

In Vitro Nitrogen Transformations by Pollen From Temperate Tree Species

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1 ***In vitro* nitrogen transformations by pollen from temperate tree species**

2

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15

16

17 **Abstract**

18 The effects of pollen on dissolved inorganic nitrogen (DIN) compounds in throughfall water are not
19 completely understood. We conducted a 7-day leaching experiment with pollen from silver birch
20 (including a sterilized control), European beech, sessile oak, Scots pine, Corsican black pine and
21 Norway spruce using an immersion medium containing nitrate ($11.295 \text{ mg N l}^{-1}$). Within 2 hours,
22 pollen released substantial amounts of potassium (K^+), phosphate (PO_3^-) and organic compounds.
23 Solute concentrations of ammonium (NH_4^+) were built up over time. In treatments with pollen from
24 birch, oak and beech, nitrate (NO_3^-) concentrations started to decrease after 24–48 hours, while
25 simultaneously nitrite (NO_2^-) emerged, but part of the inorganic nitrogen could no longer be detected
26 in solution. For birch, sterilisation of the pollen made no difference, indicating that microorganisms on
27 the pollen played no substantial role in the observed N transformations. Conditions in the samples
28 were oxic ($1.82\text{--}6.12 \text{ mg O}_2 \text{ l}^{-1}$), rendering microbial denitrification unlikely. Our findings revealed
29 that pollen from broadleaved deciduous trees can transform throughfall NO_3^- into NO_2^- and likely also
30 nitric oxide (NO), probably through the nitrate reductase pathway. The synthesis of NH_4^+ might be
31 part of a natural defence mechanism protecting reproductive organs against pathogens during
32 pollination.

33

34 **Keywords**

35 Throughfall

36 Trees

37 Pollen

38 Ammonium

39 Nitrate

40 Nitrite

41

42 **Introduction**

43 After passage through the forest canopy, the chemical composition of precipitation is altered through
44 multiple processes collectively referred to as canopy exchange (Mayer & Ulrich 1977; Lindberg et al.
45 1986). Evaporation, washing out of intercepted atmospheric gases, particles and aerosols on the
46 surface of leaves and bark and leaching of metabolites from leaves, epiphytes and phytophagous
47 insects increase throughfall element concentrations and fluxes (Mayer & Ulrich 1977; Thimonier et al.
48 2008; Pitman et al. 2010; Van Stan and Pypker 2015). Canopy exchange processes involving nitrogen
49 (N) include the uptake of dry deposited ammonia (NH₃) and oxidized N (nitric acid plus particle
50 nitrate) and biochemical/microbiological transformations, often resulting in a net retention of N by the
51 canopy (Carlisle et al. 1966; Sievering et al. 1994; Adriaenssens et al. 2012; Guerrieri et al. 2015;
52 Karlsson et al. 2019). The current understanding of many processes is still limited, hence complicating
53 the assessment of total N deposition to forests, which could be a factor 1–2 higher than throughfall
54 deposition (Staelens et al. 2008; Clarke et al. 2016). In temperate forests, throughfall deposition
55 follows a seasonal pattern with elevated fluxes during the growing season and distinct peaks in spring
56 and fall, which are more pronounced in broadleaved deciduous forests (Lindberg et al. 1986;
57 Verstraeten et al. 2012). During the spring, precipitation typically interacts also with flowers and
58 pollen, especially from anemophilous plants, which generally produce the largest pollen amounts. In
59 Europe, throughfall is monitored with open funnel systems (bulk collectors) as a part of the
60 International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on
61 Forests (ICP Forests). Due to their relatively small size (generally between 10 and 100 µm), pollen
62 grains pass through the mesh filters (0.25–1 mm²), which aim to prevent larger particles such as litter
63 and insects from entering the throughfall samplers (Carlisle et al. 1966). When pollen is rehydrated
64 within the throughfall, a mixture of organic substances including lipids and functional proteins is
65 released from the pollen coat (Rejón et al. 2016). Organic carbon and nitrogen, potassium,
66 phosphorus, sulphur and calcium are the prevailing elements that dissolve from the pollen tissues
67 (Doskey and Ugoagwu 1989; Draaijers et al. 1996; Rösel et al. 2012). That is why several authors
68 suggested that pollen might be an overlooked contributor to throughfall fluxes for these elements,
69 although it is difficult to distinguish the specific effects of pollen from other factors affecting

70 throughfall biochemistry (Ferm 1997; Carlisle et al. 1966; Le Mellec et al. 2010; Verstraeten et al.
71 2016).

72 Regarding the impacts of pollen on inorganic N compounds in throughfall, clues might be given by
73 laboratory experiments, designed to study pollination associated biochemistry. The decomposition of
74 pollen by microorganisms might lead to higher N concentrations in solution, as observed after the
75 immersion of *Pinus sylvestris* pollen in lake waters (Rösel et al. 2012). Furthermore, rehydrating
76 pollen from various angiosperm and gymnosperm plant species was observed to produce intracellular
77 gaseous nitric oxide (NO), which can migrate into the extracellular matrix where it could interact with
78 surrounding cells (McInnis et al. 2006; Bright et al. 2009; Wilson et al. 2009; Pasqualini et al. 2015;
79 Jiménez-Quesada et al. 2017). Nitric oxide is a reactive oxygen species common in plant tissues and a
80 key signalling molecule in an array of plant physiological processes (Niu et al. 2019). In plant sexual
81 reproduction, NO plays a role in pollen germination as it acts as a negative chemotropic agent of
82 pollen tube growth and orientation towards the ovule (Prado et al. 2004; Wang et al. 2009; Wilson et
83 al. 2009; Šírová et al. 2011; Domingos et al. 2015). The source of NO in plants is however still matter
84 of debate and multiple oxidative or reductive pathways might coexist (Domingos et al. 2015; Niu et al.
85 2019; León and Costa-Broseta 2020). The enzymatic transformation of nitrate (NO_3^-) by nitrate
86 reductase oxidases through a two-step reaction with nitrite (NO_2^-) as an intermediate product likely is
87 the prevailing mechanism active in plant tissues under oxic conditions. The observed accumulation of
88 NO_2^- in the extracellular medium around rehydrating pollen, which produced NO, points in this
89 direction but it is not clear whether NO_2^- accumulation is a function of pollen NO oxidation or a NO
90 independent phenomenon (Bright et al. 2009). A possible alternative are NO synthases (NOS), which
91 convert L-arginine into L-citrulline and NO, independently of NO_3^- and NO_2^- , but evidence for the
92 existence of this mechanism in land plants is lacking so far (Jeandroz et al. 2016; León and Costa-
93 Broseta 2020). Nitrite can also be reduced to NO through the mitochondrial electron transport chain
94 (mETC), but this process is sensitive to the inhibition by O_2 and therefore likely only functions under
95 anaerobic/hypoxic conditions (León and Costa-Broseta 2020).

96 We conducted an *in vitro* experiment with the aim to investigate the NO₃⁻ reduction capacity of pollen,
97 collected from three coniferous and three broadleaved deciduous tree species commonly occurring in
98 European temperate forests. Bud scales and flower stalks of beech were also investigated and a
99 sterilized control was included to examine the possible role of pollen borne microorganisms. Element
100 concentrations and dissolved oxygen (O₂) concentrations were monitored for up to seven days,
101 mimicking the relatively long residence time of throughfall samples in the field. The following
102 hypotheses were tested: 1) tree pollen is an important source of nutrients in throughfall, 2) rehydrating
103 pollen is able to transform extracellular NO₃⁻.

104

105 **Materials and Methods**

106

107 *Collection of pollen, bud scales and flower stalks*

108 Low-hanging branches of sessile oak (*Quercus robur* L.), European beech (*Fagus sylvatica* L.), Scots
109 pine (*Pinus sylvestris* L.) and Corsican black pine (*P. nigra* ssp. *laricio* var. *Corsicana* Loud.) with
110 immature flowers/catkins were collected from tree rows and forest edges in Flanders, Belgium on 15–
111 19 April 2018. In the laboratory, branches were sorted per tree species, put in containers with water
112 and arranged in separate greenhouse chambers. The branches were incubated for three weeks until the
113 flowers/catkins opened and most pollen were shed and gathered on sheets of paper placed beneath the
114 containers. Pollen was then collected with a clean brush and transferred into sterile polypropylene
115 tubes. Tubes with archived dehydrated pollen from silver birch (*Betula pendula* L.) collected in
116 Flanders, Belgium (Belgian aerobiological network) and Norway spruce (*Picea abies* L.) from
117 Trentino, Italy (Fondazione Edmund Mach) were delivered by mail. Bud scales and flower stalks of
118 beech were selected from material collected in June 2018 during the regular litterfall survey in a forest
119 monitoring (Level II) plot of the ICP Forests in Sonian forest (Flanders, Belgium) following the
120 protocol published in the ICP Forests Manual Part XIII (Ukonmaanaho et al. 2016). All tubes were
121 collectively stored in darkness at 4 °C until the start of the experiment.

122

123 *Leaching experiment*

124 The 7-day leaching experiment was initiated on 5 August 2019. For the treatments, 50 ± 0.52 mg dry
125 pollen i.e. roughly $1\text{--}10 \times 10^6$ pollen grains (6 tree species), bud scales or flower stalks (beech) were
126 added to sterile 500 ml borosilicate glass conical flasks. For silver birch, an additional treatment was
127 prepared; the pollen was first put in a 1.5 ml tube and sterilized with liquid nitrogen gas (N_2), in order
128 to exclude any interference from pollen borne microorganisms. The preparation of a sterilized control
129 was not possible for the other tree species, because the available amount of pollen was too limited. For
130 each of the nine treatments, three replicates were prepared ($n = 27$ samples). After weighing the
131 organic material, 200 ml of stock solution consisting of sodium nitrate (NaNO_3) dissolved in MilliQ
132 water ($11.295 \text{ mg N l}^{-1}$) was added to each flask, without further stirring or shaking. The flasks were
133 sealed immediately with Parafilm to avoid airborne contamination. During the experiment, all flasks
134 were stored together at a central location in the laboratory at ambient room conditions (around $21 \text{ }^\circ\text{C}$,
135 not sheltered from daylight and artificial light). Subsamples (15 ml) of the solutions were taken with a
136 pipette 2, 24, 48, 72, 96 and 168 hours after the start of the experiment, while avoiding to shake the
137 flasks and to draw in pollen. It was noticed that the pollen from deciduous trees was sedimented and
138 the pollen from coniferous trees floated on the solution surface (Fig. S1). Just before collecting the
139 subsamples, the concentrations of dissolved O_2 in the solutions were measured with a WTW FDO 925
140 Dissolved Oxygen Sensor. Upon collection, the subsamples were filtered using syringe filters with a
141 pore size of $0.45 \text{ }\mu\text{m}$ and a diameter of 25 mm (Chromafil PET Xtra-45/25) and stored in borosilicate
142 glass tubes in darkness at $4 \text{ }^\circ\text{C}$ until the start of chemical analysis.

143

144 *Chemical analysis*

145 All subsamples were analysed jointly in a single run during the week following the experiment. The
146 concentrations of NO_2^- , NO_3^- , sulphate (SO_4^{2-}), phosphate (PO_4^{3-}), chloride (Cl^-), ammonium (NH_4^+),
147 calcium (Ca^{2+}), potassium (K^+), magnesium (Mg^{2+}) and sodium (Na^+) were determined with ion
148 chromatography (Dionex ICS-3000, limit of quantification $\text{LOQ} = 0.05 \text{ mg l}^{-1}$ for NO_2^- , K^+ and Mg^{2+}
149 and 0.1 mg l^{-1} for all other ions). The concentrations of dissolved organic carbon (DOC) and total
150 nitrogen (TN) were determined with a C/N analyser (Skalar Formacs^{HT}, $\text{LOQ} = 0.5 \text{ mg l}^{-1}$ for TN and
151 1 mg l^{-1} for DOC).

152

153 *Calculations*

154 If the measured concentration of an element was lower than the LOQ, its concentration was equated to
155 50% of the LOQ for calculations and representation in graphs. The concentrations of dissolved organic
156 nitrogen (DON) were calculated as the difference between the measured TN concentrations and the
157 sum of the measured concentrations of three dissolved inorganic nitrogen (DIN) forms according to
158 formula (1):

159

$$160 \quad DON = TN - (NH_4^+ \text{ }_N + NO_2^- \text{ }_N + NO_3^- \text{ }_N) \quad (1)$$

161

162 The biomass (in mg) dissolved from pollen, bud scales and flower stalks was calculated based on the
163 sum C_i of the concentrations of all elements x in solution, correcting for the amount of added Na^+ and
164 NO_3^- and the progressive reduction of sample volume through the collection of subsamples using
165 formulas (2) and (3):

166

$$167 \quad C_i = \sum_{j=1}^x C_j \quad (2)$$

168

$$169 \quad Dissolved \ biomass = C_1 V_1 + \sum_{i=2}^6 IF((C_i - C_{i-1}) < 0; 0; (C_i - C_{i-1})(V_1 - (i - 1)\Delta V)) \quad (3)$$

170

171 with i the subsample number ($i = 1-6$), V_1 the initial sample volume (200 ml), ΔV the volume of a
172 single subsample (15 ml) and IF a logical function returning zero when solute concentrations
173 decreased between two subsequent subsamples, assuming that no elements dissolved in that case.
174 Values were ultimately converted into percentages of the amount of added organic matter.

175

176 *Data analysis*

177 The drawing of figures and statistical testing were carried out using the R software version 4.0.2 (R
178 Core Team 2020). Treatments were compared using the non-parametric Kruskalmc test (Multiple

179 comparison test after Kruskal-Wallis) included in the ‘pgirmess’ package (Giraudoux 2018). Changes
180 in the concentrations of nitrogen forms were tested with a linear model in function of time (hours)
181 elapsed since the start of the experiment using the `lm()` function included in the ‘stats’ package (R
182 Core Team 2020).

183

184 **Results**

185 In all treatments with pollen from broadleaved deciduous trees, NO_3^- concentrations in the immersion
186 medium significantly decreased (-0.050 – -0.076 $\text{mg N l}^{-1} \text{ h}^{-1}$) throughout the 7-day experiment (Fig. 1,
187 Table S1 and S2). Simultaneously, NO_2^- and NH_4^+ appeared in solution, with concentrations gradually
188 increasing over time (NO_2^- 0.011 – 0.052 $\text{mg N l}^{-1} \text{ h}^{-1}$, but not significantly for beech; NH_4^+ 0.007 –
189 0.027 $\text{mg N l}^{-1} \text{ h}^{-1}$). In contrast, the concentrations of DIN decreased over time (-0.014 – -0.045 mg N l^{-1}
190 h^{-1} , but not significantly for silver birch) and 12.88–64.63% of the N initially added to the immersion
191 medium could no longer be detected at the end of the experiment (after 168 hours). In the treatments
192 with pollen from coniferous trees, NO_3^- concentrations were stable (spruce) or only slightly decreased
193 (-0.004 – -0.007 $\text{mg N l}^{-1} \text{ h}^{-1}$) over the course of the experiment and only small amounts of NO_2^- were
194 formed (no significant change except 0.004 $\text{mg N l}^{-1} \text{ h}^{-1}$ for Scots pine). The concentrations of NH_4^+ ,
195 however, also increased in the treatments with pollen from coniferous trees (0.005 – 0.014 $\text{mg N l}^{-1} \text{ h}^{-1}$).
196 This resulted in a significant net increase in DIN concentrations at the end of the experiment with
197 8.88% for Scots pine and 21.83% for Norway spruce (0.003 – 0.013 $\text{mg N l}^{-1} \text{ h}^{-1}$, but not significantly
198 for Corsican black pine). After seven days, 75.26–98.27% of the added NO_3^- was transformed in the
199 treatments with pollen from broadleaved deciduous trees, but only 0.34–7.45% in the treatments with
200 pollen from coniferous trees (Table S3). For flower stalks and bud scales of beech, no significant
201 changes in the concentrations of inorganic N compounds were observed and concentrations of NO_2^-
202 and NH_4^+ stayed below the LOQ during the experiment.

203 Pollen released predominantly organic compounds in the immersion medium. Mean concentrations
204 over the course of the experiment were 11.72–30.67 mg DOC l^{-1} and 1.26–3.75 mg DON l^{-1} for pollen
205 from broadleaved deciduous trees and 9.54–17.16 mg DOC l^{-1} and 1.61–4.13 mg DON l^{-1} for pollen

206 from coniferous trees (Fig. 1, Fig. 2, Table S1). Among the inorganic ions, potassium (K^+) was found
207 to be the dominating base cation by far with 0.048–0.054 meq l^{-1} for pollen from broadleaved
208 deciduous trees and 0.038–0.109 meq l^{-1} for pollen from coniferous trees, followed by PO_4^{3-} (0.016–
209 0.047 meq l^{-1} for pollen from deciduous trees; 0.005–0.038 meq l^{-1} for pollen from coniferous trees),
210 Na^+ (0.005–0.019 meq l^{-1} for pollen from deciduous trees; 0.012–0.032 meq l^{-1} for pollen from
211 coniferous trees) and SO_4^{2-} (0.003–0.011 meq l^{-1} for pollen from deciduous trees; 0.004–0.018 meq l^{-1}
212 for pollen from coniferous trees) (Fig. **3a,b**; Table S1). The concentrations of Cl^- , Ca^{2+} and Mg^{2+} were
213 below the LOQ or slightly higher, indicating that the amounts leached from pollen were small. Bud
214 scales and flower stalks of beech released some DON (0.87–1.17 mg l^{-1}) (Fig. 1), K^+ (0.021–0.039
215 meq l^{-1}) and Na^+ (0.009–0.020 meq l^{-1}) (Fig. **3a**), while the concentrations were below the LOQ for all
216 other elements.

217 At the end of the experiment (i.e. after 7 days), 11.73–23.62% (mean 17.94%) of pollen biomass had
218 dissolved, from which 55–75% within the first two hours (Fig. S2). There was considerable variation
219 among individual tree species, but no systematic differences between coniferous and broadleaved
220 deciduous trees in the fraction of dissolved biomass. For K^+ and PO_4^{3-} , the highest concentrations were
221 recorded already after two hours. The fraction of dissolved biomass was much lower for flower stalks
222 (1.92%) and bud scales of beech (1.86%), and this was explained mainly by the difference in DOC.

223 Dissolved O_2 concentrations were 1.82–6.16 mg l^{-1} (Fig. S3), indicating that oxic conditions (> 1.0 mg
224 l^{-1}) prevailed in all samples during the experiment (Gerardi, 2002). In the treatments with pollen, a
225 transient decline in the dissolved O_2 concentrations was observed on days 2–4, while they were more
226 stable in the treatments with flower stalks and bud scales of beech.

227 For pollen from silver birch, the sterilisation treatment had no significant influence on the dissolved
228 element concentrations (Table S1).

229

230 **Discussion**

231

232 *Elements dissolved from pollen*

233 The water-soluble fraction of pollen is approximately 40% of total pollen biomass, as determined with
234 a soft extraction technique during 49 days (Greenfield 1999). In our experiment, slightly less than half
235 of this fraction was mobilised after immersion in ultrapure water with a high NO_3^- concentration for
236 seven days. Similar dissolved fractions were observed after immersion of Scots pine pollen in natural
237 lake waters for 16 days (Rösel et al. 2012). The amounts of elements dissolved from pollen during the
238 experiment indicate that tree pollen should be considered as an important source of DOC, DON, K^+ ,
239 PO_4^{3-} , Na^+ and SO_4^{2-} in throughfall, confirming our first hypothesis that tree pollen is an important
240 source of nutrients in throughfall. These results are in line with previous findings derived from
241 throughfall data, except for Na^+ (Carlisle et al. 1966; Draaijers et al. 1996; Le Mellec et al. 2010;
242 Verstraeten et al. 2016). Sodium in throughfall originates mainly from sea spray, which makes it
243 difficult to distinguish an input from pollen in throughfall data (Thimonier et al. 2008). Considering
244 the observed predominant leaching of cations, pollen likely also increases the concentrations of
245 bicarbonates (HCO_3^-) as these are important for safeguarding the electro-neutrality of a solution
246 (Kopáček et al. 2000).

247 Pollen is washed efficiently from the air by rainfall and enters throughfall collectors after passing
248 through the mesh filters (Draaijers et al. 1996; Lawson and Rands 2019). Upon rehydration, pollen
249 immediately becomes biochemically active, as also shown by our observation that most elements
250 dissolved within a time span of two hours (Wilson et al. 2009; Domingos et al. 2015). The latter
251 implies that nutrient inputs from pollen in throughfall could not be completely kept out by solely
252 increasing the sampling frequency. Additional deployment of wet-only samplers could only correct
253 partially this, because only dry deposition of pollen is excluded. On the other hand, additional
254 simultaneous monitoring of local airborne pollen concentrations at ground level or pollen deposition
255 spectra and fluxes, along with pollen emission and dispersion modelling results (e.g. Sofiev et al.
256 2013; Verstraeten et al. 2019) may allow a better estimate of the impact of pollen on throughfall
257 deposition.

258 The ten times higher amount of elements per unit of biomass dissolved from pollen compared to bud
259 scales and flower stalks suggests that the latter contribute only little to throughfall fluxes. The annual
260 flux of bud scales/flowers amounts, however, to 400–500 kg ha⁻¹ a⁻¹ (Table S4). This is more
261 substantial than the pollen deposition flux in forested areas, which rarely exceeds a few dozens of kg
262 ha⁻¹ a⁻¹ (Doskey and Ugoagwu 1989; Cho et al. 2003; Lee and Booth 2003). Accordingly, bud scales
263 and flowers should also be considered as a source of elements in throughfall during spring, particularly
264 for K⁺, Na⁺ and DON.

265

266 *Pollen induced nitrate reduction in deposition collectors beneath broadleaved trees*

267 Pollen itself has an N content of only 1–8% including small amounts of water-extractable NO₃⁻, and
268 NO₃⁻ is also not among the main constituents of stigma exudates (Doskey and Ugoagwu 1989;
269 Webster et al. 2008; Rejón et al. 2014). But after landing on the pistil or inside deposition collectors,
270 pollen may interact with throughfall water, in which NO₃⁻ is ubiquitous in variable concentrations
271 (Waldner et al. 2014). Our experiment showed that pollen from three broadleaved deciduous tree
272 species is able to transform extracellular NO₃⁻ *in vitro*, hence confirming our second hypothesis. The
273 applied amounts of pollen and NO₃⁻ are equivalent with a pollen deposition flux of 3.5 kg ha⁻¹ and a
274 throughfall deposition flux of 1.5 kg N l⁻¹ for the standard equipment used in throughfall monitoring in
275 ICP Forests Level II plots in Flanders, Belgium (10 bulk samplers; funnel diameter 14 cm; plot size
276 0.25 ha). Pollen transformed 75.26–98.27% of the added NO₃⁻ in a time span of seven days, which is
277 about half the maximum exposure time in the field for sampling on a semi-monthly basis. Considering
278 that a half-monthly throughfall deposition of 1.5 kg N l⁻¹ is relatively high and observed only in N-
279 polluted areas (Waldner et al. 2014), our data indicate that pollen could transform a large part of the
280 NO₃⁻ contained in throughfall samples of temperate deciduous woodlands in the period of dispersal.
281 This might explain the lower NO₃⁻ fluxes that have been measured using bulk samplers compared to
282 wet-only samplers operated in pair at the same location (Richter and Lindberg 1988). Throughfall is,
283 however, generally monitored using bulk samplers and it would be useful to check whether this signal
284 can be detected more generally in throughfall data and can be linked/correlated to the airborne pollen

285 spread and deposition timing and intensity. This could assist the validation and interpretation of
286 deposition data and enable to optimize sampling methodologies.

287 We observed that a relatively small portion of NO_3^- was also transformed in the treatments with pollen
288 from Corsican black pine and Norway spruce, as compared to broadleaved pollen. This might be
289 explained by the air sacs or wings with which gymnosperm pollen is equipped as an adaptation for
290 controlling and maximizing the success of wind pollination (Lu et al. 2011). The wings allowed the
291 coniferous pollen to float on the medium, in contrast to the broadleaved pollen, which sunk to the
292 bottom of the containers (Fig. S1). This buoyancy of coniferous pollen might reduce the contact
293 between the pollen grains and the chemical compounds in the medium, possibly with a lower (or null)
294 transformation of NO_3^- as a result.

295 Because dissolved O_2 concentrations measured in the samples were always above 1.82 mg l^{-1} , and thus
296 above the threshold of 1.0 mg l^{-1} for oxic conditions, it is unlikely that the transformation of NO_3^- in
297 the samples was the result of anaerobic denitrification (Gerardi 2002). Also, the transformation of
298 NO_3^- through dissimilatory nitrate reduction to ammonium (DNRA), which according to recent
299 findings may occur under different oxygenic conditions, seems to be improbable, since this would
300 have also affected the treatments with pollen from coniferous trees (Wang et al. 2020). The results that
301 we obtained also did not discriminate sterilized and non-sterilized pollen from silver birch. Several
302 kinds of evidence thus indicate that pollen borne microorganisms, or originated from the plants, did
303 not play an essential role in the observed N transformations. The synthesis of large quantities of NO_2^-
304 along with the consumption of NO_3^- in the treatments with pollen from broadleaved deciduous trees is
305 probably the result of the transformation of NO_3^- into NO_2^- through nitrate reductase activity, which is
306 a common enzymatic pathway in angiosperm plant tissues (Yamasaki and Sakihama 2000; Rockel et
307 al. 2002; Domingos et al. 2015). Also the partial disappearance of DIN at the end of the experiment
308 corroborates this conclusion. Previous experiments demonstrated that pollen from various angiosperm
309 plants including a number of tree species can both produce NO_2^- and NO (Bright et al. 2009). The N
310 lost in our experiment was thus probably due to volatilisation in the form of gaseous NO.

311

312 *Ammonium synthesis by pollen*

313 Free amino acids and proteins make up about 90% of the water-extractable N compounds in pollen
314 and an average 7% occurs as $\text{NH}_4^+\text{-N}$, probably contained in glutamine and asparagine (Greenfield
315 1999). The concentrations of NH_4^+ gradually increased during the experiment, which means that NH_4^+
316 must have been formed in solution through secondary N transformation. Because NH_4^+ was also
317 formed in the treatments with coniferous pollen wherein NO_3^- concentrations remained nearly stable, it
318 is most likely a by-product originating from the enzymatic degradation of proteins and free amino
319 acids that dissolved from the pollen (Hildebrandt et al. 2015). The observed decrease in DOC and
320 DON concentrations towards the end of the experiment could support this speculation.

321 Besides being a plant nutrient, NH_4^+ is known to play a role in plant defence and immunity (Marino
322 and Moran 2019). It is possible that NH_4^+ has an additional function in plant reproduction. For
323 instance, high levels of NH_4^+ found in the nectar of *Lathraea clandestina* may act as a repellent against
324 nectar thieves such as ants and birds (Prŷs-Jones and Willmer 1992). Stigmatic exudates and
325 pollination drops contain a variety of defence-related proteins (O’Leary et al. 2007; Rejón et al. 2014).
326 Whether NH_4^+ is also involved in the protection of reproductive organs during pollination should be
327 further investigated.

328

329 **Conclusion**

330 Our experiment showed that pollen from broadleaved deciduous trees is able to enzymatically
331 transform extracellular NO_3^- into NO_2^- and likely also NO *in vitro*, which may affect throughfall
332 samples, routinely collected in the forest monitoring programme of ICP Forests. In the spring, pollen
333 is an important contributor of throughfall K^+ , PO_4^{3-} , Na^+ , SO_4^{2-} and DOC. The chemical composition
334 of throughfall samples results not only from atmospheric deposition and passive leaching processes,
335 but also from enzymatically driven transformations during periods of pollen dispersal.

336

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339

340 **Conflicts of interest/Competing interests**

341 Not applicable.

342

343 **Availability of data and material**

344 All data are available as supplementary information.

345

346 **Code availability**

347 The R code used for statistical analysis and creating the graphs is available as supplementary

348 information.

349

350 **Authors' contributions**

351 A.V., E.G., N.B. and J.N. designed the study; A.V., E.G. and N.B. collected samples for the

352 experiment; A.V. and G.G. carried out the experiment and compiled the data; A.V. analysed the data

353 and drafted the manuscript; and all authors contributed critically to the drafts and gave final approval

354 for publication.

355

356 **References**

357

358 Adriaenssens S, Staelens J, Wuyts K, Van Wittenberghe S, Wuytack T, Verheyen K, Boeckx P,

359 Samson R (2012) Canopy uptake of ¹⁵NH₃ by four temperate tree species and the interaction with leaf

360 properties. *Water Air Soil Poll* 223:5643–5657. <https://doi.org/10.1007/s11270-012-1304-4>

361

362 Bright J, Hiscock SJ, James PE, Hancock JT (2009) Pollen generates nitric oxide and nitrite: a

363 possible link to pollen-induced allergic responses. *Plant Physiol Bioch* 47:49–55.

364 <https://doi.org/10.1016/j.plaphy.2008.09.005>

365

366 Carlisle A, Brown AHF, White EJ (1966) The organic matter and nutrient elements in the precipitation
367 beneath a Sessile oak (*Quercus petraea*) canopy. J Ecol 54:87–98. <https://doi.org/10.2307/2257660>
368

369 Cho Y-J, Kim IS, Kim P, Lee EJ (2003) Deposition of airborne pine pollen in a temperate pine forest.
370 Grana, 42: 178–182. <https://doi.org/10.1080/00173130310016158>
371

372 Clarke N, Žlindra D, Ulrich E, Mosello R, Derome J, Derome K, König N, Lövblad G, Draaijers GPJ,
373 Hansen K, Thimonier A, Waldner P (2016) Part XIV: Sampling and Analysis of Deposition. In:
374 UNECE ICP Forests Programme Coordinating Centre (ed.): Manual on methods and criteria for
375 harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests.
376 Thünen Institute of Forest Ecosystems, Eberswalde, Germany, 32 p. + Annex.
377

378 Domingos P, Prado AM, Wong A, Gehring C, Feijo JA (2015). Nitric oxide: a multitasked signaling
379 gas in plants. Mol Plant 8:506–520. <https://doi.org/10.1016/j.molp.2014.12.010>
380

381 Doskey PV, Ugoagwu BJ (1989) Atmospheric deposition of macronutrients by pollen at a semi-
382 remote site in northern Wisconsin. Atmos Environ 23:2761–2766. [https://doi.org/10.1016/0004-](https://doi.org/10.1016/0004-6981(89)90556-8)
383 [6981\(89\)90556-8](https://doi.org/10.1016/0004-6981(89)90556-8)
384

385 Draaijers GPJ, Erisman JW, Spranger T, Wyers GP (1996) The application of throughfall
386 measurements for atmospheric deposition monitoring. Atmos Environ 30:3349–3361.
387 [https://doi.org/10.1016/1352-2310\(96\)00030-1](https://doi.org/10.1016/1352-2310(96)00030-1)
388

389 Ferm M (1997) Improvement and validation of the throughfall technique for nitrogen deposition
390 measurements to forest ecosystems. In: Slanina S, eds. Biosphere-atmosphere exchange of pollutants
391 and trace substances. Transport and chemical transformation of pollutants in the troposphere, vol 4.
392 Springer, Berlin, Heidelberg.
393

394 Gerardi MH (2002) Nitrification and Denitrification in the Activated Sludge Process. John Wiley &
395 Sons, New York.

396

397 Giraudoux P (2018) pgirmess: spatial analysis and data mining for field ecologists. R package version
398 1.6.9. <https://CRAN.R-project.org/package=pgirmess>.

399

400 Greenfield LG (1999) Weight loss and release of mineral nitrogen from decomposing pollen. *Soil Biol*
401 *Biochem* 31:353–361. [https://doi.org/10.1016/S0038-0717\(98\)00134-5](https://doi.org/10.1016/S0038-0717(98)00134-5)

402

403 Guerrieri R, Vanguelova EI, Michalski G, Heaton THE, Mencuccini M (2015) Isotopic evidence for
404 the occurrence of biological nitrification and nitrogen deposition processing in forest canopies. *Glob*
405 *Change Biol* 21:4613–4626. <https://doi.org/10.1111/gcb.13018>

406

407 Hildebrandt TM, Nunes Nesi A, Araújo WL, Braun H-P (2015) Amino acid catabolism in plants. *Mol*
408 *Plant* 8: 1563–1579. <https://doi.org/10.1016/j.molp.2015.09.005>

409

410 Jeandroz S, Wipf D, Stuehr DJ, Lamattina L, Melkonian M, Tian Z, Zhu Y, Carpenter EJ, Wong GK,
411 Wendehenne D (2016) Occurrence, structure, and evolution of nitric oxide synthase–like proteins in
412 the plant kingdom. *Sci Signal* 9:re2. <https://doi.org/10.1126/scisignal.aad4403>

413

414 Jiménez-Quesada MJ, Carmona R, Lima-Cabello E, Traverso JA, Castro AJ, Claros MG, de Dios
415 Alché J (2017) Generation of nitric oxide by olive (*Olea europaea* L.) pollen during in vitro
416 germination and assessment of the S-nitroso- and nitroproteomes by computational predictive
417 methods. *Nitric Oxide* 68:23–37. <https://doi.org/10.1016/j.niox.2017.06.005>

418

419 Karlsson PE, Pihl Karlsson G, Hellsten S, Akselsson C, Ferm M, Hultberg H (2019) Total deposition
420 of inorganic nitrogen to Norway spruce forests – Applying a surrogate surface method across a

421 deposition gradient in Sweden. *Atmos Environ* 217:116964.
422 <https://doi.org/10.1016/j.atmosenv.2019.116964>
423
424 Kopáček J, Hejzlar J, Mosello, R (2000) Estimation of organic acid anion concentrations and
425 evaluation of charge balance in atmospherically acidified colored waters. *Water Res* 34: 3598–3606.
426 [https://doi.org/10.1016/S0043-1354\(00\)00109-3](https://doi.org/10.1016/S0043-1354(00)00109-3)
427
428 Lawson DA, Rands SA (2019) The effects of rainfall on plant–pollinator interactions. *Arthropod-Plant*
429 *Inte* 13:561–569. <https://doi.org/10.1007/s11829-019-09686-z>
430
431 Lee EU, Booth T (2003) Macronutrient input from pollen in two regenerating pine stands in southeast
432 Korea. *Ecol Res* 18:423–430. <https://doi.org/10.1046/j.1440-1703.2003.00566.x>
433
434 Le Mellec A, Meessenburg H, Michalzik B (2010) The importance of canopy-derived dissolved and
435 particulate organic matter (DOM and POM) – comparing throughfall solution from broadleaved and
436 coniferous forests. *Ann For Sci* 67:411. <https://doi.org/10.1051/forest/2009130>
437
438 León J, Costa-Broseta Á (2020) Present knowledge and controversies, deficiencies, and
439 misconceptions on nitric oxide synthesis, sensing, and signaling in plants. *Plant Cell Environ* 43:1–15.
440 <https://doi.org/10.1111/pce.13617>
441
442 Lindberg SE, Lovett GM, Richter DD, Johnson DW (1986) Atmospheric deposition and canopy
443 interactions of major ions in a forest. *Science* 231:145–145.
444 <https://doi.org/10.1126/science.231.4734.141>
445
446 Lu Y, Jin B, Wang L, Wang Y, Wang D, Jiang X-X, Chen P (2011) Adaptation of male reproductive
447 structures to wind pollination in gymnosperms: Cones and pollen grains. *Can J Plant Sci* 91:897–906.
448 <https://doi.org/10.4141/cjps2011-020>

449

450 Marino D, Moran JF (2019) Can ammonium stress be positive for plant performance? *Front Plant Sci*
451 10:1103. <https://doi.org/10.3389/fpls.2019.01103>

452

453 Mayer R, Ulrich B (1977) Acidity of precipitation as influenced by the filtering of atmospheric
454 sulphur and nitrogen compounds – its role in the element balance and effect on soil. *Water Air Soil*
455 *Poll* 7:409–416. <https://doi.org/10.1007/BF00284135>

456

457 McInnis SM, Desikan R, Hancock JT, Hiscock SJ (2006) Production of reactive oxygen species and
458 reactive nitrogen species by angiosperm stigmas and pollen: potential signaling crosstalk? *New Phytol*
459 172:221–228. <https://doi.org/10.1111/j.1469-8137.2006.01875.x>.

460

461 Niu L, Yu J, Liao W, Yu J (2019) Hydrogen peroxide and nitric oxide signaling network. In: Gupta
462 D., Palma J., Corpas F. (eds) *Nitric oxide and hydrogen peroxide signaling in higher plants*. Springer,
463 Cham. https://doi.org/10.1007/978-3-030-11129-8_2

464

465 O'Leary SJB, Poulis BAD, von Aderkas P (2007) Identification of two thaumatin-like proteins (TLPs)
466 in the pollination drop of hybrid yew that may play a role in pathogen defence during pollen
467 collection. *Tree Physiol* 27:1649–1659. <https://doi.org/10.1093/treephys/27.12.1649>

468

469 Pitman RM, Vanguelova EI, Benham SE (2010) The effects of phytophagous insects on water and soil
470 nutrient concentrations and fluxes through forest stands of the Level II monitoring network in the UK.
471 *Sci Total Environ* 409:169–181. <https://doi.org/10.1016/j.scitotenv.2010.09.029>

472

473 Pasqualini S, Cresti M, Del Casino C, Faleri C, Frenguelli G, Tedeschini E, Ederli L (2015) Roles for
474 NO and ROS signalling in pollen germination and pollen-tube elongation in *Cupressus arizonica*. *Biol*
475 *Plant* 59: 735–744. <https://doi.org/10.1007/s10535-015-0538-6>

476

477 Prado AM, Porterfield DM, Feijó JA (2004) Nitric oxide is involved in growth regulation and re-
478 orientation of pollen tubes. *Development* 131:2707–2714. <https://doi.org/10.1242/dev.01153>
479

480 Prÿs-Jones OE, Willmer PG (1992) The biology of alkaline nectar in the purple toothwort (*Lathraea*
481 *clandestina*): ground level defences. *Biol J Linn Soc* 45:373–388. <https://doi.org/10.1111/j.1095->
482 [8312.1992.tb00650.x](https://doi.org/10.1111/j.1095-8312.1992.tb00650.x)
483

484 R Core Team (2020) R: A language and environment for statistical computing. R Foundation for
485 Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
486

487 Rejón JD, Delalande F, Schaeffer-Reiss C, de Dios Alché J, Rodríguez-García MI, Van Dorsselaer A,
488 Castro AJ (2016) The pollen coat proteome: at the cutting edge of plant reproduction. *Proteomes* 4:5.
489 <https://doi.org/10.3390/proteomes4010005>
490

491 Rejón JD, Delalande F, Schaeffer-Reiss C, Carapito C, Zienkiewicz K, de Dios Alché J, Rodríguez-
492 García MI, Van Dorsselaer A, Castro AJ (2014) The plant stigma exudate: a biochemically active
493 extracellular environment for pollen germination? *Plant Signal Behav* 9:e28274.
494 <https://doi.org/10.4161/psb.28274>
495

496 Richter DD, Lindberg SE (1988) Wet deposition estimates from long-term bulk and event wet-only
497 samples of incident precipitation and throughfall. *J Environ Qual* 17:619–622.
498 <https://doi.org/10.2134/jeq1988.00472425001700040017x>
499

500 Rockel P, Strube F, Rockel A, Wildt J, Kaiser WM (2002) Regulation of nitric oxide (NO) production
501 by plant nitrate reductase *in vivo* and *in vitro*. *J Exp Bot* 53:103–110.
502 <https://doi.org/10.1093/jexbot/53.366.103>
503

504 Rösel P, Rychła A, Wurzbacher C, Grossart H-P (2012) Effects of pollen leaching and microbial
505 degradation on organic carbon and nutrient availability in lake water. *Aquat Sci* 74:87–99.
506 <https://doi.org/10.1007/s00027-011-0198-3>
507

508 Sievering H, Enders G, Kins L, Kramm G, Ruoss K, Roeder G, Zelger M, Anderson L, Dlugi R (1994)
509 Nitric acid, particulate nitrate and ammonium profiles at the Bayerischer Wald: evidence for large
510 deposition rates of total nitrate. *Atmos Environ* 28:311–315. [https://doi.org/10.1016/1352-](https://doi.org/10.1016/1352-2310(94)90106-6)
511 [2310\(94\)90106-6](https://doi.org/10.1016/1352-2310(94)90106-6)
512

513 Šírová J, Sedlářová M, Piterková J, Luhová L, Petřivalsky M (2011) The role of nitric oxide in the
514 germination of plant seeds and pollen. *Plant Sci* 181:560–572.
515 <https://doi.org/10.1016/j.plantsci.2011.03.014>
516

517 Sofiev M, Siljamo P, Ranta H, Linkosalo T, Jaeger S, Rasmussen A, Rantio-Lehtimäki A, Severova E,
518 Kukkonen J (2013) A numerical model of birch pollen emission and dispersion in the atmosphere.
519 Description of the emission module. *Int J Biometeorol* 57:45–58. [https://doi.org/10.1007/s00484-012-](https://doi.org/10.1007/s00484-012-0532-z)
520 [0532-z](https://doi.org/10.1007/s00484-012-0532-z)
521

522 Staelens J, Houle D, De Schrijver A, Neiryneck J, Verheyen K (2008) Calculating dry deposition and
523 canopy exchange with the canopy budget model: Review of assumptions and application to two
524 deciduous forests. *Water Air Soil Pollut* 191:149–169. <https://doi.org/10.1007/s11270-008-9614-2>
525

526 Thimonier A, Schmitt M, Waldner P, Schleppi P (2008) Seasonality of the Na/Cl ratio in precipitation
527 and implications of canopy leaching in validating chemical analyses of throughfall samples. *Atmos*
528 *Environ* 42:9106–9117. <https://doi.org/10.1016/j.atmosenv.2008.09.007>
529

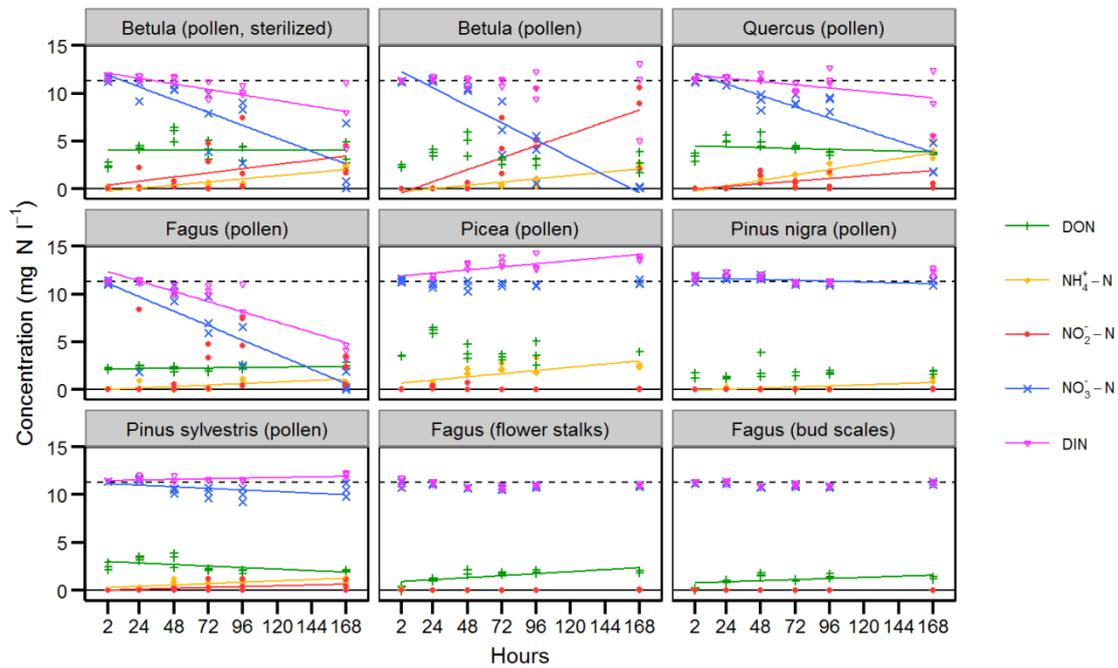
530 Ukonmaanaho L, Pitman R, Bastrup-Birk A, Breda N, Rautio P (2016) Part XIII: Sampling and
531 Analysis of Litterfall. In: UNECE ICP Forests Programme Co-ordinating Centre, eds. Manual on

532 methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of
533 air pollution on forests. Thünen Institute for Forests Ecosystems, Eberswalde, Germany, 14 p. +
534 Annex [<http://www.icp-forests.org/manual.htm>].
535
536 Van Stan JT, Pypker TG (2015) A review and evaluation of forest canopy epiphyte roles in the
537 partitioning and chemical alteration of precipitation. *Sci Total Environ* 536:813–824.
538 [10.1016/j.scitotenv.2015.07.134](https://doi.org/10.1016/j.scitotenv.2015.07.134)
539
540 Verstraeten A, Neiryck J, Genouw G, Cools N, Roskams P, Hens M (2012) Impact of declining
541 atmospheric deposition on forest soil solution chemistry in Flanders, Belgium. *Atmos Environ* 62:50–
542 63. <https://doi.org/10.1016/j.atmosenv.2012.08.017>
543
544 Verstraeten A, Verschelde P, De Vos B, Neiryck J, Cools N, Roskams P, Hens M, Louette G, Sleutel
545 S, De Neve S (2016) Increasing trends of dissolved organic nitrogen (DON) in temperate forests under
546 recovery from acidification in Flanders, Belgium. *Sci Total Environ* 553:107–119.
547 <https://doi.org/10.1016/j.scitotenv.2016.02.060>
548
549 Verstraeten WW, Dujardin S, Hoebeke L, Bruffaerts N, Kouznetsov R, Dendoncker N, Hamdi R,
550 Linard C, Hendrickx M, Sofiev M, Delcloo AW (2019) Spatio-temporal monitoring and modelling of
551 birch pollen levels in Belgium. *Aerobiologia* 35:703–717. <https://doi.org/10.1007/s10453-019-09607->
552 [w](https://doi.org/10.1007/s10453-019-09607-w)
553
554 Waldner P, Marchetto A, Thimonier A, Schmitt M, Rogora M, Granke O, Mues V, Hansen K,
555 Karlsson GP, Žlindra D, Clarke N, Verstraeten A, Lazdins A, Schimming C, Iacoban C, Lindroos A-J,
556 Vanguelova E, Benham S, Meesenburg H, Nicolas M, Kowalska A, Apuhtin V, Napa U, Lachmanová
557 Z, Kristoefel F, Bleeker A, Ingerslev M, Vesterdal L, Molina J, Fischer U, Seidling W, Jonard M,
558 O'Dea P, Johnson J, Fischer R, Lorenz M (2014) Detection of temporal trends in atmospheric

559 deposition of inorganic nitrogen and sulphate to forests in Europe. *Atmos Environ* 95:363–374.
560 <https://doi.org/10.1016/j.atmosenv.2014.06.054>
561
562 Wang S, Liu C, Wang X, Yuan D, Zhu G (2020) Dissimilatory nitrate reduction to ammonium
563 (DNRA) in traditional municipal wastewater treatment plants in China: Widespread but low
564 contribution. *Water Res* 179:115877. <https://doi.org/10.1016/j.watres.2020.115877>
565
566 Wang Y, Chen T, Zhang C, Hao H, Liu P, Zheng M, Baluska F, Samai J, Lin J (2009) Nitric oxide
567 modulates the influx of extracellular Ca²⁺ and actin filament organization during cell wall construction
568 in *Pinus bungeana* pollen tubes. *New Phytol* 182:851–862. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-8137.2009.02820.x)
569 [8137.2009.02820.x](https://doi.org/10.1111/j.1469-8137.2009.02820.x)
570
571 Webster EA, Tilston EL, Chudek JA, Hopkins DW (2008) Decomposition in soil and chemical
572 characteristics of pollen. *Eur J Soil Sci* 59:551–558. <https://doi.org/10.1111/j.1365-2389.2008.01022.x>
573
574 Wilson ID, Hiscock SJ, James PE, Hancock JT (2009) Nitric oxide and nitrite are likely mediators of
575 pollen interactions. *Plant Signal Behav* 4:416–418. <https://doi.org/10.4161/psb.4.5.8270>
576
577 Yamasaki H, Sakihama Y (2000) Simultaneous production of nitric oxide and peroxynitrite by plant
578 nitrate reductase: in vitro evidence for the NR-dependent formation of active nitrogen species. *FEBS*
579 *Letters* 468:89–92. [https://doi.org/10.1016/S0014-5793\(00\)01203-5](https://doi.org/10.1016/S0014-5793(00)01203-5)
580

581 **Figures**

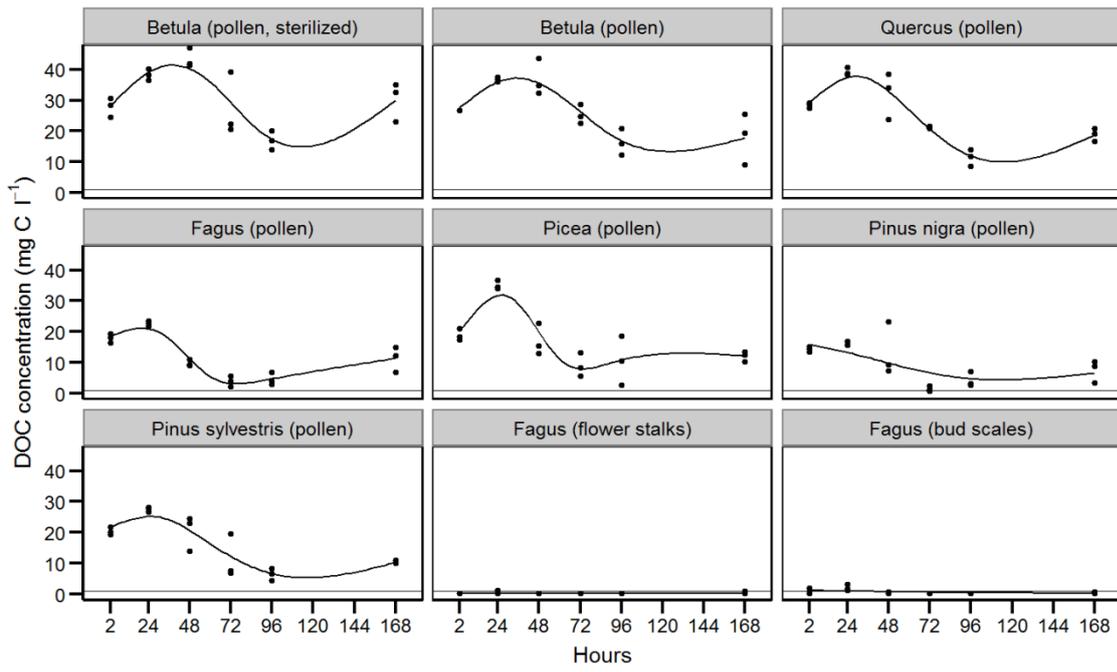
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583

584 Fig. 1 Concentrations of dissolved organic nitrogen (DON), ammonium nitrogen ($\text{NH}_4^+\text{-N}$), nitrite
585 nitrogen ($\text{NO}_2^-\text{-N}$), nitrate nitrogen ($\text{NO}_3^-\text{-N}$) and dissolved inorganic nitrogen (DIN) during the
586 experiment (mg N l^{-1}). Coloured lines show significant changes over time based on linear regression
587 (coefficients and p-values are listed in Table S2). Dotted black lines indicate the amount of added
588 $\text{NO}_3^-\text{-N}$ ($11.295 \text{ mg N l}^{-1}$). Solid black lines represent the LOQs for measured N forms ($0.015 \text{ mg N l}^{-1}$
589 for $\text{NO}_2^-\text{-N}$; $0.023 \text{ mg N l}^{-1}$ for $\text{NO}_3^-\text{-N}$; $0.078 \text{ mg N l}^{-1}$ for $\text{NH}_4^+\text{-N}$).

590



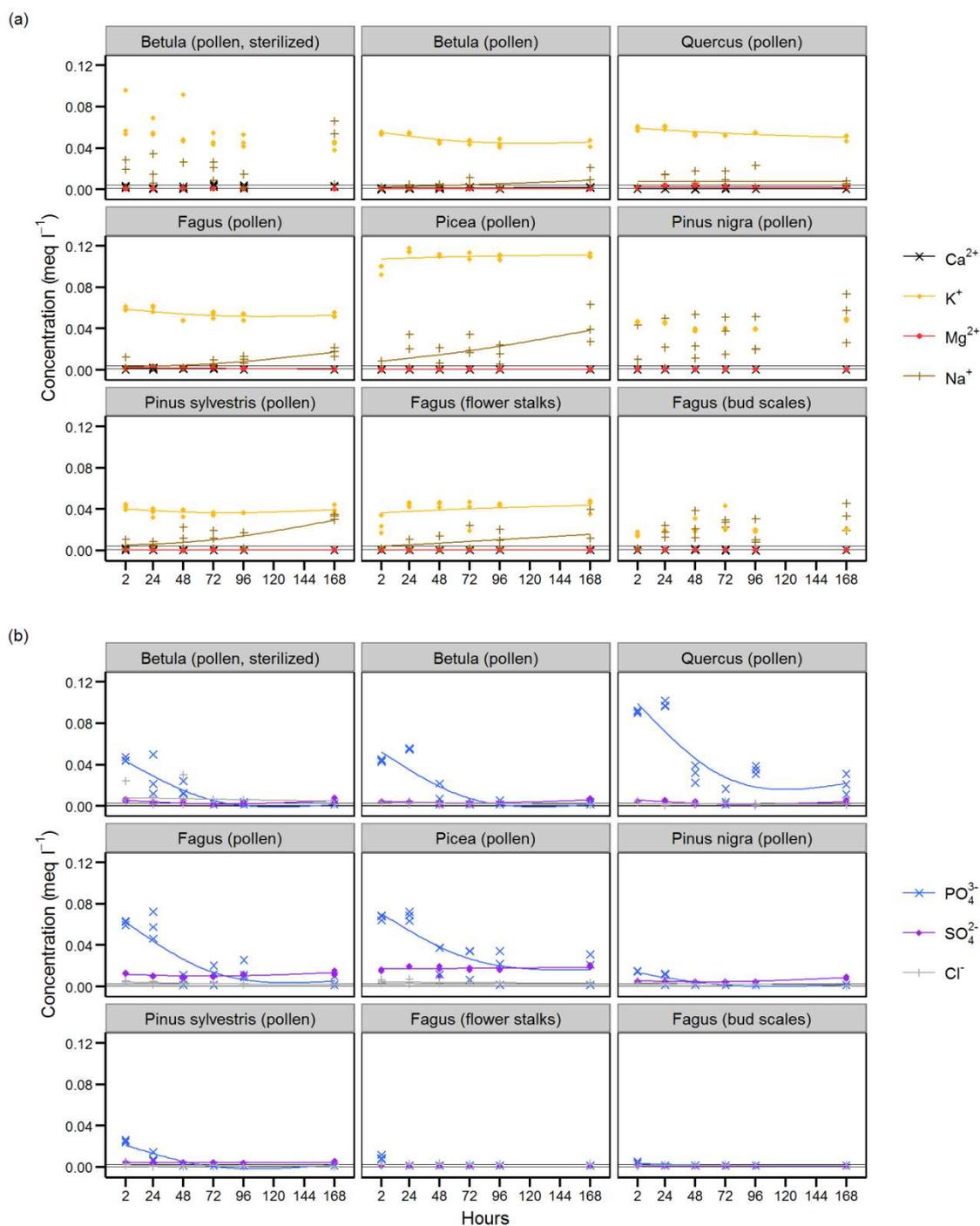
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592 Fig. 2 Concentrations of dissolved organic carbon (DOC) during the experiment (mg l⁻¹). A smoother

593 was added to the observations to aid visual interpretation. Solid black lines represent the LOQ (1 mg C

594 l⁻¹).

595



596

597 Fig. 3 Concentrations of cations (a) and anions (b) excluding N compounds and added Na⁺ during the
 598 experiment (meq l⁻¹). A smoother was added to the observations to aid visual interpretation. Solid
 599 black lines represent the LOQs (0.0012 meq l⁻¹ for Ca²⁺, 0.0013 meq l⁻¹ for K⁺, 0.0010 meq l⁻¹ for
 600 Mg²⁺, 0.0043 meq l⁻¹ for Na⁺, 0.0004 meq l⁻¹ for PO₄³⁻, 0.0005 meq l⁻¹ for SO₄²⁻, 0.0028 meq l⁻¹ for Cl⁻
 601).

602

Supplementary Files

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