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3 **P and K additions enhance canopy N retention and accelerate**

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the associated leaching

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15 **Abstract**

16 This study evaluated the interactive effects of combined phosphorus (P) and potassium
17 (K) additions on canopy nitrogen (N) retention (CNR) and subsequent canopy leaching
18 at a long-term N manipulation site on Whim bog in south Scotland. Ambient deposition
19 is 8 kg N ha⁻¹ yr⁻¹ and an additional 8, 24, and 56 kg N ha⁻¹ yr⁻¹ of either ammonium
20 (NH₄⁺) or nitrate (NO₃⁻) with or without P and K has been applied over 11 years.
21 Throughfall N deposition below *Calluna vulgaris* and foliar N and P concentrations were
22 assessed. Results showed that 60 % for low dose and 53 % for high dose of NO₃⁻ and
23 80 % for low dose and 38 % for high dose of NH₄⁺ onto *Calluna* was retained by *Calluna*
24 canopy. The CNR was enhanced by P and K addition in which 84 % of NO₃⁻ and 83 % of
25 NH₄⁺ for high dose were retained. CNR for NO₃⁻ increased the canopy leaching of
26 dissolved organic N (DON) and associated organic anions. NH₄⁺ retention increased
27 canopy leaching of magnesium and calcium through ion exchange. Even over 11-years N
28 exposure without P and K, foliage N:P ratio of *Calluna* did not increase, suggesting that
29 N exposure did not lead to N saturation of *Calluna* at this study site. Our study concluded
30 that increases in P and K availability enhance CNR of *Calluna* but accelerate the
31 associated canopy leaching of DON and base cations, depending on foliar N status.

32 **Keywords:** Manipulation experiment; peatland; *Calluna vulgaris*; dissolved organic
33 nitrogen; base cations; long-term study

34 **Introduction**

35 Atmospheric nitrogen (N) deposition has been increasing on a global scale (Galloway et
36 al. 2008) and may affect peatland ecosystems which are sensitive to increased N
37 deposition (Bobbink et al. 1998). *Calluna vulgaris*, one of the dominant canopy-
38 forming species on many peatlands, can modify throughfall deposition (Bobbink et al.,
39 1992). Understanding of canopy nitrogen (N) retention (CNR) and which factors affect
40 retention is essential to quantifying total atmospheric N deposition and to evaluate its
41 ecological effects. CNR has been widely observed in throughfall measurements (Lovett
42 and Lindberg 1993; Lovett et al., 1996; Staelens et al. 2008; Aguilhaume et al., 2017;
43 Avila et al., 2017; Pinkalski et al., 2018). In N manipulation experiments, the application
44 of aliquots of N directly to the canopy (Cape et al. 2001; Chiwa et al. 2004; Gaige et al.
45 2007), has shown that CNR can lead to underestimation of total atmospheric N deposition.
46 CNR often exceeded 75% of total N deposition to coniferous forests during the growing
47 season in the Niwot Forest with low (3 kg N ha^{-1} during the growing season) atmospheric
48 N deposition (Tomaszewski et al. 2003; Sievering et al. 2007). Even at a site with
49 moderate or high ($17\text{--}96 \text{ kg N ha}^{-1} \text{ year}^{-1}$) atmospheric N deposition, the canopy may

50 retain up to 40% (Lovett and Lindberg 1993; Chiwa et al. 2004) and 70% of total N
51 deposition (Gaige et al. 2007), respectively. CNR is an important factor affecting the
52 impact of elevated atmospheric N deposition on forest C sequestration (Tomaszewski and
53 Sievering, 2007; Dezi et al., 2010; Chiwa et al., 2012), suggesting the likelihood of an
54 important link with C sequestration in *Calluna*, a key component of peatland canopies.

55 Several researchers have explored factors affecting CNR including tree species
56 (Brumme et al., 1992; Eilers et al., 1992), canopy leaf area (Lovett et al., 1996; Wuyts et
57 al., 2008), leaf phenology (Houle et al., 1999; Hagen-Thorn et al., 2006), and leaf surface
58 condition (Boyce et al., 1991; Sase et al., 2008; Adriaenssens et al., 2011). However, little
59 information exists concerning the interactive effect of the availability of other nutrients
60 such as phosphorus (P) and potassium (K). Our first hypothesis is that increases in P and
61 K availability enhance the CNR, because P and K are also limiting in wetlands in addition
62 to N (Venterink et al., 2002).

63 The process of CNR remains elusive, especially for NO_3^- . Chiwa et al. (2016)
64 showed that NO_3^- retention by *Sphagnum* moss caused the leaching of DON and organic
65 anions from *Sphagnum*. However, it remains to be shown whether this process can be

66 applied to vascular plants. Canopy retention of NH_4^+ is usually accompanied by base
67 cation leaching (Bobbink et al., 1992; Krupa, 2003; Li et al., 2013; Staelens et al., 2008;
68 Stevens et al., 2011) and hydrogen ion (H^+) leaching (Krupa, 2003; Liu et al., 2013;
69 Manninen et al., 2011; Paulissen et al., 2004; Stevens et al., 2011; Tomassen et al., 2003).
70 Our second hypothesis is that the enhanced CNR by P and K addition accelerates canopy
71 leaching including DON and base cations.

72 To test these two hypotheses, this study aimed to 1) quantify CNR of *Calluna*
73 exposed with/without P and K addition and 2) explore the leaching from the canopy when
74 NO_3^- and NH_4^+ are taken up by a *Calluna* canopy with/without P and K. These objectives
75 were addressed using the N manipulation experiment on Whim bog in SE Scotland where
76 the theoretical input to the *Calluna* canopy can be calculated using known concentrations
77 and volumes of N treatments, giving us information about the input-output balances of
78 ions through a canopy and the possibility of exploring the processing of nitrogen retention
79 in the *Calluna* canopy.

80

81 **Materials and Methods**

82 *Study site*

83 This study was conducted at an N manipulation experiment on 3-6 m of deep peatland
84 (282m a.s.l., 3°16'W, 55° 46'N) at Whim bog located *c.* 30 km south of Edinburgh in
85 Scotland. No active management has been conducted for at least 70 years. The vegetation
86 is dominated by *Calluna vulgaris*, *Eriophorum vaginatum*, *Sphagnum capillifolium*,
87 *Hypnum jutlandicum*, *Pleurozium schreberi* and *Cladonia portentosa* which occur widely
88 on similar habitats through the northern hemisphere (Gore, 1983). Ambient N deposition
89 is 8 kg N ha⁻¹ yr⁻¹, consisting of wet deposition for NH₄⁺ (*ca.* 3 kg N ha⁻¹ yr⁻¹) and NO₃⁻
90 (*ca.* 3 kg N ha⁻¹ yr⁻¹), and dry deposition for NH₃ (2 kg N ha⁻¹ yr⁻¹) (Leith et al., 2004;
91 Sheppard et al., 2004; Sheppard et al., 2014). Mean annual values (and corresponding
92 ranges) of temperature and precipitation between 2003 and 2013 were 7.9 (5.9 – 9.0) °C
93 and 1,124 (734 – 1,486) mm, respectively.

94

95 *Treatments*

96 The experimental peatland area was divided into four replicated blocks each containing
97 eleven 12.8 m² circular plots. To avoid contamination from adjacent plots, plots are 3 m

98 apart. The eleven different N treatments (Table 1), replicated in four plots, have been
99 supplied to each plot from a central spinning disc generating fine rain droplets all year
100 round since June 2002 (Sheppard *et al.*, 2004). The wet N treatments are in addition to
101 the estimated ambient deposition of *ca.* 8 kg N ha⁻¹ yr⁻¹ and supply 10 % additional
102 rainwater (Sheppard *et al.*, 2014). Potassium hydrogen phosphate (K₂HPO₄) adjusted in
103 a 1:14 molar ratio (1:14 and 1:7 for P and K, respectively) to N was used as the P and K
104 treatments. Treatments are applied automatically when weather conditions meet the
105 following criteria: sufficient rainfall in the holding tanks, air temperature > 0 °C and wind
106 speed < 5 m s⁻¹ simulating a much more realistic frequency scenario *ca.* 120 applications
107 yr⁻¹ (Sheppard *et al.*, 2014).

108

109 *Sampling and chemical analysis*

110 Six samplings were conducted for throughfall under *Calluna vulgaris* between
111 July and October 2013 (Table 2). During the sampling period, 35 individual rainfall
112 events, defined as > 1 mm of continuous precipitation over 6 hours, occurred, of which,
113 63%, 22 rainfall events were collected in this study (Table 2). The six samplings were

114 also typical events in terms of rainfall intensity (Table 2).

115 Before the sampling occurred the *Calluna* which is mostly in the
116 mature/degenerate phase (Sheppard et al., 2011; Carfrae et al., 2007) had already received
117 11 years of N treatment. Throughfall collectors for *Calluna* were made of a silicone tube
118 cut lengthwise (width 2.8-3.2 mm × length 150 – 200 mm: *c.* 50 cm²) draining into a 150
119 ml square polyethylene bottle. Prior to sampling, the sample bottles (100 mL HDPE) and
120 tubes were placed into a 1% Decon solution (Decon 90, Decon Laboratories Limited, East
121 Sussex, UK) for at least 24 h and then washed with distilled water. After drying, the tube
122 was connected to the polyethylene bottle using duct tape. The projected area of the tube
123 was measured with a ruler.

124 One throughfall collector was randomly placed under green *Calluna vulgaris*
125 shrubs (height 30-50 cm) in control and N treated plots prior to a rain event. Precipitation
126 was also collected in the open area at the study site using three collectors, each with a 20
127 cm diameter polyethylene funnel mounted 1.5 m above ground, draining to a 2 litre
128 polyethylene bottle.

129 Collected water samples were immediately transported back to the nearby

130 laboratory where aliquots were filtered through a 0.45 µm membrane filter (Puradisc™,
131 Whatman Inc., NJ, USA), and stored in the dark at 4°C. The following chemical
132 determinations were carried out on the filtered pore water samples: pH by glass electrode
133 (MP220, Mettler Toledo, Leicester, UK), major ions (Cl⁻, NO₃⁻, SO₄²⁻, Na⁺, K⁺, Ca²⁺, and
134 Mg²⁺) by ion chromatography (CH-9101, Metrohm, Herisau, Switzerland), NH₄⁺ by
135 AMmonia Flow Injection Analyser (AMFIA, ECN; Wyers *et al.* 1993), and dissolved
136 total nitrogen by HPLC with a nitrogen specific detector (Model 8060, Antek Instruments,
137 Houston, USA). Dissolved inorganic N (DIN) concentrations were calculated as the sum
138 of NO₃⁻ and NH₄⁺. The concentrations of dissolved organic nitrogen (DON) were
139 calculated by subtracting DIN from the total N concentrations. Anion deficits were
140 calculated by subtracting total anion (Cl⁻ + NO₃⁻ + SO₄²⁻) concentration from total cation
141 (H⁺ + Na⁺ + NH₄⁺ + K⁺ + Mg²⁺ + Ca²⁺) concentration.

142

143 *Foliage sampling*

144 To evaluate the nutrient status of *Calluna* in control and N treated plots, *Calluna*
145 foliage (current year) was collected at the beginning of December 2013. One sample of

146 foliage was collected in each plot from similar green *Calluna* shoots. Collected foliage
147 samples were transported to the laboratory, then dried at 70 °C for 72 h, and ball milled.
148 Total N in foliage was determined by the combustion method (CN Corder MT-700;
149 Yanaco Co., Ltd., Tokyo, Japan). For P, the dried samples were heated at 550 °C for 2 h,
150 then digested using potassium peroxodisulfate (K₂S₂O₈), and the P content was measured
151 using the molybdenum blue (ascorbic acid) spectrophotometric method (UV mini-1240;
152 Shimadzu, Kyoto, Japan). Standard reference material (NIST Apple Leaves 1515;
153 National Institute of Standards and Technology, Gaithersburg, MD, USA) was analyzed
154 along with leaf samples to ensure accuracy within 5% of known N and P concentrations.

155

156 *Calculation and statistical analysis*

157 CNR of NO₃⁻ and NH₄⁺ by *Calluna* was calculated using the following
158 equations:

$$159 \quad NO_3^- \text{ uptake}_{plot\ x} = (Na^+_{plot\ x} - Na^+_{control}) - NO_3^-_{plot\ x}$$

$$160 \quad NH_4^+ \text{ uptake}_{plot\ y} = (Cl^-_{plot\ y} - Cl^-_{control}) - NH_4^+_{plot\ y}$$

161 where the unit is μmol m⁻² for the ions in throughfall. Na⁺_{control} and Cl⁻_{control} concentrations

162 were averaged over the 4 control plots.

163 In calculating the retention of N from the treatments in each plot, it was
164 assumed that the counter ions (ions of opposite charge in a solution) of oxidized and
165 reduced N treatments (*i.e.* Na^+ for NO_3^- and Cl^- for NH_4^+) were not retained by *Calluna*,
166 but acted as conservative tracers, allowing the total amount of applied N to be estimated
167 from the throughfall of the counter ions. This assumption is based on the premise that Na^+
168 and Cl^- in pore water are derived only from atmospheric deposition, such as rainfall, and
169 the treatment supplied in this study. Conservative tracer behaviour has been shown for
170 both Na^+ (Eppinga *et al.*, 2008; Staelens *et al.*, 2008) and Cl^- (Appelo and Postma, 1994;
171 Bragazza *et al.*, 2005).

172 Spearman's rank correlation coefficient (*r*) was used to examine the
173 relationships between CNR of NO_3^- or NH_4^+ and DON and the sum of Mg^{2+} and
174 Ca^{2+} deposition. Statistical differences in deposition of DON, Mg^{2+} and Ca^{2+} , and anion
175 deficit among the plot were determined using Tukey's honest significant difference test
176 followed by analysis of variance. Statistical analyses were carried out using SPSS 22.0J
177 (SPSS Japan Inc., Tokyo, Japan).

178

179 **Results**

180 Throughfall deposition of NO_3^- and NH_4^+ below the *Calluna* canopy was 3.5 and 5.5
181 times respectively lower than bulk precipitation to the canopy (Figure 1). Figure 2 shows
182 the relationship between throughfall NO_3^- and counter ions of Na^+ (conservative tracer)
183 (Figure 2a) and NH_4^+ and counter ions of Cl^- (conservative tracer) (Figure 2b). There
184 were linear relationships with slope value of 0.40 and 0.47 for NO_3^- of lower (8-32 kg N
185 $\text{ha}^{-1} \text{yr}^{-1}$) and higher (16-64 kg N $\text{ha}^{-1} \text{yr}^{-1}$) dose respectively (Figure 2a) and I still don't
186 understand why low is designated 8-32 and high 16=64 what do these values represent
187 why not 16 and 64 ?????0.20 and 0.62 and for NH_4^+ of lower (8-32 kg N $\text{ha}^{-1} \text{yr}^{-1}$) and
188 higher (16-64 kg N $\text{ha}^{-1} \text{yr}^{-1}$) dose respectively (Figure 2b). Calculating from the slope
189 values, 60 % for low dose and 53 % for high dose and 80 % for low dose of NO_3^- and
190 38 % for high dose of NH_4^+ total (ambient deposition plus fertilization) inputs onto
191 *Calluna* were retained by *Calluna*.

192 Slope values with P and K addition were lower for NO_3^- (0.16, Figure 2a) and
193 NH_4^+ (0.17, Figure 2b) for the higher N doses (16-64 kg N $\text{ha}^{-1} \text{yr}^{-1}$) than those without P

194 and K, indicating the positive effects of P and K additions on CNR. Based on the slope
195 values, 84 % of NO_3^- and 83 % of NH_4^+ total (ambient deposition plus fertilization) inputs
196 onto the *Calluna* canopy were retained. Therefore, PK addition increased CNR by 31%
197 (53 \rightarrow 84%) and 45% (38 % \rightarrow 83%) for NO_3^- and NH_4^+ , respectively. There were
198 significant differences in water depth, amount of deposition collected, of throughfall
199 among treatments (Figure 3).

200 There was a significant positive relationship between NO_3^- retention by the
201 *Calluna* canopy and the concentration of DON in throughfall (Figure 4a), but there was
202 no increase in throughfall DON with retention of NH_4^+ by the *Calluna* canopy (Figure
203 4b). There was a significant positive relationship, with slope value of 0.49, between NH_4^+
204 retention by the *Calluna* canopy and the sum of magnesium (Mg^{2+}) and calcium (Ca^{2+})
205 in throughfall (Figure 4d). There was a significant positive relationship between anion
206 deficit and DON concentration (Figure 5a). DON deposition (Figure 6a) and deposition
207 of anion deficits (Figure 6c) were significantly higher for Nox 56PK and Mg^{2+} and Ca^{2+}
208 deposition (Figure 6b) was significantly higher for Nred 56PK, indicating significance of
209 P and K addition. Figure 7 illustrates the total amount of N deposition collected under the

210 canopy exposed to N with and without P and K addition. P and K addition substantially
211 increased CNR for both Nox and Nred and DON leaching for Nox affecting the total N
212 deposition under the *Calluna* canopy.

213 Foliar N:P ratio in *Calluna* exposed to different levels of N addition did not
214 increase even after 11-years exposure without P and K (Figure 8a) and with P and K
215 (Figure 8b). Foliar N (Figure 8cd) and P (Figure 8ef) concentrations of *Calluna* increased
216 with increasing N deposition.

217

218 **Discussion**

219 *Calluna* CNR

220 The presence of a *Calluna* canopy in peatlands will modify throughfall N deposition
221 (Figure 1), as suggested by Bobbink et al. (1992). Lower throughfall deposition of NO_3^-
222 and NH_4^+ than bulk precipitation indicates CNR by the *Calluna* canopy. In contrast,
223 higher throughfall deposition of DON could be due to canopy leaching of dissolved
224 organic matter (DOM) and canopy processing of wet or dry-deposited inorganic nitrogen
225 (Cape et al., 2010). Quantifying CNR is of great importance for precise quantification of

226 atmospheric deposition of pollutants using throughfall collection. CNR has been widely
227 observed in throughfall measurements (Lovett and Lindberg 1993; Staelens et al. 2008;
228 Aguilhaume et al., 2017; Avila et al., 2017; Pinkalski et al., 2018) and in N manipulation
229 experiments applied directly to the tree canopy (Cape et al. 2001; Chiwa et al. 2004;
230 Gaige et al. 2007). However, these studies mostly focused on trees in upland forests,
231 although CNR has also been observed in heathland (Bobbink et al., 1992; Limpens et al.,
232 2004). Bobbink et al (1992) recorded N retention of between 40-90% of net throughfall.
233 Here we discriminated between the N forms and against a background of 11 years of
234 known deposition and measured similar amounts of retention. Our results for retention,
235 even after 11 years' N applications (Figure 2) were 60 % for low dose and 53 % for high
236 dose of NO_3^- and 80 % for low dose and 38% for the high dose of NH_4^+ total (ambient
237 deposition plus fertilization). Different slope values between high and low doses of NH_4^+
238 (Figure 2b) indicate that the capacity for canopy NH_4^+ retention varies with the dose of
239 atmospheric NH_4^+ deposition.

240

241 *Increased CNR by P and K addition*

242 Lower slope values with P and K addition than those without P and K (Figure
243 2) suggest that P and K additions enhance CNR of *Calluna*, supporting the first hypothesis.
244 84 % of NO_3^- and 83 % of NH_4^+ total (ambient plus fertilization) inputs onto *Calluna* was
245 retained by *Calluna* canopy, exceeding the 53 and 38 % of retention for NO_3^- and NH_4^+
246 by the *Calluna* canopy without P and K addition.

247 The reason for the increased CNR by P and K addition cannot be established
248 from this study, but it may have to do with enhanced N demand of *Calluna* exposed to P
249 and K treatment. Enhanced CNR could be caused by increased canopy area resulting from
250 increased biomass production and/or increased physiological activity per unit of surface
251 area. Canopy area is an important contributor to CNR (Lovett 1996 et al., 1996). However,
252 we have no evidence that the *Calluna* canopy area increased in response to P and K
253 addition. Although the morphology of the *Calluna* canopies exposed to various
254 treatments was not estimated in this study, water depth of throughfall (Figure 3) indicates
255 that the canopy area of *Calluna* exposed to N treatment with P and K addition does not
256 exceed the control or those without P and K addition, because throughfall volume is
257 inversely proportional to canopy area under the same meteorological condition, reflecting

258 canopy interception loss (Levia et al., 2006). It is possible that physiological activity per
259 unit of canopy area increased in response to P and K addition. The increased physiological
260 activity of the canopy may be the result of increased physiological activity of the shoots,
261 but also of any epiphytes living on the leaves which may be responsible for some of the
262 canopy exchange reactions observed. Measurements of photosynthesis made around the
263 same time, LICOR in the field, did not reveal significant treatment differences (Owen S.
264 pers comm). P and K addition did have positive effects on N assimilation of *Sphagnum*
265 moss, non-vascular plant (Limpens et al., 2004). However, to our knowledge, this is the
266 first study showing that increases in P and K availability enhance canopy N retention by
267 vascular plants.

268

269 *Increased DON and cation leaching with NO₃⁻ and NH₄⁺ canopy retention by P and K*
270 *addition*

271 The significant positive relationship between NO₃⁻ retention by the *Calluna*
272 canopy and DON deposition in throughfall (Figure 4a) but no increase in DON deposition
273 with NH₄⁺ retention by the *Calluna* canopy (Figure 4b) indicates the link between DON

274 leaching and *Calluna* canopy NO_3^- retention, as found for *Sphagnum* moss (Chiwa et al.
275 2016). DON leaching appears to be associated with the leaching of organic N anions to
276 retain the charge balance when NO_3^- is taken up, as suggested by the significant positive
277 relationship between anion deficit and DON concentration (Figure 5a).

278 The significant positive relationship with a slope value of 0.49 between NH_4^+
279 retention by *Calluna* canopy and the sum of Mg^{2+} and Ca^{2+} deposition of throughfall
280 (Figure 4d) indicates that NH_4^+ retention by *Calluna* canopy can be partly explained by
281 the leaching of Mg^{2+} and Ca^{2+} through ion exchange. We did not include potassium (K^+)
282 and hydrogen ion (H^+) to evaluate canopy leaching because K_2HPO_4 mist solution was
283 used for P and K addition. Canopy retention of NH_4^+ is usually accompanied by cation
284 leaching (Bobbink et al., 1992; Krupa, 2003; Li et al., 2013; Staelens et al., 2008; Stevens
285 et al., 2011) and hydrogen ion (H^+) leaching (Krupa, 2003; Liu et al., 2013; Manninen et
286 al., 2011; Paulissen et al., 2004; Stevens et al., 2011; Tomassen et al., 2003). Our result
287 was consistent with those of Bobbink et al (1992) for *Calluna*.

288 Significantly higher DON deposition for Nox 56PK (Figure 6a), higher Mg^{2+}
289 and Ca^{2+} deposition for Nred 56PK (Figure 6b), and higher deposition of anion deficits

290 for Nox 56PK (Figure 6c) indicate that P and K addition increased the DON and cations
291 leaching from the canopy associated with the increased canopy retention of NO_3^- and
292 NH_4^+ . Therefore, it is suggested that P and K additions accelerate the nutrient cycling of
293 substances leaching from the canopy. These results demonstrate that PK availability can
294 influence the cycling of N by the *Calluna* canopy (Figure 7).

295

296 *Effects of N status of Calluna on enhanced CNR and the associated canopy leaching with*
297 *P and K addition*

298 Foliar N concentrations of *Calluna* increased with increasing N deposition
299 (Figure 8cd), which is consistent with the study of Pitcairn et al. (1995) which found
300 significant increases in tissue N concentration of *Calluna* along a N deposition range from
301 15 to 30 kg N ha⁻¹ yr⁻¹ throughout Europe. Although there were not significant differences
302 for foliar P concentrations among the treatments, the stable N:P ratio (Figure 8a) must be
303 caused by increases in the foliar P concentration (Figure 8e). One plausible explanation
304 for the increases in P concentration is that *Calluna* may upregulate its P acquisition in order
305 to utilise the N, incorporate it into amino acids, rather than allow it to accumulate in toxic

306 forms, in response to increased N deposition. It is known that terrestrial plants allocate
307 excess N to proteins for enzyme production enabling increased enzyme activity, e.g. the
308 phosphatase enzymes, to enhance P availability, thus delaying the onset of P limitation
309 (Marklein and Houlton 2012; Maistry et al. 2015). If a higher leaf N:P ratio is indicative
310 of N saturation diagnosed from the leaf stoichiometry in wetlands (Koerselman and
311 Meuleman 1996; Gü ewell 2004; Chiwa et al., 2018), then the small increases in foliar
312 N:P ratio of *Calluna* exposed to different levels of N addition without P and K even after
313 11-years exposure (Figure 8a) and decreases in the N:P ratio with P and K (Figure 8b),
314 suggest that N exposure at this site does not cause N saturation in *Calluna* even for the
315 high N dose without P and K addition.

316 Chiwa et al. (2018) showed that *Sphagnum* tissue N concentration at this same
317 site also increased, whereas tissue P concentrations did not, in response to N dose.
318 Subsequently, *Sphagnum* N:P ratio did increase and *Sphagnum* exposed to NH_4^+ addition
319 of $56 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for 11 years became N-saturated. Therefore, it is suggested that
320 *Calluna* could have lower sensitivity than *Sphagnum* to elevated N deposition because
321 *Calluna* could enhance P acquisition via increased enzyme activity.

322

323 **Conclusions**

324 This study has provided the first results of enhanced canopy N retention by
325 increases in P and K availability. This study has advanced our understanding of canopy
326 N retention and the factors controlling its variation, improving our ability to evaluate
327 atmospheric N deposition and its ecological effects. It was found that DON and base
328 cation leaching are accelerated by the enhancement of canopy N retention, in which
329 canopy retention of NO_3^- and NH_4^+ was accompanied by leaching of DON and base
330 cations, respectively. Importantly this study has distinguished between the effects of
331 reduced versus oxidised N. More intensive measurements will be required to investigate
332 the seasonal effects of P and K addition on the CNR and the associated leaching.

333

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338

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480

481

482 **Figure Captions**

483 **Figure 1** Effect of *Calluna* canopy on nitrate (NO_3^-), ammonium (NH_4^+), and dissolved
484 organic nitrogen (DON) deposition. Bars represent standard deviation ($n = 3$ for rainfall
485 and $n = 4$ for throughfall).

486

487 **Figure 2** Relationship between a) throughfall sodium (Na^+) deposition and throughfall
488 nitrate (NO_3^-) deposition and between b) throughfall chloride (Cl^-) deposition and
489 throughfall ammonium (NH_4^+) deposition.

490

491 **Figure 3** Rainfall and throughfall depth (mm). Bars represent standard deviation ($n = 3$
492 for rainfall and $n = 4$ for throughfall).

493

494 **Figure 4** Relationship between throughfall deposition of dissolved organic nitrogen
495 (DON) and retention of a) NO_3^- and b) NH_4^+ . Relationship between throughfall deposition
496 of magnesium (Mg^{2+}) and calcium (Ca^{2+}) and retention of c) NO_3^- and d) NH_4^+ .

497

498 **Figure 5** Relationship between throughfall deposition of anion deficits and dissolved
499 organic nitrogen (DON) for a) Nox and b) Nred.

500

501 **Figure 6** Throughfall deposition of a) dissolved organic nitrogen (DON), b) magnesium
502 (Mg^{2+}) + calcium (Ca^{2+}), and c) anion deficit. Bars represent standard deviation ($n = 4$).

503

504 **Figure 7** Effect of P and K addition on throughfall deposition of nitrate (NO_3^-),
505 ammonium (NH_4^+), and dissolved organic nitrogen (DON) deposition. Bars represent
506 standard deviation ($n = 4$).

507

508 **Figure 8** Relationship of nitrogen (N) deposition (the sum of ambient N deposition and
509 applied N) with *Calluna* foliage of N:P ratio, N concentration, and phosphorus (P)
510 concentration. Background N deposition is *ca.* $8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Leith *et al.*, 2004;
511 Sheppard *et al.*, 2004).

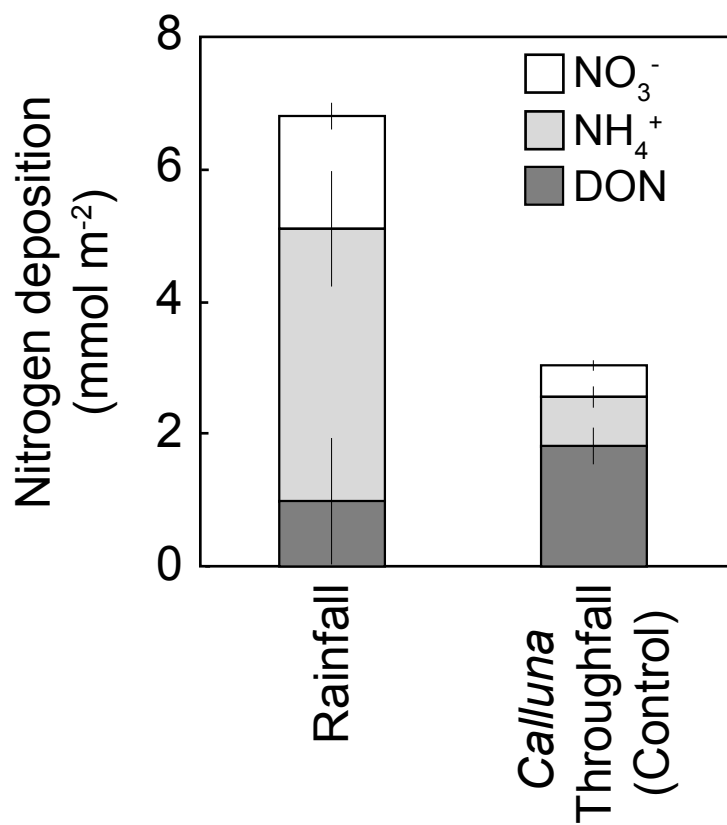


Figure 1
Chiwa et al.

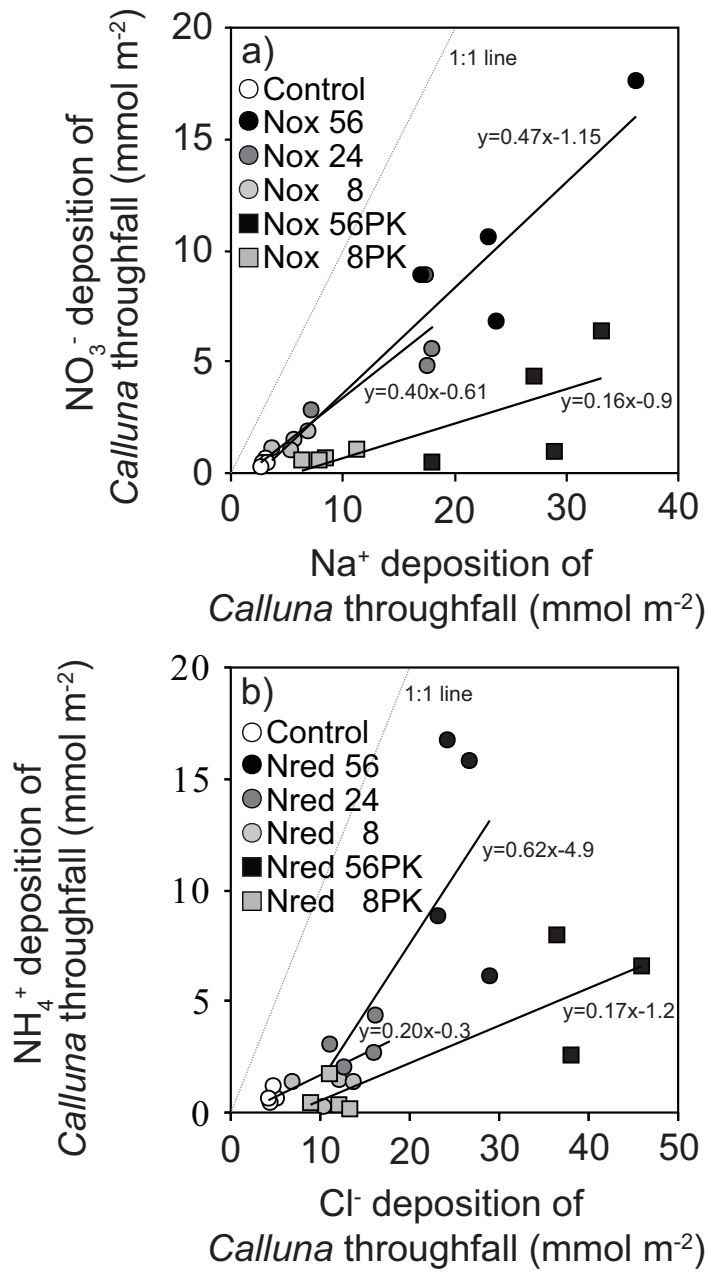


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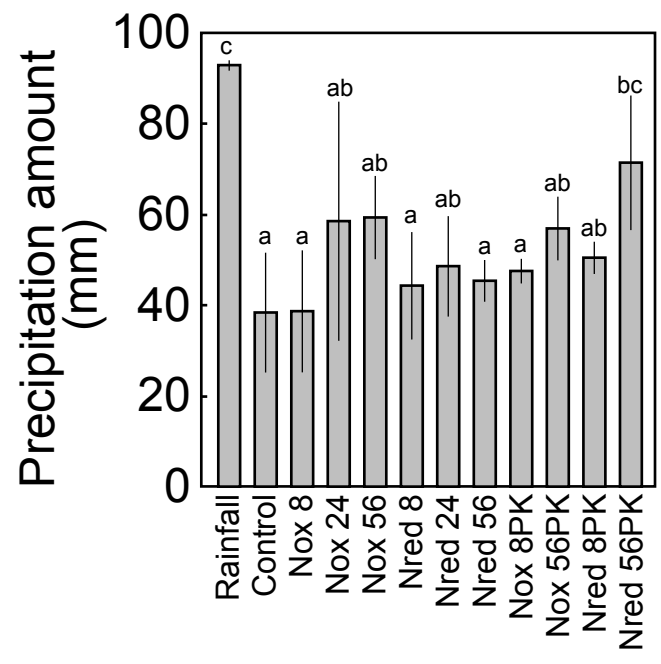


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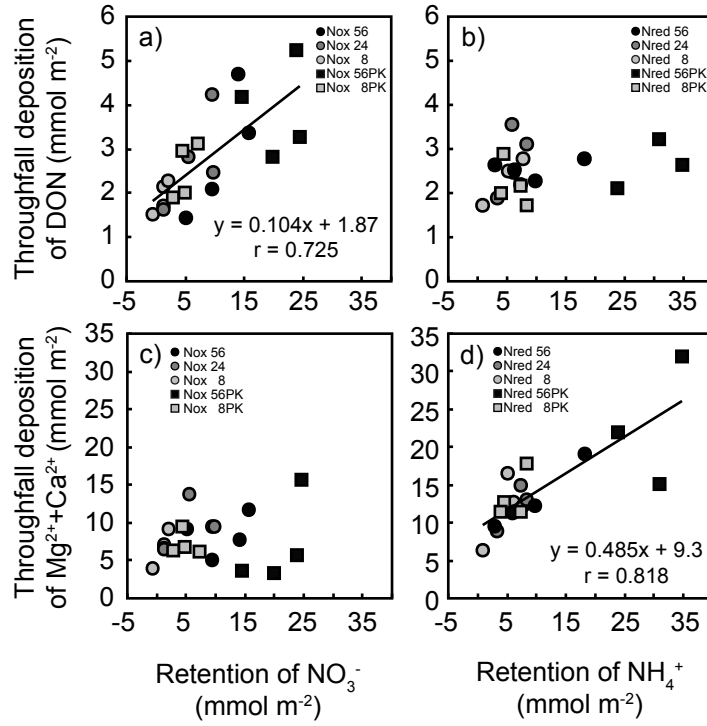


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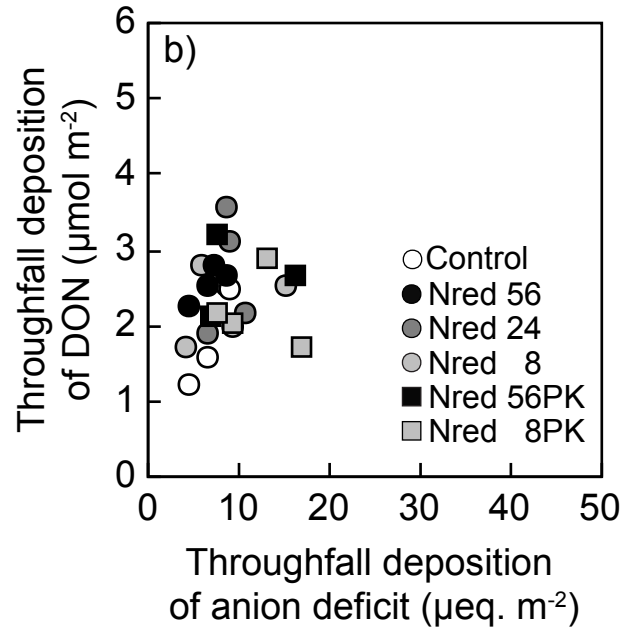
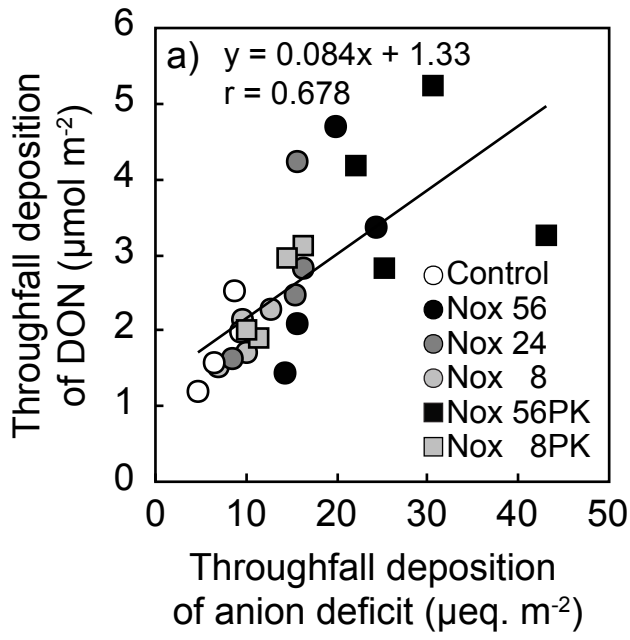


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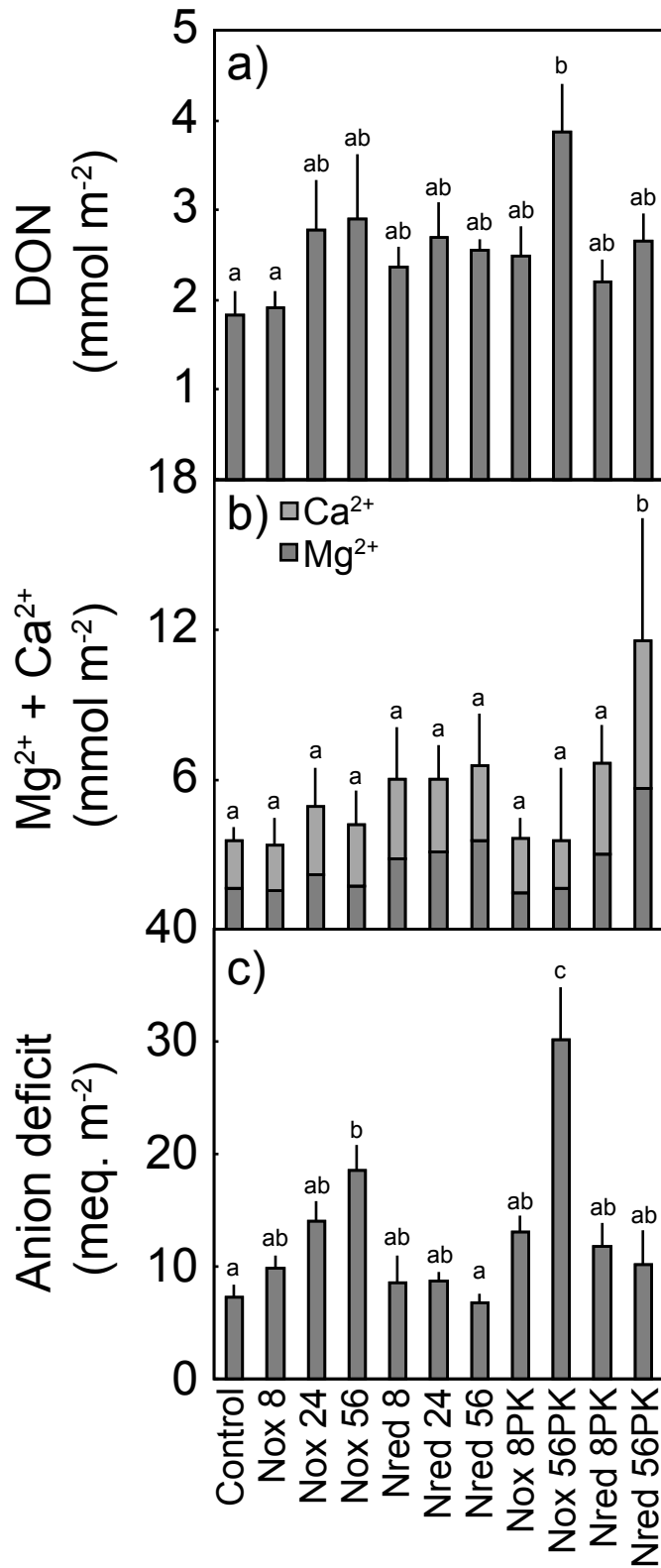


Figure 6
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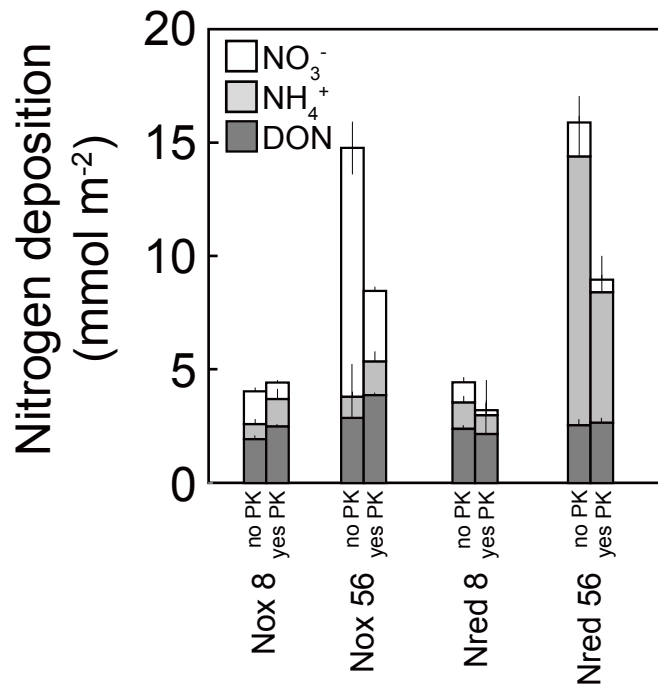


Figure 7
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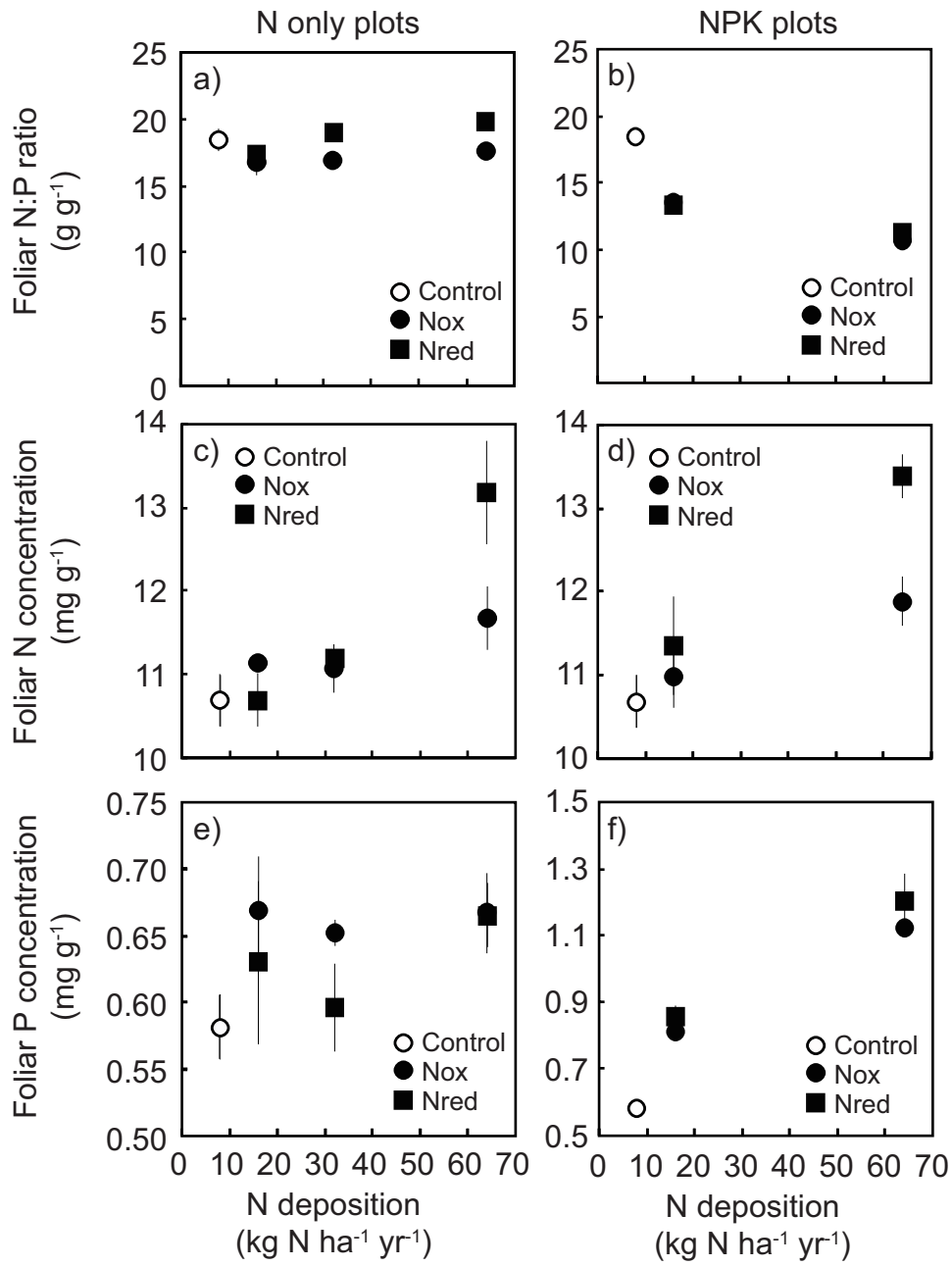


Figure 8
Chiwa et al

Table 1 Composition of the artificial mist treatments applied to the Whim bog peatland in each of the 11 different treatment plots. The total annual input is the cumulative application from *ca.* 120 treatments.

Treatment	Components	Concentration (mmol L ⁻¹)	Total annual input (kg ha ⁻¹ yr ⁻¹)
Control			8 kg N ha ⁻¹ yr ⁻¹
Nox 8	NaNO ₃	0.57	16 kg N ha ⁻¹ yr ⁻¹
Nox 24	NaNO ₃	1.71	32 kg N ha ⁻¹ yr ⁻¹
Nox 56	NaNO ₃	4.0	64 kg N ha ⁻¹ yr ⁻¹
Nred 8	NH ₄ Cl	0.57	16 kg N ha ⁻¹ yr ⁻¹
Nred 24	NH ₄ Cl	1.71	32 kg N ha ⁻¹ yr ⁻¹
Nred 56	NH ₄ Cl	4.0	64 kg N ha ⁻¹ yr ⁻¹
Nox 8PK	NaNO ₃	0.57	8 kg N ha ⁻¹ yr ⁻¹
	K ₂ HPO ₄	0.018	0.57 kg P ha ⁻¹ yr ⁻¹ 1.4 kg K ha ⁻¹ yr ⁻¹
Nox 56PK	NaNO ₃	4.0	64 kg N ha ⁻¹ yr ⁻¹
	K ₂ HPO ₄	0.13	4 kg P ha ⁻¹ yr ⁻¹ 10 kg K ha ⁻¹ yr ⁻¹
Nred 8PK	NH ₄ Cl	0.57	16 kg N ha ⁻¹ yr ⁻¹
	K ₂ HPO ₄	0.018	0.57 kg P ha ⁻¹ yr ⁻¹ 1.4 kg K ha ⁻¹ yr ⁻¹
Nred 56PK	NH ₄ Cl	4.0	64 kg N ha ⁻¹ yr ⁻¹
	K ₂ HPO ₄	0.13	4 kg P ha ⁻¹ yr ⁻¹ 10 kg K ha ⁻¹ yr ⁻¹

Table 2 Meteorological condition at each event for throughfall (TF) collection

Sampling	Setup	Collection	Number of rainfall events	Rainfall depth (mm)	Rainfall duration (h)	Rainfall intensity (mm/h)	Air Temp (°C)	TF control volume (mm)
1	1 Jul	4 Jul	2	18.0	20	1.0	11.7	8.4
2	22 Jul	25 Jul	2	25.0	8	4.3	16.5	12.2
3	5 Aug	13 Aug	4	14.8	25	1.5	12.5	5.6
4	19 Aug	2 Sep	5	15.2	60	1.3	13.4	4.7
5	10 Sep	17 Sep	4	24.8	34	0.7	9.6	9.9
6	24 Sep	8 Oct	5	21.6	52	0.5	11.5	10.1
Collected period			22	114	199	1.6	12.2	38.6
All period (1 Jul - 8 Oct)			35	250	351	1.5	13.1	NA