

Supplementary Information

Diesel exhaust rapidly degrades floral odours used by honeybees

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Poppy¹

Supplementary Tables

Supplementary Table 1 | Percentage constituents of the synthetic floral blend and their vapour pressures; ratios are based upon the natural emission rates identified from oilseed rape flowers by Blight et al.²⁰

Floral chemical	%	Vapour pressure (mm Hg ⁻¹ at 25 °C)	Purity (%)	Supplier
α -pinene	8.83	4.7500	98	Sigma Aldrich
3-carene	0.83	3.7200	≥ 98	Sigma Aldrich
α -terpinene	0.83	1.6380	≥ 95	Sigma Aldrich
<i>p</i> -cymene	2.92	1.4600	99	Sigma Aldrich
linalool	9.58	0.0905	97	Sigma Aldrich
phenylacetaldehyde	0.83	0.3680	≥ 90	Sigma Aldrich
(<i>E,E</i>)- α -farnesene	72.5	0.0067	n.a.	Contech
2-phenylethanol	4.17	0.0740	n.a.	Sigma Aldrich

Sigma Aldrich (St. Louis, MO, USA); Contech (Victoria, BC, Canada)

Supplementary Table 2 | Mean abundances (x 1000; ± s.e.m) from gas-chromatography mass-spectrometry analyses of the constituents of the synthetic floral blend, exposed to ambient ‘clean’ air or diesel exhaust polluted air at four different time points post exposure ($n = 5$).

Floral chemical	Time (min)	Ambient Air				Diesel Exhaust			
		1	30	60	120	1	30	60	120
α -pinene		948 ± 36	539 ± 39	449 ± 29	411 ± 28	845 ± 38	558 ± 25	472 ± 20	436 ± 16
3-carene		554 ± 13	494 ± 22	406 ± 19	363 ± 22	492 ± 11	511 ± 23	431 ± 19	386 ± 13
α -terpinene		305 ± 24	322 ± 35	259 ± 29	223 ± 25	-	-	-	-
<i>p</i> -cymene		1046 ± 41	1327 ± 38	1128 ± 38	1014 ± 43	1085 ± 38	1461 ± 74	1283 ± 55	1136 ± 43
linalool		380 ± 21	2369 ± 62	2782 ± 24	2768 ± 40	308 ± 36	1851 ± 45	2272 ± 88	2288 ± 145
phenylacetaldehyde		39 ± 11	178 ± 36	231 ± 43	253 ± 37	23 ± 7	125 ± 18	143 ± 20	133 ± 18
α -farnesene		36 ± 5	421 ± 27	725 ± 39	1081 ± 82	-	-	-	-
2-phenylethanol		165 ± 9	1201 ± 56	1573 ± 57	1585 ± 83	113 ± 14	633 ± 14	824 ± 31	909 ± 43

Supplementary Table 3 | Statistical comparisons of mean abundances of the constituents of the synthetic floral blend, exposed to ambient ‘clean’ air or diesel exhaust polluted air at four different time points post exposure. Data that were normally distributed for each chemical at each time point were compared by *t*-tests, those that were not normally distributed were compared by Mann-Whitney U tests.

Floral chemical	Time (min)	1		30		60		120	
		Test statistic	P	Test statistic	P	Test statistic	P	Test statistic	P
α -pinene		$t = 1.967$	0.085	$t = 0.398$	0.701	$t = 0.668$	0.523	$t = 0.762$	0.468
3-carene		$Z = 2.611$	0.008	$t = 0.551$	0.597	$Z = 0.940$	0.421	$Z = 1.149$	0.310
α -terpinene		-	-	-	-	-	-	-	-
<i>p</i> -cymene		$Z = 0.522$	0.690	$Z = 1.567$	0.151	$t = 2.302$	0.050	$t = 2.014$	0.079
linalool		$t = 1.722$	0.123	$t = 6.756$	<0.001	$t = 5.607$	0.001	$Z = 1.776$	0.095
phenylacetaldehyde		$t = 1.278$	0.237	$t = 1.332$	0.220	$t = 1.853$	0.101	$t = 2.902$	0.020
α -farnesene		-	-	-	-	-	-	-	-
2-phenylethanol		$t = 3.207$	0.012	$t = 9.782$	<0.001	$t = 11.568$	<0.001	$Z = 2.611$	0.008

Supplementary Table 4 | Statistical comparisons of mean abundances of the constituents of the synthetic floral blend, exposed to ambient ‘clean’ air or a **10:1** ratio of NO:NO₂ with NO at either 10, 1 or 0.1 ppm. Data that were normally distributed for each chemical at each time point were compared by *t*-tests, those that were not normally distributed were compared by Mann-Whitney U tests.

Floral chemical	NO concentration	10 ppm		1 ppm		0.1 ppm	
		Test statistic	P	Test statistic	P	Test statistic	P
<i>α</i> -pinene		<i>t</i> = 5.746	0.001	<i>t</i> = 5.952	0.001	<i>t</i> = 4.600	0.004
3-carene		<i>Z</i> = 2.309	0.029	<i>Z</i> = 2.309	0.029	<i>Z</i> = 2.309	0.029
<i>α</i> -terpinene		<i>t</i> = 25.394	<0.001	<i>t</i> = 5.849	0.001	<i>t</i> = 8.476	<0.001
<i>p</i> -cymene		<i>t</i> = 5.383	0.002	<i>t</i> = 4.548	0.004	<i>t</i> = 6.418	0.001
linalool		<i>t</i> = 5.007	0.005*	<i>Z</i> = 1.155	0.343	<i>Z</i> = 0.577	0.686
phenylacetaldehyde		<i>Z</i> = 2.309	0.029	<i>t</i> = 10.469	<0.001	<i>t</i> = 19.940	<0.001
<i>α</i> -farnesene		<i>t</i> = 6.875	<0.001	<i>t</i> = 2.486	0.047	<i>t</i> = 0.717	0.500
2-phenylethanol		<i>t</i> = 7.606	<0.001	<i>t</i> = 2.149	0.075	<i>t</i> = 2.254	0.065

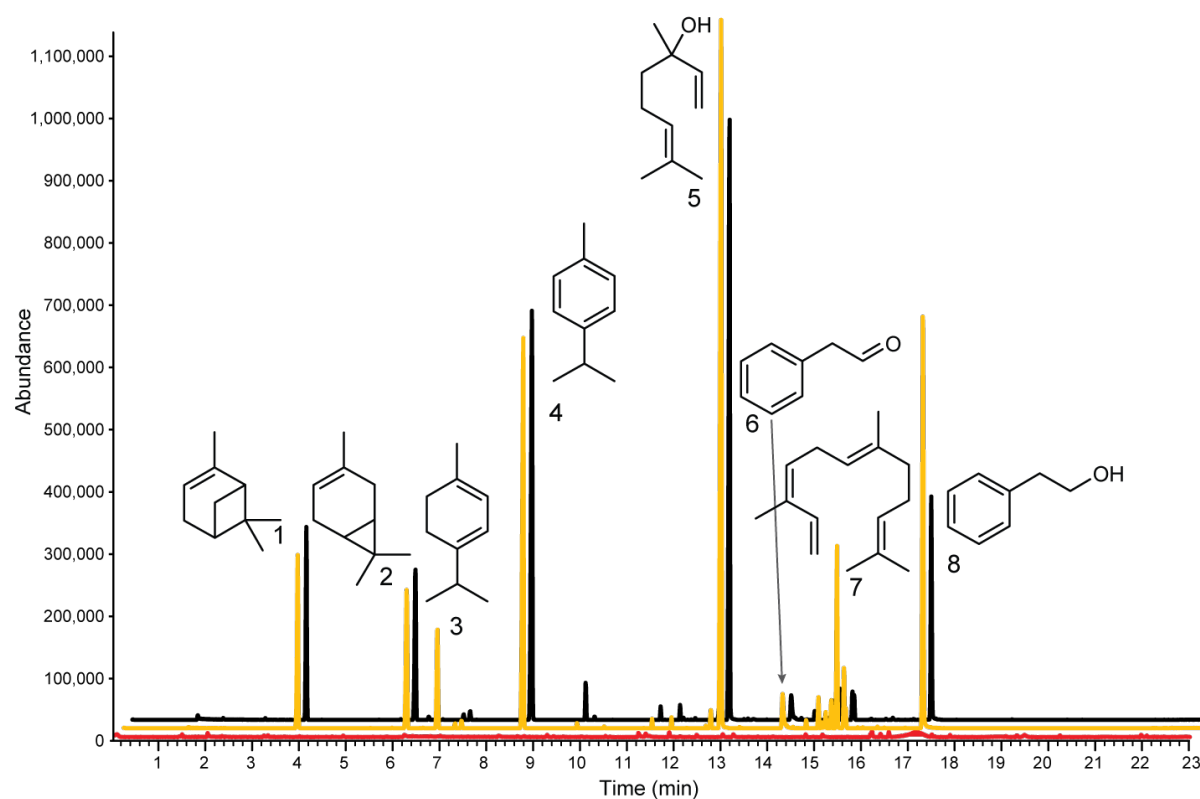
*equality of variances not assumed

Supplementary Table 5 | Statistical comparisons of mean abundances of the constituents of the synthetic floral blend, exposed to ambient ‘clean’ air or a **1:1** ratio of NO:NO₂ with NO at either 10, 1 or 0.1 ppm. Data that were normally distributed for each chemical at each time point were compared by *t*-tests, those that were not normally distributed were compared by Mann-Whitney U tests.

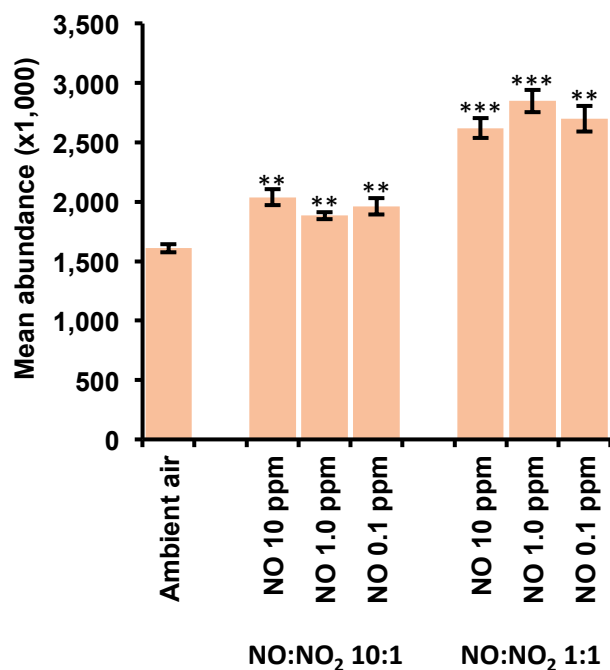
Floral chemical	NO concentration	10 ppm		1 ppm		0.1 ppm	
		Test statistic	P	Test statistic	P	Test statistic	P
<i>α</i> -pinene		<i>t</i> = 11.160	<0.001	<i>t</i> = 12.324	<0.001*	<i>t</i> = 9.694	0.001*
3-carene		<i>Z</i> = 2.309	0.029	<i>Z</i> = 2.309	0.029	<i>Z</i> = 2.309	0.029
<i>α</i> -terpinene		-	-	<i>t</i> = 8.133	0.003*	<i>t</i> = 16.076	<0.001
<i>p</i> -cymene		<i>t</i> = 19.321	<0.001	<i>t</i> = 13.555	0.001*	<i>t</i> = 5.616	0.011*
linalool		<i>t</i> = 0.133	0.898	<i>t</i> = 2.963	0.025	<i>t</i> = 0.531	0.627*
phenylacetaldehyde		<i>t</i> = 23.326	<0.001	<i>t</i> = 23.235	<0.001	<i>Z</i> = 2.309	0.029
<i>α</i> -farnesene		<i>t</i> = 8.918	<0.001	<i>Z</i> = 2.309	0.029	<i>t</i> = 3.894	0.008
2-phenylethanol		<i>t</i> = 4.368	0.005	<i>t</i> = 2.865	0.029	<i>t</i> = 5.114	0.002

*equality of variances not assumed

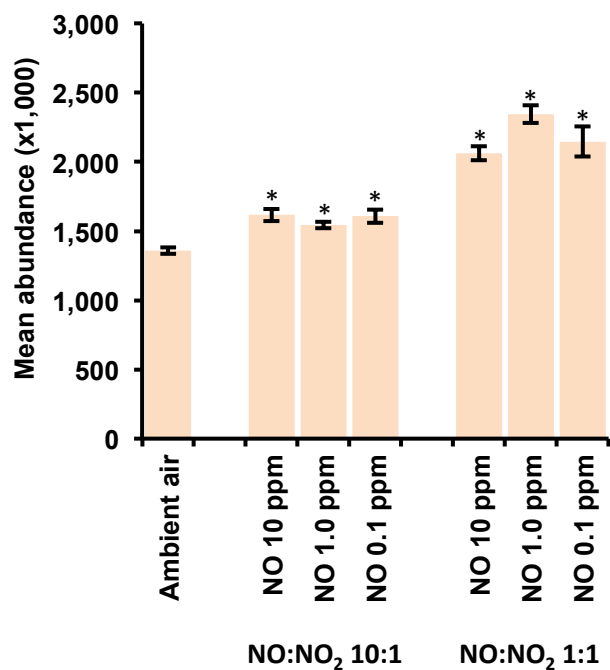
Supplementary data



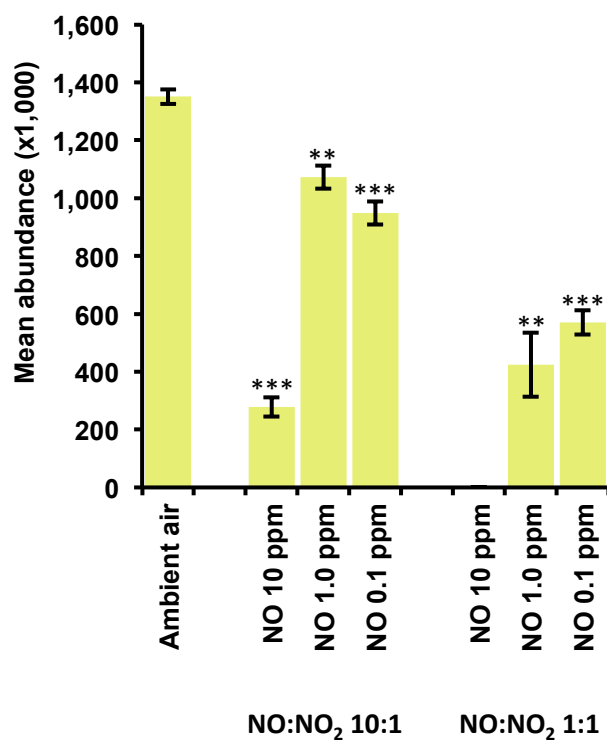
Supplementary Figure 1 | Total ion current chromatogram. Diesel exhaust polluted air (red line) in the mass ratio scanned showed no components likely to cause interference with or be mistaken for the floral chemicals used in the subsequent experiments. The constituents of the synthetic floral blend, exposed to diesel exhaust polluted air (black line) or ambient 'clean' air (yellow line): 1) α -pinene; 2) 3-carene; 3) α -terpinene; 4) *p*-cymene; 5) linalool; 6) phenylacetaldehyde; 7) α -farnesene; 8) 2-phenylethanol. Recordings were made after an exposure time of 30 min.



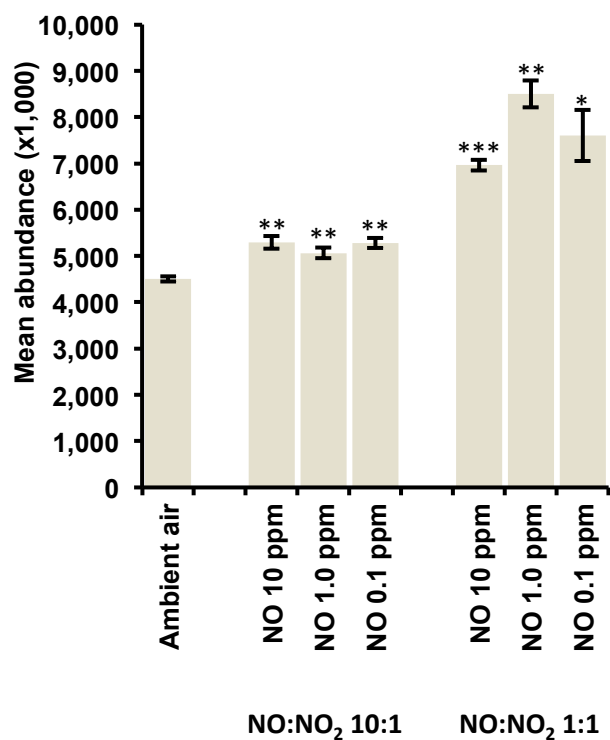
Supplementary Figure 2 | Mean abundance of α -pinene (x 1000; \pm s.e.m) from gas-chromatography mass-spectrometry analyses, when exposed to ambient ‘clean’ air or a variety of concentrations and ratios of NO and NO₂ ($n = 4$). Asterisks denote significance of difference in comparisons with ambient air (P < 0.01, *** P < 0.001).**



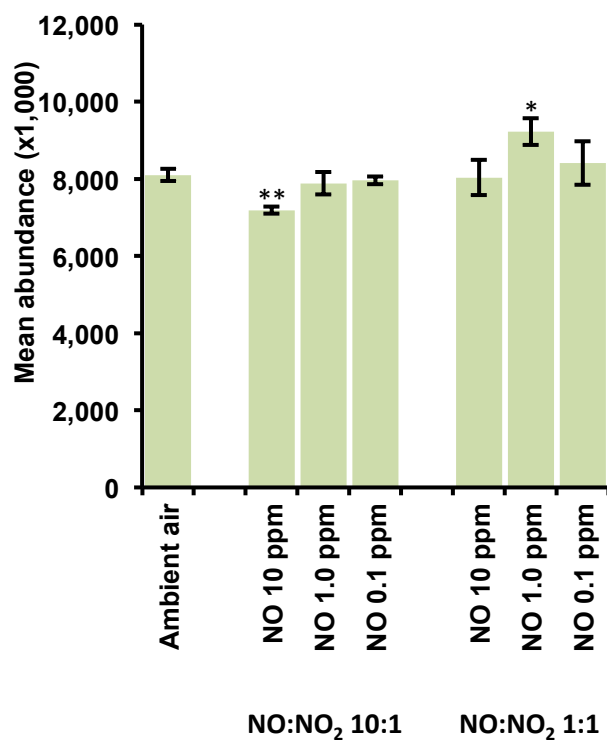
Supplementary Figure 3 | Mean abundance of 3-carene (x 1000; \pm s.e.m) from gas-chromatography mass-spectrometry analyses, when exposed to ambient ‘clean’ air or a variety of concentrations and ratios of NO and NO₂ ($n = 4$). Asterisks denote significance of difference in comparisons with ambient air (* $P < 0.05$).



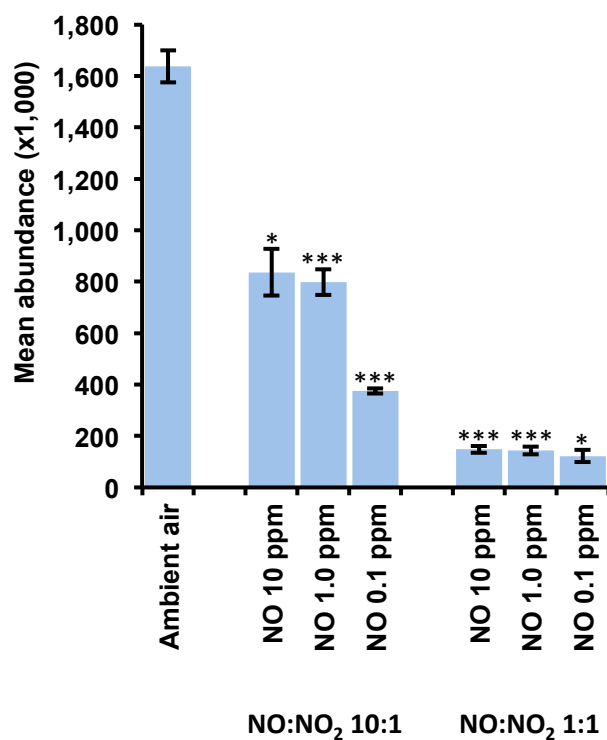
Supplementary Figure 4 | Mean abundance of α -terpinene (x 1000; \pm s.e.m) from gas-chromatography mass-spectrometry analyses, when exposed to ambient ‘clean’ air or a variety of concentrations and ratios of NO and NO₂ ($n = 4$). Asterisks denote significance of difference in comparisons with ambient air ($P < 0.01$, *** $P < 0.001$).**



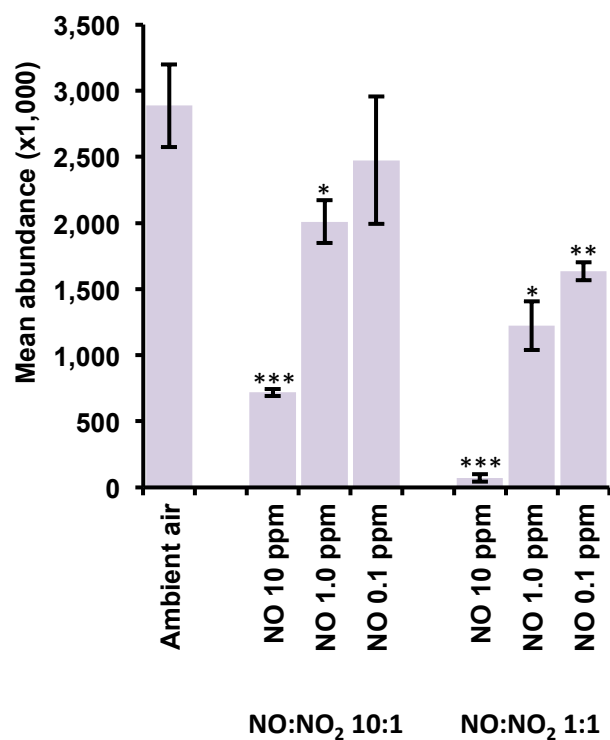
Supplementary Figure 5 | Mean abundance of *p*-cymene (x 1000; \pm s.e.m) from gas-chromatography mass-spectrometry analyses, when exposed to ambient ‘clean’ air or a variety of concentrations and ratios of NO and NO₂ ($n = 4$). Asterisks denote significance of difference in comparisons with ambient air (* $P < 0.05$, ** $P < 0.01$, * $P < 0.001$).**



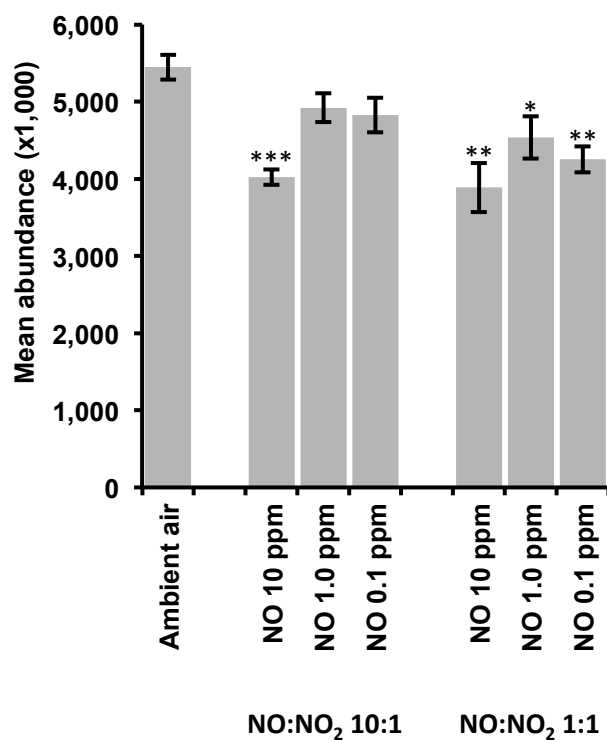
Supplementary Figure 6 | Mean abundance of linalool (x 1000; ± s.e.m) from gas-chromatography mass-spectrometry analyses, when exposed to ambient ‘clean’ air or a variety of concentrations and ratios of NO and NO₂ (*n* = 4). Asterisks denote significance of difference in comparisons with ambient air (* *P* < 0.05, ** *P* < 0.01).



Supplementary Figure 7 | Mean abundance of phenylacetaldehyde (x 1000; \pm s.e.m) from gas-chromatography mass-spectrometry analyses, when exposed to ambient ‘clean’ air or a variety of concentrations and ratios of NO and NO₂ ($n = 4$). Asterisks denote significance of difference in comparisons with ambient air (* $P < 0.05$, *** $P < 0.001$).



Supplementary Figure 8 | Mean abundance of α -farnesene (x 1000; \pm s.e.m) from gas-chromatography mass-spectrometry analyses, when exposed to ambient ‘clean’ air or a variety of concentrations and ratios of NO and NO₂ ($n = 4$). Asterisks denote significance of difference in comparisons with ambient air (* $P < 0.05$, ** $P < 0.01$, * $P < 0.001$).**



Supplementary Figure 9 | Mean abundance of 2-phenylethanol (x 1000; ± s.e.m) from gas-chromatography mass-spectrometry analyses, when exposed to ambient ‘clean’ air or a variety of concentrations and ratios of NO and NO₂ (*n* = 4). Asterisks denote significance of difference in comparisons with ambient air (* *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001).