Farm yard manure application mitigates aluminium toxicity and phosphorus deficiency for different upland rice genotypes

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Abstract

Upland rice production on acid, weathered soils is often constrained by phosphorus (P) deficiency and aluminium toxicity. Farmyard manure application (FYM) can sharply enhance yields and agronomic P fertilizer (TSP) efficiency under such abiotic stresses. We tested the hypothesis that rice genotypes differ in aluminium tolerance and in the extent of using organic P, offering distinct benefits under TSP-FYM combinations. Multiple field trials were conducted in the uplands of Madagascar, with factorial combinations of six genotypes, amendments of FYM (at a rate of 17-25 kg P ha⁻¹ versus a zero control) and TSP application (40 kg P ha⁻¹ versus a zero control), with blanket N&K additions. Rice grain yields reached a maximum of 6 t ha⁻¹ after three years of TSP+FYM application, with an average of 3.2 t ha⁻¹ over the years. Grain yields were about 1.2 t ha⁻¹ for FYM only and about 1.5 t ha⁻¹ for TSP only while crops failed under zero P input. Genotypic effects on rice yields were much smaller than the large effects of FYM, TSP, or its combination. Application of FYM increased soil pH and CaCl₂-extractable P while decreasing CaCl₂-extractable aluminium. An additional liming trial indicated that the beneficial effects of FYM over TSP relate to liming effects. The FYM application lowers aluminium toxicity which overrules potential effects of organic P supply. Hence, genotypic ranking of yields and agronomic efficiency was inconsistent, without superior genotypes under FYM versus TSP. However, Chomrong Dhan and FOFIFA 172 generally displayed superior yields. Chomrong Dhan is found to be more sensitive to aluminium toxicity whereas FOFIFA 172 is less performant under low P input. Aluminium tolerance should be considered when developing rice genotypes for high P efficiency in weathered soils.

Introduction

Upland rice is often grown by subsistence farmers in Asia, sub-Saharan Africa, and Central America; however, yield gaps of rice are larger in upland compared to lowland rice cropping systems (Chauhan, Jabran, & Mahajan, 2017). A large fraction of tropical upland systems are strongly nutrient-depleted due to high soil weathering, persistent soil degradation and continuous cropping without external inputs (Stoorvogel et al. 1993). The weathered soils are dominated by sesquioxides which, combined with high soil acidity, can cause aluminium (Al) toxicity, low phosphorus (P) availability, and hence low agronomic P use efficiency to the rice crop (Nishigaki et al., 2019). Therefore, upland rice production is often constrained by the combination of P limitations and soil acidity (i.e. Al toxicity), logically aggravated by drought (Diagne et al., 2013; Mueller et al., 2012). For example, upland rice production in Madagascar contributes largely to the country's total rice production, but a vast amount of the available upland area remains uncultivated as a result of these particular soil constraints (Andriamananjara et al., 2018; Raboin et al., 2014).

To overcome P limitations and Al toxicity in strongly weathered, acid upland soils, relatively large amounts of mineral P fertilizer are needed, which are often beyond reach to most common farmers (Nziguheba, 2007). Efforts are thus needed to increase the agronomic P fertilizer use efficiency. This can be achieved by soil management practices that increase the P availability to the crop, in combination with the use of P-efficient genotypes (Nziguheba et al., 2016).

The application of organic matter (OM) can enhance soil P availability, alter soil pH and reduce Al toxicity. Several field trials with upland rice showed that farmyard manure (FYM) applications in the planting holes largely increase the agronomic P use efficiency in fields with added N&K fertilisers (Andriamananjara et al., 2018, 2019). Organic anions released during OM decomposition compete with phosphate (PO_4) sorption and improve availability of P from mineral P fertilizers (e.g. triple super phosphate (TSP)). The

FYM applications can temporarily enhance soil pH, thereby facilitating root proliferation and plant access to soil P while, at the same time, reducing PO_4 sorption (Haynes and Mokolobate, 2001; Cong and Merckx, 2005). Finally, local high doses of FYM can enhance soil moisture content and reduce water stress. However, none of these factors have been experimentally disentangled to explain the beneficial effects on yields and P uptake for upland rice.

As for multiple abiotic stresses (Jagadish et al., 2012), rice breeding for P-efficient genotypes will be beneficial in upland rice cropping systems. The adaptation to P-limiting environments can derive either from an increased internal P utilization efficiency ($\text{PUE} = [\text{Plant Biomass}] * [\text{Plant P Uptake}]^{-1}$), i.e., how efficient the plant can use its tissue P to grow, or from an increased P acquisition efficiency ($\text{PAE} = [\text{Plant P uptake}] * [\text{Root biomass}]^{-1}$), i.e. how efficient a plant can acquire P from the soil (Vandamme, Rose, Saito, Jeong, & Wissuwa, 2016). (Akhtar, Oki, & Adachi, 2008; Vandamme, Rose, et al., 2016; Vandamme, Wissuwa, et al., 2016; Wissuwa & Ae, 2001). Genotypic variation in PAE is related to a combination of differences in effective root surface area per plant (Nestler & Wissuwa, 2016) or in P acquisition strategies (Clark, 1983). For example, it is well established that plants can mobilize soil-adsorbed P by secreting root exudates, with different underlying P-solubilisation strategies from the inorganic P pool, or that plants can access organic P forms by the excretion of phosphatases (Richardson et al., 2011; Rose et al., 2013). The potential role of the organic P pool as a source of available P in tropical weathered soils has been emphasized in previous studies (Turner, 2006), however, information on the utilization of soil organic P by rice is scarce. One recent isotope dilution study with rice (Rakotoson, 2014), and an older study with ryegrass (Thibaud et al., 1988) and wheat (Osborne & Rengel, 2002) suggested that plant roots can locally absorb P from organic sources, likely by mineralization after phosphatase secretion or by interacting with microbial communities (Mehra et al., 2017). Potentially, rice genotypes may thus differ in the extent of using organic P and some genotypes may offer distinct benefits in FYM-TSP combinations compared to TSP only. However, only limited research has been conducted to assess the interaction between upland

rice genotypes and the supply of organic soil amendments on the agronomic efficiency of P in highly weathered soils.

The objectives of this study were to evaluate the interactions between rice genotypes and supply of FYM in acid, low P soils and to identify soil chemical mechanisms explaining such interactions and ameliorating the abiotic stresses related to soil acidity and low P availability to the rice plant. We speculated that some genotypes may offer distinct benefits in FYM-TSP combinations. Multiple field trials were conducted in the uplands of Madagascar, with factorial combinations of six genotypes, FYM and TSP applications with blanket adequate N&K dosing and periodic irrigation, except for the first year. Specific attention was given to treatment effects on soluble P, Al, and soil pH by including an additional soil incubation trial and a mineral lime reference field trial for all genotypes.

Materials and methods

Summary of the experimental design

Field trials with factorial combinations of genotypes (Geno) and soil amendments were performed on three different, but adjacent fields with similar properties. In Field experiment 1, FYM-TSP-Geno interactions were tested for three subsequent seasons spread over three years, in Field experiment 2, FYM-TSP-Geno interactions for two subsequent seasons and in Field experiment 3, Lime-TSP-Geno interactions for one season. This combination of field experiments and seasons is referred to as an ensemble of six field trials, i.e. on three different field experiments, each over a period of, respectively, 3, 2, and 1 season. All plots were periodically irrigated except for the first season of field 1. In addition, a laboratory incubation trial was set up to identify soil chemical processes explaining FYM-TSP interactions and to corroborate information from soil analysis of the field, sampled at the end of the field trials.

Selected rice genotypes

Six upland rice genotypes were selected based on their contrasting performance on low P soils and differences in P acquisition capacity and efficiency (PAE). Detailed information on the selected genotypes is presented in Table 1. Two genotypes with high PAE, DJ 123 and CG 14 (Koide et al., 2013; Mori et al., 2016; Saito et al., 2015), and one control genotype with low performance in low P soils, NERICA 4 (De Bauw et al., 2019; Koide et al., 2013; Mori et al., 2016; Pujol & Wissuwa, 2018; Saito et al., 2015), were selected. Three other genotypes, locally adopted in Madagascar (i.e. Chomrong Dhan, FOFIFA 172 and FOFIFA 173) of which detailed information on their PAE is still lacking (Raboin et al., 2014), were also included.

Field trials

A three-seasonal field experiment (Field experiment 1) was conducted between November and May (2016-2019) at an upland site in Behenjy, located in the central highland region of Madagascar. Details on the locations and standard soil characteristics are presented in Table 2. During the three consecutive years, five (year 1, 2016) or six (years 2-3; 2017-2018) upland rice genotypes, two farmyard manure (FYM) treatments (0 and 12.7, 21.7, and 10 t dry weight FYM ha⁻¹ respectively for each year) and two mineral P treatments (triple super phosphate (TSP); 0 and 40 kg P ha⁻¹) were combined in a full factorial design. The FYM treatments corresponded to an addition of 0, 19.0, 25.1, and 17.0 kg P ha⁻¹, respectively, for each year as presented in Table S1 (Supplementary Information). Four large TSP × FYM treatment blocks (i.e. Ctrl, FYM, TSP, TSP+FYM) were established. The selected genotypes were sown in each treatment block (i.e. a nested design). All plots, including control, received 80 kg ha⁻¹ N as urea and 60 kg ha⁻¹ K as K₂SO₄. The selected genotypes were sown at a planting distance of 20 cm × 20 cm with two seeds per planting hole. Each genotype was sown in different subplots of 1.92 m² with 12 plants × 4 rows, randomly distributed within the treatment blocks. For the FYM treatments, fresh FYM was locally applied in the planting hole up to approximately 5-10 cm depth and mixed with the soil. For the TSP treatments, granules of triple super phosphate (2-4 mm diam.) were also locally applied in the planting hole up to

approximately 2 cm depth and mixed with the soil. For each year, the FYM was collected from local smallholder farmers; the chemical properties and corresponding nutrient doses of the applied FYM are presented in Table S1 (Supplementary Information). Plots were manually weeded. During the first year, water availability depended on natural rainfall, while the plots were irrigated after each five days without rain in year 2 (2017) and year 3 (2018) to avoid water stress masking the effects of treatments. Straw and grain yield was determined at maturity (i.e. 138-159 days after sowing (DAS) in year 1 (2016), 151 DAS in year 2 (2017) , and 130-144 DAS in year 3 (2018)), omitting one border row per plot and after sample drying at 70°C. Grain yield is reported at 14% moisture content. Straw and grain samples were ground, and composite samples of each genotype were made by combining all subplots within the treatment block for plant analysis. Soil samples were collected at the start (i.e. initial, before any treatment) and at 11 (i.e. middle) and 34 months after the first treatment application (i.e. final harvest) in Field experiment 1 (Table S2, Supplementary Information) as follows. Before starting the experiment, the topsoil (0-15 cm depth) was randomly collected from four points covering the field site. The later soil sampling was done after dividing each treatment block into 4 subplots; the same sampling depth was used at the second sampling but two soil layers (topsoil at 0-15 cm and subsoil at 15-30 cm) were collected at the final sampling. The collected samples from the different points at time zero and from different subplots were separately analysed.

The second field experiment (Field experiment 2) was initiated on a neighbouring field in 2017. This trial was repeated for two seasons, simultaneously with years 2 and 3 of Field experiment 1. All treatments were identical as in year 2 (2017) and year 3 (2018) of the first field experiment. For this second field experiment, a completely randomized design was established by including two replicate blocks of the same treatments (i.e. Ctrl, FYM, TSP, TSP+FYM), each having a size of 42.2 m². The six selected rice genotypes (Table 1) were planted in subplots which were randomly distributed within each treatment block. Plots were irrigated to field capacity after each five days without rain. All plots received N and K as

described for Field experiment 1. At maturity, straw and grain yield determination, and P analyses occurred similarly as explained for Field experiment 1. Soil samples were collected 21 months after the first treatment application (i.e. final harvest; Table S2, Supplementary Information) in Field experiment 2, as described for Field experiment 1.

The third field experiment (Field experiment 3) was performed to test Lime-TSP-Geno interactions during one season (year 3; 2018). This field trial was established in 2018, having two replicate blocks at the side of Field experiment 2, and included two liming treatments in a completely randomized block design. The combination of six rice genotypes (Table 1) and two liming treatments were randomly assigned over the two blocks. In one treatment 4 t ha⁻¹ dolomite was applied (Lime), while in the other treatment 4 t ha⁻¹ dolomite and 40 kg P ha⁻¹ (as TSP) were amended together (Lime+TSP). All plots received N and K as described for Field experiment 1. The dolomite was broadcasted and incorporated into the top soil at approximately 10-20 cm depth one week before fertilizer application and subsequent sowing. Similar grain and straw data were collected as described for Field experiment 1 and 2. Soil samples were collected 12 months after liming (i.e. final harvest; Table S2, Supplementary Information) in Field experiment 3, as described for Field experiment 1. Soils were collected from two subplots per treatment and analysed separately.

Soil incubation experiment

A soil incubation experiment was set up to monitor the effects of FYM on soil chemical properties, i.e. pH and 1 mM CaCl₂ extractable elements. Topsoil (0-20 cm) was collected from the side of Field experiment 1 (Table 2), air-dried and sieved (4 mm). The FYM was collected from different farmers in 2016, air-dried and ground into 0.2 mm. Characteristics of the FYM used in this experiment are presented in Table S1 (Supplementary Information). The FYM was added at different rates (0, 2.3, 12.0, 23.5, 46.9 g FYM kg⁻¹ soil; Table S1) and was homogeneously mixed with the soil. The FYM rates applied on the field ranged

from 10 to 21.7 ton ha⁻¹, and these were more or less equivalent to the intermediate FYM rates used in this incubation experiment (i.e. 12 to 23 g FYM kg⁻¹), as the FYM is mixed on the field into the 0-10 cm soil with a bulk density of 0.98 g cm⁻³ (Table S1, Supplementary Information).

Before incubation, the soil and FYM were thoroughly mixed and the soil moisture content was adjusted with distilled water to 20% w/w. The moist soil mixtures (equivalent of 500-g air-dried soil) were placed in a 2-L tray (19.2 cm × 16.4 cm × 9 cm ht.) and covered by a plastic lid with pinholes to keep the incubated soil aerated. These trays were subsequently placed in a dark incubation room at 25°C. A randomized complete block design was established and three tray replicates were included. These soil-FYM mixtures were not disturbed or mixed during the entire incubation period, to minimize the fluctuation of soil oxygen supply. The soil moisture content was maintained at 20% w/w by spraying distilled water once a week (weight based). Samples were taken from the undisturbed soil surface immediately after mixing and after 3, 9, 15, 22, 28, 43, 72, and 99 days.

Plant and soil analyses

The total P concentrations in the shoots, grains, and FYM were analysed after digestion in concentrated HNO₃ heated in a block digester (DigiPREP MS, SCP Science Co.) and measured by inductively coupled plasma optical emission spectrometry (ICP-OES; iCAP 7000 series, Thermo Scientific Inc.). Internal plant reference samples were included in every batch to control quality. Grain and shoot P in each genotype was analysed as a composite sample from each treatment replicate. Total P uptake after harvest was then determined by combining grain and shoot P.

The agronomic efficiency (APE; kg grain kg⁻¹ P applied) was calculated as the additional grain yield (kg ha⁻¹) per unit P applied (kg P ha⁻¹) through either or both TSP and/or FYM, when compared to the control plot (Ctrl). For the Lime+P treatment, this was also calculated in comparison with the grain yield of the Lime treatment. None of the control plots (no amendment) had rice grain yields, i.e. the second term was zero

in all trials except for Field experiment 3, where rice yield was successful in the limed plots with zero P input.

The soil pH and CaCl_2 -extractable elements were measured on an equivalent of 3 g air-dried soil, either fresh (incubation) or air-dried (field experiments) that was suspended with 15 ml of 1 mM CaCl_2 solution. These soil suspensions were equilibrated by end-over-end shaking (26 rpm) for 24h. Next, the samples were centrifuged (2000 rcf, 10 min, 20°C) and the subsample of supernatant was collected. The remaining samples were used for the pH measurement. After additional filtration (0.45 μm), the total elemental concentrations in the collected supernatant, further denoted as CaCl_2 -extractable elements, were measured with inductively coupled plasma mass spectroscopy (ICP-MS; Agilent 7700x, Agilent Technologies, Inc.) after acidification with 1% HNO_3 . The limit of quantification for P was 3 $\mu\text{g L}^{-1}$.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using R software, version 3.6.1 (R Development Core Team, 2012). The factors genotype (Geno), FYM, TSP as well as the liming treatments in Field experiment 3 were considered as fixed effects, whereas the subplot replicates were included as a random effect. All field data were analysed separately per field and season, while treatment effects on yield and P uptake were plotted per genotype, including the standard deviation to evaluate the genotypic ranking. The coefficient of variation among genotypes was additionally calculated within each treatment combination.

In order to evaluate the overall effects of genotype, P application and soil acidity and/or FYM on rice yield and P uptake, a mixed model was fitted on the ensemble of all the seasons from the three field experiments using JMP software (JMP pro 14, SAS Institute, Inc.). In this model, Y (i.e. rice grain yield, total P uptake, or APE) was explained as a function of total P applied (P_{appl} , in kg P ha^{-1}) and soil acidity, i.e. either CaCl_2 -extractable Al (Al , in $\mu\text{g Al L}^{-1}$) or pH as a proxy for the potential mechanism of FYM

application. The year of the field trial (i.e. 2016, 2017, 2018) was added as a random variable. The model reads:

$$Y = (\beta_0 + \beta_{0,Geno}) + (\beta_1 + \beta_{1,Geno}) * (P_{appl}) + (\beta_2 + \beta_{2,Geno}) * (AI) \quad (\text{Eqn. 1})$$

Genotype (*Geno*) is a categorical variable with 6 levels and assigned as the genotype-specific corrector value ($\beta_{i,Geno}$, *i* is 0, 1 or 2) to examine the overall effect of genotype and its interaction with P_{appl} and AI. The significance of this genotype-specific correctors denotes whether specific genotypes significantly differ from the mean responses in all six field trials. In other words, specific genotypes are inherently different ($\beta_{0,Geno}$) or different because of its sensitivity to P addition ($\beta_{1,Geno}$) or to soil acidity, i.e. AI toxicity ($\beta_{2,Geno}$). The incomplete soil sampling did not allow to link soil AI or pH data to corresponding response data and only data of year 3 (i.e. topsoil at final harvest from all three fields; Table S2, Supplementary Information) were used. Control yields were zero and hence these data were excluded from the modelling, while the data included those of Field 3 (Lime-TSP-Geno) in order to explain the beneficial FYM effects by soil analysis.

Results

Genotypic variation in response of rice to FYM and TSP application

Rice grain yield and shoot P uptake largely responded to the application of FYM and TSP in both Field experiments 1 and 2 in all years (Figure 1 & 2). The yield and P uptake were more affected by soil treatments than by genotype. Without P inputs (Ctrl), no grain yield was obtained throughout both field experiments (Figure 1). In this Ctrl treatment, shoot P content at 80 DAS (average 0.03 mg P per shoot) was half the seed P content at the start (average of 0.06 mg P per seed), confirming the extremely weathered, P-deficient condition of this soil (Table 2). Rice grain yields reached 6 t ha⁻¹ in year 3 (2018)

(TSP+FYM) and were lowest in year 1 (2016) when no irrigation was yet adopted. The rice grain yield and total P uptake ranked FYM+TSP>TSP~FYM>Ctrl, with the FYM either above, similar, or below the TSP, depending on field and year. Interestingly, the average grain yield in the TSP+FYM treatment was consistently higher than the sum of the TSP and FYM treatments separately, suggesting that the effect of the combined application of TSP and FYM was unlikely to be the mere P addition of each material. Similar trends were observed for P uptake.

The effects of genotypes on grain yield and P uptake were smaller than soil treatment effects, and were significant ($P<0.05$) in four of the five trials. The interactions of these endpoints between genotypes and soil treatments were significant in some, but not all trials (Figure 1 & 2). None of the genotypes consistently outperformed others in terms of P uptake or grain yield after a FYM application compared to the mineral P amendment. The ranking of genotypes in terms of total P uptake and grain yield within each treatment was inconsistent over the seasons. The two tropical genotypes (i.e. DJ 123 and CG 14) were generally inferior in both grain yield and total P uptake, while the control genotype NERICA 4 and FOFIFA 173 were intermediate. The two Malagasy highland genotypes (i.e. Chomrong Dhan and FOFIFA 172) were superior in all fertilizer treatments (Figures 1 & 2). Interestingly, the coefficient of variation of either grain yield or P uptake among the genotypes within each treatment was highest in the mineral-P-amended (TSP) plots compared to those the FYM- or the TSP+FYM-amended plots in four of the five trials (Figure 1 & 2). This means that FYM amendment decreased the variation in grain yield and P uptake among genotypes. The agronomic P efficiency (APE) of the P applied under the different treatments was consistently highest for the FYM application only (FYM), and lowest for the mineral P amendment (TSP) (Table 3).

Genotypic variation in response of rice to lime and TSP application

The limed soil without P input (Lime) had no crop failure and the average grain yield (0.6 t ha^{-1}), and total P uptake (0.8 kg P ha^{-1}) was similar to that of the FYM treatment in the first season of Field experiment 1 and both seasons of Field experiment 2 (Figure 1, 2, and 3). Combining TSP with lime (Lime+TSP) resulted in a seven-fold increase of grain yield and a six-fold increase of total P uptake, compared to applying lime only (Figure 3). These results are similar to the TSP+FYM treatment from the simultaneously conducted experiment (Field experiment 2, season 2). The liming treatment showed the highest coefficient of variation among genotypes within treatment, clearly due to the good performance of both Chomrong Dhan and FOFIFA 173 in the limed, but strongly P-deficient subplots. This coefficient of variation was then strongly reduced upon P fertilization, in combination with lime application (Figure 3). Interestingly, the APE under the Lime+TSP treatment in this experiment was higher than that of the TSP+FYM treatment in Field experiment 2 (Table 3).

Changes in soil properties to FYM and lime applications

The FYM applications to the soil in the laboratory incubation increased the soil pH from an initial value of 4.3 to 4.5-6.1, depending on the application rate. The sharp and immediate increase in pH was followed by a gradual pH decrease but all soils amended with FYM had higher pH than the unamended soil (Figure 4A). The FYM applications increased the CaCl_2 -extractable P (Figure 4B) and decreased CaCl_2 -extractable Al up to pH 5.0 (Figure 4C). Aluminium oxyhydroxides are amphoteric, i.e. reacting as acids and bases and dissolving well in acid as in alkaline conditions (Sposito, 1996). The CaCl_2 -extractable Al exhibited such a distinct amphoteric behaviour to soil pH and to FYM dose, i.e. soluble Al decreased at initial FYM doses and increased again at the two highest FYM rates for which pH increased to 5.5 and 6.0 (Figure 4C). The CaCl_2 -extractable P increased with increasing FYM doses and the semi-log trend to pH was linear (Figure 4B).

Similar, but less pronounced effects were observed in soils of the field trials. In the control treatments, the soil pH at final harvest decreased over time and CaCl₂-extractable Al increased (Table S2, Supplementary Information). In Field experiment 1, initially, both FYM and TSP treatments increased pH and decreased extractable Al, while after the final harvest, only such changes were found in the FYM treatments (FYM and TSP+FYM). These changes were observed in both surface and subsoils. Similar trends were observed in Field experiment 2 except that the pH increase and extractable Al decrease was consistently similar in all treatments. The CaCl₂-extractable P showed a clear increase compared to the control after either TSP and/or FYM application, however, no additive effect was observed in the TSP+FYM treatment compared to the single TSP and FYM treatment (Table S2, Supplementary Information). Liming strongly increased soil pH and CaCl₂-extractable P, and it strongly reduced CaCl₂-extractable Al, even in the subsoil (Table S2, Supplementary Information). Both liming treatments (Lime & Lime+TSP) resulted in the highest CaCl₂-extractable P concentrations of all treatments, taking to account that samples were taken only 12 months after application. The CaCl₂-extractable P in these soils was never above 25 µg P L⁻¹ whereas 100 µg P L⁻¹ is considered as a threshold for adequate P supply (De Bauw et al., 2019; Six et al., 2014).

Overall genotypic effects to P addition and soil acidity

Equation (1) was fitted to the ensemble of six field trials and revealed the contribution of Geno, P application, and soil acidity to the overall crop responses (Table 4 and Table S3). Including either soil pH or CaCl₂-extractable Al similarly explained grain yield, P uptake, and APE. Only the Al-based data, rather than soil pH, were further used to indicate effects of Al toxicity. The overall variation in rice grain yield explained by soil and genotypic factors rank P addition>> genotype> soil acidity> genotype interaction with P addition or soil acidity (Table 4). By including the zero-yielding control, the effects of genotype became even smaller in the mixed models (details not shown).

The model was extended to test whether the FYM application also affected crop response beyond its effect on reducing soil acidity. This was done by extending Eqn. (1) with the added FYM dose and its interaction with Geno for all field data of year 3, i.e. the TSP-FYM data of Fields 1 & 2 and the Lime trial of Field 3. This model showed that FYM had no statistically significant effect on grain yield after correcting for the effects of P input and reducing soil acidity. This suggests that the main effect of FYM on rice yield and P uptake follows from its effect on soil acidity and Al toxicity.

In the ensemble of these data, genotypes Chomrong Dhan and FOFIFA 172 performed significantly superior whereas DJ 123 and CG 14 were significantly lower (Table 4 and Table S3). The genotype-specific corrector values for either P addition or CaCl_2 -extractable Al suggest that FOFIFA 172 is more responsive to P addition than Chomrong Dhan and that the latter is more sensitive to Al (Table S3). That is also clearly visualized in the liming trial where Chomrong Dhan performs well under low P after lifting the soil acidity constraint (Figure 3 versus Figure 2).

Discussion

Yield and P uptake of upland rice genotypes after FYM application

This study could not identify genotypes that consistently outperform others after the application of organic matter, in contrast to only mineral P amendments, or vice versa. The main significant effects of genotypes (Table 4) are more affected by their growth potential, i.e. under TSP+FYM application, rather than by their inherent differences in PAE and PUE that exist among rice genotypes (De Bauw et al., 2019; Nestler and Wissuwa, 2016; Vandamme et al., 2016a). This indicates that genotype selection likely becomes inferior to management options when soil limitations are very severe. It is interesting that the coefficient of variation of yield and P uptake generally decreases after FYM application compared to mineral P amendment only (Figures 1 & 2), and that it further decreases for yield when combining FYM

with a mineral P amendment. This can be explained by the fact that the genetic variability under a mineral P amendment includes sensibility of rice genotypes to multiple factors (e.g. low pH, Al toxicity, P deficiency, cold tolerance), while FYM application ameliorates Al toxicity (Haynes & Mokolobate, 2001). However, when only FYM is applied, the genotypic variation still includes tolerance to P deficiency. Hence, when both FYM and mineral P are combined, yields are closer to their potential, with fewer environmental limitations contributing to the genotypic variation (i.e. cold tolerance) (Abera et al., 2020; Raboin et al., 2014). Under combined TSP and FYM amendments, the genotypes Chomrong Dhan and FOFIFA 172 generally outperformed other tested genotypes in terms of yields. This can be explained by their improved tolerance to cold conditions, and possibly explaining why these genotypes are widely adopted by Malagasy farmers (Raboin et al., 2014). Additionally, it was suggested that Chomrong Dhan has a high PAE, as it gives high grain yields under low input system (Raboin et al., 2014). However, such a high P acquisition under P limitations could not consistently be confirmed for Chomrong Dhan in this study. The multivariate model Eqn (1) fitted to the ensemble of data allows to interpret the genotypic variation and its interaction with treatments, i.e. quantify the so-called $G \times E$ interactions (Table S3, Supplementary Information). This showed that the most performant genotype (Chomrong Dhan) is the most sensitive to soil acidity whereas the other performant genotype, FOFIFA 172, is relatively more tolerant. Conversely, the responsiveness to P input is in the reverse order, i.e. FOFIFA 172 is more responsive (less performant under low P input) than Chomrong Dhan.

The generally P-efficient genotype DJ 123, known for its good performance on low P soils (Mori et al., 2016; Nestler & Wissuwa, 2016; Vandamme, Wissuwa, et al., 2016), was not high yielding in the low P environment of this study and it even showed comparable or lower yields than the generally P-inefficient rice genotype, NERICA 4. Hence, this indicates that this P-efficient genotype (DJ 123) is likely not adapted to the cold highlands of Madagascar while NERICA 4 seems somehow more robust in such environment, as this genotype was indeed previously classified as moderately tolerant to cold stress (Wainaina et al.,

2015). It was previously also observed that DJ 123 was extremely sensitive to Al toxicity (Famoso et al., 2011), while NERICA 4 was more moderately tolerant (Kang et al., 2012). However, no such differences in Al sensitivity among these genotypes were found here as judged from the statistical analysis (Table S3, Supplementary Information).

Effects of FYM application on P and Al dynamics in weathered soils and implications for rice yields and P uptake

This study demonstrates that FYM application improves yields up to above 5 t ha⁻¹ when additionally amended with mineral P fertilizer, and positive interactions between FYM and TSP were found. The benefits of FYM applications confirmed earlier data for rice (Andriamananjara et al., 2018; Haeefele et al., 2006; Satyanarayana et al., 2002), however, the mechanisms were never confirmed. Andriamananjara et al. (2018) suggested that the benefits of FYM application to weathered upland soils relate to the better water supply or reduced Al toxicity. By including irrigation here in years 2 & 3 of the study, the effect of FYM on water availability was excluded. This study now confirms that the application of FYM initially increases soil pH, which concomitantly reduces soluble Al and increases P availability. An initial strong liming effect of FYM application is observed in the soil incubation trial (Figure 4) but, interestingly, on the field these liming effects of FYM application on soil pH are much smaller on the longer term (Table S2, Supplementary Information). This suggests that the initial pH increase over the first two or three months of residue decomposition (Haynes & Mokolobate, 2001) is followed by a fast pH decrease resulting from mineralisation processes, i.e. nitrification, and subsequent leaching of nitrates and/or neutral cations on the field (Rowell, 2014). These observations confirm that pH effects after FYM application are indeed local and short-term (Cong & Merckx, 2005), leaving a short-term window of opportunity for crop production. In contrast to the short-term pH effects, the reduced Al and increased P availability after FYM application are more sensitive indices of a longer-term liming effect because small pH changes in the range 4-5 affect soluble Al by over one order of magnitude (Figure 4B). The amphoteric behaviour of Al solubility likely

indicates the trend of reduced Al^{3+} in the most acidic part followed by solubilisation of Al complexed with dissolved organic matter at high FYM doses and higher pH (Sposito, 1996). The increased P availability on the longer term after FYM application likely follows from (i) ligand exchange (i.e. organic sorbates competing with P for mineral binding sites), and, (ii) the slow release of organic P by mineralization (Guppy et al., 2005; Pypers et al., 2005).

Here, we now argue that the positive FYM-TSP interaction effects in acid upland soils are related to mitigated Al toxicity. This is supported by the inclusion of a liming field trial and the statistical observation that soluble Al and P dose explain crop response, i.e. that the FYM dose does not further explain the effects across trials with lime and FYM. The local and short-term pH increase after FYM application and the associated reduction in Al toxicity increase the P uptake from the TSP granules (Andriamananjara et al., 2018; Cong & Merckx, 2005). This improves initial root proliferation during seedling establishment, thereby further enhancing later root growth, P uptake, plant establishment, and final yield. A hydroponic study reported a critical Al concentration of $1350 \mu\text{g Al L}^{-1}$ for rice (Zhang et al., 2016). In our field experiments, the measured CaCl_2 -extractable Al generally exceeded this limit, but the concentration tended to be lower after FYM, TSP, or lime application, hence enabling rice yields (Table S2, Supporting Information). These observations indeed validate the hypothesis that Al toxicity plays a critical role in such P deficient, weathered soils.

The agronomic consequences of FYM applications and implications to field management

Rice genotype selection is thus inferior to soil management effects on acid, weathered soils when considering yields. However, depending on the environment, selecting cold-, drought-, and/or disease-tolerant genotypes can help further improve the yield potential after tackling soil limitations by additions of FYM and TSP. It is striking that attempting rice cultivation on such acid soils without FYM or mineral P amendments (i.e. control treatment) even further decreases soil pH and increases Al in soil solution compared to the initial condition (Table S2, Supplementary Information). This is explained by (i) the

natural tendency for soil acidification over time, (ii) an increased aeration and enhanced microbial activity due to soil cultivation, and (iii) the nitrification of the applied urea (Rowell, 2014). Farmers should recognize that cultivation and application of urea on such already acid soils, without other amendments further aggravates Al toxicity to the rice plant.

This work clearly demonstrates how liming facilitates rice cultivation on highly weathered soils, but without additional nutrient inputs yields remain very low (Figure 3). Liming only is not a sustainable way to increase upland rice production, while liming combined with mineral P amendments can quickly and strongly increase rice yields. It should, however, be regarded that both lime and TSP are often beyond reach of farmers, following market availability and costs (AGRA, 2019; Chianu et al., 2012; Raboin et al., 2016).

As FYM is often more easily accessible to farmers, the application of FYM forms a huge opportunity to more sustainably increase rice yields on weathered, acid, low P soils. However, in order to obtain short-term soil pH effects on the field, this work indicates that rates of at least 10 t ha⁻¹ of FYM are required (Figure 4), or farmers should strongly concentrate the FYM in the planting hole to facilitate local pH effects close to the seedling. Applying FYM to uncultivable fields enables rice production, but rice yields still remain very low (below 2 t ha⁻¹ even after three years of FYM application), due to remaining P limitations. This is because the applied P in the FYM is not readily available to the plant and large portions of the FYM-derived P is immobilized to the soil. Therefore, it was stated before and confirmed here that the use of organic amendments alone are unlikely to overcome P deficiency in such strongly depleted soils (Andriamananjara et al., 2018; Nziguheba, 2007; Nziguheba et al., 2016). Hence, the combination of organic resources with mineral fertilizers would offer a more sustainable way for increasing soil fertility and rice production, while reducing the use of often inaccessible mineral fertilizer. (Buresh et al., 1997; Chivenge et al., 2009; Nziguheba et al., 2016; Six et al., 2014).

436

437 **Conclusions**

438 This study demonstrated how FYM application ameliorates Al toxicity and P deficiency in severely
439 weathered upland soils by altering the dynamics in soil chemistry. The FYM amendment initially and
440 temporary increases soil pH, which concomitantly reduces soluble Al and increases P availability. In
441 contrast to this short-term pH effect of FYM application, Al toxicity is ameliorated on the longer term.

442 On the longer term, P limitations can, however, not be overcome by FYM amendments only. Hence the
443 combination of FYM with TSP amendments forms a huge opportunity to ameliorate P limitations and Al
444 toxicity, thereby strongly improving the phosphate fertilizer use efficiency.

445 This study could not confirm the hypothesis that genotypic differences exist in organic P use efficiency for
446 rice. Further research is needed to confirm the existence of such genotypic variation in organic P cycling
447 after amendments of organic matter.

448 Finally, rice genotype selection is inferior to the effects on yield by FYM or TSP application, or the
449 combination, when the soil is highly weathered and acidic. However, two performant rice genotypes could
450 indeed be identified for such low P environments (i.e. Chomrong Dhan and Fofifa 172). In addition, this
451 study suggests that Chomrong Dhan is more sensitive to soil acidity whereas FOFIFA 172 is less performant
452 under low P input. To conclude, Al toxicity should be considered when breeding new rice genotypes for
453 low P environments.

454

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Table 1. Information of the six rice genotypes used in the field experiments.

Genotype Name	Genus	Varietal subgroup	Region of Origin	Varietal type	PAE ^a	RE ^b	References
NERICA 4	<i>O. sativa</i> × <i>O. glaberrima</i>	<i>tropical japonica</i>	West Africa	Modern	low	low	De Bauw et al., 2019; Koide et al., 2013; Mori et al., 2016; Saito et al., 2015
CG 14	<i>O. glaberrima</i>	-	West Africa	Traditional	high	low	Koide <i>et al.</i> 2013 Wissuwa (personal communication)
DJ 123	<i>O. sativa</i>	<i>aus</i> subspecies	Bangladesh	Traditional	high	high	Mori et al., 2016; Saito et al., 2015
Chomrong Dhan	<i>O. sativa</i>	<i>temperate japonica</i>	Nepal	Traditional	NA	NA	Raboin <i>et al.</i> 2014
FOFIFA 172	<i>O. sativa</i>	<i>tropical japonica</i>	Madagascar	Modern	NA	NA	Raboin <i>et al.</i> 2014
FOFIFA 173	<i>O. sativa</i>	<i>tropical japonica</i>	Madagascar	Modern	NA	NA	Raboin <i>et al.</i> 2014

NA Data not available. ^aPhosphorus Acquisition Efficiency, expressed as total P uptake in mg at low P availability in the soil. ^bRoot Efficiency, expressed as total P uptake per unit root size (either root biomass in g or root surface area in cm²).

Table 2. Information on soil properties of unamended (control) soil in the field and soil incubation experiments. Mean and standard deviations are presented when applicable (n=4 for Field 1 and 2; n=3 for the incubation experiment). Field experiment 3 was conducted at the edge of Field experiment 2, and hence soil properties are similar.

Experiment	Field experiment 1	Field experiment 2	Field experiment 3	Incubation experiment [§]
Start year	2016	2017	2018	2019
Duration	3 seasons	2 seasons	1 season	99 days
Coordinate	19°12'59.3"S 47°27'10.6"E	19°13'01.5"S 47°27'09.2"E	19°13'00.4"S 47°27'09.5"E	19°12'59.3"S 47°27'10.6"E
<i>Soil properties</i>				
Soil classification ^a	Ferralsol	Ferralsol	Ferralsol	Ferralsol
Bulk Density (g cm ³)	0.98	-	-	-
Soil texture ^b	Silty clay	Clay	~	NA
Sand (%)	1.93±1.66	0.64±1.28	~	NA
Silt (%)	50.8±2.6	39.0±7.9	~	NA
Clay (%)	47.2±2.9	60.3±9.0	~	NA
SOC ^c (%)	3.0±0.5	3.0±0.3	~	3.3±0.1
Soil pH _{CaCl2} ^d	4.3±0.1	4.4±0.1	~	4.3±0.1
eCEC ^e (cmol _c kg ⁻¹ soil)	2.1±0.3	1.7±0.2	~	2.3±0.1
P-total ^f (mg kg ⁻¹ soil)	680±30	550±10	~	620±10
N-total ^c (mg kg ⁻¹ soil)	2029±360	1690±170	~	2220±90
P-ox ^g (mg kg ⁻¹ soil)	26.7±4.1	17.3±1.9	~	29.3±3.3
Fe-ox ^g (mg kg ⁻¹ soil)	1940±130	1620±80	~	1720±50
Al-ox ^g (mg kg ⁻¹ soil)	2680±210	2650±90	~	2670±601
Mn-ox ^g (mg kg ⁻¹ soil)	17.8±9.1	10.6±4.9	~	21.9±1.2

[§]Soil sampling location is indicated in this table, while incubation was performed in Belgium. ^aSoil classification (IUSS Working Group WRB, 2015). ^bSoil texture based on particle size distribution (Soil Science Division Staff, 2017) determined by laser diffraction. ^cSoil organic carbon and total N determined with elemental analyzer. ^dpH (1:10, 24h) determined in 1 mM CaCl₂. ^eEffective cation exchange capacity (eCEC) was determined in a 0.0166 M cobalt hexamine (Cohex) extract (ISO, 2007), as the difference between total Co added and Co measured in the extract with inductively coupled plasma-optical emission spectrometry (ICP-OES). ^fTotal P determined in aqua-regia digests (Walinga, Lee, Houba, Vark, & Novozamsky, 1995). ^gAmmonium-oxalate extractive P, Al, Fe and Mn (Schwertmann, 1964).

Table 3. The agronomic P efficiency (APE) [kg grain yield kg⁻¹ P application] of the mineral and organic P amendments in the three field experiments. Mean values with standard error are presented for each treatment, as also the overall mean and coefficient of variation (CV) within treatment.

Genotype	Field Experiment 1								
	Season 1 (2016)			Season 2 (2017)			Season 3 (2018)		
	FYM	TSP	P+FYM	FYM	TSP	P+FYM	FYM	TSP	P+FYM
NERICA 4	18.2 (7.3)	12.3 (5.7)	13.5 (5.1)	78.8 (6.2)	13.1 (2.0)	68.9 (10.3)	81.8 (8.9)	70.3 (13.8)	69.3 (7.2)
CG 14	26.0 (1.0)	7.7 (2.5)	16.8 (1.2)	55.0 (1.9)	25.8 (4.0)	41.4 (2.9)	99.8 (6.2)	45.9 (9.1)	52.5 (13.8)
DJ 123	10.0 (1.3)	3.8 (1.1)	7.3 (NA)	62.4 (10.5)	9.1 (2.2)	28.4 (3.2)	75.1 (23.9)	54.9 (6.8)	59.8 (4.1)
Chomrong Dhan	28.4 (15.0)	8.1 (3.2)	13.3 (1.9)	95.4 (19.7)	27.7 (4.6)	47.2 (10.0)	95.2 (19.3)	46.5 (5.5)	103.7 (6.3)
FOFIFA 172	31.3 (6.0)	9.6 (2.1)	NA	67.7 (15.0)	46.2 (5.8)	83.1 (4.0)	72.6 (13.9)	49.5 (16.3)	85.6 (12.0)
FOFIFA 173	NA	NA	NA	73.8 (12.4)	34.4 (5.2)	46.7 (5.7)	110.7 (7.1)	54.2 (12.1)	77.0 (13.5)
Overall Mean (SE)	23.0 (3.4)	8.5 (1.8)	14.2 (1.7)	72.5 (5.3)	26.4 (2.9)	54.6 (4.4)	88.0 (6.3)	53.2 (4.4)	76.2 (4.7)
CV	63.3	88.5	37.7	38.6	57.4	41.9	38.1	43.0	32.4

Genotype	Field Experiment 2						Field Experiment 3	
	Season 1 (2017)			Season 2 (2018)			Season 1 (2018)	
	FYM	TSP	P+FYM	FYM	TSP	P+FYM	Lime+TSP vs ctrl	Lime+TSP vs Liming
NERICA 4	40.6 (5.4)	14.3 (4.0)	29.1 (5.2)	38.8 (9.3)	40.1 (4.5)	72.9 (9.2)	87.9 (12.6)	NA
CG 14	38.4 (4.9)	14.2 (2.9)	17.2 (1.0)	55.0 (10.5)	79.1 (3.8)	43.3 (4.7)	133.2 (41.3)	123.2 (41.3)
DJ 123	24.5 (3.0)	7.1 (1.4)	14.9 (1.8)	41.8 (5.5)	35.2 (4.3)	64.3 (6.5)	60.6 (13.6)	48.9 (13.6)
Chomrong Dhan	51.9 (6.2)	31.2 (5.1)	47.6 (2.9)	65.7 (9.3)	80.1 (10.7)	93.3 (9.1)	123.0 (15.9)	81.3 (15.9)
FOFIFA 172	50.8 (6.3)	25.9 (3.8)	37.7 (6.2)	53.4 (8.6)	72.5 (10.4)	82.1 (10.7)	176.0 (13.5)	168.5 (13.5)
FOFIFA 173	34.2 (7.1)	26.4 (5.1)	24.4 (6.4)	95.7 (13.4)	76.3 (15.3)	99.0 (5.1)	111.9 (29.2)	92.3 (29.2)
Overall Mean (SE)	41.0 (2.6)	20.4 (2.0)	28.9 (2.4)	57.2 (4.5)	62.2 (4.5)	75.5 (4.2)	109.4 (10.2)	94.4 (13.1)
CV	42.0	64.3	55.7	52.1	47.3	36.1	40.4	52.1

Table 4. Summary of rice grain yield, shoot P uptake, and agronomic P efficiency (APE) in the six field trials. Data include all treatments (including lime treatments) but exclude the zero yielding control treatments. Data show statistical comparison (Tukey's HSD) for the least square means of the genotypes after fitting model Eqn. (1) that is presented in Table S3 (Supplementary Information). The p-values are assigned as ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$. Means in the same column followed by the same character are not significantly different (Tukey's HSD, $p < 0.05$). The effect size (% variance explained) was calculated by including year as fixed (not random) factor, while the models presented in Eqn. (1) (Table S3) present a mixed modelling with year as random factor.

Genotype	Grain yield [t ha ⁻¹]	P uptake [kg P ha ⁻¹]	Agronomic P efficiency (APE) [kg grain yield kg ⁻¹ P application]
DJ 123	1.1 ^d	2.8 ^b	28 ^b
CG 14	1.3 ^{cd}	2.8 ^b	38 ^b
NERICA 4	1.6 ^{bc}	2.8 ^b	39 ^b
FOFIFA 173	1.9 ^{ab}	3.2 ^{ab}	52 ^a
FOFIFA 172	2.1 ^a	3.5 ^{ab}	51 ^a
Chomrong Dhan	2.2 ^a	3.7 ^a	55 ^a
Factor	% variance explained		
Year	18.0***	10.9***	31.9***
P application (kg ha ⁻¹)	28.5***	43.2***	0.1 ^{n.s.}
CaCl ₂ -extractable Al in soil (µg L ⁻¹)	1.4***	<0.1 ^{n.s.}	2.2***
Geno	5.6***	1.4*	7.3***
Geno × P _{appl}	2.6***	1.5*	1.7*
Geno × Al	1.0*	0.5 ^{n.s.}	1.2 ^{n.s.}
Total model R ²	0.57	0.57	0.45

Figure Captions

Figure 1: Rice grain yields of Field Experiment 1 and Field Experiment 2, after 1, 2, or 3 seasons of continuous soil fertilization. Barplots are presented as mean with standard deviation. Treatment (Trt) effects are FYM application (FYM), mineral P fertiliser (TSP), and genotype (Geno), while complete crop failure was observed for the unamended control soil (Ctrl). The coefficient of variation of yield among genotypes is presented for each soil treatment in a box below the bars and was generally largest for the TSP treatment. Note that the scale of the Y-axis is different for each year.

Figure 2: The total P uptake in rice grain and straw at maturity in Field Experiment 1 and Field Experiment 2, after 1, 2, and 3 years of continuous soil fertilization. Barplots are presented as mean with standard deviation. Treatment (Trt) effects are FYM application (FYM), mineral P amendment (TSP), and genotype selection (Geno), while complete crop failure was observed for the unamended control soil (Ctrl). The coefficient of variation of the total P uptake among genotypes is presented for each soil treatment in a box below the bars and was generally largest for the TSP treatment. Note that the scale of the Y-axis is different for each year.

Figure 3: The grain yield and total P uptake at maturity in Field Experiment 3 (Lime and Lime+TSP). Barplots are presented as mean with standard deviation. The coefficient of variation among genotypes is given for each treatment (Trt) in a box below the bars.

Figure 4: The dynamics of (A) the soil pH (top), (B) 1 mM CaCl_2 extractable P (middle), and (C) 1 mM CaCl_2 extractable Al (bottom) of the soils incubated in laboratory conditions. Note the log scale of extractable solute concentrations. Soils were amended with four rates of FYM (0, 2.3, 12.0, 23.5, 46.9 g FYM kg^{-1} soil, $n=3$ each), and incubated at 25 °C without leaching.