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

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Abstract

Revealing belowground-aboveground relationships (BAR) is essential to drive ecological processes to address agriculture dysfunctions, especially in the management of aboveground plant diseases. Earthworms are one of the most important soil organisms involved in BAR, and silicon (Si) has been identified as a crucial element regulating aboveground plant health. How earthworm-Si interactions induce BAR in poor- and rich-nutrient soil contexts is still poorly understood, despite a growing interest in agricultural sustainability. We investigated the potential of BAR induced by the earthworm-silicon interaction to control the severity of rice blast disease in a Ferralsol in Madagascar, with or without NPK fertilization. We conducted a greenhouse microcosm experiment in which we manipulated the presence of the endogeic earthworm *Pontoscolex corethrurus* and the fungus *Pyricularia oryzae* in a Ferralsol supplied or not with Si and fertilized with macronutrients (nitrogen, phosphorus and potassium, i.e., NPK). After eight weeks of growth, plant biomass, nutrition and disease severity were measured. Our results validated the hypothesis that a dual treatment of earthworm inoculation and Si fertilization in a nutrient-poor tropical soil confers a higher tolerance of rainfed rice to *P. oryzae*, in comparison with treatments with only earthworms or Si, providing the optimal agronomic balance between a gain in biomass (and nutrition) and a reduction in disease severity. The supply of macronutrients altered this positive BAR by favouring the phenomenon of N-induced susceptibility. The aboveground plant C:N ratio of 15 is a threshold below which any increase in N per C unit likely enhances blast disease. The role of belowground interactions to counteract agricultural dysfunctions is supported by our study. To accomplish ecological intensification and provision of ecosystem services such as disease regulation, our findings recommend replacing excessive use of macronutrient fertilizer with sustained agricultural practices promoting the development of earthworm populations, such as organic matter inputs, superficial or no tillage, and the use of cover crops or conservation agriculture.

Keywords: Belowground-aboveground relationships; Disease severity; plant nutrition; *Pontoscolex corethrurus*; *Pyricularia oryzae*; Madagascar

1. Introduction

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

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

Diverse mechanisms involved in the positive role of earthworms in plant growth and plant-herbivore interactions have been proposed: (i) direct effects on pests such as predation, habitat destruction, competition for organic matter, and production of fungicides and bactericides (Brown et al., 2004); (ii) changes in soil structure driving root development and water retention (Lavelle et al., 1997); (iii) higher mineralization of soil organic matter, which makes nutrients available to plants and thus explains changes in defensive compounds such as phytosteroles and iridoid glycosides (Bezemer and van Dam, 2005; Wurst, 2010); (iv) production of signal molecules involved in the communication between soil organisms and plants, i.e., diffusive bioactive molecules interacting with the indole acetic acid (IAA)-signalling pathway (Puga-Freitas and Blouin, 2015; Blouin, 2018), which is a direct effect of earthworms on plant defence responses (Bezemer and van Dam, 2005); (v) stimulation of beneficial soil microorganisms such as plant growth-promoting rhizobacteria (Van Wees et al., 2008); (vi) modulation of the expression of plant genes known to be responsive to stress (Jana et al., 2010) and (vii) higher acquisition of Si by plants (Bityutskii et al., 2016). This last mechanism appears to be especially relevant to the efficiency of available Si in reducing plant disease (Bityutskii et al., 2016).

Silicon (Si) is known to increase plant tolerance to pathogens (Hayasaka et al., 2008; Wang et al., 2017). The mechanisms of the prophylactic role of Si were recently reviewed by Coskun et al. (2019). These authors proposed a unified model of the Si-induced functions in plants through the “apoplastic obstruction hypothesis” where Si is an “extracellular prophylactic agent against biotic and abiotic stresses”. In highly weathered soils, plant available Si can be amply supplied through the biological recycling of Si in natural ecosystems (Lucas et al., 1993; Alexandre et al., 1997; Meunier et al., 1999; Cornelis and Delvaux, 2016). In croplands, however, depletion of plant-available silicon in these soils may occur in rice cropping systems (Savant et al., 1997), as demonstrated in less weathered soils in temperate cereal cropping systems when crop residues are exported out of cultivated fields (Guntzer et al., 2012). Both natural soil desilication and intense cropping involving exportation of crop residues may deplete plant available Si. This question is particularly acute in rice cropping systems (Klotzbücher et al., 2015). Improving Si availability in such soils thus appears to be a promising approach to control blast disease (Datnoff et al., 1997; Voleti et al., 2008).

In the Central Highlands of Madagascar, despite the development of varieties resistant to cold climates (Raboin et al., 2014), rice production is still below local demand due to important agronomic constraints including the blast disease caused by the ascomycete fungus *Pyricularia oryzae* Cavara (syn. *Magnaporthe oryzae* B.C. Couch). Blast disease can be responsible for 2.5-6 (Savary et al., 2019) to 30% (Nalley et al., 2017) of global rice production losses, leading to some of the highest fungicide expenditures in fungal disease control (Illana et al., 2013). In Madagascar, the *P. oryzae* pathogen occurs in most rice growing areas (Raboin et al., 2012; Sester et al., 2019), and the disease was responsible for the abandonment of the first upland rice cold-tolerant varieties in the 2000s (Raboin et al., 2012). The current option to control blast development is to use resistant cultivar, mixtures of different cultivars (Raboin et al., 2012), with promising results in fungal disease control (Vidal et al., 2017). Other options include reducing the spread of inoculum by destroying residues (Raveloson et al., 2018), using natural bioactive products (Abed-Ashtiani et al., 2018) or reducing nitrogen (N) application rates (Long et al., 2000). The latter option is linked to the well-known crucial role of N in the development of blast disease, i.e., the phenomenon of N-induced susceptibility or NIS (Ballini et al., 2013).

Here, we investigated the potential of BAR induced by earthworms and Si to control the severity of rice blast disease in a Ferralsol from Madagascar. We have tested two hypotheses. Firstly, since earthworms can enhance the bioavailability of Si in soil (Bityutskii et al., 2016), we expected a higher tolerance of rainfed rice to *P. oryzae* following combined supply of earthworms and Si. Secondly, we expected that supplying N through NPK would alter the earthworm-Si BAR by enhancing of N-induced susceptibility. To test these hypotheses, we conducted a 8-week greenhouse microcosm experiment using earthworms, NPK, Si and *P. oryzae*.

2. Materials and Methods

2.1. Soil type

The first 10 cm of a Ferralsol under natural savanna was sampled using a metal spade at a site near Antananarivo, Madagascar (Lazaina, 18°46’5559 S, 47°32’463 N, 1274 m above sea level). The soil is characterized by a sandy-clay texture, slightly acid pH 5.5, a total organic C 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 inorganic P content of 4.7 mg kg^{-1} and a soil SiO_2 content equal to 100.1 mg kg^{-1}. The soil was air-dried until constant weight and sieved at 2 mm before use.

2.2. Biological materials

Earthworms

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

Rice cultivar

We used the B22 cultivar, originating from Brazil and introduced to Madagascar in the 2000s. The cultivar is largely used by farmers in the Highlands of Madagascar. Although it was tolerant to blast disease when it was introduced, B22 is now susceptible probably due to the adaptation of more aggressive fungus populations (Ballini et al., 2008).

Fungal strain

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

2.3. Experimental design

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

2.4. Microcosm set-up

We used 15-cm diameter plastic microcosms containing 1 kg of air-dried, 2-mm sieved soil and allowing us to monitor rice growth for 8 weeks. The bottoms of the microcosms were perforated with 5 mm-diameter holes to facilitate water circulation and then covered by thin mosquito mesh to prevent earthworms from escaping. According to treatments, the required quantity of NPK and Si was mixed with the soil before its addition to the microcosms. Mineral $N_{11}P_{22}K_{16}$ fertilizer was used at a dose of 150 mg kg^{-1} soil (i.e., 190 kg ha^{-1}). The amount of Si added was 785 mg kg^{-1}, equivalent to 1 Mg ha^{-1} in the form of mini-granules (SiO_2 silicon dioxide amorphous, CAS Number 60676-86-0, Sigma Aldridge). Microcosms were filled with 1 kg of 2-mm sieved soil and moistened to reach field capacity, i.e., 49 g water per 100 g soil. Then, three adult specimens of the earthworm *P. corethrurus* were added to appropriate ("+E") microcosms; this falls within the range of earthworm densities under favourable conditions in the Malagasy Ferralsols (Rakotomanga et al., 2016). Three rice seeds of the B22 variety were sown in each pot. The inoculation of fungal spores on rice plants was achieved 25 days after seeding. For each replication of a given treatment, a suspension of 24 ml (50,000 spores ml^{-1}) was sprayed on the plants. This operation was repeated until all microcosms received the same quantity of fungal spores. Then, microcosms were left in the incubation room for 18 hours before return to the greenhouse.

2.5. Measurements at the end of the experiment

2.5.1. Plant and soil parameters

At the end of the experiment (i.e., 8 weeks after rice sowing) the number of earthworm individuals in each microcosm was measured by sieving the soil; this was compared with the number of inoculated earthworms in order to calculate earthworm survival rates. We also counted the number of cocoons. Above- and belowground rice biomasses were measured after drying plant tissues at 65°C for 48 hours. The above-belowground biomass ratio (A:B ratio) was calculated. Total N contents in above- and belowground tissues were measured with a CHNS microanalyser (Flash 2000 Series, CHNS/O 122 Analysers Thermo Scientific, IRCOF, France). Total P contents were also measured in rice tissues following mineralization in a microwave oven and using nitric acid at 65%. Then, a colorimetric dosage was realized using the malachite green method (Ohno and Zibilske, 1991). The total amounts of N and P in above- and belowground plant (abbreviated as Above-N, Below-N, Above-P, Below-P) tissues were calculated by multiplying plant N and P contents by plant biomass. After measuring N and P in aerial biomass, the quantity of tissues was not enough to measure Si content. The only possibility was to pool the five replicates of a given treatment. This was even not possible for treatments "-NPK-Si-E" and "-NPK+Si-E") with too low tissue quantities. For the 6 remaining treatments, the amount of SiO_2 in leaves was measured by gravimetry with the method used by Horwitz (1960) and is reported as the % of SiO_2 in the dry matter. The absence of replications and of values for two treatments does not allow us to include them in the statistical analysis.

2.5.2. Severity of plant disease

We recorded symptoms 7 days after fungal inoculation and before plant harvest. We noted the severity of the leaf disease, which is the percentage of leaf surface attacked by the fungus on the total leaf surface (Sester et al., 2014). Disease severity was calculated as the mean of the severity values measured for the three plants in a given microcosm. We also assessed the number and the size of the lesions on the second leaf of one plant per microcosm, which was chosen randomly (Gallet et al., 2014). The number of lesions was counted on this leaf using a 5 cm^2 central window. From each lesion counted, its size was estimated using a scale where the template surface was known.

2.5.3. The Biomass-Disease Index (BDI)

For each treatment, the gain during the experiment in aboveground biomass relative to the development of the blast disease was calculated using an original, relative gain index called the "biomass-disease index" or "BDI". For that purpose, all variables (aboveground biomass, disease severity, lesion number and size) were first transformed to range between 0.1 and 1 using a homothetic transformation among all treatments (Velásquez et al., 2007). The mean transformed values of the three disease parameters (disease severity, lesion number and size) corresponded to the "disease index". BDI was calculated by dividing transformed values of aboveground biomass by the disease index. Hence, values of BDI greater than 1 indicate higher aboveground biomass relative to disease development, while values lower than 1 indicates dominance of fungal disease to biomass.

2.6. Statistics

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

3. Results

3.1. Earthworm survival rates

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

3.2. Plant biomass

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

3.3. Plant Si, N and P amounts

In our experiment, we noticed a positive effect of silicon supply on the SiO_2 content in plants with mean values in the range 2.27-4.12% without Si supply and in the range 5.58-6.79% with Si supply. The total plant N and P amounts were mainly affected by NPK fertilization (Tables 1 and 2). Unsurprisingly, NPK fertilization strongly increased plant N, with values for the above-, belowground and total plant biomass, respectively 4.1, 2.2 and 3.6 higher than in treatments without NPK. To a lesser extent, earthworms increased plant N amount by 1.5 times (P-value = 0.003). We found a significant interaction between the factors “NPK” and “Earthworms” on Above-N (P-value = 0.044). Indeed, the positive effect of earthworms on Above-N was more pronounced in -NPK treatments in comparison to +NPK, with increases of 81% and 50%, respectively. Conversely, the supply of Si had no significant effect on plant N (Table 2). Above-P also increased significantly, by 240%, following NPK fertilization (P-value < 0.001). For Below-P, a large effect of NPK (P-value < 0.001) was observed, with a 450% increase of total P; the Si input also induced an increase but to a lesser extent; and the interaction effect of NPK and Si was significant. Indeed, in treatments without NPK, the presence of Si increased Below-P by 55%. Conversely, the presence of Si decreased P by 22% in treatments with NPK. Regarding total P amount in total rice biomass, only NPK significantly influenced this parameter (P-value < 0.001). The presence of earthworms always induced higher P amount in plant tissues, except in the presence of both NPK and Si. Despite higher plant P amount in the presence of earthworms (irrespective of NPK and Si presence), this earthworm effect was overall not significant according to the 3-way ANOVA because of high significant effect of NPK input on plant P amount that masked the benefit effect of earthworm on this variable. We however believe that is important, despite absence of significance, to mention the positive effect on earthworm on plant P nutrition.

3.4. Disease severity

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

3.5. The biomass-disease index

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

4. Discussion

4.1. NPK fertilization

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

4.2. Silicon supply

Silicon supply, with or without NPK, had no significant impact on plant growth, indicating that Si did not impact rice growth in the Malagasy Ferralsols unlike previous observations (Velly, 1975) highlighting a pronounced positive effect of Si fertilization on rice yield. However, we observed a positive effect of Si on belowground P amount only without NPK. Combining NPK and Si supplies led to a decrease in plant P content. Actually, Si uptake decreases P absorption once the latter nutrient is unlimited while it increases under P deficiency that occurs in high P-fixing Ferralsols (Okuda and Takahashi, 1964; Velly, 1975). This increase may be explained by different mechanisms:

- Si control on the expression of genes involved in the transport of inorganic P (Pi), especially symports H^+:Pi (Pht1 family) (Kostic et al., 2017) induced by P deficiency in plants (Plassard et al., 2015);
- Si application may result in the elevation of soil pH (Owino-Gerroh and Gascho, 2005), leading to an increase in the availability of iron and aluminium phosphates (Plassard et al., 2015);
- Si stimulates the exudation of carboxylates, especially malate and citrate (Kostic et al., 2017), that can replace phosphate on adsorption sites and/or favour the complexation of metallic ions (Ca, Al, Fe) involved in P adsorption (Jones, 1998).

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

- Si in the epidermal cell walls of leaves serves as a physical barrier to pathogen penetration into cells (Ma and Takahashi, 1990; Ma et al., 2001);
- Si improves plant defence mechanisms through a rapid deposit of phenolic compounds and lignin on pathogen infection sites (Datnoff et al., 1997);
- activation of defence genes (Wang et al., 2017).

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

4.3. Effects of earthworms

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

Earthworms also promoted a reduction in disease severity in comparison to controls, except when both NPK and Si were provided. It is possible that earthworms enhanced the mobility and bioavailability of native Si (Bityutskii et al., 2016). In the NPK treatments, the disease severity increased in the presence of earthworms, despite Si fertilization. This finding may have resulted from the earthworm-induced increase in the available N and plant N content leading to a decrease in the aboveground tissue C:N ratio from 13.8 to 10.4, which should worsen disease severity. In the absence of NPK, the activity of earthworms decreased the aboveground tissue C:N ratio from 20 to 15, which probably enhanced the microbial N turnover and the net N mineralization rate. In this case, the C:N ratio remained above (or equal to) the threshold of 15, below which the blast disease severity increases exponentially with decreasing plant C:N values (see Fig. 2.A).

4.4. How N fertilization altered belowground interactions on aboveground functions

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

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

5. Conclusions

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

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

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Figure 1

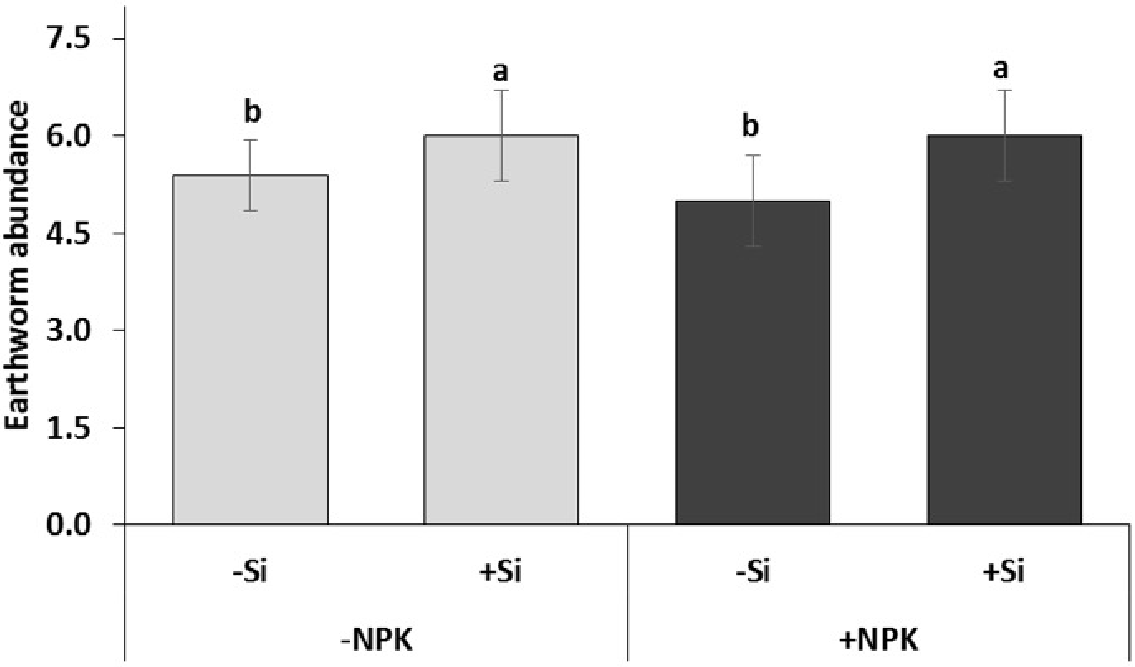
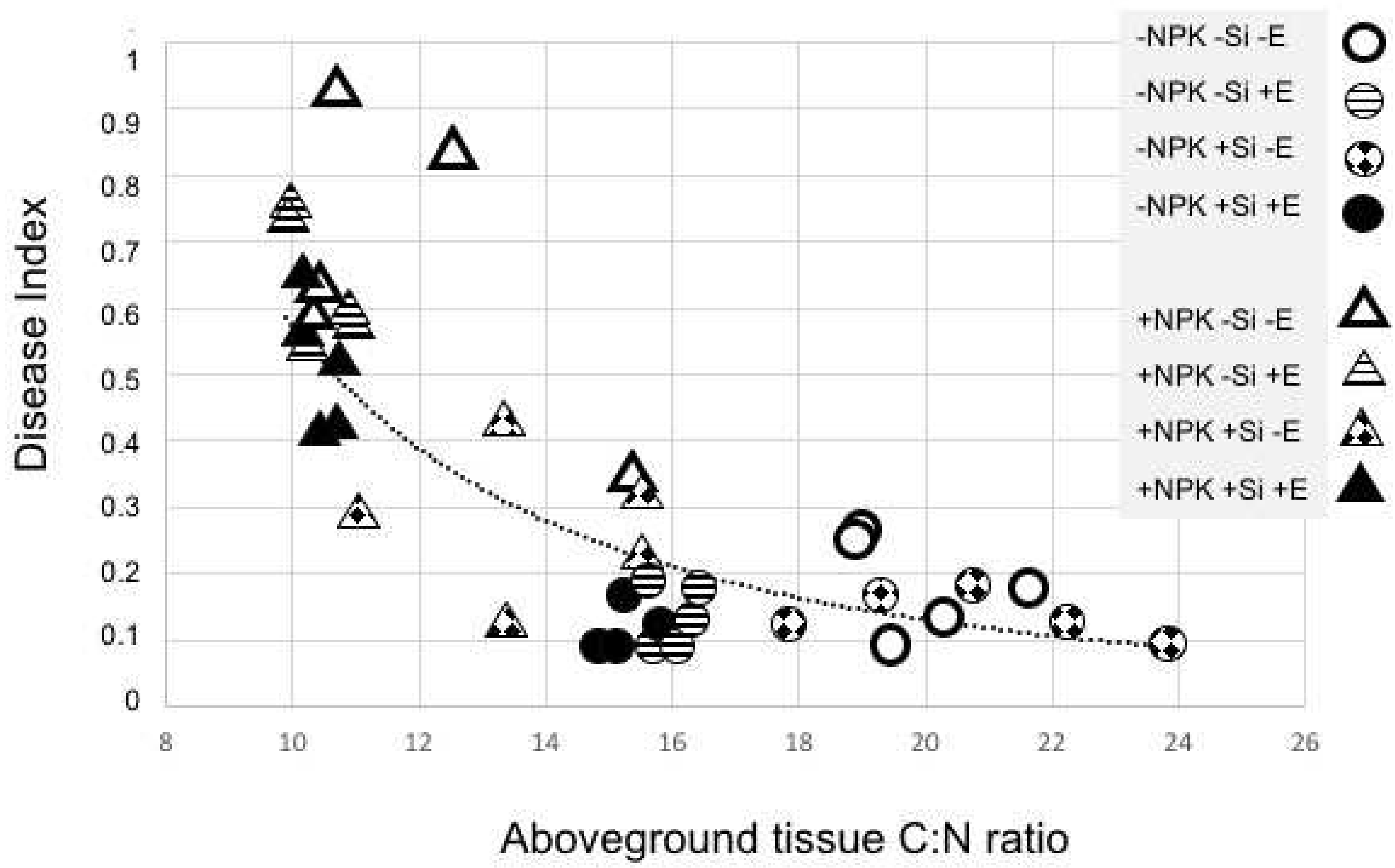
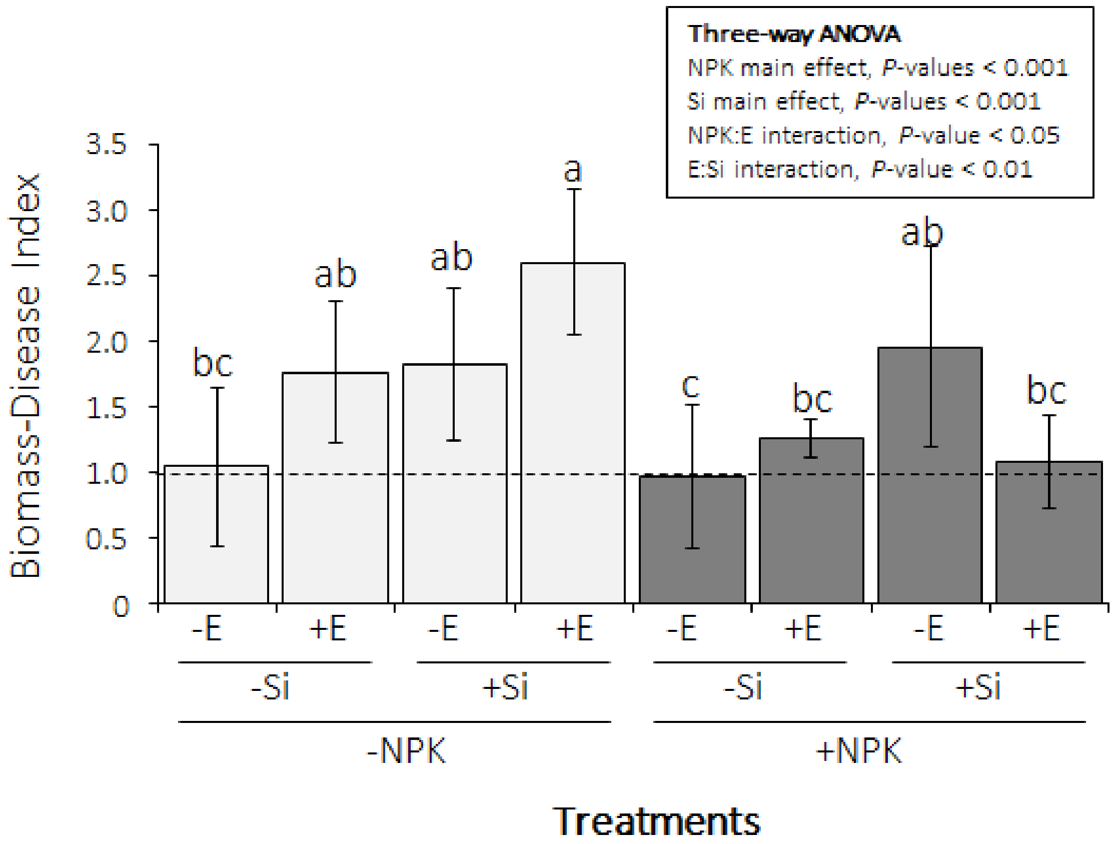


Figure 2A



-NPK -Si -E
-NPK -Si +E
-NPK +Si -E
-NPK +Si +E
+NPK -Si -E
+NPK -Si +E
+NPK +Si -E
+NPK +Si +E
Disease Index
Aboveground tissue C:N ratio
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
8
10
12
14
16
18
20
22
24
26

Figure 2B



Three-way ANOVA
NPK main effect, P-values < 0.001
Si main effect, P-values < 0.001
NPK:E interaction, P-value < 0.05
E:Si interaction, P-value < 0.01
Biomass-Disease Index
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
bc
ab
ab
a
c
bc
ab
bc
-E
+E
-E
+E
-E
+E
-E
+E
-Si
+Si
-Si
+Si
-NPK
+NPK
Treatments

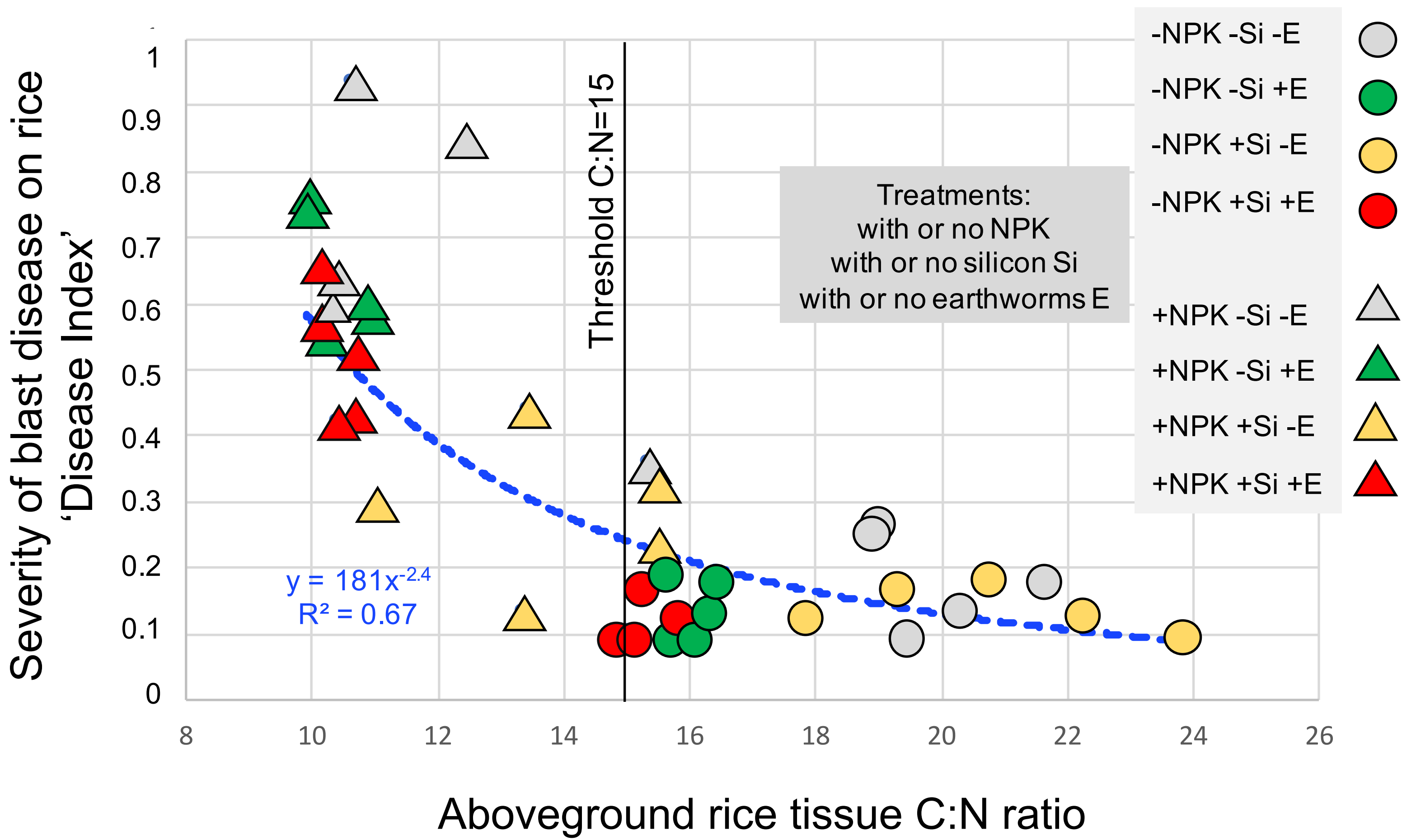
Table 1. Mean (SD) of plant variables (plant biomass, nutrient amount and disease development) measured at the end of the experiment according to treatments (NPK fertilization, silicon and earthworm supply).

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)

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

Variables	**Units**	**Three-way ANOVA factors and interactions**[$]						
		Main effects			**Interactions**			
		NPK	*Silicon (Si)*	*Earthworms €*	*NPK:Si*	*NPK:E*	*Si:E*	*NPK:Si:E*
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

[$]NS (non-significant) indicates P-values > 0.05



Severity of blast disease on rice
'Disease Index'
Aboveground rice tissue C:N ratio
Threshold C:N=15
y = 181x^-2.4
R² = 0.67
Treatments:
with or no NPK
with or no silicon Si
with or no earthworms E
-NPK -Si -E
-NPK -Si +E
-NPK +Si -E
-NPK +Si +E
+NPK -Si -E
+NPK -Si +E
+NPK +Si -E
+NPK +Si +E
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
8
10
12
14
16
18
20
22
24
26