Table S1. Cuticular hydrocarbon profiles of *M. rubra* and *M. ruginodis*

#	RI	Substance	Diagnostic ions (m/z)	M. rubra	M. ruginodis
				Mean±s.d.	Mean±s.d.
1	19.01	n-C19	*268*	0.29±0.27	0.51±0.68
2	19.59	unknown CHC	-	0.16±0.13	-
3	23.02	n-C23	*324*	0.28±0.37	-
4	23.53	5-MeC23	85/281	0.13±0.12	-
5	24.02	n-C24	*338*	0.29±0.19	0.05±0.12
6	24.77	C25-ene	*350*	0.06±0.1	-
7	24.96	C25-ene	*350*	1.1±0.92	-
8	25.03	n-C25	*352*	5.51±4.41	0.12±0.16
9	25.36	9-;11-;13-MeC25	140/252; 168/224; 196	1.7±0.75	-
10	25.43	7-MeC25	112/280	1.5±0.97	-
11	25.52	5-MeC25	84/308	1.41±0.8	-
12	25.67	8,12-DiMeC25	126/280, 196/210	0.47±0.24	-
13	25.74	3-MeC25	56/336	1.26±0.61	-
14	25.84	5,9-;5,11-DiMeC25	85/323, 154/252, 182/224	0.74±0.41	-
15	26.01	n-C26	*366*	0.61±0.26	0.05±0.07
16	26.11	3,x-DiMeC25	56/364	0.37±0.16	-
17	26.36	10-MeC26*	154/252	0.47±0.39	-
18	26.4	8-MeC26*	126/280	0.82±0.56	-
19	26.64	10,14-DiMeC26	154/266, 224/196	0.34±0.3	-
20	26.68	8,12-DiMeC26	126/294, 196/224	0.5±2.04	-
21	26.77	6,10-; 6.12-DiMeC26	98/322, 168/252 196/224	0.34±0.24	-
22	26.98	C27-ene	*378*	1.83±1.56	-
23	27.01	n-C27	*380*	3.37±1.98	2.72±2.26
24	27.2	4,8,12-TriMeC26	70/378, 140/308, 210/238	0.32±0.33	-
25	27.34	11-;13-MeC27	168/252; 196/224	-	0.18±0.15
26	27.36	9-;11-MeC27	140/280; 168/252	15.47±4.89	-

Journal of Experimental Biology • Supplementary information

#	RI	Substance	Diagnostic ions (m/z)	M. rubra	M. ruginodis
				Mean±s.d.	Mean±s.d.
27	27.43	7-MeC27	112/308	2.18±1.58	0.21±0.27
28	27.53	5-MeC27	85/337	4.93±2.2	0.21±0.23
29	27.63	11,15-DiMeC27	168/266, 238/196	5.39±2.47	-
30	27.66	9,13-DiMeC27	140/294, 210/224	-	0.02±0.05
31	27.72	7,11-DiMeC27	112/323, 183/252	8.15±3.77	-
32	27.74	3-MeC27	57/365	0.15±0.54	0.44±0.49
33	27.84	5,11-DiMeC27	85/351, 183/253	5.23±2.35	-
34	27.98	7,11,15-TriMeC27	112/337, 183/267, 253/197	1.35±1.32	-
35	28.01	7,11,21-TriMeC27	112/337, 183/267, 337/112	0.68±0.56	-
36	28.01	n-C28	*394*	-	0.33±0.32
37	28.1	5,9,13-;5,9,15-TriMeC27	85/364, 154/294, 225, 253/197	1.6±0.59	-
38	28.35	7-;10-;12-;13-;14-MeC28	112/323; 155/281; 181/253; 197/238; 210/225	1.87±0.78	-
39	28.38	3,7,11-TriMeC27	56/392, 126/322, 196/252	0.33±0.5	-
40	28.46	unknown CHC	-	0.12±0.11	-
41	28.64	3,7,11,15-TetraMeC27	57/407, 127/337, 197/267, 267/197	1.8±0.67	-
42	28.64	C29diene	*404*	-	0.42±0.61
43	28.68	C29diene	*404*	-	0.92±0.74
44	28.73	C29ene	*406*	-	1.23±0.56
45	28.76	3,x,y,z-TetraMeC27	56/406	0.36±0.29	-
46	28.79	C29-9-ene	*406*, [173, 327, 500]	-	4.18±1.52
47	28.86	C29-7-ene	*406*, [145, 355, 500]	-	2.29±1.1
48	28.9	C29ene	*406*	-	0.5±0.42
49	29.01	n-C29	*408*	1.62±1.12	5.22±4.25
50	29.18	4,8,16-TriMeC28 (position of 3rd methyl group tentative)	71/393, 141/323, 267/196	0.04±0.08	-
51	29.24	x-MeC29-diene	-	-	0.3±0.24

Journal of Experimental Biology • Supplementary information

#	RI	Substance	Diagnostic ions (m/z)	M. rubra	M. ruginodis
				Mean±s.d.	Mean±s.d.
52	29.33	9-;11-;13-;15-MeC29	141/308; 167/281; 197/252; 224	8.32±2.85	7±2
53	29.42	7-MeC29	112/336	1.06±0.64	0.16±0.44
54	29.52	5-MeC29	84/364	1.83±0.97	1.5±0.9
55	29.6	11,15-;13,17-DiMeC29	168/295, 239/224; 196/267, 267/196	3.27±1.56	0.68±0.81
56	29.7	9,13-; 7,11-DiMeC29	140/322, 210/252; 112/350, 182/280	-	0.49±0.74
57	29.74	3-MeC29	57/393	2.51±0.86	2.24±0.85
58	29.82	5,17-DiMeC29	85/379, 267/196	2.64±0.93	0.63±0.37
59	29.87	5-21-DiMeC29*	85/379, 323/140	0.05±0.11	-
60	29.98	7,11,17-TriMeC29	112/364, 183/296, 281/196	0.29±0.31	-
61	30.05	n-C30	*422*	-	0.21±0.21
62	30.07	3,11*-DiMeC29	56/406, 182/280	0.53±0.37	-
63	30.31	10-;12-;13-;14-MeC30	154/308; 182/280; 196/266; 210/252	0.46±0.25	0.59±0.31
64	30.36	unknown CHC	-	0.12±0.14	-
65	30.49	C31diene	*432*	-	0.11±0.14
66	30.55	C31diene	*432*	-	0.42±0.53
67	30.65	C31diene	*432*	-	4.76±1.94
68	30.65	2-;4-MeC30	43/420; 71/393	0.62±0.5	-
69	30.7	C31diene	*432*	-	14.35±6.61
70	30.7	C31-11-ene	*434*, [201, 327, 528]	-	2.44±3.61
71	30.8	C31diene	*432*	-	1.67±2.54
72	30.8	C31-9-ene	*434*, [173, 355, 528]	-	8.63±5.28
73	30.87	C31ene	*434*	-	0.68±0.98
74	30.91	C31ene	*434*	-	0.92±0.77
75	31	n-C31	*436*	-	0.46±0.63
76	31.16	unknown CHC	-	-	0.32±0.39

#	RI	Substance	Diagnostic ions (m/z)	M. rubra	M. ruginodis
				Mean±s.d.	Mean±s.d.
77	31.21	13-;14-MeC31ene	*448*, 194/278, 210/266	-	1.51±0.82
78	31.3	9-;11-;13-;15-MeC31	140/336; 168/308; 196/280; 224/252	-	6.33±2.37
79	31.32	13-;15-MeC31	196/280; 224/252	1.35±0.94	-
80	31.41	7-MeC31	112/364	0.19±0.18	-
81	31.44	cf. Methyltrien (unknown unsaturated)	-	-	0.42±0.45
82	31.52	5-MeC31	85/392	0.04±0.12	-
83	31.56	13,17-DiMeC31	196/295, 267/224	0.98±0.81	3.46±2.49
84	31.62	13,21-DiMeC31*	196/294, 322/168	0.07±0.12	-
85	31.65	9,17-;9,19-;9,21-DiMeC31	140/350, 266/224, 294/196, 322/168	-	2.48±2.17
86	31.7	unknown CHC	-	0.13±0.15	-
87	31.74	3-MeC31	57/420	-	0.83±0.67
88	31.8	5,15-;5,17-DiMeC31	85/407, 239/252, 267/224	0.19±0.14	1.08±0.64
89	32.04	3,15-DiMeC31	57/435, 239/252	-	0.3±0.28
90	32.3	12-;13-;14-;15-;16-MeC32	182/308; 196/294; 210/280; 224/267; 238/252	0.04±0.08	0.22±0.22
91	32.51	C33diene	*460*	-	0.54±0.52
92	32.57	C33diene	*460*	-	1.78±0.98
93	32.65	C33diene	*460*	-	4.63±1.89
94	32.73	C33diene	*460*	-	1.46±1.14
95	32.79	C33diene	*460*	-	0.87±1.11
96	32.87	C33ene	*462*	-	0.14±0.2
97	33.13	unknown CHC	-	-	0.1±0.16
98	33.3	11-;13-;15-;17-MeC33	169/336; 196/309; 224/280; 252	0.07±0.16	1.11±0.89
99	33.46	unknown CHC	-	0.05±0.13	-
100	33.54	13,17-;13,19-DiMeC33	196/323, 267/252, 295/224	-	0.62±0.59
101	33.61	9,x-;11,x-DiMeC33 [x=17 or 19]	140/379, 168/350, 267/252, 294/224	-	0.59±0.6

#	RI	Substance	Diagnostic ions (m/z)	M. rubra	M. ruginodis
				Mean±s.d.	Mean±s.d.
102	33.67	unknown CHC	-	-	0.05±0.11
103	33.78	5,21-;5,23-DiMeC33	84/434, 322/196, 350/168	-	0.43±0.35
104	34.04	3,15-DiMeC33	56/462, 238/280	-	0.12±0.24
105	34.45	C35diene	*488*	-	0.31±0.39
106	34.49	C35diene	*488*	-	0.34±0.38
107	34.55	C35diene	*488*	-	1.33±1.06
108	34.65	C35diene	*488*	-	0.22±0.35
109	35.24	13-;15-;17-MeC35	196/336; 224/338; 252/280	-	0.09±0.18
110	35.4	15,x-DiMeC35	224/322	0.02±0.07	-
111	35.45	15,19-DiMeC35	224/322, 294/252	-	0.23±0.37
112	35.51	11/13/15/17,19/21/23-DiMeC35 (combination of methyl group positions unknown)	168/378, 196/350, 224/322, 252/294, 294/252, 322/224, 350/196	-	0.12±0.16
113	36.41	C37diene	*516*	-	0.13±0.22

Overall, we detected and identified 64 hydrocarbon peaks in *M. rubra* and 69 peaks in *M. ruginodis.* The table shows the retention index (Kovats index; RI) (Carlson et al., 1998), substance name, diagnostic ions (mass peaks are printed in italics) and its mean percentage±s.d. Double bond positions for most abundant substances were determined by DMDS derivatisation (see diagnostic ions in brackets). Tentative identifications are marked by asterisks.

Table S2. Effects of acclimation conditions on the abundance of mono-, tri- and tetramethyl alkanes, as well as the overall profile. The table shows results from linear mixed effects models (LME) with temperature, humidity and caste as fixed factors and colony ID and sampling location as random factors. For the overall profiles, PERMANOVA with the same fixed and random factors was used. Test statistics are χ^2 (LME) or pseudo-F (PERMANOVA). Superscript letters denote whether data were a) log-, b) logit-, c) arcsine-square root- transformed, d) not transformed.

Species	Dependent variable	Fixed factor	N	df	χ^2 or *Pseudo-F*	p
M. rubra	**mono-methyl alkanes** [c]	Temperature	470	3	17.53	0.0005
		Humidity		1	0.72	0.40
		Caste		1	23.98	< 0.0001
	tri-methyl alkanes [d]	Temperature	466	3	114.83	< 0.0001
		Humidity		1	0.57	0.45
		Caste		1	29.42	< 0.0001
	tetra-methyl alkanes [c]	Temperature	480	3	82.00	< 0.0001
		Humidity		1	7.62	0.0006
		Caste		1	24.72	< 0.0001
	overall profile [d]	Temperature	480	3	*69.54*	0.001
		Humidity		1	*4.01*	0.007
		Caste		1	*20.01*	0.001
		Temp. x Hum.		3	*1.94*	0.015
		Temp. x Caste		3	*1.68*	0.053
		Hum. x Caste		1	*2.46*	0.025
		3-way interaction		3	*0.55*	0.920
M. ruginodis	**mono-methyl alkanes** [c]	Temperature	480	3	60.66	< 0.0001
		Humidity		1	37.01	< 0.0001
		Caste		1	19.88	< 0.0001
		Temp. x Hum.		3	16.25	0.0010
		Temp. x Caste		3	13.50	0.0037
	overall profile [d]	Temperature	480	3	*76.73*	0.001
		Humidity		1	*18.16*	0.001
		Caste		1	*23.29*	0.001
		Temp. x Hum.		3	*2.84*	0.002
		Temp. x Caste		3	*2.14*	0.008
		Hum. x Caste		1	*1.28*	0.230
		3-way interaction		3	*1.52*	0.075

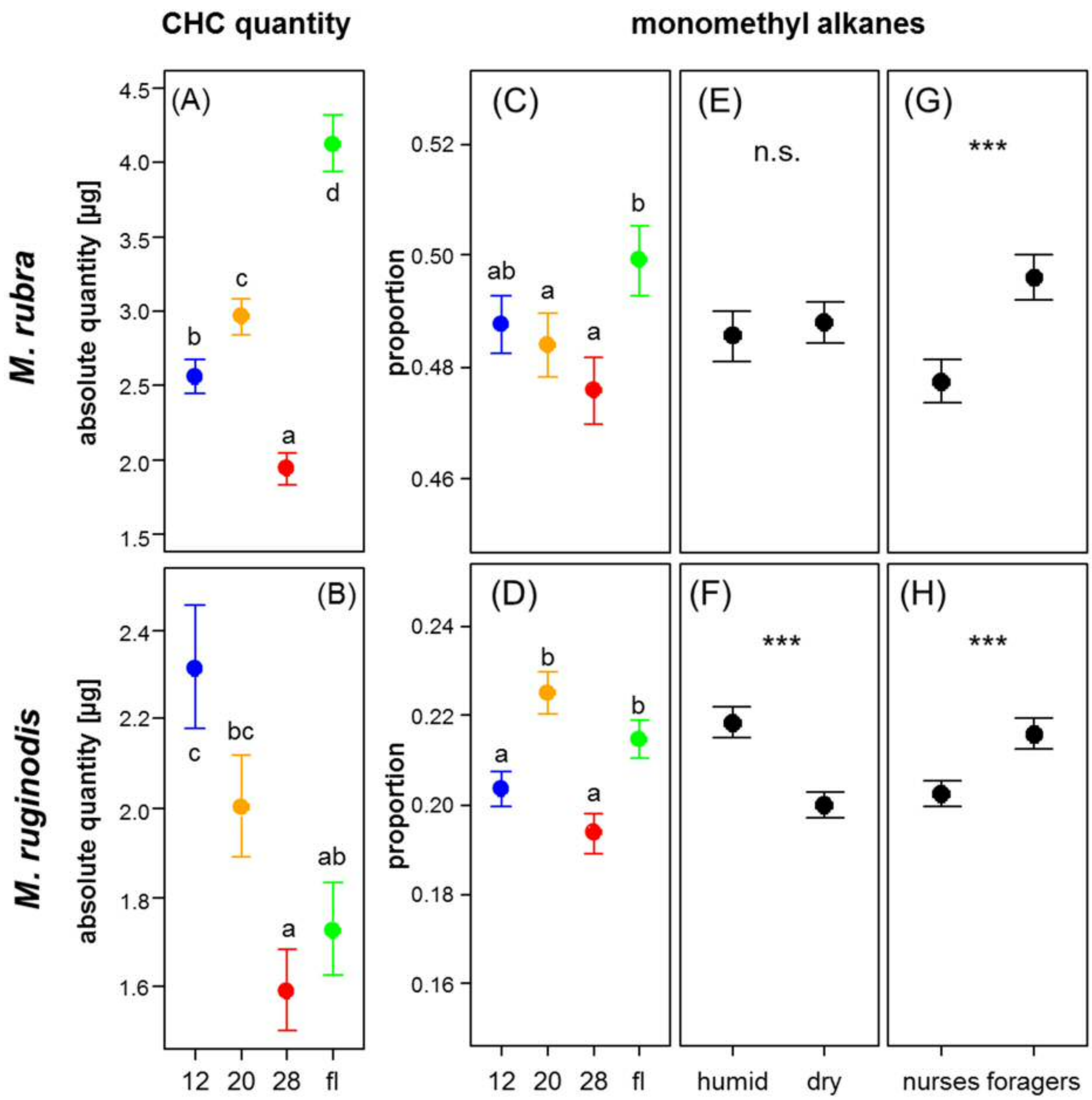

Fig. S1. Effect of temperature on absolute CHC amounts and effects on mono-methyl alkanes in *M. rubra* and *M. ruginodis*. All plots show back-transformed means ± SE. Different letters indicate statistically significant differences according to pairwise Tukey-tests on the LME data ($p < 0.05$; A-D). Significant differences are indicated by asterisks, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; LME (E-H).

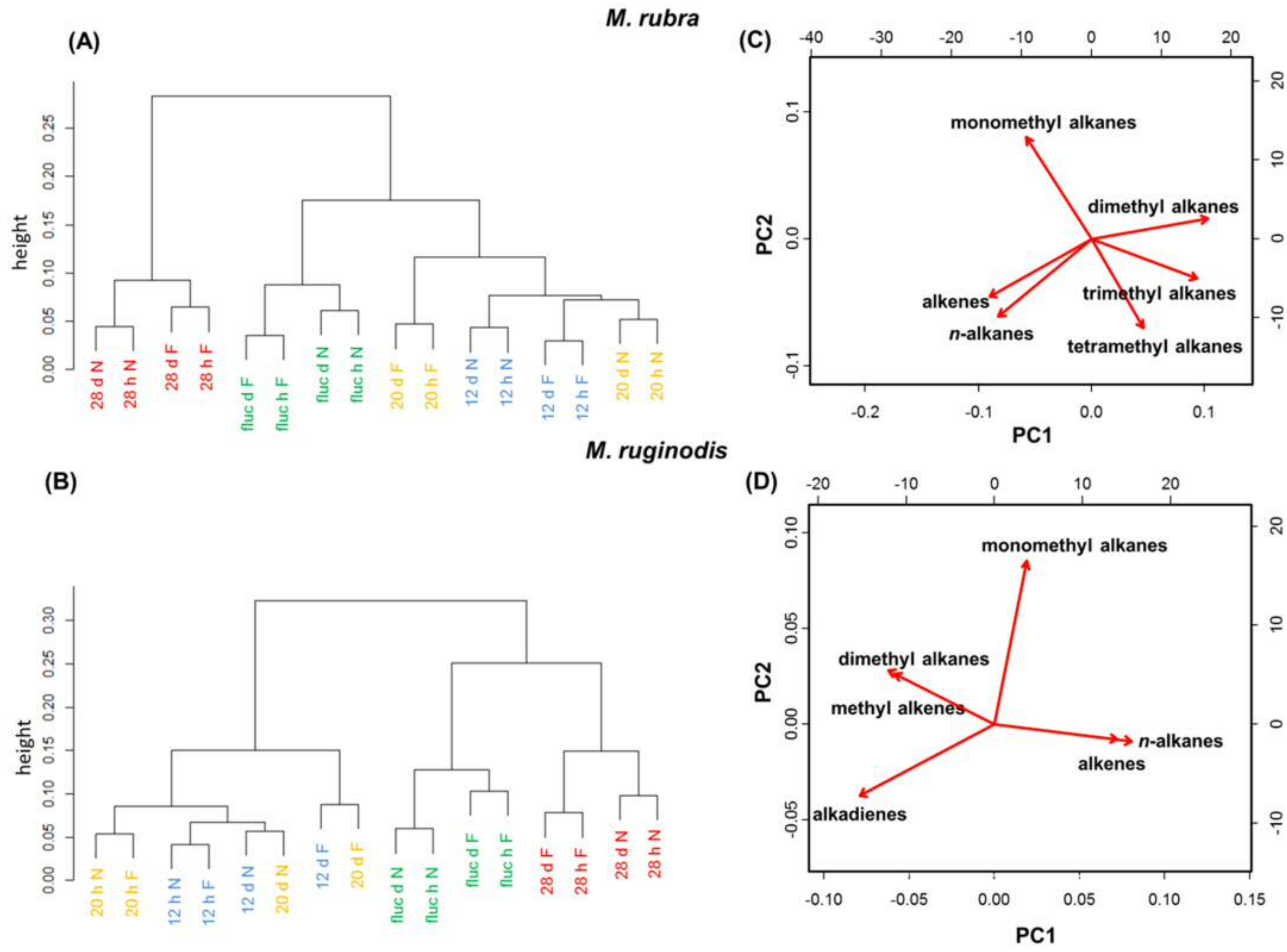

Fig. S2. Cluster dendrograms for a complete linkage hierarchical cluster analysis based on the Bray-Curtis similarity of the average chemical profiles of the different treatment groups (A+B) and co-variation of CHC classes, calculated based on factor loadings of a principal component analysis (PCs) for *M. rubra* (C) and *M. ruginodis* (D). In the cluster dendrograms (A+B) treatment groups are shown according to temperature treatment (12°C, 20°C, 28°C and fluctuating; colour-coded), humidity (d – dry and h – humid) and behavioural caste (F – forager and N – nurse). The co-variation plots (C+D) show the correlation coefficients of the different substance classes with the PC axes.

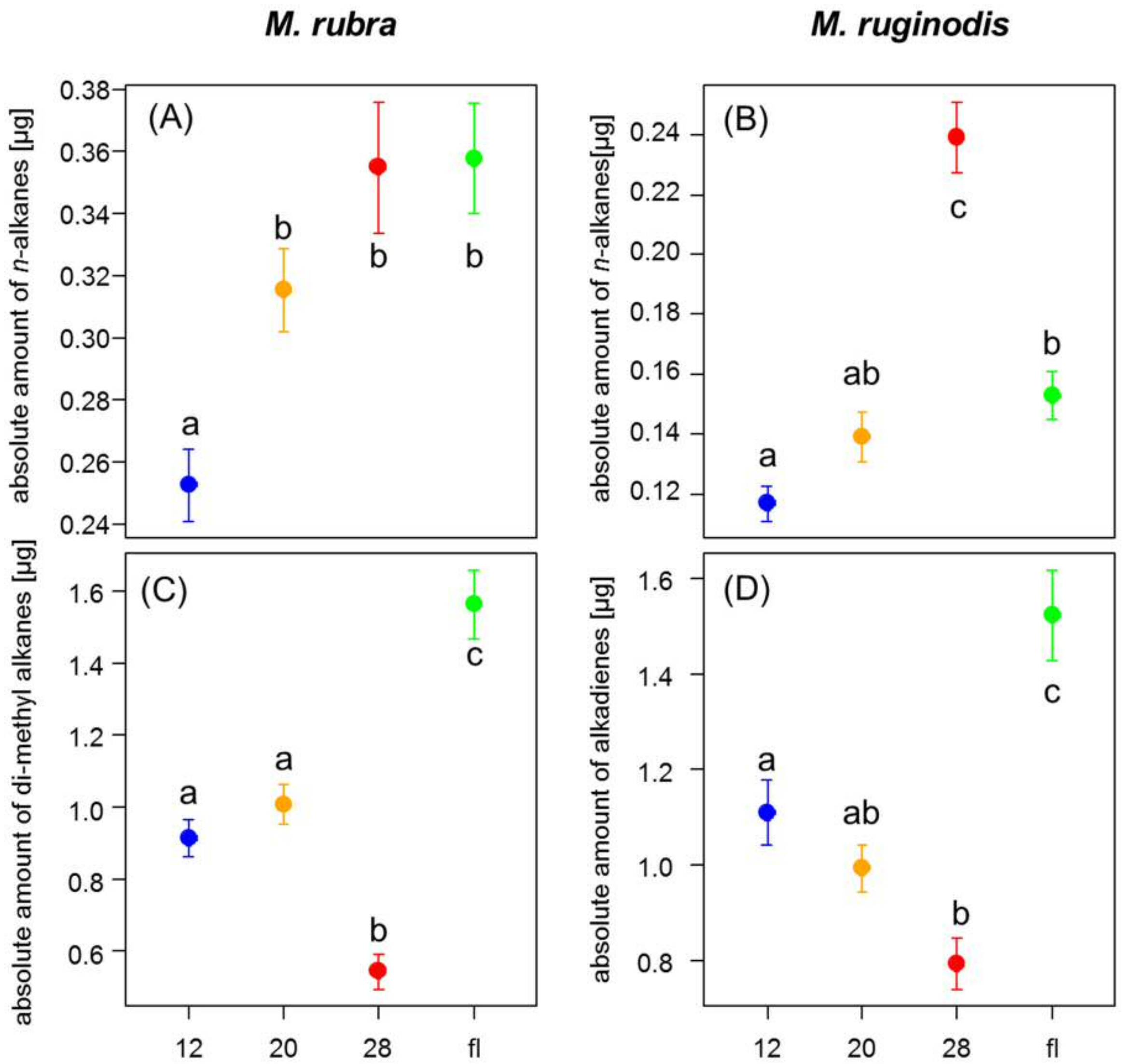

Fig S3. Absolute quantities of *n*-alkanes (A, B), di-methyl alkanes (C) and alkadienes (D) per temperature regime. All plots show means ± SE. Different letters indicate statistically significant differences according to pairwise Tukey-tests on the LME data ($p < 0.05$).

(A) In *M. rubra* the amount of *n*-alkanes was lowest in 12°C treated ants (LME: $\chi^2_3 = 38.57$, $p < 0.001$). Further, it was higher in workers from the dry treatment ($\chi^2_1 = 114.77$, $p < 0.001$) as well as foragers compared to nurses ($\chi^2_1 = 8.44$, $p = 0.004$).

(B) In *M. ruginodis* effects of temperature on the absolute amount of *n*-alkanes were significant ($\chi^2_3 = 153.36$, $p < 0.001$), as were effects of humidity ($\chi^2_1 = 103.53$, $p < 0.001$). In the dry treatments, 20°C ants had more *n*-alkanes than those at 12°C, but not so in the humid treatment (temperature x humidity: $\chi^2_3 = 16.02$, $p = 0.001$).

(C) Di-methyl alkanes in *M. rubra* were most abundant in the fluctuating temperature treatment and least common in 28°C ($\chi^2_3 = 187.78$, $p < 0.001$). They were also less abundant in the humid treatment (presumably because of the higher overall amount of CHC under dry conditions, $\chi^2_1 = 25.95$, $p < 0.001$). Also nurses possessed more di-methyl alkanes than foragers ($\chi^2_1 = 21.56$, $p < 0.001$).

(D) The absolute amount of alkadienes in *M. ruginodis* was dependent on the acclimation temperature ($\chi^2_3 = 70.89$, $p < 0.001$) as they were most abundant under fluctuating temperatures and least common under 28°C, but however not on humidity ($\chi^2_1 = 0.62$, $p = 0.43$). Finally, nurses had more alkadienes than foragers ($\chi^2_1 = 10.07$, $p = 0.002$).

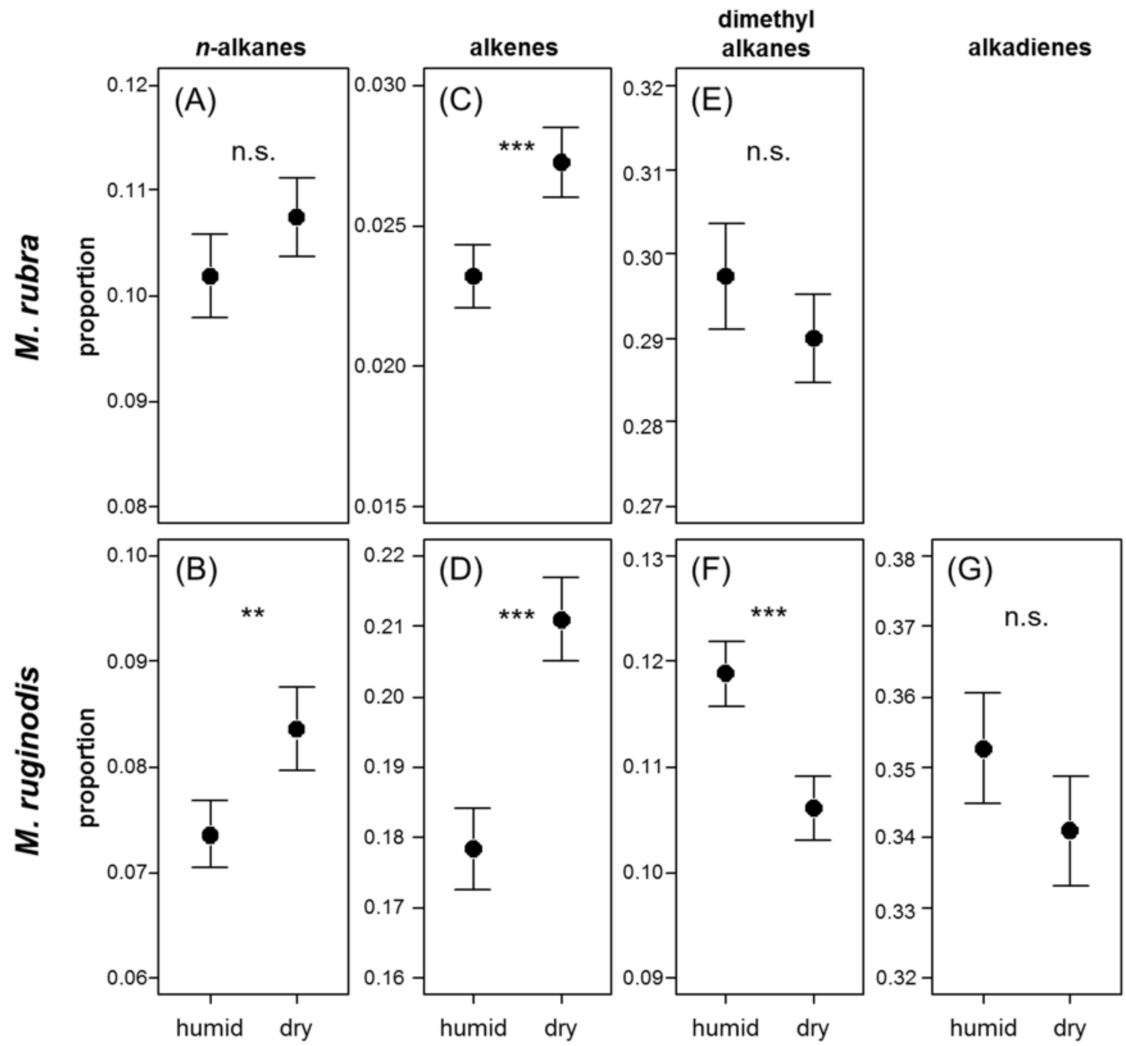

Fig. S4. Effects of humidity on the ants' CHC profiles. The upper row shows *M. rubra*, the bottom row *M. ruginodis*. Plotted are effects of the two different humidity treatments on the proportions of *n*-alkanes (A,B), di-methyl alkanes (C,D), alkenes (E,F) and alkadienes (G). All plots show back-transformed means ± SE. Significant differences are indicated by asterisks, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; LME.

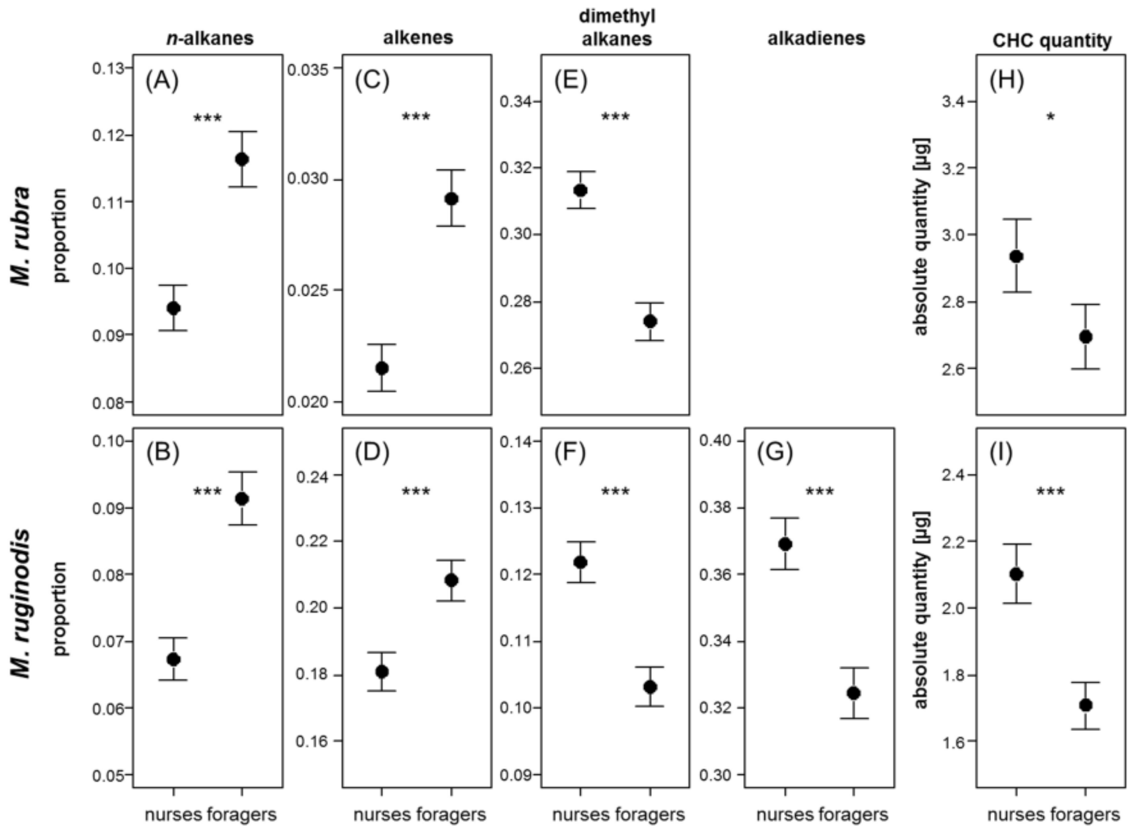

Fig. S5. Differences between nurses and foragers. The top row shows differences in *M. rubra*, the bottom row in *M. ruginodis*. Plotted are differences between nurses and foragers in the proportions of *n*-alkanes (A,B), di-methyl alkanes (C,D), alkenes (E,F), alkadienes (G) and the absolute amount of CHC (H,I). All plots show back-transformed means ± SE. Significant differences are indicated by asterisks, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; LME.