

## Anthropogenic air pollutants reduce insect-mediated pollination services

Ryalls, James M . W. ; Langford, Ben; Mullinger, Neil; Bromfield, Lisa M.; Nemitz, Eiko; Pfrang, Christian; Girling, Robbie D.

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1 Anthropogenic air pollutants reduce insect-mediated pollination  
2 services

3

4 James M.W. Ryalls<sup>a\*</sup>, Ben Langford<sup>b</sup>, Neil J. Mullinger<sup>b</sup>, Lisa M. Bromfield<sup>a</sup>, Eiko Nemitz<sup>b</sup>,  
5 Christian Pfrang<sup>c,d</sup> & Robbie D. Girling<sup>a</sup>

6 <sup>a</sup>School of Agriculture, Policy and Development, University of Reading, Whiteknights, Earley  
7 Gate, Reading, RG6 6EU, UK

8 <sup>b</sup>UK Centre for Ecology & Hydrology, Penicuik, Midlothian, EH26 0QB, UK

9 <sup>c</sup>School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston,  
10 Birmingham, B15 2TT, UK

11 <sup>d</sup>Department of Meteorology, University of Reading, Whiteknights, Earley Gate, Reading, RG6  
12 6BB, UK

13

14 \*Correspondence: [J.Ryalls@reading.ac.uk](mailto:J.Ryalls@reading.ac.uk)

15

16

17 **Abstract**

18 Common air pollutants, such as nitrogen oxides (NO<sub>x</sub>), emitted in diesel exhaust, and ozone (O<sub>3</sub>),  
19 have been implicated in the decline of pollinating insects. Reductionist laboratory assays, focused  
20 upon interactions between a narrow range of flowering plant and pollinator species, in  
21 combination with atmospheric chemistry models, indicate that such pollutants can chemically alter  
22 floral odors, disrupting the cues that foraging insects use to find and pollinate flowers. However,  
23 odor environments in nature are highly complex and pollination services are commonly provided  
24 by suites of insect species, each exhibiting different sensitivities to different floral odors.  
25 Therefore, the potential impacts of pollution-induced foraging disruption on both insect ecology,  
26 and the pollination services that insects provide, are currently unknown. We conducted *in-situ*  
27 field studies to investigate whether such pollutants could reduce pollinator foraging and as a  
28 result the pollination ecosystem service that those insects provide. Using free-air fumigation, we  
29 show that elevating diesel exhaust and O<sub>3</sub>, individually and in combination, to levels lower than is  
30 considered safe under current air quality standards, significantly reduced counts of locally-  
31 occurring wild and managed insect pollinators by 62 to 70% and their flower visits by 83 to 90%.  
32 These reductions were driven by changes in specific pollinator groups, including bees, flies,  
33 moths and butterflies, and coincided with significant reductions (14-31%) in three different metrics  
34 of pollination and yield of a self-fertile test plant. Quantifying such effects provides new insights  
35 into the impacts of human-induced air pollution on the natural ecosystem services upon which we  
36 depend.

37  
38 **Keywords:** Air pollution, diesel exhaust, tropospheric ozone, floral odor cues, insect pollinators,  
39 ecosystem services.

40

## 41 **1. Introduction**

42 Insect pollination facilitates approximately 7 to 8% of the total value of agricultural food production  
43 worldwide (Potts et al., 2016a) and 70% of all crop species rely upon insect pollination (Klein et  
44 al., 2007). However, there have been sustained declines in insect pollinator abundance and  
45 diversity over the past century, resulting from a combination of environmental pressures (Potts et  
46 al., 2016b; Powney et al., 2019; Vanbergen and the Insect Pollinators Initiative, 2013). Recent  
47 research suggests that common tropospheric pollutants, including nitrogen oxides (otherwise  
48 known as NO<sub>x</sub>, comprised of nitric oxide (NO) and nitrogen dioxide (NO<sub>2</sub>)) from diesel exhaust,  
49 and ozone (O<sub>3</sub>), may contribute to pollinator declines through: i) direct effects on insect pollinator  
50 health (Reitmayer et al., 2019) and; ii) potential reductions in pollinator foraging efficiency (Farré-  
51 Armengol et al., 2016; Fuentes et al., 2016; Girling et al., 2013; McFrederick et al., 2008).

52  
53 In foraging for a flower, odor plumes are an important stimulus for many pollinating insect  
54 species. Each flower species' plume is comprised of a unique combination of chemicals, known  
55 as volatile organic compounds (VOCs), and an insect's success in locating a flower can depend  
56 on the presence, concentration and/or ratio of these VOCs within a plume (Riffell et al., 2014;  
57 Wright and Schiestl, 2009). These plume characteristics will be altered by atmospheric pollutants  
58 (NO<sub>x</sub> and O<sub>3</sub> in particular), or by the products of these pollutants' reactions within the troposphere  
59 (especially hydroxyl radicals; McFrederick et al., 2008), either through direct reaction with the  
60 plume (Jamieson et al., 2017) or masking of its components (Riffell et al., 2014). Such changes  
61 could have consequences for the foraging efficiency of insects that use these cues. Validation of  
62 this prediction is currently based upon laboratory behavioral studies (e.g. Farré-Armengol et al.,  
63 2016; Girling et al., 2013; Lusebrink et al., 2015), and atmospheric chemistry modelling (e.g.  
64 Fuentes et al., 2016; McFrederick et al., 2008), which are focused on a narrow range of pollinator  
65 species and on individual pollutants in isolation. Therefore, the ecological impacts remain unclear  
66 (Jamieson et al., 2017).

67  
68 We investigated whether exposure to diesel exhaust (particularly NO<sub>x</sub>) and elevated O<sub>3</sub>,  
69 individually and in combination, affected the ability of naturally occurring pollinating insects to: i)  
70 locate floral resources, and ii) provide pollination services. NO<sub>x</sub> and O<sub>3</sub> commonly occur together  
71 in the troposphere, therefore sequential or simultaneous exposure of air pollutants, rather than  
72 exposure to individual pollutants in isolation, is considered an essential requirement when  
73 investigating how such pollutants modify the interactions between plants and the biotic  
74 communities they reside with (Li et al., 2016; Papazian and Blande, 2020). Globally, many of the  
75 newly approved diesel vehicles sold continue to exceed emission limits, ensuring diesel exhaust  
76 emissions will remain a problem for many decades (the average lifespan of a passenger vehicle  
77 varies between countries (9-23 years; Oguchi and Fuse, 2015)), with diesel vehicles lasting up to  
78 30 years) (ACEA, 2019; Brand, 2016). Increasing urbanization and traffic congestion is likely to  
79 result in higher NO<sub>x</sub> in peri-urban and rural areas, increasing the potential exposure of  
80 neighboring agricultural land and intensifying pollen limitation (Bennett et al., 2020). Moreover,  
81 global tropospheric background O<sub>3</sub> concentrations are rising and regular weather-induced  
82 episodic increases continue to occur (Hansen et al., 2019; Turnock et al., 2019). Depending on  
83 whether the region is VOC- or NO<sub>x</sub>-limited, reductions in NO<sub>x</sub> emission sources may also  
84 increase O<sub>3</sub> formation, and vice versa (Bae et al., 2020; Wang et al., 2019), which may influence  
85 the foraging behavior of pollinating insects (Blande, 2021). To experimentally investigate the  
86 ecological effects of simultaneous pollutants at field-scale, we designed a novel Free-Air Diesel  
87 and O<sub>3</sub> Enrichment (FADOE) facility, which allowed emission of regulated quantities of NO<sub>x</sub>  
88 (emitted in diesel exhaust) and O<sub>3</sub>.

## 91 **2. Materials and methods**

### 92 *2.1. Free-Air Diesel and Ozone Enrichment (FADOE)*

93 The FADOE facility consisted of eight 8m-diameter octagons; two octagons were assigned to  
94 each of four treatments: i) diesel exhaust (D), ii) O<sub>3</sub>, iii) diesel exhaust and O<sub>3</sub> combined (D+O<sub>3</sub>),  
95 and iv) control ambient air (Fig. 1A). The aim was to maintain fumigation levels of NO<sub>x</sub> and O<sub>3</sub>  
96 within the pollution treatment octagons at field-realistic doses, i.e. below 120 ppb (based on  
97 average concentrations adjacent to major UK roadways and urban areas; Ares and Smith, 2017)  
98 and 90 ppb (based on peak concentrations recorded in rural European sites in 1990-2012;  
99 Colette et al., 2016), respectively. The combined (D+O<sub>3</sub>) treatment octagons were maintained at  
100 the same maximum concentration as those set for each pollutant octagon individually. The  
101 FADOE system configuration is visualised in Fig. S1. The centre of each FADOE octagon was  
102 positioned 50 m from the centre of a field (decimal degrees: 51.482853° N 0.897749° W in 2018  
103 and 51.482374° N 0.895855° W in 2019) in an octagonal formation, such that each octagon was  
104 separated by a distance of at least 30 m. A diesel generator (Hyundai, DHY8000SELR 7.2 kVA,  
105 Genpower Ltd, UK) and two ozone generators (CD1500P, ClearWater Tech, USA) positioned in  
106 the centre of the field were used to deliver elevated levels of diesel exhaust and ozone to the  
107 octagons via 50 mm (ID) heavy duty conduit connected to vacuum-blower pumps (R4110-2,  
108 Gast, USA). Octagons of the same treatment were positioned opposite each other within the field  
109 to minimise spatial effects. One-hundred and twenty 5 mm-diameter holes were drilled (20 cm  
110 apart) in the pipes surrounding each octagon, which provided a diffuse plume of pollutant (or  
111 ambient air in the case of the Control treatment octagons) directed towards the centre of the  
112 octagon. The concentrations of NO, NO<sub>2</sub>, NO<sub>x</sub> (i.e. NO+NO<sub>2</sub>) and ozone (O<sub>3</sub>) at the centre of  
113 each octagon were monitored sequentially (every 120 s) via an automated switching system  
114 coupled to O<sub>3</sub> (Model 49i, Thermo Scientific, USA) and NO<sub>x</sub> (Model 42C, Thermo Scientific, USA)  
115 analysers. Three-way mixing valves (VRG131 connected to ARA600 proportional actuators,  
116 ESBE, Sweden) and UV-light controllers (CD1500P 4-20mA control board) altered the quantities  
117 of diesel exhaust and O<sub>3</sub> released into the octagons. In-line filter units (G057502, Donaldson,  
118 Czech Republic) with a RS3954 filter (Baldwin, USA) were used to remove soot deposits from the  
119 pipes before reaching the pumps and excess diesel exhaust was directed through conduit pipes  
120 away from the field site (100 m north-east or downwind; Fig. S2). The diesel exhaust was passed  
121 through metal conduit pipe directly from the generator to dissipate heat before entering a mixing  
122 barrel where collected water vapour was dispensed from a tap (three times weekly). Generators  
123 were turned on for up to 17 hours each day (between 4.30 am and 9.30 pm) to ensure that  
124 pollution treatments were applied during times of peak daily pollinator activity for all insect  
125 pollinators recorded. Wind speed and wind direction were recorded continuously from four A100R  
126 anemometers and W200P potentiometer windvanes (Vector Instruments, UK), positioned north,  
127 east, south and west of the field to ensure consistency in prevailing wind direction throughout  
128 data collection (south-westerly; Fig. S2). The FADOE octagons were positioned within a field of  
129 winter wheat (*Triticum aestivum* cv. Skyfall, sown in November 2017 at a seeding rate of 300  
130 seeds m<sup>-2</sup> and a row spacing of 166 mm) at the University of Reading's Sonning farm, UK. The  
131 wheat acted as a non-insect pollinated (i.e. non-flowering) buffer, limited plant diversity and  
132 maximised weed control. Winter wheat was re-sown in November 2018 in an adjacent field,  
133 where the FADOE facility was reassembled for a second year. Octagons were fumigated during  
134 two summer seasons (May-September 2018 and 2019).

135

## 136 2.2. Plant material

137 In May each year (2018 and 2019), 192 black mustard plants (*Brassica nigra* cv. *Abyssinica*)  
138 were grown from seed (Heirloom & Perennial Ltd., Cornwall, UK) in netted 100 mL seed wells in  
139 an open glasshouse (24.5 °C ± 2.4 SD). This self-fertile variety, with a two- to three-month  
140 flowering period, was used as a model flowering plant based on mechanistic evidence from  
141 laboratory studies that O<sub>3</sub> degrades its floral signal and changes its attraction to bumblebees  
142 (Farré-Armengol et al., 2016; Saunier and Blande, 2019). Four-week-old plants were transferred  
143 to 18 cm diameter pots containing 2.7 kg of vegetable topsoil (Quality Garden Supplies Ltd.,  
144 Staffordshire, UK) and placed in a polytunnel covered with insect mesh for one week until the  
145 seedlings had established. At five weeks old (ca. one week before flowering), 24 plants were

146 distributed evenly in each octagon and dug into the ground within the wheat crop, such that the lip  
147 of each pot was flush with the surface of the soil. These plants remained in the octagons until  
148 they had finished flowering (2-3 months) and were fumigated for the entire duration.  
149

### 150 2.3. *Insect visitation assessments*

151 Insect visitation to flowers of *B. nigra* was recorded on days conducive to insect activity (dry, >15  
152 °C, between 10:00 and 17:00). The number of insect visits to a focal patch (observation unit) of  
153 six adjacent plants (containing an average of 73 flowers) within each FADOE octagon were  
154 recorded for up to 10 minutes. The selection of six plants per observation unit enabled a clear  
155 view of the flowers upon which visits were counted (Reitan and Nielsen, 2016). The Order and  
156 group of visitor (Hymenoptera (honey bees, bumble bees, solitary bees and parasitic wasps),  
157 Lepidoptera (moths and butterflies), Diptera (hoverflies and other flies), Coleoptera (beetles) and  
158 Hemiptera (true bugs)) were recorded, along with the number of flowers that were visited by  
159 individual insects (bees, moths, butterflies and hoverflies only) and the total number of flowers  
160 within the observation unit. A visit was classified as a landing, or an attempt to feed on or collect  
161 pollen and/or nectar (Nuttman and Willmer, 2003). Observers (two field researchers trained in  
162 pollinator observation) stood a minimum of 1 m away from the observation unit and remained still  
163 during the observation period. For each day of sampling, observers were assigned, at random, to  
164 an observation unit within each octagon and the eight octagons were observed in a randomised  
165 order. Insect abundances (i.e. the number of individuals recorded on flowers for all insect  
166 pollinator groups) and flower visits (i.e. the number of flowers visited by each individual bee,  
167 hoverfly, moth and butterfly) were scaled according to the number of flowers in the observation  
168 unit and survey duration to give insect counts flower<sup>-1</sup> h<sup>-1</sup> and flower visits flower<sup>-1</sup> h<sup>-1</sup>,  
169 respectively.  
170

### 171 2.4. *Accounting for spatial changes and direct impacts on pollinator foraging*

#### 172 2.4.1. *Control experiment 1 – Accounting for spatial differences in pollinator foraging within the* 173 *field*

174 For one week (12-19 July 2019), plants and treatments were rotated between the different  
175 FADOE octagons so that control octagons became diesel exhaust-polluted octagons and ozone  
176 octagons became combined-treatment octagons (and vice versa). This enabled us to quantify the  
177 level of spatial variation associated with changes in pollinator foraging behaviour among  
178 treatments.  
179

#### 180 2.4.2. *Control experiment 2 – Determining the direct effects of air pollution on pollinator flight* 181 *activity when floral cues were absent*

182 Triple pan traps (i.e. brightly coloured visual stimuli that superficially resemble flowers), containing  
183 20% propylene glycol, were placed in the FADOE octagons for 72 hours at the beginning and end  
184 of each field season (when no *Brassica nigra* plants were present) to record background  
185 pollinator numbers and determine whether air pollutants had a direct effect on pollinators entering  
186 the octagons. Potential insect pollinators within pan traps were identified to Genus or the most  
187 precise taxonomic resolution possible, which included seven Genera (Sphecodes, Tachina,  
188 Lasioglossum, Andrena, Apis, Halictus, Hylaeus), 14 Families (Anthomyiidae, Calliphoridae,  
189 Muscidae, Sarcophagidae, Syrphidae, Tachinidae, Vespidae, Halictidae, Acartophthalmidae,  
190 Apoidea, Bibionidae, Pieridae, Tenthredinidae, Tephritidae) and one Order (Lepidoptera).  
191

### 192 2.5. *Yield assessments*

#### 193 2.5.1. *Experimental plants*

194 After plants stopped flowering, they were removed from the FADOE octagons, and left to mature  
195 in an insect mesh-covered polytunnel before being harvested. Once mature, the number of  
196 developed and undeveloped pods were counted on the third raceme up from the main stem of  
197 each plant. Pod development was calculated using equation (1).  
198

$$199 \quad \text{Pod development (\%)} = \left( \frac{\text{Number of developed pods}}{\text{Number of developed pods} + \text{Number of undeveloped pods}} \right) \times 100 \quad (1)$$

200  
201 For each plant, ten random pods were removed from adjacent racemes, before being oven dried  
202 (at 70 °C) and weighed. Their seeds were removed, counted, and weighed. The aboveground  
203 part of the plant was cut to ground level, oven dried and weighed, before being threshed to  
204 separate seeds, which were subsequently counted and weighed. 1000-seed mass was calculated  
205 for each plant using equation (2).  
206

$$207 \quad 1000 \text{ seed mass (g)} = \left( \frac{\text{Total mass of seeds (g)}}{\text{Total number of seeds}} \right) \times 1000 \quad (2)$$

208

### 209 2.5.2. Control experiment 3 – Quantifying the direct effects of air pollution on plant yield

210 In 2019, 10 additional *B. nigra* plants were netted (using 75 cm x 100 cm organza bags to  
211 exclude pollinators) and distributed evenly within each FADOE octagon. These plants acted as  
212 yield control (YC) plants to determine whether pollution treatments had a direct impact on *B. nigra*  
213 yield. They were therefore cross-pollinated with each other by hand twice weekly to ensure  
214 maximum pollination rates for all YC plants. Yield metrics were measured in the same way as the  
215 experimental plants. By separating the direct and indirect effects of air pollution on plant yield we  
216 were able to establish whether any air pollution-mediated changes in pollinator foraging would be  
217 associated with any changes in plant yield metrics.  
218

### 219 2.6. Statistical analyses

220 All analyses were performed using the R statistical interface v4.0.2. General (LMM) and  
221 generalized linear mixed effect models (GLMM) using the R package *lme4* (Bates et al., 2014)  
222 were used to determine the effects of air pollution treatments on NO<sub>x</sub> and O<sub>3</sub> concentrations  
223 (LMM), insect pollinator visitation (total number of insects that landed on a flower within the  
224 observation unit and individual flower visits flower<sup>-1</sup> h<sup>-1</sup>; GLMM), yield metrics (number of seeds  
225 per pod, pod mass, % pods developed, 1000-seed mass and plant dry mass; LMM), abundances  
226 of individual insect groups (GLMM) and background pollinator numbers recorded from triple pan  
227 traps (GLMM). 'Octagon location' nested within 'Year' were included as random effects in mixed  
228 models to minimise issues associated with pseudo-replication and account for spatial and  
229 seasonal differences. 'Observer ID' was also included as a random effect in abundance and  
230 flower visitation models to account for any bias associated with differences in identification and  
231 recording skills between individuals (Westphal et al., 2008). Negative binomial models were used  
232 for pollinator abundance and flower visitation. Models for background pollinator numbers and  
233 individual insect groups were run using a poisson error distribution. Negative binomial GLMM  
234 were also used to determine whether octagon location within the field impacted pollinator  
235 abundance and flower visitation frequencies using data collected 6-24 July 2019 (one week either  
236 side of the octagon rotation inclusive; Control experiment 1). 'Rotation' (i.e. rotated vs unrotated  
237 octagons) and 'Treatment', and their interaction, were included as fixed effects, with 'Octagon  
238 location' as a random effect to account for repeated measures. Contrasts of fixed effects (t-  
239 statistics based on Satterthwaite's approximation) from model summaries using the R package  
240 *lmerTest* (Kuznetsova et al., 2017) were provided for NO<sub>x</sub> concentrations, O<sub>3</sub> concentrations,  
241 NO:NO<sub>2</sub> and background pollinator numbers to clarify their responses to the elevated pollution  
242 treatments relative to the control treatment.

243  
244

### 245 **3. Results and discussion**

#### 246 *3.1. Pollutant concentrations within Free-Air Diesel and Ozone Enrichment octagons*

247 Fumigation in the single pollutant octagons resulted in significant increases of O<sub>3</sub> to 35.2 ±  
248 0.6 ppb ( $P < 0.001$ ) and of NO<sub>x</sub> to 59.6 ± 1.0 ppb (NO = 38.5 ± 0.8 ppb, NO<sub>2</sub> = 21.2 ± 0.3 ppb;  $P <$   
249 0.001), relative to the control octagons (values are means (± SE) over the entire experimental  
250 period during the two summer seasons of 2018 and 2019; Fig. 1B). These levels were well below  
251 the current United States Environmental Protection Agency's National Ambient Air Quality  
252 Standards (O<sub>3</sub> = 70 ppb averaged over 8 hours, NO<sub>2</sub> = 53 ppb averaged annually, values for NO  
253 are not stipulated), which specify the maximum outdoor pollutant levels for public health and  
254 environmental safety (EPA, 2021). In the combined pollutant octagons, the same amount of each  
255 pollutant was emitted as for the single pollutant octagons, yet O<sub>3</sub> concentrations achieved were  
256 equivalent to those in the control octagons. Moreover, NO<sub>x</sub> concentrations decreased in the  
257 combined pollutant octagons compared with the diesel exhaust-only octagons, associated with a  
258 significant reduction in NO:NO<sub>2</sub> (Fig. 1B; statistical results in Table S1). NO<sub>x</sub> and O<sub>3</sub> in the  
259 troposphere commonly react with each other and, depending on local quantities of NO<sub>x</sub>, VOCs  
260 and O<sub>3</sub> catalysts (in particular reactive hydrogen species such as hydroxyl and hydroperoxyl  
261 radicals), NO<sub>x</sub> emissions can lead to both the formation and destruction of O<sub>3</sub>. O<sub>3</sub> is typically  
262 lower in urban areas or next to busy roads because it reacts with NO<sub>x</sub> from vehicle exhaust  
263 emissions (Bae et al., 2020). For example, mean hourly concentrations of NO<sub>x</sub> and O<sub>3</sub> next to the  
264 M25 motorway in Staines, UK were recorded as 84.5 and 12.5 ppb, respectively, and those  
265 recorded in an M25 motorway tunnel were 479.9 and 1.5 ppb, respectively (Sayegh et al., 2016).  
266 In the current study, it appears that the reaction between NO<sub>x</sub> and O<sub>3</sub> in the combined treatment  
267 resulted in a decrease in the ratio of NO:NO<sub>2</sub> compared with the diesel exhaust-only treatment  
268 because O<sub>3</sub> reacts with NO to produce NO<sub>2</sub> (Richmond-Bryant et al., 2017), which also explains  
269 why O<sub>3</sub> concentrations were depleted in the combined treatment relative to the O<sub>3</sub>-only treatment.  
270 This complex interplay between oxidative pollutants is likely to alter the fate of biogenic VOCs  
271 and, in turn, influence the behavior of odor-dependent insects, highlighting the importance of  
272 using realistic pollution concentrations and combinations when investigating the effects of air  
273 pollution on plant–insect communication processes (Blande, 2021).

274

#### 275 *3.2. Air pollution effects on insect pollinator abundance and flower visitation*

276 In both diesel exhaust and O<sub>3</sub> treatments, individually and in combination, we recorded dramatic  
277 reductions in pollinator numbers ( $\chi^2_{3,8} = 21.52$ ,  $P < 0.001$ ,  $N_{\text{obs}} = 352$ ) and flower visitation ( $\chi^2_{3,8} =$   
278 35.74,  $P < 0.001$ ,  $N_{\text{obs}} = 352$ ), by more than 62% (Fig. 2A) and 83% (Fig. 2B), respectively.  
279 Differences in pollinator and flower visitation frequencies between octagons remained consistent  
280 when pollutants were rotated between octagons (Treatment:Rotation  $\chi^2_{3,11} = 0.36$ ,  $P = 0.949$  and  
281  $\chi^2_{3,11} = 1.30$ ,  $P = 0.729$ , respectively; Control experiment 1), indicating that placing the octagons  
282 opposite each other in the field was sufficient to account for spatial changes in insect pollinator  
283 activity. Abundances of background pollinators (i.e. those attracted to only visual stimuli when *B.*  
284 *nigra* were not present in the octagons; Control experiment 2) did not vary significantly between  
285 treatments (Table S2), providing no evidence to suggest that pollinating insects were inhibited  
286 from entering the pollution octagons when floral odor cues were not a factor. While this control  
287 does not rule out the potential for air pollution to directly impair pollinator health (Leonard et al.,  
288 2019; Reitmayer et al., 2019; Thimmegowda et al., 2020) or for higher short-term (peak)  
289 concentrations to directly impair motility (Vanderplanck et al., 2021), it suggests that the observed  
290 changes in pollinator foraging behavior between treatments were most likely to be associated  
291 with changes in their attraction to plant-emitted VOCs, including floral odors, providing field-based  
292 validation and quantification of studies previously limited to laboratory conditions (Farré-Armengol  
293 et al., 2016; Girling et al., 2013; McFrederick et al., 2008).



294

### 295 3.3. Responses of insect pollinator groups to air pollution

296 To assess the impacts of air pollution on ecological processes and natural capital, it must be  
297 understood how pollutants affect different insect groups and species at field scales (Jamieson et  
298 al., 2017; Pinto et al., 2010). We found differing responses to air pollution between insect groups  
299 (Fig. 2C and D; Table S3). Air pollution treatments reduced the abundance of seven pollinator  
300 groups, which included all bees (i.e. honey bees, solitary bees and bumblebees), all flies (i.e.  
301 hoverflies and other flies), butterflies and moths. These seven groups were responsible for driving  
302 the air-pollution mediated decreases in total pollinator abundance and each group showed similar  
303 responses to air pollution, with significantly higher abundances observed in unpolluted (control)  
304 octagons (Fig. 2C). Similar effects were observed for the number of flowers visited by bees,  
305 hoverflies, butterflies and moths (Fig. 2D). In contrast, the abundances of three groups, including  
306 beetles (the vast majority of which were pollen beetles, *Meligethes* spp.), true bugs and parasitic  
307 wasps, were not significantly affected by pollution treatments (Fig. 2C; Table S3). Such  
308 differences between groups, and likely between different species, are to be expected, because  
309 each will utilize a unique combination of different senses and stimuli during foraging. For  
310 example, those hoverfly species that possess smaller eyes and, as a result, a greater reliance on  
311 odor cues, are likely to be more negatively affected by air pollution than those hoverfly species  
312 that rely more on visual stimuli and less on the olfactory environment (McFrederick et al., 2009;  
313 Nordström et al., 2017). Furthermore, responses may differ between generalist and specialist  
314 species (McFrederick et al., 2009; Verheggen et al., 2008; Whittaker, 2001).

315

316 In general, some insect groups or species may be less reliant on those plant VOCs that are more  
317 reactive with air pollutants (Fuentes et al., 2016; Khaling et al., 2016; Nordström et al., 2017;  
318 Sprayberry, 2018) and a laboratory study has suggested that the tobacco hawkmoth, *Manduca*  
319  *sexta*, may have the capacity to learn to associate air pollution-altered floral odor blends with a  
320 food resource (Cook et al., 2020), which, if translated to the field, could potentially mitigate the  
321 negative effects of air pollution on pollinator foraging over time. Alternatively, polluted  
322 environments may release some groups from competitive constraints and/or increase the  
323 abundance of their prey species (Khaling et al., 2016; Verheggen et al., 2008). The variations in  
324 response by the different insect groups that we observed demonstrates that in order to elucidate  
325 the ecological impacts of air pollution, research in this field needs to investigate the effects of air  
326 pollution on community ecology, and move beyond studies focusing only on single species, bi- or  
327 tri-trophic interactions, using such studies instead as a tool to elucidate mechanisms once field-  
328 scale ecological effects have been identified.

329

330 The foraging behaviors of pollinating insects such as bees and hoverflies are likely to be most  
331 affected at times of peak pollution levels, such as on hot summer days and at times of peak daily  
332 traffic adjacent to major roads or in urban areas. Phillips et al. (2021), for example, demonstrated  
333 lower densities of insect pollinators closer to roads, which is also where concentrations of  
334 pollutants are greatest. High concentrations of NO<sub>x</sub> next to major roads tend to return to  
335 background concentrations at approximately 100 m away from the road (Bignal et al., 2007).  
336 While fresh emissions of NO<sub>x</sub> can slow the formation of O<sub>3</sub>, emitted NO<sub>x</sub> can also lead to O<sub>3</sub>  
337 formation later and further downwind (Bae et al., 2020; Sayegh et al., 2016), which as a result  
338 may deleteriously affect some insect species or communities, but not others. Therefore, further  
339 studies incorporating wider spatial scales across landscapes will be important to facilitate  
340 predictions of how insect communities respond to field-realistic concentrations of air pollution.  
341 Such landscape-scale studies will face additional challenges because it will be difficult to account  
342 for spatial and temporal variation, but they have the potential to provide more realistic  
343 measurements of insect foraging that could help to identify potential ecological risks. Therefore,  
344 coupling these wider field-based approaches with more controlled field studies (such as the  
345 current study) and mechanistic laboratory studies, which can identify behaviorally-important  
346 VOCs that different species or groups rely on, will be essential for developing a complete

347 understanding of how ecosystems respond to air pollution as we shift away from NO<sub>x</sub> emission  
348 sources.

349

### 350 3.4. Air pollution effects on yield metrics of a self-fertile plant

351 Reductions in flower visitation under pollution treatments coincided with significant decreases in  
352 *B. nigra* seed metrics (Table 1). *Brassica nigra* is an O<sub>3</sub>-tolerant plant (Saunier and Blande, 2019)  
353 that is commonly found alongside major roadways and has been used as a model species for  
354 investigating plant–insect responses to multiple environmental stressors (Papazian and Blande,  
355 2020 and references therein). The self-fertile variety used in this study was not reliant on  
356 pollination for seed development, yet we observed a 14-31% reduction in some seed metrics  
357 under pollution treatments. This suggests that plant species more reliant on insect pollination, and  
358 especially those that are ecologically specialized on a single pollinator taxon (Bennett et al.,  
359 2020), may be even more severely affected. Seed metrics of 'yield control' *B. nigra*, i.e. those  
360 plants placed in each octagon that were netted and hand-pollinated to ensure maximum  
361 pollination (Control experiment 3), did not vary significantly between treatments (Table 1). This  
362 indicates that being in a polluted environment was not the cause of the reductions in seed metrics  
363 observed in experimental plants and that reductions were almost certainly a result of changes in  
364 pollination rates. Air pollution can directly impact the health of many plant species, including some  
365 food crops, which can cause reduced yields (Papazian and Blande, 2020) and these results  
366 suggest that air pollution can further reduce yields through reduction of insect-mediated  
367 pollination.

368

369

## 370 4. Conclusions

371 Our study indicates that air pollutants, at levels currently deemed by legislation to be safe for the  
372 environment, can cause significant reductions in flower visitation, by key insect pollinator groups,  
373 resulting in significant changes in metrics of plant yield. That is to say, air pollutants reduce  
374 insect-provided pollination services. Our results imply that these changes are caused by the  
375 reactions of floral VOCs with air pollutants, altering pollinating insect species' perceptions of  
376 these floral VOC profiles, supporting and validating the findings of previous laboratory  
377 investigations. However, VOCs are used ubiquitously by plants and insects for communication  
378 and for perception of their environments. Therefore, the implications of our findings are  
379 anticipated to extend beyond effects on pollinators and pollination services, and future studies  
380 should prioritize investigations into the broader ecological and economic consequences of VOC-  
381 communication disruption by common tropospheric pollutants. Our findings indicate that there is  
382 an urgent need for research that investigates the wider potential of air pollutants to disrupt the  
383 many insect-mediated ecological processes and ecosystem services upon which humans and  
384 nature rely.

385

386

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390

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394

### 395 Declaration of competing interest

396 The authors declare that they have no known competing financial interests or personal  
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398

#### 399 **Data availability**

400 Data are available at the EIDC repository (DOI: 10.5285/d2e0cf65-010c-4206-8302-  
401 195449d0acba), embargoed until 31 December 2021.

402

#### 403 **Author statement**

404 **James Ryalls:** Conceptualization, Resources, Methodology, Validation, Investigation, Formal  
405 analysis, Writing – Original Draft, Visualization

406 **Ben Langford:** Funding acquisition, Conceptualization, Software, Writing – Review & Editing

407 **Neil Mullinger:** Methodology, Software, Writing – Review & Editing

408 **Lisa Bromfield:** Methodology, Investigation, Writing – Review & Editing

409 **Eiko Nemitz:** Funding acquisition, Conceptualization, Supervision, Writing – Review & Editing

410 **Christian Pfrang:** Funding acquisition, Conceptualization, Supervision, Writing – Review &  
411 Editing

412 **Robbie Girling:** Funding acquisition, Conceptualization, Resources, Methodology, Validation,  
413 Supervision, Writing – Original Draft

414

415

#### 416 **References**

417 ACEA, 2019. European Automobile Manufacturers Association Access to Euro 6 RDE data  
418 [<https://www.acea.be/publications/article/access-to-euro-6-rde-monitoring-data>] (1 July 2020)

419 Ares, E., Smith, L., 2017. Air pollution: meeting nitrogen dioxide targets (Commons Library  
420 Briefing). House of Commons Library, p. 26.

421 Bae, C., Kim, H.C., Kim, B.-U., Kim, S., 2020. Surface ozone response to satellite-constrained  
422 NO<sub>x</sub> emission adjustments and its implications. *Environmental Pollution* 258, 113469.

423 Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting linear mixed-effects models using  
424 lme4. *Journal of Statistical Software*. ArXiv preprint arXiv:1406.5823.

425 Bennett, J.M., Steets, J.A., Burns, J.H., Burkle, L.A., Vamosi, J.C., Wolowski, M., Arceo-Gómez,  
426 G., Burd, M., Durka, W., Ellis, A.G., Freitas, L., Li, J., Rodger, J.G., Ştefan, V., Xia, J., Knight,  
427 T.M., Ashman, T.-L., 2020. Land use and pollinator dependency drives global patterns of pollen  
428 limitation in the Anthropocene. *Nature Communications* 11, 3999.

429 Bignal, K.L., Ashmore, M.R., Headley, A.D., Stewart, K., Weigert, K., 2007. Ecological impacts of  
430 air pollution from road transport on local vegetation. *Applied Geochemistry* 22, 1265-1271.

431 Blande, J.D., 2021. Effects of air pollution on plant–insect interactions mediated by olfactory and  
432 visual cues. *Current Opinion in Environmental Science & Health* 19, 100228.

433 Brand, C., 2016. Beyond 'Dieselgate': Implications of unaccounted and future air pollutant  
434 emissions and energy use for cars in the United Kingdom. *Energy Policy* 97, 1-12.

435 Colette, A., Aas, W., Banin, L., Braban, C.F., Ferm, M., Gonzalez Ortiz, A., Ilyin, I., Mar, K.,  
436 Pandolfi, M., Putaud, J.-P., 2016. Air pollution trends in the EMEP region between 1990 and  
437 2012. Joint Report of the EMEP Task Force on Measurements and Modelling (TFMM), Chemical

438 Co-ordinating Centre (CCC), Meteorological Synthesizing Centre-East (MSC-E), Meteorological  
439 Synthesizing Centre-West (MSC-W).

440 Cook, B., Haverkamp, A., Hansson, B.S., Roulston, T.a., Lerdau, M., Knaden, M., 2020.  
441 Pollination in the Anthropocene: a moth can learn ozone-altered floral blends. *Journal of*  
442 *Chemical Ecology* 46, 987-996.

443 EPA, 2021. United States Environmental Protection Agency: Air Topics  
444 [<http://www.epa.gov/air/criteria.html>] (31 March 2021)

445 Farré-Armengol, G., Peñuelas, J., Li, T., Yli-Pirilä, P., Filella, I., Llusia, J., Blande, J.D., 2016.  
446 Ozone degrades floral scent and reduces pollinator attraction to flowers. *New Phytologist* 209,  
447 152-160.

448 Fuentes, J.D., Chamecki, M., Roulston, T., Chen, B., Pratt, K.R., 2016. Air pollutants degrade  
449 floral scents and increase insect foraging times. *Atmospheric Environment* 141, 361-374.

450 Girling, R.D., Lusebrink, I., Farthing, E., Newman, T.A., Poppy, G.M., 2013. Diesel exhaust  
451 rapidly degrades floral odours used by honeybees. *Scientific Reports* 3, 2779.

452 Hansen, E.M.Ø., Hauggaard-Nielsen, H., Launay, M., Rose, P., Mikkelsen, T.N., 2019. The  
453 impact of ozone exposure, temperature and CO<sub>2</sub> on the growth and yield of three spring wheat  
454 varieties. *Environmental and Experimental Botany* 168, 103868.

455 Jamieson, M.A., Burkle, L.A., Manson, J.S., Runyon, J.B., Trowbridge, A.M., Zientek, J., 2017.  
456 Global change effects on plant–insect interactions: the role of phytochemistry. *Current Opinion in*  
457 *Insect Science* 23, 70-80.

458 Khaling, E., Li, T., Holopainen, J.K., Blande, J.D., 2016. Elevated ozone modulates herbivore-  
459 induced volatile emissions of *Brassica nigra* and alters a tritrophic interaction. *Journal of*  
460 *Chemical Ecology* 42, 368-381.

461 Klein, A.-M., Vaissiere, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C.,  
462 Tschamntke, T., 2007. Importance of pollinators in changing landscapes for world crops.  
463 *Proceedings of the Royal Society B-Biological Sciences* 274, 303-313.

464 Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear  
465 mixed effects models. *Journal of Statistical Software* 82, 1 - 26.

466 Leonard, R.J., Pettit, T.J., Irga, P., McArthur, C., Hochuli, D.F., 2019. Acute exposure to urban air  
467 pollution impairs olfactory learning and memory in honeybees. *Ecotoxicology* 28, 1056-1062.

468 Li, T., Blande, J.D., Holopainen, J.K., 2016. Atmospheric transformation of plant volatiles disrupts  
469 host plant finding. *Scientific Reports* 6, 33851.

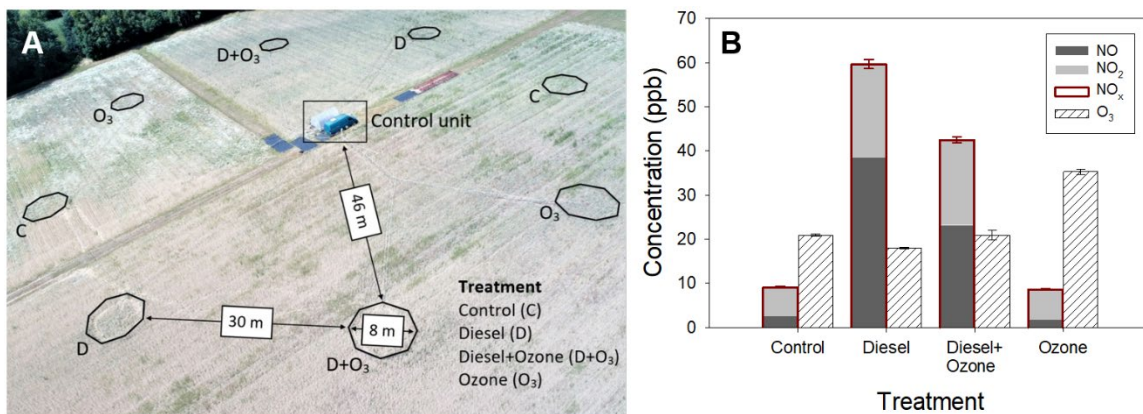
470 Lusebrink, I., Girling, R.D., Farthing, E., Newman, T.A., Jackson, C.W., Poppy, G.M., 2015. The  
471 effects of diesel exhaust pollution on floral volatiles and the consequences for honey bee  
472 olfaction. *Journal of Chemical Ecology* 41, 904-912.

473 McFrederick, Q.S., Fuentes, J.D., Roulston, T., Kathilankal, J.C., Lerdau, M., 2009. Effects of air  
474 pollution on biogenic volatiles and ecological interactions. *Oecologia* 160, 411-420.

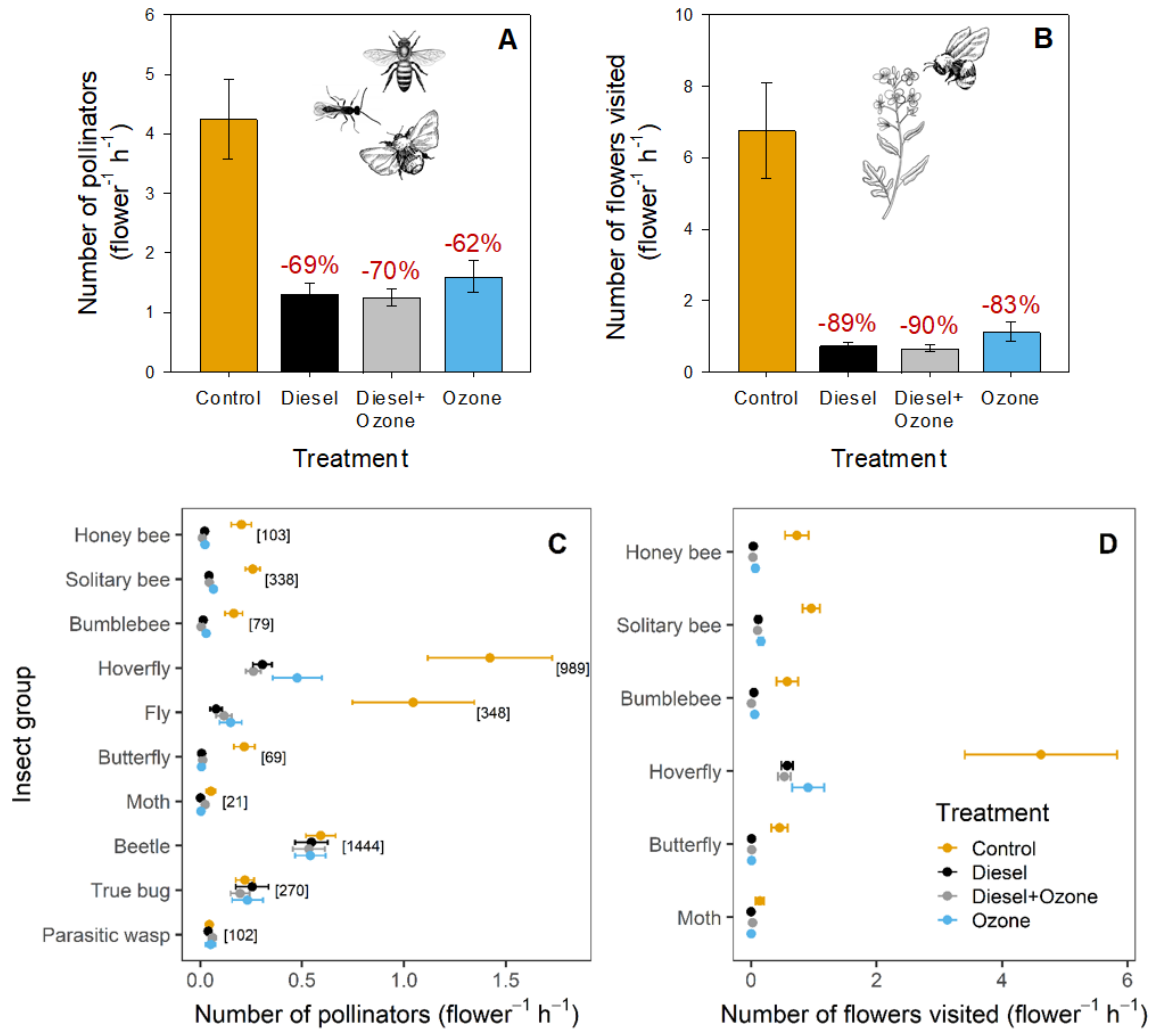
- 475 McFrederick, Q.S., Kathilankal, J.C., Fuentes, J.D., 2008. Air pollution modifies floral scent trails.  
476 *Atmospheric Environment* 42, 2336-2348.
- 477 Nordström, K., Dahlbom, J., Pragadheesh, V.S., Ghosh, S., Olsson, A., Dyakova, O., Suresh,  
478 S.K., Olsson, S.B., 2017. In situ modeling of multimodal floral cues attracting wild pollinators  
479 across environments. *Proceedings of the National Academy of Sciences* 114, 13218-13223.
- 480 Nuttman, C., Willmer, P., 2003. How does insect visitation trigger floral colour change? *Ecological*  
481 *Entomology* 28, 467-474.
- 482 Oguchi, M., Fuse, M., 2015. Regional and longitudinal estimation of product lifespan distribution:  
483 a case study for automobiles and a simplified estimation method. *Environmental Science &*  
484 *Technology* 49, 1738-1743.
- 485 Papazian, S., Blande, J.D., 2020. Dynamics of plant responses to combinations of air pollutants.  
486 *Plant Biology* 22, 68-83.
- 487 Phillips, B.B., Bullock, J.M., Gaston, K.J., Hudson-Edwards, K.A., Bamford, M., Cruse, D., Dicks,  
488 L.V., Falagan, C., Wallace, C., Osborne, J.L., 2021. Impacts of multiple pollutants on pollinator  
489 activity in road verges. *Journal of Applied Ecology* 58, 1017-1029.
- 490 Pinto, D.M., Blande, J.D., Souza, S.R., Nerg, A.-M., Holopainen, J.K., 2010. Plant volatile organic  
491 compounds (VOCs) in ozone (O<sub>3</sub>) polluted atmospheres: the ecological effects. *Journal of*  
492 *Chemical Ecology* 36, 22-34.
- 493 Potts, S.G., Imperatriz-Fonseca, V., Ngo, H., Biesmeijer, J.C., Breeze, T., Dicks, L., Garibaldi, L.,  
494 Settele, J., Vanbergen, A.J., Aizen, M.A., 2016a. Summary for policymakers of the assessment  
495 report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services  
496 (IPBES) on pollinators, pollination and food production.
- 497 Potts, S.G., Imperatriz-Fonseca, V., Ngo, H.T., Aizen, M.A., Biesmeijer, J.C., Breeze, T.D., Dicks,  
498 L.V., Garibaldi, L.A., Hill, R., Settele, J., Vanbergen, A.J., 2016b. Safeguarding pollinators and  
499 their values to human well-being. *Nature* 540, 220.
- 500 Powney, G.D., Carvell, C., Edwards, M., Morris, R.K.A., Roy, H.E., Woodcock, B.A., Isaac,  
501 N.J.B., 2019. Widespread losses of pollinating insects in Britain. *Nature Communications* 10,  
502 1018.
- 503 Reitan, T., Nielsen, A., 2016. Do not divide count data with count data; a story from pollination  
504 ecology with implications beyond. *PLOS ONE* 11, e0149129.
- 505 Reitmayer, C.M., Ryalls, J.M.W., Farthing, E., Jackson, C.W., Girling, R.D., Newman, T.A., 2019.  
506 Acute exposure to diesel exhaust induces central nervous system stress and altered learning and  
507 memory in honey bees. *Scientific Reports* 9, 5793.
- 508 Richmond-Bryant, J., Owen, R.C., Graham, S., Snyder, M., McDow, S., Oakes, M., Kimbrough,  
509 S., 2017. Estimation of on-road NO<sub>2</sub> concentrations, NO<sub>2</sub>/NO<sub>x</sub> ratios, and related roadway  
510 gradients from near-road monitoring data. *Air Quality, Atmosphere & Health* 10, 611-625.
- 511 Riffell, J.A., Shlizerman, E., Sanders, E., Abrell, L., Medina, B., Hinterwirth, A.J., Kutz, J.N., 2014.  
512 Flower discrimination by pollinators in a dynamic chemical environment. *Science* 344, 1515-1518.

- 513 Saunier, A., Blande, J.D., 2019. The effect of elevated ozone on floral chemistry of Brassicaceae  
514 species. *Environmental Pollution* 255, 113257.
- 515 Sayegh, A., Tate, J.E., Ropkins, K., 2016. Understanding how roadside concentrations of NO<sub>x</sub> are  
516 influenced by the background levels, traffic density, and meteorological conditions using Boosted  
517 Regression Trees. *Atmospheric Environment* 127, 163-175.
- 518 Sprayberry, J.D.H., 2018. The prevalence of olfactory- versus visual-signal encounter by  
519 searching bumblebees. *Scientific Reports* 8, 14590.
- 520 Thimmegowda, G.G., Mullen, S., Sottolare, K., Sharma, A., Mohanta, S.S., Brockmann, A.,  
521 Dhandapany, P.S., Olsson, S.B., 2020. A field-based quantitative analysis of sublethal effects of  
522 air pollution on pollinators. *Proceedings of the National Academy of Sciences* 117, 20653-20661.
- 523 Turnock, S.T., Wild, O., Sellar, A., O'Connor, F.M., 2019. 300 years of tropospheric ozone  
524 changes using CMIP6 scenarios with a parameterised approach. *Atmospheric Environment* 213,  
525 686-698.
- 526 Vanbergen, A.J., the Insect Pollinators Initiative, 2013. Threats to an ecosystem service:  
527 pressures on pollinators. *Frontiers in Ecology and the Environment* 11, 251-259.
- 528 Vanderplanck, M., Lapeyre, B., Lucas, S., Proffit, M., 2021. Ozone induces distress behaviors in  
529 fig wasps with a reduced chance of recovery. *Insects* 12, 995.
- 530 Verheggen, F.J., Arnaud, L., Bartram, S., Gohy, M., Haubruge, E., 2008. Aphid and plant volatiles  
531 induce oviposition in an aphidophagous hoverfly. *Journal of Chemical Ecology* 34, 301-307.
- 532 Wang, N., Lyu, X., Deng, X., Huang, X., Jiang, F., Ding, A., 2019. Aggravating O<sub>3</sub> pollution due to  
533 NO<sub>x</sub> emission control in eastern China. *Science of The Total Environment* 677, 732-744.
- 534 Westphal, C., Bommarco, R., Carré, G., Lamborn, E., Morison, N., Petanidou, T., Potts, S.G.,  
535 Roberts, S.P.M., Szentgyörgyi, H., Tscheulin, T., Vaissière, B.E., Woyciechowski, M., Biesmeijer,  
536 J.C., Kunin, W.E., Settele, J., Steffan-Dewenter, I., 2008. Measuring bee diversity in different  
537 European habitats and biogeographical regions. *Ecological Monographs* 78, 653-671.
- 538 Whittaker, J.B., 2001. Insects and plants in a changing atmosphere. *Journal of Ecology* 89, 507-  
539 518.
- 540 Wright, G.A., Schiestl, F.P., 2009. The evolution of floral scent: the influence of olfactory learning  
541 by insect pollinators on the honest signalling of floral rewards. *Functional Ecology* 23, 841-851.
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544 **Figures and Tables**  
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547  
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 549 **Fig. 1. Free-Air Diesel and Ozone Enrichment octagon layout during 2018 (A) and mean**  
 550 **concentrations ( $\pm$  SE) of nitrogen oxides ( $\text{NO}_x = \text{NO} + \text{NO}_2$ ) and ozone ( $\text{O}_3$ ) within**  
 551 **treatments (B).** Octagons were distributed in an octagonal formation within a field of wheat  
 552 (drone image by UoR SAGES UAV; A), which acted as a non-flowering buffer. In B, red bars  
 553 ( $\text{NO}_x$  concentrations) include stacked concentrations of nitric oxide (NO) and nitrogen dioxide  
 554 ( $\text{NO}_2$ ).  
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**Fig. 2. The effects of diesel exhaust and ozone pollution on pollinator foraging behavior.** Means (± SE) of pollinator abundance (A), flower visitation frequencies (B), abundances per insect group (C) and flower visits per insect group (D) were scaled according to the number of flowers within each observation unit and survey duration. For part C, numbers in square brackets represent the total number of individuals counted for each group. If an insect landed on a flower within the observation unit, that insect was counted as '1' for abundance. If that same insect landed on five flowers within the observation unit, the number of flower visits was recorded as '5'. Flower visitation (B and D) was recorded for bees, hoverflies, butterflies and moths only.



570 **Table 1. The effects of diesel exhaust and ozone pollution on yield metrics of *Brassica***  
 571 ***nigra*.** Statistical values in brackets represent pollution treatment effects on yield control (YC)  
 572 plants. Values in bold indicate statistical significance ( $P < 0.05$ ).  $N_{\text{obs}} = 383$  and 80 for  
 573 experimental plants and YC plants, respectively. Random models include 'Year/Octagon location'  
 574 ( $N = 16$ ) for experimental plants and 'Octagon location' for YC plants ( $N = 8$ ).  
 575

Response variable	Treatment				Statistical analysis	
	Control	Diesel	Diesel+Ozone	Ozone	$\chi^2_{3,7(3,6)}$	$P$
Seeds per pod	9.49 $\pm 0.28$	6.74 $\pm 0.21$	6.59 $\pm 0.22$	7.36 $\pm 0.24$	<b>15.05</b> (3.27)	<b>0.002</b> (0.352)
Pod mass (g)	0.058 $\pm 0.003$	0.041 $\pm 0.002$	0.042 $\pm 0.002$	0.043 $\pm 0.002$	<b>11.13</b> (1.96)	<b>0.011</b> (0.581)
Pods developed (%)	51.46 $\pm 1.40$	43.92 $\pm 1.10$	42.59 $\pm 1.19$	44.51 $\pm 1.11$	<b>16.76</b> (1.53)	<b>&lt; 0.001</b> (0.676)
1000-seed mass (g)	2.40 $\pm 0.08$	2.50 $\pm 0.08$	2.44 $\pm 0.08$	2.29 $\pm 0.08$	4.04 (0.95)	0.258 (0.814)
Plant dry mass (g)	14.31 $\pm 0.45$	12.93 $\pm 0.50$	12.98 $\pm 0.77$	12.88 $\pm 0.70$	0.71 (2.48)	0.870 (0.478)

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