Original Research Article

Earthworm functional groups, residue quality and management impact on upland rice growth and yield – An experimental study in the Madagascar Highlands

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ABSTRACT

Aims: In Madagascar, agroecological practices to increase and sustain upland rice productivity are based on an intensification of soil ecological processes.
Study design: The effects of earthworm presence and identity (*Pontoscolex corethrurus*, *Dichogaster saliens*, or no earthworms), residue presence and identity(*Crotalaria grahamiana* (Fabaceae), *Desmodium uncinatum* (Fabaceae), *Stylosanthes guianensis* (Fabaceae), *Eleusine coracana* (Poaceae), *Zea mays* (Poaceae) or no residues) and residue location (mulched or buried) on nutrient availability and rice growth and yield were investigated in outdoor mesocosms. 33 treatments were managed in a completely random design.
Place and duration of study: The experiment was conducted at Andranomanelatra near Antsirabe, Vakinankaratra region, in the highlands of Madagascar (19°46'45"S, 47°06'25"E, 1600 m above sea level) in 2016.
Results: Earthworms had no effect on soil nutrient availability and opposite effects on plant biomass. Nevertheless, the presence of earthworms increased the shoot:root ratio.The main significant effects on soil properties and crop yields were due to the presence, identity and location of the residues. The addition of *Desmodium* residues enhanced the total plant biomass, rice grain yields, soil nitrate content and total P uptake by rice. No significant interactive effect was foundbetween earthworms and residues on plant and soil properties.
Conclusion: The most striking finding of the present studystudy was that the identity and location of the residues were the most important factors influencing soil nutrient content, plant growth and crop production, irrespective of earthworm presence.

Keywords: Pontoscolex corethrurus*,* Dichogaster saliens*, Plant growth, Resource allocation, Soil nitrogen, Organic matter decomposition*

1. INTRODUCTION

Agroecology is a recent paradigm that provides major importance to ecological processes occurring in agrosystems. A critical challenge of agroecological practices is to stimulate soil processes so that ecosystem goods and services will be provided in a way beneficial to farmers and society [1]. These soil processes are driven by the large soil biodiversity responsible for delivering ecosystem services [2, 3]. Indeed, soil organisms act and interact in very complex webs that control the main soil ecological functions at the basis of crop productivity: the maintenance of soil structure, recycling of soil nutrients, decomposition of organic materials, regulation of pests and pathogens [4].

There is an increasing interest in the possibility of manipulating soil biodiversity in order to optimize soil ecological functions. Soil invertebrates are well known to be major actors for many of these ecosystem services [3]. Some of these invertebrates have been defined as ecosystem engineers, i.e.,organisms that directly or indirectly modulate the availability of resources to other species by causing changes in the physical states of biotic or abiotic materials [5]. Earthworms, the highest animal biomass in the majority of terrestrial ecosystems, belong to this functional group [6]. They play an important role in the incorporation of organic residues into the soil and are greatly involved in the initial stages of residue decomposition [7]. Earthworms contribute to the release and recycling of nutrients by mixing organic and mineral matter, by ingesting soil and plant debris, by stimulating microbial activity, and by egesting casts into the soil or at the soil surface [8]. Numerous studies have shown that freshly egested earthworm casts are hotspots of microbial activity generally characterized by an intense mineralization of organic matter and the release of nutrients available for plants [9, 10, 11]. In laboratory experiments, recent research has shown or confirmed that the presence of earthworms affects the diversity and activity of microorganisms [12], increases both the decomposition of organic matter (in the short term) and its long-term storage [13], increases the availability of soil phosphorus [14] and increases plant growth [15, 16]. Thus, the management of earthworms is of great agricultural interest, especially for the restoration of ecosystems, and represents an excellent potential resource for managing ecosystem services [8, 17, 18]. Earthworm species are classified into ecological categories that have functional significance: (i) epigeic (feed on surface litter and live in the upper layers of soils); (ii) anecic (feed on surface litter and make permanent vertical burrows); and (iii) endogeic species (feed on soil more or less enriched with organic matter and live in deeper soil layers) [19]. However, some species showing intermediate characteristics between two groups can be classified as epi-endogeic, epi-anecic or endo-anecic. Based on their behavior, earthworms of different ecological categories may contribute differently to ecosystem processes and thus, ecosystem services. They may affect nutrient mineralization and plant growth in different ways [20, 21]. Nevertheless, earthworms are generally absent or rare in conventional tilled systems [22, 23] leading to soil ecological dysfunction [8].

Previous experiments indicated that the manipulation of soil engineers is possible only when coupled with the introduction of organic amendments [24]. Amendments serve as food for soil engineers; there is scientific evidence that earthworms will modulate the dynamics of organic amendments in a different way than when soil engineers are absent [25]. However, little is known about the relationship between the potential of earthworm functional groups with residue quality at different locations (mulched or buried) in the perspective of manipulating earthworm activity to enhance plant growth and productivity.

In a mesocosm field experiment in the highlands of Madagascar, the potential to manage earthworms and residues in a way beneficial to crop production and yieldwas explored. These agroecological innovative practices are of great importance for the development of sustainable and productive rainfed rice production in the highlands of Madagascar.

The objective of this study was to assess the distinct and synergistic effects of (i) two functionally different earthworm species, (ii) five residue types, and (iii) two residue locations (mulched vs buried), on upland rice (*Oryza sativa*) growth and productivity and soil nutrient (nitrogen and phosphorus) availability. The residues came from plants commonly used in rainfed rice cropping systems in Madagascar, generally in rotation with rice. They were used because of their known interest in agroecological systems. Both legume and grass residues were tested because of their different biochemical compositions and decomposition kinetics [26]. Both residue locations were expected to impact the activities of earthworm functional groups since they have different habitats and food resources.

2. MATERIAL AND METHODS

2.1 Study site and soil sampling

The experiment was conducted at Andranomanelatra near Antsirabe, Vakinankaratra region, in the highlands of Madagascar (19°46'45"S, 47°06'25"E, 1600 m above sea level). The climate is an altitude tropical climate, with a dry and cold season from May to October and a wet and hot season from November to April. The mean annual rainfall is 1300 mm and the mean annual temperature is 16 °C. The soil is classified as a Ferralsol (FAO classification) with 62% kaolinitic clay, 19% silt and 19% sand. Bulk density is 0.9 $g.m^{-2}$ for the 0–10 cm layer and the pH_{H2O} is 5.7. The soil contained 29.4 gC kg^{-1} and 1.77 gN kg^{-1}. The available (resin) P content was 0.71 mg kg^{-1}. The contents of iron and aluminium oxides were 47 and 17 g kg^{-1}, respectively [14]. The soil was collected from an adjacent savanna area. The topsoil layer (0–10 cm depth) was collected using a spade, then air-dried for 5 days, gently hand-crushed and mixed thoroughly. Most of the roots and vegetation debris were removed.

2.2 Experimental design

In a completely random design, 33 treatments were managed crossing (i) three earthworm treatments (endogeic *Pontoscolex corethrurus*, epi-endogeic *Dichogaster saliens* and no earthworms), (ii) six residue treatments (*Crotalaria grahamiana* (Fabaceae), *Desmodium uncinatum* (Fabaceae), *Stylosanthes guianensis* (Fabaceae), *Eleusine coracana* (Poaceae), *Zea mays* (Poaceae) and no residues), and (iii) two residue locations: mulched or partly buried in the first 5 cm of soil. Logically, when treatments without residues were applied, no data concerning the location of the residues were available. In total, there were eleven treatment combinations of residue management (*Crotalaria* mulched, *Crotalaria* buried, *Desmodium* mulched, *Desmodium* buried, *Stylosanthes* mulched, *Stylosanthes* buried, *Eleusine* mulched, *Eleusine* buried, *Zea* mulched, *Zea* buried, no residues) combined with three earthworm treatments. This explains the 33 treatments (11 × 3), and each was replicated 4 times to give a total of 132 mesocosms. Both earthworm species were collected in the fields near the experiment.

P. corethrurus (Glossoscoloscidae) is a medium-size endogeic geophagous species; this peregrine species has been studied all over the tropics. In Madagascar, it is present in all pedoclimatic regions [27]. It can ingest large amounts of soil, creates a macroaggregate structure and affects microbial activity, nutrient cycling, and soil organic matter dynamics [12, 13, 14].

D. saliens (Acanthodrilidae) is a small epi-endogeic earthworm that lives between the roots of plants, especially grasses. It has been shown to strongly stimulate the priming effect in the rhizosphere, thus leading to an increased release of nutrients to plants (Bernard et al. unpub. data). Recently, a field trial in Madagascar showed that its introduction in soil led to a significant increase in rice yield (higher number of full grains compared to the absence of earthworms) (Bernard et al. unpub. data).

2.3 Mesocosm set-up

The mesocosms consisted of 15 L plastic buckets with a top diameter of 28 cm. Drains at the bottom of the mesocosms were drilled (6 holes with 1 cm diameter) to let the water flow. Each hole was filled with a cotton mesh so that water could easily flow down. The bottoms of the mesocosms were covered with a mosquito net to prevent earthworms from escaping. A Velcro® hook-and-loop fastener was pasted around the top of the mesocosms to prevent earthworms from scaping as well. Mesocosms were filled with 12 kg of air-dried soil and were then introduced into the soil in the field so that surface level was similar inside and outside the mesocosms. They were randomly placed outside in natural weather conditions during the experiment.

Residues of five plant species were collected from agricultural fields in the same area. In the present experiment, *Desmodium* residues were collected from plants at a young stage of growth and predominantly taken in leaf material, while *Stylosanthes* residues mostly consisted of stem material (high stem:leaf ratio) taken from mature plants. *Crotalaria* residues were essentially in the form of twigs, whereas *Eleusine* and *Zea* residues were constituted by straw. The characteristics of the residues were extracted from the TSBF (Tropical Soil Biology and Fertility Programme) database and kindly provided by Dr. Bernard Vanlauwe (IITA, Kenya); they are given in Table 1. Oven-dried residues were cut into debris approximately 2-3 cm length and were then added at a rate of 30 g dry mass per mesocosm, corresponding to the annual input made by famers in no-till systems, i.e., 5 Mg dry mass ha^{-1}. Residues were either mulched (left at the soil surface) or partly buried (manually mixed into the upper 5 cm of soil). Then, mesocosms were irrigated to moisten the soil and reach field capacity at the beginning of the experiment.

Table 1. Characteristics of plant materials

Plant materials	C (%)	N (%)	P (%)	Lignin (L) (%)	Total Polyphenol (PP) (%)	C:N	C:P	(L+PP) : N	Source
Crotalaria grahamiana	37.8	3.04	0.14	7.05	2.00	12.4	273	2.98	database TSBF
Desmodium uncinatum	65	3.32	0.18	10.49	4.78	19.7	361	4.60	database TSBF
Stylosanthes guianensis	63.9	1.93	0.14	9.54	4.57	33.01	456	7.31	database TSBF
Eleusine coracana	-	-	-	-	-	82.1	-	-	database TSBF
Zea mays	42.8	0.73	0.07	9.18	0.93	57.7	626	13.76	database TSBF

Earthworm species were sampled near the study site. Six adults of the species *P. corethrurus*(equivalent to about 100 $ind.m^{-2}$) and twenty adults of the species *D. saliens*(equivalent to about 300 $ind.m^{-2}$) were added to each mesocosm.

At the time of sowing, each mesocosm received a small amount of fertilization with the compound fertilizer $N_{11}P_{22}K_{16}$ at a rate of 300 mg per container, i.e. 18 $kg.ha^{-1}$ equivalent to the dose used by local farmers. NPK was used as a starter fertilizer for the seedling growth. Finally, five seeds of rice (variety FOFIFA 161) were sown in each mesocosm. After 2 weeks, two seedlings were kept in each mesocosm. The experiment started in mid-November 2014 with the introduction of soil, earthworms, residues, and rice seeds, and lasted until mid-May 2015 with rice harvest.

2.4 Plant growth

Rice growth was assessed by measuring the height at different stages during the course of the experiment (tillering, panicle initiation, flowering and maturity) (data not shown). Moreover, the presence of pests was monitored regularly until rice harvest. The results of rice height were not shown in this study in order to focus on plant parameters at the end of the experiment.

2.5 Plant and soil analyses

At the end of experiment (rice harvest), the aerial parts were cut at the soil surface. The soil was removed from mesocosms and separated into three layers: 0–5, 5–10 and 10–20 cm. The soil of each layer was gently, manually disaggregated to check for earthworm presence. All analyses were performed in the 0–5 cm layer. After homogenization of the soil (each layer separately), an aliquot was sampled and stored at 4 ℃ for mineral N and available P analyses, while another aliquot was dried for classical analyses (total soil C and N).

Plant shoots and seeds were manually separated. Roots were carefully removed from each soil layer and washed to eliminate adhering soil particles. Shoot biomass and root biomass (sum of the root biomass in each layer) were weighed after drying at 60 °C for 72 h. Rice yield components were calculated by using the number of panicles, the number of grains per panicle, the percentage of filled grains, and the weight of a thousand grains [28].

The P concentrations in shoots (StrawP) and seeds (SeedsP) were determined after digestion with chlorhydric acid (HCl) and analyzed with a spectrometer at 882 nm after a reaction with an ammonium molybdate solution. The total N and C contents in soil were measured by using a CHN microanalyzer (Fisons / Carlo Erba NA 2000), while the available soil P content in soil was measured using the resin method. Indeed, resin membranes function as plant roots in the extraction of soil-available P and therefore provide a close estimate [29].The resin-exchangeable P content was measured by extracting 2 g of soil for 16 h with 30 ml of ultra-pure water and an anion exchange resin charged with $NaHCO_3$, eluting the resin with 30 ml of 0.1M HCl / 0.1M NaCl for two hours.Phosphorus concentrations in the extract solutions were measured with the malachite green method [30]. Mineral N was extracted with 1M KCl.

2.6 Statistical analyses

All statistical analyses were done with the R software [31] with a P-value threshold set at 5%. Three-way ANOVA models were used to test the effects of earthworms and residues on untransformed soil and plant variables. The three factors were: (1) the presence and species identity of earthworms coded “E” (no earthworms, *P. corethrurus, D. saliens*), (2) the presence and identity of the residues coded “R” (no residues, *C. grahamiana*, *D. uncinatum*, *S. guianensis*, *E. coracana* and *Z. mays*) and (3) the location of the residues coded “L” (mulched or buried). For each variable, a full (with all factor levels) ANOVA model was first performed using the “aov” functions from the “ade4” package (by default, it implements a sequential sum of squares). The normality of the data and the homogeneity of variance were checked using Shapiro and Levene’s tests, respectively. When there was no significant interaction effect, the type II sum of squares (SS) test was chosen with the function “Anova” from the package “car” in order to improve the initial model because it was more powerful in this case. If an interaction was present, a type III SS was used with the same function. The significance of the interactions and main effects was provided by these full improved models. The type of SS used in the improved models was indicated in the results section. The contrasts were then specified within the improved ANOVA model in order to distinguish the significant effects of the presence from that of the identity of both “E” and “R” factors. The significant differences among levels within factors were detected using the Tukey HSD post hoc test (function and package “TukeyC”).

3. RESULTS AND DISCUSSION

3.1 Earthworm presence

At the end of the experiment, the densities of *P. corethrurus* and *D. saliens* had decreased on average by 76% and 78%, respectively, in all treatments. The presence of residues increased the survival rate of earthworms 4-fold in comparison to treatments without residues (24% vs. 6%).The low density of living earthworms at the end of the experiment can be explained by the fact that the experiment lasted up to the harvest in mid-May 2015 at a time when rainfall had stopped for 5-6 weeks. As a consequence, the soil was dry when sampled, and it is likely that earthworms did not survive this drought. The very low survival rates of earthworms in treatments without residues were probably attributed to the lack of food in addition to soil drought; it is likely that the presence of residues maintained the water content for a longer period. Despite the low earthworm abundance at the end of the experiment, visual observations of physical soil characteristics (burrows, macroaggregates) confirm that earthworms were present and active during the rainy period. Moreover, earthworm presence (irrespective of species) affected some soil and plant parameters; for example, they increased the rice height at maturity (p=0.074, data not shown). However, this positive earthworm effect on plant height was not confirmed by the rice grain yields, which suggests that earthworms probably died before grain filling. Similarly, in a review article, [32] noticed that earthworm presence did not significantly increase crop yields in experiments with survival rates lower than 50%, despite the fact that earthworm weight loss or gain was responsible for smaller variations in the size of the effect.

3.2 Soil properties

3.2.1 Effect of earthworm presence and species on soil properties

The analyses of variance and the contrast analysis showed that neither earthworm presence nor species identity significantly changed total soil carbon (C), soil ammonium (NH_4), nitrate (NO_3) and inorganic phosphorus content (Pi) (Table 2). However, it was observed that the NO_3 content tended to be lower in the presence of both earthworm species than in their absence. It decreased by 9% and 8%, respectively, in the presence of *D. saliens* and *P. corethrurus* ($p = 0.096$).

No enrichment of mineral N and available P in the soil was observed in presence of earthworms, as usually found in other earthworm experiments [6, 14, 15, 21, 33, 34]. Earthworm presence decreased the NO_3 content in the 0-5 cm upper soil layer although this was not significant. This might be because earthworms increased the N uptake for plant growth and production. In another experiment, [15] observed that the presence of earthworms increased the total N acquired by chickpea by 17 %. Another explanation for the decrease of soil nitrate is that earthworms could have increased microbial activity and biomass [12], which could in turn increase microbial N immobilization [35]. Nevertheless, microbial biomass was not measured.

Table 2. ANOVA and contrast table of p-value showing the main effects of earthworm presence and species identity, residue presence and identity and residue location and their interaction on soil properties. Legend: total soil carbon (TotC), ammonium (NH_4), nitrate (NO_3), inorganic phosphorus (Pi).

Factors		**Soil variables**			
		C_tot	**NH_4**	**NO_3**	**Pi**
Main effects	*Earthworms (E)*	0.493 ns	0.203 ns	0.096 ns	0.746 ns
	Residues (R)	0.000***	0.646 ns	0.000***	0.079 ns
	Location (L)	0.000***	0.782 ns	0.131 ns	0.820 ns
Interactions	*E:R*	0.106 ns	0.728 ns	0.224 ns	0.559 ns
	E:L	0.789 ns	0.110 ns	0.966 ns	0.882 ns
	R:L	0.941 ns	0.726 ns	0.634 ns	0.878 ns
	E:R:L	0.730 ns	0.984 ns	0.076 ns	0.991 ns
Contrasts	*E:Input*	/	/	/	/
	E:Species	/	/	/	/
	R:Input	0.002 **	/	0.000***	/
Tukey HSD	*R:identity*				
	R:Cro	27.8 a	/	80.4 a	/
	R:Des	28.2 a	/	82.8 a	/
	R:Sty	27.4 a	/	64.7 b	/
	R:Ele	27.5 a	/	52.1 b	/
	R:Mai	27.7 a	/	58.4 b	/
	NR	25.5 b	/	58.2 b	/
Type of SS		II	II	II	II

ns: not significant at 5%. *P < 0.05; **P < 0.01; ***P < 0.001. "/" not tested in the model if significant interaction or absence of both significant interaction and main effect.

3.2.2 Effect of residue presence, identity and location on soil properties

The presence and identity of the residues strongly affected the NO_3 content ($p < 0.001$). It was significantly higher with legume residues than with grass residues. The highest values were found in the treatments that received *Desmodium* (82.8 mg kg^{-1}) and *Crotalaria* residues (80.4 mg kg^{-1}), while the lowest values were found in the treatments that received *Zea* (58.4 mg kg^{-1}) and *Eleusine* residues (52.1 mg kg^{-1}). Total soil C was significantly higher with than without residues (27.8 vs. 25.6 g kg^{-1}, $p < 0.001$). Regarding the location of the residues, total soil C was significantly higher with buried than with mulched residues (28.0 vs. 27.3 g kg^{-1}, $p < 0.001$).

In the present studystudy, the NO_3 contents were strongly affected by the identity of the residues. *Desmodium* and *Crotalaria* residues increased the soil NO_3 content, which suggests high N mineralization and microbial activity in those treatments. Generally, organic matter inputs with a low C:N ratio promote nitrogen release in soil, whereas organic matter with a high C:N ratio induces the immobilization of soil N by microorganisms [36, 37]. Legumes can fix substantial quantities of N by symbiotic fixation with soil bacteria (rhizobia) and are characterized by high N content with a narrow C/N ratio reducing the competition for available N by microorganisms and consequently enhancing the decomposition and nutrient release [38, 39]. In contrast, cereals are characterized by lower N content with a higher C:N ratio, resulting in N immobilization after incorporation [40]. However, the soil nitrate content in the treatment with *Stylosanthes* residues tended to be similar to those with cereal residues, suggesting microbial N immobilization in this treatment. Similar results have been reported in other studies [41]. This general pattern could be due to differences in the rate of residue decomposition, which is mainly driven by the biochemical quality of plant material [42]. In general, water-soluble fractions are degraded faster [43] followed by structural polysaccharides (hemicellulose and cellulose) [44] and then lignin [45]. In parallel, the (lignin+polyphenol):N ratio also determines the nitrogen release dynamics ([46]. It is also important to note that changes in biochemical composition during the growth period of most crop plants [47] affect residue quality; older plants (such as *Stylosanthes* in the presentexperiment) are characterized by a decrease in water-soluble constituents, whereas the amount of hemicellulose, cellulose, and lignin increases. As a result, the residues of young plants (such as *Desmodium* and *Crotalaria* in the present experiment) generally decompose more readily than those of older plants [48] and release more nutrients [47]. Consequently, based on their biochemical composition, *Desmodium* and *Crotalaria* residues were of higher quality, while *Stylosanthes*, *Eleusine* and *Zea* residues were of lower quality.

The statistical analyses showed that the total soil C was higher for buried than for mulched residues. After weighing the residues at the end of experiment, it was noticed that the loss of litter for mulched residues was lower than for buried residues. When residues are placed on the surface, they are less associated with mineral soil and protected from microbial attack [49]; they thus decompose more slowly than when buried [50, 51].

3.3 Plant biomass

3.3.1 Effect ofearthworm presence and species on plant growth

Shoot biomass, root biomass, total biomass and the shoot:root ratio were significantly affected by earthworm presence and species identity ($p = 0.021$, $p = 0.005$, $p = 0.013$, $p = 0.011$, respectively) (Table 3). In the presence of *D. saliens*, both shoot and root biomass were significantly lower (10.7 g and 5.3 g, respectively) than in the control without earthworms (11.1 g and 6.3 g, respectively) and in the presence of *P. corethrurus* (12.3 g and 6.5 g, respectively). Consequently, the total biomass was lower (16.2 g) in the presence of *D. saliens* compared to treatment with no earthworms, with a decrease by 7%. The highest biomass was found in the presence of *P. corethrurus* (18.8 g)

The shoot:root ratio increased in the presence of earthworms, with a more pronounced effect in the presence of *D. saliens* (2.07) than in the presence of *P. corethrurus* (1.91) compared to treatment without earthworms (1.82).

Table 3. ANOVA and contrast table of p-value showing the main effects of earthworm presence and species identity, residue presence and identity and residue location and their interaction on plant properties. Legend: SB: shoot biomass in g, RB: root biomass in g, TB: total biomass in g, SR: shoot:root ratio, GY: grain yields in Mg ha^{-1}, StrawP: phosphorus accumulated in straw in mg kg^{-1}, SeedsP: phosphorus accumulated in seeds in mg kg^{-1}, TotalP: total phosphorus uptake by rice in mg.

Factors		Plant variables							
		SB	RB	TB	S:R	GY	StrawP	SeedsP	TotalP
Main effects	*Earthworms (E)*	0.021 *	0.005 **	0.013 *	0.011 *	0.581 ns	0.482 ns	0.566 ns	0.355 ns
	Residues (R)	0.000 ***	0.000 ***	0.000 ***	0.915	0.007 **	0.655 ns	0.001**	0.043*
	Location (L)	0.000 ***	0.011 *	0.000***	0.046 *	0.000 ***	0.360 ns	0.639 ns	0.000 ***
Interactions	*E:R*	0.919 ns	0.621 ns	0.893 ns	0.175 ns	0.946 ns	0.376 ns	0.409 ns	0.959 ns
	E:L	0.445 ns	0.751 ns	0.480 ns	0.753 ns	0.336 ns	0.309 ns	0.137 ns	0.747 ns
	R:L	0.077 ns	0.153 ns	0.108 ns	0.146 ns	0.077 ns	0.696 ns	0.987 ns	0.348 ns
	E:R:L	0.624 ns	0.412 ns	0.511 ns	0.687 ns	0.382 ns	0.833 ns	0.449 ns	0.301 ns
Contrasts	*E:Input*	0.013 *	0.087 ns	0.026 *	0.004 **	/	/	/	/
	E:Species	0.522 ns	0.014 *	0.175 ns	0.298 ns	/	/	/	/
	R:Input	0.000***	0.000***	0.000***	/	0.000***	/	0.006 **	0.004 **
Tukey HSD	*R:identity*								
	R:Cro	13.41 a	6.97 ab	20.37 a	/	1.10 ab	/	2000 a	0.013 ab
	R:Des	14.42 a	7.70 a	22.12 a	/	1.46 a	/	2091 a	0.015 a
	R:Sty	10.42 b	5.49 bc	15.91 b	/	0.99 b	/	1964 ab	0.011 ab
	R:Ele	10.43 b	5.46 bc	15.89 b	/	1.13 ab	/	1834 ab	0.013 ab
	R:Mai	9.63 b	5.25 c	15.09 b	/	1.09 ab	/	1653 b	0.010 b
	NR	7.99 b	4.77 c	12.76 b	/	0.72 b	/	1629 b	0.008 b
Type of SS		II	II	II	II	II	III	II	II

*ns: not significant at 5%. *P < 0.05; **P < 0.01; ***P < 0.001. "/" not tested in the model if significant interaction or absence of both significant interaction and main effect.*

The presence of earthworms increased the shoot:root ratio, as already reported in several earthworm experiments [15, 52, 53]. Regarding the identity of earthworms, the shoot:root ratio was higher in the presence of both earthworm species, whereas a significant difference was observed only between the treatment with *D. saliens* and the treatment without earthworms. This finding suggests that the modification of biomass allocation depends on the earthworm species. The impact of *D. saliens* on biomass allocation may be explained by both trophic and non-trophic interactions between earthworms and plants [15]. These interactions are respectively based on:

(i) the strategy of plants in optimizing resource allocation to the root system to efficiently take up nutrients [53]. It is well established that earthworms can increase the availability of soil nutrients [11]. Plants, in the presence of earthworms, would then produce less root biomass per shoot unit [53]. This explanation may also confirm thehypothesis on the decrease of the soil NO_3 content in the presence of *D. saliens*, probably because of higher N uptake;

(ii) the release of phytohormones [16, 20, 54]. Earthworms are known to trigger the release of molecules recognized as phytohormones by plants, in particular, an auxin-like effect [55], which may affect negatively root elongation so that root biomass decreases [56].

On the other hand, the presence of *D. saliens* reduced plant biomass (-7%), while *P. corethrurus* promoted higher total biomass (+16%) compared to the treatment without earthworms. Our results are consistent with a previous study by Jouquet et al. [57], who found a lower plant biomass when *Dichogaster bolaui* (a small epi-endogeic earthworm with similar functions to *D. saliens*) were present in vermicompost-treated soil. Observed differences between the effects of earthworm species are often attributed to variations in their feeding and burrowing behaviors [49]. However, the identification of the mechanisms responsible for the differential performance of earthworms needs further investigation.

3.3.1 Effect ofresidue presence, identity and location on plant growth

With regards to the effect of identity and location of the residues on plant growth, the highest plant biomass (shoot, root and total biomass) was found in treatments including *Desmodium* and *Crotalaria* residues. The plant biomass was significantly higher with mulched than with buried residues ($p < 0.001$ for shoot biomass; $p = 0.011$ for root biomass and $p < 0.001$ for total biomass).
A similar trend was found between the effect of the identity of the residues on soil and plant properties, showing a stronger effect of legumes compared to cereals. The positive effect of *Desmodium* and *Crotalaria* residues on plant growth could be attributed to improved N and P supply. This is corroborated by the highest soil NO_3 concentration and P accumulated in rice seeds observed in those treatments in comparison to treatments with *Stylosanthes* and cereal residues. On the other hand, we observed that the rice grain yield was higher in the treatment with *Desmodium* residues and lower with *Stylosanthes*. As explained above, the addition of residues with low C:N ratio and (lignin+polyphenol):N ratios increases the soil nutrient availability, which also affects nutrient uptake [58] and then crop yields. Moreover, the low C:P ratio for *Desmodium* and *Crotalaria* increases P availability.
The smallest plants and lowest grain yields were found in treatments in which the residues were buried (mixed in the upper 5 cm of soil) compared to treatments with mulched residues. These results confirmed the work of Bonkowski et al. [59], who studied the effect of organic substrate heterogeneity in soil on ryegrass growth. They observed that plant growth was reduced when the organic substrate was homogeneously mixed into the soil. Basically, thisresult might be explained by two reasons: (i) with mulched residues, the moisture content of the soil was maintained (water conservation), and (ii) with buried residues, competition between plant roots and microbes for available nutrients increased. The effect of mulching on moisture conservation and crop productivity has been reported in previous studies [60]. It seems well established that conserving moisture through mulching is very impactful to plants during stress [61]. Conserving water in soil might have been useful to crops during grain filling [62]. This finding corresponds with theresult on rice grain yield, which increased by 84% with mulched residues compared to buried residues.

3.4 Rice grain yields and phosphorus acquisition

Statistical analyses showed that neither the presence of earthworms nor the species affected rice grain yields ($p = 0.581$, Table 3) or phosphorus acquisition ($p = 0.482$ for StrawP; $p = 0.566$ for SeedsP and $p = 0.355$ for TotalP). However, there was a significant effect of the presence and identity of the residues and their location on rice grain yields ($p = 0.007$). When residues were added, the rice grain yields increased by 1.6-fold (1.15 Mg ha^{-1}) compared to treatments without residues (0.72 Mg ha^{-1}) ($p < 0.001$). The highest increase was observed in the treatment that received *Desmodium* residues (1.46 Mg ha^{-1}), while the lowest increase was obtained in the treatment with *Stylosanthes* residues (0.99 Mg ha^{-1}). Considering all types of residues, we found that the rice grain yield was significantly higher for mulched (1.34 Mg ha^{-1}) than for buried residues (0.97 Mg ha^{-1}).
With regards to P acquisition, the identity of the residues affected significantly the P accumulated in seeds and total P uptake by rice ($p = 0.001$ and $p = 0.043$, respectively). *Desmodium* and *Crotalaria* increased the P accumulated in seeds across all treatments. For

total P uptake, the highest value was observed in the treatment with *Desmodium* residues; it increased 1.8 fold (0.015 mg) compared to the treatment without residues (0.008 mg).

3.5 Effect of interaction between earthworms and residues

In this study, crop residues were used as food for earthworms so that earthworm activity increased and earthworms could increase crop production by increasing nutrient release in their casts. Thus, a synergy of the combination of earthworms (presence and species) and residues (identity and location) on soil and plant properties was expected. However, no significant interacting effects were found. This could be explained by the magnitude of the effects of earthworms, which seems to depend not only on the presence of crop residues, earthworm density and type but also on the rate of residue application [32]. It has been reviewed that the positive effect of earthworms becomes larger when more residues are returned to the soil (application rate ≥ 6000 kg c $ha^{-1}yr^{-1}$) but greatly decreases at zero and very low residue application rates (0 – 2999 kg c $ha^{-1}yr^{-1}$) [32]. In the presentexperiment, the residue application rate was typical of low input systems in the tropics, which could lead to a smaller effect of earthworms on soil and plant properties. Moreover, the droughtat the end of the experiment was most likely the constraining factor for reaching the full potential of earthworm activity. Pashanasi et al. [63] found thatplant biomass production and grain yield in the presence of *P. corethrurus*increased during rainy seasons and decreased during dry seasons. Another experiment by Blouin et al. [64]showed that the shoot biomass of rice did not increase in the presence of earthworms under drought conditions. Nevertheless, in rice rainfed cropping systems in the highlands of Madagascar, the dry season occurs generally after grain filling and during the whole period of maturity. Thus, the effects of earthworms are expected to strongly impact soil properties (release of nutrients, modification of the soil structure) at least during the rainy season, which could influence subsequent plant production. Interestingly, since residues improve moisture conservation in soil, high input systems (with high residue application rate) might provide excellent conditions for earthworm activity.

4. CONCLUSION

The aim of the present study was to manipulate earthworms and residues under field conditions in order to propose innovative practices to manage agricultural production in a sustainable manner. In this experiment, a positive effect of earthworm species on the modification of plant biomass allocation was found. However, no significant interactive effect between earthworms and residues was found. The most striking finding of the presentstudy was that the identity and location of the residues were the most important factors influencing soil nutrient content, plant growth and crop production, irrespective of earthworm presence. Adding fast-decomposing and high-quality residues such as legumes increased nutrient release, enhanced N-mineralization in the soil and then positively affected plant growth. The lack of evidence of the positive effect of earthworms and their interaction with residue input could be due to the low residue application rate and the drought that occurred at the end of the experiment. However, the effect of earthworms under drought conditions seemed to depend on the earthworm species. *D. saliens* induced a negative effect on rice total biomass, while a positive effect of *P. corethrurus* was observed. This result suggests that endogeic species such as *P. corethrurus* are better adapted to a water deficit than epi-endogeic species such as *D. saliens*, especially when residues are mulched. Controlling the population of introduced earthworm species is difficult under field conditions, requiring continuous introduction. Indeed, further research on the long-term effects of the management of earthworms and plant residues is of great importance for sustainable agriculture in different agro-pedo-climatic areas.

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