Short Title: Steel slag improves soil fertility and rice yield

STEEL SLAG AMENDMENT INCREASES NUTRIENT AVAILABILITY AND RICE YIELD IN A SUBTROPICAL PADDY FIELD IN CHINA

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SUMMARY

Rice is the main food for most of the human population, so sustainable rice production is very important for food security. The fertility of the soil in paddy fields is the key factor controlling rice growth and production. Steel slag amendment is becoming an effective method to increase the soil fertility, stabilize rice production, and reduce greenhouse-gas emissions in Asiatic paddy fields (i.e. Korea, Japan, Bangladesh, and China). We studied the relationships of steel slag amendment with plant-soil nutrient allocation, stoichiometry, and rice yield in a paddy field in subtropical China. Amendment was associated with higher soil N and P availability, lower available-N:available-P ratio, and higher available Ca and Si concentrations. Increases in P, Ca, and Mg availability were correlated with high yields. High yields under steel slag amendment were also associated with high foliar and stem N and P concentrations and lower N:P ratios and with high shoot/root N and P concentration ratios, traits that are typically associated with productive ecosystems able to support species with high growth rates. The positive correlation between steel slag application and yield was partially due to an indirect effect (35% of the total effect) of enhancement of soil Ca, Si, and P availability, which were positively correlated with yield. Steel slag amendment in this paddy field increased plant growth and yield by enhancing nutrient availability, altering soil and plant stoichiometry, and shifting stem:root nutrient allocation.

Keywords: Nitrogen; N:P; Phosphorus; Soil; Stoichiometry.

INTRODUCTION

Rice is the major cereal crop for more than half of the world's population. FAO (2009) estimated that rice production needed to increase by 40% by 2030 to meet the rising demand from the increasing population. Soil fertility improvement is an important factor for increasing rice production (Ali *et al*., 2014). Several strategies for agricultural management are being tested for achieving both sustainable rice production and adequate soil fertility such as straw biochar (Zhao *et al*., 2014), anaerobically digested cattle manure (Nishikawa *et al*., 2012), rice straw (Zhang *et al*., 2013), crop rotation (Nishida et al., 2013), and silicate fertilization (Lee *et al*., 2012). Rice is the base of the diet for 60% of the Chinese population, so the protection of rice production has a pivotal role for food security (Zhu, 2006). Rice cultivation accounts for approximately 28% of the total area of crop cultivation, and total rice production accounts for approximately 38% of the total China crop production (Zhu, 2006). Ninety percent of the paddy fields in China are located in subtropical regions, such as the provinces of Fujian, Jiangxi, and Hunan. The development of methods for increasing soil fertility and sustaining productivity without jeopardizing environmental health in subtropical regions of China is therefore important.

The accumulation of industrial waste is a major environmental problem in urban areas and its rational use is the most effective way to solve such problem (Montejo *et al*., 2013). Steel slag is an important waste product of the steel industry, with a total production of 90 x 10^6 tons in China (Yi *et al*., 2012) and only 22% of this amount used for other economical or environmental activities (Yi *et al*., 2012). Slag contains mainly calcium (Ca) and silicon (Si), which can reach more than 70% of the total weight, whereas the N and P concentrations are 10.33 and 0.07 mg g^{-1}, respectively (Lan *et al*., 2015). Previous studies have found that steel slag amendment increased soil fertility and rice growth, yield, and grain quality (Wang *et al*., 2013). The effects of slag application on soil fertility and rice growth and yield in subtropical paddy fields, however, have been less well studied. C, N and P interact strongly and other nutrients such as Ca and Mg can be limiting in some terrestrial ecosystems (Baribault *et al*., 2012; Lapenis *et al*., 2013; Naples and Fisk, 2010). Thus extra Ca and Si inputs as well as moderate amounts of N and P would change concentration, availability and stoichiometry of soil nutrients and then affect plant growth (Sardans *et al*., 2012a; 2012b). Plant growth is generally N limited in Chinese wetlands (Zhang *et al.*, 2013), especially in paddy fields as periodic inundation limits the access to nutrients due to impaired root metabolism under hypoxia (Kirwan and Guntenspergen, 2012), there is reduction of mineralization rates (Adame *et al*., 2010) and increases in nutrient leaching (Kobayashi *et al*., 2009). The improvement of nutrient availability in paddy fields by sustainable practices would substantially improve rice production.

Increases in rice production due to the application of steel slag have been found (Carvalho-Pupatto *et al*., 2004; Ning *et al*., 2014; Wang *et al*., 2015), with increases in root growth (Carvalho-Pupatto *et al*., 2004) and decreases in the bioavailability of soil trace elements (Gu *et al*., 2011). Steel slag application can increase the resistance against diseases (Gu *et al*., 2011; Ning *et al*., 2014) and decrease the emission of greenhouse gases (Wang *et al*., 2015). Data, however, are not available for the effects of steel slag application on C, N, and P concentrations and stoichiometry of the plant-soil system in rice croplands. Specifically, it is not known if steel slag amendment affects rice production and nutrient stoichiometry by altering the soil nutrient concentrations. We hypothesized that changes of nutrient concentrations in different plant organs and in the entire plant could provide a more realistic view of the role of nutrient concentrations and stoichiometric relationships on growth and other ecophysiological traits than focusing only on foliar stoichiometry. Several studies have noticed that the inclusion of more organs in ecological stoichiometry studies can drastically affect the relationships of stoichiometry with other ecophysiological traits (Elser *et al*., 2003; Sardans *et al*., 2012a).

The present study was carried out in subtropical paddy fields in Fujian Province, China. We chose steel slag from the steel industry as the test material to improve soil nutrients and rice production. We previously reported that steel slag was an effective amendment for increasing rice yields in a subtropical paddy field in Fujian Province (Wang *et al*., 2013), but the effect of steel slag application on plant and soil nutrient concentrations and stoichiometries and their relationships with the increasing yields remain unknown. The present study examined the effect of steel slag amendment on soil and rice nutrient concentrations and stoichiometries in a subtropical paddy field in southeastern China. Specifically, we studied the effects during the growing season of different amounts of steel slag amendments on soil nutrient concentrations and C:N, C:P, and N:P ratios in rice leaves, stems, and roots; and examined the relationships between yield and changes in nutrient uptake, allocation to different organs, and concentrations and stoichiometries in the plant-soil system.

MATERIALS AND METHODS

Study site

All field experiments were carried out in the Wufeng Agronomy Field of the Fujian Academy of Agricultural Sciences (Supplementary Material Fig S1) in a subtropical region of southeastern China. The characteristics and historical management of the field site have been described by Wang *et al*. (2015). The soil of the paddy field was moist, poorly drained, and had a ratio of sand:silt:clay of 28:60:12 (Wang *et al*., 2013). The bulk density of the soil before the study was 1.1 g cm^{-3}. The soil had a pH (1:5 with H_2O) of 6.5 and concentrations of organic carbon, total nitrogen, and total phosphorus were 18.1, 1.2, and 1.1 g kg^{-1}, respectively (Wang *et al*., 2012). The water level was maintained at 5-7 cm above the soil surface during the periods of rice growth by an automatic water-level controller, and the paddy field was drained two weeks before harvesting. Fertilization consisted of a mix of NPK ($N:P_2O_5:K_2O$ at 16:16:16%, Keda Fertilizer Co., Ltd., Shandong, China) and urea (46% N) fertilizers applied to the crops at rates of 95, 70, and 70 kg ha^{-1} of N, P_2O_5, and K_2O, respectively, in each of three growth phases: before transplantation, at tillering, and at panicle formation (Wang *et al*., 2015).

Experimental design and treatments

Our study period was from 16 April to 17 July 2011, which is the first of the two yearly periods of rice crop. The field was plowed to a depth of 15 cm with a moldboard plow on 12 April 2011 and flooded on 14 April 2011. The rice (scientific name?) variety was Hesheng 10, and *the spacing between rows was 28 cm and between plants in the row was 14 cm*. Three replicates of four treatment plots (50 m^2 each) were arranged in a randomized block design. The slag was granular (< 2 mm in diameter), with a pH of 8.5 (when dissolved in water) and composed mainly of CaO (34.9%), SiO_2 (40.7%), and Fe_2O_3 (4.8%) (Wang *et al*., 2012), which was similar to slags used in previous studies (Wang *et al*., 2013). The slag was applied to the paddy field at 0 (control), 2, 4, and 8 Mg ha^{-1}, equivalent to the addition of 0, 67.2, 134, and 269 kg Fe ha^{-1}, respectively, two days before rice transplantation. All control and amended plots followed the same scheme of crop management, including conventional fertilization. A total of 84 soil samples (four treatments × three replicates × seven evaluation dates) were collected. Rice stems, leaves, and root (0-15 cm) biomasses were collected from random 0.25 × 0.25 m quadrats in all plots immediately after transplantation and then every two weeks until harvest. Plant material was gently washed with water. A total of 252 plant samples (four treatments × three replicates × seven evaluation dates × three organs) were thus collected. Shoot, root, and total biomasses and yield were determined at harvest (15 July 2011).

Sampling and chemical analyses of plant and soil samples

Both above- and below-ground samples were oven-dried (80 °C for 24-36 h) to a constant weight and weighed. Plant C and N concentrations were determined using a Vario EL III Elemental Analyzer (Elementar Scientific Instruments, Hanau, Germany), and P concentration was measured by perchloric-acid digestion followed by molybdate-blue reaction using a UV-2450 spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan) (Lu, 1999).

Core soil samples were collected from the top layers (0-30 cm) of all plots the day after transplantation and then every two weeks until harvest. The samples were divided into two parts, one maintained fresh for evaluating the microbial biomass carbon (MBC) and dissolved organic carbon (DOC), and the other air-dried (in the shade) for measuring all other physicochemical characteristics after removing all roots and other plant components. Soil properties were determined following the methods of Lu (1999). Soil MBC concentration was determined by fumigation-extraction (Lu, 1999). DOC concentration was determined by extraction with 1:5 soil:deionized water (Lu, 1999) and measured using an TOC-V CPH total carbon analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). Available-N concentrations were determined by diffusion using alkaline hydrolysis (Lu, 1999), while available-P concentrations were determined by extraction with 0.5 mol L^{-1} $NaHCO_3$ and then measured by Mo-Sb colorimetry (Lu, 1999). Available-K concentrations were determined by extraction with 1 mol L^{-1} NH_4Ac (Lu, 1999) and then measured by FP 640 flame photometry (Shanghai Electronic Technology Instruments, Shanghai, China).”Available-Si concentrations were determined by silicon molybdenum blue colorimetry (Lu, 1999). Available-Ca and -Mg concentrations were determined by EDTA titration (Lu, 1999).

Statistical analyses

All statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, USA). The differences of all plant and soil variables among control and different amendments were analyzed by repeated-measures analyses of variance. The relationships among the concentrations and stoichiometries of the various nutrients in the plant above- and below-ground samples, and the relationships of rice yield and shoot, root, and total biomasses with nutrient concentrations and stoichiometries in the soil and plant samples, were examined by Pearson correlation analysis. For these relationships data from all the experimental treatments were used, except for the correlations with yield in which only the final data was used. We used Hochberg and Benjanimi (1990) correction for the correction of type I error in multiple regressions. We performed multivariate statistical analyses using functional discriminant analysis (FDA) to determine the importance of soil Ca, Si, K, Mg, N, and P availabilities; DOC and MBC concentrations; the ratios of DOC:available N, DOC:available P, and available N:available P; foliar, stem, and root N, P, and C concentrations and C:N, C:P, and N:P concentration ratios; foliar, stem, root, and total biomasses; and rice yield as variables. The FDA was performed using Statistica 6.0 (StatSoft Inc., Tulsa, USA). C:N, C:P, and N:P ratios were calculated as mass ratios. We selected the soil and plant variables strongly correlated with slag amendment and rice yield by structural equation modeling. We thus analyzed the total, direct, and indirect effects of slag amendment on rice yield by the relationships between amendment and the chemical traits of soil and plants. We fitted the various models using the sem R package (Fox *et al*., 2013) and determined the minimum adequate model using Akaike's information criterion (Akaike, 1974). Standard errors and significance levels (P) of the total, direct, and indirect effects were calculated using bootstrapping (1200 repetitions).

RESULTS

Effect of slag amendment on the soil and plant nutrient concentrations and ratios

Soil available-N, -P, -Si, and -Ca concentrations were significantly higher under slag amendment than in the control, whereas soil DOC:available-P ratios (Table 1, Fig. S2 and S3) and foliar C concentrations (Table 1, Figs. S4 and S5) were on average lower under slag application throughout the experimental period.

Relationships of C:N, C:P, and N:P ratios between soil and rice plants

Foliar, stem, and root C:N ratios were positively correlated with the soil DOC:available-N ratio (Table S1), whereas foliar, stem, and root N:P ratios were positively correlated with the soil available-N:available-P ratio (Table S1). On the other hand, foliar, stem, and root C:P ratios were negatively correlated with the soil DOC:available-P ratio (Table S1).

Relationships of crop yield with C, N, and P availabilities

Rice yield was correlated positively with soil available-P, -Si, and -Ca concentrations, foliar and stem N and P concentrations, and root C:N ratio (Table 2). Negative correlations were found between yield and soil available-Mg concentration. Shoot, root and total biomass were correlated positively with soil available-N, -P, -Si, and -Ca concentrations, foliar and stem N and P concentrations, and root C:P and N:P ratios. Yield, and shoot, root and total biomass were positively correlated with N and P concentrations in leaves and stems but not with the corresponding concentrations in roots that in the case of N concentrations were negatively correlated with yield, and root and total plant biomass. Stem C:P, C:N and N:P ratio and root C:N ratio were also positively correlated with yield and plant biomasses except root C:N ratio and shoot biomass. Root C:P and N:P ratios were positively correlated with shoot biomass.

The application of slag did not change the C content of whole plants but it was associated with a higher allocation of C to roots (Table 3). In contrast, slag application caused increases in N and P contents of whole plants and higher allocation of these nutrients to aboveground plant organs at intermediate levels of application (Table 3).

Multivariate and SEM analyses

Soils amended with the various levels of slag were well separated in the DFA analyses (Table S2, Figure 1). Rice yield (RY) best explained the DFA grouping factors (different plots of slag amendment). Changes in soil P, Ca, N, and Si availabilities, foliar N and P concentrations, and soil and foliar N:P ratios were other variables explaining the separation among samples receiving different levels of slag (Table S3, Figure 1). The best structural model explaining the variance in rice yield ($R^2 = 0.69$, $P<0.0001$) identified a positive relationship between slag application and yield, partially due to an indirect effect (32% of the total effect) from the enhancement of soil Ca, Si, and P availabilities, which were also positively correlated with yield (Figure 2).

DISCUSSION

Effect of steel slag amendment on soil and plant nutrients

Application of steel slag has increased soil Ca-, Si-, N, and P-availability (Table 1), which are consistent with results of previous reports (Wang *et al*., 2013; Ali *et al*., 2014). The addition of silicate ions could also increase N and P availabilities by displacing nitrogenous ions (NO_3^- and NH_4^+) and phosphates from ligand exchange sites (Lee *et al*., 2004) and/or by decreasing their sorption on soil colloids (Shariatmadari *et al*., 1999). The observed increases in soil pH due to the alkalinity of the slag should also decrease the mobilization of most heavy metals and thus reduce the risk of soil toxicity. There was a strong impact of steel slag fertilization not only in the soil availability of N, P, Ca and Si, but also on crop yield and plant biomass (Table 3). Thus the results strongly suggest that this sub-tropical paddy that had historically been managed by conventional fertilization in Fujian had its rice production capacity limited by P, Ca and Si (Figure 2).

Slag can absorb and retain nutrients, slowing nutrient release, preventing leaching and consequent water eutrophication, and improving the capacity of soil to provide nutrients for plant uptake (Zhao, 2012). In fact, the total amount of nutrients stored in plants increased (Table 3) due to the increase of plant growth related to the improved nutrient availability under steel slag fertilization. Ca and Si are involved in several metabolic and physiological processes linked to growth, fruit development and defense against pathogens in rice plants (Datnoff *et al*., 1991; Frattini *et al*., 1999; Rodrigues et al., 2001,2003; Guntzer *et al*., 2012; Cacique et al., 2013; Sano *et al*., 2015). In addition, simultaneous increases in soil availability of several nutrients at once can have positive synergies in crop growth and yield production because the fertilization of one nutrient increases the plant uptake and content of other nutrients (Deren, 1997; He *et al*., 1999; Kim *et al*., 2005; Pati *et al*., 2016).

Soil DOC concentrations decreased with the steel slag application and this decrease is likely related to stabilization (fraction in non-soluble C forms) through combination with Fe_2O_3 (Song *et al*., 2012). In our study, the application of 2, 4, and 8 Mg ha^{-1} of steel slag added 96, 192, and 384 kg Fe_2O_3 ha^{-1}, respectively.

Changes in soil and plant nutrient stoichiometry

The results of this study are in agreement with previous ones in paddy fields, where N is limiting. In the Minjiang estuary N limitation have been previously descrived (Wang *et al*., 2012). The success of the invasive Phragmites australis in a nearby wetland was associated with its higher capacity to resorb N and increase N-use efficiency (Wang et al., 2014). Consistently with these previous studies the paddy soils in our study had particularly low available-N:available-P ratios (1.6-3.3 on mass basis), which contrasted with the global average of 5.9 (Cleveland and Liptzin, 2007), suggesting that since the point of view of soil N:P ratio, N should be more limiting in our experimental conditions. However, observing the low availability of N and P in this soils and the fact that steel slag fertilization increase the concentrations of both nutrients, this should be more important to improve rice growth than the changes of soil available-N:available-P ratio. Moreover, above-/belowground N ratio (in mass basis) from 11.7 in control plots to 15.9 in plots receiving 2 MG ha-1 of steel slag together with above-/belowground P ratio (in mass basis) from 2.32 in control plots to 2.94 in plots receiving 4 MG ha^{-1} of steel slag, suggest a greater proportional allocation of N and P to leaves versus roots when steel slag fertilization was applied (Tables 1 and 3). Thus steel slag application produces higher N and P uptake associated to higher biomass production but not to larger N and P concentrations and promotes higher proportional allocation of nutrient sources directly to production when certain levels of steel slag are applied as fertilizer. However, the concentrations of N and P observed in this study in different rice plant organs are in the same range than those observed in previous studies (Deren, 1997; Lavakush *et al*., 2014). This together with the fact that steel slag did not increase C:N and C:P ratios, allow to conclude that the observed enhancement of rice plant biomass associated to steel slag was not due to an increase of efficiency in N- and P-use. Thus, despite N and P soil availability and rice plants N and P contents (but not concentrations) have increased with steel slag fertilization the overall analysis showed that N and P soil availability rise was not the main or at least the unique cause associated with the rise in yield and growth of rice plants receiving steel slag fertilization. In fact, a previous study in this area reported that rice plants retained more N in plots amended with slag than in the control plots (Zhao, 2012). Our study thus found that steel slag fertilization was not associated with higher N and P concentrations but with increases in N and P accumulation, and also of Ca and Si all them associated with higher availability of all four nutrients in soil by steel slag amendment. Finally, the increases in plant growth and yield associated to steel slag fertilization were mainly related to the increases in soil P-, Ca- and Mg-availability (Figure 2). Thus, this wetland area can also be P, Si and Ca limited as SEM analysis showed.The multivariate and SEM analyses also confirmed the positive relationships of slag amendment with plant growth and yield: these relationships were associated with an enhancement of nutrient availability in soil, higher N and P contents in plant tissues, and a shift in nutrient allocation between stems and roots. The novelty of our results is that slag application did not alter plant N:P ratios, but it changed the nutrient allocation and improved resources use and growth rates. Specifically, changes in soil P, Ca, N, and Si availabilities, foliar N and P concentrations, and soil available-N:available-P ratio explained the separation among samples receiving different levels of slag (Figure 1, Tables1-3) and then higher rice yields were related with higher dose of slag application (Figure 1). However, we can not discard the possible potential impacts of slag on soil, groundwater and wildlife or food quality, which must be monitored in agricultural systems. Until now, we have not detected significant impacts of steel slag when considering the most dangerous trace elements (Wang *et al*., 2015, 2016). *However, some negative environmental impacts associated with the use of steel slag at soil and superficial water level have been detected in other regions of China (Meng and Liu, 2000). This last study showed an increase of some trace elements in paddy soils and rice plants mostly in northern regions of China in sites with long-term application of great amounts of steel slag. The establishment of an adequate dose/year to attain optimum results (maximum benefices without negative effects) at medium and long-term warrants further studies*.

CONCLUSION

Steel slag increased soil N, P, Ca, and Si availabilities and rice plant biomass and nutrient contents. The allocation of N and P to aboveground organs also increased, at least at intermediate levels of slag application. This method of fertilization could be applied on a large scale at low cost, because 7.82 × 10^8 t of steel were produced in China in 2013, and the amount of slag generated was 0.46 t per ton of steel produced (Xie and Xie, 2003). The total amount of steel slag produced in 2013 was thus 3.60 × 10^8 t, so its application to paddy fields to easily improve rice production and soil conservation in China would be very cost effective..

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Table 1. Soil and plant nutrient concentrations and stoichiometries (mean ± SE). Significant (*P*<0.05) values are highlighted in bold type. Different letters among treatments indicate significant differences. MBC = Soil microbial biomass carbon. DOC = Soil dissolved organic carbon

	Variable	Rate of steel slag amendment				Treatments
		0 Mg ha^{-1}	2 Mg ha^{-1}	4 Mg ha^{-1}	8 Mg ha^{-1}	
Soil	MBC concentration (mg kg^{-1})	609±64	719±39	670±41	624±45	*F*=1.071; *P*=0.366
	DOC concentration (mg kg^{-1})	240±35	277±33	255±32	263±29	*F*=0.227; *P*=0.878
	Available-N concentration (mg kg^{-1})	7.90±1.31b	10.9±1.7ab	16.6±3.6a	8.96±1.59b	***F*=3.046; *P*=0.033**
	Available-P concentration (mg kg^{-1})	3.13±0.34b	4.57±0.56ab	5.06±0.56a	5.54±0.69a	***F*=3.575; *P*=0.018**
	Available-K concentration (mg kg^{-1})	141±12	137±10	142±11	141±9	*F*=0.046; *P*=0.987
	Available-Si concentration (mg kg^{-1})	330±10c	921±35b	1093±93ab	1295±143a	***F*=22.893; *P*<0.001**
	Available-Ca concentration (cmol kg^{1})	0.033±0.002a	0.033±0.002a	0.046±0.003b	0.047±0.004b	***F*=8.231; *P*<0.001**
	Available-Mg concentration (cmol kg^{1})	0.046±0.005	0.051±0.006	0.049±0.005	0.039±0.002	*F*=1.417; *P*=0.244
	DOC:available-N ratio	30.4±7.3	25.4±3.9	15.4±5.5	29.4±4.8	*F*=0.842; *P*=0.475
	DOC:available-P ratio	76.7±11.5a	60.6±7.9ab	50.4±3.6b	47.5±3.7b	***F*=3.320; *P*=0.024**
	Available N:available-P ratio	2.52±0.14ab	2.39±0.14ab	3.28±0.33a	1.62±0.13b	***F*=2.306; *P*=0.083**
Plant	Foliar C concentration (mg g^{-1})	411±3a	396±4b	398±5b	391±5b	***F*=4.294; *P*=0.007**
	Foliar N concentration (mg g^{-1})	32.3±2.9	33.3±3.0	33.2±3.3	35.0±3.2	*F*=0.127; *P*=0.994
	Foliar P concentration (mg g^{-1})	1.16±0.22	1.33±0.27	1.36±0.26	1.44±0.26	*F*=0.212; *P*=0.888
	Foliar C:N ratio	12.7±0.8	11.9±0.8	12.0±0.7	11.2±0.6	*F*=0.432; *P*=0.731
	Foliar C:P ratio	354±40	298±38	293±37	272±34	*F*=0.731; *P*=0.536
	Foliar N:P ratio	27.8±4.7	25.0±5.2	24.4±5.0	24.3±4.6	*F*=0.048; *P*=0.986
	Stem C concentration (mg g^{-1})	384±4a	374±4ab	375±4ab	372±4b	***F*=1.989; *P*=0.122**
	Stem N concentration (mg g^{-1})	15.5±2.3	16.6±2.3	17.4±2.3	17.6±2.3	*F*=0.176; *P*=0.912
	Stem P concentration (mg g^{-1})	1.43±0.24	1.57±0.27	1.58±0.27	1.76±0.28	*F*=0.261; *P*=0.853
	Stem C:N ratio	24.8±4.1	22.5±3.1	21.6±2.3	21.1±2.4	*F*=1.154; *P*=0.333
	Stem C:P ratio	269±35	238±33	237±32	211±32	*F*=0.396; *P*=0.756
	Stem N:P ratio	10.8±2.7	10.6±2.7	11.0±2.6	10.0±2.6	*F*=0.009; *P*=0.999
	Root C concentration (mg g^{-1})	340±9	340±8	336±9	347±11	*F*=0.257; *P*=0.856
	Root N concentration (mg g^{-1})	11.3±1.1	9.70±1.09	10.2±1.0	9.83±0.99	*F*=0.474; *P*=0.701
	Root P concentration (mg g^{-1})	3.39±0.43	3.12±0.40	2.82±0.47	3.33±0.44	*F*=0.344; *P*=0.793
	Root C:N ratio	30.1±2.4	35.1±2.6	32.9±2.3	35.3±2.8	*F*=0.522; *P*=0.669
	Root C:P ratio	100±23	109±21	119±24	104±23	*F*=0.427; *P*=0.734
	Root N:P ratio	3.33±1.21	3.11±1.19	3.62±1.40	2.95±1.11	*F*=0.495; *P*=0.687

Table 2. Pearson correlations among rice yield and biomasses with soil and plant nutrient concentrations and ratios. Significant correlations after applying the Benjamini & Hoghberg correction of false discovery rate (*R*> ± 0.642, *P*<0.0025) are highlighted in bold type (n=12).

Variable	Yield	Shoot biomass	Root biomass	Total biomass
Soil				
MBC concentration	0.047	-0.142	-0.161	0.002
DOC concentration	0.431	0.079	0.398	0.375
Available-N concentration	0.337	0.552	-0.089	0.350
Available-P concentration	**0.972***	**0.837***	**0.838***	**0.959***
Available-K concentration	0.260	0.605	0.158	0.319
Available-Si concentration	**0.968***	**0.824***	**0.839***	**0.954***
Available-Ca concentration	**0.902***	**0.997***	**0.746***	**0.927***
Available-Mg concentration	**-0.516**	-0.470	**-0.764***	-0.533
DOC:available-N ratio	-0.530	-0.358	-0.279	-0.495
DOC:available-P ratio	**-0.943***	**-0.919***	**-0.719***	**-0.944***
Available-N:available-P ratio	-0.291	0.010	**-0.660***	-0.266
Plant				
Foliar C concentration	**-0.896***	**-0.652***	**-0.835***	**-0.867***
Foliar N concentration	**0.906***	**0.687***	**0.993***	**0.890***
Foliar P concentration	**0.966***	**0.794***	**0.871***	**0.949***
Foliar C:N ratio	**-0.948****	**-0.735***	**-0.966***	**-0.930***
Foliar C:P ratio	**-0.976***	**-0.802***	**-0.959***	**-0.963****
Foliar N:P ratio	**-0.879***	**-0.825***	**-0.946***	**-0.891***
Stem C concentration	**-0.874***	**-0.643***	**-0.770***	**-0.845***
Stem N concentration	**0.975***	**0.905***	**0.800***	**0.971***
Stem P concentration	**0.950***	**0.750***	**0.979***	**0.935***
Stem C:N ratio	**-0.937***	**-0.853***	**-0.728***	**-0.927***
Stem C:P ratio	**-0.965***	**-0.779***	**-0.971***	**-0.951***
Stem N:P ratio	**-0.797***	-0.507	**-0.952***	**-0.771***
Root C concentration	0.443	0.189	**0.778***	0.427
Root N concentration	**-0.727***	-0.440	**-0.637**	**-0.685***
Root P concentration	-0.288	-0.429	0.134	-0.293
Root C:N ratio	**0.700***	0.358	**0.709***	**0.654***
Root C:P ratio	0.384	**0.699***	0.017	0.425
Root N:P ratio	0.133	**0.489***	-0.244	0.175

*, significant correlation at *P*<0.05; **, significant correlation at *P*<0.0022 R=±0.642 (after applying the Benjamini & Hoghberg correction of false discovery rate).

MBC = Soil microbial biomass carbon DOC = Soil dissolved organic carbon

Table 3. Plant nutrient contents and stoichiometries in the various treatments (mean ± SE).

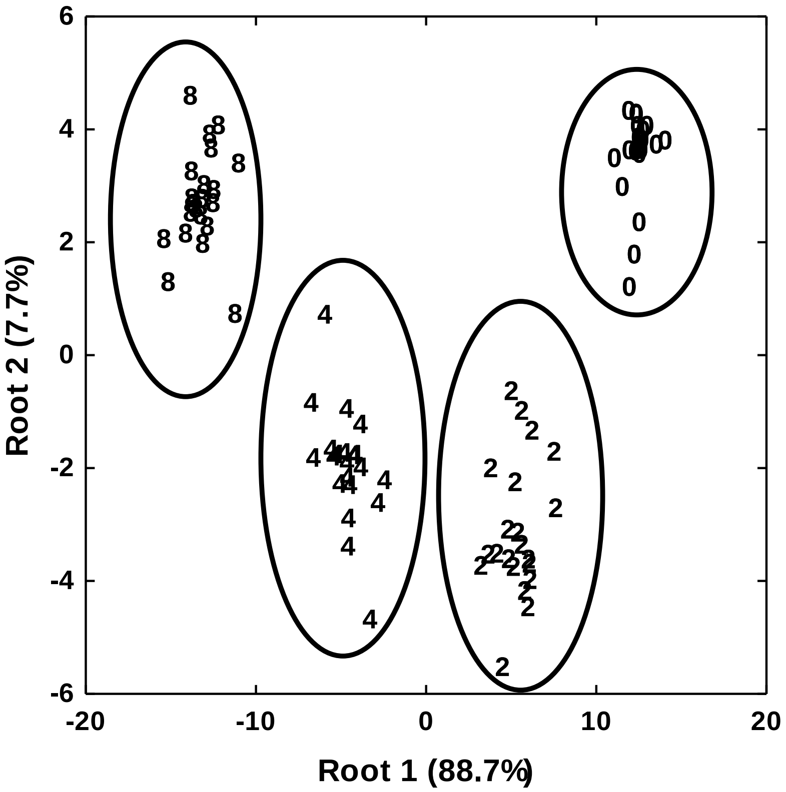
Variable	Steel slag amendment rate			
	0 Mg ha^{-1}	2 Mg ha^{-1}	4 Mg ha^{-1}	8 Mg ha^{-1}
Above-/belowground C ratio	7.12±0.28a	6.52±0.15b	6.46±0.52b	6.49±0.12b
Above-/belowground N ratio	11.7±0.3b	15.9±2.0a	12.1±0.2b	12.8±0.2ab
Above-/belowground P ratio	2.32±0.07b	2.56±0.07ab	2.94±0.03a	2.46±0.07b
Total plant C content (kg ha^{-1})	6539±112	6462±181	6710±142	6820±157
Total plant N content (kg ha^{-1})	169±5c	187±5ab	178±6bc	198±5a
Total plant P content (kg ha^{-1})	35.8±1.9ab	34.4±0.8b	34.3±1.0b	38.9±1.1a
Total plant C:N content	38.7±0.2b	34.6±0.3c	37.7±0.4a	34.4±0.4c
Total plant C:P content	183±9ab	188±4ab	196±1a	175±4b
Total plant N:P content	4.72±0.09	5.44±0.1	5.19±0.12	5.09±0.15

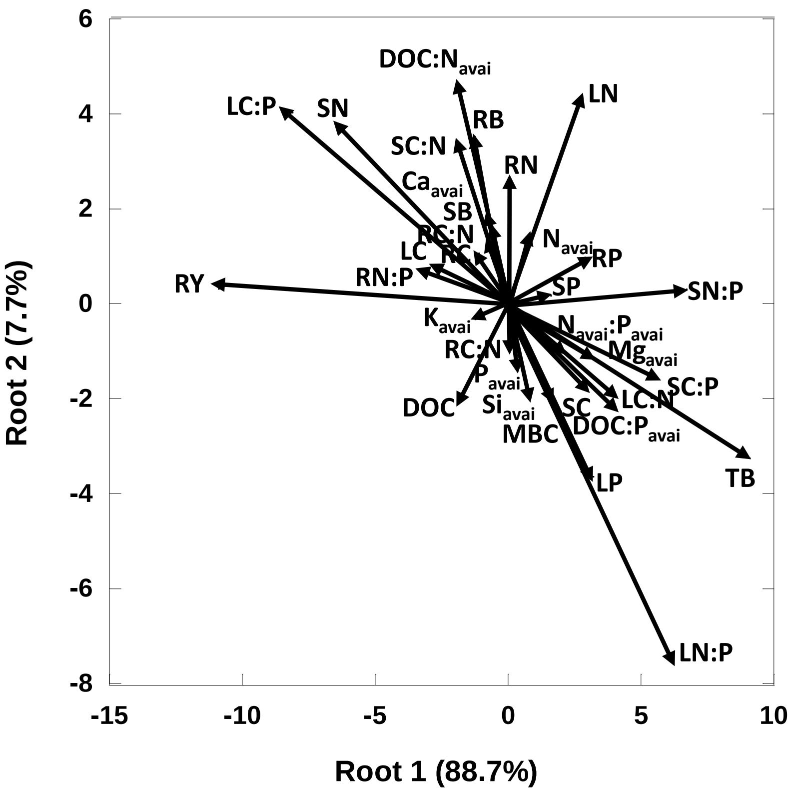
Different letters among treatments indicate significant differences (P<0.05).

Figure legends

Figure 1. Biplots of the standardized canonical discriminate function coefficients for the first two roots representing the soil and plant variables as independent variables and the various grouping dependent factors corresponding to the levels of steel slag amendment (0, 2, 4, and 8 Mg ha^{-1}). Ca_{avai}, soil Ca availability; Si_{avai}, soil Si availability; K_{avai}, soil K availability; Mg_{avai}, soil Mg availability; N_{avai}, soil N availability; P_{avai}, soil P availability; DOC, dissolved organic C; DOC:N_{avai}, DOC:soil available-N ratio; DOC:P_{avai}, DOC:soil available-P ratio; N_{avai};P_{avai}, soil available-N:soil available-P ratio; LN, foliar N concentration; LP, foliar P concentration; LC, foliar C concentration; SN, stem N concentration; SP, stem P concentration; SC, stem C concentration; RN, root N concentration; RP, root P concentration; RC, root C concentration; LC:N, foliar C:N concentration ratio; LC:P, foliar C:P concentration ratio; LN:P, foliar N:P concentration ratio; SC:N, stem C:N concentration ratio; SC:P, stem C:P concentration ratio; SN:P, stem N:P concentration ratio; RC:N, root C:N concentration ratio; RC:P, root C:P concentration ratio; RN:P, root N:P concentration ratio; MBC, soil microbial C; RC, rice yield; LB, foliar biomass; SB, stem biomass; RB, root biomass; TB, total biomass.

Figure 2. Diagrams of the structural equation models that best explained the maximum variance of rice yield with steel slag application and soil P, Si and Ca availability as endogenous variables. Blue and red arrows indicate negative and positive relationships, respectively.


Root 2 (7.7%)
Root 1 (88.7%)


DOC:N$_{avai}$
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SP
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N$_{avai}$:P$_{avai}$
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LC:N
DOC
Si$_{avai}$
SC
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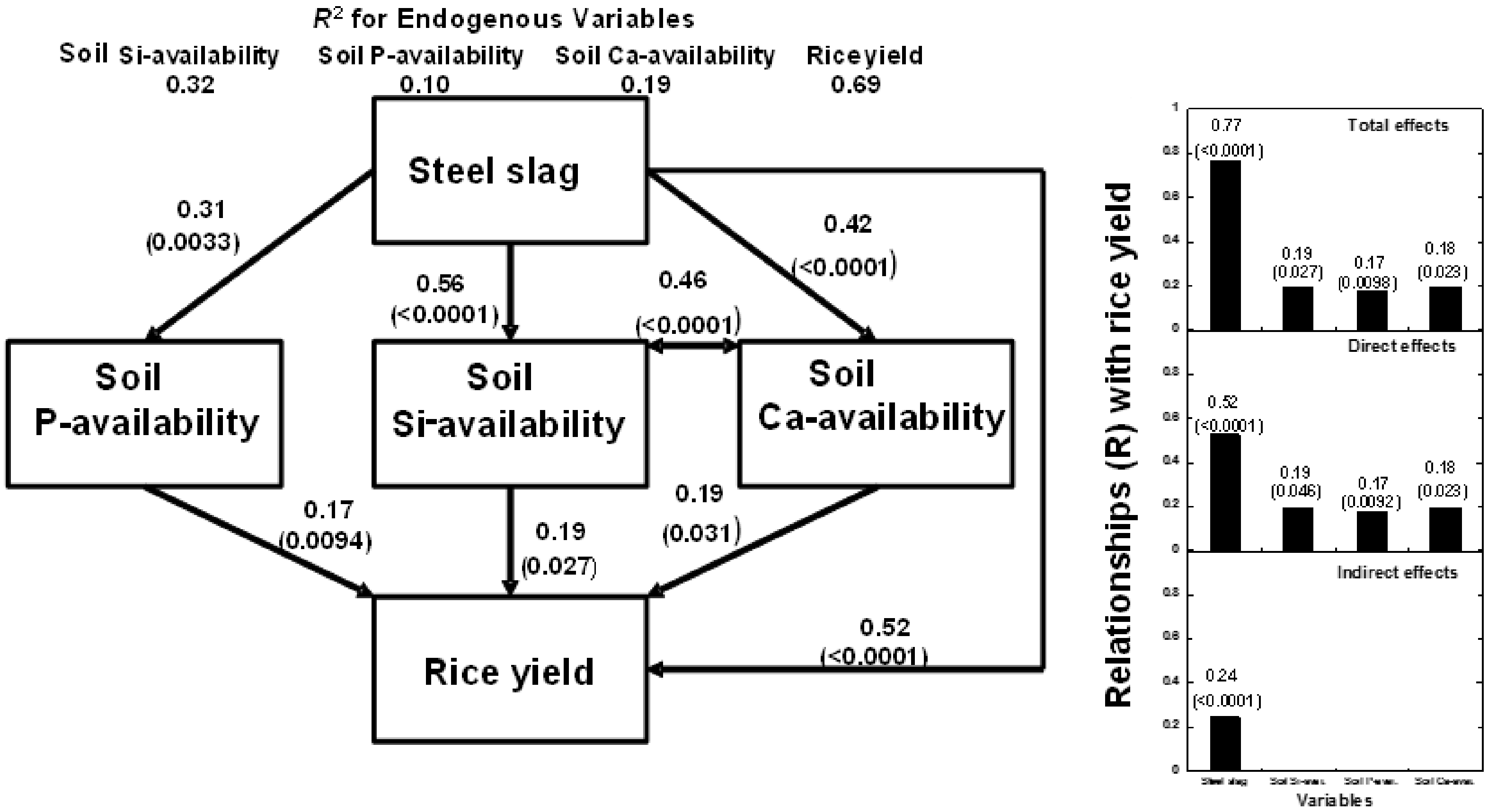
MBC
TB
LP
LN:P
Root 2 (7.7%)
Root 1 (88.7%)

Figure 1



R2 for Endogenous Variables
Soil Si-availability 0.32
Soil P-availability 0.10
Soil Ca-availability 0.19
Rice yield 0.69
Steel slag
0.31 (0.0033)
0.56 (<0.0001)
0.46 (<0.0001)
0.42 (<0.0001)
Soil P-availability
Soil Si-availability
Soil Ca-availability
0.17 (0.0094)
0.19 (0.027)
0.19 (0.031)
0.52 (<0.0001)
Rice yield
Relationships (R) with rice yield
Total effects
0.77 (<0.0001)
0.19 (0.027)
0.17 (0.0098)
0.18 (0.023)
Direct effects
0.52 (<0.0001)
0.19 (0.046)
0.17 (0.0092)
0.18 (0.023)
Indirect effects
0.24 (<0.0001)
Variables

Figure 2

Supplementary material

Table S1. Pearson correlations among soil and plant nutrient concentrations and ratios. Significant correlations after applying the Benjamini & Hoghberg correction of false discovery rate (*R*> ± 0.302, *P*<0.008) are highlighted in bold type (n=84).

Variable	DOC	Available-N concentration	Available-P concentration	DOC:available-N ratio	DOC:available-P ratio	Available-N:available-P ratio
Foliar C concentration	0.062	**0.485***	0.238	-0.164	-0.211	**0.399***
Foliar N concentration	-0.217	**0.545***	**0.823***	**-0.511***	**-0.515***	0.197
Foliar P concentration	**0.638***	**-0.490***	-0.243	**0.768***	**0.325***	**-0.485***
Foliar C:N ratio	0.061	**-0.500***	**-0.698***	**0.320***	**0.421***	-0.181
Foliar C:P ratio	**-0.741***	**0.609***	**0.424***	**-0.836***	**-0.542***	**0.498***
Foliar N:P ratio	**-0.602***	**0.643***	**0.765***	**-0.782***	**-0.616***	**0.305***
Stem C concentration	0.246	**0.311***	0.007	0.081	-0.106	0.292
Stem N concentration	**-0.374***	**0.670***	**0.813***	**-0.620***	**-0.624***	**0.328***
Stem P concentration	**0.645***	**-0.592***	**-0.301***	**0.847***	**0.446***	**-0.552***
Stem C:N ratio	0.116	**-0.552***	**-0.688***	**0.353***	**0.543***	-0.213
Stem C:P ratio	**-0.609***	**0.708****	0.289	**-0.809***	**-0.555***	**0.657***
Stem N:P ratio	**-0.554***	**0.791****	**0.605***	**-0.790***	**-0.636***	**0.577***
Root C concentration	**0.482***	-0.109	**-0.565***	**0.448***	**0.365***	0.091

Root N concentration	**-0.557***	**0.712***	**0.573***	**-0.670***	**-0.622***	**0.492***
Root P concentration	**0.549***	**-0.736***	**-0.540***	**0.746***	**0.635***	**-0.510***
Root C:N ratio	**0.458***	**-0.600***	**-0.599***	**0.616***	**0.606***	**-0.364***
Root C:P ratio	**-0.516***	**0.767***	**0.349***	**-0.622***	**-0.523***	**0.616***
Root N:P ratio	**-0.581***	**0.755***	**0.433***	**-0.634***	**-0.553***	**0.568***

* significant correlation at $P<0.008$ (after applying the Benjamini & Hoghberg correction of false discovery rate).

DOC = Soil dissolved organic carbon

Table S2. Test statistics for squared Mahalanobis distances among treatments with different levels of steel slag amendment with soil Ca, Si, K, Mg, N, and P availabilities; DOC and MBC concentrations; the ratios of DOC:available N, DOC:available P, and available N:available P; foliar, stem, and root N, P, and C concentrations and C:N, C:P, and N:P concentration ratios; foliar, stem, root, and total biomasses; and rice yield as variables.

Mg steel slag ha^{-1}	Mg steel slag ha^{-1}		
	2	4	8
0	SM= 102 *F*=19.5 *P*<0.0001	SM = 327 *F*=62.4 *P*<0.0001	SM = 663 *F*=126 *P*<0.0001
2		SM = 127 *F*=24.3 *P*<0.0001	SM = 102 *F*=380 *P*<0.0001
4			SM = 102 *F*=113 *P*<0.0001

SM=Squared Mahalanobis distances.

Table S3. Main effects of the variables in the GDA analysis. Statistics (Wilks' λ and *P*) of the discriminant functional analysis among treatments with soil Ca, Si, K, Mg, N, and P availabilities; DOC and MBC concentrations; the ratios of DOC:available N, DOC:available P, and available N:available P; foliar, stem, and root N, P, and C concentrations and C:N, C:P, and N:P concentration ratios; foliar, stem, root, and total biomasses; and rice yield as variables. Significant effects of a variable in the model are highlighted in bold type (*P*<0.05).

Independent variable	Wilks' λ	*P*
MBC concentration	0.646	**<0.0001**
DOC concentration	0.830	**0.028**
Available-N concentration	0.816	**0.019**
Available-P concentration	0.832	**0.034**
Available-K concentration	0.962	0.60
Available-Si concentration	0.631	**<0.0001**
Available-Ca concentration	0.595	**<0.0001**
Available-Mg concentration	0.451	**<0.0001**
DOC:available-N ratio	0.594	**<0.0001**
DOC:available-P ratio	0.525	**<0.0001**
Available-N:available-P ratio	0.851	**0.050**
Foliar C concentration	0.656	**0.00014**
Foliar N concentration	0.826	**0.026**
Foliar P concentration	0.765	**0.0046**
Stem C concentration	0.718	**0.0011**
Stem N concentration	0.867	0.074
Stem P concentration	0.973	0.73
Root C concentration	0.682	**0.00034**
Root N concentration	0.875	0.091
Root P concentration	0.791	**0.0098**
Foliar C:N ratio	0.946	0.44
Foliar C:P ratio	0.769	**0.0052**
Foliar N:P ratio	0.737	**0.0020**
Stem C:N ratio	0.681	**0.00033**
Stem C:P ratio	0.851	**0.049**
Stem N:P ratio	0.891	0.13
Root C:N ratio	0.949	0.47
Root C:P ratio	0.828	**0.027**
Root N:P ratio	0.852	0.051
Rice yield	0.180	**<0.0001**
Shoot biomass	0.775	**0.0062**
Root biomass	0.654	**0.00013**
Total biomass	0.356	**<0.0001**

Figure legends

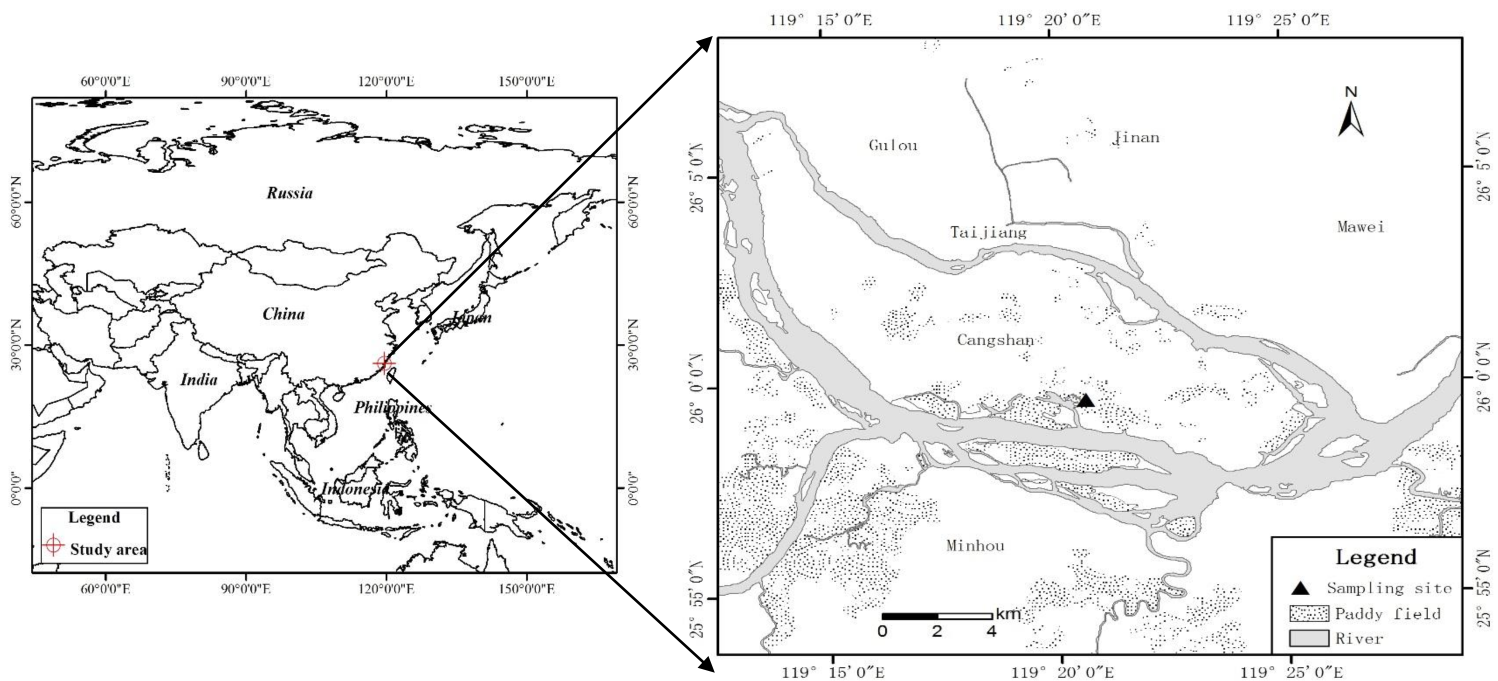
Figure S1. The location of the study area and sampling sites (▲) in Fujian Province, southeastern China.

Figure S2. Concentrations of MBC (A), DOC (B), available N (C), available P (D), available K (E), available Si (F), available Ca (G), and available Mg (H) during the growing season in the soils of the control and various treatments of steel slag application. Different letters indicate significant differences between treatments (P<0.05).

Figure S3. Ratios of soil DOC:available N (A), soil DOC:available P (B), and soil available N:available P (C) during the growing season in the soils of control and the various treatments of steel slag application. Different letters indicate significant differences between treatments (P<0.05).

Figure S4. Concentrations of foliar C (A), foliar N (B), foliar P (C), stem C (D), stem N (E), stem P (F), root C (G), root N (H), and root P (I) during the growing season in control and the various treatments of steel slag application. Different letters indicate significant differences between treatments (P<0.05).

Figure S5. Foliar C:N (A), foliar C:P (B), foliar N:P (C), stem C:N (D), stem C:P (E), stem N:P (F), root C:N (G), root C:P (H), and root N:P (I) ratios during the growing season in the plant organs from control and the various treatments of steel slag application. Different letters indicate significant differences between treatments (P<0.05).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120°0'0"E
150°0'0"E
Russia
China
India
Japan
Philippines
Indonesia
60°0'0"N
30°0'0"N
0°0'0"
Legend
Study area
119° 15' 0"E
119° 20' 0"E
119° 25' 0"E
26° 5' 0"N
26° 0' 0"N
25° 55' 0"N
N
Gulou
Jinan
Taijiang
Mawei
Cangshan
Minhou
0 2 4 km
Legend
Sampling site
Paddy field
River

Figure S1

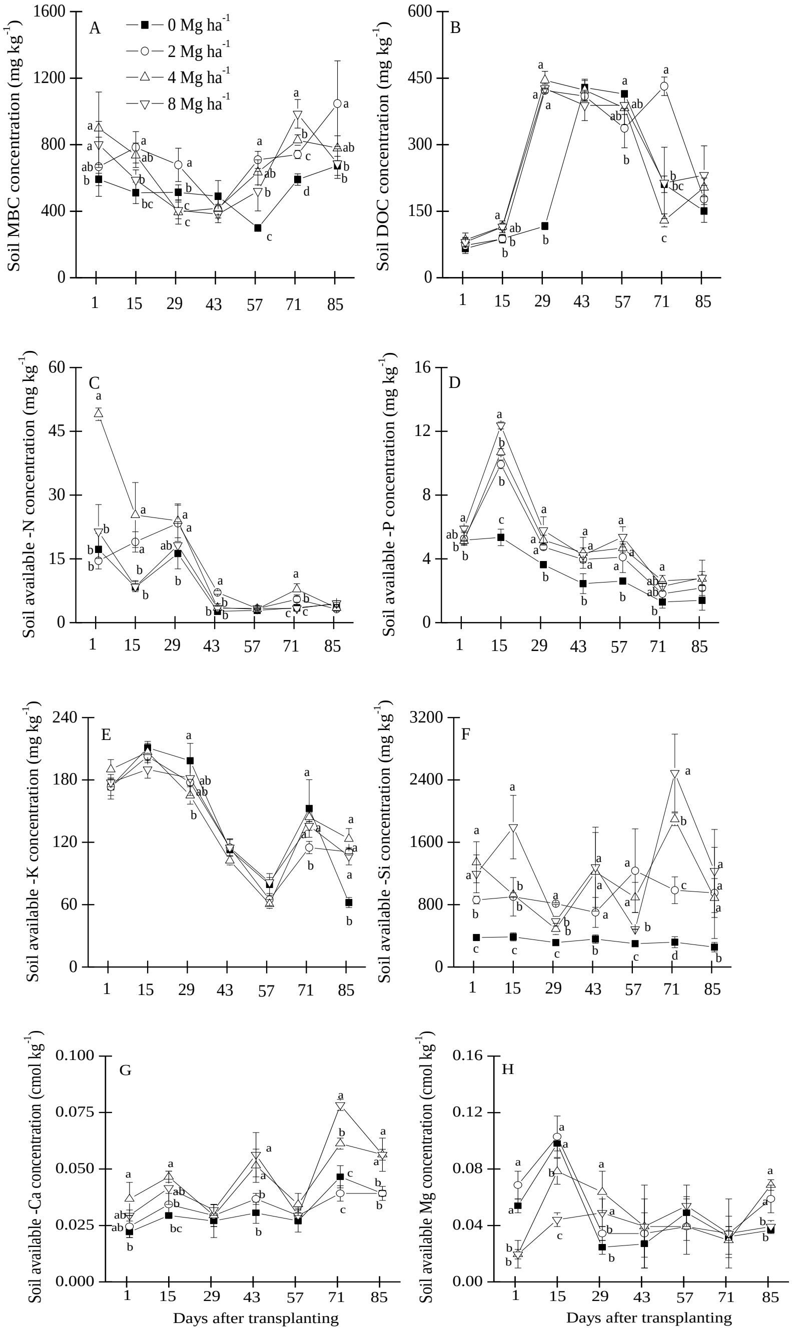
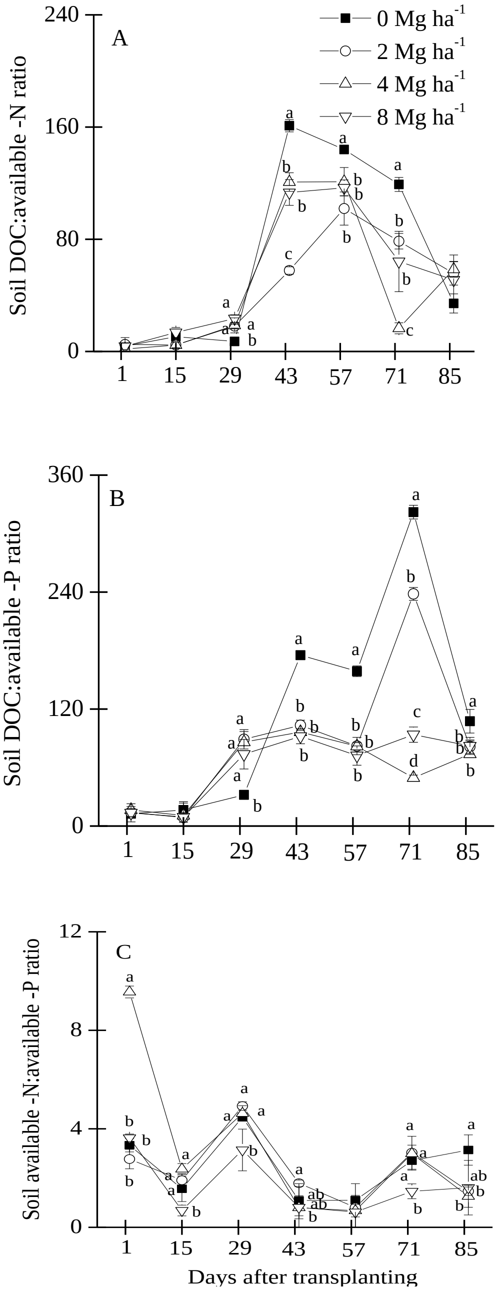
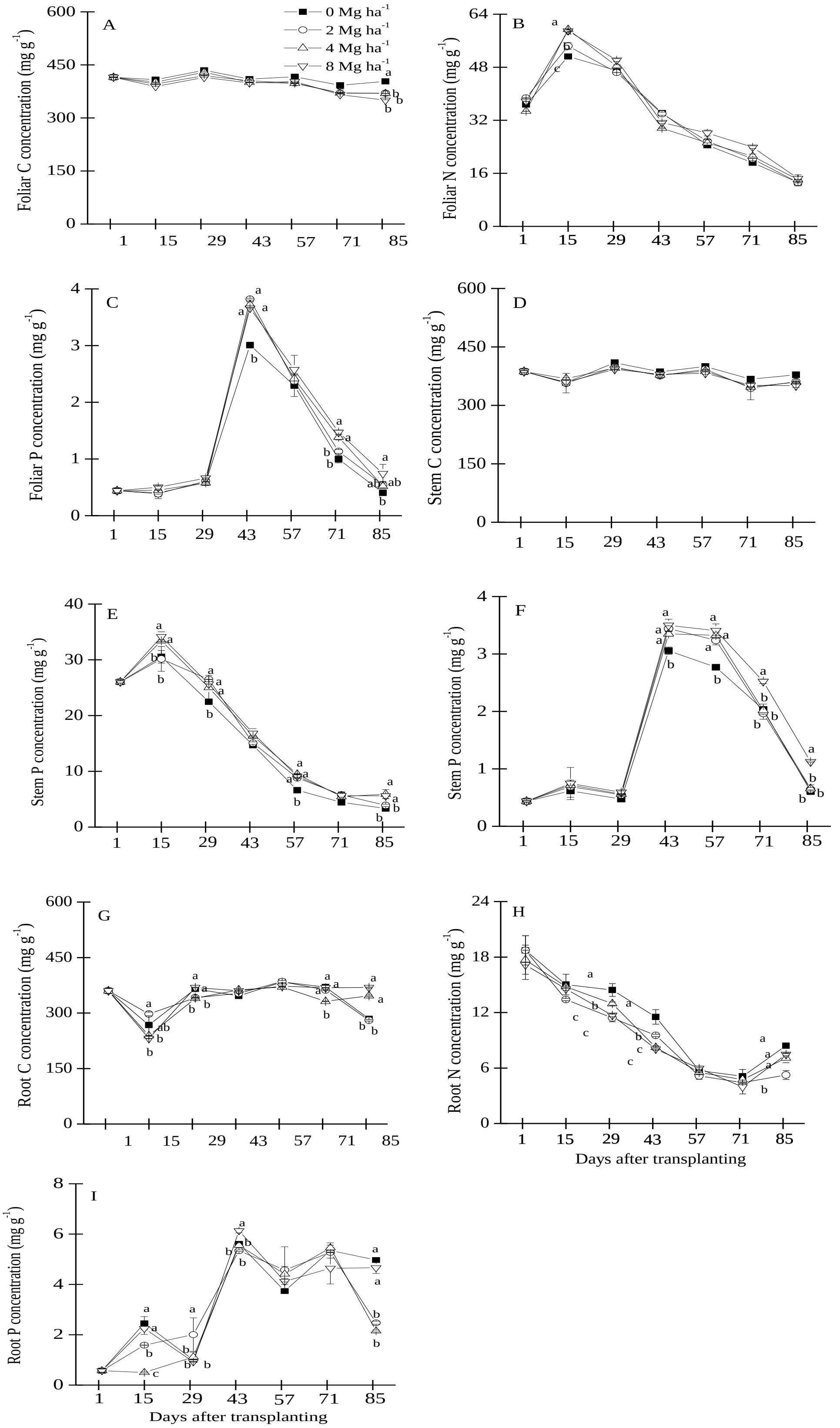

A
0 Mg ha-1
2 Mg ha-1
4 Mg ha-1
8 Mg ha-1
Soil MBC concentration (mg kg-1)
0
400
800
1200
1600
1 15 29 43 57 71 85
B
Soil DOC concentration (mg kg-1)
0
150
300
450
600
1 15 29 43 57 71 85
C
Soil available -N concentration (mg kg-1)
0
15
30
45
60
1 15 29 43 57 71 85
D
Soil available -P concentration (mg kg-1)
0
4
8
12
16
1 15 29 43 57 71 85
E
Soil available -K concentration (mg kg-1)
0
60
120
180
240
1 15 29 43 57 71 85
F
Soil available -Si concentration (mg kg-1)
0
800
1600
2400
3200
1 15 29 43 57 71 85
G
Soil available -Ca concentration (cmol kg-1)
0.000
0.025
0.050
0.075
0.100
1 15 29 43 57 71 85
Days after transplanting
H
Soil available Mg concentration (cmol kg-1)
0.00
0.04
0.08
0.12
0.16
1 15 29 43 57 71 85
Days after transplanting

Figure S2



A
B
C
0 Mg ha-1
2 Mg ha-1
4 Mg ha-1
8 Mg ha-1
Soil DOC:available -N ratio
Soil DOC:available -P ratio
Soil available -N:available -P ratio
Days after transplanting

Figure S3



A
0 Mg ha-1
2 Mg ha-1
4 Mg ha-1
8 Mg ha-1
Foliar C concentration (mg g-1)
B
Foliar N concentration (mg g-1)
C
Foliar P concentration (mg g-1)
D
Stem C concentration (mg g-1)
E
Stem P concentration (mg g-1)
F
Stem P concentration (mg g-1)
G
Root C concentration (mg g-1)
H
Root N concentration (mg g-1)
Days after transplanting
I
Root P concentration (mg g-1)
Days after transplanting

Figure S4

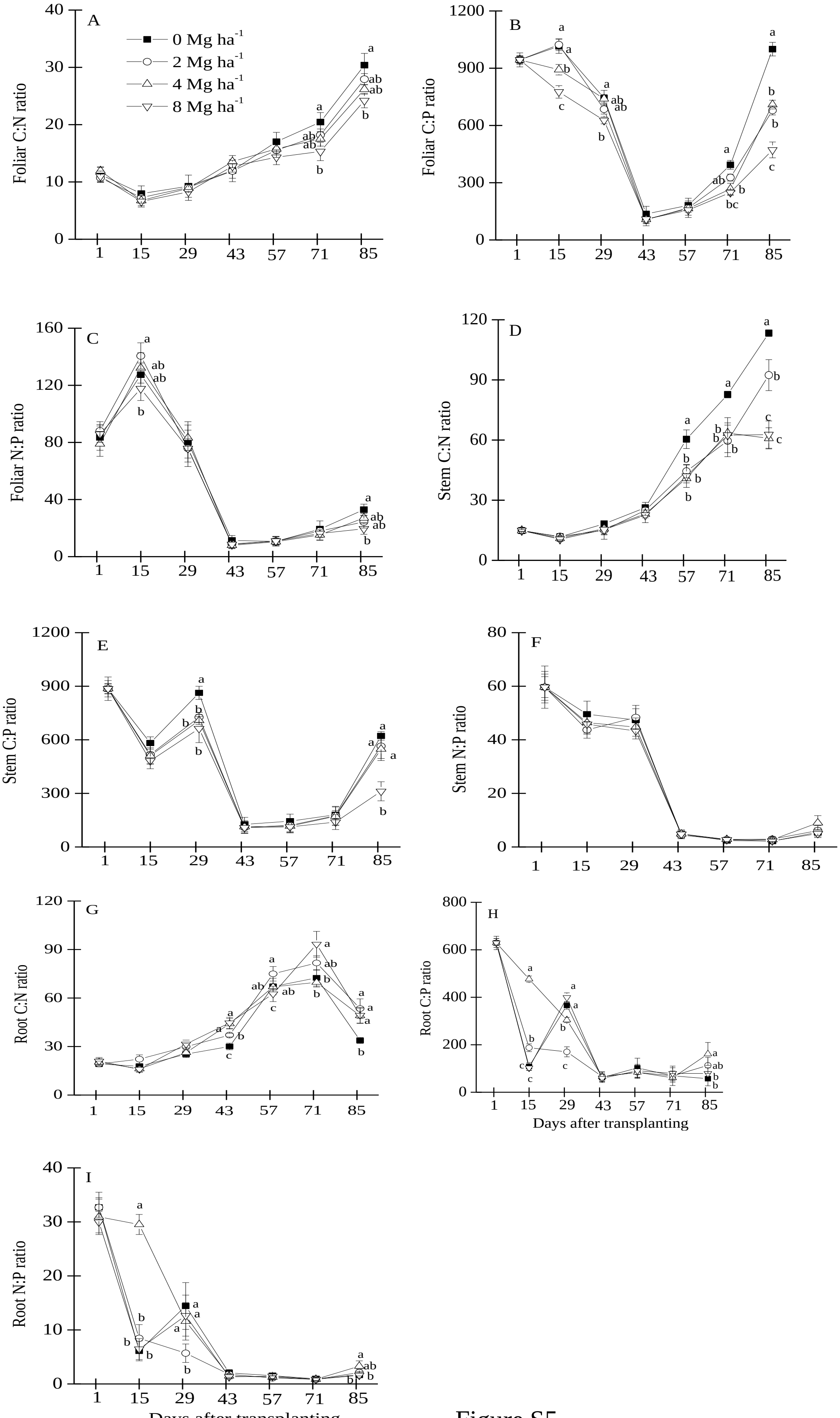

A
B
C
D
E
F
G
H
I
0 Mg ha-1
2 Mg ha-1
4 Mg ha-1
8 Mg ha-1
Foliar C:N ratio
Foliar C:P ratio
Foliar N:P ratio
Stem C:N ratio
Stem C:P ratio
Stem N:P ratio
Root C:N ratio
Root C:P ratio
Root N:P ratio
Days after transplanting

Figure S5

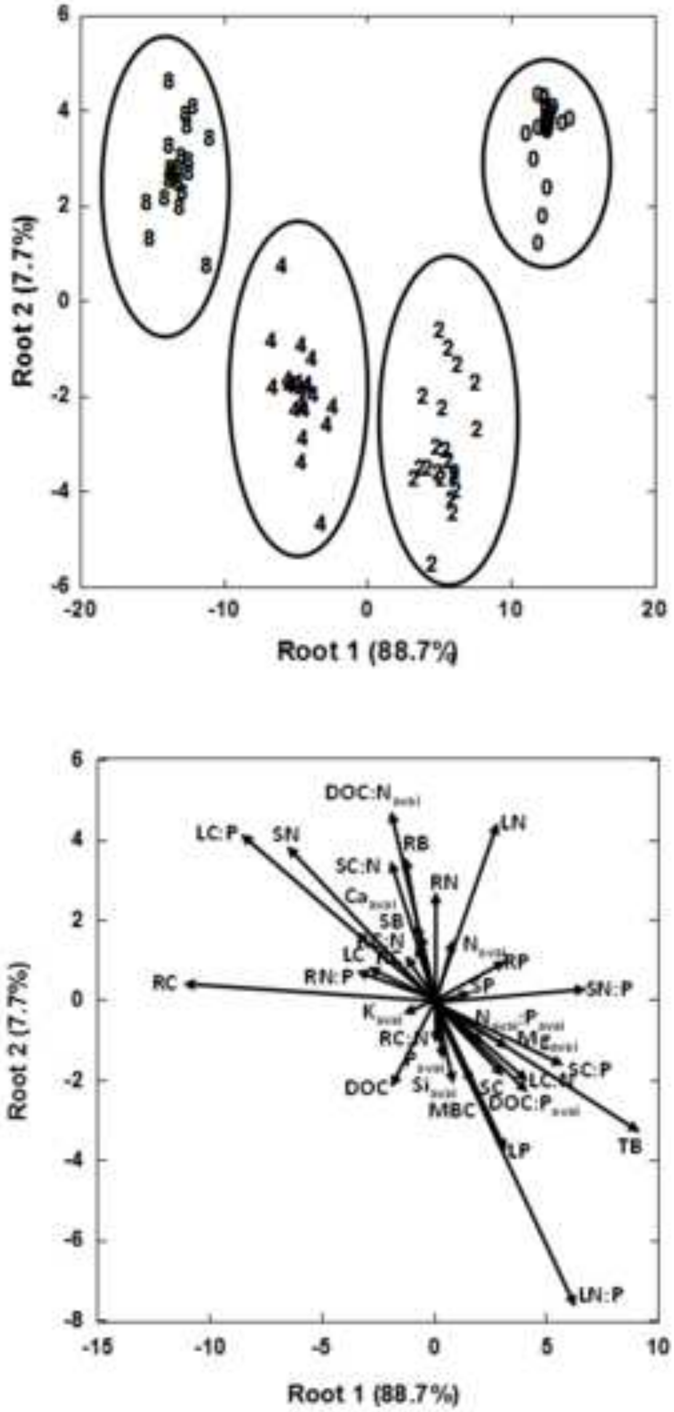
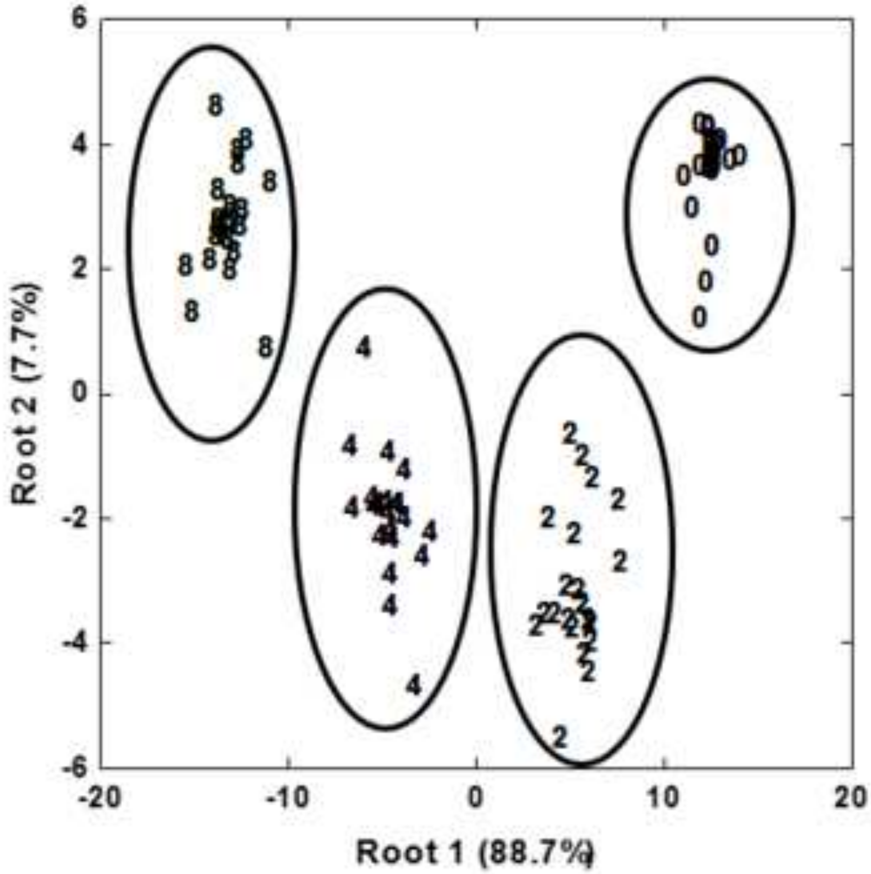
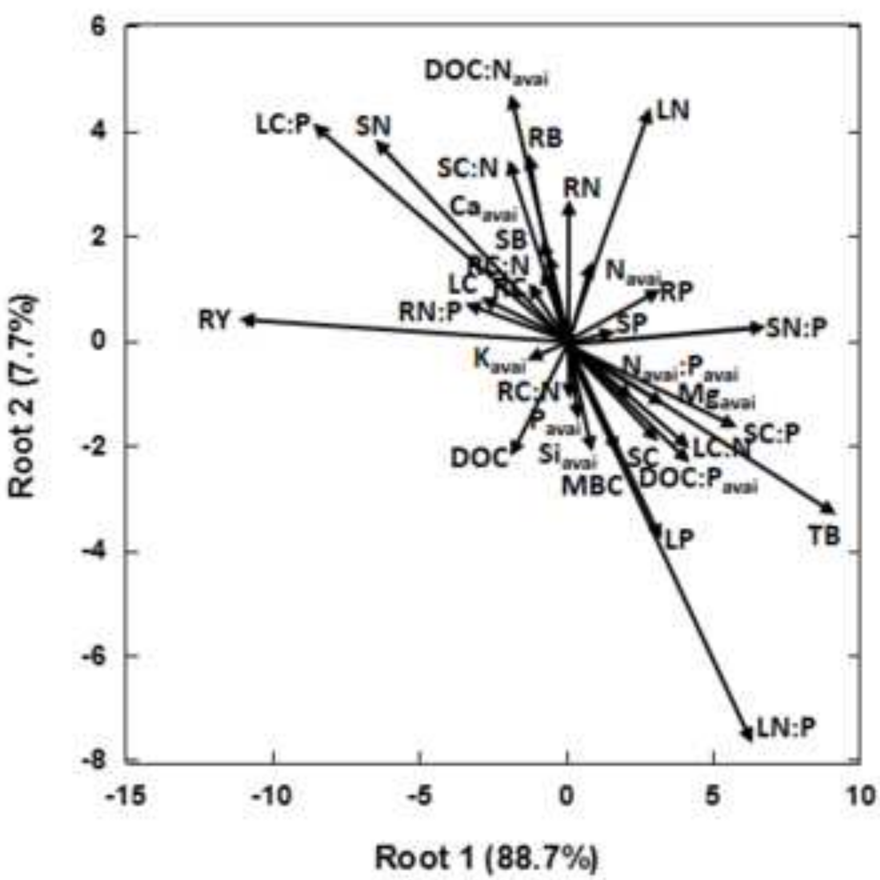

Root 2 (7.7%)
Root 1 (88.7%)
DOC:N
LC:P
SN
RB
LN
SC:N
RN
RC
RN:P
SN:P
RP
SP
DOC
MBC
SC:P
TB
LP
LN:P
Root 2 (7.7%)
Root 1 (88.7%)

Figure 6

Figure

[Click here to download Figure Figure 2.tif](#)





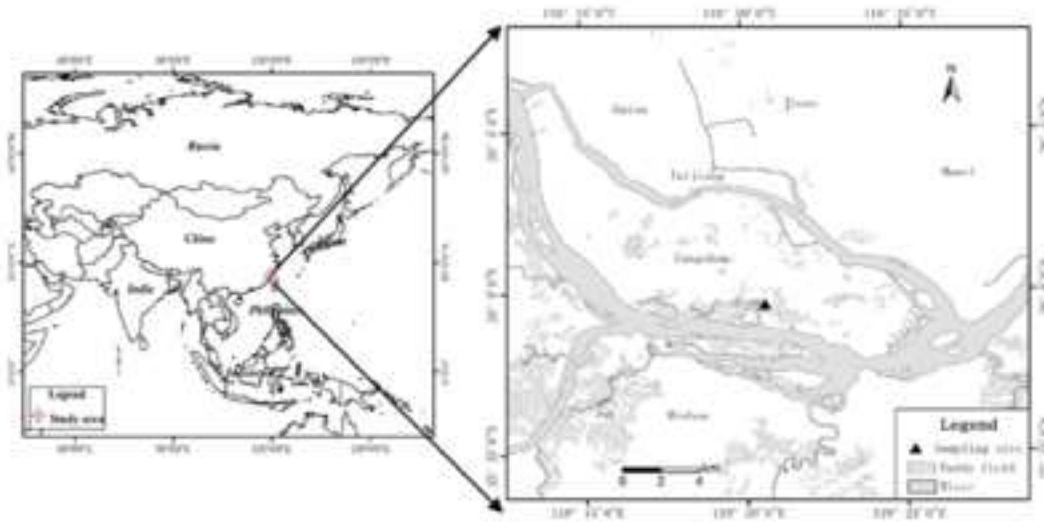


Figure 1

Figure

Click here to download Figure Figure S2.tif

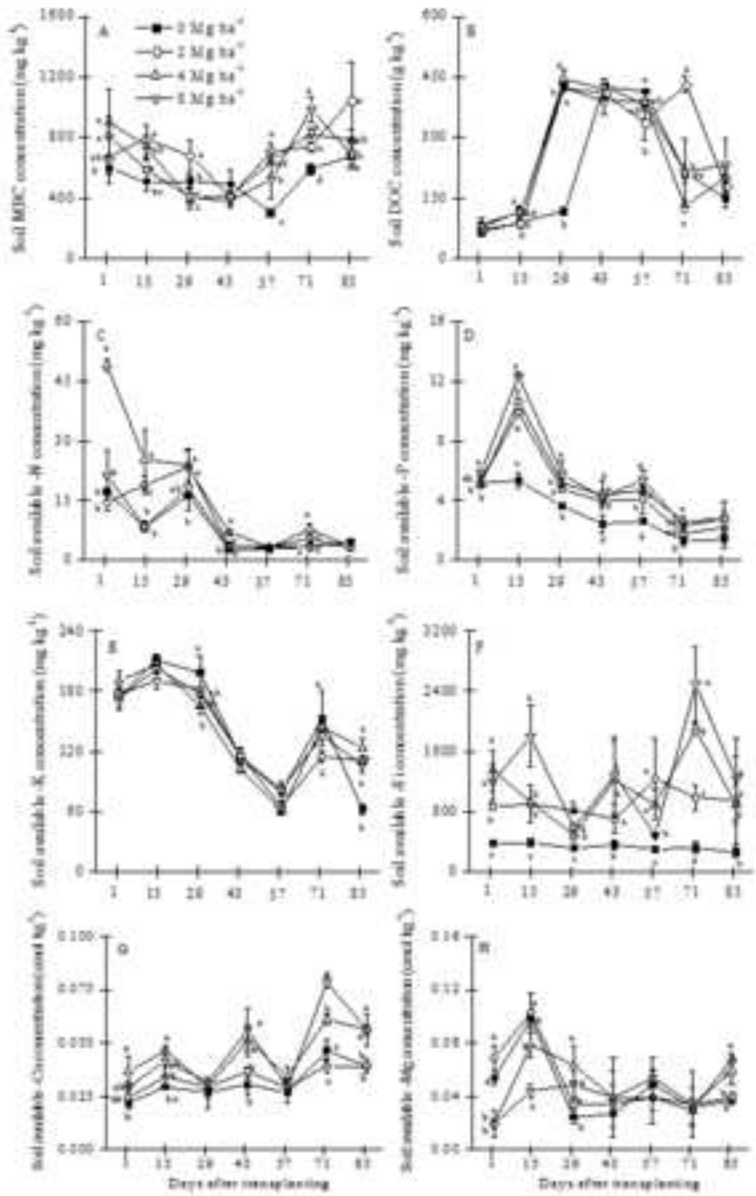
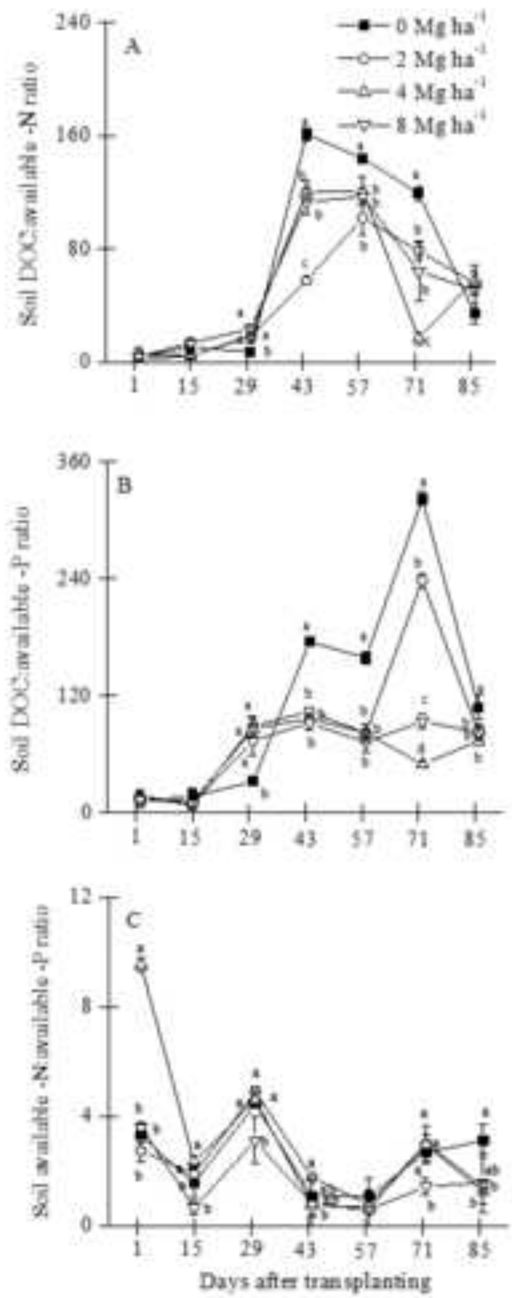


Figure 2



A
B
C
0 Mg ha⁻¹
2 Mg ha⁻¹
4 Mg ha⁻¹
8 Mg ha⁻¹
Soil DOC:available -N ratio
Soil DOC:available -P ratio
Soil available -N:available -P ratio
Days after transplanting

Figure 3

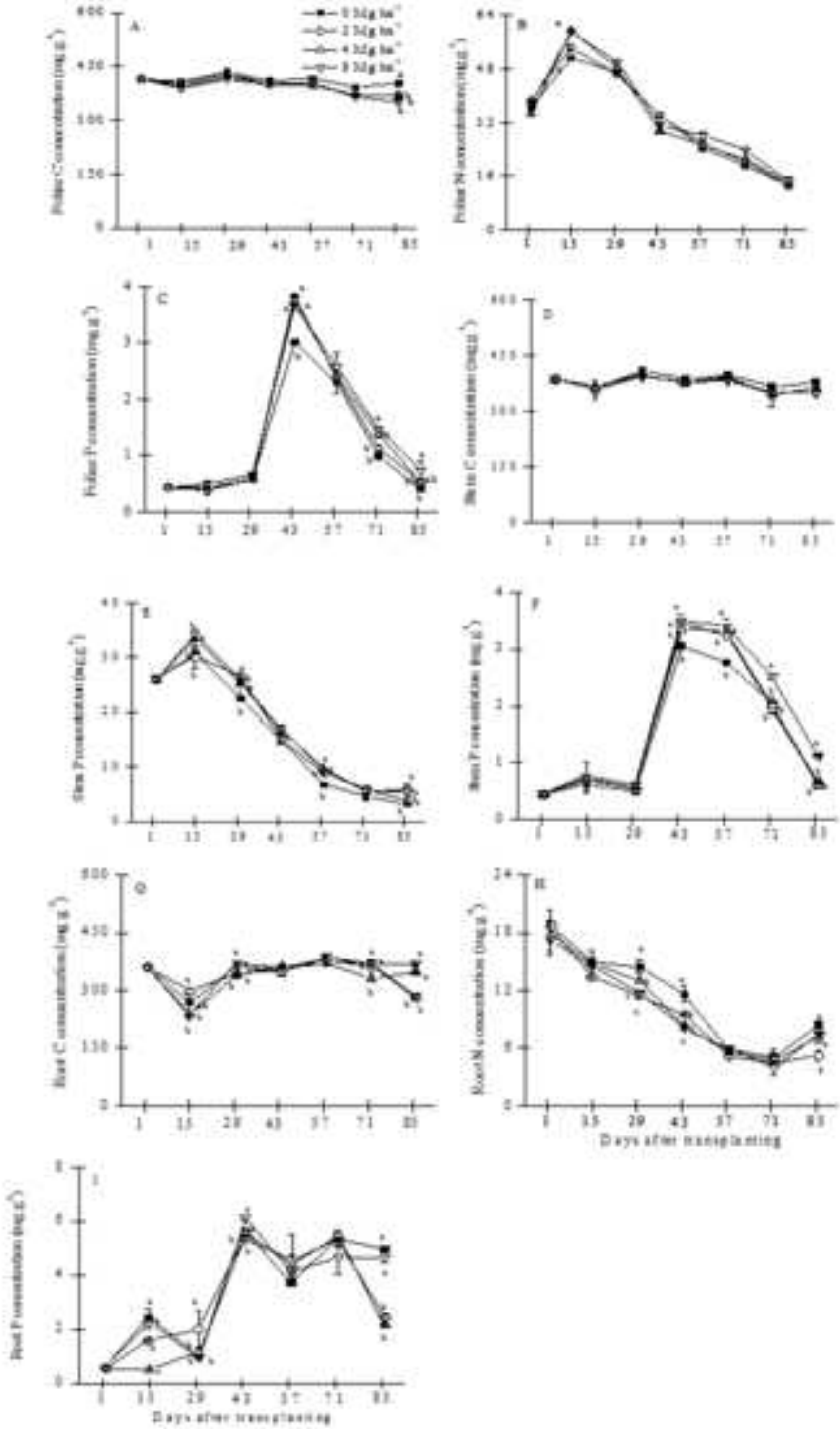


Figure 4

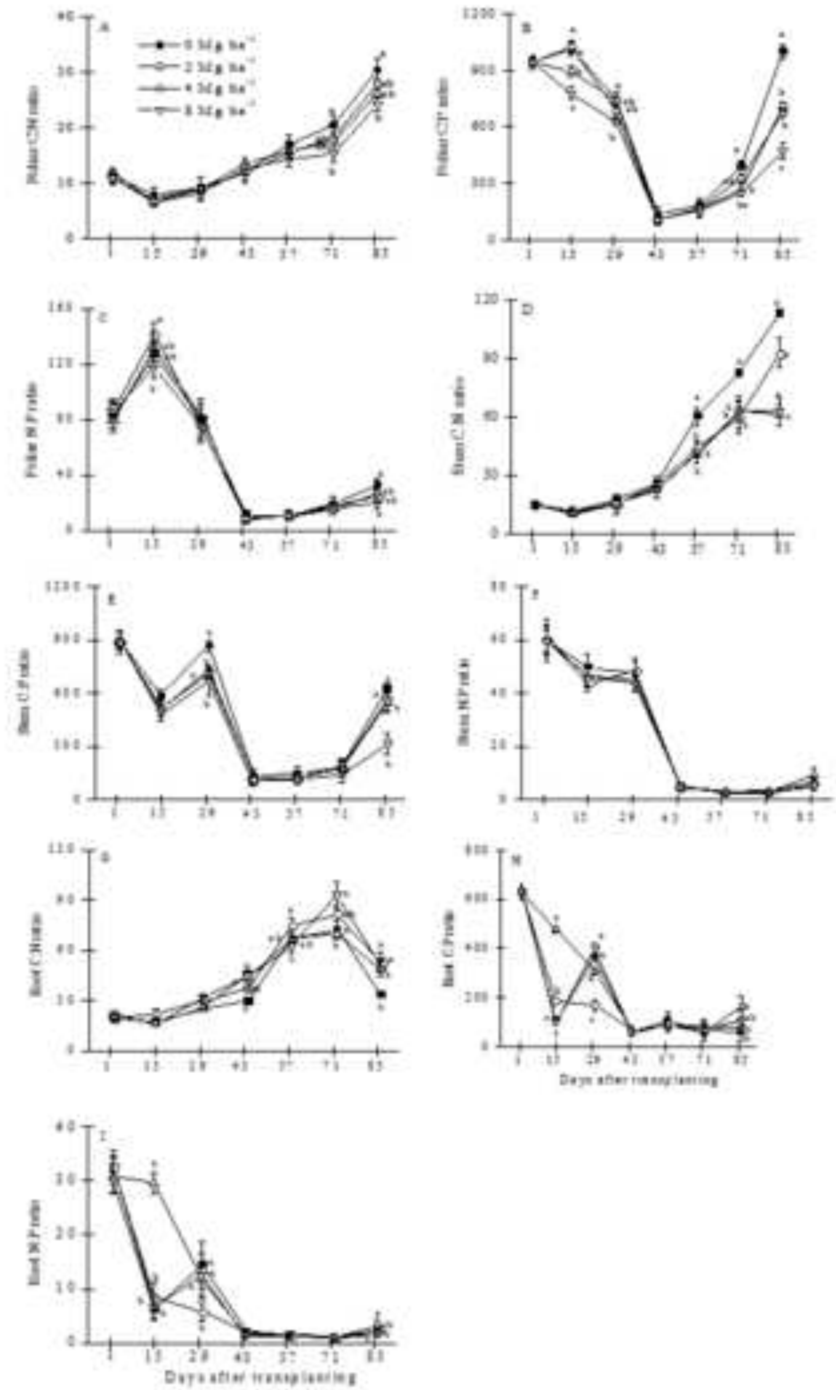


Figure 5