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Cheating belowground interactions : diversity, ecology and distribution of mycoheterotrophy

Gomes, S.I.F.

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Author: Gomes, S.I.F.

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CHAPTER 4

Environmental drivers for cheaters of arbuscular mycorrhizal symbiosis in tropical rainforests

SOFIA I.F. GOMES^{1,2}, PETER M. VAN BODEGOM¹, VINCENT S.F.T. MERCKX²,

NADEJDA A. SOUDZILOVSKAIA¹

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¹Institute of Environmental Sciences (CML), University of Leiden, the Netherlands

²Naturalis Biodiversity Center, Leiden, 2300 RA, the Netherlands

ABSTRACT

Hundreds of non-photosynthetic mycoheterotrophic plant species cheat the arbuscular mycorrhizal symbiosis. Their patchy local occurrence suggests a constraint by abiotic factors. Yet, little is known about the ecological conditions under which mycoheterotrophy is able to occur.

Here, we examine the edaphic drivers predicting the local-scale distribution of mycoheterotrophic plants in two lowland rainforests in South America. We compared soil chemistry and nutrients in plots where mycoheterotrophic plants were present to those without these plants. Soil pH, soil nitrate, and the interaction between soil potassium and nitrate concentrations were the best predictors for the occurrence of mycoheterotrophic plants in these tropical rainforests. Mycoheterotrophic plant occurrences decreased with a rise in each of these predictors. This indicates that these plants avoid high fertility patches. Such low-fertility conditions coincide with conditions that potentially favor a weak mutualism between plants and arbuscular mycorrhizal fungi according to the trade balance model. Hence, when local-scale mutualism is weak, cheating is likely to occur.

Our study uncovers the mechanisms favoring the cheating of arbuscular mycorrhizal networks in tropical forests. The patchy occurrence of mycoheterotrophic plants suggests that local soil heterogeneity causes the stability of arbuscular mycorrhizal networks to vary at a very small scale.

INTRODUCTION

Mycorrhizal symbiosis is one of the most widespread mutualisms on Earth (van der Heijden *et al.* 2015). Typically, it is a mutually beneficial interaction where plants transfer photosynthesized carbon to their mycorrhizal fungal partners, which in turn facilitate the uptake of mineral nutrients from the soil, enhancing plant nutrition (Smith & Read, 2008). The symbiosis is therefore extremely important in soils of low nutrient availability or where the distribution of nutrients is heterogeneous (Cavagnaro *et al.* 2005). Yet, mycoheterotrophic plants evolved a strategy where the carbon flux is reversed from their fungal partners to themselves so that the plants depend exclusively on their mycorrhizal partners to obtain carbohydrates (Leake 1994). It has been argued that mycoheterotrophic plants may provide other benefits to the fungi in exchange for the carbon, such as vitamins or protection but such evidence has not been yet presented (Sellesse & Rousset, 2011). Thus, these plants managed to subvert the “biological market” established between plants and mycorrhizal fungi (Kiers *et al.* 2011), avoiding the costs of obtaining resources from symbiotic partners. There are over 500 fully mycoheterotrophic plant species, of which about half is associated with arbuscular mycorrhizal (AM) fungi (Merckx, 2013). Since these plants require the presence of an established mycorrhizal network to support their carbon demands during the entire life cycle, ultimately relying on the surrounding photosynthetic plants, mycoheterotrophy can be regarded as a mechanism enabling cheating on the mycorrhizal symbiosis.

Many species of mycoheterotrophic plants have remarkably widespread distributions, yet at local-scale their distribution is often highly patterned (Cheek & Williams, 1999; Bergman *et al.* 2006; Yamato *et al.* 2016). As general characteristics of mycoheterotrophic plants' habitat at global scale, Leake (1994) described that these plants often occur in humid forests with dense overstory in deep shade, with a thick layer of leaf litter on the forest floor and restricted occurrence of herbaceous plants. This leads to the hypotheses that mycoheterotrophic plants are probably adapted to low-light conditions, occurring mostly inside forests with close canopies, where autotrophic plants fail to grow (Bidartondo *et al.* 2004). Alternatively, mycoheterotrophs may require a particular forest floor microclimate, with potentially high vapor pressure, low evapotranspiration and small diurnal temperature variation (Leake 1994; Cheek & Williams, 1999; Klooster & Culley 2009). Furthermore, the availability of water seems to be a consistent feature of habitats of mycoheterotrophs and potentially explains their

preference for the humid tropics and moist temperate regions, and swamps periodically inundated or moist humus rich soils (Merckx, 2013).

The patchy occurrence of these plants suggests that besides fulfilling general requirements such as humidity, light availability or temperature suitability, the presence of mycoheterotrophic plants in a community is constrained by particular local-scale factors. Due to the reliance of these plants on mycorrhizal networks, both biotic (interactions with their fungi) and abiotic (soil conditions) factors can potentially contribute to their occurrence at local-scale. Previous studies showed highly species-specific interactions between these plants and their fungal partners from local to global scale (Yamato *et al.* 2016; Renny *et al.* 2017). This could indicate that the occurrence of their fungal associates may determine the distribution of mycoheterotrophic plants (Bougoure *et al.* 2009; Yamato *et al.* 2016). However, Merckx *et al.* (2017) suggested that the distribution of AM fungi does not drive the distribution of highly specialized mycoheterotrophic plants in the genus *Thismia*, since their specific fungal associates were found to occur beyond the range of the plants' distribution. Also other studies indicated inconsistent trends between the local occurrence of mycoheterotrophic plants and the abundance of their associated fungal partners (Yamato *et al.* 2016; Sheldrake *et al.* 2017). Hence the presence of specific fungi is not enough for cheaters to establish. Sheldrake *et al.* (2017) tested the impact of nitrogen and phosphorus on the occurrence of mycoheterotrophic plants along a fertility gradient across a 65-Km forest in Panama. Their results suggested that the occurrence of these plants is limited by high phosphorus concentration in the soil, which simultaneously reduces the presence and abundance of the AM fungi associated with mycoheterotrophic species. Hence, soil nutrient availability may have an impact on the occurrence of mycoheterotrophic plants by affecting them directly, or indirectly via the AM networks that these plants rely upon. Yet, which soil characteristics influence the occurrence of mycoheterotrophic plants at local scale, either directly or indirectly, is not known.

Here, we examine which soil drivers lead to the patchy distribution of AM mycoheterotrophic plants in tropical rainforests. We explore how the edaphic preferences of mycoheterotrophs reflect the stability of the AM symbiosis upon conditions under which cheating arises.

MATERIALS AND METHODS

Study area

This study was conducted in two forest sites in Colombia in the beginning of the wet season, where mycoheterotrophic plant species are known to occur. We spent five days sampling in each of the sites. The first site consisted of wet tropical lowland forest on terra firme, part of the Amazon rainforest near Leticia ('Amazon'; 4°00'30"S 70°06'12"W). The second site consisted of wet tropical coastal forest on terra firme, part of the Chocó rainforest, near Buenaventura ('Coast'; 3°55'24"N 77°18'56"W). Both sites have no human influence.

Large scale patterns of soil properties do not necessarily reflect the high heterogeneous profiles of soil at local scale, thus we opted for a paired plot sampling strategy where a "positive" plot with mycoheterotrophic plants was simultaneously selected alongside with a nearby "negative" plot without mycoheterotrophic plants. Through this design we were able to identify the effects of specific local differences in soil properties on the patchy occurrences of mycoheterotrophy, within the presumable large-scale variation in soil parameters among sites. We established a total of 16 pairs of plots of 4 x 4 m in the two forests, with five pairs of plots in the Amazon and eleven in the Coast. Positive and negative plots were 5-10 m apart. Pairs of plots were separated by at least 30 m. The number of mycoheterotrophic plants in the positive plots varied between 1 and 22 individuals, and we found up to 6 species per plot (Supporting Information Table S1).

Within each plot, we randomly collected six soil cores, and combined them into a 250 g composite sample per plot. The soil in both sites had clay texture. Soil cores were taken in the shallow top layer of the soil (0-5 cm depth) because we were interested in the chemical properties and nutrient abundance in the soil layer where the roots of the mycoheterotrophic plants are found. Big stones and roots were removed from the samples. The soil was homogenized and preserved on ice immediately after collection until transportation to the laboratory for further processing.

Soil chemistry and nutrient analyses

Soil chemical and nutrient properties were assessed for all 32 plots. Each composite sample was analyzed for soil pH. Total amounts of nitrogen (N_{TOT}) and phosphorus

(P_{top}) were estimated by the Kjeldahl method (Bremner, 1960). The available nitrogen (NH_4^+ and NO_3^-) in the soil was determined by spectrophotometry using 1 N potassium chloride (Maynard & Kalra, 1993). The available phosphorus (P_{av}) was extracted using Bray II solution (Murphy & Riley, 1962). Exchangeable bases (Na, K, Ca and Mg) were measured by the ammonium acetate method (Hanway & Heidel, 1952) and determined by atomic absorption spectrometry. The available micronutrients (Cu, Zn, Mn and Fe), available bore (B), sulfur (S), aluminum (Al), cation exchange capacity (CEC), and soil moisture (Humidity) were determined according to Carter & Gregorich (2006). Organic matter (OM) content in the soil was determined according to Walkley & Black (1934). All analyses were performed by the Centro Internacional de Agricultura Tropical in Colombia. Total soil C and N (on air-dried soil), and abundance of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were analyzed at the UC-Davis (University of California). To evaluate the influence of nutrient stoichiometry on soil processes, we calculated the N:P, N:K, C:P and C:N ratios.

Data analysis

We described and compared the general soil characteristics from the Amazon and Coast using a principal component analysis (PCA). We tested for differences in overall soil composition among positive and negative plots across both sites using a one-way permutational multivariate analysis of variance (perMANOVA with 999 permutations). We tested for homogeneity of dispersion among groups before performing the perMANOVA, and confirmed the assumption of homogeneous dispersion among sites ($P = 0.753$), and between negative and positive plots within the Amazon ($P = 0.198$) and the Coast ($P = 0.873$).

Because we were interested in the effect of soil properties that drive the presence of mycoheterotrophic plants, we calculated the difference in the soil parameter values within each paired negative and positive plot, which hereafter we refer to as delta (Δ). A negative delta indicates that the parameter is lower in the plots where mycoheterotrophic plants were absent, and a positive delta indicates that a specific parameter is lower in the plot where these plants were present. We tested whether there were significant differences across all deltas of the soil properties among sites using perMANOVA (homogeneity of dispersion: $P = 0.713$). We examined whether the individual delta of soil properties varied across and within sites using ANOVAs with “Site” as fixed factor. We also tested for differences in the individual deltas of soil properties while

considering the density of mycoheterotrophic plants found in each plot as a weighting factor to evaluate the effects on both occurrence and abundance of mycoheterotrophic plants simultaneously.

To assess which combination of soil properties mycoheterotrophic plants were selecting for, we selected all soil properties with significantly different deltas. Each predictor was standardized to mean = 0 and SD = 1 to avoid scaling variance issues due to different measurement scales. With all predictors, we built generalized mixed-effects models (GLMMs) to understand the soil parameters that mycoheterotrophic plants have preference for, with 'Plot' as a random effect term to account for the heterogeneity of soil reflected in the spatial clustering of the paired plots. Model selection was performed by adding terms to the model, including interactions between variables, and selecting the terms that gave the greatest improvement to the model likelihood, as assessed by the lowest Bayesian Information Criteria (BIC; Aho et al. 2014). The variables included in the final model were retained if they were significant, and had a variance inflation factor (VIF) < 4 (Zuur *et al.* 2010) and showed a Pearson correlation with all other modelled predictors < |0.70| (Dormann *et al.* 2013). Furthermore, to quantitatively examine the nature of the observed relationships, we used general linear models (GLMs) to tests for relationships between the predictors retained in the best model and the density of mycoheterotrophic plants found in each plot using the deltas of the soil variables.

All analyses were performed in R 3.4.1 (R Core Team, 2016), using the packages NLME, MULTCOMP and VEGAN.

RESULTS

Soil characteristics

We obtained 21 soil parameters from the soil analyses (Table 1). Overall, soil characteristics were significantly different between the two sites ($F = 28.338$, $R^2 = 0.49$, $P = 0.001$; Supporting Information Fig. S1). Soil characteristics were not significantly different between positive and negative plots in the Amazon ($F = 0.738$, $R^2 = 0.08$, $P = 0.627$; Fig. 1a), but they did differ in the Coast ($F = 3.079$, $R^2 = 0.13$, $P = 0.044$; Fig. 1b). Yet, when considering each soil property individually, in the Amazon site the availability of NO_3 , P_{AV} and CEC was significantly lower (Supporting Information Table S2) in the positive plots compared to the respective negative plots, while the main soil

Table 1 | Variation of the soil parameters measured in the plots within the Amazon and Coast calculated by the difference between negative and positive plots.

Soil parameters	Amazon mean Δ	P	Coast mean Δ	P
pH	0.226	0.278	0.183	0.172
MO	-15.936	0.790	-39.811	0.074
P _{TOTAL}	-39.600	0.279	-12.727	0.727
P _{AV}	1.257	0.900	-3.834	0.161
Ca	0.707	0.105	-0.376	0.223
Mg	0.163	0.482	-0.222	0.081
Al	0.392	0.541	0.032	0.990
CICE	1.262	0.083	-0.798	0.106
S	2.378	0.977	-13.095	0.252
B	-0.356	0.743	-0.740	0.097
Zn	-2.721	0.351	-3.723	0.031
Mn	70.543	0.011	-12.612	0.635
Fe	-42.925	0.531	-6.253	0.969
N _{TOTAL}	-487.674	0.402	-310.000	0.442
NH ₄	9.260	0.459	-9.354	0.199
NO ₃	21.504	0.000	0.278	0.991
Humidity	-35.408	0.846	-132.661	0.024
K	0.027	0.966	-0.232	0.020
Cu	-0.073	0.895	-0.042	0.923
$\delta^{13}\text{C}$	2.328	0.116	-0.034	0.999
$\delta^{15}\text{N}$	0.026	0.997	0.610	0.053
N:P	2.058	0.316	1.598	0.228
N:K	53.925	0.023	17.158	0.344
K:P	-0.007	0.961	-0.003	0.982
K:C	0.000	0.928	-0.001	0.158
C:P	-6.560	0.879	13.978	0.311
C:N	-1.434	0.946	-8.116	0.050

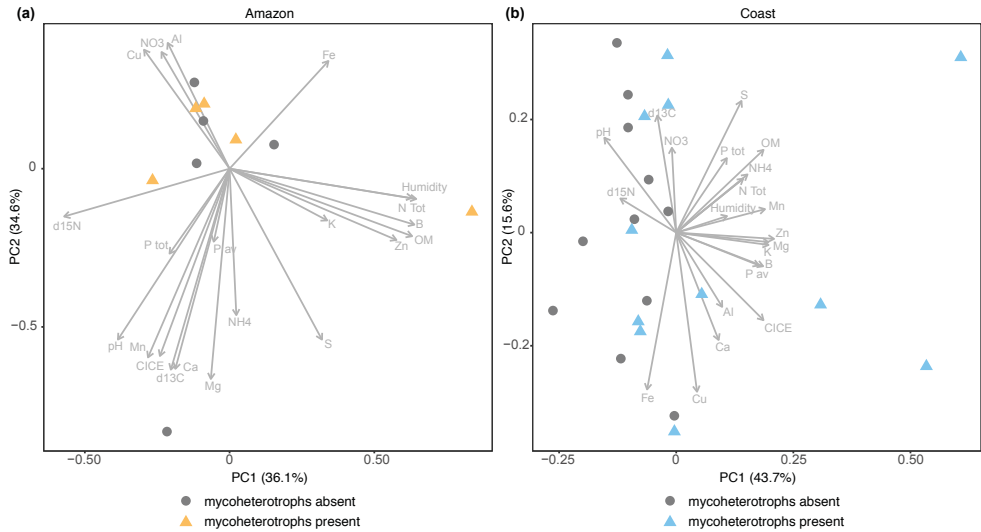


Figure 1 | Principal Components Analysis of the soil properties in the positive plots (triangles) and negative plots (circles) present in the Amazon (a) and the Coast (b). Length of the arrows represents the relative importance of predictors.

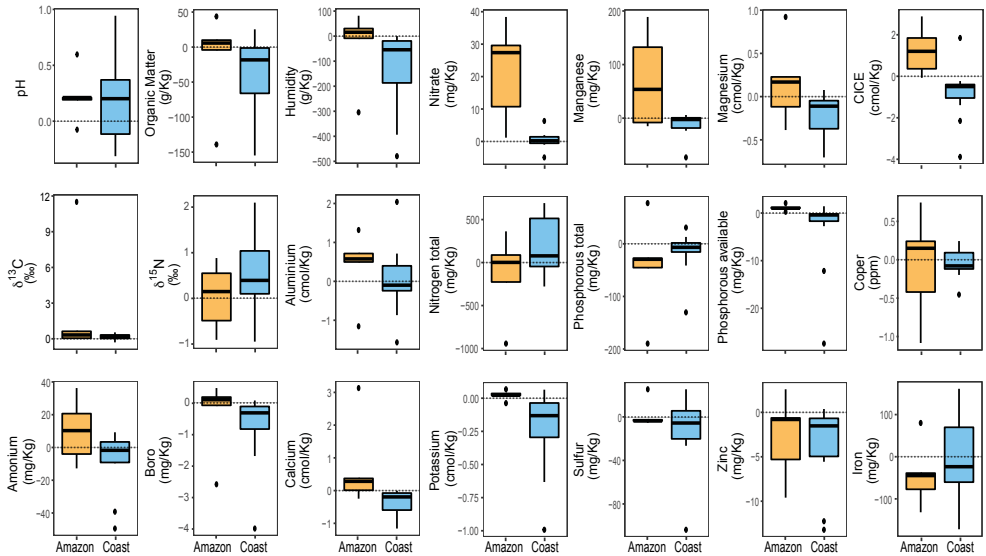


Figure 2 | Variation of soil properties between the negative and positive plots in the Amazon (yellow) and the Coast (blue). Positive values indicate higher availability of a soil property in the negative plots, while negative values indicate a higher availability in the positive plots.

properties that mostly vary within the whole site were OM, Humidity, N_{TOT} , $\delta^{15}N$, B and Zn (Fig. 1a). This indicates that the nutrients that are more variable within this site did not provide a clear separation between positive and negative plots. At the Coast site, positive plots had significantly higher availability of OM, Humidity, CEC, N_{TOT} , and positive ions such as K, Ca, Mg, B, Zn, Mn; and lower availability of $\delta^{15}N$ (Supporting Information Table S2), which correspond to the same soil properties that had more variation within this site (Fig. 1b).

The deltas of the soil characteristics observed between negative and positive plots showed a trend of difference among sites ($F = 2.266$, $R^2 = 0.14$, $P = 0.051$; Fig. 2). The deltas of OM, pH, NO_3 , Humidity and Zn were significantly different for both sites combined. The deltas of NO_3 and Mn differed significantly within the Amazon plots, while the deltas of Humidity, Zn and K differed significantly within the Coast plots (Table 1). Thus, OM, pH, NO_3 , Humidity, Zn, Mn and K were selected as predictors to build generalized mixed-effects models. 'Site' was also considered as predictor due to the interaction of some predictors with either the Amazon or the Coast plots.

Model selection

The best model showed a significant effect of NO_3 , pH and the interaction between NO_3 and K (GLMM: $R^2 = 0.53$, BIC = 40.3; Table 2). We selected this model from a set of undistinguishable models which included the effect of the interaction of pH with Site, the interaction of pH with K, or the interaction of NO_3 with Site. The second best model showed a significant effect of Humidity and pH and the interaction between Humidity and OM (GLMM: $R^2 = 0.35$, BIC = 46.3; Table 2). OM was highly correlated with K (Pearson correlation: $R^2 = 0.67$) and Zn (Pearson correlation: $R^2 = 0.72$), and K correlated with Zn (Pearson correlation: $R^2 = 0.74$), not allowing to separate their impacts. Therefore we did not include these factors in the same model.

The ΔNO_3 was the best predictor for the density of mycoheterotrophic plants (GLM: $Z = -4.827$, $df = 15$, $R^2 = 0.48$, $P < 0.001$; Fig. 3). The number of mycoheterotrophic plants decreased with the increasing difference in concentration of NO_3 , corresponding to an increase in total concentration of NO_3 (Pearson correlation between ΔNO_3 and NO_3 : $R^2 = 0.77$; Fig. 4).

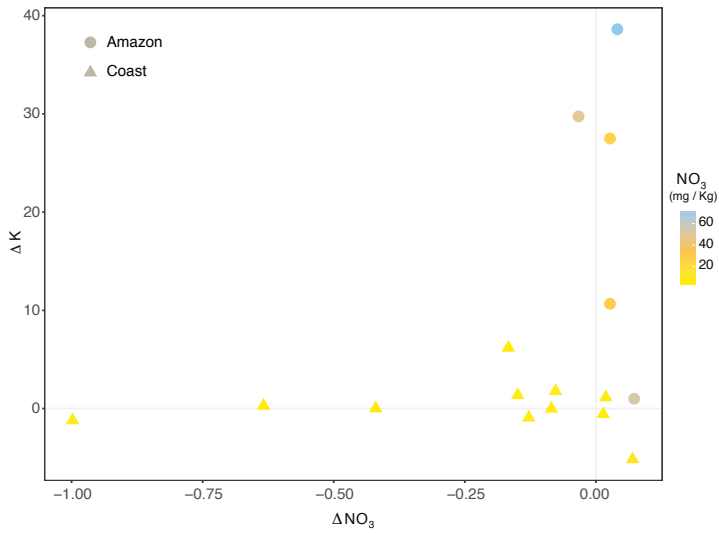


Figure 3 | Variation of K in relation to NO_3 per sample. Positive values indicate higher values of a soil property in the negative plots, while negative values indicate a higher values in the positive plots.

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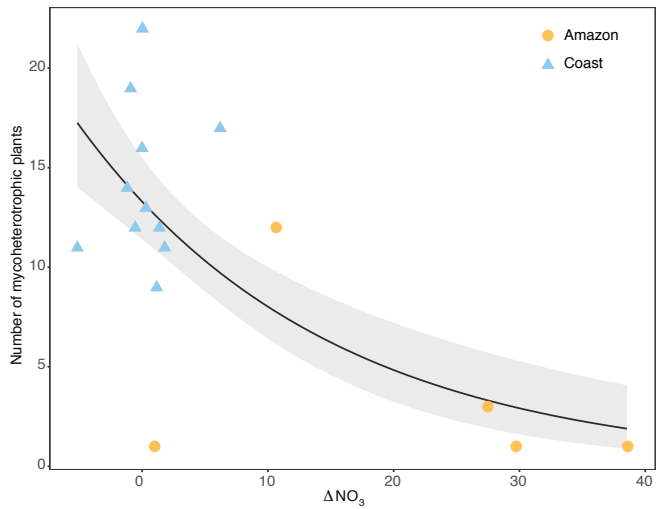


Figure 4 | Relationship between the number of mycoheterotrophic plants observed in the positive plots and the ΔNO_3 (negative plots minus positive plots).

Table 2| Outcomes of the Generalized Linear Mixed Effect modeling aimed to explain the occurrence of mycoheterotrophic plants. 1 is the best model. 2 is the best alternative model ($\Delta BIC = 6.0$).

Model	Terms	Coefficient	SE	z value	P value
1	Intercept	1.038	0.710	1.463	0.144
	NO ₃	-3.432	1.347	-2.548	0.011
	pH	-1.405	0.820	-1.713	0.087
	NO ₃ : K	-11.165	4.264	-2.618	0.009
2	Intercept	-0.474	0.614	-0.772	0.440
	Humidity	5.052	2.377	2.126	0.034
	pH	-2.157	1.137	-1.897	0.058
	Humidity : OM	4.538	2.226	2.038	0.042

DISCUSSION

In this study, we compared soil characteristics of paired plots with and without mycoheterotrophic plants to search for local-scale drivers that influence the occurrence of cheaters in the AM mycorrhizal symbiosis in tropical rainforests. We found that the strongest edaphic predictors of mycoheterotrophic species occurrences involved the interaction between NO₃ and K, and the individual effect of NO₃ and pH. The interaction between soil NO₃ and K is well known in crop responses. Crop response to added nitrogen fertilizers decreases when the exchangeable potassium content of a soil is below an optimal level, because plants deficient in potassium content are not able to produce proteins despite an abundance of available nitrogen (Ranade-Malvi, 2011). In addition, several studies have shown a negative impact of nitrogen addition in agriculture systems on the AM symbiosis performance (Kabir *et al.* 1998; Galvez *et al.* 2001; Oehl *et al.* 2004), because an increased availability of nitrogen to plant roots can lead to carbon limitation in the AM fungal network, which can in turn induce phosphorus deficiency due to carbon limitation to the fungi (Olsson *et al.* 2005). Moreover, excessive amounts of N reduce the plant uptake of P, K and other micronutrients (Ranade-Malvi, 2011). While the stoichiometry of nutrients in soils does not sufficiently reflect nutrient deficiency levels, nutrient stoichiometry in soils has been shown to be crucial in determining the relative availability of nutrients for plant uptake and the stability of the AM symbiosis (Johnson, 2010; Khan *et al.* 2015). Additionally, pH strongly influences the availability of nutrients in the soil, which in turn also impacts the efficiency of nutrient uptake by plants (Rippy *et al.* 2004).

Our study shows a distinct response of the occurrence of mycoheterotrophic plants to NO_3 and K according to the degree of soil fertility, which is also linked to pH variations. In the Amazon, where fertility is higher and heterogeneous, mycoheterotrophic plants avoid high NO_3 conditions while K does not vary. In contrast, in the Coast, where fertility is lower and more homogeneous among the plots, plants select for higher availability of K. While there seems to be a consistent avoidance of higher fertility patches, also reflected in the lower density of plants found with increasing NO_3 availability, it remains unclear how K influences the distribution of these plants. A possible explanation for the overall increased availability and patchiness of K in the Coast can be the effect of salt spray from sea that is close by. The uptake of K by photosynthetic plants is enhanced by the association with AM fungi, which require a minimum availability of K in the soil for the stability of the AM symbiosis (Khan *et al.* 2015).

Mycoheterotrophic plants preferred patches with lower N:K ratios compared to their paired negative plot in the Amazon (Table 1), and the positive plots in the coast had a trend of lower N:K ratios than the negative plots (Table S2, $P < 0.1$). The N:K ratio is highly correlated with NO_3 concentrations, not allowing to disentangle the effect of both predictors in explaining the occurrence of mycoheterotrophic plants in this study. We speculate that in the Amazon, the preference for a lower N:K ratio is mostly related to the avoidance of fertile conditions, while in the Coast, mycoheterotrophic plants prefer higher K availability.

Despite the known importance of N:P ratios in the occurrence and distribution of plants and AM fungi, the N:K ratio and not the N:P ratio appeared to be the relevant predictor for the local distribution of mycoheterotrophic plants. According to the trade balance model (Johnson, 2010), a stable mutualistic mycorrhizal interaction is expected at high N:P ratios. At the same time, a strong positive correlation between the accumulation of P and K has been reported during the AM symbiosis (Olsson *et al.* 2011), while the accumulation of K in AM fungi has been related to low carbon supply from the host plant (Hammer *et al.* 2011). Together, this suggests that accumulation of K may be a consequence of the accumulation of the P that AM fungi do not transfer to plants at low carbon availability conditions (Garcia & Zimmermann, 2014). Thus, it is likely that the performance of the AM symbiosis is affected in a similar manner by the N:P and N:K ratios. Following the trade balance model, our results indicate that in comparison with the negative plots, the lower N:K ratio in the positive plots could indicate that mycorrhizal networks between photosynthetic plants and mycorrhizal

fungi are potentially stronger in the negative plots, and thus mycoheterotrophic plants avoid patches with conditions that favor a strong mutualism between plants and AM fungi.

As indicated above, phosphorus did not relate to local mycoheterotrophic occurrences in this study while available phosphorus – together with soil moisture - has been suggested to be the strongest environmental predictor of plant species distributions in tropical forests (Condit *et al.* 2013), and while phosphorus is considered to be the main limiting element in tropical forests for microbial processes, including mycorrhizal fungi (Camenzind *et al.* 2017). Sheldrake *et al.* (2017) reported a threshold of 2 mg P Kg⁻¹ beyond which mycoheterotrophic plants did not occur across a natural fertility gradient in Panama. Yet, in our study, we found mycoheterotrophic plants growing at much higher concentrations of P in the Coast ranging from 1 to 29 mg P Kg⁻¹ (mean = 5.2 mg P Kg⁻¹, sd = 8.7), and in the Amazon from 4 to 5 mg P Kg⁻¹ (mean = 4.8 mg P Kg⁻¹, sd = 0.2). In fact, in our study, phosphorus did not significantly vary between the positive and negative plots. This suggests that there is not a direct selection for particular concentrations of phosphorus, and that the availability of other nutrients in the soil may play an important role on the impact of phosphorus in this system, at least under the range of available phosphorus of our sampling sites, and for the species considered in our study. Instead, our results highlight that the nutrient stoichiometry rather than the actual concentration of each nutrient drives the occurrence of mycoheterotrophic plants. Particularly the balance between NO₃ and K is key for the prevalence of AM mycoheterotrophic plants in tropical rainforests. Because the influence of individual nutrient concentrations can vary due to the stoichiometry effect of other nutrients, we propose that absolute concentrations of elements may give poorer information about the probability of cheating the AM symbiosis.

Soil moisture appeared to influence mycoheterotrophic plants' occurrence, but to a lesser extent than soil fertility. Soil moisture has been hypothesized to be the main limiting factor to the occurrence of these plants, due to their sensitivity to desiccation (Leake, 1994; Klooster & Culley, 2009), but our study shows that mycoheterotrophic plants have a stronger selection for other soil conditions. The absolute values of humidity are significantly lower in the Amazon comparing to the Coast, yet our study shows a preference for patches with higher humidity only in the Coast. This finding implies that while humidity still contributes for to the occurrence probability of mycoheterotrophic plants, it is not as limiting as previously thought, or at least not at this fine scale. Possibly,

soil moisture determines the occurrence of mycoheterotrophic plants at a broader scale such as between forests (Leake, 1994), while at a finer scale of the 4 x 4 m plots that we used in this study, the heterogeneous fertility conditions within forests play a major role.

In the Amazon site, mycoheterotrophic plants were relatively rare. Three established plots only contained one individual, in one plot we found three and in another one we detected 12 specimens. At the Coast, mycoheterotrophic plants were quite abundant throughout the forest but displaying the characteristic patchy distribution and co-occurrence of distantly related species. Possibly, the lower abundance of mycoheterotrophs in the Amazon reflects less favorable environmental conditions for their occurrence in general, or in particular, at the time of sampling. The differences in success in finding these plants at both sites suggests that differences in soil properties between the two forest sites (Supporting Information Fig. S1) may explain this difference in mycoheterotrophs abundances. Even though the abundance of mycoheterotrophic plants differed between the two sites, within both sites the N:K ratio still determined their local occurrence patterns.

In conclusion, our results provide empirical support to the view that mycoheterotrophic plants avoid high fertility conditions, where fungi are prone to parasitism (high N, high P or K). We also suggest that these plants seem to avoid conditions that could favor a strong AM mutualism (high N, low P or K), according to the trade balance model (Johnson, 2010). In accordance, our findings show a negative response of the abundance of mycoheterotrophs individuals to an increase in N availability. Therefore, we propose that mycoheterotrophy is more prone to occur in conditions of weak mutualistic strength (low N, low P or K) between plants and AM fungi according to the trade balance model (Johnson, 2010). In conditions leading to commensalism between plants and AM fungi (low N, high P or K), cheating is less likely to occur, since both partners are exchanging limited resources, and therefore it is theoretically more difficult for mycoheterotrophic plants to obtain carbon from these fungi.

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Author Contribution:

All authors designed the study. S.I.F.G. and V.S.F.T. conducted fieldwork. S.I.F.G. analysed the data. S.I.F.G. wrote the manuscript. All authors contributed to discussion and to earlier versions of the manuscript.

SUPPORTING INFORMATION

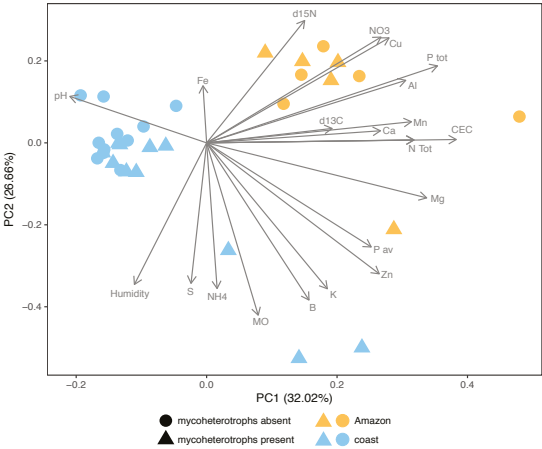


Figure S1 | Principal Components Analysis of the soil properties in the Amazon (yellow) and the Coast (blue). Positive plots (triangles) and negative plots (circles) within each site are represented. Length of the arrows represents the relative importance of predictors.

Table S1 | Mycoheterotrophic plant species present in the 16 plots. Number of individuals from each species is presented

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8	Plot 9	Plot 10	Plot 11	Plot 12	Plot 13	Plot 14	Plot 15	Plot 16
<i>Apteria aphylla</i>																
<i>Gymnosiphon brachycephalus</i>		2	1		3	7		4			3					
<i>Gymnosiphon divaricatus</i>		8	8	8	7	5		6	5	6	4		3			
<i>Sciaphila</i> sp.	11															
<i>Sciaphila purpurea</i>										4	8					
<i>Soridium spruceanum</i>				1	6		6		5	3	3					
<i>Voyria aphylla</i>				1		8	6		1							
<i>Voyria chionea</i>													1	1	2	
<i>Voyria pittieri</i>															10	
<i>Voyria tenella</i>		6						3				1				

Table S2 | Overall soil parameters in the negative and positive plots within the Amazon and Coast sites.

	Amazon			Coast		
	Neg plots mean	Pos plots mean	P	Neg plots mean	Pos plots mean	P
pH	4.26	4.03	0.106	4.49	4.31	0.156
OM	77.77	93.71	0.648	88.73	128.54	0.038**
P _{TOTAL}	441.80	481.40	0.411	199.82	212.55	0.344
P _{AV}	6.10	4.85	0.013*	1.38	5.21	0.175
Ca	1.68	0.97	0.319	0.31	0.69	0.008**
Mg	0.64	0.48	0.502	0.18	0.40	0.023**
Al	2.94	2.55	0.319	1.42	1.39	0.898
CEC	5.26	3.99	0.077*	2.09	2.89	0.066*
S	47.61	45.23	0.709	59.71	72.8	0.230
B	0.65	1.01	0.563	0.62	1.36	0.071*
Zn	4.49	7.21	0.274	2.31	6.03	0.029**
Mn	114.20	43.66	0.154	4.10	16.71	0.094*
Fe	226.49	269.41	0.289	195.04	201.29	0.850
N _{TOTAL}	3366.48	3854.20	0.526	1841.82	2151.82	0.058*
NH ₄	34.65	25.40	0.296	38.90	48.25	0.136
NO ₃	67.99	46.48	0.035**	5.61	5.33	0.741
Humidity	409.85	445.25	0.636	521.84	654.5	0.019**
K	0.30	0.27	0.191	0.18	0.42	0.030**
Cu	1.80	1.87	0.829	0.55	0.59	0.507
δ ¹³ C	-27.07	-29.40	0.365	-29.31	-29.27	0.691
δ ¹⁵ N	5.35	5.33	0.942	4.30	3.69	0.053*
N:P	11.78	9.72	0.217	5.07	3.48	0.170
N:K	241.93	188.01	0.157	34.98	17.82	0.074*
K:P	0.05	0.06	0.205	0.18	0.18	0.903
K:C	0.004	0.004	0.848	0.002	0.003	0.017**
C:P	12.74	19.30	0.396	84.94	70.97	0.270
C:N	1.36	2.80	0.383	18.46	26.57	0.074*

The soil parameters are significantly different between the negative and positive plots within the respective site (* $P < 0.10$; ** $P < 0.05$).