

Bottom-up effects of soil quality on a coffee arthropod interaction web

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Abstract. Nutrient availability and soil quality influence herbivores through changes in plant traits and can have cascading effects on herbivore interactions. In complex systems, with many positive and negative interactions, the consequences of these bottom-up effects are still not well established. We carried out a set of studies to determine the impact of soil quality (organic compost amendments) on a hemipteran herbivore (*Coccus viridis*), two ant mutualists, predators, pathogens, parasitoids of *C. viridis*, and an arboreal arthropod community on coffee (*Coffea arabica*). We also determined the impact of *Azteca instabilis* ants on the arthropod community with an exclusion experiment. In an observational study, the carbon to nitrogen ratio (C:N) of leaf tissue correlated negatively with *C. viridis* density, however caffeine content did not correlate with *C. viridis*. In a field experiment with coffee seedlings, both *C. viridis* and total arthropod abundance were greater on high-quality plants than on low-quality plants. Excluding *A. instabilis* resulted in higher *C. viridis* abundance and parasitism rate, and higher spider and total arthropod abundance. Although *A. instabilis* attendance of *C. viridis* only marginally differed across soil quality treatments, in a second experiment, *Pheidole synanthropica* ants recruited more workers per *C. viridis* individual on high-quality relative to low-quality plants. Soil quality treatments did not impact predator abundance or fungal pathogen prevalence. These results suggest soil quality impacts *C. viridis* herbivores, *P. synanthropica* ants, and total abundance of arthropods on coffee, but did not impact the third trophic level. Thus this study provides a complex case study of pathways in which bottom-up effects influence arthropod interaction webs.

Key words: ant-hemipteran mutualism; *Azteca*; *Azya*; Chiapas, Mexico; *Coccus*; *Coffea*; indirect interactions; nutrients; *Pheidole*; plant quality.

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INTRODUCTION

Ecologists have long debated how biotic and abiotic factors control populations and provide structure to ecological communities (Hairston et

al. 1960, Hunter and Price 1992). For many terrestrial systems, nutrient availability can have important consequences on communities through direct effects on the quality and quantity of primary producers (plants), and indirect

effects on herbivores that consume plants (Awmack and Leather 2002). Theory also suggests that increased productivity can increase the length of food chains (Fretwell 1977, Oksanen et al. 1981, Abrams 1993). At the same time, the presence of some organisms at higher trophic levels, such as top-predators and mutualists of herbivores, can have strong community wide effects that cascade down to influence plant abundance and quality (Gratton and Denno 2003, Gruner 2004, Kay et al. 2004, Müller et al. 2005). It is now commonly acknowledged that both bottom-up and top-down forces work together to shape ecological communities, yet our understanding of how these forces interact with one another is still lacking because systems are often only studied from one point of view (Hunter and Price 1992).

Nutrient enrichment typically increases populations of herbivores that are limited by elements such as nitrogen (N) and other limiting nutrients (Mattson 1980). For instance, plant nutritional traits that are altered by fertilization or soil quality have strong effects on hemipterans because hemipterans feed on plant phloem, which is low in amino acid content (Ponder et al. 2000, Awmack and Leather 2002, Stadler et al. 2002, Morales and Beal 2006, Nowak and Komor 2010). Increases in herbivore density from enrichment can result in increases in predator density and pathogen prevalence (Forkner and Hunter 2000, Raubenheimer and Simpson 2009). Nutrient enrichment also impacts other interactions like mutualisms between ants and plants, as well as between ants and honeydew-producing insects (Baylis and Pierce 1991, Heil et al. 2002). Ant-hemipteran mutualisms, in particular, structure tropical arboreal communities (Floren et al. 2002, Davidson et al. 2003) and can be mediated by the indirect effects of nutrient availability on host-plant quality (Baylis and Pierce 1991, Cushman 1991). Ant abundance increases on plants with high nutrient availability or with high plant quality due to increases in honeydew-producing herbivore abundance (Strauss 1987) or subsequent increases in the quality of honeydew rewards.

Despite this knowledge of nutrient influences on species interactions, many food webs are complicated and complexity may attenuate the effects of nutrient enrichment or productivity. For

instance, fertilization can increase defenses in some plants, which can actually make plants more toxic to some herbivores, thereby preventing higher productivity at consumer levels (Dyer et al. 2004). Predators can also exhibit intra-guild predation that may attenuate fertilization effects at higher trophic levels (Letourneau et al. 2009). Additionally, defense mutualists of herbivores can guard herbivores from predators and thereby prevent the third trophic level from becoming more productive. Understanding how complex ecological communities respond to enrichment is therefore a goal of future studies.

In coffee plantations of southern Mexico, the complex interaction web of arthropods on coffee has been well described (Vandermeer et al. 2010). However, few have investigated the bottom-up effects of fertilization, soil quality, or plant quality on the interactions that structure the web. Here, we investigate the direct and indirect influence of soil quality on the interactions of an arthropod community on coffee (*Coffea arabica* L. [Rubiaceae]) (Fig. 1). First, in an observational study, we examined if coffee plant traits are correlated with the abundance of a honeydew-producing hemipteran (*Coccus viridis* Green [Hemiptera: Coccidae]) throughout the coffee agroecosystem. Second, in a seedling experiment, we determined whether improved soil quality would increase the quality of coffee host plants, abundance of *C. viridis*, and in turn the recruitment of *Azteca instabilis* F. Smith [Hymenoptera: Dolichoderinae] ants, abundance of predators, pathogens, and parasitoids of *C. viridis*, as well as the overall abundance of the arthropod community. In a second experiment, we tested if soil quality would mediate the recruitment of a second ant-mutualist (*Pheidole synanthropica* Longino [Hymenoptera: Myrmecinae]) to *C. viridis*. We hypothesized that increased soil quality would lead to increased plant quality and result in higher densities of *C. viridis*. We predicted that the presence of *A. instabilis* ants would also increase *C. viridis* density because previous studies have suggested a positive relationship between *A. instabilis* and *C. viridis* (Vandermeer and Perfecto 2006). We hypothesized that tending ants (*A. instabilis* and *P. synanthropica*) would be more abundant on higher-quality plants and have a higher per capita rate of attendance to *C. viridis* on high-

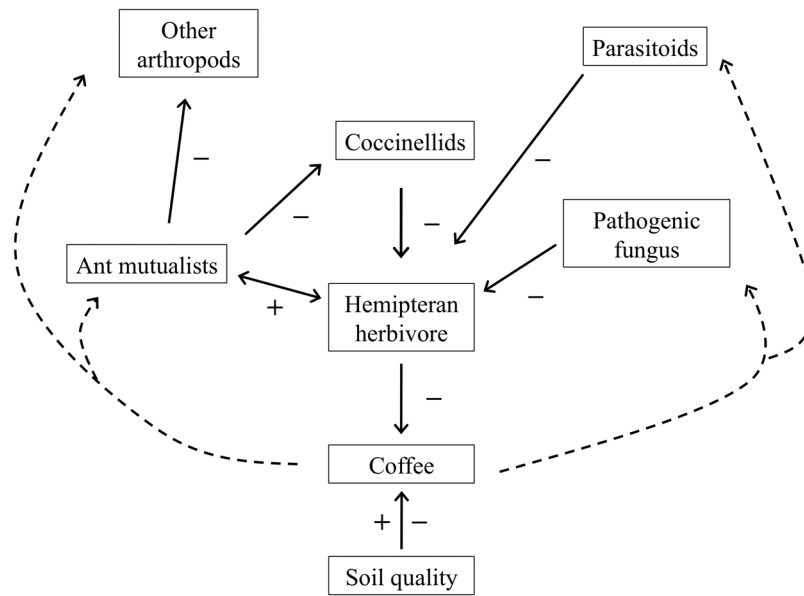


Fig. 1. Coffee interaction web and conceptual pathway of positive and negative interactions between species. Indirect interactions are shown as dotted lines and direct interactions are shown as solid lines.

quality plants relative to low-quality plants because of the increased quality of honeydew rewards. We hypothesized that predators and parasitoids would exhibit an interaction between soil quality and ant exclusion treatments, where they would be more abundant with increased soil quality, but only on ant exclusion plants. In the presence of ants, predators and parasitoids would be lower under high-quality treatments because of increased ant guard of *C. viridis*. We expected that pathogens of *C. viridis* would be more prevalent in the presence of ants and under increased soil quality, where *C. viridis* would be more abundant. Finally, overall abundance of other arthropods within the community should also exhibit an interaction between soil quality and ant treatment, where there are more arthropods on high-quality plants, but only in the absence of ants. High-quality plants should provide more resources to host more arthropods, however *A. instabilis* indiscriminately removes arthropods from foliage (Liere and Perfecto 2008) limiting the effect of soil quality on plants with *A. instabilis*.

METHODS

Study system

We conducted all research at Finca Irlanda (15°11' N, 92°20' W; 900 m asl; 4500 mm/yr rain; hereafter Irlanda), a shade coffee plantation in the Soconusco region of Chiapas, Mexico in February to July of 2008. There, *C. viridis* feeds on the phloem of coffee and forms mutualisms with ants, including the dominant arboreal ant, *A. instabilis* and the ground-nesting *P. synanthropica* (Vandermeer et al. 2010). *Coccus viridis* reaches its highest densities near to shade trees with *A. instabilis* nests (Vandermeer and Perfecto 2006). Many natural enemies attack *C. viridis*, including parasitoid wasps, adults and larvae of *Azya orbiger* Mulsant (Coleoptera: Coccinellidae), and entomopathogenic fungus (*Lecanicillium lecanii* [Zimmerman] Zare and Gams) (Vandermeer et al. 2010). There are also a number of rare or low-density coffee herbivores that include aphids (*Toxoptera* sp. [Hemiptera: Aphidae]), Lepidoptera, Orthoptera, and Coleoptera. Coffee also produces the xanthine alkaloid caffeine that has insecticidal properties in some herbivores, but weak effects on specialists (Nathanson 1984, Guerreiro Filho and Mazzafera 2000, Magalhães et al. 2008). Research suggests

caffeine is induced by *C. viridis* infestations and it may deter *C. viridis* in feeding assays (Lemes Fernandes et al. 2011).

Field survey

To investigate the hypothesis that plant quality can impact *C. viridis* distribution, we determined the relationship between plant nitrogen and caffeine and *C. viridis* in coffee plantations. We located 11 shade trees housing *A. instabilis* nests and surveyed nearby coffee plants for *C. viridis* ($n = 38$ coffee plants). In each site, we counted *C. viridis* individuals (both healthy and infected with *L. lecanii*) on three randomly selected branches on at least one plant with high densities and one plant with low density of *C. viridis* to capture variation in density. All coffee plants were within 5m of ant nests. To correlate caffeine content and *C. viridis*, we collected phloem exudates from 2-3 leaves from each plant using a method modified from King and Zeevart (1974) because *C. viridis* is a phloem feeder. Following 1 h of darkness, we cut the petiole of one newly expanding leaf and submerged the petiole tip in 5 ml of 20 mmol EDTA for 8 h and then collected the solution for analysis. We also collected newly expanding leaf samples for leaf level caffeine, N, and C analysis.

Soil quality–*Azteca instabilis* experiment

To investigate hypotheses related to how soil quality impacts the arthropod interaction web, we conducted a factorial designed field experiment with coffee seedlings crossing soil quality with ant exclusion treatments (3×2). We obtained 8-month old coffee (*C. arabica* var. *Catuai* and *Catimor*) seedlings (mean 16 leaves) from the Irlanda plant nursery (Feb 2009) and repotted seedlings in pots (11.5 cm diam. by 15 cm height) with one of three treatments: (1) low quality that contained a 3:1 mixture of sand and soil, (2) intermediate quality that contained a 1:2:1 mixture of sand, soil, and organic compost, and (3) high quality that contained a 1:3 mixture of soil and organic compost. We obtained soil from the study site, sand from a nearby riverbank, and organic compost (worm vermiculture of coffee parchment, chicken manure, and calcium carbonate) from the Irlanda composting facility. Weekly, the high- (100 ml) and intermediate-treatments (50 ml) received compost ‘tea’

(stewed compost). Watering was unnecessary because rainfall saturated potted soil almost daily. We confirmed the quality of soil treatments by running soil analyses ($N = 3$) at Universidad Autonoma de Chiapas in Huehuetan, Mexico (Appendix A: Table A1). After two months, we infested seedlings with *C. viridis* by placing them in contact with other heavily infested seedlings. Adults of *C. viridis* are sessile, but nymphs (crawlers) are mobile and readily colonize new plant tissues (Fredrick 1943). Prior to the experiment, we thinned *C. viridis* to 30 individuals per plant with cotton swabs. We placed six randomly selected seedlings, two of each soil quality treatment, in random order 1 m from the tree base of 19 selected independent *A. instabilis* nests on *Inga micheliana* trees. Then we randomly excluded ants from half of plants (one per soil treatment) by painting Tanglefoot (Grand Rapids, MI) around the stem 3 cm above soil. To control for any effect of Tanglefoot, we painted a half circle of it on control plant stems. We re-applied Tanglefoot and removed encroaching vegetation as necessary. Each week, for five weeks after set-up, we counted the number of *C. viridis* (excluding crawlers and individuals infected with *L. lecanii*), *Toxoptera* sp., *A. instabilis* workers, *A. orbigera*, *L. lecanii* infected *C. viridis* (only last 3 wk), parasitized *C. viridis* (only last 3 wk), and all orders of arthropods on plants; except Diptera because of the difficulty of observing them. All measurements were taken at sites between 6:30 AM and 1:30 PM under similar weather conditions.

To determine the effect of treatments on plant traits we examined plant growth rates by dividing the number of leaves at the end of experiment by the number at the onset of treatments. At the completion of the experiment, we also collected phloem exudates from 2-3 coffee leaves per plant and collected old leaves for caffeine and nutritional analysis as described below.

Soil quality–*Pheidole synanthropica* experiment

To further investigate the hypothesis that soil quality can indirectly alter the per capita attendance of ants to *C. viridis* on coffee, we conducted a second field experiment. Upon completion of the *A. instabilis* field experiment described above, we moved all plants to the outdoor plant nursery

and investigated the effect of soil quality on recruitment of *P. synanthropica* ants to *C. viridis*. We removed Tanglefoot from all plants, divided them into two areas (blocks) of the nursery (separated by 30 m), and evenly interspersed them in rows (0.25 m apart). Independent colonies of *P. synanthropica* often occur at small distances (3–4 m; K. Ennis, *personal observation*), so we assumed the two blocks were foraged by independent *P. synanthropica* colonies. On two dates (a week apart), we counted *P. synanthropica* workers on each plant and calculated means across the two sample dates.

Plant trait analyses

To aid our understanding of how soil quality alters plant quality, we measured several plant nutritional and chemical traits. To prepare samples for both leaf caffeine and nitrogen analysis, we dried tissues at 40–45°C for 48 h and ground them in liquid nitrogen with a mortar and pestle. We analyzed caffeine using a maximized ultrasonic assisted extraction method at the University of Toledo. Briefly, we combined 0.1 g of prepped leaf sample with 20 ml of methanol and extracted caffeine by ultra-sonication for 4 h at 65°C and passed extracts through 13 mm (0.45 µm pores) filter paper. We diluted aliquots with methanol within the linear working range of the instrument. We spiked samples with an internal standard (¹³C₃-caffeine) to control for instrumental error. We analyzed samples using liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS, Model Varian 1200L, Agilent, Santa Clara, CA). For the field survey, we compared new leaves; however for the soil quality experiment we used old leaves because there were too few new leaves. We ran each leaf sample in triplicate to control for variability within plant. For phloem caffeine, we filtered un-diluted liquid exudates and analyzed samples with LC-ESI-MS/MS. To determine leaf nitrogen and carbon, we analyzed samples with C-H-N combustion analysis (Perkin Elmer model 2400) at the Application Technology Research Unit at the United States Department of Agriculture's research facility in Toledo, OH.

Statistical analysis

To determine if plant traits influenced *C. viridis* within the field survey, we performed general

linear mixed models (GLMM). *Azteca instabilis* nests were treated as a random factor within model and the carbon to nitrogen ratio (C:N), and phloem and leaf caffeine concentration were treated as fixed covariates in model. We performed type III F-tests of significance for main effects with restricted maximum likelihood (REML) to estimate the fixed effect parameters and the variance of random effects (West et al. 2007). Non-significant factors were removed from the model and we compared all possible models with Akaike Information Criteria (AIC). We centered the dependent variable (*C. viridis* abundance) around the mean to improve the fit of GLMM.

To examine treatment effects within the field experiment, we performed GLMM as described above. Specifically, models considered soil quality, exclusion, and sampling date as fixed factors, and interactions between factors. We included nest as a random effect. We corrected for multiple comparisons by performing Bonferroni corrections. To do so, we grouped the arthropod community into four assemblies: mutualists (*C. viridis*, *Toxoptera* sp., *A. instabilis*, and *A. instabilis* per *C. viridis*), *C. viridis* enemies (*A. orbiger*, parasitized scales (per *C. viridis*), and *L. lecanii* infected scales (per non-infected *C. viridis*), other arthropods (spiders, hemipterans [without *C. viridis* and *Toxoptera* sp.], beetles, other ants), and total arthropods (minus ants and hemipteran mutualists). Then we corrected *P*-values by dividing them by the number of dependent variables compared within each group. To meet assumptions of normality, we transformed all variables with the natural log (variable + 0.001); except we square root transformed total arthropods. We also compared plant growth rate, leaf C:N, and caffeine (phloem and leaf) using GLMM as above. To meet the assumptions of normality, we natural log transformed (value + 0.001) growth rate, and phloem caffeine. We used regression analysis to predict which plant traits were important to *C. viridis* abundance. Small sample size and lack of adequate plant material resulted in many missing cases and prevented multiple regression analysis from being conducted. Instead, we performed individual regressions between independent variables C:N, leaf caffeine, and phloem caffeine against *C. viridis* abundance (natural log transformed [+0.001] for all analy-

ses).

For the soil quality–*Pheidole synanthropica* experiment, we compared mean *P. synanthropica* per plant and *P. synanthropica* per *C. viridis* across soil quality treatment with GLMM. Because we were using plants from the previous experiment, we ensured that the previous exclusion treatment did not impact results by including this factor in the model. Area (block) was included as a random effect. We natural log transformed variables (value + 0.001) to meet assumptions of normality. We analyzed all statistics with SPSS (16.0).

RESULTS

Field survey

The field survey generally supported the hypothesis that plant traits alter *C. viridis* distribution. Coffee C:N ratio ($F_{1,34} = 4.4$, $P = 0.043$, parameter estimate -18.1 ± 8.6 [mean \pm SE]) correlated negatively with *C. viridis* density, but phloem ($F_{1,10} = 2.5$, $P = 0.147$, parameter estimate 9.2 ± 35.1) and leaf caffeine ($F_{1,34} = 0.069$, $P = 0.794$, parameter estimate -9.4 ± 6) did not correlate with *C. viridis* (all factors included in best fit model).

Soil quality–*A. instabilis* experiment

Hemipteran mutualists and A. instabilis ants.—*Coccidius viridis* was influenced by soil quality, ant-treatment, and time (Fig. 2A, Table 1). Pair-wise comparisons revealed high-quality plants had 64% more *C. viridis* than low-quality plants ($P < 0.001$), and 19% more *C. viridis* than intermediate-quality plants ($P = 0.001$). There was 61% more *C. viridis* on ant-excluded plants relative to controls. There was no clear pattern of *C. viridis* abundance over time, yet pair-wise comparisons revealed lower *C. viridis* abundance in the second ($P < 0.001$) and the last time points ($P = 0.019$) compared with the first. Unlike *C. viridis*, *Toxoptera* sp. aphids were not strongly influenced by soil quality, exclusion treatment, or time effects (Table 1).

Azteca instabilis abundance per plant was significantly influenced by exclusion and marginally influenced by soil quality (Fig. 2B, Table 1). Control treatment plants had 26 times more *A. instabilis* than exclusion treatment plants. *Azteca instabilis* abundance tended to be higher on high-

quality plants relative to low-quality plants. Only the obvious effect of exclusion treatment influenced the number of *A. instabilis* per *C. viridis* individual (Table 1).

Enemies of C. viridis.—Ant exclusion and time impacted enemies of *C. viridis*, however there was no effect of soil quality treatment (Fig. 3, Table 2). *Azya orbiger* larvae abundance increased over time on ant-excluded plants, but did not change with time on control plants (Fig. 3A). The proportion of *L. lecanii* infected *C. viridis* to un-infected *C. viridis* was not influenced by soil quality or exclusion treatments, however it increased significantly over the three weeks sampled (Fig. 3B, Table 2). The proportion of parasitized *C. viridis* to un-parasitized *C. viridis* varied by exclusion treatment and time, but at no time were more than 3% of *C. viridis* parasitized, suggesting parasitism was rare (Fig. 3C, Table 2).

Community level.—Soil quality, exclusion, and time influenced the arthropod community. Arthropod abundance tended to increase with time on high- and intermediate-quality plants, but declined over time on low-quality plants ($F_{8,209} = 2.7$, $P = 0.0077$; Fig. 4). Ant-excluded plants had 8% more arthropods than control plants ($F_{1,531} = 6.4$, $P = 0.0118$). For specific groups, there was 4.7 times greater abundance of other ants (excluding *A. instabilis*) on control plants relative to ant-excluded plants (species observed included: *Brachymyrmex heeri*, *Brachymyrmex* sp. 2, *Pheidole protensa*, *Pheidole synanthropica*, *Xenomyrmex* sp., *Pseudomyrmex ejectus*, *Procryptocerus hylaeus*, *Cephalotes atratus*, *Wasmannia auropunctata*; Appendix A: Tables A2 and A3). On the contrary, there were twice as many spiders on ant-excluded relative to control plants (Appendix A: Fig. A1, Tables A2 and A3). Coleoptera abundance per plant increased with time, and Hemipteran abundance did not vary by any factor (Appendix A: Tables A2 and A3).

Plant traits.—Soil quality treatments impacted coffee seedling traits (Table 3). High-quality plants grew 79% more than low-quality plants ($P < 0.001$) and intermediate-quality plants grew 60% more than low-quality plants ($P < 0.001$). The C:N ratio of low-quality plants was 28% ($P < 0.001$) greater than high- and 24% ($P < 0.001$) greater than intermediate-quality plants. Phloem exudates of high-quality ($P = 0.007$) and intermediate-quality plants ($P = 0.046$) contained 2

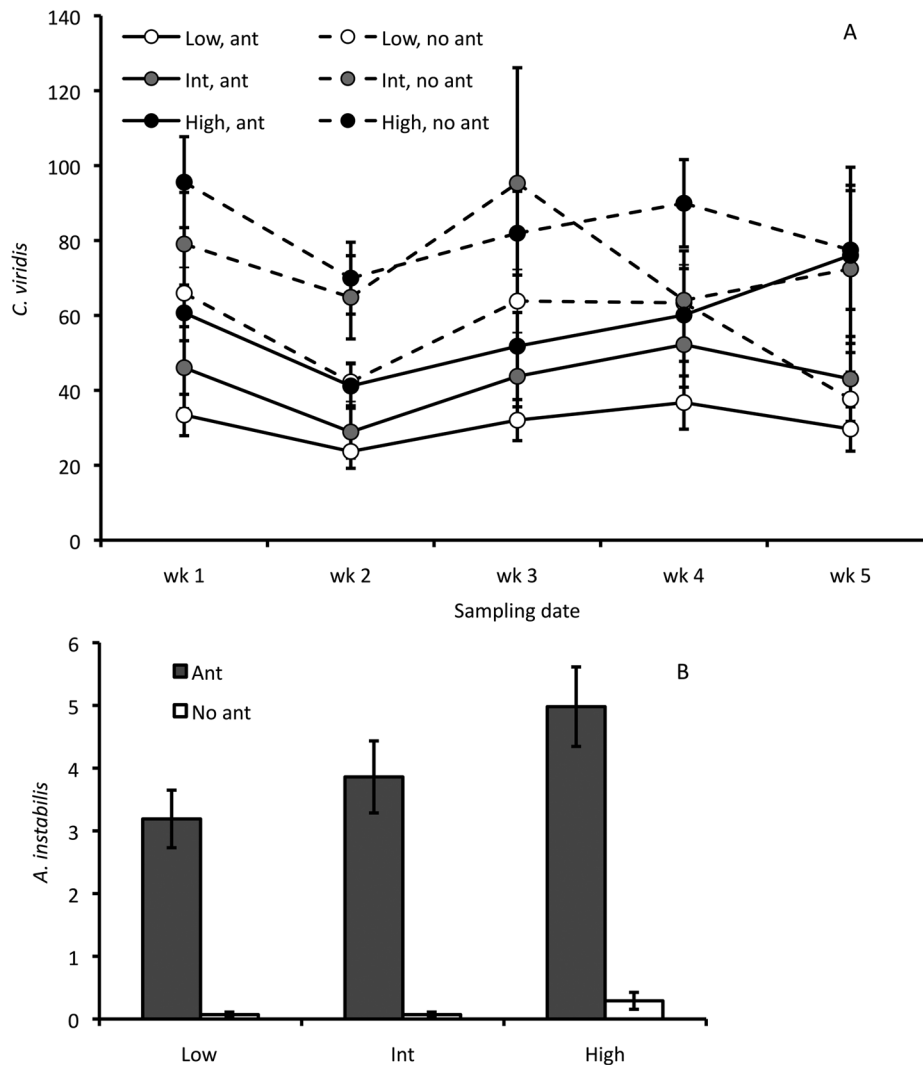


Fig. 2. Soil quality, exclusion treatment, and time effects on the mean (\pm SE) abundance of *C. viridis* (A) and *A. instabilis* recruitment per plant (B).

times more caffeine relative to low-quality plants. Leaf caffeine did not differ with soil quality. Across all plant traits there was no effect of exclusion (Table 3, Appendix A: Table A4). Plant C:N ratio negatively correlated with *C. viridis* density (mean across five time points) ($R^2 = 0.062$, $F_{1,63} = 4.17$, $P = 0.045$). However, leaf ($R^2 = 0.050$, $F_{1,21} = 1.1$, $P = 0.306$) and phloem caffeine ($R^2 = 0.016$, $F_{1,43} = 0.69$, $P = 0.410$) did not correlate with *C. viridis*.

Soil quality–*Pheidole synanthropica* experiment

Pheidole synanthropica recruitment per plant

was three times greater on high-quality relative to low-quality plants ($P < 0.001$) (Fig. 5A, $F_{2,106} = 9.8$, $P < 0.001$). Neither high- ($P = 0.059$) nor low-quality plants ($P = 0.098$) differed from intermediate-quality plants in terms of *P. synanthropica* per plant. There was no difference in *P. synanthropica* recruitment on plants previously assigned to exclusion and control treatments in the *A. instabilis* experiment ($F_{2,106} = 0.25$, $P = 0.622$). This suggests there was no effect of previous experimental legacies (ant pheromones or Tanglefoot residues). There was no correlation between the number of *C. viridis* per plant and

Table 1. Effects of soil quality, exclusion, and time treatment on *C. viridis*, *Toxoptera* sp. aphids, and *A. instabilis* ants.

Source	df	F	P†
<i>C. viridis</i>			
Intercept	1, 19	373.6	0.000004
Soil quality	2, 421	16.4	0.000004
Ant	1, 421	0.3	0.000004
Time	4, 184	6.2	0.000412
Soil × Ant	2, 421	0.02	>0.999
Soil × Time	8, 184	0.3	>0.999
Ant × Time	4, 184	1.1	>0.999
Soil × Ant × Time	8, 184	0.4	>0.999
<i>Toxoptera</i> sp.			
Intercept	1, 18	15.9	0.003472
Soil quality	2, 332	3.2	0.174792
Ant	1, 332	6	0.060796
Soil × Ant	2, 332	3.5	0.12188
<i>A. instabilis</i>			
Intercept	1, 18	44	0.000012
Soil quality	2, 523	4.4	0.050928
Ant	1, 523	383.4	0.000004
<i>A. instabilis</i> (per <i>C. viridis</i>)			
Intercept	1, 18	42.3	0.000016
Ant	1, 473	222.4	0.000004

† Bonferroni-corrected *P* value.

the number of *P. synanthropica* suggesting that although we did not control for the number of *C. viridis* per plant this was not an important factor. On high-quality plants there were 3 times as many *P. synanthropica* workers per *C. viridis* relative to low-quality plants ($P = 0.04$) (Fig. 5B, $F_{2,104} = 3.9$, $P = 0.024$). The number of workers per *C. viridis* on intermediate-quality plants did not differ from high- ($P = 0.056$) or low-quality ($P = 0.987$) plants.

DISCUSSION

Our hypothesis that *C. viridis* would increase with soil quality was supported, which suggests changes in soil quality indirectly influenced *C. viridis* growth. This effect was likely channeled through changes in plant quality or productivity. In the field experiment, high-quality plants had greater growth rates and lower C:N than low-quality plants. Growth of phloem-feeding insects is often limited by amino acid content in the phloem (Ponder et al. 2000, Awmack and Leather 2002, Nowak and Komor 2010), which is correlated with leaf N in some studies (Nowak and Komor 2010). The field survey data also supported this hypothesis because C:N was correlated with *C. viridis* density across adult coffee plants

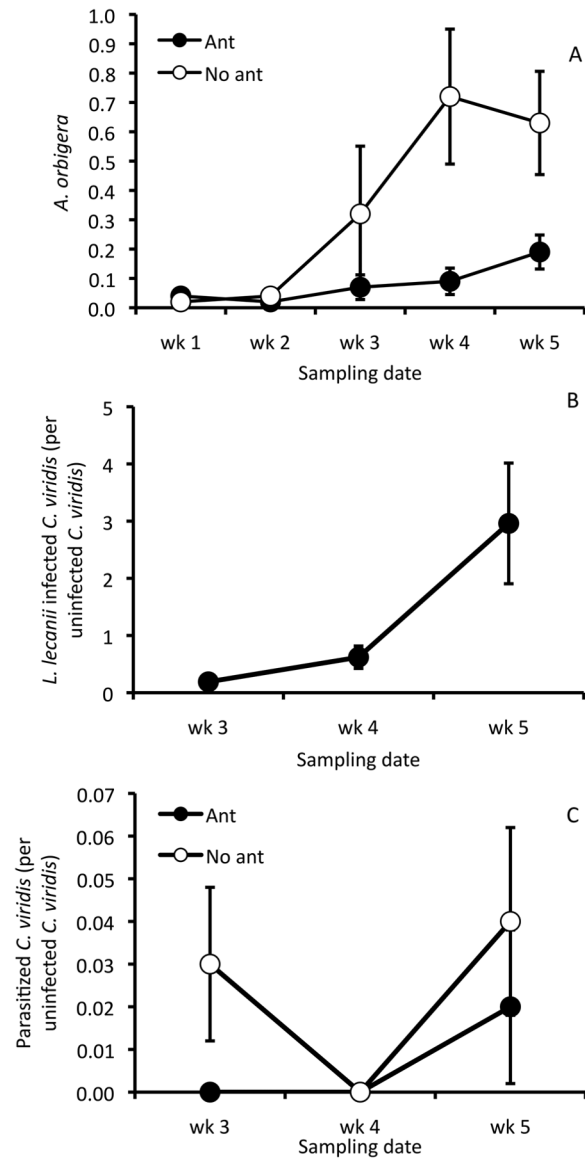


Fig. 3. Soil quality, exclusion treatment, and time impacts on the mean (\pm SE) abundance of *A. orbigera* larvae (A), *L. lecanii* (per *C. viridis*) (B), and parasitized *C. viridis* (per *C. viridis*) (C).

(Table 1), although there was only a marginal correlation between C:N and *C. viridis* in the experimental study. Other greenhouse experiments support this finding (Lemes Fernandes 2007). However, unlike in Lemes Fernandes et al. (2011), caffeine concentration was not correlated with *C. viridis* abundance in our study. Caffeine may not have impacted *C. viridis* in our study

Table 2. Effects of soil quality, exclusion, and time treatment on natural enemies of *C. viridis*.

Source	df	F	P†
<i>A. orbiger</i>			
Intercept	1, 55	40.9	0.000003
Soil quality	2, 240	2.5	0.261771
Ant	1, 344	11.6	0.00219
Time	4, 176	9.6	0.000003
Soil × Ant	2, 240	0.6	>0.999
Ant × Time	4, 176	3.2	0.043788
<i>L. lecanii</i> (per <i>C. viridis</i>)			
Intercept	1, 119	11.8	0.002415
Soil quality	2, 117	1.6	0.619926
Ant	1, 117	0.1	>0.999
Time	2, 127	5.1	0.02217
Soil × Ant	2, 117	0.6	1.64142
Soil × Time	4, 127	0.8	>0.999
Ant × Time	2, 127	0.03	>0.999
Soil × Ant × Time	4, 127	0.4	>0.999
Parasitoids (per <i>C. viridis</i>)			
Intercept	1, 89	17.4	0.000216
Ant	1, 109	9.9	0.006336
Time	2, 154	7.9	0.001632

† Bonferroni-corrected *P* value.

because variation between treatments (0.4 to 10 mg g⁻¹ leaf caffeine) was low compared to the range (4.2 to 28 mg g⁻¹) found in Lemes Fernandes (2007). It is also possible that the presence of predators, pathogens, parasitoids,

and ant-mutualists attenuated caffeine effects.

Surprisingly, *C. viridis* was more abundant on seedlings without ants than on plants with ants, rejecting our hypothesis that *C. viridis* density would increase in the presence of *A. instabilis*. Other studies have supported (Philpott et al. 2008) and contested (Reimer et al. 1993; Uno, unpublished data) this finding. The response of *C. viridis* to exclusion may depend on the given ant mutualist and predators within the community. Lower density of *C. viridis* on plants with *A. instabilis* may be due to predation by *A. instabilis* ants, as has been observed in other ant-hemipteran mutualisms (Offenberg 2001). It also could be that exclusion treatments lowered the abundance of non-flying predators, leading to increased *C. viridis* densities (Mueller et al. 1988, Piñol et al. 2009). Other combined factors, such as parasitism and infection, may have also contributed to differences observed between exclusion and control treatments.

Our results support our hypothesis that soil quality influences ant-hemipteran mutualisms because *Azteca instabilis* marginally responded to soil quality (at the per plant level) and *P. synanthropica* responded strongly to treatments

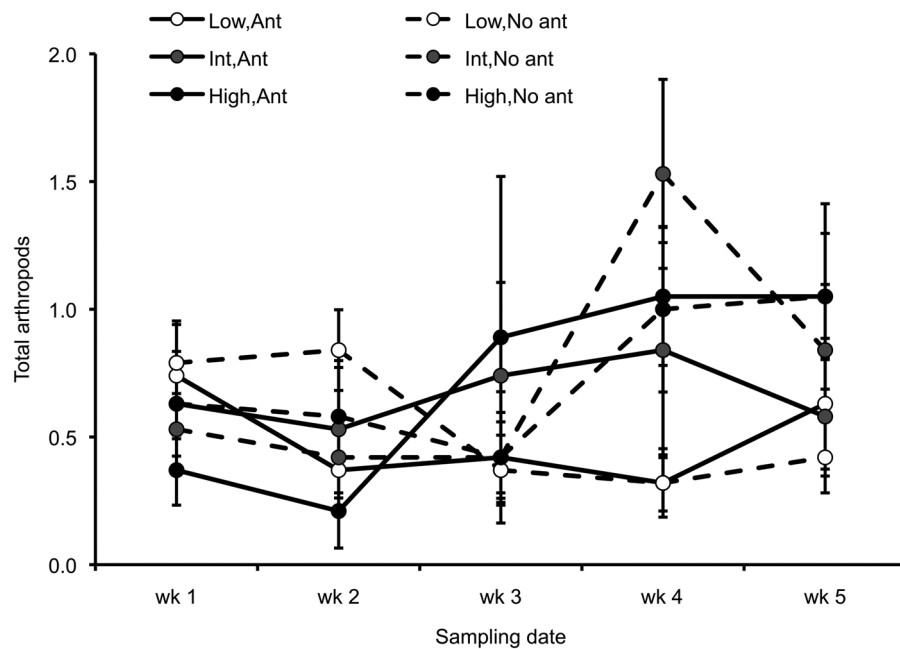


Fig. 4. Soil quality, exclusion treatment, and time impacts on the mean (\pm SE) abundance of total arthropods (without ants and their mutualists).

Table 3. Type III effects of soil quality and exclusion fixed factors on plant traits.

Source	df	F	P
Growth rate			
Soil quality	2, 88	29.9	<0.001
Ant	1, 88	1.7	0.195
Soil × Ant	1, 88	1.5	0.236
Carbon:nitrogen			
Soil quality	2, 48	40.6	<0.001
Ant	1, 50	0.3	0.566
Soil × Ant	2, 52	0.7	0.502
Phloem caffeine			
Soil quality	2, 34	3.7	0.036
Ant	1, 34	0.03	0.836
Soil × Ant	2, 33	0.148	0.836
Leaf caffeine			
Soil quality	2, 17	0.85	0.447
Ant	1, 16	0.38	0.549
Soil × Ant	2, 16	0.8	0.468

(at the per plant and per *C. viridis* levels). For *P. synanthropica* and *C. viridis*, this is evidence that soil quality caused an interaction modification, which has important ecological and evolutionary implications (Wootton 1994, Mooney and Agrawal 2008). Several other studies have demonstrated increased ant recruitment to honeydew-producing insects on fertilized host plants (Baylis and Pierce 1991, Billick et al. 2005, Morales and Beal 2006) or on plants varying by genotype or hybridization (Wimp and Whitham 2001, Mooney and Agrawal 2008). Here, the likely mechanism behind the increased recruitment per *C. viridis* on high- relative to low-quality plants is the increased N in honeydew that likely limits ants like *P. synanthropica* (Baylis and Pierce 1991, Davidson et al. 2003). There are

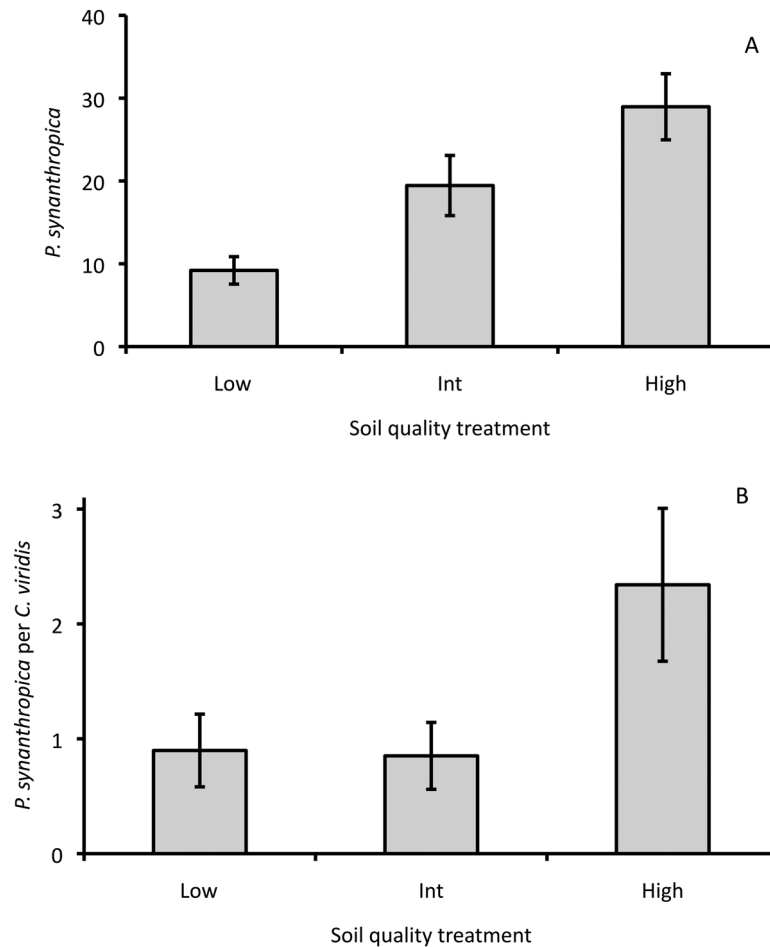


Fig. 5. Soil quality treatment effect on the mean (\pm SE) number of *P. synanthropica* per coffee seedling (A) and number of *P. synanthropica* (per *C. viridis*) (B).

several possibilities for why we observed differences between the recruitment in the two ant species. First, the *A. instabilis* study had much greater variation between site locations and a smaller number of replications were used relative to the *P. synanthropica* experiment, therefore the former experiment might have had more environmental variation and reduced statistical power to capture treatment effects. *Coccus viridis* densities on our seedlings may not have been high enough to attract *A. instabilis* away from other honeydew-producing insects on adjacent plants (Livingston et al. 2008). Differences in ant response to soil quality could also be a result of differences in species dominance, discovery ability, or foraging strategy between the two ant species.

Our hypotheses that soil quality would impact organisms at the third trophic level (*C. viridis* enemies) and that there would be an interaction between plant quality and ant exclusion treatments were rejected. Nonetheless the predator *A. orbiger*a increased in abundance after the third week on ant-excluded plants. Many of the *A. orbiger*a individuals observed on the ant-excluded plants were early instar larvae, suggesting that they were eggs that hatched after adult oviposition. The higher abundance of *A. orbiger*a on ant-excluded plants is particularly surprising because *A. orbiger*a larvae have waxy extensions that deter ants from removing them, and parasitism of *A. orbiger*a larvae is lower in the presence of *A. instabilis* relative to the absence of this ant (Liere and Perfecto 2008). The number of *C. viridis* exhibiting symptoms of infection from *L. lecanii* relative to the number of healthy *C. viridis* also increased with time, however there was no differences across exclusion or soil quality treatment. *Lecanicillium lecanii* was the most important top-down regulating agent of *C. viridis* in this study. By the end of the final week, there were three times more *L. lecanii*-infected *C. viridis* individuals than healthy *C. viridis* individuals. Although parasitism rates of *C. viridis* varied by time and exclusion treatment, rates were so low that these results may not be biologically relevant.

Soil quality, exclusion, and time influenced total arthropod abundance, however we did not observe a hypothesized interaction between soil quality and ant exclusion. Arthropod abundance

likely increased on high-quality plants because these plants had greater leaf area than low-quality plants, which likely led to more arthropods per plant due to more plant resources per plant. The presence of *A. instabilis* also led to fewer total arthropods on coffee. Similarly, the number of *C. viridis*, *Toxoptera* sp., *A. orbiger*a larvae, spiders, and parasitized scales was also reduced in the presence of *A. instabilis*. Other studies have demonstrated that *A. instabilis* reduces the abundance of specific arthropod groups on coffee (Philpott et al. 2008) or on shade trees (Philpott et al. 2004, Gonthier et al. 2010), however this study is the first to suggest that *A. instabilis* reduces overall arthropod abundance. This finding corroborates the work of others that show ants have community wide impacts on arboreal arthropod communities (Wimp and Whitham 2001, Floren et al. 2002, Kaplan and Eubanks 2005, Styrsky and Eubanks 2007, Rosumek et al. 2009).

Although our soil quality treatments impacted plant quality traits and the arthropod community, increased fertilization may not have been solely responsible. Increased N in the plant tissues of high-soil-quality plants might suggest that fertilization was important, but other soil characteristics, like organic matter, water retention, pH, and the microbial community may have also influenced plant-animal interactions. Dissecting the components of soil quality and evaluating their impact on plant-animal interactions remains an important research avenue with relevance to both ecologists and agricultural producers.

Bottom-up effects of soil quality, nutrient fertilization, and plant quality traits can have far reaching effects on higher trophic levels within ecological communities. Improved plant quality can increase abundance at the second trophic level and in some instances the third trophic level (Forkner and Hunter 2000, Gruner 2004). Although we found strong impacts of soil quality on herbivores and some impacts on ant-mutualists, we did not find any effects on predators, parasitoids, and pathogens. Also, we did not observe interactions between soil quality and ant exclusion despite both treatments having important effects on some components of the arthropod community. Perhaps it is not surprising that our results are complicated given the

complexity of the coffee arthropod interaction web studied. Interactions between herbivores and mutualists may have attenuated the transfer of productivity from herbivores to predators or perhaps our study was too short to observe these effects. We conclude that bottom-up effects are important factors to consider within the coffee agroecosystem, but further research is needed in these systems and other systems where there is substantial variation in soil quality. Few other studies have investigated the bottom-up effects of soil quality on arthropod communities in human-dominated ecosystems, such as coffee plantations. Our results will lend important insights into the consequences of changes in soil quality given land-use change, agricultural intensification, nitrogen deposition, and forms of natural variation in soil quality.

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SUPPLEMENTAL MATERIAL

APPENDIX A

Table A1. The effect of soil quality treatment on soil quality traits (means \pm SE).

Soil quality treatment	N (%)	P (ml/l)	K (ml/l)	Organic matter (%)
Low	0.17 ^a \pm 0.01	16 ^a \pm 2	49 ^a \pm 3	4.6 ^a \pm 0.1
Intermediate	0.21 ^a \pm 0.02	68 ^b \pm 17	88 ^a \pm 1	5.3 ^b \pm 0.1
High	0.34 ^b \pm 0.03	29 ^{a,b} \pm 1	189 ^b \pm 19	10.7 ^c \pm 0.1

Note: Common letters denote means that are not significantly different ($P=0.05$) from one another, as determined by Tukey's HSD.

Table A2. Numbers of organisms (mean \pm SE) within the coffee community in soil quality and ant treatments averaged across time.

Organism	Low, ant	Low, no ant	Int, ant	Int, no ant	High, ant	High, no ant
<i>C. viridis</i>	31.12 \pm 2.562	54.62 \pm 3.631	42.79 \pm 4.414	75.14 \pm 8.69	57.96 \pm 5.918	82.98 \pm 5.445
<i>Toxoptera</i> sp.	0.34 \pm 0.285	1.84 \pm 0.824	5.46 \pm 4.218	7.98 \pm 5.407	0.46 \pm 0.228	5.85 \pm 2.138
<i>A. instabilis</i>	3.19 \pm 0.459	0.07 \pm 0.04	3.86 \pm 0.574	0.07 \pm 0.04	4.98 \pm 0.634	0.29 \pm 0.135
<i>A. instabilis</i> (per <i>C. viridis</i>)	0.151 \pm 0.027	0.008 \pm 0.006	0.208 \pm 0.077	0.007 \pm 0.004	0.205 \pm 0.047	0.011 \pm 0.006
<i>A. orbigera</i>	0.04 \pm 0.026	0.48 \pm 0.179	0.09 \pm 0.03	0.41 \pm 0.119	0.11 \pm 0.038	0.14 \pm 0.071
<i>L. lecanii</i> infected <i>C. viridis</i> (per uninfected <i>C. viridis</i>)	0.39 \pm 0.154	1.38 \pm 0.681	1.94 \pm 1.364	2.38 \pm 1.407	1.17 \pm 0.675	0.28 \pm 0.085
Parasitized <i>C. viridis</i> (per <i>C. viridis</i>)	0.02 \pm 0.018	0.01 \pm 0.004	0 \pm 0.001	0.05 \pm 0.028	0 \pm 0.001	0.01 \pm 0.003
Total arthropods†	0.49 \pm 0.093	0.55 \pm 0.067	0.66 \pm 0.136	0.75 \pm 0.111	0.72 \pm 0.161	0.74 \pm 0.1
Coleoptera	0.21 \pm 0.075	0.52 \pm 0.174	0.41 \pm 0.109	0.62 \pm 0.148	0.38 \pm 0.141	0.25 \pm 0.096
Hemiptera	0.18 \pm 0.047	0.15 \pm 0.037	0.13 \pm 0.037	0.17 \pm 0.046	0.18 \pm 0.065	0.31 \pm 0.058
Other ants	5.53 \pm 2.311	0.8 \pm 0.558	5.64 \pm 2.076	0.21 \pm 0.088	7.66 \pm 2.806	2.91 \pm 2.155
Spiders	0.11 \pm 0.032	0.2 \pm 0.044	0.11 \pm 0.035	0.29 \pm 0.052	0.14 \pm 0.046	0.21 \pm 0.06

† Total arthropods without ants and their hemipteran mutualists (*C. viridis* and *Toxoptera* sp.).

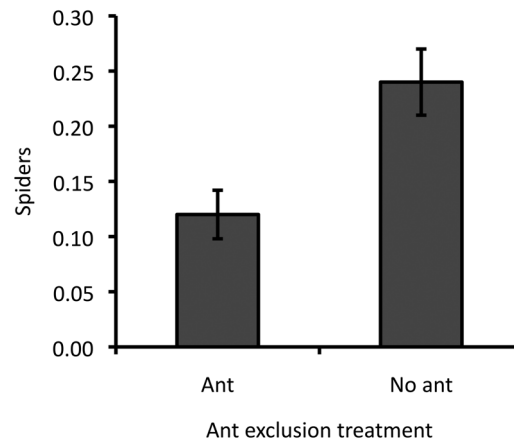
Table A3. Effects of soil quality, exclusion, and time treatments on arthropod groups.

Source	df	F	P†
Other ants			
Intercept	1, 19	7.2	0.06
Soil quality	1, 316	39.9	0.000004
Ant	2, 316	0.8	>0.999
Time	4, 168	3	0.076
Soil × Ant	2, 316	0.1	>0.999
Soil × Time	8, 168	0.2	>0.999
Ant × Time	4, 168	0.1	>0.999
Soil × Ant × Time	8, 168	0.1	>0.999
Spiders			
Intercept	1, 20	56.2	0.000004
Ant	1, 494	14.9	0.000524
Time	4, 196	3.1	0.070856
Hemiptera (non-ant mutualists)			
Intercept	1, 18	48.8	0.000008
Ant	1, 497	1.7	0.77896
Coleoptera			
Intercept	1, 21	41.4	0.000008
Soil quality	2, 387	4.1	0.069364
Ant	1, 387	0.4	>0.999
Time	4, 212	8.6	0.000008

† Bonferroni-corrected *P* value.Table A4. The effect of soil quality and ant exclusion treatments on mean (\pm SE) plant traits.

Source	Low	Intermediate	High
Plant growth rate†			
Control	1.6 \pm 0.1	2.5 \pm 0.2	2.6 \pm 0.2
Ant exclusion	1.5 \pm 0.1	2.6 \pm 0.2	3.1 \pm 0.2
C:N ratio			
Control	23.3 \pm 0.4	18.3 \pm 0.6	17.5 \pm 0.6
Ant exclusion	22.1 \pm 0.9	18.1 \pm 0.6	17.8 \pm 0.6
Phloem caffeine (μg/l)			
Control	0.13 \pm 0.07	0.28 \pm 0.05	0.23 \pm 0.05
Ant exclusion	0.12 \pm 0.02	0.25 \pm 0.08	0.22 \pm 0.06
Leaf caffeine (mg/g)			
Control	5.05 \pm 0.94	5.74 \pm 1.00	5.03 \pm 1.45
Ant exclusion	5.04 \pm 1.67	3.67 \pm 0.90	7.26 \pm 0.09

† Plant growth rate was equal to the division of final by initial total number of leaves.

Fig. A1. Influence of exclusion treatment on the abundance of spiders (mean \pm SE).