Supporting Information

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SI Materials and Methods

Experimental Organisms. Mosquitoes (*Anopheles stephensi*) were obtained from the Penn State Insectary. All studies were conducted in accordance with Penn State policies and oversight regarding ethical treatment and health and safety considerations.

Behavioral Assays. ***Wind tunnel experiments.*** Clean air was pushed through an activated charcoal filter and then humidified and split into two streams, each with a flow rate of 1.2 L/min. Each airstream passed through a glass chamber containing an odor source (e.g., an infected or healthy mouse or a rubber septum releasing extracted volatiles). In some assays (as noted), CO_2 (0.4 L/min) was added to the airflow before it exited into a trapping chamber at the end of the 1.5 × 0.5 × 0.5-m wind tunnel. The two trapping chambers were each 120 mm in length and 80 mm in diameter, set 30 cm apart, with an internal mesh screen set 30 mm away from the airflow exit. Except for the trapping chambers, the upwind end of the wind tunnel was opaque to minimize extraneous visual cues. During each trial, a fan pulled air from the downwind end of the wind tunnel through a mesh barrier (creating a push/pull system). An air filtration and exhaust system ensured that the air in the room where these assays was conducted did not become saturated with odors.

Treatments were randomly assigned to the left or right side of the wind tunnel. An initial trial comparing a healthy mouse vs. CO_2 was carried out each day to confirm mosquito responsiveness before conducting the other assays (day 11 after infection was not included in the analyses due to lack of response to these positive controls on that date). Additional assays were conducted in random order. After each experiment, clean air was blown through the wind tunnel for 10 min, and the inside of the wind tunnel was cleaned with 70% ethanol. Fresh trapping chambers were used for each treatment.

For trials conducted with extracted volatiles, rubber septa were first treated with 200 μL dichloromethane, which was left to be absorbed for 10 min, and then with 30 μL of volatile extract. Septa were covered with parafilm until the sample was absorbed and then immediately sealed and frozen (−20 °C) until used. Each septum was subsequently used in behavioral experiments for no longer than 1.5 h.

Selection of individual compounds for manipulation in behavioral trials. Statistical analyses were conducted using R v. 3.02 (1). Because the concentrations of most compounds were not normally distributed, compounds were rank transformed and reevaluated using the linear mixed model of the package nlme (2), and significant differences among days of infection were identified using a Tukey test with Bonferroni adjustments.

Chemical Analyses. ***Sample preparation.*** Volatile samples were eluted into vials using 150 μL of dichloromethane (Honeywell, Burdick and Jackson); 200 ng of n-octane and 400 ng of nonylacetate (Sigma-Aldrich) were added to each sample as internal standards.

Compound quantification by GC equipped with a flame ionization detector. Compounds were separated on a VOCOL capillary column (30 m × 0.25 mm ID × 1.5-μm film thickness; Supelco) using the following temperature program: Starting at 35 °C (for 5 min), the temperature was raised by 3.75 °C/min to a final temperature of 240 °C (for 4 min). The injector and detector were held at 250 °C. Injection volume was 1 μL, and the carrier gas was helium at a constant flow of 1.1 mL/min. Compounds were quantified based on their integrated area relative to the area of the internal standards (200 ng of n-octane and 400 ng of nonyl acetate).

Compound identification by GC-MS. Compounds were separated under the same analytical conditions listed above. The MS transfer line was held at 240 °C, and the MS operated in electron impact mode (70 eV: ion source 230 °C: quadropole 150 °C, mass scan range: 30–550 amu). Deconvolution algorithms (extraction and correlation) were applied to the total ion chromatograms (TICs) of the samples (MassHunter Workstation, Qualitative Analysis software B.06.00; Agilent Technologies). Compounds were then identified by comparing deconvoluted mass spectra to spectra in the NIST08 spectral library (National Institute of Standards and Technologies), and identities were confirmed by comparison with mass spectra and retention times of commercially available standards. A list of definitively identified compounds is presented in Table S2.

1. R Development Core Team (2008) *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna).

2. Pinheiro J, Bates D, DebRoy S, Sarkar D R Core Team (2013) *NLME: Linear and Nonlinear Mixed Effects Models, R Package Version 3.1-108* (The R Foundation for Statistical Computing, Vienna).

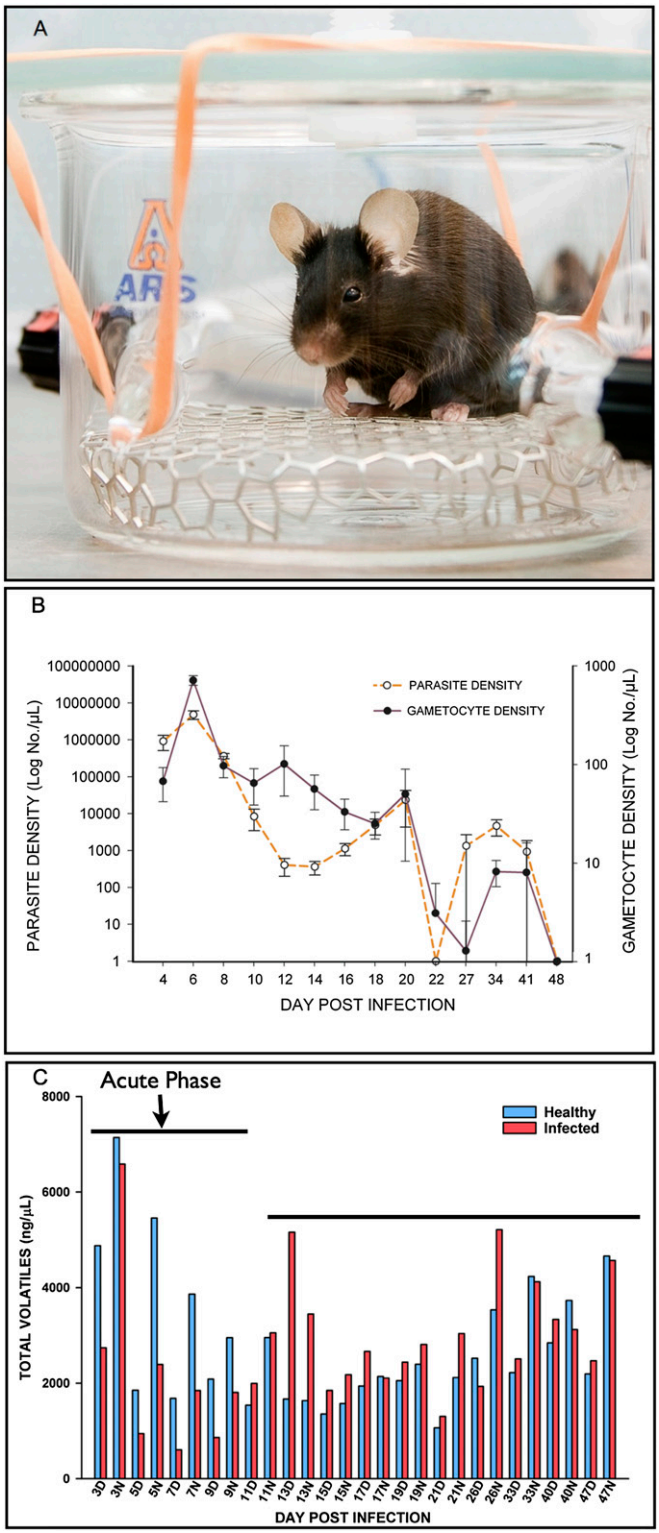

Fig. S1. Overview of the first mouse volatiles study. (*A*) Apparatus for volatile collections from individual mice. (*B*) Gametocyte and parasite densities (*Plasmodium chabaudi*) for each infected mouse through time. (*C*) Average total volatiles produced by healthy mice (blue) and malaria-infected mice (red) in day and night collections during the acute and chronic phases of infection. (Photograph by Nick Sloff, Pennsylvania State University, University Park, PA.)

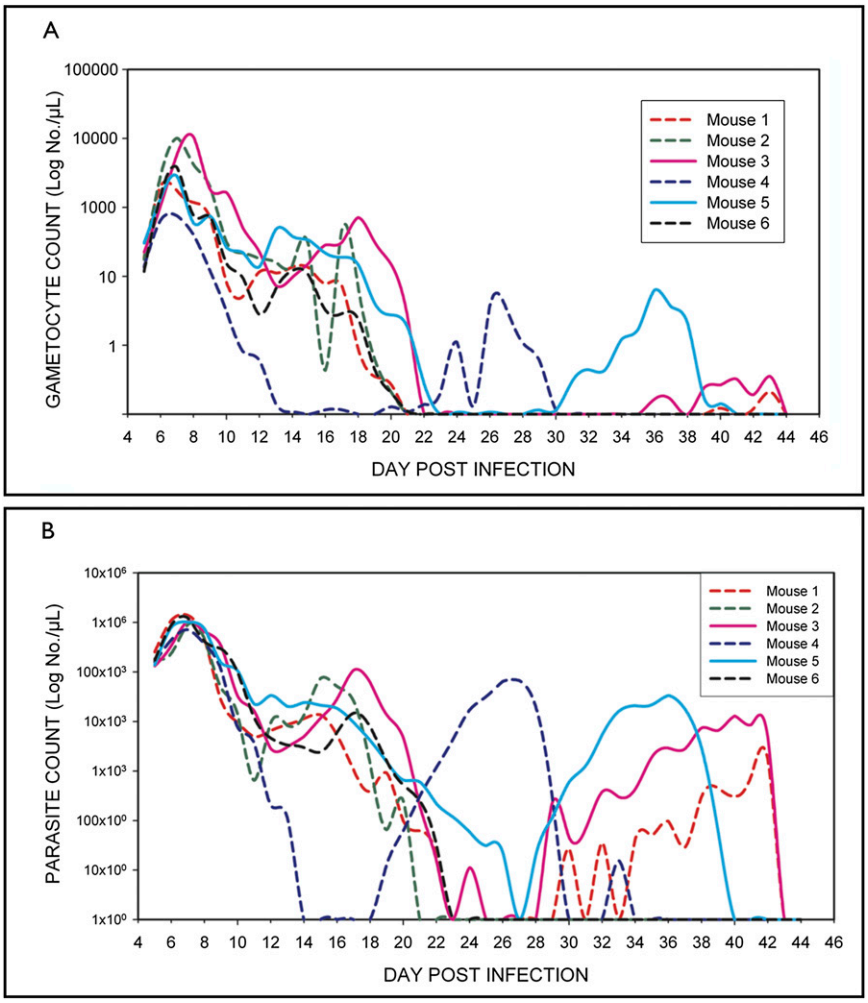

Fig. S2. *P. chabaudi* gametocyte (*A*) and parasite (*B*) densities over the course of infection for each infected mouse in the second volatile collection study.

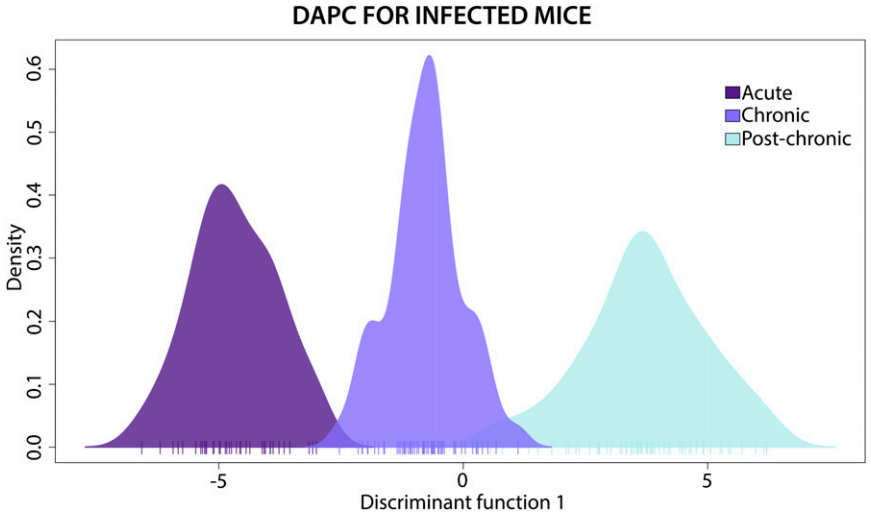

Fig. S3. Density of the first discriminant function showing separation between acute, chronic, and postchronic phases for infected mice.

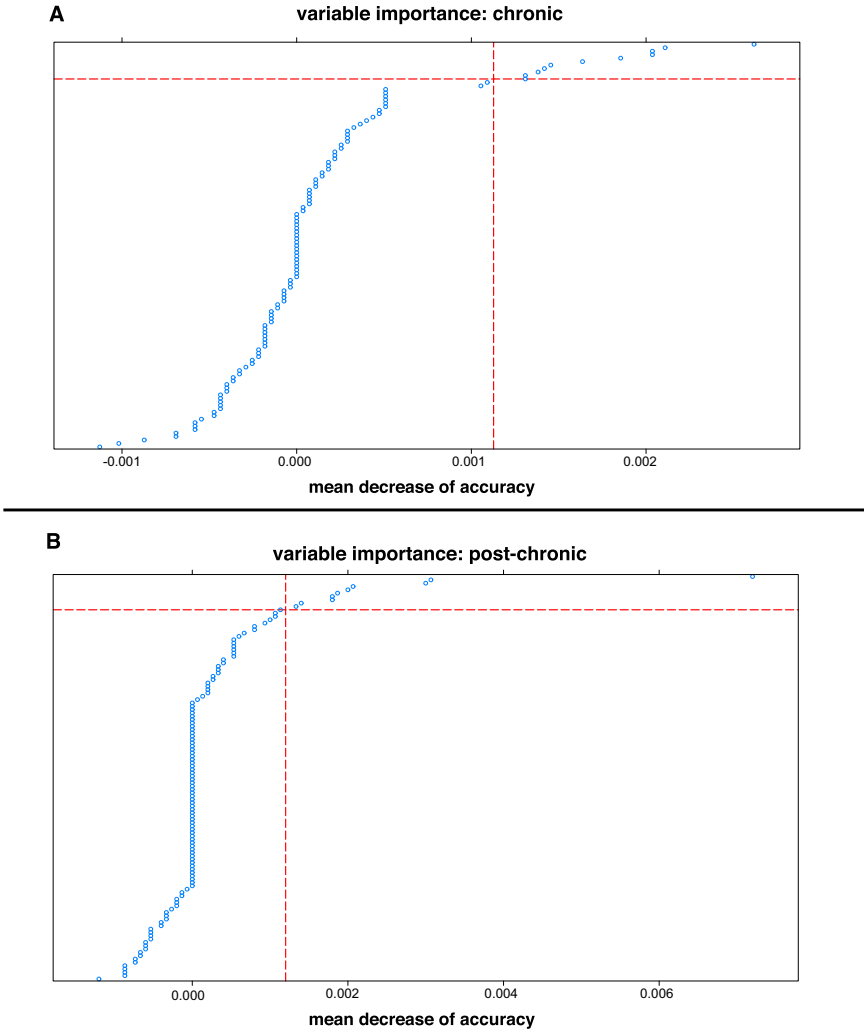

Fig. S4. Random Forest selection of relevant compounds (conditional variable importance score for each compound). A compound was considered to be informative and important if its mean decrease of accuracy value was above the absolute value of the lowest negative score (this threshold is indicted by the vertical dotted line). (*A*) Compounds selected in the chronic phase: M94, 1-tridecane; M16, 2-hexanone; M80, 2-pyrrolidone; M58, benzaldehyde; M13, 3-methyl-2-buten-1-ol; M104, *N*,*N*-dibutylformamide; M20, 3-methyl butanoic acid; and the unidentified compounds M129, M125, M84, and M43. (*B*) Compounds selected in the postchronic phase: M46, hexanoic acid; M86, 4-ethyl phenol; M18, 2,3-butanediol; M16, 2-hexanone; and the unidentified compounds M97, M147, M139, M93, M17, and M38.

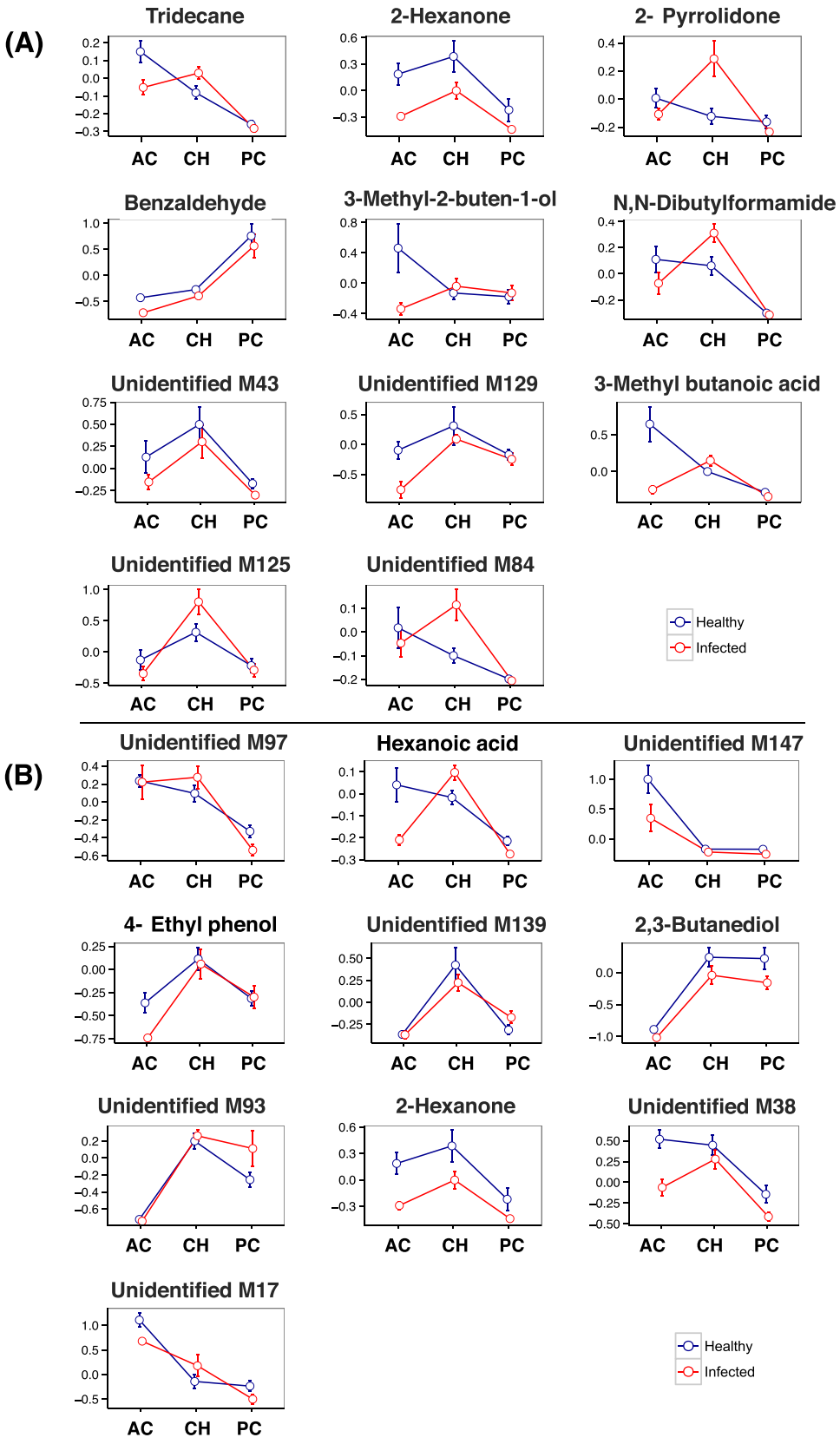

Fig. S5. Mean emission levels of select compounds, with SE, for healthy and infected mice during the acute (AC), chronic (CH), and postchronic (PC) phases. Values were mean centered using a z-score transformation (zero on the vertical axis reflects the overall mean for a given compound across all individuals and dates). (*A*) Compounds identified by Random Forest analysis as important predictors of infection status in the chronic phase. (*B*) Compounds identified by Random Forest analysis as important predictors of infection status in the postchronic phase.

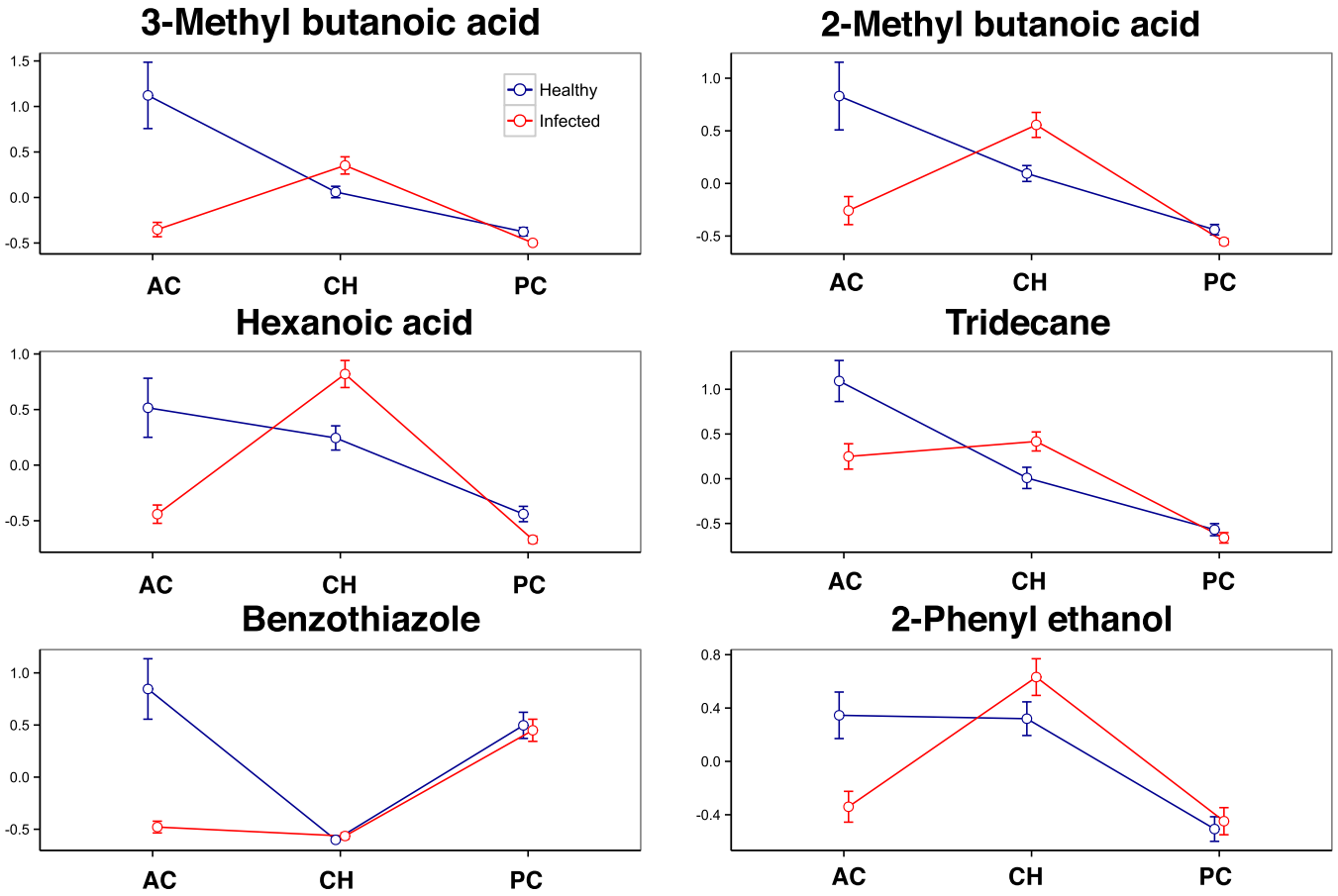

Fig. S6. Plot of mean compound emission levels during the acute, chronic, and postchronic phases for compounds tested individually in behavioral trials.

Table S1. Details of post hoc *t*-tests for compounds selected for manipulation in behavioral trials

Compound	Phase	*t*	df	*P* value
3-Methyl butanoic acid	Acute	3.66	31.88	0.001
	Chronic	−1.78	139.15	0.076
	Postchronic	1.79	68.14	0.079
2-Methyl-butanoic acid	Acute	3.13	46.70	0.003
	Chronic	−3.28	100.05	0.001
	Postchronic	3.13	46.70	0.003
Hexanoic acid	Acute	3.16	34.51	0.003
	Chronic	−1.81	142.12	0.072
	Postchronic	2.74	60.24	0.008
Tridecane	Acute	2.75	50.24	0.008
	Chronic	−2.75	147.40	0.007
	Postchronic	0.83	81.98	0.408
Benzothiazole	Acute	3.94	31.32	0.000
	Chronic	0.13	148.70	0.897
	Postchronic	0.16	82.00	0.876
2-Phenyl ethanol	Acute	2.02	49.65	0.049
	Chronic	−2.01	140.83	0.046
	Postchronic	−0.26	75.41	0.799

Table S2. List of definitively identified compounds, with retention times and Kovats retention indices

Compounds	CAS number	Retention time (min)	Retention index
3-Methyl-2-butanone	563-80-4	14.1	716
Propanoic acid	79-09-4	15.19	737
2-Pentanone	107-87-9	15.28	738
2-Methyl-propanoic acid	79-31-2	17.36	778
Butanoic acid	107-92-6	19.07	811
3-Methyl-2-buten-1-ol	556-82-1	19.73	824
2-Hexanone	591-78-6	20.81	845
2,3-Butanediol	24347-58-8	21.16	851
Ethyl butyrate	105-54-4	21.4	856
3-Methyl butanoic acid	503-74-2	21.97	867
2-Methyl butanoic acid	116-53-0	22.46	877
Pentanoic acid	109-52-4	24.37	915
p-Xylene	106-42-3	24.7	921
2-Heptanone	110-43-0	25.8	944
Hexanoic acid	142-62-1	28.89	1,009
1-Octen-3-ol	3391-86-4	29.49	1,022
6-Methyl-5-hepten-2-one	110-93-0	30.58	1,046
Phenol	108-95-2	30.81	1,051
Dimethyl sulfone	67-71-0	31.03	1,056
Benzaldehyde	100-52-7	31.27	1,062
Benzyl alcohol	100-51-6	34.22	1,129
Urea	57-13-6	35.15	1,152
4-Methyl-phenol	106-44-5	35.19	1,153
Nonanal	124-19-6	35.49	1,160
Acetophenone	98-86-2	36.02	1172
o-Toluidine	95-53-4	36.45	1,183
2-Methoxy-phenol	90-05-1	36.84	1,192
Dodecane	112-40-3	37.24	1,202
2-Pyrrolidone	616-45-5	37.49	1,208
2-Phenyl ethanol	60-12-8	38.36	1,230
Benzyl methyl ketone	103-79-7	38.99	1,246
4-Ethyl phenol	123-07-9	39.26	1,253
Tridecane	629-50-5	41.41	1,309
Benzothiazole	95-16-9	43.6	1,368
N,*N*-Dibutylformamide	761-65-9	45.19	1,413
Indole	120-72-9	46.6	1,454