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The effects of short- and long-term air pollutants on plant phenology and leaf characteristics

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1. Introduction

Ambient air pollution was estimated to cause 3.7 million premature deaths worldwide in 2012 (WHO, 2014). Although the concentrations of some industry-related pollutants such as sulfur dioxide (SO₂) have decreased in recent decades in Europe, concentrations of traffic-related pollutants, such as nitrogen oxides (NO_x) and particulate matter (PM), have even increased (Kreyling et al., 2003; Fenger, 2009).

Air quality can be improved by urban vegetation which absorbs

or filters gaseous and particulate air pollutants (Leung et al., 2011). On the other hand, plants are threatened by pollution whereby different agents lead to different effects (Mudd and Kozlowski, 1975; Taylor, 1978; Gratani et al., 2000; Beck et al., 2013).

Ozone (O₃), a secondary pollutant, is considered to affect plants severely, ranging from visible injuries to higher susceptibility to pathogens and to a reduction of plant productivity (Krupa et al., 2000; Gregg et al., 2003; Karlsson et al., 2003; Ainsworth et al., 2012). By entering leaves through the stomata, O₃ produces reactive oxygen species and causes oxidative stress, implying a reduction of photosynthesis, plant growth and biomass accumulation (Ainsworth et al., 2012). Oxides of nitrogen (NO_x) were found to affect plants depending on the concentration, length of exposure, species, stage of plant development and site characteristics leading to visible injury such as necrosis, wilting or even defoliation (Taylor

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et al., 1975). PM constitutes a diverse mixture of particles of different origin and chemical composition with diverse effects on plants and ecosystems (Grantz et al., 2003): they have an indirect influence by altering soil chemistry and thus nutrient cycling and plant nutrient uptake. PM in the air decreases the amount of incoming radiation (Kozlov and Berlina, 2002) and is therefore associated with lower temperature and carbon assimilation. However, PM is also responsible for direct effects, for example on leaf surfaces. The effects of PM deposited on leaves are related to their acidity, salinity, nutrients, trace metal content and surfactant properties (Grantz et al., 2003).

In addition, plant phenology, the timing of recurring natural events, was found to be altered by air pollution. Some studies analyzed phenology along roadsides (Bhatti and Iqbal, 1988; Shafiq and Iqbal, 2003) or at other polluted areas such as in the proximity of nickel-copper smelters or iron pellet plants (Kozlov et al., 2007). Other studies were experiment based: for example by exposing seedlings to polluted/unpolluted soil (Kozlov et al., 2007) or by fumigation of herbaceous plants using a diesel generator to simulate urban air pollution (Honour et al., 2009). In these studies, plant phenology was generally delayed due to pollution (e.g., Honour et al., 2009); however, depending on the species or pollutants, no effects were observed in some cases (e.g., Kozlov et al., 2007; Honour et al., 2009). Overall, the relative importance of single pollutants and the susceptibility of different species are not satisfactorily understood yet. Furthermore, short-term experiments might lead to unrealistic results, since the impact of pollutant exposure on plant growth is likely to become relevant only after a longer fumigation period (Honour et al., 2009).

Moreover, in order to avoid or compensate cellular damage (Dineva, 2004), leaf density and thickness are altered when exposed to environmental stressors. Morphological, structural or biochemical characteristics of plant leaves can act as bio-indicators for air pollution; numerous studies (e.g., Kardel et al., 2010; Wuytack et al., 2010, 2011; Khavaninzadeh et al., 2014) have focused on the suitability of different herbs or trees for bio-monitoring.

In this study, we conducted a vast field survey in the greater area of Munich, Germany, in order to analyze the effects of O_3 , nitrogen dioxide (NO_2), nitrogen oxides (NO_2 and NO ; NO_x), PM with an aerodynamic diameter $< 2.5 \mu m$ ($PM_{2.5}$) and $< 10 \mu m$ (PM_{10}) and $PM_{2.5}$ absorbance (which is a proxy for elemental carbon (Cyrys et al., 2003); $PM_{2.5}$ abs) on flowering and leaf unfolding onset dates of the tree species: silver birch (*Betula pendula* Roth), common hazel (*Corylus avellana* L.) and horse chestnut (*Aesculus hippocastanum* L.). Cumulated atmospheric concentrations of O_3 and NO_2 in the spring season 2010 derived from passive sampling (representing short-term exposure) and respective concentrations derived from Land Use Regression (LUR) models (representing long-term exposure) were analyzed. In addition, we assessed the influence of those pollutants on leaf morphology of birch and we were thus able to test whether leaf thickness, area, weight and specific leaf area (SLA) were useful functional traits for bio-monitoring air pollution.

2. Materials and methods

2.1. Study sites and observed plants

Munich ($48^{\circ}8' N$, $11^{\circ}35' E$; 515 m a.s.l.) is located in southern Bavaria, Germany (see Fig. 1), on the Isar river north of the Bavarian Alps and has a population size of 1.38 million. Its climate (1971–2000) is characterized by an annual mean temperature of $9.5^{\circ}C$ ($0.3^{\circ}C$ in January, $18.9^{\circ}C$ in July) and a mean sum of precipitation of 954 mm (125 mm in July, 46 mm in January) (data

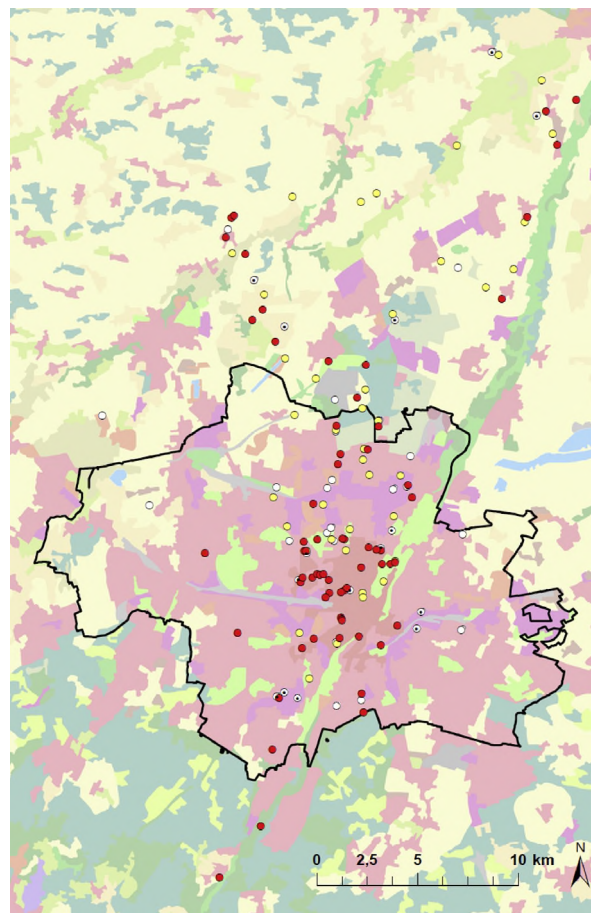


Fig. 1. Study sites of silver birch (*Betula pendula* Roth, white dots), common hazel (*Corylus avellana* L., yellow dots) and horse chestnut (*Aesculus hippocastanum* L., red dots) in the greater area of Munich, Germany. Small black dots within white dots symbolize the sites where O_3 and NO_2 passive sampling was performed in 2010. Background: CORINE Land Cover 2000 (EEA, 2010), major classes: red = urban fabric, green = forest and pastures, yellow = arable land, blue = rivers, lakes (see www.eea.europa.eu/themes/landuse/interactive/clc-download for a complete legend).

source: DWD, German Meteorological Service).

We observed flowering and leaf unfolding of silver birch and flowering of hazel and horse chestnut in 2010 (see Fig. 1 and Table 1). The phenological development was assessed every third day and assigned to the BBCH code (Meier, 2001). For analyses we selected the onset dates of BBCH 61 (beginning of flowering: 10% of flowers open/emitting pollen), BBCH 65 (full flowering: > 50% of flowers open/emitting pollen, first petals falling), BBCH 10 (mouse-ear stage: green leaf tips 10 mm above the bud scales) and BBCH 11 (first leaves unfolded).

2.2. Temperature measurements

Air temperature was recorded at each of the 38 birch observation sites using loggers (HOBO U23-001, Onset Computer Corporation, Bourne, MA, USA) which were placed in a radiation shield and mounted at a height of 3 m on the northern side of the tree. In contrast, no site-specific temperatures for hazel and horse chestnut flowering were measured; temperature data of the nearest birch monitoring site was used instead. The distance of hazel sites to the next meteorological station ranged between 20.0 m and 5.8 km and was on average 1.4 km. For flowering of horse chestnut the distance was on average 1.6 km (min = 70.0 m, max = 9.5 km).

Table 1
Observed species, number of sites and trees and analyzed phenophases in Munich in 2010. BBCH 61: beginning of flowering: 10% of flowers open/emitting pollen, BBCH 65: full flowering: >50% of flowers open/emitting pollen, first petals falling, BBCH 10: mouse-ear stage: green leaf tips 10 mm above the bud scales, BBCH 11: first leaves unfolded.

Species	Latin name	Number of sites (urban/rural)	Number of trees (urban/rural)	Analyzed phenophases
Silver birch	<i>Betula pendula</i> Roth	38 (25/13)	136 (84/52)	BBCH 61, 65, 10 and 11
Common hazel	<i>Corylus avellana</i> L.	40 (19/21)	129 (59/70)	BBCH 61 and 65
Horse chestnut	<i>Aesculus hippocastanum</i> L.	65 (45/20)	256 (201/55)	BBCH 61 and 65

2.3. Short-term air pollution exposure

Passive sampling for O₃ and NO₂ was carried out at 15 birch sites during the one-week period from May 11th to 18th 2010, i.e. roughly two weeks after the last birch trees started to flower. Most of the sites were equipped with two samplers (in total $N = 24$) and mean values were calculated from these measurements.

Passive samplers for O₃ were provided and analyzed by PASSAM AG (Männedorf, Switzerland). The NO₂ concentration was measured according to Palmes' principle (Palmes et al., 1976): a triethanolamine-aceton mixture was applied to stainless steel meshes which were subsequently air-dried for ten minutes. For each location three coated meshes were brought into an air-tight tube and fixed at the tree at a height of 3 m. Since NO₂ binds to the coated meshes by forming a triethanolamine–NO₂–complex, the adsorption of NO₂ could be determined photometrically after the exposure to ambient air.

These site-specific pollution data were only recorded at birch sites. Since we expected a high spatial variation of O₃ and NO₂ concentrations, we did not allocate these values to hazel or horse chestnut locations.

2.4. Long-term air pollution exposure

Long-term air pollution exposure was estimated by Land Use Regression (LUR) models which are often applied in epidemiological studies (Hoek et al., 2008). These models use multivariable linear regressions to analyze the associations between measured atmospheric pollution concentration and predictor variables. These predictors range from background variables such as land use, altitude or population density to traffic variables such as distance to the nearest road or traffic intensity (Briggs et al., 1997, 2000).

The concentrations of O₃ for all sites were obtained from freely available European maps with a resolution of 1 km (<http://www.integratedassessment.eu/node/831>). These maps were developed for the 15 member states (EU-15) for the year 2001 as part of the Air Pollution Modeling for Support to Policy on Health and Environmental Risks in Europe project (APMoSPHERE; <http://www.apmosphere.org/>). O₃ was modeled by kriging and LUR techniques using routine monitoring data in Airbase, a European database of air quality based on routine air pollution monitoring in the EU member states. Separate models were developed for the global, rural, and urban scales, and composite maps were prepared (Beelen et al., 2009). The concentrations of NO₂, NO_x, PM_{2.5}, PM₁₀ and PM_{2.5} abs were estimated using a combination of measurements and modeling as a part of the European Study of Cohorts for Air Pollution Effects (ESCAPE; <http://www.escapeproject.eu/>). Measurements of PM were conducted at 20 monitoring sites and of NO₂ and NO_x at 40 monitoring sites distributed throughout Upper Bavaria and Swabia regions during three periods of two weeks, each in cold, warm, and intermediate temperature seasons from October 2008 to July 2009. The annual mean concentrations of the pollutants were estimated for all sites using the ESCAPE LUR models. A more detailed description of the measurement methods, quality control, data analysis and the LUR models development in the ESCAPE study has been given in Beelen et al. (2013), Cyrys et al. (2012) and Eeftens et al. (2012a,b).

2.5. Leaf morphological characteristics of birch

Since shade is known to influence specific leaf area (SLA; Wuytack et al., 2011) we collected birch leaves from different branches of the sun crown in the end of July 2010 when leaves were fully developed and not yet affected by senescence. Nine to 20 leaves per tree ($N = 95$) at each location ($N = 38$) were used in a mixed sample to characterize site conditions, resulting in total $N = 1119$ leaves. A site consists of one to four trees which are located in the nearest proximity and are subjected to equivalent environmental conditions. Coarse particles adhered to the leaves were washed off using demineralized water. We weighted each leave and measured the thickness with a thickness tester. Leaf area was analytically determined from scanning. The specific leaf area (SLA) was calculated as the ratio of leaf area to leaf mass in cm²/g.

2.6. Statistical analyses

Since nearly all air pollution models (except for O₃) were based mainly on measurements from sites located in populated areas and therefore are believed to be more reliable within the city of Munich compared to its surroundings, we conducted our analyses also for solely urban sites. We therefore calculated an index describing the degree of urbanization for each site using the proportion of urban land use with predominantly sealed soil (according to CORINE land cover data, EEA, 2010) within a radius of 2 km. A site was classified as "urban" when the index exceeded the value of 0.5 (see also Jochnner et al., 2012, 2013).

We calculated descriptive statistics for the analyzed short- and long-term air pollutants and assessed differences between urban and rural means using t-test (for normally distributed variables) and Mann–Whitney test (for non-normally distributed variables).

In phenological studies the air temperature of the previous months is commonly related to phenological onset dates (e.g., Sparks et al., 2000). Thus, we selected the mean temperature of January and February for flowering of hazel and the mean temperature of March and April for flowering and leaf unfolding of birch and flowering of horse chestnut. Since most of the variability in onset dates of spring phenophases can be explained by air temperature (see Table S1), we selected this meteorological factor as a control variable in partial correlation analyses in order to investigate the association between air pollutants and phenology in detail. The relationship of pollutants and leaf morphological characteristics of birch were analyzed solely using bivariate correlation analyses since no association with temperature was detected (see Table S2). Stepwise linear regression was used to further investigate the relative importance of environmental variables in predicting the onset date of full flowering of the selected species.

All statistical analyses were conducted using IBM SPSS 22.0.

3. Results

3.1. Short- and long-term O₃ and NO₂ data

When comparing pollution data derived from our passive sampling campaign in 2010 with the modeled long-term concentrations from ESCAPE and APMoSPHERE (see Fig. 2a and b), we

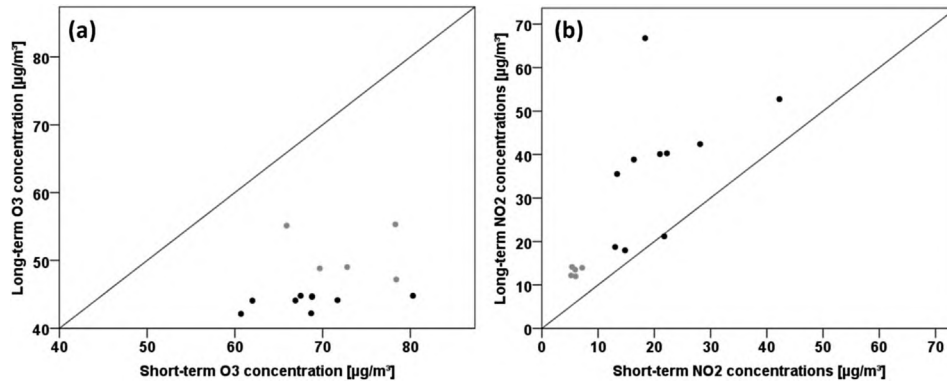


Fig. 2. Scatterplots of short-term and long-term atmospheric concentrations for (a) O_3 and (b) NO_2 . Black dots symbolize urban sites ($N = 10$), gray dots rural sites ($N = 5$).

found no significant correlation for O_3 ($r_s = 0.435$, $p > 0.05$), but a significant and high correlation for NO_2 ($r_s = 0.868$, $p < 0.001$). Short-term O_3 concentrations ranged between 60.7 and 80.3 $\mu\text{g}/\text{m}^3$; however, long-term exposure levels were much lower and ranged between 38.8 and 55.5 $\mu\text{g}/\text{m}^3$. For NO_2 , instead, short-term data (5.2–42.2 $\mu\text{g}/\text{m}^3$) underestimated long-term data (11.5–66.8 $\mu\text{g}/\text{m}^3$).

Most of the air pollutants showed higher levels in urban compared to rural areas (Table 2). The only exception was O_3 which was significantly enhanced at rural sites when considering long-term data. Except O_3 and $PM_{2.5}$, all pollutants were more variable (higher standard deviation) in the urban environment. O_3 (short-term) and $PM_{2.5}$ (long-term) did also not differ significantly between urban and rural sites.

3.2. Influence of short- and long-term pollution exposure on phenology

We did not find any significant correlations of phenological onset dates with short-term pollution concentrations for the analyzed species and phases (Table 3). Instead, we found a few significant correlations when long-term pollution levels were considered (Table 3). Interestingly, atmospheric O_3 did not affect phenological onset dates of birch when all sites were analyzed; however, when restricting the analyses to urban sites, birch phenophases (except for BBCH 10) were significantly delayed with increasing O_3 concentrations. The highest correlation coefficient was obtained for BBCH 61 ($r = 0.589$, $p < 0.01$). The urban sites,

however, were associated with a generally lower variation of O_3 (see Table 2). An example for the spatial variability of O_3 along with the onset date of flowering of birch and the corresponding temperatures can be seen in Fig. S1.

Hazel flowering was not affected by variations of O_3 (Table 3). However, NO_2 , NO_x and $PM_{2.5}$ abs were significantly and positively correlated with onset dates of full flowering (delayed phenology with increasing pollution), especially when solely urban sites were regarded. In this case the coefficients were higher and even significant correlations with PM_{10} and PM_{10} were revealed. These correlations were especially high for PM_{10} ($r = 0.634$, $p < 0.01$). Since the pollutants are intercorrelated (see Table S3) we also calculated partial correlations with NO_x , NO_2 and temperature as control variable. Here, no significant correlations with $PM_{2.5}$, PM_{10} and $PM_{2.5}$ abs could be detected (not shown).

We did not find any correlations with flowering onset dates of horse chestnut and pollutant concentrations of NO_2 , NO_x , $PM_{2.5}$, PM_{10} and $PM_{2.5}$ abs (Table 3). However, we obtained significant correlation coefficients for O_3 that were again higher (up to $r = 0.509$, $p < 0.01$) when rural sites were excluded.

Stepwise linear regression analyses were used to identify the best predictors for a parsimonious model, regardless of possible intercorrelations between variables (e.g., temperature and O_3 , see Table S3). The analyses for full flowering of birch resulted in one significant model with air temperature as sole predictor (see Table 4). For common hazel two models were obtained: one model with air temperature and one model with air temperature and NO_x . However, the inclusion of NO_x only increased R^2 by 3.7%. For horse

Table 2

Descriptive statistics of short- and long-term air pollutant concentrations at all sites and separated for urban and rural sites. SD = standard deviation, Min = minimum, Max = maximum, all values in $\mu\text{g}/\text{m}^3$. Equality of means (tested by t-test or Mann–Whitney test) refers to differences between urban and rural sites, significance levels: *** $p \leq 0.001$, ** $p \leq 0.01$, ns: not significant ($p > 0.05$).

	Urban and rural sites				Urban sites				Rural sites				Equality of means	
Short-term	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	p	Test
O ₃	70.0	5.6	60.7	80.3	68.5	5.4	60.7	80.3	73.0	5.4	65.9	78.4	ns	t-test
NO ₂	16.1	10.2	5.2	42.2	21.1	8.8	13.0	42.2	5.9	0.8	5.2	7.2	**	t-test
	Urban and rural sites				Urban sites				Rural sites				Equality of means	
Long-term	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	p	Test
O ₃	45.2	4.2	38.8	55.5	43.0	1.9	38.8	45.2	49.0	4.2	43.2	55.5	***	Mann–Whitney test
NO ₂	23.2	10.8	11.5	66.8	27.6	10.8	15.3	66.8	16.0	6.1	11.5	43.9	***	Mann–Whitney test
NO _x	39.8	21.2	19.7	131.8	46.5	22.4	23.5	131.8	28.8	13.3	19.7	90.8	***	Mann–Whitney test
PM _{2.5}	14.1	1.4	11.3	18.4	14.2	1.3	11.5	17.9	13.9	1.5	11.3	18.4	ns	Mann–Whitney test
PM ₁₀	19.7	3.7	14.8	34.9	20.7	3.8	14.8	34.9	18.1	2.8	14.8	27.8	***	Mann–Whitney test
PM _{2.5} abs	1.8	0.5	1.3	3.6	1.9	0.5	1.3	3.6	1.6	0.4	1.3	3.4	***	Mann–Whitney test

Table 3

Partial correlations (control variable: air temperature) between phenological onset dates (BBCH 61: beginning of flowering: 10% of flowers open/emitting pollen), BBCH 65: full flowering: > 50% of flowers open/emitting pollen, first petals falling, BBCH 10: mouse-ear stage: green leaf tips 10 mm above the bud scales, BBCH 11: first leaves unfolded) of silver birch (*Betula pendula* Roth), common hazel (*Corylus avellana* L.) and horse chestnut (*Aesculus hippocastanum* L.) and short-term exposure of pollutants (O₃, NO₂, only for silver birch) and long-term exposure of pollutants (O₃, NO₂, NO_x, PM_{2.5}, PM₁₀ and PM_{2.5} abs) in Munich (urban and rural sites as well as solely urban sites) in 2010, significance levels: ** $p \leq 0.01$, * $p \leq 0.05$.

Silver birch	Urban and rural sites				Urban sites			
	BBCH 61	BBCH 65	BBCH 10	BBCH 11	BBCH 61	BBCH 65	BBCH 10	BBCH 11
Short-term								
O ₃	-0.359	-0.273	-0.175	-0.138	-0.290	-0.119	-0.207	-0.249
NO ₂	0.166	0.058	-0.058	0.050	0.340	0.171	-0.029	0.157
Long-term								
O ₃	-0.028	-0.103	-0.263	-0.260	0.589**	0.467*	0.140	0.433*
NO ₂	-0.012	0.008	-0.027	-0.151	0.075	0.065	0.053	-0.098
NO _x	-0.026	-0.002	-0.052	-0.169	0.074	0.068	0.028	-0.101
PM _{2.5}	0.093	0.064	0.008	-0.026	0.106	-0.016	-0.084	-0.089
PM ₁₀	-0.127	-0.226	-0.226	-0.314	0.028	-0.074	-0.137	-0.211
PM _{2.5} abs	-0.002	0.012	-0.046	-0.178	0.078	0.071	0.026	-0.134
Common hazel	BBCH 61	BBCH 65			BBCH 61	BBCH 65		
O ₃	-0.130	-0.058			0.079	-0.172		
NO ₂	0.271	0.345*			0.319	0.564*		
NO _x	0.281	0.354*			0.365	0.577*		
PM _{2.5}	0.224	0.208			0.350	0.498*		
PM ₁₀	0.118	0.206			0.341	0.634**		
PM _{2.5} abs	0.282	0.341*			0.247	0.473*		
Horse chestnut	BBCH 61	BBCH 65			BBCH 61	BBCH 65		
O ₃	0.408**	0.396***			0.509**	0.482***		
NO ₂	-0.142	-0.098			-0.136	-0.034		
NO _x	-0.095	-0.016			-0.107	0.038		
PM _{2.5}	-0.118	0.039			-0.075	0.059		
PM ₁₀	-0.076	-0.017			-0.151	0.005		
PM _{2.5} abs	-0.007	0.091			-0.040	0.106		

chestnut also two models were obtained: one model with air temperature and one model with air temperature and O₃. The model with two predictors resulted in an increase of R^2 by 6.3%.

When only focusing on urban sites (Table 5) full flowering of birch was associated with O₃ as significant predictor in the first model and O₃ and temperature in the second model. The model with another parameter increases R^2 by 8.1%. O₃ was the only significant predictor for flowering of horse chestnut. Common hazel, however, was associated with PM₁₀ in the first model and with PM₁₀ and O₃ in the second model, resulting in an increase of 7.1%. Linear regressions with solely temperature as predictor yielded in an R^2 of 34.4% (birch), 6.5% (hazel) and 18.8% (horse chestnut), respectively (data not shown).

3.3. Influence of pollution on leaf morphological characteristics of birch

We did not find any significant correlation of the leaf morphological characteristics (mass, area and thickness, specific leaf areas (SLA)) of birch and short-/long-term pollutants when all sites were considered (Table 6). When excluding rural sites we found one

significant correlation with NO_x and SLA indicating that higher levels of this pollutant increase SLA.

4. Discussion

4.1. Air pollution and phenology

Short-term O₃ concentration was not related to phenology (see Table 3). This may be due to the fact that O₃ is highly variable from one week to another (Schipa et al., 2009). The selected period for passive sampling in our study was characterized by warm and dry weather conditions which probably led to an overestimation of long-term O₃ concentrations. This is also confirmed by Fig. S2 which shows the annual course of O₃ at two monitoring stations in Munich: here, most of the year is characterized by lower values than the mean value of our passive sampling campaign. These results suggest some caution when interpreting short-term pollution data collected in field studies.

By analyzing long-term data we found that increasing levels of O₃ delayed plant phenology of birch and horse chestnut (see Table 3). Birch species are typically regarded as O₃ sensitive species

Table 4

Stepwise linear regression for the explanatory variable BBCH 65 (full flowering: > 50% of flowers open/emitting pollen, first petals falling) for silver birch (*Betula pendula* Roth), common hazel (*Corylus avellana* L.) and horse chestnut (*Aesculus hippocastanum* L.) in Munich in 2010. Significant predictors: T = air temperature (common hazel: mean of January and February; silver birch and horse chestnut: mean of March and April), O₃: long-term exposure of ozone, NO_x: long-term exposure of nitrogen dioxide, significance levels: *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, R^2 : = goodness of fit.

Species	Model no.	Predictor variables (p)	(Adjusted) R^2	Model p	Formula
Silver birch	1	T (***)	39.0	***	BBCH 65 = -3.5 T + 136.8
Common hazel	1	T (***)	51.9	***	BBCH 65 = -12.2 T + 53.1
	2	T (***), NO _x (*)	55.6	***	BBCH 65 = 0.1 NO _x - 14.1 T + 46.7
Horse chestnut	1	T (***)	51.0	***	BBCH 65 = -6.3 T + 174.9
	2	T (***), O ₃ (***)	57.3	***	BBCH 65 = 0.4 O ₃ - 4.4 T + 143.8

Table 5

Stepwise linear regression for the explanatory variable BBCH 65 (full flowering: > 50% of flowers open/emitting pollen, first petals falling) for silver birch (*Betula pendula* Roth), common hazel (*Corylus avellana* L.) and horse chestnut (*Aesculus hippocastanum* L.) in solely urban sites of Munich in 2010. Significant predictors: T = air temperature (common hazel: mean of January and February; silver birch and horse chestnut: mean of March and April), O₃: long-term exposure of ozone, PM₁₀: long-term exposure of particulate matter < 10 µm, significance levels: ****p* ≤ 0.001, ***p* ≤ 0.01, **p* ≤ 0.05, R² = goodness of fit.

Species	Model no.	Predictor variables (<i>p</i>)	(Adjusted) R ²	Model <i>p</i>	Formula
Silver birch	1	O ₃ (**)	35.9	**	BBCH 65 = 1.3 O ₃ + 52.2
	2	O ₃ (*), T (*)	44.0	***	BBCH 65 = 0.9 O ₃ - 3.4 T + 94.4
Common hazel	1	PM ₁₀ (***)	42.2	**	BBCH 65 = 1.0 PM ₁₀ + 44.6
	2	PM ₁₀ (***), O ₃ (*)	49.3	**	BBCH 65 = 1.1 PM ₁₀ - 1.0 O ₃ + 87.6
Horse chestnut	1	O ₃ (***)	34.6	***	BBCH 65 = 0.9 O ₃ + 90.9

(Matyssek, 2001); thus, we suggest that the delay in spring phenology with increasing O₃ levels might be a consequence of the species' sensitivity. Although the variation of O₃ is lower at urban compared to rural sites (see Table 2), this environmental factor was only relevant for full flowering of birch in stepwise regression analyses when rural sites were excluded (see Table 5). We did not find an influence of O₃ on birch phenology when urban and rural sites were jointly considered. This was somewhat unexpected since the lowest concentrations of O₃ are typically found in urban areas (here: minimum: 38.8 µg/m³, mean: 43.0 ± 1.9 µg/m³) and the highest in rural areas (here: maximum: 55.5 µg/m³, mean: 49.0 ± 4.2 µg/m³, see Table 2). The importance of O₃ for full flowering of hazel and for horse chestnut was particularly evident when urban sites were solely considered. Interestingly, the predictive power of O₃ was found to outweigh that of temperature (likely by combining the effects of temperature and O₃), especially for horse chestnut and birch.

In our study, we additionally found that NO₂ and NO_x were positively correlated with full flowering of common hazel (see Table 3). The correlation was especially high when rural sites were excluded. LUR were generally developed for urban environments in order to mirror the within-city variability of air pollutants (Briggs et al., 1997). Therefore, we suggest that the modelled data also represents the pollutant concentrations in urban areas better than adjacent rural sites. This is probably related to the fact that the variability of NO₂ and NO_x is especially high in urban areas (see Table 2). It can be assumed that later flowering stages are more vulnerable to the exposure of NO₂ and NO_x since we did not observe significant influences on earlier flowering stages. The importance of NO_x for hazel flowering was also underlined with stepwise linear regression analyses, increasing R² by more than 6% (see Table 4).

Delayed flowering onset of some herbaceous species was also demonstrated by Honour et al. (2009) who installed a diesel generator to produce NO_x concentrations representative of urban conditions in a fumigation experiment. The authors also

documented an accelerated senescence and therefore provided evidence for harmful effects of traffic pollution on plant phenology. However, it was also shown that the species' response differed considerably indicating a species-specific susceptibility to air pollution.

The effects of PM on foliar processes are believed to be small or even non-existent except when the exposure is considerably high (Grantz et al., 2003). This might be the reason why the amount of PM₁₀ (maximum: 34.9 µg/m³, mean: 19.7 ± 3.7 µg/m³) and PM_{2.5} (maximum: 18.4 µg/m³, mean: 14.1 ± 1.4 µg/m³, see also Table 2) estimated for our study sites did not have an effect on most of the phenophases. However, full flowering of common hazel was delayed with increasing PM levels when solely urban areas were considered (Table 3). Stepwise regression analyses revealed that PM was only important for full flowering of hazel when rural sites were excluded (see Table 5). The exclusion of temperature yielded in superior models which were only based on estimates of PM₁₀ or PM₁₀ along with O₃. Thus, these predictors might not only mirror the effect of temperature but also of pollution. A foliar uptake of chemicals is not plausible, since leaves only develop after flowering and it is more likely that PM might have exerted an indirect effect via altering soil chemistry. This consequence is also believed to be the major effect of PM on plants (Grantz et al., 2003). In general, there are high correlations with PM and other pollutants (see Table S3); however, the relative abundance and importance of single chemicals within PM could not be evaluated in our study. The majority of identified direct effects of PM on phenology was reported to occur in severely polluted areas, for example around factories which melt or produce heavy metals (Kozlov et al., 2007).

4.2. Pollution and leaf morphology

With respect to leaf morphological characteristics we only found a significant and positive correlation between NO_x and SLA (Table 6) indicating that higher levels of this pollutant increase the surface to weight ratio (which is linked to a greater leaf area and

Table 6

Spearman rank correlations between leaf morphological characteristics (M: mass, F: area, THK: thickness, SLA: specific leaf area) of silver birch (*Betula pendula* Roth) and pollutants (O₃, NO₂, NO_x, PM_{2.5}, PM₁₀ and PM_{2.5} abs) in Munich (urban and rural sites as well as solely urban sites) in 2010, significance level: **p* ≤ 0.05.

	Urban and rural sites				Urban sites			
	M	F	THK	SLA	M	F	THK	SLA
Short-term								
O ₃	-0.320	-0.193	-0.241	0.232	-0.340	-0.371	-0.188	-0.006
NO ₂	0.068	-0.118	0.336	-0.339	0.224	0.297	0.236	-0.164
Long-term								
O ₃	-0.196	-0.187	-0.272	0.040	-0.277	-0.156	-0.381	0.142
NO ₂	0.025	-0.021	0.101	-0.048	-0.036	0.104	-0.058	0.220
NO _x	0.004	-0.018	0.035	0.031	-0.098	0.128	-0.194	0.398*
PM _{2.5}	0.077	0.114	-0.032	0.140	-0.093	0.090	-0.116	0.343
PM ₁₀	-0.152	-0.166	-0.045	0.094	-0.114	0.036	-0.038	0.272
PM _{2.5} abs	-0.151	-0.109	-0.038	0.161	-0.239	-0.045	-0.182	0.378

less density and/or thickness).

In general, the sensitivity of plants to O₃ varies between species, cultivars or clones and is generally assessed using different measures: e.g., growth, visible injury, senescence of leaves or stomatal responses (Pääkkönen et al., 1997). A greater tolerance of birch clones was linked to higher stomatal density and thicker leaves, characteristics which improve the detoxification of O₃ (Pääkkönen et al., 1997).

There exist a number of studies which demonstrated effects of pollution on plant leaves: mean leaf size, number of leaves and foliage area of silver birch was found to be reduced with higher O₃ levels (Pääkkönen et al., 1997). In addition, Oksanen et al. (2005) found that elevated O₃ led to thinner leaves of silver birch but CO₂ decreased the total leaf thickness and SLA, pointing to counteracting effects of different atmospheric gases. Antagonistic but also synergistic and additive effects could also explain the non-significant correlations with most of the pollutants measured (short-term) and estimated (long-term) in our study. Thus, we suggest that controlled fumigation experiments may be supportive for disentangling the effects of different air pollutants on leaf morphology of birch.

The influence of air pollution on SLA of other species varies greatly (Wuytack et al., 2011). Pooerter et al. (2009) suggested that SLA of monocots increases under higher O₃ levels but decreases for dicots. Wuytack et al. (2011), for example, found that NO_x and O₃ influenced SLA of *Salix alba* L., however, the authors could not separate the relative importance of both pollutants and suggested that amplifying and extenuating effects are likely which also stresses the need for studies using experimental designs.

We conclude that *in situ* measurements of foliar characteristics of birch are not suitable for bio-monitoring of air pollution. We only detected a significant relationship with SLA and NO_x, however, the correlation coefficient was too low ($r_s = 0.398$) to allow for an adequate estimation of NO_x exposure. It is also likely that the pollution within our study area is far too less to involve substantial changes in leaf morphology of birch.

4.3. Ecological consequences of pollution

The influence of pollution on phenology may lead to a failure of fingerprinting climate change. Generally, phenology is regarded as an excellent bio-indicator for climate change, since air temperature is able to explain a huge amount of the variability in phenological onset dates of temperate species in spring (Menzel and Fabian, 1999). However, the associations with air pollution found in our study might also be attributable to other environmental conditions that are statistically correlated with pollution. Although we excluded the influence of air temperature on phenological onset dates in partial correlation analyses (Table 3), there might be other factors (e.g., radiation, soil nutrients) that are altered by pollution/urbanization.

In general, delays in phenology affect a range of ecological processes such as the start of CO₂ uptake via photosynthesis and the start of pollination which is also important for human health when allergenic plants are considered.

5. Conclusions

Our study demonstrated that concentrations of air pollutants were significantly associated with delays in spring phenology. However, inconsistencies between long- and short-term exposure effects of pollution suggest some caution when interpreting results. Since ongoing global change will be associated with an increase in air pollution (Pozzer et al., 2012), especially in the city, further monitoring of direct and indirect effects on vegetation, also within

controlled experiments, seems inevitable.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2015.07.040>.

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