides useful information about the vulnerability to loss of the species. Therefore, the range-restricted species will also include the Brazilian endemic species (which do not occur anywhere else than in Brazil).

2.1.5. Correlation between total species richness and range-restricted species richness

Species richness for all species and for range-restricted species alone were considered as potential indicators of biodiversity in this study. The latter was tested as a biodiversity indicator by evaluating the fraction of range-restricted species (FRS) out of the total species in each of the ecoregions and biomes in Brazil, and by assessing variations across the regions.

2.2. Soil pH data

Data on soil pH were accessed through SoilGrids1km, which is an automated system for global soil mapping and is part of the Global Soil Information Facilities (GSIF), a platform developed by the International Soil Reference and Information Centre (ISRIC) for collating and predicting soil properties and soil classes in 3D at 1km resolution (Hengl et al., 2014). The soil pH maps were generated from point observations and covariation layers, and for Brazil, the Brazilian national soil profile database was used as the main source of points of observation (Hengl et al., 2014). The Brazilian national soil profile database consists of 5086 profiles with a total of 10034 soil horizons (i.e. distinct layers running parallel to the surface) with information on 31 variables covering soil morphological, physical and chemical attributes, one of them being the soil pH (Cooper et al., 2005). The collected data were processed using ArcGIS v.10.3.1. Brazil was divided into approximately 10.2 million grid cells of 1km x 1km and a soil pH value was extracted for each grid cell. Soil horizons representing a depth range of 0-60 cm were assumed relevant for plant exposure to acidifying or alkalizing substances. To obtain a representative pH value, an arithmetic mean of the average proton concentrations was calculated across this depth range for each grid cell. In the use of these pH data, inconsistencies present in the database were found and addressed. Details are provided in Supporting Methods C (ESM-1).

2.3. Processing of species richness

2.3.1. Species richness distribution

The georeferenced locations for occurrences of plant species (Section 2.1) were matched with the soil pH 1-km² grid cells (Section 2.2) to create an inventory of occurrences of plant species at different soil pH values within each of the ecoregions and biomes. From this information, each species could be attributed a range of soil pH, delimited by the lowest and highest pH values at which it has been reported within a given ecoregion or biome. A species may thus not necessarily have been recorded at all intermediary pH values within its range. It may also exist outside the defined pH range even

though it has not been recorded. It was however not possible to check the latter, and considering the very high number of observations reported for most ecoregions, it was assumed that the minimum and maximum pH values defining the range are representative of the occurrence of the species. Based on the observed ranges, the species richness distributions as functions of the soil pH were determined at the biome level and at the ecoregion level for both the entire list of species and the list of range-restricted species.

2.3.2. Correlation between species richness and soil pH

Regression analyses were performed to analyze the relationships between soil pH and species richness as determined in Section 2.3.1. Parametric fitting models such as logistic and lognormal distributions are commonly used for species richness distributions (Guisan et al., 2002; Longino et al., 2002; Volkov et al., 2003; McGill et al., 2007; Azevedo et al., 2013; Colwell and Coddington, 1994). Both logistic and lognormal distribution models were tested to identify the one that statistically provided the best fit.

2.4. Uncertainties

Four factors were identified as possible sources of uncertainty in this study: (i) inaccurate georeferencing of occurrences; (ii) errors in taxonomic identification; (iii) classification and/or selection of plant species with terrestrial habitat; and (iv) estimates of soil pH values.

Georeferencing errors in records used in databases such as GBIF can be associated with the lack of accuracy when digitizing the recorded samples from old collections, for which the geographical coordinates are not available or are not easily readable (Maldonado et al., 2015). Inaccurate georeferencing may lead to a species erroneously recorded in the neighboring ecoregion. For these cases, we assumed that the boundaries between ecoregions can change gradually due to the difficulty in limiting the transition zones between the different types of vegetation. Thus, even if a species is counted in the neighboring ecoregion, considering that the transition zones might have similar environmental characteristics and considering the high number of species occurrences for most ecoregions, overall the resulting error is very likely negligible.

Adopting the correct species name is often associated to cases of taxonomic disagreement regarding species delimitations, synonymisation and nomenclatural problems, making such an identification not trivial (Maldonado et al., 2015). The taxonomic alignment step was performed to minimize errors when selecting the accepted species name and its consistency relies on the set of high-quality

taxonomy sources (TPL, Tropicos, GCC and ILDIS). The authority of the names and the list of accepted names and synonyms may vary according to the order of consultation of the taxonomy sources. Given the large data set and the large number of species, this source of uncertainty is likely to be negligible.

Regarding the selection of terrestrial plant species, 25223 out of 33166 identified species have the habitat classification available and approximately 86% of these species have explicitly been associated with a terrestrial habitat classification (i.e. 21738 out of the 25223 species root in soil). The uncertainties come from the remaining species for which the habitat was not reported (7943 species out of 33166). Due to the high percentage of terrestrial species in a land-based environment as Brazil, it was assumed that all species with unknown habitat were terrestrial to avoid arbitrary choices on which species to be disregarded as a non-terrestrial among the set of species with unknown habitat. Species with known habitat that do not root in soil, such as aquatic, epiphytic, parasitic and saprophytic species, were disregarded as non-terrestrial species.

The uncertainties related to the estimates of soil pH values come from the statistical modelling of the distribution of soil pH values. The pH values are modelled based on extrapolation from a limited number of measured values. The predictions for each layer are based on the predicted value (mean) and the 90% prediction interval, which can be used to propagate uncertainties in models where soil property maps are used. In this study, only the mean value was used. The width of the prediction interval varies from 1.5 to 1.6 pH units for all regions and is not expected to impact the observed relationships between species richness and soil pH since there is the same probability of the correct value being higher or lower than the average. Specifically for the pH values the uncertainty about the data can also come from laboratory errors, but this is not considered as a source of uncertainty in the prediction models.

3. RESULTS AND DISCUSSION

3.1. Inventory of terrestrial plant species in Brazil

3.1.1. Inventory for entire Brazil

Disregarding habitat type, this study initially addressed 976345 occurrences of plants in Brazil, representing 33166 different species of Angiosperms, Bryophytes, Gymnosperms, Ferns and Lycophytes. For the same groups of plants, a total of 35639 species have been catalogued in the latest published update of the Brazilian List system (Zappi et al., 2015; Costa and Peralta, 2015; Prado et al., 2015), thus suggesting a high representativeness in the developed inventory. A comparison

between the content of the present dataset and the information provided by the Brazilian Flora Checklist (Brazil Flora G, 2014) shows that the Brazilian Flora Checklist has catalogued 97% of the occurrences of plants, which represent 79% of the reported species, addressed in the present inventory. The differences between the two lists may be explained by choices related to the taxonomic alignment, i.e. the authority given to each of the considered taxonomy sources and the different levels of updating (see Section 2.1.2).

After the habitat selection, the inventory of plant species in Brazil with terrestrial habitat comprises 891313 occurrences of plants covering 29712 species. Among these, a total of 8242 plant species were identified as range-restricted (see definition in Section 2.1.4), corresponding to 28% of the total number of plant species in the country. The inventory of terrestrial plant species at country, biome and ecoregion levels, with differentiation of range-restricted species, is given in the Electronic Supplementary Material 2 (ESM-2; Excel file). To summarize the data associated with the inventory, Table S1 in ESM-1 presents an overview of the species number for each biome and ecoregion of Brazil.

3.1.2. Biome level

The plant species counts for the six biomes in Brazil (Table S1, ESM-1) show that the biome Tropical and subtropical moist broadleaf forests is the most species-rich biome with 25774 species, representing 87% of the total number of terrestrial plant species registered in Brazil (see Figure 1a, in dark red). It is also the biome with the highest number of range-restricted species, with 5373 species, representing 65% of the total number of range-restricted species identified in this study (see Figure 1c). The high number of species present in this single biome is consistent with the literature (Costa and Peralta, 2015; Prado et al., 2015 and Zappi et al., 2015). This biome indeed covers the region of the Amazon forest – a known source of great biodiversity in the world located in the North Brazil – and the Southeastern Brazil that concentrates most of the species according to the Brazilian Flora Checklist (Brazil Flora G, 2014). While the Amazon Rainforest is reported to have the highest number of species for Gymnosperms, the Southeastern Brazil has been identified as hosting the highest number of species and endemic species for Angiosperms, Bryophytes, Ferns and Lycophytes (Costa and Peralta, 2015; Prado et al., 2015 and Zappi et al., 2015).

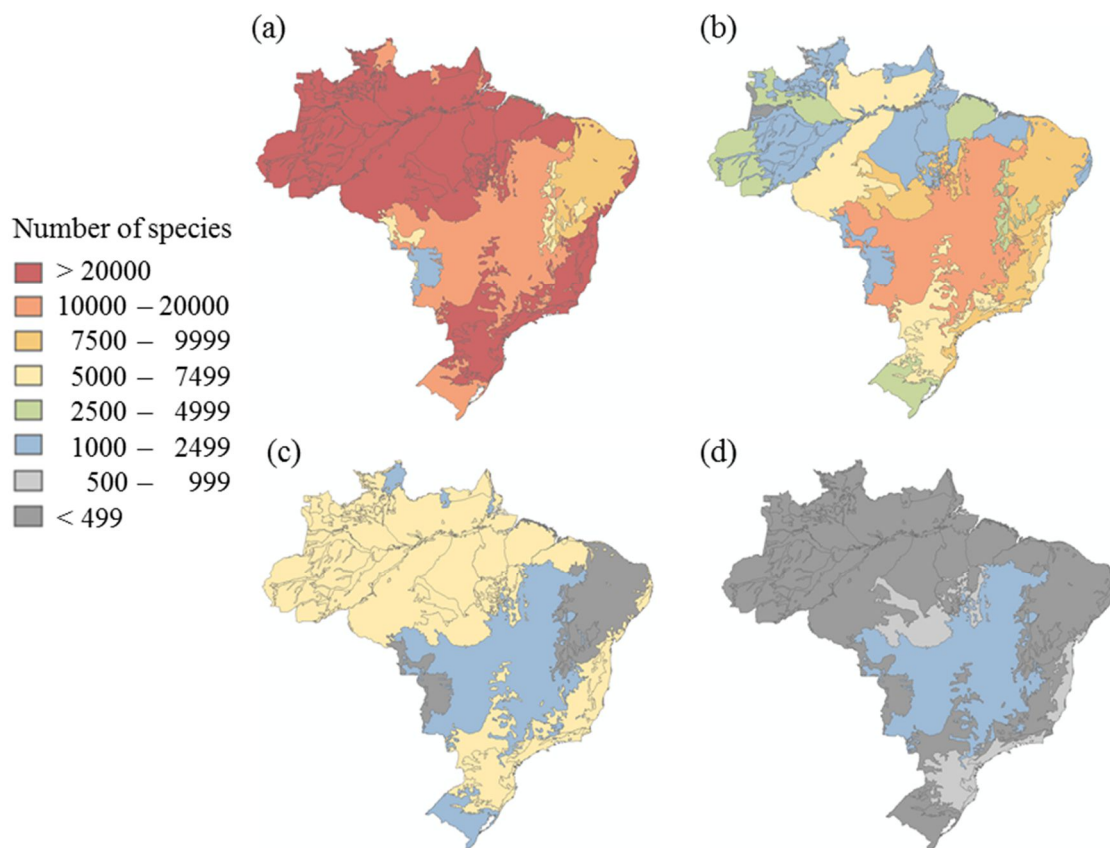


Figure 1. Terrestrial plant species in Brazil: (a) Total species per biome (six biomes), (b) Total species per ecoregion (47 ecoregions), (c) Range-restricted species per biome (six biomes) and (d) Range-restricted species per ecoregion (47 ecoregions).

3.1.3. Ecoregion level

At ecoregion level, the plant species counts indicate that out of the 47 ecoregions, the ecoregion Cerrado presents the highest number of terrestrial plant species in Brazil, with 12751 species, thus capturing ca. 43% of all terrestrial species in Brazil and 54% of the species within the biome Tropical and subtropical grasslands, savannas and shrublands, to which it belongs (see Figure 1b). Besides being the ecoregion with the highest number of species in Brazil, Cerrado presents the highest number of range-restricted species, with 1573 species corresponding to 19% of the total number of range-restricted species identified in Brazil (see Table S1, ESM-1). The detailed information for the remaining ecoregions can be found in Table S1 in ESM-1.

3.1.4. Influence of data collection distribution on the species count

The larger the area, the larger the number of collection spots, and the higher the number of species (species richness) in an ecoregion or biome (see Figures S1-S2 and Table S2, ESM-1). This apparent bias is reduced when the number of collection spots is expressed relative to the total area of the ecoregion or biome. The biome Tropical and subtropical moist broadleaf forests (Figure 1a, in red) is a notable example on how the non-balanced geographic distribution of data collection influences the count of species. Despite having the highest number of species and the highest number of collection spots, the biome Tropical and subtropical moist broadleaf forests presents only 0.01 spot/km² (based on the data given in Table S1, ESM-1). Most of the ecoregions, which compose this biome and are located in the North Brazil (Amazon rainforest), have a low number of collection spots per area (< 0.01 spots/km²), while the number is higher for ecoregions located on the coast or in the southeast of Brazil, as Serra do Mar coastal forests (0.12 spots/km²) and Bahia coastal forests (0.09 spots/km²). The comparison between the distribution of collection spots per biome and ecoregion shows that the concentration of collection spots is higher in the central and southeastern areas of Brazil (see Figure 2). This high concentration of collection spots is not necessarily related to high numbers of species, but rather to the high number of groups of experts and research institutions located in these regions, while access to the Amazon forest regions is limited. Thus, even if the Southeastern region is the largest contributor in numbers of recorded species for the biome Tropical and subtropical moist broadleaf forests, it is not possible to decide whether or not the Southeastern region actually has the highest species richness or whether the North region of Brazil has been underestimated due to limited access to new areas for collection of plant species.



Figure 2. Distribution of collection spots in Brazil (891313 occurrences of terrestrial plants covering 29712 species).

3.1.5. Relationships between species richness and range-restricted species richness

Endemism has already been combined with species richness to result in an endemic species richness indicator (Kier and Barthlott, 2001; Kier et al., 2009, Crisp et al., 2001). In this context the concepts of species richness and range-restricted species were combined to result in a biodiversity indicator called range-restricted species richness. The existence of a relationship between the total species richness and the range-restricted species richness was tested by using the fraction of range-restricted species (FRS) and the total species richness for all biomes and ecoregions. FRS is found to vary from 1 to 13% at ecoregion level (see Figure 3 and Table S1, ESM-1). For more than 50% of ecoregions – mainly the ones with small number of species – the FRS values range between 1 and 3%, and the results presented in Figure 3 suggest a weak correlation (correlation coefficients below 0.562 for Spearman rank-order and Pearson correlation tests, $P < 0.05$, see Table S3a, ESM-1) between the total number of species in a certain ecoregion and the number of range-restricted species.

The weak relationships between species richness and range-restricted species richness suggest that the use of a single biodiversity indicator must be considered with caution. Conserving an area of high biodiversity in terms of plant species richness does not guarantee that the largest number of range-restricted species will be conserved. Furthermore, despite the correlation between plant species richness and area shown in Section 3.1.4, even small areas with a low number of species may hold a high number of range-restricted species, which is likely attributable to other factors such as the isolation of an area with special living conditions (e.g. islands) (Kruckeberg and Rabinowitz, 1985). The identification of range-restricted species from a more continuous mapping of species occurrences, using fine GIS grid cell resolutions instead of ecoregion or biome differentiations, would eliminate interference from the shape of a region and the presence of species at the boundaries. It would, however, also increase uncertainties due to the lack of data, especially in areas with comparatively few collection spots, like in the Amazon forest. Alternatively, the definition of range-restricted species used in this study could be adjusted to also include species that are present only in transition zones between ecoregions, which may thus not be captured in our definition. Using different species richness indicators in combination may contribute to an effective species conservation, with a combined focus on extinction of unique species (through the range-restricted-based indicator) and preservation of the overall diversity of occurring species (through the total species richness indicator).

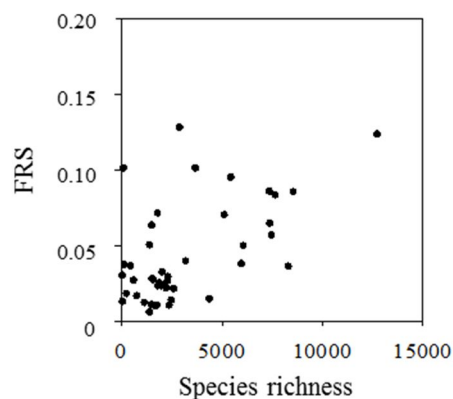


Figure 3. Relationships between the fraction of range-restricted species (FRS) and total species richness (correlation coefficients < 0.562 and $P < 0.05$). Statistical analysis data given in Table S3 in ESM-1.

3.2. Relationships between plant species richness and soil pH

3.2.1. Overall species richness and soil pH

Figure 4 and Figure S3 (ESM-1) show the distribution of terrestrial plant species richness as a function of soil pH at biome and whole country levels, respectively. At biome level, a very strong correlation was found with R^2 above 0.920 regardless of which type of regression fit (log normal or logistic) was used (see Figure 4). Lognormal distribution curves showed a slightly better fit to the collected data (higher R^2) for biomes and were chosen as the preferred approach for this study. At ecoregion and whole-Brazil levels, soil pH and species richness also demonstrate statistically significant correlations, with R^2 ranging 0.830-1.000 for 40 out of 45 ecoregions (two additional ecoregions could not be described by the regression model) and $R^2 = 0.999$ for Brazil as a country, assuming a lognormal distribution model (see Table S3, ESM-1).

Data availability clearly influences the regression analysis, as demonstrated by the observation that the remaining five ecoregions with weaker correlations have relatively small data sets. A limited number of species occurrence data points thus results in uncertain boundaries for the pH ranges, potentially leading to species richness distribution curves with several peaks.

The pH range and the lognormal distributions observed for all spatial resolution levels are consistent with the general pattern of physiological tolerance behavior of plants observed for different environmental conditions (Pärtel, 2002). The lognormal distribution curves observed for nearly all regions indicate that for each ecoregion or biome, there is an optimum pH associated with the highest species richness within the region. Disregarding other environmental factors that may co-vary with soil pH, it indicates that decreasing or increasing pH from the optimum points may lead to a reduction in the number of species that occur. This interpretation should however be cautioned by the fact that the composition of the ecosystems may change as a result of change in soil pH; for example, a decreasing pH in an ecoregion with an initial pH above optimum would lead to an increase in total number of species, although the species composition and nature of the ecosystems may have dramatically changed. Such possible changes could not be included in the current study; they nevertheless constitute a topic worth exploring in future research. The distribution of the optimum pH across biomes and ecoregions in Brazil is further discussed in Section 3.3.

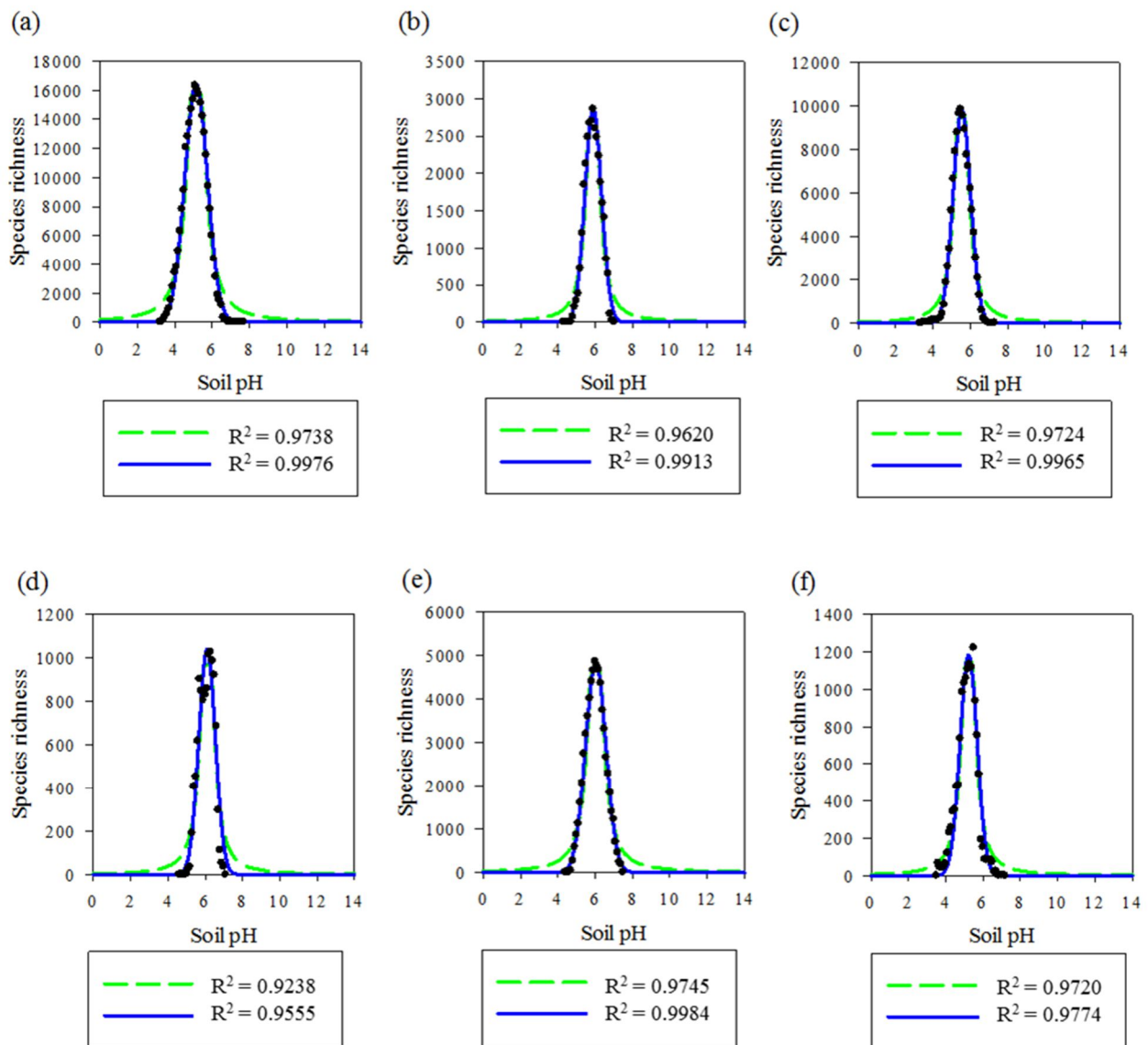


Figure 4. Comparison between continuous logistic regression (in green dotted curve) and lognormal regression (in blue thick curve) for the six Brazilian biomes: (a) Tropical and subtropical moist broadleaf forests, (b) Tropical and subtropical dry broadleaf forests, (c) Tropical and subtropical grasslands, savannas and shrublands, (d) Flooded grasslands and savannas, (e) Deserts and xeric shrublands and (f) Mangroves. Dots represent the collected data (number of species present at each 0.1 unit of pH).

3.2.2. Range-restricted species richness and soil pH

The correlations between the range-restricted species richness and the soil pH at country, biome and ecoregion levels are shown in Table S4 in ESM-1. The two variables demonstrate statistically significant correlations, with $R^2 = 0.982$ at country level (see Figure S3, ESM-1), R^2 ranging 0.855-0.995 at biome level (except for one biome, Figure 5), and R^2 ranging 0.700-0.995 for 32 out of 41 ecoregions (six ecoregions could not be described by the regression model; data not shown). When comparing with the total species richness distributions (Section 3.2.1), the range-restricted species richness distributions present slightly decreased R^2 values for most biomes, except for Mangroves ($R^2 = 0.583$). Out of the 41 ecoregions with regression results, only five ecoregions had R^2 lower than 0.500 due to small and unrepresentative data sets. This observation is consistent with previous results from Section 3.2.1, where ecoregions with lowest numbers of data points showed poor correlations.

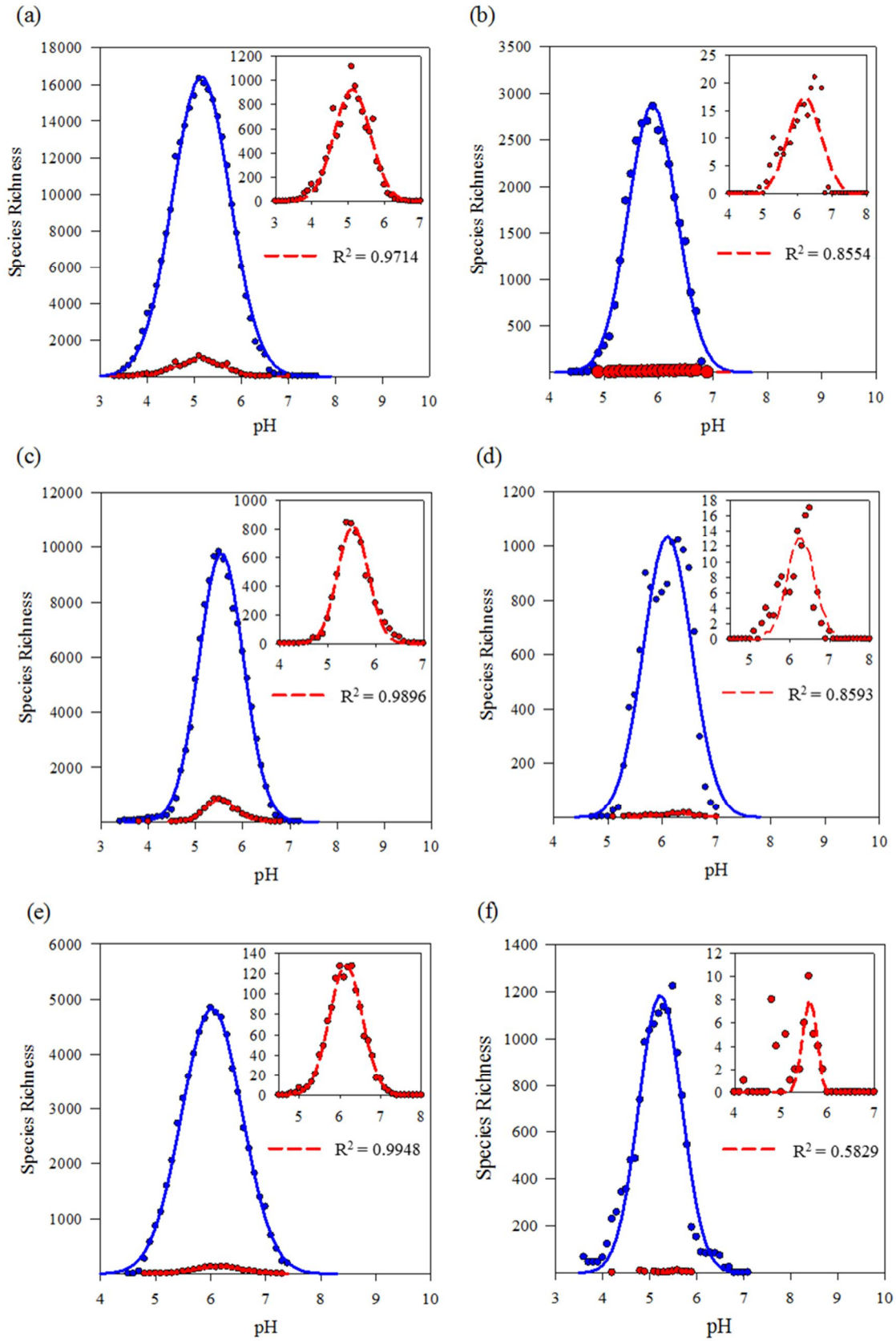


Figure 5. Species richness distribution for the entire list of species (thick curve, in blue) and for range-restricted species (dotted curve in red) for the six Brazilian biomes (inserts show distributions

for range-restricted species in higher resolution): (a) Tropical and subtropical moist broadleaf forests, (b) Tropical and subtropical dry broadleaf forests, (c) Tropical and subtropical grasslands, savannas and shrublands, (d) Flooded grasslands and savannas, (e) Deserts and xeric shrublands and (f) Mangroves. Dots represent the collected data (number of species present at each 0.1 unit of pH).

3.3. Variability of the species richness distributions across ecoregions

Within a biome, different ecoregions show different patterns in terms of optimum pH for the species richness distribution. Two distinct behaviors were observed among biomes in Brazil. Some biomes may include several ecoregions with different profiles, such as the Tropical and subtropical moist broadleaf forests, with 34 ecoregions and a high variability of optimum pH across the grouped ecoregions (see Figure 6a). Others may include few ecoregions and be well represented by just one or two of them (see Figure 6b, 6c and 6d). The disparities of species richness distributions and optimum pH values that can be observed at the ecoregion level are thus not observed at the biome level, reflecting an averaging effect across the larger areas and more variable conditions, as observed in Figures 6 and 7.

Pärtel and co-workers have previously demonstrated that, for low latitudes, the pool of plant species suited for low-pH soil ($\text{pH} < 5.5$) is larger than the pool of species suited for high-pH soil ($\text{pH} > 5.5$) (Pärtel, 2002; Pärtel et al., 2004). This is confirmed by our findings at country level, with entire Brazil having an optimum pH of 5.2. At the levels of biomes (Figure 7a) and ecoregions (Figure 7b), it can be observed that some specific biomes and ecoregions are associated with optimum pH above 5.5, hence showing a larger pool of species suited for high pH soil.

However, vegetation development and soil properties have a complex relationship that also depends on external stress factors, like droughts or fires (e.g. Folster et al. 2001). Our study furthers the understanding of these relationships and of the vegetation development at large, supporting biodiversity assessment. Yet, the influence of external factors like climate change and fires are not considered here, and are recommended to be investigated in future works.

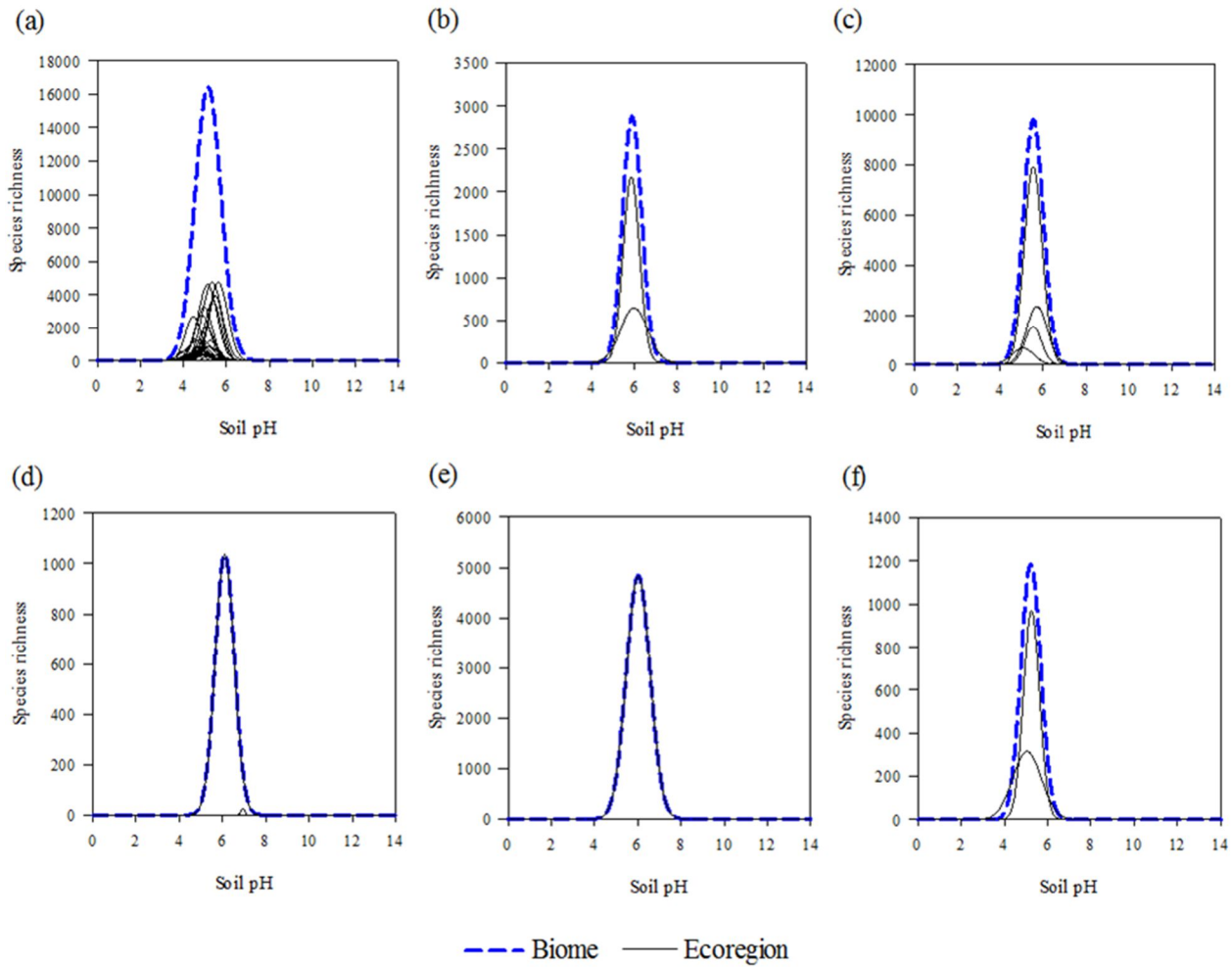


Figure 6. Species richness distribution: grouping of ecoregions within each biome (a) Tropical and subtropical moist broadleaf forests (incl. 34 ecoregions), (b) Tropical and subtropical dry broadleaf forests (incl. two ecoregions), (c) Tropical and subtropical grasslands, savannas and shrublands (incl. six ecoregions), (d) Flooded grasslands and savannas (incl. two ecoregions), (e) Deserts and xeric shrublands (incl. one ecoregion) and (f) Mangroves (incl. two ecoregions).

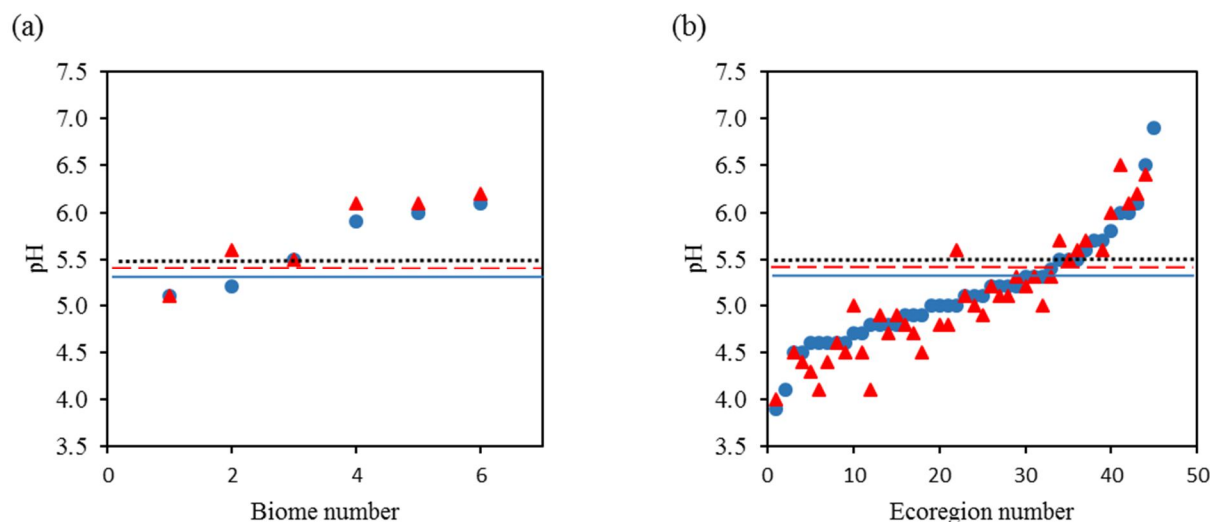


Figure 7. Optimum pH distribution at (a) biome level (total of 6 biomes) and (b) ecoregion level (total of 45 ecoregions). Circles (in blue) represent optimum pH for total species; Triangles (in red) represent optimum pH for range-restricted species; Thick line (in blue) represents optimum pH for total species in Brazil; and dashed line (in red) represents optimum pH for range-restricted species in Brazil. Dotted line (in black) represents a boundary between acidic and non-acidic soils, with pH=5.5 (Pärtel, 2002; Pärtel et al., 2004).

4. CONCLUSIONS AND IMPLICATIONS FOR BIODIVERSITY ASSESSMENT

An inventory of terrestrial plants species is provided for Brazil, listing 29712 species with a harmonized nomenclature, spatially differentiated into biomes and ecoregions and identification of range-restricted species (species only occurring in one ecoregion). Range-restricted species richness is found to correlate poorly with total species richness and hence proposed as a complementary indicator of biodiversity. Besides preserving the diversity of species, the former also focuses on the preservation of unique species, avoiding their disappearance in the considered region. For future work it is suggested to compare the list of range-restricted species with the lists of endemics and The IUCN Red List of Threatened Species (IUCN, 2017) since endemism and threatened species can also be used as indicators of biodiversity that prioritize unique species to the region and indicate potential risks of extinction, respectively.

Additionally, the integration of physicochemical properties of the environment, in this case soil pH, contributes to the analysis of specificities within ecosystems that may be related to species vulnerability, supporting the identification of areas of high conservation priority. Regardless of the

spatial resolution, the species richness variation as a function of soil pH indicates that decreasing or increasing pH from the optimum pH may be associated with a reduction in the number of species that occur. The relationship between terrestrial plant species richness and soil pH can thus support the assessment of impacts related to terrestrial acidification (Crespo-Mendes et al., 2018). The substantial empirical data presented here offer additional opportunities to estimate species loss per unit of land use that can be used to assess land use impacts. Such developments can help improve sustainability assessment approaches, such as Life Cycle Assessment, and support biodiversity conservation through planning and management of soil usage.

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Article II: Electronic Supplementary Material 1

Relationships between plant species richness and soil pH at the level of biome and ecoregion in Brazil

Natalia Crespo Mendes^{1}, Alexis Laurent¹, Hans Henrik Bruun² and Michael Zwicky Hauschild¹*

¹ Division for Quantitative Sustainability Assessment (QSA), Department of Management Engineering, Technical University of Denmark (DTU), 2800 Kgs. Lyngby, Denmark

² Department of Biology, University of Copenhagen (KU), 2100 Copenhagen Ø, Denmark

* To whom correspondence should be addressed; e-mail: cm.natalia@gmail.com

This document includes:

- Supporting Figures S1 to S4
- Supporting Tables S1 to S4
- Supporting Methods
- Supporting References

Supporting Figures (Figures S1 – S4)

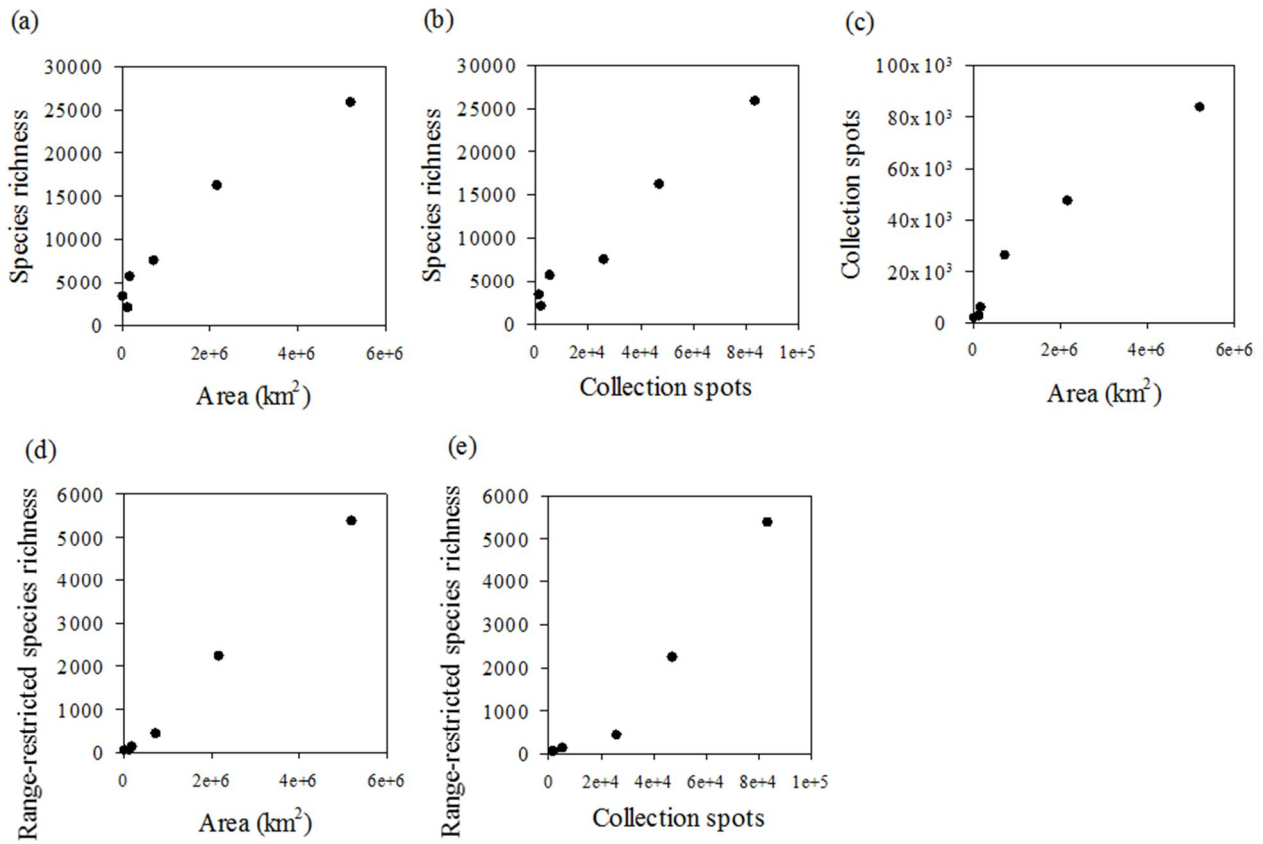


Figure S1. Correlation tests at biome level.

The tested variables are species richness (total and range-restricted), area and collection spots. A correlation was found between all tested variables with $P < 0.05$. Corresponding statistical data (Spearman rank-order and Pearson correlation tests) are fully reported in Table S2a.

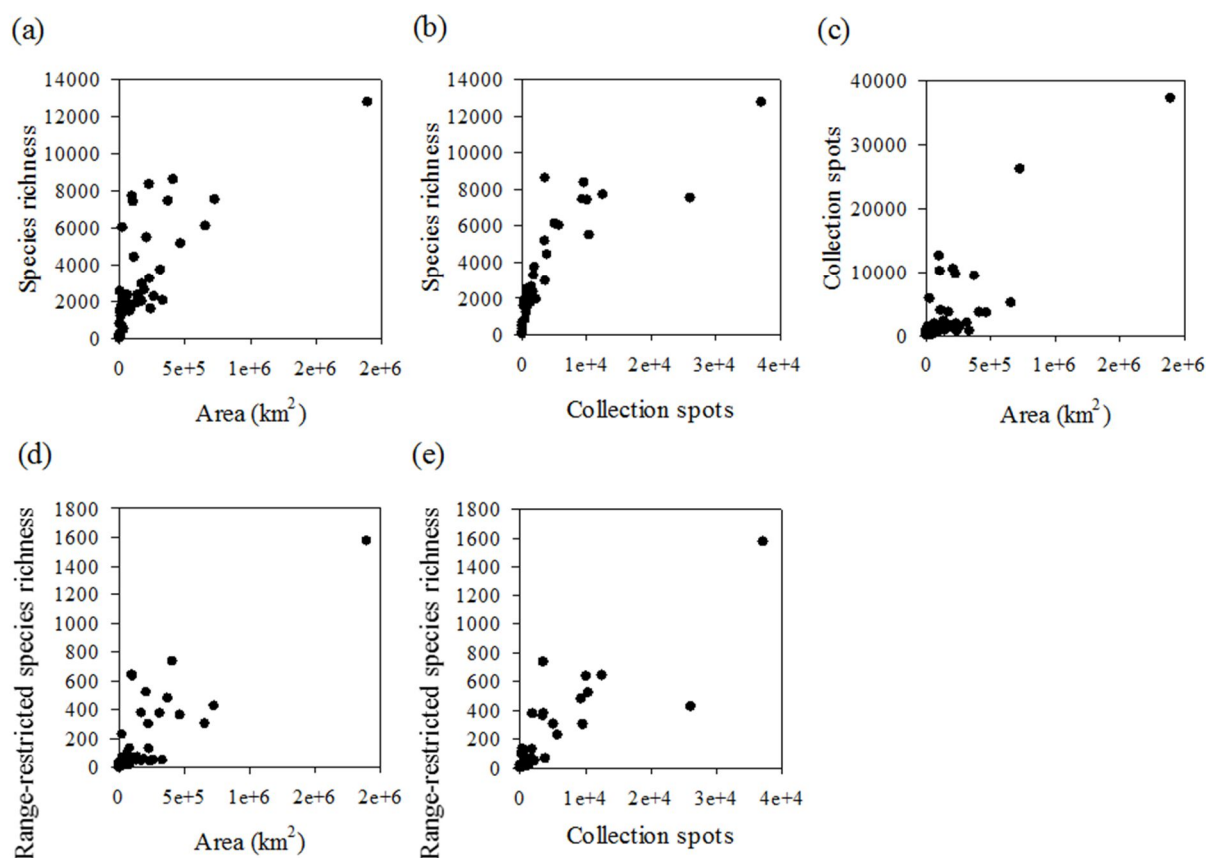


Figure S2: Correlation tests at ecoregion level.

The tested variables are species richness (total and range-restricted), area and collection spots. A correlation was found between all tested variables with $P < 0.05$. Corresponding statistical data (Spearman rank-order and Pearson correlation tests) are fully reported in Table S2b.

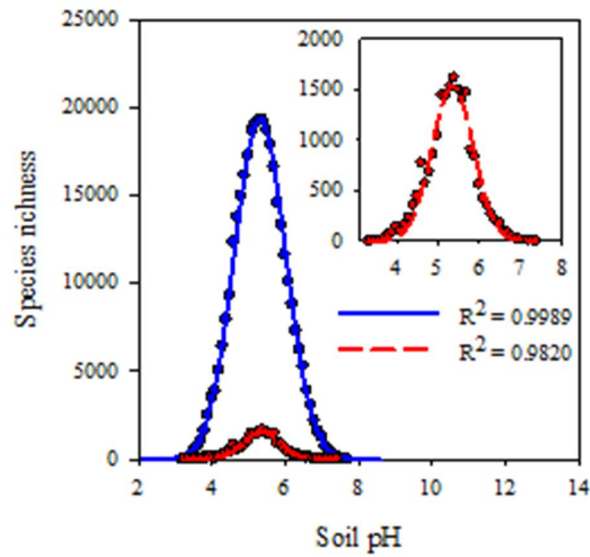


Figure S3.

Distribution of terrestrial plant species richness as a function of soil pH in Brazil, at the country level: the entire list of species (line, in blue) and only range-restricted species (dashes, in red). Insert shows distribution for range-restricted species in higher resolution. Dots represent the collected data. Corresponding statistical data are reported in Table S4.

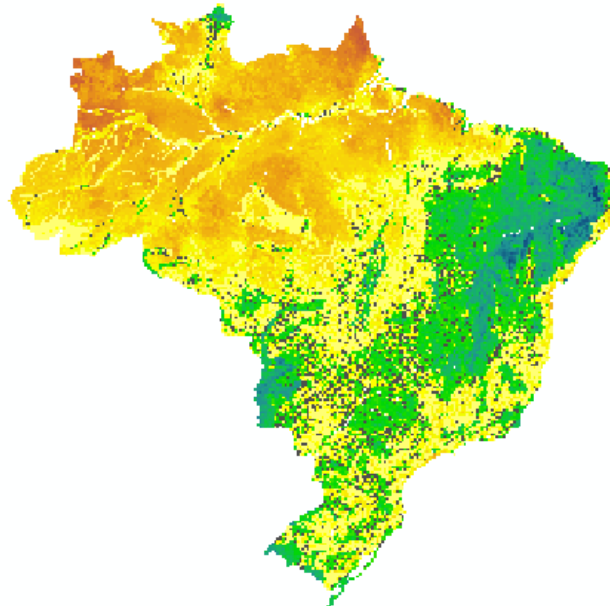


Figure S4.

Soil pH distribution in Brazil: The soil pH distribution in Brazil is analyzed adopting pH=5.5 as a boundary between acidic and non-acidic soils (Pärtel, 2002; Pärtel et al., 2004). Acidic soil in Brazil corresponds to 67% of the country area (in yellow-red), 27% of the country area are non-acidic soil (in green-blue) and 6% of the area is considered neutral area with pH = 5.5 (in black).

Supporting Tables (Tables S1 – S4)

Table S1. Overview of the number of terrestrial plant species at country, biome and ecoregion level in Brazil.

	Area (km ²)	Number of collection spots	Number of species	Number of range- restricted species	Fraction of range- restricted species
Brazil	8456372	166531	29712	8242	28%
Biome: Tropical and subtropical moist broadleaf forests	5213434	83706	25774	5373	21%
Ecoregion:					
Alto Paraná Atlantic forests	374601	9347	7423	477	6%
Araucaria moist forests	211216	10430	5471	519	9%
Atlantic Coast restingas	4966	611	1433	8	1%
Bahia coastal forests	106873	10110	7390	632	9%
Bahia interior forests	228905	9605	8333	299	4%
Caatinga Enclaves moist forests	4776	478	796	13	2%
Caqueta moist forests	12672	18	80	1	1%
Guianan Highlands moist forests	24875	101	636	17	3%
Guianan moist forests	66900	281	1539	97	6%
Guianan piedmont and lowland moist forests	84867	427	1829	130	7%
Gurupa varzea	9881	31	84	---	---
Iquitos varzea	31104	675	2058	66	3%
Japurá-Solimoes-Negro moist forests	232505	1806	3230	127	4%
Juruá-Purus moist forests	241492	626	1599	43	3%
Madeira-Tapajós moist forests	658348	5085	6097	302	5%
Marajó varzea	86897	442	1528	16	1%
Maranhão Babaçu forests	141590	1442	1999	48	2%
Mato Grosso seasonal forests	412312	3575	8593	732	9%
Monte Alegre varzea	66506	847	1793	18	1%
Negro-Branco moist forests	48574	648	1552	43	3%
Northeastern Brazil restingas	9435	79	281	5	2%
Pantepui	4461	33	129	13	10%
Pernambuco coastal forests	17157	1320	1725	16	1%
Pernambuco interior forests	21432	1360	2409	24	1%
Purus-Madeira moist forests	173254	1231	2005	46	2%
Purus varzea	143705	779	2346	68	3%
Rio Negro campinarana	80377	788	1438	72	5%
Serra do Mar coastal forests	100381	12543	7689	639	8%
Solimões-Japurá moist forests	35529	52	469	17	4%

Southwest Amazon moist forests	315731	1917	3708	374	10%
Tapajós-Xingu moist forests	335098	690	2041	48	2%
Tocantins/Pindare moist forests	192447	1438	2620	55	2%
Uatuma-Trombetas moist forests	469497	3526	5143	360	7%
Xingu-Tocantins-Araguaia moist forests	265070	1365	2249	48	2%
<hr/>					
Biome: Tropical and subtropical dry broadleaf forests	180289	5608	5656	124	2%
Ecoregion:					
Atlantic dry forests	114660	3863	4400	63	1%
Chiquitano dry forests	65629	1745	2328	61	3%
<hr/>					
Biome: Tropical and subtropical grasslands, savannas and shrublands	2175904	47286	16172	2223	14%
Ecoregion:					
Campos Rupestres montane savanna	26313	5701	6002	226	4%
Cerrado	1895808	37171	12751	1573	12%
Dry Chaco	126	3	2	1	50%
Guianan savanna	78121	720	1840	42	2%
Humid Chaco	1009	99	163	6	4%
Uruguayan savanna	174527	3592	2932	375	13%
<hr/>					
Biome: Flooded grasslands and savannas	136642	2226	1965	50	3%
Ecoregion:					
Pantanal	136273	2219	1918	48	3%
Southern Cone Mesopotamian savanna	369	7	67	2	3%
<hr/>					
Biome: Deserts and xeric shrublands	729906	26128	7505	424	6%
Ecoregion:					
Caatinga	729906	26128	7505	424	6%
<hr/>					
Biome: Mangroves	20197	1577	3268	48	1%
Ecoregion:					
Amazon-Orinoco-Southern Caribbean mangroves	13691	656	1183	14	1%
Southern Atlantic mangroves	6506	921	2521	34	1%

Table S2. Statistical data - Spearman rank-order and Pearson correlation tests (Section 3.1.4)

(a)

Biome level (N=6)	Spearman rank-order correlation		Pearson correlation	
	Coefficients	P-values	Coefficients	P-values
Area vs Species richness (Figure S1a)	0.943	0.017	0.980	5.75E-04
Collection spots vs Species richness (Figure S1b)	0.943	0.017	0.989	1.80E-04
Area vs Collection spots (Figure S1c)	1.000	0.003	0.980	5.87E-04
Area vs Range-restricted species richness (Figure S1d)	1.000	0.003	0.980	4.35E-04
Collection spots vs Range- restricted species richness (Figure S1e)	1.000	0.003	0.971	1.24E-03

(b)

Ecoregion level (N=47)	Spearman rank-order correlation		Pearson correlation	
	Coefficients	P-values	Coefficients	P-values
Area vs Species richness (Figure S2a)	0.769	2.00E-07	0.733	4.79E-09
Collection spots vs Species richness (Figure S2b)	0.944	2.00E-07	0.829	6.30E-13
Area vs Collection spots (Figure S2c)	0.724	2.00E-07	0.838	1.96E-13
Area vs Range-restricted species richness ^a (Figure S2d)	0.790	2.00E-07	0.821	2.92E-12
Collection spots vs Range- restricted species richness ^a (Figure S2e)	0.841	2.00E-07	0.858	2.57E-14

^a N=46

Table S3: Statistical data - Spearman rank-order and Pearson correlation tests (Section 3.1.5)

(a)

Ecoregion level (N=45)	Spearman rank-order correlation		Pearson correlation	
	Coefficients	P-values	Coefficients	P-values
Species richness vs FRS (Figure 2)	0.448	2.14E-03	0.562	5.96E-05
	0.356 ^a	0.016 ^a	0.091 ^a	0.550 ^a

^a Including an outlier at 50% (an ecoregion for which only two species were reported, the one being range-restricted), N=46.

Table S4. Relationships between plant species richness and soil pH: lognormal distribution model.

	R ²	Soil pH range	Optimum pH	Number of species at the optimum pH	R ² (range-restricted species)	Soil pH range (range-restricted species)	Optimum pH (range-restricted species)	Number of range-restricted species at the optimum pH
Brazil	0.999	2.2 - 8.5	5.3	19310	0.982	3.4 - 7.3	5.4	1532
Biome: Tropical and subtropical moist broadleaf forests	0.998	2.4 - 7.9	5.1	16399	0.971	3.2 - 7.0	5.1	927
Ecoregion:								
Alto Paraná Atlantic forests	0.984	4.1 - 6.9	5.5	3937	0.922	5.6 - 5.8	5.7	287
Araucaria moist forests	0.993	3.8 - 6.9	5.4	3619	0.978	4.2 - 6.5	5.3	180
Atlantic Coast restingas	0.935	3.0 - 7.5	5.2	339	0.699	5.2 - 5.3	5.2	4
Bahia coastal forests	0.994	3.3 - 7.0	5.1	4610	0.986	3.8 - 6.4	5.1	258
Bahia interior forests	0.994	3.8 - 7.4	5.6	4731	0.947	4.6 - 6.8	5.7	65
Caatinga Enclaves moist forests	0.927	4.4 - 5.9	5.2	333	0.798	4.9 - 5.5	5.1	3
Caqueta moist forests	---	---	---	---	---	---	---	---
Guianan Highlands moist forests	0.843	4.2 - 4.7	4.5	323	0.929	4.4 - 4.6	4.5	10
Guianan moist forests	0.787	3.1 - 4.8	3.9	542	0.932	3.9 - 4.1	4.0	59
Guianan piedmont and lowland moist forests	0.890	3.2 - 6.2	4.7	645	0.794	4.5 - 5.6	5.0	47
Gurupa varzeá	0.289	4.1 - 6.1	5.0	8	---	---	---	---
Iquitos varzeá	0.936	4.0 - 6.4	5.2	826	0.692	4.3 - 6.0	5.1	12
Japurá-Solimoes-Negro moist forests	0.937	2.4 - 7.2	4.8	1251	0.754	3.3 - 5.0	4.1	21
Juruá-Purus moist forests	0.856	3.1 - 6.2	4.7	459	0.709	3.7 - 5.7	4.5	5
Madeira-Tapajós moist forests	0.938	3.3 - 6.4	4.9	2784	0.759	4.0 - 5.5	4.8	62
Marajó varzeá	0.901	2.9 - 6.3	4.6	503	0.653	4.0 - 4.8	4.3	3
Maranhão Babaçu forests	0.986	3.7 - 6.7	5.2	846	0.863	4.5 - 6.0	5.3	10
Mato Grosso seasonal forests	0.859	3.4 - 6.5	5.0	3251	0.325	3.7 - 5.9	4.8	122
Monte Alegre varzeá	0.898	3.4 - 6.4	4.9	582	0.807	4.6 - 4.8	4.7	7
Negro-Branco moist forests	0.919	2.4 - 6.9	4.6	470	0.726	3.0 - 5.9	4.1	6

Northeastern Brazil restingas	0.498	4.3 - 7.2	5.7	50	---	---	---	---
Pantepui	0.539	3.6 - 5.7	4.6	15	0.204	4.3 - 5.5	4.4	1
Pernambuco coastal forests	0.974	3.9 - 6.1	5.0	816	0.313	4.5 - 5.7	4.8	2
Pernambuco interior forests	0.950	3.8 - 6.7	5.3	1190	0.694	4.9 - 5.5	5.2	4
Purus-Madeira moist forests	0.935	3.7 - 5.5	4.6	837	0.779	4.2 - 4.9	4.6	13
Purus varzea	0.947	3.1 - 6.5	4.8	794	0.803	4.4 - 5.4	4.9	13
Rio Negro campinarana	0.830	2.7 - 7.1	4.9	328	0.367	3.1 - 6.4	4.5	6
Serra do Mar coastal forests	0.989	3.4 - 7.3	5.3	4719	0.952	3.8 - 6.8	5.3	155
Solimões-Japurá moist forests	0.561	3.2 - 5.0	4.1	101	---	---	---	---
Southwest Amazon moist forests	0.980	3.6 - 6.5	5.1	2031	0.961	4.0 - 6.1	5.0	112
Tapajós-Xingu moist forests	0.900	3.4 - 6.2	4.8	692	0.867	4.4 - 4.9	4.7	15
Tocantins/Pindare moist forests	0.967	3.2 - 5.9	4.6	1265	0.747	3.8 - 5.5	4.5	7
Uatuma-Trombetas moist forests	0.922	2.9 - 6.0	4.5	2662	0.889	3.8 - 4.9	4.4	136
Xingu-Tocantins-Araguaia moist forests	0.969	3.4 - 6.1	4.8	870	0.853	4.6 - 5.2	4.9	16

Biome: Tropical and subtropical dry broadleaf forests

0.991 4.1 - 7.7 5.9 2.879 0.855 5.0 - 7.4 6.1 17

Ecoregion:

Atlantic dry forests	0.978	4.3 - 7.4	5.8	2157	0.969	5.1 - 6.8	6.0	11
Chiquitano dry forests	0.849	3.6 - 8.3	6.0	648	0.783	6.0 - 7.0	6.5	16

Biome: Tropical and subtropical grasslands, savannas and shrublands

0.996 3.5 - 7.6 5.5 9764 0.990 4.3 - 6.7 5.5 823

Ecoregion:

Campos Rupestres montane savanna	0.967	3.7 - 7.7	5.7	2358	0.741	4.4 - 6.9	5.6	26
Cerrado	0.995	3.7 - 7.3	5.5	7920	0.995	4.5 - 6.6	5.5	696
Dry Chaco	---	---	---	---	---	---	---	---
Guianan savanna	0.963	3.1 - 7.0	5.1	669	0.608	3.9 - 6.3	4.9	4
Humid Chaco	0.964	6.2 - 6.8	6.5	81	0.972	6.2 - 6.6	6.4	4
Uruguayan savanna	0.960	4.0 - 7.1	5.5	1519	0.913	4.3 - 6.9	5.6	112

Biome: Flooded grasslands and savannas	0.955	4.4 - 7.8	6.1	1038	0.859	5.4 - 7.1	6.2	13
Ecoregion:								
Pantanal	0.953	4.4 - 7.8	6.1	1035	0.853	5.4 - 7.1	6.2	13
Southern Cone Mesopotamian savanna	0.879	6.7 - 7.1	6.9	26	---	---	---	---
Biome: Deserts and xeric shrublands	0.998	3.7 - 8.3	6.0	4835	0.995	4.8 - 7.4	6.1	128
Ecoregion:								
Caatinga	0.998	3.7 - 8.3	6.0	4835	0.995	4.8 - 7.4	6.1	128
Biome: Mangroves	0.977	3.5 - 7.0	5.2	1184	0.583	5.3 - 5.9	5.6	8
Ecoregion:								
Amazon-Orinoco-Southern Caribbean mangroves	0.861	2.7 - 7.4	5.0	314	0.898	5.4 - 5.9	5.6	4
Southern Atlantic mangroves	0.979	3.8 - 6.7	5.3	963	0.439	4.3 - 6.1	5.0	3

Supporting Methods

A. Global Biodiversity Information Facility (GBIF) database

Global Biodiversity Information Facility is an international platform to connect and access biodiversity databases around the world (GBIF, 2015). Its open data infrastructure provides users with free access to more than 14000 datasets published by over 750 institutions, covering over 450 million geo-referenced species occurrence records (GBIF, 2015). Data downloaded from GBIF provided the following information on each record: taxon names (kingdom, phylum, class, order, family, genus, species and infra specific epithet), taxon rank, scientific name, locality (name of the region), decimal latitude and longitude, event date (day, month, year), identified by, recorded by and possible observed issues.

B. Configurations set for the taxonomic alignment supported by the use of the Taxonomic Name Resolution

The list of plant species was submitted to the Taxonomic Name Resolution Service v4.0 (TNRS; Boyle et al., 2013). Below are the configurations adopted in the fields “Name processing settings” and “Downloading results”:

- Name processing settings
 1. Processing mode:
 - (X) Perform name resolution
 - () Parse names only
 2. Classification:
 - (X) APGIII
 - () NCBI
 3. Sources:
 - (X) GCC
 - (X) ILDIS
 - (X) TPL
 - (X) TROPICOS
 - () USDA
 - () NCBI

4. Match accuracy:

Allow partial matching

When selected this option allows the indication of the plant genus name when the species name is not found. This option was not selected since the scope of this study includes only plants that have the name of the species available for inspection.

- Downloading results

1. Best match settings:

Constrain by higher taxonomy

Constrain by source

2. Results to download:

Best matches only

All matches

3. Download format:

Simple

Detailed

Based on the species list provided by the TNRS the following adjustments were made: (1) all the species classified as ‘accepted name’ were maintained on the list; (2) names classified as ‘illegitimate’, ‘invalid’ or ‘no opinion’ were deleted; and (3) names classified as synonyms were identified and replaced by their accepted names.

Similar species names with only one or two different letters were identified and investigated in the main botanical databases in order to refine the taxonomic alignment and eliminate possible spelling errors. When both names were found in different databases the priority was given to the name indicated by The Plant List (TPL). When both names were found in the TPL, the name with higher confidence level was prioritized. In total 236 names of species were identified in this step and 47 names were replaced.

C. Soil pH data

Soil pH data were collected in SoilGrids1km (2015). The pH values that correspond to areas within the Brazilian territory have been downloaded according to the following set of configurations:

1. Projection: Geographic

2. Horizontal Datum: WGS84

3. Vertical Datum: EGM96

4. Latitude: reported in decimal degrees
5. Longitude: reported in decimal degrees
6. Date associated with the value estimate: year
7. Depth range: 0-5 cm, 5-15 cm, 15-30 cm and 30-60 cm

Inconsistencies were present in the soil pH data with grid cells being reported to have pH of zero. These grid cells were assumed to be in areas with rivers, which can be verified through maps of the hydrographic basins of Brazil. New pH values were estimated for 9120 grid cells based on the statistic of the values around the grid cells with reported pH of zero.

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Article III

Effect factors of terrestrial acidification in Brazil for use in Life Cycle Impact Assessment

Crespo-Mendes, N., Laurent, A., & Hauschild, M. Z.

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Effect factors of terrestrial acidification in Brazil for use in Life Cycle Impact Assessment

Natalia Crespo Mendes*, Alexis Laurent and Michael Zwicky Hauschild

Division for Quantitative Sustainability Assessment (QSA), Department of Management Engineering, Technical University of Denmark (DTU), 2800 Kgs. Lyngby, Denmark

* To whom correspondence should be addressed; e-mail: cm.natalia@gmail.com

Abstract

Purpose: In Life Cycle Impact Assessment, atmospheric fate factors, soil exposure factors and effect factors are combined to characterize potential impacts of acidifying substances in terrestrial environments. Due to the low availability of global datasets, effect factors (EFs) have been reported as the major contributors to statistical uncertainties of characterization factors and they are the focus of this study. We aim to develop spatially differentiated EFs taking Brazil as case, and explore new methodological ways to derive them.

Methods: EFs are calculated based on a comprehensive database reporting observations of approximately 30000 plant species at biome and ecoregion levels. Species richness distributions as function of soil pH are developed and translated into Potentially Not Occurring Fraction (PNOF) of species, which can be equated to the more commonly-used Potentially Disappeared Fraction of species, to assess effects of changes in soil hydrogen ion concentration on terrestrial plant species. Potentially Extinct Fraction (PXF) of species is proposed as a complementary metric for LCIA models based on distributions of range-restricted species (species only occurring in one ecoregion of Brazil). Different approaches for determining EFs from the species richness distributions are evaluated. Area-weighted EFs are explored to determine potential effects when considering both acid and alkaline sides of species richness curves, thus integrating potentially positive effects of acidification on biodiversity.

Results and discussion: Spatially differentiated EFs are provided for 6 biomes and 45 ecoregions composing Brazil. Comparisons with previous EFs demonstrate that data availability might significantly influence regression analyses and the use of more representative data can lead to more consistent EFs. Moreover, consideration of the entire species richness

curves yields positive and negative EFs. Adding acidifying substances onto specific soils in Brazilian ecoregions may therefore be associated with increased species richness if the pH approaches the optimum pH from the alkaline side of the curve. The meaningfulness of species richness as indicator of acidification stress is discussed based on this finding, as is the inclusion of the metric PXF, highlighting species whose loss could cause irreversible damages to the environment.

Conclusions: We recommend the calculation of area-weighted EFs to be integrated into characterization models for terrestrial acidification, and we therefore advocate that similar work be done for other regions in the world than Brazil to enhance the consistency of the EFs and reduce their uncertainties. We additionally recommend that LCIA method developers further explore the application of PXF for other impact categories than acidification.

Keywords: Species richness, Endemism, Extinction, Biodiversity loss, Biome, Ecoregion.

1. INTRODUCTION

As an impact category in Life Cycle Impact Assessment (LCIA), terrestrial acidification is primarily caused by the atmospheric emissions and depositions of nitrogen oxides (NO_x), sulfur dioxide (SO₂) and ammonia (NH₃) (EC-JRC 2010). To characterize the potential impacts that these acidifying substances can cause in the environment, the LCIA methodologies rely on characterization factors (CF), which are generally composed of an atmospheric fate factor (FF), an exposure factor (XF) and an effect factor (EF) (Udo de Haes et al. 2002). Existing CF calculated at midpoint level are usually given by the FF or its combination with the XF. They can express the potential acidification impacts in terms of hydrogen ions (H⁺) released into the environment (as in CML 1992 (Heijungs et al. 1992), EDIP 97 (Wenzel et al. 1997; Hauschild and Wenzel 1998) and MEEuP (Kemna et al. 2005) LCIA methodologies), H⁺ ions deposited on land (as in TRACI (Norris 2003), EPS (Steen 1999) and LIME (Hayashi et al. 2004) methodologies), relative risk ratio (CML 2002, Guinee et al. (2002)), affected ecosystem areas due to exposure over its critical load, in which the ecosystem sensitivity is considered but not in terms of species loss (EDIP 2003 (Potting et al. 1998) methodology), accumulative critical load exceedance (Accumulated Exceedance, Seppälä et al. 2006) methodology) or soil acidity change (ReCiPe (Van Zelm et al. 2007; Goedkoop et al. 2009) (Van Zelm et al. 2015)). The EF are included in the calculations and the CF can express the potential damages to the ecosystem through net primary productivity (LIME methodology, Hayashi et al. 2004) or occurrences of

plants species, which can address damages to all plant species (ReCiPe 2016 (Huijbregts et al. 2017), Impact World+ (2018) and LC-Impact (2018) methodologies) or to specific target plant species (Eco-indicator 99 methodology) (Van Zelm et al. 2015).

According to the guidance for LCIA in the European context offered by the International Reference Life Cycle Data System (ILCD) Handbook (EC-JRC 2011), among the evaluated endpoint methodologies for acidification there is a lack of global fate models and effect models for other continents than Europe. The ILCD Handbook however does not address the recent developments with LCIA methods like Impact World+ (2018), LC-Impact (2018) and ReCiPe 2016 (Huijbregts et al. 2017), that bring advances regarding spatial differentiation and provide spatially differentiated characterization factors on a global scale. These methodologies adopt the same models for the calculations of fate factor (Roy et al. 2012a), exposure factor (Roy et al. 2012b) and effect factor (Azevedo et al. 2013; Roy et al. 2014). While the fate factor and, to a lesser extent, the exposure factor have been reported to be the main source of spatial variability, the effect factors have been identified as the main source of statistical uncertainties of the characterization factors due to the low availability of global data sets (Roy et al. 2014; Van Zelm et al. 2015). In Azevedo et al. (2013) spatially-resolved EFs are calculated based on the relationships between changes in plant species richness and soil pH variations, taking occurrences of 2409 plant species worldwide attributed to 13 terrestrial global biomes. Considering the species diversity in a country like Brazil, which has more than 30000 species of plants (Forzza et al. 2012; Crespo-Mendes et al. 2018), one may question the representativeness of the EF calculated in Azevedo et al. (2013) and Roy et al. (2014), thus calling for more comprehensive studies.

In this study, taking Brazil as a case study, we therefore aim to (1) provide updated spatially differentiated EFs for terrestrial acidification in Brazil, based on the use of a comprehensive botanic database and investigate their spatial variability (ecoregion, biome and whole country levels); (2) assess the influence of data completeness and representativeness, and increased spatial resolution, on EF values by comparing the obtained EFs with those developed in recent LCIA methodologies; and (3) discuss the appropriateness of the metrics used in LCIA to address biodiversity loss caused by acidification. The selection of Brazil was motivated by the extensive territorial area and great variations in terms of population density, anthropogenic activities and environmental characteristics throughout the country.

2. METHODS

2.1. Data sources

2.1.1. Botanical data

A comprehensive and harmonized data inventory of terrestrial plants in Brazil reported by Crespo-Mendes et al. (2018) was used to estimate the potential losses of terrestrial plants species richness as a result of exposure to acidifying substances. Data were collected based on records provided by the Global Biodiversity Information Facility (GBIF 2017) and processed by using the Geographic Information System (GIS) into a georeferenced inventory of 29712 terrestrial plant species with a harmonized nomenclature. The number of different species observed in a certain region, defining the region's species richness, was quantified for the six biomes and 47 ecoregions in Brazil delineated by Olson et al. (2001) (see Figure 1). Further details on the construction of the plant species inventories are available in Crespo-Mendes et al. (2018).

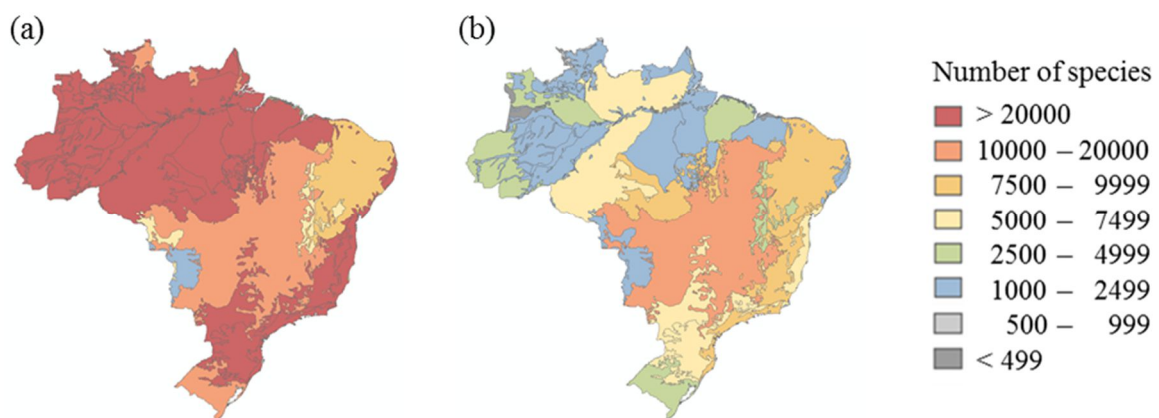


Figure 1. Terrestrial plant species in Brazil: (a) per biome (six biomes) and (b) per ecoregion (47 ecoregions) (from Crespo-Mendes et al., 2018).

2.1.2. pH data

Soil pH was taken as an indicator of soil acidity. The pH data used in this study were extracted for each unit of 1-km² grid cells from SoilGrids1km (Hengl et al. 2014) and processed to represent a depth range of 0-60 cm, which was assumed relevant for plant root exposure to

acidifying or alkalizing substances. Figure 2 presents the soil pH distribution in Brazil. Details are provided in Crespo-Mendes et al. (2018).

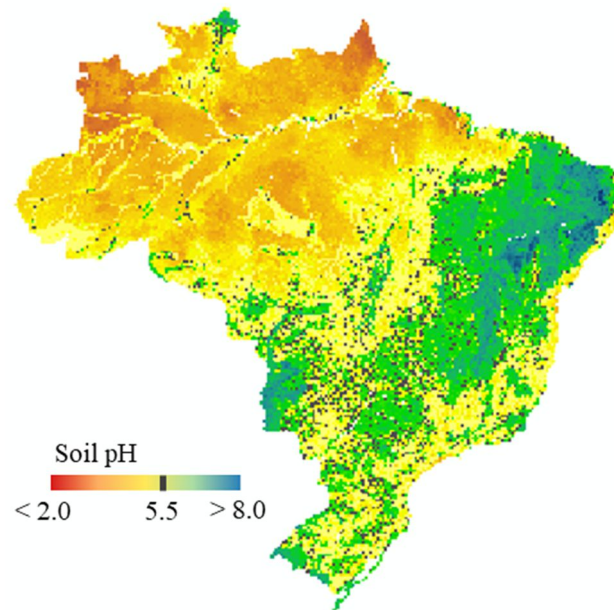


Figure 2. Soil pH distribution in Brazil: acidic soil in Brazil corresponds to 67% of the country area ($\text{pH} < 5.5$, in yellow-red), 27% of the country area are non-acidic soil ($\text{pH} > 5.5$, in green-blue) and 6% of the area is considered neutral area ($\text{pH} = 5.5$, in black) (from Crespo-Mendes et al., 2018)

2.1.3 Species richness distribution data

Data about species richness distributions as function of the soil pH at country, biome and ecoregion levels were reported in Crespo-Mendes et al. (2018). The distributions were obtained by matching the georeferenced locations of terrestrial plant species (Section 2.1.1) with their associated soil pH values (Section 2.1.2) and by assigning to each species a range of soil pH defined by the lowest and highest pH values at which the species has been recorded. Even though a species has not been recorded for all intermediate pH values and may also exist (unobserved) outside the determined range, the thus determined soil pH range was assumed to be representative of the occurrence of the species due to the high number of observations reported in the plant inventory (Crespo-Mendes et al. 2018).

2.2. Potentially Not Occurring Fraction (PNOF)

The Potentially Not Occurring Fraction (PNOF) of species is a zero-to-one measure representing the presence or absence of species (Equation 1) used for determining the effect factors (Azevedo et al. 2013) (see Section 2.3). It is an alternative metric to the more commonly-used Potentially Disappeared Fraction (PDF) of species, as it focuses on the fraction of species that potentially do not occur, i.e. potential losses of species in a given region due to changes in soil pH. To be consistently integrated within the LCIA methodologies, PNOF can be converted to PDF through equalization of PNOF in PDF (PNOF = PDF) (LC-Impact, 2018; Huijbregts et al. 2017). Note that the PDF provided in this study is calculated locally for Brazil. A global PDF (PDF_{global}) accounts for the vulnerability of the species, reflecting that not all species are equally affected depending on their adaptive capacity and recovery potential. The PDF_{global}, which combines the actual species richness with vulnerability scores (VS), has been used to assess biodiversity effects from land and water use within LCIA (Verones et al. 2015) and should ideally be sought in future works that extend this study.

The species richness distributions obtained in Section 2.1.3 were translated into modeled PNOF by using lognormal regression analysis (Equation 2). A lognormal distribution was considered since it showed a slightly better fit to the collected data than a logistic distribution, with R² values of 0.999 for entire Brazil, R² > 0.955 for all six biomes and R² values of 0.830-1.000 for 40 out of 45 ecoregions (two additional ecoregions could not be described by the regression model; data not shown) (Crespo-Mendes et al. 2018). The lognormal regression fit also made it easier to capture both sides of the species richness curve (termed “SR curve” in the following), where the optimum pH (i.e. the pH value at which the highest species richness occurs within a region) was taken as a boundary between acid (pH < pH_{opt}) and alkaline (pH > pH_{opt}) sides of the curve. Thus, this approach also shows potential effects on plant species richness by decreasing soil pH towards the optimum pH from the alkaline side of the SR curve. To the authors’ knowledge, such possible environmental mechanism has not been addressed in any of the previous studies with regard to acidification although positive effects have been included in assessment of other impact categories, like water stress (e.g. Scherer and Pfister, 2016).

$$PNOF_{ij} = 1 - \frac{SR_{ij}}{SR_{\max j}} \quad (\text{Equation 1})$$

where SR_{ij} is the predicted value of species richness present at pH i in biome or ecoregion j , and $SR_{\max,j}$ is the highest species richness occurring at any pH value in the biome or ecoregion j .

$$SR_{ij} = \frac{a}{C} \exp \left[-0.5 \left(\frac{\ln(C/x_0)}{b} \right)^2 \right] \quad (\text{Equation 2})$$

where C is the soil concentration of H^+ [$\text{mol } H^+ \cdot L^{-1}$] relative to the SR_{ij} and a , b and x_0 are regression parameters derived from the lognormal distribution model. Associated R^2 values and regression coefficients are given in Table S1, Electronic Supplementary Material, ESM.

2.3. Potentially Extinct Fraction (PXF)

Following the approach used for modeling the Potentially Not Occurring Fraction (PNOF) of all species (see Section 2.2), the Potentially Extinct Fraction (PXF) of species is a zero-to-one measure representing the presence or absence of range-restricted species. We propose this new metrics based on previous works having focused on endemic species in other impact categories (e.g. de Baan et al. 2013; Verones et al. 2013). Here, PXF were modeled based on the selection of plant species that were found to occur in only one of Brazil's ecoregions (i.e. range-restricted species). Some of them may also occur outside Brazil and thus not be truly endemic to the ecoregion, but due to the size of the country and its high species diversity, many of the thus defined range-restricted species will be truly endemic (Crespo-Mendes et al. 2018). Decreasing the species richness of range-restricted plant species thus indicates the potential extinction of unique species.

Although both PXF and PDF_{global} (see Section 2.2; Verones et al. 2015) aim to reflect a differentiation of species – trying to emphasize those species most likely to be extinct – PXF only includes the range-restricted species and thus omits all other species, which may be functionally-important for the ecosystems. Unlike PXF, in a global PDF this information could still be retained and included. Ideally, extending to a global scale the procedures for collecting

and processing data on species occurrence and soil pH would allow the calculation of PDF_{global} and the comparison between the list of range-restricted species and endemic species to the world. However, it requires important computational resources, which could not be met in this work.

2.4. Effect factors (EF)

Effect factors (EF) were calculated based on the state-of-the-art characterization models for terrestrial acidification (Azevedo et al. 2013; Roy et al. 2014), which is the approach adopted by Impact World+ (2018), LC-Impact (2018) and ReCiPe 2016 (Huijbregts et al. 2017) methodologies. The effect factors express the effect on the plant species occurrence from a change in soil hydrogen ion concentration. They were defined by the slope of the SR curve (see Section 2.1.3) at the relevant soil pH value, which was translated into PNOF (Section 2.2) or PXF (Section 2.3) by the integration of Equation 2 into Equation 1 (see Section 2.2), thus, the derivative was calculated as a function of C (soil concentration of H^+ [$\text{mol } H^+ \cdot L^{-1}$]) as described by Equation 3.

$$EF_{ij} = \frac{dPNOF_{ij}}{dC} = \frac{(1 - PNOF_{ij})}{C} \left[1 + \frac{\ln(C/x_0)}{b^2} \right] \quad (\text{Equation 3})$$

We used different settings to enable the calculation of EFs for biomes and ecoregions and the comparison with the existing factors. Table 1 gives an overview of the settings used for the calculations. To show the potential differences with previous studies due to the use of different species occurrence data sets, the EFs were first calculated using logistic regression distributions (Table 1, sets 1a and 1b) to be compatible with the EFs calculated from Roy et al. (2014) (Table 1, set 2) and Azevedo et al. (2013) (Table 1, set 3). Effect factors calculated in Azevedo et al. (2013) and Roy et al. (2014) are based on the same plant inventory reported in Azevedo et al. (2013) but adopt different approaches to relate species richness to soil pH. Roy et al. (2014) bases the pH-dependent species richness distributions on the lowest pH at which each species was recorded (termed “pH min” approach in the following) while Azevedo et al. (2013)

considers the whole interval defined by the lowest and highest pH value at which each species was recorded (termed “pH range” approach). Both approaches base the calculation of EFs on the pH value where 50% of plant species potentially do not occur (PNOF = 0.5), which is similar to the approach applied in the calculation of effect factors for ecotoxicity based on a species sensitivity distribution curve representing the potentially affected fraction (PAF) of species as a function of the toxicant concentration (Larsen and Hauschild 2007; Pennington et al. 2004).

Acidification impacts on vegetation differ mechanistically from impacts caused by toxic chemicals and the analogy with ecotoxicological effects may be inadequate for estimating impacts caused by acidifying substances as illustrated by the bell-shaped form of the PNOF curves. In order to analyze the behavior of species throughout their entire distribution curves, we also applied a pH range approach and propose as an alternative calculation of EF the area-weighted EF (EF_{aw}). EF_{aw} is defined as the weighted average of the EF for each pH unit, using as weighting factor the area that each pH unit represents relative to the total area of the studied region (e.g. ecoregion or biome) (Equation 4). Sets 4a and 4b allow the comparison between an EF calculated based on PNOF = 0.5 and an area-weighted EF for the acid and alkaline sides of the SR curves separately, at country, biome and ecoregion levels. Additionally, sets 5a and 5b allow the comparison between area-weighted EF (EF_{aw}) calculated from the complete list of plant species and on the list of range-restricted species, at country, biome and ecoregions levels.

$$EF_{aw} = \sum \frac{EF_{ij} \cdot A_{ij}}{A_{totj}} \quad (\text{Equation 4})$$

where EF_{ij} and A_{ij} are, respectively, the EF and the area (km^2) for each pH unit i in biome or ecoregion j , and A_{totj} is the total area (km^2) of biome or ecoregion j .

Table 1. Settings for effect factor calculations.

Set	Approach	Curve side	Regression	Resolution	Data set	Data source	
1a 1b	PNOF = 0.5	pH min. pH range	Acid	Logistic	Biome	All species	Crespo-Mendes et al. (2018)
2	PNOF = 0.5	pH min.	Acid	Logistic	Biome	All species	Roy et al. (2014) ^a
3	PNOF = 0.5	pH range	Acid	Logistic	Biome	All species	Azevedo et al. (2013) ^a
4a 4b	PNOF = 0.5 Area-weighted	pH range	Acid / Alkaline	Lognormal	Biome/ Ecoregion	All species /	Crespo-Mendes et al. (2018)
5a 5b	Area-weighted	pH range	Entire curve	Lognormal	Biome/ Ecoregion	All species Range-restricted species	Crespo-Mendes et al. (2018)

^a The coefficients used to calculate the effect factors for each study are found in Table S2, Electronic Supplementary Material (ESM).

3. RESULTS AND DISCUSSION

3.1. Influence of more comprehensive data set

Table 2 shows the comparison of EFs based on different datasets, using previous approaches for calculations (see Table 1). As previously described, scenarios 1a and 2 refer to the species count based on pH min (Roy et al. 2014), i.e. the lowest pH where each species occurs in a given biome, while scenarios 1b and 3 use the pH range defined by the lowest and highest pH value at which each species occurs in a given biome (Azevedo et al. 2013). The ratios between our EF and the existing factors presented in Table 2 show significant discrepancies for the 6 biomes, ranging from 0.07 to 9.27 for the EF calculated from the pH range approach and from 0.06 to 75.13 for the EF based on the minimum pH (comparison of set 1a vs. 2 and 1b vs. 3; see Table 2). Some consistency can be observed where our study yields higher EFs (for the biomes Tropical and subtropical moist broadleaf forests and Mangroves) or considerably lower EF (Deserts and xeric shrublands) compared to both previous studies. For two biomes (Tropical and subtropical grasslands, savannas and shrublands, and Flooded grasslands and savannas) the difference is more inconsistent, with Roy et al. (2014) > The current study > Azevedo et al. (2013), and for the last biome (Tropical and subtropical dry broadleaf forests) the current study

gives much higher EF than Roy et al. (2014) while Azevedo et al. (2013) do not report any value.

The applied database is the primary difference between the three scenarios and hence the cause of differences between the EFs. The EFs by Azevedo et al. (2013) and by Roy et al. (2014) were based on data collected from a critical review of the scientific literature (reported by Azevedo et al., 2013). Although the critical review may result in more accurate pH values for the species at a given location, this eventually limited the number of studies addressed to determine the species richness and soil pH per biome (i.e. 140 studies listing a total of 2409 plant species worldwide). In addition, the data available in Azevedo et al., 2013 represent the number of species per biomes distributed throughout the world, without possibility of differentiating data specifically referring to the Brazilian territory. In our study we considered compiled information from separate databases of plant occurrences and soil pH in Brazil, combined using the Geographic Information System (GIS). The higher representativeness of the data set (an inventory of 29712 plants species) is evident when comparing the number of species per biome and the number of species at the optimum pH between the three studies. For example, the number of species covered by Azevedo et al. (2013) for the biome Deserts and xeric shrublands is approximately 5% of the number of species reported in the current study (Crespo-Mendes et al. 2018), and for the remaining biomes this number decreases to 1-2% (see Table 3), thus indicating a very low coverage of species in Azevedo et al. (2013). Table 3 also shows the pH range of species occurrence. The study by Azevedo et al. (2013) with lowest number of species reports maximum pH values of species occurrence higher than those observed in Crespo-Mendes et al. (2018) for 4 out of 6 biomes (see Table 3). The broader pH range does not guarantee that the highest values of pH are related to soils of Brazil, since the data set reported by Azevedo et al. (2013) covers biomes on a global scale without the differentiation of specific data for Brazil. The reported high pH values of species occurrence may thus relate to soils in other countries than Brazil, where the same biomes are represented. The differences shown in terms of ratios between EFs (Table 2) result from the use of a more comprehensive and representative data set, demonstrating that the much lower number of reported plant species (see Table 3), which yielded lower species richness, greatly influences the determined EF.

Table 2. Effect factors at PNOF=0.5 [PNOF.(mol H⁺. L⁻¹)⁻¹]: comparison with previous approaches at the biome level (sets 1a, 1b, 2 and 3).

Biome	(pH min)			(pH range)		
	This study [PNOF.(mol H ⁺ . L ⁻¹) ⁻¹]	Roy et al. (2014) [PNOF.(mol H ⁺ . L ⁻¹) ⁻¹]	This study/ Roy et al. (2014)	This study [PNOF.(mol H ⁺ . L ⁻¹) ⁻¹]	Azevedo et al. (2013) [PNOF.(mol H ⁺ . L ⁻¹) ⁻¹]	This study/ Azevedo et al. (2013)
	(set 1a)	(set 2)		(set 1b)	(set 3)	
Tropical and subtropical moist broadleaf forests	1.43E+04	2.00E+03	7.16	1.12E+04	2.14E+03	5.23
Tropical and subtropical dry broadleaf forests	1.61E+05	2.14E+03	75.13	1.72E+05	-	-
Tropical and subtropical grasslands, savannas and shrublands	6.62E+04	8.33E+04	0.80	6.25E+04	2.41E+04	2.60
Flooded grasslands and savannas	2.37E+05	2.45E+06	0.10	2.56E+05	6.72E+04	3.80
Deserts and xeric shrublands	1.39E+05	2.31E+06	0.06	1.18E+05	1.74E+06	0.07
Mangroves	3.61E+04	5.03E+03	7.18	2.11E+04	2.28E+03	9.27

Table 3. Overview of plant species richness data for each study.

Biome	Azevedo et al. (2013) and Roy et al. (2014) (data extracted from Azevedo et al. 2013 for both studies)			This Study (data extracted from Crespo-Mendes et al. 2018)		
	Number of species	Number of species at the optimum pH	pH range of species occurrence	Number of species	Number of species at the optimum pH	pH range of species occurrence
Tropical and subtropical moist broadleaf forests	533	358	3.0-8.2	25774	16399	2.4-7.9
Tropical and subtropical dry broadleaf forests	139	65	5.5-8.5	5656	2879	4.1-7.7
Tropical and subtropical grasslands, savannas and shrublands	131	107	4.5-6.1	16172	9764	3.5-7.6
Flooded grasslands and savannas	18	18	5.3-6.9	1965	1038	4.4-7.8
Deserts and xeric shrublands	350	293	5.1-10.5	7505	4835	3.7-8.3
Mangroves	25	25	3.4-7.2	3268	1184	3.5-7.0

^a Data extracted from Azevedo et al. (2013) represent the number of species per biomes distributed throughout the world, whereas data extracted from Crespo-Mendes et al. (2018) represent the number of species per biomes within Brazil.

3.2. Integrating both acid and alkaline sides of the SR curves

Table 4 shows calculated EFs for terrestrial acidification at country and biome levels in Brazil. The EFs were calculated with the settings presented in sets 4a, 4b, 5a and 5b, as reported in Table 1 (see Section 2.4). Analyzing the acid and alkaline sides of the SR curve, two soil pH values are obtained corresponding to PNOF=0.5 instead of only one – when considering the acid part of the distribution curve only, as done in previous studies by Azevedo et al. (2013) and Roy et al. (2014). Positive values for EFs are obtained when considering pH values on the acid side of the SR curve. For the alkaline side, negative EF values are obtained (see Table 4) meaning that adding acidity to a soil on the alkaline side of the optimum pH will be associated with an increase in species richness as pH approaches the optimum pH from the right side of the curve. In this context, decreasing soil pH may not necessarily be associated with damages to the environment, depending on the soil pH where the acidifying substance is deposited. Increasing spatial differentiation helps to better capture the specificities in this regard. Using EFs calculated on the basis of ecoregions in Brazil can better capture the disparities between ecoregions that belong to the same biome, which is discussed in Section 3.4.

The previous models for terrestrial acidification (Azevedo et al. 2013; Roy et al. 2014) focus on the acid side of the SR curve and define EFs based on PNOF = 0.5, i.e. where 50% of plant species potentially do not occur. This is inspired by the approach that is currently taken in ecotoxicity effect modelling (Pennington et al. 2004; Larsen and Hauschild 2007) to represent the impact of a toxic substance on the occurrence of species in the exposed ecosystem. Protons from acidification are thus treated in the same way as a toxic agent, but this approach ignores the bell shape of the species richness distributions and PNOF with an optimum pH value in terms of species richness meaning that addition of acidity may be associated with a higher species diversity as well as a lower one. The probability of either of the two may be represented by the fraction of areas within the region that have soil pH on either side of the optimum pH, thus reinforcing the relevance of area-integrated EF. Moreover, it is assumed that the larger the area of land with a certain soil pH, the greater the probability of this area to receive acidifying substances. Thus, as long as species richness is the basis of the indicator for ecosystem damage, the area-weighted effect factors (EF_{aw}) considering both sides of the curve should be preferred in calculation of EF for acidification since they reflect (i) how the acidifying emissions move the region away from or towards the optimum soil pH, and (ii) the contribution of each area of

land with a specific pH value within a region. Adopting this approach implies the possibility of obtaining negative characterization factors to be applied in the LCA studies.

Table 4. Effect factors for terrestrial acidification per biome and for total Brazil, for all species [PNOF.(mol H⁺. L⁻¹)⁻¹] and range-restricted species [PXF.(mol H⁺. L⁻¹)⁻¹]. Between parentheses the EFs with uncertainty ranges (EFs calculated considering the 95% confidence interval on the SR curves).

Biome	EF [PNOF.(mol H ⁺ . L ⁻¹) ⁻¹]		EF _{aw} [PNOF.(mol H ⁺ . L ⁻¹) ⁻¹]			EF _{aw-rr} [PXF.(mol H ⁺ . L ⁻¹) ⁻¹]
	(PNOF=0.5)		(area-weighted)			(range-restricted)
	Acid side	Alkaline side	Acid side	Alkaline side	Entire curve	Entire curve
	(set 4a)	(set 4a)	(set 4b)	(set 4b)	(set 5a)	(set 5b)
Tropical and subtropical moist broadleaf forests	1.12E+04 (1.09E+04; 1.16E+04)	-3.05E+05 (-3.07E+05; -3.04E+05)	9.06E+03	-3.04E+04	-2.13E+04 (-2.14E+04; -2.12E+04)	-2.61E+04 (-2.64E+04; -2.55E+04)
Tropical and subtropical dry broadleaf forests	1.32E+05 (1.26E+05; 1.39E+05)	-1.48E+06 (-1.48E+06; -1.48E+06)	7.14E+04	-3.64E+05	-2.93E+05 (-2.95E+05; -2.91E+05)	-1.07E+05 (-9.98E+04; -8.35E+04)
Tropical and subtropical grasslands, savannas and shrublands	5.29E+04 (5.12E+04; 5.47E+04)	-6.84E+05 (-6.85E+05; -6.85E+05)	3.20E+04	-1.42E+05	-1.10E+05 (-1.11E+05; -1.10E+05)	-1.23E+05 (-1.23E+05; -1.21E+05)
Flooded grasslands and savannas	2.18E+05 (1.95E+05; 2.48E+05)	-2.41E+06 (-2.44E+06; -2.42E+06)	1.22E+05	-6.19E+05	-4.98E+05 (-5.10E+05; -4.83E+05)	-5.03E+05 (-5.33E+05; -4.71E+05)
Deserts and xeric shrublands	1.11E+05 (1.08E+05; 1.14E+05)	-2.17E+06 (-2.18E+06; -2.17E+06)	4.61E+04	-7.74E+05	-7.28E+05 (-7.31E+05; -7.25E+05)	-7.62E+05 (-7.65E+05; -7.60E+05)
Mangroves	2.71E+04 (2.50E+04; 2.97E+04)	-3.25E+05 (-3.27E+05; -3.27E+05)	8.58E+03	-9.79E+04	-8.94E+04 (-9.03E+04; -8.80E+04)	1.88E+03 (-1.41E+04; -7.82E+03)
Brazil	1.14E+04 (1.11E+04; 1.16E+04)	-5.04E+05 (-5.07E+05; -5.02E+05)	8.11E+03	-1.02E+05	-9.37E+04 (-9.37E+04; -9.36E+04)	-8.89E+04 (-8.98E+04; -8.78E+04)

3.3. Spatial variability of the effect factors

In addition to providing EFs for the whole country and for the six biomes in Brazil (see Table 4), EFs were also calculated for 45 ecoregions in Brazil (see Table S3, in Electronic Supplementary Material, ESM) to use the large georeferenced species richness database to investigate the spatial variability of effect factors and discuss what the relevant level of spatial differentiation is in a country like Brazil. Table 4 shows significant variations of area-weighted EFs from $-2.13\text{E}+04$ to $-7.28\text{E}+05$ for total species and from $1.88\text{E}+03$ to $-7.62\text{E}+05$ for range-restricted species at biome level, while Table S3 and Figure 3 show EFs variations from $5.69\text{E}+04$ to $-3.13\text{E}+06$ for total species and from $5.16\text{E}+04$ to $-1.20\text{E}+06$ for range-restricted species at ecoregion level. Having EFs in fine resolutions thus helps to better understand the effects that soil pH changes may have on plant diversity, since variations in species richness distribution observed at the ecoregion level may no longer be visible at the scale of biomes or the whole country (Crespo-Mendes et al. 2018).

No consistent pattern was found in the distribution of EFs of ecoregions within the biome to which they belong. The variability of species richness distributions across the grouped ecoregions determined in Crespo-Mendes et al. (2018) is reflected in the EFs presented in Figure 4, with the EFs at the biome level and the spread of EFs for the subordinated ecoregions around it. The number of ecoregions that each biome comprises is not a good predictor of the range of variation around the biome EF. Tropical and subtropical moist broadleaf forests is the biome with by far the largest number of ecoregions (34), and it does have the broadest range of ecoregion EFs both for all species and for range-restricted species. The other five Brazilian biomes have considerably fewer ecoregions (between one and six) but the biome Flooded grasslands and savannas, a biome with only two ecoregions, shows a wide range of ecoregion EFs, in contrast to Mangroves – also a biome with only two ecoregions, as observed in Figure 4. There is poor correspondence between patterns observed for all species and for range-restricted species. For two ecoregions (Caqueta moist forests and Dry Chaco) it was not possible to determine an EF since these ecoregions could not be described by the regression model (data not shown, Crespo-Mendes et al. 2018).

To define at which geographical level the EF should be determined (i.e. at country, biome or ecoregion level), other elements that compose the impact characterization for terrestrial acidification should be considered. The level of spatial differentiation of a characterization model also relies on the spatial resolution adopted by the atmospheric fate models due to the

source-receptor relationship that the fate factors (FF) represent (i.e. climatic conditions and mechanisms from the emission of acidifying substances to their deposition (Roy et al. 2012a)) and on the exposure models, which express environmental conditions of the receiving environment through the exposure factors (XF). LC-Impact, Impact World+ and ReCiPe 2016 methodologies currently have adopted worldwide $2^{\circ} \times 2.5^{\circ}$ grid resolution fate and exposure factors developed by Roy et al. (2012a, b). Considering that (i) areas of the ecoregions in Brazil vary from 126 to 1895808 km² and (ii) 29 out of 47 ecoregions have an area larger than the grid size used in the fate and exposure factor models (assumed to be a maximum of 61738 km² to regions close to the equator), the EF at ecoregion level should be preferred since they show how EFs vary at a smaller scale and reflect the variability that is unknown when we operate at biome level (see Figure 4). In cases where it is not possible to determine an EF at the ecoregion level, we recommend as default to use the EF of the biome to which the ecoregion belongs. Moreover, the use of EFs determined at biome level is also recommended for countries composed mostly of ecoregions with an area smaller than the $2^{\circ} \times 2.5^{\circ}$ grid resolution used for the FF and XF, as these EFs are a good proxy of the area-weighted average of the ecoregion EFs (see Table 5).

At the country level, the EF_{aw} based on the SR curve for whole Brazil ($EF_{aw} = -9.37E+04$ PNOF.(mol H⁺. L⁻¹)⁻¹, see Table 4) can also be used as a proxy of the EF_{aw} based on the area-weighted average of the biome EF_{aw} 's for biomes inside the country ($EF_{aw} = -1.19E+05$ PNOF.(mol H⁺. L⁻¹)⁻¹), being recommended if a country-specific effect factor is needed.

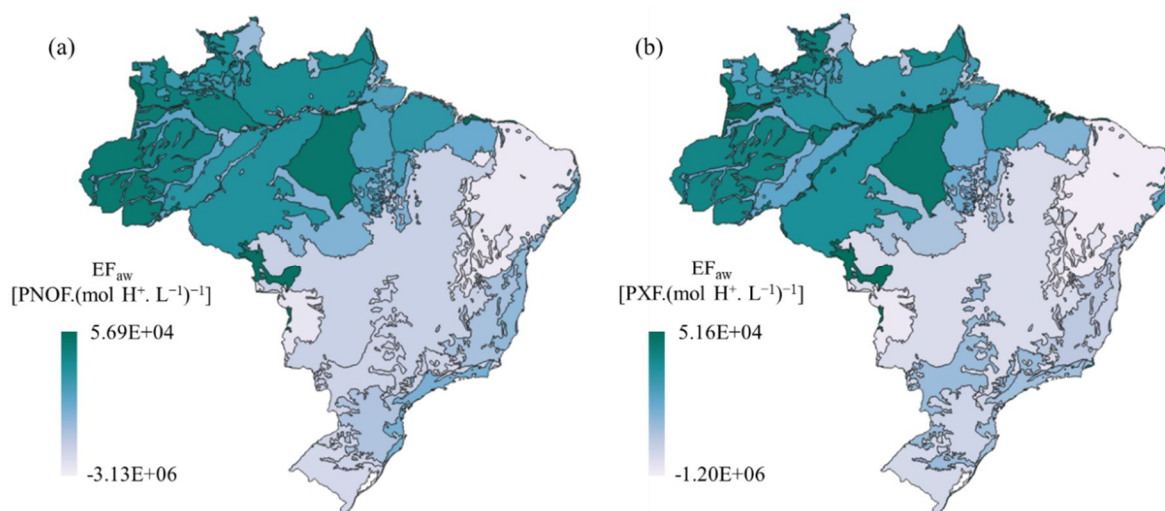


Figure 3. Area-weighted effect factors (EF_{aw}) for ecoregions in Brazil: (a) EF_{aw} [PNOF.(mol H⁺. L⁻¹)⁻¹] for all species and (b) EF_{aw} [PXF.(mol H⁺. L⁻¹)⁻¹] for range-restricted species.

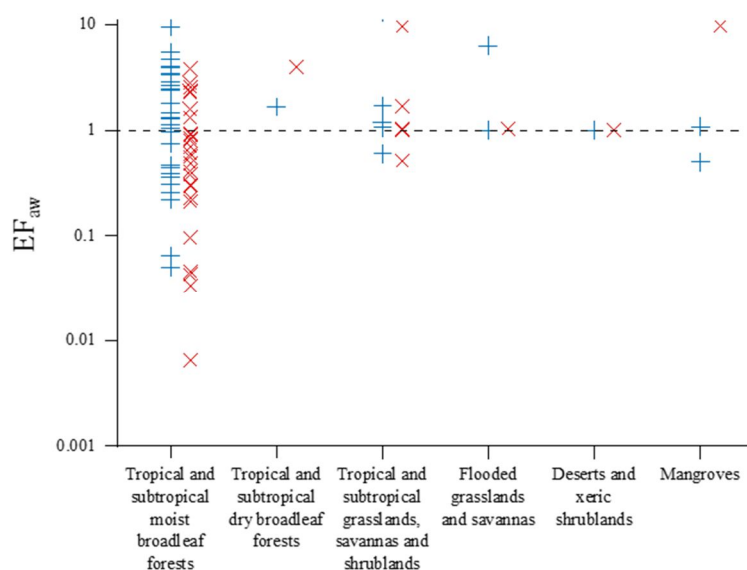


Figure 4. Overview of EF_{aw} [$\text{PNOF} \cdot (\text{mol H}^+ \cdot \text{L}^{-1})^{-1}$] for ecoregions normalized against the EF_{aw} of the biome to which they belong, for all species (cross (+)) and for range-restricted species (cross (x)). Dotted line represent the EF_{aw} at biome level. An overview of EF_{aw} calculated for the acid side and the alkaline side of the SR curve separately is presented in Figure S1, ESM.

Table 5. Comparison for biomes between EF_{aw} [$\text{PNOF} \cdot (\text{mol H}^+ \cdot \text{L}^{-1})^{-1}$] based on the biome SR curves and EF_{aw} [$\text{PNOF} \cdot (\text{mol H}^+ \cdot \text{L}^{-1})^{-1}$] based on the area-weighted average of the ecoregion EF_{aw} 's for ecoregions inside the biome.

Biome	(I)	(II)	II/I Ratio
	EF based on SR curve from Table 4 [$\text{PNOF} \cdot (\text{mol H}^+ \cdot \text{L}^{-1})^{-1}$]	EF based on area- weighted ecoregion EFs [$\text{PNOF} \cdot (\text{mol H}^+ \cdot \text{L}^{-1})^{-1}$]	
Tropical and subtropical moist broadleaf forests	-2.13E+04	-2.83E+04	1.33
Tropical and subtropical dry broadleaf forests	-2.93E+05	-3.01E+05	1.03
Tropical and subtropical grasslands, savannas and shrublands	-1.10E+05	-1.18E+05	1.07
Flooded grasslands and savannas	-4.98E+05	-5.07E+05	1.02
Deserts and xeric shrublands	-7.28E+05	-7.28E+05	1.00
Mangroves	-8.94E+04	-8.07E+04	0.90

3.4. Potentially Extinct Fraction (PXF) as a complementary metric for terrestrial acidification in LCIA

Range-restricted species richness as a potential complementary indicator of biodiversity was integrated into the effect factors through the use of the Potentially Extinct Fraction (PXF) of species (set 5b, Section 2.4). PXF curves are narrower than PNOF curves for all biomes in Brazil, in particular for the biome Mangroves (see Figure 5), reflecting that range-restricted species occur across a smaller range of soil pH values. In terms of optimum pH, i.e. the pH value at which the highest number of range-restricted species occur (PXF=0), two behaviors are observed at the biome level. Some biomes have the same optimum pH value for all species and for range-restricted species. This is the case for Tropical and subtropical moist broadleaf forests and Tropical and subtropical grasslands, savannas and shrublands. The remaining biomes present a slight displacement of the PXF curve towards more alkaline pH, showing an optimum pH for range-restricted species that is higher than the optimum pH for the entire list of species. For the first group of biomes, relying the EF on PNOF or PXF brings similar results since the maximum number of species in the biome coincides in terms of soil pH with the maximum number of range-restricted species. For the second group of biomes where the PXF curve is displaced relative to the PNOF curve, changes in the soil caused by acidifying substances may be associated with a decrease in the number of range-restricted species that is not necessarily reflected by a decrease in the total species richness. These two behavior patterns observed at biome level were also observed at ecoregion level (see Figure S1, ESM). For most ecoregions (mainly those that compose the biomes Tropical and subtropical moist broadleaf forests and Tropical and subtropical grasslands, savannas and shrublands) the optimum pH for range-restricted species is lower than the optimum pH for the entire list of species (Crespo-Mendes et al. 2018), causing a slight displacement of the PXF curves towards acid pH.

Since PNOF curves are often not a good proxy for PXF curves, the two should be considered as complementary metrics to assess the effects of terrestrial acidification on biodiversity. The first focusing on the total quantity of species of a region and the last prioritizing the conservation of unique species, thus preventing the extinction of potentially endemic species of Brazil or, possibly, of the world. To support the application of the PXF concept in the LCIA methodologies, EFs were calculated following the scenario recommended in Section 3.2. Area-weighted EF for the entire curve at country, biome and ecoregion levels are presented in Table 4 and Table S3 (ESM), respectively. For six ecoregions (see Table S3, ESM), for which EFs

were not attributed due to lack of data for their regression model (Crespo-Mendes et al. 2018), it is recommended to use the EF of the biome to which they belong.

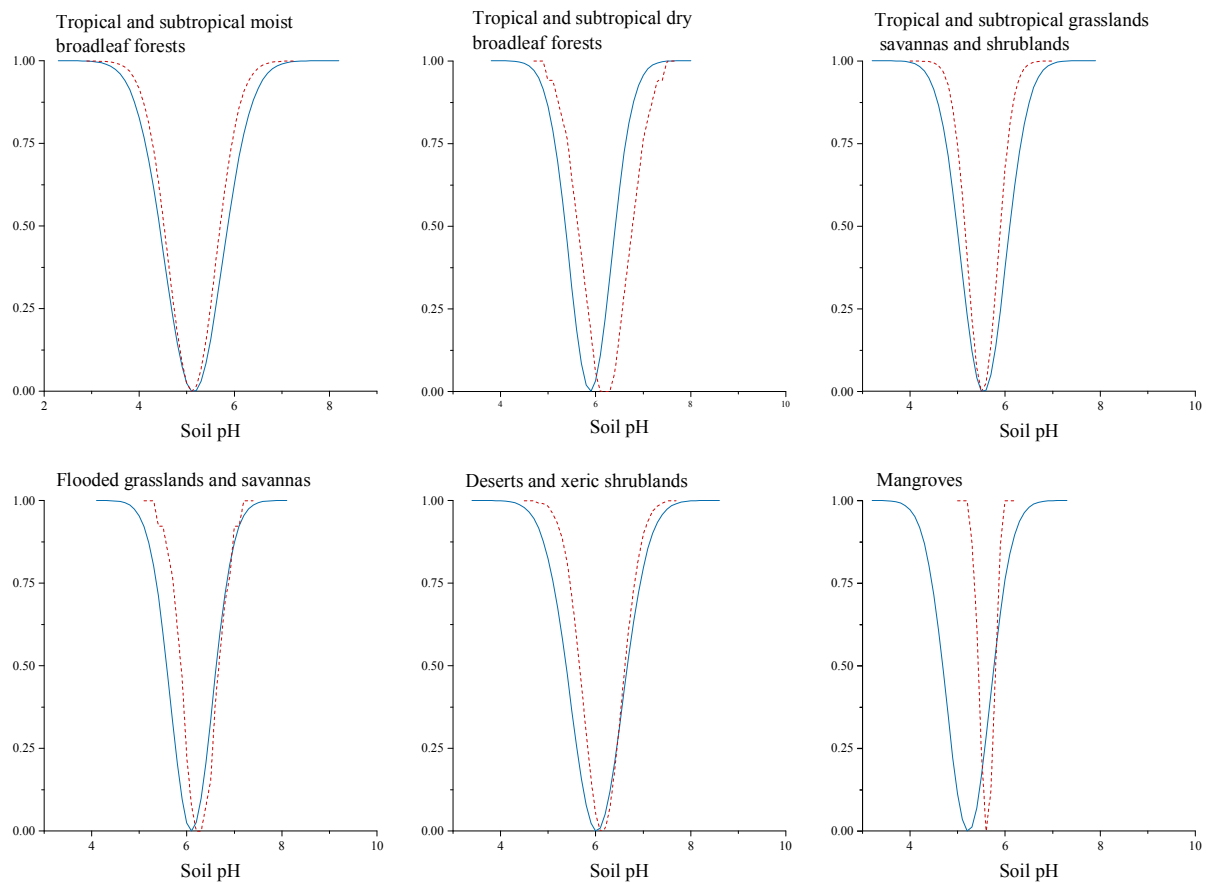


Figure 5. Potentially not occurring fractions (PNOF) of species (thick curve) and Potentially Extinct Fraction (PXF) of species (dotted curve) per biome (6) in Brazil. More PNOF and PXF curves at the country, biome and ecoregion levels in Brazil are presented in Figures S2, S3 and S4 (ESM), respectively.

3.5. Uncertainties

Sources of uncertainty in this study stem mainly from the data sets used to build the species richness curves as a function of soil pH. Crespo-Mendes et al. (2018) have identified the main sources as: inaccurate georeferencing of plant occurrences, errors in taxonomic identification, classification and/or selection of plant species with terrestrial habitat and estimates of soil pH values. Initiatives taken to minimize their effects are detailed in Crespo-Mendes et al. (2018). The first three sources refer directly to the botanical inventory used to represent the species richness of Brazil. However, given the large data set, the high number of species occurrences

for all biomes and most ecoregions in Brazil these sources of uncertainty are assumed to be negligible (Crespo-Mendes et al. 2018). Uncertainties related to the statistical modeling of the distribution of soil pH values are the most relevant for this study since pH is used as an indicator of soil acidity. Considering that the width of the prediction interval used to propagate the uncertainties in models presents the same variation for all regions, there is a same probability that the correct value is higher or lower than the average one used in this study. Thus, it is not expected a significant influence on species richness curves (Crespo-Mendes et al. 2018).

Regarding the uncertainties introduced by the modeling of the PNOF and PXF curves, the parameters provided by the lognormal regression analysis are used to estimate the 95% confidence interval and propagate them to the effect factor values. These EFs with uncertainty ranges can be used for comparisons with existing factors, e.g. EFs by Azevedo et al. (2013). At country and biome levels, the uncertainty ranges related to the area-weighted EFs are lower than 3% (see Table 4) and for most ecoregions the uncertainty ranges are lower than 10%, as shown in Table S4, ESM.

4. CONCLUSIONS AND RECOMMENDATIONS

Effect factors to assess terrestrial acidification in LCIA are provided for all six biomes and 45 ecoregions in Brazil based on a representative data set of approximately 30000 terrestrial plant species. Comparisons with previous studies (Azevedo et al. 2013; Roy et al. 2014) show that using more comprehensive data sets avoids underestimating the species richness, thus yielding more accurate EFs. Additionally, having EFs in refined spatial resolutions such as ecoregions allows us to observe variations in the species richness distribution that are not necessarily observed at the country or biome level. Besides providing EFs that are compatible with those adopted by the most recent LCIA methodologies, we also provide area-weighted EFs for the whole country, biomes and ecoregions that enable the consideration of both acid and alkaline sides of the species richness curves. We recommend these latter EF values for application in LCIA of terrestrial acidification.

The metrics to assess damages from terrestrial acidification are presented as key points of this study. Species richness is the metric that is generally used to represent damage to ecosystems in current LCIA methods at endpoint level, and previous effect factor models have focused exclusively on the acid side of the species richness or PNOF curves for determination of the

effect factor (Azevedo et al. 2013; Roy et al. 2014). This approach, which has been adopted for terrestrial acidification, is based on the species sensitivity distributions (SSD) method currently used for ecotoxicological impact assessment in LCIA (see Section 3.3). Our recommended area-weighted effect factors (EF_{aw}) however do not consider the protons from acidification as toxic agents, and the two sides of the SR curves are thus included in its calculation. As visible from Tables 4 and S3, acidification EF_{aw} values for Brazil at biome and ecoregion levels are mostly negative indicating that soils in Brazil are too alkaline (“under-acidified”) and that acidification overall brings benefits to plant species since lowering of soil pH will be associated with a higher species richness. However, biodiversity is inadequate as a sole indicator of ecosystem health since decreasing or increasing the number of species as a result of soil pH variance does not automatically reflect, for example, the functions of the species in the ecosystem (i.e. functional diversity). By focusing on species richness only, a number of species with unique functions in the ecosystem eventually can be lost, even if the number of species increases. The current situation may be improved by introducing further spatial differentiation to capture the differences in EFs among ecoregions inside a given biome that receive acid inputs, but the chosen level of spatial differentiation should respect the size of the deposition area for the emission.

An improvement potential also lies in introduction of the range-restricted species richness as a complementary metric – expressed as PXF – to represent the conservation of species that are unique to ecoregions in Brazil and could cause irreversible damage to the environment if lost. This is a relevant metric for the assessment of species vulnerability that could be integrated into terrestrial acidification characterization models. Another key point of the prevailing method for effect factor assessment, also applied in this study, concerns the lack of proven causality in the relation between soil pH and species occurrence. The work of Crespo-Mendes et al. (2018) and the resulting species richness curves demonstrate a very strong correlation between these two variables, but it does not prove that soil pH is the driver for the species occurrence. Many other environmental factors are potentially influential (nutrients, light, local climate, etc.) and although the correlations are strong, there may be confounding factors like the fact that the soils with the most frequent pH values represent the largest surface of the region and are hence also the soils most likely to be observed and have their plant species reported in the database. All these above findings therefore point out to the inadequacy of species richness to comprehensively represent damage to ecosystems and call for complementary indicators.

Overall, based on the methodological elements developed in this study, that rely on the use of comprehensive databases such as the botanical inventory reported by Crespo-Mendes et al. (2018), we recommend the integration into LCIA methodologies of area-weighted effect factors that consider the entire species richness distribution curves (both acid and alkaline parts of the curves) and the new complementary metric Potentially Extinct Fraction (PXF) of species, which could be used for characterizing terrestrial acidification as well as other impact categories. To do so, further research is however needed to expand such work to other regions than Brazil and reach a complete global coverage, thus enabling to combine the EF with atmospheric fate factors and soil exposure factors to derive characterization factors to be used in LCIA.

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Article III: Electronic Supplementary Material 1

Effect factors of terrestrial acidification in Brazil for use in Life Cycle Impact Assessment

Natalia Crespo Mendes, Alexis Laurent and Michael Zwicky Hauschild*

Division for Quantitative Sustainability Assessment (QSA), Department of Management Engineering, Technical University of Denmark (DTU), 2800 Kgs. Lyngby, Denmark

* To whom correspondence should be addressed; e-mail: cm.natalia@gmail.com

This document includes:

- Supporting Tables S1 to S4
- Supporting Figures S1 to S4
- Supporting References

Supporting material (Tables S1 – S4)

Table S1. Coefficients and R^2 values provided by Crespo-Mendes et al. (2018), at country, biome and ecoregion levels.

	Species richness				Range-restricted species richness			
	R^2	a	b	x_0	R^2	a	b	x_0
Brazil	0.9989	3.41E-01	1.6104	6.46E-05	0.9820	1.32E-02	1.1590	1.68E-05
Biome: Tropical and subtropical moist broadleaf forests	0.9976	3.16E-01	1.4014	5.13E-05	0.9714	1.39E-02	1.1508	2.90E-05
Ecoregion:								
Alto Paraná Atlantic forests	0.9844	1.72E-02	0.7758	5.89E-06	0.9216	6.00E-04	0.1157	2.07E-06
Araucaria moist forests	0.9926	2.26E-02	0.8588	9.00E-06	0.9777	1.10E-03	0.7982	8.58E-06
Atlantic Coast restingas	0.9352	5.90E-03	1.4719	5.17E-05	0.6995	3.23E-05	0.0875	5.93E-06
Bahia coastal forests	0.9936	5.78E-02	1.0142	2.09E-05	0.9857	3.10E-03	0.8697	1.76E-05
Bahia interior forests	0.9940	1.85E-02	1.0004	6.45E-06	0.9466	2.00E-04	0.8413	4.43E-06
Caatinga Enclaves moist forests	0.9273	2.60E-03	0.5134	8.76E-06	0.7980	2.57E-05	0.4043	8.08E-06
Caqueta moist forests								
Guianan Highlands moist forests	0.8434	1.33E-02	0.1792	3.60E-05	0.9288	4.00E-04	0.1387	3.51E-05
Guianan moist forests	0.7866	7.64E-02	0.5598	2.00E-04	0.9318	6.10E-03	0.1342	1.00E-04
Guianan piedmont and lowland moist forests	0.8904	2.01E-02	0.9507	4.90E-05	0.7944	5.00E-04	0.4691	1.11E-05
Gurupa varzea	0.2890	1.00E-04	1.0134	2.08E-05				
Iquitos varzea	0.9361	7.50E-03	0.7388	1.19E-05	0.6916	1.00E-04	0.8176	1.28E-05
Japurá-Solimoes-Negro moist forests	0.9366	5.94E-02	1.4143	1.00E-04	0.7542	1.90E-03	0.7417	1.00E-04
Juruá-Purus moist forests	0.8562	1.67E-02	1.0028	6.00E-05	0.7090	2.00E-04	1.0696	6.45E-05
Madeira-Tapajós moist forests	0.9376	5.59E-02	0.8917	2.98E-05	0.7592	1.20E-03	0.5753	2.31E-05
Marajó varzea	0.9007	2.23E-02	1.0689	7.85E-05	0.6532	2.00E-04	0.5062	5.33E-05
Maranhão Babaçu forests	0.9856	8.10E-03	0.9047	1.44E-05	0.8626	6.78E-05	0.7332	9.15E-06
Mato Grosso seasonal forests	0.8588	5.36E-02	0.8934	2.44E-05	0.3252	2.60E-03	0.7734	2.92E-05
Monte Alegre varzea	0.8981	1.20E-02	0.9318	3.17E-05	0.8071	2.00E-04	0.1464	2.13E-05
Negro-Branco moist forests	0.9195	2.89E-02	1.4154	2.00E-04	0.7257	7.00E-04	1.4856	3.00E-04
Northeastern Brazil restingas	0.4985	2.00E-04	1.1385	6.24E-06				
Pantepui	0.5387	5.00E-04	0.9541	5.12E-05	0.2044	2.54E-05	1.0820	3.97E-05
Pernambuco coastal forests	0.9737	9.90E-03	0.6708	1.51E-05	0.3131	2.46E-05	0.8952	1.93E-05
Pernambuco interior forests	0.9503	9.70E-03	0.8766	1.19E-05	0.6943	2.61E-05	0.4341	7.48E-06

Purus-Madeira moist forests	0.9347	2.32E-02	0.5562	3.21E-05	0.7794	4.00E-04	0.3781	3.12E-05
Purus varzea	0.9471	2.26E-02	1.0275	4.82E-05	0.8030	2.00E-04	0.5259	1.66E-05
Rio Negro campinarana	0.8301	1.19E-02	1.4568	1.00E-04	0.3675	4.00E-04	1.7126	3.00E-04
Serra do Mar coastal forests	0.9888	3.80E-02	1.0542	1.40E-05	0.9517	1.30E-03	1.0187	1.41E-05
Solimões-Japurá moist forests	0.5614	1.01E-02	0.6606	1.00E-04				
Southwest Amazon moist forests	0.9799	2.45E-02	0.8290	1.70E-05	0.9606	1.40E-03	0.7615	1.64E-05
Tapajós-Xingu moist forests	0.9004	1.62E-02	0.8603	3.39E-05	0.8671	3.00E-04	0.2626	2.17E-05
Tocantins/Pindare moist forests	0.9673	4.88E-02	0.8120	5.33E-05	0.7467	3.00E-04	0.9055	5.21E-05
Uatuma-Trombetas moist forests	0.9224	1.36E-01	0.8600	7.34E-05	0.8892	6.00E-03	0.4144	4.85E-05
Xingu-Tocantins-Araguaia moist forests	0.9694	2.07E-02	0.8407	3.38E-05	0.8533	2.00E-04	0.2902	1.37E-05
Biome: Tropical and subtropical dry broadleaf forests	0.9913	6.30E-03	1.0262	3.72E-06	0.8554	1.96E-05	1.0950	2.09E-06
Ecoregion:								
Atlantic dry forests	0.9780	4.60E-03	0.8837	3.09E-06	0.9690	1.57E-05	0.8273	2.07E-06
Chiquitano dry forests	0.8486	1.90E-03	1.4515	8.55E-06	0.7830	5.55E-06	0.4474	3.71E-07
Biome: Tropical and subtropical grasslands, savannas and shrublands	0.9965	5.05E-02	1.0841	9.27E-06	0.9896	3.20E-03	0.7283	5.07E-06
Ecoregion:								
Campos Rupestres montane savanna	0.9674	8.70E-03	1.1208	6.91E-06	0.7410	9.65E-05	1.0525	6.35E-06
Cerrado	0.9948	3.70E-02	0.9654	7.41E-06	0.9949	2.70E-03	0.6701	4.78E-06
Dry Chaco								
Guianan savanna	0.9631	1.19E-02	1.1778	3.53E-05	0.6078	7.97E-05	1.4037	5.43E-05
Humid Chaco	0.9641	2.93E-05	0.2413	3.58E-07	0.9719	1.79E-06	0.2516	4.02E-07
Uruguayan savanna	0.9603	6.80E-03	0.9097	6.70E-06	0.9133	5.00E-04	0.9487	6.42E-06
Biome: Flooded grasslands and savannas	0.9555	1.40E-03	1.0205	2.26E-06	0.8593	9.76E-06	0.8017	1.02E-06
Ecoregion:								
Pantanal	0.9534	1.40E-03	1.0118	2.24E-06	0.8533	9.70E-06	0.7791	9.88E-07
Southern Cone Mesopotamian savanna	0.8793	3.38E-06	0.1940	1.22E-07				

Biome: Deserts and xeric shrublands	0.9984	1.02E-02	1.2627	4.68E-06	0.9948	1.00E-04	0.9286	1.72E-06
Ecoregion: Caatinga	0.9984	1.02E-02	1.2627	4.68E-06	0.9948	1.00E-04	0.9286	1.72E-06
Biome: Mangroves	0.9774	1.23E-02	1.0538	1.81E-05	0.5829	2.17E-05	0.3418	2.79E-06
Ecoregion: Amazon-Orinoco-Southern Caribbean mangroves	0.8607	9.10E-03	1.5301	9.33E-05	0.8981	9.00E-06	0.3483	2.51E-06
Southern Atlantic mangroves	0.9794	7.80E-03	0.8597	1.17E-05	0.4394	3.36E-05	1.0921	1.92E-05

Table S2. Coefficients for biomes provided by previous studies.

Biome	Azevedo et al. (2013)		Roy et al. (2014) ^a	
	α	β	α	β
Tropical and subtropical moist broadleaf forests	-3.55	0.18	-4.11	0.7
Tropical and subtropical dry broadleaf forests	Model did not fit the empirical data		-3.55	0.18 ^b
Tropical and subtropical grasslands, savannas and shrublands	-4.55	0.16	-4.73	0.07
Flooded grasslands and savannas	-5.31	0.33	-6.80	0.28
Deserts and xeric shrublands	-6.76	0.36	-6.79	0.29
Mangroves	-3.72	0.25	-3.87	0.16

^a "The data on lowest tolerable pH per species were reported by Azevedo et al. (2013)"

^b Biome whose coefficients were approximated by other biomes based on similar climate conditions

Table S3. Effect factors for terrestrial acidification per ecoregion in Brazil, for all species [PNOF.(mol H⁺. L⁻¹)⁻¹] and range-restricted species [PXF.(mol H⁺. L⁻¹)⁻¹].

	EF [PNOF.(mol H ⁺ . L ⁻¹) ⁻¹]		EF [PNOF.(mol H ⁺ . L ⁻¹) ⁻¹]			EF [PXF.(mol H ⁺ . L ⁻¹) ⁻¹]
	(PNOF=0.5)		(area-weighted)			(range-restricted)
	Acid side	Alkaline side	Acid side	Alkaline side	Entire curve	Entire curve
Biome: Tropical and subtropical moist broadleaf forests	1.12E+04	-3.05E+05	9.06E+03	-3.04E+04	-2.13E+04	-2.61E+04
Ecoregion:						
Alto Paraná Atlantic forests	9.44E+04	-5.89E+05	4.17E+04	-1.60E+05	-1.18E+05	-4.17E+04
Araucaria moist forests	5.79E+04	-4.40E+05	2.43E+04	-1.08E+05	-8.39E+04	-1.00E+05
Atlantic Coast restingas	1.19E+04	-3.83E+05	2.88E+03	-2.04E+05	-2.01E+05	1.84E+04
Bahia coastal forests	2.35E+04	-2.57E+05	9.14E+03	-6.95E+04	-6.04E+04	-7.39E+04
Bahia interior forests	7.64E+04	-8.05E+05	5.42E+04	-1.28E+05	-7.38E+04	-6.69E+04
Caatinga Enclaves moist forests	9.33E+04	-3.15E+05	1.39E+04	-9.97E+04	-8.57E+04	-7.32E+04
Caqueta moist forests						
Guianan Highlands moist forests	8.23E+04	-1.31E+05	8.66E+03	-1.00E+04	-1.37E+03	-2.49E+03
Guianan moist forests	4.54E+03	-1.70E+04	1.47E+03	-6.17E+03	-4.70E+03	-1.19E+03
Guianan piedmont and lowland moist forests	1.02E+04	-9.53E+04	8.22E+03	-1.48E+04	-6.57E+03	-1.70E+02
Gurupa varzea	2.35E+04	-2.73E+05	4.12E+03	-1.05E+05	-1.01E+05	
Iquitos varzea	4.85E+04	-2.79E+05	2.38E+04	-5.47E+04	-3.09E+04	-2.10E+04
Japurá-Solimoes-Negro moist forests	4.51E+03	-1.27E+05	5.87E+03	-4.60E+03	1.26E+03	-5.96E+03
Juruá-Purus moist forests	8.21E+03	-8.75E+04	8.59E+03	-9.65E+03	-1.06E+03	-8.74E+02
Madeira-Tapajós moist forests	1.72E+04	-1.41E+05	1.28E+04	-2.04E+04	-7.67E+03	-5.56E+03
Marajó varzea	6.24E+03	-7.73E+04	3.05E+03	-2.68E+04	-2.37E+04	-1.07E+04
Maranhão Babaçu forests	3.52E+04	-2.97E+05	2.24E+04	-6.03E+04	-3.79E+04	-2.44E+04
Mato Grosso seasonal forests	2.09E+04	-1.73E+05	8.03E+03	-6.10E+04	-5.29E+04	-6.15E+04
Monte Alegre varzea	1.59E+04	-1.43E+05	7.59E+03	-6.42E+04	-5.66E+04	1.18E+04

Negro-Branco moist forests	3.49E+03	-9.85E+04	3.10E+03	-1.29E+04	-9.83E+03	-1.27E+04
Northeastern Brazil restingas	7.92E+04	-1.16E+06	9.96E+04	-4.27E+04	5.69E+04	
Pantepui	9.73E+03	-9.35E+04	4.79E+03	-3.23E+04	-2.75E+04	-1.47E+04
Pernambuco coastal forests	4.13E+04	-2.01E+05	2.04E+04	-4.07E+04	-2.04E+04	-9.91E+03
Pernambuco interior forests	4.33E+04	-3.44E+05	1.85E+04	-8.98E+04	-7.13E+04	-3.46E+04
Purus-Madeira moist forests	2.34E+04	-8.76E+04	1.02E+04	-1.84E+04	-8.23E+03	-1.83E+04
Purus varzea	1.02E+04	-1.15E+05	5.39E+03	-3.33E+04	-2.80E+04	-2.37E+04
Rio Negro campinarana	5.79E+03	-1.78E+05	4.51E+03	-2.03E+04	-1.58E+04	-1.56E+04
Serra do Mar coastal forests	3.51E+04	-4.23E+05	2.35E+04	-7.51E+04	-5.16E+04	-5.96E+04
Solimões-Japurá moist forests	5.09E+03	-2.41E+04	3.94E+03	-1.79E+03	2.16E+03	
Southwest Amazon moist forests	3.14E+04	-2.22E+05	2.10E+04	-1.78E+04	3.21E+03	-1.12E+03
Tapajós-Xingu moist forests	1.54E+04	-1.17E+05	1.40E+04	-5.54E+03	8.46E+03	1.54E+04
Tocantins/Pindare moist forests	1.01E+04	-6.90E+04	5.87E+03	-1.53E+04	-9.44E+03	-7.78E+03
Uatuma-Trombetas moist forests	7.10E+03	-5.44E+04	3.94E+03	-9.43E+03	-5.48E+03	-7.92E+03
Xingu-Tocantins-Araguaia moist forests	1.56E+04	-1.14E+05	8.89E+03	-3.13E+04	-2.25E+04	-2.20E+04
Biome: Tropical and subtropical dry broadleaf forests	1.32E+05	-1.48E+06	7.14E+04	-3.64E+05	-2.93E+05	-1.07E+05
Ecoregion:						
Atlantic dry forests	1.66E+05	-1.35E+06	6.66E+04	-5.57E+05	-4.91E+05	-4.28E+05
Chiquitano dry forests	7.05E+04	-2.16E+06	6.61E+04	-4.10E+04	2.51E+04	5.16E+04
Biome: Tropical and subtropical grasslands, savannas and shrublands	5.29E+04	-6.84E+05	3.20E+04	-1.42E+05	-1.10E+05	-1.23E+05
Ecoregion:						
Campos Rupestres montane savanna	7.13E+04	-9.99E+05	3.83E+04	-2.25E+05	-1.86E+05	-2.06E+05
Cerrado	6.70E+04	-6.55E+05	3.67E+04	-1.54E+05	-1.17E+05	-1.27E+05
Dry Chaco						
Guianan savanna	1.41E+04	-2.29E+05	8.47E+03	-7.46E+04	-6.61E+04	-6.34E+04
Humid Chaco	5.55E+06	-9.95E+06	1.29E+06	-2.75E+06	-1.46E+06	-1.20E+06

Uruguayan savanna	7.57E+04	-6.49E+05	4.78E+04	-1.79E+05	-1.31E+05	-1.24E+05
Biome: Flooded grasslands and savannas	2.18E+05	-2.41E+06	1.22E+05	-6.19E+05	-4.98E+05	-5.03E+05
Ecoregion:						
Pantanal	2.19E+05	-2.38E+06	1.22E+05	-6.17E+05	-4.95E+05	-5.22E+05
Southern Cone Mesopotamian savanna	2.14E+07	-3.48E+07	3.41E+06	-6.54E+06	-3.13E+06	
Biome: Deserts and xeric shrublands	1.11E+05	-2.17E+06	4.61E+04	-7.74E+05	-7.28E+05	-7.62E+05
Ecoregion:						
Caatinga	1.11E+05	-2.17E+06	4.60E+04	-7.74E+05	-7.28E+05	-7.62E+05
Biome: Mangroves	2.71E+04	-3.25E+05	8.58E+03	-9.79E+04	-8.94E+04	1.88E+03
Ecoregion:						
Amazon-Orinoco-Southern Caribbean mangroves	7.06E+03	-2.61E+05	2.14E+03	-9.86E+04	-9.65E+04	1.84E+04
Southern Atlantic mangroves	4.46E+04	-3.41E+05	2.33E+04	-6.82E+04	-4.49E+04	-4.04E+04

Table S4. Recommended effect factors for terrestrial acidification at country, biome and ecoregion level in Brazil, for all species [PNOF.(mol H⁺. L⁻¹)⁻¹] and range-restricted species [PXF.(mol H⁺. L⁻¹)⁻¹]. Between parentheses the EFs with uncertainty ranges (EFs calculated considering the 95% confidence interval on the SR curves).

	EF [PNOF.(mol H ⁺ . L ⁻¹) ⁻¹] (PNOF=0.5)		EF [PNOF.(mol H ⁺ . L ⁻¹) ⁻¹] (area-weighted)	EF [PXF.(mol H ⁺ . L ⁻¹) ⁻¹] (range-restricted)
	Acid side	Alkaline side	Entire curve	Entire curve
Brazil	1.14E+04 (1.11E+04; 1.16E+04)	-5.04E+05 (-5.07E+05; -5.02E+05)	-9.37E+04 (-9.37E+04; -9.36E+04)	-8.89E+04 (-8.98E+04; -8.78E+04)
Biome: Tropical and subtropical moist broadleaf forests	1.12E+04 (1.09E+04; 1.16E+04)	-3.05E+05 (-3.07E+05; -3.04E+05)	-2.13E+04 (-2.14E+04; -2.12E+04)	-2.61E+04 (-2.64E+04; -2.55E+04)
Ecoregion:				
Alto Paraná Atlantic forests	9.44E+04 (8.89E+04; 1.01E+05)	-5.89E+05 (-5.93E+05; -5.83E+05)	-1.18E+05 (-1.19E+05; -1.17E+05)	-4.17E+04 (-3.95E+04; -3.63E+04)
Araucaria moist forests	5.79E+04 (5.55E+04; 6.06E+04)	-4.40E+05 (-4.43E+05; -4.39E+05)	-8.39E+04 (-8.48E+04; -8.29E+04)	-1.00E+05 (-1.02E+05; -9.75E+04)
Atlantic Coast restingas	1.19E+04 (1.04E+04; 1.49E+04)	-3.83E+05 (-4.10E+05; -3.93E+05)	-2.01E+05 (-2.06E+05; -1.98E+05)	1.84E+04 (-3.58E+04;)
Bahia coastal forests	2.35E+04 (2.25E+04; 2.46E+04)	-2.57E+05 (-2.57E+05; -2.57E+05)	-6.04E+04 (-6.09E+04; -5.98E+04)	-7.39E+04 (-7.47E+04; -7.31E+04)
Bahia interior forests	7.64E+04 (7.33E+04; 7.98E+04)	-8.05E+05 (-8.10E+05; -8.08E+05)	-7.38E+04 (-7.37E+04; -7.36E+04)	-6.69E+04 (-6.63E+04; -6.44E+04)
Caatinga Enclaves moist forests	9.33E+04 (8.33E+04; 1.05E+05)	-3.15E+05 (-3.27E+05; -3.04E+05)	-8.57E+04 (-9.24E+04; -8.01E+04)	-7.32E+04 (-5.55E+04; -2.48E+04)
Caqueta moist forests				
Guianan Highlands moist forests	8.23E+04 (7.12E+04; 9.63E+04)	-1.31E+05 (-1.45E+05; -1.20E+05)	-1.37E+03 (-9.07E+02; -1.97E+03)	-2.49E+03 (-1.96E+03; 7.14E+01)
Guianan moist forests	4.54E+03 (3.08E+03; 4.68E+03)	-1.70E+04 (-1.51E+04; -1.36E+04)	-4.70E+03 (-5.04E+03; -4.56E+03)	-1.19E+03 (-8.25E+02; -6.11E+02)
Guianan piedmont and lowland moist forests	1.02E+04 (8.62E+03; 1.26E+04)	-9.53E+04 (-9.90E+04; -9.66E+04)	-6.57E+03 (-6.50E+03; -6.10E+03)	-1.70E+02 (-9.33E+01; 5.17E+02)
Gurupa varzea	2.35E+04 (1.47E+04; 1.69E+05)	-2.73E+05 (-8.07E+05; -3.92E+05)	-1.01E+05 (-4.19E+04; 1.08E+05)	
Iquitos varzea	4.85E+04 (4.30E+04; 5.54E+04)	-2.79E+05 (-2.86E+05; -2.75E+05)	-3.09E+04 (-3.14E+04; -2.95E+04)	-2.10E+04 (-1.89E+04; -1.66E+04)
Japurá-Solimoes-Negro moist forests	4.51E+03 (4.81E+03; 7.93E+03)	-1.27E+05 (-1.84E+05; -1.64E+05)	1.26E+03 (1.96E+03; 2.65E+03)	-5.96E+03 (-3.97E+03; -2.79E+03)
Juruá-Purus moist forests	8.21E+03 (6.77E+03; 1.07E+04)	-8.75E+04 (-9.10E+04; -9.01E+04)	-1.06E+03 (-1.60E+03; 2.39E+02)	-8.74E+02 (-7.61E+02; 1.19E+03)
Madeira-Tapajós moist forests	1.72E+04 (1.52E+04; 1.99E+04)	-1.41E+05 (-1.44E+05; -1.41E+05)	-7.67E+03 (-8.27E+03; -6.61E+03)	-5.56E+03 (-7.61E+03; -2.79E+03)
Marajó varzea	6.24E+03 (5.32E+03; 7.73E+03)	-7.73E+04 (-7.97E+04; -7.94E+04)	-2.37E+04 (-2.40E+04; -2.32E+04)	-1.07E+04 (-4.89E+03; -1.47E+04)
Maranhão Babaçu forests	3.52E+04 (3.32E+04; 3.76E+04)	-2.97E+05 (-2.99E+05; -2.96E+05)	-3.79E+04 (-3.84E+04; -3.73E+04)	-2.44E+04 (-2.97E+04; -1.68E+04)

Mato Grosso seasonal forests	2.09E+04 (1.73E+04; 2.66E+04)	-1.73E+05 (-1.80E+05; -1.74E+05)	-5.29E+04 (-5.40E+04; -4.97E+04)	-6.15E+04 (-6.05E+04; -5.63E+04)
Monte Alegre varzea	1.59E+04 (1.35E+04; 1.94E+04)	-1.43E+05 (-1.47E+05; -1.44E+05)	-5.66E+04 (-5.85E+04; -5.47E+04)	1.18E+04 (1.36E+04; 1.41E+04)
Negro-Branco moist forests	3.49E+03 (2.61E+03; 3.51E+03)	-9.85E+04 (-9.11E+04; -7.92E+04)	-9.83E+03 (-1.09E+04; -1.01E+04)	-1.27E+04 (-1.07E+04; -9.30E+03)
Northeastern Brazil restingas	7.92E+04 (5.60E+04; 2.28E+05)	-1.16E+06 (-1.83E+06; -1.48E+06)	5.69E+04 (4.38E+04; 6.33E+04)	
Pantepui	9.73E+03 (6.80E+03; 1.95E+04)	-9.35E+04 (-1.14E+05; -1.00E+05)	-2.75E+04 (-3.09E+04; -2.14E+04)	-1.47E+04 (; 5.98E+03)
Pernambuco coastal forests	4.13E+04 (3.84E+04; 4.47E+04)	-2.01E+05 (-2.05E+05; -1.98E+05)	-2.04E+04 (-2.08E+04; -1.96E+04)	-9.91E+03 (-4.43E+03; 2.40E+04)
Pernambuco interior forests	4.33E+04 (3.88E+04; 4.90E+04)	-3.44E+05 (-3.49E+05; -3.43E+05)	-7.13E+04 (-7.58E+04; -6.70E+04)	-3.46E+04 (-5.50E+04; -3.64E+04)
Purus-Madeira moist forests	2.34E+04 (2.10E+04; 2.64E+04)	-8.76E+04 (-9.12E+04; -8.45E+04)	-8.23E+03 (-8.70E+03; -7.56E+03)	-1.83E+04 (-1.85E+04; -1.48E+04)
Purus varzea	1.02E+04 (9.04E+03; 1.18E+04)	-1.15E+05 (-1.16E+05; -1.16E+05)	-2.80E+04 (-2.83E+04; -2.74E+04)	-2.37E+04 (-2.68E+04; -2.23E+04)
Rio Negro campinarana	5.79E+03 (4.93E+03; 1.01E+04)	-1.78E+05 (-2.22E+05; -2.15E+05)	-1.58E+04 (-1.28E+04; -1.20E+04)	-1.56E+04 (; -8.94E+02)
Serra do Mar coastal forests	3.51E+04 (3.31E+04; 3.73E+04)	-4.23E+05 (-4.24E+05; -4.23E+05)	-5.16E+04 (-5.17E+04; -5.10E+04)	-5.96E+04 (-5.97E+04; -5.83E+04)
Solimões-Japurá moist forests	5.09E+03 (4.28E+03; 1.10E+04)	-2.41E+04 (-3.85E+04; -2.81E+04)	2.16E+03 (2.71E+03; 1.49E+03)	
Southwest Amazon moist forests	3.14E+04 (2.92E+04; 3.38E+04)	-2.22E+05 (-2.24E+05; -2.21E+05)	3.21E+03 (3.31E+03; 3.24E+03)	-1.12E+03 (-1.13E+03; -8.11E+02)
Tapajós-Xingu moist forests	1.54E+04 (1.32E+04; 1.85E+04)	-1.17E+05 (-1.20E+05; -1.16E+05)	8.46E+03 (9.96E+03; 7.58E+03)	1.54E+04 (1.24E+04; 1.16E+04)
Tocantins/Pindare moist forests	1.01E+04 (9.26E+03; 1.11E+04)	-6.90E+04 (-7.00E+04; -6.86E+04)	-9.44E+03 (-9.68E+03; -9.10E+03)	-7.78E+03 (-6.91E+03; -4.51E+03)
Uatuma-Trombetas moist forests	7.10E+03 (6.20E+03; 8.33E+03)	-5.44E+04 (-5.56E+04; -5.42E+04)	-5.48E+03 (-5.80E+03; -4.74E+03)	-7.92E+03 (-8.47E+03; -7.24E+03)
Xingu-Tocantins-Araguaia moist forests	1.56E+04 (1.43E+04; 1.72E+04)	-1.14E+05 (-1.15E+05; -1.13E+05)	-2.25E+04 (-2.24E+04; -2.23E+04)	-2.20E+04 (-2.24E+04; -1.77E+04)
Biome: Tropical and subtropical dry broadleaf forests	1.32E+05 (1.26E+05; 1.39E+05)	-1.48E+06 (-1.48E+06; -1.48E+06)	-2.93E+05 (-2.95E+05; -2.91E+05)	-1.07E+05 (-9.98E+04; -8.35E+04)
Ecoregion:				
Atlantic dry forests	1.66E+05 (1.54E+05; 1.80E+05)	-1.35E+06 (-1.36E+06; -1.34E+06)	-4.91E+05 (-4.92E+05; -4.88E+05)	-4.28E+05 (-4.67E+05; -4.22E+05)
Chiquitano dry forests	7.05E+04 (5.87E+04; 1.07E+05)	-2.16E+06 (-2.46E+06; -2.40E+06)	2.51E+04 (2.76E+04; 3.09E+04)	5.16E+04 (4.95E+04; 4.97E+04)
Biome: Tropical and subtropical grasslands, savannas and shrublands	5.29E+04 (5.12E+04; 5.47E+04)	-6.84E+05 (-6.85E+05; -6.85E+05)	-1.10E+05 (-1.11E+05; -1.10E+05)	-1.23E+05 (-1.23E+05; -1.21E+05)
Ecoregion:				

Campos Rupestres montane savanna	7.13E+04 (6.48E+04; 8.01E+04)	-9.99E+05 (-1.01E+06; -1.01E+06)	-1.86E+05 (-1.90E+05; -1.81E+05)	-2.06E+05 (-2.05E+05; -1.79E+05)
Cerrado	6.70E+04 (6.45E+04; 6.97E+04)	-6.55E+05 (-6.57E+05; -6.54E+05)	-1.17E+05 (-1.17E+05; -1.17E+05)	-1.27E+05 (-1.27E+05; -1.26E+05)
Dry Chaco				
Guianan savanna	1.41E+04 (1.28E+04; 1.61E+04)	-2.29E+05 (-2.32E+05; -2.29E+05)	-6.61E+04 (-6.84E+04; -6.42E+04)	-6.34E+04 (-8.13E+04; -7.67E+04)
Humid Chaco	5.55E+06 (5.21E+06; 5.91E+06)	-9.95E+06 (-1.03E+07; -9.62E+06)	-1.46E+06 (-1.42E+06; -1.31E+06)	-1.20E+06 (-1.16E+06; -6.62E+05)
Uruguayan savanna	7.57E+04 (6.85E+04; 8.48E+04)	-6.49E+05 (-6.60E+05; -6.47E+05)	-1.31E+05 (-1.32E+05; -1.29E+05)	-1.24E+05 (-1.23E+05; -1.21E+05)
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Biome: Flooded grasslands and savannas	2.18E+05 (1.95E+05; 2.48E+05)	-2.41E+06 (-2.44E+06; -2.42E+06)	-4.98E+05 (-5.10E+05; -4.83E+05)	-5.03E+05 (-5.33E+05; -4.71E+05)
<hr/>				
Ecoregion:				
Pantanal	2.19E+05 (1.96E+05; 2.51E+05)	-2.38E+06 (-2.41E+06; -2.39E+06)	-4.95E+05 (-5.07E+05; -4.80E+05)	-5.22E+05 (-5.38E+05; -4.61E+05)
Southern Cone Mesopotamian savanna	2.14E+07 (1.86E+07; 2.46E+07)	-3.48E+07 (-3.83E+07; -3.09E+07)	-3.13E+06 (-3.37E+06; -2.97E+06)	
<hr/>				
Biome: Deserts and xeric shrublands	1.11E+05 (1.08E+05; 1.14E+05)	-2.17E+06 (-2.18E+06; -2.17E+06)	-7.28E+05 (-7.31E+05; -7.25E+05)	-7.62E+05 (-7.65E+05; -7.60E+05)
<hr/>				
Ecoregion:				
Caatinga	1.11E+05 (1.08E+05; 1.14E+05)	-2.17E+06 (-2.18E+06; -2.17E+06)	-7.28E+05 (-7.31E+05; -7.25E+05)	-7.62E+05 (-7.65E+05; -7.60E+05)
<hr/>				
Biome: Mangroves	2.71E+04 (2.50E+04; 2.97E+04)	-3.25E+05 (-3.27E+05; -3.27E+05)	-8.94E+04 (-9.03E+04; -8.80E+04)	1.88E+03 (-1.41E+04; -7.82E+03)
<hr/>				
Ecoregion:				
Amazon-Orinoco-Southern Caribbean mangroves	7.06E+03 (5.95E+03; 1.08E+04)	-2.61E+05 (-3.00E+05; -2.90E+05)	-9.65E+04 (-9.90E+04; -9.18E+04)	1.84E+04 (8.23E+03; 4.67E+04)
Southern Atlantic mangroves	4.46E+04 (4.15E+04; 4.82E+04)	-3.41E+05 (-3.45E+05; -3.40E+05)	-4.49E+04 (-4.49E+04; -4.44E+04)	-4.04E+04 (-2.23E+04; 7.13E+03)

Supporting Figures (S1-S4)

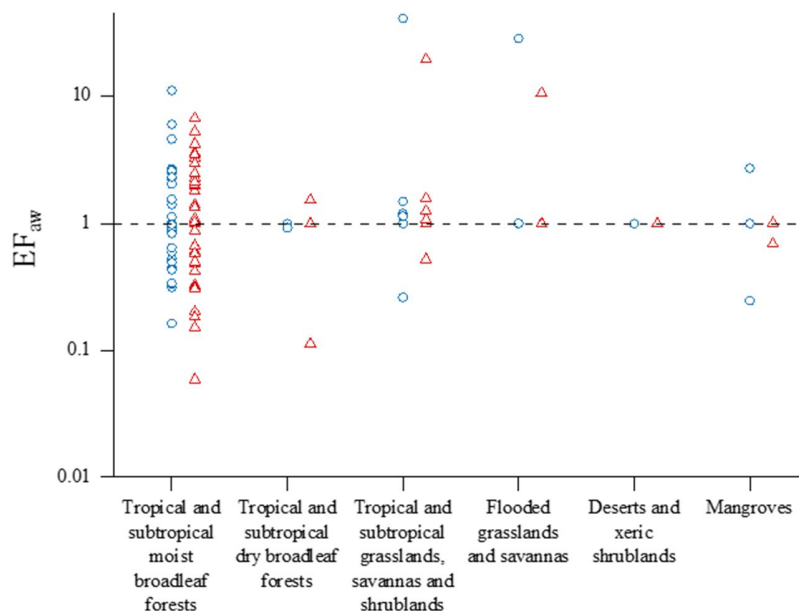


Figure S1. Overview of EF_{aw} calculated for the acid side and the alkaline side of the SR curve separately. The EF_{aw} for ecoregions were normalized against the EF_{aw} of the biome to which they belong. Circles (in blue) represent the EFs [PNOF.(mol H⁺. L⁻¹)⁻¹] calculated for all species and triangles (in red) represent the EFs [PXF.(mol H⁺. L⁻¹)⁻¹] calculated for range-restricted species. Dotted line represent the EF_{aw} at biome level.

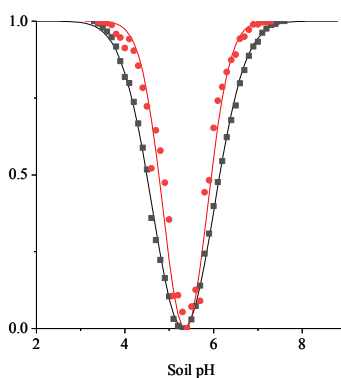


Figure S2. PNOF curve (in black) and PXF curve (in red) for Brazil. Dots represent the collected data.

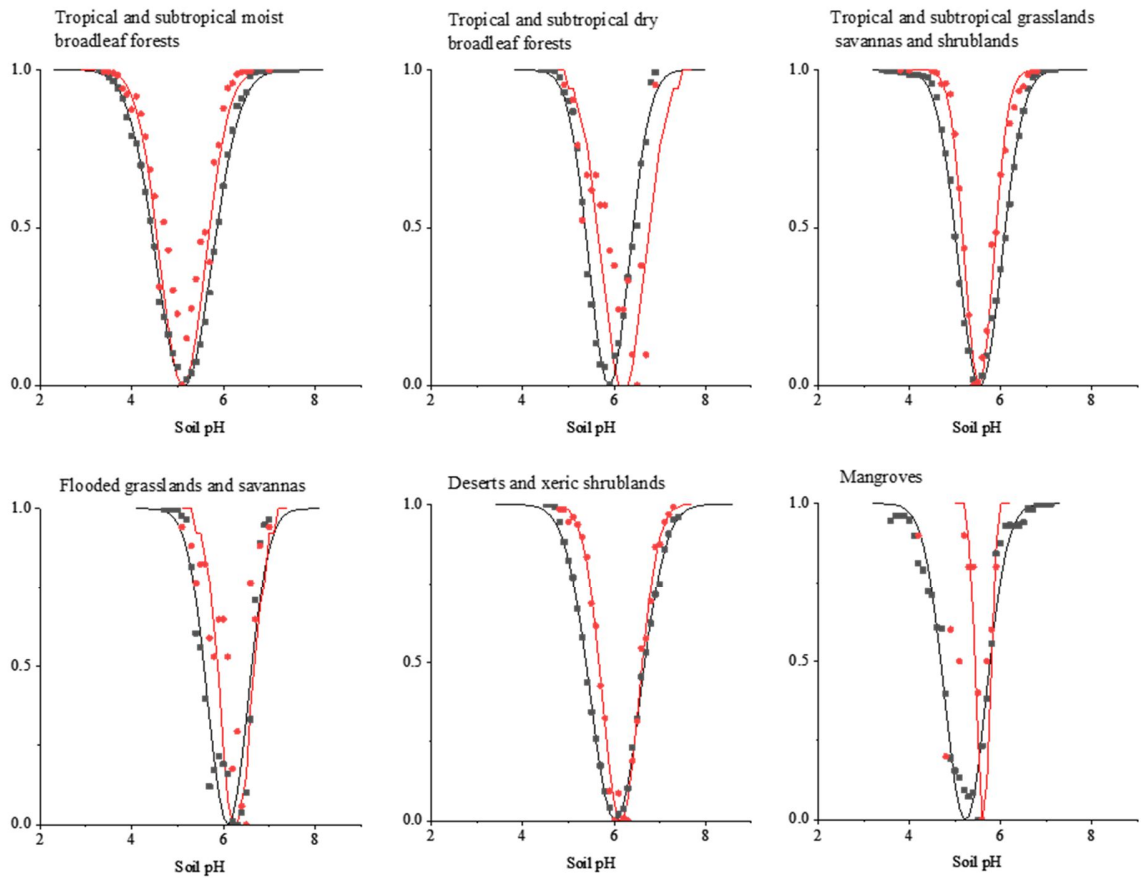
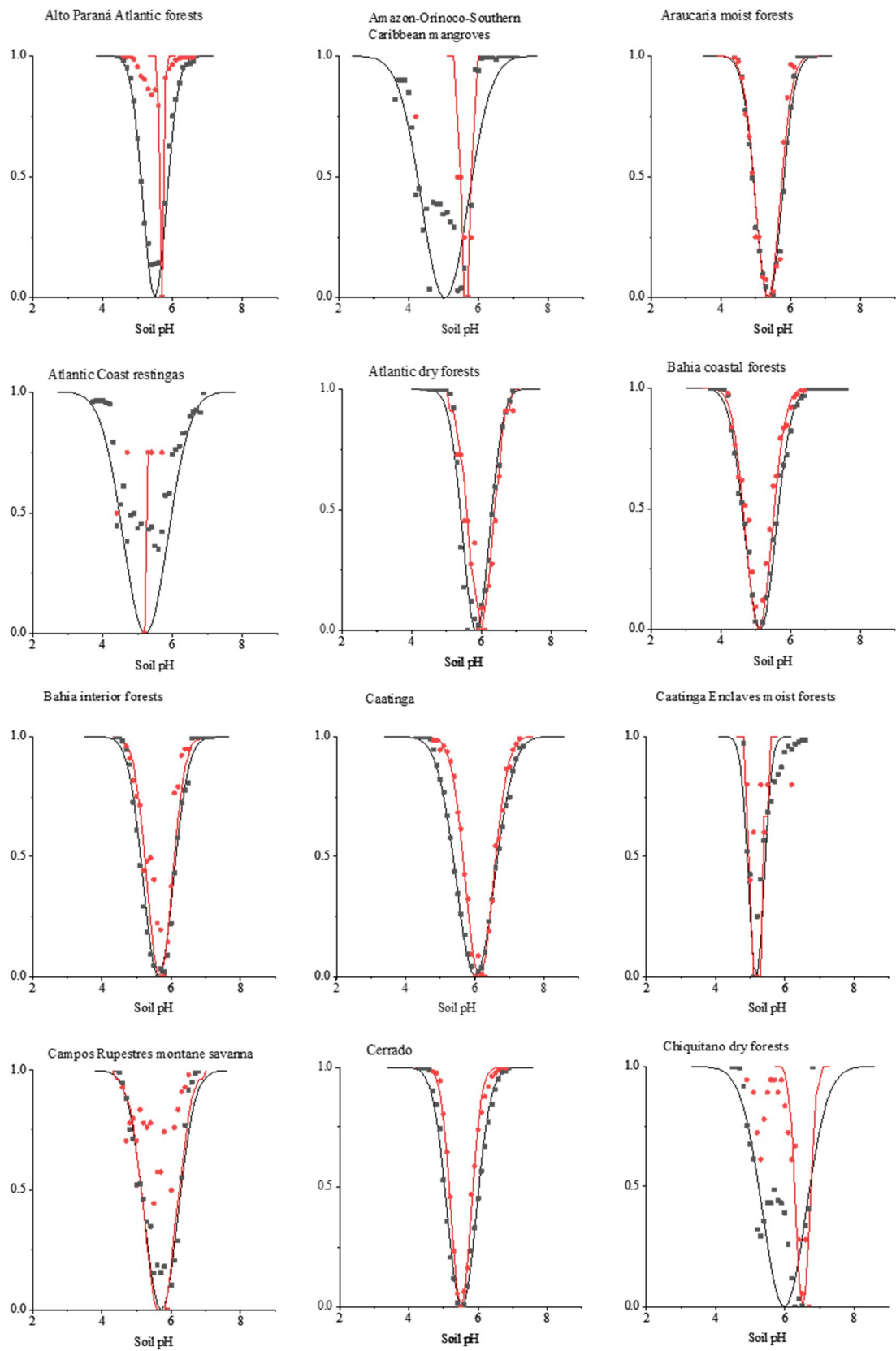
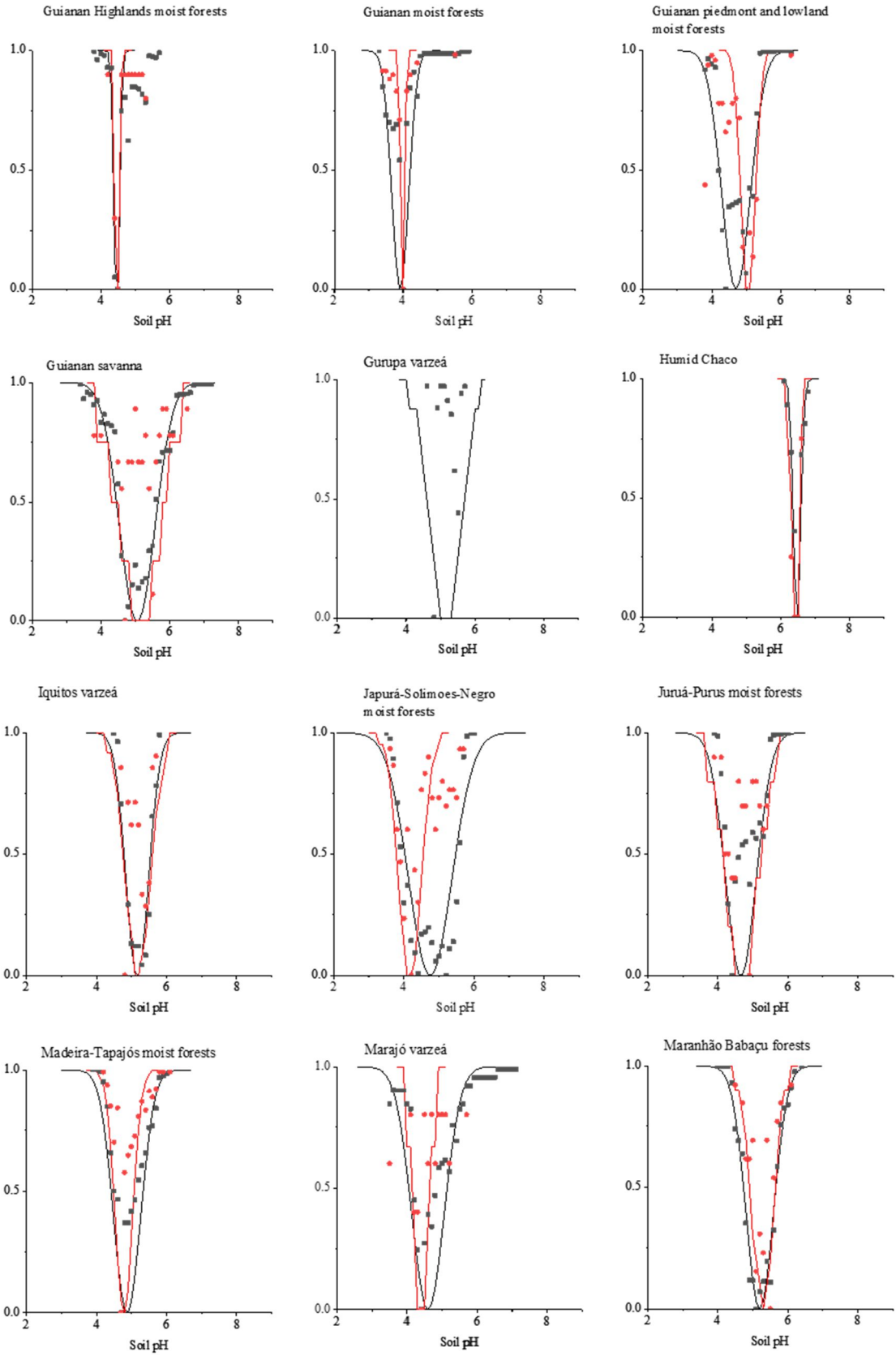
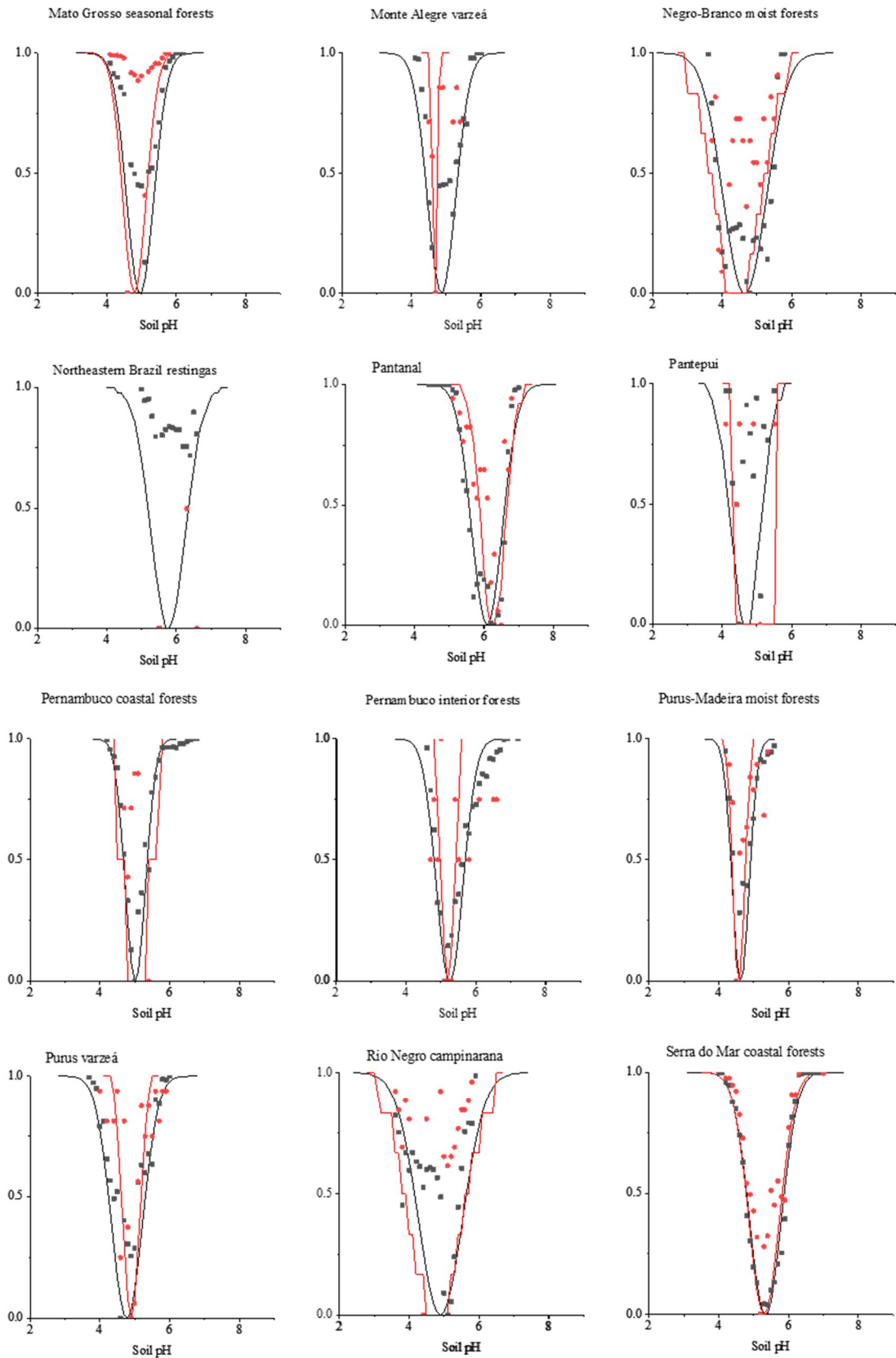


Figure S3. PNOF curves (in black) and PXF curves (in red) for biomes in Brazil. Dots represent the collected data.







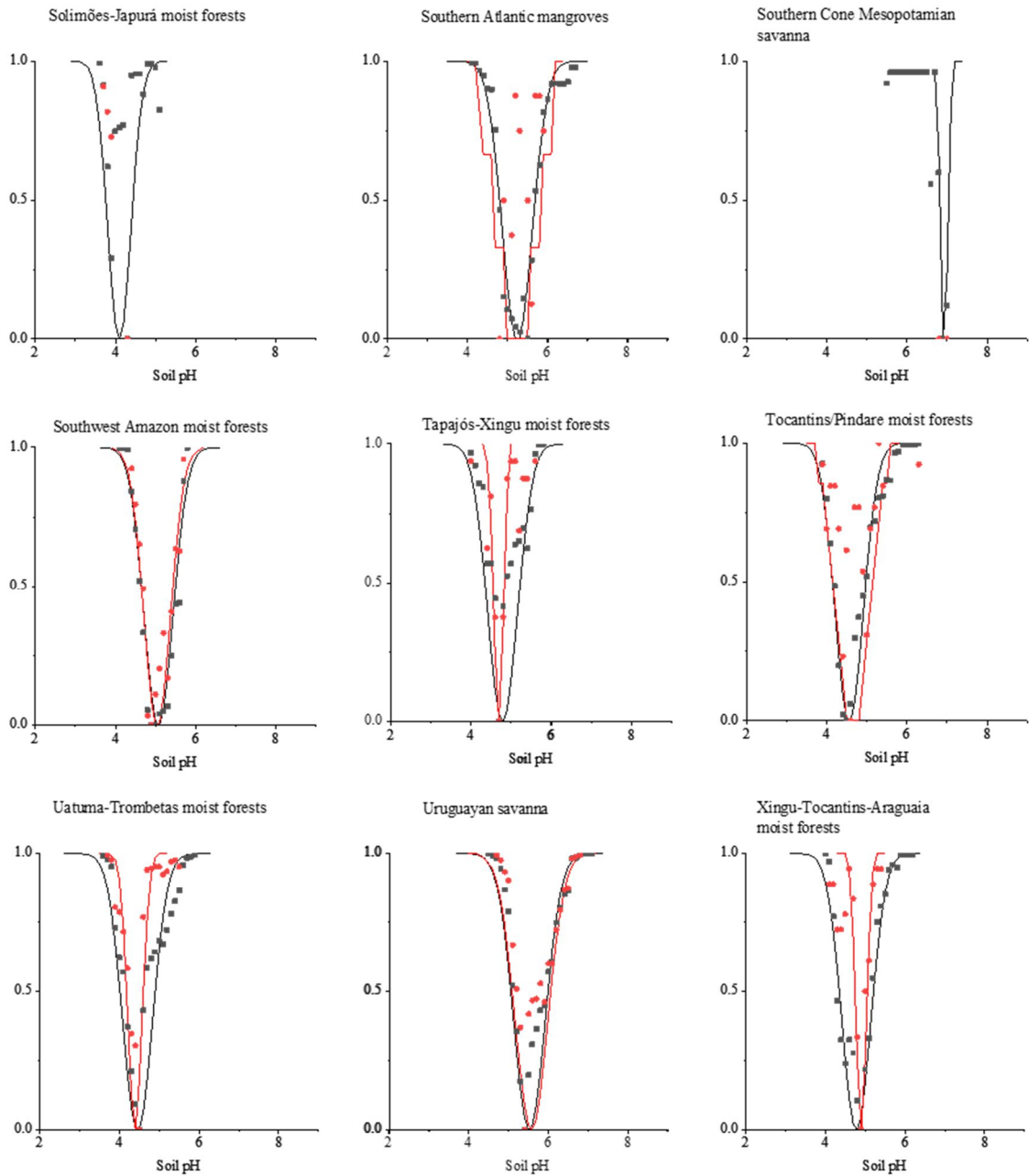


Figure S4. PNOF curves (in black) and PXF curves (in red) for ecoregions in Brazil. Dots represent the collected data.

Supporting References

Azevedo, L. B., van Zelm, R., Hendriks, A. J., Bobbink, R., Huijbregts, M. A. (2013). Global assessment of the effects of terrestrial acidification on plant species richness. *Environmental pollution*, 174, 10-15.

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