

RESEARCH PAPER

Nutrient status: a missing factor in phenological and pollen research?

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Abstract

Phenology ranks among the best ecosystem processes for fingerprinting climate change since temperature explains a high percentage of the interannual or spatial variation in phenological onset dates. However, roles of other environmental variables, such as foliar nutrient concentrations, are far from adequately understood. This observational study examined the effects of air temperature and 11 nutrients on spring phenology of *Betula pendula* Roth (birch) along an urban–rural gradient in Munich, Germany, during the years 2010/2011. Moreover, the influence of temperature, nutrients, and air pollutants (NO₂ and O₃) on the amounts of pollen and catkin biomass in 2010 was evaluated. In addition to the influence of higher temperatures advancing phenological onset dates, higher foliar concentrations of potassium, boron, zinc, and calcium were statistically significantly linked to earlier onset dates. Since flushing of leaves is a turgor-driven process and all the influential nutrients are involved in cell extension, membrane function, and stability, there might be a reasonable physiological interpretation of the observed association. The amounts of pollen were negatively correlated with temperature, atmospheric NO₂, and foliar iron concentration, suggesting that these variables restrict pollen production. The results of this study suggested an influence of nutritional status on both phenology and pollen production. The interaction of urbanization and climate change should be considered in the assessment of the impact of global warming on ecosystems and human health.

Key words: *Betula pendula* Roth, birch, catkin biomass, Munich, nutrients, phenology, pollen, temperature, urban heat island.

Introduction

Temperature is the most important driver of plant phenology in temperate and boreal regions (Menzel and Fabian, 1999; Wielgolaski, 1999). Analyses of long-term phenological series have revealed that more than two-thirds of the variance in onset dates can be explained by temperature alone, with higher temperatures typically associated with earlier onset dates (Menzel and Fabian, 1999).

In addition to temperature, external and internal factors, ranging from soil humidity, diseases, pests, competition, and pollutants to individual genes and plant age, have also been implicated as influencing plant phenology (Menzel, 1999). Sophisticated modelling approaches characterizing the effects of these variables independent of temperature on phenology have resulted in few statistically significant effects (Sparks *et al.*, 1997).

Long-term aerobiological data (e.g. Spieksma *et al.*, 1995; Ziello *et al.*, 2012) and experimental studies using urban-rural gradients as surrogates for climate change (e.g. Ziska *et al.*, 2003) have revealed associations between warmer conditions and/or atmospheric CO₂ concentrations and increases in the amounts of pollen of various plant species. However, little is known about the role of additional factors, such as by nutrients and pollutants, in the modification of pollen production.

There has been little research on the effect of nutrients on timing of phenological events, with most studies predominantly related to agriculture (Dobermann and Cassman, 2002; Steiner *et al.*, 2007) and horticulture (Reickenberg and Pritts, 1996; Yang *et al.*, 2011). There are only a few studies in forest science (e.g. Sigurdsson, 2001; Weih and Karlsson, 2001), some with focus on seedlings and physiological parameters such as growth rates (Iivonen *et al.*, 2001; Weih and Karlsson, 2001). The studies have typically analysed soil nutrients, either by manipulation experiments using fertilizers (Weih and Karlsson, 2001) or by quantifying the actual availability of a small number of nutrients in the soil (Wielgolaski, 2001).

However, nutrient availability in the soil is not related to nutrient concentrations in the leaves in a straightforward way; there are environmental cues that influence nutrient uptake and translocation rates. Variations in soil temperature modify the activity of soil microorganisms and nutrient mineralization (White and Haydock, 1970; Fisher, 1980; Marschner, 1995; Radrizzani *et al.*, 2011). Through the effect of air temperature on transpiration, higher temperatures can support greater nutrient uptake and translocation rates (Marschner, 1995; Wielgolaski, 2001). Therefore, foliar nutrient concentrations are most suitable for the assessment of the environmental conditions to which plants are exposed. Finally, traditional soil analyses may not account for heterogeneous soil conditions, especially in urban areas, where sealed surfaces, such as concrete and tarmac, impede access to the soil.

This study aimed to evaluate the impact of nutritional status on phenology and pollen/catkin characteristics (i.e. amount of pollen, catkin weight) using foliar nutrient analyses for *Betula pendula* Roth (silver birch) in an observation network characterized by different degrees of urbanization within the greater area of Munich. In contrast to many other studies, air temperature and humidity were measured directly at the trees. This observational study is believed to be the first to investigate the effects of foliar nutrient concentrations on plant phenology and pollen/catkin characteristics at the mesoscale.

Materials and methods

Study area

The study was conducted in Munich (48°8'N, 11°35'E), a city located in southern Bavaria, Germany, on the Isar river north of the Bavarian Alps at an altitude of ~515 m a.s.l. (Fig. 1). Only observation sites that were located on calcareous gravel and covered with loamy river sediments or calcareous low level moor were included in the analysis.

Phenological data

Flowering and leaf phenophases of birch were recorded every third day during the spring seasons of 2010 and 2011 in the greater Munich area (Fig. 1) using the extended BBCH scale (Biologische Bundesanstalt, Bundessortenamt and CHEMical industry scale) for development stages of mono- and dicotyledonous agricultural plants (Meier, 2001). The scale allows the evaluation of principal growth stages, such as flowering and leaf unfolding, and substages, such as buds showing a green tip and flower buds visible, that are passed through consecutively during the development process. This study focused on four stages: beginning of flowering (BBCH 61), full flowering (BBCH 65), leaves at mouse-ear stage (BBCH 10), and first leaves unfolded (BBCH 11). Phenological onset dates of 2–6 trees were averaged at each of the 34 analyzed sites (see black dots in Fig. 1).

Pollen data

Additionally, up to 200 birch catkins per tree were collected during the flowering season in spring 2010 from 40 different individual trees across 22 different sites located in urban and rural areas of Munich (see yellow dots in Fig. 1). Catkins were collected between when they emit pollen sporadically (phase BBCH 60) and the beginning of flowering. They were dried for 24 h, and pollen was extracted by sieving (sieve particle size of 100 µm followed by 72 µm). Catkin biomass was assessed by weighing, and the amount of pollen was quantified in g⁻¹ of catkin.

Temperature data

Temperature data were recorded at each phenological observation site ($n=34$, black dots in Fig. 1) using sensors (HOBO U23-001, Onset Computer Corporation, Bourne, MA, USA) that were installed in a radiation shield on the northern side of one representative birch tree at 3 m above ground level.

NO₂ and O₃ determination

Nitrogen dioxide (NO₂) and ozone (O₃) concentrations were also measured directly at the stem of the birch trees ($n=22$, yellow dots in Fig. 1). Passive sampling of these pollutants lasted 1 week in spring 2010 in order to assess the general characteristics of air quality of the study sites. NO₂ passive samplers were photometrically analyzed for NO₂ adsorption. For this purpose, stainless steel meshes were immersed in a triethanolamine–acetone mixture and dried for 10 min in ambient air. Three meshes were attached in a hermetic tube and NO₂ was fixed on these meshes by forming a triethanolamine–NO₂ complex. O₃ passive samplers were provided and analyzed by Passam AG (Männedorf, Switzerland).

Assessment of nutritional status

Foliar samples were collected according to common practice from different branches of the sun crown at the optimum time for sampling (end of July 2010) when leaves were fully developed and not yet affected by senescence or relocation of nutrients (Wellbrock *et al.*, 2006). Ten leaves from every tree at each location were used in a mixed sample to characterize conditions at the site.

Adhering coarse particles were washed off using demineralized water. The leaves were dried (65 °C) for 2 d in an oven and subsequently milled. After digestion with freshly distilled concentrated HNO₃, the elements phosphorus (P), potassium (K), sulphur (S), boron (B), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn) were determined using ICP-OES (Spectro, Model Genesis; Kleve, Germany). Total nitrogen (N) was measured using an elemental analyzer (Elementar; Hanau, Germany). The nutritional status of birch was assessed by the same method as used by Göttlein *et al.* (2011) based on the receptive data

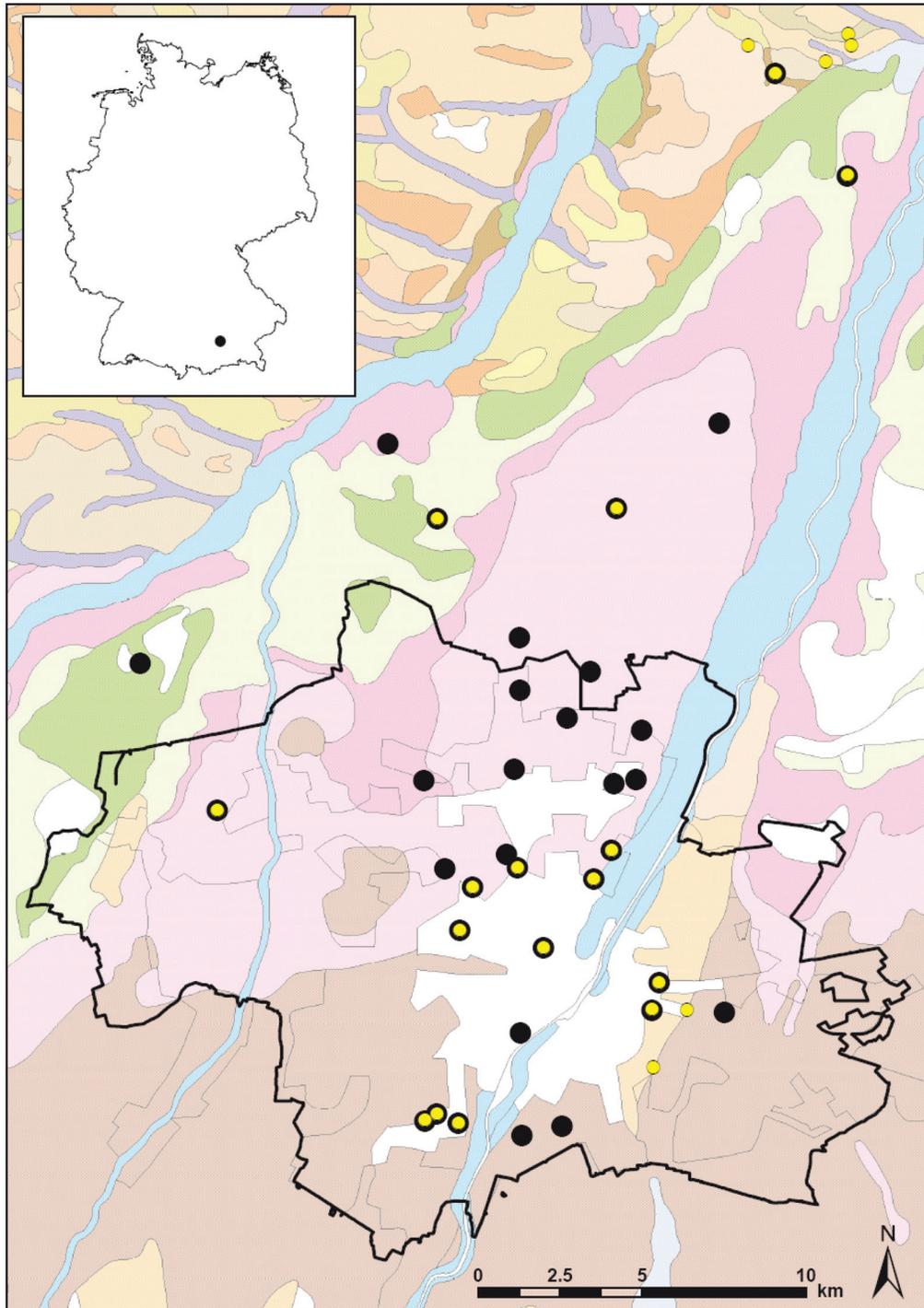


Fig. 1. Locations of the selected birch sites in the greater area of Munich, Germany (city outline denoted by the solid black line; source: Landeshauptstadt München). Black dots denote sites where meteorological measurements, nutrient analyses, and phenological observations were collected ($n=34$); yellow dots indicate measurements of amounts of pollen/catkin weights, nutrient analyses, and measurements of pollutant ($n=22$). Background: terrain map BK200 (1:200 000; source: Bayerisches Landesamt für Umwelt); beige, primarily Calcaric Regosols derived from deposited humic material above carbonatic gravel or building site rubble; light pink, Calcaric Regosols derived from carbonatic gravel, mostly covered with a shallow layer of marly or loamy river sediments; dark pink, primarily Calcaric Regosols very rich in humus derived from carbonatic gravel, mostly covered with a layer of marly or loamy river sediments; brown, primarily Luvisols and their transitional forms to Cambisols derived from carbonatic gravel covered with layers of loamy river sediments; dark green, primarily low-level moor, some of them calcareous; light green, primarily Humic Gleysols derived from fluvial marl over carbonatic gravel. For a complete legend see www.geoportal.bayern.de. Inset: location of the study area (dot) within Germany.

listed in the literature compilation of van den Burg (1985, 1990). Nutrient threshold values for normal, excess, and deficient nutrient supply were obtained. The data set was corrected for outliers, and the respective threshold values were only calculated if at least three data records were available.

Statistical analyses

Partial correlation analyses

For a general overview of the relationships between phenology, pollen/catkin characteristics, and the environmental variables temperature, nutrients, and pollutants, partial correlations were calculated using the urban index as a control variable. The urban index describes the degree of urbanization and was calculated for each site using the method of Jochner et al. (2012). It is based on land use data derived from CORINE Land Cover (CLC) 2006 data (www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster) with a spatial resolution of 100 m. Particular elements of artificial surfaces, such as continuous and discontinuous urban fabric, and industrial and commercial units, that are characterized by a high degree of impervious surfaces were extracted using ArcGIS 10 (ESRI 2009, Redlands, CA, USA). The proportion of urban land use was subsequently calculated within a 2 km radius. Since this index is an estimate of the degree of urbanization and therefore includes *inter alia* temperature and pollution effects, artificial correlations attributable to the site selection are eliminated. For example, the inner city of Munich is characterized not only by a greater amount of calcareous soil but also by higher temperatures compared with the northern countryside. A plain correlation analysis might therefore only reveal that the nutrient Ca is positively correlated with temperature and therefore negatively correlated with onset dates.

Since this study incorporates two different data sets [phenology data ($n=34$) with nutrients and temperature; and pollen data ($n=22$) with nutrients, pollutants, and temperature] that comprise 2 years and 1 year, respectively, further statistical analyses are based on different methods. For the phenology data set, linear mixed effects (LME) models to account for multiple years were used; for the pollen data set, linear regression models sufficed for the single year measurements.

Linear mixed effects models (LMEs)

The influence of air temperature and leaf nutrients on phenological onset dates was assessed using LME models fitted by maximum likelihood estimation. Stepwise variable selection for the different nutrients was performed using the Bayesian information criterion (BIC) (Schwarz, 1978); the commonly used Akaike's information criterion (AIC) was also reported.

Plots of the four birch phenophases versus temperature indicated dependence on the year (Fig. 2a–d), which could be separated into global yearly temperature effects (red versus blue) and location- and year-specific site effects (individual dots). To account for the global effect of yearly averaged March–April temperature, the variable $avgT$, which was computed as the average March–April temperature across all sites for each of the two years (2010=7.08 °C; 2011=8.99 °C), was included as a fixed effect in the model. Additionally, site- and year-specific March–April temperatures (T) were included as fixed effects in the model, along with interactions between these and the year-average temperatures. All of these covariates were modelled as continuous variables. In its most general form, taking into account effects of all 11 nutrients, the following mixed model was used:

$$ph = b_0 + b_1T + b_2 avg T + b_3 avgT \times T + \sum_{k=1}^{11} b_{3+k} nutr_k + site + \varepsilon, \quad (1)$$

where ph is the onset date of the phenophase, $nutr_k$ is the site-specific concentration of the k -th plant nutrient (measured once, in 2010), $site$ is the random effect of each individual site assumed

independently and normally distributed with mean 0 and variance σ_{site}^2 [$\sim N(0, \sigma_{site}^2)$], and $\varepsilon \sim N(0, \sigma^2)$ are the independent errors. The regression parameters (b) and variance parameters ($\sigma_{site}^2, \sigma^2$) are estimated using maximum likelihood and stepwise model selection to choose the optimal subset of nutrients among $nutr_1, \dots, nutr_k$ for inclusion in the model.

Spearman's correlation coefficients were calculated for each pair of nutrients. To account for possible multicollinearity among nutrients destabilizing the model selection procedure, alternative models were also run beginning with only one nutrient at a time and testing for additional inclusion of other nutrients using the likelihood ratio test (LRT). BICs of all models were calculated and compared with the BIC from the stepwise procedure.

Linear models (LMs)

To determine the influence of the nutrients on the amount of pollen and catkin biomass, data from just 2010 were available, so a linear model without random effects was used. In addition to foliar nutrients, site-specific temperature (March–April) and pollutant data (O_3 and NO_2) were incorporated. Variable selection was performed using BIC-based stepwise selection as above. The adjusted R^2 was used to assess the amount of variance explained by the covariates. The general form of the models for the amount of pollen and catkin biomass was:

$$pollen/catkin = b_0 + b_1T + b_2O_3 + b_3NO_2 + \sum_{k=1}^{11} b_{3+k} nutr_k + \varepsilon_i, \quad (2)$$

All calculations and graphics were performed using the statistical software R (R Development Core Team) and the mixed model package lme4 (Bates and Mächler, 2010).

Results

Nutritional status

Table 1 reports the nutritional status of the analyzed birch trees according to the threshold values derived from the van den Burg data set. For N, the well-known 'thinning effect' in forest nutrition studies (Larcher, 1994) was confirmed, with a threshold value for extreme deficit slightly higher than the threshold for trees included in the next deficiency category. Dominance of shallow soils on calcareous gravel was reflected by the nutritional status of the trees. Most trees showed an excessive supply of Ca, and, consequently, for the elements Fe, Mn, and Zn, the majority of trees were classified at the lower end and below the adequate range. Most trees were classified as latent deficient for S and N, while for K, P, Mg, Cu, and B, most trees fell within the range of adequate nutrition. Extreme nutrient deficiencies were not found, and only a few trees showed a marked deficiency for some of the elements.

Influence of nutrient concentrations on onset dates

Table 2 shows average (across 2010 and 2011) partial correlation coefficients (adjusted for urban index) between foliar nutrient concentrations and phenological onset dates. The most important variables for phenology (according to the size

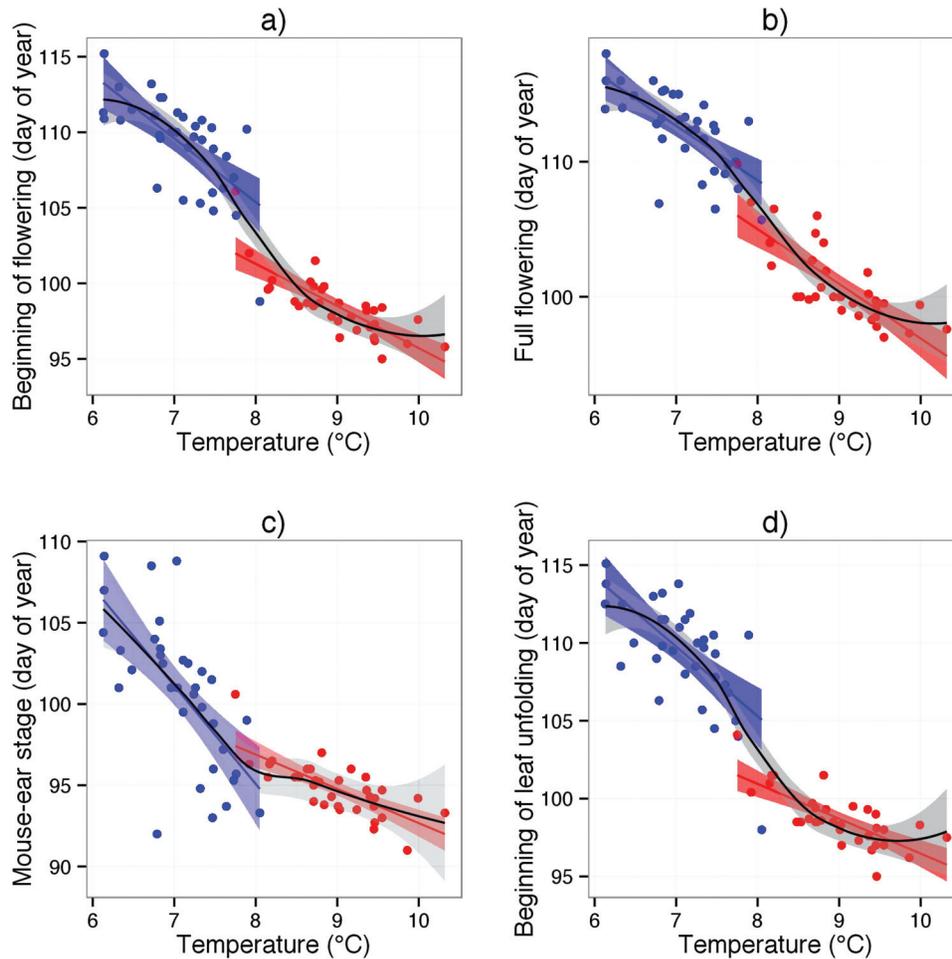


Fig. 2. Phenological onset dates (day of year) versus mean March and April temperatures ($^{\circ}\text{C}$) for birch in 2010 (blue) and 2011 (red) with pointwise 95% confidence bands (shaded). The black line represents the trend with both years grouped together. The black line with its confidence band is an estimation using local weighted scatterplot smoothing (LOWESS). The blue and red lines are estimates of linear models and the corresponding confidence bands. (a) Beginning of flowering (BBCH 61); (b) full flowering (BBCH 65); (c) mouse-ear stage (BBCH 10); and (d) leaf unfolding (BBCH 11).

Table 1. Foliar threshold values of nutrient supply of birch (deficit, adequate, and excess range) derived from the van den Burg data set, and number (n) of trees that fell into the respective category.

Nutrient ^a	Deficiency range ^b			Normal range			Surplus range ^c					
	Deficiency	n	Latent	n	Lower	n	Central	n	Upper	n	Luxury	n
N	<19.2	1	19.2–24.6	18	24.6–25.8	6	25.8–30.2	9	30.2–33.4	0	33.4–37.9	0
P	<1.3	1	1.3–1.9	6	1.9–2.4	3	2.4–3.5	20	3.5–4.2	4	4.2–5.3	0
K	<5.4	1	5.4–7.2	4	7.2–8.9	10	8.9–11.9	14	11.9–13.1	4	13.1–31	1
Ca			<4.4	0	4.4–6.2	0	6.2–9.5	3	9.5–11.1	4	>11.1	27
Mg	<1.2	0	1.2–2	2	2–2.5	5	2.5–3.5	14	3.5–4.1	11	>4.1	2
S	<1.4	2	1.4–1.9	20	1.9–2	7	2–2.6	5	2.6–3.1	0	>3.1	0
Fe			<85	32	85–99	2	99–144	0	144–175	0	>175	0
Mn			<142	33	142–377	1	377–783	0	783–954	0	>954	0
Cu			<5	4	5–6	6	6–8	24	8–9	0	>9	0
Zn			<176	16	176–374	16	374–773	2	773–973	0	>973	0
B			<26	1	26–38	10	38–53	15	53–56	2	56–300	6

^a Units are mg g^{-1} for N through S, and $\mu\text{g g}^{-1}$ for all others.

^b There were no trees in the extreme deficiency ranges defined for N (<19.4), K (<3.6), Mg (<1.1), and Mn (<12).

^c There were no trees in the extreme surplus ranges defined for N (>37.9), P (>5.3), K (>1), and B (>300).

Table 2. Correlations (adjusted for urban index) between different phenophases of birch (2010 and 2011) and foliar nutrient concentrations in Munich.

BBCH	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
61	-0.061 ^{NS}	-0.174 ^{NS}	-0.559 ^{***}	-0.274 ^{NS}	-0.147 ^{NS}	-0.220 ^{NS}	-0.126 ^{NS}	0.094 ^{NS}	-0.106 ^{NS}	-0.330 ^(*)	-0.459 ^{**}
65	-0.087 ^{NS}	-0.239 ^{NS}	-0.554 ^{***}	-0.383 [*]	-0.275 ^{NS}	-0.193 ^{NS}	-0.344 [*]	-0.067 ^{NS}	-0.164 ^{NS}	-0.390 [*]	-0.472 ^{**}
10	0.051 ^{NS}	-0.108 ^{NS}	-0.598 ^{***}	-0.434 [*]	-0.114 ^{NS}	0.117 ^{NS}	-0.392 [*]	0.005 ^{NS}	-0.093 ^{NS}	-0.420 [*]	-0.479 ^{**}
11	0.086 ^{NS}	-0.196 ^{NS}	-0.600 ^{***}	-0.495 ^{**}	-0.316 ^(*)	-0.127 ^{NS}	-0.295 ^(*)	-0.045 ^{NS}	-0.029 ^{NS}	-0.474 ^{**}	-0.576 ^{***}

BBCH 61, beginning of flowering; BBCH 65, full flowering; BBCH 10, mouse-ear stage; BBCH 11, first leaves unfolded.
^{***} $P \leq 0.001$; ^{**} $P \leq 0.01$; ^{*} $P \leq 0.05$; ^(*) $P \leq 0.1$; NS, not significant.

of the correlation coefficients and their significance) were K and B, followed by Zn and Ca. For K, the coefficients were highly significant ($P \leq 0.001$) and ranged between $r = -0.554$ and $r = -0.600$, indicating that higher K concentrations were associated with earlier flowering and leaf unfolding onset dates. This relationship also applied for B; higher concentrations were linked to earlier phenophases. For B, the coefficients ranged between $r = -0.459$ and $r = -0.576$ (all $P \leq 0.01$). In particular, correlation coefficients for Zn and Ca were also negative and in most cases significant ($P \leq 0.05$), but somewhat smaller.

The LME models revealed that among the 11 considered nutrients, only K was predictive for birch phenology (Table 3). This nutrient was a statistically significant predictor for the phenological phases mouse-ear stage ($P \leq 0.01$), beginning of leaf unfolding ($P \leq 0.01$), and beginning of flowering ($P \leq 0.05$), but was not associated with full flowering. The impact of K ranged between -0.26 and -0.47 d mg^{-1} K in 1 g of leaves. This means that—in the case of constant temperatures—phenological onset dates were advanced by 1–2 d if the K concentration was raised by 4 mg g^{-1} .

Figure 3a illustrates the relationship between beginning of flowering and K, as well as temperature. For an average foliar K concentration of $\sim 9 \text{ mg g}^{-1}$, a temperature increase from 7°C to 9°C advanced the phenological onset by 6 d from 20 April [day of year (DOY) 110] to 14 April (DOY 104). When considering a constant temperature of 8°C , the increase in the K concentration from 6 mg g^{-1} to 10 mg g^{-1} still resulted in a 1 d earlier flowering onset date. However, the mouse-ear stage (Fig. 3b) was more strongly influenced by K since advances amounted to 1.9 d using the scenario of an increase in K concentration by 4 mg g^{-1} . For leaf unfolding (Fig. 3c), the advance was 1.5 d. Comparing the mouse-ear stage (Fig. 3b) and beginning of flowering (Fig. 3a), it was obvious that the K concentration was almost twice as important for the leaf phenophase. The greater influence on leaf phenophases in general was also demonstrated by the fact that K was not significant for the model incorporating full flowering.

As anticipated, there were high correlations between many of the nutrients (Table 4), especially between K and B ($r = 0.672$), Ca and Mg ($r = 0.772$), and N and S ($r = 0.828$) (all $P \leq 0.001$). The evaluation of a single nutrient at a time to avoid multicollinearity using LRT also demonstrated that the optimal model included K in addition to site and temperature effects. Besides, the analyses showed that there were mostly

non-significant or minor correlations between nutrients and temperature (Table 4).

Influence of nutrient concentrations on pollen/catkin characteristics

The amount of pollen was negatively correlated not only with temperature ($r = -0.770$), but also with K ($r = -0.497$), indicating that this nutrient does not promote pollen production (Table 5). In addition, the amount of pollen was also negatively influenced by Fe ($r = -0.575$). The same effect was observed with atmospheric NO_2 concentration ($r = -0.479$). Catkin weight was particularly influenced by the same nutrients, most influencing phenology: high concentrations of K, B, Zn, and Ca significantly increased catkin biomass ($r \sim 0.5$).

To identify meaningful influential factors further, linear regression models were calculated based on stepwise selection. The models revealed the variables that were most suitable in statistical modelling of pollen/catkin characteristics (Table 6). The amount of pollen was mostly influenced by mean temperature and Fe, achieving an adjusted R^2 value of 72.5%; this equates to an increase in model fit of 14.3% compared with the model using temperature alone. Also the values of AIC and BIC confirmed that the model with Fe is superior to the model solely based on air temperature (Table 6; values in parentheses). An increase of $100 \mu\text{g Fe g}^{-1}$ of leaves would lead to a decrease in the amount of pollen by 0.1 g and is hence almost comparable with a temperature increase of 1°C .

In contrast, the best model for catkin weight was based on more explanatory variables (Table 6). The inclusion of temperature, Ca, N, and P achieved an adjusted R^2 value of 73.8% and is therefore far higher than the value of 11.8% that was calculated for the linear model incorporating only temperature.

Discussion

Phenological onset dates

Air temperature measured at the birches was able to explain between 55.7% and 83.7% of the variance in onset dates (Table 3). Recent studies in urban phenology, however, reported lower values of R^2 calculated from linear regression analyses. For example, modelling of *Prunus yedoensis* (Yoshino cherry) flowering onset dates in Tokyo, Japan, by

Table 3. LME model results and coefficients: Percentage of explained variance due to explanatory variables in the linear mixed effects models and their respective AIC and BIC values (here, values in parentheses refer to linear mixed effects models solely based on temperature; lower AICs and BICs indicate better fitting models), estimated regression coefficients and their standard deviations (SD), and the SD of random site effects.

BBCH	Explained variance				Criterion			Covariates				Random effect	
	T	avgT	avgT:T	K	AIC	BIC	Intercept (SD)	T (SD)	avgT (SD)	avgT:T (SD)	K (SD)	SD	
61	83.7***	2.6***	Not included	0.6*	282.3 (299.7)	295.5 (310.0)	151.38 (2.09)	-2.973** (0.47)	-2.63*** (0.52)	Not included	-0.26* (0.12)	0.625	
65	81.5***	1.3***	Not included	Not included	291.8 (301.2)	302.9 (308.5)	154.30 (1.78)	-3.99*** (0.51)	-1.94*** (0.55)	Not included	Not included	1.068	
10	55.7***	0.3 ^{NS}	5.8***	3.0**	314.8 (321.0)	330.3 (303.9)	262.26 (31.81)	-20.37*** (4.12)	-16.91 ^{NS} (3.98)	2.13*** (0.50)	-0.47** (0.16)	0.946	
11	81.4***	3.0***	1.1**	1.1**	284.8 (290.6)	300.2 (334.2)	228.98 (25.64)	-12.39*** (3.32)	-12.53*** (3.21)	1.20** (0.40)	-0.37** (0.12)	0.715	

K, potassium; T, mean March and April temperature; avgT:T, interaction of factor variable year and temperature; BBCH 61, beginning of flowering; BBCH 65, full flowering; BBCH 10, mouse-ear stage; BBCH 11, first leaves unfolded.

*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; (^{NS}) $P \leq 0.1$; NS, not significant.

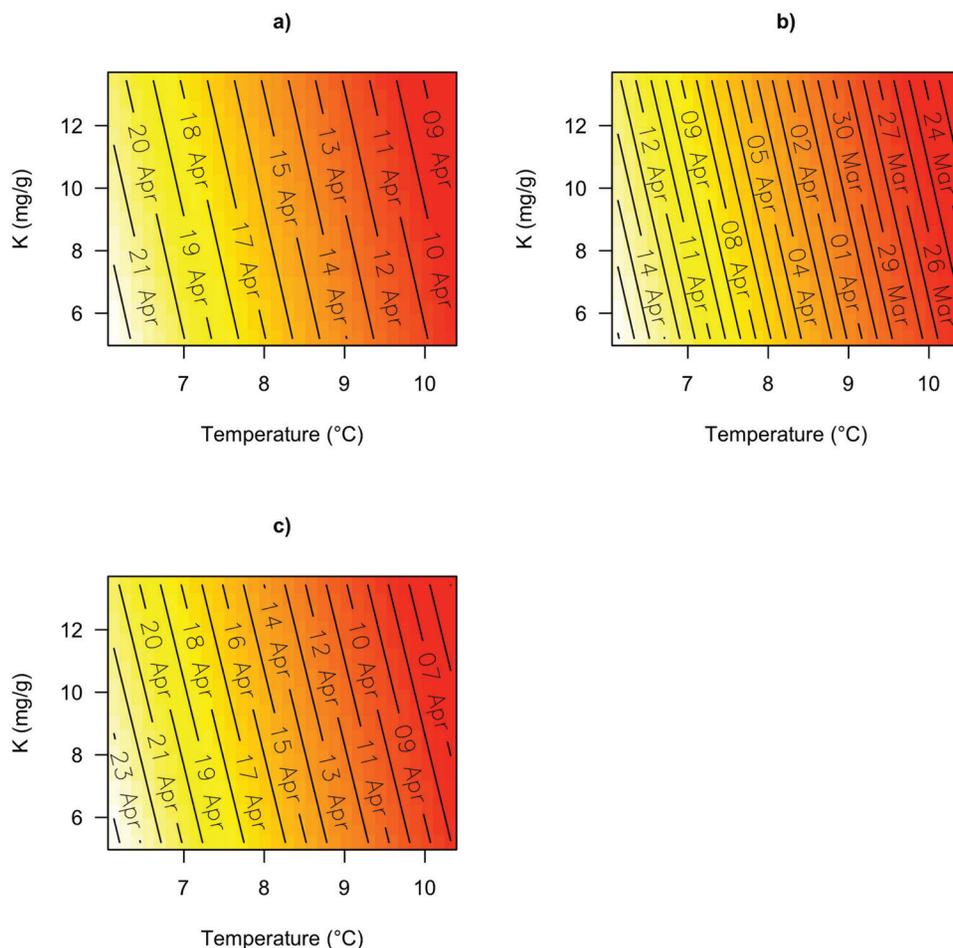


Fig. 3. Illustration of the relationship between onset dates, and mean March and April temperatures ($^{\circ}\text{C}$) as well as foliar K (potassium) concentration (mg g^{-1}). The contour lines with corresponding label denote the onset date. (a) Beginning of flowering (BBCH 61); (b) mouse-ear stage (BBCH 10); and (c) leaf unfolding (BBCH 11).

mean March temperature in 2004 revealed an R^2 value of 52% (Matsumoto *et al.*, 2009). Lakatos and Gulyás (2003) calculated an R^2 value of 42% for onset dates of full flowering of *Forsythia suspensa* (forsythia) and urban heat island intensity in Debrecen, Hungary, in spring 2003. These relatively

modest R^2 values may probably be related to smaller temperature variations between the selected sites within one observation year.

Besides the obvious relationship between temperature and phenology, this study was the first that showed the statistical

Table 4. Spearman correlation matrix for mean March–April temperature (T) and foliar nutrient concentrations.

	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
T	–0.089 ^{NS}	0.220*	0.223*	0.069 ^{NS}	0.079 ^{NS}	–0.025 ^{NS}	0.134 ^{NS}	0.151 ^{NS}	0.071 ^{NS}	0.246*	0.269*
N		0.303*	0.336**	–0.081 ^{NS}	–0.067 ^{NS}	0.828***	–0.041 ^{NS}	–0.086 ^{NS}	0.483***	–0.152 ^{NS}	0.197 ^{NS}
P			0.484***	0.141 ^{NS}	0.405***	0.273*	0.418***	0.534***	0.481***	0.382**	0.529***
K				0.289*	0.155 ^{NS}	0.461***	0.302*	0.189 ^{NS}	0.335**	0.407***	0.672***
Ca					0.772***	0.020 ^{NS}	0.302*	0.436***	–0.134 ^{NS}	0.501***	0.346**
Mg						0.023 ^{NS}	0.446***	0.642***	0.079 ^{NS}	0.603***	0.415***
S							–0.099 ^{NS}	–0.124 ^{NS}	0.610***	0.010 ^{NS}	0.370**
Fe								0.468***	0.226(*)	0.450***	0.468***
Mn									0.077 ^{NS}	0.479***	0.293*
Cu										0.22(*)	0.353**
Zn											0.443***

*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; (*) $P \leq 0.1$; NS, not significant.

Table 5. Correlations (adjusted for urban index) between pollen amounts, catkin biomass, and mean March–April temperature (T), foliar nutrient concentrations, and air pollutants in Munich 2010.

Variable	T	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B	O ₃	NO ₂
Amount of pollen	−0.770***	0.152 ^{NS}	0.172 ^{NS}	−0.490*	−0.425(*)	−0.144 ^{NS}	−0.106 ^{NS}	−0.575**	0.325 ^{NS}	−0.286 ^{NS}	−0.244 ^{NS}	−0.332 ^{NS}	−0.044 ^{NS}	−0.479*
Catkin biomass	0.429(*)	0.185 ^{NS}	−0.037 ^{NS}	0.527*	0.440(*)	0.228 ^{NS}	0.342 ^{NS}	0.176 ^{NS}	0.008 ^{NS}	−0.138 ^{NS}	0.519*	0.526*	0.071 ^{NS}	0.194 ^{NS}

*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; (*) $P \leq 0.1$; NS, not significant.

Table 6. Percentage explained variance according to explanatory variables in the linear regression models and their respective AIC and BIC values (here, values in parentheses refer to linear regression models solely based on temperature; lower AIC and BIC values indicate better-fitting models).

Variable	Explained variance					Criterion		Coefficients					
	T	Fe	Ca	N	P	AIC	BIC	Intercept	T	Fe	Ca	N	P
Amount of pollen	58.2***	14.3*	Not included	Not included	Not included	−65.12 (−59.22)	−62.03 (−56.90)	0.619***	−0.057***	−0.001*	Not included	Not included	Not included
Catkin biomass	11.8***	Not included	24.5***	23.44**	14.0*	−54.08 (−36.83)	−49.45 (−34.51)	−1.190***	0.092***	Not included	0.013***	0.269**	−0.041*

T, March and April temperature; Fe, iron; Ca, calcium; N, nitrogen; P, phosphorus.
*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; (*) $P \leq 0.1$; NS, not significant.

association of plant nutrients and birch onset dates in detailed analyses. All the elements with a higher number of significant partial correlations—K, B, Zn, and Ca (Table 2)—have an influence, directly or indirectly, on cell extension as well as on membrane function and stability (Marschner, 1995). Their influence varied between flowering and leaf unfolding phenophases. However, in all cases, the observed relationship showed that an increase of the foliar nutrient concentrations of K, B, Zn, and Ca was associated with an advance in phenological onset dates, with K being the nutrient having the strongest correlations with phenology, especially with leaf unfolding.

K is characterized by a high mobility in plants and, in addition to other physiological functions, it contributes most to the osmotic potential of cells and tissues (Marschner, 1995). Increasing the osmotic potential in the vacuoles is essential for cell extension, and it is a long known phenomenon in forest nutrition that K-deficient plants show a clearly reduced growth (Becker-Dillingen, 1939). In contrast to the present findings, where it could be demonstrated that K had the strongest effect on phenological onset dates, Nord and Lynch (2009) proposed that deficiencies in K are less likely to affect the phenology or growth of plants. However, flushing of leaves is a turgor-driven process that is initiated in spring by bud burst whereby a high amount of K might be supportive of rapid development. Catkins of birch, however, are already formed in the previous year, long before leaf expansion. With increasing temperature, anthers will extend and consequently pollen sacs will emit pollen, whereas relative humidity plays an important role in the dehiscence of the sacs (Laaidi et al., 2003). Thus, K should have a greater effect on leaf unfolding phenophases of birch than on flowering phenophases, as

shown in this study. Note that these different phenophases occur within the same week.

Zn deficiency results in growth depression, mainly inhibition of internode elongation and reduced leaf size (Bergmann, 1993; Marschner, 1995). In the present study, about half of the investigated birch trees showed Zn deficiencies (Table 1). The observed correlation of Zn concentration and phenology confirms the beneficial role of Zn for functioning and growth of shoots. Although Zn and phenological onset dates of birch were strongly related according to the partial correlation analysis (Table 2), Zn was not selected within the LME models.

During cell elongation, Ca stimulates the synthesis of cell wall precursors. Furthermore, Ca is of high importance for membrane stability and functionality (Marschner, 1995). Although a relationship between Ca and phenological phases was indicated by the partial correlation (Table 2), its importance in phenology at the mesoscale was not confirmed using the LME approach.

B is involved in a number of metabolic pathways and plays a major role in cell wall biosynthesis and structure as well as plasma membrane integrity, with B deficiency in the shoot inducing symptoms similar to Zn deficiency. Both elements, Zn and B, have an influence on the metabolism of indole-3-acetic acid (IAA), a phytohormone which plays an important role in cell division and elongation (Marschner, 1995). The relationship of phenological onset dates of birch and the nutrient B became obvious in the partial correlation analysis. However, B was always excluded from the LME models.

In the end, K showed the highest predictive power in LME models and in many cases prevented the other elements from

being included in the models. Correlations with nutrients and temperature (Table 4) suggest that regression analyses cannot untangle direct causal effects of nutrients and temperatures on phenological onset dates from indirect effects, such as the effect of temperature on nutrients and the effect of nutrients on onset dates. The fact that K remained statistically significantly associated with phenological onset dates after adjustment for temperature is typically regarded as evidence that it has independent predictive value; hence, the indirect effect of temperature on nutrients is accounted for. In addition, likelihood ratio tests revealed that K was associated with the highest importance, and a direct relationship between phenology and K concentrations seems reasonable.

A literature search revealed conflicting findings on the effects of soil fertilization on phenology. Roberntz (1999) and Amundson *et al.* (1995), for example, found no effect on bud burst of Norway and Red spruce growing on fertilized soils. However, Chandler and Dale (1990) reported an advancement of bud burst of fertilized Sitka spruces by 3 weeks. Earlier bud burst was also demonstrated by Amundson *et al.* (1995). In contrast, Wielgolaski (2001), who incorporated analyses of available soil nutrients without additional fertilizers, reported high levels of P, K, Mg, and Ca in the soil that delayed plant development. This was especially true for early phenophases, but not for phases that occurred later in the season (e.g. cultivated plants), and strongly depended on high temperatures. Therefore, the author concluded that the influence of nutrients measured in the soil was moderated by climatic factors. This finding is also in agreement with Nord and Lynch (2009) who proposed that higher temperatures, via altering soil moisture, lead to higher mineralization rates and therefore affect nutrient availability. This confirms the present suggestion that soil nutrient availability cannot adequately reflect the physiological performance of a tree and supports the evaluation of foliar nutrient concentrations.

Amount of pollen and catkin biomass

The present results showed that the amount of pollen was significantly reduced at sites with higher temperatures ($r = -0.770$, Table 5). However, the relationship between temperature and amount of pollen in the existing literature appears not to be straightforward. Some studies suggested that higher temperatures resulted in higher pollen loads. For example, experimental studies showed that an increase in temperature (Ziska *et al.*, 2003) and in atmospheric CO₂ concentration (Ziska and Caulfield, 2000; Wayne *et al.*, 2002; Ziska *et al.*, 2003) also increased the amount of *Ambrosia artemisiifolia* (common ragweed) pollen. Field observations in the study area of Munich in 2009 (Jochner *et al.*, 2011) showed no significant difference in the amounts of urban and rural birch pollen per catkin. In addition, there is evidence from studies analysing long-term pollen trap data that amounts of pollen have increased over time, most probably as a consequence of temperature increase (e.g. Rasmussen, 2002; Frei and Gassner, 2008) or rising CO₂ concentration (Ziello *et al.*, 2012). In contrast, Emberlin *et al.* (1999) proposed that an urban polluted environment exerts more stressful conditions

for plant species, resulting in decreased amounts of pollen. This is also in accordance with Guedes *et al.* (2009) who found decreased pollen production, along with smaller pollen sizes, of *Chenopodium alba* (white goosefoot) in urban Porto, Portugal. Furthermore, drought conditions in southern Switzerland in the extreme warm summer of 2003 were associated with remarkably small airborne amounts of pollen of *Rumex* spp., *Urtica* spp., and *Artemisia* spp. (Gehrig, 2006). The present finding—that the amounts of pollen were negatively influenced by temperature—might also be related to adverse high urban temperatures. Since birch mainly grows at lower temperatures in mid to high latitudes, an increase in temperature might restrict the plant's physiological performance, for example via a decrease in pollen production (Ziello *et al.*, 2012).

In addition, the present study showed that higher temperatures were associated with a greater weight of birch catkins (Table 5). This is in accordance with Ziska *et al.* (2003) who found a significant increase in catkin length of common ambrosia in urban areas of Baltimore. However, in the present study, multiple regression analysis showed that Ca, N, and P had more predictive power than temperature alone. In previous studies, the increase in urban biomass was also attributed to an increase in CO₂ concentration (ragweed; Ziska and Caulfield, 2000) or decrease in tropospheric O₃ concentration (cottonwood; Gregg *et al.*, 2003). In the present study, however, an O₃ effect on catkin biomass could not be detected.

Conclusions

The interaction of urbanization and climate change has to be considered in the assessment of impacts on ecosystems. Although this study was observational, it demonstrated that not only temperature but also leaf nutrient concentration was statistically associated with the timing of birch phenology. Of all the nutrients considered, it was shown that leaf unfolding phenophases were most associated with K. In addition, birch trees growing under warmer conditions were characterized by heavier catkins. However, the amount of pollen was less compared with cooler locations. It can be assumed that cities with their urban heat island and air and soil pollution might already create stressful conditions for plants, especially for birch, resulting in a decrease in pollen production and therefore pollination and reproductive success. Ongoing global change and temperature increase might result in major changes for pollen productivity and, in turn, for public health. Experimental studies are scarce and lack a range of allergenic plants, especially trees. Further research in this field is required to disentangle the main influential factors, for example on pollen production, and the different responses by different species. There is a need to consider the role of both air pollutants and plant nutrients on plant fitness in future research.

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