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

DOI: 10.1016/j.envpol.2022.118847

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Document Version Peer reviewed version

Citation for published version (Harvard):

Ryalls, JMW, Langford, B, Mullinger, N, Bromfield, LM, Nemitz, E, Pfrang, C & Girling, RD 2022, 'Anthropogenic air pollutants reduce insect-mediated pollination services', *Environmental Pollution*, vol. 297, 118847. https://doi.org/10.1016/j.envpol.2022.118847

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Anthropogenic air pollutants reduce insect-mediated pollination services

- 3
- James M.W. Ryalls^{a*}, Ben Langford^b, Neil J. Mullinger^b, Lisa M. Bromfield^a, Eiko Nemitz^b,
 Christian Pfrang^{c,d} & Robbie D. Girling^a
- ^aSchool of Agriculture, Policy and Development, University of Reading, Whiteknights, Earley
 Gate, Reading, RG6 6EU, UK
- ^bUK Centre for Ecology & Hydrology, Penicuik, Midlothian, EH26 0QB, UK
- School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston,
- 10 Birmingham, B15 2TT, UK
- ^dDepartment of Meteorology, University of Reading, Whiteknights, Earley Gate, Reading, RG6 6BB, UK
- 12 6BB, 13
- 14 *Correspondence: <u>J.Ryalls@reading.ac.uk</u>
- 15

17 Abstract

18 Common air pollutants, such as nitrogen oxides (NO_x), emitted in diesel exhaust, and ozone (O₃),

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

floral odors, disrupting the cues that foraging insects use to find and pollinate flowers. However, odor environments in nature are highly complex and pollination services are commonly provided

by suites of insect species, each exhibiting different sensitivities to different floral odors.

Therefore, the potential impacts of pollution-induced foraging disruption on both insect ecology,

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

result the pollination ecosystem service that those insects provide. Using free-air fumigation, we

show that elevating diesel exhaust and O_3 , individually and in combination, to levels lower than is

30 considered safe under current air quality standards, significantly reduced counts of locally-

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

of pollination and yield of a self-fertile test plant. Quantifying such effects provides new insights

into the impacts of human-induced air pollution on the natural ecosystem services upon which wedepend.

37

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

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_x, comprised of nitric oxide (NO) and nitrogen dioxide (NO₂)) from diesel exhaust, 49 and ozone (O_3) , may contribute to pollinator declines through: i) direct effects on insect pollinator 50 health (Reitmaver et al., 2019) and; ii) potential reductions in pollinator foraging efficiency (Farré-Armengol et al., 2016; Fuentes et al., 2016; Girling et al., 2013; McFrederick et al., 2008). 51

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_x and O₃ 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., 2016; Girling et al., 2013; Lusebrink et al., 2015), and atmospheric chemistry modelling (e.g. 63 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

We investigated whether exposure to diesel exhaust (particularly NO_x) and elevated O_3 , 68 69 individually and in combination, affected the ability of naturally occurring pollinating insects to: i) 70 locate floral resources, and ii) provide pollination services. NOx and O3 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 communities they reside with (Li et al., 2016; Papazian and Blande, 2020). Globally, many of the 74 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 NOx 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 O₃ 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_x-limited, reductions in NO_x emission sources may also 84 increase O_3 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_3 Enrichment (FADOE) facility, which allowed emission of regulated quantities of NO_x 88 (emitted in diesel exhaust) and O₃.

89 90

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_3 , iii) diesel exhaust and O_3 combined (D+O₃), 95 and iv) control ambient air (Fig. 1A). The aim was to maintain fumigation levels of NOx and O₃ 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 $(D+O_3)$ 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 the centre of the field were used to deliver elevated levels of diesel exhaust and ozone to the 106 107 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 ambient air in the case of the Control treatment octagons) directed towards the centre of the 111 octagon. The concentrations of NO, NO₂, NO_x (i.e. NO+NO₂) and ozone (O₃) at the centre of 112 each octagon were monitored sequentially (every 120 s) via an automated switching system 113 114 coupled to O₃ (Model 49i, Thermo Scientific, USA) and NO_x (Model 42C, Thermo Scientific, USA) 115 analysers. Three-way mixing valves (VRG131 connected to ARA600 proportional actuators, ESBE, Sweden) and UV-light controllers (CD1500P 4-20mA control board) altered the quantities 116 117 of diesel exhaust and O₃ 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 anemometers and W200P potentiometer windvanes (Vector Instruments, UK), positioned north, 126 127 east, south and west of the field to ensure consistency in prevailing wind direction throughout data collection (south-westerly; Fig. S2). The FADOE octagons were positioned within a field of 128 129 winter wheat (Triticum aestivum cv. Skyfall, sown in November 2017 at a seeding rate of 300 130 seeds m⁻² 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 O₃ 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

distributed evenly in each octagon and dug into the ground within the wheat crop, such that the lip
of each pot was flush with the surface of the soil. These plants remained in the octagons until
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 view of the flowers upon which visits were counted (Reitan and Nielsen, 2016). The Order and 155 156 group of visitor (Hymenoptera (honey bees, bumble bees, solitary bees and parasitic wasps), Lepidoptera (moths and butterflies), Diptera (hoverflies and other flies), Coleoptera (beetles) and 157 158 Hemiptera (true bugs)) were recorded, along with the number of flowers that were visited by individual insects (bees, moths, butterflies and hoverflies only) and the total number of flowers 159 160 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 during the observation period. For each day of sampling, observers were assigned, at random, to 163 164 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⁻¹ h⁻¹ and flower visits flower⁻¹ h⁻¹, 169 respectively.

170

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

- 2.4.1. Control experiment 1 Accounting for spatial differences in pollinator foraging within the
 field
- 174 For one week (12-19 July 2019), plants and treatments were rotated between the different
- FADOE octagons so that control octagons became diesel exhaust-polluted octagons and ozone 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 among178 treatments.
- 179

2.4.2. Control experiment 2 – Determining the direct effects of air pollution on pollinator flight 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, Lasioglossum, Andrena, Apis, Halictus, Hylaeus), 14 Families (Anthomyiidae, Calliphoridae, 188 189 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

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

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

200

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

206

207 $1000 \text{ seed mass } (g) = \left(\frac{\text{Total mass of seeds } (g)}{\text{Total number of seeds}}\right) \times 1000$ (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 experimental plants. By separating the direct and indirect effects of air pollution on plant yield we 215 216 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 Ime4 (Bates et al., 2014) 222 were used to determine the effects of air pollution treatments on NO_x and O₃ 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⁻¹ h⁻¹; GLMM), yield metrics (number of seeds 225 per pod, pod mass, % pods developed, 1000-seed mass and plant dry mass; LMM), abundances of individual insect groups (GLMM) and background pollinator numbers recorded from triple pan 226 traps (GLMM). 'Octagon location' nested within 'Year' were included as random effects in mixed 227 228 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 location' as a random effect to account for repeated measures. Contrasts of fixed effects (t-238 239 statistics based on Satterthwaite's approximation) from model summaries using the R package 240 *ImerTest (Kuznetsova et al., 2017)* were provided for NO_x concentrations. O_3 concentrations. 241 NO:NO₂ 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_3 to $35.2 \pm$ 248 0.6 ppb (P < 0.001) and of NO_x to 59.6 ± 1.0 ppb (NO = 38.5 ± 0.8 ppb, NO₂ = 21.2 ± 0.3 ppb; P <249 0.001), relative to the control octagons (values are means (\pm 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 ($O_3 = 70$ ppb averaged over 8 hours, $NO_2 = 53$ 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 O₃ concentrations achieved were equivalent to those in the control octagons. Moreover, NO_x concentrations decreased in the 256 257 combined pollutant octagons compared with the diesel exhaust-only octagons, associated with a 258 significant reduction in NO:NO₂ (Fig. 1B; statistical results in Table S1). NO_x and O₃ in the 259 troposphere commonly react with each other and, depending on local quantities of NOx, VOCs 260 and O_3 catalysts (in particular reactive hydrogen species such as hydroxyl and hydroperoxyl 261 radicals), NO_x emissions can lead to both the formation and destruction of O_3 . O_3 is typically 262 lower in urban areas or next to busy roads because it reacts with NO_x from vehicle exhaust 263 emissions (Bae et al., 2020). For example, mean hourly concentrations of NO_x and O₃ 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_x and O_3 in the combined treatment resulted in a decrease in the ratio of NO/NO₂ compared with the diesel exhaust-only treatment 267 because O_3 reacts with NO to produce NO₂ (Richmond-Bryant et al., 2017), which also explains 268 269 why O_3 concentrations were depleted in the combined treatment relative to the O_3 -only treatment. This complex interplay between oxidative pollutants is likely to alter the fate of biogenic VOCs 270 271 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 O₃ treatments, individually and in combination, we recorded dramatic 277 reductions in pollinator numbers ($\chi^2_{3,8}$ = 21.52, *P* < 0.001, *N*_{obs} = 352) and flower visitation ($\chi^2_{3,8}$ = 278 35.74, P < 0.001, $N_{obs} = 352$), 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 guantification 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 responses to air pollution, with significantly higher abundances observed in unpolluted (control) 303 304 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 beetles (the vast majority of which were pollen beetles, Meligethes spp.), true bugs and parasitic 306 wasps, were not significantly affected by pollution treatments (Fig. 2C; Table S3). Such 307 308 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 odor cues, are likely to be more negatively affected by air pollution than those hoverfly species 311 312 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; Sprayberry, 2018) and a laboratory study has suggested that the tobacco hawkmoth, Manduca 318 319 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 abundance of their prey species (Khaling et al., 2016; Verheggen et al., 2008). The variations in 323 324 response by the different insect groups that we observed demonstrates that in order to elucidate the ecological impacts of air pollution, research in this field needs to investigate the effects of air 325 326 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_x next to major roads tend to return to 335 background concentrations at approximately 100 m away from the road (Bignal et al., 2007). While fresh emissions of NO_x can slow the formation of O_3 , emitted NO_x can also lead to O_3 336 337 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 predictions of how insect communities respond to field-realistic concentrations of air pollution. 340 341 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

understanding of how ecosystems respond to air pollution as we shift away from NO_x emission
 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 O₃-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 pollination for seed development, yet we observed a 14-31% reduction in some seed metrics 356 357 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., 2020), may be even more severely affected. Seed metrics of 'yield control' B. nigra, i.e. those 359 360 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 indicates that being in a polluted environment was not the cause of the reductions in seed metrics 362 observed in experimental plants and that reductions were almost certainly a result of changes in 363 364 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 ubiguitously 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

387 Acknowledgments

We thank Richard Casebow, Caroline Hadley, Kaan Alkan, Tom Staton and Fiona Gierer for help with FADOE construction, and AMOF for the loan of NO_x and O₃ analyzers.

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391 Funding

This research was funded by a Natural Environment Research Council grant awarded to RDG (NE/P002404/1) and EN (NE/P001971/2).

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395 Declaration of competing interest

- 396 The authors declare that they have no known competing financial interests or personal
- relationships that could have appeared to influence the work reported in this paper.
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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

- James Ryalls: Conceptualization, Resources, Methodology, Validation, Investigation, Formal
 analysis, Writing Original Draft, Visualization
- Ben Langford: Funding acquisition, Conceptualization, Software, Writing Review & Editing
 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

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544 Figures and Tables

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Fig. 1. Free-Air Diesel and Ozone Enrichment octagon layout during 2018 (A) and mean concentrations (\pm SE) of nitrogen oxides (NO_x = NO + NO₂) and ozone (O₃) within

treatments (B). Octagons were distributed in an octagonal formation within a field of wheat
 (drone image by UoR SAGES UAV; A), which acted as a non-flowering buffer. In B, red bars
 (NO_x concentrations) include stacked concentrations of nitric oxide (NO) and nitrogen dioxide
 (NO₂).



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560 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.

 Table 1. The effects of diesel exhaust and ozone pollution on yield metrics of Brassica nigra.

 Statistical values in brackets represent pollution treatment effects on yield control (YC)

plants. Values in bold indicate statistical significance (P < 0.05). $N_{obs} = 383$ and 80 for experimental plants and YC plants, respectively. Random models include 'Year/Octagon location' (N = 16) for experimental plants and 'Octagon location' for YC plants (N = 8).

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