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Effects of tree pollen on throughfall element fluxes in European forests

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Abstract The effects of tree pollen on precipitation chemistry are not fully understood and this can lead to misinterpretations of element deposition in European forests. We investigated the relationship between forest throughfall (TF) element fluxes and the Seasonal Pollen Integral (SPIn) using linear mixed-effects modelling (LME). TF was measured in 1990–2018 during the main pollen season (MPS, arbitrary two months) in 61 managed, mostly pure, even-aged *Fagus*, *Quercus*, *Pinus*, and *Picea* stands which are part of the ICP Forests Level II network. The SPIn for the dominant tree genus was observed at 56 aerobiological monitoring stations in nearby cities. The net contribution of pollen was estimated as the TF flux in the MPS minus the fluxes in the preceding and succeeding months. In stands of *Fagus* and *Picea*, two genera that do not form large amounts of flowers every year, TF fluxes of

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potassium (K^+), ammonium-nitrogen (NH_4^+-N), dissolved organic carbon (DOC), and dissolved organic nitrogen (DON) showed a positive relationship with SPIn. However- for *Fagus*- a negative relationship was found between TF nitrate-nitrogen (NO_3^--N) fluxes and SPIn. For *Quercus* and *Pinus*, two genera producing many flowers each year, SPIn displayed limited variability and no clear association with TF element fluxes. Overall, pollen contributed on average 4.1–10.6% of the annual TF fluxes of $K^+ >$ DOC > DON > NH_4^+-N with the highest contribution in *Quercus* > *Fagus* > *Pinus* > *Picea* stands. Tree pollen appears to affect TF inorganic nitrogen fluxes both qualitatively and quantitatively, acting as a source of NH_4^+-N and a sink of NO_3^--N. Pollen appears to play a more complex role in nutrient cycling than previously thought.

Keywords Throughfall · Airborne pollen concentrations · Nitrogen · Potassium · Dissolved organic carbon · ICP Forests

Introduction

The current understanding of canopy exchange processes and particularly microbiological interactions affecting the chemical composition of precipitation, is still limited as for example indicated by recent evidence for nitrifying micro-organisms in the phyllosphere affecting TF inorganic nitrogen (N) fluxes (Ulrich 1983; Lindberg et al. 1986; Guerrieri et al. 2015, 2021). Temperate and boreal forests are dominated by anemophilous tree species that flourish and release pollen in the air mainly during spring (Faegri and van der Pijl 1979; Tormo–Molina et al. 1996). Some tree genera with anemophilous pollination strategy, including *Pinus* and *Quercus*, produce vast amounts of flowers and pollen each year, while *Fagus*, *Betula* and *Picea* switch between years with few and abundant inflorescences (Kelly and Sork 2002; Geburek et al. 2012; Grewling et al. 2012; Lebourgeois et al. 2018; Nussbaumer et al. 2020). Only a tiny fraction of the emitted pollen is effectively involved in pollination and the majority is washed out from the air by precipitation and dry deposition (Kluska et al. 2020). When pollen rehydrates, either on the pistil of a flower or in the TF, it becomes biochemically active and releases a mixture of organic substances, including lipids and functional proteins from the so-called pollen coat (Rejón et al. 2016). During in vitro leaching experiments, rehydrating pollen from temperate tree species discharged mainly the elements carbon (C), nitrogen (N), potassium (K) and phosphorus (P) up to one fifth of the initial pollen biomass (Doskey and Ugoagwu 1989; Rösel et al. 2012). Studies purely

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based on TF monitoring data reported peaks in the fluxes of the aforementioned elements during spring but didn't separate the effects induced by pollen from those controlled by other factors, such as passive leaching, insect infestations and microbial activities on forest canopies (Carlisle et al. 1966; Draaijers et al. 1996; Ferm 1997; Le Mellec et al. 2010; Pitman et al. 2010; Verstraeten et al. 2016).

A possible way to disentangle the effects of pollen on TF chemistry is to evaluate TF element fluxes in combination with local pollen concentrations in the air. Both types of data are available from national/regional or international monitoring networks. TF deposition is monitored at the pan-European scale by the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests, see http://icp-forests.net) under the UNECE Air Convention and in parallel by several national networks since the early 1990s. TF is sampled continuously in permanent intensive monitoring (Level II) plots and analysis is carried out by the individual regions/countries on a weekly to monthly basis, according to harmonized methods (Clarke et al. 2022). TF sampling is traditionally performed with bulk collectors, which are open-funnel systems equipped with mesh filters, typically sized 0.25–1 mm^2 to keep out larger particles such as litter and insects, without obstructing the water flow. Pollen grains, however, are smaller (generally between 20 and 80 µm, but up to 150 µm for *Picea*), allowing them to pass through these filters and enter the TF collectors. Airborne pollen concentrations from various plants, including several common and widely distributed tree genera (e.g., *Betula*, *Alnus*, *Quercus*), are monitored daily in densely populated areas across the globe because of their allergenicity (Buters et al. 2018; Biedermann et al. 2019). The Seasonal Pollen Integral (SPIn), defined as the sum of the observed daily mean airborne pollen concentration during the MPS, is a standard measure for quantifying and comparing the yearly airborne pollen abundances (Galán et al. 2017).

In the present study, we used TF monitoring data from the past three decades collected in managed, mostly pure, even-aged stands of *Fagus sylvatica*, *Quercus* sp., *Pinus* sp. and *Picea abies* across Europe, to assess the impact of tree pollen on TF element fluxes. We focused on K^+, NH_4^+-N, NO_3^--N, dissolved organic carbon (DOC), and dissolved organic nitrogen (DON) because these compounds are known or suspected to be influenced by pollen amount and activity (as stated above) and their concentrations in TF are available for most regions and countries. We also examined the relationship between TF element fluxes and airborne pollen concentrations (SPIn) of the dominant tree species observed at aerobiological monitoring stations located in the surroundings of Level II plots using LME. We hypothesize that there is a significant relationship between airborne pollen concentrations and TF element fluxes of the aforementioned compounds.

Materials and methods

Intensive forest monitoring (level II) plots

A total of 61 forest monitoring plots located in 12 European countries were selected for this study based on the dominant tree species at the site, tree age and the availability of data from nearby aerobiological monitoring stations (as stated below). The permanent monitoring plots are part of the UNECE ICP Forests Level II network and the Swedish Throughfall Monitoring Network (SWETHRO). Sites are geographically distributed from 43.2 to 69.6° N and 0.9° W to 29.3° E with an altitudinal range of 0 to 1900 m above sea level, thus covering temperate, boreal, continental, Alpine and Mediterranean climate zones (Fig. 1, Table S1). Plots were originally installed in mature, managed, mostly pure, even-aged forest stands of European beech (*Fagus sylvatica* L.), oak (*Quercus robur* L., *Q. petraea* (Matt.) Liebl., *Q. cerris* L.), pine (*Pinus sylve*stris L., *P. mugo* Turra, *P. cembra* L., *P. nigra* Arnold subsp. *laricio* Maire) and Norway spruce (*Picea abies* (L.) Karst.). The terms pure and even-aged refer to the dominant tree layer, disregarding any scattered dominant individuals and suppressed ingrowth of secondary tree species. For statistical analyses, plots were aggregated into four groups at tree genus level, i.e. *Fagus* (n = 22), *Quercus* (n = 12), *Pinus* (n = 10) and *Picea* (n = 17) (Fig. S1).

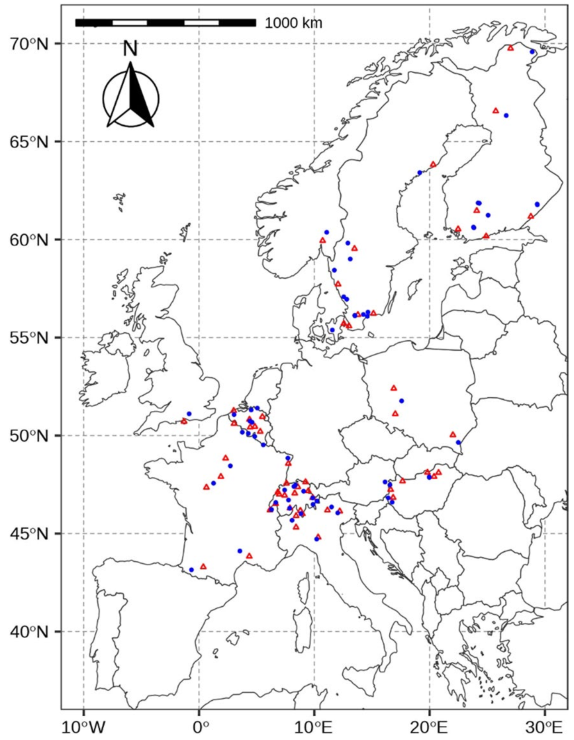


Fig. 1 Locations of the 61 selected Level II plots (blue dots) based on ICP Forests data and 56 aerobiological monitoring stations (red triangles)

Throughfall fluxes

The temporal range of available TF deposition fluxes measured in selected Level II plots varied in extent from 6 to 29 years, covering the period 1990–2018 (Table S1). For each plot, the longest possible time series was used in the calculations and statistical analyses, including only those years for which corresponding data on airborne pollen concentrations from nearby stations for the dominant tree genus were available (see below). After selection, a total of 1091 plot-year combinations (annual TF fluxes) was included in the analysis. TF sampling and chemical analyses were carried out by the countries/regions according to harmonized methods following the ICP Forests Manual Part XIV (Clarke et al. 2022). Quality assurance in the programme is ascertained through regular revision of the procedures for sampling and analysis by the expert panels, ring tests for laboratories and data validation procedures as described in the ICP Forests Manual Part III (Ferretti et al. 2021).

Airborne pollen concentrations

A total of 56 aerobiological monitoring stations from regional and national monitoring networks were selected for this study using their distance to the Level II plots as the main criterion. Trees of the same species show synchronicity of masting, i.e. high pollen and seed production, across relatively large areas (Ranta et al. 2008; Nussbaumer et al. 2016). This may explain why in rather flat areas like the Swiss plateau temporal patterns of the MPS can be very similar at distances of 50 km and still well comparable at distances of 150–200 km, while in mountainous areas the correlation often is much lower at these distances (Gehrig 2019). To ensure a certain level of representativeness, we applied an upper limit of 100 km distance in flat areas and 75 km in mountainous areas (the mean distance being 54.2 km) regardless of the prevailing wind direction (Fig. 1, Table S2). We used data on the SPIn, being the sum of all average daily pollen air concentrations over a single season (pollen grains * day * m^{-3}) for the respective tree genera (Galán et al. 2017). For each Level II plot, mean annual values of SPIn for the dominant genus were calculated using data from the nearest aerobiological monitoring stations, with a maximum of four stations. For each Level II plot also the MPS for the dominant genus was determined. In the context of this study, MPS was defined as the average period covering the annual peak of airborne pollen concentrations (arbitrary two months, i.e. from one month before to one month after the pollen peak day; semi-monthly time step starting on the 1st or the 15th day of the month), based on data with daily resolution available for a limited number of aerobiological monitoring stations (Table 1). If data on a daily level were not available for a site, the MPS was determined as the best possible estimate considering its location (latitude, longitude, altitude) and the MPS for nearby plots with the same dominant tree genus.

Calculations and statistical analysis

The production of figures and statistical analysis were carried out in RStudio v2022.07.2 with the R software version 4.2.1 (RStudio Team 2022, R Core Team 2022). Linear mixed-effects models (LME) for the TF fluxes of K^+, nitrate-nitrogen (NO_3^--N), ammonium-nitrogen (NH_4^+-N), DOC, and DON during the MPS (Table 1)

Table 1 Number of Level II plots (n) with different timing of the MPS as derived from the days when maximum airborne pollen concentrations were registered

Tree genus	April		May		June		July	
Fagus (n = 22)	17							
		5						
Quercus (n = 12)	10							
		2						
Pinus (n = 10)		1						
			5					
				3				
					1			
Picea (n = 17)		2						
			8					
				5				
					2			

as a function of SPIn were evaluated for the four genera using the R package 'lme4' (Bates et al. 2015). A random intercept term for plot was included in the model to account for variance heterogeneity among sites (e.g., related to differences in local environmental conditions and in sampling and chemical analyses carried out by the individual countries/regions). This resulted in the following modelling function:

$$\mathrm{TF(MPS)}_{ij} = \alpha + \beta_1 \times Plot_i + \beta_2 \times SPIn_{ij} + \varepsilon \quad (1)$$

$Plot_i$ is a factor with n levels ($i = 1, \ldots, n$) and j is the number of years. Bootstrapped (n = 1000 simulations) 95%-confidence intervals for predicted values were calculated using the add_ci function included in the R package 'ciTools' (Haman and Avery 2020). Model validity was judged based on the homogeneity of residual variance across levels of the predictor variable (SPIn) and the fitted values (Zuur et al. 2009). All variables had a skewed distribution and were therefore $\log_e$-transformed prior to modelling. For SPIn, TF DON, TF NH_4^+-N and TF NO_3^--N the value of 0.5 had to be added before transforming because the data contained zeros (McDonald 2014).

The contribution of pollen to TF (in kg ha^{-1} yr^{-1}) for each compound was calculated as the difference between the mean annual TF flux during the MPS (Table 1) and the sum of the mean TF flux during the previous and the following month for each Level II plot:

$$\mathrm{TF(pollen)} = \mathrm{TF(MPS)} - \mathrm{TF(previous + following\ month)} \quad (2)$$

The contribution of pollen to TF fluxes was also expressed in terms of a percentage (%) of annual mean TF deposition. Values were compared among the four genera and among the five compounds using the non-parametric multiple comparison test after Kruskal–Wallis (R function 'Kruskalmc') included in the R package 'pgirmess' (Giraudoux 2018).

For *Fagus*, the genus with probably the most distinct masting behaviour (Nussbaumer et al. 2016, 2020), we additionally applied the non-parametric Wilcoxon test from the R package 'stats' (R Core Team 2022) to test the difference in input from pollen to TF fluxes between years with massive pollen dispersal (SPIn > 80% of the annual mean) and all years, and between years with massive pollen dispersal and years with low pollen production (SPIn ≤ 80% of the annual mean).

For each tree species, we applied the one-sided *t*-test included in the R package 'stats' (R Core Team 2022) to test whether the contribution of pollen to TF fluxes was different from zero. For *Fagus*, the same was also done for years with massive pollen dispersal and years with low pollen production separately.

Results

Relationship between SPIn and TF element fluxes

For *Fagus*, the LME models revealed a significant positive relationship between TF fluxes of K^+, NH_4^+-N, DON, DOC and SPIn (Fig. 2; Table 2). However, a significant negative relationship was found between TF fluxes of NO_3^--N and SPIn for *Fagus*. For *Quercus*, the TF element fluxes showed

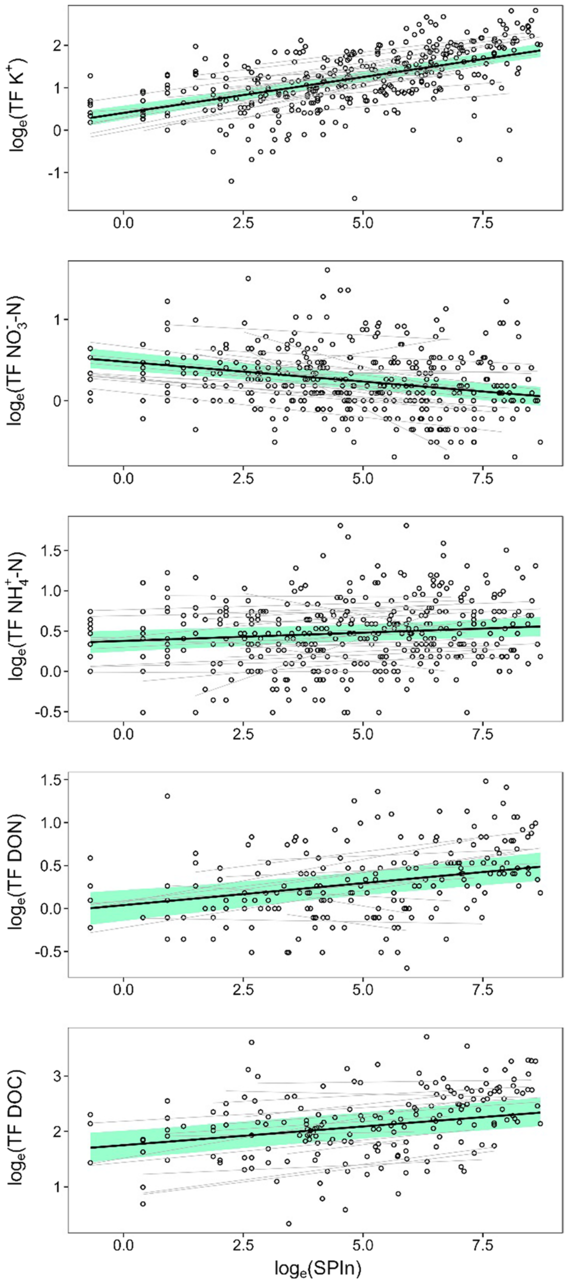


Fig. 2 Output of the mixed-effects models (LME) for *Fagus* stands. Left: $\log_e$-transformed throughfall deposition fluxes (kg ha^{-1}) of K$^+$, NO$_3^-$-N, NH$_4^+$-N, DON, and DOC during the MPS (as in Table 1) in function of $\log_e$ transformed SPIn (pollen grains * day * m^{-3}). Black lines represent the response curves and green fields are the 95% confidence intervals. Circles represent individual plot-years. Grey lines were added to show the relationship for individual plots using simple linear regression (also when not significant). Based on ICP Forests data

no relationship with SPIn. For *Pinus*, a significant positive relationship was found between TF fluxes of K$^+$ and SPIn, but not for the other elements. For *Picea*, TF fluxes of K$^+$ and NH$_4^+$-N showed a significant positive relationship with SPIn, but not the other elements.

Impact of pollen on TF

Mean annual contribution of pollen to the TF fluxes of K$^+$ was significantly higher in *Quercus* (3.67 kg K$^+$ ha^{-1} yr^{-1}) and *Fagus* stands (2.20 kg K$^+$ ha^{-1} yr^{-1}) than in *Pinus* (0.79 kg K$^+$ ha^{-1} yr^{-1}) and *Picea* stands (0.62 kg K$^+$ ha^{-1} yr^{-1}) (Fig. 3a, Table S3–6). The input from pollen covered a significantly lower mean part of annual TF K$^+$ fluxes in *Picea* stands (4.8%) than in *Quercus* stands (15.0%) and *Fagus* stands (10.5%), but there was no significant difference to the mean for *Pinus* (10.9%) (Fig. 3f). Pollen added significantly more DOC to TF in *Quercus* stands (9.54 kg C ha^{-1} yr^{-1}) than in *Fagus* (3.21 kg C ha^{-1} yr^{-1}) and *Picea* stands (2.91 kg C ha^{-1} yr^{-1}), but the difference with the mean for *Pinus* (3.29 kg C ha^{-1} yr^{-1}) was not significant (Fig. 3e). The input from pollen covered a significantly higher mean part of annual TF DOC fluxes in *Quercus* stands (12.0%) than in *Picea* stands (4.3%), but the difference with the mean for *Fagus* (7.0%) and *Pinus* (6.3%) was not significant (Fig. 3j). The contribution of pollen to TF DON was significantly higher in *Quercus* stands (0.53 kg N ha^{-1} yr^{-1}) than in *Picea* (0.14 kg N ha^{-1} yr^{-1}) and *Pinus* stands (0.13 kg N ha^{-1} yr^{-1}), but the difference with the mean for *Fagus* (0.33 kg N ha^{-1} yr^{-1}) was not significant (Fig. 3d). The input from pollen covered a significantly higher mean part of annual TF DON fluxes in *Quercus* stands (13.1%) than in *Picea* stands (3.9%), but the difference with the mean for *Fagus* (8.0%) and *Pinus* (4.9%) was not significant (Fig. 3i). The mean annual input of NH$_4^+$-N to TF by pollen was considerable in *Quercus* stands (0.68 kg N ha^{-1} yr^{-1} or 10.4% of annual TF fluxes), limited in *Fagus* (0.12 kg N ha^{-1} yr^{-1} or 2.1% of annual TF fluxes) and *Pinus* stands (0.10 kg N ha^{-1} yr^{-1} or 1.9% of annual TF fluxes), and negligible in *Picea* stands (0.01 kg N ha^{-1} yr^{-1} or 0.3% of annual TF fluxes, Fig. 3c and h). Pollen removed more NO$_3^-$-N from TF in *Quercus* stands (0.15 kg N ha^{-1} yr^{-1}) than in *Picea* (0.02 kg N ha^{-1} yr^{-1}) and *Pinus* stands (where even an addition of 0.01 kg N ha^{-1}

Table 2 Modelling results (LME) for TF element fluxes (kg ha^{-1}) in function of SPIn (pollen grains * day * m^{-3}) during the MPS, with the number of included plot-years (n) and estimated slope of the relationship

Tree genus		K^+	NO_3^-–N	NH_4^+–N	DON	DOC
Fagus	Slope	0.169 (12.9)	– 0.049 (– 6.7)	0.021 (2.6)	0.051 (4.5)	0.069 (4.9)
	n	371	382	382	196	186
Quercus	Slope	ns	ns	ns	ns	ns
	n	208	210	210	133	97
Pinus	Slope	0.123 (2.5)	ns	ns	ns	ns
	n	192	192	192	175	146
Picea	Slope	0.074 (5.1)	ns	0.048 (5.1)	ns	ns
	n	280	307	307	142	137

The value of the *t*-test is shown between brackets when significant ($t > \pm 1.96$, ns: not significant). Based on ICP Forests data

yr^{-1} was found), but the difference to the removal in *Fagus* stands (0.12 kg N ha^{-1} yr^{-1}) was not significant (Fig. 3b). The percentage of annual TF NO_3^--N fluxes removed by pollen was, however, not statistically different among tree species (Fig. 3g). Overall, the estimated contribution of pollen to TF fluxes was highest in *Quercus* > *Fagus* > *Pinus* > *Picea*, covering on average 4.1–10.6% of the annual TF fluxes of K^+ > DOC > DON > NH_4^+-N, while pollen removed on average 1.8% of annual TF NO_3^--N fluxes in European forests (significant differences among elements are listed in Table S7). The impact of pollen on TF element fluxes was significantly different from zero for all tree genera, except for NH_4^+-N and NO_3^--N in *Pinus* and *Picea* stands (Fig. 3).

For *Fagus*, the contribution of pollen to TF K^+ fluxes was about three times higher ($p < 0.0001$) in years with massive pollen dispersal (3.70 kg ha^{-1} yr^{-1} or 15.3% of annual TF fluxes) than in other years (1.15 kg ha^{-1} yr^{-1} or 6.1% of annual TF fluxes) (Fig. 4a and f). In *Fagus* stands, pollen also added more than twice the amount of DOC to TF ($p < 0.01$) in years with massive pollen dispersal (4.78 kg ha^{-1} yr^{-1} or 9.8% of annual TF fluxes) than in other years (1.89 kg ha^{-1} yr^{-1} or 4.4% of annual TF fluxes) (Fig. 4e and j). In absolute terms, the contribution of pollen to TF DON fluxes in *Fagus* stands was almost two times higher in years with massive pollen dispersal (0.45 kg ha^{-1} yr^{-1}, 10.4% of annual TF fluxes) than in other years (0.25 kg ha^{-1} yr^{-1} or 6.3% of annual TF fluxes), but the difference was not significant (Fig. 4d and i). The difference in the contribution of pollen to TF NH_4^+-N fluxes in *Fagus* stands between years with massive pollen dispersal (0.22 kg ha^{-1} yr^{-1} or 3.8% of annual TF fluxes) and other years (0.05 kg ha^{-1} yr^{-1} or 1.0% of annual TF fluxes) was also not significant (Fig. 4c and h). *Fagus* pollen removed over three times more NO_3^--N from TF ($p < 0.0001$) in years with massive pollen dispersal (0.23 kg ha^{-1} yr^{-1} or 4.2% of annual TF fluxes) than in other years (0.06 kg ha^{-1} yr^{-1} or 1.0% of annual TF fluxes) (Fig. 4b and g). In years with massive pollen dispersal, the impact of pollen on TF fluxes was significantly different from zero for all elements. In years with low pollen dispersal, the impact of pollen on TF fluxes was significantly different from zero for K^+, DOC and DON, but not for NH_4^+-N and NO_3^--N (results not shown).

Discussion

Effects of pollen on TF element fluxes

This study, to our knowledge the first of its kind performed at large spatial scale, yielded a quantitative estimate of the impact of airborne pollen on TF fluxes of K^+, NO_3^--N, NH_4^+-N, DOC, and DON based on long-term measurements in managed, mostly pure, even-aged forest stands across Europe. Isolating the effects of pollen during the MPS enabled to minimize other factors influencing TF fluxes year-round or throughout the growing season, including atmospheric deposition, leachates from foliage and associated nitrifying microorganisms (negligible in broadleaved stands in the period of leaf emergence), stems and branches, phytophagous insects and epiphytic mosses and lichens (Pitman et al. 2010; Van Stan

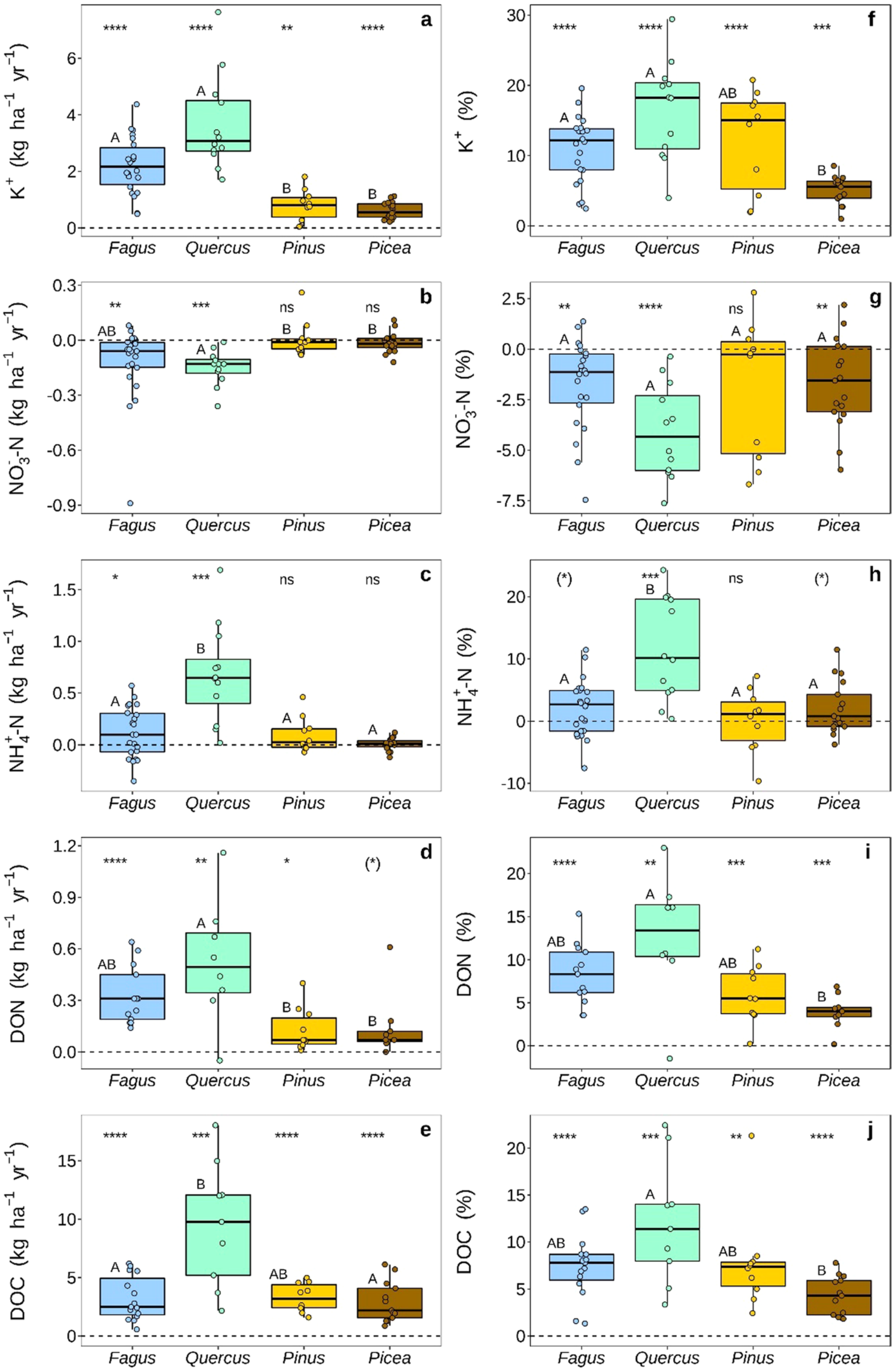
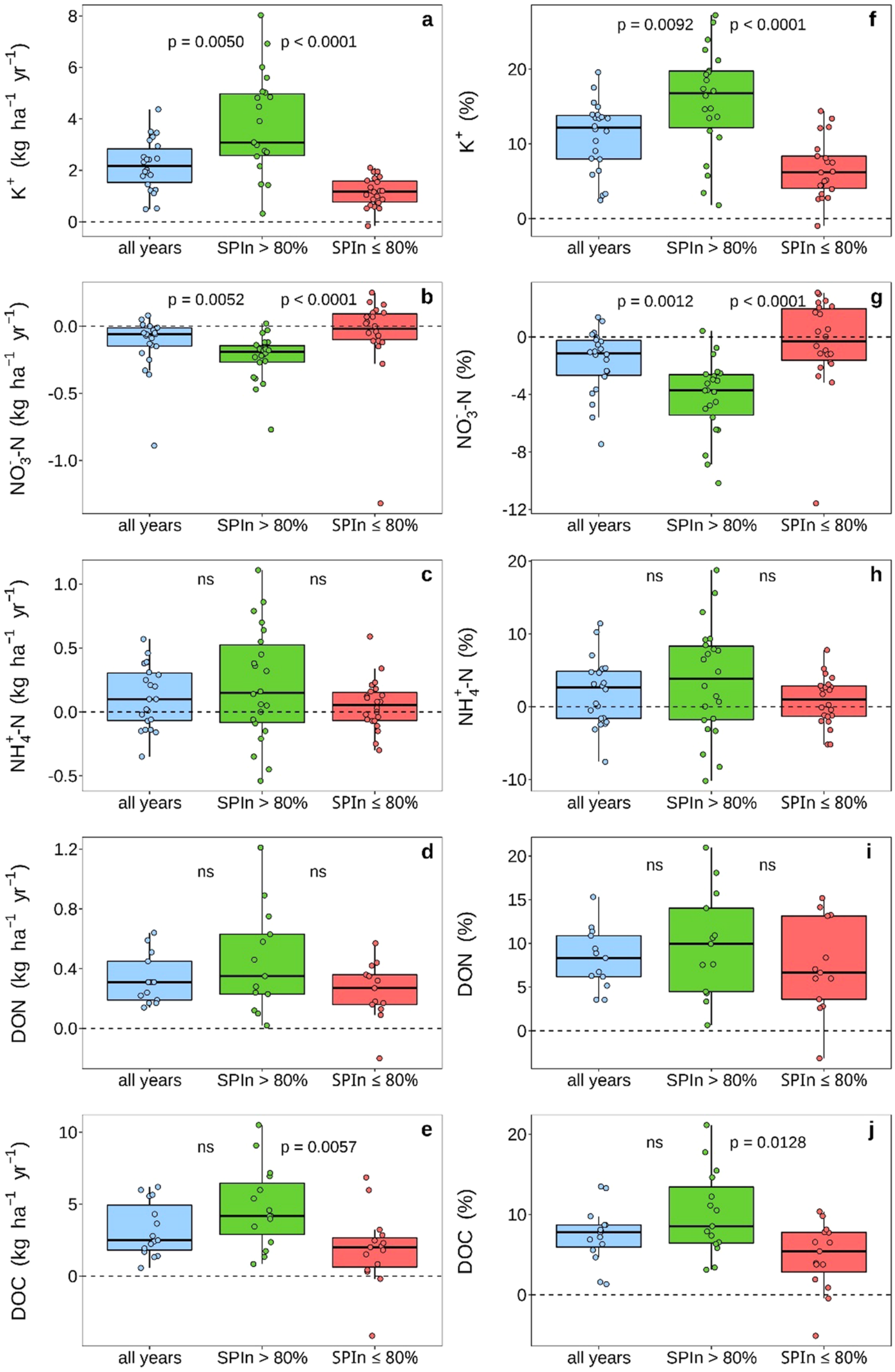


Fig. 3 Input from pollen to TF fluxes in *Fagus*, *Quercus*, *Pinus* and *Picea* stands as derived from TF monitoring data (a positive value means that pollen added elements, a negative value that pollen removed elements). Dots represent individual plots. **a**–**e**: mean annual contribution (kg ha^{-1} yr^{-1}). **f**–**j**: expressed as percentage of mean annual TF flux. Capital letters (A, B) denote significantly different groups ($p < 0.05$). Symbols indicate whether the mean of all values is different from zero (****: $p < 0.0001$; ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; (*): $p < 0.1$; ns: not significant). Based on ICP Forests data

et al. 2012; Wagenhoff et al. 2014; Guerrieri et al. 2021). Nevertheless, outbreaks of certain phytophagous insects like *Melolontha melolontha* and *Tortrix viridana* may impact TF fluxes in spring particularly in *Quercus* stands and might have partly obscured the effects of pollen in this group of forests. Another factor that might influence atmospheric fluxes specifically during the MPS more generally is leaching from flowers (i.e. flower parts other than pollen), but as far as we know this has never been quantified. The latter would require a laboratory dissolution experiment, and prior removal of any residual pollen from the

Fig. 4 Input from pollen to TF fluxes in *Fagus* stands as derived from monitoring data ($n=22$) for all years compared with years for which SPIn > 80% of the annual mean, and years for which SPIn ≤ 80% of the annual mean. Dots represent individual plots. **a**–**e**: mean annual contribution (kg ha^{-1} yr^{-1}). **f**–**j**: expressed as percentage of mean annual TF flux. The significance level of the difference between groups is indicated by the p-value of the Wilcoxon test (ns = not significant). Based on ICP Forests data



flower parts, which is probably difficult to achieve. In this study, we assumed that leaching from flower parts is negligible, considering that flowers from *Fagus*, *Quercus*, *Pinus* and *Picea* decompose relatively slowly. Because the total productions of inflorescences, flowers, anthers and pollen grains in trees are all logically correlated (Molina et al. 1996), any leaching from flower parts other than pollen will not obscure but rather amplify the biochemical signal from pollen, and does not significantly affect the conclusions of our study. The significantly higher fluxes that we found in *Fagus* stands during years with massive pollen dispersal, provided further evidence that the observed effects were induced by pollen. Pollen, however, hosts various microorganisms, including bacteria and fungi, that might affect pollen germination and tube elongation or pollen defence and further study is needed to elucidate their possible role in pollen-associated biochemistry (Madmony et al. 2005; Obersteiner et al. 2016; Cullen et al. 2021).

Many unknowns exist about the complex processes governing forest canopy N cycling (Guerrieri et al. 2021). However, our results support the findings from previous TF studies at local/regional level and in vitro experiments suggesting that pollen is an important source of K^+ and organic C and N in TF, confirming our main hypothesis (Table 3). Moreover, we found that pollen also may affect TF inorganic N fluxes by adding NH_4^+-N and removing NO_3^--N. The latter provides evidence for an effect that, to date, was demonstrated only at the intracellular level, i.e. the enzymatic reduction of NO_3^- through the 2-step nitrate reductase pathway (Bright et al. 2009). Furthermore, it might explain why NO_3^- concentrations at a North American watershed were found to be lower in precipitation and TF collected with bulk samplers compared to wet-only samplers, the latter excluding dry deposition (Richter and Lindberg 1988). Hence, pollen dispersal appears to be an important factor also in canopy inorganic N cycling. If NO_3^- in TF is enzymatically reduced by pollen, then short-lived NO_2^- peaks are expected in TF, at the moment when intermediately formed NO_2^- is not yet further reduced into NO, or converted back into NO_3^- by nitrifiers, which appears to be common in the phyllosphere (Guerrieri et al. 2015, 2021). Unfortunately, it was impossible to include NO_2^- in the present study because few countries analyse this compound in TF samples and the available data were too limited. However, in a mixed *Quercus robur–Fagus sylvatica* stand in Gontrode (Flanders, Belgium), which was not included in this study where we focused on single-species even-aged stands, remarkable peaks in the TF NO_2^- fluxes of variable magnitude were indeed observed during the spring in most years (Fig. S2, unpublished results INBO). This indicates the possible effects of pollen and/or associated microorganisms on TF inorganic N fluxes. Specific laboratory experiments and a more detailed analysis of inorganic N forms in TF might help to shed light on the abilities of pollen to reduce extracellular NO_3^-.

We found an overall higher impact of pollen on TF composition in deciduous broadleaved stands (*Quercus*, *Fagus*) compared to evergreen coniferous stands (*Pinus*, *Picea*). It was rather expected that *Quercus* and *Pinus* would alter the fluxes the most because these genera produce many flowers and high amounts of pollen each year. We think this discrepancy could be explained by interference from pollen originating from broadleaved trees growing in the understorey or direct surroundings of coniferous stands. A large part of the *Pinus* and *Picea* plots is located in the boreal zone of Fennoscandia (40% of the *Pinus* stands and 76% of the *Picea* stands, Fig. S1), where *Betula* is a naturally widespread and abundant genus (Beck et al. 2016). The MPS of *Betula* in Fennoscandia ranges from late April to early June and is partly overlapping with that of *Pinus* and *Picea* (Biedermann et al. 2019). Given the method (Eq. 2) applied to calculate the contribution of

Table 3 Overview of the effects of rehydrating pollen on elemental concentrations in solution reported by various studies

Study	Tree species	Effect
Analysis of TF data		
Carlisle et al. (1966)	*Quercus petraea*	K^+, P (increase)
Ferm (1997)	*Picea abies*	K^+, organic N (increase)
Le Mellec et al. (2010)	*Fagus sylvatica*, *Picea abies*	organic C and N (increase)
Verstraeten et al. (2016)	*Fagus sylvatica*, *Quercus robur*	organic N (increase)
In vitro experiments with pollen		
Doskey and Ugoagwu (1989)	*Pinus resinosa*, *Pinus strobus*	K^+, P, organic C (increase)
Bright et al. (2009)	*Magnolia delavayi*, *Protea nitida*, *Banksia serata*, *Ulmus scabra*	intracellular: NO_3^- (decrease), NO_2^-, NO (increase)
Rösel et al. (2012)	*Pinus sylvestris*	P, organic C, N (increase)

pollen to TF fluxes, admixture of *Betula* pollen may have resulted in an underestimation of the impact in coniferous stands. To verify this, it would be necessary to study local pollen spectra at the stand level, as explained below.

Although according to previous studies TF P fluxes are increased by pollen (Table 3), it was not possible to include analysis of P in this study because the available data were too limited.

Indicative value of SPIn for the impact of pollen on TF element fluxe

For *Fagus* stands, we found a clear relationship between SPIn and TF element fluxes of K^+, NO_3^--N, NH_4^+-N, DOC, and DON, confirming our main hypothesis, but this was not the case for *Picea*, *Pinus* and *Quercus* stands. Differences in the intrinsic dispersal abilities of pollen at the tree genus level may partly explain this result. The effective transport distance of pollen from wind-pollinated trees is inversely related to its size and weight, but other factors also seem to play a role, like pollen morphology and local topography (Erdtman 1969; Damialis et al. 2017). For *Fagus* pollen (Ø 30–50 µm), the effective transport distance is generally limited to a few hundred meters (Wagner et al. 2010), but this might be overcome by the common occurrence of *Fagus* in large parts of Europe. For genera with larger and heavier pollen grains such as *Abies* and *Picea* (Ø 80–150 µm), airborne pollen concentrations in residential areas are generally less representative for those in forests because few pollen grains are able to reach the samplers, although the pollen grains of both taxa are equipped with air sacs ('sacci') that facilitate air transport. In contrast, pollen from *Pinus* (Ø 40–85 µm, also equipped with two sacci) and also *Betula* (Ø 20–25 µm) and *Quercus* (Ø 26–40 µm) can float in the atmosphere, allowing it to travel much further (Wrońska–Pilarek et al. 2016; Damialis et al. 2017; Bogawski et al. 2019). This means that *Pinus, Betula* or *Quercus* pollen collected at a particular location can originate from both local and remote sources, depending on the meteorological conditions, particularly wind, rainfall and solar radiation (Szczepanek et al. 2017; Bruffaerts et al. 2018; Verstraeten et al. 2019). Measurements of airborne pollen concentrations are not included in the standard monitoring programme for Level II plots, so we had to rely on data from aerobiological monitoring stations installed predominantly in urban environments to assess the risk for associated respiratory allergies (Buters et al. 2018; Biedermann et al. 2019). This difference in location means that the representativeness of SPIn for the airborne pollen concentrations in the forest may vary, depending on the distance, difference in altitude, and other factors. In general, SPIn as well as pollen accumulation rates in sediments are positively related to the abundance and proximity of local pollen sources, especially forests, but also trees growing along streets and in parks (Verstraeten et al. 2019; Abraham et al. 2021). To get more representative airborne pollen concentration data for forests, passive pollen traps (gravitational fluxes) would have to be installed under the canopy. Alternatively, the TF filtration residue using filters with an adequate pore size (5 µm) could be analysed. Ground-level samplers within the forest plots might have allowed to relate more directly local pollen emissions to throughfall biochemistry, but it would also have been more influenced by the pollination behaviour of some individual trees and might be less representative of the forest masting that this study aims to relate at larger scale. Elaboration of pollen dispersion models based on the spatial distribution and abundance of tree species combined with meteorological data may provide further insights into the emission sources of pollen, but these models are currently available only for a limited number of tree genera, e.g. *Betula* and *Pinus* (Sofiev et al. 2013; Szczepanek et al. 2017; Verstraeten et al. 2019).

Besides variation in the representativeness of airborne pollen concentrations, differences in flowering strategies among the tree genera likely affect the results. Trees belonging to the genera *Fagus* and *Picea* exhibit a distinct masting behaviour and produce much more flowers during years with successful development of fruits and seeds and vice versa. On the contrary, *Quercus* and *Pinus* produce large amounts of flowers every year resulting in airborne pollen concentrations that are higher and more constant over the years (Geburek et al. 2012; Lebourgeois et al. 2018; Bogdziewicz et al. 2019). This contrast is reflected in the overall higher SPIn and lower coefficient of variation (CV) of SPIn for *Quercus* and *Pinus* stands compared to *Fagus* and *Picea* stands (Fig. 5a and b). Limited ability to catch the annual variations in constantly very high pollen loads with the available pollen predictors is probably the second main reason why we could not find a clear relationship between TF element fluxes and SPIn for *Quercus* and *Pinus*.

Conclusions

Tree pollen was found to make a substantial contribution to the annual TF fluxes of K^+ > DOC > DON > NH_4^+-N in managed, mostly pure, even-aged forest stands across Europe, an effect that appeared to be stronger in deciduous broadleaved stands than in evergreen coniferous stands (*Quercus* > *Fagus* > *Pinus* > *Picea*). On the other hand, we obtained evidence that pollen removes a small part of annual TF NO_3^--N fluxes. The estimation of throughfall fluxes is thus significantly altered by pollen. Airborne pollen concentrations (SPIn) measured in urban areas were able to predict TF fluxes for tree genera with distinct masting behaviour like *Fagus* and *Picea*, but not for genera with a more constant pollen production like *Quercus* and *Pinus*. Pollen dispersal seems to play a more prominent and complex role in forest nutrient cycling than previously thought, also with regard to inorganic N.

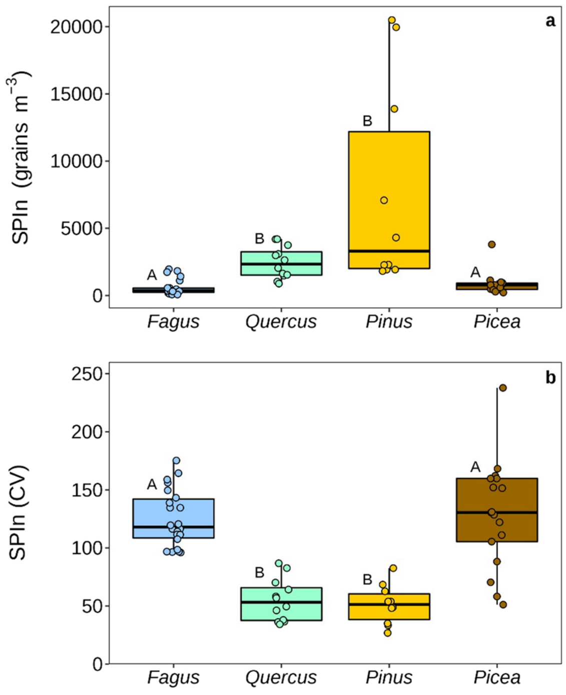


Fig. 5 Boxplots of mean annual SPIn (grains m^{-3}) per plot **a** and coefficient of variation (CV) of SPIn per plot **b** for the four tree genera. Capital letters (A–C) denote significantly different groups ($p < 0.05$)

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Author's contributions AV, EG and N.B. designed the study; AV compiled the data, analysed the data and drafted the manuscript; and all other authors provided data and/or commented critically to the drafts and gave final approval for publication.

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Data availability The TF data can be obtained by submitting an official data request through the ICP Forests website http://icp-forests.net/. Data on airborne pollen concentrations can be requested from the national/regional monitoring networks representatives (https://www.zaum-online.de/pollen/pollen-monitoring-map-of-the-world/).

Code availability Not applicable.

Declarations

Conflicts of interest The authors declared that they have no conflict of interest.

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