A phosphorus threshold for mycoheterotrophic plants in tropical forests

Merlin Sheldrake[*,1,2], Nicholas P. Rosenstock[3], Daniel Revillini[2,4], Pål Axel Olsson[5], S. Joseph Wright[2] & Benjamin L. Turner[2]

[1] *Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK*

[2] *Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Republic of Panama*

[3] *Center for Environmental and Climate Research, Lund University, 22362 Lund, Sweden*

[4] *Department of Biological Sciences, Northern Arizona University, PO BOX 5640, Flagstaff, AZ 86011, USA*

[5] *Department of Biology, Lund University, 22362 Lund, Sweden*

Correspondence and requests for materials should be addressed to M.S. (merlinsheldrake@gmail.com)

Additional materials enclosed in submission:

1. **Supplementary Figures S1-S9**
2. **Supplementary Tables S1-S3**
3. **Supplementary Methods**
4. **Supplementary Discussion**
5. **Raw data as .xlsx file**

6. **Unpublished manuscript cited in discussion of our findings:** Turner, B. L., Brenes-Arguedas, T. & R. Condit, Pervasive phosphorus limitation of tropical tree species, *Unpublished Manuscript*

ABSTRACT

The majority of terrestrial plants associate with arbuscular mycorrhizal (AM) fungi, which typically facilitate the uptake of limiting mineral nutrients by plants in exchange for plant carbon. However, hundreds of non-photosynthetic plant species — mycoheterotrophs — depend entirely on AM fungi for carbon as well as mineral nutrition. Mycoheterotrophs can provide insight into the operation and regulation of AM fungal relationships, but little is known about the factors, fungal or otherwise, that affect mycoheterotroph abundance and distribution. In a lowland tropical forest in Panama, we conducted the first systematic investigation into the influence of abiotic factors on the abundance and distribution of mycoheterotrophs, to ask whether the availability of nitrogen and phosphorus altered the occurrence of mycoheterotrophs and their AM fungal partners. Across a natural fertility gradient spanning the isthmus of Panama, and also in a long-term nutrient addition experiment, mycoheterotrophs were entirely absent when soil exchangeable phosphate concentrations exceeded 2 mg P kg^{-1}. Experimental phosphorus addition reduced the abundance of AM fungi, and also reduced the abundance of the specific AM fungal taxa required by the mycoheterotrophs, suggesting that the phosphorus sensitivity of mycoheterotrophs is underpinned by the phosphorus sensitivity of their AM fungal hosts. The soil phosphorus concentration of 2 mg P kg^{-1} also corresponds to a marked shift in tree community composition and soil phosphatase activity across the fertility gradient, suggesting that our findings have broad ecological significance.

Keywords

Arbuscular mycorrhizal fungi, mycoheterotroph, tropical forest, epiparisitism, phosphorus

MAIN TEXT

1. Introduction

The 400-million-year-old symbiotic relationship between plants and arbuscular mycorrhizal (AM) fungi is a fundamental component of terrestrial ecosystems [1,2]. Plants supply the fungi with up to 20% of photosynthetically-derived carbon in return for improved access to mineral nutrients, with up to 90% of plant phosphorus being derived from AM fungal partners [3-6]. AM fungi are thus major players in global carbon and nutrient cycles [7-9]. The functioning of plant–AM fungal symbioses in highly diverse tropical forests is poorly understood, partly because tropical plant–AM fungal associations are understudied compared with agricultural and temperate systems [10,11], and partly because tropical plant–AM fungal relations are more complicated than in temperature systems, with the majority of tropical trees depending on AM fungi for establishment and growth [2,12].

Over 400 species of plant are non-photosynthetic and depend entirely on fungi for carbon as well as mineral nutrition [13-16] (Figure 1). These plants are known as full mycoheterotrophs because they depend on fungi for their entire carbon and nutrient supply over their lifecycle. The mycoheterotrophic habit has evolved independently more than 40 times across many plant phyla [17]. Because AM fungi are themselves obligate symbionts and derive all of their carbohydrate from photosynthetic plants, the carbohydrates acquired by

mycoheterotrophs are ultimately derived from other plants via common mycorrhizal networks, thus demonstrating that AM fungi can facilitate biologically significant plant-to-plant carbon transfer, a hotly contested topic [6]. Mycoheterotrophs also demonstrate that plant–AM fungal relations are not always based on the reciprocal exchange of resources. In light of recent efforts to portray plant-AM fungal relations as a 'biological market' in which plant-AM fungal relations are determined by the mutual evaluation of a range of trading partners [18], mycoheterotrophs thus represent important exceptions.

Little is known about factors, fungal or otherwise, that determine mycoheterotroph distributions [17]. Changes in the relative availability of resources, notably nitrogen and phosphorus, strongly determine the operation of AM fungal symbioses [19], affecting AM fungal abundance, community composition and diversity, resource allocation and mutualistic quality of plant and fungal partners [20-23]. Here, we performed the first systematic investigation into the effects of abiotic factors on the abundance and distribution of mycoheterotrophs to ask whether the relative availability of nitrogen and phosphorus altered the occurrence of mycoheterotrophs and their AM fungal partners.

2. Methods

We investigated the most common mycoheterotrophic species — *Voyria tenella* and *Voyria corymbosa* (Figure 1) — across: i) a well-characterised fertility gradient across the isthmus of Panama [24,25], and ii) a fully factorial NPK nutrient-addition experiment that had been running for fifteen years (Figure S1) [26]. We asked which environmental factors determined mycoheterotroph abundance by combining mycoheterotroph census data and mycoheterotroph tissue nutrient data with a range of environmental metrics along the fertility gradient. We then used the nutrient-addition experiment to ask whether i) the patterns of

mycoheterotroph occurrence across the gradient could be experimentally recreated, ii) experimental nutrient addition affected the net abundance of AM fungi in both soil and in the roots of autotrophic plants, and iii) experimental nutrient addition affected the specific AM fungal hosts of the mycoheterotrophs in the soil, and in the roots of autotrophic plants.

(a) Fertility gradient

(i) Site descriptions

The natural fertility gradient consisted of 37 sites near the Panama Canal in closed canopy forest, including undisturbed old growth and secondary stands (60-100 years old) [27]. Sites spanned a rainfall and edaphic gradient at low elevation (< 200 m above sea level; SI methods). Concentrations of readily exchangeable phosphate extracted by anion exchange membranes — a measure of biologically available phosphorus — vary more than 300-fold across these sites and represent a range comparable to that of the entire lowland tropics [25]. Most of the sites were 1-ha permanent census plots [26].

(ii) Mycoheterotroph census and sampling

We censused all sites over a two-week period between 4th and 18th October 2013. This fell in the middle of the wet season and the peak of mycoheterotroph flowering (based on our observations and a preliminary census between August and December 2012 at the Barro Colorado Nature Monument; BCNM). Counts for each plot are the average of three independent counts by the same three-person team who combed each plot in 3-m wide bands (SI methods). In 1-ha plots, we surveyed a randomly selected 40-m × 40-m area. When we found no mycoheterotrophs in the 40-m × 40-m area we combed the entire 1-ha plot to

confirm their absence. Plots where mycoheterotrophs were entirely absent were re-checked in late October, mid-November and mid-December).

Mycoheterotroph samples from the fertility gradient were collected for tissue phosphorus analysis. We sampled 6-10 individuals of the common *Voyria tenella* and 3-6 individuals of the less common *Voyria corymbosa* (Figure 1) at each site, where present. Plants were located away from gaps, and separated from each other by at least 3 m. Roots and stems for tissue nutrient analysis (fertility gradient samples) were dried at 60°C and stored at room temperature.

(iii) Soil chemistry

The fertility gradient is naturally occurring, not experimental, and multiple variables were required to model mycoheterotroph occurrence. All soil chemistry data used was obtained by the methods described by Condit et al. (2013) [25] (SI methods).

(iv) Tissue nutrient analysis

We asked whether increasing levels of soil phosphorus corresponded to an increase in mycoheterotroph tissue phosphorus across the fertility gradient. Mycoheterotroph stem and root tissue was ground to a fine powder in a homogeniser (TissueLyser II, Qiagen) and then pooled by plot, each individual contributing an equal mass to the composite sample. Samples were ashed at 550°C, dissolved in 1 M HCl, and phosphorus was detected by automated molybdovanadate colorimetry on a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO). Values are expressed on a 60°C dry mass basis.

(b) Nutrient addition experiment

148

149 *(i) Site descriptions*

150 The nutrient-addition experiment is a factorial NPK fertilisation experiment with eight
151 treatments (N, P, K, NP, NK, PK, NPK, and unfertilised controls) and a ninth micronutrient
152 treatment, each replicated four times across the 38.4-ha study site (a total of 36 plots). The
153 plots measured 40-m × 40-m. Starting in 1998, fertilisers were applied by hand in four equal
154 doses a year, equally spaced across the wet season. The annual doses were 125 kg N ha^{-1} yr^{-1}
155 as urea, 50 kg P ha^{-1} yr^{-1} as triple superphosphate, 50 kg K ha^{-1} yr^{-1} as potassium chloride,
156 and a mixture of micronutrients (B, Ca, Cu, Fe, Mg, Mn, Mo, S, Zn; SI methods). The
157 nutrient-addition experiment is located within the BCNM in Panama [26] (see SI methods for
158 a detailed site description).

159

160 *(ii) Mycoheterotroph census and sampling*

161 Across the nutrient-addition experiment, we surveyed entire 40-m × 40-m experimental plots
162 (total area = 1,600 m^2) across all treatments (a total of 36 plots). In all other ways, the
163 mycoheterotroph census and sampling was performed as described for the fertility gradient.
164 Since mycoheterotroph samples from the nutrient-addition experiment were collected for 454
165 sequencing of AM fungal communities in their roots, we wiped down and flame-sterilised all
166 equipment in between samples, handled all samples with fresh latex gloves, and double-
167 bagged samples in sealed Ziploc^{TM} bags. We rinsed root systems in filtered deionised water,
168 and removed soil particles with a fine brush. All brushes and containers were sterilised with
169 boiling water between samples to prevent cross-contamination. Roots were surface sterilised
170 by immersion for 1 minute in 70% ethanol then 1 minute in 1% bleach (NaOCl), rinsed with
171 sterile deionised water, dried separately over silica gel in 4 ml tubes, and stored at -20°C.

172

(iii) Soil sampling

We collected soil samples for i) AM fungal DNA extraction, and ii) lipid analysis across the control, N, P, K, NP and NK treatments (24 plots). We collected 81 soil samples (9 × 9 grid) from 0-10 cm depth within the inner 20-m × 20-m of each plot, using a volumetric spoon to ensure that there were equal volumes of soil in each subsample. We chose the surface layer (0-10 cm) because the majority of fine roots are located in this layer [28,29]. We composited and thoroughly mixed the samples for each plot, sieved the soil to remove large roots, and subsampled the mixed soil 10 times to make two replicate samples (*c.* 10 g per sample), for DNA extraction and lipid analysis. We froze these samples at –80°C for twelve hours, lyophilised them, and stored them dry at –80°C until further processing.

(iv) Autotrophic seedling sampling

We harvested autotrophic seedlings to analyse i) AM fungal community composition, and ii) AM fungal root colonization. We sampled seedlings of seven of the most common tree species across the plots: *Alseis blackiana* Hemsl. (Rubiaceae), *Desmopsis panamensis* (B.L. Rob.) Saff. (Annonaceae), *Heisteria concinna* Standl. (Olacaceae), *Sorocea affinis* Hemsl. (Moraceae), *Simarouba amara* Aubl. (Simaroubaceae), *Tetragastris panamensis* (Engl.) Kunze. (Burseraceae), and *Virola sebifera* Aubl. (Myristicaceae). These species span a range of life history strategies and maximum adult heights [30] (SI methods).

We harvested 4-6 seedlings of each of the seven species from each plot. Seedlings were sampled from the same treatments as soil with the exception of NK, due to time constraints (a total of 20 plots). This resulted in *c.* 35 seedlings for each of the 20 plots, making a total of *c.* 700 seedlings. Seedlings were 15-20 cm tall, were located away from gaps, and separated

from each other by at least 3 m. From each seedling, we subsampled healthy fine roots for DNA extraction as described above, and stored subsamples in 70% ethanol for microscopic analysis.

(v) AM fungal abundance

We used the neutral lipid fatty acid (NLFA) 16:1ω5 as a biomarker for extra-radical AM fungal biomass in soils from the nutrient-addition experiment. We performed lipid extraction and analysis following Frostegård et al. (1993) [31], with modifications described by Nilsson et al. (2007) [32] (SI methods). The mean NLFA:PLFA ratio across samples was 2.3, indicating that NLFA 16:1ω5 is an effective AM fungal biomarker in these soils [33].

We quantified root colonisation to assess intra-radical AM fungal abundance in the roots of autotrophic seedlings sampled from the nutrient-addition experiment. To measure AM fungal colonisation of roots, we observed cleared and stained roots (SI methods) using a compound light microscope at 200 × magnification, and quantified AM fungal colonization following McGonigle et al. [34], with at least 100 intersections for one sample per seedling. Mycorrhizal colonisation was expressed as the percentage fine root length colonised by AM fungal hyphae, vesicles or arbuscules.

(vi) DNA extraction and sequencing

Mycoheterotroph and autotrophic seedling root samples were individually pulverised in a homogeniser prior to DNA extraction (TissueLyser II, Qiagen), and an equal mass of each root sample was pooled to make one composite sample per species per plot. Soil samples were pulverised by the same method. We extracted DNA from 50 mg of pulverised root and

25 mg of pulverised soil using MoBio PowerPlant and PowerSoil DNA isolation kits and manufacturer's instructions (MoBio Laboratories Inc., Carlsbad, CA, USA).

We amplified the partial small subunit (SSU) region of 18S ribosomal DNA (*c.* 550 bp) with universal eukaryotic primer NS31 [35] and the AM fungal-specific primer AM1 [36]. The primers were modified by the addition of the 454 pyrosequencing adaptors A and B, in addition to a 10 bp multiplex identifier (MID) on the forward primer (NS31). We conducted duplicate polymerase chain reactions (PCRs) in 25 µl sample volume using Phire hot start II DNA polymerase (Life Technologies LTD, Paisley, UK). Conditions were: 98°C for 1 minute; 32 cycles of 98°C for 10 s and 72°C for 15 s; and a final extension phase of 72°C for 2 minutes. We gel-purified PCR products using MinElute PCR purification kits (Qiagen Ltd, West Sussex, UK) and pooled the samples in equimolar concentrations.

Amplicon libraries were sequenced by the Cambridge DNA Sequencing Facility (Department of Biochemistry, University of Cambridge, UK) on an FLX Titanium system (Roche, Basel, Switzerland). No sequences were detected in the blanks included as negative controls at each of the extraction, PCR, gel purification, quantification, and sequencing stages.

(vii) Bioinformatic analyses

All bioinformatic analysis was performed using the software mothur [37] unless otherwise stated. Reads were removed from the dataset if they did not contain the 10 bp MID, had > 1 error in the barcode sequence, > 2 errors in the forward primer, or were shorter than 200 bp in length.

Clustering was performed using the algorithm Clustering 16S rRNA for Operational Taxonomic Unit (OTU) Prediction (CROP). Sequence alignment was performed with the software MAFFT v7.149b [38] and improved with MUSCLE [39] using the –refine option. Trees were built using RAxML v. 8.0 [40] with GTR GAMMA implementation, and bootstrap values based on 1000 runs. We used the Basic Local Alignment Search Tool (BLAST [60]; minimum e-value 10^{-30}) on one representative sequence from each cluster iteratively against three databases in the following order of preference: i) sequences from Krüger et al. (2012); ii) all virtual taxa (VT) from the MaarjAM AM fungal sequence database (www.maarjam.botany.ut.ee); and iii) all 18S Glomeromycotan sequences from the SILVA database. Non-Glomeromycotan clusters were removed when the highest blast match did not correspond to an AM fungal sequence in any of the three datasets.

Clusters were named based on matches to database entries at > 97% covering a minimum of 80% of the query sequence. We used the generic names from Krüger et al. (2012), and VT numbers from the MaarjAM database. Where clusters did not match a VT at > 97%, we assigned a name based on the highest VT match and phylogeny (eg. Glomus_OTU1). Clusters that occurred in < 2 samples, and with < 5 reads total were removed from the dataset. A breakdown of the sequencing results is provided in the Supplementary Discussion. For detailed description of bioinformatic procedures see SI methods.

(c) Statistical analyses

All statistical analysis was conducted in R version 3.1.2 (R Development Core Team, 2014).

(i) Mycoheterotroph census (fertility gradient and nutrient-addition experiment)

We analysed the results of mycoheterotroph censuses across the fertility gradient and nutrient-addition experiment using generalised linear models (GLMs) with negative binomial error structures with glm.nb from the package MASS [41]. We analysed only the most abundant species (*V. tenella* across the gradient, and *V. tenella* and *V. corymbosa* across the nutrient-addition experiment), and built separate models for each species. Significance of model terms was assessed using likelihood-ratio chi-square tests.

The fertility gradient is not a controlled experiment. Consequently, multiple regression was required to model mycoheterotroph occurrence. We worked with a subset of environmental variables selected and described by Condit et al. (2013) [25] (dry-season moisture, inorganic N, P, Ca, Zn, K; see SI methods). P, Ca, Zn, and K had extreme values and were log-transformed to reduce the influence of outliers. All predictors were standardized to zero mean and unit variance.

Across the nutrient-addition experiment, we modelled counts of mycoheterotrophs in NPK factorial models and tested for all two-way interactions. The experimental design of the nutrient-addition experiment includes four replicates of an incomplete block design, and 'replicate' was used as a spatial blocking term to control for natural variation across the site [26].

(ii) Mycoheterotroph tissue phosphorus (fertility gradient)

We used linear models to analyse the relationship between soil exchangeable phosphorus and root and stem tissue phosphorus concentration of *V. tenella.*

(iii) AM fungal abundance in roots and soil (nutrient-addition experiment)

We used linear models to analyse: i) the concentration of NLFA 16:1ω5 (a proxy for AM fungal biomass) in the soil and, ii) the percentage of tree seedling root length colonised by AM fungi, pooling six of the seven seedling species (*Tetragastris* roots were damaged during the clearing process and omitted from analysis). In both cases we tested for N × P interactions (omitting the K treatment) using factorial ANOVA, and for the significance of K in a separate model with a single 'treatment' term. The spatial blocking term was included in all models.

(iv) AM fungal taxa in roots and soil (nutrient-addition experiment)

We used the number of DNA sequences as a measure of relative abundance of OTUs (Supplementary Discussion). We analysed the relative abundance, in the soil, of the five AM fungal taxa dominating (constituting > 97%) the AM fungal communities in the roots of *V. tenella* and *V. corymbosa* using factorial GLMs with negative binomial errors (using glm.nb), building a separate model for each fungal taxon, including the spatial blocking term, and testing for N × P and N × K interactions using likelihood-ratio chi-square tests (soil samples were not collected from PK and NPK plots).

We analysed the relative abundance of the AM fungal taxon most strongly associated with the roots of *V. tenella* and *V. corymbosa* (constituting > 90% of their AM fungal taxa; Sclerocystis_VTX00126) in the roots of the seven autotrophic plant species with generalised linear mixed models (GLMMs; glmer.nb from the package lme4) with 'P' (0 versus 1), 'species', and the spatial blocking term as fixed effects, and 'plot' as a random effect to control for the pseudoreplication arising from having seven species per plot. Main treatment effects (P, 'species' and P × 'species' interaction) were assessed by comparing nested models using likelihood-ratio chi-square tests.

3. Results

(a) Fertility gradient

(i) Mycoheterotroph census

Across the fertility gradient, the abundance of *V. tenella* precipitously declined with increasing soil exchangeable phosphorus; numbers of plants fell from 3500 ha^{-1} at the lowest soil phosphorus concentrations to 0 plants ha^{-1} above 2 mg P kg^{-1} ($\chi^2 = 55.1$, $P < 0.001$, Figure 2a). The abundance of *V. tenella* was not related to any other environmental variable included in the analysis (dry-season moisture, inorganic N, and exchangeable Ca, Zn and K; Figure S2, Table S1). Furthermore, we found no mycoheterotrophs of any other species growing in plots with exchangeable soil phosphorus above 2 mg P kg^{-1}.

(ii) Mycoheterotroph tissue phosphorus analysis

Stem and root tissue phosphorus concentrations of *Voyria tenella* did not respond to increasing levels of soil exchangeable phosphorus across the network of forest plots at the lower end of the fertility gradient (< 2 mg P kg^{-1}) where they were found (stem: $F_{1,21} = 1.8$, $P = 0.20$; root: $F_{1,17} = 0.45$, $P = 0.51$; Figure S7). Some root samples did not yield sufficient material for analysis, meaning that sample sizes for stem and root analyses differed (n = 23 and n = 19 for stem and root, respectively).

(b) Nutrient-addition experiment

(i) Mycoheterotroph census

In the nutrient-addition experiment, phosphorus addition in all nutrient combinations (P, NP, PK, NPK) completely eliminated mycoheterotrophs of any species. In the case of *V. tenella*, numbers were reduced from 1000 plants ha^{-1} (in no-P treatments) to 0 ($\chi^2 = 497$, $P < 0.001$), and in the case of *V. corymbosa* from 45 plants ha^{-1} (in no-P treatments) to 0 ($\chi^2 = 205$, $P < 0.001$, Figure 2b and Figure S3). Micronutrient addition did not affect numbers of either *V. tenella* or *V. corymbosa* confirming that phosphorus and not the calcium counterion of the phosphorus fertiliser (triple superphosphate, $Ca(H_2PO_4)_2.H_2O$) was responsible for the elimination of mycoheterotrophs (Figure S8). Numbers of *V. tenella* were reduced by nitrogen addition and restored to control levels when nitrogen and potassium were added together, as indicated by a significant nitrogen × potassium interaction ($\chi^2 = 7.94$, $P = 0.005$; Figure S3), while the abundance of *V. corymbosa* was increased by N addition ($\chi^2 = 7.68$, $P < 0.006$; Figure S3).

(ii) AM fungal abundance

Phosphorus addition reduced the biomass of AM fungi in the soil by *c.* 25% ($F_{1,11} = 5.02$, $P = 0.04$, Figure S6a) and AM fungal colonisation in tree seedling roots, by *c.* 12% ($F_{1,11} = 9.39$, $P = 0.01$, Figure S6b). AM fungal biomass in the soil was also reduced by nitrogen addition ($F_{1,15} = 5.05$, $P = 0.04$; Figure S6a), as was AM fungal colonisation of tree seedling roots ($F_{1,9} = 18.4$, $P = 0.002$; Figure S6b).

(iii) AM fungal community composition

Five AM fungal families were represented across root and soil samples (Acaulosporaceae, Archaeosporaceae, Diversisporaceae, Gigasporaceae, Glomeraceae). Rarefaction curves for each sample approached asymptotes indicating that sequencing intensity was sufficiently

high to detect the majority of OTUs and that sampling effort was sufficient to capture the range of AM fungal diversity across the sites (Figure S9). 95.9% of all sequences were Glomeromycotan, and 80 OTUs remained after blasting 80 OTUs remained after blasting, filtering, merging, and trimming, representing a total of 288,139 sequences. Samples contained a mean of 13 OTUs (range: 1–40), and the mean number of sequences per sample was 1055 (range: 201–2442). Sclerocystis_VTX00126 was the most dominant taxon across the dataset.

Experimental phosphorus addition reduced the relative abundance of the AM fungal taxon most strongly associated with the roots of *V. tenella* and *V. corymbosa* in soil communities (constituting > 90% of their AM fungal communities ; Sclerocystis_VTX00126) by *c.* 65% ($\chi^2 = 24.8$, $P < 0.001$, Figure 3 and Figure S4). In contrast, in the roots of photosynthetic seedlings the relative abundance of Sclerocystis_VTX00126 was reduced in some species but not others (phosphorus × species interaction, $\chi^2 = 26.8$, $P < 0.001$, Figure S5). Although reduced, the AM fungal taxa colonising mycoheterotrophs were still present in the soil in P-fertilised plots, and remained part of intact networks with the photosynthetic plant species that they partnered with in unfertilised controls (Figure 4).

4. Discussion

We found that the abundance and distribution of *V. tenella* and *V. corymbosa* were strongly dependent on soil phosphorus availability, and that numbers of *V. tenella* increased sharply below a phosphorus threshold of 2 mg P kg^{-1}. Long-term experimental phosphorus addition not only eliminated *Voyria*, but also reduced the net abundance of AM fungi in soil and roots of autotrophic plants (Figure S6), and the relative abundance of the specific AM fungal taxa

394 hosting both species of *Voyria* (Figure 3). We observed a moderate response of *V. tenella* and
395 *V. corymbosa* abundance to experimental nitrogen addition, although this was not consistent
396 between the two species (Figure S3). The abundance of AM fungi in soil and roots of
397 autotrophic plants was also reduced by nitrogen addition (Figure S6).
398

(a) Explaining *Voyria's* phosphorus sensitivity

Although we cannot resolve the mechanism by which phosphorus impacts *Voyria*, we can evaluate several possibilities.

i) Phosphorus toxicity

It is unlikely that phosphorus is directly toxic to either species of *Voyria*. Across the plant kingdom, phosphorus toxicity occurs at tissue phosphorus concentrations of 10–40 mg phosphorus g^{-1} dry mass (from the extremely phosphorus-sensitive Proteaceae to arable crops) [42]. Across the phosphorus gradient where *V. tenella* occurred (from 0–2 mg P kg^{-1} soil exchangeable phosphorus), tissue phosphorus concentrations of *V. tenella* never exceeded 3.2 mg P g^{-1}, well below the documented lower limit of phosphorus toxicity, and did not increase in response to increasing soil phosphorus (Figure S7).

ii) Shifts in plant species distributions

Plant species distributions shift markedly across the fertility gradient [25]. In some cases, mycoheterotrophs can specialise on fungi linked to a specific set of autotrophic host trees [43], and the change in AM fungal host might influence the occurrence of mycoheterotrophs across this gradient. However, both species of *Voyria* were eliminated by experimental phosphorus addition, whereas tree species distribution has not been altered by phosphorus addition, strongly suggesting that the sensitivity of mycoheterotrophs to phosphorus is not mediated by autotrophic plant host identity.

iii) Reduced abundance of mycoheterotrophs' AM fungal partners

Both the overall abundance of AM fungi in soil and roots, and the relative abundance of the AM fungal taxa required by *V. tenella* and *V. corymbosa* in soil were reduced by phosphorus

addition (Figures 3 and 4). This is likely to contribute to reduced *Voyria* abundance with increasing soil phosphorus, and strongly suggests that the phosphorus sensitivity of *Voyria* can be explained in terms of the phosphorus sensitivity of *Voyria's* AM fungal symbionts. The impact of phosphorus availability on AM fungal abundance and function is well-documented and is caused by changes in the exchange relationships between AM fungi and their autotrophic hosts [19]. This finding raises the interesting possibility that changes in the pattern of resource exchange between autotrophs and AM fungi may affect the abundance and distribution of mycoheterotrophs.

iv) Shift in AM fungal function

Although the AM fungal taxa required by both species of *Voyria* were reduced by phosphorus addition across the nutrient-addition experiment, these taxa were not eliminated (Figure 3 and 4). Furthermore, in three of the +P plots, where mycoheterotrophs were absent, both the proportional abundance of the dominant Sclerocystis_VTX00126 and net AM fungal biomass in the soil was comparable or exceeded that in three no-P plots, where mycoheterotrophs were present (Table S2). This indicates that at least in some cases, phosphorus addition eliminated mycoheterotrophs without reducing the prevalence of their preferred fungal partners in the soil, or reducing net soil AM fungal biomass. We speculate that this could indicate a phosphorus-dependent shift in the underlying exchange relationships between photosynthetic plants and their AM fungal partners. Although elucidation of the exact mechanism falls outside the scope of this study, we hypothesise that such a functional shift could be underpinned by a reduction in carbon allocation from photosynthetic plants to AM fungal partners at increasing concentrations of soil phosphorus [44], either inhibiting the release of chemical factors from the fungi that stimulate the germination of mycoheterotrophs [45], or causing the fungi to restrict carbon flow to mycoheterotrophs [46]. Alternatively, it

449 could be that changes in AM fungal community dynamics (such as competition) at elevated
450 soil phosphorus (indicated by changes in soil AM fungal community composition; Figure 4)
451 may affect mycoheterotroph abundance. These possibilities merit further investigation.
452
453 **(b) Broader implications**
454 Mycoheterotrophs are necessarily connected to other plants via common mycorrhizal
455 networks and cannot exist without fungal support [17]. If, as seems likely, the sensitivity of
456 *Voyria* to phosphorus is due to the sensitivity of their AM fungal symbionts to phosphorus,
457 we might expect to see phosphorus-dependent responses in not only mycoheterotrophic plant
458 species, but in autotrophic species too.
459
460 Indeed, soil exchangeable phosphorus has been identified as the most important mineral
461 nutrient driving tree species distribution across the fertility gradient, with more than half of
462 the 550 measured tree species showing significant affinity with either high or low phosphorus
463 soils [25]. The point at which species with high or low phosphorus affinity dominate the tree
464 community shifts at a concentration of 2.2 mg P kg^{-1} exchangeable soil phosphorus.
465 Strikingly, this concentration corresponds to a marked shift in tree growth rates and the
466 activity of soil phosphatase enzymes (responsible for the hydrolysis of the majority of
467 organic phosphorus compounds in soil [24]); below 2 mg P kg^{-1} tree growth responses to
468 phosphorus increased markedly, and phosphatase activity increased exponentially (Turner *et*
469 *al.* Unpublished Manuscript – *enclosed with submission*). This suggests that the
470 mycoheterotrophic response we describe here corresponds to an ecosystem-wide threshold
471 below which phosphorus demand increases markedly above- and below-ground.
472

5. Conclusions

We show that i) the occurrence of two species in the mycoheterotrophic genus *Voyria* is strongly determined by levels of soil phosphorus, and ii) the effects of phosphorus on *Voyria* are likely to be underpinned by the phosphorus sensitivity of their AM fungal symbionts. We identify a critical concentration of soil phosphorus for *V. tenella* (2 mg P kg^{-1}), which corresponds to broad shifts in plant species distributions and growth responses. Our findings suggest that the well-documented effects of phosphorus availability on plant species distributions [25,47] may act by altering the dynamics of resource exchange in mycorrhizal networks, and highlight the importance of further investigation into the functioning of plant–AM fungal relationships under natural conditions.

AUTHOR CONTRIBUTIONS

M.S. conceived and carried out the study, analysed the data, and drafted the manuscript with input from B.T., N.R., P.A.O., and S.J.W.. N.R. conducted bioinformatic analysis. D.R. provided lab support. P.A.O. oversaw lipid analysis. S.J.W. established the nutrient addition experiment and supported field work. B.T. conducted nutrient analyses and supported the field work.

ACKNOWLEDGEMENTS

We thank R. Gonzales, O. Hernandez, J. Rodriguez, N. Fossatti, D. Herrera, and J. Castillo for their assistance in the field. M. Rath and S. Imhof supported confocal microscopy, and H. Wallander supported lipid analysis. Dayana Agudo and Aleksandra Bielnicka assisted in the analysis of soils. I. Henderson provided advice on sequencing. R. Condit and A. Tanentzap

provided advice on statistics. A. Herre, U. Paszkowski, E. Tanner, E. Leigh, C. Zalamea, E. Verbruggen, and E. Sayer provided comments on the manuscript. The Smithsonian Tropical Research Institute (STRI) served as the base of operations and provided logistical support.

DATA

Supporting data is available in the electronic supplementary material. Raw sequence data are available in the International Nucleotide Sequence Database Sequence Read Archive (accession no. SRP076949).

FUNDING

M.S. was funded by a STRI predoctoral fellowship, Cambridge University, and the Department of Plant Sciences, Cambridge. N.R. was funded by the Swedish Research Council. P.A.O. was funded by Lund University and the Swedish Research Council.

REFERENCES

1. Remy, W., Taylor, T. N., Hass, H. & Kerp, H. 1994 Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 11841–11843. (doi:10.1073/pnas.91.25.11841)

2. Alexander, I. & Lee, S. 2005 Mycorrhizas and ecosystem processes in tropical rain forest: implications for diversity. In *Biotic Interactions in the Tropics* (eds D. Burslem M. Pinard & S. Hartley), pp. 165–203. Cambridge, UK: Cambridge. (doi:10.1017/CBO9780511541971)

3. Jakobsen, I. & Rosendahl, L. 1990 Carbon Flow into Soil and External Hyphae from Roots of Mycorrhizal Cucumber Plants. *New Phytologist* **115**, 77–83. (doi:10.1111/j.1469-8137.1990.tb00924.x)

4. Drigo, B. et al. 2010 Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO2. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 10938–10942. (doi:10.1073/pnas.0912421107)

5. Smith, S. E. & Read, D. J. 2008 *Mycorrhizal Symbiosis 3rd Edn*. Academic Press.

528 6. Van Der Heijden, M. G. A., Martin, F. M., Selosse, M.-A. & Sanders, I. R. 2015
529 Mycorrhizal ecology and evolution: the past, the present, and the future. *New*
530 *Phytologist* **205**, 1406–1423. (doi:10.1111/nph.13288)

531 7. Bago, B., Pfeffer, P. E. & Shachar-Hill, Y. 2000 Carbon metabolism and transport in
532 arbuscular mycorrhizas. *Plant Physiol.* **124**, 949–958. (doi:http://dx.doi.org/10.1104/
533 pp.124.3.949)

534 8. Rillig, M. C. 2004 Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecol.*
535 *Lett.* **7**, 740–754. (doi:10.1111/j.1461-0248.2004.00620.x)

536 9. Johnson, N. C., Angelard, C., Sanders, I. R. & Kiers, E. T. 2013 Predicting community
537 and ecosystem outcomes of mycorrhizal responses to global change. *Ecol. Lett.* **16**,
538 140–153. (doi:10.1111/ele.12085)

539 10. Alexander, I. & Selosse, M.-A. 2009 Mycorrhizas in tropical forests: a neglected
540 research imperative. *New Phytologist* **182**, 14–16. (doi:10.1111/j.1469-
541 8137.2009.02798.x)

542 11. Mohan, J. E. et al. 2014 Mycorrhizal fungi mediation of terrestrial ecosystem
543 responses to global change: mini-review. *Fungal Ecology* **10**, 3–19.
544 (doi:10.1016/j.funeco.2014.01.005)

545 12. McGuire, K. L., Henkel, T. W., Granzow de la Cerda, I., Villa, G., Edmund, F. &
546 Andrew, C. 2008 Dual mycorrhizal colonization of forest-dominating tropical trees
547 and the mycorrhizal status of non-dominant tree and liana species. *Mycorrhiza* **18**,
548 217–222. (doi:10.1007/s00572-008-0170-9)

549 13. Leake, J. R. 1994 The biology of myco-heterotrophic ('saprophytic') plants. *New*
550 *Phytologist* **127**, 171–216. (doi:10.1111/j.1469-8137.1994.tb04272.x)

551 14. Bidartondo, M. I., Redecker, D., Hijri, I., Wiemken, A., Bruns, T. D., Domínguez, L.,
552 Sérsic, A., Leake, J. R. & Read, D. J. 2002 Epiparasitic plants specialized on
553 arbuscular mycorrhizal fungi. *Nature* **419**, 389–392. (doi:10.1038/nature01054)

554 15. Bidartondo, M. I. 2005 The evolutionary ecology of myco-heterotrophy. *New*
555 *Phytologist* **167**, 335–352. (doi:10.1111/j.1469-8137.2005.01429.x)

556 16. Leake, J. R. & Cameron, D. D. 2010 Physiological ecology of mycoheterotrophy. *New*
557 *Phytologist* **185**, 601–605. (doi:10.1111/j.1469-8137.2009.03153.x)

558 17. Merckx, V., Bidartondo, M. I. & Hynson, N. A. 2009 Myco-heterotrophy: when fungi
559 host plants. *Annals of Botany* **104**, 1255–1261. (doi:10.1093/aob/mcp235)

560 18. Wyatt, G. A. K., Kiers, E. T., Gardner, A. & West, S. A. 2014 A biological market
561 analysis of the plant-mycorrhizal symbiosis. *Evolution* **68**, 2603–2618.
562 (doi:10.1111/evo.12466)

563 19. Johnson, N. C. 2010 Resource stoichiometry elucidates the structure and function of
564 arbuscular mycorrhizas across scales. *New Phytologist* **185**, 631–647.
565 (doi:10.1111/j.1469-8137.2009.03110.x)

20. Johnson, N. 1993 Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications*. **3**, 749–757. (doi: 10/2307/1942106)

21. Johnson, N. C., Wilson, G. W. T., Bowker, M. A., Wilson, J. A. & Miller, R. M. 2010 Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 2093–2098. (doi:10.1073/pnas.0906710107)

22. Hoeksema, J. D. et al. 2010 A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters* **13**, 394–407. (doi:10.1111/j.1461-0248.2009.01430.x)

23. Grman, E. & Robinson, T. M. P. 2013 Resource availability and imbalance affect plant-mycorrhizal interactions: a field test of three hypotheses. *Ecology* **94**, 62–71. (doi: 10.2307/23435669)

24. Turner, B. L. & Engelbrecht, B. M. J. 2011 Soil organic phosphorus in lowland tropical rain forests. *Biogeochemistry* **103**, 297–315. (doi:10.1007/s10533-010-9466-x)

25. Condit, R., Engelbrecht, B. M. J., Pino, D., Perez, R. & Turner, B. L. 2013 Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 5064–5068. (doi:10.1073/pnas.1218042110)

26. Wright, S. J. et al. 2011 Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* **92**, 1616–1625. (doi:http://dx.doi.org/10.1890/10-1558.1)

27. Pyke, C. R., Condit, R., Aguilar, S. & Lao, S. 2001 Floristic Composition across a Climatic Gradient in a Neotropical Lowland Forest. *J Veg Sci* **12**, 553–566. (doi:10.2307/3237007)

28. Yavitt, J. B. 2001 Drought and Irrigation Effects on Fine Root Dynamics in a Tropical Moist Forest, Panama. *Biotropica*, 1–14. (doi:http://dx.doi.org/10.1646/0006-3606(2001)033[0421:DAIEOF]2.0.CO;2)

29. Yavitt, J. B., Harms, K. E., Garcia, M. N., Mirabello, M. & Wright, S. J. 2011 Soil fertility and fine root dynamics in response to 4 years of nutrient (N, P, K) fertilization in a lowland tropical moist forest, Panama. *Austral Ecology* **36**, 433–445. (doi:10.1111/j.1442-9993.2010.02157.x)

30. Gilbert, B., Wright, S. J., Muller-Landau, H. C., Kitajima, K. & Hernandéz, A. 2006 Life history trade-offs in tropical trees and lianas. *Ecology* **87**, 1281–1288. (doi:10.1890/0012-9658(2006)87[1281:LHTITT]2.0.CO;2)

31. Frostegård, Å., Tunlid, A. & Bååth, E. 1993 Phospholipid Fatty Acid Composition, Biomass, and Activity of Microbial Communities from Two Soil Types Experimentally Exposed to Different Heavy Metals. *Applied and Environmental Microbiology* **59**, 3605–3617.

32. Nilsson, L. O., Bååth, E., Falkengren-Grerup, U. & Wallander, H. 2007 Growth of ectomycorrhizal mycelia and composition of soil microbial communities in oak forest soils along a nitrogen deposition gradient. *Oecologia* **153**, 375–384.

(doi:10.1007/s00442-007-0735-x)

33. Olsson, P. L. A. 1999 Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology* **29**, 303–310. (doi:10.1111/j.1574-6941.1999.tb00621.x)

34. McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L. & Swan, J. A. 1990 A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **115**, 495–501. (doi:10.1111/j.1469-8137.1990.tb00476.x)

35. Simon, L., Lalonde, M. & Bruns, T. D. 1992 Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. *Applied and Environmental Microbiology* **58**, 291–295.

36. Helgason, T., Daniell, T. J., Husband, R., Fitter, A. H. & Young, J. P. W. 1998 Ploughing up the wood-wide web? **394**, 431–431. (doi:10.1038/28764)

37. Schloss, P. D. et al. 2009 Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology* **75**, 7537–7541. (doi:10.1128/AEM.01541-09)

38. Katoh, K., Misawa, K., Kuma, K.-I. & Miyata, T. 2002 MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**, 3059–3066. (doi: 10.1093/nar/gkf436)

39. Edgar, R. C. 2004 MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**, 1792–1797. (doi:10.1093/nar/gkh340)

40. Stamatakis, A. 2014 RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313. (doi:10.1093/bioinformatics/btu033)

41. Venables, W. N. & Ripley, B. D. 2002 *Modern Applied Statistics with S*. Springer.

42. Shane, M. W., McCully, M. E. & Lambers, H. 2004 Tissue and cellular phosphorus storage during development of phosphorus toxicity in *Hakea prostrata* (Proteaceae). *Journal of Experimental Botany* **55**, 1033–1044. (doi:10.1093/jxb/erh111)

43. Bougoure, J., Brundrett, M., Brown, A. & Grierson, P. F. 2008 Habitat characteristics of the rare underground orchid Rhizanthella gardneri. *Aust. J. Bot.* **56**, 501–11. (doi:10.1071/BT08031)

44. Olsson, P. A., Rahm, J. & Aliasgharzad, N. 2010 Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits. *FEMS Microbiology Ecology* **72**, 125–131. (doi:10.1111/j.1574-6941.2009.00833.x)

45. Bruns, T. D. & Read, D. J. 2000 In vitro germination of nonphotosynthetic, myco-heterotrophic plants stimulated by fungi isolated from the adult plants. *New Phytologist* **148**, 335–342. (doi:10.1046/j.1469-8137.2000.00766.x)

644 46. Fellbaum, C. R., Mensah, J. A., Pfeffer, P. E., Kiers, E. T. & Bücking, H. 2012 The
645 role of carbon in fungal nutrient uptake and transport: Implications for resource
646 exchange in the arbuscular mycorrhizal symbiosis. *psb* **7**, 1509–1512.
647 (doi:10.4161/psb.22015)

648 47. Wassen, M. J., Venterink, H. O., Lapshina, E. D. & Tanneberger, F. 2005 Endangered
649 plants persist under phosphorus limitation. *Nature* **437**, 547–550.
650 (doi:10.1038/nature03950)

651

652 **FIGURE CAPTIONS**

653

654 **Figure 1.** The mycoheterotrophs *Voyria tenella* (**a**), and *V. corymbosa* (**b**) in a lowland
655 tropical forest in Panama. The root system of the mycoheterotroph *V. tenella* is intensely
656 colonised by arbuscular mycorrhizal (AM) fungi (**d**). In **c-i**, fungal material is visible as the
657 light-coloured ring surrounding the central vasculature. In **c-ii,** fungal material (hyphae and
658 coils) is rendered in red and plant material is not shown. In **d,** plant material is displayed in
659 grey, and fungal material in red. The same image stack is displayed in **d-i–iv** with the plant
660 material made increasingly transparent. Confocal micrographs (**c-ii**) and (**d-i–iv**) were
661 obtained by differential staining of plant and fungal tissues, shown as 3D projections
662 (AMIRA™). Photos **a** and **b** courtesy of Christian Ziegler. In **a** and **b** scale bar = 20 mm; in **c**
663 scale bar = 1 mm; in **d** scale bar = 100 µm.

664

665 **Figure 2.** (**a**) Numbers of the mycoheterotroph *Voyria tenella* sharply decline with increasing
666 soil exchangeable phosphorus (P) across a naturally occurring gradient in lowland tropical
667 forests in Panama. The solid line depicts the fitted response of a generalised linear model
668 with negative binomial errors (n = 37). Red dashed lines indicate the 95% confidence
669 interval. The blue shaded region represents the concentrations of soil exchangeable
670 phosphorus found in +P plots in the nearby factorial nutrient-addition experiment (Figure

671 S1). (**b**) The abundant mycoheterotroph *V. tenella* (**b-i**) and less common congener *V.*
672 *corymbosa* (**b-ii**) are eliminated by phosphorus (P) addition in a long-term factorial nutrient-
673 addition experiment in a lowland tropical forest in Panama. Figure contrasts 16 no-P plots
674 (control, N, K, NK treatments) with 16 +P plots (P, NP, KP, NPK treatments). Values are
675 fitted responses of generalised linear model with negative binomial errors and show 95%
676 confidence intervals. The effects of individual fertilisation treatments on numbers of *V.*
677 *tenella* and *V. corymbosa* are presented in Figure S3.
678
679 **Figure 3.** The relative abundance — in the soil — of the AM fungal taxa most strongly
680 associated with *Voyria tenella* (**a-i**) and *Voyria corymbosa* (**b-i**) are reduced but not
681 eliminated in +P treatments (**a-ii**, **b-ii**) in a long-term factorial nutrient-addition experiment in
682 a lowland tropical forest in Panama. Upper bars (**a-i** and **b-i**) represent the relative abundance
683 of AM fungal taxa in the roots of *V. tenella* and *V. corymbosa* (averaged across control, N, K,
684 NK treatments; n = 16). Lower bars (**a-ii** and **b-ii**) illustrate the effect of experimental
685 phosphorus addition on the proportional abundance of AM fungal taxa in the soil. Figure
686 contrasts 16 no-P plots (control, N, K, NK treatments) with 8 +P plots (P, NP treatments).
687 Values are fitted responses of generalised linear model with negative binomial errors and
688 show 95% confidence intervals. Significant effects of phosphorus addition are asterisked.
689 Data are based on read counts from 454-sequencing. See Figure S4 for the effects of
690 individual fertilisation treatments on the relative abundance of AM fungal taxa in the soil.
691
692 **Figure 4.** The AM fungal partners of mycoheterotrophs *Voyria tenella* and *Voyria corymbosa*
693 under unfertilised conditions (**a-i**) were present both in the soil and in the roots of autotrophic
694 plants when fertilised with phosphorus (**b-ii, b-iii**), although the relative abundance of AM
695 fungal taxa shifted in response to phosphorus addition. Interactions between

mycoheterotrophs, AM fungi, and autotrophs in unfertilised control plots are represented in (**a**), and in phosphorus-addition plots in (**b**). (**b**) displays *potential* linkages (indicated by shaded blue region) between mycoheterotrophs and AM fungi based on their partners in unfertilised control plots; there were no mycoheterotrophs actually found in phosphorus-addition plots. Values are based on the mean of 4 unfertilised control plots, and 4 phosphorus-addition plots. The widths of bars representing AM fungal OTUs (**ii**) are scaled to the relative abundance of OTUs in soil communities in control (**a-ii**) and phosphorus-addition (**b-ii**) treatments respectively. The thickness of the linkages is scaled to reflect the proportion of the AM fungal community constituting the linkages. AM fungal OTUs found in the roots of either species of *Voyria* and their linkages are depicted in colour (see legend), and those AM fungal OTUs not interacting with mycoheterotrophs in grey. Full list of OTU codes are given in Table S3. Only the 50 most abundant AM fungal OTUs (in the soil) are plotted, and AM fungal OTUs making up less than 1% of the total number of sequences in a sample type are omitted for clarity. Data are based on read counts from 454-sequencing. V. ten = *Voyria tenella*, V. cor = *Voyria corymbosa*, ALSB = *Alseis blackiana*, DESP = *Desmopsis panamensis*, HEIC = *Heisteria concinna*, SIMA = *Simarouba amara*, SORA = *Sorocea affinis*, TET2 = *Tetragastris panamensis*, VIR1 = *Virola sebifera*.

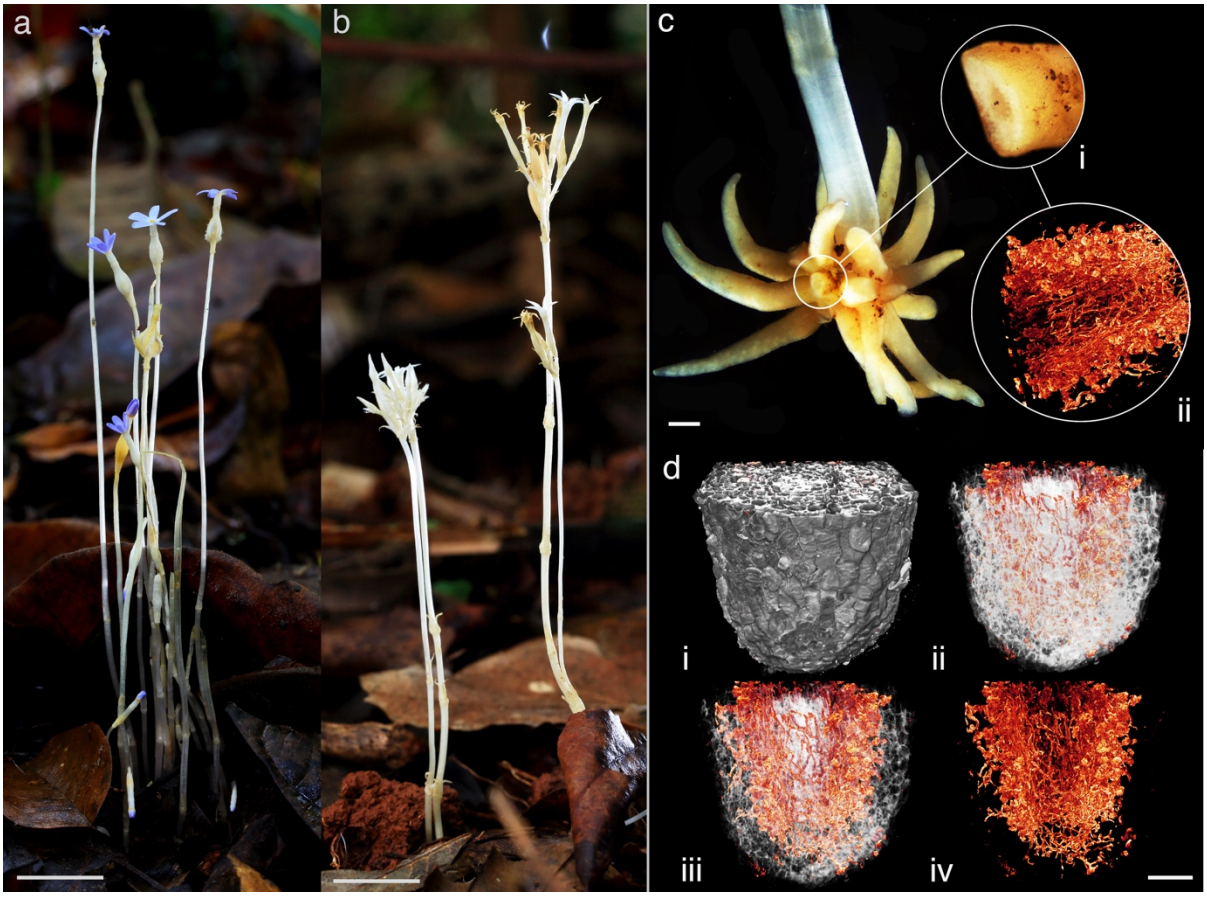

Figure 1. The mycoheterotrophs *Voyria tenella* (**a**), and *Voyria corymbosa* (**b**) in a lowland tropical forest in Panama. The root system of the mycoheterotroph *V. tenella* is reduced (**c**) and intensely colonised by arbuscular mycorrhizal (AM) fungi (**d**). In (**c-i**), fungal material is visible as the light-coloured ring surrounding the central vasculature. In (**c-ii**) fungal material (hyphae and coils) is rendered in red and plant material is not shown. In (**d**) plant material is displayed in grey, and fungal material in red. The same image stack is displayed in (**d-i–iv**) with the plant material made increasingly transparent. (**c-ii**) and (**d-i–iv**) are confocal micrographs obtained by differential staining of plant and fungal tissues, shown as 3D projections (AMIRA™). Photos (**a**) and (**b**) courtesy of Christian Ziegler. In (**a**) and (**b**) scale bar = 20 mm; in (**c**) scale bar = 1 mm; in (**d**) scale bar = 100 μm.

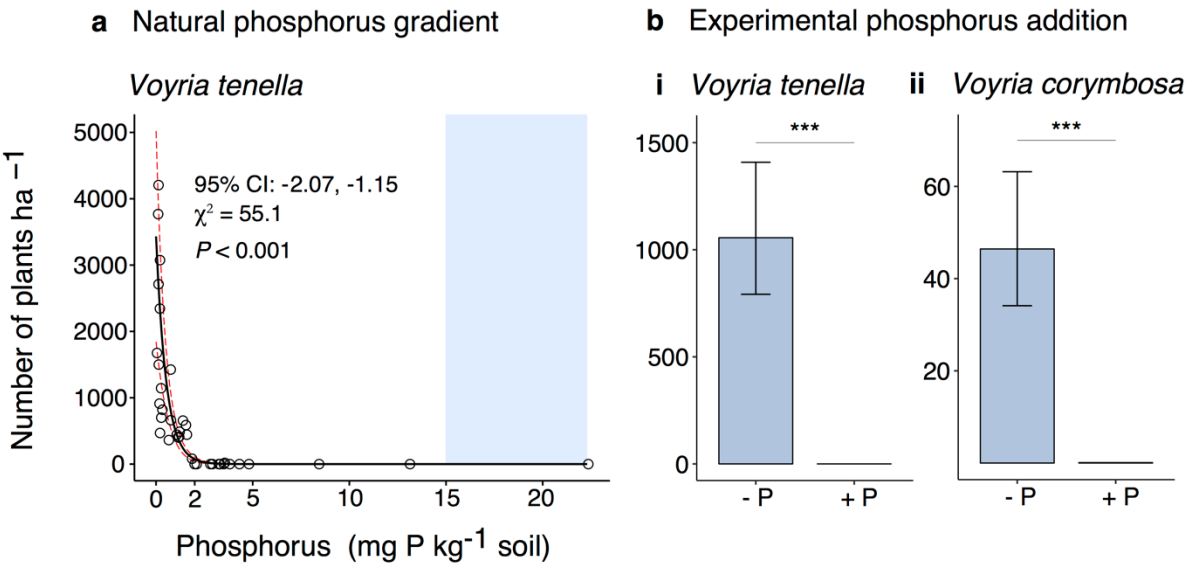

Figure 2. (**a**) Numbers of the mycoheterotroph *Voyria tenella* sharply decline with increasing soil exchangeable phosphorus (P) across a naturally occurring gradient in lowland tropical forests in Panama. The solid line depicts the fitted response of a generalised linear model with negative binomial errors (n = 37). Red dashed lines indicate the 95% confidence interval. The blue shaded region represents the concentrations of soil exchangeable phosphorus found in +P plots in the nearby factorial nutrient-addition experiment (the Gigante Fertilisation Project, Figure S1). (**b**) The abundant mycoheterotroph *Voyria tenella* (**b-i**) and less common congener *Voyria corymbosa* (**b-ii**) are eliminated by phosphorus (P) addition in a long-term factorial nutrient-addition experiment in a lowland tropical forest in Panama. Figure contrasts 16 no-P plots (control, N, K, NK treatments) with 16 +P plots (P, NP, KP, NPK treatments). Values are fitted responses of generalised linear model with negative binomial errors and show 95% confidence intervals. The effects of individual fertilisation treatments on numbers of *V. tenella* and *V. corymbosa* are presented in Figure S3.

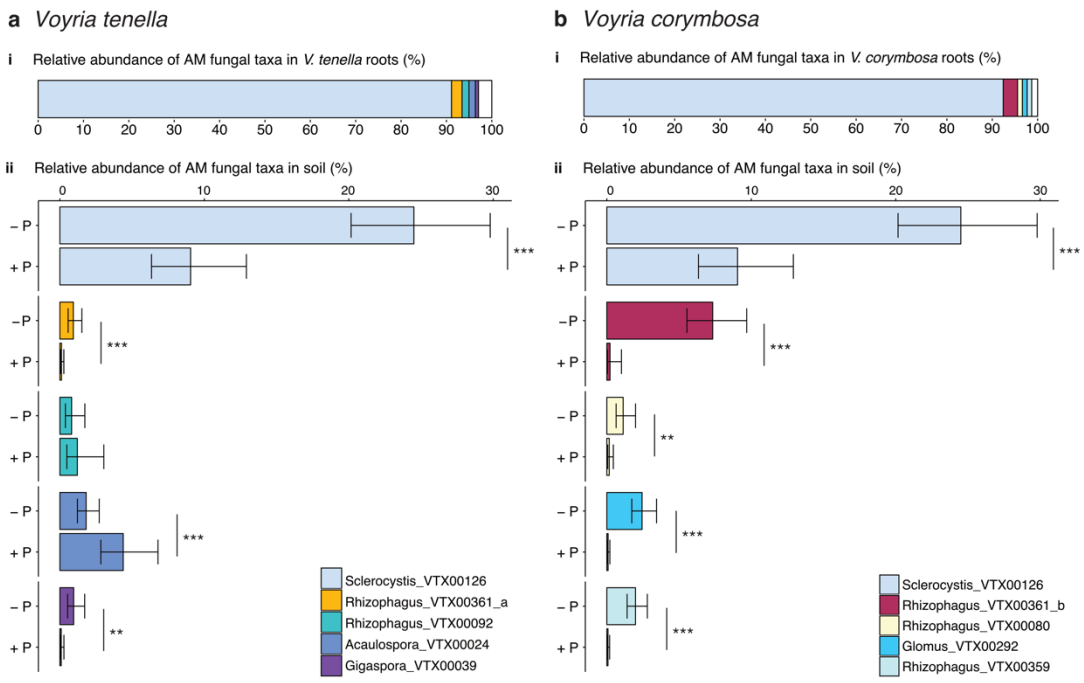

Figure 3. The relative abundance — in the soil — of the AM fungal taxa most strongly associated with *Voyria tenella* (**a-i**) and *Voyria corymbosa* (**b-i**) are reduced but not eliminated in +P treatments (**a-ii**), (**b-ii**) in a long-term factorial nutrient-addition experiment in a lowland tropical forest in Panama. Upper bars (**a-i**) and (**b-i**) represent the relative abundance of AM fungal taxa in the roots of *V. tenella* and *V. corymbosa* (averaged across control, N, K, NK treatments; n = 16). Lower bars (**a-ii**) and (**b-ii**) illustrate the effect of experimental phosphorus addition on the proportional abundance of AM fungal taxa in the soil. Figure contrasts 16 no-P plots (control, N, K, NK treatments) with 8 +P plots (P, NP treatments). Values are fitted responses of generalised linear model with negative binomial errors and show 95% confidence intervals. Significant effects of phosphorus addition are asterisked. See Figure S4 for the effects of individual fertilisation treatments on the relative abundance of AM fungal taxa in the soil.

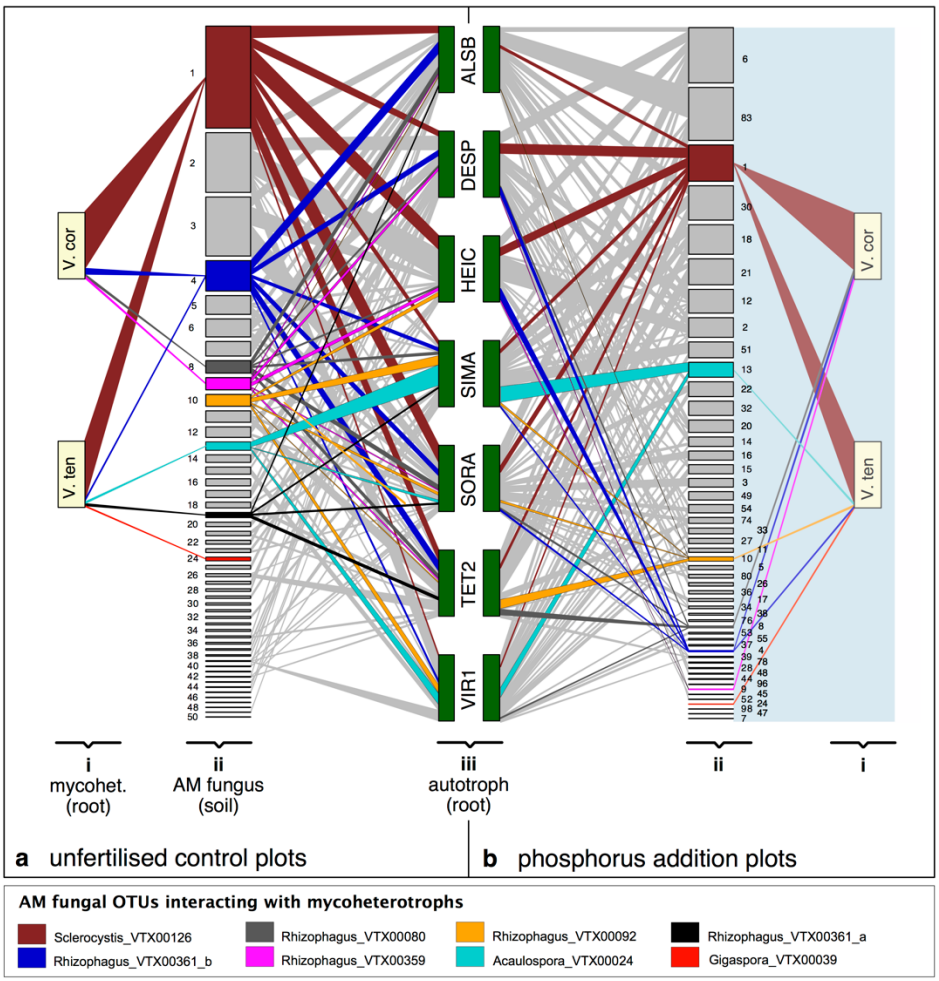

Figure 4. The AM fungal partners of mycoheterotrophs *Voyria tenella* (V. ten) and *Voyria corymbosa* (V. cor) under unfertilised conditions (**a-i**) were present both in the soil and in the roots of autotrophic plants when fertilised with phosphorus (**b-ii, b-iii**), although the relative abundance of AM fungal taxa shifted in response to phosphorus addition. Interactions between mycoheterotrophs, AM fungi, and autotrophs in unfertilised control plots are represented in (**a**), and in phosphorus-addition plots in (**b**). (**b**) displays *potential* linkages (indicated by shaded blue region) between mycoheterotrophs and AM fungi based on their partners in unfertilised control plots; there were no mycoheterotrophs actually found in phosphorus-addition plots. Values are based on the mean of 4 unfertilised control plots, and 4 phosphorus-addition plots. The widths of bars representing AM fungal OTUs (**ii**) are scaled to the relative abundance of OTUs in soil communities in control (**a-ii**) and phosphorus-addition (**b-ii**) treatments respectively. The thickness of the linkages are scaled to reflect the proportion of the AM fungal community constituting the linkages. AM fungal OTUs found in the roots of either species of *Voyria* and their linkages are depicted in colour (see legend); AM fungal OTUs not interacting with mycoheterotrophs are depicted in grey. Full list of OTU codes are given in Table S3. Only the 50 most abundant AM fungal OTUs (in the soil) are plotted, and AM fungal OTUs making up less than 1% of the total number of sequences in a sample type are omitted for clarity. V. ten = *Voyria tenella*, V. cor = *Voyria corymbosa*, ALSB = *Alseis blackiana*, DESP = *Desmopsis panamensis*, HEIC = *Heisteria concinna*, SIMA = *Simarouba amara*, SORA = *Sorocea affinis*, TET2 = *Tetragastris panamensis*, VIR1 = *Virola sebifera*.

SUPPLEMENTARY METHODS

(a) Detailed site description: Gigante Fertilisation Project

The Gigante Fertilisation Project (GFP) is located on the Gigante Peninsula, within the Barro Colorado Nature Monument (BCNM) in Panama [1]. Tree species composition and canopy height are characteristic of mature secondary forest [1]. Nearby Barro Colorado Island (c. 5 km from the study site) has a mean annual rainfall of 2600 mm, with a strong dry season between January and April and a mean annual temperature of 26 ^{o}C {Sayer:2011vi}. The soils are predominantly moderately acidic Oxisols [2] and are comparable to the plateau soils, on andesite, on nearby Barro Colorado Island (BCI) [3]. Compared to soils worldwide, the Gigante soils are infertile and strongly weathered, with low concentrations of available phosphorus and moderate concentrations of base cations [4,5]. The GFP is a factorial NPK fertilisation experiment consisting of nine fertiliser treatments (N, P, K, NP, NK, PK, NPK, micronutrients, and unfertilised controls) replicated four times across the 26.6-ha study site. The 32 experimental plots measured 40-m × 40-m, and were separated by a minimum distance of 40 m, with the exception of two plots separated by 20 m and a 3 m deep stream bed. Starting in 1998, fertilisers were applied by hand in four equal doses a year, equally spaced across the wet season. The annual doses were 125 kg N ha^{-1} yr^{-1} as urea, 50 kg P ha^{-1} yr^{-1} as triple superphosphate, and 50 kg K ha^{-1} yr^{-1} as potassium chloride.

The micronutrient treatment consisted of HBO2, CuSO4, FeSO4, MnSO4, ZnSO4 and (NH4)6Mo7O24 at 25 kg ha-1 year-1 besides dolomitic limestone CaMg(CO3)2 (36.8 kg year-1) at 230 kg ha-1 year-1.

(b) Detailed rationale for use of natural rainfall and edaphic gradient alongside long-term experimental manipulation

It is possible that the artificially large amounts of added phosphorus in the experiment may have induced an exaggerated response in the mycoheterotrophs. However, the natural gradient allowed us to model the response of mycoheterotrophs to soil exchangeable phosphorus ranging from concentrations lower than those in experimental no-P treatments to concentrations as high or higher than the experimental +P treatments. Conversely, the response of mycoheterotrophs along the natural phosphorus gradient could be caused by

correlated variables such as pH. However, the experimental data allowed us to observe the response of mycoheterotrophs to soil phosphorus alone. In addition, patterns in mycoheterotroph abundance across the natural gradient with plots spanning a wide geographic area could be confounded by dispersal effects. However, the blocked design of the nutrient-addition experiment provided control over spatial variation and allowed us to quantify the effect of soil phosphorus on mycoheterotroph abundance accounting for natural variation in dispersal. Finally, the micronutrient plots, which received dolomitic limestone to provide Ca, allowed us to confirm that phosphorus rather than the calcium counterion of the phosphorus fertiliser (triple superphosphate; $Ca(H_2PO_4)_2.H_2O$) was responsible for the observed effects (Figure S8).

(c) Autotrophic seedling species

Alseis is a medium-sized canopy species recruiting from very small wind dispersed seeds in canopy gaps [6]; *Desmopsis* is a small understory tree; *Heisteria* is a small understory tree that sometimes reaches the canopy; *Sococea* is an understory shrub to small tree; *Simarouba* is fast-growing canopy tree associated with gaps; *Tetragastris* is a shade-tolerant canopy species; and *Virola* is a shade-tolerant mid-storey tree [7]. *Sorocea*, *Desmopsis* and *Heisteria* reproduce under closed canopies of taller trees [8].

(d) Soil chemistry

All soil chemistry data used was obtained by the methods described by Condit et al. (2013) [9]. Briefly, nitrogen was extracted within 6 h of collection in 0.5 M K_2SO_4, with ammonium and nitrate determined by automated colorimetry on a Lachat Quickchem 8500 (Hach Ltd, Loveland, CO, USA). Soil pH was determined in deionized water and 0.01 M $CaCl_2$ with a glass electrode (Hach Ltd, Loveland, Colorado) in a 1:2 solution to solution ratio. Readily-exchangeable phosphate (resin P) was determined by extraction with anion-exchange membranes, with phosphate detection by automated molybdate colorimetry [10]. All other inorganic nutrients were extracted in Mehlich-3 solution [11] with detection by inductively-coupled plasma optical-emission spectrometry on an Optima 7300DV (PerkinElmer, Waltham, MA, USA).

(e) AM fungal abundance

We used the neutral lipid fatty acid (NLFA) 16:1ω5 as a biomarker for extra-radical AM fungal biomass. We performed lipid extraction and analysis according to Frostegård et al. (1993) [12], with modifications described by Nilsson et al. (2007) [13]. Briefly, extracted lipids, from 4 g lyophilised soil per plot were fractionated into neutral lipids, glycolipids, and polar lipids on silica columns by successive elution with chloroform, acetone and methanol. Methyl nonadecanoate (FAME 19:0) was added as an internal standard, and neutral and polar fractions were converted to fatty acid methyl esters (FAMEs) prior to analysis on a gas chromatograph with a flame ionisation detector and a 50 m HP5 capillary column (Hewlett Packard, Wilmington, DE, USA).

We analysed both NLFAs and phospholipid fatty acids (PLFAs) to ascertain the ratio of NLFA 16:1ω5 to PLFA 16:1ω5, as a ratio > 1 indicates that NLFA 16:1ω5 is a sensitive AM fungal biomarker [14]. Because PLFA 16:1ω5 is known to be produced by bacteria, and is not a reliable AM fungal biomarker in field soils [15], we used NLFA 16:1ω5 as a general proxy for extra-radical AM fungal biomass. The mean NLFA:PLFA ratio across the samples analysed in this study was 2.3, suggesting that NLFA 16:1ω5 is an effective AM fungal biomarker in these soils [14].

To measure AM fungal colonisation of roots, we soaked and rinsed the root samples with distilled water to remove the ethanol. Roots were then cleared by autoclaving in 5% KOH for 5-60 minutes; bleached in solution of ammonia in 3% H_2O_2 for 15-60 minutes depending on the composition of roots in the sample [16]; acidified in 2% HCl for 30 minutes; and stained with 0.05% trypan blue (in a 1:1:1 solution of distilled water, glycerol and lactic acid) for 20 minutes at 60 ^{o}C. The optimum clearing and bleaching time varied depending on the thickness and pigmentation of the roots. We quantified AM fungal colonisation using a compound light microscope at 200 × magnification, according to the method of McGonigle et al. [17], with at least 100 intersections per sample, and one sample per seedling. Mycorrhizal colonisation was expressed as the percentage fine root length colonised by AM fungal hyphae, vesicles and arbuscules.

(h) Detailed bioinformatic analyses

All bioinformatic analysis was performed using the software mothur [18] unless otherwise stated. Sequence filtering was performed with the sff.multiple quality filtering protocol. Reads were removed from the dataset if they did not contain the 10 bp MID, had > 1 error in the barcode sequence, > 2 errors in the forward primer, or were shorter than 200 bp in length.

Clustering was performed using the algorithm Clustering 16S rRNA for Operational Taxonomic Unit (OTU) Prediction (CROP). CROP is an unsupervised Bayesian clustering method that forms clusters based on the actual organisation of sequences without setting a hard similarity cutoff [19]. To capture species level diversity as far as possible, we set the *i* and *u* parameters to 2% cluster difference rather than the conventional 3% because the SSU region has relatively low variation [20,21]. The centre sequence from each cluster was used as a representative sequence in subsequent analyses.

Sequence alignment was performed with the software MAFFT v7.149b [22] using the L-INS-i algorithm (iterative refinement using local pairwise alignment) and the alignment from Krüger et al. (2012) [23] as a backbone. Alignments were improved with MUSCLE [24] using the –refine option. Trees were built using RAxML v. 8.0 [25] with GTR GAMMA implementation, and bootstrap values based on 1000 runs.

We used the Basic Local Alignment Search Tool (BLAST [60]; minimum e-value 10^{-30}) on one representative sequence from each cluster iteratively against three databases in the following order of preference: i) sequences from Krüger et al. (2012); ii) all virtual taxa (VT) from the MaarjAM AM fungal sequence database (www.maarjam.botany.ut.ee); and iii) all 18S Glomeromycotan sequences from SILVA database. Non-Glomeromycotan clusters were removed when the highest blast match did not correspond to an AM fungal sequence in any of the three datasets.

Clusters were named based on matches to database entries at > 97% covering a minimum of 80% of the query sequence. We used the generic names from Krüger et al. (2012), and VT numbers from the MaarjAM database. Where clusters did not match a VT at > 97%, we assigned a name based on the highest VT match and phylogeny (eg. Glomus_OTU1). We fused clusters based on matches to database sequences > 97% and the tree topology obtained from RaXML. Clusters that occurred in < 2 samples, and with < 5 reads total were removed

from the dataset. A breakdown of the sequencing results is provided in the Supplementary Discussion. Raw sequence data were deposited in the International Nucleotide Sequence Database Sequence Read Archive (accession no. SRP076949).

(i) Statistical analyses: mycoheterotroph numbers across the rainfall and edaphic gradient

We analysed the results of all mycoheterotroph censuses using generalised linear models (GLMs). Count data (integer abundances) can be modelled using Poisson or negative binomial error structures [26]. Because the variance >> mean we used negative binomial error structures with glm.nb from the package MASS [27]. We analysed only the most abundant species (*V. tenella* across the rainfall and edaphic gradient, and *V. tenella* and *V. corymbosa* across the GFP), and built separate models for each species. Significance of model terms was assessed using likelihood-ratio chi-square tests.

When modelling mycoheterotroph counts across the 37 plots spanning the rainfall and edaphic gradient we worked with a subset of environmental variables selected and described by Condit et al. (2013) [9]. Briefly, the subset was the largest set of variables within which all pairs were weakly correlated ($r^2 < 0.40$). Variables consisted of dry-season moisture, soil phosphorus (P), calcium (Ca), zinc (Zn), potassium (K), aluminium (Al), iron (Fe), and inorganic nitrogen (N). Magnesium, manganese, organic nitrogen and pH were closely correlated with calcium, and therefore excluded. Resin extractable phosphorus was chosen above Mehlich-3 and total phosphorus measures because resin extractable phosphorus better corresponds to the plant-available phosphorus fraction. Mehlich-3 iron and aluminium data were unavailable for five of the plots (Gigante Leaf Litter Manipulation Project control plots), and omitted from further analysis. We did not consider this to be a problem: Condit et al. (2013) [9] identified dry-season moisture, phosphorus, potassium and calcium as the most important variables in predicting tree distributions across this gradient, suggesting that these are the predictors that vary enough to matter. All of the predictors apart from dry-season moisture and inorganic nitrogen had extreme values (ie. P, Ca, Zn, K) and were log-transformed to reduce the influence of outlying values. All predictors were standardized to zero mean and unit variance.

1. Wright, S. J. et al. 2011 Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* **92**, 1616–1625. (doi:http://dx.doi.org/10.1890/10-1558.1)

2. Turner, B. L. & Wright, S. J. 2013 The response of microbial biomass and hydrolytic enzymes to a decade of nitrogen, phosphorus, and potassium addition in a lowland tropical rain forest. *Biogeochemistry* **117**, 115–130. (doi:10.1007/s10533-013-9848-y)

3. Dieter, D., Elsenbeer, H. & Turner, B. L. 2010 Phosphorus fractionation in lowland tropical rainforest soils in central Panama. *Catena* **82**, 118–125. (doi:10.1016/j.catena.2010.05.010)

4. Yavitt, J. B., Harms, K. E., Garcia, M. N., Mirabello, M. & Wright, S. J. 2011 Soil fertility and fine root dynamics in response to 4 years of nutrient (N, P, K) fertilization in a lowland tropical moist forest, Panama. *Austral Ecology* **36**, 433–445. (doi:10.1111/j.1442-9993.2010.02157.x)

5. Turner, B. L., Yavitt, J. B., Harms, K. E., Garcia, M. N., Romero, T. E. & Wright, S. J. 2013 Seasonal Changes and Treatment Effects on Soil Inorganic Nutrients Following a Decade of Fertilizer Addition in a Lowland Tropical Forest. *Soil Science Society of America Journal* **77**, 1357. (doi:10.2136/sssaj2012.0128)

6. Dalling, J. W., Winter, K., Nason, J. D., Hubbell, S. P., Murawski, D. A. & Hamrick, J. L. 2001 The Unusual Life History of Alseis blackiana: A Shade-Persistent Pioneer Tree? *Ecology* **82**, 933–945. (doi:10.1890/0012-9658(2001)082[0933:TULHOA]2.0.CO;2)

7. Uriarte, M., Condit, R., Canham, C. D. & Hubbell, S. P. 2004 A Spatially Explicit Model of Sapling Growth in a Tropical Forest: Does the Identity of Neighbours Matter? *Journal of Ecology* **92**, 348–360. (doi:10.1111/j.0022-0477.2004.00867.x)

8. Santiago, L. S., Wright, S. J., Harms, K. E., Yavitt, J. B., Korine, C., Garcia, M. N. & Turner, B. L. 2011 Tropical tree seedling growth responses to nitrogen, phosphorus and potassium addition. *Journal of Ecology* **100**, 309–316. (doi:10.1111/j.1365-2745.2011.01904.x)

9. Condit, R., Engelbrecht, B. M. J., Pino, D., Perez, R. & Turner, B. L. 2013 Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 5064–5068. (doi:10.1073/pnas.1218042110)

10. Turner, B. L. & Romero, T. E. 2009 Short-Term Changes in Extractable Inorganic Nutrients during Storage of Tropical Rain Forest Soils. *Soil Science Society of America Journal* **73**, 1972. (doi:10.2136/sssaj2008.0407)

11. Mehlich, A. 1984 Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Communications in Soil Science & Plant Analysis* **15**, 1409–1416. (doi:10.1080/00103628409367568)

12. Frostegård, Å., Tunlid, A. & Bååth, E. 1993 Phospholipid Fatty Acid Composition, Biomass, and Activity of Microbial Communities from Two Soil Types Experimentally Exposed to Different Heavy Metals. *Applied and Environmental*

Microbiology **59**, 3605–3617.

13. Nilsson, L. O., Bååth, E., Falkengren-Grerup, U. & Wallander, H. 2007 Growth of ectomycorrhizal mycelia and composition of soil microbial communities in oak forest soils along a nitrogen deposition gradient. *Oecologia* **153**, 375–384. (doi:10.1007/s00442-007-0735-x)

14. Olsson, P. L. A. 1999 Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology* **29**, 303–310. (doi:10.1111/j.1574-6941.1999.tb00621.x)

15. Frostegård, Å., Tunlid, A. & Bååth, E. 2011 Use and misuse of PLFA measurements in soils. *Soil Biology and Biochemistry* **43**, 1621–1625. (doi:10.1016/j.soilbio.2010.11.021)

16. Koske, R. E. & Gemma, J. N. 1989 A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* **92**, 486–488. (doi:10.1016/S0953-7562(89)80195-9)

17. McGonigle, Miller, Evans, FairchildSwan 1990 A new method which gives an objective measure of colonization of roots by vesicular- arbuscular mycorrhizal fungi. *New Phytologist* **115**, 495–501. (doi:10.1111/j.1469-8137.1990.tb00476.x)

18. Schloss, P. D. et al. 2009 Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology* **75**, 7537–7541. (doi:10.1128/AEM.01541-09)

19. Hao, X., Jiang, R. & Chen, T. 2011 Clustering 16S rRNA for OTU prediction: a method of unsupervised Bayesian clustering. *Bioinformatics* **27**, 611–618. (doi:10.1093/bioinformatics/btq725)

20. Öpik, M. et al. 2013 Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. *Mycorrhiza* **23**, 411–430. (doi:10.1007/s00572-013-0482-2)

21. Davison, J. et al. 2015 Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science* **349**, 970–973. (doi:10.1126/science.aab1161)

22. Katoh, K., Misawa, K., Kuma, K.-I. & Miyata, T. 2002 MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**, 3059–3066. (doi: 10.1093/nar/gkf436)

23. Krüger, M., Krüger, C., Walker, C., Stockinger, H. & Schüßler, A. 2012 Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytologist* **193**, 970–984. (doi:10.1111/j.1469-8137.2011.03962.x)

24. Edgar, R. C. 2004 MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**, 1792–1797. (doi:10.1093/nar/gkh340)

25. Stamatakis, A. 2014 RAxML version 8: a tool for phylogenetic analysis and post-

analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313. (doi:10.1093/bioinformatics/btu033)

26. Crawley, M. J. 2012 *The R Book*. Chichester, UK: John Wiley & Sons. (doi:10.1002/9781118448908)

27. Venables, W. N. & Ripley, B. D. 2002 *Modern Applied Statistics with S*. Springer.

SUPPLEMENTARY TABLES S1-S3

Table S1. Numbers of *Voyria tenella* modelled against six environmental variables across the natural phosphorus gradient (generalized linear model with negative binomial errors).

Variable	Parameter estimate	95% CI	χ^2	*P*
log P	**-1.594**	**-2.07 , -1.15**	**55.07**	**< 0.001**
log Ca	-0.268	-0.72 , 0.16	1.468	0.226
dry season moisture	0.282	-0.17 , 0.76	1.455	0.228
log K	-0.365	-0.82 , 0.07	2.744	0.098
log Zn	-0.045	-0.40 , 0.31	0.062	0.804
inorganic N	-0.181	-0.53 , 0.17	1.053	0.305

Table S2. Comparison of three +P plots and three no-P plots with comparable proportional abundance of the dominant Sclerocystis_VTX00126 net AM fungal biomass in the soil.

P status	Plot	Treatment	Sclerocystis_VTX00126 (%)	AM fungal biomass (nmol g^{-1} dry soil NLFA 16:1ω5)	Number of *V. tenella*
-	18	K	13.55	73.19	61
-	32	K	14.28	94.76	115
-	23	N	14.79	59.27	216
+	24	P	12.94	113.11	0
+	22	NP	13.75	72.24	0
+	3	NP	14.92	47.01	0

Table S3. AM fungal OTUs corresponding to numbers in Figure 4.

#	OTU name	#	OTU name	#	OTU name
1	Sclerocystis_VTX00126	45	Glomus_VTX00368	89	Rhizophagus_OTU4
2	Rhizophagus_VTX00089	46	Glomus_VTX00093_a	90	Rhizophagus_OTU5
3	Rhizophagus_VTX00399_c	47	Rhizophagus_VTX00404	91	Rhizophagus_OTU6
4	Rhizophagus_VTX00361_b	48	Glomus_OTU12	92	Rhizophagus_OTU7
5	Glomus_VTX00103_a	49	Glomus_VTX00137_a	93	Rhizophagus_OTU8
6	Glomus_VTX00093_b	50	Scutellospora_OTU1	94	Rhizophagus_VTX00084
7	Rhizophagus_VTX00070	51	Acaulospora_VTX00227	95	Rhizophagus_VTX00090
8	Rhizophagus_VTX00080	52	Glomus_OTU10	96	Rhizophagus_VTX00099
9	Rhizophagus_VTX00359	53	Glomus_VTX00096	97	Rhizophagus_VTX00223
10	Rhizophagus_VTX00092	54	Glomus_VTX00183_a	98	Rhizophagus_VTX00399_b
11	Glomus_VTX00166	55	Rhizophagus_VTX00235	99	Scutellospora_VTX00254
12	Glomus_VTX00120	56	Scutellospora_VTX00041		
13	Acaulospora_VTX00024	57	Acaulospora_OTU1		
14	Glomus_VTX00121	58	Acaulospora_OTU2		
15	Glomus_VTX00203	59	Acaulospora_OTU3		
16	Glomus_VTX00199	60	Acaulospora_OTU4		
17	Rhizophagus_VTX00074	61	Acaulospora_OTU5		
18	Glomus_VTX00122_b	62	Acaulospora_VTX00012		
19	Rhizophagus_VTX00361_a	63	Acaulospora_VTX00014		
20	Glomus_OTU13	64	Acaulospora_VTX00030		
21	Sclerocystis_VTX00269	65	Acaulospora_VTX00328		
22	Glomus_VTX00101_b	66	Archaeospora_OTU1		
23	Glomus_VTX00167	67	Archaeospora_VTX00004		
24	Gigaspora_VTX00039	68	Archaeospora_VTX00005		
25	Glomus_VTX00292	69	Gigaspora_OTU1		
26	Rhizophagus_VTX00399_a	70	Glomus_OTU5		
27	Glomus_OTU1	71	Glomus_OTU6		
28	Glomus_VTX00194	72	Glomus_OTU7		
29	Glomus_VTX00175	73	Glomus_OTU8		
30	Sclerocystis_VTX00069	74	Glomus_VTX00075		
31	Rhizophagus_VTX00253	75	Glomus_VTX00109		
32	Acaulospora_VTX00026	76	Glomus_VTX00122_a		
33	Glomus_OTU9	77	Glomus_VTX00135		
34	Glomus_VTX00209	78	Glomus_VTX00137_b		
35	Glomus_VTX00189	79	Glomus_VTX00146		
36	Glomus_OTU2	80	Glomus_VTX00183_b		
37	Glomus_OTU11	81	Glomus_VTX00366		
38	Glomus_OTU3	82	Glomus_VTX00370		
39	Rhizophagus_VTX00397	83	Glomus_VTX00410		
40	Glomus_OTU4	84	Glomus_VTX00420		
41	Glomus_VTX00103_b	85	Redeckera_VTX00262		
42	Glomus_VTX00186	86	Rhizophagus_OTU1		
43	Acaulospora_VTX00231	87	Rhizophagus_OTU2		
44	Glomus_VTX00101_a	88	Rhizophagus_OTU3		

SUPPLEMENTARY FIGURES S1-S9

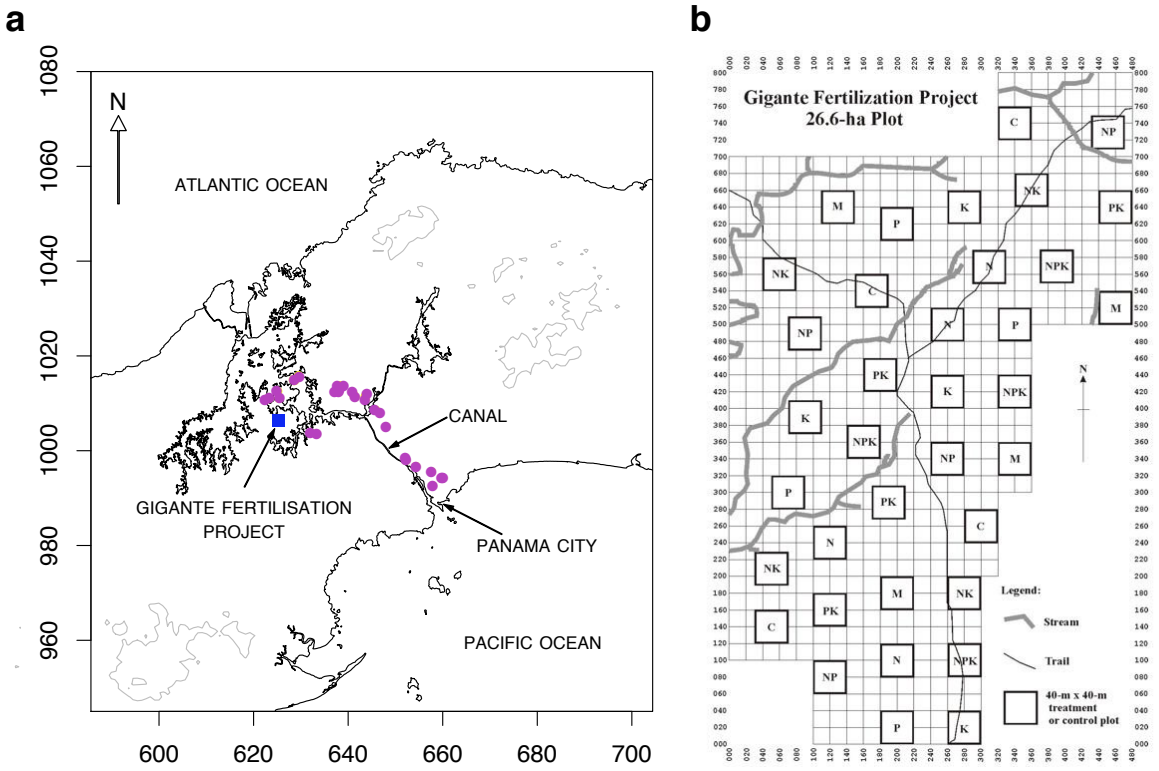

Figure S1. Maps of the study sites in the Panama Canal area. Units on the axes are UTM (Universal Transverse Mercator) coordinates (zone 17) in kilometers. Sites across the edaphic gradient are marked as purple points. The blue square marks the long-term nutrient addition experiment (the Gigante Fertilisation Project, GFP). In (**b**): the placements of treatments in the GFP are shown. Treatments are represented by the combination of nutrients added (N = nitrogen, P = phosphorus, K = potassium, C = unfertilised control, M = micronutrients).

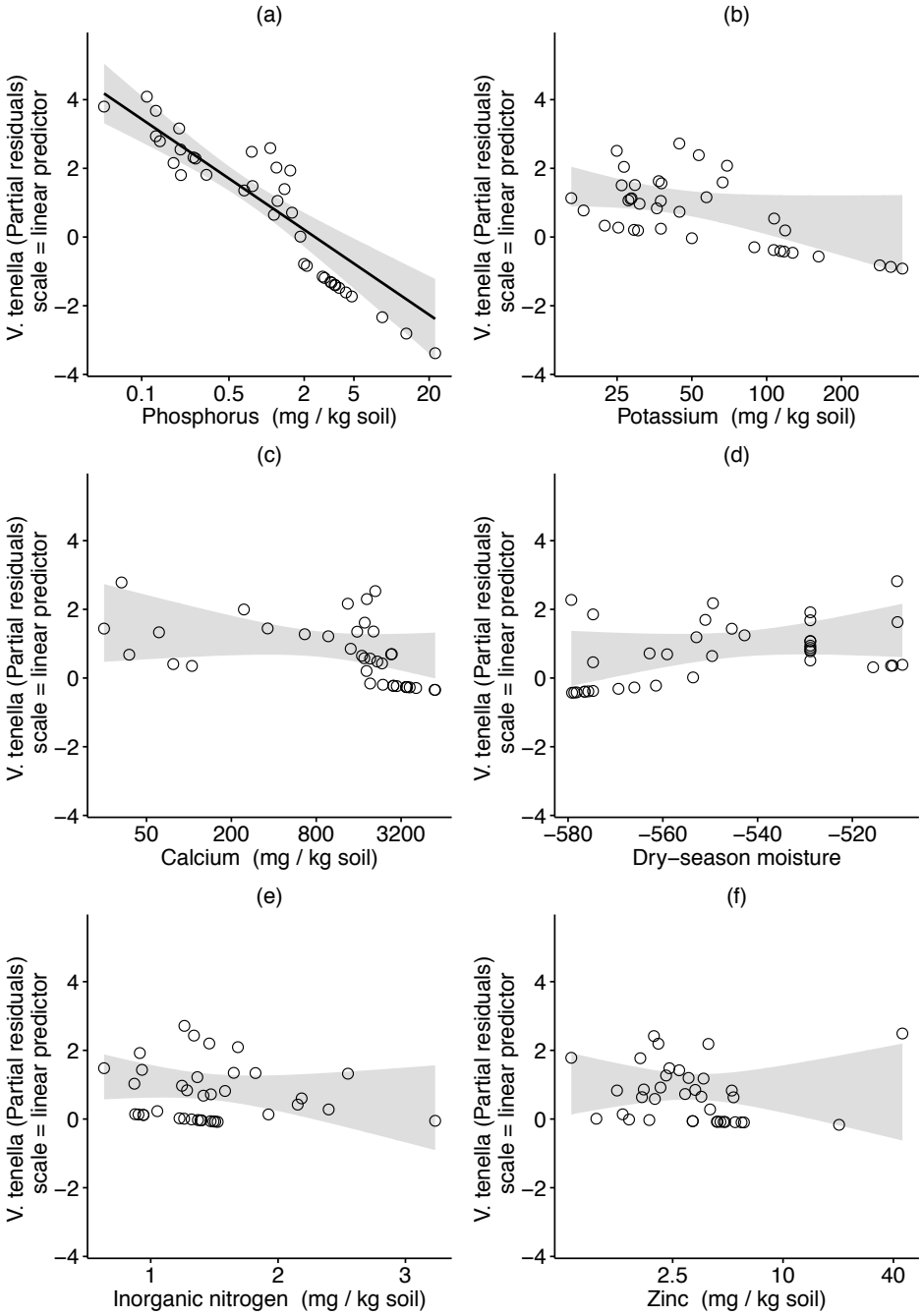

Figure S2. Numbers of the mycoheterotroph *Voyria tenella* modelled against six environmental variables across a naturally occurring rainfall and edaphic gradient in lowland tropical forests in Panama. Solid lines indicate a significant relationship, and represent the fitted response of *V. tenella* to a given predictor in the generalised linear model with negative binomial errors (n = 37). Figures are plotted on the scale of the linear predictor. Points are partial residuals (the proportion of the response explained by a given term). Shaded regions are 95% confidence bands. Parameter estimates are reported in Supplementary Table 1. Although in the models all predictors were standardised to a mean of zero and standard deviation of 1, predictors are here plotted on the original scale of the predictor to facilitate interpretation.

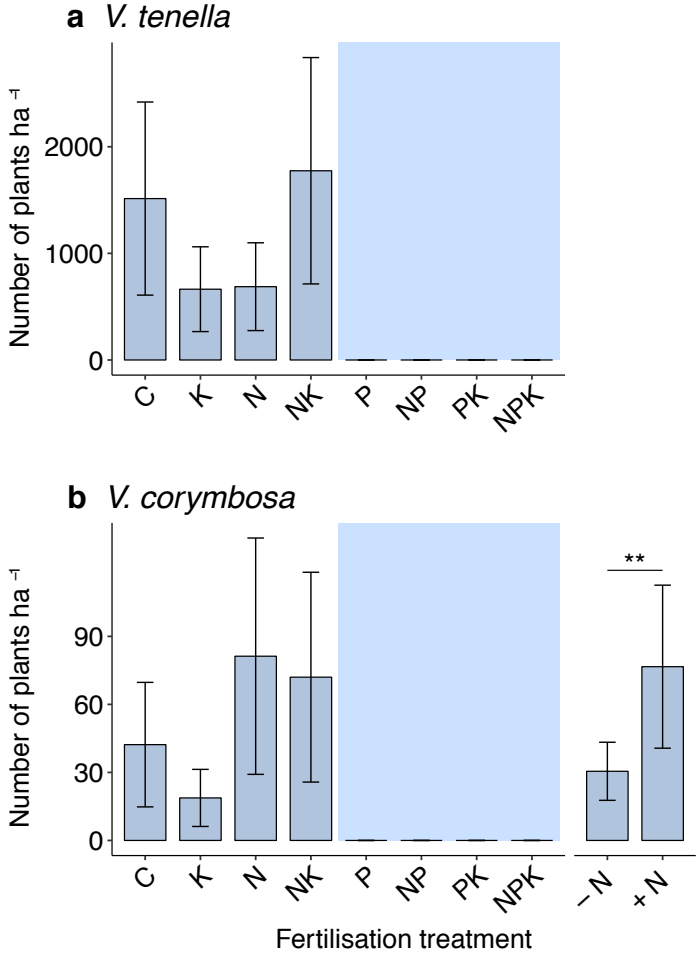

Figure S3. The abundant mycoheterotroph *Voyria tenella* (**a**) and less common congener *Voyria corymbosa* (**b**) are eliminated by phosphorus (P) addition in a long-term factorial nutrient-addition experiment in a lowland tropical forest in Panama. Shaded regions highlight +P treatments. In (**a**) the significant N × K interaction is marked, and in (**b**) the significant effect of N is shown, contrasting 16 no-N plots with 16 +N plots. Values are fitted responses of generalised linear model with negative binomial errors and show 95% confidence intervals. C is control, K is potassium, N is nitrogen, P is phosphorus.

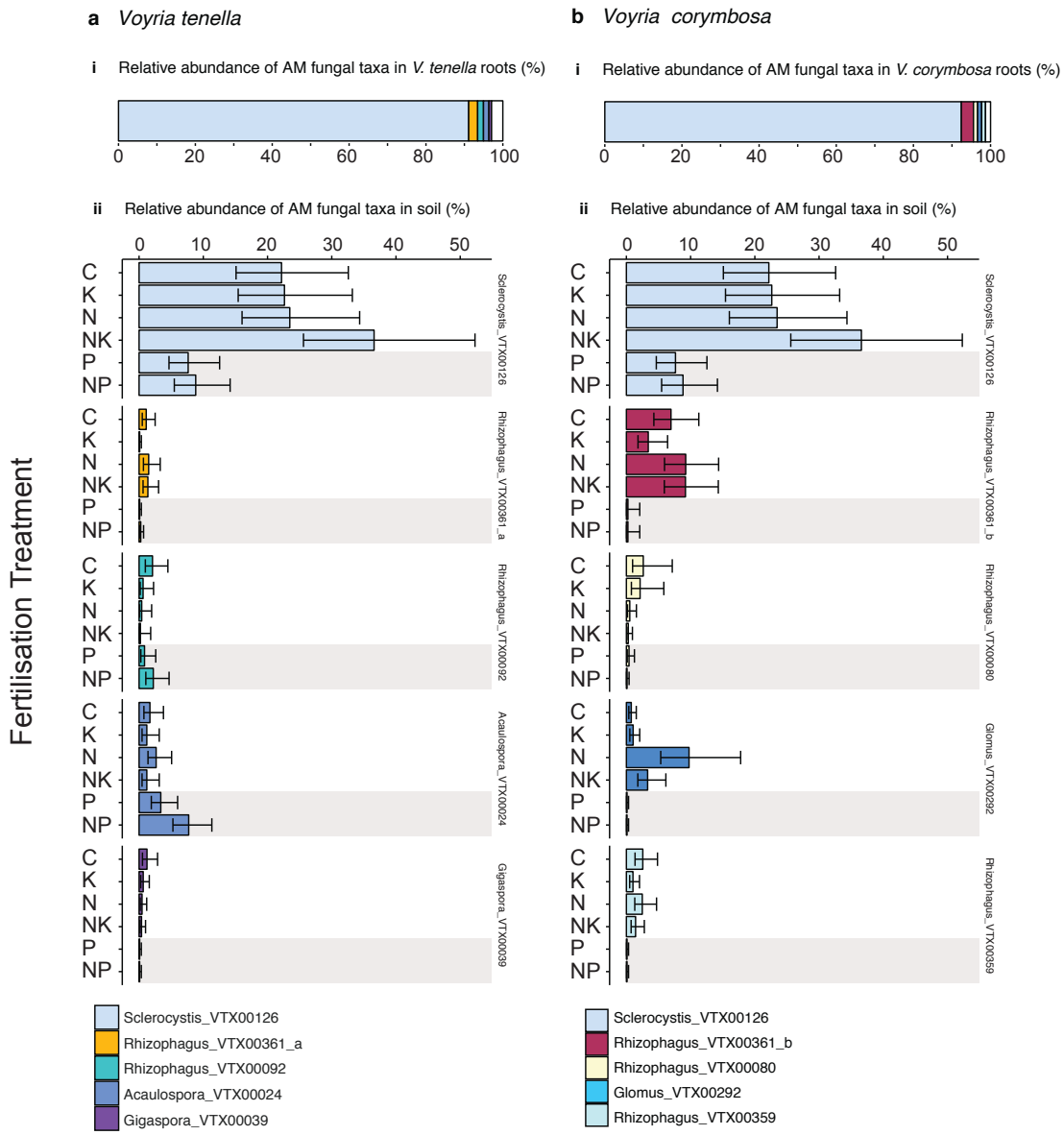

Figure S4. The relative abundance — in the soil — of the AM fungal taxa most strongly associated with the mycoheterotrophs *Voyria tenella* (**a-i**) and *Voyria corymbosa* (**b-i**) are reduced but not eliminated in treatments with added phosphorus (**a-ii**) and (**b-ii**) in a long-term factorial nutrient-addition experiment in a lowland tropical forest in Panama. Upper horizontal bars (**a-i**) and (**b-i**) represent the relative abundance of AM fungal taxa in the roots of *V. tenella* and *V. corymbosa* (averaged across control, N, K, NK treatments; n = 16). Lower bars (**a-ii**) and (**b-ii**) illustrate the effect of fertilisation treatments on the relative abundance of AM fungal taxa in the soil. Grey shaded bands highlight fertilisation treatments with added phosphorus. Values are fitted responses of generalised linear model (n = 4) with negative binomial errors and show 95% confidence intervals. C is control, K is potassium, N is nitrogen, P is phosphorus.

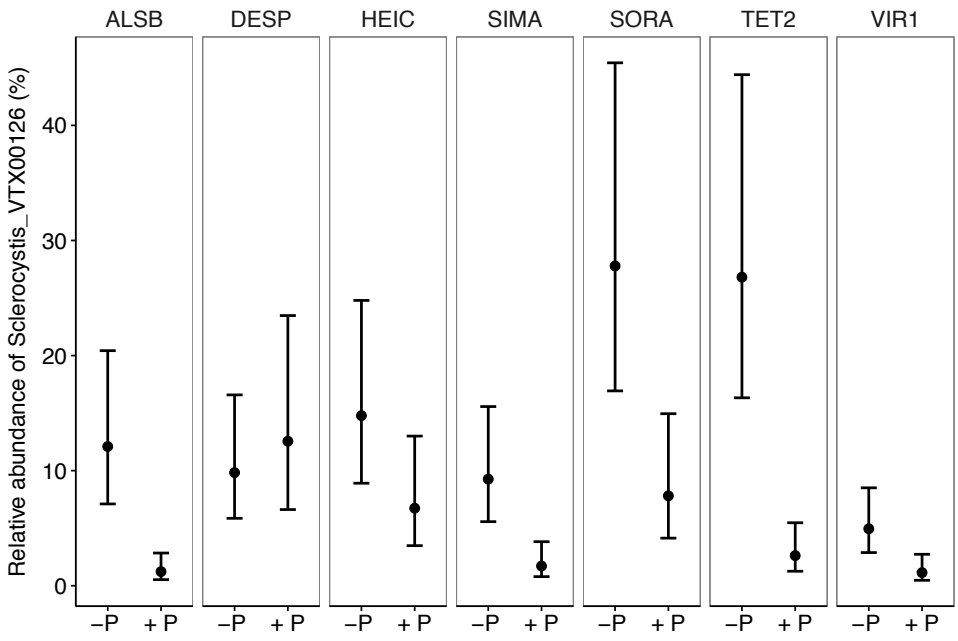

Figure S5. The relative abundance — in the roots of seven species of tree seedlings — of the AM fungal taxon making up > 90% of the AM fungal community in the roots of the mycoheterotrophs *V. tenella* and *V. corymbosa* (Sclerocystis_VTX00126) was reduced in some species but not others (significant phosphorus × species interaction, $\chi^2 = 26.8$, $P < 0.001$). Values are fitted responses of generalised linear mixed model with negative binomial errors and show 95% confidence intervals obtained by parametric bootstrapping with 10,000 simulations. The Figure contrasts 12 no-P plots (control, N, K treatments) with 8 +P plots (P, NP treatments). ALSB = *Alseis blackiana*, DESP = *Desmopsis panamensis*, HEIC = *Heisteria concinna*, SIMA = *Simarouba amara*, SORA = *Sorocea affinis*, TET2 = *Tetragastris panamensis*, VIR1 = *Virola sebifera*.

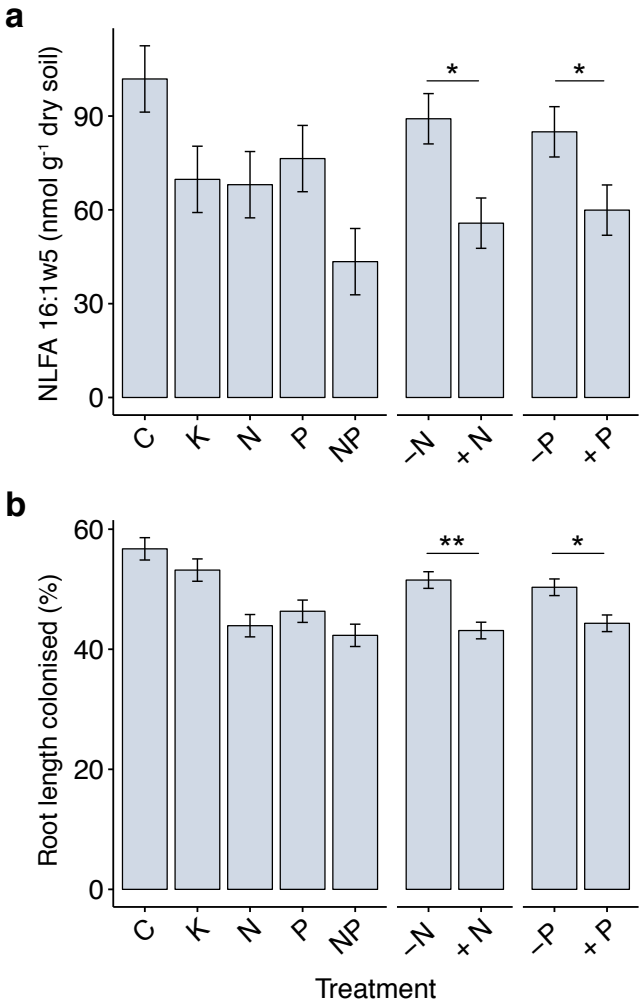

Figure S6. Net AM fungal biomass was reduced by long-term nitrogen (N) and phosphorus (P) addition in a lowland tropical forest in Panama. (**a**) Levels of the neutral lipid fatty acid (NLFA) 16:1ω5, a commonly used proxy for net AM fungal biomass, in forest soil. (**b**) Percentage of root length colonised in six species of tree seedling. Significant effects of N and P addition are shown, contrasting eight –N / –P plots with eight +N / +P plots. Values are fitted responses of linear models ± one standard error. In (**a**), effect of nitrogen: $F_{1,11} = 8.61$, $P = 0.01$; effect of phosphorus: $F_{1,11} = 4.84$, $P = 0.05$. In (**b**) effect of nitrogen: $F_{1,11} = 18.43$, $P = 0.01$; effect of phosphorus: $F_{1,11} = 9.39$, $P = 0.01$. C is control, K is potassium, N is nitrogen, P is phosphorus.

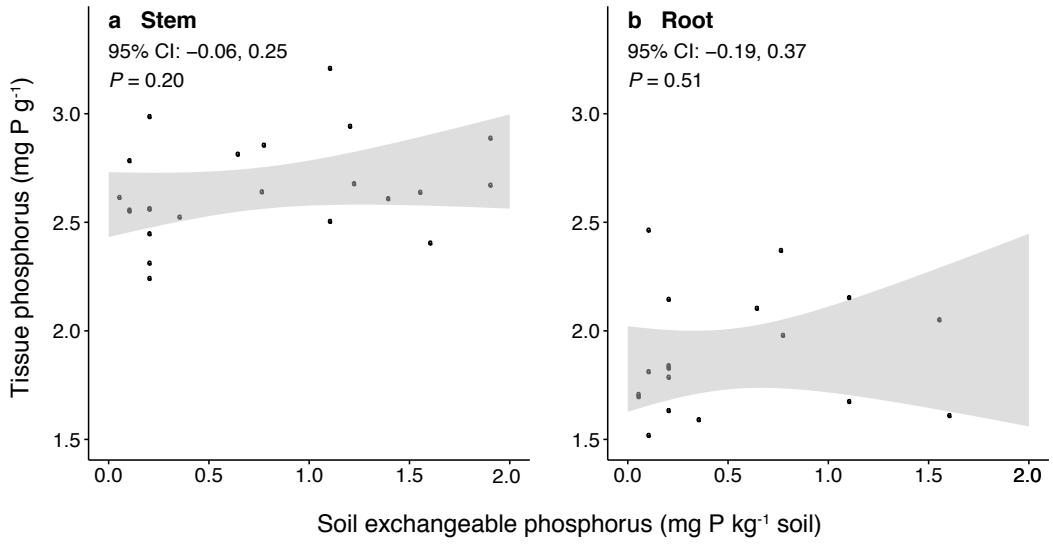

Figure S7. Stem (**a**) and root (**b**) tissue phosphorus concentrations of the mycoheterotroph *Voyria tenella* do not respond to increasing levels of soil exchangeable phosphorus across a natural phosphorus gradient in lowland tropical forests in Panama. Shaded regions represent 95% confidence bands.

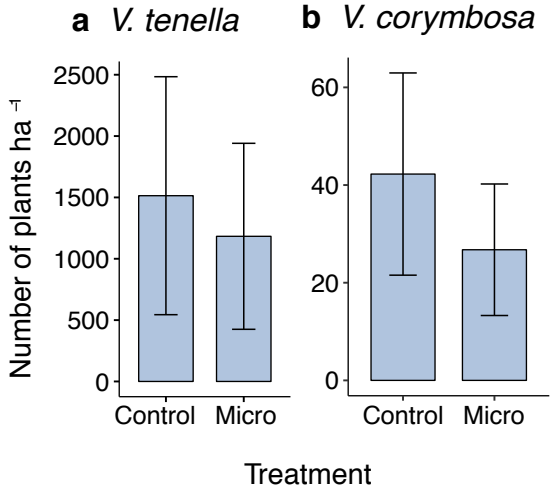

Figure S8. The abundance of the mycoheterotrophs *Voyria tenella* (**a**) and *Voyria corymbosa* (**b**) was unaffected by long-term micronutrient addition in a lowland tropical forest in Panama, confirming that phosphorus and not the calcium counterion of the phosphorus fertiliser (triple superphosphate, $Ca(H_2PO_4)_2.H_2O$) was responsible for the elimination of mycoheterotrophs. The micronutrient treatment contained dolomitic limestone to provide Ca and Mg, and a micronutrient fertiliser that contained B, Cu, Fe, Mn, Mo, S, and Zn. Values are fitted responses of generalised linear model with negative binomial errors (n = 4) and show 95% confidence intervals.

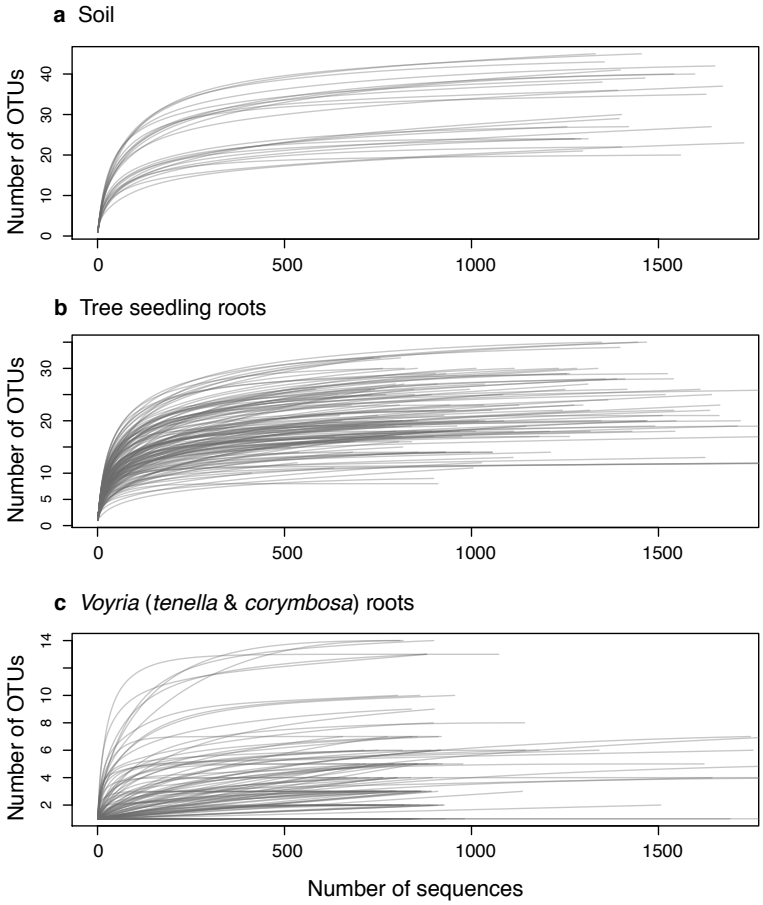

Figure S9. Rarefaction curves for each sample approached asymptotes indicating that sequencing intensity was sufficiently high to detect the majority of OTUs and that sampling effort was sufficient to capture the range of AM fungal diversity across the sites.