

Supporting Information

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SI Methods

Focal Populations of *M. smithii*. From May 21, 2014 to June 11, 2014, we harvested *Mycocepurus smithii* colonies within the Soberanía National Park, Panamá (N 9.13528, W 79.72141), locating nests entrances (holes in the ground <3 mm diameter) by dispersing oat-polenta bait in the leaf litter and following laden workers back to the nest. Back in the laboratory, we established colonies in plastic containers with ad libitum water and ground polenta, and acclimated them to laboratory conditions at 24 °C for 6.5 mo. Vouchers of ants (identified by D. Donoso) are stored at the Universidad de Loja, Ecuador, and fungus from these colonies used in barcoding analyses are stored in the collection of P.W.K. at the University of Copenhagen, Denmark.

Fungal Isolation, Growth Rate, and No-Choice Diet Experiment. After acclimating colonies to laboratory conditions, we used sterile forceps to remove 2-cm³ pieces of fungus from colonies. Under the fume hood, these isolates were subdivided into 2-mm³ pieces with a sterile dissecting needle and forceps, placed on 150-mm × 15-mm Petri dishes containing PDA medium (potato dextrose agar without antibiotics; Difco), and sealed with parafilm. Fungal isolates were monitored daily for contamination and infected portions were removed with a sterile surgical scalpel. To generate libraries of axenic fungi, healthy isolates were reisolated after 3 wk into smaller (60 mm × 15 mm) Petri dishes containing PDA and stored at 24 °C (51).

We performed a no-choice diet experiment on fungus isolated from a colony of *M. smithii* (177198) (Table S9). We developed a bacteriological version of the diet, modified from ref. 32, to prepare nine P:C diets (9:1, 6:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:6, 1:9), with protein + carbohydrates diluted to 8, 20, 40, and 60 g/L (36 total diets) (Table S1). Diets were prepared in 200-mL batches with distilled water and 3.2 g [1.6% (wt:vol)] bacteriological agar (Amresco), and had pH of 6.9. Carbohydrates were provided by equal parts sucrose (Doradita cane sugar) and starch (from potato, Sigma-Aldrich), and protein was provided from equal parts bacto peptone (enzymatic digest of protein; Becton Dickinson), trypticase peptone (pancreatic digest of casein; Becton Dickinson), and bacto tryptone (pancreatic digest of casein; Becton Dickinson). Micronutrients were provided by crushed vitamin (2% of the mass of protein + carbohydrates; Centrum). These ingredients were mixed with 200 mL distilled (d) water on a stirring plate for 5 min and then sterilized by autoclaving at 121 °C.

We aseptically inoculated 60-mm × 15-mm Petri dishes containing 12 mL of P:C diet with 19.6-mm² cylindrical plugs of PDA and fungus, sealed these with parafilm (12 plates per diet by dilution combination). Every few days, we checked plates for contamination, removing infected agar from some plates, and discarding plates if overrun with contaminants. After 32 d, we photographed surviving plates and estimated fungal expansion rate (final area, square millimeters), using ImageJ (NIH Image; v1.49g) to produce threshold contrast-adjusted grayscale images and analyze particles (with pixel² = 0.02) (as per ref. 32). It was not feasible to measure change in fungal mass because fine fungal hyphae could not be accurately separated from the agar. After 80 d, we photographed all instances of mushroom growth (*n* = 47) and used percent surviving plates with mushrooms for each diet treatment as the dependent variable in subsequent analyses.

We used least-square regressions with both linear and quadratic terms to evaluate how fungal expansion rates and percentages of surviving fungal plates with mushrooms varied across the 36 experimental diets (32). Nonparametric thin-plate splines that do not

constrain the shape of the response surface were determined with the “fields” package v.2.14.0 in R and used to generate a heat map of fungus growth and mushroom production (32). Topological resolution of response surfaces were set to $\lambda = 0.001$ as a smoothing parameter.

DNA Extraction Methods for the Comparative Fungus Growth Rate Experiment. We next compared fungal growth rates across *M. smithii* colonies by first preserving small fungal samples in 95% ethyl alcohol for barcoding analyses, and then growing pure fungal cultures from nine *M. smithii* colonies on sealed Petri dishes with 12 mL of PDA. After 30 d, we measured fungal growth rate as described in the P:C diet experiment. DNA from fungal symbionts was obtained by adding ~1 mm² fungus garden material to a 10% (wt/vol) Chelex Tris solution (47). Samples were boiled at 100 °C for 1 h and stored in the freezer for further analyses. One conserved region was selected to be amplified by PCR: the nuclear LSU, using the universal primers LR0R (5'-ACC CGC TGA ACT TAA GC-3') and LR5 (5'-TCC TGA GGG AAA CTT CG-3'). Amplification was performed with PCR with 25 μ L VWR Red Taq DNA polymerase Master Mix (VWR International), 2.5 μ L forward and reverse primer each, 18 μ L ddH₂O, and 2 μ L DNA, and a program of 5 min denaturing at 94 °C, followed by 40 cycles of 30-s denaturing at 94 °C, 30-s annealing at 54 °C, and 60-s extension at 72 °C, and finally a 10-min extension at 72 °C. All PCR products were sequenced at BGI Europe and sequences were deposited in GenBank (Table S9).

Phylogenetic Analyses. We used DNA barcoding data (Table S9) to create a chronogram and test for phylogenetic signal in fungal growth rate across the nine *M. smithii* colonies using the *ape* package in R v3.0-8 (phylosig) (48). Specifically, we estimated Blomberg's *K*, which indicates the amount of phylogenetic signal in branch tip data relative to a null expectation of Brownian motion for trait evolution in the chronogram. The DNA sequences were aligned with an iterative approach (i.e., simultaneous alignment and tree estimation) using SATé-II v2.2.2 (52). These alignments were used to estimate the fungal phylogeny. Before the tree estimation, the molecular model of the aligned sequences was determined using jModelTest v0.1.1 (53) and the best model was used for subsequent phylogenetic analyses. We performed a maximum-likelihood (ML) and a Bayesian estimation of the phylogeny with Garli v2.0 (54) and MrBayes v3.4 (55). The Bayesian approaches were performed with default settings for all priors. The Markov chain-Monte Carlo analysis was run six times independently, each one with two chains of 75 million generations with a sampling rate of 1,500 generations. The convergence of the runs was determined using Tracer v1.4 (56). Nodal support was calculated after 200 nonparametric bootstrap searches for both the ML approach and in the posterior probabilities for the Bayesian estimation. Both methodologies gave similar tree topologies and only the ML phylogenies are provided.

Field Substrate Collection. We cataloged substrates harvested by *M. smithii* workers in the field from November 2013 to December 2013 (the rainy season) by performing >20 h of foraging observations at 22 focal colonies in a Panamanian rainforest within Soberanía National Park (Table S7). Substrate pieces were dried at 60 °C for 24 h, weighed to the nearest 1 μ g on a Sartorius CP2P microbalance, and cataloged into five categories: insect frass, wood, flower fragment, small seed, and other (miscellaneous plant

pieces and unknown items). We also recorded foraging rates (number of laden workers returning to their nests with substrate in their mandibles), and leaf litter surface temperature ($^{\circ}\text{C}$) with an infrared thermometer from approximately 1 m above the ground.

Elemental Nitrogen Analysis. From October 2014 to December 2014, we harvested additional substrate from 21 colonies of *M. smithii* for analysis of elemental nitrogen (%N) (Table S8). We homogenized sorted samples of substrates taken from laden returning workers, occasionally pooling tiny frass fragments across colonies to have sufficient mass (approximately 0.5 mg) for elemental analyses (Table S8). We measured %N with a Flash EA1112 analyzer (CE Elantech), in the laboratory of Ben Turner at the Smithsonian Tropical Institute, Panama. We also dried and homogenized samples of fungus garden collected on the first and last days of the P:C diet experiment, or on the day that colonies with failed crops were removed from the experiment, and analyzed 1-mg subsamples for %N using the analytical techniques described above.

Whole-Colony P:C Diet Experiments. In the days before the feeding experiments, we measured the initial mass of the colonies (adult ants + brood + fungus gardens). It was not possible to initially count workers, larvae, and pupae because they were embedded inside the garden matrix, and pilot trials indicated that attempts to do so stressed the ants and caused them to discard large fractions of their gardens. We thus estimated initial worker number as the sum of dead workers found during the 29-d experiment and the final surviving workers. Although it was also not possible to standardize the initial size of colonies at the start of the experiment, colony sizes were evenly distributed across diet treatments ($F_{4, 20} = 0.74$; $P = 0.578$).

We used a modified protocol from ref. 50 to prepare agar-based diets of 20 g/L dilution of protein + carbohydrates and P:C ratios from 1:6–6:1, spanning the range of P:C selected by ant species in other studies (33, 43, 44). The 20-g/L macronutrient concentrations used in these experimental diets were lower than 100 g/L–300 g/L concentrations used in similar geometric framework experiments with nonfarming ants (33, 43, 44). However, these low nutrient concentrations were appropriate for the present study based on pilot studies and based on experiments from the present study linking performance of isolated fungi with substrate protein + carbohydrate concentrations. Moreover, these concentrations matched the low overall nutritional concentrations in detrital substrates harvested by *M. smithii* in the field (Tables S7 and S8).

Protein in these diets came from whole egg powder, calcium caseinate, and whey protein. The digestible carbohydrate was sucrose. Crushed multivitamin tablets added to the diets provided micronutrients. For example, we prepared the 6:1 P:C diet by boiling 150 mL dH₂O with 4.50 g agar (Acros Organics). In a separate mixture, we combined 150 mL dH₂O, 1.80 g whole egg powder (Great American Spice Co.), 2.71 g whey protein (GNC), 2.48 g calcium caseinate (TrueProtein), 0.16 g of crushed multivitamin (Centrum), and 0.59 g sucrose (cane sugar, Doradita). We mixed the agar and nutritive solutions, poured the mixture into Petri dishes, and stored them at 4 $^{\circ}\text{C}$ until the diets were provided to colonies in 1-g (wet mass) cubes. We kept the mass of whole egg powder constant for both diets to standardize dietary fat content (50). We repeated this protocol for each diet, modifying ingredient amounts to adjust P:C, while maintaining a 20 g/L protein + carbohydrate dilution (Table S5).

At the start of feeding experiments, colonies ranged in initial wet mass from 0.24 g to 2.15 g (mean: 0.70 g \pm 0.37 g) and had from 23 workers to 121 workers (mean: 49.76 \pm 25.60). We divided colonies between diet treatments in a stratified random manner, selecting colonies randomly from within classes of colony-farm mass to ensure that initial colony-farm mass did not

differ between diet types ($F_{4, 20} = 0.74$; $P = 0.578$). We used a no-choice experiment that confined colony-farms to a 1:6, 1:3, 1:1, 3:1, or 6:1 P:C diet, with five colonies per diet treatment. We collected diets every day during the 22-d feeding experiments, dried them for 24 h at 60 $^{\circ}\text{C}$, and weighed them to the nearest 0.1 mg. Initial dry diet mass was estimated with dry:wet ratios from control diets (four per diet). The cumulative sum of daily macronutrient harvest per colony was calculated from dietary P:C ratios.

After 29 d, we performed three measurements of colony demography: final worker number, final colony mass (adult ants + brood + fungus garden), and final brood mass. Because brood mass was only measured when fungus gardens were disassembled following the experiment, final brood mass represented diet effects on brood maintenance, rather than brood production, per se. We analyzed the proportion of colonies with failed crops at the end of the experiment using a GLM with a binomial distribution and a logit link function, including diet protein availability as an ordinal variable, and using a Firth bias adjustment because all crops failed in some treatments (6:1) and all survived in others (1:6). Initial colony mass was not a significant covariate and was not included in this analysis.

Estimating Carbohydrates Harvested to Fuel Worker Maintenance Metabolism. To interpret the observed intake targets, we determined the fraction of harvested carbohydrates that ant workers would require to fuel their maintenance metabolic demands. To do this we estimated two quantities: (i) the elemental carbon in dietary sucrose harvested by workers over 22 d confined to 1:6 or 6:1 P:C diets, and (ii) the elemental carbon respired by workers in CO₂ over 22 d in the feeding experiment. To calculate the mass of carbon harvested by each colony, we measured total sucrose (milligrams) consumed over 22 d in both 1:6 and 6:1 diet treatments, and calculated the fraction of this mass represented by carbon based on the mass composition by element for sucrose (C₁₂H₂₂O₁₁) in milligrams per mole.

To calculate the elemental carbon excreted in CO₂ by *M. smithii* colonies over 22 d, we multiplied a published value for individual *M. smithii* worker metabolic rate at 25 $^{\circ}\text{C}$ (0.232 $\mu\text{L CO}_2 \text{ h}^{-1}$) (39) by the number of workers in each colony on day 1 of the experiment. We estimated the CO₂ emission over 22 d by multiplying this hourly rate by 528 h. We converted these values from microliter to liter and estimated the milligrams of carbon in this volume of CO₂ by converting it from liters to moles, assuming that a mole of the gas at standard conditions for temperature (0 $^{\circ}\text{C}$) and pressure (0.986 atm; standard temperature and pressure) has a volume of 22.4 L. We next converted moles of carbon excreted over 22 d to milligrams of carbon excreted over 22 d based on the mass composition by element of CO₂. This method likely slightly overestimates colony-level CO₂ emission rates because it assumes that metabolic rate is additive (isometric) from workers to the whole colony, even though previous studies suggested a decline in per-ant metabolic rate (i.e., allometry or hyperometry) as workers accumulate in larger groups and colonies (39, 57, 58). We then calculated the fractional mass of carbon in respired CO₂ relative to the total mass of carbon in harvested sucrose.

Protein Harvested by Ants in the Field and Laboratory and Protein Availability for Plated Fungi. We evaluated whether and how *M. smithii* protein foraging strategies would enable colonies to navigate the fungal growth-reproduction trade-off depicted in Fig. 1. To do this, we estimated three quantities: (i) rates of protein harvest in the field from estimates of substrate %N, (ii) amounts of protein available to fungal inoculates in Petri dishes, and (iii) rates of protein harvest in laboratory experiments from defined amounts of protein in diet recipes.

To estimate field protein harvest rates, we first calculated substrate percent crude protein by multiplying %N in harvested substrate pieces by 6.25, assuming that proteins contain, on average, 16% N (49). In total, we analyzed 12 frass samples (mean: 1.67, SD: 1.28% N, corresponding to mean: 10.43, SD: 8.02% crude protein) and three wood fragment samples (mean: 0.67, SD: 0.36% N, corresponding to mean: 4.17, SD: 2.27% crude protein) from 20 colonies (Table S8). We then multiplied the mean hourly mass of substrate harvested by colonies (1.3 mg) (Table S7) by the overall mean percent crude protein in harvested substrate (9.17%) (Table S8), which yielded 0.12 mg crude protein harvested per colony per hour. We finally obtained two estimates of daily crude protein harvest, a lower estimate assuming workers only forage during approximately 12 daylight hours per day (1.4 mg) and an upper estimate assuming workers forage during 20 rain-free hours per day (2.4 mg).

We next estimated protein availability for fungal inoculates across P:C diets in the Petri dish experiments. We estimated the amount of protein available to fungal inoculates in the P:C diets with extremely low (8 g/L 1:9) and extremely high (60 g/L 9:1) protein levels. These calculations were possible because each 6-cm \times 1.5-cm (28.26 cm²) Petri dish had 12 mL of diet with

defined amounts of protein and carbohydrates added per 100 mL of water. For example, fungal inoculates confined to the 8 g/L, 1:9 P:C diet had 0.096 g protein + carbohydrates per dish (0.8 g protein + carbohydrates per 100 mL), and 3.17 mg protein. Overall, Petri dishes contained protein masses ranging from 3.17 mg (1:9 P:C 8g/L) to 482.24 mg (9:1 P:C 60 g/L). However, this protein was dispersed across the entire dish, such that the fungal hyphae likely accessed only a small fraction of this protein mass on a daily basis. We estimated this daily amount by calculating protein availability per square centimeter, which indicated that growing hyphae expanding out from the central inoculate plug likely encountered in between 0.11 mg/cm² of protein on a daily basis in the 1:9 8 g/L 1:9 P:C ratio treatment and 17.06 mg/cm² in the 9:1 P:C 60-g/L treatment.

We next calculated the daily protein amounts harvested by workers across the P:C diets during 22 d in the controlled laboratory experiment. From the data in Fig. 2B, colonies harvested the following amounts of protein per day: 0.70 mg (1:6 P:C), 1.10 mg (1:3 P:C), 1.01 mg (1:1 P:C), 1.25 mg (3:1 P:C), and 1.57 mg (6:1 P:C). This finding indicates that colonies typically regulated daily protein harvest levels at amounts similar to 1.4mg–2.4 mg crude protein harvested by workers in the field.

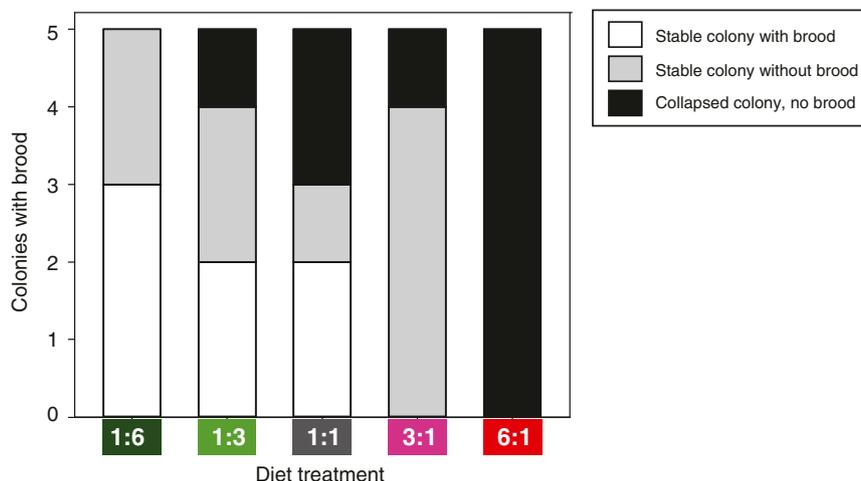


Fig. S1. Colonies were less likely to contain brood (and had lower brood mass) (Table S6) after being confined to increasingly protein-biased diets. These results indicate reductions in colony growth potential as a major cost of protein consumption. Colonies with collapsed gardens always also lacked brood.

Table S1. Recipes for 36 synthetic diets varying in P:C ratio (9:1, 6:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:6, 1:9) and protein + carbohydrate concentration (8, 20, 40, and 60 g/L)

PC	Bacto peptone	Trypticase peptone	Bacto tryptone	Sucrose	Starch	Vitamin	Agar
Diet concentration: 8 g/L							
9:1	0.517	0.511	0.511	0.076	0.076	0.032	3.2
6:1	0.492	0.486	0.487	0.111	0.111	0.032	3.2
3:1	0.426	0.426	0.431	0.197	0.197	0.032	3.2
2:1	0.379	0.379	0.384	0.263	0.263	0.032	3.2
1:1	0.284	0.284	0.287	0.398	0.398	0.032	3.2
1:2	0.189	0.189	0.191	0.533	0.533	0.032	3.2
1:3	0.142	0.142	0.144	0.599	0.599	0.032	3.2
1:6	0.081	0.081	0.082	0.685	0.685	0.032	3.2
1:9	0.057	0.057	0.057	0.720	0.720	0.032	3.2
Diet concentration: 20 g/L							
9:1	1.277	1.278	1.293	0.191	0.191	0.080	3.2
6:1	1.216	1.216	1.231	0.277	0.277	0.080	3.2
3:1	1.064	1.065	1.077	0.492	0.492	0.080	3.2
2:1	0.947	0.948	0.959	0.658	0.658	0.080	3.2
1:1	0.709	0.710	0.718	0.995	0.995	0.080	3.2
1:2	0.471	0.472	0.477	1.332	1.332	0.080	3.2
1:3	0.355	0.355	0.359	1.497	1.497	0.080	3.2
1:6	0.203	0.203	0.205	1.712	1.712	0.080	3.2
1:9	0.142	0.142	0.144	1.799	1.799	0.080	3.2
Diet concentration: 40 g/L							
9:1	2.554	2.555	2.585	0.381	0.381	0.16	3.2
6:1	2.431	2.422	2.480	0.554	0.554	0.16	3.2
3:1	2.128	2.129	2.154	0.984	0.984	0.16	3.2
2:1	1.895	1.896	1.918	1.315	1.315	0.16	3.2
1:1	1.419	1.419	1.436	1.990	1.990	0.16	3.2
1:2	0.943	0.942	0.954	2.664	2.664	0.16	3.2
1:3	0.709	0.710	0.718	2.995	2.995	0.16	3.2
1:6	0.406	0.406	0.411	3.425	3.425	0.16	3.2
1:9	0.284	0.284	0.287	3.598	3.598	0.16	3.2
Diet concentration: 60 g/L							
9:1	3.830	3.833	3.878	0.572	0.572	0.24	3.2
6:1	3.647	3.649	3.692	0.832	0.832	0.24	3.2
3:1	3.192	3.194	3.232	1.477	1.477	0.24	3.2
2:1	2.842	2.844	2.877	1.973	1.973	0.24	3.2
1:1	2.128	2.129	2.154	2.984	2.984	0.24	3.2
1:2	1.414	1.415	1.432	3.996	3.996	0.24	3.2
1:3	1.064	1.065	1.077	4.492	4.492	0.24	3.2
1:6	0.609	0.609	0.616	5.137	5.137	0.24	3.2
1:9	0.426	0.426	0.431	5.397	5.397	0.24	3.2

All diets were prepared in 200 mL distilled water and all amounts are in grams.

Table S2. Univariate tests of parameter estimates

Dependent variable		Degrees of freedom	<i>F</i>	<i>P</i>
Expansion rate	P	1	56.02	0.0001
	P ²	1	3.45	0.0639
	C	1	13.07	0.0003
	C ²	1	6.05	0.0144
	P × C	1	11.45	0.0008
	Error	354		
Mushroom production	P	1	16.62	0.0003
	P ²	1	14.99	0.0005
	C	1	4.67	0.0388
	C ²	1	3.46	0.0431
	P × C	1	0.76	0.3901
	Error	30		

Mapping two opposing measures of fungal performance, production of edible hyphae (square millimeter of growth after 32 d) and inedible mushrooms (percent of surviving plates with mushroom after 80 d) across a nutritional landscape of 36 diets varying across nine P:C ratios (between 1:9 and 9:1) and four protein and carbohydrate concentrations (8, 20, 40, and 60 g/L). Shown are the results of least-square regressions estimating parametric nonlinear response surfaces with the linear and quadratic components of protein (P) and carbohydrate (C) composition in diets, and the P × C interaction. Plates with contaminated inoculates were excluded from surface plots and analyses. Percent mushroom production was arcsine square-root-transformed before analysis. See *Methods* for experimental details.

Table S3. Significance test for univariate models

Model	Degrees-of-freedom model	Degrees-of-freedom error	<i>R</i> ²	MS model	<i>F</i>	<i>P</i>
Expansion rate	5	354	0.597	415653.19	105.60	0.0001
Mushroom production	5	30	0.622	0.50	9.88	0.0001

Mapping two opposing measures of fungal performance, production of edible hyphae (square millimeter of growth after 32 d) and inedible mushrooms (percent of surviving plates with mushroom after 80 d) across a nutritional landscape of 36 diets varying across nine P:C ratios (between 1:9 and 9:1) and four protein and carbohydrate concentrations (8, 20, 40, and 60 g/L). Plates with contaminated inoculates were excluded from surface plots and analyses. Percent mushroom production was arcsine square-root transformed before analysis. See *Methods* for experimental details.

Table S4. Estimated coefficients

Dependent variable	Diet component	Linear β (SE)	Quadratic β (SE)	Correlational P × C β (SE)
Expansion rate	P	−7.612 (1.017)****	0.032 (0.017)	0.102 (0.030)***
	C	−3.665 (1.014)***	0.042 (0.017)*	
Mushroom production	P	−0.466 (0.011)***	0.001 (0.000)***	−0.000 (0.000)
	C	0.025 (0.011)*	−0.000 (0.000)*	

Mapping two opposing measures of fungal performance, production of edible hyphae (mm² growth after 32 d) and inedible mushrooms (percent of surviving plates with mushroom after 80 d) across a nutritional landscape of 36 diets varying across nine P:C ratios (between 1:9 and 9:1) and four protein and carbohydrate concentrations (8, 20, 40, and 60 g/L). Shown are the results of least-square regressions estimating parametric nonlinear response surfaces with the linear and quadratic components of protein (P) and carbohydrate (C) composition in diets. Plates with contaminated inoculates were excluded from surface plots and analyses. Percent mushroom production was arcsine square-root-transformed before analysis. See *Methods* for experimental details. Significance of estimated coefficients: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

Table S5. Diet recipes for the colony feeding experiment adapted from ref. 50

P:C	Whey protein	Calcium caseinate	Whole egg powder	Sucrose	Vitamin	Water (mL)	Agar
6:1	2.71 (2.03)	2.48 (2.27)	1.80 (0.85)	0.59	0.16	300	4.50
3:1	2.3 (1.73)	2.11 (1.93)	1.80 (0.85)	1.26	0.16	300	4.50
1:1	1.39 (1.04)	1.28 (1.17)	1.80 (0.85)	2.84	0.16	300	4.50
1:3	0.41 (0.31)	0.38 (0.35)	1.80 (0.85)	4.43	0.16	300	4.50
1:6	0.0078 (0.0059)	0.0072 (0.0066)	1.80 (0.85)	5.11	0.16	300	4.50

Numbers in parentheses represent the amount of protein in the total mass of each ingredient (g). Diets were diluted to 20 g/L protein + carbohydrates. As indicated by manufacturer's labels, whey protein was 75.0% protein and 7.1% carbohydrates, calcium caseinate was 91.5% protein and 1.8% carbohydrates, and whole egg powder was 47.0% protein and 2.0% carbohydrates.

Table S6. GLM model analyses of P:C diet effects on nutrients harvested by workers

Test	Dependent variable	Treatment	df	F	P
Diet harvest					
Total diet harvest	Diet		4	8.04	0.0006
	Initial mass		1	2.91	0.104
	Error		19		
Protein harvest	Diet		4	1.94	0.145
	Initial mass		1	0.74	0.402
	Error		19		
Carbohydrate harvest	Diet		4	28.75	0.0001
	Initial mass		1	4.20	0.054
	Error		19		
Colony demography					
Final worker number	Diet		4	3.08	0.041
	Initial mass		1	21.46	0.0002
	Error		19		
Final colony mass	Diet		4	3.51	0.026
	Initial mass		1	60.23	0.0001
	Error		19		
Final brood mass	Diet		4	3.74	0.021
	Initial mass		1	8.61	0.009
	Error		19		

GLM model analyses of P:C diet effects on nutrients harvested by workers, and the resulting demographic (colony size: mass and worker number; colony growth: final brood mass) consequences of these collective foraging decisions. We compared diet harvest and demography across P:C diet treatments (single P:C diets of 1:6, 1:3, 1:1, 3:1, 6:1), with colony ID included as a random factor and initial colony mass as a covariate. Diet harvest was cumulative across 22 d and colony demography data were measured after 29 experimental days.

Table S7. Raw data for *M. smithii* substrate collection

Site	Collection date	ID	Start time	Duration (min)	No. laden workers	Insect frass	Wood piece	Flower	Seed	Other	Total	
LS1	12/9/13	37820	8:30	60	23.6	2	0.114	0.145			0.259	
JG1	11/16/13	176700	11:46	60	26.7	7	1.51	6.24			7.75	
JG1	11/18/13	176701	10:02	30	26	3	0.369	0.015			0.384	
JG1	11/18/13	176704	10:33	30	26.1	3	0.464				0.464	
GP1	11/27/13	176765	10:22	60	26.6	1		1.015			1.015	
GP1	11/29/13	176796	12:00	60	28.3	2	0.08	0.032		0.056	0.168	
GP1	12/4/13	176801	13:18	60	26.3	19			3.333	0.334	3.667	
GP1	11/29/13	176804	16:42	30	26.4	1	0.096				0.096	
LS1	12/13/13	176819	10:16	60	25.5	1	0.642				0.642	
LS1	12/2/13	176865	8:10	60	24	3	0.235				0.235	
GP1	11/26/13	176956	9:03	60	25.8	4	1.188	0.021			1.209	
JG1	11/22/13	176963	9:38	60	26.2	4	1.084	0.155		0.016	1.255	
GP1	11/27/13	177010	10:08	60	26.5	5	0.997			0.071	1.068	
JG1	11/22/13	177013	8:22	60	26	3	0.869			0.029	0.898	
GP1	11/30/13	177040	11:30	60	26.9	5	0.04	0.036	0.172	0.01	0.258	
GP1	11/26/13	177070	11:50	60	26.6	5	0.208				0.208	
GP1	12/4/13	177100	12:08	60	27.9	4	0.402	0.009	0.288		0.699	
GP1	12/5/13	177103	14:10	60	27.6	3	2.878		0.405	0.015	3.298	
LS1	12/12/13	177126	11:28	60	25.6	1				0.033	0.033	
LS1	12/12/13	177136	11:28	60	25.6	4	1.104				1.104	
LS1	12/9/13	177153	9:48	60	25.2	3	1.078				1.078	
LS1	12/23/13	177221	14:27	60	25.1	4	0.205	0.207		0.121	0.533	
Total (average)				1,230 (56)	26.1	87 (4)	13.563 (0.714)	7.668 (0.852)	0.612 (0.306)	3.793 (1.264)	0.685 (0.031)	26.321 (1.196)

All substrate masses are dry weights (milligrams) weighed to the nearest 1 μg . In total, we observed >20 h of worker foraging. The "other" substrate category includes small fragments of vegetation and other unidentifiable bits of detritus. Number of laden workers indicates the number of workers returning to their nests carrying substrate in their mandibles. Collection sites, located in Soberania Park, included La Seda Creek (LS1), Juan Grande Creek (JG1), and Gamboa (GP1). Temperature ($^{\circ}\text{C}$) measures are of the ant's foraging milieu measured with an infrared thermometer from approximately 1 m above the leaf litter surface.

Table S8. Raw nitrogen (%N) data used to estimate the crude protein content of substrate detritus harvested by *M. smithii* workers in natural populations

<i>n</i>	Substrate type	Sample mass (mg)	%N	% Crude protein	Crude protein mass (mg)
2	Insect frass	0.710	0.106	0.665	0.005
2	Insect frass	0.505	0.586	3.661	0.018
1	Insect frass	1.942	0.863	5.394	0.105
3	Insect frass	0.526	0.920	5.748	0.030
2	Insect frass	0.615	0.991	6.193	0.038
1	Insect frass	1.370	1.059	6.620	0.091
2	Insect frass	0.363	1.643	10.272	0.037
1	Insect frass	0.258	1.772	11.077	0.029
1	Insect frass	0.267	1.776	11.097	0.030
2	Insect frass	0.206	2.034	12.711	0.026
1	Insect frass	0.150	3.965	24.780	0.037
1	Insect frass	2.225	4.317	26.983	0.600
2	Wood	0.301	0.255	1.593	0.005
1	Wood	0.509	0.804	5.022	0.026
3	Wood	0.163	0.941	5.881	0.010

All substrate masses are dry weights (mg) measured to the nearest 1 μg . Substrates were harvested from 20 colonies in the focal study area from October 2014 to December 2014. In total, we analyzed 12 frass samples ($1.67 \pm 1.28\%$ N; $10.43 \pm 8.02\%$ crude protein) and 3 wood fragment samples ($0.67 \pm 0.36\%$ N; $4.17 \pm 2.27\%$ crude protein). We calculated crude protein content by multiplying %N by 6.25, assuming that proteins contain, on average, 16% N (49). Some samples were pooled across colonies because of the small mass, and *n* in the first column indicates the number of pooled colonies per sample.

Table S9. Information (GenBank accession numbers, collection code and collection locality) about colonies providing fungal samples for the growth rate experiment

<i>Mycocrepurus</i> Colony ID	BankIt number	Mean growth (mm ²)	SE	Number of plates	Collection locality
177299	Seq5	216.77	92.26	2	Gamboa
177321	Seq8	308.87	12.44	4	Gamboa
177311	Seq6	278.32	41.88	5	Gamboa
177287	Seq3	262.26	26.77	3	Gamboa
177312	Seq7	276.93	33.84	4	Gamboa
177347	Seq9	338.19	17.63	4	Gamboa
177106	Seq1	268.39	8.42	3	Gamboa
177198	Seq2	461.55	37.99	5	Gamboa
177288	Seq4	276.13	N/A	1	Gamboa

This table also provides growth data after 30 d (Fig. 5). All sequences listed in GenBank in the genus *Leucocoprinus* under the file name BankIt1941687. GenBank accession numbers refer to sequences used for PCR amplification of the conserved nuclear large subunit rRNA (LSU) to infer the chronogram of *M. smithii* cultivars. All sequences are previously unpublished. N/A, not applicable.