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

Susanne Jochner, Josef Höfler, Isabelle Beck, Axel Göttlein, Donna Pauler Ankerst, Claudia Traidl-Hoffmann, and Annette Menzel

1 Department of Ecology and Ecosystem Management, Ecoclimatology, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 2, D-85354 Freising, Germany
2 Mathematical Statistics, Technische Universität München, Boltzmannstraße 3, D-85748 Garching b. München, Germany
3 Center of Allergy and Environment (ZAUM), Technische Universität München and Helmholtz Center, Biedersteiner Str. 29, D-80802 Munich, Germany
4 Department of Ecology and Ecosystem Management, Forest Nutrition and Water Resources, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 2, D-85354 Freising, Germany
5 Department of Dermatology and Allergy, Technische Universität München, Biedersteiner Str. 29, D-80802 Munich, Germany

*To whom correspondence should be addressed. E-mail: jochner@wzw.tum.de

Received 10 December 2012; Revised 11 February 2013; Accepted 18 February 2013

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. Spiksma 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 Casman, 2002; Steiner et al., 2007) and horticulture (Reichenberg 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 g⁻¹ of catkin.

Temperature data
Temperature data were recorded at each phenological observation site (n=34, black dots in Fig. 1) using sensors (HOB0 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 respective 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 a common, 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 $\text{avgT}$, 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:

$$ ph = b_0 + b_T \text{avgT} + b_3 \text{avgT} \times T + \sum_{k=1}^{11} b_{3+k} \text{nutr}_k + \text{site} + \epsilon, \quad (1) $$

where $ph$ is the onset date of the phenophase, $\text{nutr}_k$ is the site-specific concentration of the $k$-th plant nutrient (measured once, in 2010), $\text{site}$ is the random effect of each individual site assumed independently and normally distributed with mean 0 and variance $\sigma^2_\text{site} \sim N(0, \sigma^2_\text{site})$, and $\epsilon \sim N(0, \sigma^2)$ are the independent errors. The regression parameters ($b$) and variance parameters ($\sigma^2_\text{site}, \sigma^2$) are estimated using maximum likelihood and stepwise model selection to choose the optimal subset of nutrients among $\text{nutr}_1, \ldots, \text{nutr}_{11}$ 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:

$$ \text{pollen/catkin} = b_0 + b_T \text{avgT} + b_3 \text{avgT} \times T + \sum_{k=1}^{11} b_{3+k} \text{nutr}_k + \epsilon,$$

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
Nutrients, phenology, and pollen | 2085

Fig. 2. Phenological onset dates (day of year) versus mean March and April temperatures (°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 | Deficiency range | Normal range | Surplus range |
|----------|------------------|--------------|--------------|
|          | Deficiency n | Latent n | Lower n | Central n | Upper n | Luxury n | 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 | 2  |
| 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 | 20  |
| 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 | 3  |
| Ca       | <4.4  0 | 4.4–6.2  0 | 6.2–9.5  3 | 9.5–11.1  4 | >11.1  1 | 11  |
| 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 | 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 | 3  |
| Fe       | <85  32 | 85–99  2 | 99–144  0 | 144–175  0 | >175  0 | 175  | 0  |
| Mn       | <142  33 | 142–377  1 | 377–783  0 | 783–954  0 | >954  0 | 954  | 0  |
| Cu       | <6  4 | 5–6  6 | 6–8  24 | 8–9  0 | >9  0 | >9  | 0  |
| Zn       | <176  16 | 176–374  16 | 374–773  2 | 773–973  0 | >973  0 | >973  | 0  |
| B        | <26  1 | 26–36  10 | 36–53  15 | 53–56  2 | >56  0 | >56  | 0  |

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

\[\text{b} \quad \text{There were no trees in the extreme deficiency ranges defined for N (<19.4), K (<3.6), Mg (<1.1), and Mn (<12).}
\]

\[\text{c} \quad \text{There were no trees in the extreme surplus ranges defined for N (>37.9), P (>5.3), K (>1), and B (>300).}
\]
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 $\approx 9$ 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 ($r \approx 0.5$).

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 µg 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** | 5.8*** | 3.0** | 314.8 (321.0) | 330.3 (303.9) | 262.26 (25.64) | -20.37*** (4.12) | -16.91*** (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 ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05; (*)P ≤ 0.1; NS, not significant.
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<sup>NS</sup> | 0.220* | 0.223* | 0.069<sup>NS</sup> | 0.079<sup>NS</sup> | -0.025<sup>NS</sup> | 0.134<sup>NS</sup> | 0.151<sup>NS</sup> | 0.071<sup>NS</sup> | 0.246* | 0.269* |
| N   | 0.303* | 0.336** | -0.081<sup>NS</sup> | -0.067<sup>NS</sup> | 0.828*** | -0.041<sup>NS</sup> | -0.086<sup>NS</sup> | 0.483*** | -0.152<sup>NS</sup> | 0.197<sup>NS</sup> |
| P   | 0.484*** | 0.141<sup>NS</sup> | 0.405*** | 0.273* | 0.418*** | 0.534*** | 0.481*** | 0.382** | 0.529*** |
| K   | 0.289* | 0.150<sup>NS</sup> | 0.461*** | 0.302* | 0.189<sup>NS</sup> | 0.335** | 0.407*** | 0.672*** |
| Ca  | 0.772*** | 0.020<sup>NS</sup> | 0.302* | 0.020<sup>NS</sup> | 0.466*** | 0.642*** | 0.079<sup>NS</sup> | 0.603*** | 0.415*** |
| Mg  | -0.099<sup>NS</sup> | -0.124<sup>NS</sup> | -0.134<sup>NS</sup> | 0.023<sup>NS</sup> | -0.099<sup>NS</sup> | -0.124<sup>NS</sup> | 0.010<sup>NS</sup> | 0.370** |
| S   | 0.077<sup>NS</sup> | 0.479*** | 0.293* | 0.22(*) | 0.450*** | 0.468*** |
| Fe  | 0.468*** | 0.144** | 0.078*** | 0.22(*) | 0.450*** | 0.468*** |
| Mn  | 0.22(*) | 0.353** |
| Cu  | 0.443*** |
| Zn  | 0.22(*) | 0.353** |

***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05; (*)P ≤ 0.1; NS, not significant.
assocation 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 for- mation 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. 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.
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 advance- ment 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 fertil- izers, 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 CO$_2$ 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 sig- nificant 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 conse- quence of temperature increase (e.g. Rasmussen, 2002; Frei and Gassner, 2008) or rising CO$_2$ 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 pol- len 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 tem- peratures were associated with a greater weight of birch cat- kins (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 CO$_2$ concentration (cottonwood; Ziska and Caulfield, 2000) or decrease in tropospheric O$_3$ concentration (cottonwood; Gregg et al., 2003). In the present study, however, an O$_3$ 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 charac- terized 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 pub- lic health. Experimental studies are scarce and lack a range of allergic 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.

Acknowledgements

The research conducted in this study was supported by the grant ME 179/3-1 and TR 467/8-1 of the Deutsche
Forschungsgemeinschaft (DFG), the EUROPA MÖBEL-Umweltstiftung, and Christine Kühne-Center for Allergy Research and Education (CK-Care). The authors thank Jörg Prietzel (Chair of Soil Science, Technische Universität München) and Klaus Erdle (Chair of Plant Nutrition, Technische Universität München) for fruitful discussions.

References

Amundson RG, Kohut RJ, Laurence JA. 1995. Influence of foliar N on foliar soluble sugars and starch of red spruce saplings exposed to ambient and elevated ozone. *Tree Physiology* 15, 167–174.

Bates D, Mächler M. 2010. lme4: linear mixed-effects models using S4 classes. R package ver. 0.999375–31, http://cran.r-project.org/web/packages/lme4/index.html.

Becker-Dillingen J. 1939. *Die Ernährung des Waldes*. Berlin: Verlagsgesellschaft für Ackerbau.

Bergmann W. 1993. *Ernährungsstörung bei Kulturpflanzen*, 3rd edn. Jena: Gustav Fischer Verlag.

Chandler JW, Dale JE. 1990. Needle growth in Sitka spruce (*Picea sitchensis*): effects of nutrient deficiency and needle position within shoots. *Tree Physiology* 6, 41–56.

Dobermann A, Cassman KG. 2002. Plant nutrient management for enhanced productivity in intensive grain production systems of the United States and Asia. *Plant and Soil* 247, 153–175.

Emberlin J, Mullins J, Corden J, Jones S, Millington W, Brooke M, Savage M. 1999. Regional variations in grass pollen seasons in the UK, long-term trends and forecast models. *Clinical and Experimental Allergy* 29, 347–356.

Fisher MJ. 1980. The influence of water stress on nitrogen and phosphorus uptake and concentrations in Townsville stylo (*Stylosanthes humilis*). *Australian Journal of Experimental Agriculture* 20, 175–180.

Frei T, Gassner E. 1998. Climate change and its impacts on birch pollen quantities and the start of the pollen season an example from Switzerland for the period 1969–2006. *International Journal of Biometeorology* 52, 667–674.

Gehrig R. 2006. The influence of the hot and dry summer 2003 on the pollen season in Switzerland. *Aerobiologia* 22, 27–34.

Göttlein A, Baier R, Mellert KH. 2011. Neue Ernährungskennwerte für die forstlichen Hauptbaumarten in Mitteleuropa—Eine statistische Herleitung aus van den Burg’s Literaturzusammenstellung. *Allgemeine Forst- und Jagdzeitung* 182, 173–186.

Gregg JW, Jones CG, Dowson TE. 2003. Urbanization effects on tree growth in the vicinity of New York City. *Nature* 424, 183–187.

Guedes A, Ribeiro N, Ribeiro H, Oliveira M, Noronha F, Abreu I. 2009. Comparison between urban and rural pollen of *Chenopodium album* and characterization of adhered pollutant aerosol particles. *Aerosol Science* 40, 81–86.

Iivonen S, Rikala R, Vapaavuori E. 2001. Seasonal root growth of Scots pine seedlings in relation to shoot phenology, carbohydrate status, and nutrient supply. *Canadian Journal of Forest Research* 31, 1569–1578.

Jochner S, Beck I, Behrendt H, Traidl-Hoffmann C, Menzel A. 2011. Effects of extreme spring temperatures on urban phenology and pollen production: a case study in Munich and Ingolstadt. *Climate Research* 49, 101–112.

Jochner S, Sparks TH, Estrella N, Menzel A. 2012. The influence of altitude and urbanisation on trends and mean dates in phenology (1980–2009). *International Journal of Biometeorology* 56, 387–394.

Laaidi M, Thibaudon M, Besancenot J-P. 2003. Two statistical approaches to forecasting the start and duration of the pollen season of *Ambrosia* in the area of Lyon (France). *International Journal of Biometeorology* 48, 65–73.

Lakatos L, Gulyás Á. 2003. Connection between phenological phases and urban heat island in Debrecen and Szeged, Hungary. *Acta Climatologica et Chorologica* 36–37, 79–83.

Larcher W. 1994. *Ökophysiologie der Pflanzen. Leben, Leistung und Stressbewältigung der Pflanze in der Umwelt*. Stuttgart: Ulmer Verlag.

Marschner H. 1995. *Mineral nutrition of higher plants*, 2nd edn. London: Academic Press.

Matsumoto F, Mikami T, Fukuoka Y. 2009. Effects of urban heat island on flowering dates of Somei Yoshino (cherry trees)—a case study in the wards area of Tokyo. In: Mayer H, Matzerakis A, eds. *5th Japanese–German Meeting on Urban Climatology. Berichte des Meteorologischen Instituts der Albert-Ludwigs-Universität Freiburg Nr. 18*. University of Freiburg: Freiburg, 101–106.

Meier U. ed. 2001. *Entwicklungsstadien mono- und dikotyler Pflanzen*. BBCH-Monograph. Berlin, Braunschweig: Biologische Bundesanstalt für Land und Forstwirtschaft.

Menzel A. 1999. Veränderungen der phänologischen Jahreszeiten. In: Deutscher Wetterdienst, ed. *Klimastatusbericht 1999*. Offenbach am Main: Deutscher Wetterdienst, 99–106.

Menzel A, Fabian P. 1999. Growing season extended in Europe. *Nature* 397, 659.

Nord EA, Lynch JP. 2009. Plant phenology: a critical controller of soil resource acquisition. *Journal of Experimental Botany* 60, 1927–1937.

Radrizzani A, Dalzell SA, Shelton HM. 2011. Effect of environment and plant phenology on prediction of plant nutrient deficiency using leaf analysis in *Leucaena leucocephala*. *Crop and Pasture Science* 62, 248–260.

Rasmussen A. 2002. The effects of climate change on the birch pollen season in Denmark. *Aerobiologia* 18, 253–265.

Reichenberg RL, Pritts MP. 1996. Dynamics of nutrient uptake from foliar fertilizers in red raspberry (*Rubus idaeus L*). *Journal of the American Society for Horticultural Science* 121, 158–163.

Robenrtz P. 1999. Effects of long-term CO2 enrichment and nutrient availability in Norway spruce. I. Phenology and morphology of branches. *Trees* 13, 188–198.

Schwarz G. 1978. Estimating the dimension of a model. *Annals of Statistics* 6, 461–464.

Sigurdsson BD. 2001. Elevated [CO2] and nutrient status modified leaf phenology and growth rhythm of young *Populus trichocarpa* trees in a 3-year field study. *Trees* 15, 403–413.
Sparks TH, Carey PD, Combes J. 1997. First leafing dates of trees in Surrey between 1947 and 1996. London Naturalist 76, 15–20.
Spieksma FTM, Emberlin JC, Hjelmroos M, Jäger S, Leuschner RM. 1995. Atmospheric birch (Betula) pollen in Europe: trends and fluctuations in annual quantities and the starting dates of the seasons. Grana 34, 51–57.
Steiner C, Teixeira WG, Lehmann J, Nehls T, de Macêdo JLV, Blum WEH, Zech W. 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. Plant and Soil 291, 275–290.
van den Burg J. 1985. Foliar analysis for determination of tree nutrient status—a compilation of literature data. Report no. 414. Wageningen: De Dorschkamp.
van den Burg J. 1990. Foliar analysis for determination of tree nutrient status—a compilation of literature data; 2. Literature 1985–1989. Report no. 591. Wageningen: De Dorschkamp.
Wayne P, Foster S, Connolly J, Bazzaz F, Epstein P. 2002. Production of allergenic pollen by ragweed (Ambrosia artemisiifolia L.) is increased in CO2-enriched atmospheres. Annals of Allergy, Asthma and Immunology 88, 279–282.
Weih M, Karlsson PS. 2001. Growth response of mountain birch to air and soil temperature: is increasing leaf-nitrogen content an acclimation to lower air temperature? New Phytologist 150, 147–155.
Weilbrock N, Aydin C-T, Block J, et al. 2006. Bodenzustandserhebung im Wald (BZE II), Arbeitsanleitung für die Außenaufnahmen, 2nd edn. Bonn: Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz.
White RE, Haydock KP. 1970. Phosphate concentration in Siratro as a guide to its phosphate status in the field. Australian Journal of Experimental Agriculture and Animal Husbandry 10, 426–430.
Wielgolaski FE. 1999. Starting dates and basic temperatures in phenological observations of plants. International Journal of Biometeorology 42, 158–168.
Wielgolaski FE. 2001. Phenological modifications in plants by various edaphic factors. International Journal of Biometeorology 45, 196–202.
Yang Q, Zhang F, Li F. 2011. Effect of different drip irrigation methods and fertilization on growth, physiology and water use of young apple tree. Scientia Horticulatiae 129, 119–126.
Ziello C, Sparks TH, Estrella N, et al. 2012. Changes to airborne pollen counts across Europe. PLoS One 7, e34076.
Ziska LH, Caulfield FA. 2000. Rising atmospheric carbon dioxide and ragweed pollen production: implications for public health. Australian Journal of Plant Physiology 27, 893–898.
Ziska LH, Gebhard DE, Frenz DA, Faulkner S, Singer BD, Straka JG. 2003. Cities as harbingers of climate change: common ragweed, urbanization, and public health. Journal of Allergy and Clinical Immunology 111, 290–295.