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Appendix 1

Table A1. Characteristics of the farms (blocks) and sites where inocula for the greenhouse experiment were collected from and where the field experiment was set up. At each farm there were three habitats: a pasture (P), a sun-exposed coffee plantation (C), and a forest fragment (F).

Farm (block)	Geographic coordinates	Altitude (m a.s.l)	Mean annual precipitation (mm year^{-1})	Mean annual temperature (ºC)	Habitat (site)	Habitat patch size (ha)
Cenicafé	05º00′N,75º36′W	1380	2733	20.9	P	0.5
					C	0.5
					F	40
Playa rica	05º00′N,75º36’W	1290	2750	20.7	P	10
					C	60
					F	30
Alto español*	04º56′N,75º42′W	1720	3140	18.8	P	1.0
					C	2.0
					F	0.3
Naranjal	04º59′N,75º39′W	1400	3137	21.4	P	22
					C	38
					F	27
La Argentina	05º02′N,75º41′W	1354	2935	19.9	P	0.5
					C	100
					F	1.5

* this block was not used in the field experiment.

Table A2. Mean (SE) soil pH, organic matter (OM) content and nutrient content of the pot soil used in the greenhouse experiment, as well as soils of the three habitats where the field experiment was set up.

Habitat	pH[†]	OM[‡] (%)	N[¶] (%)	P[§] (mg kg^{-1})	K[††] (cmol kg^{-1})	Ca[††] (cmol kg^{-1})	Mg[††] (cmol kg^{-1})
Greenhouse pot soil	5.4	1.9	0.1	7.0	0.37	3.4	1.1
Pastures	5.6 (0.1)	7.2 (1.4)	0.3 (0.04)	54.4 (25.3)*	0.6 (0.2)	5.6 (0.7)*	2.1 (0.4)
Coffee plantations	5.0 (0.3)	9.6 (1.7)	0.4 (0.06)	42.0 (16.7)*	0.3 (0.1)	4.4 (1.1)*	1.7 (0.6)
Forest fragments	5.5 (0.2)	12.4 (2.1)	0.5 (0.06)	5.6 (0.4)*	0.4 (0.05)	8.3 (2.3)*	2.4 (0.7)

At each of the 15 sites, five samples of 200 g of soil (0–15 cm in depth) each were taken and pooled in a composite sample from which a subsample was analyzed. Log-transformed data was analyzed with one-way ANCOVA including block as a random factor and habitat as a fixed factor.
[†] pH: determined with 1:1 mix of soil:water
[‡] OM: organic matter was determined with the Walkley–Black method and colorimetry
[¶] N: total nitrogen per unit dry mass
[§] P: phosphorus was determined with the Bray test 2 (Bray II followed by Bray–Kurtz colorimetry)
[††] K, Ca, Mg: potassium, calcium, and magnesium extracted with 1N ammonium acetate
* significant differences between habitat types (field soil) $p < 0.05$

Table A3. Results of ANOVA for the dependency of AMF proportion root colonization (arcsine-root square transformed) of 10 plant species on inoculum source (pastures, coffee plantations, and forest fragments), plant species and their interaction. Only plants grown with 'live' inocula were included in the analysis, as plants in the sterile treatments had no AMF root colonization.

		AMF colonization	
Source	DF	F	p
Inoculum source	2	3.6	0.034
Species	9	10.5	<0.001
Inoculum source × Species	18	1.7	0.06
Error	60		

Table A4**.** Percent survival of seedlings of six plant species (mean SE) of four farms for each habitat-treatment combination) in the field experiment. Sixteen seedlings from each species (except *R. rospigliosii* for which there were 10 seedlings) were transplanted to each site; half served as control and half were treated once a month with fungicide (benomyl).

	Pastures		Coffee plantations		Forest fragments	
Species	Control	Fungicide	Control	Fungicide	Control	Fungicide
Brachiaria brizantha (grass)	78.5 (9.3)	81.5 (8.0)	53.3 (19.4)	53.5 (21.4)	31.5 (16.5)	31.8 (12.0)
Cecropia angustifolia (pioneer)	87.8 (5.1)	81.5 (7.8)	62.8 (16.9)	56.5 (16.5)	53.3 (20.7)	66.0 (22.0)
Solanum aphyodendron (pioneer)	31.5 (8.0)	37.8 (13.4)	9.8 (3.3)	3.3 (3.3)	0.0 (0.0)	19.0 (8.1)
Coffea arabica (shade tolerant)	97 (3.0)	100 (0.0)	100 (0.0)	97 (3.0)	100 (0.0)	100 (0.0)
Garcinia madrunno (shade tolerant)	97 (3.0)	94 (3.5)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
Retrophyllum rospigliosii (shade tolerant)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	90 (5.8)

Table A5. Results of proportional hazard model analysis of dependency of seedling survival on factors that may potentially affect survival in the field experiment.

Source	DF	Log-ratio χ^2	p
Habitat	2	0.0	1.00
Shade tolerance class (STC)	1	99.7	<0.001
STC[Species]	4	70.2	<0.001
Fungicide	1	4.0	0.046
Habitat × STC[Species]	8	22.5	0.004
STC × Habitat	2	0.0	1.00
STC × Fungicide	1	4.2	0.041
Fungicide × STC[Species]	4	6.0	0.20
Habitat × Fungicide	2	4.1	0.13
Habitat × Fungicide × STC	2	3.7	0.16
Block	3	12.6	0.006
Light	1	0.0	0.860
Initial leaf area	1	40.7	<0.001
Plot species richness	1	0.1	0.78
Proportion vegetation cover	1	1.0	0.32

The eight plant species are categorized to shade tolerance class (STC: fast growing grass and pioneer tree vs. shade tolerant trees including coffee), and species identity was examined as a factor nested within STC. Also included in the model are farm (treated as the random block factor), habitat type (pastures, coffee plantations, forest fragments), fungicide treatment (control, fungicide benomyl), their interactions, and covariates including light, initial leaf area, plot species richness and vegetation cover determined for each seedling plot (one seedling transplanted per plot).

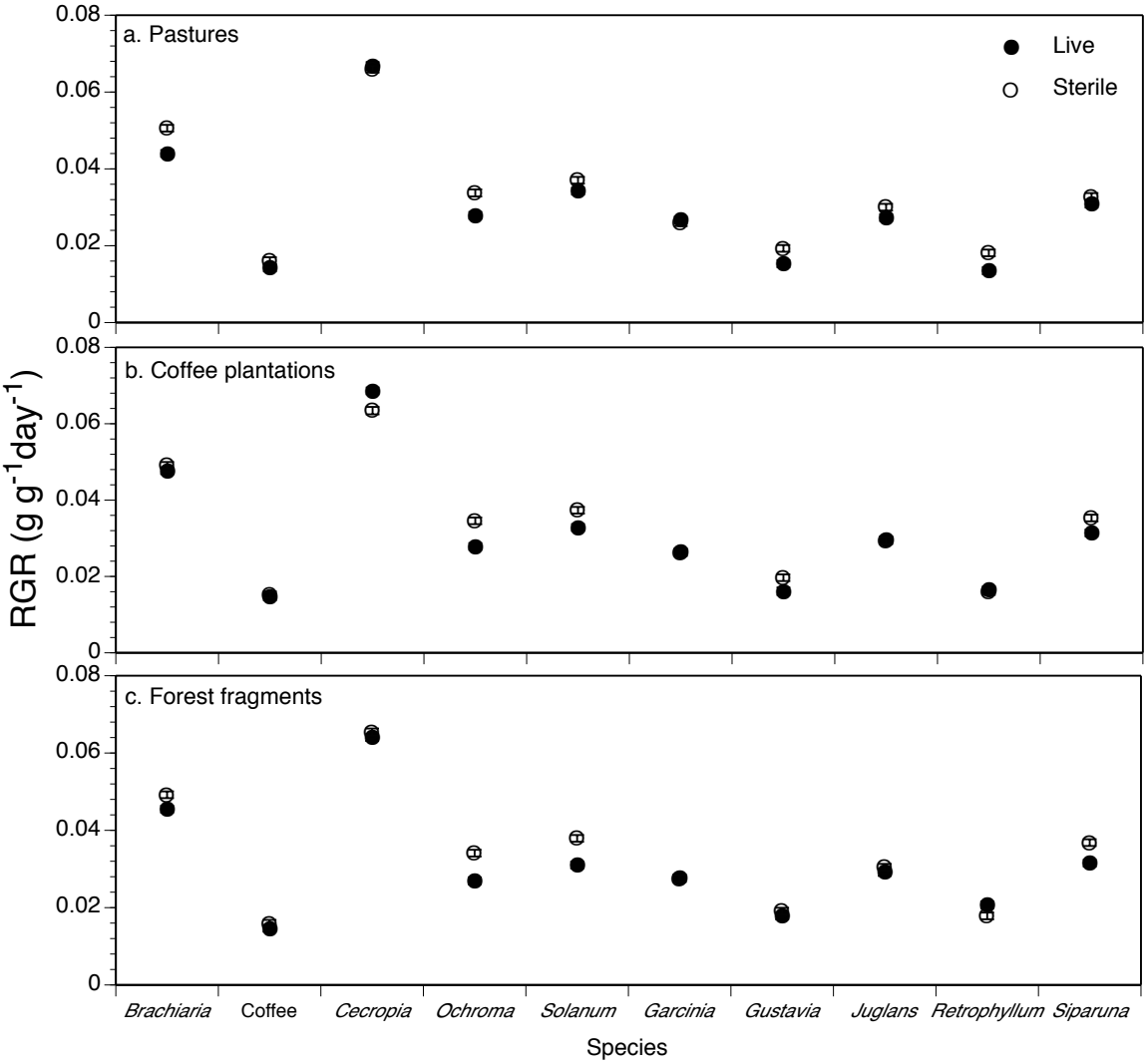

Figure A1. Seedling relative growth rate (RGR) of seedlings from 10 plant species in the greenhouse with live (closed circles) and sterilized (open circles) soil inocula from pastures (a), coffee plantations (b), and forest fragments (c). Least square means (± SE) of four farms. All non-overlapping means differ at $p < 0.05$.

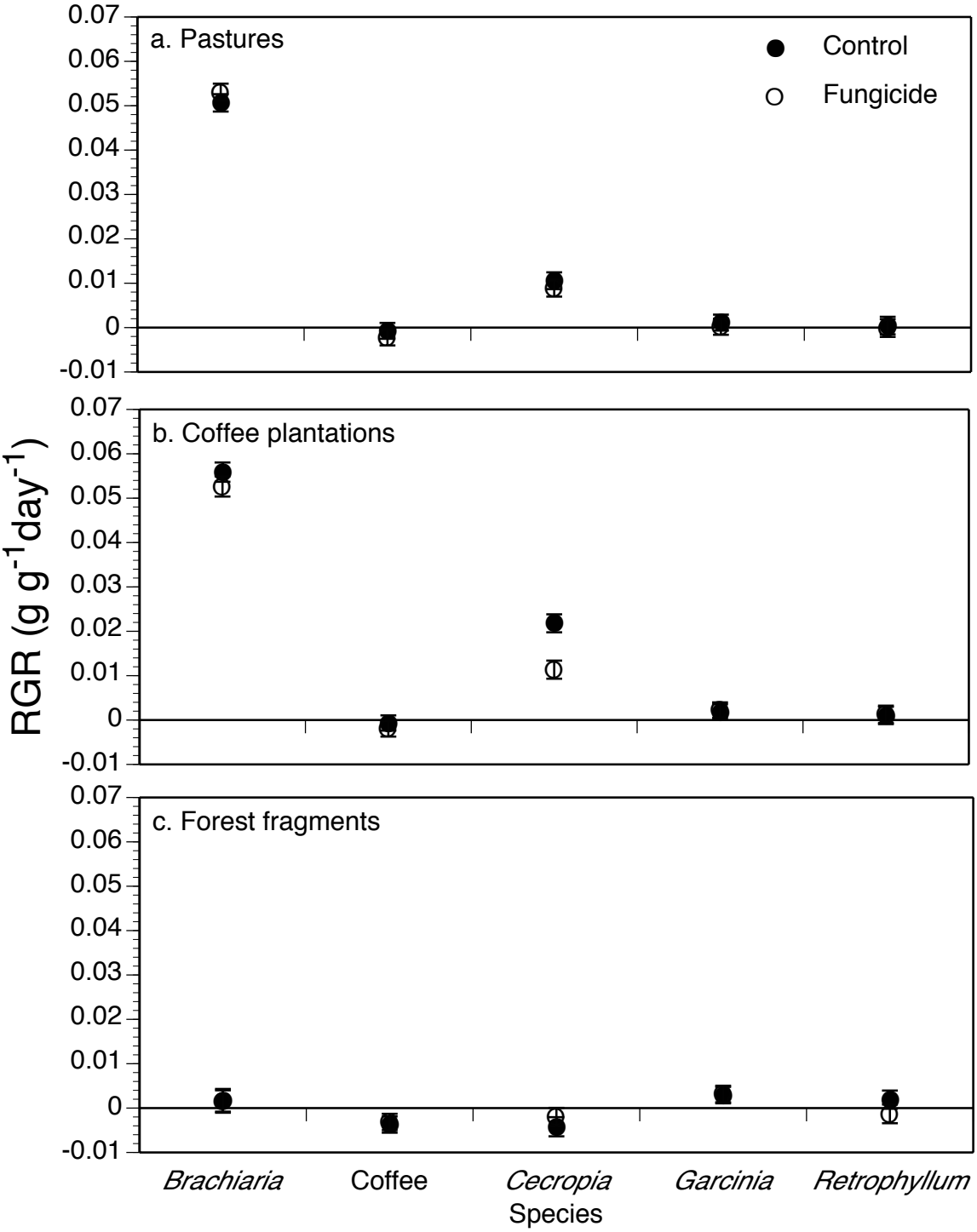

Figure B1**.** Seedling relative growth rate (RGR) of five plant species transplanted to pastures (a), coffee plantations (b), and forest fragments (c) and grown without (closed circles) and with the fungicide benomyl (open circles). Least square means (± SE) of four farms. All non-overlapping means differ at $p < 0.05$.

Appendix 2

Description of inoculum collection for the greenhouse experiment and set up of the field experiment

Greenhouse experiment

To obtain soil inoculum that would incorporate soil heterogeneity at the habitat level, we collected soil from five random locations within each pasture, coffee plantation, and forest fragment at each of the five farms (Supplementary material Appendix 1 Table A1). Soils were collected from random locations within pastures entirely dominated by *Brachiaria*, at intermediate distance between coffee bushes in coffee plantations (9500 plants ha^{-1}), and at intermediate distance between random combinations of native plants (including species that we worked with; Table 1) in forest fragments. Each soil sample was taken using a small shovel from the top 15 cm consisting of humus layer, mineral soil, roots, rhizosphere soil and associated organisms. Soils from the five sites were pooled by habitat type, so that we had one pooled inoculum for each habitat type. After mixing well, half of each pooled sample was used as live inoculum, while the other half was used as sterile inoculum after autoclaving for 2 h.

Field experiment

Seeds of *Brachiaria*, coffee, two pioneer tree species, and two shade tolerant forest tree species (Table 1) were germinated in sterilized soil as in the greenhouse experiment. Prior to transplanting to the field, seedlings were grown for two months (coffee and shade tolerant trees), one month (pioneer trees), or two weeks (*Brachiaria*) to allow seedlings to reach a similar size (at least two fully expanded leaves). The field plots were set up at four of the five farms used as the inoculum sources for the greenhouse experiment (Supplementary material Appendix 1 Table A1, A2). At each of twelve sites (three habitat types × four farms), 90 1 × 1 m plots were prepared one month prior to transplanting. Number of species and % vegetation ground cover were quantified in each

plot, and plots with ground cover greater than 60% were cleared with a machete. Then we made a 20 × 20 cm and 30 cm deep hole in the center of each plot. Plots were randomly assigned to either control or fungicide treatment with benomyl (1-[(Butylamino) carbonyl]-1H-benzimidazol-2-yl] carbamic acid methyl ester) at a concentration of 1.125 g l^{-1} (Helgason et al. 2007). Benomyl fungicide is expected to suppress a wide range of soil fungi including many AMF species as well as pathogenic ascomycetes and basidiomycete fungi (Helgason et al. 2007, Nijjer et al. 2007). Seedlings were randomly assigned to site-treatment combinations, and marked with individual tin tags. The total initial leaf area was estimated from leaf number and length. In the morning before transplantation to the field, each seedling (with as much soil around the roots as possible) was placed in an individual plastic bag to which we added 100 ml of either distilled water or benomyl. Bagged seedlings were taken to the field in a cooler box, and each seedling was transplanted into an individual plot. For each site-treatment combination (four farms × three habitat types × six plant species × two treatments), eight seedlings were transplanted (except five seedlings for *R. rospigliosii* due to limited germination success of this species), totaling 1080 seedlings. At the time of transplanting we added 1 l of either water to control plots, or benomyl solution to fungicide treatment plots. Every month throughout the three-month experiment, we repeated the fungicide treatments, and recorded seedling survival. Control plots were not watered as the experiment was conducted during the rainy season and soils were saturated with water. Photosynthetic active radiation (PAR) was measured above each seedling relative to PAR at an open site, twice during the experiment at different times of the day with a light meter. After three months all seedlings were harvested (with roots) and dried for three days at 60°C, for determination of total dry mass. Roots from three randomly selected plants from each treatment combination were cleared and stained to examine the presence of AMF and non-AMF soil microbes.

References:

Helgason, T. et al. 2007. Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. − J. Ecol. 95: 623–630.

Nijjer, S. et al. 2007. Negative plant–soil feedbacks may limit persistence of an invasive tree due to rapid accumulation of soil pathogens. − Proc. R. Soc. B 274: 2621–2627.

Appendix 3

Results on seedling survival in field experiment

Seedling survival was higher for the three shade tolerant species (Table 1*)* than for *Brachiaria* and woody pioneer plant species across different habitat types and treatments (Supplementary material Appendix 1 Table A4, A5). Only seven seedlings of shade tolerant species died during the experiment, and there were no obvious differences among habitats. In contrast, survival of fast growing species varied significantly among habitats (Supplementary material Appendix 1 Table A5). Seedling survival was higher in pastures (80–82%) than in shadier habitats of coffee plantations and forest fragments (31–60%) for *Brachiaria* (Wilcoxon $\chi^2 = 23.7$, $p < 0.001$) and the pioneer tree *C. angustifolia* (Wilcoxon $\chi^2 = 12.0$, $p = 0.0025$) (Supplementary material Appendix 1 Table A5). The second pioneer tree, *S. aphyodendron* had high mortality across habitats, with only 34.6% surviving in pastures, and less than 10% survival in the other two habitats (Wilcoxon $\chi^2 = 8.4$, $p = 0.015$). Therefore, this species was excluded from the growth analyses. Fungicide had a marginal but significant effect on seedling survival, affecting fast growing and pioneer species more than shade tolerant species (Supplementary material Appendix 1 Table sA4, A5). Seedlings treated with fungicide had marginally lower survival for *C. angustifolia* (C: 67.9% ± 9.3; F: 66.9% ± 9.0), but higher survival for *S. aphyodendron* (C: 13.8% ± 4.8; F: 20% ± 6.4) across the three habitat types.