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Soil fertility regulates invasive herbivore performance and top-down control in tropical agroecosystems of Southeast Asia

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Running head: Soil fertility shapes invader success

Soil fertility regulates invasive herbivore performance and top-down control in tropical agroecosystems of Southeast Asia

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38 **Abstract**

39 In terrestrial ecosystems, changes in soil nutrient availability, plant growth or natural enemies
40 can generate important shifts in abundance of organisms at various trophic levels. In
41 agroecosystems the performance of (invasive) herbivores and their impacts on crops is of
42 particular concern. Scientists are presently challenged with making reliable inferences on invader
43 success, natural enemy performance and efficacy of biological control, particularly in tropical
44 agroecosystems. In this study, we assess how trophic regulatory forces (bottom-up vs. top down)
45 influence the success of three globally important pests of cassava. We examine the mealybug
46 species (Hemiptera: Pseudococcidae) of differing host breadth and invasion history:
47 *Phenacoccus manihoti*, *Paracoccus marginatus*, and *Pseudococcus jackbeardsleyi*. Potted plant
48 fertilizer trials were combined with a regional survey in Vietnam, Laos and Cambodia of 65
49 cassava fields of similar size and age, but with varying soil fertility. Relative abundance of each
50 mealybug invader was mapped along a soil fertility gradient, and contrasted with site-specific
51 measures of parasitism. Potted plant trials revealed strong bottom-up effects for *P. manihoti*,
52 such that impacts of nitrogen and potassium additions were propagated through to higher trophic
53 levels and substantially boost development and fitness of its specialist parasitoid, *Anagyrus*
54 *lopezi* (Hymenoptera: Encyrtidae). Field surveys indicate that mealybug performance is highly
55 species-specific and context-dependent. For example, field-level abundance of *P. jackbeardsleyi*
56 and *P. marginatus*, was related to measures of soil fertility parameters, soil texture and plant
57 disease incidence. Furthermore, for *P. manihoti*, in-field abundance is equally associated with
58 soil texture (i.e., silt content). Principal component analysis (PCA) and regression suggested that
59 *P. manihoti* and *P. marginatus* are disproportionately favored in low-fertility conditions, while *P.*
60 *jackbeardsleyi* prospers in settings with high organic carbon and phosphorus. Parasitism of *P.*
61 *manihoti* by *A. lopezi* varied greatly with field and soil fertility conditions, and was highest in

62 soils with intermediate fertility levels and where management practices include the addition of
63 fertilizer supplements. Our characterization of the relative performance of invasive mealybugs
64 and strength of parasitism across variable soil fertility conditions will help guide parasitoid
65 release programs and soil management practices that enhance mealybug biological control.

66

67 **Keywords:** classical biological control, trophic dynamics, soil fertility, biotic resistance,
68 invasive species

69 **1. Introduction**

70 Around the globe, impacts of human-mediated biodiversity loss, land-use change, and global
71 warming are proceeding at an unrelenting pace, with profound effects on ecosystems and
72 associated food webs (e.g., Vitousek et al., 1997; Newbold et al., 2016). Such changes are
73 particularly relevant for smallholder agriculture in the tropics, where soil fertility (and resulting
74 plant communities) is drastically altered by a variety of management practices and larger-scale
75 patterns in land-use change. In Southeast Asia, cassava (*Manihot esculenta*) production has
76 expanded considerably over the past few decades and now occupies more than 4 million ha
77 throughout the region (Cramb et al., 2016; Mahanty & Milne, 2016). Cassava is typically
78 managed as an annual crop and grown under a range of biophysical and socio-economic
79 conditions, from shifting cultivation in the uplands of Laos and Cambodia, to large-scale
80 monocultures in the lowlands of southern Vietnam (e.g., Howeler et al., 2011). This crop
81 produced especially well in the early years of cultivation, due to an overall absence of limiting
82 pests and diseases. However, over the past decade, a series of non-native mealybug (Hemiptera:
83 Pseudococcidae) species have colonized Asia's prime cassava-growing regions (Graziosi et al.,
84 2016). These include (1) *Phenacoccus manihoti* Matile-Ferrero, a Neotropical parthenogenetic,
85 oligophagous herbivore (9 host records) with broad climatic adaptability (Yonow et al., 2017)
86 and global distribution (33 countries); (2) *Paracoccus marginatus* Williams & Granara de
87 Willink, a Nearctic sexual, polyphagous herbivore (133 host genera), reported from 33 different
88 countries; and (3) *Pseudococcus jackbeardsleyi* Gimpel & Miller, a Neotropical polyphagous
89 species (98 host genera), found in 46 countries worldwide. Invasion history is variable between
90 species, with respective colonization processes in mainland SE Asia presumably initiated around
91 2008, 2010, and 1987 respectively (Ben-Dov et al., 2016), and biological control with parasitic

92 wasps such as *Anagyrus lopezi* De Santis (for *P. manihoti*, released in 2009), *Acerophagus*
93 *papayae* Noyes & Schauff (for *P. marginatus*, colonized post-2010) and a set of endemic and
94 exotic generalists wasps for *P. jackbeardsleyi* (Muniappan et al., 2009).

95 Soil fertility and overall quality has been shown to be a principal determinant of plant health
96 and resistance to pests and disease (e.g., Amtmann et al. 2008), however, the impact of
97 belowground processes on aboveground interactions is varied and often difficult to predict
98 (Wardle et al. 2004). Understanding how these invasive herbivores and their associated
99 parasitoids interact and respond to soil fertility conditions offers a number of possible benefits
100 for managing pests. For example, such information could help target parasitoid releases, identify
101 context-specific needs for integrated pest management and help improve our overall
102 understanding of linkages between above and below-ground processes. So far, little research has
103 been conducted on trophic regulation and associated invader success along gradients of
104 ecosystem productivity or soil fertility (e.g., Zarnetske et al., 2013). While some suggest that
105 highly fertile sites disproportionately favor invaders, regardless of top-down forces such as
106 parasitoids (see Hovick & Carson, 2015), evidence also exists to the contrary.

107 Alterations in resource availability or species abundance are transmitted through trophic
108 chains, and affect the relative role of resource (“bottom-up”) versus consumer (“top-down”)
109 forces in the structuring of ecological communities (Hunter & Price, 1992; Ives & Carpenter,
110 2007). Changes in top predators or basal resources, e.g., through fertilizer addition, can shift the
111 equilibrium abundances of various trophic levels and affect the relative success of certain species
112 (native or exotic) Comparatively few empirical studies have concurrently assessed the relative
113 effect of top-down, bottom-up and interactive processes on ecological communities (Moran &
114 Scheidler, 2002; Gruner, 2004; Garibaldi et al., 2010) and population-level processes under field

115 conditions are rarely considered in addressing such issues (Walker et al., 2008; Zaugg et al.,
116 2013; Rzanny et al., 2013). Success rates of invasive species are explained through a range of
117 hypotheses linked to trophic processes, in which community productivity, disturbance, species
118 diversity and natural enemy action are all posed as important determinants. As these hypotheses
119 are non-exclusive, interactions between mechanisms are increasingly employed to predict
120 invasion outcomes and invader success (e.g., Parepa et al., 2013; Mallon et al., 2015; Peltzer et
121 al., 2016). Certain theories simultaneously account for the role of resource availability (e.g., soil
122 fertility) and natural enemies (Blumenthal, 2005; Center et al., 2014).

123 Particularly for sessile invasive herbivores, such as mealybugs, plant nutritional quality
124 strongly determines species abundance and performance, shaping entire herbivore feeding guilds
125 (Shurin et al., 2002; Carcamo et al., 2005; Rzanny et al., 2013). Also, soil fertility and plant
126 nutrients may lead to differential, species-specific responses amongst invaders (Peltzer et al.,
127 2016). Invader success and trophic regulation have previously been linked to single-nutrient
128 (e.g., soil N, P, K, Zn) measures (e.g., Walter & DiFonzo, 2007; Chen et al., 2010). However,
129 increasing attention is being paid to overall plant quality and more universal measures of soil
130 fertility (e.g., Ode, 2006; Bardgett & van der Putten, 2014). Thus, composite soil fertility indices
131 potentially can help explain relative success of invasive mealybugs and associated biological
132 control processes in fields with differing resource availability.

133 In this study, we assess soil-plant-herbivore-parasitoid interactions through both manipulative
134 and observational approaches to better understand the relative influence of top-down vs. bottom
135 up forces on herbivore pest performance. We evaluate the effect of resource quality on the
136 success of invasive mealybug species, in a controlled laboratory setting as well as, in cassava
137 fields along a soil fertility gradient. More specifically, we address the following three research

138 questions: (1) do fertilizer supplement studies reveal the effects of single-element additions of
139 nitrogen (N) and potassium (K) on *P. manihoti* performance and top-down forces (i.e., parasitism
140 by a recently-introduced natural enemy); (2) does abundance of different invasive species vary
141 along a soil fertility gradient, and do particular measures of soil fertility explain invader success;
142 (3) do top-down forces (i.e., parasitism by a recently-introduced natural enemy) shift in
143 importance between contexts of varying resource quality, as determined by soil fertility.

144

145 **2. Materials and Methods**

146

147 2.1. Potted plant fertilizer trials

148 2.1.1. *Plant cultivation*

149 In this set of trials, we assessed the extent to which single-element nutrient additions affected
150 different development parameters of *P. manihoti*, and its primary parasitoid, *A. lopezi*. During
151 2014-2015, assays were established at Hue University of Agriculture and Forestry (HUAF), in
152 Hue, Vietnam. Soil was collected from an uncultivated plot at the HUAF experimental campus,
153 and was homogenized for use in a controlled pot experiment. A sub-sample of this soil analyzed
154 at the HUAF laboratory of Agronomy was determined to have a pH of 5.2, an organic carbon (C)
155 content of 1.5%, and available concentrations of K₂O, N and P₂O₅ of 4.59 mg, 0.65 mg and 10.5
156 mg per 100 g of soil, respectively. Approximately 10 kg of this soil was placed in pots (30 dia. x
157 20 cm deep) and a single vegetative cutting (approx. 20 cm in length) of cassava (variety KM94,
158 a popular cassava variety, widely cultivated across the region) was planted vertically in each pot.
159 KM94 is. Pots were placed outside in a screen-house and watered daily. After two weeks, plants
160 were randomly assigned to five fertilizer treatments: 1) no fertilizer (i.e., untreated controls), 2)

161 low N addition (90 kg N ha⁻¹), 3) high N addition (180 kg N ha⁻¹), 4) low K addition (90 kg K₂O
162 ha⁻¹), and 5) high K addition (180 kg K₂O ha⁻¹). This was equivalent to application rates of 0.65
163 g and 1.30 g N and 0.50 and 1.00 g K₂O per pot (respectively for medium and high fertilizer
164 treatments) and represents fertilizer rates commonly applied by Asian cassava growers (e.g.,
165 Howeler, 2011). We focused on N and K additions in the potted plant fertilizer trials, as cassava
166 is most responsive to these nutrients and has comparatively high capacity to mobilize P from
167 tropical soils via root association with mycorrhizae and other mechanisms (Nguyen et al., 2007;
168 Howeler, 2011). Both N (as urea) and K (as K₂O) were dissolved in water and applied in liquid
169 form. After six weeks, the plants were moved into a climate-controlled chamber (ambient RH, 30
170 ± 1°C and 12L: 12D).

171

172 2.1.2. *Mealybug and parasitoid colony maintenance*

173 In mid-2014, a starter laboratory culture of *P. manihoti* was established from field-collected
174 individuals from Hue and Quang Tri, central Vietnam and reared on cassava stems grown in
175 glass jars with a diluted fertilizer solution inside 60 x 160 x 180 cm cages. Prior to initiation of
176 the trials, a total of five *P. manihoti* sub-colonies were concurrently established in a climate-
177 controlled chamber, on plants subjected to each of the above five experimental fertilizer
178 treatments. Each sub-colony was initiated at the same time with approx. 100 mealybugs (mixed-
179 age population), as obtained from the starter colony. Mealybug populations were maintained on
180 these plants for two to three generations prior to use in experiments, to mitigate the influence of
181 parental trophic feeding history and eventual other maternal effects. A colony of *A. lopezi* was
182 established with field collected individuals obtained in mid-2015 from fields near Hue, Vietnam.
183 The laboratory colony was kept on cassava plantlets infected with *P. manihoti* in cages with the

184 following dimensions: 40 x 50 x 60 cm. All of the mealybug and parasitoid colonies were
185 maintained at $30 \pm 1^\circ\text{C}$ and 12L: 12D photoperiod, and colonies were regularly refreshed by
186 adding (unspecified, yet small numbers of) field-collected individuals. Voucher specimens of
187 mealybugs and *A. lopezi* wasps were deposited at Hue University of Agriculture and Forestry
188 (VNUA), Vietnam.

189

190 2.1.3. *Experimental assays & data processing*

191 At the onset of the experiment, a *P. manihoti* ovisac (egg batch) was collected from each
192 mealybug sub-colony and allowed to hatch. Upon emergence, ten first-instar nymphs were
193 transferred to the 3rd youngest leaf of an experimental plant grown in the same fertilizer
194 treatment as the sub-colony. We used 20 un-fertilized control plants and 10 plants for each of the
195 N and K-addition treatments (N= 60). Nymphs on each experimental plant were enclosed in one
196 single 5 x 10 x 20 cm clip-cage. Clip-cages were constructed out of transparent, plastic
197 polypropylene (PP) containers and equipped with a mesh lid to allow sufficient ventilation.
198 Development and embryonic mortality of all nymphs (N= 600) was thus assessed within each
199 fertility regime, following protocols by Tertuliano et al. (1993). Development parameters were
200 thus recorded for ten different cohorts (i.e., replicate clip-cages) under each fertilizer treatment,
201 and cohort trials for the separate treatments were run simultaneously. Size and weight measures
202 were recorded for young females (stage L4, prior to oviposition) obtained from ten unfertilized
203 plants and five plants for each of the N and K-addition treatments (N= 300).

204 Adult reproduction and mortality were recorded for each of the plant fertilizer treatments in a
205 separate experiment, using a new set of experimental potted cassava plants. Young females
206 (stage L4) were collected from each of the five sub-colonies, and transferred to clip cages on the

207 3rd youngest leaf of an experimental plant with the same fertilizer treatment as the sub-colony,
208 thus establishing a cohort of 10 females per clip-cage. Daily reproduction and mortality were
209 recorded for all females per fertilizer treatment (N= 600). Furthermore, duration of the pre-
210 reproductive period, fecundity and adult weight were assessed on 10 unfertilized control plants
211 and five plants for each of the fertilizer treatments (N= 300) (e.g., Tertuliano et al., 1993).
212 Reproductive output for each adult was recorded on a daily basis by removing newly-laid ovisacs
213 from the clip cage and counting the number of eggs under a stereomicroscope.

214 In a second set of laboratory assays, we assessed *A. lopezi* parasitoid fitness, development and
215 survival rates under the same fertilizer treatments used above. *P. manihoti* ovisacs were collected
216 from each mealybug colony/fertilizer treatment combination, and one ovisac was placed in a 5 x
217 10 x 20 cm clip-cage on 10 unfertilized control plants and five plants for each of the fertilization
218 treatments (N= 300). At nymphal emergence, a total of 70 first-instar nymphs were allowed to
219 establish within the cage, and the number was then reduced to 50 at the L3 stage (e.g., Van
220 Driesche et al., 1987). Subsequently, a 1-day old adult, mated and naïve female *A. lopezi* wasp
221 was introduced into each clip-cage together with one adult male *A. lopezi* for a 24 h period and
222 allowed to oviposit. After the allotted time, the adult female parasitoid was transferred to another
223 clip cage with another 50 third-instar nymphs on a different plant, but at the same fertilizer
224 treatment. By transferring each parasitoid on a daily basis to a new clip-cage with ample new
225 hosts, we were able to assess total lifetime reproductive output. A total of 20 female *A. lopezi*
226 were assessed on unfertilized controls, and 10 for each of the fertilization treatments (N= 60).
227 This process was repeated on a daily basis until death of the female, regularly replacing male
228 wasps that had died. Daily parasitism rate was calculated as the average parasitism (number of
229 mummies/50 nymphs) within each cage every day, until death of the female. For each wasp,

230 lifetime fecundity (# mummies), oviposition period (d) and rate (mummy/d) were calculated.
231 After removal of the parasitoid from the clip-cage, each cassava plant was incubated at 30°C and
232 mealybug mortality, parasitoid development time (egg deposition-mummification, and adult
233 emergence) and sex ratio were recorded. A total of 10 replicate female wasps were assayed from
234 each fertilizer treatment and 20 replicates for the non-fertilized controls. Upon offspring
235 emergence, a subset of 60 wasps of each sex for the fertilized treatments, and 120 for the
236 controls, were isolated in Eppendorf vials and provided daily access to honey mixed with water
237 (50%). To assess longevity of wasps from each fertilizer treatment, we recorded daily mortality
238 rates

239

240 2.2. Observational studies

241 2.2.1. *Field-level arthropod survey*

242 In a second experiment, a geographically widespread survey was conducted to assess the extent
243 to which mealybug abundance relates to soil fertility in a set of cassava fields, representative of
244 each of the target regions. During February-March 2015, a total of 65 fields were randomly
245 chosen across three countries, with 20 fields in southern Vietnam, 18 in eastern Cambodia, and
246 27 in southern and south-central Laos. Fields were chosen within primary cassava-growing
247 regions in each of the above countries, with assistance from local extension personnel. Plants
248 within each field were 6-9 months old, and were located in the countries' primary cassava-
249 growing regions. Survey activities covered two provinces in Vietnam (Binh Thuan / Ba Ria
250 Vung Tau, Dak Lak), two in Cambodia (Kracheh, Tboung Khmum), and four in Laos
251 (Bolikhamxay, Vientiane, Salavanh, Champasak). All survey work was carried out during the
252 region's main dry season, when mealybug populations are generally increasing (e.g., Graziosi et

253 al., 2016). Nearly 80% of the fields were planted with one of two popular cassava varieties
254 (KM94 and Rayong 72), while in the other fields, less common varieties were cultivated or
255 varietal mixes were used. Five representative linear transects (approx. 10 m in length; covering
256 10 plants) were assessed for the presence of arthropods, and the number of resident mealybug
257 species per field transect. We also examined plants for symptoms of cassava witches broom
258 disease (CWB), a phytoplasma disease that is commonly found in local fields (Alvarez et al.,
259 2013; Graziosi et al., 2016). Mealybug species identity was determined according to
260 morphological characteristics such as coloration and presence of abdominal waxy filaments (i.e.,
261 short- or long-tailed). This permitted field identifications of the most common invasive
262 mealybugs in Asia's cassava crops, including *P. manihoti*, *P. marginatus*, and *P. jackbeardsleyi*.
263 Also, in mixed-species infestations of long-tailed mealybugs, *P. jackbeardsleyi* tends to be the
264 prevalent species in Vietnam and Laos (Graziosi et al., 2016). Average abundance or incidence
265 levels for each of the different species were then calculated at a field level and used for
266 subsequent analyses.

267

268 2.2.2. Soil sampling and sample analysis

269 Leaf chemistry of mid- to late-season cassava is largely reflective of soil fertility status at early
270 growth stages (e.g., Schulthess et al., 1997). In this experiment, we relate mealybug abundance
271 to soil texture and fertility measures from samples collected at the time of the arthropod surveys
272 (see Section 2.2.1; on 7-9 month old plants in the dry season). By doing so, we likely overlook
273 eventual impacts of fertilizer supplementation at the time of planting, but do capture the effect of
274 background soil fertility. This approach though is suitable given that fertilization practices are
275 relatively uniform across the study region (except for Tay Ninh, Vietnam). One soil sample was

276 collected along each of the five survey transects in each field. For each transect, two soil sub-
277 samples were collected from within the planting row (5-10 m apart) at two depths (0-20, 20-40
278 cm) using a 5 cm dia. corer. Soils from each transect were composited by depth, while rocks,
279 roots and other debris were removed prior to air-drying of each composite sample. Once all
280 samples were collected and dry, they were submitted to the soil diagnostics laboratory of the Soil
281 and Fertilizer Research Institute (SFRI) in the Vietnam Academy of Agricultural Sciences
282 (VAAS), in Hanoi, for nutrient analysis.

283 A suite of measurements of soil fertility and texture were conducted. Soil texture was assessed
284 according to the Bouyoucos method (Gee & Bauder, 1986). Other variables include pH (1:1,
285 soil:water solution), electrical conductivity (EC; 1:5, soil:water solution), and exchangeable Ca,
286 Mg and K (extracted with ammonium acetate ($\text{NH}_4\text{CH}_3\text{CO}_2$) at pH 7 and measured by atomic
287 absorption spectrometry (AAS, Perkin Elmer 3100; Perkin Elmer, Norwalk, CT) and flame
288 photometry (Elex 6361; Eppendorf, Hamburg, Germany) (Herrmann, 2005). Additionally, we
289 measured total organic C using the Walkley-Black method and total N using the Kjeldahl
290 method. Total P was measured using sulfuric acid-hydrogen peroxide-hydrofluoric acid digestion
291 with 18M H_2SO_4 , while total K was determined using hydrofluoric acid (HF) and either H_2SO_4
292 or HClO_4 (Sparks, 1996). Finally, available P was determined using Bray and Kurt (Bray II)
293 method and acidity (Al^{3+} , H^+) was measured through titration with a KCl 1M solution (Sparks,
294 1996).

295

296 2.3.Comparative evaluation of *P. manihoti* parasitism

297 2.3.1. Field sites

298 In a third set of experiments, we conducted a comparative field-level assessment of the strength
299 of top-down regulation of *P. manihoti* by parasitism. Cassava fields of different developmental
300 stages and varying soil fertility were selected. During 2014 and 2016, targeted sampling was
301 performed during the dry season (January-March) in plots in Tay Ninh, Ba Ria Vung Tau and
302 Binh Thuan (Vietnam) and Kracheh (Cambodia). In 2014, fields were visited as part of a larger,
303 region-wide survey of *P. manihoti* parasitism, in which no particular attention was paid to soil
304 variables. Fields that were visited in 2016 took into account observed trends in soil texture and
305 fertility from earlier site visits (Section 2.2). In these surveys, soil fertility and crop
306 intensification schemes were not specifically evaluated but rather inferred based on province- or
307 district-level trends (as equally reflected in the PCA; Fig. 1). Cassava crops in Ba Ria Vung Tau
308 and Binh Thuan were 7-9 months old, established at low-fertility sites with sandy soils. Cassava
309 crops in Tay Ninh were either 2-3 months old (crop development status similar to the potted
310 plant fertilizer assays) or 7-9 months old, and established at intermediate-fertility sites under
311 intensified cropping schemes (i.e., with substantial input of fertilizer and herbicides). Lastly,
312 crops in Kracheh were 7-9 months old, established under relatively high soil fertility conditions
313 and with low-intensity management schemes (i.e., little or no fertilizer supplementation at
314 planting). Multiple of the fields in Kracheh were equally visited for the 2015 arthropod survey
315 (see Section 2.2).

316

317 2.3.2. *Sample collection and assessment of parasitism levels*

318 Within each region, up to eight different fields were visited and 10-20 mealybug-infested tips
319 were collected from each field. Plant tips were placed in sealed paper bags and transferred to the
320 laboratory. Before bagging, apparent predators such as ladybeetles and lacewing larvae were

321 removed (see Meyhofer & Klug, 2002). Sample bags with plant material were kept in a cooler
322 while being transported to the laboratory. In the laboratory, cassava tips were examined, the total
323 number of *P. manihoti* was counted, and tips with >10 individuals were further processed. Other
324 mealybug species were discarded. Mealybug individuals from each tip were gently brushed onto
325 a young cassava plant, placed in a transparent, 40 x 25 cm polypropylene (PP) plastic container
326 that was provided with a mesh screen on the side. Daily collections of emerging parasitoids were
327 made with an aspirator for 18 days. In Ba Ria Vung Tau and Binh Thuan, local collaborators
328 adopted a slightly modified methodology to record field-level parasitism rates that proved
329 equally effective. Specifically, field-collected cassava tips were transferred to 95 mm diameter
330 transparent PET plastic cups (390 ml), with each tip inserted into humidified floral foam. Each
331 cup was closed with a lid, provided with a mesh screen to permit air circulation. Cassava tips
332 with >10 *P. manihoti* individuals were transferred to the cups, placed within a field laboratory at
333 ambient temperature, and kept for 14 days (until full emergence of parasitoids). Parasitoid
334 emergence was evaluated on a regular basis, and emerged wasps were removed from cups. For
335 each site and field, *P. manihoti* abundance on field-collected cassava tips was recorded, and
336 field-level parasitism rates and parasitoid sex ratio were subsequently computed.

337

338 2.4. Statistics

339 In experiment #1, *P. manihoti* and *A. lopezi* development, reproductive and survival measures
340 were tested for normality (PROC UNIVAR) and Analysis of Variance (ANOVA) on log-
341 transformed data was used to evaluate the effect of fertilization on these parameters (PROC
342 GLM; SAS version 9.4).

343 In the first field study, bivariate relationships between each mealybug species, corresponding
344 soil fertility parameters and *CWB* infection status were investigated (using Kendall’s rank
345 correlation analysis). Next, regression was performed to model the combined effect of soil
346 fertility parameters and plant quality measures on the incidence of individual mealybug species.
347 For *P. manihoti*, a general linear model based on negative binomial distribution was adopted, as
348 the incidence data for this species was significantly zero-inflated. Simultaneous forward and
349 reverse stepwise selection on all 14 parameters up to 2-way interaction was performed on a
350 saturated model, starting with a null model (i.e., a model containing only the intercept), so as to
351 select the best models for each species. This analysis yielded only two models in total for *P.*
352 *marginatus* and *P. manihoti*, while for *P. jackbeardsleyi*, three models were identified. To select
353 the best model, among the ones identified in the previous step, we employed a similar strategy as
354 in Noma et al. (2010). A model with the lowest Akaike information criterion (AIC) (Model 1, i.e.
355 “best-fit” model in Table 4), and another model (Model 2, i.e. “competing-model” in Table 4),
356 with an AIC score that is within 2 units of the AIC score of Model 1 were selected. Diagnostic
357 checks such as assessment of heteroscedasticity (using Non-constant Variance Score Test) and
358 auto-correlating factors was also performed on the selected models. Correlation analysis, was
359 performed using the base function “cor” with the Kendall tau b method in R (version 3.3.1)
360 statistics environment (R Development Core Team, 2016). Regression modeling and the
361 associated model fitting diagnostics was performed using the base function “step”, the MASS
362 package (<http://cran.r-project.org/web/packages/MASS>) and the CAR package ([https://cran.r-](https://cran.r-project.org/web/packages/car)
363 [project.org/web/packages/car](https://cran.r-project.org/web/packages/car)) in R (version 3.3.1) statistics environment (R Development Core
364 Team, 2016).

365 Since individual soil fertility measures tend to be strongly correlated (e.g., Fujita et al., 2013),
366 we conducted Principal Component Analysis (PCA) to extract the main axes of variation. The
367 dataset that was subjected to multivariate analysis was composed of a total of 13 soil fertility
368 measures (i.e., crude sand, W silt, fine sand, pH, EC, Al, K, Ca, Mg, C, N, P, avail P) and field-
369 level incidence of cassava witches broom (CWB) disease, the latter as an additional index for
370 plant resource quality. Systemic pathogens can bring about important shifts in plant quality and
371 secondary chemistry, which rarely get taken into account (Tack & Dicke, 2013). From the PCA,
372 factor loading scores (i.e. scores for each field) were extracted for the two main axes of variation
373 (PCA axis 1 and 2). A general linear model based on negative binomial distribution was used to
374 relate field-level abundance measures of *P. manihoti*, *P. jackbearsleyi* and *P. marginatus* with
375 the factor loading scores. In addition, a Chi-square based test was performed on residual
376 deviance measures from the selected models, in order to obtain a goodness-of-fit measure.
377 Multivariate analyses were performed using the base function “princomp”, and the resulting
378 biplot was visualized with the ggbiplot package (<https://github.com/vqv/ggbiplot>), within the R
379 statistical environment. Eigen values for each component were extracted using the nFactors
380 package (<https://cran.r-project.org/web/packages/nFactors/>). Components with an Eigen value
381 lower than 2 were disregarded for further analysis. Regression modelling and model
382 visualization was performed using the MASS and CAR packages within the R statistical
383 environment. In order to identify the effect of aggregated groups, each consisting of multiple
384 fertility measures on mealybug abundance, multivariate analysis of the 13 soil fertility measures
385 and CWB incidence was performed.

386 In experiment #3, ANOVA or non-parametric tests (e.g., Kruskal-Wallis, for data that did not
387 meet normality assumptions) was used to compare parasitism rates, sex ratio and mealybug

388 abundance rates between different sites. Normality and homoscedasticity of the dataset was
389 checked, and the necessary data transformations (i.e., SQRT) were conducted prior to statistical
390 analysis.

391

392 **3. Results**

393 3.1. Potted plant fertilizer trials

394 Mealybugs feeding on fertilized plants developed more rapidly than those reared on the controls,
395 with the high N and medium K addition treatments having the strongest effects (Table 1a).
396 Nutrient addition did not affect *P. manihoti* survival, but N and the lower K regimes had positive
397 effects on both insect weight and length. Total fecundity was highest for females on plants
398 treated with the lower N dose and lowest on control plants (Table 1a), while oviposition rate did
399 not vary among treatments. However, both pre-oviposition and oviposition periods were strongly
400 affected by nutrient additions. Nitrogen additions led to the shortest pre-oviposition, followed by
401 K and then the controls, but no differences were observed between the two application rates for
402 either nutrient. Similarly, N applications extended the insects' oviposition period, but no clear
403 impact of K on this parameter was recorded (Table 1a).

404 On N-fertilized plants, parasitism rates were significantly higher than for control or K-amended
405 treatments (Table 1b). Parasitoid females in the fertilized treatments attained higher fecundity
406 levels than with the un-fertilized controls, regardless of nutrient type and application rate.
407 Parasitoid oviposition rate was significantly higher on N-fertilized plants. Fertilizer treatments
408 also affected *A. lopezi* offspring, such that emergence rates were significantly higher in all
409 fertilizer treatments, and sex ratio was far more female-biased for either nutrient supplement,
410 particularly on N-fertilized plants (Table 1b).

411

412 3.2.Observational studies

413 All three species of mealybug were found in cassava fields across the surveyed region, with
414 field-level incidence and abundance exhibiting significant differences between fields and
415 countries. Mealybug incidence significantly varied between countries for *P. jackbeardsleyi* ($F_{2, 62} = 7.431, p < 0.001$) and *P. marginatus* ($F_{2, 62} = 11.832, p < 0.001$), while plant-level abundance
416 differed between countries only for *P. marginatus* ($F_{2, 58} = 3.532, p = 0.036$) (Table 2). On
417 average, 31.2 ± 27.9 plants per field were affected and symptomatic for CWB, with disease
418 incidence levels significantly different between countries ($F_{2, 62} = 7.556, p = 0.001$).

420 Bivariate correlations were found between field-level abundance levels of a given mealybug
421 species, and a set of single soil fertility measures (Table 3). More specifically, statistically-
422 significant negative correlations were found between *P. manihoti* abundance and silt content,
423 organic C, N and available P, while crude sand content demonstrated a significant positive
424 relationship. For *P. jackbeardsleyi*, in-field abundance was positively correlated with CWB
425 incidence, soil pH, EC, and available P, but negatively correlated with Al^{3+} content. For *P.*
426 *marginatus*, significant negative correlations were observed with Ca^{2+} content, Mg^{2+} content, soil
427 organic C and total N. Above values correspond to Kendall tau b (τ_B) correlation coefficients
428 (Table 3), indicating a measure of concordance between the measured variables.

429 When evaluating the combined effect of multiple soil fertility and plant quality measures,
430 different species-specific patterns were found (Table 4). Both the best-fitting model, and the
431 competing model showed that abundance of *P. marginatus* was negatively related to total soil N,
432 available P and the fine sand soil fraction, and positively related to CWB incidence. Similar to *P.*
433 *marginatus*, both CWB incidence (positive) and N (negative) were found to be related to *P.*

434 *jackbeardsleyi* abundance. However, unlike in the case of *P. marginatus*, soil C was also
435 positively associated with the abundance of *P. jackbeardsleyi*. In the stepwise multiple
436 regression analysis, only silt+clay content of the soil was negatively related to the abundance of
437 *P. manihoti*. Multiple R^2 values were not obtained for the selected models of *P. manihoti*, as the
438 models were built using a negative binomial regression approach. For the regression analyses,
439 variation partitioning showed that, for *P. marginatus*, 34% of the variability was explained by N,
440 15% by Ca^{2+} and 13-14% each by EC and fine sand. Meanwhile, for *P. jackbeardsleyi*, 31% of
441 the variability was explained by CWB infection, 25% by Al^{3+} content, 20% by P, and <10% each
442 by N and K.

443 Principal component analysis extracted important degrees of variation and the associated
444 loading values (Table 5) in a combined dataset of soil fertility profiles and CWB incidence levels
445 (reflecting overall plant resource quality), with the first two components representing 55.8% of
446 the overall variance. The first PCA axis represented 36.3% of variance and was largely reflective
447 of overall nutrient availability, as determined by major (N, P, Ca, Mg) elements, organic C and
448 soil texture (Fig. 1). Fields in the left side of the PCA panel (i.e., with negative PC1 values) were
449 characterized by conditions of high soil fertility, including recently cleared and burned plots in
450 Cambodia, where cassava had been grown for 2-5 years with limited external inputs. On the right
451 side of the PCA were fields from Binh Thuan provinces with sandy soils. Plots with
452 intermediate soil fertility, staggered growing cycles and occasionally more intensive agro-
453 production schemes (i.e., ample N usage, high-quality planting materials, herbicide use) were
454 located towards the center of the fertility gradient defined by PC1, and included fields from Dak
455 Lak (Vietnam) and Laos.

456 The second PCA axis represented 19.5% of variance, and was largely reflective of Ca, Mg, Al,
457 EC, sand content and pH. Fields in the upper side of the biplot (i.e., positive values) were
458 characterized by sandy soils with high EC and high levels of elements such as Ca or Mg, while
459 more weathered fine textured (silt or clay) soils with high levels of Al oxides were found in the
460 lower part of the PCA panel. Incidence of CWB (as additional determinant of plant resource
461 quality) was associated with N content, organic C levels and % silt and clay fractions. The PCA
462 analysis also differentiated fields from the different countries, with Cambodia's flat alluvial soils
463 generally high pH, Ca and Mg content, Vietnamese plots typified by sandy texture and low soil
464 fertility, and Lao soil differentiated by comparatively higher levels of Al oxides (Fig. 1).

465 Principal component regression was then carried out on aggregate measure of soil fertility (as
466 reflected by PC1 and PC2 axes). Given that several soil fertility measures (and plant disease
467 infection status) exhibited high levels of correlation, abundance of specific mealybug invaders
468 appears to be associated with a combination of several variables. Field-level incidence patterns
469 of all three mealybug species were significantly related to the first PCA axis (Fig. 2), with
470 positive relationships for *P. marginatus* and *P. manihoti*, and negative trends for *P.*
471 *jackbeardsleyi*. The second PCA axis showed significantly positive relationship with only *P.*
472 *manihoti* abundance and not with the other mealybug species.

473

474 3.3.Comparative evaluation of top-down pressures

475 In the third experiment, we specifically examined parasitism rates of *P. manihoti* for three
476 different sites as positioned along the soil fertility spectrum, reflective of the PC1 axis. Nearly
477 400 *P. manihoti*-infected cassava tips were collected over the course of the 2014 and 2016 dry
478 seasons. Out of these, 205 tips had >10 mealybug individuals and were monitored for parasitoid

479 emergence. In 2-month old crops at intermediate soil fertility, mealybug abundance showed high
480 levels of variability between individual tips, with a coefficient of variation (CV) of 1.77,
481 especially as compared to the CV values of 0.73 in fields in low-fertility settings. Mealybugs
482 reached average abundance levels of 41.0 ± 30.0 , 50.6 ± 89.7 , 71.5 ± 123.7 and 101.2 ± 158.9
483 individuals per tip in Binh Thuan on 7-9 month old cassava, Tay Ninh on 2-3 month old cassava,
484 Tay Ninh on 7-8 month cassava, and Kracheh on 7-9 month old cassava, respectively.
485 Associated parasitism levels (proportion) were 0.10 ± 0.15 , 0.52 ± 0.40 , 0.57 ± 0.32 and $0.32 \pm$
486 0.27 , respectively, for the four samplings. Parasitoid communities were largely dominated by the
487 parasitic wasp *A. lopezi*. Mealybug numbers did not differ between sites and field conditions
488 (Kruskal-Wallis, $X^2= 5.96$, $p=0.114$), but parasitism rates were significantly higher in the
489 intermediate fertility site (i.e., Tay Ninh), than at other locales (Kruskal-Wallis, $X^2= 74.41$,
490 $p<0.001$; Fig. 3).

491 Total number of parasitoids (per tip) significantly differed between sites (Kruskal-Wallis, $X^2=$
492 52.05 , $p<0.001$). Regression analyses revealed that parasitoid abundance was closely related to
493 *P. manihoti* infestation pressure, with different patterns of density-dependence for each of the
494 soil fertility conditions as such: Kracheh ($F_{1,18}= 10.781$, $p< 0.01$), Tay Ninh 2 months ($F_{1,85}=$
495 77.183 , $p< 0.01$), Tay Ninh 8 months ($F_{1,38}= 83.602$, $p<0.001$) and Binh Thuan ($F_{1,54}= 8.625$, $p<$
496 0.01 ; Fig. 3). Positive density dependence was recorded in all sites, with the strongest parasitoid
497 response at intermediate fertility sites (i.e., Tay Ninh). Although no data were obtained from
498 Binh Thuan and Ba Ria Vung Tau provinces, parasitoid sex ratio from sites in Cambodia and
499 Tay Ninh were not significantly different (Kruskal-Wallis, $X^2= 3.13$, $p=0.209$).

500

501 4. Discussion

502 Much remains to be learned about the regulatory forces that shape ecological communities in
503 terrestrial systems (Gruner, 2004; Borer et al., 2006; Allen & Wesner, 2016). The
504 agroecosystems studied here provide a unique and highly-relevant opportunity to evaluate
505 trophic regulation processes. In tropical agroecosystems in particular, soils tend to be highly
506 weathered and thus plant resource or bottom-up effects can exert strong effects on herbivore
507 communities that may either overshadow the role of top-down regulation (Ritchie, 2000) or
508 influence herbivores by affecting the strength of top-down forces. While past research has
509 examined how fertilizer addition impacts particular feeding guilds and plant-herbivore
510 interactions (Sipura, 1999; Forkner & Hunter, 2000; Ritchie, 2000; Garibaldi et al., 2010;
511 Rzanny et al., 2013), much of this work has been conducted in perennial ecosystems. Our study
512 is unique in the extent to which it relies upon community ecology approaches, to assess invader
513 success mitigated by plant resource constraints in a rapidly-expanding tropical agricultural
514 system.

515

516 4.1 *Soil fertility impacts on herbivore and parasitoid performance*

517 Plant chemistry, largely determined by soil fertility, affects life history and physical
518 characteristics of herbivores as well as higher trophic orders (Ode, 2006; Chen et al., 2010; Stam
519 et al., 2014). Plant stoichiometry, defense mechanisms (constitutive or induced), and primary
520 productivity are all factors that affect plant-herbivore interactions and impacts on parasitoids or
521 predators. Through our manipulated “microcosm” studies, we gained an initial appreciation of
522 how soil fertility and/or availability of key limiting nutrients impacts such interactions between
523 plants, the herbivore, *P. manihoti*, and the parasitoid, *A. lopezi*. Nitrogen fertilization led to a
524 reduction in *P. manihoti* development time along with an increase in body size, adult weight and

525 total fecundity. Meanwhile, K addition had less pronounced effects, and only intermediate
526 fertilization levels seemed to benefit *P. manihoti* growth and reproduction. These findings are in
527 line with results from other herbivore-plant systems, in which sap-feeders such as aphids
528 increase their populations on short-season crops when soils are deficient in K, or under N
529 supplements (Noma et al., 2010). While both nutrients influence phloem content of dietary N, a
530 limiting nutrient for the development of homopterans such as mealybugs (Dixon, 1998), the
531 effect of K fertilization may only be apparent when plants are K-stressed (e.g., Walter &
532 DiFonzo, 2007). For *A. lopezi*, laboratory trials corroborate previous findings that its
533 development rate and sex ratio are shaped by the size of its host (van Dijken et al., 1991;
534 Schulthess et al., 1997). The microcosm studies conducted here, thus indicate that *P. manihoti*
535 (and indirectly *A. lopezi*) abide to Price's (1991) 'plant vigor hypothesis', in which vigorously-
536 growing plants are better hosts for herbivores and N disproportionately supports herbivore growth
537 as it is the basis for protein synthesis. This is aligned with *P. manihoti* feeding habits, as this
538 insect prefers nutrient-rich, actively growing tissues (White, 2009). Laboratory findings reported
539 here are also reflected by the strong parasitoid response to high *P. manihoti* population levels
540 that was evident from the field samplings in Tay Ninh (i.e., at settings with intermediate soil
541 fertility and N enrichment, based on farmer discussions; Fig. 3). Our microcosm studies could
542 thus constitute a first step towards defining a crop-specific range of N concentrations that benefit
543 plant growth and boost a plant's immune responses or optimize biological control (Chen et al.,
544 2010).

545 While microcosm studies are valuable and informative, it's often difficult to extrapolate their
546 results to field conditions particularly when considering short-term pot assays for semi-perennial
547 woody plants, such as cassava, which can have 2-m long roots (Connor et al., 1981). Also,

548 performance of herbivores such as mealybugs is determined by the nutritional content and
549 quality of above-ground plant parts, but addition of single-element fertilizer does not necessarily
550 affect those. For example, K fertilization regularly causes minor variation in leaf nutrient
551 content, while leaf N content can concurrently be controlled by multiple soil fertility parameters,
552 e.g., soil pH, P content or water availability (e.g., Lower & Orians, 2003; Fujita et al., 2013).
553 Hence, the effect of a single nutrient addition on host-parasitoid systems can easily be obscured
554 by other plant growth-limiting factors. In fertilizer supplement studies, the strongest effects are
555 often found when nutrient limitation is alleviated (see Elser et al., 2007). As our microcosm
556 studies were conducted using relatively fertile, nutrient-rich soil instead of inert media (e.g.,
557 sand), thus some of the envisaged effects on herbivore-parasitoid complexes may have been
558 dampened (see Gutierrez et al., 1988). Similar to work by Denno et al. (2002), we observe a
559 correspondence between laboratory studies and field surveys for *P. manihoti*, both of which
560 suggest a dominance of bottom-up controls. While we cannot draw conclusions for the other two
561 species of mealybugs, bottom-up impacts are likely determined by their particular traits and
562 associated parasitoid (complex). We can infer that improved mealybug performance is related to
563 increased leaf N content, though this was not measured, so this conclusion is somewhat
564 speculative. In the meantime, it should be noted that individual-level measurements of the effect
565 of soil fertility and plant nutritional quality on herbivore or parasitoid performance may not
566 necessarily translate into population effects (Zaugg et al., 2013).

567 In our study, field observations indicated that population levels for the three invasive
568 herbivores across the various sampling sites were correlated to a soil fertility with distinct soil
569 fertility parameters. Our findings suggest species-specific relationships with single measures of
570 soil fertility, texture and disease infection status. While correlations don't necessarily entail

571 causality, observed patterns do differ substantially between the three invaders. For *P. manihoti*,
572 in-field incidence was positively correlated with sand content, and negatively with silt, soil C, N
573 and P (Table 3). This supports previous work in Africa suggesting that *P. manihoti* achieves high
574 population levels on low-fertility, sandy soils, even in the presence of effective parasitoids
575 (Neuenschwander et al., 1990). On the other hand, the abundance of *P. jackbeardsleyi* appeared
576 to follow a distinct pattern and was largely associated with pH, EC and CWB-infestation status.
577 Given the multi-dimensional usage of resources by plants, herbivores and parasitoids, an
578 aggregate measure (e.g., obtained through PCA) may be more suitable to capture species'
579 responses to soil- or resource-based conditions (see also Drenovsky et al., 2012; Fujita et al.,
580 2013). Nevertheless, various single soil fertility measures did correlate significantly with field-
581 level abundance for all three mealybug species (Table 3) and both approaches are likely valuable
582 for understanding soil impacts on herbivore performance.

583

584 *4.2 Life history and field management drivers for invader success*

585 Differential life history traits and invasion history of the three invaders might help explain
586 some of observed disparate response trends. As globally successful invaders, all three mealybug
587 species could benefit from similar high phenotypic plasticity or adaptation potential (e.g.,
588 Dawson et al., 2012), but may still differ in myriad other aspects. Although there's no single trait
589 that reflects invasiveness, so-called 'invader attributes' tend to comprise competitive ability,
590 phenotypic plasticity, niche construction and phenological niche separation (e.g., Perkins &
591 Nowak, 2013). Amongst others, the outcome of invasions is set by the interaction of the above
592 species' traits with nutrient availability over short and long time periods (Mata et al., 2013).
593 Competitively-inferior invaders capable of rapid population growth, such as *P. manihoti*, can

594 capitalize on short-term nutrient pulses, while competitively-superior or dominant invaders are
595 easily disrupted by disturbance-related resource heterogeneity. In our study, such disturbance-
596 related heterogeneity was indirectly measured through the soil fertility measurements in the field
597 survey, but only qualitatively inferred for the specific case of *P. manihoti* and *A. lopezi*. Life
598 history traits and feeding behavior can also explain comparative performance of specific
599 herbivores in given varying resource quality or soil fertility (White, 2009). For *P. manihoti*, the
600 ‘plant vigor hypothesis’ possibly may apply, with this species benefiting greatly under crop
601 management schemes with important levels of nutrient addition. On the other hand, species such
602 as *P. jackbeardsleyi* that feed preferentially on older senescing tissues and on CWB-affected
603 plants, might follow the ‘plant stress hypothesis’ and experience a niche opportunity on
604 debilitated plants with sub-optimum nutrition for other mealybug species. In studies with plant
605 hoppers, Denno et al. (2002) also pointed at mobility as a prime mediator of top-down vs.
606 bottom-up impacts for a given species. However, for largely sedentary species such as
607 mealybugs, mobility and specifically a species’ ability to elude aggregative responses of natural
608 enemies may be of limited relevance. Lastly, history of the invasion process can bring about
609 species-specific shifts in top-down forces. For a long-time invader such as *P. jackbeardsleyi*
610 (first reported from Asia in 1987), parasitoid communities possibly have had comparatively more
611 time to assemble, diversify or adapt (e.g., Shea & Chesson, 2002), and exert stronger top-down
612 pressures. Also, recent invaders relatively less burdened by natural enemies, such as *P. manihoti*
613 or *P. marginatus*, may outperform long-time invaders in high-resource settings (Blumenthal,
614 2005), or cassava might simply be a far superior host for them as compared to *P. jackbeardsleyi*.
615 Sites along the soil fertility continuum vary in historic land-use, current management practices,
616 as well as a range of important environmental parameters (e.g., climate, landscape

617 heterogeneity). More specifically, sites on the left side of the continuum (Fig. 1) include
618 recently-cleared swidden agriculture plots and fields under rotation with other crops (e.g.,
619 soybean). Under less intensive agricultural systems, C storage and soil quality can be
620 substantially higher than under continuous annual cropping systems (Bruun et al., 2009),
621 although these patterns can also be greatly influenced by soil fertility management and inherent
622 properties, such as soil texture. Land-use legacies can persist for decades and have profound
623 impacts on herbaceous species composition, biodiversity and resulting parasitoid communities
624 (Stahlheber et al., 2015; Stuhler & Orrock, 2016). This may be particularly relevant, as Lao and
625 Cambodian fields under swidden agriculture regimes and less intensive management had far
626 greater weed cover (Wyckhuys, unpublished). On the other hand, several plots in the central
627 portion of the soil fertility continuum (Fig. 1) were under more intensive crop management
628 practices (e.g., tillage, herbicide use, high-quality planting materials) and frequent additions of
629 N-P-K fertilizers. Actions such as N fertilization are not necessarily reflected in soil fertility
630 metrics, and fertilizer N additions are even likely to amplify N-loss pathways (see Lu et al.,
631 2011). Nevertheless, they can augment plant nutritional status, and subsequent herbivore
632 population growth and/or parasitoid development. Invader performance appeared to differ under
633 these varying contexts, and suggests that soil fertility (measured), disturbance frequency
634 (inferred, but not measured), and community composition or maturity (inferred, but not
635 measured) all contributed to shape invasion and invader dynamics (e.g., Mattingly & Orrock,
636 2013). While certain species were more successful in plots with high soil fertility, others thrived
637 under more resource-limited settings (see also Funk & Vitousek, 2007). Increased abundance of
638 *P. marginatus* and *P. manihoti* at sites with intensified agro-production and intermediate (or low)
639 soil fertility (Fig. 2) may hint that these species benefit primarily from nutrient pulses (inferred

640 from pot trials, but not measured in the field) and disturbance regimes (largely inferred, but not
641 measured). In contrast, enhanced presence of *P. jackbeardsleyi* in high-fertility settings suggests
642 a strong bottom-up effect and a substantially shortened “window of vulnerability” to resident
643 natural enemies (see ‘slow growth – high mortality’ phenomenon; Benrey & Denno, 1997). For
644 *P. manihoti*, bottom-up effects were evident and top-down forces appeared to be strengthened in
645 high-fertility soils or plots with external nutrient enrichment (Fig. 3). Such increases of top-down
646 forces with resource inputs have been recorded in several other systems (e.g., Hunter & Price,
647 1992; Forkner & Hunter, 2000; Walker et al., 2008). Our results also echo those of Ritchie
648 (2000), in which bottom-up influences are quite pronounced in resource-limited settings, while
649 some herbivores can experience far stronger top-down forces within environments with fertile
650 soils and N-rich plant tissue. Though patterns are highly species-specific, soil nutrient profiles
651 and fertilizer additions shape host plant quality and either enhance or reduce an individual
652 mealybug’s relative niche opportunity (Stiling & Moon, 2005).

653

654 **5. Conclusions**

655 Our work points at differential trophic regulation for three invasive mealybugs in tropical
656 agroecosystems, and elucidates important species-specific and context patterns. The findings
657 presented here emphasize how biological control is strongly dependent upon site fertility for the
658 particular case of *P. manihoti* (see Hovick & Carson, 2015), and illuminate resource-mediated
659 performance of two other key mealybug invaders. Microcosm experiments clearly emphasized
660 the potential of plant nutrient availability, N in particular, for regulating the performance of
661 multiple trophic levels in cassava systems. Meanwhile, results from regional field studies show
662 important species-specific responses of invasive herbivores (and their parasitoids) to a gradient

663 in soil fertility and management intensity. Our results support the notion that soil fertility and
664 plant quality variables, either singly or as composite indices, should be taken into consideration
665 when setting priorities for invasive species management or planning biological control
666 interventions (Mace & Mills, 2016). Last but not least, our findings from smallholder systems in
667 the developing-world tropics provide renewed impetus for earlier calls to address agricultural
668 pest management in a more holistic and integrated fashion (e.g., Lewis et al., 1997).

669

670

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681

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886

Table 1a. Development and reproductive outputs of the cassava mealybug (*P. manihoti*) feeding on plants treated with different fertilizer treatments. Means (\pm SD) followed by the same letter do not differ (ANOVA, $\alpha = 0.05$).

Parameter (<i>P. manihoti</i>)	N	control	N90	N180	K ₂ O90	K ₂ O180	Test statistic; P-value
Development time (d)	527	15.5 \pm 0.06 a	15.0 \pm 0.06 b	14.2 \pm 0.07 d	14.3 \pm 0.07 d	14.8 \pm 0.09 c	F _{4,522} =61.24; P<0.0001*
Survival per plant (%)	60	85.5 \pm 0.02	87.0 \pm 0.03	91.0 \pm 0.03	89.0 \pm 0.02	89.0 \pm 0.03	F _{4,55} =0.8; P=0.53
Adult ^a weight (mg)	300	4.76 \pm 0.26 b	5.85 \pm 0.52 a	5.54 \pm 0.69 ab	6.4 \pm 0.16 a	5.00 \pm 0.24 b	F _{4,295} =3.78; P=0.016*
Adult length (mm)	300	2.01 \pm 0.02 c	2.15 \pm 0.02 b	2.14 \pm 0.02 b	2.32 \pm 0.04 a	1.98 \pm 0.05 c	F _{4,295} =18.43; P<0.0001*
Total fecundity (eggs)	279	369.0 \pm 8.6 c	438.0 \pm 11.3 a	419.6 \pm 9.0 ab	403.8 \pm 10.3 b	395.8 \pm 10.8 b	F _{4,274} =7.75; P<0.0001*
Oviposition rate (eggs/d)	279	23.4 \pm 0.5	24.4 \pm 0.5	24.0 \pm 0.5	23.9 \pm 0.8	23.6 \pm 0.5	F _{4,274} =1.04; P=0.389
Pre-oviposition period (d)	279	5.4 \pm 0.1 a	4.4 \pm 0.1 c	4.0 \pm 0.1 c	5.2 \pm 0.2 b	4.9 \pm 0.1 b	F _{4,274} =20.51; P<0.0001*
Oviposition period (d)	279	16.0 \pm 0.3 b	18.0 \pm 0.3 a	17.7 \pm 0.4 a	17.0 \pm 0.3 ab	16.8 \pm 0.4 b	F _{4,274} =5.13; P=0.0005*

^a Young females (stage L4, prior to oviposition)

Table 1b. Parasitism levels and reproductive output of *A. lopezi*, when developing on *P. manihoti* nymphs reared on plants with different fertilizer treatments. Means followed by the same letter do not differ (ANOVA, $\alpha = 0.05$), while an asterisk indicates statistical significance.

Parameter (<i>A. lopezi</i>)	N	control	N90	N180	K2O90	K2O180	Test statistic; P-value
Parasitism rate (proportion)	413	0.27 \pm 0.005 c	0.29 \pm 0.008 ab	0.30 \pm 0.008 a	0.27 \pm 0.008 bc	0.27 \pm 0.006 bc	F _{4,408} =3.42 P=0.009*
Lifetime fecundity (# mummies)	60	87.3 \pm 2.0 b	99.4 \pm 2.9 a	100.6 \pm 3.3 a	96.6 \pm 3.8 a	96.4 \pm 2.8 a	F _{4,55} =18.43; P<0.0001*
Oviposition period (d)	60	6.5 \pm 0.24	7 \pm 0.21	6.8 \pm 0.20	7.1 \pm 0.23	7.1 \pm 0.18	F _{4,55} =2.07 P=0.097
Oviposition rate (mummy/d)	60	13.5 \pm 0.3 b	14.2 \pm 0.2 ab	14.8 \pm 0.3 a	13.6 \pm 0.2 b	13.6 \pm 0.2 b	F _{4,55} =5.05 P=0.002*
Emergence rate (adults/mumm)	413	0.64 \pm 0.01 c	0.72 \pm 0.01 ab	0.71 \pm 0.01 a	0.89 \pm 0.02 b	0.73 \pm 0.01 ab	F _{4,408} =10.84 P<0.0001*
Offspring sex ratio (f/m)	413	0.60 \pm 0.02 c	0.69 \pm 0.03 ab	0.71 \pm 0.02 a	0.65 \pm 0.03 b	0.67 \pm 0.02 b	F _{4,408} =3.06 P=0.017*
Offspring longevity (d)	360	10.41 \pm 0.2	9.97 \pm 0.3	9.97 \pm 0.2	9.92 \pm 0.2	10.35 \pm 0.2	F _{4,355} =1.05 P=0.38*

Table 2. Incidence frequency (% sampled plants) and plant-level infestation rates (mealybug densities per plant) of three invasive mealybug species (*P. jackbeardsleyi*, *P. manihoti*, *P. marginatus*) for 65 cassava plots that were visited during the 2015 dry season in Vietnam, Cambodia and Laos. Incidence and infestation levels are indicated as mean \pm SD. Means followed by the same letter do not differ between countries (ANOVA, $\alpha = 0.05$; Tukey post-hoc test).

Mealybug species	Field-level incidence (% sampled plants)			
	Vietnam	Cambodia	Lao PDR	Regional
	<i>n</i> = 20	<i>n</i> = 18	<i>n</i> = 27	<i>n</i> = 65
<i>P. jackbeardsleyi</i>	12.8 \pm 9.1a	33.7 \pm 24.3b	15.6 \pm 18.4a	19.7 \pm 20.0
<i>P. manihoti</i>	10.4 \pm 10.9a	3.2 \pm 5.0a	8.6 \pm 13.7a	7.7 \pm 11.3
<i>P. marginatus</i>	56.5 \pm 25.7a	24.0 \pm 23.5b	26.9 \pm 21.9b	35.2 \pm 27.3
Plant-level infestation (individuals/plant)				
<i>P. jackbeardsleyi</i>	3.2 \pm 3.5a	61.6 \pm 137.1a	2.6 \pm 2.1a	10.1 \pm 47.8
<i>P. manihoti</i>	5.1 \pm 8.2a	56.8 \pm 136.8a	14.6 \pm 21.5a	17.4 \pm 57.3
<i>P. marginatus</i>	29.0 \pm 21.6a	38.7 \pm 76.4ab	5.7 \pm 11.3b	22.0 \pm 43.2

Table 3. Bivariate relationships between the three invasive mealybug species and single soil fertility parameters and CWB pathogen infection status. Values correspond to Kendall *tau b* (τ_B) correlation coefficients, which indicate a measure of concordance between corresponding variables. A negative sign before the value indicates negative relationship, while no sign indicates positive correlation. Values marked with an asterisk are coefficients that were statistically significant (**P-value** < **0.05**) for that particular comparison, when tested against a null hypothesis of no correlation ($\tau_B = 0$) between the variables.

	<i>P. manihoti</i>	<i>P. jackbeardsleyi</i>	<i>P. marginatus</i>
crude_sand	0.15*	-0.07	0.04
Silt	-0.30*	0.08	-0.13
fine_sand	0.21	-0.04	0.08
pH	0	0.27*	-0.15
EC	-0.02	0.10*	0
Al	-0.09	-0.30*	0
K	-0.18	0.10	-0.18
Ca	-0.14	0.26	-0.25*
Mg	-0.13	0.28	-0.20*
OC	-0.17*	0.07	-0.17*
N	-0.24*	0	-0.25*
P	-0.25*	0.16*	-0.16
P ₂ O ₅	0.10	0.10	0.08
CWB ^a	-0.04	0.18*	-0.13

Soil fertility parameters are abbreviated as follows: coarse sand corresponds to % of > 0.02 mm soil particles, silt is well-graded silt + clay ; fine sand is % soil particles 0.02-0.2 mm); pH is pH of soil suspension; EC is soil electron conductivity; Al is Al 3+ mg/100 g soil; K is K+ mg/100 g soil; Ca is Ca2+ mg/100 g soil; Mg is Mg2+ mg/100 g soil; OC is % of soil organic carbon); N is % nitrogen); P is % P₂O₅; P₂O₅ is mg P₂O₅ / 100 g soil.

^a CWB corresponds to field-level incidence of cassava witches broom disease, as recorded during transect walks.

Table 4. Stepwise multiple regression models for abundance of three different species of mealybug, in relation to soil fertility measures and pathogen-infection status (both measures reflecting plant resource quality). For each of the invasive mealybug species, two statistical models are represented, with their respective Akaike information criterion (AIC) and R².

<i>Mealybug species</i>	<i>Model equation*</i>	<i>AIC</i>	<i>Multiple R² value</i>
<i>Paracoccus marginatus</i>	Model I ^{**} : 45.02 – 125.46 x <i>N</i> – 0.36 x <i>fine_sand</i> – 40.37 x <i>P</i> + 0.26 x <i>CWB</i> + 184.63 x <i>EC</i> – 1.09 x <i>Ca</i>	315.47	36.1%
	Model II ^{**} : 48.61 – 153.53 x <i>N</i> – 0.37 x <i>fine_sand</i> – 41.36 x <i>P</i> + 0.25 x <i>CWB</i> + 139.79 x <i>EC</i>	316.27	33.1%
<i>Pseudococcus jackbeardsleyi</i>	Model I ^{**} : 10.27 – 102.78 x <i>N</i> + 0.29 x <i>CWB</i> + 5.39 x <i>OC</i> + 21.42 x <i>K</i>	270.27	41.0%
	Model II ^{**} : 12.55 – 10.54 x <i>Al</i> + 0.27 x <i>CWB</i> + 24.77 x <i>P</i> – 87.75 x <i>N</i> + 4.61 x <i>OC</i>	270.42	38.9%
<i>Phenacoccus manihoti</i>	Model I ^{**} : 3.21 – 6.23 x <i>silt</i> – 15 x <i>EC</i>	293.31	--***
	Model II ^{**} : 2.70 – 5.82 x <i>silt</i>	293.46	--***

* explanatory variables: *N*: nitrogen %; *fine_sand*: (0.02-0.2 mm fraction)%; *P*: P₂O₅ %; *CWB*: field-level incidence of cassava witches broom disease; *EC*: soil electron conductivity; *OC*: soil organic carbon %; *silt*: W silt + clay
 ** Model 1 corresponds to the model with the least AIC score (“best-fit model”), and model 2 (“competing model”) corresponds to the model that obtained an AIC score, that is within 2 units of the AIC score of the “best-fit model”.

*** Multiple R² values for the selected models of *P. manihoti* are not available, as the models were built using a negative binomial regression approach (see Materials & Methods section for more details).

Table 5. Loading values of all the measured soil variables and cassava witches broom –CWB- infestation (per field) obtained from the first two PCA components (PC1 and PC2) (see Figure 1 legend for explanation of the variables)

Variables	PC1	PC2
CWB	0.192	0.141
Crude sand (>0.02mm)	-0.133	-0.225
Wsilt and clay	0.326	0.355
Fine sand (0.02-0.2mm)	-0.302	-0.278
pH	0.302	-0.335
EC	-0.341	0.161
Al	-0.206	0.429
K	0.226	-0.157
Ca	0.324	-0.29
Mg	0.362	-0.275
OC	0.35	0.171
N	0.359	0.231
P	0.272	0.49
mgP2O5	-0.201	0.668

Figure legends

Figure 1. Principal component analysis (PCA) of 65 cassava fields, based upon soil fertility status and CWB pathogen-infection of host plants. Soil fertility status was determined through a total of 13 different measures, and CWB infection was assessed by recording disease symptomatology. Fields are classified per country, and soil fertility measures are abbreviated as follows: crude sand (% particles 630 μ m – 2mm); W silt (well-graded silt + clay); fine sand (% particles 63 μ m – 200 μ m); pH (pH of soil suspension); EC (soil electron conductivity); Al (Al 3+ mg/100 g soil); K (K+ mg/100 g soil); Ca (Ca2+ mg/100 g soil); Mg (Mg2+ mg/100 g soil); OC (% soil organic carbon); N (% nitrogen); P (% P₂O₅); P₂O₅ (mg P₂O₅ / 100 g soil).

Figure 2. Relationships between field-level abundance of *P. jackbeardsleyi*, *P. marginatus* and *P. manihoti*, as obtained through principal component regression. For each mealybug species, regression patterns are represented with the first and second PCA axis (i.e., PC1, PC2). Lines represent regression curves, with associated p values. Goodness-of-fit was assessed for each regression model, by testing for significant difference between the residual deviances of an ideal model (where the predicted values are identical to the observed) and the selected model using a chi-square test. None of the models yielded a significant p-value in the chi-square test, indicating that the selected model fit the data well.

Figure 3. Relationship between per-plant *P. manihoti* infestation level and parasitism rate in three different soil fertility contexts in four locations, as positioned along the PC1 axis. Locations include Kracheh (Cambodia) and Tay Ninh, Binh Thuan / Ba Ria Vung Tau (Vietnam), covering a high-fertility and intermediate fertility site, and a low-fertility site with sandy soils respectively. For the site in Tay Ninh, *P. manihoti* abundance and *A. lopezi* parasitism rates were assessed on 2-month and 8-months old crops. Regression curves represent statistically significant patterns, with the following coefficients of determination: Kracheh 7-9 months ($R^2= 0.375$), Tay Ninh 2 months ($R^2= 0.476$), Tay Ninh 8 months ($R^2= 0.688$), and Binh Thuan 7-9 months ($R^2= 0.138$). Parameter estimates for slopes of the regression curves were as follows: 56.44, 43.35, 24.87, and 7.95 respectively.

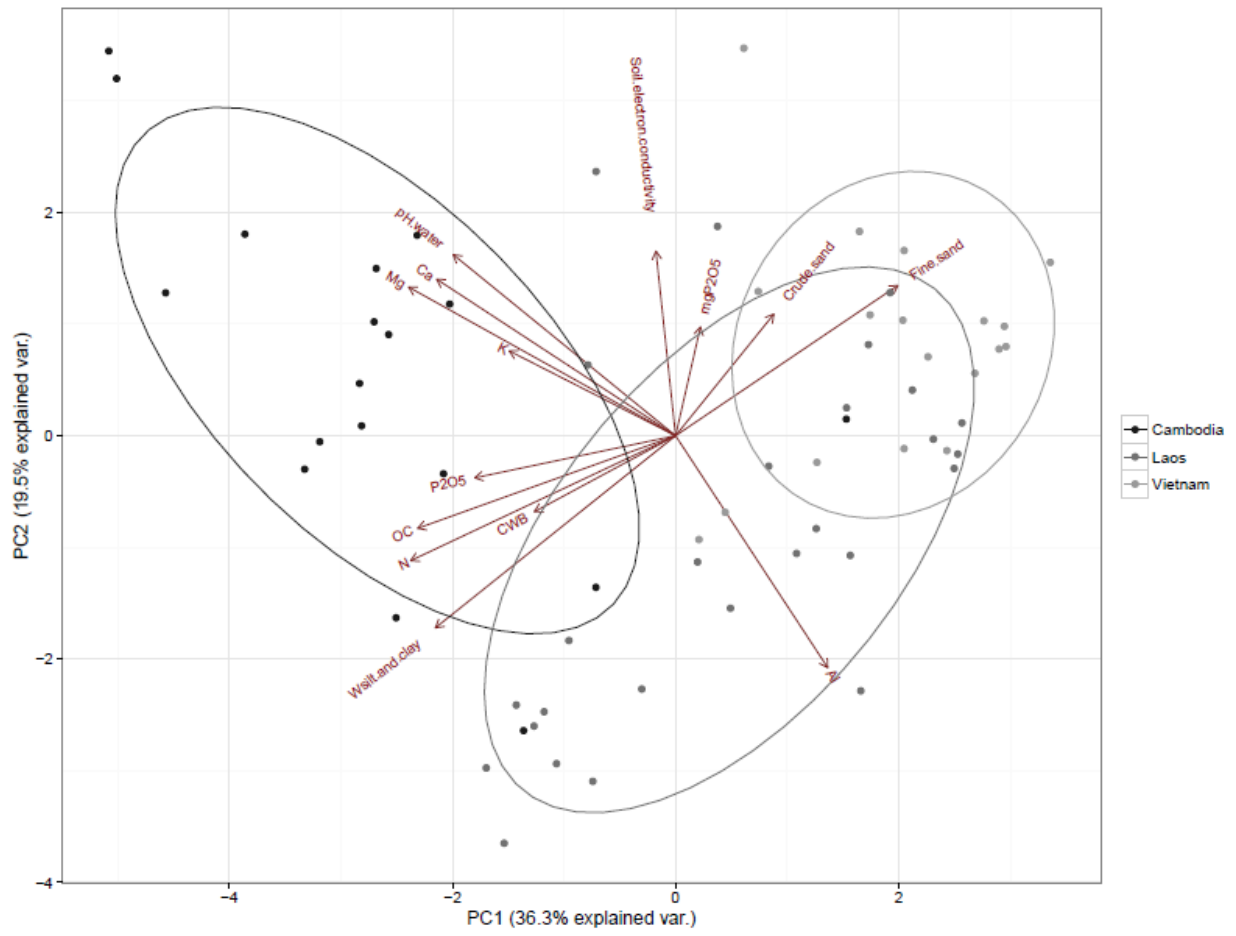


Figure 1.

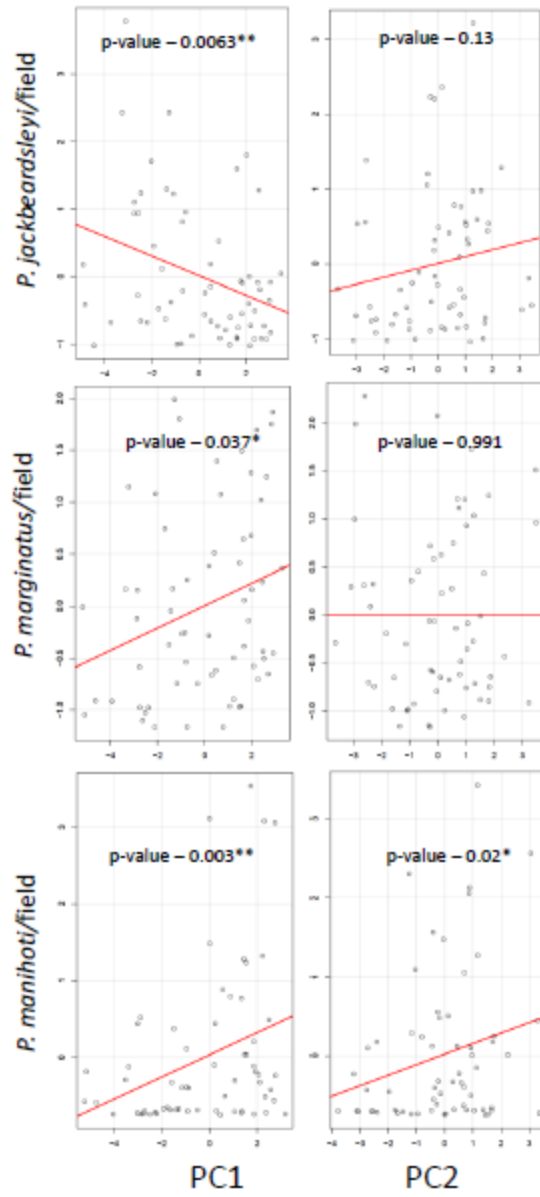


Figure 2.

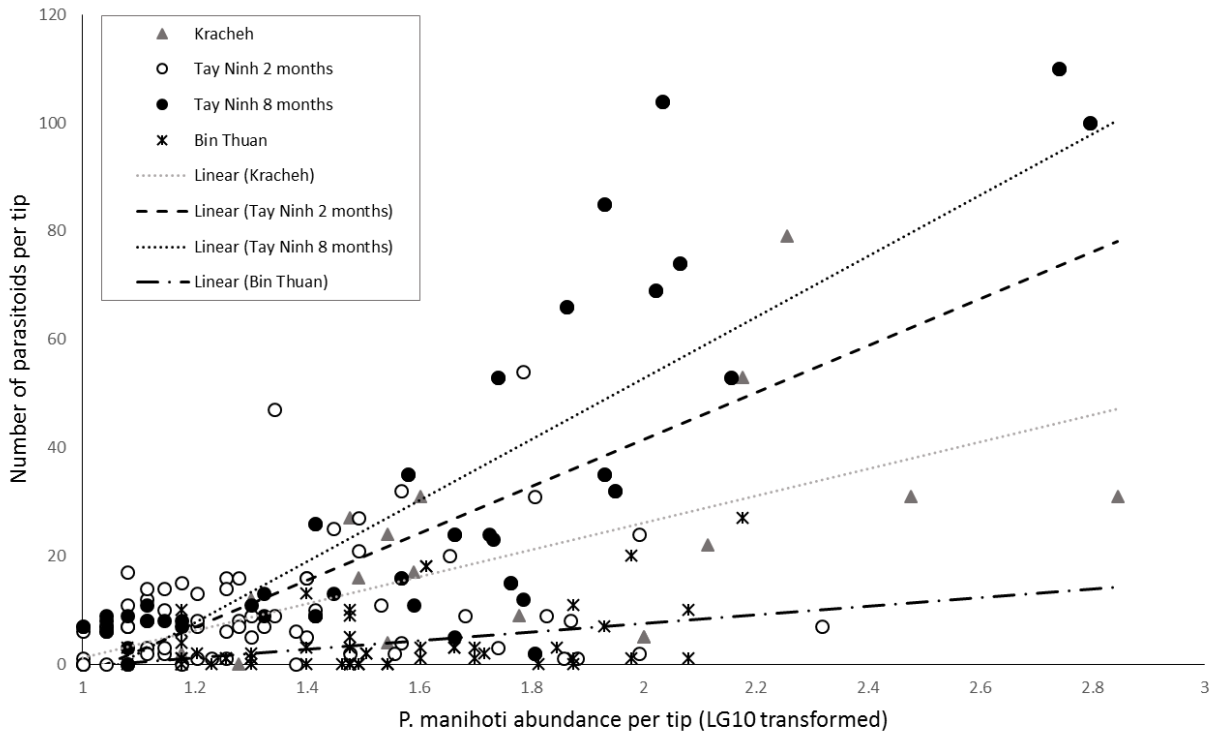


Figure 3.