Linking plant-soil feedback to the effect of frugivore treatment on seed germination

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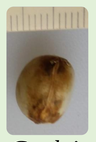
INTRODUCTION

- Frugivores contribute to increase germination success by removing seeds away from conspecifics – which reduces density-dependent mortality (Janzen-Connell model) [1] – and by cleaning the seeds[2], which reduces pathogen infestation.
- High mortality under parent trees has been attributed to the soil microbiota, a process called "negative plant-soil feedback"[3].
- Here, we tested how two plant species, *Castilla ulei* (Moraceae) and *Hymenaea parvifolia* (Fabaceae), are affected by the manipulation – seed cleaning and removal from conspecifics – of the gray woolly monkey (*Lagothrix cana*). This frugivorous primate is an efficient seed disperser, both quantitatively and qualitatively[2], of the Amazon rainforest.

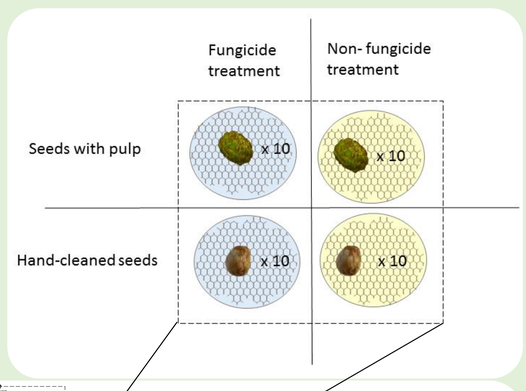
METHODS

- We collected 1600 fruits from trees of *H. parvifolia* and *C. ulei* and set up a factorial field experiment with four treatments per plot (10 seeds/ treatment unit) excluding medium and large predators:
  → hand-cleaned seeds with and without fungicide,
  → seeds with pulp with and without fungicide.
- We set up four groups of plots per tree under 5 paired conspecific and heterospecific trees (figure 2).



C. ulei

H. parvifolia



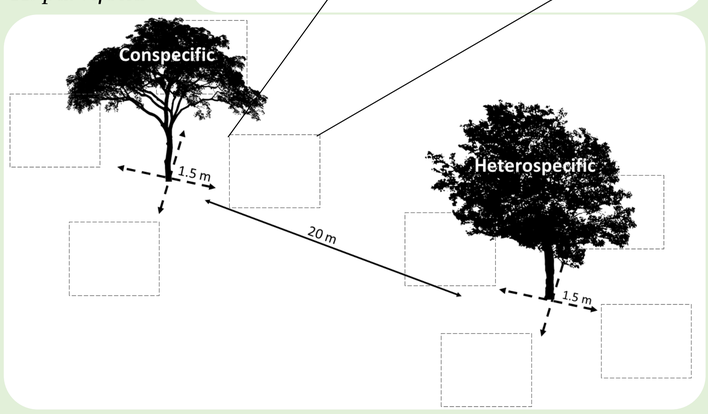


Fig. 2: Factorial design of the field experiment for each study species.

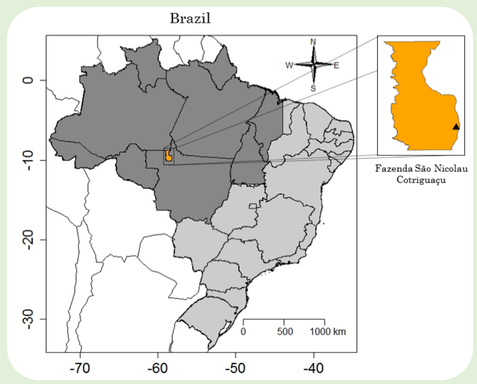


Fig. 1: Study area: Amazon rainforest in the Fazenda São Nicolau, Cotriguaçu, MT, Brazil.

✖ 5 experimental groups

We cleaned the seeds under running water and used sodium hypochlorite (NaClO 2.5%) as fungicide that we applied every 10 days when germination and seedling establishment were checked.

- We compared the germination success (number of seeds germinated) among treatments with a mixed effect model with the individual adult trees as random factor and treatment as fixed effect (GLMM).

RESULTS

Table 1: Overall results for *Castilla ulei* and *Hymenaea parvifolia:*

	C. ulei (Moraceae)	*H. parvifolia* (Fabaceae)	n
Maximum number of germination per treatment unit	7 (70%)	7 (70%)	10 seeds
Maximum number of seedlings per treatment unit	7 (70%)	2 (20%)	10 seeds
Number of treatment unit with successful seeds	60 (37.50%)	56 (35%)	160 treatment units
Overall germination success	180 (11.2%)	82 (5.12%)	1600 seeds
Overall seedling establishment	142 (8.9%)	18 (1.12%)	1600 seeds
Viable seeds at the end of experiment	0 (0%)	427 (26.6%)	1600 seeds

***Castilla ulei* (Moraceae)**

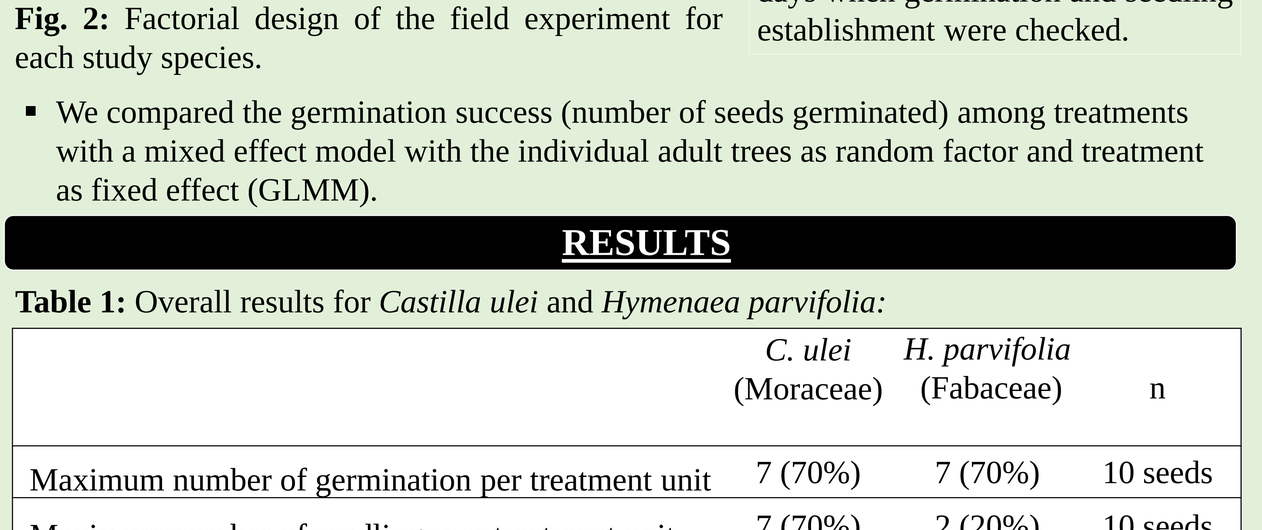
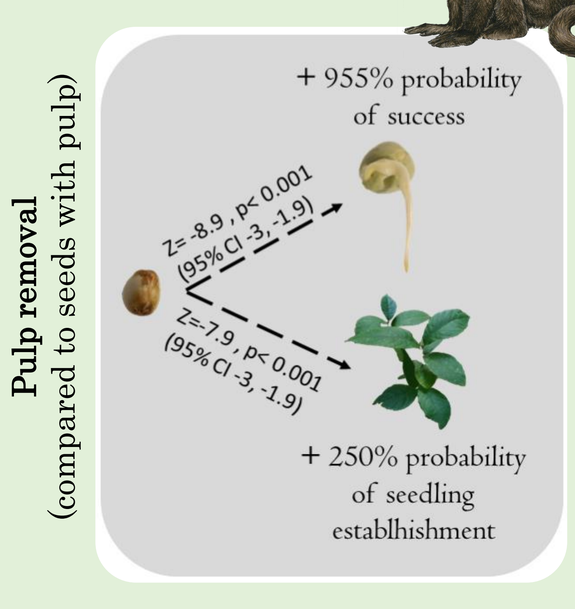
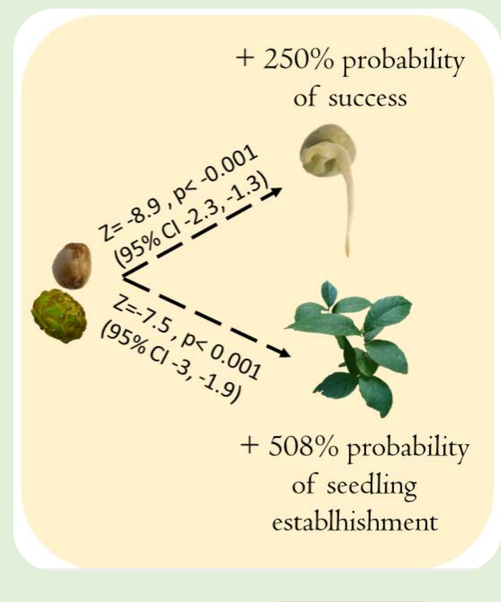


Fig. 3: The average number of successful seeds of *C. ulei* per treatment unit among three treatments after 20 days in the field. Being under conspecific did not affect germination success of *C. ulei.* (z= -0.7, *p*= 0.5). Treatments pulp and fungicide, however, did affect the germination success (z= -8.9 p<0.001 and z= -6.9, *p*<0.005 respectively).





***Hymenaea parvifolia* (Fabaceae)**

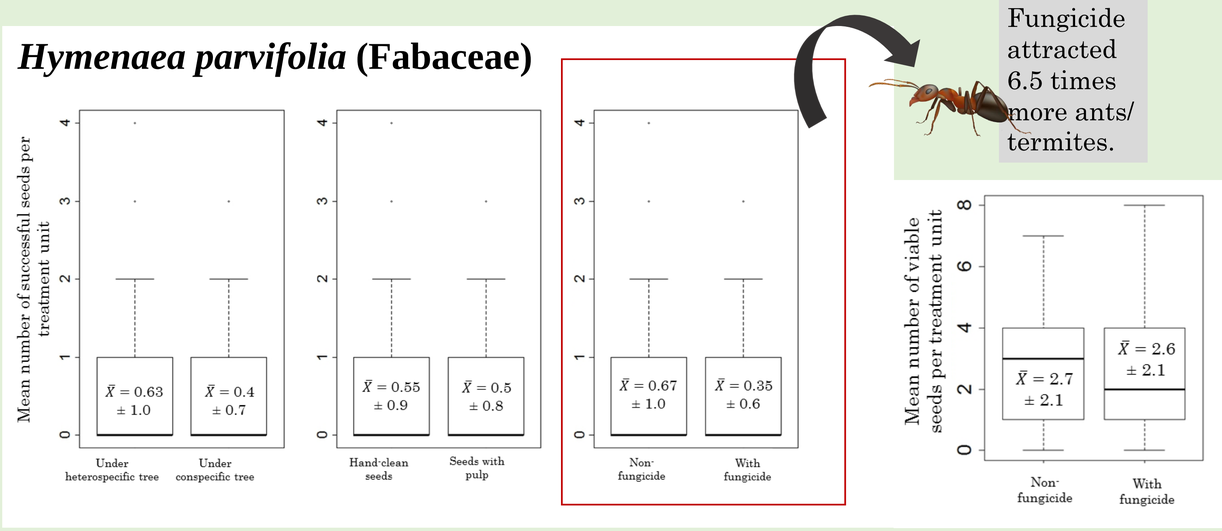
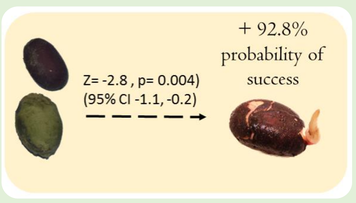


Fig. 6: The average number of successful seeds per treatment unit among six treatments after 40 days in the field. Although few seeds germinated in general, only fungicide treatment showed significant statistical difference according to the GLMM (z= -2.8 *p=0.004*). However, it did not affect seed viability (z= -1.6, *p=0.1*). Being under conspecific and having pulp did not affect the germination success according to the model (z= -1.5, *p=0.1* and z= -0.7, *p=0.5* respectively).

Non-fungicide treatment:
(compared to seeds with fungicide)



DISCUSSION

- Our results showed that Janzen–Connell effects were not the main driver of seed mortality for these species. Pulp removal was more important to increase germination success of *C. ulei* regardless of where the seeds were deposited.

  → The *C. ulei* has a highly adhered fleshy pulp that attracts a great number of agents of mortality on the forest floor. The fruit rots easily when falls on the soil. Therefore, frugivores such as *Lagothrix cana* have great importance as they potentially increase germination success by 955% by removing the seed pulp.

  → The *H. parvifolia*, in contrast, has a farinaceous pulp, easily unleashed from seeds by rain, which can explain the absence of pulp removal effect. Breaking seed dormancy by disrupting seed coat could be more important to germination success than seed cleaning and deposition site.

- In contrast to our expectation, fungicide reduced germination probability in both species. Unexpectedly, the NaClO attracted ants/termites. They may prefer sterilized seeds over seeds contaminated by fungi[4]. However, a tetrazolium test for viability showed that insects did not affect the embryo of *H. parvifolia* seeds, but reduced germination probability by burying them (up to 30cm below ground).
- Most studies on the plant-soil feedback are conducted on laboratory conditions and semi-arid environment[5]. Our field experiment in tropical forest was not able to test the fungi effect on seed germination as insects dissembled the outcome. However, our results highly suggest that frugivore manipulation is important to reduce seed mortality of fleshy fruits in early stages.

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