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Klaus Steenberg Larsen, Louise C. Andresen, Claus Beier, Sven Jonasson, Kristian R. Albert, Per Ambus, Karen Stevnbak, Marie Frost Arndal, Mette S. Carter, Soren Christensen, et al.

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Reduced N cycling in response to drought, warming, and elevated CO₂ in a Danish heathland: Synthesizing results of the CLIMAITE project after two years of treatments

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Keywords:	<p>Climate driver interactions, C/N ratio, multi-factor climate change experiment, nitrogen cycling, nitrogen mineralization, N₂O, soil fauna</p>
Abstract:	<p>Field-scale experiments simulating realistic future climate scenarios are important tools for investigating the effects of current and future climate changes on ecosystem functioning and biogeochemical cycling. We exposed a semi-natural Danish heathland ecosystem to elevated atmospheric carbon dioxide (CO₂), warming, and extended summer drought in all combinations. Here, we report on the short-term responses of the nitrogen (N) cycle after two years of treatments. Elevated CO₂ significantly affected above-ground stoichiometry by increasing the carbon to nitrogen (C/N) ratios in the leaves of both co-dominant species (<i>Calluna vulgaris</i> and <i>Deschampsia flexuosa</i>), as well as the C/N ratios of <i>Calluna</i> flowers and by reducing the N concentration of <i>Deschampsia</i> litter. Below-ground, elevated CO₂ had only minor effects, whereas warming increased N turnover, as indicated by increased rates of microbial NH₄⁺-N consumption, gross mineralization, potential nitrification, denitrification and N₂O emissions. Drought reduced below-ground gross N mineralization and decreased fauna N mass and N mineralization. Leaching was unaffected by treatments but was significantly higher across all treatments in the second year than in the much drier first year indicating that ecosystem N loss is highly sensitive to changes and variability in amount and timing of precipitation. Interactions between treatments were common and although some synergistic effects were observed, antagonism dominated the interactive responses in treatment combinations, i.e. responses were smaller in combinations than in single treatments. Nonetheless, increased C/N ratios of photosynthetic tissue, decreased litter N production, and decreased fauna N mineralization prevailed in the full treatment combination indicating reduced N turnover in the simulated future climate scenario, which could act to reduce the potential growth response of plants to elevated atmospheric CO₂ concentration. In conclusion, effects observed in single-factor studies should be evaluated with caution. Multi-factor climate experiments are needed for improving realistic estimation of the combined ecosystem responses to future climate changes.</p>

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6 2 heathland: Synthesizing results of the CLIMAITE project after two years of treatments
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10 4 **Running title:** Effects of climate change on N cycling
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55 23 **Keywords:** Climate driver interactions, C/N ratio, multi-factor climate change experiment,
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57 24 nitrogen cycling, nitrogen mineralization, N₂O, soil fauna
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67 **Abstract**

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10 27 Field-scale experiments simulating realistic future climate scenarios are important tools for
11
12 28 investigating the effects of current and future climate changes on ecosystem functioning and
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14 29 biogeochemical cycling. We exposed a semi-natural Danish heathland ecosystem to elevated
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16 30 atmospheric carbon dioxide (CO₂), warming, and extended summer drought in all
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18 31 combinations. Here, we report on the short-term responses of the nitrogen (N) cycle after two
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20 32 years of treatments.
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25 33 Elevated CO₂ significantly affected above-ground stoichiometry by increasing the
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27 34 carbon to nitrogen (C/N) ratios in the leaves of both co-dominant species (*Calluna vulgaris*
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29 35 and *Deschampsia flexuosa*), as well as the C/N ratios of *Calluna* flowers and by reducing the
30
31 36 N concentration of *Deschampsia* litter. Below-ground, elevated CO₂ had only minor effects,
32
33 37 whereas warming increased N turnover, as indicated by increased rates of microbial NH₄⁺-N
34
35 38 consumption, gross mineralization, potential nitrification, denitrification and N₂O emissions.
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37 39 Drought reduced below-ground gross N mineralization and decreased fauna N mass and N
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47 44 observed, antagonism dominated the interactive responses in treatment combinations, *i.e.*
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9 50 In conclusion, effects observed in single-factor studies should be evaluated with
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13 52 combined ecosystem responses to future climate changes.
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7 54 **Introduction**
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10 55 Climate change with elevated atmospheric CO₂, increased temperature and altered
11 56 precipitation will fundamentally affect key drivers for ecosystem functioning and lead to
12 57 adverse effects in terrestrial ecosystems across the globe (IPCC, 2007). These climate-driven
13 58 changes may affect ecosystem functioning directly, *e.g.* through CO₂-stimulated
14 59 photosynthesis and growth (Luo *et al.* 2006; de Graaff *et al.* 2006), warming-induced
15 60 increased nutrient mineralization (Emmett *et al.* 2004) or drought-induced growth limitation
16 61 (Penuelas *et al.* 2007). They may also act indirectly *e.g.* through impacts on species
17 62 composition (Penuelas *et al.* 2007).
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29 63 Growth in many natural and semi-natural terrestrial ecosystems is limited by nitrogen
30 64 and characterized by strong competition for nitrogen between plants and the soil communities
31 65 of fungi and bacteria (Marion *et al.* 1989; Jonasson *et al.* 1996). Consequently, ecosystem N
32 66 availability is an important factor controlling the carbon uptake in terrestrial ecosystems and
33 67 therefore also plays a dominant role in controlling the feedback between the biosphere and the
34 68 atmosphere in a warmer and more CO₂ enriched world. The theory of nitrogen as a limiting
35 69 factor constraining the carbon uptake in terrestrial ecosystems in response to elevated CO₂ is
36 70 conceptualized in the PNL framework (Progressive Nitrogen Limitation) (Luo *et al.* 2004)
37 71 describing how increased carbon sequestration driven by elevated CO₂ requires increased
38 72 access to nitrogen in order to balance the carbon input. Accordingly, without additional inputs
39 73 of plant-available N, nitrogen will progressively limit further carbon uptake. But increased N
40 74 availability has also been shown as a consequence of increased availability of labile soil
41 75 carbon substrate (Zak *et al.* 1993) and increased soil moisture content due to improved plant
42 76 water use efficiency (WUE) (Zak *et al.* 1993; Hungate 1999), as well as increased N
43 77 mineralization in response to warming (Schmidt *et al.* 2004; Emmett *et al.* 2004). The
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3 78 interaction between the carbon and nitrogen cycles clearly illustrates how sensitive
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5 79 ecosystems are to changes in nitrogen pools and turnover, current input of N, and other
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8 80 nutrients, and changes in the water regime. This is particularly important for N limited
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10 81 systems, such as natural and semi-natural ecosystems with relatively low nitrogen inputs and
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12 82 internal N turnover rates of the mineral N pool as fast as a few days (Davidson *et al.* 1990;
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14 83 Lipson & Näsholm 2001; Schimel & Bennett 2004; Vervaet *et al.* 2004). In systems with very
15
16 84 high internal turnover rates of nitrogen, the N turnover has also been shown to be very
17
18 85 sensitive to changes in natural climatic variations such as reduced water availability
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20 86 (Jamieson *et al.* 1999), potentially leading to a high sensitivity of these systems to climate-
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22 87 driven changes in the internal processes regulating the demand or supply of nitrogen.
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27 88 Most of the experimental evidence of PNL and the role of nitrogen in constraining
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29 89 carbon uptake is based on modeling studies (Rastetter *et al.* 1997) or on experiments
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31 90 involving elevated CO₂ alone or in combination with various levels of nitrogen addition to
32
33 91 demonstrate the role of N in stimulating CO₂ sequestration responses (Lutze & Gifford 2000;
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35 92 Lutze *et al.* 2000; van Heerwaarden *et al.* 2005; de Graaff *et al.* 2006; Reich *et al.* 2006).
36
37 93 However, in a future CO₂ enriched world, the nitrogen limitation and the CO₂ response in N
38
39 94 limited systems will to a large extent be regulated by the climate change-associated responses
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41 95 of the internal nitrogen cycle, which are not well captured in a CO₂ alone experiment. For
42
43 96 instance, it has been demonstrated that warming can stimulate internal nitrogen
44
45 97 mineralization, leading to increased N availability (Emmett *et al.* 2004) and even increased N
46
47 98 leaching (Lukewille & Wright 1997; Schmidt *et al.* 2004). Correspondingly, changes in water
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49 99 availability, such as water limitations by droughts, can reduce N mineralization and N
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51 100 availability (Emmett *et al.* 2004). On the other hand, increased water availability due to
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53 101 increased precipitation or to CO₂ stimulated increase in WUE can increase N mineralization
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55 102 and N availability under water limited conditions (Hungate 1999). Since the future climate
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3 103 change will involve changes in all of these factors, it is important to understand the possible
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5 104 interactions between different climate drivers on N availability and nitrogen limitation.
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8 105 Therefore, studies involving all factors simultaneously are needed.
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10 106 In the present study, we aim to synthesize the overall impacts of changes in the nitrogen
11
12 107 cycle in response to warming, elevated atmospheric CO₂ concentration and increased summer
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14 108 drought events after two years of treatments. We compare the responses of the three-factor
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16 109 treatment with responses to treatments including only one, or pair wise combinations of the
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20 110 factors.

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22 111 In response to elevated CO₂, we expected to observe increased plant tissue and litter
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24 112 C/N ratios (Paterson *et al.* 1999; Lutze & Gifford 2000; van Heerwaarden *et al.* 2005; Finzi *et*
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26 113 *al.* 2006; Chen *et al.* 2007; Hovenden *et al.* 2008). In the short term, we further expected that
27
28 114 the increased water use efficiency under elevated CO₂ (Ainsworth & Long 2005; Leuzinger &
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30 115 Körner 2007; Ainsworth & Rogers 2007; Albert *et al.* 2010) would stimulate plant growth,
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32 116 and increase plant N demand and biomass, and that concomitant increased rhizodeposition
33
34 117 would lead to increased microbial biomass activity and mineralization. Warming was
35
36 118 expected to increase plant growth and increase mineralization (Rustad *et al.* 2001), whereas
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38 119 extended summer drought was expected to reduce plant N demand and decrease N
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40 120 mineralization and nitrification rates (Emmett *et al.* 2004; Schmidt *et al.* 2004; Sowerby *et al.*
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42 121 2008) as a product of direct or indirect effects on microbial activity. Assuming simple
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44 122 addition of these single treatment effects, we expected that drought effects would be reduced
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46 123 in combination with both warming and elevated CO₂ and, consequently, that increased N
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48 124 mineralization and plant growth in response to both warming and elevated CO₂ would
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50 125 dominate over decreases caused by drought in the full combination of treatments.
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127 Materials and methods*128 Experimental site and climate change manipulations*

129 The CLIMAITE experimental site is a dry, temperate heathland situated approximately 50 km
130 NW of Copenhagen, Denmark (55°53' N, 11°58'E). The vegetation is dominated by the
131 evergreen dwarf shrub *Calluna vulgaris* (L.), the perennial grass *Deschampsia flexuosa* (L.)
132 and various mosses and herbs. The soil is a well-drained, nutrient-poor sandy deposit with a
133 pH of 4-5 and an organic top layer ranging from 2 to 5 cm in depth. Long-term annual mean
134 air temperature is 8.0 °C, annual mean precipitation is 613 mm (Danish Meteorological
135 Institute 2009) and the total N bulk deposition at the site was 1.27 ± 0.07 and 1.35 ± 0.04 g N m⁻²
136 y⁻¹ in 2006 and 2007, respectively (Larsen KS, unpublished data). The experimental area
137 covers approximately 2 ha and consists of twelve 7m diameter octagons laid out pair-wise in 6
138 blocks (= 6 replicates). Each block consists of two octagons, one with ambient (A)
139 atmospheric CO₂ and one receiving elevated atmospheric CO₂ concentration (CO₂) (510 ppm
140 in a free air CO₂ enrichment setup (FACE)). Each octagon is separated into 4 subplots
141 receiving in addition to the ambient or elevated CO₂ 1) one month summer drought (D) by
142 rain-out shelters, 2) passive warming (T) of air and soil by night time warming curtains 50 cm
143 above ground, 3) a combination of drought and warming (TD) or 4) no drought or
144 temperature treatment. In total, the experiment provides a full factorial design with all 7
145 combinations of D, T and CO₂ and an untreated control for reference (A). Details on the
146 experimental setup are described by Mikkelsen *et al.* (2008).

147 The treatments were initiated in October 2005 and the first summer drought was
148 applied in July/August 2006. The warming treatment elevated temperature at 5 cm depth by
149 0.3 °C in the winter to 0.7 °C in the summer months, on average. The maximum mean daily
150 temperature elevation was 1.2, 2.1 and 2.8 °C in the 5 cm depth, soil surface and 20 cm height

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3 151 sensors, respectively (4 October 2005 - 31 December 2007). The drought campaigns removed
4
5 152 52 mm and 94 mm of precipitation, resulting in peak reductions of soil water content of 11
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8 153 and 13 % v/v over 0-20 cm soil depth in 2006 (3-20 July and 26 July-4 August) and 2007 (21
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10 154 May-22 June), respectively . The drought campaigns were stopped when soil water content
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12 155 reached about 5 % v/v over 0-20 cm soil depth.
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19 157 *N stocks in plants, litter, microbes and soil, C/N ratios, extractable N concentrations*

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22 158 Above-ground biomasses N of *Calluna* and *Deschampsia* were estimated from non-
23
24 159 destructive point intercept measurements in two 50 x 50 cm subplots within each plot
25
26
27 160 combined with a linear regression model linking point intercept measurements and biomass
28
29 161 obtained from destructive harvesting of similar plots outside the treatment plots (Jonasson
30
31 162 1988; Kongstad *et al.* 2010; Riis-Nielsen & Schmidt 2010). To compensate for pre-treatment
32
33 163 differences between the initial biomass values in individual sub-plots and the subsequent
34
35 164 development during the experiment, treatment effects on above-ground plant biomasses were
36
37 165 normalized and scaled to the average biomass development in the ambient plots while
38
39 166 maintaining the observed relative variance. Briefly, a regression was fitted between the pre-
40
41 167 treatment plant biomass and the treatment biomass at a given time point, within each
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43 168 treatment. The slope of the regression in the ambient treatment defined the ambient or control
44
45 169 response; the normalized treatment effect was determined as the difference between the
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47 170 predicted control response and the predicted treatment response for a given treatment. See van
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49 171 der Linden *et al.* (2010) for further description.
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56 172 *Calluna* and *Deschampsia* plants as well as mosses from the experimental plots were
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58 173 harvested in August 2007. The C and N concentrations measured in green tissue, flowers,
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60 174 wood/stem and roots or whole plants (mosses) (Andresen *et al.* 2010a) were used to calculate

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3 175 C/N ratios. The N concentrations were used in combination with the non-destructive above-
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6 176 ground biomass estimates to calculate the total N stock of these pools. Concurrent with plant
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8 177 harvest, soil samples from each plot (0-10 cm soil depth) were taken to measure plant root
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10 178 biomasses, microbial biomass N and, soil organic matter N (SOM-N) and soil extractable
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13 179 $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and dissolved organic nitrogen (DON) (Andresen *et al.* 2010a). Litter
14
15 180 production by *Deschampsia* was estimated from point intercept-based difference between
16
17 181 seasonal minimum and maximum biomass of live and dead fractions of *Deschampsia* leaves
18
19 182 multiplied with N concentrations in the litter (Kongstad *et al.* 2010; Riis-Nielsen & Schmidt
20
21 183 2010).

184

185 *Soil fauna*

186 Soil cores within each plot (0-10 cm soil depth) were sampled in October and November 2007
187 for biomass estimation of protozoa/nematodes and enchytraeids/microarthropodes,
188 respectively. For estimation of protozoan biomass, a soil suspension was prepared by
189 blending 5 g of soil with 100 ml of Amoeba Saline (AS, Page 1988) for 1 min. Three-fold
190 dilution series of the soil suspension were prepared in microtiter plates being inspected for
191 protozoa after one and three weeks of growth at 15°C with a modified version of the Most
192 Probable Number method (Darbyshire *et al.* 1974; Rønn *et al.* 1995). Nematodes were
193 extracted from between 10 and 11 g (fresh weight) of soil by a modified Baermann tray
194 extraction method (Georgieva *et al.* 2005). Samples were extracted for 48 h, and nematodes
195 were then counted at 40× magnification using a dissecting microscope. Biomass N of
196 protozoa and bacterivorous nematodes were calculated according to Stout & Heal (1967) and
197 Sohlenius & Sandor (1987), respectively. N mineralization (excretion and turnover) of
198 microfauna was calculated assuming bacterivore turnover rate of 0.16 d⁻¹ (Zwart *et al.* 1994)

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3 199 for both nematodes and protozoans and setting yield ratios of protozoans and nematodes on
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5 200 bacteria to 0.4 and 0.04 (Coleman *et al.* 1978). The total biomass of enchytraeids was
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7 201 determined as described by Maraldo *et al.* (2008). The density of microarthropods was
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9 202 determined as described by Holmstrup *et al.* (2007), and the biomass of dominating
10
11 203 taxonomic groups was estimated using representative values from Petersen & Luxton (1982).
12
13 204 The pool of N contained in the biomass was calculated assuming nitrogen concentrations of
14
15 205 11.5 % and 10 % of dry weight in enchytraeids and microarthropods, respectively (Persson
16
17 206 1983). The yearly turnover rate of biomass N was assumed to be 1.2 for enchytraeids
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19 207 (Standen 1973), and 1.8 for microarthropods (De Ruiter *et al.* 1993). The annual
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21 208 mineralization (excretion and turnover) of NH_4^+ -N by enchytraeids and microarthropods was
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23 209 estimated based on biomass as described by Persson (1983).
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211 *N₂O emissions and N₂O reductase*

212 Fluxes of N_2O were measured with a static chamber method using white PVC chambers
213 (height 15 or 45 cm depending on vegetation height) placed on metal collars (60 × 60 cm)
214 permanently installed in the soil. Enclosure times were 2 to 2.5 h, during which headspace air
215 was sampled four times and later analyzed for N_2O concentration by gas chromatography.
216 The N_2O flux was calculated using linear regression. Nine N_2O measuring campaigns
217 including all eight treatments were carried out from June 2006 to March 2007, and from April
218 to June 2007 five campaigns were conducted in five of the treatments, *viz.* A, CO_2 , D, DCO_2
219 and TDCO_2 . Annual cumulative N_2O fluxes were derived by interpolation between
220 measurement days and by extrapolating the emission from the treatments T, TD and TCO_2 for
221 the period March- June 2007. Further methodological description is found in Carter *et al.*
222 (2010).

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3 223 To determine N₂O reductase activity, nitrate was removed from sub samples of the
4
5 224 soil cores taken in November 2007 for fauna biomass estimation by vortexing 10 g fresh soil
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8 225 and 30 ml of phosphate buffered saline (PBS) for 5 sec followed by centrifugation for 10 min
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10 226 at 3500 x G and 5 °C. The supernatant was discarded and the pellet resuspended in 30 ml
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12 227 PBS. After the third centrifugation the pellet was resuspended in 15 ml of 0.5 mM glucose,
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15 228 0.5 mM sodium acetate, and 0.5 mM sodium succinate and transferred to a 100 ml incubation
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17 229 bottle. The bottle was sealed with a butyl rubber stopper, flushed with N₂ for 2 min, added
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19 230 100 ppm N₂O (final concentration), placed horizontally on a shaker (200 rpm), and incubated
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21 231 at 22 °C. After 0, 1, 3, 6 and 24 h, 3 ml of headspace was transferred to a pre-evacuated 3 ml
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23 232 Venoject vial before analysis of N₂O by gas chromatography. The N₂O reductase activity was
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25 233 calculated from the consumption of N₂O during the incubation.
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33 235 *Nitrogen mineralization and immobilization*

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36 236 Gross rates of N mineralization and immobilization were determined by the isotope pool-
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38 237 dilution approach applied in laboratory incubations. Soil cores within each plot were sampled
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40 238 in September 2007 (0-5 and 5-10 cm soil depth, respectively), sieved and stored at 5 °C for
41
42 239 about one week until incubations were initiated. Fresh samples (10 g) were weighed in 250 ml
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44 240 poly-ethylene bottles and mixed thoroughly with 400 µl of a ¹⁵N-NH₄Cl solution (5 atm %
45
46 241 ¹⁵N) to provide 5 µg N g⁻¹ soil. One set of samples was analyzed immediately upon substrate
47
48 242 application, and a second set was analyzed after 7 days of incubation at 20 °C. Contents of
49
50 243 mineral N and the ¹⁵N contents were determined upon extraction and micro-diffusion
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52 244 (Sørensen & Jensen 1991). Gross daily rates of mineralization and immobilization were
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54 245 calculated according to equations given by Kirkham & Bartholomew (1954). Upscaling to
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3 246 annual rates was done using the mean annual soil temperatures in 5 cm depth (10 °C) at the
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6 247 experimental site and assuming a Q_{10} of 2.
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12 249 *Potential nitrification and denitrification*
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15 250 Subsamples from the soil cores taken in November 2007 were gently homogenized by hand
16
17 251 and major roots were removed. Soil samples were stored at 4 °C until analysis (less than 24
18
19 252 h). Potential nitrification was determined in 3 g soil samples by a modification of the method
20
21 253 described by Belser & Mays (1980) but without adding chlorate. Potential nitrification rates
22
23 254 were estimated from the increase in nitrite+nitrate concentrations. Potential denitrification
24
25 255 rates (Wolsing & Prieme 2004) in 10 g soil samples were estimated from linear regression of
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27 256 increase in headspace N_2O concentration during 180 min incubation.
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36 258 *Water percolation and nitrogen leaching*
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39 259 Leachate water was collected monthly from passive PVC soil water draining collectors below
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41 260 the organic soil layer (approximately 5 cm depth) and polytetrafluoroethylene (PTFE) suction
42
43 261 cups with continuous sub-atmospheric pressure (Prenart Super Quarz, Frederikberg, DK)
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45 262 below the main rooting zone (60 cm depth). Concentrations of NH_4^+ -N, NO_3^- -N, and total N
46
47 263 (by UV-persulphate oxidation) were analyzed on an Autoanalyzer 3 (Bran+Luebbe GmbH,
48
49 264 Germany). DON was calculated as total N minus inorganic N. Precipitation was measured at
50
51 265 the site and the volume of percolating water was estimated by simulation with the CoupModel
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53 266 model (Jansson & Moon 2001; Svensson *et al.* 2008) – a coupled ecosystem model with a
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55 267 balance of abiotic and biotic processes in the soil-plant-atmosphere system. The basic
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57 268 structure of the model is a depth profile of the soil, and calculations of water and heat flows
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3 269 are based on common soil properties. The model was parameterised using measured soil layer
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6 270 properties and against time series of soil temperature and moisture content, using on site
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8 271 measurements of radiation, precipitation and wind speed as driving data and a generalised
9
10 272 likelihood uncertainty evaluation approach. Percolation of water below 60 cm was calculated
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12 273 as an output of the simulations and leaching of nitrogen at that depth was calculated as the
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15 274 product of the sum of water percolation and the concentration measured in the soil water for
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17 275 each water collection period (Schmidt *et al.* 2004).
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24 277 *Statistical analyses*
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27 278 Statistical analyses were conducted using the multiple linear regression procedure PROC
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29 279 MIXED of SAS (SAS Institute 2003). The statistical model included a random statement that
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31 280 accounted for the experimental design (*Random* Block octagon octagon*D octagon*T). The
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33 281 same model was used for all tested variables and included the three main factor effects (CO₂,
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35 282 D, and T) as well as all possible interactions (D*CO₂, T*CO₂, T*D, and T*D*CO₂). P-values
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37 283 ≤ 0.05 were considered significant. Data were transformed when necessary to obtain
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39 284 normality and homogeneity of variance. Differences of Least Squares Means (DLSM) were
40
41 285 used to interpret significant treatment interactions. During the DLSM evaluation, each
42
43 286 significant interaction was categorized as either *antagonistic*, *i.e.* the combined effect led to a
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45 287 reduction of the observed individual effects or *synergistic*, *i.e.* the combined effect led to
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47 288 either a) an amplification of the observed individual effect(s) or b) to a significant effect only
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49 289 when treatments were combined. The DLSM evaluation also revealed if interactions were due
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51 290 to significant single treatment effects, *i.e.* effects observe in single treatment plots only, which
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53 291 disappear in combination with other treatments. Furthermore, DLSM evaluation of significant
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55 292 interactions revealed if significant main factor effects were effects observed only when in
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3 293 combination with other treatments or if it was significant also as a single treatment effect.
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5 294 Finally, we noted simple additive effects when two of the treatments both showed significant
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8 295 main factor effects but with no interactions.
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For Review Only

296

297 **Results**298 *Ecosystem N balance*

299 The N cycle at the site is dominated by higher internal N turnover in comparison to the inputs
300 and losses by leaching and gaseous emission to the atmosphere (Fig. 1). Gross mineralization
301 ($6.7 \pm 0.96 \text{ g N m}^{-2}\text{y}^{-1}$) is five times higher than bulk deposition ($1.35 \pm 0.04 \text{ g N m}^{-2}\text{y}^{-1}$), an
302 order of magnitude higher than losses by leaching ($0.80 \pm 0.37 \text{ g N m}^{-2}\text{y}^{-1}$) and two orders of
303 magnitude higher than gaseous losses in form of N_2O ($0.031 \pm 0.006 \text{ g N m}^{-2}\text{y}^{-1}$). Negligible
304 N_2O reductase activity was observed in the soil samples with N_2O uptake rates less than 1 ng
305 $\text{N g}^{-1} \text{ dw h}^{-1}$ in all samples and N_2 production is therefore assumed to be negligible (data not
306 shown). Above ground, about 90 % of N is found in the two dominant species *Calluna* (~60
307 %) and *Deschampsia* (~30 %). Below ground, most N is stored in the SOM pool (~90 %) with
308 *Deschampsia* roots (~4 %), *Calluna* roots (~3 %) and soil microbial (~1.5 %) pools
309 dominating the remaining fraction. Soil fauna biomass was ~10 % of microbial biomass but
310 their mineralization of N is estimated to $5.0 \pm 0.6 \text{ g NH}_4^+\text{-N m}^{-2}\text{y}^{-1}$, *i.e.* an amount of labile N
311 much larger than the N input to the system by N bulk deposition. The system retained 0.53 g
312 $\text{N m}^{-2}\text{y}^{-1}$ of bulk deposition in the ambient, non-manipulated plots in 2007 as estimated by
313 difference between bulk deposition and sum of total N leaching below 60 cm soil depth and
314 N_2O emission. While bulk deposition in 2006 ($1.27 \pm 0.07 \text{ g N m}^{-2}\text{y}^{-1}$) was similar to the
315 deposition in 2007 ($1.35 \pm 0.04 \text{ g N m}^{-2}\text{y}^{-1}$), total N leaching across all treatments was
316 significantly lower ($P < 0.0001$) in 2006 ($0.13 \pm 0.06 \text{ g N m}^{-2}\text{y}^{-1}$) than in 2007 ($0.56 \pm 0.20 \text{ g}$
317 $\text{N m}^{-2}\text{y}^{-1}$) with no significant treatment effects in either 2006 (data not shown) or in 2007
318 (Table 1). The major cause of this interannual difference was a wet summer in 2007 (202 mm

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3 319 precipitation in July) with extensive leaching compared to a dry summer in 2006 (60 mm
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5 320 precipitation in July) when summer leaching was absent.
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11 322 *Plant responses to climate manipulations*
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15 323 The above-ground biomass N of the dominant plant species *Calluna* and *Deschampsia* did not
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17 324 respond to the climate manipulations (Table 1). Root biomass N of *Deschampsia* was also
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19 325 unaffected by the treatments, whereas root biomass N of *Calluna* increased in response to
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21 326 drought (Table 1, $P = 0.04$) indicating a change in the root:shoot N allocation pattern. While
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23 327 N in above-ground biomasses were unaffected by treatments, the C/N ratios of *Calluna*
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25 328 flowers as well as leaves from both *Calluna* and *Deschampsia* increased in response to
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27 329 elevated CO₂ (Table 1, $P = 0.02$, 0.04, and 0.01, respectively). The evaluation by DLSM of
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29 330 the three-way interaction for the C/N ratio of mosses (Table 1, $P = 0.003$) showed that the
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31 331 interaction was due to significantly higher C/N ratios in the single treatments of drought and
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33 332 elevated CO₂, while ratios were unchanged when drought and elevated CO₂ were combined
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35 333 with each other as well as when they were combined with warming. The observed increases in
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37 334 C/N ratios in new plant tissue of leaves and flowers were not seen in whole-plant C/N ratios
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39 335 of *Deschampsia* or in the green tissue or wood fractions of *Calluna*. Yet, the N concentration
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41 336 of *Deschampsia* litter decreased under elevated CO₂ (Table 1, $P = 0.05$), indicating that the
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43 337 changes in C/N ratios starting in the leaves, are already beginning to cascade through the
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45 338 system, after only two years of treatments (Fig. 2a).
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53 339 *Deschampsia* leaves and also mosses (single treatment only) responded to drought
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55 340 with increased C/N ratios (Table 1, $P = 0.002$ and $P \leq 0.05$, respectively) whereas *Calluna*
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57 341 leaves were unaffected (Table 1, Fig. 2b). Also, the pool of N in the annually produced
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59 342 *Deschampsia* litter decreased in response to drought (Table 1, $P = 0.01$). The warming
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3 343 treatment had fewer effects on above-ground processes than the other treatments, showing
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5 344 only an increase of N concentration in *Deschampsia* litter (Table 1, P = 0.03, Fig 2c).
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9 345 When all treatments were combined (Fig. 2d), the lack of interactions between single
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11 346 treatment effects for leaf level C/N ratios indicates that the effect is found also in treatment
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13 347 combinations. Consequently, increased C/N ratios of leaves as well as decreased
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15 348 *Deschampsia* litter N production are found in the full combination of treatments, *i.e.* in the
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17 349 simulation of the projected future climate scenario. In contrast, the C/N ratio of mosses
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19 350 increases in two of the three single treatments and N concentrations in *Deschampsia* litter
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21 351 either increase or decrease depending on treatment but these effects disappear when all
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23 352 treatments are combined.
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29 354 *Responses of below-ground processes to climate manipulations*

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32 355 The below-ground N turnover was significantly affected by the climate manipulations,
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34 356 primarily by the warming and drought treatments (Table 1, Fig. 3) showing changes
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36 357 cascading through pools and processes. Elevated CO₂ as a main factor had no effects below
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38 358 ground but the analysis of DLSP when evaluating the interactions revealed reductions in
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40 359 leachate concentrations in 5 cm soil depth of NO₃⁻-N, NH₄⁺-N and DON (Table 1, Fig. 3a) in
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42 360 the single CO₂ treatment. These effects were reduced when elevated CO₂ was combined with
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44 361 other treatments causing the significant interactions for these three variables (Table 1).
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52 362 The drought treatment reduced the N pools of enchytraeids and protozoans and their
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54 363 NH₄⁺-N mineralization (Table 1, P = 0.03 and 0.04, respectively), decreased gross
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56 364 mineralization (Table 1, P = 0.03), and reduced the pool of NH₄⁺-N (Table 1, P = 0.02), thus
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58 365 indicating an overall decrease in the N turnover in response to drought (Fig. 3b). In contrast,
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60 366 warming stimulated microbial ammonium consumption in the top 5 cm of soil (Table 1, P =

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3 367 0.04) as well as the gross mineralization rate (single treatment only, Table 1, $P \leq 0.05$) and
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5 368 nitrification rates (Table 1, $P = 0.004$), leading to both increased denitrification (Table 1, $P =$
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8 369 0.02) and N_2O emission (Table 1, $P = 0.05$). Overall, the below-ground response to the
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10 370 warming treatments was therefore an increase in N turnover (Fig. 3c).
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13 371 While no direct main effects of elevated CO_2 were observed, it influenced the
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15 372 responses to drought and warming primarily by reducing some of the significant individual
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17 373 effects in the three-factor combination. Still, the drought-induced decrease of fauna-N and
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19 374 fauna N mineralization as well as the warming-induced increased denitrification did not
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21 375 interact significantly with the other treatments and consequently remained in the full
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23 376 treatment combination.
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30 31 32 378 *Interactions between climate manipulations*

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35 379 Across the 47 variables reported (Table 1) the drought treatment lead to the highest number of
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37 380 significant main effects (11) followed by warming and CO_2 treatments (6 and 4, respectively).
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39 381 Simultaneously there were a large number of interactions between the three treatments (14).
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41 382 Interestingly, the analysis of the interactions revealed a dominance of antagonistic effects
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43 383 (Fig. 4) with more than twice as many antagonistic effects as synergistic effects. Furthermore,
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45 384 simple additive effects were less frequent than both antagonistic and synergistic effects.
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386 **Discussion**387 *Overall ecosystem N turnover*

388 The N cycle at the experimental site is characterized by higher internal N turnover than bulk
389 N deposition and losses by leaching and N₂O production as generally seen in natural and
390 semi-natural ecosystems. In comparison to our estimated annual gross mineralization rate (6.7
391 ± 0.96 g N m⁻²y⁻¹), Emmett *et al.* (2004) reported net mineralization rates of 3.8 to 6.6 g N m⁻²
392 y⁻¹ from another Danish dry heathland and Aerts (1993) reported rates ranging from 4.4 to
393 12.6 g N m⁻²y⁻¹ under different plant species in wet and dry Dutch heathlands, where
394 atmospheric deposition is 2-3 times higher than in Denmark. Inter-annual variability in N
395 leaching was significant, while treatment differences after two years of treatments were not
396 significant. The observed difference in leaching between 2006 and 2007 shows that the
397 ecosystem N loss by leaching is highly dependent on the amount and timing of precipitation.
398 In a future climate with more precipitation distributed in fewer, but more severe rain events, N
399 leaching is likely to increase in this ecosystem. The inter-annual variability in leaching may
400 also indicate that the ecosystem is close to N saturation as also suggested from an adjacent
401 short-term experiment where N and phosphorus (P) addition resulted in only a modest
402 increase in fine root biomass of *Deschampsia*, and no effects on *Calluna* roots (Nielsen *et al.*
403 2009). Furthermore, *Deschampsia* responded more when both N and P were added indicating
404 that this species is currently more limited by P than N availability. If nitrogen availability
405 should decrease due to progressive nitrogen limitation as the treatment exposure continues,
406 this, in combination with the P limitation for *Deschampsia*, would be beneficial to the slower
407 growing *Calluna*. However, other climate factors, *i.e.* water availability and temperature

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3 408 increase, are likely to influence the overall competitive relationship, potentially disturbing the
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5 409 balance of the equilibrium, especially if the rates of mortality of *Calluna* are increased.
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9 410 The mineralization of $\text{NH}_4^+\text{-N}$ by soil fauna is often neglected but contributes
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11 411 significantly to the pool of plant available N in the soil (Osler & Sommerkorn 2007). In the
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13 412 present study, we calculated fauna N mineralization from estimated biomasses and literature
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15 413 values of the turnover times and yields of microfauna and mesofauna. Obviously, upscaling of
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17 414 *e.g.* enumeration of bacterivorous protozoa and nematodes to gross nitrogen mineralization
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19 415 should be evaluated with caution. Values for bacterivore biomass, turnover, and growth yield
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21 416 on bacteria are all needed to perform the upscaling and very few references exist in the
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23 417 literature on such values. This said, the value for fauna-related N mineralization in the
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25 418 ambient treatment based on this calculation seems trustworthy based on the gross N
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27 419 mineralization from the pool dilution assay of $6.7 \text{ g N m}^{-2}\text{y}^{-1}$. The assumption that most
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29 420 bacterial mineralization is facilitated through predation and mineralization by the soil fauna
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31 421 implies that N mineralization by bacteria is responsible for approximately 75% of the total
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33 422 activity, the rest being due to fungi.
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43 424 *Responses to elevated CO₂, warming and drought*

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46 425 Elevated atmospheric CO₂ concentration is known to induce down-regulation of
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48 426 photosynthetic capacity, *e.g.* via reduced content of Rubisco in the leaves (Ainsworth & Long
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50 427 2005; Ainsworth & Rogers 2007) and reduction of leaf nitrogen content and increased C/N
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52 428 ratios of leaves (Paterson *et al.* 1999; Lutze & Gifford 2000; van Heerwaarden *et al.* 2005;
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54 429 Finzi *et al.* 2006; Chen *et al.* 2007; Hovenden *et al.* 2008). At our experimental site, we
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56 430 observed reduced photosynthetic capacity (Albert *et al.* 2010) and leaf nitrogen under
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58 431 elevated CO₂ for the dominant species, *Calluna* and *Deschampsia*, as well as increased leaf
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3 432 C/N ratios. However, the increased C/N ratios observed in leaves, and for *Calluna* also
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5 433 observed in flower biomass were after two years of treatments not large enough to affect
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8 434 whole plant biomass C/N ratios, which were not affected by the treatments. Previous studies
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10 435 have found increasing C/N ratios in other plant tissues than leaves (McGuire *et al.* 1995;
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12 436 Cotrufo *et al.* 1998), but the change often is much weaker than that observed for leaves
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15 437 (McGuire *et al.* 1995), indicating that the C/N effect of elevated CO₂ is closely linked to the
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17 438 processes involved in the carbon sequestration in the photosynthetic apparatus (Körner 2000).
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19 439 As hypothesized, however, N concentration in *Deschampsia* litter after two years of
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21 440 treatments was also reduced by elevated CO₂, possibly showing that the effect is beginning to
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23 441 cascade from leaf level to other ecosystem N pools, although the effect disappears in the full
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25 442 treatment combination. Together with the observed increased concentration of tannins
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27 443 (Schmidt *et al.* 2007) in plant tissues, we anticipate that the changes in C/N ratios will affect
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29 444 litter decomposition rates as the experimental treatments continue.
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34 445 In contrast to our expectations, the above- and below-ground plant N pools were not
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36 446 increased by elevated CO₂. However, total root biomass N of *Calluna* increased unexpectedly
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38 447 in response to drought. It is inherently difficult to estimate total roots from small soil samples
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40 448 due to the large spatial heterogeneity in root distribution and, at the same time, collection of
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42 449 larger samples is problematic due to the destruction of the experimental plots. We are
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44 450 therefore reluctant to put too much emphasis on this result and conclude overall that total
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46 451 biomass N of both dominant species as well as mosses after two years of treatments were only
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48 452 marginally affected by the applied climate drivers.
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54 453 Increased rhizodeposition is a common plant response to elevated CO₂ (Pendall *et al.*
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56 454 2004; Allard *et al.* 2006), and strong effects on below-ground C cycling have been observed
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58 455 in response to elevated CO₂ in the current experiment, *e.g.* increased soil respiration rates
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60 456 (Selsted *et al.* 2010) and increased production of dissolved organic carbon (Andresen *et al.*

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3 457 2010a). However, in contrast to our expectation, we observed very few direct effects of
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5 458 elevated CO₂ on below-ground N processes. Still, the decreased concentrations of organic and
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7 459 inorganic N leachate water at 5 cm soil depth imply that mineralization processes are affected
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10 460 or possibly that root N uptake has increased. In contrast to the lack of direct changes in
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12 461 response to elevated CO₂, soil processes were more sensitive to the drought and warming
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14 462 treatments. Our study supports previous observations of increased below-ground N turnover
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16 463 in response to warming (Rustad *et al.* 2001; Schmidt *et al.* 2004). We observed increased
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18 464 microbial NH₄⁺-N consumption, gross mineralization, potential nitrification, and
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20 465 denitrification from lab incubations of soils, as well as increased *in situ* N₂O emissions. Soil
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22 466 and litter incubations *in situ* also showed that warming promoted N cycling (Andresen *et al.*
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24 467 2010b). Previous field studies with artificial warming also showed no warming effect on N₂O
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26 468 emission rates (Peterjohn *et al.* 1994; Mchale *et al.* 1998). However, we interpret our
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28 469 observation of five variables that all responded in the same direction to warming as a strong
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30 470 indicator that the warming effect on N turnover was real.
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37 471 The below-ground N turnover was reduced by drought as observed by reduced gross
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39 472 N mineralization and fauna N mineralization. Reduced N mineralization in response to
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41 473 drought has previously been observed (Emmett *et al.* 2004; Schmidt *et al.* 2004; Sowerby *et*
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43 474 *al.* 2008) but the negative effects of drought on N mineralization are often attributed directly
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45 475 to reduced microbial activity. The importance of soil fauna to the N mineralization shown in
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47 476 this study, together with the negative effects of drought on top soil layer populations of
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49 477 enchytraeids (Maraldo *et al.* 2010) and other fauna groups and their decreased N
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51 478 mineralization rates, indicate that the fauna response plays a significant role in the observed
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53 479 decrease in N turnover in drought-exposed plots. Furthermore, while the warming effects
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55 480 were generally reduced in combination with drought and elevated CO₂, the negative effect of
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57 481 drought on fauna N biomass and N mineralization was not reduced by interactions.
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3 482 Consequently, the effect prevailed in the full combination of treatments, indicating that N
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5 483 mineralization will be reduced in the full future climate scenario. Therefore, despite the fact
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8 484 that this ecosystem currently show no clear sign of N limitation of plant growth, we cannot
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10 485 rule out the possibility that progressive nitrogen limitation will play a role in the longer term,
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12 486 as the impacts of increased CO₂ on C/N ratios of leaves combined with the drought induced
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15 487 reduction of mineralization and litter N production continues to reduce the availability of N in
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17 488 the ecosystem.
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22 23 24 490 *Importance of climate driver interactions*

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27 491 We thoroughly investigated the background for all significant interactions observed in the 47
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29 492 variables reported in Table 1 by evaluating the DLSP from the mixed model statistical
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31 493 output. This analysis revealed a clear dominance of antagonistic effects over synergistic and
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33 494 simple additive effects when several treatments were combined (Table 1 and Fig. 4), *i.e.* the
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35 495 ecosystem is more robust when more than one factor is changed. For instance, the C/N ratio
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37 496 of mosses increased in both single treatments of drought and elevated CO₂ but was unaffected
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39 497 when treatments were combined. Similarly, the increased gross mineralization rate observed
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41 498 in the single warming treatment was reduced especially in combination with elevated CO₂.
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43 499 Furthermore, two of the three additive effects observed (*Deschampsia* litter N concentration
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45 500 and N₂O emissions) were additive responses with opposing directions, which therefore acts to
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47 501 reduce the observed effects in treatment combinations.
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54 502 Previous studies have investigated interactions between elevated CO₂ and *e.g.* N
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56 503 supply (Lutze & Gifford 2000; Lutze *et al.* 2000; van Heerwaarden *et al.* 2005; Reich *et al.*
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58 504 2006), warming (Peltola *et al.* 2002; Tingey *et al.* 2003; Hovenden *et al.* 2008; Dijkstra *et al.*
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60 505 2010), biodiversity (Reich *et al.* 2001; Niklaus *et al.* 2001) and water relations (Morgan *et al.*

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3 506 2004; Nowak *et al.* 2004). A review by Barnard *et al.* (2005) of 25 multi-factor studies
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5 507 concluded that interactions were generally rare. These 25 studies were dominated by
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7 508 experimentally elevated CO₂ combined with increased N supply. The lack of interactions
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9 509 could be due to the common trend that most ecosystems respond strongly to increased N
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11 510 supply, which hides weaker interactive responses. Luo *et al.* (2008) analyzed and modeled
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13 511 interactions between elevated CO₂, warming and changes in precipitation in 7 multi-factor
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15 512 studies and found that three-way interactions were rare while two-way interactions were more
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17 513 common. Generally, interactions were positive between elevated CO₂ and warming and
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19 514 negative between elevated CO₂ and reduced precipitation with respect to net primary
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21 515 production (NPP).
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28 516 A general pattern of most published studies is the focus on a few important variables,
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30 517 *i.e.* overall changes in NPP or biomasses or specific studies focusing on processes, *e.g.*
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32 518 nitrification and denitrification. As interactions are only sometimes significant, investigating a
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34 519 small number of variables prevents a more general interpretation of the importance of
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36 520 interactions. Furthermore, assuming that three-way interactions are more uncommon than
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38 521 two-way interactions, important three-way interactions could be missed when fewer variables
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40 522 are analyzed. Out of 14 interactions observed across 47 N related variables in the present
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42 523 study, 5 were three-way interactions. This suggests that insufficient interactions between
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44 524 elevated CO₂, warming and changes in precipitation were included in the ecosystem models
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46 525 applied by Luo *et al.* (2008) and reinforces the need for further multi-factor ecosystem
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48 526 manipulation experiments focusing on interactive effects.
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54 527 When analyzing the interactions, we found a predominance of antagonism in the
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56 528 combined responses. The consequence of the dominance of antagonistic interactions in
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58 529 combined climate driver responses is that ecosystem changes caused by climate change may
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60 530 be less pronounced than judged from single factor experiments. Based on these results we

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3 531 raise the hypothesis that combined effects of all future climate change are dampened
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6 532 compared to expected effects based on simple addition of single treatment responses. If this
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8 533 pattern prevails for ecosystem responses to future climate change in general it highlights 1)
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10 534 that single factor studies are likely to overestimate responses and should be evaluated with
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12 535 caution, and 2) that multi-factor field-scale ecosystem experiments are critical in order to be
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15 536 able to predict realistic response strengths as well as for developing a conceptual framework
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17 537 for understanding interactions among climate drivers. Our multi-variable approach may aid
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20 538 the latter point if tested in other multi-factor studies and by including variables not only
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22 539 constraint to the nitrogen cycle.
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56 541 **Conclusions**
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10 542 The drought treatment effects dominated over effects of warming and elevated CO₂ in
11
12 543 observed responses of the nitrogen cycle after two years of treatments. In contrast to our
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14 544 expectations, we did not observe increased growth of the N pools of the dominant plant
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16 545 species, *Calluna* and *Deschampsia* in response to elevated CO₂. While previous studies have
17
18 546 usually found interactions to be rare, interactions were commonly observed and antagonism
19
20 547 dominated over synergism in combined responses, *i.e.* the ecosystem response to the
21
22 548 combination of several climate drivers is dampened compared to single treatment responses.
23
24 549 Still, the observed negative response to drought of soil fauna biomass and N mineralization
25
26 550 and *Deschampsia* litter N production prevailed in the full treatment combination and so did
27
28 551 the increased leaf C/N ratios of the dominant species *Calluna* and *Deschampsia*. If persistent,
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30 552 these changes are likely to lead to reduced N availability and possibly to progressive nitrogen
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32 553 limitation as the experimental treatments continue.
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16
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24 562 Commission.
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52
53
54
55
56
57
58
59
60

1
2
3 563
4
5
67 **Reference List**

8
9
10 565 Aerts R (1993) Biomass and nutrient dynamics of dominant plant species from heathlands. In:
11 566 *Heathlands: Patterns and Processes in a Changing Environment* (eds Aerts R, Heil GW), pp.
12 567 51-84. Kluwer Academic Publishers, Dordrecht, The Netherlands.

13
14
15
16
17 568 Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO₂
18 569 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New*
19 570 *Phytologist*, **165**, 351-371.

20
21
22
23
24
25 571 Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to
26 572 rising [CO₂]: mechanisms and environmental interactions. *Plant Cell and Environment*, **30**,
27 573 258-270.

28
29
30
31
32
33 574 Albert KR, Ro-Poulsen H, Mikkelsen TN, Michelsen A, Beier C (2010) Water status interacts
34 575 strongly with the effect of elevated CO₂, warming and drought episodes on plant carbon
35 576 uptake in a temperate heath ecosystem. Submitted July 2010 to *Plant Cell Environment*.

36
37
38
39
40
41 577 Allard V, Robin C, Newton PCD, Lieffering M, Soussana JF (2006) Short and long-term
42 578 effects of elevated CO₂ on *Lolium perenne* rhizodeposition and its consequences on soil
43 579 organic matter turnover and plant N yield. *Soil Biology & Biochemistry*, **38**, 1178-1187.

44
45
46
47
48
49 580 Andresen, L. C., Michelsen, A., Ambus, P., and Beier, C. (2010a) Belowground heathland
50 581 responses after 2 years of combined warming, elevated CO₂ and summer drought.
51 582 *Biogeochemistry*, in press.

52
53
54
55
56
57
58
59
60

- 1
2
3 583 Andresen LC, Michelsen A, Jonasson S, Schmidt IK, Mikkelsen TN, Ambus P, Beier C
4
5 584 (2010b) Plant nutrient mobilization in temperate heathland responds to elevated CO₂,
6
7 585 temperature and drought. *Plant and Soil*, **328**, 381-396.
8
9
10
11 586 Barnard R, Leadley PW, Hungate BA (2005) Global change, nitrification, and denitrification:
12
13 587 A review. *Global Biogeochemical Cycles*, **19**, 1-13, DOI: 10.1029/2004GB002282.
14
15
16
17 588 Belser LW, Mays EL (1980) Specific-Inhibition of Nitrite Oxidation by Chlorate and Its Use
18
19 589 in Assessing Nitrification in Soils and Sediments. *Applied and Environmental Microbiology*,
20
21 590 **39**, 505-510.
22
23
24
25 591 Carter MS, Ambus P, Albert K, et al (2010) Effects of elevated atmospheric CO₂, prolonged
26
27 592 summer drought and temperature increase on N₂O and CH₄ fluxes in a temperate heathland.
28
29 593 Submitted July 2010 to *Biogeochemistry*.
30
31
32
33 594 Chen X, Tu C, Burton MG, Watson DM, Burkey KO, Hu SJ (2007) Plant nitrogen acquisition
34
35 595 and interactions under elevated carbon dioxide: impact of endophytes and mycorrhizae.
36
37 596 *Global Change Biology*, **13**, 1238-1249.
38
39
40
41 597 Coleman DC, Anderson RV, Cole CV, Elliott ET, Woods L, Campion MK (1978) Trophic
42
43 598 Interactions in Soils As They Affect Energy and Nutrient Dynamics .4. Flows of Metabolic
44
45 599 and Biomass Carbon. *Microbial Ecology*, **4**, 373-380.
46
47
48
49 600 Cotrufo MF, Ineson P, Scott A (1998) Elevated CO₂ reduces the nitrogen concentration of
50
51 601 plant tissues. *Global Change Biology*, **4**, 43-54.
52
53
54 602 Danish Meteorological Institute (2009) www.dmi.dk.
55
56
57
58
59
60

- 1
2
3 603 Darbyshire JF, Wheatley RE, Greaves MP, Inkson RHE (1974) Rapid micromethod for
4
5 604 estimating bacterial and protozoan populations in soil. *Revue D Ecologie et de Biologie du*
6
7 605 *Sol*, **11**, 465-475.
8
9
10
11 606 Davidson EA, Stark JM, Firestone MK (1990) Microbial production and consumption of
12
13 607 nitrate in an annual grassland. *Ecology*, **71**, 1968-1975.
14
15
16
17 608 de Graaff MA, van Groenigen KJ, Six J, Hungate B, van Kessel C (2006) Interactions
18
19 609 between plant growth and soil nutrient cycling under elevated CO₂: a meta-analysis. *Global*
20
21 610 *Change Biology*, **12**, 2077-2091.
22
23
24
25 611 De Ruiter PC, Moore JC, Zwart KB, *et al.* (1993) Simulation of nitrogen mineralization in the
26
27 612 belowground food webs of 2 winter-wheat fields. *Journal of Applied Ecology*, **30**, 95-106.
28
29
30 613 Dijkstra FA, Blumenthal D, Morgan JA, Pendall E, Carrillo Y, Follett RF (2010) Contrasting
31
32 614 effects of elevated CO₂ and warming on nitrogen cycling in a semiarid grassland. *New*
33
34 615 *Phytologist*, **187**, 426-437.
35
36
37
38 616 Emmett BA, Beier C, Estiarte M, *et al.* (2004) The response of soil processes to climate
39
40 617 change: Results from manipulation studies of shrublands across an environmental gradient.
41
42 618 *Ecosystems*, **7**, 625-637.
43
44
45
46 619 Finzi AC, Moore DJP, DeLucia EH, *et al.* (2006) Progressive nitrogen limitation of
47
48 620 ecosystem processes under elevated CO₂ in a warm-temperate forest. *Ecology*, **87**, 15-25.
49
50
51
52 621 Georgieva S, Christensen S, Petersen H, Gjelstrup P, Thorup-Kristensen K (2005) Early
53
54 622 decomposer assemblages of soil organisms in litterbags with vetch and rye roots. *Soil Biology*
55
56 623 *& Biochemistry*, **37**, 1145-1155.
57
58
59
60

- 1
2
3 624 Holmstrup M, Maraldo K, Krogh PH (2007) Combined effect of copper and prolonged
4
5 625 summer drought on soil Microarthropods in the field. *Environmental Pollution*, **146**, 525-533.
6
7
8
9 626 Hovenden MJ, Newton PCD, Carran RA, *et al.* (2008) Warming prevents the elevated CO₂-
10
11 627 induced reduction in available soil nitrogen in a temperate, perennial grassland. *Global*
12
13 628 *Change Biology*, **14**, 1018-1024.
14
15
16
17 629 Hungate BA (1999) Ecosystem responses to rising atmospheric CO₂: Feed backs through the
18
19 630 nitrogen cycle. In: *Carbon dioxide and environmental stress* (eds Luo YQ, Mooney HA), pp.
20
21 631 265-285. Academic Press, San Diego, USA.
22
23
24
25 632 IPCC (2007) Climate Change 2007: The Physical Science Basis. Contribution of Working
26
27 633 Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.
28
29 634 (eds Solomon S, Qin D, Manning M *et al.*), pp. 1-996. Cambridge University Press,
30
31 635 Cambridge, UK and New York, NY, USA.
32
33
34
35 636 Jamieson N, Monaghan R, Barraclough D (1999) Seasonal trends of gross N mineralization in
36
37 637 a natural calcareous grassland. *Global Change Biology*, **5**, 423-431.
38
39
40
41 638 Jansson PE, Moon DS (2001) A coupled model of water, heat and mass transfer using object
42
43 639 orientation to improve flexibility and functionality. *Environmental Modelling & Software*, **16**,
44
45 640 37-46.
46
47
48
49 641 Jonasson S (1988) Evaluation of the Point Intercept Method for the Estimation of Plant
50
51 642 Biomass. *Oikos*, **52**, 101-106.
52
53
54 643 Jonasson S, Michelsen A, Schmidt IK, Nielsen EV, Callaghan TV (1996) Microbial biomass
55
56 644 C, N and P in two arctic soils and responses to addition of NPK fertilizer and sugar:
57
58 645 implications for plant nutrient uptake. *Oecologia*, **106**, 507-515.
59
60

- 1
2
3 646 Kirkham D, Bartholomew WV (1954) Equations for following nutrient transformations in
4
5
6 647 soil, utilizing tracer data. *Soil Science Society of America Proceedings*, **18**, 33-34.
7
8
9 648 Kongstad J, Schmidt IK, Riis-Nielsen T, Beier C, Arndal MF, Mikkelsen TN (2010) Species
10
11 649 specific responses in plant growth to changes in temperature, drought and CO₂ in
12
13 650 combination: results from the CLIMAITE experiment. Submitted May 2010 to Global
14
15
16 651 Change Biology.
17
18
19 652 Körner C (2000) Biosphere responses to CO₂ enrichment. *Ecological Applications*, **10**, 1590-
20
21 653 1619.
22
23
24 654 Leuzinger S, Körner C (2007) Water savings in mature deciduous forest trees under elevated
25
26
27 655 CO₂. *Global Change Biology*, **13**, 2498-2508.
28
29
30 656 Lipson D, Näsholm T (2001) The unexpected versatility of plants: organic nitrogen use and
31
32 657 availability in terrestrial ecosystems. *Oecologia*, **128**, 305-316.
33
34
35
36 658 Lukewille A, Wright RF (1997) Experimentally increased soil temperature causes release of
37
38 659 nitrogen at a boreal forest catchment in southern Norway. *Global Change Biology*, **3**, 13-21.
39
40
41 660 Luo Y, Su B, Currie WS, et al. (2004) Progressive nitrogen limitation of ecosystem responses
42
43 661 to rising atmospheric carbon dioxide. *BioScience*, **54**, 731-739.
44
45
46
47 662 Luo YQ, Gerten D, Le Maire G, et al. (2008) Modeled interactive effects of precipitation,
48
49 663 temperature, and [CO₂] on ecosystem carbon and water dynamics in different climatic zones.
50
51 664 *Global Change Biology*, **14**, 1986-1999.
52
53
54
55 665 Luo YQ, Hui DF, Zhang DQ (2006) Elevated CO₂ stimulates net accumulations of carbon and
56
57 666 nitrogen in land ecosystems: A meta-analysis. *Ecology*, **87**, 53-63.
58
59
60

- 1
2
3 667 Lutze JL, Gifford RM (2000) Nitrogen accumulation and distribution in *Danthonia*
4
5 668 *richardsonii* swards in response to CO₂ and nitrogen supply over four years of growth. *Global*
6
7
8 669 *Change Biology*, **6**, 1-12.
9
10
11 670 Lutze JL, Gifford RM, Adams HN (2000) Litter quality and decomposition in *Danthonia*
12
13 671 *richardsonii* swards in response to CO₂ and nitrogen supply over four years of growth. *Global*
14
15 672 *Change Biology*, **6**, 13-24.
16
17
18
19 673 Maraldo K, Schmidt IK, Beier C, Holmstrup M (2008) Can field populations of the
20
21 674 enchytraeid, *Cognettia sphagnetorum*, adapt to increased drought stress? *Soil Biology &*
22
23 675 *Biochemistry*, **40**, 1765-1771.
24
25
26
27 676 Maraldo K, van der Linden L, Christensen B, Mikkelsen TN, Beier C, Krogh PH, Holmstrup
28
29 677 M (2010) The counteracting effects of elevated atmospheric CO₂ concentrations and drought
30
31 678 episodes: studies of enchytraeid communities in dry heathland. *Soil Biology and*
32
33 679 *Biochemistry*, in press.
34
35
36
37 680 Marion GM, Hastings SJ, Oberbauer SF, Oechel WC (1989) Soil-plant element relationships
38
39 681 in a tundra ecosystem. *Holarctic Ecology*, **12**, 296-303.
40
41
42
43 682 McGuire AD, Melillo JM, Joyce LA (1995) The role of nitrogen in the response of forest net
44
45 683 primary production to elevated atmospheric carbon-dioxide. *Annual Review of Ecology and*
46
47 684 *Systematics*, **26**, 473-503.
48
49
50
51 685 Mchale PJ, Mitchell MJ, Bowles FP (1998) Soil warming in a northern hardwood forest: trace
52
53 686 gas fluxes and leaf litter decomposition. *Canadian Journal of Forest Research-Revue*
54
55 687 *Canadienne de Recherche Forestiere*, **28**, 1365-1372.
56
57
58
59
60

- 1
2
3 688 Mikkelsen TN, Beier C, Jonasson S, et al. (2008) Experimental design of multifactor climate
4
5 689 change experiments with elevated CO₂, warming and drought: the CLIMAITE project.
6
7
8 690 *Functional Ecology*, **22**, 185-195.
9
10
11 691 Morgan JA, Pataki DE, Körner C, et al. (2004) Water relations in grassland and desert
12
13 692 ecosystems exposed to elevated atmospheric CO₂. *Oecologia*, **140**, 11-25.
14
15
16
17 693 Nielsen PL, Andresen LC, Michelsen A, Schmidt IK, Kongstad J (2009) Seasonal variations
18
19 694 and effects of nutrient applications on N and P and microbial biomass under two temperate
20
21 695 heathland plants. *Applied Soil Ecology*, **42**, 279-287.
22
23
24
25 696 Niklaus PA, Leadley PW, Schmid B, Körner C (2001) A long-term field study on biodiversity
26
27 697 x elevated CO₂ interactions in grassland. *Ecological Monographs*, **71**, 341-356.
28
29
30 698 Nowak RS, Ellsworth DS, Smith SD (2004) Functional responses of plants to elevated
31
32 699 atmospheric CO₂ – do photosynthetic and productivity data from FACE experiments support
33
34 700 early predictions? *New Phytologist*, **162**, 253-280.
35
36
37
38 701 Osler GHR, Sommerkorn M (2007) Toward a complete soil C and N cycle: Incorporating the
39
40 702 soil fauna. *Ecology*, **88**, 1611-1621.
41
42
43
44 703 Page FC (1988) A New Key to Freshwater and Soil Gymnamoebae. Freshwater Biological
45
46 704 Association, Cumbria, England.
47
48
49
50 705 Paterson E, Hodge A, Thornton B, Millard P, Killham K (1999) Carbon partitioning and
51
52 706 rhizosphere C-flow in *Lolium perenne* as affected by CO₂ concentration, irradiance and
53
54 707 below-ground conditions. *Global Change Biology*, **5**, 669-678.
55
56
57
58
59
60

- 1
2
3 708 Peltola H, Kilpelainen A, Kellomaki S (2002) Diameter growth of Scots pine (*Pinus*
4
5 709 *sylvestris*) trees grown at elevated temperature and carbon dioxide concentration under boreal
6
7
8 710 conditions. *Tree Physiology*, **22**, 963-972.
9
10
11 711 Pendall E, Mosier AR, Morgan JA (2004) Rhizodeposition stimulated by elevated CO₂ in a
12
13 712 semiarid grassland. *New Phytologist*, **162**, 447-458.
14
15
16
17 713 Penuelas J, Prieto P, Beier C, *et al.* (2007) Response of plant species richness and primary
18
19 714 productivity in shrublands along a north-south gradient in Europe to seven years of
20
21 715 experimental warming and drought: reductions in primary productivity in the heat and
22
23 716 drought year of 2003. *Global Change Biology*, **13**, 2563-2581.
24
25
26
27 717 Persson T (1983) Influence of soil animals on nitrogen mineralisation in a northern Scots pine
28
29 718 forest. In: *New Trends in Soil Biology* (eds Lebrun P, André H, De Medts A, Grégoire-Wibo
30
31 719 C, Wauthy G), pp. 117-126. Dieu Bricart, Louvain-la-Neuve, Belgium.
32
33
34
35 720 Peterjohn WT, Melillo JM, Steudler PA, Newkirk KM, Bowles FP, Aber JD (1994)
36
37 721 Responses of trace gas fluxes and N availability to experimentally elevated soil temperatures.
38
39 722 *Ecological Applications*, **4**, 617-625.
40
41
42
43 723 Petersen H, Luxton M (1982) A comparative-analysis of soil fauna populations and their role
44
45 724 in decomposition processes. *Oikos*, **39**, 287-388.
46
47
48
49 725 Rastetter EB, Agren GI, Shaver GR (1997) Responses of N-limited ecosystems to increased
50
51 726 CO₂: A balanced-nutrition, coupled-element-cycles model. *Ecological Applications*, **7**, 444-
52
53 727 460.
54
55
56 728 Reich PB, Hobbie SE, Lee T, *et al.* (2006) Nitrogen limitation constrains sustainability of
57
58 729 ecosystem response to CO₂. *Nature*, **440**, 922-925.
59
60

- 1
2
3 730 Reich PB, Knops J, Tilman D, et al. (2001) Plant diversity enhances ecosystem responses to
4
5 731 elevated CO₂ and nitrogen deposition. *Nature*, **410**, 809-812.
6
7
8
9 732 Riis-Nielsen T, Schmidt IK (2010) Non-destructive plant biomass and cover estimates from
10
11 733 point intercept analysis – a case study of *Calluna vulgaris* and *Deschampsia flexuosa*.
12
13 734 Submitted May 2010 to *Functional Ecology*.
14
15
16
17 735 Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for mpn-
18
19 736 enumeration of naked amoebae and heterotrophic flagellates in soil. *Pedobiologia*, **39**, 10-19.
20
21
22 737 Rustad LE, Campbell JL, Marion GM, et al. (2001) A meta-analysis of the response of soil
23
24 738 respiration, net nitrogen mineralization, and aboveground plant growth to experimental
25
26 739 ecosystem warming. *Oecologia*, **126**, 543-562.
27
28
29
30 740 SAS Institute (2003) SAS/STAT User's Guide. Statistical Analysis System Institute, Cary,
31
32 741 NC, USA.
33
34
35
36 742 Schimel JP, Bennett J (2004) Nitrogen mineralization: Challenges of a changing paradigm.
37
38 743 *Ecology*, **85**, 591-602.
39
40
41 744 Schmidt IK, Beier C, Kongstad J, et al. (2007) Klimaændringer og processer og funktion i
42
43 745 terrestriske økosystemer. *Flora og Fauna*, **113**, 121-132.
44
45
46
47 746 Schmidt IK, Tietema A, Williams D, Gundersen P, Beier C, Emmett BA, Estiarte M (2004)
48
49 747 Soil solution chemistry and element fluxes in three European heathlands and their responses
50
51 748 to warming and drought. *Ecosystems*, **7**, 638-649.
52
53
54
55 749 Selsted MB, Ibrom A, Ambus P, et al. (2010) Soil respiration in a temperate heathland
56
57 750 responds strongly to elevated temperature, extended summer drought and elevated CO₂.
58
59 751 Submitted July 2010 to *Global Change Biology*.
60

- 1
2
3 752 Sohlenius B, Sandor A (1987) Vertical distribution of nematodes in arable soil under grass
4
5 753 (*Festuca pratensis*) and Barley (*Hordeum vulgare*). *Biology and Fertility of Soils*, **3**, 19-25.
6
7
8
9 754 Sørensen P, Jensen ES (1991) Sequential diffusion of ammonium and nitrate from soil
10
11 755 extracts to a polytetrafluoroethylene trap for N-15 determination. *Analytica Chimica Acta*,
12
13 756 **252**, 201-203.
14
15
16
17 757 Sowerby A, Emmett BA, Tietema A, Beier C (2008) Contrasting effects of repeated summer
18
19 758 drought on soil carbon efflux in hydric and mesic heathland soils. *Global Change Biology*, **14**,
20
21 759 2388-2404.
22
23
24
25 760 Standen V (1973) Production and respiration of an enchytraeid population in blanket bog.
26
27 761 *Journal of Animal Ecology*, **42**, 219-245.
28
29
30 762 Stout JD, Heal OW (1967) Protozoa. In: *Soil Biology* (eds Burges A, Raw F), pp. 149-195.
31
32 763 Academic Press, New York, USA.
33
34
35
36 764 Svensson M, Jansson PE, Gustafsson D, Kleja DB, Langvall O, Lindroth A (2008) Bayesian
37
38 765 calibration of a model describing carbon, water and heat fluxes for a Swedish boreal forest
39
40 766 stand. *Ecological Modelling*, **213**, 331-344.
41
42
43
44 767 Tingey DT, McKane RB, Olszyk DM, Johnson MG, Rygiewicz PT, Lee EH (2003) Elevated
45
46 768 CO₂ and temperature alter nitrogen allocation in Douglas-fir. *Global Change Biology*, **9**,
47
48 769 1038-1050.
49
50
51
52 770 van der Linden L, Beier C, Mikkelsen TN, et al (2010) The effects of warming, drought and
53
54 771 elevated CO₂ on the Carbon balance of a Danish heathland: the Climaite project carbon
55
56 772 synthesis. Expected submitted September 2010 to *Global Change Biology*.
57
58
59
60

- 1
2
3 773 van Heerwaarden LM, Toet S, van Logtestijn RSP, Aerts R (2005) Internal nitrogen dynamics
4
5 774 in the graminoid *Molinia caerulea* under higher N supply and elevated CO₂ concentrations.
6
7 775 *Plant and Soil*, **277**, 255-264.
8
9
10
11 776 Vervaet H, Boeckx P, Boko AMC, Van Cleemput O, Hofman G (2004) The role of gross and
12
13 777 net N transformation processes and NH₄⁺ and NO₃⁻ immobilization in controlling the mineral
14
15 778 N pool of a temperate mixed deciduous forest soil. *Plant and Soil*, **264**, 349-357.
16
17
18
19 779 Wolsing M, Prieme A (2004) Observation of high seasonal variation in community structure
20
21 780 of denitrifying bacteria in arable soil receiving artificial fertilizer and cattle manure by
22
23 781 determining T-RFLP of nir gene fragments. *Fems Microbiology Ecology*, **48**, 261-271.
24
25
26
27 782 Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL (1993) Elevated
28
29 783 atmospheric CO₂ and feedback between carbon and nitrogen cycles. *Plant and Soil*, **151**, 105-
30
31 784 117.
32
33
34
35 785 Zwart KB, Kuikman PJ, van Veen JA (1994) Rhizosphere Protozoa: their significance in
36
37 786 nutrient dynamics. In: *Soil Protozoa* (ed Darbyshire JF), CAB International, Wallingford, UK.
38
39
40
41
42
43
44
45
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788 **Figure captions**

789 Figure 1

790 Measured N pools in August 2007 (**bold**, g N m⁻²) and annual fluxes (*italic*, g N m⁻²y⁻¹) in
791 ambient plots down to 10 cm soil depth (leaching below 60 cm depth) in 2007. Abbreviations:
792 *Calluna vulgaris* (Cal), *Deschampsia flexuosa* (Des), dissolved organic nitrogen (DON),
793 microbial biomass nitrogen (Mic-N), soil organic matter nitrogen (SOM-N), Gross
794 mineralization (Gross min). Data of litter N mass and litter N production was only available
795 for *Deschampsia*. Estimates of fauna biomass and mineralization includes enchytraeids,
796 microarthropods, nematodes, and protozoans. Dashed line indicates the major components
797 involved in N mineralization. The annual N balance was +0.53 g N m⁻²y⁻¹.

798

799 Figure 2

800 Observed above-ground changes in N pools, C/N ratios, litter N production of *Deschampsia*
801 and percentage N in litter of *Deschampsia* caused by single treatments factors (a – c) and
802 when all treatments are combined (d). *Calluna vulgaris* (Cal), *Deschampsia flexuosa* (Des).
803 See Table 1 for statistical significance of observed changes.

804

805 Figure 3

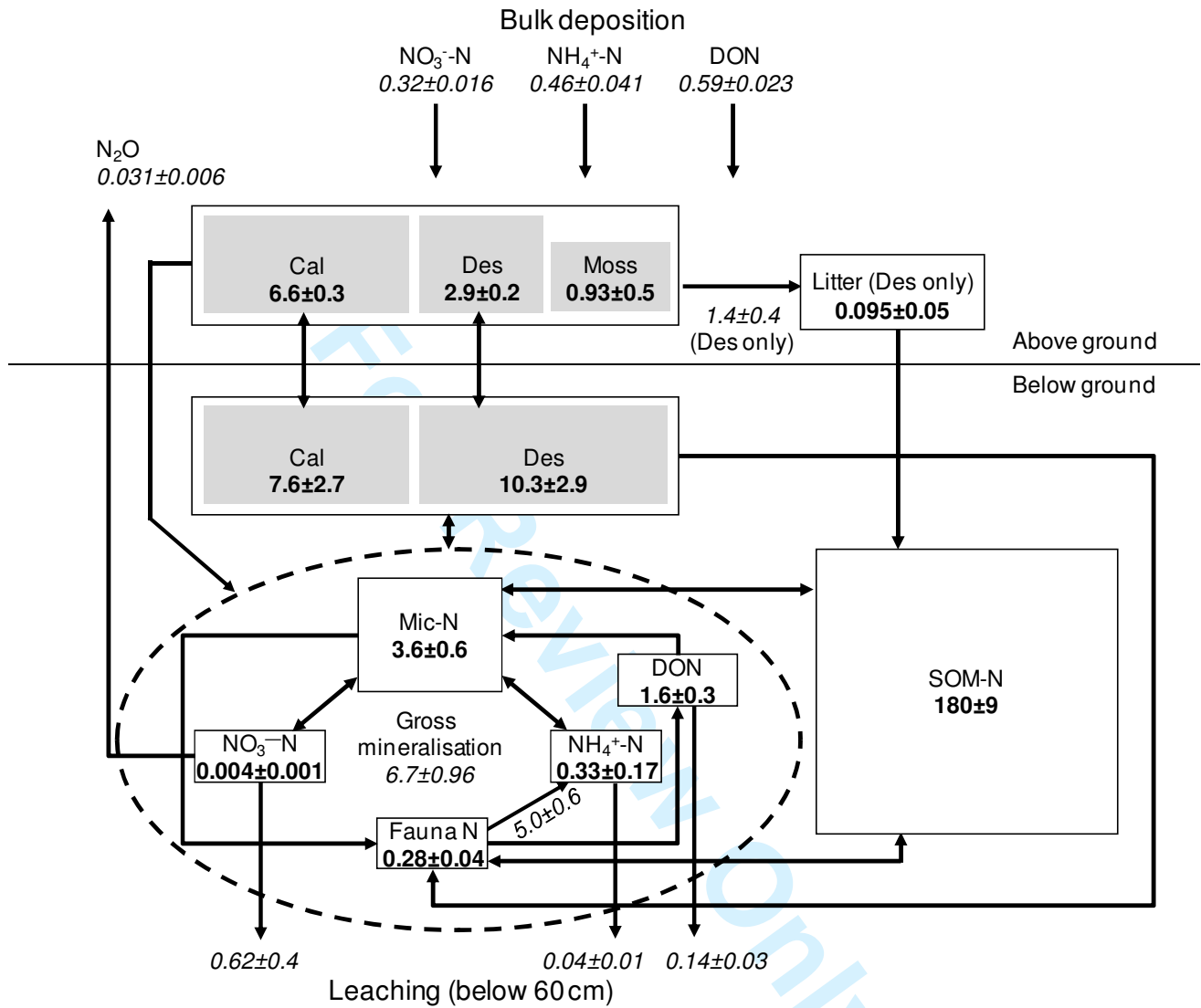
806 Observed below-ground changes in N pools, N fluxes/processes and N concentrations caused
807 by single treatments factors (a – c) and when all treatments are combined (d). Dissolved
808 organic nitrogen (DON), microbial biomass nitrogen (Mic-N), mineralization (min). See

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3 809 Table 1 for statistical significance of observed changes.
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For Review Only

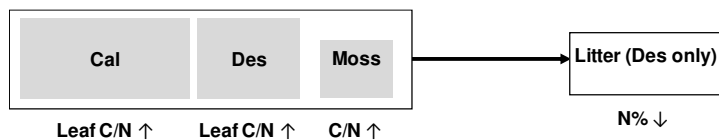
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10 812 Observed significant interactions ($P \leq 0.05$) from the PROC MIXED analysis of 47
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12 813 individually tested variables (see Table 1) followed by analysis of Differences of Least
13
14 814 Squares Means (DLSM) were categorized as antagonistic, *i.e.* combination leads to reduction
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16 815 of effects, or as synergistic, *i.e.* combination leads to amplification of single effects or
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18 816 significant effect is only observed in combination. Number of observed simple additive
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20 817 effects is also shown, *i.e.* cases where two significant individual effects were observed
21
22 818 without significant interaction. Note that the sum of antagonistic and synergistic effects are 15
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24 819 because one three-way interaction (potential nitrification – see Table 1) was caused by both
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26 820 antagonistic and synergistic effects.
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Figure 1

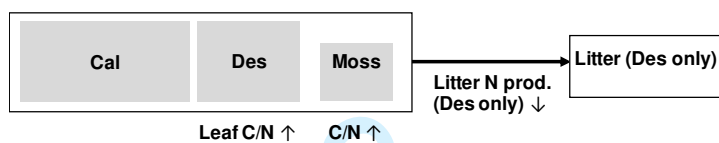


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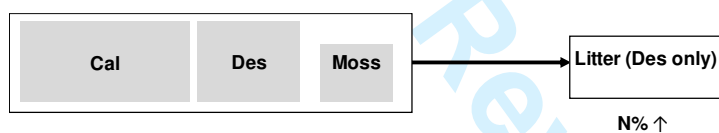
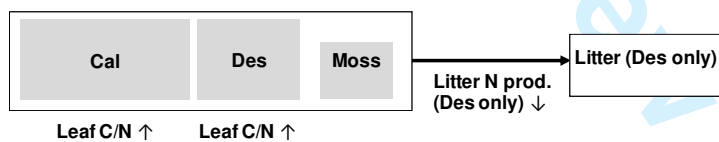
Figure 2

(a) CO₂

(b) Drought



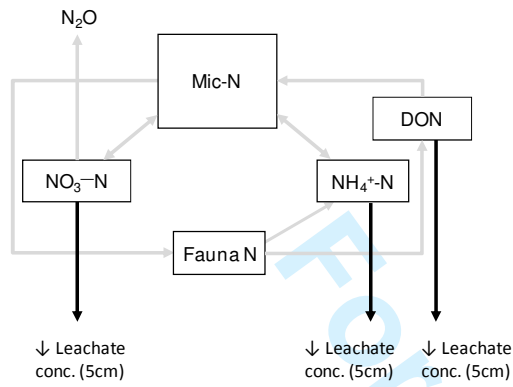
(c) Warming

(d) CO₂, drought and warming1
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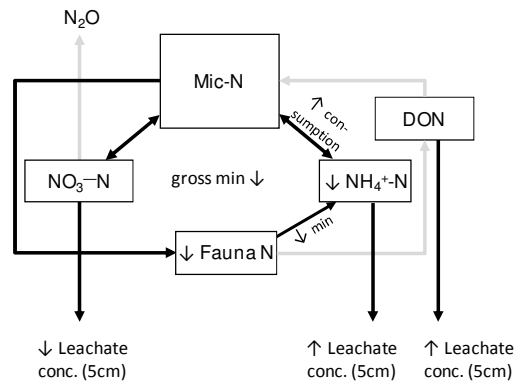
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Figure 3

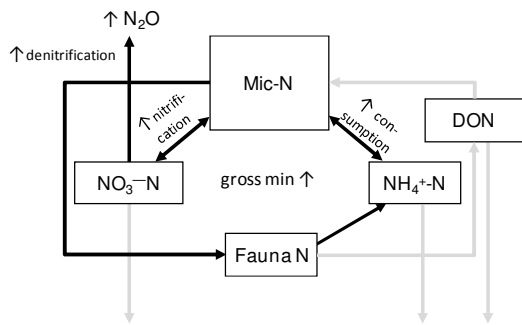
(a) CO₂



(b) Drought



(c) Warming



(d) CO₂, drought and warming

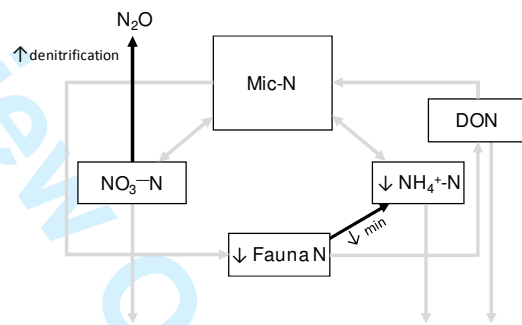
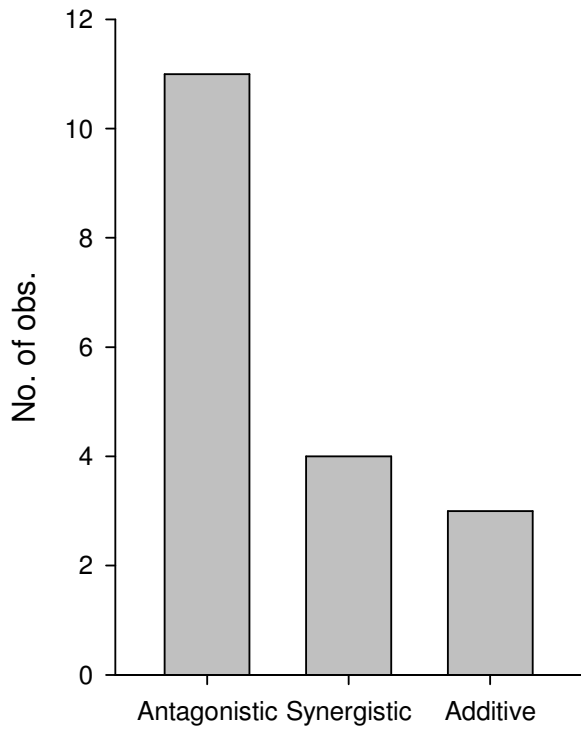


Figure 4



view Only