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Anthropogenic air pollutants reduce insect-mediated pollination services

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Abstract

Common air pollutants, such as nitrogen oxides (NO_x), emitted in diesel exhaust, and ozone (O₃), have been implicated in the decline of pollinating insects. Reductionist laboratory assays, focused upon interactions between a narrow range of flowering plant and pollinator species, in 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* 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 O₃, individually and in combination, to levels lower than is 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%. These reductions were driven by changes in specific pollinator groups, including bees, flies, 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 we depend.

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

1. Introduction

Insect pollination facilitates approximately 7 to 8% of the total value of agricultural food production worldwide (Potts et al., 2016a) and 70% of all crop species rely upon insect pollination (Klein et al., 2007). However, there have been sustained declines in insect pollinator abundance and diversity over the past century, resulting from a combination of environmental pressures (Potts et al., 2016b; Powney et al., 2019; Vanbergen and the Insect Pollinators Initiative, 2013). Recent research suggests that common tropospheric pollutants, including nitrogen oxides (otherwise known as NO_x, comprised of nitric oxide (NO) and nitrogen dioxide (NO₂)) from diesel exhaust, and ozone (O₃), may contribute to pollinator declines through: i) direct effects on insect pollinator health (Reitmayer 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).

In foraging for a flower, odor plumes are an important stimulus for many pollinating insect species. Each flower species' plume is comprised of a unique combination of chemicals, known as volatile organic compounds (VOCs), and an insect's success in locating a flower can depend on the presence, concentration and/or ratio of these VOCs within a plume (Riffell et al., 2014; Wright and Schiestl, 2009). These plume characteristics will be altered by atmospheric pollutants (NO_x and O₃ in particular), or by the products of these pollutants' reactions within the troposphere (especially hydroxyl radicals; McFrederick et al., 2008), either through direct reaction with the plume (Jamieson et al., 2017) or masking of its components (Riffell et al., 2014). Such changes could have consequences for the foraging efficiency of insects that use these cues. Validation of 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. Fuentes et al., 2016; McFrederick et al., 2008), which are focused on a narrow range of pollinator species and on individual pollutants in isolation. Therefore, the ecological impacts remain unclear (Jamieson et al., 2017).

We investigated whether exposure to diesel exhaust (particularly NO_x) and elevated O₃, individually and in combination, affected the ability of naturally occurring pollinating insects to: i) locate floral resources, and ii) provide pollination services. NO_x and O₃ commonly occur together in the troposphere, therefore sequential or simultaneous exposure of air pollutants, rather than exposure to individual pollutants in isolation, is considered an essential requirement when 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 newly approved diesel vehicles sold continue to exceed emission limits, ensuring diesel exhaust emissions will remain a problem for many decades (the average lifespan of a passenger vehicle varies between countries (9-23 years; Oguchi and Fuse, 2015)), with diesel vehicles lasting up to 30 years) (ACEA, 2019; Brand, 2016). Increasing urbanization and traffic congestion is likely to result in higher NO_x in peri-urban and rural areas, increasing the potential exposure of neighboring agricultural land and intensifying pollen limitation (Bennett et al., 2020). Moreover, global tropospheric background O₃ concentrations are rising and regular weather-induced episodic increases continue to occur (Hansen et al., 2019; Turnock et al., 2019). Depending on whether the region is VOC- or NO_x-limited, reductions in NO_x emission sources may also increase O₃ formation, and vice versa (Bae et al., 2020; Wang et al., 2019), which may influence the foraging behavior of pollinating insects (Blande, 2021). To experimentally investigate the ecological effects of simultaneous pollutants at field-scale, we designed a novel Free-Air Diesel and O₃ Enrichment (FADOE) facility, which allowed emission of regulated quantities of NO_x (emitted in diesel exhaust) and O₃.

2. Materials and methods

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

The FADOE facility consisted of eight 8m-diameter octagons; two octagons were assigned to each of four treatments: i) diesel exhaust (D), ii) O₃, iii) diesel exhaust and O₃ combined (D+O₃), and iv) control ambient air (Fig. 1A). The aim was to maintain fumigation levels of NO_x and O₃ within the pollution treatment octagons at field-realistic doses, i.e. below 120 ppb (based on average concentrations adjacent to major UK roadways and urban areas; Ares and Smith, 2017) and 90 ppb (based on peak concentrations recorded in rural European sites in 1990-2012; Colette et al., 2016), respectively. The combined (D+O₃) treatment octagons were maintained at the same maximum concentration as those set for each pollutant octagon individually. The FADOE system configuration is visualised in Fig. S1. The centre of each FADOE octagon was positioned 50 m from the centre of a field (decimal degrees: 51.482853° N 0.897749° W in 2018 and 51.482374° N 0.895855° W in 2019) in an octagonal formation, such that each octagon was separated by a distance of at least 30 m. A diesel generator (Hyundai, DHY8000SELR 7.2 kVA, 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 octagons via 50 mm (ID) heavy duty conduit connected to vacuum-blower pumps (R4110-2, Gast, USA). Octagons of the same treatment were positioned opposite each other within the field to minimise spatial effects. One-hundred and twenty 5 mm-diameter holes were drilled (20 cm 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 octagon. The concentrations of NO, NO₂, NO_x (i.e. NO+NO₂) and ozone (O₃) at the centre of each octagon were monitored sequentially (every 120 s) via an automated switching system coupled to O₃ (Model 49i, Thermo Scientific, USA) and NO_x (Model 42C, Thermo Scientific, USA) 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 of diesel exhaust and O₃ released into the octagons. In-line filter units (G057502, Donaldson, Czech Republic) with a RS3954 filter (Baldwin, USA) were used to remove soot deposits from the pipes before reaching the pumps and excess diesel exhaust was directed through conduit pipes away from the field site (100 m north-east or downwind; Fig. S2). The diesel exhaust was passed through metal conduit pipe directly from the generator to dissipate heat before entering a mixing barrel where collected water vapour was dispensed from a tap (three times weekly). Generators were turned on for up to 17 hours each day (between 4.30 am and 9.30 pm) to ensure that pollution treatments were applied during times of peak daily pollinator activity for all insect pollinators recorded. Wind speed and wind direction were recorded continuously from four A100R anemometers and W200P potentiometer windvanes (Vector Instruments, UK), positioned north, 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 winter wheat (*Triticum aestivum* cv. Skyfall, sown in November 2017 at a seeding rate of 300 seeds m⁻² and a row spacing of 166 mm) at the University of Reading's Sonning farm, UK. The wheat acted as a non-insect pollinated (i.e. non-flowering) buffer, limited plant diversity and maximised weed control. Winter wheat was re-sown in November 2018 in an adjacent field, where the FADOE facility was reassembled for a second year. Octagons were fumigated during two summer seasons (May-September 2018 and 2019).

2.2. Plant material

In May each year (2018 and 2019), 192 black mustard plants (*Brassica nigra* cv. Abyssinica) were grown from seed (Heirloom & Perennial Ltd., Cornwall, UK) in netted 100 mL seed wells in an open glasshouse (24.5 °C ± 2.4 SD). This self-fertile variety, with a two- to three-month flowering period, was used as a model flowering plant based on mechanistic evidence from laboratory studies that O₃ degrades its floral signal and changes its attraction to bumblebees (Farré-Armengol et al., 2016; Saunier and Blande, 2019). Four-week-old plants were transferred to 18 cm diameter pots containing 2.7 kg of vegetable topsoil (Quality Garden Supplies Ltd., Staffordshire, UK) and placed in a polytunnel covered with insect mesh for one week until the 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.

2.3. Insect visitation assessments

Insect visitation to flowers of *B. nigra* was recorded on days conducive to insect activity (dry, >15 °C, between 10:00 and 17:00). The number of insect visits to a focal patch (observation unit) of six adjacent plants (containing an average of 73 flowers) within each FADOE octagon were 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 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 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 within the observation unit. A visit was classified as a landing, or an attempt to feed on or collect pollen and/or nectar (Nuttman and Willmer, 2003). Observers (two field researchers trained in 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 an observation unit within each octagon and the eight octagons were observed in a randomised order. Insect abundances (i.e. the number of individuals recorded on flowers for all insect pollinator groups) and flower visits (i.e. the number of flowers visited by each individual bee, hoverfly, moth and butterfly) were scaled according to the number of flowers in the observation unit and survey duration to give insect counts flower⁻¹ h⁻¹ and flower visits flower⁻¹ h⁻¹, respectively.

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

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 level of spatial variation associated with changes in pollinator foraging behaviour among treatments.

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

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

2.5. Yield assessments

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).

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

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).

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

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

In 2019, 10 additional *B. nigra* plants were netted (using 75 cm x 100 cm organza bags to exclude pollinators) and distributed evenly within each FADOE octagon. These plants acted as yield control (YC) plants to determine whether pollution treatments had a direct impact on *B. nigra* yield. They were therefore cross-pollinated with each other by hand twice weekly to ensure 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 were able to establish whether any air pollution-mediated changes in pollinator foraging would be associated with any changes in plant yield metrics.

2.6. Statistical analyses

All analyses were performed using the R statistical interface v4.0.2. General (LMM) and generalized linear mixed effect models (GLMM) using the R package *lme4* (Bates et al., 2014) were used to determine the effects of air pollution treatments on NO_x and O₃ concentrations (LMM), insect pollinator visitation (total number of insects that landed on a flower within the observation unit and individual flower visits flower⁻¹ h⁻¹; GLMM), yield metrics (number of seeds 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 traps (GLMM). 'Octagon location' nested within 'Year' were included as random effects in mixed models to minimise issues associated with pseudo-replication and account for spatial and seasonal differences. 'Observer ID' was also included as a random effect in abundance and flower visitation models to account for any bias associated with differences in identification and recording skills between individuals (Westphal et al., 2008). Negative binomial models were used for pollinator abundance and flower visitation. Models for background pollinator numbers and individual insect groups were run using a poisson error distribution. Negative binomial GLMM were also used to determine whether octagon location within the field impacted pollinator abundance and flower visitation frequencies using data collected 6-24 July 2019 (one week either side of the octagon rotation inclusive; Control experiment 1). 'Rotation' (i.e. rotated vs unrotated 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-statistics based on Satterthwaite's approximation) from model summaries using the R package *lmerTest* (Kuznetsova et al., 2017) were provided for NO_x concentrations, O₃ concentrations, NO:NO₂ and background pollinator numbers to clarify their responses to the elevated pollution treatments relative to the control treatment.

3. Results and discussion

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

Fumigation in the single pollutant octagons resulted in significant increases of O_3 to 35.2 ± 0.6 ppb ($P < 0.001$) and of NO_x to 59.6 ± 1.0 ppb ($NO = 38.5 \pm 0.8$ ppb, $NO_2 = 21.2 \pm 0.3$ ppb; $P < 0.001$), relative to the control octagons (values are means (\pm SE) over the entire experimental period during the two summer seasons of 2018 and 2019; Fig. 1B). These levels were well below the current United States Environmental Protection Agency's National Ambient Air Quality Standards ($O_3 = 70$ ppb averaged over 8 hours, $NO_2 = 53$ ppb averaged annually, values for NO are not stipulated), which specify the maximum outdoor pollutant levels for public health and environmental safety (EPA, 2021). In the combined pollutant octagons, the same amount of each pollutant was emitted as for the single pollutant octagons, yet O_3 concentrations achieved were equivalent to those in the control octagons. Moreover, NO_x concentrations decreased in the combined pollutant octagons compared with the diesel exhaust-only octagons, associated with a significant reduction in $NO:NO_2$ (Fig. 1B; statistical results in Table S1). NO_x and O_3 in the troposphere commonly react with each other and, depending on local quantities of NO_x , VOCs and O_3 catalysts (in particular reactive hydrogen species such as hydroxyl and hydroperoxyl radicals), NO_x emissions can lead to both the formation and destruction of O_3 . O_3 is typically lower in urban areas or next to busy roads because it reacts with NO_x from vehicle exhaust emissions (Bae et al., 2020). For example, mean hourly concentrations of NO_x and O_3 next to the M25 motorway in Staines, UK were recorded as 84.5 and 12.5 ppb, respectively, and those recorded in an M25 motorway tunnel were 479.9 and 1.5 ppb, respectively (Sayegh et al., 2016). 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 $NO:NO_2$ compared with the diesel exhaust-only treatment because O_3 reacts with NO to produce NO_2 (Richmond-Bryant et al., 2017), which also explains 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 and, in turn, influence the behavior of odor-dependent insects, highlighting the importance of using realistic pollution concentrations and combinations when investigating the effects of air pollution on plant-insect communication processes (Blande, 2021).

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

In both diesel exhaust and O_3 treatments, individually and in combination, we recorded dramatic reductions in pollinator numbers ($\chi^2_{3,8} = 21.52$, $P < 0.001$, $N_{obs} = 352$) and flower visitation ($\chi^2_{3,8} = 35.74$, $P < 0.001$, $N_{obs} = 352$), by more than 62% (Fig. 2A) and 83% (Fig. 2B), respectively. Differences in pollinator and flower visitation frequencies between octagons remained consistent when pollutants were rotated between octagons (Treatment:Rotation $\chi^2_{3,11} = 0.36$, $P = 0.949$ and $\chi^2_{3,11} = 1.30$, $P = 0.729$, respectively; Control experiment 1), indicating that placing the octagons opposite each other in the field was sufficient to account for spatial changes in insect pollinator activity. Abundances of background pollinators (i.e. those attracted to only visual stimuli when *B. nigra* were not present in the octagons; Control experiment 2) did not vary significantly between treatments (Table S2), providing no evidence to suggest that pollinating insects were inhibited from entering the pollution octagons when floral odor cues were not a factor. While this control does not rule out the potential for air pollution to directly impair pollinator health (Leonard et al., 2019; Reitmayer et al., 2019; Thimmegowda et al., 2020) or for higher short-term (peak) concentrations to directly impair motility (Vanderplanck et al., 2021), it suggests that the observed changes in pollinator foraging behavior between treatments were most likely to be associated with changes in their attraction to plant-emitted VOCs, including floral odors, providing field-based validation and quantification of studies previously limited to laboratory conditions (Farré-Armengol et al., 2016; Girling et al., 2013; McFrederick et al., 2008).

3.3. Responses of insect pollinator groups to air pollution

To assess the impacts of air pollution on ecological processes and natural capital, it must be understood how pollutants affect different insect groups and species at field scales (Jamieson et al., 2017; Pinto et al., 2010). We found differing responses to air pollution between insect groups (Fig. 2C and D; Table S3). Air pollution treatments reduced the abundance of seven pollinator groups, which included all bees (i.e. honey bees, solitary bees and bumblebees), all flies (i.e. hoverflies and other flies), butterflies and moths. These seven groups were responsible for driving 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) octagons (Fig. 2C). Similar effects were observed for the number of flowers visited by bees, 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 wasps, were not significantly affected by pollution treatments (Fig. 2C; Table S3). Such differences between groups, and likely between different species, are to be expected, because each will utilize a unique combination of different senses and stimuli during foraging. For 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 that rely more on visual stimuli and less on the olfactory environment (McFrederick et al., 2009; Nordström et al., 2017). Furthermore, responses may differ between generalist and specialist species (McFrederick et al., 2009; Verheggen et al., 2008; Whittaker, 2001).

In general, some insect groups or species may be less reliant on those plant VOCs that are more 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 sexta*, may have the capacity to learn to associate air pollution-altered floral odor blends with a food resource (Cook et al., 2020), which, if translated to the field, could potentially mitigate the negative effects of air pollution on pollinator foraging over time. Alternatively, polluted 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 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 pollution on community ecology, and move beyond studies focusing only on single species, bi- or tri-trophic interactions, using such studies instead as a tool to elucidate mechanisms once field-scale ecological effects have been identified.

The foraging behaviors of pollinating insects such as bees and hoverflies are likely to be most affected at times of peak pollution levels, such as on hot summer days and at times of peak daily traffic adjacent to major roads or in urban areas. Phillips et al. (2021), for example, demonstrated lower densities of insect pollinators closer to roads, which is also where concentrations of pollutants are greatest. High concentrations of NO_x next to major roads tend to return to 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 O₃, emitted NO_x can also lead to O₃ formation later and further downwind (Bae et al., 2020; Sayegh et al., 2016), which as a result may deleteriously affect some insect species or communities, but not others. Therefore, further 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. Such landscape-scale studies will face additional challenges because it will be difficult to account for spatial and temporal variation, but they have the potential to provide more realistic measurements of insect foraging that could help to identify potential ecological risks. Therefore, coupling these wider field-based approaches with more controlled field studies (such as the current study) and mechanistic laboratory studies, which can identify behaviorally-important 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.

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

Reductions in flower visitation under pollution treatments coincided with significant decreases in *B. nigra* seed metrics (Table 1). *Brassica nigra* is an O₃-tolerant plant (Saunier and Blande, 2019) that is commonly found alongside major roadways and has been used as a model species for investigating plant–insect responses to multiple environmental stressors (Papazian and Blande, 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 under pollution treatments. This suggests that plant species more reliant on insect pollination, and 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 plants placed in each octagon that were netted and hand-pollinated to ensure maximum 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 observed in experimental plants and that reductions were almost certainly a result of changes in pollination rates. Air pollution can directly impact the health of many plant species, including some food crops, which can cause reduced yields (Papazian and Blande, 2020) and these results suggest that air pollution can further reduce yields through reduction of insect-mediated pollination.

4. Conclusions

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

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Declaration of competing interest

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.

Data availability

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

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Figures and Tables

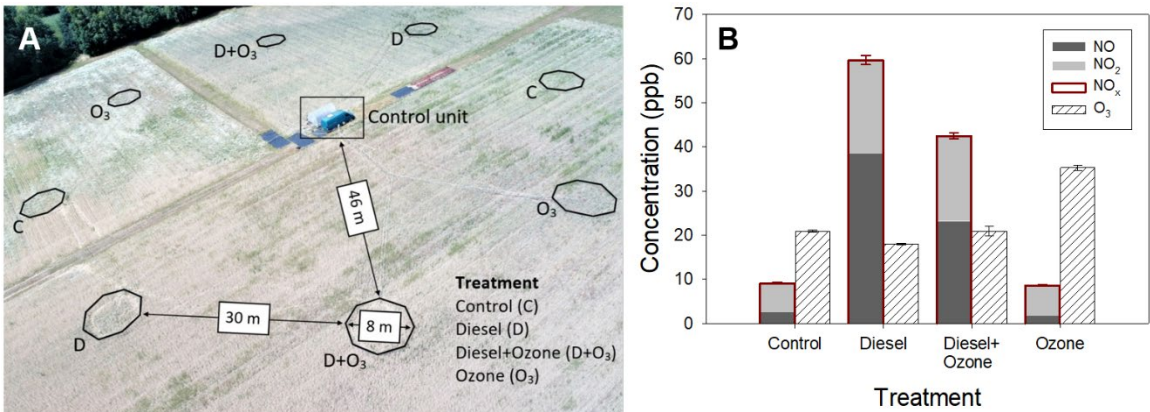


Fig. 1. Free-Air Diesel and Ozone Enrichment octagon layout during 2018 (A) and mean concentrations (\pm SE) of nitrogen oxides ($\text{NO}_x = \text{NO} + \text{NO}_2$) and ozone (O_3) within treatments (B). Octagons were distributed in an octagonal formation within a field of wheat (drone image by 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 (NO_2).

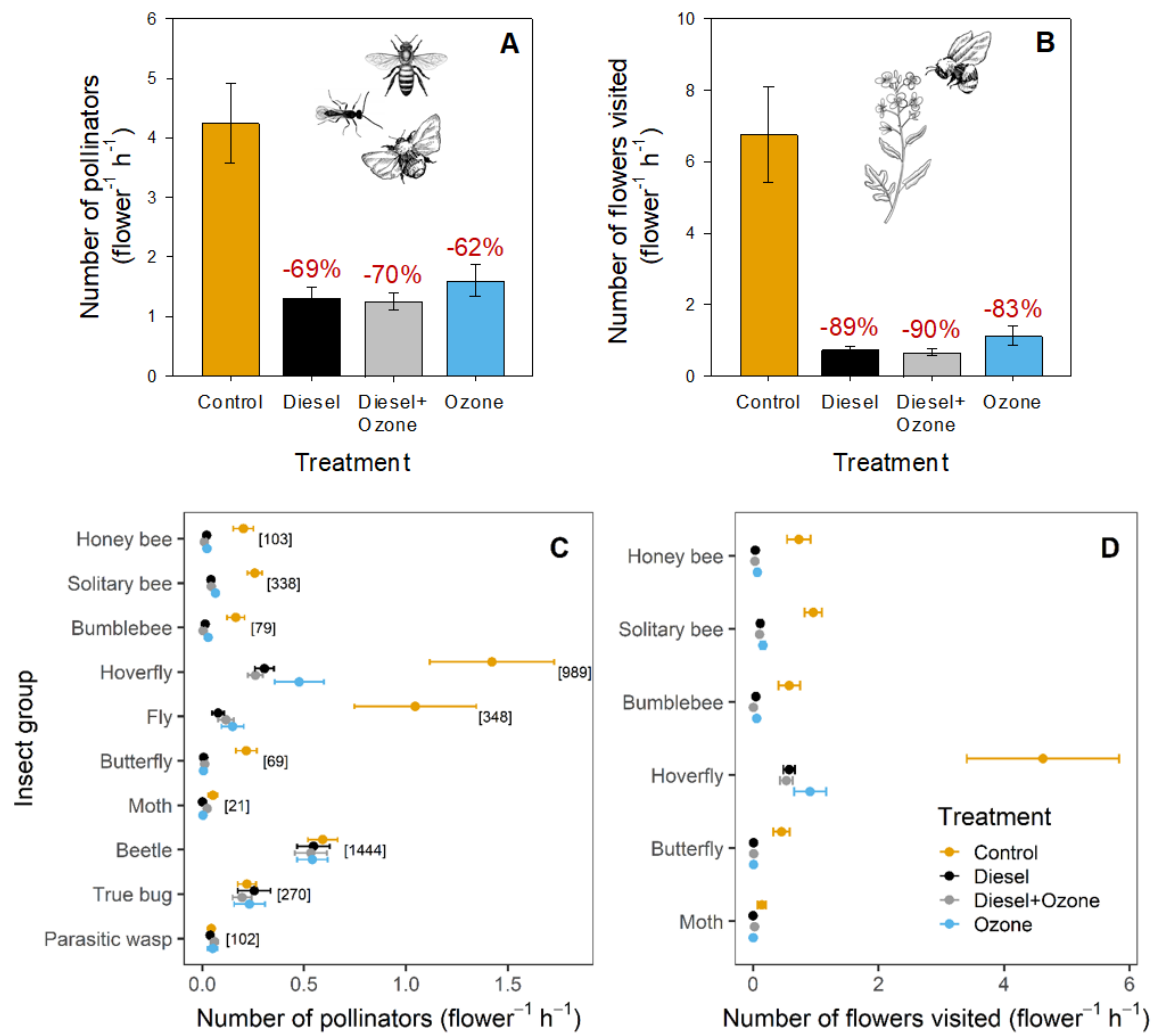


Fig. 2. The effects of diesel exhaust and ozone pollution on pollinator foraging behavior. Means (\pm SE) of pollinator abundance (A), flower visitation frequencies (B), abundances per insect group (C) and flower visits per insect group (D) were scaled according to the number of flowers within each observation 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 ($P < 0.05$). $N_{\text{obs}} = 383$ 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				Statistical analysis	
	Control	Diesel	Diesel+Ozone	Ozone	$\chi^2_{3,7(3,6)}$	P
Seeds per pod	9.49 ± 0.28	6.74 ± 0.21	6.59 ± 0.22	7.36 ± 0.24	15.05 (3.27)	0.002 (0.352)
Pod mass (g)	0.058 ± 0.003	0.041 ± 0.002	0.042 ± 0.002	0.043 ± 0.002	11.13 (1.96)	0.011 (0.581)
Pods developed (%)	51.46 ± 1.40	43.92 ± 1.10	42.59 ± 1.19	44.51 ± 1.11	16.76 (1.53)	< 0.001 (0.676)
1000-seed mass (g)	2.40 ± 0.08	2.50 ± 0.08	2.44 ± 0.08	2.29 ± 0.08	4.04 (0.95)	0.258 (0.814)
Plant dry mass (g)	14.31 ± 0.45	12.93 ± 0.50	12.98 ± 0.77	12.88 ± 0.70	0.71 (2.48)	0.870 (0.478)