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Does rice breeding affect the ability of plants to interact with earthworms in nutrient-depleted Ferralsols?

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Abstract

Agroecology is based on the optimization of ecological processes occurring in agrosystems, especially by improving beneficial interactions among organisms. Recent studies have provided evidence that the ability of plants to interact with free-living soil organisms can be influenced by plant breeding. The upland rice breeding programme in the Highlands of Madagascar aims to develop cultivars that are adapted to local climatic, soil fertility and pest constraints. However, it is still unknown whether this genetic improvement alters rice cultivar abilities to interact with free-living soil plant-mutualistic organisms such as earthworms. We assessed in mesocosms the response of six upland rice cultivars commonly used by farmers in the Highlands of Madagascar to the presence of the endogeic earthworm *Pontoscolex corethrurus*. Plant nutrition and growth-related traits were measured after eight weeks of growth. These traits were significantly improved in the presence of *P. corethrurus* for all cultivars compared to the control treatment. However, the magnitudes of earthworm effects on plant traits were strongly variable and were dependent on specific rice cultivars. Agronomic and phylogenetic distance matrices were computed using agronomic data and available phylogenetic data of the rice cultivars. We did not detect significant correlations between cultivar responses to earthworm inoculation and agronomic or phylogenetic distances. Our results suggest that (i) the ability of rice to exploit beneficial interactions involving free-living soil organisms is influenced by its genetic background, but (ii) the loss of earthworm-interactive abilities of rice crops is independent of the genetic distance among cultivars and breeders' agronomic criteria. Our findings are significant for attempts to use rice cultivars that optimize plant-soil interactions within agroecological practices and highlight the need to integrate free-living soil partners as life-long plant partners in future strategies for plant breeding.

Keywords: Agroecology, plant-mutualistic free-living organisms, plant-soil interactions, breeding programme

1. Introduction

Plant breeding programmes mainly focus on crop adaptation to environmental and biotic stresses to increase yields. In the Highlands of Madagascar, breeding was used to develop high yielding and adapted upland rice varieties due to (i) cold climate at the high altitudes (Raboin et al., 2014), (ii) poor mineral fertility of Ferralsols (Raminoarison et al., 2019), and (iii) blast disease caused by the ascomycete fungus *Pyricularia oryzae* Cavara (Dusserre et al., 2017). However, because wild plants have evolved over time with specific soil biota as their plant-mutualistic partners, plant breeding is also a practice that may affect interactions between cultivated plants and soil organisms (Barot et al., 2010; Litrico and Violle, 2015). Indeed, by developing genetically homogenous crops with higher yields, resistance to pests and higher tolerance to climatic stresses, plant breeding may have considerable effects on plant-soil interactions (Bakker et al., 2012; Mariotte et al., 2018). For instance, the response of crop plants to soil life is known to vary with the presence of bacteria (Briones et al., 2002; Hardoim et al., 2011), mycorrhizal fungi (Declerck et al., 1995; Hetrick et al., 1996; Vallino et al., 2009), protists (Somasundaram et al., 2008), or earthworms (Barot et al., 2010; Junaidi et al., 2018; Noguera et al., 2011). To date, plant breeding strategies with mutualistic free-living soil organisms as direct selection targets are poorly developed (Gopal and Gupta, 2016; Wei and Jousset, 2017). Knowing that soil organisms can significantly promote plant functions, this lack of consideration is seen as a significant failure (Altieri, 1999; Barrios, 2007).

Earthworms present a worldwide-distributed soil biota (Phillips et al., 2019) and are involved in key soil functions (Lavelle, 1997; Lavelle et al., 2006; Jouquet et al., 2006, 2008). They are known to increase plant growth via different mechanisms (Brown et al., 2004; Scheu, 2003): (i) increased mineralization of soil organic matter (Bernard et al., 2012; Coq et al., 2007); (ii) production of plant growth substances via stimulation of microbial activity (Blouin, 2018);

(iii) control of pests and parasites (Blanchart et al., 2019; Loranger-Merciris et al., 2012); (iv) stimulation of symbionts (Huhta, 2006) and (v) modifications of soil porosity, aggregation and water retention (Blanchart et al., 1999). Breeding modern high-yielding cultivars takes place under high input conditions (i.e., mineral fertilizers and chemical treatment of pests) in soils that can be unfavourable to earthworms (Doran and Zeiss, 2000; Ponge et al., 2013). Therefore, it is possible that these cultivars have lost some of the plant functional traits involved in interactions with earthworms, which may result in contrasting responses of plants to earthworm presence. For instance, Noguera et al. (2011) showed that the effects of *Pontoscolex corethrurus* on rice growth varied significantly for different cultivars and to the presence of biochar. However, the factors e.g., genetic background, agronomic selection criteria and targeted plant traits that determine this variability in plant responses to earthworms remain unidentified.

In the Ferralsols of Madagascar, upland crops face several edaphic constraints. Ferralsols are very infertile with numerous limiting nutrients, especially P but also N, Ca, Mg (Raminoarison et al., 2020). They have very low carbon contents and a low biological activity leading to a very poor productivity of the native vegetation. In the Highlands, the main earthworm species (i.e. *P. corethrurus* (Rhinodrilidae), *Dichogaster bolaui*, *Dichogaster saliens* (Acanthodrilidae), and *Amynthas corticis* (Megascolecidae)) can deeply modify soil structure and soil C and N cycles and P availability (Trap et al., 2021). This last function is especially important for P-fixing soils such as the Ferralsols.

In the present study, by using a mesocosm greenhouse experimental approach, we aimed to assess the effects of earthworms on the growth and nutrition of six locally widespread upland rice cultivars that are adapted to the nutrient-depleted Ferralsols in Madagascar. We also tested whether agronomic selection criteria or the genetic signals among cultivars may explain the various responses of rice cultivars to earthworm inoculation. For this purpose, the relationships between the response of rice cultivars to earthworm inoculation and agronomic or phylogenetic dissimilarity among the cultivars were assessed.

2. Materials and methods

2.1 Soil sampling

The upper 10 cm layer of a Ferralsol (FAO classification) in a natural grassland was collected from the experimental station of the "Laboratoire des Radio-Isotopes" (LRI) in Lazaina (18°46'55 59° S, 47°32'46 3°N, 1,274 m altitude, Madagascar) in October 2016 from a 2 m² area. In the laboratory, the soil sample was dried at room temperature for 7 days and was then mixed using a shovel and then by hand to homogenize the sample. Most of the roots and vegetation debris were removed. The soil was then sieved to 2 mm and stored at room temperature in the dark for further analysis of soil properties.

2.2 Soil analyses

Total soil organic carbon and nitrogen were measured by dry combustion in a Flash 2000 CHNS analyser (Flash 2000 Series, CHNS/O 122 Analyser Thermo Scientific, IRCOF, France). The Olsen method (Olsen 1954) was used to determine the plant-available P content. Total P was evaluated by digestion (4 h at 600°C) with perchloric acid (HClO4) for extraction and by using a spectrophotometer and the colorimetry method (molybdenum blue) to analyse dosage. Cation exchange capacity (CEC), exchangeable K, exchangeable Mg, and exchangeable Ca were analysed using cobaltihexamine extraction and measured using an atomic absorption spectrophotometer (iCE 3000 Series AA spectrometer, Belgium). The exchangeable Al ions were extracted with a 1 N KCl solution and assayed by titrimetry with NaOH. The soil pH in water and in 1 M KCl were measured using a glass electrode pH metre (pH 211 microprocessor pH metre, Hanna Instruments, Romania) with a volume ratio of

1:2.5, and particle sizes were determined with the hydrometer method (Okalebo et al., 2002). The soil had a sandy-clay texture, pH ($H2O$) of 5.5, total organic C content of 20.8 g kg^{-1}, total N-content of 1.3 g kg^{-1}, C:N ratio of 16, total P content of 380 mg kg^{-1}, Olsen P content of 7.12 mg kg^{-1}, and exchangeable K, Ca and Mg contents of 30.7, 120.7 and 28.3 mg kg^{-1}, respectively. The CEC was 1.34 cmol+ kg^{-1}, and the water-holding capacity was 0.49 g g^{-1} dry soil.

2.3. Biological materials

Individuals of *P. corethrurus* (Rhinodrilidae) were used in the experiment. This endogeic, geophagous, peregrine species is dominant in all pedo-climatic regions in Madagascar (Razafindrakoto et al., 2010). It is a 5-8cm in length, weighing 0.7 g in average at the adult stage. This earthworm is able to live in an exceptionally substantial range of soil and climate environments. *P. corethrurus* individuals were collected near the experimental station in Lazaina and were stored in the same soil before use. Six upland rice (*Oryza sativa* L.) cultivars commonly used by farmers in the Highlands were studied: “B22”, “FOFIFA-161”, “FOFIFA-172”, “FOFIFA-182”, “NERICA-4” and “WAB 880-1-32-1” (Supplementary Table S1). Seeds were provided by the Madagascar National Centre for Applied Research on Rural Development (FOFIFA) and the French Agricultural Research Institute for Development (CIRAD)

All cultivars are fully described in the official catalogue of rainfed rice cultivars in Madagascar (Raboin et al., 2013; Radanielina et al., 2013). These rice cultivars cover the narrow current genetic diversity panel that can be found in the Highlands of Madagascar. To test the relationship between the ability of these cultivars to respond to earthworms and their agronomic characteristics, we collected agronomic data for each cultivar from the official catalogue. These agronomic data (e.g., optimal altitude for cropping, maximal grain yield, tolerance to the root parasite *Striga asiatica* or the disease *Pyricularia oryzae*, cycle duration, grain weight, length of panicle and cold tolerance) of each cultivar described in the catalogue are the result of several years of field research on the genetic improvement of rainfed rice in the Highlands of Madagascar as part of the plant genetic programme led by CIRAD and FOFIFA (Supplementary Table S1) (Raboin et al., 2012; Raboin et al., 2013; Raboin et al., 2014; Raboin et al., 2010; Radanielina et al., 2013). The rice agronomic data from the programme were averaged over 6 years of upland rice cropping under high mineral fertilization levels (e.g., 150 kg ha^{-1} of N:P:K = 11:22:16 and 80 kg ha^{-1} of urea) and organic fertilization levels (5 t ha^{-1} of cattle manure).

2.4. Experimental design

The experiment was conducted at the Radio-Isotopes Laboratory (LRI) of the University of Antananarivo (Madagascar). Two treatments were considered: (i) an earthworm treatment including two modalities, i.e., without earthworms (-EW) and with earthworms (+EW) and (ii) a rice cultivar treatment that included six modalities. Each treatment was replicated five times.

2.5. Mesocosm setup

The mesocosms consisted of 1 L plastic pots that were previously pierced (2 holes) at the bottom to avoid water accumulation. Each hole was filled with a cotton mesh to facilitate water flow. To prevent earthworms from escaping from the mesocosm, a mosquito net was placed at the bottom of the mesocosms. A total of 60 mesocosms (2 earthworm modalities × 6 rice cultivars × 5 replicates) were filled with 1 kg of sieved dry soil. At the beginning of the experiment, mesocosms were watered with 350 ml of rainwater to mimic field conditions. Three seeds of each rice cultivar were sown. Three adults of *P. corethrurus* with a welldeveloped clitellum and similar fresh mass (mean fresh weight of adults ~0.8 g) were introduced into each mesocosm. This earthworm density (~150 individuals per m²) corresponds to field density (Rakotomanga et al., 2016). During the experiment, the mesocosms were placed outside under natural weather conditions. The soil moisture content was maintained at 80% water holding capacity by weekly regular weighing. The mesocosm placements were changed every two weeks and used a new randomization. The experiment lasted 8 weeks.

2.6. Measurements of earthworm survival rates and rice traits

At the end of the experiment, the soil was gently removed from the mesocosms to check for earthworm presence. All earthworm individuals (adults, juveniles, cocoons) were collected by hand sorting. We calculated the earthworm survival rate based on abundance, expressed as the initial percentage. To evaluate the photosynthetic activity of rice leaves, the photochemical reflectance index (PRI) and normalized difference vegetation index (NDVI) of each rice cultivar were measured using PlantPen PRI 200 & NDVI 300 (Gamon et al., 2015). Five measurements were randomly performed on the largest leaf in each mesocosm every week and at the end of the experiment. Only the maximum values among the five were considered for data analysis. We also scanned the same largest leaf at the end of the experiment at 600 ppi to obtain the leaf area using the "Easy Leaf Area" software (Department of Plant Sciences, University of California). The final leaf dry matter content (LDMC) was determined after drying the leaves at 65°C for 48 h, and the specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry mass. Roots were separated from the soil by carefully washing the root system under flowing water to remove soil particles. The shoots and roots were dried at 65°C for 48 h and weighed. Total N contents in shoots and roots were measured by a CHNS/O elemental analyser (Flash 2000 Series, CHNS/O 122 Analysers Thermo Scientific, IRCOF, France). P contents in shoots and roots were determined after digestion of plant tissues with nitric acid 65%. P was measured in the digested solutions by colorimetry. The totals of N and P amounts in rice tissues were calculated by multiplying the N and P contents by biomass. N and P uptake efficiencies (NUE and PUE) were calculated as the ratio of P amounts in shoots by root biomass.

2.7. Statistical analyses

2.7.1. Ecological distance

To obtain a statistical distance among cultivars that can be used as the response to earthworm inoculation, we used the Cohen’s d effect size (Cohen, 1988): d = (Mi_+EW - Mi_-EW)/(SDi-pooled) for the “i” cultivar, where M+EW is the mean of the rice trait for the +EW treatment, M-EW is the mean of the rice trait for the -EW treatment and SD-pooled is the pooled standard deviation of the rice trait. The Cohen’s d effect size was thus calculated for each rice trait and for each cultivar using the five replications to determine the means and standard deviations (n= 5). We then computed the ecological distance using Cohens’ d-values between all pairs of rice cultivars based on the Euclidean distance using the “dist” function from the R package “stats” (Supplementary Table S2).

2.7.2. Phylogenetic distances

We assessed the phylogenetic distance among all pairs from the six cultivars (Supplementary Table S2). The molecular information is stored and managed through a web-based tool, Gigwa-Genotype investigator, for genome-wide analyses (Sempéré et al., 2019). In all cases, the complete sequence of the Nipponbare rice cultivar (Os-Nipponbare-Reference-IRGSP-1.0) was used as a reference for alignments. The Tassel GBS pipeline v3.0 (Glaubitz et al., 2014) allowed us to filter the raw data. Data were filtered for minor allele frequencies (MAF

≥ 5%) and missing rates (< 20%). A total of 36,744 Single Nucleotide Polymorphisms (SNPs) were obtained. TASSEL software version 5.0 (Bradbury et al., 2007) was used to estimate the genetic distance matrix. These analyses considered only 7,338 non-missing loci.

2.7.3. Agronomic distances

We computed the agronomic distance matrix (Supplementary Table S2) by using data collected in previous plant genetic programmes performed in Madagascar (Supplementary Table S1). We used the following agronomic criteria: optimal altitude ranges for cropping (m), maximal grain yield in fertile field situations (t ha^{-1}), tolerance to the root parasite *Striga asiatica*, cycle duration (days), grain weight (g), panicle length (cm) and cold tolerance from spikelet fertility (%). All data were collected from the cultivar catalogue that was created by the rice genetic programme of CIRAD and FOFIFA. The data were available in published studies (Raboin et al., 2013; Raboin et al., 2014; Raboin et al., 2010) or from unpublished technical sources but with free access on the internet (www.dp-spad.org/productions2/documents-techniques). The Euclidean distances for all cultivar pairs were calculated with the “dist” function from the R package “stats”. We did not weight the different agronomic parameters.

2.7.4. Data analyses

Means and standard deviations (n=5) for each variable were computed for each treatment. The normality of the data was checked using the Shapiro-Wilk test. Data were transformed with the Box-Cox transformation when required. Two-way analyses of variance (ANOVA) were then conducted with the two main factors and their interactions: earthworm inoculation (“EW”) including two modalities (absent or present) and rice cultivars covering six modalities. The significance threshold was set to 5%. We also computed the eta-squared (eta²) from ANOVA as a measure of effect size (% of variance explained) (Cohen, 1973). We detected the significant differences among the cultivars using the Tukey HSD post hoc test. The agronomic, phylogenetic and ecological distances among all pairs of cultivars were transformed to fit between 0.1 and 1.0 using a homothetic transformation; $Y = 0.1 + ((x – b/(a – b)) \times 0.9$, where Y is the value of a variable after transformation, x is the distance to transform, a is the maximum distance and b is the minimum distance among the cultivars. Then, the linear relationships between the ecological, phylogenetic and agronomic distances among cultivars were tested using parametric Pearson's product-moment correlation coefficients at the 5% significance threshold.

3. Results

3.1. Earthworm survival rate

At the end of the experiment, the earthworm survival rate ranged on average from 93% to 107% for all rice cultivar treatments (Supplementary Fig. S1). We found no significant differences ($P > 0.05$) in the earthworm survival rates among rice cultivars.

3.2. Effect of cultivars on rice traits

After eight weeks of growth, all leaf traits, except for NDVI, were significantly different among rice cultivars (Table 1). On average, F161 had the highest leaf area, LDMC and SLA, regardless of earthworm treatments (Table 2). A significant effect for rice cultivars was found for shoot biomass and shoot P and N amounts (Table 1). The highest values for shoot biomass and shoot P and N amounts were found for cultivars B22 and F161, respectively (Table 2). The lowest values were observed for F172 and N4.

3.3. Effect of earthworms on rice traits

The inoculation of *P. corethrurus* (+EW) had a strong significant effect on rice traits, except for LDMC, SLA, shoot:root ratio and PUE (Table 1). On average, rice cultivars had higher PRI (+57%), NDVI (+34%), shoot biomass (+99%), root biomass (+93%), total biomass (+96%), shoot P amount (+87%), root P amount (82%), total rice P amount (+84%) and shoot N amount (+267%) in the presence of earthworms than in their absence (Table 2).

3.4. Interaction effects of earthworms and cultivars on rice traits

We found significant interactive effects of earthworms and rice cultivars on root biomass, shoot:root ratio, and N and P acquisition efficiency (Table 1). Considering the eta² of the different sources of variation from the ANOVA models, we identified eight rice traits that were mostly affected by earthworms (eta² ranged from 49% to 68%), including physiological traits (e.g., SLA, PRI, and NDVI), growth traits (e.g., shoot biomass and total biomass) and nutrition traits (e.g., shoot P amount, total rice P amount and shoot N amount). For instance, in the presence of earthworms, SLA increased by 146% and 126% for F161 and F172, respectively, whereas it increased only by 28% for W880 (Table 2) (Fig. 1). Likewise, the positive effect of *P. corethrurus* on rice root biomass exhibited a 323% increase for N4 while this effect exhibited only a 19% increase for B22. A similar trend was observed for root P levels (Table 2). Regarding the shoot:root ratio, the effect of earthworms was highly variable and depended on specific rice cultivars (Fig. 1). It was either positive (B22, F161, and F182), neutral (F172) or negative (N4 and W880). Similarly, the response of rice cultivars to earthworms in terms of P acquisition efficiency was positive (B22, F161, and F172) or negative (F182, N4, and W880). It is interesting to note that, even if not significant according to two-way ANOVA, the magnitude of the increase induced by earthworms on shoot biomass, shoot P and shoot N amount also varied depending on rice cultivar (Fig. 1). For instance, the increase in shoot biomass induced by earthworms ranged from 40% (W880) to 129% (B22) according to the cultivars being examined. Likewise, the increase in shoot P amount in the presence of earthworms ranged from 37% (W880) to 145% (F161), and the increase in shoot N amount varied from 116% (W880) to 355% (B22) in the presence of earthworms.

Rice cultivars in the present experiment can be classified into three categories (Fig. 1): (i) rice cultivars with high average Cohen's d-values (response to earthworms), such as N4 and F182, with average d-values of 0.95 and 0.64, respectively; (ii) rice cultivars with intermediate responses, such as F172, B22, and F161, with average d-values of 0.52, 0.43 and 0.32, respectively; and (iii) rice cultivars with low average d-values, such as W880 ($d = 0.20$).

3.5. Phylogenetic, agronomic and ecological relationships

We did not find significant linear relationships for the ecological distances among rice cultivars (i.e., their ability to respond to earthworm inoculation), agronomic ($R^2 = 0.15$, P-value 0.144), and phylogenetic ($R^2 = 0.10$, P-value 0.242) distances (Fig. 2). However, the agronomic and phylogenetic distances were significantly correlated ($R^2 = 0.56$, P-value 0.001).

4. Discussion

4.1. Earthworms as plant promoters irrespective of rice cultivars

The earthworm survival rate, production of cocoons (1 to 4 cocoons per mesocosm) and surface casts in the mesocosms indicated that the conditions were favourable for the development and activity of earthworms during the experiment. Under these conditions, we demonstrated that the earthworm *P. corethrurus* strongly increased plant growth and nutrition regardless of rice cultivar. Increased rice growth was expressed by higher shoot biomass (99% increase), root biomass (93% increase) and total biomass (96% increase). We also found positive earthworm effects on leaf physiological traits, which indicated that earthworms enhanced photosynthetic light use efficiency and chlorophyll content in plants. The only plant traits that exhibited negative Cohen's d values in some cultivars were the leaf dry matter content, specific leaf area and P acquisition efficiency. Brown et al. (1999; 2004) reviewed 246 experiments conducted in tropical countries and concluded that in 43% of these studies, earthworms enhanced biomass production by more than 20%. The same observation was noted by van Groenigen et al. (2014) in a meta-analysis of 58 studies published within one century (1910–2013). These authors pointed out that increased N mineralization was the main pathway for the positive effects of earthworms on plant growth. Likewise, in the present study, earthworm inoculation strongly increased shoot N amounts (+267%), and this plant trait had the highest percentage of variance explained by earthworms (79%).

A multiple nutrient-omission experiment that was conducted with the same soil showed that P was the main nutrient which limited rice growth (Raminoarison et al., 2020). It is thus more likely that the positive effects of earthworms on rice growth are mainly driven by improved P availability (Chapuis-Lardy et al., 1998; Ros et al., 2017; Vos et al., 2019). This hypothesis is supported by higher shoot P (87% increase) when *P. corethrurus* was inoculated. We also observed significant effects of earthworms on plant P acquisition efficiency. In our experiment, a large number of casts were observed (not quantified) at the end of the experiment, which support the hypothesis formulated by Vos et al., (2014) and suggest that the increased plant P uptake was probably induced by the increase in available P in earthworm casts. The positive effect of earthworms on plant growth via increased soil P availability has been reported many times (Graff, 1970; Kuczak et al., 2006; Ros et al., 2017; Sharpley and Syers, 1976; Sharpley and Syers, 1977; Van Groenigen et al., 2019; Vos et al., 2019; Vos et al., 2014), including in Ferralsols (Chapuis-Lardy et al., 1998; Chapuis-Lardy et al., 2009).

4.2. Plant breeding and rice ability to interact with earthworms

Despite the overall positive effects of earthworms on plants, the ability of rice to interact with earthworms varied by cultivar. These findings are in agreement with Noguera et al. (2011), who conducted a mesocosm experiment in Colombia with the same earthworm species but in a nutrient-rich soil and with other rice cultivars.

From our data, it is difficult to identify the mechanisms involved in the response variability of rice cultivars to earthworms. For instance, the P acquisition efficiency (PUE) was the plant trait for which the percentage of variance explained by earthworm-rice cultivar interaction was greatest (29%). The role of P, as mentioned earlier, thus appears to be central in earthworm-rice interactions in nutrient-depleted tropical soils (Coulis et al., 2014; Le Bayon and Milleret, 2009). It would then be judicious to examine this trait. Vos et al. (2019) listed the potential pathways for earthworm-induced enhanced soil P availability: (i) elevated pH (higher pH in casts) that affects concentrations of P ions in solution, (ii) dissolved organic carbon production by worms with similar effects through competitive adsorption, (iii) stimulated mineralization of organic P (enzymes) and (iv) selective feeding. In our study, given the equal survival rates of earthworms for the various treatments, it is very unlikely that the differences observed in the plant response to earthworms could arise from these mechanisms, which are specific to worms. In contrast, we believe that these differences could come from root trait variability, which is known to be affected by earthworms (Arnone III and Zaller, 2014; Junaidi et al., 2018). We can thus suppose that the colonization of casts by roots and their fungal endomycorrhizal symbionts could affect plant nutrition (Milleret et al., 2009; Van Groenigen et al., 2014; Zaller et al., 2013), especially plant P uptake (Bolan, 1991; George et al., 1995; Smith et al., 2011). These root traits could vary according to rice cultivars. Knowing the crucial roles of these parameters in plant P acquisition (Bolan, 1991; George et al., 1995) and their interaction with earthworms (Milleret et al., 2009), it would be interesting to further investigate these root traits, e.g., root surface, length, number of tips or mycorrhizal fungal colonization, in further studies to identify the mechanisms involved in the variability in earthworm-plant interactions with plant cultivars. It would also be interesting to monitor the P nutrition of rice during its growth, in the presence or absence of earthworms, for a reduced number of contrasting varieties. This could allow us to test whether the response of varieties to the presence of rice takes place at key phenological periods.

Considering the agronomic characteristics of rice cultivars (Table 2), we did not find evidence of relationships between some agronomic criteria and rice response to earthworms. Accordingly, we did not observe any significant relationship between the agronomic distances among cultivars and their ability to interact with inoculated earthworms. These results suggest that, regardless of the agronomic criteria focused on in the breeding programme, plant breeding may affect the ability of rice crops to exploit the beneficial functions achieved by earthworms. As plant breeding affects the genetic inheritance of rice cultivars with contrasting NUE or PUE, the cultivars can therefore exploit the presence of earthworms differently. However, before taking this for granted, it would be beneficial to test this hypothesis with a larger panel of rice cultivars.

4.3. Ecological implications for agroecology and limits of the study

This experimental study showed that the upland rice cultivars commonly used by farmers in the Highlands of Madagascar have varying abilities to interact with *P. corethrurus*. This result is noteworthy because the cross effects of earthworms and rice cultivars were highly significant despite the relatively short duration of the study. To confirm these patterns and to measure rice yield, it would be beneficial to continue with an earthworm inoculation experiment in the field. Also, the low genetic diversity within our narrow cultivar panel could also be a factor that limited our conclusions. However, the criteria for choosing rice cultivars in this study were constrained by the genetic pool that was adapted to the environmental and agronomic conditions (climate, soil, uses and producer preferences) of the Highlands of Madagascar (Raboin et al., 2014). This genetic diversity is low and confers significant risks for the production of rainfed rice, particularly against pests. In view of the major role of biological interactions between soil organisms and rice for the tolerance of rice to pests and diseases (Blanchart et al., 2019), considering the capability of varieties to interact with earthworms seems to be essential. In these conditions, however, we succeeded in identifying the rice cultivars that exhibited strong (N4 and F182), intermediate (F172, B22, and F161) and low (W880) abilities for interacting with earthworms. Integrating the most appropriate rice cultivars into relevant agroecological practices has been a concern of several soil ecologists and agronomists (Loeuille et al., 2013; Noguera et al., 2011). We therefore propose to consider the plant genetic material that best interacts with earthworms to intensify soil ecological processes in low-input tropical agrosystems, either for direct use with relevant agro-ecological practices or for inclusion in plant breeding programs. Such innovative practices may be a suitable approach to meet the constraints of the low input production systems of smallholder farmers in the Highlands of Madagascar.

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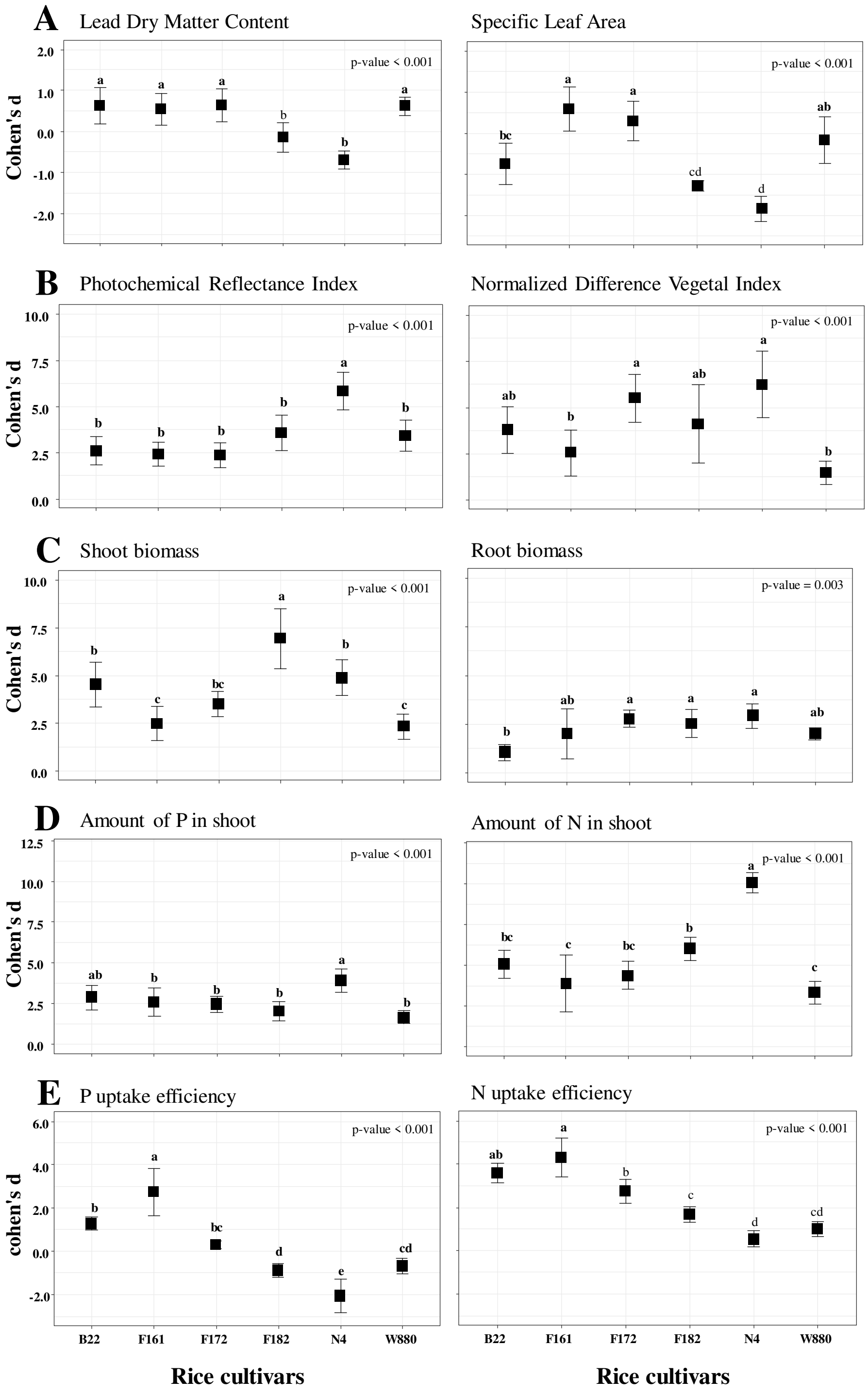
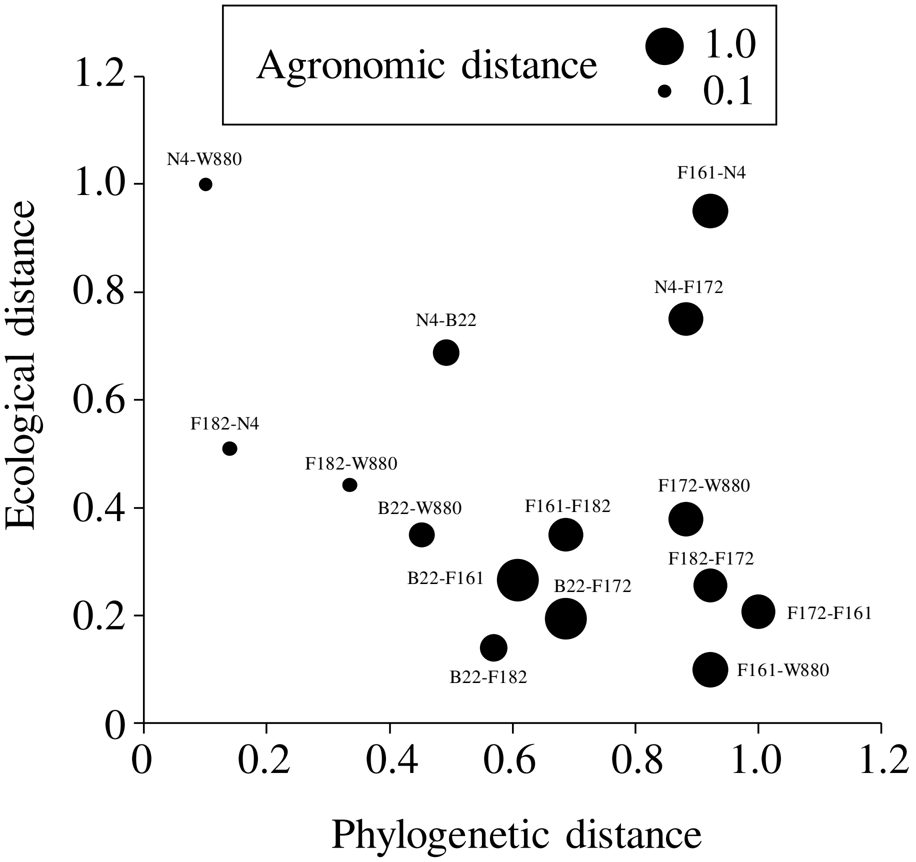
Fig. 1. Effect sizes (Cohen's d) of earthworms on different plant traits according to rice cultivars. (A) Leaf traits, (B) leaf physiological traits, (C) biomass traits, (D) nutrition traits, and (E) nutrient acquisition efficiencies. Whiskers correspond to standard deviation (SD). Different letters (a, b, c and d) indicate significant differences between rice cultivars according to Tukey HSD test (P-value < 0.05).

Fig. 2. Relationships between phylogenetic, agronomic and ecological distances. The phylogenetic distance among cultivars were computed using TASSEL software based on Single Nucleotide Polymorphisms. The Euclidean agronomic distance is based on seven unweighted agronomic criteria (Table S2). The ecological distance is the Euclidean distance computed from the response of cultivars to earthworm inoculation using Cohen's d effect size for each rice trait (Fig. 1). All distances were bounded between 0.1 and 1 using homothetic transformation. N4, W880, F161, B22, N172 and F182 refers to rice cultivars (Table S1).


A
Lead Dry Matter Content
Specific Leaf Area
B
Photochemical Reflectance Index
Normalized Difference Vegetal Index
C
Shoot biomass
Root biomass
D
Amount of P in shoot
Amount of N in shoot
E
P uptake efficiency
N uptake efficiency
Cohen's d
cohen's d
p-value < 0.001
p-value = 0.003
a
ab
b
bc
c
cd
d
e
2.0
1.0
0.0
-1.0
-2.0
10.0
7.5
5.0
2.5
12.5
6.0
4.0
B22
F161
F172
F182
N4
W880
Rice cultivars



Agronomic distance
1.0
0.1
Ecological distance
Phylogenetic distance
0
0.2
0.4
0.6
0.8
1.0
1.2
N4-W880
F161-N4
N4-F172
N4-B22
F182-N4
F182-W880
B22-W880
F161-F182
F172-W880
F182-F172
B22-F161
B22-F172
F172-F161
F161-W880
B22-F182

Table 1. Results of two-way ANOVA (eta^2 in % and p-value) for the main effects (rice cultivars and earthworms) and their interactions for each plant variable.

	Rice cultivars		**Earthworms**		**Interaction**		**Residuals**
	Eta$^{2\$}$	p-value	Eta^2	p-value	Eta^2	p-value	Eta^2
Leaf Dry Matter Content	34	***	1	ns	4	ns	61
Specific Leaf Area	20	*	1	ns	9	ns	71
Photochemical Reflectance Index	7	*	64	***	3	ns	25
Normalized Difference Vegetation Index	4	ns	68	***	5	ns	23
Shoot biomass	18	***	59	***	3	ns	20
Root biomass	3	ns	40	***	12	*	44
Shoot:root ratio	8	ns	2	ns	26	**	64
Shoot P amount	13	**	52	***	6	ns	30
Shoot N amount	7	***	79	***	2	ns	12
P acquisition efficiency	8	ns	0	ns	29	**	63
N acquistion efficiency	1	ns	33	***	17	*	49

[\$] Eta-squared (eta^2) is a measure of effect size from ANOVA (% of variance explained)

P-value thresholds: *** < 0.001; ** < 0.01; * < 0.05, ns: not significant

Table 2. Mean (+-SD) for each plant variable according to treatments without earthworms (-EW) and with earthworms (+EW) after eight weeks of growth.

Variables	**Unit**	**Rice cultivars**																							
		B22				**F161**				**F172**				**F182**				**N4**				**W880**			
		-EW		**+EW**		**-EW**		**+EW**		**-EW**		**+EW**		**-EW**		**+EW**		**-EW**		**+EW**		**-EW**		**+EW**	
Leaf Dry Matter Content	mg.g^{-1}	2.4	(0.7)	2.8	(0.5)	2.7	(0.2)	2.9	(0.5)	2.1	(0.5)	2.4	(0.3)	2.1	(0.6)	2.0	(0.5)	2.3	(0.4)	2.1	(0.3)	2.0	(0.5)	2.2	(0.3)
Specific Leaf Area	mm^2.mg^{-1}	11.9	(1.4)	10.7	(1.9)	14.3	(1.5)	17.9	(8.9)	12	(2.2)	12.7	(2.7)	14.2	(1.6)	12.3	(1.4)	14.0	(2.5)	10.4	(1.6)	14.7	(3.9)	13.9	(3.0)
Normalized Difference Vegetation Index	Abs	0.025	(0.009)	0.039	(0.003)	0.029	(0.005)	0.039	(0.004)	0.029	(0.009)	0.043	(0.005)	0.030	(0.007)	0.049	(0.005)	0.028	(0.006)	0.051	(0.002)	0.030	(0.005)	0.047	(0.005)
NDVI	Abs	0.55	(0.1)	0.76	(0.02)	0.54	(0.07)	0.70	(0.08)	0.49	(0.04)	0.7	(0.04)	0.51	(0.08)	0.71	(0.04)	0.50	(0.05)	0.75	(0.03	0.61	(0.08)	0.70	(0.07)
Shoot biomass	mg	59.4	(4.7)	136	(29.2)	47.4	(8.3)	104.0	(40.4)	44.6	(10.8)	89.1	(15.7)	38.7	(5.9)	81.9	(11.0)	35.0	(9.5)	73.7	(5.6)	61.9	(16.1)	86.7	(8.0)
Root biomass	mg	83.9	(29.2)	99.6	(9.8)	54.0	(5.5)	90.6	(35.8)	48.3	(18.5)	94.1	(18.5)	48.3	(4.4)	107.0	(44.7)	33.9	(2.7)	143.2	(73.7)	66.7	(18.5)	113.8	(30.3)
Shoot:root ratio	ratio	0.78	(0.25)	1.37	(0.3)	0.85	(0.13)	1.16	(0.22)	0.98	(0.2)	0.98	(0.27)	0.81	(0.18)	0.9	(0.4)	1.03	(0.26)	0.68	(0.41)	0.96	(0.22)	0.81	(0.24)
Shoot P amount	µg-P.pot^{-1}	46.7	(4.5)	95.8	(31.1)	35.2	(8.1)	86.1	(34.0)	30.0	(7.9)	66.7	(23.3)	39.3	(9.3)	56.0	(8.2)	31.2	(3.0)	58.6	(11.7)	42.8	(8.4)	58.4	(11.4)
Shoot N amount	mg-N.pot^{-1}	0.65	(0.06)	2.94	(0.88)	0.58	(0.16)	2.39	(0.88)	0.48	(0.16)	1.88	(0.52)	0.45	(0.06)	1.72	(0.37)	0.40	(0.15)	1.68	(0.11)	0.82	(0.31)	1.78	(0.29)
P uptake efficiency	µg-P.g^{-1} of dry root	0.61	(0.23)	0.97	(0.33)	0.64	(0.11)	0.97	(0.15)	0.66	(0.18)	0.73	(0.25)	0.83	(0.27)	0.60	(0.24)	0.92	(0.04)	0.54	(0.34)	0.67	(0.14)	0.55	(0.18)
N uptake efficiency	mg-N.g^{-1} of dry root	8.56	(3.07)	29.68	(8.82)	10.56	(2.55)	27.50	(5.68)	10.20	(1.86)	20.42	(5.81)	9.46	(2.02)	18.92	(9.28)	11.72	(4.15)	15.52	(9.80)	12.38	(2.95)	16.84	(6.22)