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1     Factors affecting leaching of dissolved organic carbon after  
2     tree dieback in an unmanaged European mountain forest

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9     **Abstract**– Forest disturbances affect ecosystem biogeochemistry, water quality and carbon  
10    cycling. We analysed water chemistry before, during and after a dieback event at a  
11    headwater catchment in the Bohemian Forest (central Europe), together with an  
12    unimpacted reference catchment, focusing on drivers and responses of dissolved organic  
13    carbon (DOC) leaching. We analysed data on carbon input to the forest floor via litter and  
14    throughfall, changes in soil moisture and composition, stream water chemistry, discharge  
15    and temperature. We observed that: (i) In the first three years following dieback, DOC  
16    production from dead biomass led to increased concentrations in soil, but DOC leaching  
17    did not increase due to chemical suppression of its solubility by elevated concentrations of  
18    protons and polyvalent cations, and elevated microbial demand for DOC associated with

19 high ammonium ( $\text{NH}_4^+$ ) concentrations. (ii) DOC leaching remained low during the next  
20 two years, because its availability in soils declined, which also left more  $\text{NH}_4^+$  available for  
21 nitrifiers, increasing  $\text{NO}_3^-$  and proton production that further increased chemical  
22 suppression of DOC mobility. (iii) After five years, DOC leaching started to increase as  
23 concentrations of  $\text{NO}_3^-$ , protons, and polyvalent cations started to decrease in soil water.  
24 Our data suggest that disturbance-induced changes in N cycling strongly influence DOC  
25 leaching via both chemical and biological mechanisms, and that the magnitude of DOC  
26 leaching may vary over periods following disturbance. Our study adds insights to why the  
27 impacts of forest disturbances are sometime observed at the local soil scale but not  
28 simultaneously on the larger catchment scale.

29

## 30 INTRODUCTION

31 The susceptibility of forest ecosystems to large disturbance events such as insect  
32 infestations and wildfires is increasing globally.<sup>1,2</sup> Although such disturbances are to some  
33 extent natural, their frequency and/or severity has tended to be exacerbated by land-  
34 management factors such as single-species or single-age stand management, increased  
35 human presence (fires), and climate change.<sup>3</sup> Large-scale tree mortalities have recently  
36 occurred in Europe and the western USA and Canada, and have been shown to  
37 detrimentally impact downstream water quality.<sup>4-6</sup> Similar increases in frequency of  
38 occurrence and severity of impacts on water quality have been recorded for wildfires.<sup>2</sup> The  
39 impacts of such disturbances typically include temporally elevated leaching of ions and  
40 nutrients (notably nitrogen) to receiving waters. However, the impacts of disturbance on

41 dissolved organic carbon (DOC) are less clear, despite its significance for ecosystem  
42 carbon budgets and (due to its high removal costs during treatment) for water supplies.<sup>5-9</sup>

43 Concentrations of dissolved organic carbon (DOC) have increased in numerous European  
44 and North American surface waters since the 1990s.<sup>10-12</sup> These increases have been  
45 attributed to: (i) declining atmospheric deposition of strong acid anions (SAAs, sum of  
46  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ , and  $\text{Cl}^-$ ) and their effect on soil water pH and ionic strength;<sup>13,14</sup> (ii) factors  
47 related to climate change including warming, increased frequency of high precipitation  
48 events and rising atmospheric carbon dioxide concentrations;<sup>15-17</sup> and (iii) elevated N  
49 deposition.<sup>18,19</sup> These general long-term trends in DOC leaching can be further magnified  
50 or weakened by changes in land-use, disturbances, and management practice in  
51 catchments.<sup>5-9,20,21</sup>

52 Major sources of dissolved organic carbon (DOC) in forest soils are incomplete microbial  
53 decomposition of litter and dead biomass (roots, understory vegetation, microbes), root  
54 exudates, and leaching from canopies.<sup>22-24</sup> Major DOC sinks are mineralization, microbial  
55 and chemical immobilization in soils, and leaching to receiving waters. The proportion of  
56 DOC immobilized in new microbial biomass, oxidized to  $\text{CO}_2$  or reduced to  $\text{CH}_4$  depends  
57 on its composition (bio-availability), residence time in soils, availability of electron  
58 acceptors and nutrients, and soil temperature and moisture, all of which affect efficiency  
59 and pathways of C microbial use.<sup>25-28</sup> Chemical properties of DOC, soil water, and surfaces  
60 of soil particles further affect solubility and mobility of DOC via a complex of physico-  
61 chemical processes that affect DOC dissociation, coagulation, precipitation, and adsorption  
62 on Al and Fe hydroxides.<sup>10,29-31</sup> The amount of DOC leached from soils is further affected  
63 by soil hydrology and physical properties that control water residence time, moisture,

64 hydraulic connection of DOC resources with their microbial sinks, and flow pathways  
65 through shallow vs. deep soil horizons.<sup>23,33,34</sup> Consequently, DOC fate in soils and its  
66 mobility and leaching from terrestrial to aquatic systems is controlled by a complex of  
67 biogeochemical factors, including plant productivity as the original source of organic C  
68 (ref. 35), hydrology, soil moisture and temperature, microbial activity, and chemical  
69 composition of soil and soil water.

70 Observed trends in DOC leaching from mountain catchments of the Bohemian Forest  
71 lakes (central Europe) combine most of the above causes.<sup>36,37</sup> The DOC concentrations  
72 have been increasing in the Bohemian Forest lakes since the late 1980s as a response to  
73 rapidly decreasing atmospheric deposition of  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$ .<sup>38</sup> In addition, stream water  
74 DOC concentrations exhibit a pronounced seasonal variation and a high DOC leaching  
75 accompanying hydrological events.<sup>36</sup> In recent years, DOC concentrations have increased  
76 in some streams following insect infestation and tree dieback in their catchments.<sup>37</sup> The  
77 aim of this study is to evaluate mechanisms affecting terrestrial export of DOC following  
78 this forest disturbance. In particular, we evaluate changes in C input to the forest floor with  
79 litter and throughfall, soil moisture and composition, chemical properties of surface water,  
80 physico-chemical factors contributing to DOC variations in stream water, and possible  
81 effects of soil N cycle on DOC availability for leaching. Finally, we suggest a general  
82 conceptual model and evaluate risks of elevated DOC leaching from disturbed forests.

83

## 84 METHODS

85 **Study site.** Plešné and Čertovo lakes are situated at 13.2–13.9 °E and 48.8–49.2 °N at  
86 elevations of 1,087 and of 1,027 m, respectively, in the Bohemian Forest (Supporting

87 Information, SI, Fig. SI-1). The Plešné and Čertovo bedrocks are formed by granite and  
88 mica-schist, their catchments are 64 and 89 ha in size and are steep, with maximum local  
89 relief of 291 and 316 m, respectively. Both catchments are mostly covered by shallow  
90 acidic forest soils (leptosol, podsol and dystric cambisol) and proportions of wetlands and  
91 bare rocks in their areas are <5%. Forest vegetation occupies >90% of both catchments and  
92 is dominated by Norway spruce (*Picea abies*). The catchments are part of a protected  
93 unmanaged area (Šumava National Park), with restricted access and land use activities.  
94 Both catchments were strongly acidified and N-saturated by high atmospheric deposition of  
95  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  since the early 1960s.<sup>39</sup> Acidic deposition dramatically decreased in  
96 the 1990s, then its decline continued at a lower rate, and its present level (10, 31 and 37  
97  $\text{mmol m}^{-2} \text{yr}^{-2}$  for  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , respectively, in precipitation) is similar to the  
98 late 19<sup>th</sup> century for  $\text{SO}_4^{2-}$  and  $\text{NH}_4^+$ , and to the 1960s for  $\text{NO}_3^-$ .<sup>37,39</sup> The Plešné and  
99 Čertovo lakes have three (PL-I to PL-III) and seven (CT-I to CT-VII) surface tributaries,  
100 respectively. These are small, first- to second-order streams, except for PL-III, which is  
101 partly subsurface and receives a high proportion of its flow from groundwater.

102 Between autumn 2004 and autumn 2008, almost all adult Norway spruce trees in the  
103 Plešné catchment were killed by a bark beetle (*Ips typographus*) outbreak.<sup>37</sup> Dead trees lost  
104 their needles during several months after infestation, and subsequently they continuously  
105 lost twigs, bark, and branches.<sup>40</sup> Most of the dead trees had been blown over by 2016, but  
106 all dead biomass has been left in place as part of the minimum-management policy within  
107 the area. Natural forest regeneration started within 1–3 years of tree dieback, and the  
108 average number of seedlings increased from 47 and 670 trees  $\text{ha}^{-1}$  during the following  
109 decade.<sup>37</sup>

110 The Čertovo forest has experienced a low level of disturbance, limited to windthrows in  
111 2007 and 2008 along the south-western ridge of the catchment, mostly in the upper parts of  
112 the CT-IV to CT-VII sub-catchments. Altogether, the total area of damaged forest (with >  
113 50% dead trees) increased from ~4 to 18% in the whole Čertovo catchment between 2000  
114 and 2015.<sup>36</sup>

115 **Soil, litterfall and throughfall.** In this study, we synthesize trends in soil chemistry,  
116 litterfall, and throughfall amount and composition that were published elsewhere. Sampling  
117 and analyses are described in detail in Part SI-1. Briefly:

118 (i) Soils were sampled from 9–21 pits in 1997–2001 and 2010 in both catchments, and in  
119 the Plešné catchment also in 2015. We use data on pH, exchangeable base cations (BCs,  
120 sum of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ , and  $\text{K}^+$ ),  $\text{Al}_i$ , and  $\text{H}^+$ , and base saturation (percent proportion of  
121 BCs in the cation exchange capacity) in the upper soil horizons (O, litter; and A, the  
122 uppermost organic rich horizon). In addition, soils were sampled at 6-week intervals at one  
123 research plot in each catchment (PL-plot and CT-plot) from 2007–2017.<sup>41,42</sup> Trees at the  
124 PL-plot were killed by bark beetles in 2006–2007, while the control CT-plot was not  
125 affected. Trends in soil chemistry at the PL- and CT-plots are mass weighted means for O-  
126 and A-horizons. From this 6-week survey, we use data on soil moisture, exchangeable BCs,  
127  $\text{Al}_i$ , and  $\text{H}^+$ , water extractable DOC,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , and concentrations of C and N in  
128 microbial biomass ( $C_{\text{MB}}$  and  $N_{\text{MB}}$ , respectively).

129 (ii) Litterfall was sampled at three and two plots in the Plešné and Čertovo catchments,  
130 respectively, from 2003 to 2016.<sup>40</sup> Trends in carbon fluxes associated with litterfall (the  
131 sum for needles, twigs, bark, cones, lichen and a mixture of poorly identifiable fragments)

132 used in this study are averages for all plots in individual catchments. Large branches (> 2  
133 cm in diameter), trunks and roots were not included in this flux.

134 (iii) Throughfall amount and chemical composition have been studied at two plots in each  
135 catchment since 1997.<sup>36,37</sup> Here, we use annual averages for amounts and concentrations of  
136 DOC and SAAs.

137 **Stream water.** Tributaries were sampled from May 1997 to April 2017 in three-week  
138 intervals. Discharge ( $Q$ ,  $L\ s^{-1}$ ) was estimated using a stopwatch and calibrated bucket at  
139 small natural waterfalls or rapids. Water temperature ( $T$ ,  $^{\circ}C$ ) was measured during  
140 sampling.

141 Details on water analyses are given in Part SI-2. Briefly: DOC was analysed as  $CO_2$  after  
142 sample mineralization in carbon analysers, with a detection limit of  $<4\ \mu mol\ L^{-1}$ .

143 Concentrations of  $NH_4^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Na^+$ ,  $K^+$ ,  $NO_3^-$ ,  $Cl^-$ ,  $SO_4^{2-}$ , and  $F^-$  were determined by  
144 ion chromatography. Concentrations of ionic Al and Fe forms ( $Al_i$ ,  $Fe_i$ ) were analysed  
145 colorimetrically after their fractionation.<sup>43</sup> Concentrations of organic acid anions ( $A^-$ ) were  
146 calculated from concentrations of DOC and pH.<sup>44</sup>

147 Linear regression was used to evaluate the significance of relationships between  
148 concentrations of DOC and other water constituents, including ionic strength, in each  
149 tributary. We used the seasonal Mann-Kendall test<sup>45</sup> to determine if long-term trends in  
150 water chemistry were significantly different from zero. Multiple linear regression with  
151 forward stepwise selection (using SigmaPlot 11.0, Systat Software, San Jose, California,  
152 USA) was used to determine what variables explained variations in DOC concentrations.  
153 For this analysis, we selected seven variables ( $Q$ ,  $T$  and concentrations of  $SO_4^{2-}$ ,  $NO_3^-$ ,  $Cl^-$ ,  
154  $H^+$ , and  $Al_i$ ) that were previously shown to play important roles in seasonal and long-term



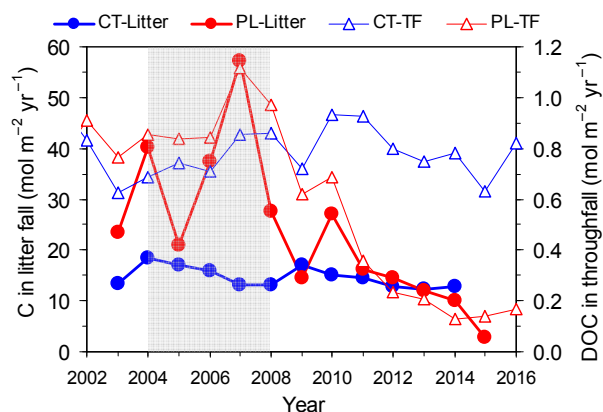
155 variations in DOC concentrations.<sup>13,34,38</sup> We did not use ionic strength and concentrations  
156 of BCs as independent variables in the forward stepwise regression, because ionic strength  
157 mostly depended on the chemical variables already included in the statistical analysis, and  
158 concentrations of BCs mostly depended on concentrations of SAAs as counter-ions.<sup>36,37</sup>  
159 Concentrations of  $H^+$  and  $Al_i$  were considered as independent variables due to their  
160 important roles in DOC dissociation and coagulation.<sup>29,31,46,47</sup> Data on all tributaries were  
161 evaluated for the whole study period (May 1997 to April 2017) and also for periods prior to  
162 (1997–2003), during (2004–2008), and after (2009–2017) the bark beetle outbreak in the  
163 Plešné catchment. In the statistical tests, values of  $p < 0.05$  were considered significant  
164 throughout the study.

165

## 166 RESULTS

167 **Litterfall carbon and throughfall DOC fluxes.** Annual inputs of organic C to the  
168 Čertovo catchment were stable throughout the study, with average ( $\pm$  standard deviation)  
169 fluxes in litterfall and throughfall of  $15 \pm 2$  and  $0.80 \pm 0.11 \text{ mol m}^{-2} \text{ yr}^{-1}$ , respectively (Fig.  
170 1). Throughfall fluxes of DOC were only slightly higher in the Plešné than Čertovo  
171 catchment prior to and during the tree dieback, but then rapidly decreased to  $\sim 0.15 \text{ mol m}^{-2}$   
172  $\text{yr}^{-1}$  during 2014–2016. The litterfall fluxes of C were similar in both catchments prior to  
173 the bark beetle attack, then increased in the Plešné catchment in 2004 and peaked at  $57 \text{ mol}$   
174  $\text{m}^{-2} \text{ yr}^{-1}$  in 2007. Since 2009, litterfall C fluxes have been similar in both catchments. The  
175 Plešné litterfall was dominated by needles, with a large proportion of green, non-senescent  
176 needles and bark in the first year after the tree infestation, and by twigs, small branches and

177 bark in the following years. In contrast, composition of the Čertovo litter was stable  
 178 throughout the study and was dominated by needles and twigs.<sup>40</sup>  
 179



180

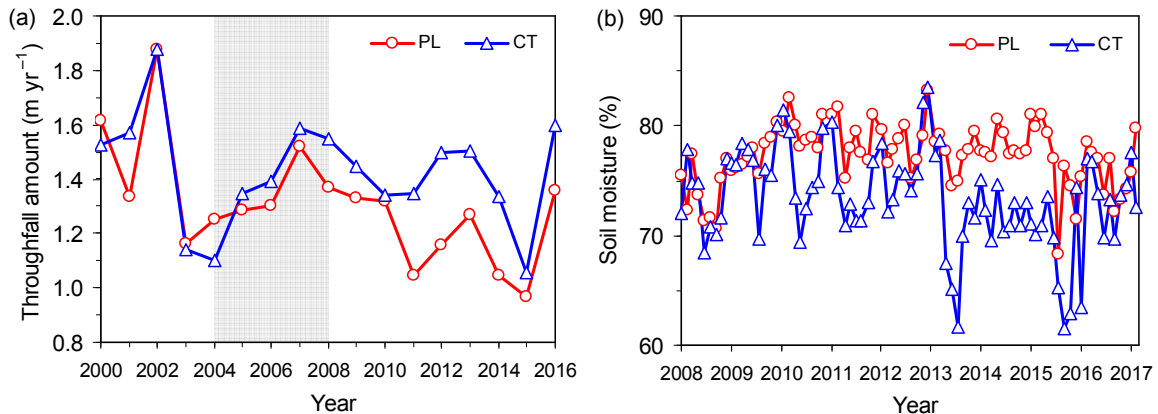
181 Fig. 1. Time series of inputs of organic carbon to the forest floor of Čertovo (CT) and  
 182 Plešné (PL) catchments in litterfall (Litter) and throughfall (TF). Grey area indicates the  
 183 period of bark beetle outbreak in the Plešné catchment.

184 The thinning of dead canopies caused significant changes in throughfall composition,  
 185 leading to decreased fluxes of BCs and SAAs to forest floor; throughfall inputs of  $\text{SO}_4^{2-}$ ,  
 186  $\text{NO}_3^-$  and  $\text{Cl}^-$  decreased on average by 41, 43, and 55%, respectively, in the Plešné relative  
 187 to Čertovo catchment during 2009–2016 (Fig. SI-3).

188 **Throughfall amount, soil moisture and discharge.** Tree dieback affected throughfall  
 189 amount and soil moisture (Fig. 2). Throughfall amounts were almost similar in both  
 190 catchments till 2007, but then became consistently lower in the Plešné than Čertovo  
 191 catchment (Fig. 2a). Soil moisture was similar at both research plots immediately after the  
 192 tree dieback at the PL-plot in 2008, but became consistently higher than at the CT-plot  
 193 from autumn 2009 (Fig. 2b), despite lower throughfall amounts in the Plešné catchment

194 (Fig. 2a). This difference was especially apparent in summer months and during years with  
 195 low precipitation.

196



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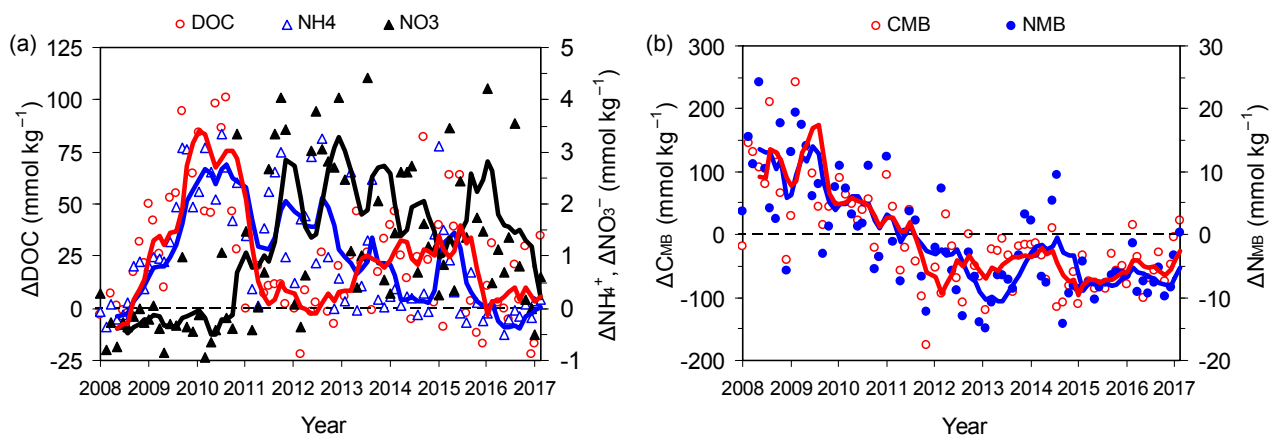
198 Fig. 2. Time series of (a) annual throughfall amount in Plešné (PL) and Čertovo (CT)  
 199 catchments, and (b) soil moisture (mass weighted mean for O- and A-horizons) at the PL  
 200 and CT research plots. Grey area indicates the period of bark beetle outbreak in the Plešné  
 201 catchment (note that soil moisture data cover the post-disturbance period only).

202 The 1997–2017 average discharges of tributaries ranged over an order of magnitude with  
 203 long-term averages from  $0.6\text{--}11 \text{ L s}^{-1}$ . During the study, water Q decreased in most  
 204 tributaries due to decreasing precipitation (Fig. 2a), but this decrease only was significant  
 205 in CT-VI, CT-VII, and PL-II (Table SI-3). Water discharge had no clear seasonality, except  
 206 for elevated Q values during snowmelt periods.

207 **Soil chemistry.** Base saturation of the Plešné soils increased dramatically after the tree  
 208 dieback, from 39 to 65% in the O-horizon, and from 21 to 38% in the A-horizon between  
 209 2000 and 2015 (Table SI-1). This increase in base saturation was accompanied by a  
 210 decrease in concentrations of exchangeable  $\text{Al}_i$ , and also  $\text{H}^+$  in the O-horizon. These

211 changes were especially pronounced at the research PL-plot, while no significant long-term  
 212 trends occurred in the control CT-plot (Fig. SI-3).

213 Concentrations of water extractable DOC and  $\text{NH}_4^+$  were higher in the upper soil  
 214 horizons at the PL- than CT-plot throughout 2008–2016, but this difference was most  
 215 pronounced during the first three years after the tree dieback (Fig. 3a). In contrast, soil  
 216  $\text{NO}_3^-$  concentrations were lower at the PL- than CT-plot until 2011, then abruptly increased  
 217 (reciprocally to the DOC decline) and exhibited higher concentrations and variation than at  
 218 the CT-plot until the end of study. Concentrations of  $\text{C}_{\text{MB}}$  and  $\text{N}_{\text{MB}}$  were higher in the  
 219 Plešné than Čertovo soils until 2011, and generally lower thereafter (Fig. 3b). Microbial C  
 220 to N ratios in soil exhibited similar molar averages ( $\sim 12$ ) and temporal patterns (not shown)  
 221 at both research plots.



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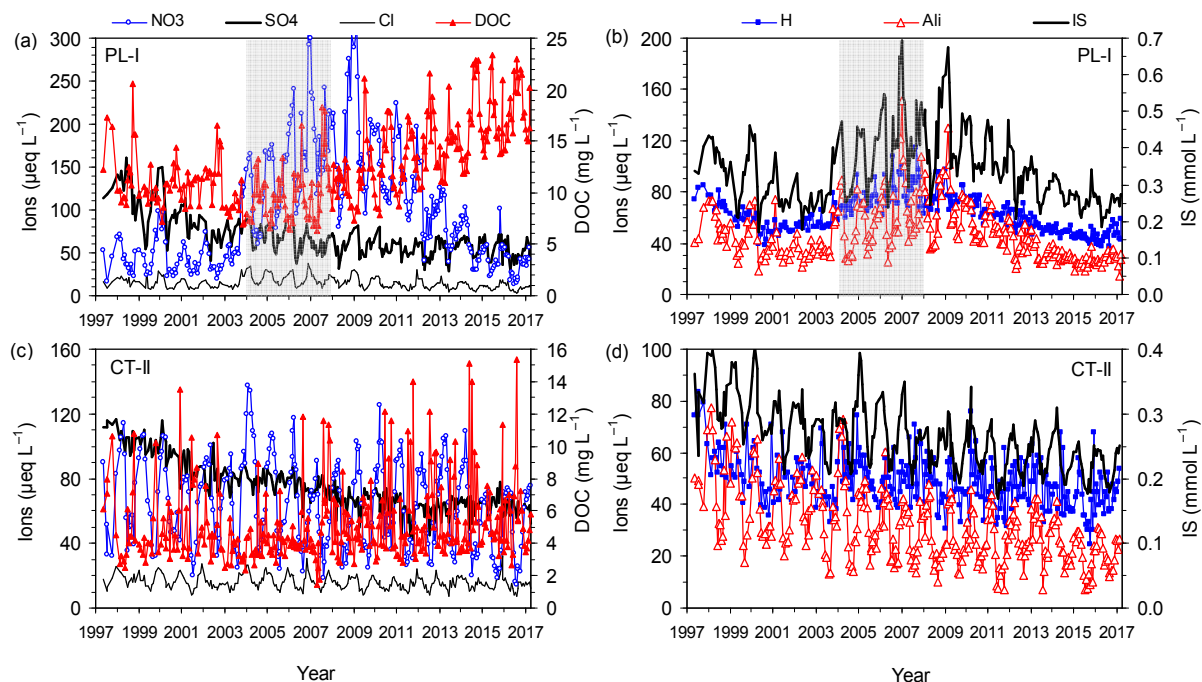
223 Fig. 3. Time series of differences ( $\Delta$ ) between soil properties observed at the Plešné (PL;  
 224 beginning of tree dieback in 2007) and Čertovo (CT; unaffected control) research plots for:  
 225 (a) concentrations of DOC,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in water extracts, and (b) concentrations of C  
 226 and N in microbial biomass ( $\text{C}_{\text{MB}}$  and  $\text{N}_{\text{MB}}$ , respectively). Lines are moving averages ( $n =$   
 227 5). For absolute values see Fig. SI-4.

228 **Water chemistry.** The Čertovo and Plešné tributaries were strongly acidic, with 1997–  
229 2017 average pH values of 4.1–4.6. Concentrations of  $\text{SO}_4^{2-}$  and  $\text{Cl}^-$  varied in relatively  
230 narrow ranges, while those of  $\text{NO}_3^-$  and DOC varied by an order of magnitude (Figs. 4 and  
231 SI-5).

232 Concentrations of DOC significantly increased in almost all Čertovo tributaries in 1997–  
233 2017. The only tributaries with no trends in DOC concentrations during 1997–2017 were  
234 CT-VI and CT-VII, where  $\text{NO}_3^-$  concentrations increased following tree damage by  
235 windthrows in 2007 and 2008. The chemistry of Čertovo tributaries slowly recovered from  
236 atmospheric acidification during our study, exhibiting decreasing concentrations of  $\text{SO}_4^{2-}$ ,  
237  $\text{Cl}^-$ ,  $\text{H}^+$ ,  $\text{Al}_i$  and ionic strength in all streams.

238 Chemistry of the Plešné tributaries exhibited similar trends to the Čertovo tributaries  
239 prior to the tree dieback (Tables SI-3 and SI-4), but strongly diverged thereafter (Fig. 4). In  
240 the period immediately following dieback there were increases in  $\text{NO}_3^-$ ,  $\text{H}^+$ ,  $\text{Al}_i$ , divalent  
241 base cations and ionic strength, whilst  $\text{SO}_4^{2-}$  and  $\text{Na}^+$  were unaffected. Concentrations of  
242 DOC did not immediately respond to dieback, but steep increases occurred from 2008–  
243 2017 as concentrations of initially affected ions and ionic strength decreased (Fig. 4).

244 Seasonal variations in water composition were highest for  $\text{NO}_3^-$  and DOC (as well as  $\text{A}^-$ )  
245 concentrations and T, and exhibited inverse seasonal patterns, with the lowest  $\text{NO}_3^-$  and the  
246 highest DOC concentrations and T values in the growing season. Concentrations of  $\text{Al}_i$  and  
247 ionic strength (and also BCs, not shown) exhibited similar seasonal variations as SAAs,  
248 while the lowest seasonal variation occurred for  $\text{H}^+$  concentrations (Fig. 4).



249

250

Fig. 4. Time series of concentrations of DOC, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup>, H<sup>+</sup>, ionic aluminium (Al<sub>i</sub>),

251

and ionic strength (IS) in the major surface tributary of (a, b) Plešné Lake (PL-I) and (c, d)

252

Čertovo Lake (CT-II). Grey areas indicate the period of bark beetle outbreak in the PL

253

catchment. For other tributaries see Fig. SI-5.

254

**Relationships between DOC and physico-chemical properties of stream water.** In the

255

Čertovo catchment, concentrations of DOC correlated positively with Q and T, and

256

negatively with BCs, Al<sub>i</sub>, and ionic strength in most tributaries. Results of forward stepwise

257

regression showed that majority of the long-term DOC variations in stream waters could be

258

explained by 5–7 of the selected variables. Among them, either T or Q (climate variables)

259

played the dominant role in the most Čertovo tributaries during the whole 2009–2017

260

period, while SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>, and H<sup>+</sup> concentrations (i.e., chemical variables) contributed to

261

explaining DOC variations in the CT-I and CT-II tributaries (Tables 1, SI-4, SI-5).

262

263 Table 1. Results of forward stepwise regression (FSR) between DOC vs. Q, T, and  
 264 concentrations of  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{H}^+$ , and  $\text{Al}_i$  in major surface tributaries to Čertovo  
 265 (CT-II, CT-VI and CT-VII) and Plešné (PL-I and PL-II) lakes, combined with correlation  
 266 coefficients of linear regressions between DOC and these variables (Table SI-4). Values for  
 267 DOC (given in brackets) represent Kendall's tau values of temporal trends for each time  
 268 period (Table SI-3). Bold numbers indicate significant relationships at  $p < 0.05$ , negative  
 269 values indicate inverse relationships. Asterisks indicate variables selected by the FSR.  
 270 Coefficient of determination ( $R^2$ ) of FSR are given for all variables selected by FSR (Table  
 271 SI-5). The most important variable in FSR is given in the last row. All values are related to  
 272 the given periods. For other tributaries and periods see Tables SI-3 to SI-5.

273

	----- 1997–2003 -----					----- 2009–2017 -----				
	CT-II	CT-VI	CT-VII	PL-I	PL-II	CT-II	CT-VI	CT-VII	PL-I	PL-II
n	74	67	67	74	56	144	143	144	143	123
DOC trend	(0.07)	(0.10)	(-0.18)	(0.05)	(0.11)	(0.16)	(0.10)	<b>(0.26)</b>	<b>(0.58)</b>	<b>(0.37)</b>
Q	<b>0.54*</b>	<b>0.51*</b>	0.16*	<b>0.55*</b>	0.27*	<b>0.54</b>	<b>0.50*</b>	<b>0.43*</b>	0.08	<b>0.24*</b>
T	0.14*	<b>0.39</b>	<b>0.66*</b>	<b>0.33*</b>	<b>0.44*</b>	<b>0.38</b>	<b>0.54*</b>	<b>0.63*</b>	<b>0.56*</b>	<b>0.51*</b>
$\text{NO}_3^-$	<b>-0.51*</b>	<b>-0.49*</b>	<b>-0.60*</b>	<b>-0.35</b>	<b>-0.34</b>	<b>-0.48*</b>	<b>-0.43*</b>	<b>-0.61*</b>	<b>-0.75*</b>	<b>-0.81*</b>
$\text{SO}_4^{2-}$	-0.21	-0.11*	<b>-0.29</b>	-0.07	-0.07	<b>-0.59*</b>	-0.07*	<b>-0.54*</b>	<b>-0.63*</b>	<b>-0.45</b>
$\text{Cl}^-$	-0.06	<b>-0.31*</b>	0.00*	-0.05	-0.22	<b>-0.20*</b>	<b>-0.22*</b>	-0.02*	<b>-0.72*</b>	<b>-0.74</b>
$\text{H}^+$	<b>0.63*</b>	<b>0.33*</b>	0.24	<b>0.30</b>	<b>0.31*</b>	<b>0.54</b>	<b>0.22*</b>	0.03	<b>-0.45*</b>	<b>-0.49</b>
$\text{Al}_i$	<b>-0.29</b>	-0.06	<b>-0.43</b>	0.01	-0.11	<b>-0.21</b>	-0.12	-0.12	<b>-0.58*</b>	<b>-0.70*</b>
FSR ( $R^2$ )	0.84	0.70	0.73	0.54	0.43	0.81	0.75	0.83	0.81	0.83
Variables	$\text{H}^+$	Q	T	Q	T	$\text{SO}_4^{2-}$	T	T	$\text{NO}_3^-$	$\text{NO}_3^-$

274

275 Climate variables were also important for the Plešné tributaries during the whole study,  
 276 but chemical variables dominated after the tree dieback (Tables 1, SI-5). During 1997–  
 277 2003, most of DOC variation was explained by Q and T. During 2004–2008,  $\text{H}^+$  and  $\text{Cl}^-$   
 278 dominated in the relationships. DOC concentrations did not, however, exhibit significant  
 279 trends during these two periods, and the selected variables mostly contributed to the

280 explanation of seasonal DOC variations. In contrast, significant DOC increases during  
281 2009–2017 were mostly explained by decreasing  $\text{NO}_3^-$  and  $\text{Al}_i$  concentrations (Table 1). A  
282 surprising positive correlation between DOC and  $\text{H}^+$  occurred in Plešné tributaries during  
283 1997–2008, but became negative in both PL-I and PL-II (the tributaries with the highest  
284 and most steeply increasing DOC concentrations) in 2009–2017 (Table 1). Relationships  
285 between DOC concentrations and SAAs, BCs,  $\text{Al}_i$ , and ionic strength were mostly negative  
286 and significant in all Plešné tributaries during all tested periods.

287

## 288 DISCUSSION

289 **Effects of soil physico-chemical properties on DOC leaching.** Water discharge and  
290 temperature explained most of the DOC variations in most Čertovo tributaries, especially  
291 during 2009–2017, and also in Plešné tributaries prior to the tree dieback in its catchment  
292 (Table 1). These relationships reflected similar seasonality of DOC concentrations and  
293 water T, and elevated DOC leaching during high flow events, and thus do not indicate that  
294 climate variations are responsible for longer-term DOC trends. Decreasing concentrations  
295 of SAAs,  $\text{H}^+$ , and  $\text{Al}_i$  contributed to the significant long-term increase in DOC  
296 concentrations in most Čertovo tributaries, especially during 1997–2008, with the  
297 exception of the two tributaries where  $\text{NO}_3^-$  increases following windthrow events in  
298 2007–2008 appeared to offset the rising DOC trend (Fig. SI-5).

299 The importance of Q and T in explaining DOC variation in the Plešné tributaries  
300 decreased after the tree dieback, while chemical variables became more important (Table  
301 1). The close positive DOC vs.  $\text{H}^+$  relationships prior to and immediately after the tree



302 dieback probably resulted from a coincidence, because it is not probable that decreasing pH  
303 would elevate DOC mobility; more likely, variations in  $H^+$  concentrations were affected by  
304 variations of  $A^-$  leaching. The most plausible reasons for the absence of trends in DOC  
305 leaching immediately after the tree dieback are increased concentrations of  $H^+$  and  
306 polyvalent cations (especially  $Al_i$ ), which increase protonation and coagulation of DOC and  
307 thereby reduce its mobility<sup>29,46</sup>, and elevated microbial DOC uptake (see next section).  
308 Chemical suppression was also found to reduce DOC leaching from a Northern Irish  
309 moorland catchment during several years following a wildfire<sup>9</sup>, suggesting that this  
310 mechanism may be a consistent short-term response to ecosystem disturbances in acidic  
311 catchments.

312 The increase in  $H^+$  concentrations in soil water and streams was fast (preceded peaks in  
313 BCs and  $Al_i$ )<sup>37</sup>, and peaked ~3 years after the tree dieback (Fig. 4), as  $H^+$  was displaced  
314 from the soil sorption complex by other cations in the upper (mostly O) horizons, and  
315 leached to surface waters along with  $NO_3^-$ .<sup>41</sup>

316 The steepest increasing trends in DOC leaching occurred in the Plešné tributaries during  
317 2009–2017, some years after the initial dieback event. During this time, the amount of litter  
318 in the catchment was still high, but concentrations of  $NO_3^-$ ,  $H^+$  and  $Al_i$  in stream water had  
319 declined, perhaps enabling more of the DOC produced through decomposition to leach to  
320 surface waters. The negative correlations between DOC and  $H^+$ ,  $Al_i$  and  $NO_3^-$  in the  
321 surface Plešné tributaries during this period (Table 1) are all consistent with an effect of  
322 decreasing soil water acidity and/or ionic strength on DOC mobility. Because changes in Q  
323 and T were negligible in the Plešné tributaries during 2009–2017 vs. previous periods, we  
324 conclude that these climatic variables did not contribute to the observed DOC increase in

325 the latter phase following tree dieback. Our data thus support the idea of multiple temporal  
326 scale drivers involved in trends of DOC leaching, indicating that seasonal and inter-annual  
327 variation can be explained by climate variables whereas long-term variation are more  
328 probably associated with changes in soil biogeochemistry.<sup>11,34,48,49</sup>

329 **Effects of soil microbial community on DOC leaching.** Changes in soil microbial  
330 biomass and tight links between C and N cycling could also have contributed to low DOC  
331 leaching immediately after the tree dieback, which occurred despite elevated DOC  
332 concentrations in soils (Fig. 3a), and to the subsequent increase as  $\text{NO}_3^-$  leaching declined  
333 (Fig. 4a). Štursová et al.<sup>50</sup> have shown that soil microbial community significantly changed  
334 in the Plešné catchment after the tree dieback, which has also been observed elsewhere.<sup>51</sup>  
335 At Plešné, fungal community biomass decreased, despite a relative increase in saprotrophic  
336 taxa, due mostly to the disappearance of mycorrhizal fungi following tree death. In  
337 contrast, bacterial biomass increased or remained unaffected after the disturbance, which  
338 resulted in a substantial decrease in the soil fungi-to-bacteria ratio.<sup>50</sup> Bacteria are  
339 distributed heterogeneously in small-scale habitats, physically connected by water, or along  
340 preferential flow paths, and their growth depends on DOC and nutrients passively  
341 transported to their surfaces.<sup>23,52</sup> This causes bacteria to be more dependent on water  
342 content and the presence of soluble compounds in soil than hyphal fungi.<sup>23</sup> The elevated  
343 soil moisture after the tree dieback (Fig. 2b) thus probably further supports development of  
344 bacterial vs. fungal biomass and their increasing role in soil C and N cycling.

345 The Plešné catchment was already N-saturated, and exhibited significant  $\text{NO}_3^-$  leaching,  
346 even before forest disturbance (Fig. 4). The elevated  $\text{NH}_4^+$  concentrations in soils  
347 immediately after the tree dieback (Fig. 3a) further increased availability of inorganic N for

348 microbial and plant communities. Consequently, N saturation in the Plešné catchment  
349 rapidly progressed to an advanced stage in which excess  $\text{NH}_4^+$  leads to elevated  
350 nitrification.<sup>53</sup> The supply of the surplus  $\text{NH}_4^+$  to nitrifiers may, however, have remained  
351 relatively low immediately after the tree dieback due to elevated soil concentrations of bio-  
352 available DOC from decaying dead biomass (e.g., fine roots), enabling immobilization of  
353 DOC and  $\text{NH}_4^+$  into  $C_{\text{MB}}$  and  $N_{\text{MB}}$  (Fig. 3b). This situation lasted for ~3 years, until DOC  
354 availability in soils decreased (Fig. 3a). The absence of elevated DOC leaching despite its  
355 production from dead biomass was therefore probably partly related to its immobilization  
356 into soil microbial biomass (as well as to the solubility controls discussed above) during  
357 this period. Even though stream water  $\text{NO}_3^-$  concentrations increased immediately after the  
358 three dieback (Fig. 4), this trend might have been steeper without abundant bio-available  
359 DOC in soils.

360 When C and N immobilization in microbial biomass decreased, more  $\text{NH}_4^+$  remained for  
361 nitrifiers, and soil water  $\text{NO}_3^-$  concentrations rapidly increased (Fig. 3a).<sup>42</sup> This  $\text{NO}_3^-$   
362 production increased concentrations of electron acceptors in the system, available for  $\text{NO}_3^-$   
363 reducing microbes (denitrification and dissimilatory nitrate reduction to ammonium) in  
364 anoxic soil micro-sites, which could further reduce the pool of DOC available for  
365 leaching.<sup>25,26,38</sup>

366 Decreasing  $\text{NO}_3^-$  availability in soils due to reduced excess N supply from mineralization  
367 of dead biomass and increasing N uptake by re-growing vegetation, and the reduced  $\text{SO}_4^{2-}$   
368 and  $\text{NO}_3^-$  throughfall deposition could contribute to the reduced availability of these  
369 electron acceptors for DOC mineralization in anoxic soil micro-sites and the increasing  
370 DOC leaching during 2009–2017 (Fig. 4). Moreover, the elevated DOC leaching also could

371 be associated with lower DOC bioavailability, connected with reduced input of fresh dead  
372 biomass.

373 **Effects of catchment characteristics and soil moisture on DOC leaching.** Long-term  
374 increasing trends in DOC leaching after tree dieback caused by bark beetle attack occurred  
375 in mountain catchments with > 50% impacted stands in western North America.<sup>8</sup> DOC  
376 concentrations increased in soil water of dead (compared to intact) mountain Norway  
377 spruce stands in Germany, as well as in streams draining clear-cut Norway spruce forests in  
378 Finland.<sup>4,21</sup> In contrast, some other studies (for review see ref. 7) found negligible effects of  
379 forest disturbance on DOC exports from soil to streams. Piirainen et al.<sup>20</sup> observed elevated  
380 DOC leaching from the organic soil horizon, but effective DOC retention in the mineral  
381 soil horizons. The spatial differences in DOC leaching after forest dieback thus also seem  
382 to reflect differences in catchment characteristics. The effect of forest dieback on DOC  
383 leaching is probably smaller when vertical water flow through well developed mineral soil  
384 horizons represents the dominant water pathway. In contrast, steep catchments (like that of  
385 Plešné Lake), with young organic-rich soils, poorly developed mineral horizons, and short  
386 water residence time in soils are more sensitive to elevated DOC leaching after tree  
387 dieback.

388 This leaching is further magnified by lateral flows during high-flow events, when water  
389 passes horizontally through the organic rich soil horizons. The probability of lateral flows  
390 and the associated risk of elevated DOC leaching increase during high precipitation and  
391 runoff events.<sup>17,54</sup> This may have been amplified following tree dieback by reduced water  
392 uptake and evapotranspiration beneath dead trees<sup>55,56</sup>, leading to increased soil moisture

393 (Fig. 2b), shallower groundwater, and therefore greater water fluxes through shallow,  
394 organic-rich soils.<sup>5,6</sup>

395 Another factor potentially affecting DOC leaching after tree dieback is pre-disturbance  
396 soil base saturation, which also appears to influence DOC response to wildfire.<sup>9</sup> In poorly  
397 buffered catchments, increases in  $\text{NO}_3^-$  (and  $\text{H}^+$ ) are likely to exceed the increases in BCs,  
398 thereby acidifying soil water and reduce DOC mobility, whereas in well-buffered  
399 catchments, acidity may be unchanged or even decrease (if BC increases exceed acid anion  
400 increases).

401 **Interplay of soil C and N responses to tree mortality.** Our data suggest a complex  
402 contribution of chemical and microbial variables to ecosystem responses to tree dieback,  
403 manifested by initial  $\text{NO}_3^-$  leaching followed by increased DOC leaching. We observed a  
404 stable level of DOC leaching in the first years after the tree dieback (Fig. 4a), despite the  
405 rapid increase in litter available for decomposition (Fig. 1) and elevated DOC  
406 concentrations in soil water (Fig. 3a). In contrast, DOC leaching increased from the system  
407 in the latter phase, when litter input to the forest floor had already ceased. These trends can  
408 be explained by changes in soil water chemistry, soil microbial community, and links  
409 between C and N cycles. Our data suggest that changes in N cycling could play an  
410 important role in DOC leaching. We conceptualise ecosystem C and N responses to tree  
411 mortality in three stages (Fig. 5):

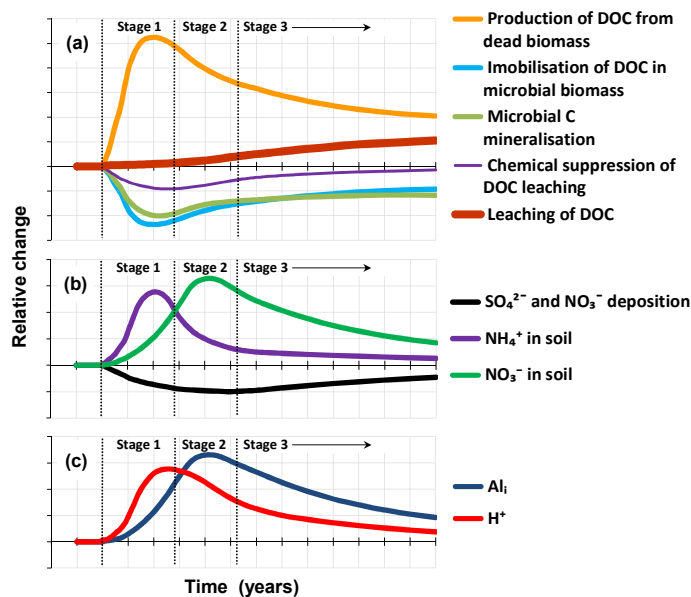
412 *Stage 1:* Immediately after tree dieback, tree-associated fungi decline, and bacterial  
413 populations increase. Elevated N availability for free bacteria enables them to utilize  
414 available DOC for transformation into bacterial biomass (Fig. 3b) and energy  
415 (mineralization to  $\text{CO}_2$ ). DOC mobility is suppressed by increasing soil water acidity due to

416  $H^+$  displacement from the soil sorption complex by other cations, and by microbial  $NH_4^+$   
417 use (Fig. 4b). Nitrification remains low relative to  $NH_4^+$  availability, and  $NO_3^-$  leaching is  
418 therefore limited despite a steep increase in soil  $NH_4^+$  concentrations after tree dieback  
419 (Fig. 3a). Elevated microbial DOC utilisation and chemical suppression of its mobility  
420 restrict DOC leaching to waters (Fig. 4a).

421 *Stage 2:* In the second stage, microorganisms became C-limited as litter inputs decrease,  
422 and their capacity to assimilate  $NH_4^+$  becomes saturated. At this point, nitrification rates  
423 increase, reducing the pool of available  $NH_4^+$  and increasing  $NO_3^-$  production and leaching.  
424 The associated production of  $H^+$  and mobilisation of polyvalent cations (including  $Al_i$  from  
425 dissolution of soil  $Al(OH)_3$ ) maintain suppression of DOC mobility. In addition,  
426 nitrification increases the availability of electron acceptors for  $NO_3^-$  reducing  
427 microorganisms. These processes together act to delay DOC leaching despite its continued  
428 liberation from the dead biomass.

429 *Stage 3:* In the final stage of ecosystem response to forest dieback, the available N pool  
430 begins to decline as the supply of  $NH_4^+$  from organic matter mineralisation is exhausted,  
431 and N uptake by re-growing trees begins to occur. During this phase, DOC leaching  
432 increases due to: (i) reduced chemical suppression of DOC mobility as soil solution  $H^+$  and  
433  $Al_i$  decline, following the decrease in  $NO_3^-$  production and leaching (Fig. 5c); (ii)  
434 decreasing availability of  $NO_3^-$  (Fig. 5b) for DOC mineralization by  $NO_3^-$  reducing  
435 microorganisms in anoxic soil micro-sites; and (iii) decreasing DOC immobilization in  
436 microbial biomass (Fig. 5a). The elevated DOC production can be considered as the net  
437 difference between ongoing DOC production from dead wood biomass by saprotrophic  
438 fungi, and the decreasing utilisation of DOC by free bacteria as the N supply declines. To

439 the extent that the increase in DOC leaching is due to the alleviation of chemical  
 440 suppression, we would expect DOC concentrations to return to the ‘baseline’  
 441 concentrations represented by the Čertovo reference site (note that DOC has been  
 442 increasing in both catchments due to the ongoing decline of atmospheric S and N  
 443 deposition<sup>38</sup>, therefore this baseline is not flat). However, the enhanced production of DOC  
 444 from dead biomass has the potential to increase DOC leaching above this reference level,  
 445 and previous work has suggested that this production can continue (albeit with decreasing  
 446 intensity) for up to three decades after a mortality event.<sup>57,58</sup>  
 447



448  
 449 Fig. 5. Conceptual graph showing significant processes and chemical changes that can  
 450 influence DOC leaching during forest dieback, based on observations from the Plešné  
 451 catchment: (a) Changes in production, microbial use, chemical suppression and leaching of  
 452 DOC. (b) Changes in availability of inorganic N and electron acceptors (NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup>)  
 453 for microbial C mineralization in anoxic soil micro-sites. (c) Changes in soil water H<sup>+</sup> and

454  $\text{Al}_i$  concentrations that affect chemical suppression of DOC mobility. Grey areas indicate  
455 period of tree dieback.

456 Our results contribute to the growing body of evidence<sup>25,38,59,60</sup> that an integrated  
457 understanding of ecosystem C and N cycles is required in order to evaluate and predict  
458 DOC and  $\text{NO}_3^-$  leaching from terrestrial ecosystems, especially their responses following  
459 forest disturbances.

460

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464

#### 465 ASSOCIATED CONTENT

##### 466 **Supporting Information Available**

467 Part 1: Detailed description of the Plešné and Čertovo catchments, including details on  
468 soil, throughfall and litter sampling and composition; Part 2: Details on water sampling and  
469 analyses; Part 3: Details on water composition and statistics. This information is available  
470 free of charge via the Internet at <http://pubs.acs.org>.

471

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475 **Notes**

476 The authors declare no competing financial interests.

477

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658 **Table of Contents graphic:**

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