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);

71 (iii) control of pests and parasites (Blanchart et al., 2019; Loranger-Merciris et al., 2012); (iv)
72 stimulation of symbionts (Huhta, 2006) and (v) modifications of soil porosity, aggregation
73 and water retention (Blanchart et al., 1999). Breeding modern high-yielding cultivars takes
74 place under high input conditions (i.e., mineral fertilizers and chemical treatment of pests) in
75 soils that can be unfavourable to earthworms (Doran and Zeiss, 2000; Ponge et al., 2013).
76 Therefore, it is possible that these cultivars have lost some of the plant functional traits
77 involved in interactions with earthworms, which may result in contrasting responses of plants
78 to earthworm presence. For instance, Noguera et al. (2011) showed that the effects of
79 *Pontoscolex corethrurus* on rice growth varied significantly for different cultivars and to the
80 presence of biochar. However, the factors e.g., genetic background, agronomic selection
81 criteria and targeted plant traits that determine this variability in plant responses to
82 earthworms remain unidentified.
83 In the Ferralsols of Madagascar, upland crops face several edaphic constraints. Ferralsols are
84 very infertile with numerous limiting nutrients, especially P but also N, Ca, Mg
85 (Raminoarison et al., 2020). They have very low carbon contents and a low biological activity
86 leading to a very poor productivity of the native vegetation. In the Highlands, the main
87 earthworm species (i.e. *P. corethrurus* (Rhinodrilidae), *Dichogaster bolaui*, *Dichogaster*
88 *saliens* (Acanthodrilidae), and *Amynthas corticis* (Megascolecidae)) can deeply modify soil
89 structure and soil C and N cycles and P availability (Trap et al., 2021). This last function is
90 especially important for P-fixing soils such as the Ferralsols.
91 In the present study, by using a mesocosm greenhouse experimental approach, we aimed to
92 assess the effects of earthworms on the growth and nutrition of six locally widespread upland
93 rice cultivars that are adapted to the nutrient-depleted Ferralsols in Madagascar. We also
94 tested whether agronomic selection criteria or the genetic signals among cultivars may explain
95 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

121 1:2.5, and particle sizes were determined with the hydrometer method (Okalebo et al., 2002).
122 The soil had a sandy-clay texture, pH (H2O) of 5.5, total organic C content of 20.8 g kg^{-1},
123 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
124 of 7.12 mg kg^{-1}, and exchangeable K, Ca and Mg contents of 30.7, 120.7 and 28.3 mg kg^{-1},
125 respectively. The CEC was 1.34 cmol+ kg^{-1}, and the water-holding capacity was 0.49 g g^{-1}
126 dry soil.
127
128 ***2.3. Biological materials***
129 Individuals of *P. corethrurus* (Rhinodrilidae) were used in the experiment. This endogeic,
130 geophagous, peregrine species is dominant in all pedo-climatic regions in Madagascar
131 (Razafindrakoto et al., 2010). It is a 5-8cm in length, weighing 0.7 g in average at the adult
132 stage. This earthworm is able to live in an exceptionally substantial range of soil and climate
133 environments. *P. corethrurus* individuals were collected near the experimental station in
134 Lazaina and were stored in the same soil before use. Six upland rice (*Oryza sativa* L.)
135 cultivars commonly used by farmers in the Highlands were studied: "B22", "FOFIFA-161",
136 "FOFIFA-172", "FOFIFA-182", "NERICA-4" and "WAB 880-1-32-1" (Supplementary
137 Table S1). Seeds were provided by the Madagascar National Centre for Applied Research on
138 Rural Development (FOFIFA) and the French Agricultural Research Institute for
139 Development (CIRAD)
140 All cultivars are fully described in the official catalogue of rainfed rice cultivars in
141 Madagascar (Raboin et al., 2013; Radanielina et al., 2013). These rice cultivars cover the
142 narrow current genetic diversity panel that can be found in the Highlands of Madagascar. To
143 test the relationship between the ability of these cultivars to respond to earthworms and their
144 agronomic characteristics, we collected agronomic data for each cultivar from the official
145 catalogue. These agronomic data (e.g., optimal altitude for cropping, maximal grain yield,

146 tolerance to the root parasite *Striga asiatica* or the disease *Pyricularia oryzae*, cycle duration,
147 grain weight, length of panicle and cold tolerance) of each cultivar described in the catalogue
148 are the result of several years of field research on the genetic improvement of rainfed rice in
149 the Highlands of Madagascar as part of the plant genetic programme led by CIRAD and
150 FOFIFA (Supplementary Table S1) (Raboin et al., 2012; Raboin et al., 2013; Raboin et al.,
151 2014; Raboin et al., 2010; Radanielina et al., 2013). The rice agronomic data from the
152 programme were averaged over 6 years of upland rice cropping under high mineral
153 fertilization levels (e.g., 150 kg ha^{-1} of N:P:K = 11:22:16 and 80 kg ha^{-1} of urea) and organic
154 fertilization levels (5 t ha^{-1} of cattle manure).
155
156 ***2.4. Experimental design***
157 The experiment was conducted at the Radio-Isotopes Laboratory (LRI) of the University of
158 Antananarivo (Madagascar). Two treatments were considered: (i) an earthworm treatment
159 including two modalities, i.e., without earthworms (-EW) and with earthworms (+EW) and
160 (ii) a rice cultivar treatment that included six modalities. Each treatment was replicated five
161 times.
162
163 ***2.5. Mesocosm setup***
164 The mesocosms consisted of 1 L plastic pots that were previously pierced (2 holes) at the
165 bottom to avoid water accumulation. Each hole was filled with a cotton mesh to facilitate
166 water flow. To prevent earthworms from escaping from the mesocosm, a mosquito net was
167 placed at the bottom of the mesocosms. A total of 60 mesocosms (2 earthworm modalities × 6
168 rice cultivars × 5 replicates) were filled with 1 kg of sieved dry soil. At the beginning of the
169 experiment, mesocosms were watered with 350 ml of rainwater to mimic field conditions.
170 Three seeds of each rice cultivar were sown. Three adults of *P. corethrurus* with a well-

171 developed clitellum and similar fresh mass (mean fresh weight of adults ~0.8 g) were
172 introduced into each mesocosm. This earthworm density (~150 individuals per m²)
173 corresponds to field density (Rakotomanga et al., 2016). During the experiment, the
174 mesocosms were placed outside under natural weather conditions. The soil moisture content
175 was maintained at 80% water holding capacity by weekly regular weighing. The mesocosm
176 placements were changed every two weeks and used a new randomization. The experiment
177 lasted 8 weeks.
178
179 ***2.6. Measurements of earthworm survival rates and rice traits***
180 At the end of the experiment, the soil was gently removed from the mesocosms to check for
181 earthworm presence. All earthworm individuals (adults, juveniles, cocoons) were collected by
182 hand sorting. We calculated the earthworm survival rate based on abundance, expressed as the
183 initial percentage. To evaluate the photosynthetic activity of rice leaves, the photochemical
184 reflectance index (PRI) and normalized difference vegetation index (NDVI) of each rice
185 cultivar were measured using PlantPen PRI 200 & NDVI 300 (Gamon et al., 2015). Five
186 measurements were randomly performed on the largest leaf in each mesocosm every week
187 and at the end of the experiment. Only the maximum values among the five were considered
188 for data analysis. We also scanned the same largest leaf at the end of the experiment at 600
189 ppi to obtain the leaf area using the "Easy Leaf Area" software (Department of Plant
190 Sciences, University of California). The final leaf dry matter content (LDMC) was determined
191 after drying the leaves at 65°C for 48 h, and the specific leaf area (SLA) was calculated as the
192 ratio of leaf area to leaf dry mass. Roots were separated from the soil by carefully washing the
193 root system under flowing water to remove soil particles. The shoots and roots were dried at
194 65°C for 48 h and weighed. Total N contents in shoots and roots were measured by a
195 CHNS/O elemental analyser (Flash 2000 Series, CHNS/O 122 Analysers Thermo Scientific,

196 IRCOF, France). P contents in shoots and roots were determined after digestion of plant
197 tissues with nitric acid 65%. P was measured in the digested solutions by colorimetry. The
198 totals of N and P amounts in rice tissues were calculated by multiplying the N and P contents
199 by biomass. N and P uptake efficiencies (NUE and PUE) were calculated as the ratio of P
200 amounts in shoots by root biomass.
201
202 ***2.7. Statistical analyses***
203 *2.7.1. Ecological distance*
204 To obtain a statistical distance among cultivars that can be used as the response to earthworm
205 inoculation, we used the Cohen's d effect size (Cohen, 1988): d = (Mi_+EW - Mi_-
206 EW)/(SDi-pooled) for the "i" cultivar, where M+EW is the mean of the rice trait for the +EW
207 treatment, M-EW is the mean of the rice trait for the -EW treatment and SD-pooled is the
208 pooled standard deviation of the rice trait. The Cohen's d effect size was thus calculated for
209 each rice trait and for each cultivar using the five replications to determine the means and
210 standard deviations (n= 5). We then computed the ecological distance using Cohens' d-values
211 between all pairs of rice cultivars based on the Euclidean distance using the "dist" function
212 from the R package "stats" (Supplementary Table S2).
213
214 *2.7.2. Phylogenetic distances*
215 We assessed the phylogenetic distance among all pairs from the six cultivars (Supplementary
216 Table S2). The molecular information is stored and managed through a web-based tool,
217 Gigwa-Genotype investigator, for genome-wide analyses (Sempéré et al., 2019). In all cases,
218 the complete sequence of the Nipponbare rice cultivar (Os-Nipponbare-Reference-IRGSP-
219 1.0) was used as a reference for alignments. The Tassel GBS pipeline v3.0 (Glaubitz et al.,
220 2014) allowed us to filter the raw data. Data were filtered for minor allele frequencies (MAF

221 ≥ 5%) and missing rates (< 20%). A total of 36,744 Single Nucleotide Polymorphisms (SNPs)
222 were obtained. TASSEL software version 5.0 (Bradbury et al., 2007) was used to estimate the
223 genetic distance matrix. These analyses considered only 7,338 non-missing loci.
224
225 *2.7.3. Agronomic distances*
226 We computed the agronomic distance matrix (Supplementary Table S2) by using data
227 collected in previous plant genetic programmes performed in Madagascar (Supplementary
228 Table S1). We used the following agronomic criteria: optimal altitude ranges for cropping
229 (m), maximal grain yield in fertile field situations (t ha^{-1}), tolerance to the root parasite *Striga*
230 *asiatica*, cycle duration (days), grain weight (g), panicle length (cm) and cold tolerance from
231 spikelet fertility (%). All data were collected from the cultivar catalogue that was created by
232 the rice genetic programme of CIRAD and FOFIFA. The data were available in published
233 studies (Raboin et al., 2013; Raboin et al., 2014; Raboin et al., 2010) or from unpublished
234 technical sources but with free access on the internet (www.dp-
235 spad.org/productions2/documents-techniques). The Euclidean distances for all cultivar pairs
236 were calculated with the "dist" function from the R package "stats". We did not weight the
237 different agronomic parameters.
238
239 *2.7.4. Data analyses*
240 Means and standard deviations (n=5) for each variable were computed for each treatment. The
241 normality of the data was checked using the Shapiro-Wilk test. Data were transformed with
242 the Box-Cox transformation when required. Two-way analyses of variance (ANOVA) were
243 then conducted with the two main factors and their interactions: earthworm inoculation
244 ("EW") including two modalities (absent or present) and rice cultivars covering six
245 modalities. The significance threshold was set to 5%. We also computed the eta-squared (eta^2)

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

271 The inoculation of *P. corethrurus* (+EW) had a strong significant effect on rice traits, except
272 for LDMC, SLA, shoot:root ratio and PUE (Table 1). On average, rice cultivars had higher
273 PRI (+57%), NDVI (+34%), shoot biomass (+99%), root biomass (+93%), total biomass
274 (+96%), shoot P amount (+87%), root P amount (82%), total rice P amount (+84%) and shoot
275 N amount (+267%) in the presence of earthworms than in their absence (Table 2).
276
277 ***3.4. Interaction effects of earthworms and cultivars on rice traits***
278 We found significant interactive effects of earthworms and rice cultivars on root biomass,
279 shoot:root ratio, and N and P acquisition efficiency (Table 1). Considering the eta² of the
280 different sources of variation from the ANOVA models, we identified eight rice traits that
281 were mostly affected by earthworms (eta² ranged from 49% to 68%), including physiological
282 traits (e.g., SLA, PRI, and NDVI), growth traits (e.g., shoot biomass and total biomass) and
283 nutrition traits (e.g., shoot P amount, total rice P amount and shoot N amount). For instance,
284 in the presence of earthworms, SLA increased by 146% and 126% for F161 and F172,
285 respectively, whereas it increased only by 28% for W880 (Table 2) (Fig. 1). Likewise, the
286 positive effect of *P. corethrurus* on rice root biomass exhibited a 323% increase for N4 while
287 this effect exhibited only a 19% increase for B22. A similar trend was observed for root P
288 levels (Table 2). Regarding the shoot:root ratio, the effect of earthworms was highly variable
289 and depended on specific rice cultivars (Fig. 1). It was either positive (B22, F161, and F182),
290 neutral (F172) or negative (N4 and W880). Similarly, the response of rice cultivars to
291 earthworms in terms of P acquisition efficiency was positive (B22, F161, and F172) or
292 negative (F182, N4, and W880). It is interesting to note that, even if not significant according
293 to two-way ANOVA, the magnitude of the increase induced by earthworms on shoot biomass,
294 shoot P and shoot N amount also varied depending on rice cultivar (Fig. 1). For instance, the
295 increase in shoot biomass induced by earthworms ranged from 40% (W880) to 129% (B22)

296 according to the cultivars being examined. Likewise, the increase in shoot P amount in the
297 presence of earthworms ranged from 37% (W880) to 145% (F161), and the increase in shoot
298 N amount varied from 116% (W880) to 355% (B22) in the presence of earthworms.
299 Rice cultivars in the present experiment can be classified into three categories (Fig. 1): (i) rice
300 cultivars with high average Cohen's d-values (response to earthworms), such as N4 and F182,
301 with average d-values of 0.95 and 0.64, respectively; (ii) rice cultivars with intermediate
302 responses, such as F172, B22, and F161, with average d-values of 0.52, 0.43 and 0.32,
303 respectively; and (iii) rice cultivars with low average d-values, such as W880 (d = 0.20).
304
305 ***3.5. Phylogenetic, agronomic and ecological relationships***
306 We did not find significant linear relationships for the ecological distances among rice
307 cultivars (i.e., their ability to respond to earthworm inoculation), agronomic (R^2 = 0.15, P-
308 value 0.144), and phylogenetic (R^2 = 0.10, P-value 0.242) distances (Fig. 2). However, the
309 agronomic and phylogenetic distances were significantly correlated (R^2 = 0.56, P-value
310 0.001).
311
312 **4. Discussion**
313 ***4.1. Earthworms as plant promoters irrespective of rice cultivars***
314 The earthworm survival rate, production of cocoons (1 to 4 cocoons per mesocosm) and
315 surface casts in the mesocosms indicated that the conditions were favourable for the
316 development and activity of earthworms during the experiment. Under these conditions, we
317 demonstrated that the earthworm *P. corethrurus* strongly increased plant growth and nutrition
318 regardless of rice cultivar. Increased rice growth was expressed by higher shoot biomass (99%
319 increase), root biomass (93% increase) and total biomass (96% increase). We also found
320 positive earthworm effects on leaf physiological traits, which indicated that earthworms

321 enhanced photosynthetic light use efficiency and chlorophyll content in plants. The only plant
322 traits that exhibited negative Cohen's d values in some cultivars were the leaf dry matter
323 content, specific leaf area and P acquisition efficiency. Brown et al. (1999; 2004) reviewed
324 246 experiments conducted in tropical countries and concluded that in 43% of these studies,
325 earthworms enhanced biomass production by more than 20%. The same observation was
326 noted by van Groenigen et al. (2014) in a meta-analysis of 58 studies published within one
327 century (1910–2013). These authors pointed out that increased N mineralization was the main
328 pathway for the positive effects of earthworms on plant growth. Likewise, in the present
329 study, earthworm inoculation strongly increased shoot N amounts (+267%), and this plant
330 trait had the highest percentage of variance explained by earthworms (79%).
331 A multiple nutrient-omission experiment that was conducted with the same soil showed that P
332 was the main nutrient which limited rice growth (Raminoarison et al., 2020). It is thus more
333 likely that the positive effects of earthworms on rice growth are mainly driven by improved P
334 availability (Chapuis-Lardy et al., 1998; Ros et al., 2017; Vos et al., 2019). This hypothesis is
335 supported by higher shoot P (87% increase) when *P. corethrurus* was inoculated. We also
336 observed significant effects of earthworms on plant P acquisition efficiency. In our
337 experiment, a large number of casts were observed (not quantified) at the end of the
338 experiment, which support the hypothesis formulated by Vos et al., (2014) and suggest that
339 the increased plant P uptake was probably induced by the increase in available P in earthworm
340 casts. The positive effect of earthworms on plant growth via increased soil P availability has
341 been reported many times (Graff, 1970; Kuczak et al., 2006; Ros et al., 2017; Sharpley and
342 Syers, 1976; Sharpley and Syers, 1977; Van Groenigen et al., 2019; Vos et al., 2019; Vos et
343 al., 2014), including in Ferralsols (Chapuis-Lardy et al., 1998; Chapuis-Lardy et al., 2009).
344

345 ***4.2. Plant breeding and rice ability to interact with earthworms***

346 Despite the overall positive effects of earthworms on plants, the ability of rice to interact with
347 earthworms varied by cultivar. These findings are in agreement with Noguera et al. (2011),
348 who conducted a mesocosm experiment in Colombia with the same earthworm species but in
349 a nutrient-rich soil and with other rice cultivars.
350 From our data, it is difficult to identify the mechanisms involved in the response variability of
351 rice cultivars to earthworms. For instance, the P acquisition efficiency (PUE) was the plant
352 trait for which the percentage of variance explained by earthworm-rice cultivar interaction
353 was greatest (29%). The role of P, as mentioned earlier, thus appears to be central in
354 earthworm-rice interactions in nutrient-depleted tropical soils (Coulis et al., 2014; Le Bayon
355 and Milleret, 2009). It would then be judicious to examine this trait. Vos et al. (2019) listed
356 the potential pathways for earthworm-induced enhanced soil P availability: (i) elevated pH
357 (higher pH in casts) that affects concentrations of P ions in solution, (ii) dissolved organic
358 carbon production by worms with similar effects through competitive adsorption, (iii)
359 stimulated mineralization of organic P (enzymes) and (iv) selective feeding. In our study,
360 given the equal survival rates of earthworms for the various treatments, it is very unlikely that
361 the differences observed in the plant response to earthworms could arise from these
362 mechanisms, which are specific to worms. In contrast, we believe that these differences could
363 come from root trait variability, which is known to be affected by earthworms (Arnone III and
364 Zaller, 2014; Junaidi et al., 2018). We can thus suppose that the colonization of casts by roots
365 and their fungal endomycorrhizal symbionts could affect plant nutrition (Milleret et al., 2009;
366 Van Groenigen et al., 2014; Zaller et al., 2013), especially plant P uptake (Bolan, 1991;
367 George et al., 1995; Smith et al., 2011). These root traits could vary according to rice
368 cultivars. Knowing the crucial roles of these parameters in plant P acquisition (Bolan, 1991;
369 George et al., 1995) and their interaction with earthworms (Milleret et al., 2009), it would be
370 interesting to further investigate these root traits, e.g., root surface, length, number of tips or

371 mycorrhizal fungal colonization, in further studies to identify the mechanisms involved in the
372 variability in earthworm-plant interactions with plant cultivars. It would also be interesting to
373 monitor the P nutrition of rice during its growth, in the presence or absence of earthworms,
374 for a reduced number of contrasting varieties. This could allow us to test whether the response
375 of varieties to the presence of rice takes place at key phenological periods.
376 Considering the agronomic characteristics of rice cultivars (Table 2), we did not find evidence
377 of relationships between some agronomic criteria and rice response to earthworms.
378 Accordingly, we did not observe any significant relationship between the agronomic distances
379 among cultivars and their ability to interact with inoculated earthworms. These results suggest
380 that, regardless of the agronomic criteria focused on in the breeding programme, plant
381 breeding may affect the ability of rice crops to exploit the beneficial functions achieved by
382 earthworms. As plant breeding affects the genetic inheritance of rice cultivars with contrasting
383 NUE or PUE, the cultivars can therefore exploit the presence of earthworms differently.
384 However, before taking this for granted, it would be beneficial to test this hypothesis with a
385 larger panel of rice cultivars.
386
387 ***4.3. Ecological implications for agroecology and limits of the study***
388 This experimental study showed that the upland rice cultivars commonly used by farmers in
389 the Highlands of Madagascar have varying abilities to interact with *P. corethrurus*. This result
390 is noteworthy because the cross effects of earthworms and rice cultivars were highly
391 significant despite the relatively short duration of the study. To confirm these patterns and to
392 measure rice yield, it would be beneficial to continue with an earthworm inoculation
393 experiment in the field. Also, the low genetic diversity within our narrow cultivar panel could
394 also be a factor that limited our conclusions. However, the criteria for choosing rice cultivars
395 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.

Acknowledgements

We want to thank Eric Randrianasolo, Andrianantenaina Hilaire Damase Razafimahafaly and Marie-Paule Razafimanantsoa (Laboratoire des RadioIsotopes) for their technical assistance. This study was funded by the Agropolis Foundation (Investissements d'Avenir Programme, ANR-10-LABX-0001-01) under the framework of the SECuRE (ID 1605-007) project. This work was also supported by the French Ministry Europe and Foreign Affairs under the programme 'Make Our Planet Great Again" for postdoctoral researchers.

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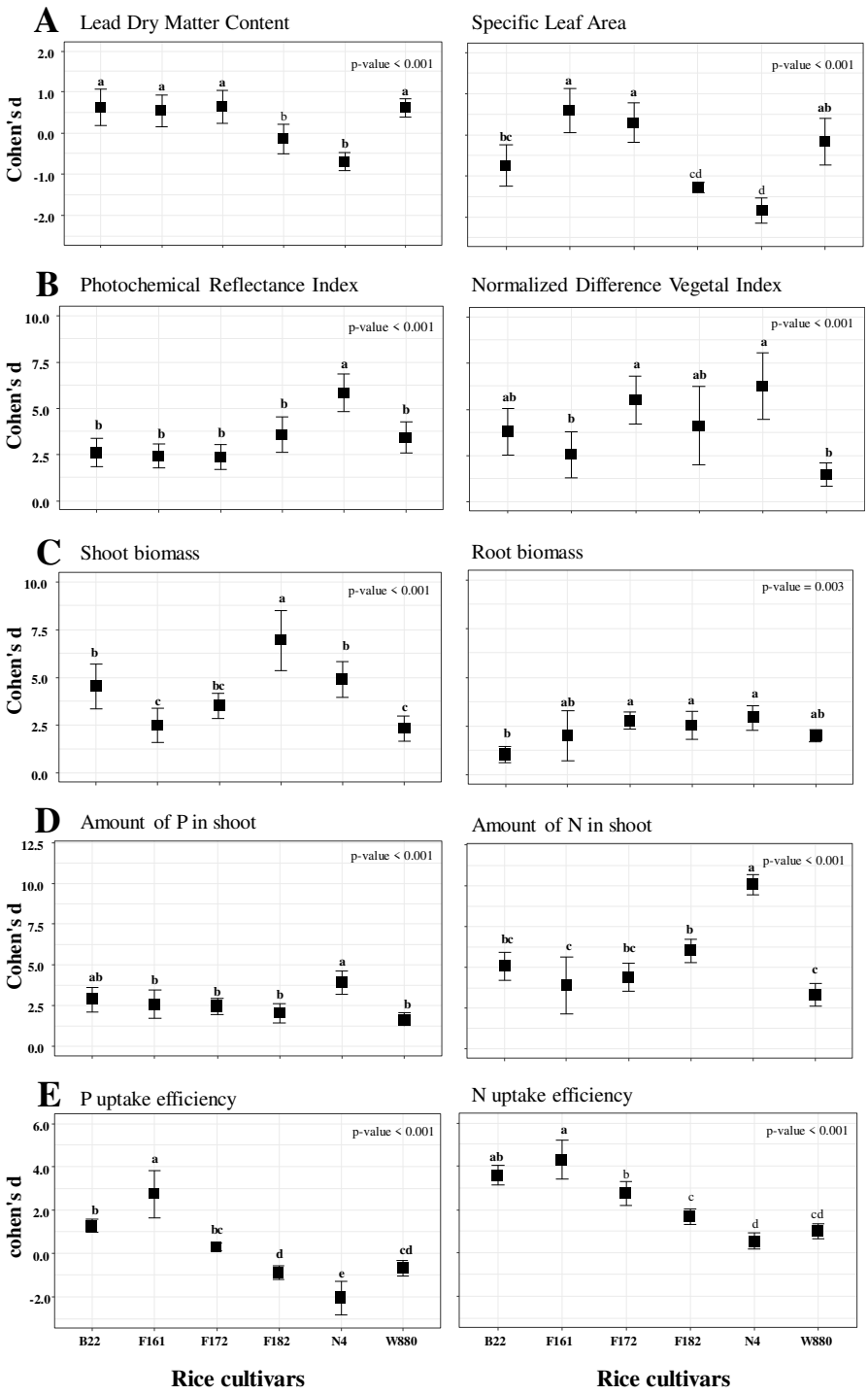
674 **Fig. 1.** Effect sizes (Cohen's d) of earthworms on different plant traits according to rice
675 cultivars. (A) Leaf traits, (B) leaf physiological traits, (C) biomass traits, (D) nutrition traits,
676 and (E) nutrient acquisition efficiencies. Whiskers correspond to standard deviation (SD).
677 Different letters (a, b, c and d) indicate significant differences between rice cultivars
678 according to Tukey HSD test (*P*-value < 0.05).
679
680 **Fig. 2.** Relationships between phylogenetic, agronomic and ecological distances. The
681 phylogenetic distance among cultivars were computed using TASSEL software based on
682 Single Nucleotide Polymorphisms. The Euclidean agronomic distance is based on seven
683 unweighted agronomic criteria (Table S2). The ecological distance is the Euclidean distance
684 computed from the response of cultivars to earthworm inoculation using Cohen's d effect size
685 for each rice trait (Fig. 1). All distances were bounded between 0.1 and 1 using homothetic
686 transformation. N4, W880, F161, B22, N172 and F182 refers to rice cultivars (Table S1).
687
688


A
Lead Dry Matter Content
Specific Leaf Area
p-value < 0.001
p-value < 0.001
Cohen's d
B
Photochemical Reflectance Index
Normalized Difference Vegetal Index
p-value < 0.001
p-value < 0.001
Cohen's d
C
Shoot biomass
Root biomass
p-value < 0.001
p-value = 0.003
Cohen's d
D
Amount of P in shoot
Amount of N in shoot
p-value < 0.001
p-value < 0.001
Cohen's d
E
P uptake efficiency
N uptake efficiency
p-value < 0.001
p-value < 0.001
cohen's d
B22
F161
F172
F182
N4
W880
Rice cultivars
Rice cultivars

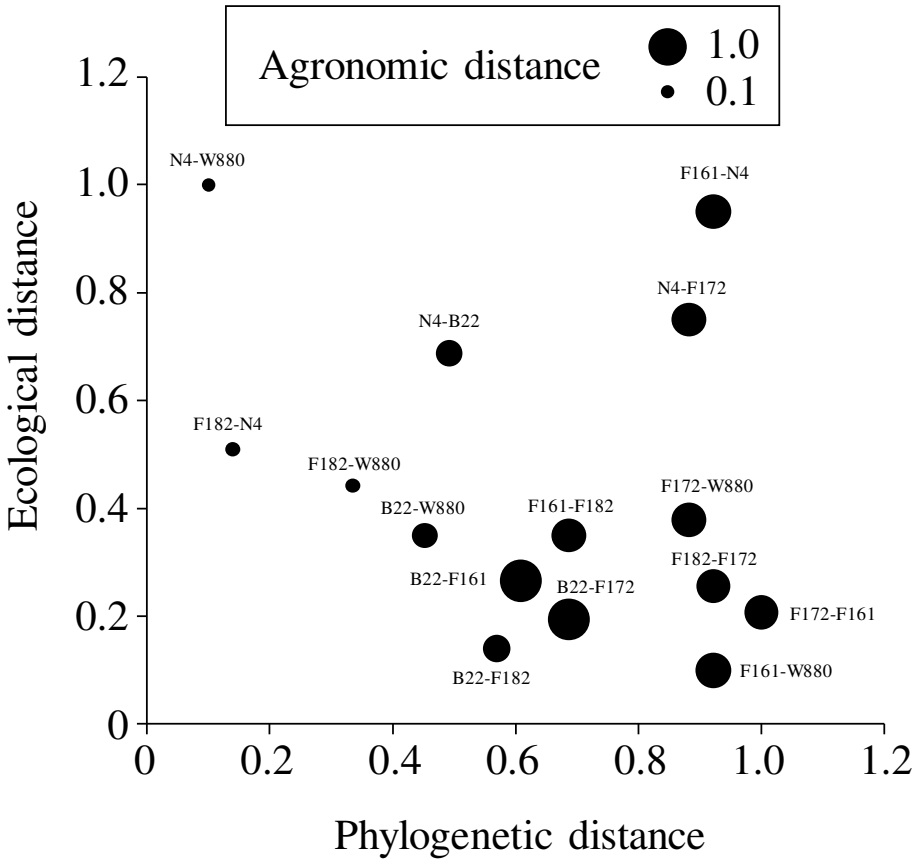

Agronomic distance
1.0
0.1
Ecological distance
Phylogenetic distance
0
0.2
0.4
0.6
0.8
1.0
1.2
N4-W880
F161-N4
N4-F172
N4-B22
F182-N4
F182-W880
B22-W880
F161-F182
F172-W880
F182-F172
B22-F161
B22-F172
F172-F161
B22-F182
F161-W880

Table 1. Results of two-way ANOVA (eta² 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 (eta²) 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	$\mu g\text{-}P.pot^{-1}$	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	$mg\text{-}N.pot^{-1}$	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	$\mu g\text{-}P.g^{-1}$ 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	$mg\text{-}N.g^{-1}$ 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)