

Article

Earthworm Diversity, Forest Conversion and Agroforestry in Quang Nam Province, Vietnam

Rachmat Mulia¹, Sam Van Hoang^{2,*}, Van Mai Dinh³, Ngoc Bich Thi Duong⁴, Anh Duc Nguyen^{5,6}, Dang Hai Lam⁷, Duyen Thu Thi Hoang⁸ and Meine van Noordwijk⁹

¹ World Agroforestry (ICRAF), Hanoi 100000, Vietnam; r.mulia@cgiar.org

² Department of Forest Plant, Vietnam National University of Forestry, Hanoi 100000, Vietnam

³ Department of Soil, Vietnam National University of Forestry, Hanoi 100000, Vietnam; vandm@vnuf.edu.vn

⁴ Department of Environmental Engineering, Vietnam National University of Forestry, Hanoi 100000, Vietnam; ngocdtb@vnuf.edu.vn

⁵ Department of Soil Ecology, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi 100000, Vietnam; ducanh@iebr.ac.vn

⁶ Department of Ecology and Biological Resources, Graduate University of Technology and Science, Vietnam Academy of Science and Technology, Hanoi 100000, Vietnam

⁷ Department of Biology, School of Education, Can Tho University, Can Tho 900000, Vietnam; Lhdang@ctu.edu.vn

⁸ Climate Change and Development Program, Vietnam-Japan University, Vietnam National University, Hanoi 100000, Vietnam; htt.duyen@vju.ac.vn

⁹ World Agroforestry (ICRAF), Bogor 16155, Indonesia; m.vannoordwijk@cgiar.org

* Correspondence: samhv@vnuf.edu.vn; Tel.: +84-983-33-78-98

Abstract: The conversion of natural forests to different land uses still occurs in various parts of Southeast Asia with poor records of impact on ecosystem services and biodiversity. We quantified such impacts on earthworm diversity in two communes of Quang Nam province, Vietnam. Both communes are situated within buffer zones of a nature reserve where remaining natural forests are under threat of continued conversion. We identified 25 different earthworm species, out of which 21 were found in natural forests, 15 in agroforestry, 14 in planted forests, and seven each in annual croplands and home gardens. Out of the six species that were omnipresent inhabitants of all observed habitats, *Pontoscolex corethrurus* largely dominated habitats with intensive anthropogenic activities but was rare in natural forests. Natural and regenerated forests had a much denser earthworm population in the top 10 cm of soil rather than in deeper soil layers. We conclude that the conversion of natural forests into different land uses has reduced earthworm diversity which can substantially affect soil health and ecosystem functions in the two communes. Protection of the remaining natural forests is urgent, while the promotion of a tree-based farming system such as agroforestry can reconcile earthworm conservation and local livelihoods.

Keywords: land-use change; belowground biodiversity; soil engineers; *Pontoscolex corethrurus*; natural habitats; planted forest



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1. Introduction

The inclusion of 'planted forest' in forest statistics has been a major factor in the 'forest transition' concept: the reversal after long periods of decline towards a net gain in forest area [1,2]. Forest statistics can thus mask the ongoing loss of natural forest by larger gains in planted forest area, even though in terms of ecosystem services old-growth natural forests are very different from young planted forests [3]. Beyond the direct benefits that land-use change brings (or is expected to bring) to land users, a wide range of 'externalities' are generated, changes that do not play a role in the land users' decision making [4]. Where such externalities are negative for others in the landscape, corrective action is needed

in the form of regulations or incentives; where they are positive, forms of incentives are appropriate [5].

Many of the ecosystem services that, as externalities of decision-making, are influenced by land use are controlled by soil conditions [6]. Beyond vegetation with its aboveground litter and plant root systems and turnover, microbes, fungi, and soil fauna are important for modifying the soil conditions that in turn shape soil structure (pores, aggregates) influencing water flows and storage and net greenhouse gas emissions that determine ecosystem services [7]. As part of the global loss of biodiversity, loss of belowground biodiversity and its impact on ecosystem services is still poorly understood [8,9]. Changes in the soil can be relatively slow to be noticed by soil biota, as the system is buffered in many aspects, but once undesirable changes take place, they are also hard to correct, especially if they reach soil layers below the surface [10], as thresholds are past where both roots and earthworms have difficulties penetrating [11]. Better knowledge of changes in belowground biodiversity in the context of ‘forest transition’ patterns of land-use change is urgent for many parts of the tropics, especially.

Over the past decades, Vietnam’s forest cover has continued to expand from 28% in 1993 to 42% in 2018 [12]. Policy reforms and nation-wide forest protection and replanting programs have been the main drivers [13,14]. Forest expansion, including planted forests, has substantially contributed to economic growth, job creation, and poverty alleviation in the country [12]. However, despite the increasing trend, the conversion and degradation of natural forests still occur in different parts of the country. The existing natural forests in Vietnam are largely in poor condition or regenerating [12]. The forest cover rates overlook forest quality. Competing land uses, exploitation of forest resources, and weak forest governance are serious challenges for the country to protect the remaining and regenerating poor natural forests [12]. Massive conversion of natural forests still occurs for example in the Central Highlands and Central Annamites region of the country. The expansion of commercial crops and infrastructure development have been the main drivers of forest conversion in Central Highlands [15]. In the Central Annamites, considered as one of the largest continuous natural forest areas and key biodiversity landscapes in Asia, forest resources and wildlife are continuously under threat of intensive exploitation by surrounding forest-dependent populations [16,17].

Concerns over the impacts of forest conversions on ecosystem services and biodiversity have recently become more prominent in Vietnam [18,19]. Several sub-national and national policies to include the 2011–2020 National Biodiversity Strategy with vision to 2030 underline the need of taking up existing scientific- and evidence-based information and undertaking further research on such impacts while strengthening forest protection efforts. Several studies from other parts of the world have reported impacts of forest conversions on biodiversity including belowground biodiversity (e.g., [20,21]). Notwithstanding, accounts of such impacts in Vietnam are limited.

The diversity and activities of earthworms as one of the most important soil biotas influence the physical, chemical, and biological properties of soils [22,23]. Their physical movement creates soil pores which ease nutrient and water dynamic in the soils. As recyclers of organic materials, earthworms facilitate microorganisms such as fungi and bacteria to undertake further decomposing process [24]. Through these activities, earthworms influence soil health and the provision of ecosystem services [25]. The population and diversity of earthworm species vary across land habitats due to variation in soil moisture, soil temperature, soil properties, the abundance of surface litter, vegetation types, land use management, and human interventions [20,26–30]. Earthworms are thus sensitive to change in land use. In general, earthworm diversity is reduced in habitats with more intensive anthropogenic interventions [23,31].

Here, we describe the results of a study on earthworm diversity in natural forests and different land uses in two communes of Quang Nam province, South Central Coast region of Vietnam. The two communes are within the buffer zones of the Song Thanh Nature Reserve, one of the main reserves within the Central Annamites mountains, and parts of

main watersheds in the region. In both communes, the conversion of natural forests into shifting cultivations and agricultural systems, such as agroforestry and annual croplands, has been taking place for decades. The purpose of the study was to compare the diversity of earthworm species among natural forest and different land uses to investigate the impact of forest conversion and to recommend necessary measures related to earthworm conservation to maintain soil health and ecosystem functions in the two buffer-zone communes.

2. Material and Methods

2.1. Study Sites

The study was conducted in Phuoc My commune of Phuoc Son district and Ta Bhing commune of Nam Giang district, Quang Nam province ($15^{\circ}35'0''$ N– $107^{\circ}55'0''$ E), South Central Coast of Vietnam (Figure 1a). The two communes are within the buffer zones of Song Thanh Nature Reserve and parts of main watersheds in South Central Coast region which supply water for several cities including Da Nang as the biggest city in Central Vietnam. For example, Phuoc My is part of Dak Mi watershed (Figure 1b), while Ta Bhing is part of Vu Gia–Thu Bon as a bigger watershed [32]. Phuoc My is situated at 223–446 m above sea level while Ta Bhing is approximately 100 m above sea level. Both communes are mountainous with flat areas concentrated at the feet of the mountains, along riverbanks.

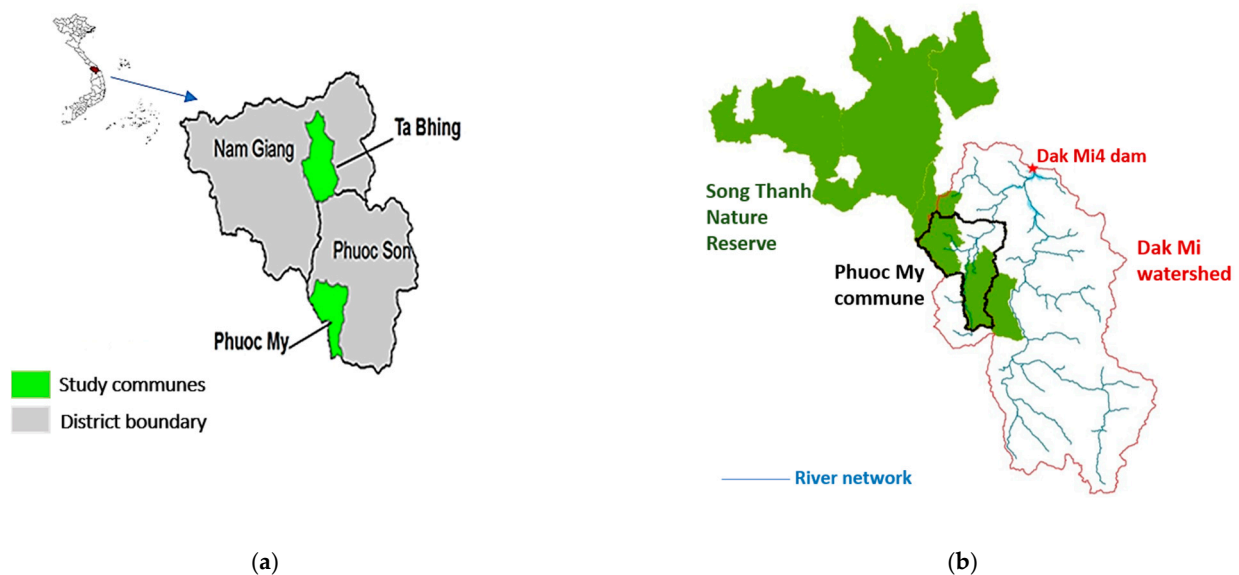


Figure 1. (a) Location of Phuoc My and Ta Bhing communes as study sites in Quang Nam province of Vietnam, (b) the boundary of Song Thanh Nature Reserve and Dak Mi watershed.

The two communes have a tropical monsoon climate. The annual rainfall is about 2650 mm in Phuoc My and 2300 mm in Ta Bhing [33]. In both communes, the rainy season usually peaks between September and November, and the dry season lasts from January until March. The highest temperature is usually recorded between May and July. The two communes have similar soil physical and chemical properties, dominated by sandy-loam and sandy-clay-loam textures [33].

Phuoc My has a smaller land size, but it has a larger area of rich natural forest than Ta Bhing in 2016. The total area of medium, poor, and regenerated natural forests accounts for 21% and 41.5% of the total land size in Phuoc My and Ta Bhing respectively. Land cover in the two communes has been classified into eleven types [16]. The area of each land cover in the two communes are described in Table A1.

2.2. Land-Use History

In both communes, massive conversion of natural forests took place more than 20 to 30 years ago [16]. Over the past two decades, natural forests have regenerated in some

areas of the communes, thanks to enhanced protection efforts by the local authorities. Planted forests have become popular in the past decade, especially the short-rotation (3–4 year) of *Acacia* (mainly the hybrid *Acacia mangium x auriculiformis*) plantation for pulp and paper. Two types of agroforestry have developed in the two communes: a *taungya* or temporary agroforestry in which annual crops such as cassava or maize become intercroops in young planted forests, usually in the first 1–2 years after tree planting before tree canopy closure; and more permanent agroforestry in which timber tree species, such as *Melia azedarach*, *Vernicia montana*, or *Ficus racemosa*, or fruit trees such as *Dimocarpus longan*, were intercropped with annual crops such as peanut, beans, or cassava. In sloping lands, agroforestry practices with rows of trees and rows of pineapple to limit soil erosion also existed.

2.3. Sample Plots by Habitat

The earthworm's observations were carried out in seven land cover types (hereafter called habitats). Three represent habitats with less anthropogenic interventions namely natural forests, regenerated forests, and grasslands (hereafter referred to as 'natural habitats'); and four with more intensive anthropogenic interventions namely planted forests, agroforestry, mixed annual crops, and home gardens (hereafter referred to as 'human-disturbed habitats'). Among the four, the most frequent and intensive anthropogenic interventions occurred in mixed annual crops and home gardens.

In total, we took 28 and 53 sample plots from the different habitats in Phuoc My and Ta Bhing commune respectively. More sample plots were taken in natural and regenerated forests to anticipate a possibility of higher species diversity in those habitats. Although both communes have similarities in many of the factors that can influence earthworm diversity such as climate condition, soil properties, and landcover types, we took sample plots in both communes, but more samples plots in Ta Bhing than Phuoc My merely for time and resource efficiency. Most of the local assistants involved in the sampling activities in both communes were from Ta Bhing. The aim was to have replications from both communes, not to compare earthworm diversity between the two communes. The number of sample plot by habitat in the two communes are given in Table A2. Figure 2 illustrates the vegetation covers and types in the selected habitats.



Figure 2. Cont.



Figure 2. Vegetation cover and types in (a) natural forest, (b) regenerated forest, (c) agroforestry, (d) plantation of *Acacia mangium x auriculiformis*, (e) plantation of *Melia azedarach*, (f) plantation for *Machilus odoratissima* Nees, (g) home garden, (h) grasslands, and (i) upland annual crops.

2.4. Identification of Earthworm Species

Following [34], at each sample plot, earthworm specimens were collected from a 50 cm × 50 cm soil block of 30 cm depth (please see Figure 2e,h,i). A 50 cm × 50 cm supporting frame and a woody measure of 30 cm long were used to ensure identical surface areas and thicknesses of all soil blocks. The specimens were collected by carefully segregating the soils per 10 cm soil depth. All specimens were stored in cloth bags to keep them alive for further treatments in the laboratory.

To facilitate species identification, all earthworms were separated from soils by cleansing with water. They were subsequently immersed in liquid containing 4% formalin for 6–12 h. Thereafter, all earthworms were transferred to fresh liquid of 4% formalin for long-term storage and morphological studies. We investigated morphological features of collected earthworms, such as intestinal caeca (presence and shape), male pores (presence of copulatory pouches), genital markings (type, location, and number), spermathecal pores (location, number, and type), septa (thin, thick or absent), and additional characteristics such as prostomium (probilobous, epilobous or tanylobous), first dorsal pore (location), intestinal origin, last heart, ovaries, and testes. Earthworm samples were scrutinized using stereo microscope Motic DM143-FBGG-C (Motic Company, Hongkong) with drawing tube and camera attached to the monitor. All specimens were compared and identified using original papers as described in [35–43]. All specimens with different morphological characters compared to all known species were named using their genera and sp. 1, 2, and so on. For biomass measurement, we used paper tissues to remove all liquids (formalin, water) and cast from the preserved earthworms before weighting using the electronic weight Sartorius GM612.

2.5. Indicators for Earthworm Dominance and Diversity

We used seven indicators to assess the dominance, density, and diversity of earthworm species. Following [34], five indicators represent dominance and density, namely density and biomass per soil layer, quantity and biomass dominance, and occurrence frequency. Two indicators, the Shannon–Wiener index [44] and similarity index [45], measure the level of diversity of earthworm species within habitat and similarity in species composition among habitats, respectively. For the similarity index, we omitted the sub-species component in the calculation. The seven indicators are described in Table A3.

2.6. Aboveground Litter Biomass as A Covariate

In this study, we focused on the impact of conversion from natural to human-disturbed habitats on earthworm diversity. Notwithstanding, to consider the effect of aboveground plant litter biomass on earthworm density (e.g., [21,46,47]), we used the annual production rate of aboveground plant litter biomass (hereafter referred to as “annual litter production”) (Table 1) as a covariate when comparing indicators among habitats. Due to a lack of data, we estimated the annual litter production from the annual production rate of aboveground

plant biomass (hereafter referred to as “annual biomass production”) of the habitats. We used a factor of 0.49 calculated from [48] for the biomass-litter conversion and assumed the same factor applies for all habitats. The biomass production data were obtained from [16] that estimated the standing aboveground plant biomass of all habitats in the two study communes, except NF and AF, under different ages using the Rapid Carbon Stock Appraisal approach [49]. The annual biomass production was calculated from the standing biomass and plot ages, assuming a constant biomass production across the year. We used an annual litter production of $9.3 \text{ ton ha}^{-1} \text{ year}^{-1}$ from [50] for NF and $2.4 \pm 0.4 \text{ ton ha}^{-1} \text{ year}^{-1}$ calculated from [51] for AF. The earthworm and plant biomass assessment were not conducted using the same sample plots. Therefore, to create a variation of annual litter biomass among earthworm sample plots, we used random numbers from a uniform distribution to generate higher or lower litter biomass by a maximum of one standard error from the mean. We applied this for all habitats except for NF that lacks input data on the standard error of the mean.

Table 1. Annual production of aboveground plant litter biomass by habitat (source: calculated from [16]).

Habitat	Number of Sample Plots	Litter Production ($\text{ton ha}^{-1} \text{ year}^{-1}$)	
		Average	SE
Regenerated Forest (RF)	4	5.8	0.26
Grassland (GL)	18	0.7	0.05
Planted Forest (PF)	37	4.4	0.35
Upland Crops (UC)	11	2.7	0.45
Home Garden (HG)	8	3.4	0.87

2.7. Statistical Analysis

We used the IBM SPSS Statistics version 23 to test the difference in earthworm indicators among habitats using one-way analysis of covariance (ANCOVA). For the statistical test, we assumed an independency among sample plots. For all data and statistical analysis, we combined the earthworm data from the two study communes and the difference between the two communes was not investigated. All graphs were produced using the Microsoft Excel.

3. Results

3.1. Species Occupancy by Habitat

A total of 25 earthworm species from 3 families and 6 genera were found in the two study communes. Of these, 23 species belong to the *Megascolecidae* family and 17 species to the *Amyntas* genus. Only one genus and one species were found in each of the other two families, *Moniligastridae* and *Rhinodrilidae*. Seven out of the 25 species, namely *Pontoscolex corethrurus*, *Amyntas aspergillum*, *Amyntas divotopapillatus*, *Amyntas modiglianii*, *Amyntas* sp.1, *Metaphire houlleti*, and *Polypheretima taprobanae* were omnipresent. However, only *P. corethrurus* dominated the habitats, except in NF and RF. The average frequency of occurrence of this species in human-disturbed habitats was 87%. Based on the occurrence frequency, *P. corethrurus* is classified as rare species in NF and uncommon species in RF. In NF, only *Drawida beddardi* had occurrence frequency above 25%. The occurrence frequency of earthworm species by habitat is given in Table A4.

3.2. Earthworm Density and Biomass by Habitat

The NF tends to have a lower earthworm density compared to other habitats (Figure 3a). However, no significant difference (p -value > 0.05) was found among habitats due to large standard errors. In terms of biomass, the natural habitats had higher earthworm biomass compared to human-disturbed habitats (p -value < 0.001), likely because of the larger earthworm’s physical size. The ratio between earthworm biomass and density was much lower in the human-disturbed habitats (p -value < 0.001) with an average of $0.73 \pm 0.14 \text{ g per}$

individual compared to 1.62 ± 0.23 g per individual in natural habitats. Related to quantity and biomass dominance, *P. corethrurus* was much more dominant in human-disturbed than natural habitats (p -value < 0.01) particularly due to extremely low dominance in NF (Figure 3b). The quantity and biomass dominance of all species by habitat is provided in Table A5.

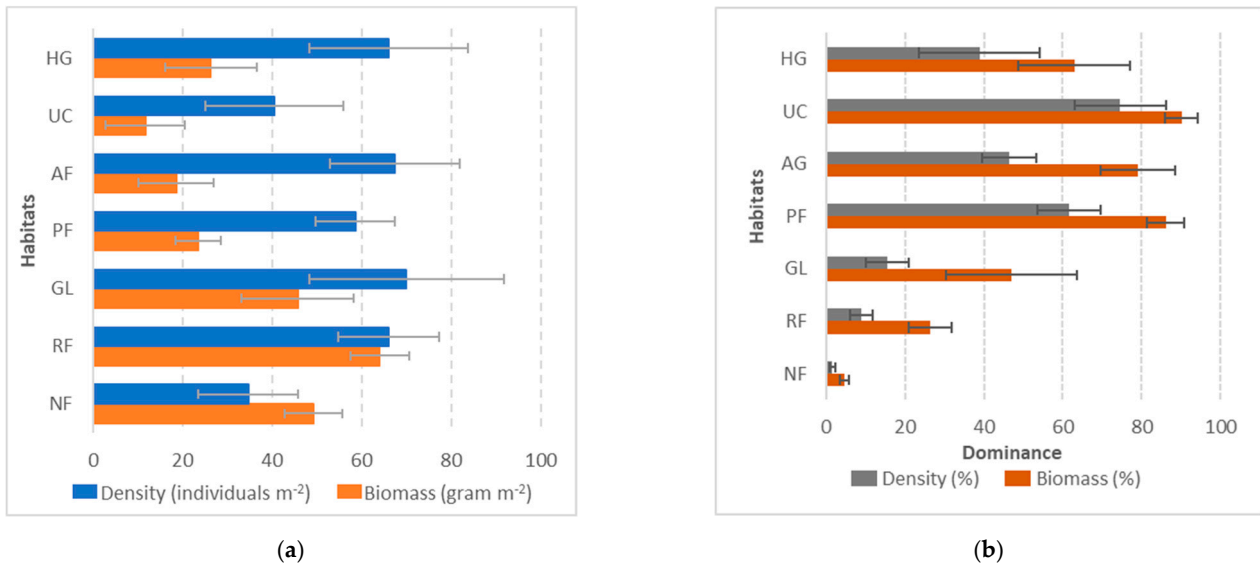


Figure 3. (a) Earthworm density and biomass and (b) quantity and biomass dominance of *P. corethrurus*.

3.3. Earthworm Density and Biomass by Soil Depth

Both the natural and human-disturbed habitats generally had higher density of earthworm in the first 10 cm than deeper soil layers (p -value = 0.001) (Figure 4a). However, the decrease in the density by soil layer was more pronounced in natural habitats, especially in NF and RF, than human-disturbed habitats. In terms of biomass, both natural and human-disturbed habitats generally had comparable earthworm biomass in the first two soil layers, and lower biomass (p -value < 0.002) in the 20–30 cm soil depth (Figure 4b).

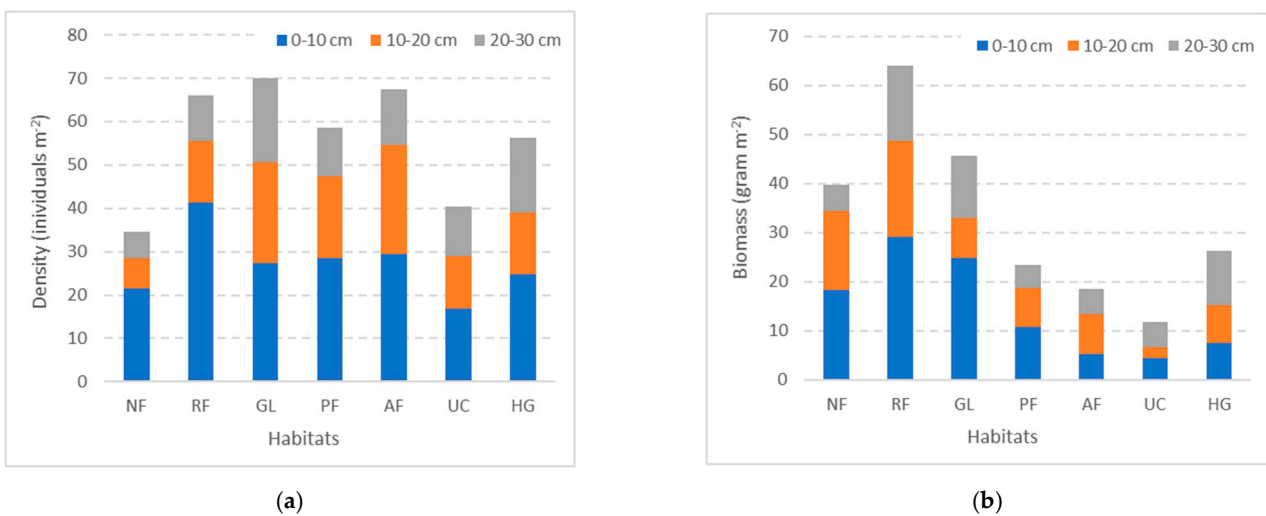


Figure 4. Earthworm (a) density and (b) biomass by soil depth.

3.4. Species Diversity and Similarity Among Habitats

The natural habitats had a higher species diversity than the human-disturbed habitats (p -value < 0.001) (Figure 5a). The weak dominance of *P. corethrurus* especially in NF and RF was associated with higher species diversity. Among the human-disturbed habitats, the number of earthworm species found in UC and HG was equal, however, the Shannon–Wiener index of UC was much lower due to the strong dominance of few species.

The NF and RF had a similar earthworm species composition (Figure 5b). Among the four human-disturbed habitats, only UC and HG had a level of similarity. In general, the species composition in natural and human-disturbed habitats was different, except between RF and AF.

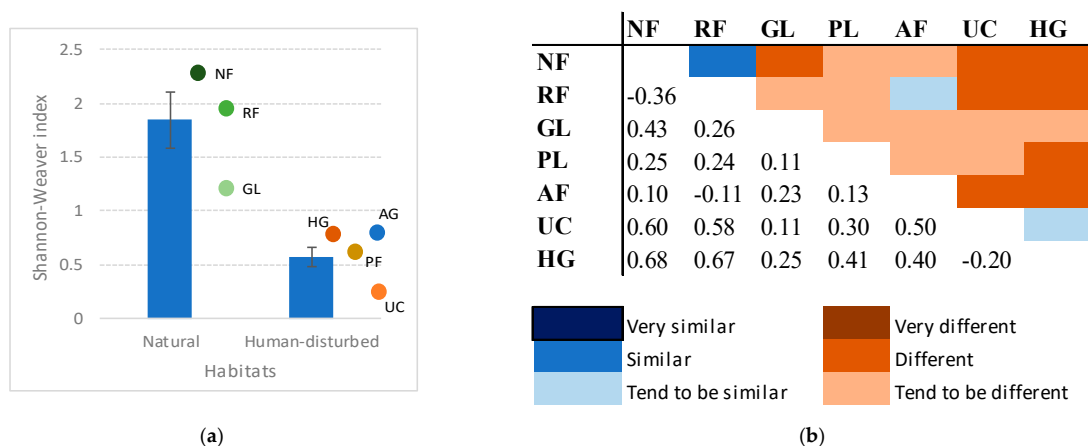


Figure 5. (a) Shannon-Wiener and (b) similarity index among habitats.

3.5. Variation among Types of Planted Forests

The Acacia plantation had more diverse earthworm species than Melia or Machilus plantation (Table 2). However, *P. corethrurus* was less dominant in Melia plantation because two other species, *Amyntas aspergillum*, and *Metapheretima tiencanhensis*, had an occurrence frequency above 25%. Also, earthworms in Melia plantation had a higher biomass and density ratio that reached 1.58 g per individual, compared to 0.3 and 0.5 g per individual in Acacia and Machilus plantations, respectively.

Table 2. Earthworm indicators comparing three types of planted forest.

Earthworm Indicators	Acacia	Melia	Machilus
No. of sample plots	15	5	4
No. of species	13	7	9
Frequency of occurrence of <i>P. corethrurus</i> (%)	97	72	100
No. of species with occurrence frequency $\geq 25\%$	1	3	2
Density (individuals m^{-2})	80	17	59
Biomass (gram m^{-2})	23	27	27
Shannon-Wiener index	0.48	0.95	0.5

4. Discussion

4.1. Earthworm Diversity in Agroforestry

In our study, among the human-disturbed habitats, higher earthworm diversity was found in tree-based farming systems like AF and PF. Other studies [52–57] reported similar evidence. Improved micro-climate, e.g., lower soil temperature and higher soil humidity, minimum plot management practices such as tillage, and a higher supply of organic matter from above- and below-ground litter all generate higher earthworm diversity in tree-based systems [58,59]. These factors also create contrast in earthworm diversity between areas nearby and far from the trees within AF, for example between tree row and crop alley in

the alley-cropping system. In addition, minimal application of chemical inputs such as fertilizer and pesticide contribute to generating higher earthworm diversity within tree rows [58,60]. The variation in micro-climate and plot management practices also influence earthworm density. For example, in poplar AF in Canada, the average earthworm density within tree row was 182 individuals m^{-2} , compared to 117 and 95 individuals m^{-2} within the crop alley at two and six meters away from tree row, respectively [60]. In our study, some of the observed AFs were alley cropping, and others had scattered trees over the plots. In both cases, soil blocks for earthworm observation were taken relatively close to the trees, to avoid causing damage to annual crops. This likely explains the comparable earthworm diversity between AF and PF. AF can become a solution if larger areas of annual crops are needed without further converting NF or RF which otherwise will further decline the ecosystem health and functions in the two communes.

4.2. Potential Impact of *P. corethrurus* Dominance

The dominance of *P. corethrurus* in human-disturbed habitats has also been reported in other studies especially in tropical areas, e.g., see review in [61]. The species was exotic to Vietnam and initially inhabited hilly areas of the country [62,63]. Nowadays, it has a large distribution area including in the coastal areas of the country [64]. Compared to other exotic species which deliberately or inadvertently colonize new habitats, *P. corethrurus* has a high survival rate thanks to its tolerance to a range of biotic and abiotic environment [24,65]. Many other species either fail to survive or survive but not invasive [66].

P. corethrurus could bring favorable or unfavorable impacts to new habitats, although generally considered harmful to the environment and native species. As summarized in [61], as favorable impacts, the species could for example stimulate nutrient release in soil and enhance plant's resistance to phyto-parasitic nematodes. However, as unfavorable impacts, it can create soil compaction and alter biogeochemical processes to affect plants, native earthworms, and microbial communities. *P. corethrurus* can compact the soils through its feeding activity which accumulates small soil aggregates. In the absence of intervention from other soil biota, the small aggregates will progressively transform into large aggregates and become compact [67,68]. In addition, layers of cast produced by this species on the soil surface, can turn into thick crust preventing water and air penetration to the soils [69]. The role of *P. corethrurus* in increasing soil bulk density and reducing soil porosity were reported in (e.g., [52,67,70,71]). Due to the unfavorable impacts, the presence and dominance of *P. corethrurus* have been considered as an indicator for soil health and level of disturbance to ecosystem and ecosystem services [72].

Our study shows the dominance of *P. corethrurus* in human-disturbed habitats namely PF, AF, UC, and HG which occupy about 17% and 20% of total land area in Phuoc My and Ta Bhing commune, respectively. These land uses potentially expand in the two communes driven by augmenting population and livelihood pressure. The two communes are therefore under a serious threat of further loss of earthworm diversity and stronger dominance of *P. corethrurus*, which can further reduce their ecosystem health and functions. Under the increasing dominance of the cosmopolitan species, the soil porosity and ecosystem functions in the two communes will mainly rely on tree roots. Therefore, tree-based farming systems such as AF are preferable to reconcile livelihood and provision of ecosystem services. Moreover, this land-use system generally has higher earthworm diversity than sole annual crop systems. Also, there is a need to select more suitable tree species for PF because of its popularity as land use in the two communes. Although Acacia is currently the most popular type of PF supported by local pulp and paper industries, Melia is worth to get further attention and research due to the lower dominance of *P. corethrurus* in the PF using this tree species. Based on the unpublished data as part of a study by [16], Melia in the communes likely had a superficial rooting system (Figure 6) which can create micro- and macropores in superficial soil layers through its coarse and fine roots. It has a root shallowness index of 0.83, higher than Acacia (0.69), and Machilus (0.63). The index measures the ratio of cross-sectional areas of horizontal and all proximal roots. Horizontal proximal

roots are defined as those with an angle of less than 45 degrees relative to the soil surface. The number of replications in the root study was however limited, only involved three different trees, and worth for further investigation. Therefore, promoting this species in the communes can contribute to maintaining soil porosity with less dominance of *P. corethrurus*. Also, in terms of topographical, soil, and climate condition, *Melia* is suitable for the two study communes [16].



Figure 6. Distribution of proximal roots of *Melia azedarach* in (a) home garden and (b) forest plantation.

4.3. Caveats in Assessing Earthworm-Habitat Linkage

In this study, we could demonstrate that natural- and human-disturbed habitats have different levels of earthworm density and diversity. However, we could not do in-depth analysis on the effect of environmental factors to the differences among habitats due to lack of data such as on soil physical and chemical properties by sample plot. Several studies utilized the results of correlation analysis between earthworm and soil indicators for further purpose, for example considering earthworm density or diversity as proxy for levels of soil quality or pollution (e.g., [73–75]). Some of these studies also assessed the level of metal pollution in the soils and in the earthworm tissue or casts [74,76]. Further researches on earthworm in the two study communes are therefore necessary and should focus on assessing the impacts of soil and terrestrial conditions on the earthworm density and diversity. The terrestrial condition includes type and abundance of plant litter biomass. The researches can also associate the results of soil-earthworm analysis with the potential role of earthworm in the two study communes on watershed service.

4.4. Further Studies for the Unidentified Species

In our study, seven out of nine unidentified species belong to *Amyntas* genus. The seven species were mostly found in NF and RF with only *Amyntas* sp. 1 was an omnipresent inhabitant of all observed habitats. Also, *Amyntas* sp. 1 had a relatively high occurrence frequency in human-disturbed habitats such as AF and HG. Compared to the eleven identified species of *Amyntas* genus, three out of the eleven were omnipresent inhabitants, namely *Amyntas aspergillum*, *Amyntas divitopapillatus*, and *Amyntas modiglianii*, while the other eight were inhabitants of some habitats only, or one habitat such as the case for *Amyntas wui*. Therefore, earthworm species of *Amyntas* genus likely have different land colonization patterns or perhaps levels of resistance to the new habitat's condition.

The seven unidentified species have different external and internal morphological features compared to 115 earthworm species of *Amyntas* genus that have been identified from past studies across regions in Vietnam. To ascertain if all the seven species are new to the country, a comparison with four species of the genus *Amyntas* Kinberg, 1867 recently identified in the southeastern part of the country [77] is however necessary. Further studies can also compare morphological features of the unidentified species with those of *Amyntas* species identified in other countries. For example, earthworm species of the genus *Amyntas* Kinberg, 1867 of family Megascolecidae, the same family with *Amyntas* earthworms found in our study, had been known as common inhabitants of natural forests in the northern part of Laos [78]. Recently, three new earthworm species belonging to this genus were found in that part of the country [78]. Apart from morphological features, further studies should compare living habitat and land colonization pattern between the unidentified and known *Amyntas* species. Similar efforts are necessary for the unidentified species of other genus found in the current study, namely one each of *Metaphire* and *Polypheretima* genus of the Megascolecidae family.

5. Conclusions

The conversion of natural forests to different land uses in the two buffer-zone communes of Song Thanh Nature Reserve had reduced earthworm diversity which can substantially affect soil health and ecosystem functions in the two communes. Also, among the identified species, *P. corethrurus* was omnipresent and largely dominated human-disturbed habitats. The dominance of this species can bring unfavorable impacts such as soil compaction and continuous threat to native earthworm species. This will further affect soil porosity and related ecosystem functions and reduce the role of the two communes to the nature reserve and watershed of which the two communes are part of. The area of human-disturbed habitats in the two communes potentially expand driven by augmenting population and livelihood pressures. This will lead to further loss of earthworm diversity and stronger dominance of *P. corethrurus*. To avoid the further decline of earthworm diversity and rampant expansion of *P. corethrurus* in the two study communes, protection of the remaining natural and regenerated forests is urgent, and tree-based farming systems such as agroforestry should be promoted to reconcile earthworm conservation and local livelihoods. The results of this study enrich current limited knowledge on impacts of forest and land use conversion on earthworm density and diversity in Vietnam. Furthermore, the results can generate stronger concerns with respect to the degradation of belowground diversity in the country and call attention to the urgent need for strengthened forest protection efforts.

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Appendix A

Table A1. Land cover types and areas in the study communes in 2016.

Land Cover Types	Phuoc My		Ta Bhing	
	Ha	%	Ha	%
Natural forest—rich	7788	61.4	5766	36.5
Logged over natural forest—medium	1447	11.4	5307	33.6
Logged over natural forest—poor	492	3.9	917	5.8
Regenerated natural forest	704	5.6	328	2.1
Planted forest	798	6.3	1687	10.7
Agroforestry	267	2.2	149	1.0
Home garden	28	0.2	56	0.4
Grasslands	641	5.1	774	4.9
Mixed annual crops	390	3.1	693	4.4
Paddy rice	63	0.5	52	0.3
Settlement, built up areas	59	0.5	89	0.6
Total (ha)	12,677		15,818	

Data source: [16].

Table A2. Selected habitats for earthworm observations and number of sample plots.

Habitat	Tree Cover (%)	No. of Sample Plots		Vegetation in Both Communes
		PM *	TB *	
Natural forest (NF)	>60	5	10	Fagaceae, Lauraceae, Meliaceae, Moraceae, Euphorbiaceae, Dipterocarpaceae, Sapindaceae
Regenerated forest (RF)	10–30	5	10	Bambusoideae such as <i>Bambusa natans</i> , <i>Dendrocalamus patellaris</i> , <i>Neohouzeana dulloo</i> , and shrubs
Grassland (GL)	<10	1	3	Shrub and grass
Planted forest (PF)	Young: <30, mature: >30	9	15	In Phuoc My: <i>Acacia</i> (5 sample plots), <i>Machilus odoratissima</i> Nees (4); in Ta Bhing: <i>Acacia</i> (10), <i>Melia azedarach</i> (5). In both communes, <i>Acacia</i> variety is mostly the hybrid <i>Acacia mangium x auriculiformis</i>
Agroforestry (AF)	Young: <30, mature: >30	4	5	<i>Acacia</i> -based with cassava, banana, or herbal plants (4), <i>Melia</i> -based with banana or cassava (2), agroforestry with mixed tree species such as <i>Vernicia montana</i> , <i>Ficus racemose</i> , <i>Dimocarpus longan</i> and annual crops such as cassava, banana, vegetables (3)
Upland annual crops (UC)	<10	3	5	Key crops such as paddy rice, maize, and cassava
Home garden (HG)	>30	1	5	Mostly diverse vegetable, annual crop and fruit trees such as mango, jackfruit, and longan trees

* PM: Phuoc My commune, TB: Ta Bhing commune.

Table A3. Indicators for earthworm's dominance and diversity.

No	Indicators	Formula	Unit	Remark
1	Density per soil layer	$\frac{n_{ijk}}{N_{jk}} \times 4$	Individual m ⁻²	n _{ijk} = number of individual of species i in soil block k layer j N _{kj} = total number of individuals from all species in soil block k layer j
2	Biomass per soil layer	$\frac{b_{ijk}}{N_{jk}} \times 4$	g m ⁻²	b _{ijk} = total biomass of species i in soil block k layer j
3	Quantity dominance	$\frac{n_i}{N} \times 100\%$	%	n _i = number of individuals of species i, N = total number of individuals of all species in the habitat
4	Biomass dominance	$\frac{b_i}{B} \times 100\%$	%	b _i = total biomass of species i, B = total biomass of all species in the habitat
5	Occurrence frequency	$\frac{S_i}{S} \times 100\%$	%	s _i = number of sample plots having species i, S = total number of sample plots for the habitat. Range of values: >75% = very common species, >50–75% = common species, 25–50% = uncommon species, <25% = rare species
6	Shanon- Wiener index	$-\sum \left(\frac{n_i}{N} \times \ln \left(\frac{n_i}{N} \right) \right)$	-	n _i = total of individuals of species i, N = total of individuals of all species in the habitat
7	Similarity index	$Rs = \frac{(2Rs + Rss)}{2 + 1}$ Where: $Rs = \frac{x + y - z}{x + y + z}, \text{ and}$ $Rss = \frac{x' + y' - z'}{x' + y' + z'}$	-	Rs = similarity level of species Rss = similarity of subspecies x (x'); y (y') = number of species (number of subspecies) found only in one habitat z (z') = number of species (number of subspecies) found in both habitats. Range of values: −1 to −0.7 = very similar, <−0.7 to −0.35 = similar, <−0.35 to 0 = tend to be similar, 0 to <0.35 = tend to be different, 0.35 to <0.7 = different, 0.7 to 1.0 = very different

Table A4. Occurrence frequency of earthworm species by habitat (codes as in Table A2).

Species	Occurrence Frequency by Habitat (%)							No. of Habitat *
	NF	RF	GL	PF	AF	UC	HG	
Family Rhinodrilidae								
<i>Pontoscolex corethrus</i>	16	48	74	92	89	76	94	7
Family Moniligastridae								
<i>Drawida beddardi</i>	34	30	3.5		4.5			4
Family Megascolecidae								
<i>Amyntas alluxus</i>	7	3		3	2.5			4
<i>Amyntas aspergillum</i>	25	32	20	18	6	2	10	7
<i>Amyntas cortices</i>	9			2	2			3
<i>Amyntas divitopapillatus</i>	12	12	12	12	12	12	12	7
<i>Amyntas exiguus austrinus</i>	10			2				2
<i>Amyntas exiguus chomontis</i>		2	10	2	5			4
<i>Amyntas falcipapillatus</i>	4							1
<i>Amyntas infantiloides</i>	4	3						2
<i>Amyntas modiglianii</i>	13	34	30	6	8	7	10	7
<i>Amyntas zoysiae</i>			10		2			2
<i>Amyntas wui</i>		6						1
<i>Amyntas sp.1</i>	9	32	50	7	23	4	40	7
<i>Amyntas sp.2</i>	24	18		2	2.5			4
<i>Amyntas sp.3</i>	9	3		3	2			4
<i>Amyntas sp.4</i>	9	1						2
<i>Amyntas sp.5</i>	20	11		2				3

Table A4. Cont.

Species	Occurrence Frequency by Habitat (%)							No. of Habitat *
	NF	RF	GL	PF	AF	UC	HG	
<i>Family Megascolecidae</i>								
<i>Amyntas</i> sp.6	6	6						2
<i>Amyntas</i> sp.7	4	1						2
<i>Metapheretima tiencanensis</i>	13	30	20	12	12			5
<i>Metaphire houlleti</i>	20	20	20	20	20	20	20	7
<i>Metaphire</i> sp.1				1				1
<i>Polypheretima</i> sp.1	4	14	30	4				4
<i>Polypheretima taprobanae</i>	20	32	17	8	9	2	14	7
Total number of species	21	20	12	14	15	7	7	

* Number of habitats where the species was found.

Table A5. Quantity (n') and biomass (b') dominance (%) of earthworm species by habitat and soil depth (NF: natural forest, RF: regenerated forest, GL: grasslands, PF: planted forest, AF: agroforestry, UC: upland annual crops, HG: home gardens).

Species	NF		RF		GL		PF		AF		UC		HG	
	n'	b'	n'	b'	n'	b'	n'	b'	n'	b'	n'	b'	n'	b'
<i>Pontoscolex corethrurus</i>														
0–10	1.7	1.2	35.7	13.6	48.6	13.8	91.1	51.8	90.1	61.8	85.4	70.6	88.4	65.8
10–20	6.7	2.2	30.7	7.3	47.4	19.0	85.6	76.6	86.8	43.4	95.6	92.6	44.3	10.5
20–30	3.2	0.5	12.9	6.0	45.4	13.7	82.0	56.2	60.7	33.9	89.6	60.8	56.5	40.5
<i>Drawida beddardi</i>														
0–10	2.3	0.9	11.7	3.7					0.4	1.1				
10–20	10.5	18.9	9.1	2.7	0.4	0.0								
20–30	4.7	1.4	9.1	1.1					2.2	0.4				
<i>Amyntas alluxus</i>														
0–10	0.4	0.0	1.2	0.3										
10–20			7.6	3.4			0.7	0.7						
20–30	6.3	2.3												
<i>Amyntas aspergillum</i>														
0–10	2.3	16.1	1.4	19.2	2.4	29.7	2.1	34.0					2.0	26.6
10–20	6.7	30.9	6.7	59.8	1.3	25.7	0.2	8.5	0.7	48.1			2.2	34.0
20–30	8.4	72.1	7.8	70.9	1.7	35.4	0.8	20.4	0.7	29.3	0.6	13.4	2.0	17.8
<i>Amyntas corticis</i>														
0–10	1.5	2.6												
10–20	1.4	1.6							0.3	1.1				
20–30	0.5	0.4												
<i>Amyntas divitopapillatus</i>														
0–10	0.8	1.3												
10–20	0.5	0.2												
20–30	0.5	0.2												
<i>Amyntas exiguus austrinus</i>														
0–10	10.5	3.5	0.5	0.1										
10–20	5.8	2.7			2.4	0.1	0.7	0.2	3.3	0.3				
20–30	18.8	1.5							18.2	2.8	0.6	0.1		
<i>Amyntas exiguus chomontis</i>														
0–10														
10–20					2.4	0.1			3.3	0.3				
20–30			1.2	0.0					18.2	2.8				

Table A5. Cont.

Species	NF		RF		GL		PF		AF		UC		HG	
	n'	b'	n'	b'	n'	b'	n'	b'	n'	b'	n'	b'	n'	b'
<i>Metapheretima tiencanhensis</i>														
0–10	0.3	1.5	4.2	6.8	6.1	2.2	0.9	1.9						
10–20			3.9	4.1			1.6	2.5	1.0	1.8				
20–30			1.2	3.0	16.7	21.5								
<i>Metaphire houletti</i>														
0–10	7.4	10.4	0.3	0.4	3.2	4.5	0.5	1.2						
10–20	0.5	0.0					1.3	3.2						
20–30	1.1	0.2												
<i>Metaphire sp1</i>														
0–10							0.1	0.9						
10–20														
20–30														
<i>Polypheretima sp.1</i>														
0–10			0.5	1.0	10.5	14.7	0.3	1.4						
10–20	1.9	0.9	0.4	0.6	14.3	45.4								
20–30	3.1	0.5	1.4	0.3	16.7	28.0	1.0	3.8						
<i>Polypheretima taprobanae</i>														
0–10	3.9	8.3	4.8	6.8	9.1	8.7	0.5	0.3	0.6	1.8			3.1	3.3
10–20	4.3	1.2	4.5	4.0	4.4	5.4	2.3	3.4	0.7	1.7			1.8	2.9
20–30	1.1	0.6	3.0	3.6	1.7	0.8	0.2	0.7	1.1	12.5	0.6	0.8	1.2	1.4

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