Impact of pollen on throughfall biochemistry in European temperate and boreal forests

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Background

- Throughfall (TF) biochemistry is still poorly understood
- **In the spring, trees bloom and disperse pollen => TF gets a yellow/brown color**
- Unexpected peaks in potassium (K^+), dissolved organic carbon (DOC) and nitrite (NO_2^-) show up **<->** some nitrate (NO_3^-) goes missing!
- Most obvious (but not exclusive) in broadleaved stands (beech, oak)
- Impact is greater in 'mast years'
- Many TF samples are rated as 'contaminated' and results are often corrected or even not submitted to the deposition database

  => systematic error

  => lack of reliable TF data for the spring period

Objectives of this study

1. **Dissolution experiment**

- Investigating the qualitative (quantitative) impact of pollen on TF.

2. **Inter-annual study (data evaluation)**

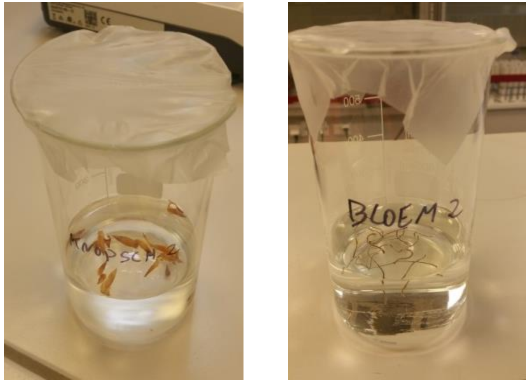
- Trying to find a link between TF deposition fluxes and airborne pollen concentrations.
- Calculating the quantitative impact of pollen on TF deposition fluxes.

3. **Intra-annual study (TF samples)**

- Gathering information on TF pollen spectra and investigating the link with TF fluxes / DOC characteristics.

1. Dissolution experiment

- A 7-day dissolution experiment was conducted with:
  - fresh pollen of *F. sylvatica* , *Quercus robur*, *Pinus sylvestris* and *Pinus nigra* (FL),
  - archived/dehydrated pollen of *Picea abies* (IT) and *Betula pendula* (FL), including a liquid N_2-sterilized control for *B. pendula*.,
  - bud scales and flower stalks of *Fagus sylvatica* (from litterfall traps in FL)



F. sylvatica

F. sylvatica

Q. robur

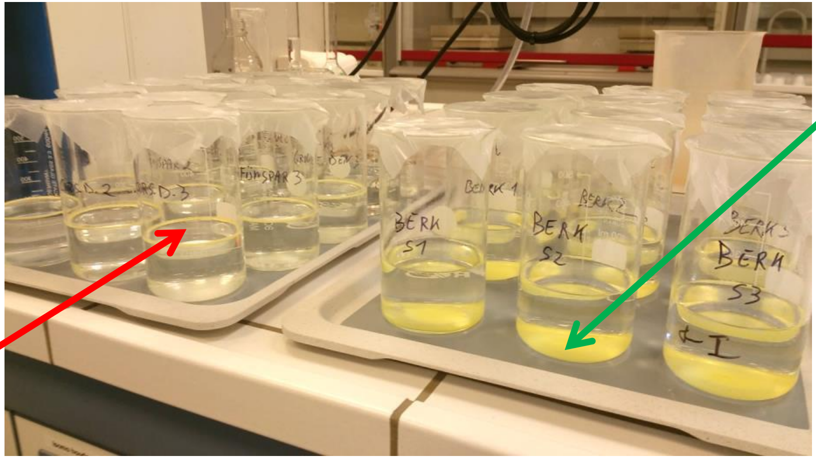
P. nigra

P. sylvestris

(Verstraeten et al., subm.)

1. Dissolution experiment

- 9 treatments, 3 replicates
- 50±0.52 mg OM was added to 200 ml $NaNO_3$ solution (50 mg NO_3^- l^{-1})
- Samples (15 ml) for chemical analysis were taken after 2h, 24h, 48h, 72h, 96h and 168h.
- Analysis:
  - Ca^{2+}, K^+, Mg^{2+}, Na^+, Cl^-, NO_3^-, NO_2^-, NH_4^+, SO_4^{2-}, PO_4^{3-} (ion chromatography, Dionex)
  - DOC and TN (=> DON) (Formax^{HT} C/N analyzer)
  - Alkalinity (HCO_3^-) (titration)
  - EC and O_2 (electrode)



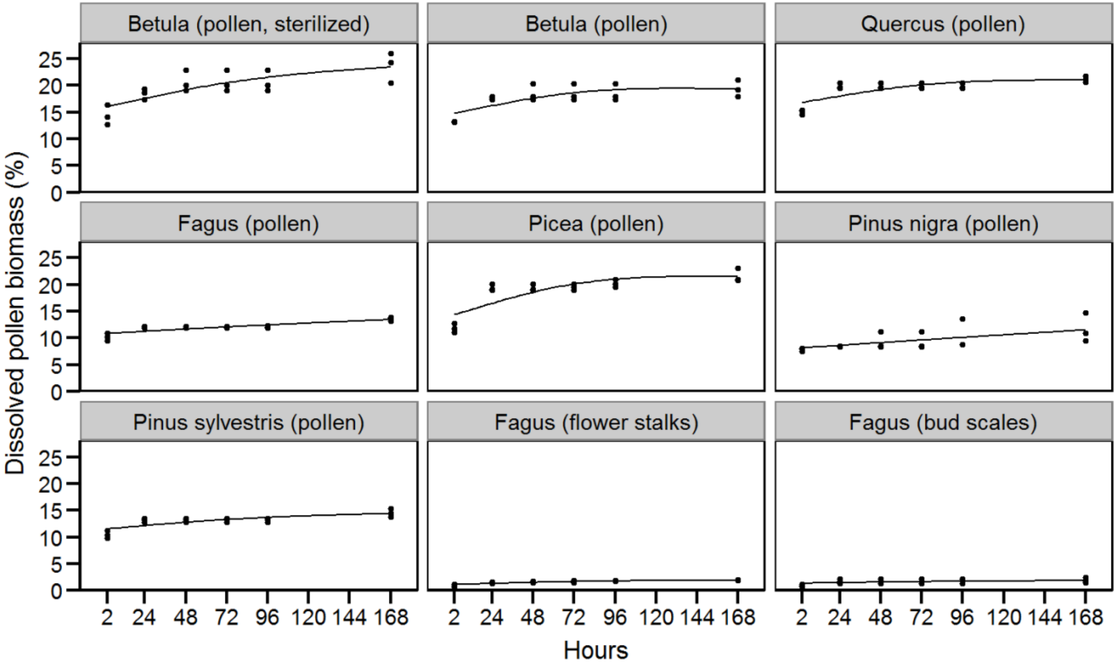
Conifers: Pollen floats

Broadleaves: Pollen sinks

(Verstraeten et al., subm.)

1. Dissolution experiment - Results

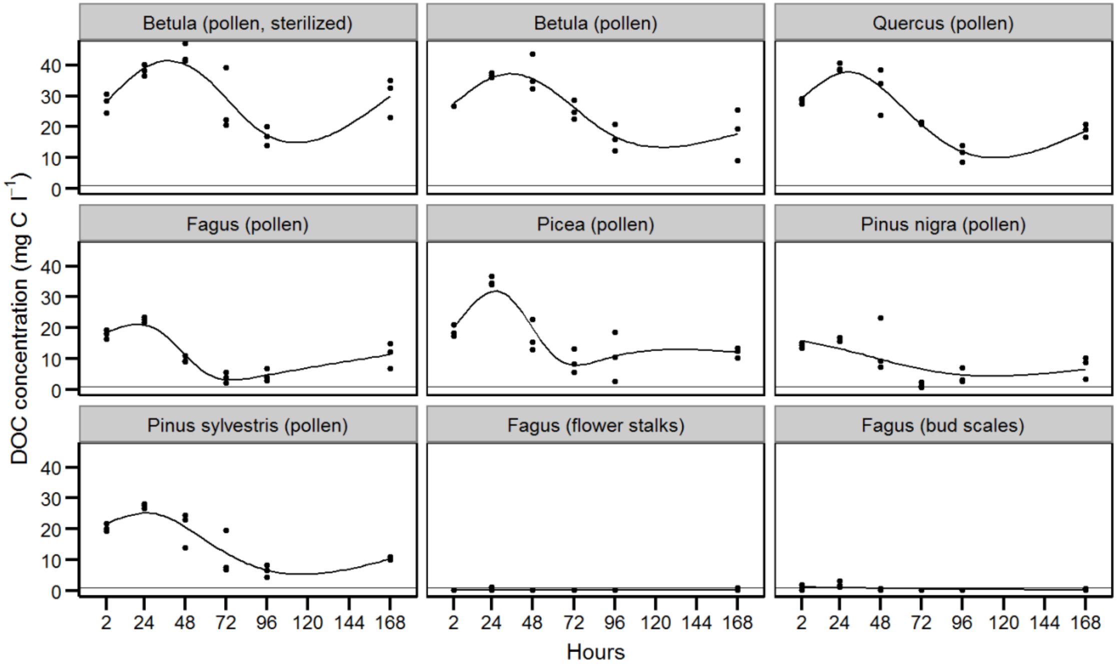
- 12 – 24% of pollen biomass dissolved
- <2% of bud scales and flower stalks



(Verstraeten et al., subm.)

1. Dissolution experiment - Results

- Pollen released **DOC**, K^+, PO_4^{3-}, and small amounts of SO_4^{2-} and Na^+



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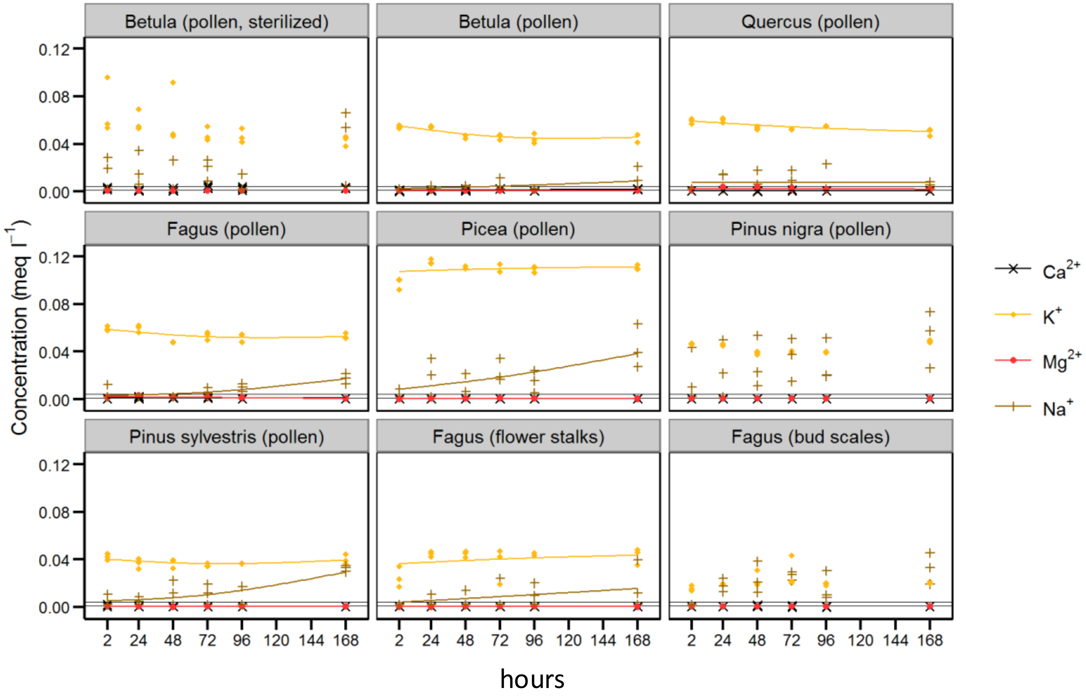

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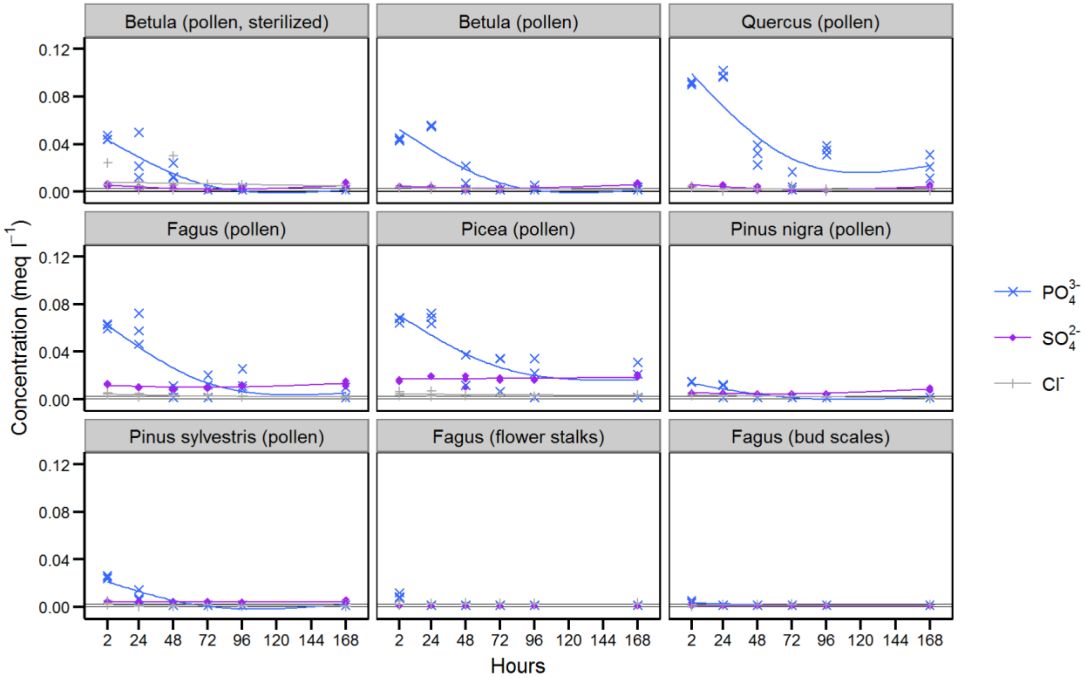
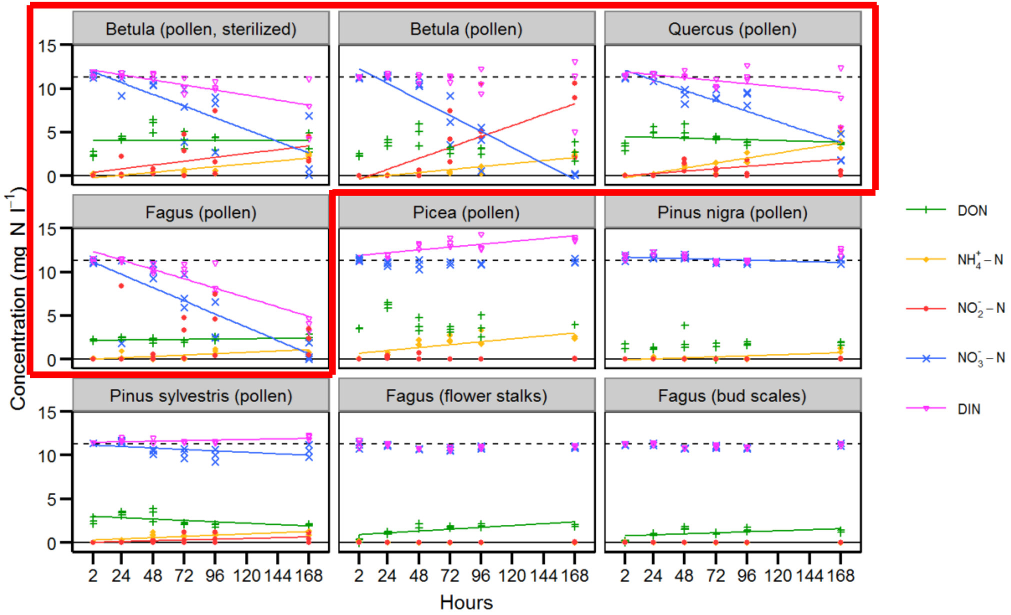


(Verstraeten et al., subm.)

1. Dissolution experiment - Results

N compounds - pollen of broadleaves

- NO_3^- decreased (-0.050 – -0.076 mg N l^{-1} h^{-1}), 75 – 98% of added NO_3^- was reduced after 7 days.
- Simultaneously, accumulation of NO_2^- (0.011 – 0.052 mg N l^{-1} h^{-1}) and NH_4^+ (0.007 – 0.027 mg N l^{-1} h^{-1}).
- Inorganic N decreased (-0.014 – -0.045 mg N l^{-1} h^{-1}) and 13 – 65% of added N was lost after 7 days, probably as gaseous nitric oxide (NO). NO is a key signaling molecule involved e.g., in pollen germination and pollen tube growth (Domingos et al., 2015 *Mol. Plant*). NO was probably formed via the nitrate reductase pathway because conditions were oxic (>1 mg O_2 l^{-1}).
- Sterilization (*Betula*) made no difference, indicating that pollen induces the observed effects and not microorganisms.

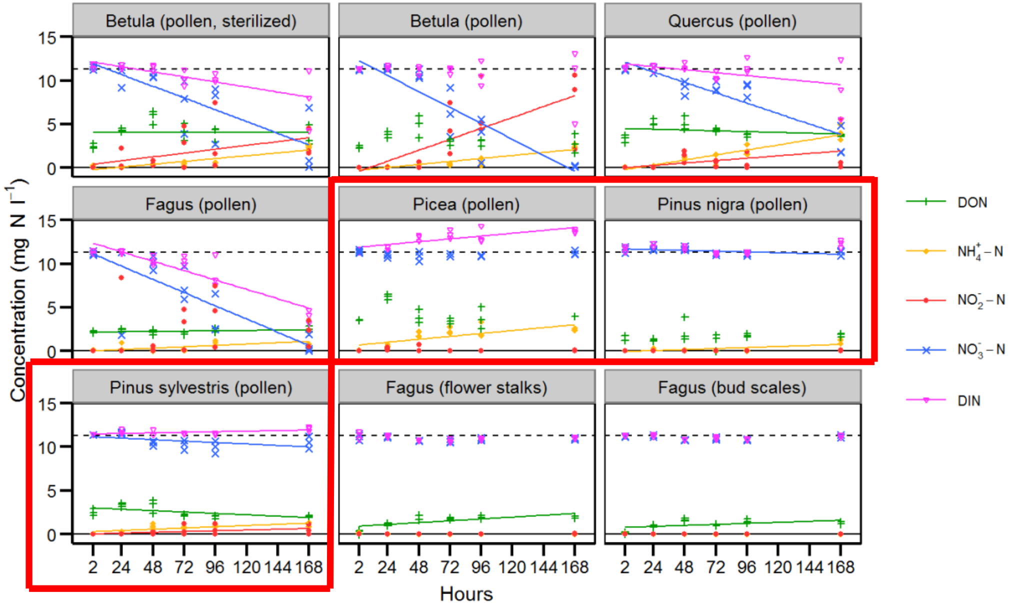


(Verstraeten et al., subm.)

1. Dissolution experiment - Results

N compounds - pollen of conifers

- NO_3^- was stable (*Picea*) or only slightly decreased (-0.004 – -0.007 mg N l^{-1} h^{-1}).
- Only small amounts of NO_2^- were formed.
- NH_4^+ accumulated (0.005 – 0.014 mg N l^{-1} h^{-1}), which might be the result of enzymatic degradation of amino acids as observed in the nectar of certain plants and might be a defense mechanism (Prŷs-Jones and Willmer, 1992 *Biol. J. Linn. Soc.*).
- Inorganic N increased with 9% for *Pinus sylvestris* and 22% for *Picea* (stable for *Pinus nigra*).

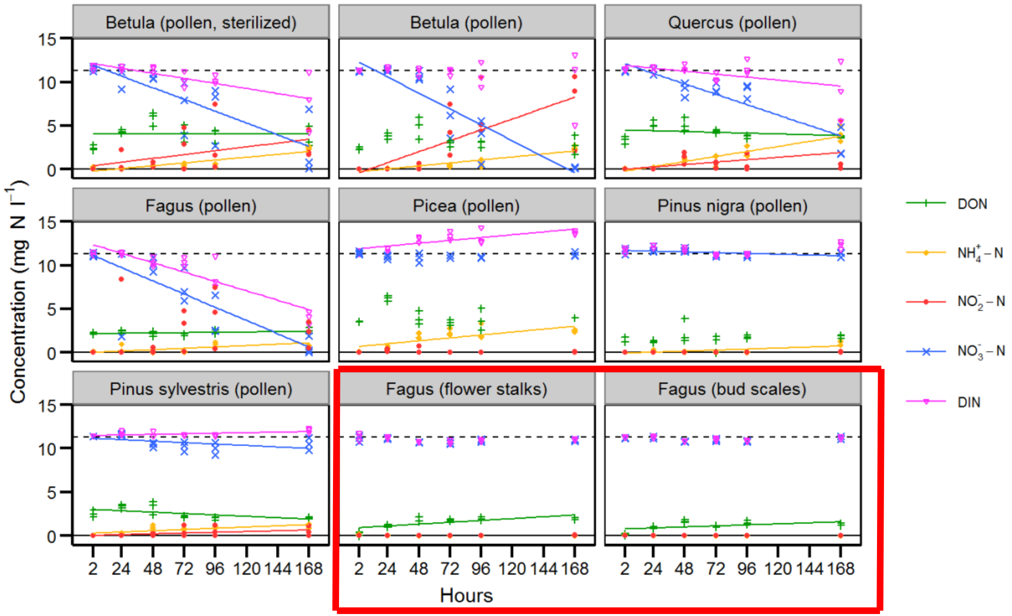


(Verstraeten et al., subm.)

1. Dissolution experiment - Results

N compounds - flower stalks and bud scales (*F. sylvatica*)

- No changes in the concentrations of inorganic N compounds were observed.
- Concentrations of NO_2^- and NH_4^+ stayed below the LOQ during the experiment.
- Some DON was released



(Verstraeten et al., subm.)

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- Trying to find a link between TF deposition fluxes and airborne pollen concentrations.
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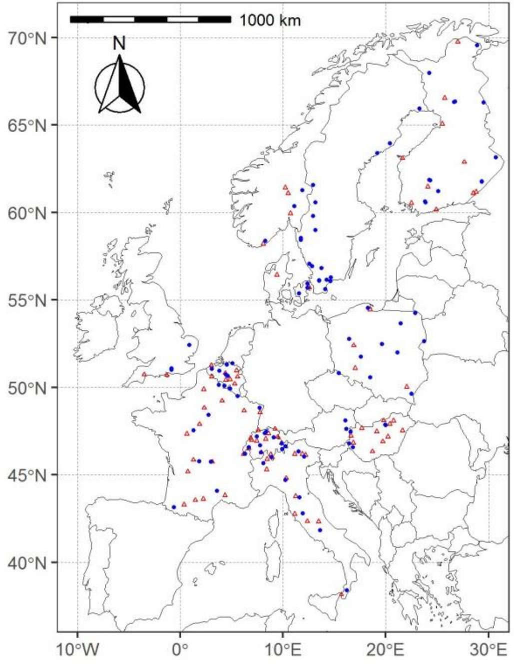
3. **Intra-annual study (TF samples)**

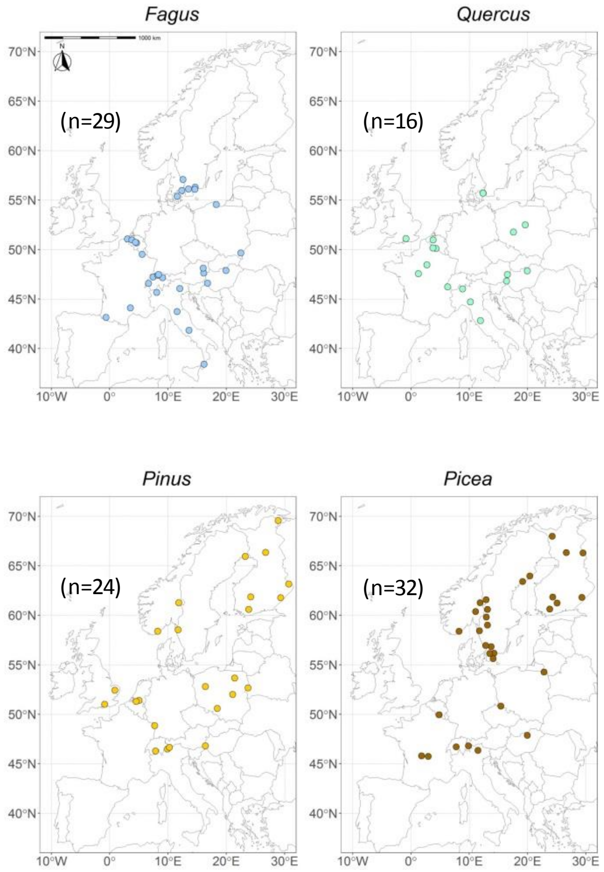
- Gathering information on TF pollen spectra and investigating the link with TF fluxes / DOC characteristics.

2. Inter-annual study (data evaluation)

- TF data from 98 Level II plots
- Airborne pollen concentrations (Seasonal Pollen Integral, SPIn) from 80 nearby (<200 km) aerobiological monitoring stations
- Grouped by tree genus (*Fagus, Quercus, Pinus, Picea*) (3 mixed)



- Level II plots
- Aerobiological monitoring stations



(Verstraeten et al., in prep.)

2. Inter-annual study (data evaluation)

- Generalized additive mixed models (GAMM's) were run for K^+, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^+$, DOC and DON, e.g.:
  TF K^+_{pollen} = s(SPIn, bs="cr", k=3) + VarIdent(Country)
- For each plot the main period of pollen dispersal during the spring* (arbitrary 2 months; halfmonthly window) was determined based on daily airborne pollen concentrations available for a limited number of sites and TF data:

	1 Apr-31 May	15 Apr-15 Jun	1 May-30 Jun	15 May-15 Jul	1 Jun-31 Jul	# plots
Fagus	24	5				**29**
Quercus	13	3				**16**
Pinus		4	10	6	4	**24**
Picea		4	9	14	5	**32**

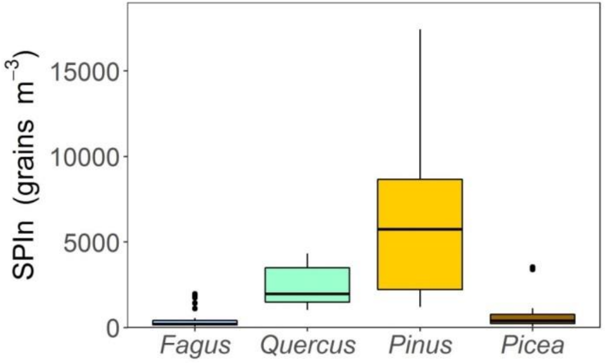
- The contribution of pollen/flowers to TF fluxes (absolute and in % of annual mean flux) was calculated as:
  INPUTpollen = TF two months pollen distribution period – TF (previous + following month)

**Pinus and Picea also disperse pollen during the autumn and winter*

(Verstraeten et al., in prep.)

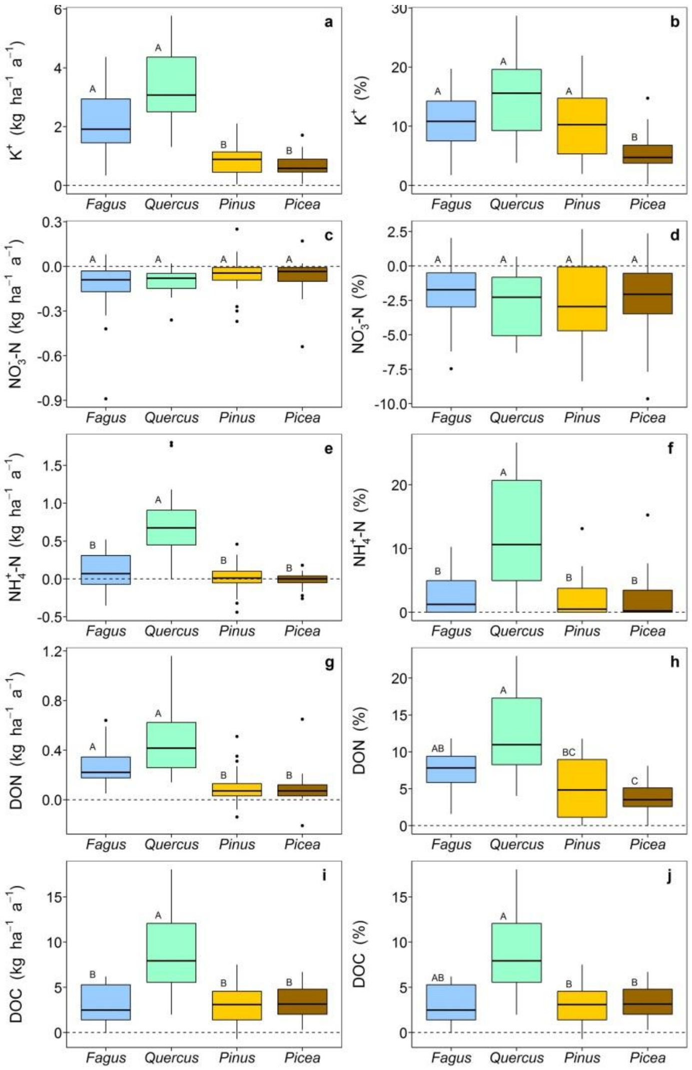
2. Inter-annual study (results)

- Pollen/flowers contributed to spring TF most in *Quercus* > *Fagus* > *Pinus* > *Picea* stands.
- Differences might be related to pollen reactivity and to the amount of pollen distributed.



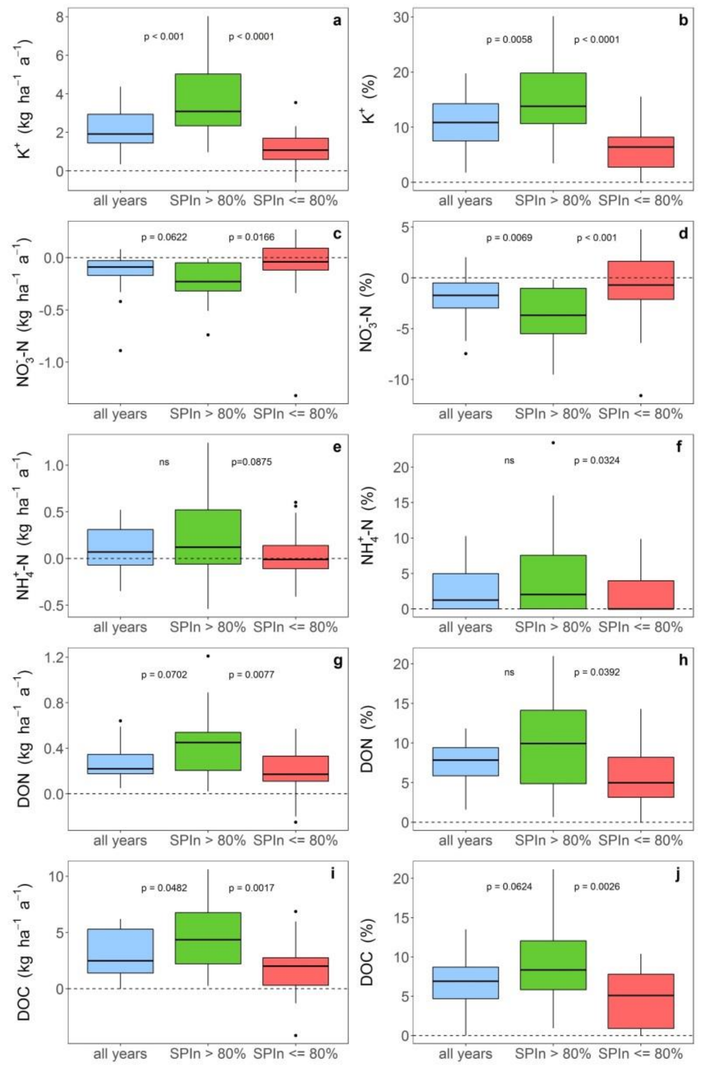
- In spring, pollen 'eats' 2-3% of annual TF N-NO_3^- deposition! More than indicated by the experiment, this was also observed for *Pinus* and *Picea*, presumably due to admixture of pollen from broadleaves (e.g., *Betula*).
- For *Quercus*, more than 10% of annual TF K^+, N-NH_4^+ and DON derives from pollen/flowers.

(Verstraeten et al., in prep.)



2. Inter-annual study (results)

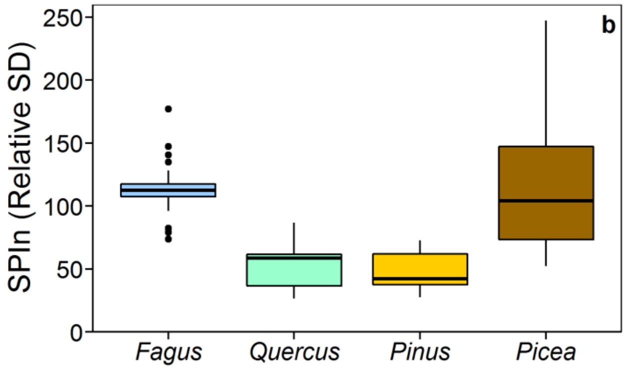
- For *Fagus*, the input from pollen/flowers to TF was significantly higher in ‘masting years’, i.e. with SPIn > 80% of the annual mean.



(Verstraeten et al., in prep.)

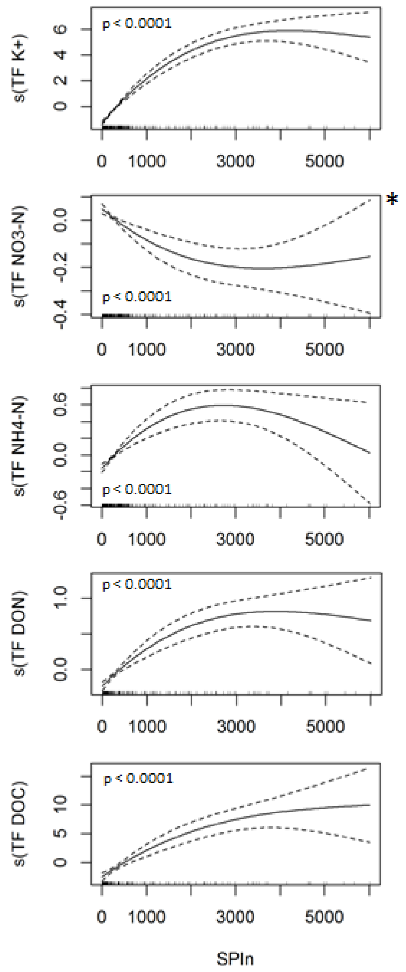
2. Inter-annual study (results)

- For ***Fagus and Picea***, the GAMM's broadly confirmed the results of the experiment, but model uncertainty was high for sparse higher values of SPIn.
- For ***Quercus* and *Pinus*,** most GAMM's were not valid because pollen is dispersed each year and <u>low variability of SPIn</u> makes it difficult to show the relationship.



- For N-NO_2^- (instable), the GAMM's were not valid

Fagus



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***15 April – 15 May**

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3. **Intra-annual study (TF samples)**

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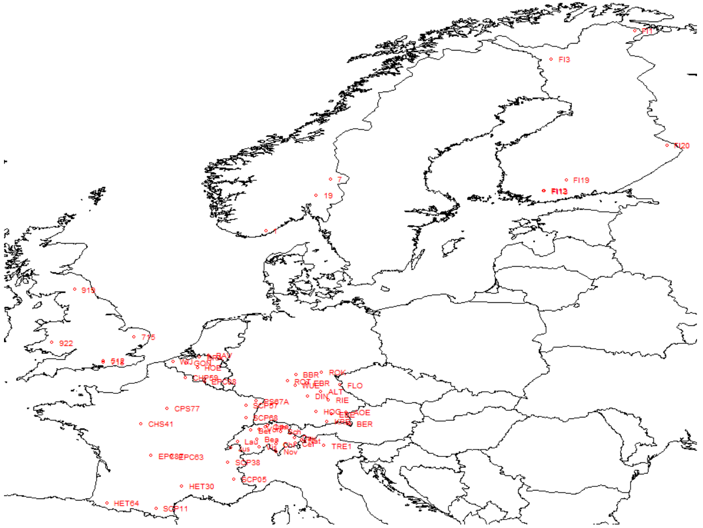
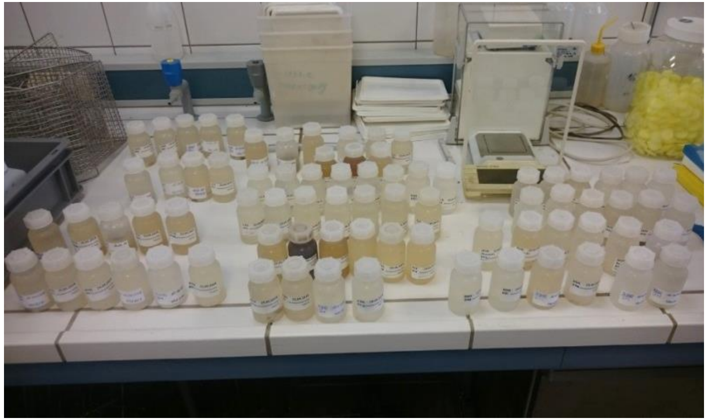
3. Intra-annual study (ongoing)

- During the spring of 2018, TF samples were collected in 60 Level II plots.

Country	Filters	Plots	Sampling frequency
Italy	11	1	weekly
UK	12	5	monthly
Switzerland	22	14	halfmonthly
Norway	11	3	monthly
Belgium, Flanders	25	5	halfmonthly
Germany, Bavaria	79	14	weekly
Finland	17	6	weekly
France	24	12	monthly
TOTAL	**201**	**60**	

- Samples were filtered (5 µm, 0.45 µm) and chemical analysis was carried out.
- 201 filters (5 µm) are analysed to determine the pollen spectra (pollen microscopy).
- A further analysis is carried out to determine DOC characteristics and degradability.



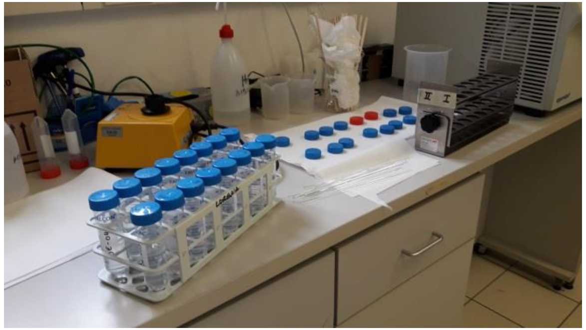


(Gottardini et al., in prep.)

3. Intra-annual study (ongoing)

Pollen microscopy

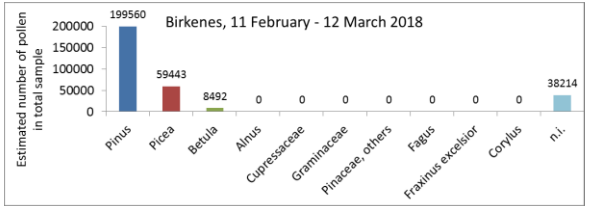
- Filters dissolved in acetone, washed, centrifuged.
- Known amount of 10-µm microspheres added for quantitative analysis.
- Fuchsine added for pollen staining.
- Slide preparation.
- Microscopic analysis (400x) for pollen identification and count (min 100 pollen grains per sample).

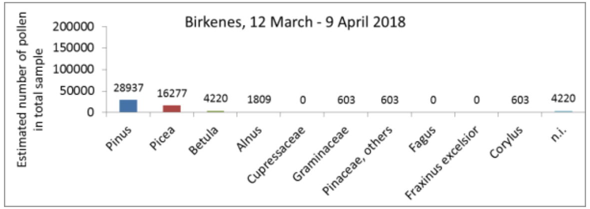


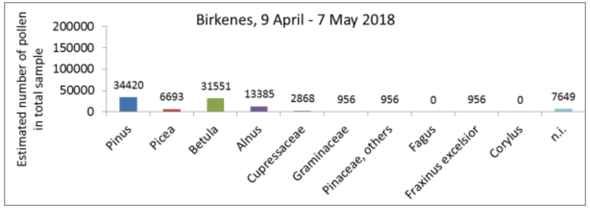
(Gottardini et al., in prep.)

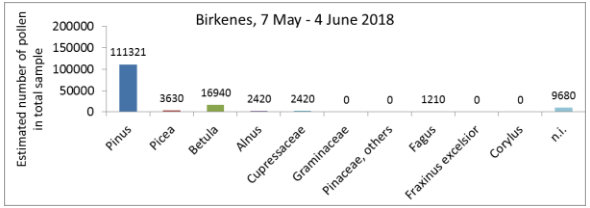
An example of results: Birkenes (NO)

Pollen composition changes over the season









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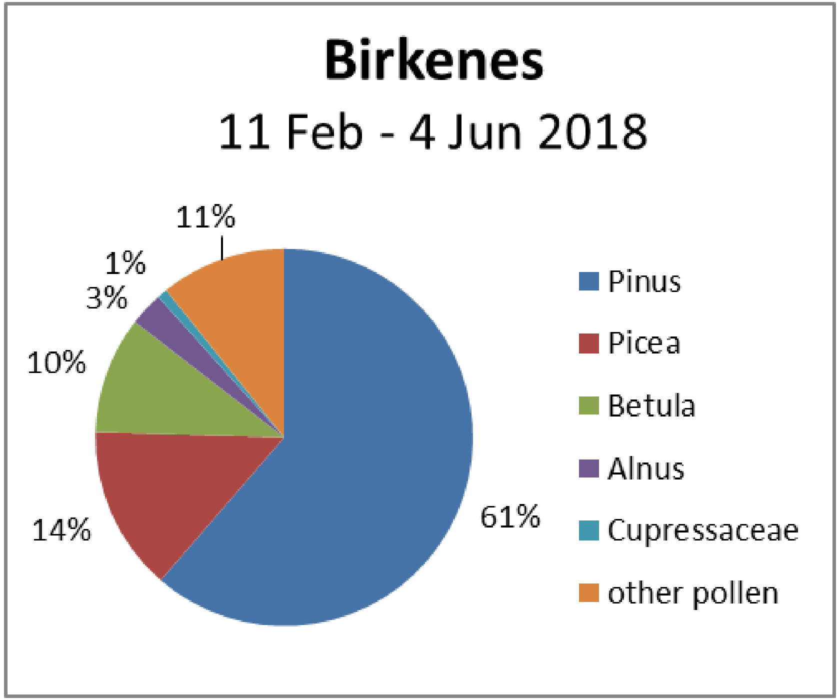
An example of results: Birkenes (NO)

Pollen spectrum on the entire period

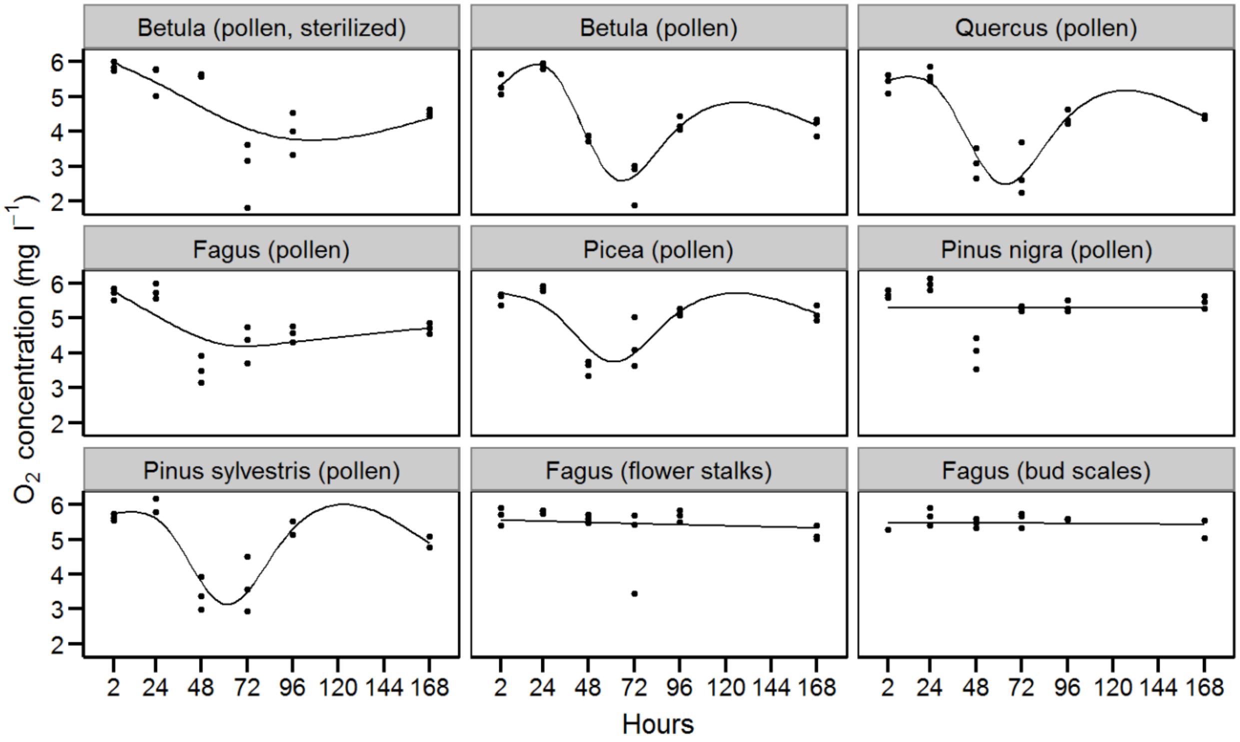


(Gottardini et al., in prep.)

Conclusions

- Pollen is an overlooked factor in forest nutrient cycling, particularly for K^+, DOC, N and PO_4^{3-}.
- Pollen induces complex N transformations involving inorganic N species as well as DON.
- These effects occur immediately or shortly after pollen is immersed in water and therefore can not be excluded.
- Accordingly, pollen biochemistry should be considered an inherent aspect of canopy exchange rather than contamination.
- The results of this study can be used to assist the validation of deposition data, the calculation of TF deposition and canopy budget modeling.

Thank you for your attention!



Betula (pollen, sterilized)
Betula (pollen)
Quercus (pollen)
Fagus (pollen)
Picea (pollen)
Pinus nigra (pollen)
Pinus sylvestris (pollen)
Fagus (flower stalks)
Fagus (bud scales)
O_2 concentration (mg l^{-1})
2 3 4 5 6
2 24 48 72 96 120 144 168
Hours