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Amplifying the benefits of agroecology by using the right cultivars

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Abstract. Tropical soils are particularly vulnerable to fertility losses due to their low capacity to retain organic matter and mineral nutrients. This urges the development of new agricultural practices to manage mineral nutrients and organic matter in a more sustainable way while relying less on fertilizer inputs. Two methods pertaining to ecological engineering and agroecology have been tested with some success: (1) the addition of biochar to the soil, and (2) the maintenance of higher earthworm densities. However, modern crop varieties have been selected to be adapted to agricultural practices and to the soil conditions they lead to and common cultivars might not be adapted to new practices. Using rice as a model plant, we compared the responsiveness to biochar and earthworms of five rice cultivars with contrasted selection histories. These cultivars had contrasted responsivenesses to earthworms, biochar, and the combination of both. The mean relative increase in grain biomass, among all treatments and cultivars, was 94% and 32%, respectively, with and without fertilization. Choosing the best combination of cultivar and treatment led to a more than fourfold increase in this mean benefit (a 437% and a 353% relative increase in grain biomass, respectively, with and without fertilization). Besides, the more rustic cultivar, a local landrace adapted to diverse and difficult conditions, responded the best to earthworms in terms of total biomass, while a modern common cultivar responded the best in term of grain biomass. This suggests that cultivars could be selected to amplify the benefit of biochar- and earthworm-based practices. Overall, selecting new cultivars interacting more closely with soil organisms and soil heterogeneity could increase agriculture sustainability, fostering the positive feedback loop between soils and plants that has evolved in natural ecosystems.

Key words: agroecology; biochar; crop breeding; cultivars; earthworm; International Center for Tropical Agriculture, Colombia; Oryza glaberrima; Oryza sativa; *rice; tropical agriculture; tropical soils.*

Introduction

Tropical soils are particularly vulnerable to fertility losses due to their low capacity to retain organic matter and mineral nutrients (Lal 1987, Tiessen et al. 1994). This is, in particular, due to high decomposition rates, the quality of clays (fewer negative charges than temperate clays), and the strength and irregularity of rains. This subsequently leads to problems of sustainability for tropical agricultures. These problems can be partially solved by the use of mineral fertilizers, but local populations do not always have access to such fertilizers for their subsistence crops. Conversely, tropical cash crops use mineral fertilizers, but nitrogen fertilizers are produced mostly using nonrenewable energy sources (Woods et al. 2010) and phosphate is produced in mines that are likely to get exhausted during the next century (Cordell et al. 2009). This shows that reducing the need for mineral fertilization and increasing the efficiency of mineral fertilization in the tropics is a crucial issue. This necessitates the development of new agricultural practices to manage mineral nutrients and organic matter in a more sustainable way. In this context, two methods pertaining to ecological engineering and agroecology have been tested with some success: (1) the addition of biochar to the soil (Glaser et al. 2001, Marris 2006) and

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(2) the maintenance of higher earthworm densities (Lavelle et al. 2001, 2006).

Earthworms (Blanchart et al. 1999, Brown et al. 2000, 2004) and biochar (Glaser et al. 2002, Steiner et al. 2008) have been shown to improve soil physicochemical and biological properties and agriculture sustainability. However, developing new agricultural practices and, especially, agroecological or ecological engineering practices requires the use of cultivars that are adapted to these practices (Murphy et al. 2007, Lammerts van Bueren 2008, Wolfe et al. 2008). In the case of biochar and earthworms, it is even possible that choosing or breeding cultivars that respond particularly well to the biochar- and earthworm-based practices would allow the amplification of their benefit through a synergy between soil properties and crop response. It is already known that plant species respond differently to earthworms (Wurst et al. 2005, Laossi et al. 2009). They might also respond in contrasted ways to biochar. It is, however, not known at all how conservative the response is between different genotypes within the same species.

Effects of earthworms on plant growth have been extensively studied. They have been shown to be generally positive (Brown et al. 1999, Scheu 2003). Although the use of biochar to increase the fertility of tropical soils is probably an old practice, this idea has recently been rediscovered (Marris 2006). Few studies have been published on the effect of biochar on plant growth (but see Glaser et al. 2002, Yamato et al. 2006, Steiner et al. 2007, Major et al. 2010), but the use of biochar in agriculture is deemed promising (Marris 2006, Ogawa and Okimori 2010). Besides, earthworms and biochar influence plant growth partially through the same interacting mechanisms (Glaser et al. 2002, Brown et al. 2004, Noguera et al. 2010). They both improve soil aggregation and soil microbial community. Earthworms increase mineralization, while biochar increases the retention of mineral nutrients. For these reasons, earthworms and biochar are likely to interact in the way they influence plant growth. This could synergistically increase the availability of mineral nutrients to plants as suggest by models of nutrient cycling (Barot et al. 2007*b*). Moreover, earthworms have been shown to ingest charcoal and incorporate it in the soil profile, which could increase its effect on fertility (Topoliantz and Ponge 2005, Ponge et al. 2006). All these arguments and a preliminary experiment (Noguera et al. 2010) suggest that combining earthworm- and biochar-based agroecological practices and using cultivars that respond particularly well to both earthworms and biochar could be fruitful.

A greenhouse experiment was thus implemented to compare the response of five rice cultivars to biochar, earthworms, and the combination of both. These cultivars have been chosen to represent a wide gradient of selection histories. This gradient both encompasses a very traditional local landrace and cultivars selected for their high yields in the conditions of modern agriculture. Three factors were combined in a complete factorial design: with/without earthworms, with/without biochar, and with/without fertilizer. This last treatment aimed at mimicking agricultural practices. It is particularly relevant because the availability of nutrients (hence the use of fertilizers) may change plant responsiveness to earthworms and biochar. On the one hand, fertilizers could decrease the impact of earthworms and biochar on plant growth if they mainly act via an increase in mineral nutrient availability (Blouin et al. 2006, Laossi et al. 2010*a*). On the other hand, biochar and fertilization could interact in a synergetic way to build up fertility (Lehmann et al. 2003), and thus fertilization could increase plant responsiveness to biochar.

Overall we tested the following hypotheses: (1) Cultivars differ in their response to biochar, earthworms, and the combination of both. (2) Choosing the right combination of treatment (biochar, earthworm, and fertilization) and cultivar allows a notable multiplication of the production of rice grains as compared to random combinations. (3) More rustic cultivars respond better to earthworms and biochar.

Materials and Methods

Experimental design

The experiment was conducted at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia. Plants were submitted to the four possible combinations of two factors: with and without earthworms (E, NE, respectively) and with and without biochar (B, NB, respectively). All treatment combinations were implemented for the African rice (*Oryza glaberrima*, IRGC 103544) and four Asian rice cultivars (*Oryza sativa*): *Line 30* (accession CIRAD 409), *Azucena* (accession IR64), *Nipponbare* (accession IRGC 12731) and *Donde lo tiren*. *O. glaberrima* has been selected and cultivated in parts of West Africa for >3500 years. It has developed adaptive mechanisms for resisting major biotic and abiotic stresses. *Line* 30 has been developed conventionally by hybridization. It is well adapted to Colombian savannas and has been widely used since the 1990s. *Azucena* is a semidwarf type derived from extensive intercrossing of improved lines, and has good yield potential and resistance to numerous biotic stresses. *Nipponbare* is the outcome of successive selection on Japonica cultivars and is characterized by a good sucker growth. *Donde lo tiren* is a Colombian local rustic landrace. These cultivars have been chosen to represent a wide gradient of selection history, from very local landraces to modern cultivars.

For each combination of treatments (E × B × rice cultivar), two fertilization treatments were implemented: without or with mineral fertilization. Five replicates were implemented for each treatment, resulting in 200 microcosms. Rice was grown in a greenhouse for four months (see Plate 1). Containers (microcosms) consisted of polyvinyl chloride (PVC) pots. They were filled with

1500 g of sieved (2 mm) dry soil. Microcosms were arranged in a completely randomized design. The soil was collected from a long-term field experiment that aimed at comparing coffee production with and without the addition of biochar. It was established in 2004 in the Andean hillsides of the Cauca Department (Pescador; 2°48′ N, 76°33′ W, Colombia). Soil was collected in the control treatments of this experiment for our microcosm control treatments, and from their biochar treatments for our microcosm B treatments. The soil of this treatment contained 25 g of biochar per dry kg of soil. Biochar was produced from logs of *Eucalyptus deglupta*: Temperature was maintained at 350°C and the oxygen level at 15%, and the charring time was 1 h (see details in Rondon et al. 2007). This led to the following characteristics for the biochar: total C, 82.4%; total N, 0.57%; P, 580 mg/g; CEC, 46.9 mmol/kg.

The "Pescador" soil is a volcanic-ash soil, an inceptisol (Soil Survey Staff 1999). The soil is moderately acid (pH [H_2O] = 5.1). It is relatively rich in organic matter MO (11.5%), and mineral nitrogen (12.9 mg NH_4^+-N/kg, 27 mg NO_3^--N/kg). The CEC is relatively high (6.0 cmol/kg). Texture is dominated by clay (24.06% sand, 27.56% silt, and 48.38% clay). The soil bulk density was 0.8 g/cm^3. The fertility treatment consists in an application of a N (urea), P ($Ca(H_2PO_4)_2$), K (KCL) and S ($(NH_4)_2SO_4$) fertilizer (77.6 mg N, 37.5 mg P, 26.2 mg K and 18.75 g S per microcosm) which corresponds to 41.4 kg N, 20 kg P, 40 kg K and 10 kg S per hectare (considering a 10 cm deep soil layer). The fertilizer was placed at the soil surface at the beginning of the experiment.

We used five adults for each microcosm (initial fresh mass 5 ± 0.5 g) of *Pontoscolex corethrurus* (Glossoscolecidae) that were gathered in the field in Pescador. This earthworm is a peregrine species that has spread in all the tropics. Three days after introducing earthworms, five rice seeds were sown. Two weeks later, a single plant per microcosm was kept (the other seedlings were removed). Microcosms were regularly weeded during the experiment and were maintained at 80% of the soil field capacity (checked through regular weighing of the pots). Conditions in the greenhouse were as follows: relative humidity 65–95%, temperature 27–29°C, light intensity 600 mmol·m^{-2}· s^{-1}, and a 12-h photoperiod.

Measurements

After 16 weeks, plants were harvested and separated into grains, leaves, and stems. Roots were collected by wet sieving. All vegetal biomasses (grains, leaves, and stems) were dried in an oven at 40°C for 2 d. Grains were counted. Subsamples of each plant material were analyzed for total carbon and total nitrogen using a Flash EA 1112 elemental analyzer (ThermoFinnigan, Milan, Italy). The total biomass was calculated as the sum of all dried biomasses (grains, leaves, stems, and roots). The shoot : root ratio was calculated as the sum of above ground dried biomasses (grains, leaves, and stems) divided by the root dried biomass.

Statistical analyses

We first analyzed our results using full ANOVA models testing for the earthworm, biochar, fertilization, and cultivar effects as well as for all interactions between these factors (Table 1). Numerous significant interactions between earthworm and biochar treatments, and rice cultivars and fertilization were found. This shows that earthworm and biochar effects on rice growth do change with rice cultivars and fertilization, which in turn, justifies the use of effect sizes (see next paragraph) to describe and analyze in a more pedagogic way the responsiveness of each cultivar to the different treatments. Histograms of the raw means corresponding to these ANOVAs are displayed for all treatments in the Appendix B.

We then based the analysis of our results on the effect sizes of the different treatments (Nakagawa and Cuthill 2007). This approach permits focusing on the magnitude of the effects of the different treatments. It compares the magnitude of these effects (with the control treatment as a reference) in different cases: here, the rice cultivars and the fertilization treatment. This also helped display results in a more synthetic way. We used a standardized statistic, Cohen's *d* (Cohen 1988), which is the difference between the treatment and control mean values divided by the pooled standard deviation. The *d* value was displayed together with its standard deviation, which was computed using the sample size and the estimated *d* value (Hedges 1981). Effect sizes were calculated separately for each rice cultivar and for each fertilization treatment (with and without). In these analyses, the control was thus without fertilization for the fertilized plants, and with fertilization for the non-fertilized plants.

Results

The full statistical model (Table 1, see also Appendix B: Fig. B1) for the total biomass, the shoot : root ratio, and the grain biomass showed that there were significant interactions (either in double or triple interactions) between rice cultivar and biochar, and between rice cultivar and earthworms. This result proves that the effects of the treatments do change with cultivars. Hereafter, we base the description of our results on the calculation of the effect sizes (ES). In the text, effect sizes are given together with the corresponding relative percentage changes.

In all cases, i.e., all cultivars and with or without fertilization, treatments increased the total rice biomass (positive ES; Fig. 1). This biomass tended to respond more to earthworms and biochar without fertilization than with fertilization. With fertilization, the strongest effect size was obtained with both biochar and earthworms in *Nipponbare* (+128%, ES ≈ 5) and *O. glaberrima* (+44%, ES ≈ 5). Without fertilization, the

TABLE 1. ANOVA table displaying *F* values for the full statistical model with the four factors and all interactions.

Model	df	*F*		
		Total biomass	Shoot : root ratio	Total grain biomass
V	4	89.1***	60.77***	191.1***
B	1	114.9***	0.1	46.8***
E	1	177.9***	41.2***	16.9***
F	1	184.0***	0.0	53.3***
V × B	4	0.9	0.8	7.2***
V × E	4	5.6***	5.9***	6.0***
V × F	4	7.0***	1.3	11.1***
B × F	1	0.1	0.3	1.6
B × E	1	0.2	1.0	2.9
E × F	1	29.2***	4.0*	3.2
V × B × E	4	2.9*	0.5	1.1
B × E × F	1	0.0	0.2	0.1
V × B × F	4	1.7	1.1	1.0
V × E × F	4	11.7***	3.1*	1.4
V × B × E × F	4	2.0	0.7	0.3
R^2		0.86	0.68	0.86

Notes: The total df is 199 for all variables. The denominator df of the *F* ratio is is the residual df and can easily be calculated: total df – model df. Abbreviations are: V, rice cultivar; B, biochar; E, earthworms; and F, fertilization.
* $P < 0.05$; *** $P < 0.001$.

strongest effect was obtained with earthworms in *O. sativa cv. Azucena* (+233%, ES ≈ 7) and *Donde lo tiren* (+119%, ES ≈ 7) and with both biochar and earthworms in *Donde lo tiren* (+171%, ES ≈ 9).

Effects of earthworms and biochar on the shoot : root ratio were different with and without fertilization. Earthworms increased the shoot : root ratio, but in a much stronger way without fertilization. Biochar tended to decrease the shoot : root ratio, but only without fertilization. The effect size depended on the rice cultivar and the on the interaction between biochar and earthworms. With fertilization, the highest effect size was obtained with earthworms in *O. glaberrima* (+160%, ES ≈ 4). Without fertilization, the highest effect was observed with the combination of earthworms and biochar in *O. glaberrima* (+51%, ES ≈ 3), and the lowest effect size was obtained with biochar in *Donde lo tiren* (−25%, ES ≈ −1).

Overall, the pattern of responsiveness of the total grain biomass was the same in the two fertilization treatments: *Nipponbare* had the highest responsiveness to biochar (+206% with fertilization, +141% without fertilization, ES ≈ 3) and earthworms (+76% with fertilization, ES ≈ 1.5; and +93% without fertilization, ES ≈ 2) and *Line 30* had the highest responsiveness to the combination of biochar and earthworms (+74% with fertilization, ES ≈ 5; and +141% without fertilization, ES ≈ 4). In some cultivars, biochar or earthworms had a negative impact on the production of grain biomass (negative ES). This pattern is different from the one observed for the total biomass, which, together with results on the shoot : root ratio, the leaf C:N, and the number of grains (for these last two variables see Appendix C: Fig. C1) shows that earthworms and biochar influence rice resource allocation in terms of biomass and nitrogen, and that these effects on resource allocation differ between rice cultivars.

DISCUSSION

The complete mechanistic interpretation of the observed effects on rice growth and resource allocation goes beyond the objective of the present article. Another experiment (Noguera et al. 2010), which focused on one rice cultivar (*Line 30*) but compared different soil treatments, suggested that earthworms and biochar mainly act through their positive effect on the availability of mineral nutrients. Indeed, in this precedent experiment and in the present experiment (results not displayed; in this case, the pattern also depended on the cultivar ability to uptake mineral nitrogen), both earthworms and biochar increased the availability of nitrate and ammonium. However, the earthworm effect could not be fully understood without assuming that they lead to the release of plant growth factors, such as auxins, in the soil through the stimulation of particular bacterial groups (Muscolo et al. 1999, Blouin et al. 2006). Here, the key point is that the pattern of response of rice cultivars is complex: The responsiveness to biochar, earthworms, and the combination of the two generally depended on the cultivar, and was often different with and without fertilization. Beyond this complexity, it is noteworthy that: (1) The same maximum total biomass could be obtained with (in *O. glaberrima*) or without fertilization (in *Donde lo tiren*), but with both earthworms and biochar. (2) The highest absolute grain biomasses were obtained with the combination of fertilization, earthworms and biochar (in *O. glaberrima* and *Line 30*). (3) With fertilization, choosing the best combination of cultivar and treatment allowed a 353% relative increase in the total grain biomass (biochar and earthworms in *Nipponbare*). (4)

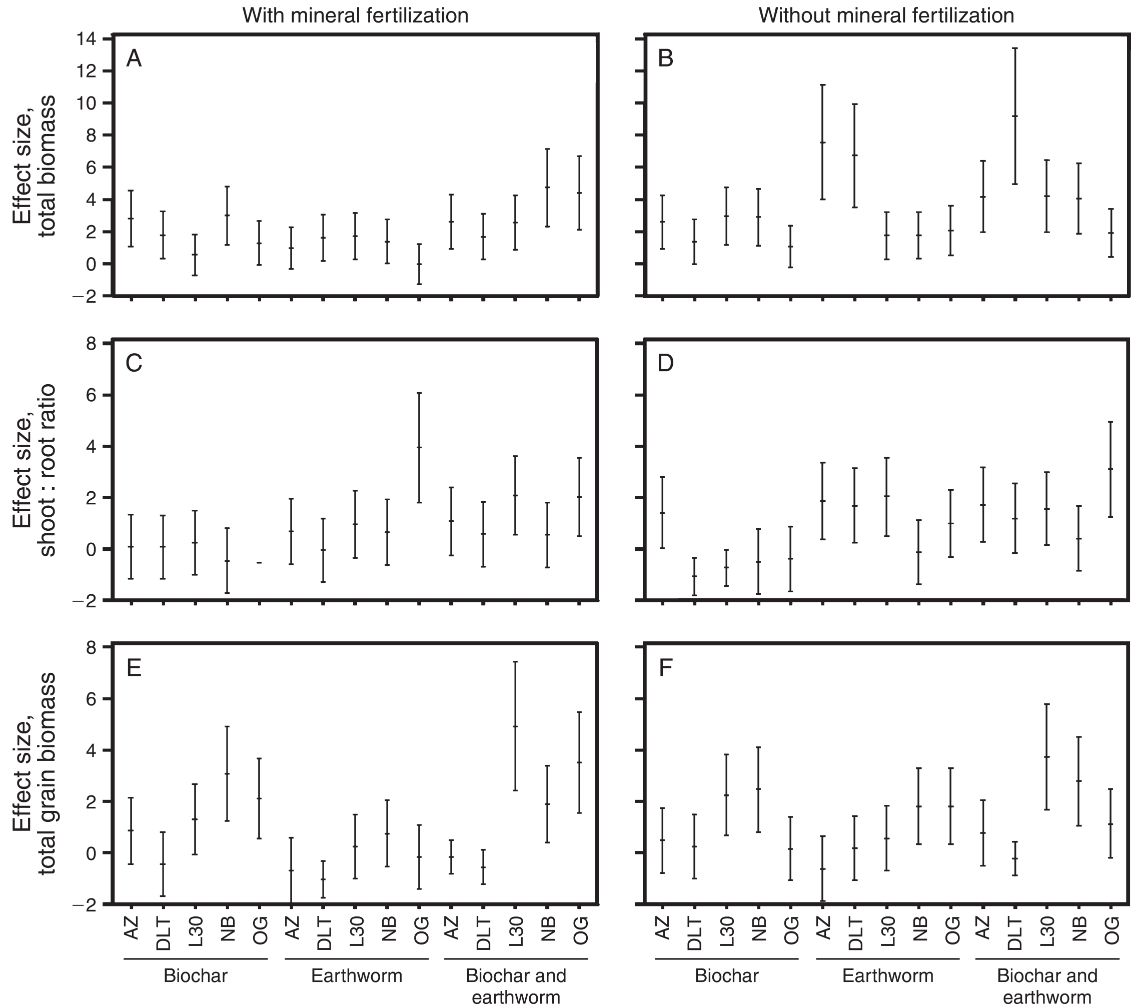

FIG. 1. Effect sizes (see *Statistical analyses* for details) of the different treatments (biochar, earthworm, and combination of biochar and earthworm) on (A, B) the total plant biomass, (C, D) the shoot : root ratio, and (E, F) the total biomass of grains. In each case, effect sizes are displayed for the mineral fertilization treatment (left-hand column of panels) and the non-fertilized treatment (right-hand column of panels), and for each Asian rice cultivar (AZ, *Azucena*; DLT, *Donde lo tiren*; L30, *Line 30*; NB, *Nipponbare*) and the African rice (OG, *Oryza glaberrima*).

Without fertilization, choosing the best combination of cultivar and treatment allowed a 437% relative increase in the total grain biomass (biochar earthworms in *Azucena*). (5) Overall, choosing the best combination of treatments and cultivar multiplied by more than four the mean relative increase in grain production calculated over all the combinations of treatments and cultivars (with and without fertilization).

The responsiveness of the cultivars to the treatments highly depended on the examined variable. As it is, so far, not possible to predict the responsiveness of different plant species to earthworms (Laossi et al. 2009) and biochar, it seems impossible to predict the responsiveness of different rice cultivars. This is not a surprise, due to the complexity and abundance of underlying mechanisms and also, in the case of biochar, because few studies have analyzed in details its effects on plant growth. Indeed, though most studies that tested the effect of biochar on plant growth found a positive effect (Glaser et al. 2002, Yamato et al. 2006, Steiner et al. 2007, Major et al. 2010, Ogawa and Okimori 2010), none of these studies tried to correlate the importance of this effect with plant traits. In fact, small differences between genotypes in their root-foraging strategies, root exudation, general resource allocation strategy, and physiological regulations are probably sufficient to trigger important changes in their responsiveness to earthworms and biochar. Here, we already show that the different cultivars respond differently to biochar and earthworms in terms of shoot : root ratio (Table 1 and Fig. 1) and C:N (see Appendix A: Table A1 and Appendix C: Fig. C1). Elements of explanation could probably be found examining, for example, the precise root architecture of the different rice cultivars. Collect-

PLATE 1. General view of the experiment, 45 days after germination. Photo credit: D. Noguera.

ing new data on the physiological and molecular response of rice cultivars to earthworms and biochar would probably be also useful to predict their responsiveness in terms of yield (see, e.g., Jana et al. 2010).

Crop breeding often focuses on improving cultivar resistance to pathogens and parasites, their capacity to resist to drought, and their capability to benefit from mineral fertilization (Hoisington et al. 1999, Witcombe et al. 2008). Our results are thus original, but they may appear useless to agronomists and plant breeders. Why focus on cultivar responsiveness to biochar and earthworms? For biochar, the answer is very clear: If we want to develop new agricultural practices, we also need to develop the suitable cultivars that highly benefit from these practices. For earthworms, the answer is threefold. First, modern agricultural practices such as tillage and the subsequent negative effect of these practices on soil organic matter content have often a negative impact on earthworm populations (Fragoso et al. 1997). Conversely, alternative agricultural practices aimed at increasing sustainability often have a positive impact on soil fauna and earthworms (Mäder et al. 2002). Second, practices are proposed to directly increase earthworm biomass to restore soil fertility (Lavelle et al. 1989). All these practices would particularly benefit from the use of responsive cultivars. Third, soil organisms are more and more thought to play an essential role in the sustainability of soil capacity to support vegetal production because of their involvement in positive feedback loops between soil properties and plant production (Lavelle et al. 1989, Wardle et al. 2004). It might thus be possible to foster further these positive effects on sustainability through the use of responsive cultivars. Such an approach is currently in development for mycorrhizae (Sawers et al. 2008). They are an obvious place to start because of their symbiotic association with plants. However, most soil organisms interact directly or indirectly with plants (Wardle et al. 2004). Therefore, this approach might be extended to many other organisms. This would be a way to green the green revolution and to favor the ecological intensification of crop production (Cassman 1999).

The next key issue is to find genotypes having a high responsiveness to the desired organisms or to biochar. It is probable that intensively selected modern cultivars have lost some of the traits that allow natural species to interact efficiently with soil organisms and fine-scale sources of soil heterogeneity (such as biochar). Indeed, agricultural practices tend to be unfavorable to many groups of soil organisms, and modern cultivars have been developed to grow efficiently when provided with an abundant mineral fertilization that tends to homogenize soil fertility (at all scales). Consequently, subtle mechanisms allowing plants to access mineral nutrients through complex rhizospheric interactions (involving interactions with soil organisms, see, e.g., Bonkowski 2004) might have been selected against or might have been stochastically lost during the selection of high-yielding cultivars. A solution might thus be to find lost traits in local landraces or nondomesticated ancestors (McCouch 2004). Our results support these views in the sense that *Donde lo tiren* is the more rustic rice cultivar of our experiment and responds well to earthworms in terms of total biomass without mineral fertilization (+119%, ES $\approx$ 6; Fig. 1). However, due to interactions with the resource allocation strategy, *Donde lo tiren* does not respond well to earthworms in terms of grain biomass. On the contrary, a modern cultivar such as *Nipponbare* very significantly increases its grain biomass in presence of earthworms, even without mineral fertilization (+93%, ES $\approx$ 2). This is probably due to the fact that modern cultivars have been selected to allocate more resources to grains.

Finally, it is known that plant species have diverse responses to earthworms (Wurst et al. 2005, Laossi et al. 2009), and we have shown here that different plant genotypes, within the same species, also differ in their responsiveness to earthworms. This shows that earthworms could represent a selection pressure for plants. To do so, earthworms not only need to influence the biomass production of different genotypes in contrasting ways, but they also need to differently influence their fitness. Our results on the number of grains produced (see Appendix A: Table A1 and Appendix C: Fig. C1) support this view. Other studies have shown that earthworms have contrasting effects on the demography, and thus, on the fitness of plant species via various mechanisms (Decaëns et al. 2003, Laossi et al. 2009, 2010*b*) that could also lead to fitness differences between genotypes. Of course, genetic differences between rice cultivars have arisen through artificial selection and might not quantitatively and qualitatively reflect differences arising between the genotypes of conspecific plant individuals within a natural population. The effect of such differences on the responsiveness to earthworms should thus be investigated in the future. Nevertheless, the Darwinian evolution of the interactions between plants and belowground organisms has mostly been studied from the point of view of symbiotic relations (e.g., Denison 2000). Our results suggest that it is relevant to extend this approach to non-symbiotic soil organisms, such as earthworms, that influence plants through their ecosystem engineering activities, modifications of the community of microorganisms, effects on soil food web, and the spatial and temporal patterns of mineral nutrient release. This would also be a way to extend evolutionary thinking in soil ecology (Barot et al. 2007*a*).

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APPENDIX A

A table showing ANOVAs for leaf C:N and the number of grains (*Ecological Archives* A021-106-A1).

APPENDIX B

Means and standard deviations for the five documented variables (*Ecological Archives* A021-106-A2).

APPENDIX C

Effect sizes for leaf C:N and the number of grains (*Ecological Archives* A021-106-A3).

Appendix A. Table A1. ANOVA table for the full statistical model with the four factors and all interactions. F values are displayed. The total degree of freedom is 199 for all variables but the leaf C/N. In this last case, it is 116 (three repetitions were analysed instead of 5 for each treatment combination). *, P<0.05 ; **, P<0.01 ; ***, P<0.001. V, rice cultivar; B, biochar; E, earthworms; F, fertilization.

	df	Leaf C/N	Grain number
V	4	**74.7*** **	**117.0*** **
B	1	**20.9*** **	**36.0*** **
E	1	**8.34** **	**33.0*** **
F	1	**7.0** **	**56.0*** **
VxB	4	**22.7*** **	**5.1*** **
VxE	4	**6.2*** **	**10.7*** **
VxF	4	**3.3* **	**11.0*** **
BxF	1	0.13	0.8
BxE	1	0.62	0.8
ExF	1	0.0	2.9
VxBxE	4	1.4	0.8
BxExF	1	1.95	0.8
VxBxF	4	2.2	**2.75* **
VxExF	4	0.8	2.2
VxBxExF	4	1.4	1.8
R^2		0.86	0.82

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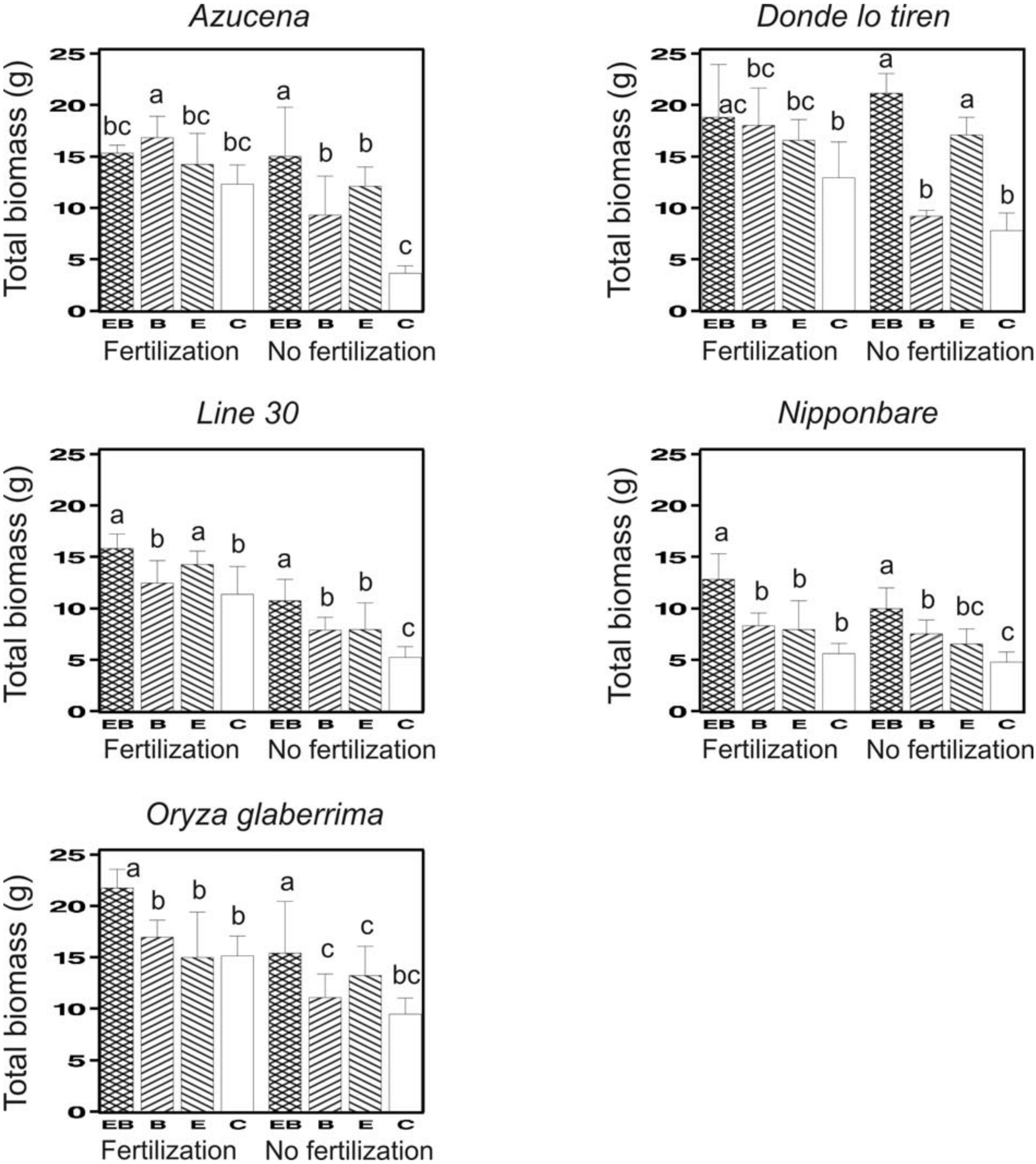
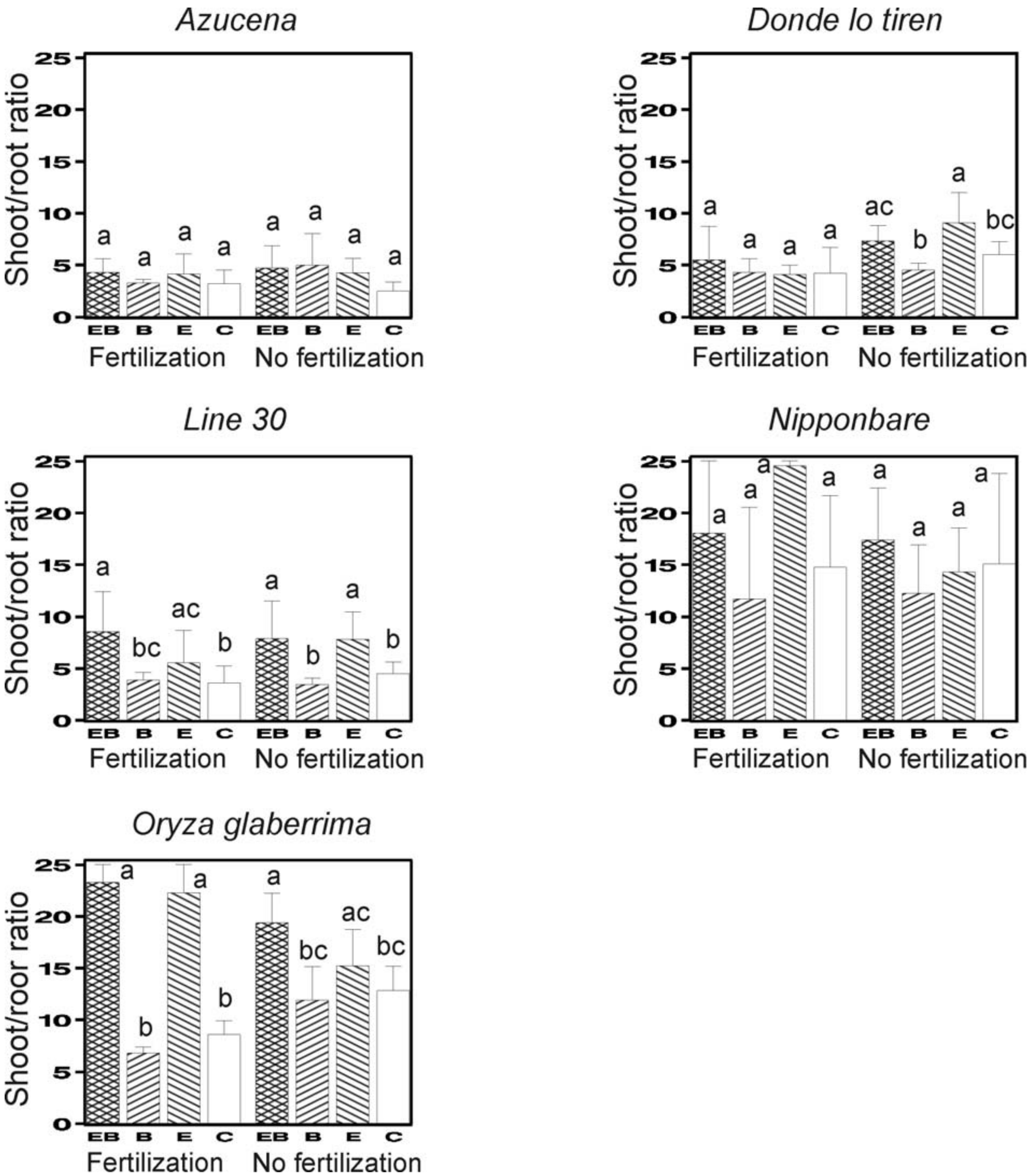
Appendix B. Figure B1-B5. Mean values for the total biomass (SM Fig. 1), the shoot/root ratio (SM Fig. 2), the total grain biomass (SM Fig. 3), the leaf C/N (SM Fig. 4), and the number of grains per plant (SM Fig. 5). Means are displayed for all combinations of the four factors: biochar, earthworm, fertilization and rice cultivar. Error bars denote standard deviations. Bars that do not share any common letter correspond to significantly different means (least square mean comparison, $P<0.05$ with a Tukey adjustment for multiple comparisons, SAS LSMEANS SAS statement) between combinations of treatments (earthworms and biochar) for each combination of cultivar and fertilization treatment.

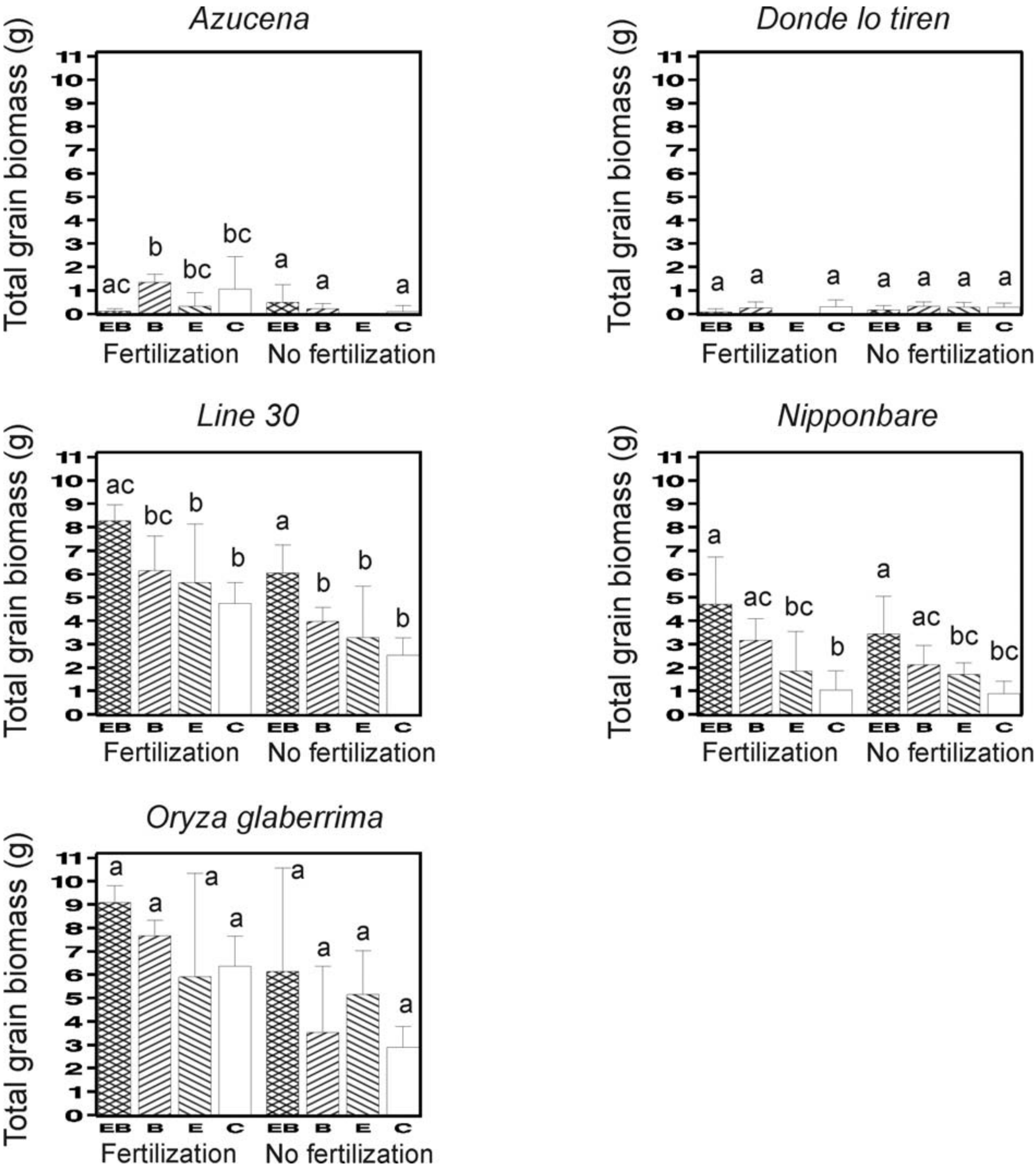
Appendix B. Figure B1



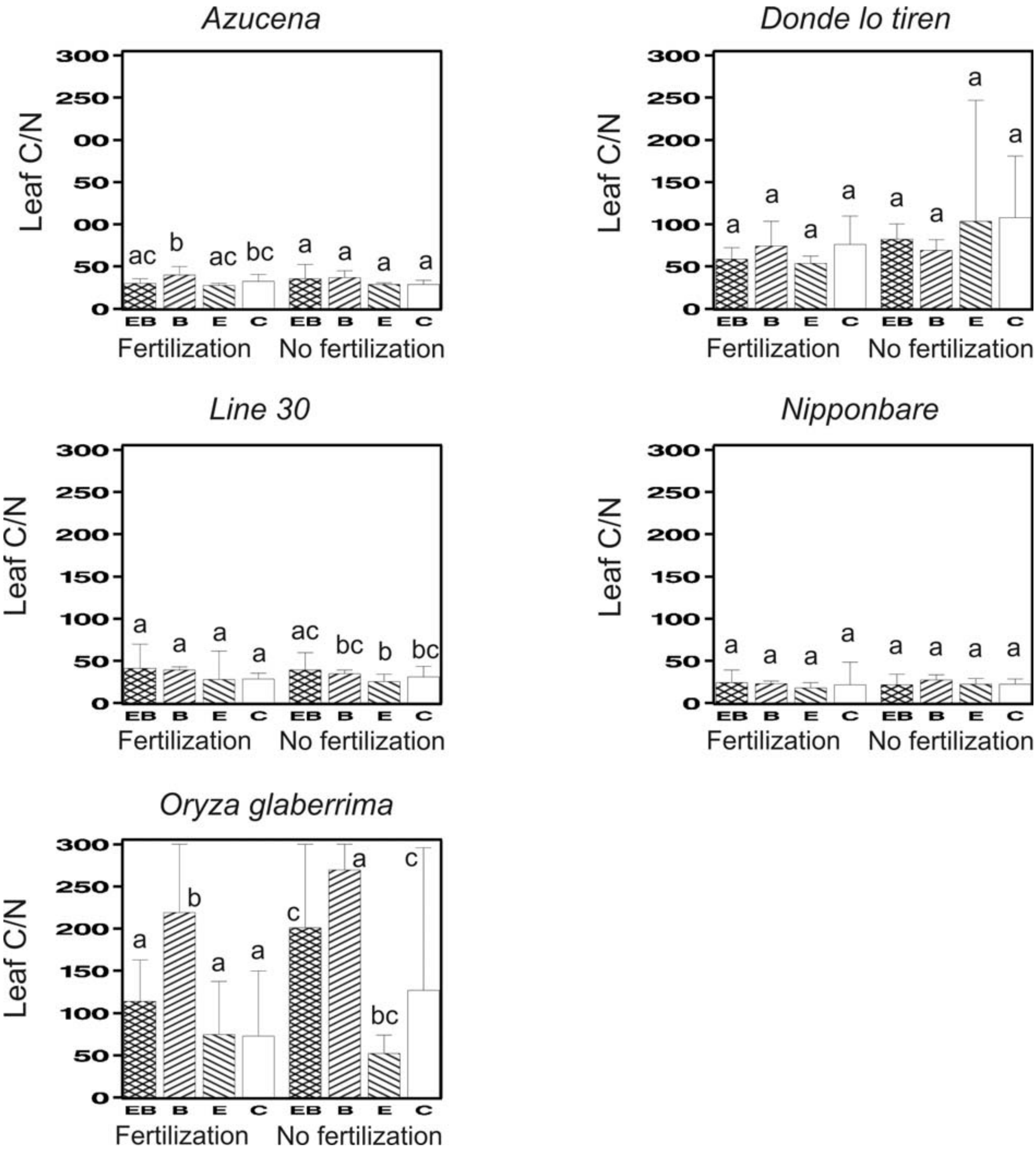
Appendix B. Figure B2



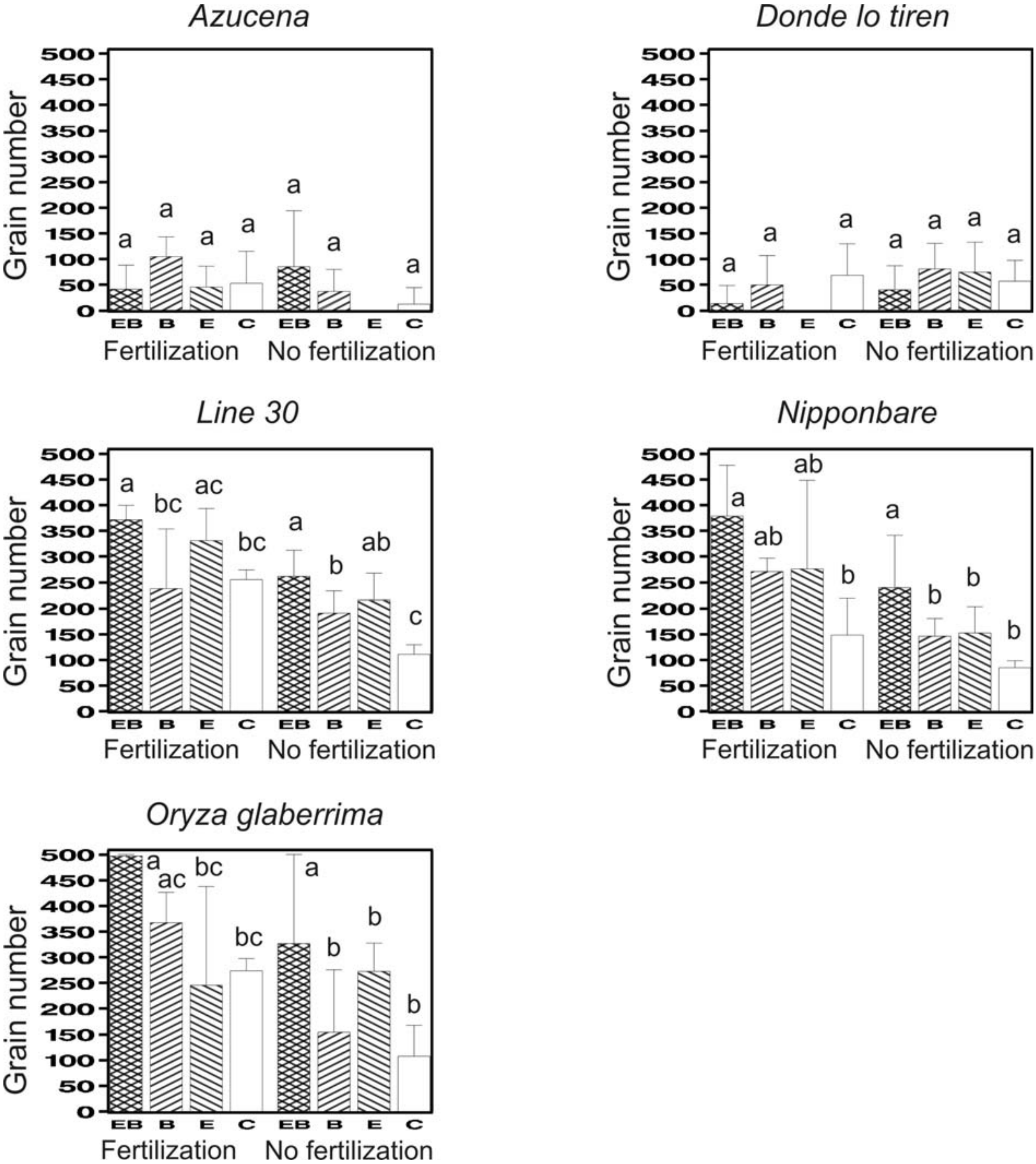
Appendix B. Figure B3



Appendix B. Figure B4



Appendix B. Figure B5



Appendix C. Figure C1. Effect sizes of the biochar, earthworm, and combination of biochar and earthworm treatments on the leaf C/N (A-B) and the grain number (C-D). In each case effect sizes are displayed for the mineral fertilization treatment (left-hand column of panels) and the non-fertilized treatment (right-hand column of panels), and for each Asian rice cultivar (AZ, *Azucena*; DLT, *Donde lo tiren*; L30, *Line 30*; NB, *Nipponbare*) and the African rice (OG, *Oryza glaberrima*).