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1 **Nitrogen supply reduces the earthworm-silicon control on rice blast disease in a Ferralsol**

2

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16

17 **Abstract**

18 Revealing belowground-aboveground relationships (BAR) is essential to drive ecological

19 processes to address agriculture dysfunctions, especially in the management of aboveground

20 plant diseases. Earthworms are one of the most important soil organisms involved in BAR, and

21 silicon (Si) has been identified as a crucial element regulating aboveground plant health. How

22 earthworm-Si interactions induce BAR in poor- and rich-nutrient soil contexts is still poorly

23 understood, despite a growing interest in agricultural sustainability. We investigated the

24 potential of BAR induced by the earthworm-silicon interaction to control the severity of rice

25 blast disease in a Ferralsol in Madagascar, with or without NPK fertilization. We conducted a
26 greenhouse microcosm experiment in which we manipulated the presence of the endogeic
27 earthworm *Pontoscolex corethrurus* and the fungus *Pyricularia oryzae* in a Ferralsol supplied
28 or not with Si and fertilized with macronutrients (nitrogen, phosphorus and potassium, i.e.,
29 NPK). After eight weeks of growth, plant biomass, nutrition and disease severity were
30 measured. Our results validated the hypothesis that a dual treatment of earthworm
31 inoculation and Si fertilization in a nutrient-poor tropical soil confers a higher tolerance of
32 rainfed rice to *P. oryzae*, in comparison with treatments with only earthworms or Si, providing
33 the optimal agronomic balance between a gain in biomass (and nutrition) and a reduction in
34 disease severity. The supply of macronutrients altered this positive BAR by favouring the
35 phenomenon of N-induced susceptibility. The aboveground plant C:N ratio of 15 is a
36 threshold below which any increase in N per C unit likely enhances blast disease. The role of
37 belowground interactions to counteract agricultural dysfunctions is supported by our study.
38 To accomplish ecological intensification and provision of ecosystem services such as disease
39 regulation, our findings recommend replacing excessive use of macronutrient fertilizer with
40 sustained agricultural practices promoting the development of earthworm populations, such
41 as organic matter inputs, superficial or no tillage, and the use of cover crops or conservation
42 agriculture.

43

44 **Keywords:** Belowground-aboveground relationships; Disease severity; plant nutrition;

45 *Pontoscolex corethrurus*; *Pyricularia oryzae*; Madagascar

46

47 **1. Introduction**

48 Elucidating belowground-aboveground relationships (BAR) is an exciting area of research that
49 the ecological community has pursued for over 20 years (Bardgett & Wardle 2010; Bardgett
50 2018). Although the mechanisms of how BAR affects agriculture dysfunctions are still
51 uncertain, this major theme in ecology is now viewed as a potential way to address the
52 current agroecological challenges (Scheu, 2003; Lavelle et al., 2004; Bezemer and van Dam,
53 2005; Mariotte et al., 2018). This theme is particularly relevant to the goal of managing
54 aboveground plant diseases, the control of which by belowground biodiversity has been
55 widely tested as an environmentally friendly alternative (Lavelle et al., 2004; Wurst, 2010;
56 Puga-Freitas and Blouin, 2015).

57 Earthworms are among the most important soil organisms found in the extensive
58 belowground biodiversity involved in BAR (Lavelle et al., 2006; Jana et al., 2010; Wurst, 2010;
59 Blouin et al., 2013). Earthworms can reduce the severity of several diseases caused by both
60 below- and aboveground pathogens, e.g., wheat eyespot disease caused by the fungus
61 *Oculimacula yallundae* (Bertrand et al., 2015), wheat take-all caused by the fungus
62 *Gaeumannomyces graminis* (Stephens et al., 1994), *Fusarium oxysporum* and *F. proliferatum*
63 on Asparagus, *Verticillium dahliae* on eggplant and *F. oxysporum* on tomato (Elmer, 2009),
64 clubroot disease of crucifers caused by the protist *Plasmodiophora brassicae* (Friberg et al.,
65 2008), infection of banana roots by the nematode *Radopholus similis* (Lafont et al., 2007),
66 *Rhizoctonia* bare patch caused by *R. solani* on wheat (Stephens et al., 1993), and thrip attacks
67 on tomato plants (Xiao et al., 2019). Earthworms thus merit deeper investigation as
68 prospective candidates for plant disease control.

69 Diverse mechanisms involved in the positive role of earthworms in plant growth and plant-
70 herbivore interactions have been proposed: (i) direct effects on pests such as predation,
71 habitat destruction, competition for organic matter, and production of fungicides and

72 bactericides (Brown et al., 2004); (ii) changes in soil structure driving root development and
73 water retention (Lavelle et al., 1997); (iii) higher mineralization of soil organic matter, which
74 makes nutrients available to plants and thus explains changes in defensive compounds such
75 as phytosterols and iridoid glycosides (Bezemer and van Dam, 2005; Wurst, 2010); (iv)
76 production of signal molecules involved in the communication between soil organisms and
77 plants, i.e., diffusible bioactive molecules interacting with the indole acetic acid (IAA)-signalling
78 pathway (Puga-Freitas and Blouin, 2015; Blouin, 2018), which is a direct effect of earthworms
79 on plant defence responses (Bezemer and van Dam, 2005); (v) stimulation of beneficial soil
80 microorganisms such as plant growth-promoting rhizobacteria (Van Wees et al., 2008); (vi)
81 modulation of the expression of plant genes known to be responsive to stress (Jana et al.,
82 2010) and (vii) higher acquisition of Si by plants (Bityutskii et al., 2016). This last mechanism
83 appears to be especially relevant to the efficiency of available Si in reducing plant disease
84 (Bityutskii et al., 2016).

85 Silicon (Si) is known to increase plant tolerance to pathogens (Hayasaka et al., 2008; Wang et
86 al., 2017). The mechanisms of the prophylactic role of Si were recently reviewed by Coskun et
87 al. (2019). These authors proposed a unified model of the Si-induced functions in plants
88 through the “apoplastic obstruction hypothesis” where Si is an “extracellular prophylactic
89 agent against biotic and abiotic stresses”. In highly weathered soils, plant available Si can be
90 amply supplied through the biological recycling of Si in natural ecosystems (Lucas et al., 1993;
91 Alexandre et al., 1997; Meunier et al., 1999; Cornelis and Delvaux, 2016). In croplands,
92 however, depletion of plant-available silicon in these soils may occur in rice cropping systems
93 (Savant et al., 1997), as demonstrated in less weathered soils in temperate cereal cropping
94 systems when crop residues are exported out of cultivated fields (Guntzer et al., 2012). Both
95 natural soil desilication and intense cropping involving exportation of crop residues may

96 deplete plant available Si. This question is particularly acute in rice cropping systems
97 (Klotzbücher et al., 2015). Improving Si availability in such soils thus appears to be a promising
98 approach to control blast disease (Datnoff et al., 1997; Voleti et al., 2008).

99 In the Central Highlands of Madagascar, despite the development of varieties resistant to cold
100 climates (Raboin et al., 2014), rice production is still below local demand due to important
101 agronomic constraints including the blast disease caused by the ascomycete fungus
102 *Pyricularia oryzae* Cavara (syn. *Magnaporthe oryzae* B.C. Couch). Blast disease can be
103 responsible for 2.5-6 (Savary et al., 2019) to 30% (Nalley et al., 2017) of global rice production
104 losses, leading to some of the highest fungicide expenditures in fungal disease control (Illana
105 et al., 2013). In Madagascar, the *P. oryzae* pathogen occurs in most rice growing areas
106 (Raboin et al., 2012; Sester et al., 2019), and the disease was responsible for the
107 abandonment of the first upland rice cold-tolerant varieties in the 2000s (Raboin et al., 2012).

108 The current option to control blast development is to use resistant cultivar, mixtures of
109 different cultivars (Raboin et al., 2012), with promising results in fungal disease control (Vidal
110 et al., 2017). Other options include reducing the spread of inoculum by destroying residues
111 (Raveloson et al., 2018), using natural bioactive products (Abed-Ashtiani et al., 2018) or
112 reducing nitrogen (N) application rates (Long et al., 2000). The latter option is linked to the
113 well-known crucial role of N in the development of blast disease, i.e., the phenomenon of N-
114 induced susceptibility or NIS (Ballini et al., 2013).

115 Here, we investigated the potential of BAR induced by earthworms and Si to control the
116 severity of rice blast disease in a Ferralsol from Madagascar. We have tested two hypotheses.
117 Firstly, since earthworms can enhance the bioavailability of Si in soil (Bityutskii et al., 2016),
118 we expected a higher tolerance of rainfed rice to *P. oryzae* following combined supply of
119 earthworms and Si. Secondly, we expected that supplying N through NPK would alter the

120 earthworm-Si BAR by enhancing of N-induced susceptibility. To test these hypotheses, we
121 conducted a 8-week greenhouse microcosm experiment using earthworms, NPK, Si and
122 *P. oryzae*.

123

124 **2. Materials and Methods**

125 **2.1. Soil type**

126 The first 10 cm of a Ferralsol under natural savanna was sampled using a metal spade at a site
127 near Antananarivo, Madagascar (Lazaina, 18°46'5559 S, 47°32'463 N, 1274 m above sea
128 level). The soil is characterized by a sandy-clay texture, slightly acid pH 5.5, a total organic C
129 content of 20.8 g kg⁻¹, a total N content of 1.3 g kg⁻¹, a C to N ratio equal to 16, a low Olsen
130 inorganic P content of 4.7 mg kg⁻¹ and a soil SiO₂ content equal to 100.1 mg kg⁻¹. The soil was
131 air-dried until constant weight and sieved at 2 mm before use.

132 **2.2. Biological materials**

133 *Earthworms*

134 Adult earthworms from the species *Pontoscolex corethrurus* (Rhinodrilidae) were collected
135 from the Lazaina-Ferralsol site. The earthworms were hand-sorted and maintained in a
136 bucket with soil. *P. corethrurus* is a peregrine endogeic species widely distributed in
137 Madagascar (Razafindrakoto et al., 2010). The species is known for its effect on nutrient
138 availability in soil and plant growth (e.g., Pashanasi et al., 1996; Lafont et al., 2007; Chapuis-
139 Lardy et al., 2009; Bernard et al., 2012).

140 *Rice cultivar*

141 We used the B22 cultivar, originating from Brazil and introduced to Madagascar in the 2000s.
142 The cultivar is largely used by farmers in the Highlands of Madagascar. Although it was

143 tolerant to blast disease when it was introduced, B22 is now susceptible probably due to the
144 adaptation of more aggressive fungus populations (Ballini et al., 2008).

145 *Fungal strain*

146 The strain of *Pyricularia oryzae* used in the experiment was isolated from B22 rice crops in the
147 highlands. *P. oryzae* was grown for 10 days under white light (12 hours per day) on a rice flour
148 agar medium (rice flour: 20 g, yeast extract: 2 g, agar-agar: 15 g, distilled water: 1000 ml, and
149 500 000 IU of Penicillin G: 1 ml added after autoclaving for 20 min at 120°C) (Talukder et al.,
150 2005; Gallet et al., 2014). For the inoculum preparation, the surface of cultures was gently
151 scraped and suspended with distilled water. Then, the suspension was filtered to obtain
152 conidia without mycelial fragments. Inoculum concentration was adjusted to 50,000 conidia
153 ml⁻¹ and mixed with 1% gelatine to aid spore fixation on leaves.

154 **2.3. Experimental design**

155 The greenhouse experiment was conducted in March-April 2017 at the FOFIFA greenhouse
156 (Antsirabe, Madagascar). Three factors were tested in this experiment: (i) the addition of
157 mineral NPK fertilizer with two modalities: with NPK (“+NPK”) or without NPK fertilizer (“-
158 NPK”), (ii) the addition of Si with two modalities: with Si (“+Si”) or without Si (“-Si”), and (iii)
159 the addition of earthworms with two modalities: with earthworms (“+E”) or without
160 earthworms (“-E”). Eight treatments crossing these three factors were thus set up and
161 monitored during the experiment in a full factorial design with 5 replicates per treatment, i.e.,
162 40 microcosms in total.

163 **2.4. Microcosm set-up**

164 We used 15-cm diameter plastic microcosms containing 1 kg of air-dried, 2-mm sieved soil
165 and allowing us to monitor rice growth for 8 weeks. The bottoms of the microcosms were
166 perforated with 5 mm-diameter holes to facilitate water circulation and then covered by thin

167 mosquito mesh to prevent earthworms from escaping. According to treatments, the required
168 quantity of NPK and Si was mixed with the soil before its addition to the microcosms. Mineral
169 $N_{11}P_{22}K_{16}$ fertilizer was used at a dose of 150 mg kg^{-1} soil (i.e., 190 kg ha^{-1}). The amount of Si
170 added was 785 mg kg^{-1} , equivalent to 1 Mg ha^{-1} in the form of mini-granules (SiO_2 silicon
171 dioxide amorphous, CAS Number 60676-86-0, Sigma Aldridge). Microcosms were filled with 1
172 kg of 2-mm sieved soil and moistened to reach field capacity, i.e., 49 g water per 100 g soil.
173 Then, three adult specimens of the earthworm *P. corethrurus* were added to appropriate
174 (“+E”) microcosms; this falls within the range of earthworm densities under favourable
175 conditions in the Malagasy Ferralsols (Rakotomanga et al., 2016). Three rice seeds of the B22
176 variety were sown in each pot. The inoculation of fungal spores on rice plants was achieved
177 25 days after seeding. For each replication of a given treatment, a suspension of 24 ml
178 ($50,000 \text{ spores ml}^{-1}$) was sprayed on the plants. This operation was repeated until all
179 microcosms received the same quantity of fungal spores. Then, microcosms were left in the
180 incubation room for 18 hours before return to the greenhouse.

181 2.5. Measurements at the end of the experiment

182 2.5.1. Plant and soil parameters

183 At the end of the experiment (i.e., 8 weeks after rice sowing) the number of earthworm
184 individuals in each microcosm was measured by sieving the soil; this was compared with the
185 number of inoculated earthworms in order to calculate earthworm survival rates. We also
186 counted the number of cocoons. Above- and belowground rice biomasses were measured
187 after drying plant tissues at 65°C for 48 hours. The above-belowground biomass ratio (A:B
188 ratio) was calculated. Total N contents in above- and belowground tissues were measured
189 with a CHNS microanalyser (Flash 2000 Series, CHNS/O 122 Analysers Thermo Scientific,
190 IRCOF, France). Total P contents were also measured in rice tissues following mineralization in

191 a microwave oven and using nitric acid at 65%. Then, a colorimetric dosage was realized using
192 the malachite green method (Ohno and Zibilske, 1991). The total amounts of N and P in
193 above- and belowground plant (abbreviated as Above-N, Below-N, Above-P, Below-P) tissues
194 were calculated by multiplying plant N and P contents by plant biomass. After measuring N
195 and P in aerial biomass, the quantity of tissues was not enough to measure Si content. The
196 only possibility was to pool the five replicates of a given treatment. This was even not possible
197 for treatments “-NPK-Si-E” and “-NPK+Si-E”) with too low tissue quantities. For the 6
198 remaining treatments, the amount of SiO₂ in leaves was measured by gravimetry with the
199 method used by Horwitz (1960) and is reported as the % of SiO₂ in the dry matter. The
200 absence of replications and of values for two treatments does not allow us to include them in
201 the statistical analysis.

202 *2.5.2. Severity of plant disease*

203 We recorded symptoms 7 days after fungal inoculation and before plant harvest. We noted
204 the severity of the leaf disease, which is the percentage of leaf surface attacked by the fungus
205 on the total leaf surface (Sester et al., 2014). Disease severity was calculated as the mean of
206 the severity values measured for the three plants in a given microcosm. We also assessed the
207 number and the size of the lesions on the second leaf of one plant per microcosm, which was
208 chosen randomly (Gallet et al., 2014). The number of lesions was counted on this leaf using a
209 5 cm² central window. From each lesion counted, its size was estimated using a scale where
210 the template surface was known.

211 *2.5.3. The Biomass-Disease Index (BDI)*

212 For each treatment, the gain during the experiment in aboveground biomass relative to the
213 development of the blast disease was calculated using an original, relative gain index called
214 the “biomass-disease index” or “BDI”. For that purpose, all variables (aboveground biomass,

215 disease severity, lesion number and size) were first transformed to range between 0.1 and 1
216 using a homothetic transformation among all treatments (Velásquez et al., 2007). The mean
217 transformed values of the three disease parameters (disease severity, lesion number and size)
218 corresponded to the “disease index”. BDI was calculated by dividing transformed values of
219 aboveground biomass by the disease index. Hence, values of BDI greater than 1 indicate
220 higher aboveground biomass relative to disease development, while values lower than 1
221 indicates dominance of fungal disease to biomass.

222 2.6. Statistics

223 Statistical analyses were performed with R software (3.3.1 version) with the packages
224 “agricolae”, “stats” and “ggplot2” (R Core Team, 2014). To test earthworm survival at the end
225 of the experiment, a two-way ANOVA was performed with NPK fertilization and Si supply as
226 factors. To test the three main effects (mineral NPK fertilizer or “NPK” factor, silicon input or
227 “Si” factor and earthworm inoculation or “E” factor) and their interactions, three-way ANOVA
228 was used on all plants, disease parameters and BDI. A post hoc Tukey HSD test was used after
229 three-way ANOVA to isolate the significant differences. The normality of the data was tested
230 using the Shapiro test. For disease severity, lesion number and size, a general linear mixed
231 model with a Poisson distribution was used to test the three factors and their interactions. A
232 non-linear least squares model was used to test the relationship between the disease index
233 (response variable) and the aboveground tissue C:N ratio (explanatory variable), using the
234 “nls” package. All tests were performed at the significance level of 0.05.

235

236 3. Results

237 3.1. Earthworm survival rates

238 At the end of the experiment, introduced earthworms had survived in all treatments. On
239 average, earthworm abundance even almost doubled compared to the number of
240 earthworms introduced, from 3 to 5.6 per microcosm (Fig. 1). This result indicates a high
241 reproduction rate and significant earthworm activity throughout the experiment. Earthworm
242 abundance was significantly affected by the Si input (P -value = 0.031); earthworm abundance
243 increased by 15% in the presence of Si in comparison to treatments without Si. We did not
244 find earthworms in microcosms that did not receive *P. corethrurus* at the beginning of the
245 experiment, indicating no contamination among microcosms.

246 3.2. Plant biomass

247 After the 8-week period of growth, pronounced differences in above- and belowground
248 biomass among treatments were measured (Table 1). The most significant effect was
249 observed when we provided NPK fertilizer (Table 2), with aboveground, belowground and
250 total plant biomass respectively 2.5, 1.7 and 2.2 times higher than in control treatments
251 without NPK. In contrast, Si input had no significant effect on plant biomass (Table 2).
252 However, we noticed that the supply of Si increased aboveground biomass in treatments that
253 did not receive NPK. Earthworm inoculation initiated intermediate effects; i.e., it significantly
254 increased aboveground biomass by 27%, while it had no effect on belowground (P -value =
255 0.883) nor on total plant biomass (P -value = 0.112) in comparison to treatments without
256 earthworms (Table 2). Consequently, the above-belowground biomass ratio also significantly
257 increased in the presence of earthworms. The lowest plant biomass values were observed in
258 the control (“-NPK-Si-E”) whereas the highest value was observed for the treatment with NPK

259 fertilizer and earthworms but without Si. However, there was no significant interaction
260 among the factors on plant biomass (Table 2).

261 3.3. Plant Si, N and P amounts

262 In our experiment, we noticed a positive effect of silicon supply on the SiO₂ content in plants
263 with mean values in the range 2.27-4.12% without Si supply and in the range 5.58-6.79% with
264 Si supply. The total plant N and P amounts were mainly affected by NPK fertilization (Tables 1
265 and 2). Unsurprisingly, NPK fertilization strongly increased plant N, with values for the above-,
266 belowground and total plant biomass, respectively 4.1, 2.2 and 3.6 higher than in treatments
267 without NPK. To a lesser extent, earthworms increased plant N amount by 1.5 times (*P*-value
268 = 0.003). We found a significant interaction between the factors “NPK” and “Earthworms” on
269 Above-N (*P*-value = 0.044). Indeed, the positive effect of earthworms on Above-N was more
270 pronounced in -NPK treatments in comparison to +NPK, with increases of 81% and 50%,
271 respectively. Conversely, the supply of Si had no significant effect on plant N (Table 2).

272 Above-P also increased significantly, by 240%, following NPK fertilization (*P*-value < 0.001).

273 For Below-P, a large effect of NPK (*P*-value < 0.001) was observed, with a 450% increase of
274 total P; the Si input also induced an increase but to a lesser extent; and the interaction effect
275 of NPK and Si was significant. Indeed, in treatments without NPK, the presence of Si increased
276 Below-P by 55%. Conversely, the presence of Si decreased P by 22% in treatments with NPK.

277 Regarding total P amount in total rice biomass, only NPK significantly influenced this

278 parameter (*P*-value < 0.001). The presence of earthworms always induced higher P amount in
279 plant tissues, except in the presence of both NPK and Si. Despite higher plant P amount in the
280 presence of earthworms (irrespective of NPK and Si presence), this earthworm effect was

281 overall not significant according to the 3-way ANOVA because of high significant effect of NPK

282 input on plant P amount that masked the benefit effect of earthworm on this variable. We

283 however believe that is important, despite absence of significance, to mention the positive
284 effect on earthworm on plant P nutrition.

285

286 **3.4. Disease severity**

287 Disease severity was strongly affected by the three factors (Table 1 and 2). NPK fertilization
288 significantly increased disease severity by ca. 720% in comparison with the absence of NPK
289 treatment. In contrast, both fertilization with Si and addition of earthworms significantly
290 decreased disease severity. The number and the surface area of lesions were also significantly
291 affected by both NPK and Si inputs. While NPK induced higher lesion number and surface
292 area, the addition of Si always reduced these parameters. We also observed a significant
293 interaction effects of earthworm and Si addition on these parameters (Table 2). Indeed, the
294 presence of earthworms tended to reduce the positive effect on Si on lesion number and
295 surface area, especially when NPK was added (Table 1).

296 **3.5. The biomass-disease index**

297 When the disease index was plotted as a function of aboveground tissue C:N ratio, a power
298 relationship was revealed (Fig. 2.A). We observed a sharp and exponential increase in DI with
299 decreasing values of tissue C:N below an apparent threshold value of 15. Considering the BDI,
300 a strong positive interactive effect of earthworm and Si input was observed in absence of
301 NPK; i.e., the highest BDI values were observed when both earthworms and Si were supplied
302 (Fig. 2.B). In contrast, when we provided NPK, this ratio was weak, and only the addition of Si
303 increased the BDI. We also noticed that the presence of earthworms limited the positive
304 effect of Si on the BDI when NPK was supplied.

305

306 4. Discussion

307 4.1. NPK fertilization

308 In the present study, NPK supply largely increased plant growth and leaf blast disease. Our
309 results are consistent with previous studies demonstrating the strong effect of mineral
310 fertilization, especially N fertilization, on the development of blast disease (Long et al., 2000;
311 Dusserre et al., 2017). Any increase in available N increases the susceptibility of plants to blast
312 disease (Ballini et al., 2013). However, it is important to emphasize that this susceptibility
313 depends on the availability of N relative to other nutrients and whether the availability of N
314 exceeds plant N requirements (Huber and Thompson, 2007). Excess N leads to shifts in the
315 host plant metabolism, in particular, to a reduction of enzymatic activity responsible for the
316 biosynthesis of phenol and lignin in cell walls (Hoffland et al., 1999), constituents involved in
317 the defence mechanisms of plant cells against pathogens. In our experiment, the NPK amount
318 used (150 mg kg^{-1} soil, i.e., 190 kg ha^{-1}) increased N availability, which likely led to a
319 weakening of plant tolerance mechanisms and increased the development of blast disease.
320 The increase in N availability following NPK application is confirmed by the high content of N
321 in plant biomass, which was 4 times higher with NPK than without NPK. Furthermore, Filippi
322 and Prabhu (1998) showed that N and P contents in plant tissues are positively correlated
323 with disease severity. We also found that P content in aboveground portion of plants was 3
324 times greater with than without NPK fertilization; this could also favour disease severity in
325 treatments with NPK.

326 4.2. Silicon supply

327 Silicon supply, with or without NPK, had no significant impact on plant growth, indicating that
328 Si did not impact rice growth in the Malagasy Ferralsols unlike previous observations (Velly,
329 1975) highlighting a pronounced positive effect of Si fertilization on rice yield. However, we

330 observed a positive effect of Si on belowground P amount only without NPK. Combining NPK
331 and Si supplies led to a decrease in plant P content. Actually, Si uptake decreases P absorption
332 once the latter nutrient is unlimited while it increases under P deficiency that occurs in high
333 P-fixing Ferralsols (Okuda and Takahashi, 1964; Velly, 1975). This increase may be explained
334 by different mechanisms:

335 - Si control on the expression of genes involved in the transport of inorganic P (Pi), especially
336 symports H⁺:Pi (Pht1 family) (Kostic et al., 2017) induced by P deficiency in plants (Plassard et
337 al., 2015);

338 - Si application may result in the elevation of soil pH (Owino-Gerroh and Gascho, 2005),
339 leading to an increase in the availability of iron and aluminium phosphates (Plassard et al.,
340 2015);

341 - Si stimulates the exudation of carboxylates, especially malate and citrate (Kostic et al.,
342 2017), that can replace phosphate on adsorption sites and/or favour the complexation of
343 metallic ions (Ca, Al, Fe) involved in P adsorption (Jones, 1998).

344 Here, Si supply decreased the severity of leaf blast disease, regardless of NPK fertilization as
345 previously observed (Seebold et al.; 2000; 2001). A negative correlation between the number
346 of lesions on rice leaves and Si content in leaves was observed by Volk et al. (1958). Different
347 mechanisms can explain this positive effect (review in Coskun et al., 2019):

348 - Si in the epidermal cell walls of leaves serves as a physical barrier to pathogen penetration
349 into cells (Ma and Takahashi, 1990; Ma et al., 2001);

350 - Si improves plant defence mechanisms through a rapid deposit of phenolic compounds and
351 lignin on pathogen infection sites (Datnoff et al., 1997);

352 - activation of defence genes (Wang et al., 2017).

353 Our study also shows that Si application reduced plant N content despite of NPK supply,
354 hence reducing disease severity even after NPK application. This observation could explain
355 the reduced susceptibility of rice to blast disease when the plant is sufficiently supplied with
356 Si (Sester et al., 2019).

357 **4.3. Effects of earthworms**

358 Earthworms significantly increased aboveground (A) biomass, without significant effects on
359 the belowground (B) portion, resulting in a significant increase in the A:B ratio, a frequent
360 pattern for cereals (Kreuzer et al., 2004; Laossi et al., 2010; Coulis et al., 2014). Changes in
361 plant biomass allocation are generally explained by the investment of energy in organs
362 involved in the acquisition of the most limiting elements. The increase in A:B ratio following
363 earthworm inoculation suggests that earthworms make soil nutrients more available for
364 plants. This observation is supported by the increase in plant N amounts after inoculation
365 with earthworms, especially without NPK. Previous studies have shown that earthworms have
366 a more positive effect on plant growth in nutrient-poor than in rich soils (Brown et al., 2004;
367 Noguera et al., 2010), possibly because of the capacity of earthworms to decompose soil
368 organic matter and release nutrients (Blouin et al., 2006).

369 Earthworms also promoted a reduction in disease severity in comparison to controls, except
370 when both NPK and Si were provided. It is possible that earthworms enhanced the mobility
371 and bioavailability of native Si (Bityutskii et al., 2016). In the NPK treatments, the disease
372 severity increased in the presence of earthworms, despite Si fertilization. This finding may
373 have resulted from the earthworm-induced increase in the available N and plant N content
374 leading to a decrease in the aboveground tissue C:N ratio from 13.8 to 10.4, which should
375 worsen disease severity. In the absence of NPK, the activity of earthworms decreased the
376 aboveground tissue C:N ratio from 20 to 15, which probably enhanced the microbial N

377 turnover and the net N mineralization rate. In this case, the C:N ratio remained above (or
378 equal to) the threshold of 15, below which the blast disease severity increases exponentially
379 with decreasing plant C:N values (see Fig. 2.A).

380 4.4. How N fertilization altered belowground interactions on aboveground functions

381 Interaction effects of Si and earthworms were observed both for the decrease in disease
382 severity and for the increase in plant biomass through the biomass-disease index. This was
383 particularly clear in treatments without NPK fertilization, where the interaction between the
384 two factors resulted in a strong increase in aboveground biomass. This treatment (-NPK, +Si,
385 +E) offered the optimal agronomic balance between a gain in biomass and a reduction in
386 disease severity. Two main reasons can explain this pattern. First, earthworms have the ability
387 to increase macronutrient (N and P) availability over short and long terms (Van Groenigen et
388 al., 2019). Unlike chemical fertilizers that provide large quantities of nutrients at their
389 application, leading to sharp increases in plant nutrient contents and therefore to a rapid
390 decrease in the C:N ratio, earthworms provide nutrients that are available continuously over
391 time and in smaller amounts per unit of time. As a result, nutrient uptake by plants is
392 continuous and quickly used to make biomass. In our study, regardless of NPK treatment,
393 earthworms induced a decrease in the C:N ratio of approximately 4 units. However, this
394 decrease in the C:N ratio only affects the disease when it occurs for a C:N below 15, that is,
395 when NPK is delivered. Second, earthworms can also increase the availability of native and
396 added Si (Bityutskii et al., 2016), which has been shown to be important for plant defence (Ma
397 et al., 2001; Hayasaka et al., 2008; Coskun et al., 2019). For instance, the amount of water-
398 extractible Si in casts of *Aporrectodea caliginosa* (endogeic species) and *Lumbricus terrestris*
399 (epi-anecic species) was 2 to 12 times higher than in non-ingested soil, and monosilicic acid
400 (Si(OH)_4) was 1.3 to 3.5 times higher in casts than in non-ingested soil (Bityutskii et al., 2016).

401 The earthworm-induced availability of N and Si thus appears crucial in the control of
402 aboveground disease. Quantifying the plant N:Si ratio according to the different treatments
403 could be a further interesting step to better understand the mechanisms involved in
404 aboveground pest regulation by belowground interactions. Our partial non-replicated data on
405 plant Si (%) prevent us to go deeper in this issue although the N:Si atomic ratio indicated a
406 variation between treatments, from 1.80 in the “-NPK+Si+E” to 4.71 in the “-NPK-Si+E”;
407 treatments with NPK fertilization ranged between these extremes, from 1.87 in “+NPK+Si-E”
408 to 4.24 in “+NPK-Si+E”.

409

410 5. Conclusions

411 Our results validated the hypothesis that the combined supply of earthworms and Si in a
412 highly weathered Ferralsol confers a higher tolerance of rainfed rice to *P. oryzae*, leading to
413 the highest biomass-disease index values, i.e., the optimal agronomic balance between a gain
414 in biomass and a reduction in disease severity. However, the supply of nitrogen through NPK
415 application alters this positive below-aboveground relationship by favouring the N-induced
416 susceptibility, as tested through our second hypothesis. The aboveground plant C:N ratio of
417 15 is a threshold below which any increase in N content would enhance blast disease more
418 than plant biomass, therefore reducing the biomass-disease index. Manipulating the
419 aboveground-belowground relationships to counteract agricultural dysfunctions is supported
420 by our findings. This conclusion is particularly true for blast disease when genetic breeding
421 seems limited by the rapid adaptation of fungal strains to disseminated resistant varieties.
422 However, further studies should be conducted to better understand the effects of the
423 earthworm-silicon interactions, not only on leaf disease but also on panicle blast disease,
424 which leads to the greatest losses in rice production. Since silicon is known to protect plant

425 (Coskun et al., 2019) and since silicon is driven by soil processes (Cornelis and Delvaux, 2016),
426 these ecological interactions should be explored both at a broad scale, i.e., the farm level with
427 the use of mixtures of tolerant and resistant rice cultivars and at a small scale to identify their
428 mechanisms. Finally, in the attempt to intensify ecological processes and ecosystem services
429 such as pest regulation, our results support the replacement of excessive use of nitrogen
430 fertilizers with agricultural practices involving the development of earthworm populations,
431 such as organic matter inputs, no- or superficial tillage, and use of cover crops or
432 conservation agriculture coupled with micronutrient fertilization.

433

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445

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651

Figure 1

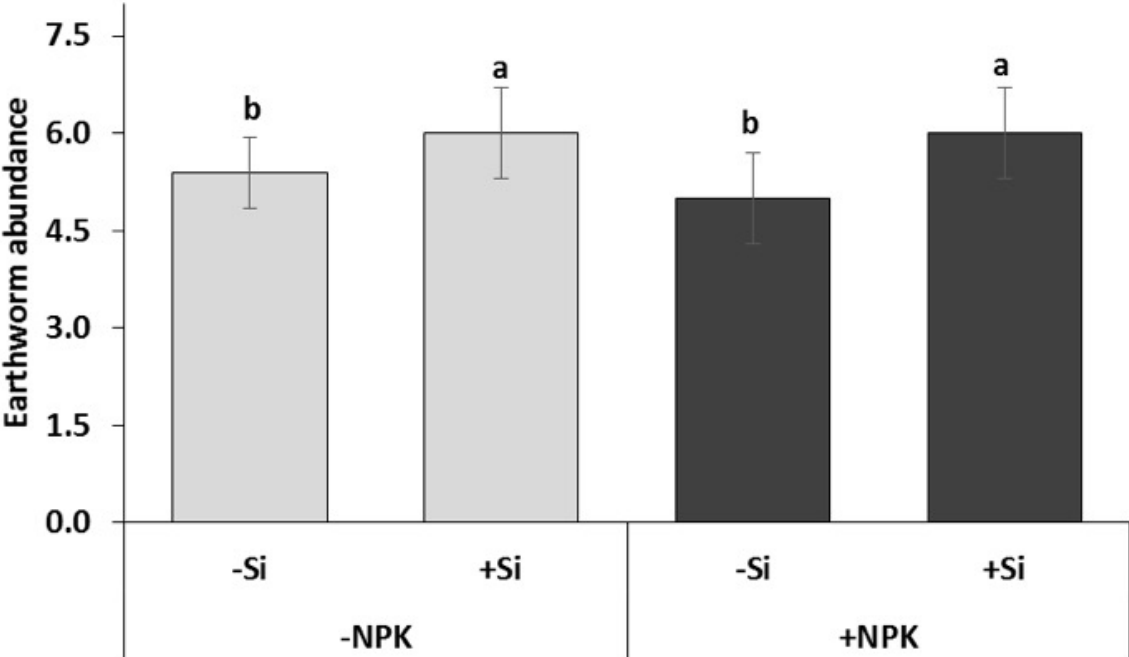


Figure 2A

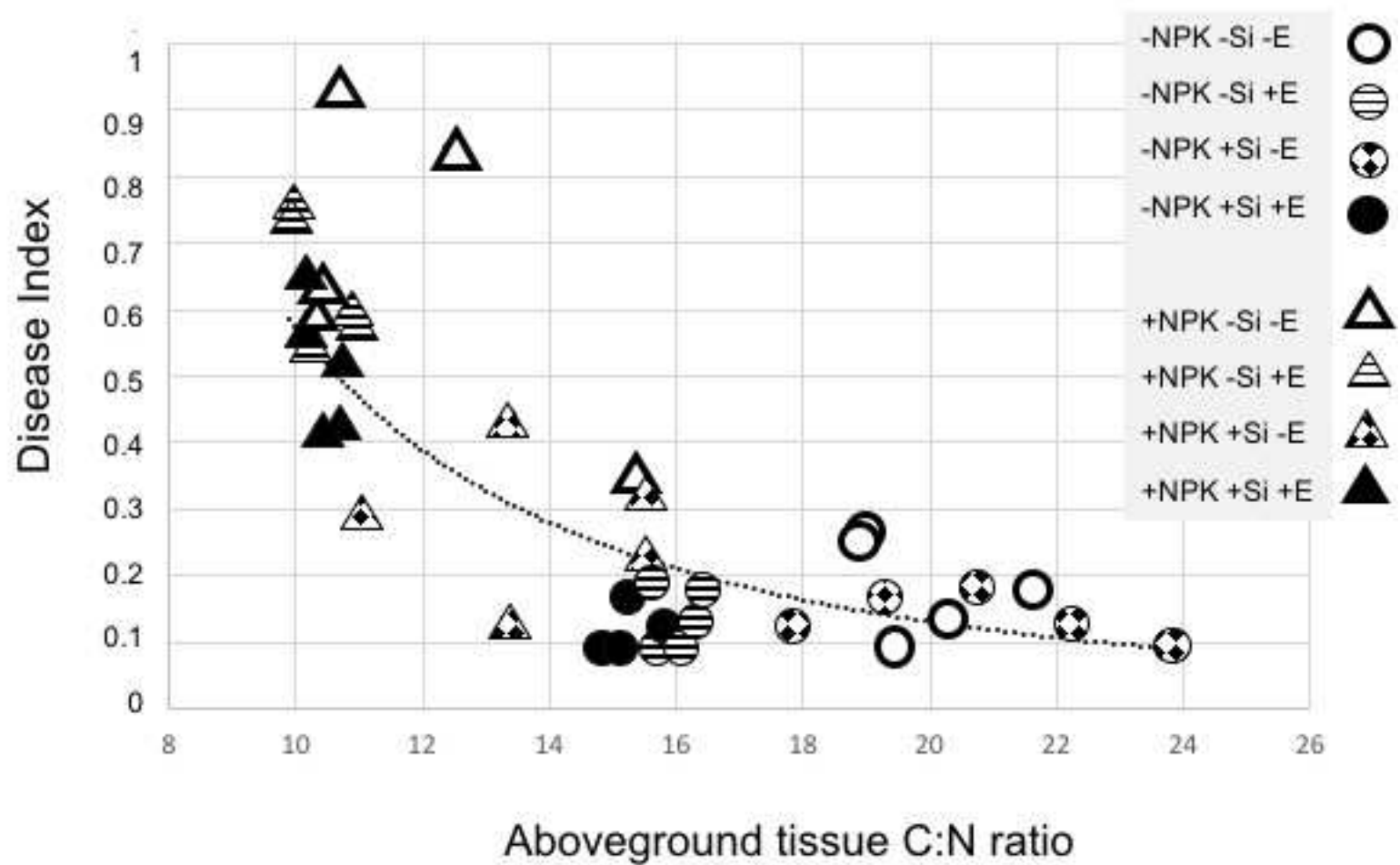
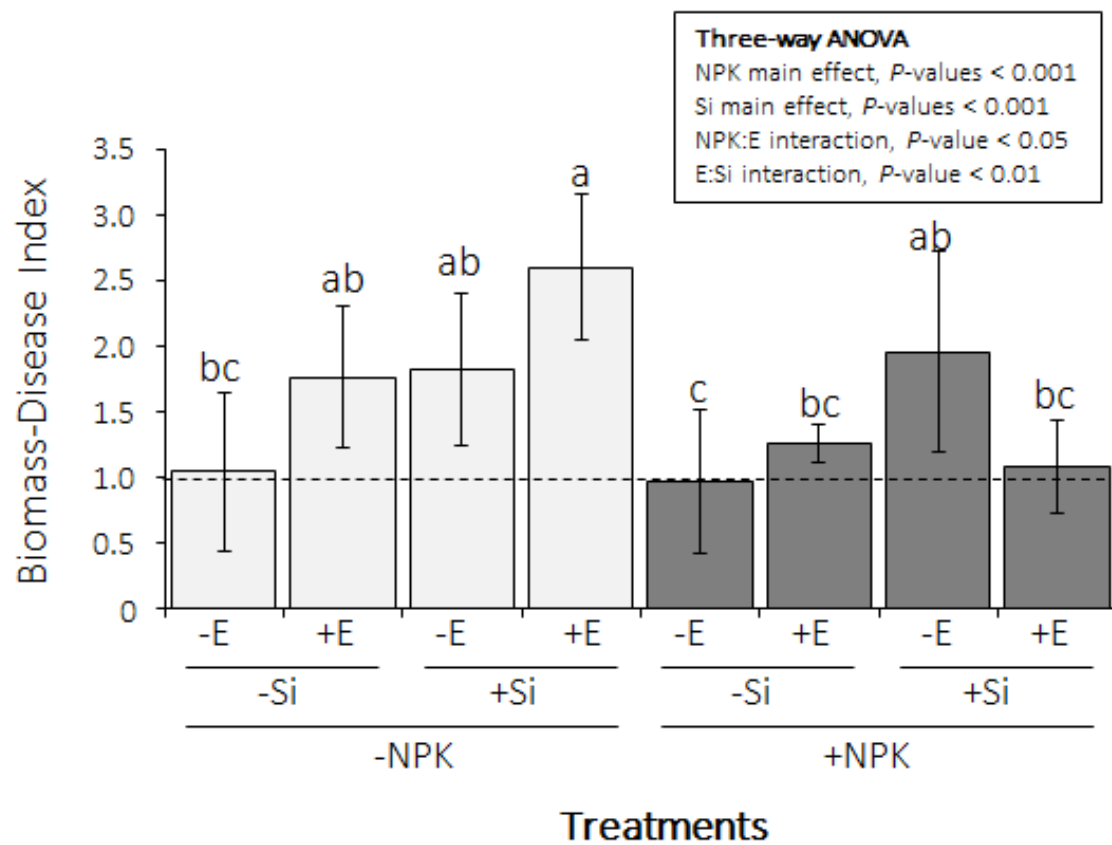


Figure 2B



1 **Table 1.** Mean (SD) of plant variables (plant biomass, nutrient amount and disease development) measured at the end of the experiment
 2 according to treatments (NPK fertilization, silicon and earthworm supply).

3

Plant variables	Units	Treatments							
		-NPK				+NPK			
		-Silicon		+Silicon		-Silicon		+Silicon	
		-Earthworm	+Earthworm	-Earthworm	+Earthworm	-Earthworm	+Earthworm	-Earthworm	+Earthworm
Plant growth									
Aboveground	mg microcosm ⁻¹	62 (17)	92 (19)	97 (24)	122 (21)	215 (46)	308 (56)	214 (121)	212 (78)
Belowground	mg microcosm ⁻¹	56 (23)	76 (14)	108 (22)	90 (42)	149 (36)	153 (38)	137 (65)	125 (55)
Total biomass	mg microcosm ⁻¹	118 (39)	168 (22)	205 (40)	212 (62)	364 (68)	462 (85)	351 (170)	337 (129)
A:B ratio	ratio	1.15 (0.17)	1.26 (0.48)	0.91 (0.18)	1.50 (0.41)	1.48 (0.39)	2.08 (0.45)	1.58 (0.54)	1.77 (0.39)
Plant N amount									
Aboveground	mg-N microcosm ⁻¹	1.18 (0.35)	2.30 (0.55)	1.85 (0.69)	3.28 (0.65)	7.63 (1.66)	12.62 (2.62)	6.32 (3.57)	8.34 (3.05)
Belowground	mg-N microcosm ⁻¹	0.54 (0.26)	0.70 (0.10)	0.97 (0.23)	0.84 (0.32)	1.84 (0.23)	1.65 (0.51)	1.64 (0.72)	1.58 (0.60)
Total biomass	mg-N microcosm ⁻¹	1.71 (0.61)	3.01 (0.53)	2.82 (0.83)	4.12 (0.97)	9.46 (1.57)	14.27 (2.92)	7.96 (4.14)	9.92 (3.60)
Plant P amount									
Aboveground	mg-P microcosm ⁻¹	0.47 (0.16)	0.75 (0.14)	0.76 (0.36)	0.94 (0.14)	2.29 (0.35)	2.79 (1.10)	2.81 (1.74)	2.07 (0.66)
Belowground	mg-P microcosm ⁻¹	0.05 (0.02)	0.06 (0.02)	0.10 (0.03)	0.07 (0.03)	0.48 (0.12)	0.39 (0.10)	0.35 (0.14)	0.33 (0.12)
Total biomass	mg-P microcosm ⁻¹	0.52 (0.18)	0.82 (0.16)	0.85 (0.38)	1.02 (0.14)	2.77 (0.29)	3.18 (1.19)	3.17 (1.82)	2.40 (0.69)
Blast disease									
Disease severity	%	0.40 (0.37)	0.19 (0.21)	0.17 (0.12)	0.13 (0.16)	3.04 (0.90)	1.98 (0.21)	1.09 (0.58)	1.24 (0.32)
Lesion number	lesions microcosm ⁻¹	3.60 (2.61)	1.80 (2.17)	2.00 (1.87)	0.75 (0.96)	17.60 (6.88)	18.00 (3.67)	4.60 (3.36)	13.00 (3.00)
Lesion surface	mm ² microcosm ⁻¹	2.30 (1.99)	1.00 (1.06)	1.10 (0.89)	1.00 (1.41)	18.90 (8.42)	21.20 (4.78)	5.70 (4.10)	18.40 (5.37)

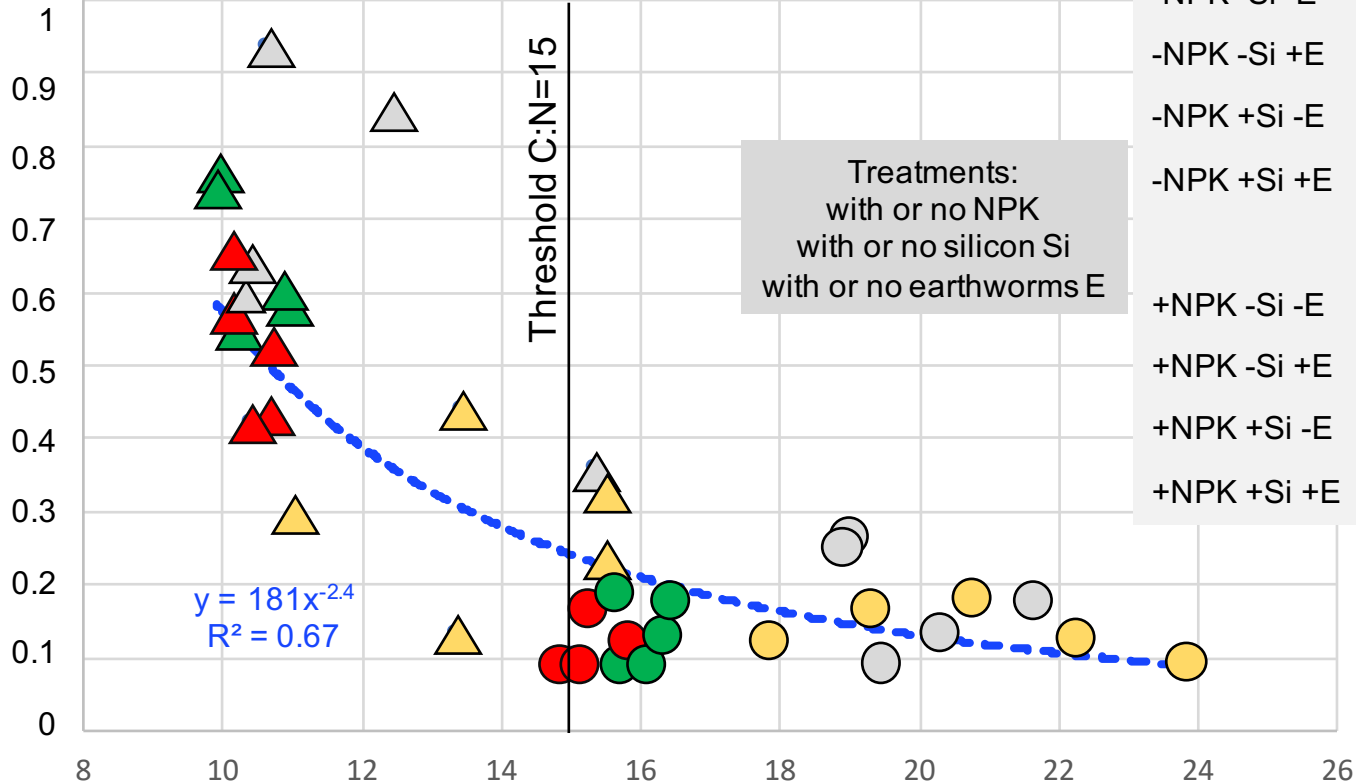
4

- 1 **Table 2.** Three-way ANOVA *P*-values for the main effects (NPK, Si and earthworm fertilization) and their interactions on plant and disease
 2 parameters.

Variables	Units	Three-way ANOVA factors and interactions [§]						
		Main effects			Interactions			
		<i>NPK</i>	<i>Silicon (Si)</i>	<i>Earthworms €</i>	<i>NPK:Si</i>	<i>NPK:E</i>	<i>Si:E</i>	<i>NPK:Si:E</i>
Plant growth								
Aboveground	mg microcosm ⁻¹	< 0.001	NS	0.018	NS	NS	NS	NS
Belowground	mg microcosm ⁻¹	< 0.001	NS	NS	NS	NS	NS	NS
Total biomass	mg microcosm ⁻¹	< 0.001	NS	NS	NS	NS	NS	NS
A:B ratio	ratio	NS	NS	0.023	NS	NS	NS	NS
Plant N amount								
Aboveground	mg-N microcosm ⁻¹	< 0.001	NS	< 0.001	NS	0.044	NS	NS
Belowground	mg-N microcosm ⁻¹	< 0.001	NS	NS	NS	NS	NS	NS
Total biomass	mg-N microcosm ⁻¹	< 0.001	NS	0.003	NS	NS	NS	NS
Plant P amount								
Aboveground	mg-P microcosm ⁻¹	< 0.001	NS	NS	NS	NS	NS	NS
Belowground	mg-P microcosm ⁻¹	< 0.001	0.025	NS	0.030	NS	NS	NS
Total biomass	mg-P microcosm ⁻¹	< 0.001	NS	NS	NS	NS	NS	NS
Blast disease								
Disease severity	%	< 0.001	< 0.001	0.014	NS	NS	NS	NS
Lesion number	lesions microcosm ⁻¹	< 0.001	< 0.001	NS	NS	NS	0.006	NS
Lesion surface	mm ² microcosm ⁻¹	< 0.001	< 0.001	NS	NS	NS	0.002	NS

- 3 [§]NS (non-significant) indicates *P*-values > 0.05

Severity of blast disease on rice
'Disease Index'



Aboveground rice tissue C:N ratio