



37 *Fig. S1: The mean stand basal area (BA) of dieback stages of the gradient plots. Standard error bars are* 

- 38 *shown in red.*
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40 *Table S1: Basal area (BA) statistics. Mean, standard deviation (SD), standard error (SE), confidence interval* 

41 *(CI), minimum (Min) size of BA and maximum (Max) size of BA for each of the stages of dieback.*

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## Supplementary Methods: SM1

*Plot set-up*

56 Each plot was 20 x 20 m (400 m<sup>2</sup>; 0.04 ha). The edges were delineated with measuring tapes. A compass was used to confirm that the adjacent angles were at 90˚ angles. A nested sub-plot 58 of 10 x 10 m (100 m<sup>2</sup>) was set up in the centre of each plot, laid out in the same orientation as the full plot. The centre and the corners of the sub-plot were marked with wooden stakes for easy identification on return visits. The mid-points of each plot were recorded using a handheld GPS (GPSMAP 60CSx; Garmin, USA).

#### *Structural survey*

 The diameters at breast height (dbh) of both living and dead standing trees (snags) were measured at 1.3 m using a diameter tape pulled taut horizontally to the trunk. Following 66 advice and procedures from Husch *et al.<sup>1</sup>* and van Laar and Akça<sup>2</sup>, specific instructions were followed when using diameter tapes for difficult trees. The combined dbhs were used to 68 calculate the overall  $BA^3$ , forming the basis of the primary criterion.

#### *Crown condition*

 Living beech trees were further assessed for their condition, undertaken using binoculars at several points around each tree where visibility was good. The condition attributes were the potential crown loss, live growth loss, condition of the current branches and discolouration of the crown. Potential crown loss and leave loss were recorded as a percentage based on the average values provided by two observers. Similarly, condition was recorded as number (1-4) based on the descriptions. Any pathogens present were also recorded after a thorough search of the lower sections of each tree.

### *Canopy openness*

 At each corner of the 10 x 10 m sub-plot four readings were taken using a spherical 81 densiometer, one in each cardinal direction, giving an overall average for that  $plot<sup>4</sup>$ .

## *Understorey openness*

 Understorey openness was determined the same way as canopy openness, but only for trees less than approximately 6 m in height.

#### *Forest biomass*

88 Following Jenkins *et al.<sup>5</sup>*, oven-dry biomass was determined in four different components of the stand; the roots, the tree stems, the branches and foliage. To calculate the total biomass of a single species, the stem biomass, crown biomass and root biomass were summed together and multiplied by the number of that species present in the plot. The total biomass of all species was then calculated by summating all individual species' biomass values. The oven- dry biomass was calculated based on specific values for broadleaves, taken from McKay *et al<sup>6</sup> .*

*Carbon assessment for trees*

 Carbon content of a plot was calculated by multiplying the oven-dry matter biomass by 0.5, 98 the carbon fraction of biomass<sup>7</sup>.

*Herbivore pressure metrics*

 To account for the relative presence and influence of herbivores, understorey crown condition, browseline, sward height, seedling and sapling abundance, browsing intensity, dung counts, and presence of a shrub layer were recorded.

 For living trees in the understorey, crown condition (average of two different observers) was recorded based on deviation from perceived 'pristine' condition (i.e. 100%). Percentage of discolouration, percentage of leaves remaining, potential crown structure, empty branches and position of the tree were taken into account.

 The browse lines of palatable (e.g. beech, oak, birch) and unpalatable (e.g. holly, hawthorn) trees were recorded if they were within the edges of the plot. Using a marked range pole, any branches that were higher than 1.8 m (a deer's maximum browse height), but lower than 2.3 m (based on an average drop of 50 cm in the winter), were counted as browsed. Any branches that retained leaves below 1.8 m were counted as unbrowsed. A percentage ratio of browsed to unbrowsed was calculated. The sward height was measured using a measuring stick, based 116 on the findings of Stewart *et al.*<sup>8</sup> This was measured in the centre and at the four corners of the sub-plot, and a mean value was recorded.

 The percentages cover of mosses, bare ground, bracken, trampling and ground flora were recorded from a detailed visual assessment of each plot. Similarly, seedling (< 1.3 m in  height) and sapling (> 1.3 m and dbh < 10 cm) abundances were assessed through a manual search of the entire 20 x 20 m plot. Seedlings were any counted if they were older than a year, based on physical aspects.

 Partial defoliation or complete consumption of plants occur through herbivore browsing, the intensity of which is commonly determined by counts of un-browsed and browsed 127 branches<sup>9,10</sup>. This was undertaken using a random stratified design. Initially, a 2 x 2 m quadrat was placed in the most south-westerly corner of the sub-plot, continuing clockwise (NW, NE, SE) around the corners, until 100 stems had been assessed. The same technique 130 was used for assessing bramble browsing, following Bazely *et al* <sup>11</sup>.

 For estimating herbivore abundance from dung, the faecal standing crop (FSC) method, the 133 most commonly used and efficient technique<sup>12,13</sup>, was used. A manual dung count was carried out in the sub-plot; the amount, condition and the species recorded. Following Jenkins and 135 Manly<sup>14</sup>, the individual pellets/ bolus and their condition were recorded. The faecal matter of different animals (deer, *Equus* species, rabbits and cattle) were recorded separately.

*Soil survey*

139 Following the methods of DeLuca *et al.*<sup>15</sup>, ten separate soil samples were taken in randomly-140 stratified positions, two from the centre and two at each corner of the nested 10 x 10 m sub- plot, for both the O horizon and A horizon soil layer (0-15 cm below the O horizon). The vegetation the sample was taken under (e.g. bracken, grass) was noted.

144 For bulk density (BD) measurements, three 100 cm<sup>3</sup> stainless steel rings were inserted into the soil to ensure a known volume. These were taken from the SW and NE corners and from the mid-point.

148 For analyses of  $NO_3^-$  and  $NH_4^+$ , 5 g of sieved, field-moist soil was placed into a labelled tube with 25 ml of 1 M KCl added. The soils were shaken by hand and placed horizontally on a rotary shaker for 30 minutes at 250 rev/min. The extracts were immediately filtered through a Fisher QT 210 filter paper into a labelled polypropylene vial. The filtrates were then frozen 152 immediately and analysed two months later. Both  $NH<sub>4</sub>$ <sup>+</sup> and  $NO<sub>3</sub>$ <sup>-</sup> were analysed using the 153 microplate-colorimetric technique, with the salicylate-nitroprusside method for NH<sub>4</sub><sup>+</sup>, 154 following Mulvaney<sup>16</sup> and the vanadium method for  $NO<sub>3</sub><sup>-17</sup>$ .

 To determine the potential mineralisable nitrogen concentrations, 5 g of sieved, field-moist soil was placed into a labelled tube with 25 ml of ultrapure water added. The headspace was 158 then flushed with N<sub>2</sub> (g). The tube was sealed and incubated for 7 days at  $40^{\circ}$ C <sup>18</sup>. Immediately after incubation, 1.75 g of KCl was added to each tube. The tubes were shaken 160 (1 hr at 200 rev/min), centrifuged and filtered immediately, using the process as for  $NO<sub>3</sub>$  and 161 NH<sub>4</sub><sup>+</sup>. The pH and electrical conductivity of soil was determined using a 2:1 deionized water to soil ratio.

#### *Net N mineralisation and nitrification:*

 To enable analysis of in-situ of nitrification and N mineralisation rates, following DeLuca *et al.*<sup>15</sup>, a polyester mesh ionic resin capsule (Unibest, Walla Walla, WA, USA) was buried in the centre of each plot, 10 cm deep into the mineral layer. The capsules were placed between 168 9<sup>th</sup> October and 12<sup>th</sup> November, 2014 and were removed from the ground four months later.

 The nitrogen mineralisation and nitrification of a plot were analysed through leaching of resin capsules (RC). Initially, 10 mL of 1 M KCl was placed into each tube containing a RC, which was then shaken horizontally for 30 minutes at 250 rpm. The extractant was poured into a clean storage tube. This process was repeated two more times, making a total of 30 mL of the extractant. The extractant was centrifuged at 4000 rpm for 10 minutes. 20 mL of the supernatant was then pipetted into a 30 mL polypropylene tube and frozen prior to colorimetric analysis as described above.

#### *Soil respiration rate:*

 Soil respiration rate was measured using a SR-1 closed chamber Infra-red gas analyser (PP Systems, Amesbury, MA, USA). All measurements were recorded between 10:00 and 14:00 on sunny days within a month of each other. After automatic flushing and calibration of the chamber, the PVC chamber was inserted 2 cm into the soil after any vegetation had been 183 removed from the surface. The CO<sub>2</sub> concentration was measured continuously for 2 minutes. Five measurements were taken from each survey plot and then averaged to produce a mean soil respiration rate for the whole plot. Soil respiration rate was calculated as in (PP Systems<sup>19</sup>:

188  $R=V/A \times ((Cn-Co)/(Tn))$ 

 Where *R* is the respiration rate, *V* is the volume of the chamber, *A* is the area of soil exposed, *Cn* is the CO<sup>2</sup> concentration at time 0, and *Co* is the CO2 concentration at time, *Tn* (120 seconds in this study).

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- *Soil moisture*

 Soil moisture was measured as the difference in weight of a 5 g moist soil sample before and 196 after oven-drying. Sieved mineral and organic samples were oven-dried at 105 °C and 80 °C, respectively, until they remained a constant weight. To measure the soil organic matter (SOM), the oven-dried samples were then placed in a 500 °C furnace overnight (12 hours), 199 the final weight recorded after being cooled in a desiccator.  $LOI = 100 \text{ x}$  (mass of oven-dry 200 soil-mass of ignited soil)/ mass of oven-dry soil = g per 100g oven-dry soil<sup>20</sup>. The soil was 201 dried at 105 °C for 24 h and then sieved (2 mm) to remove stones and other non-soil material ( $>2$  mm diameter). Bulk density was calculated by dividing soil mass (less stone mass) by core volume (less stone volume).

#### *Soil content and structure*

 The Forest Research (FR) team at Alice Holt Lodge, Surrey, measured the exchangeable cations/anions of K, S, Ca, Mg, Na, Al, Mn and F; total N and C, organic and inorganic C; the plant-available P; and the particle sizes of the soil from air-dried samples. Following FR 209 methods, the exchangeable cations/anions were analysed using  $BaCl<sub>2</sub>$  extraction (FR Reference method: ISO 11260 & 14254). First, a soil suspension of 3 g soil and 36 ml of 0.1 211 M BaCl<sub>2</sub> was shaken for 60 minutes, centrifuged and filtered with 0.45 µm syringe filter. Extracts were then acidified and analysed using a dual view ICP-OES (Thermo ICap 6500 duo). The Olsen P method with ADAS index was used to determine the amount of phosphorus available (FR Reference method: The analysis of Agricultural Materials MAFF 3rd Edition RB427). A suspension of 5 g soil with 100 ml of sodium bicarbonate solution was buffered at pH 8.5. The solution was shaken for 30 min on an orbital shaker, centrifuged and filtered with 0.45µm syringe filters. Extracts were then acidified with 1.5 M sulphuric acid and mixed with a solution of ascorbic acid and ammonium molybdate for 10 min and then measured at 880 nm with a Shimadzu UV sprectrophotometer. Total C and N were analysed using a Carlo Erba CN analyser (Flash1112 series) and combustion method (FR Reference method: ISO 10694 & 13878). Samples were ball-milled for homogenisation and then around 30 mg weighed in tin capsules, pressed and measured using the analyser.  Following, 30 g of soil was placed in a silver capsule to quantify inorganic C. The silver 224 capsule was put furnace at  $500^{\circ}$ C for 2 hours, which removed the organic carbon. The organic carbon fraction was calculated as the difference between total carbon and inorganic carbon. The soil particle size distribution was determined using a Laser Diffraction Particle Sizer (FR Reference method: Laser diffraction); 30 g of soil were suspended in water and 228 passed through the flow cell of the analyser (Beckman Coulter LS13320).

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#### 230 *Data analysis*

 Random intercepts and slopes were included for each site. All the variables were tested for normal distribution with the Shapiro–Wilk test and for homogeneity of variances for 233 Bartlett's test<sup>21</sup>. Data that did not fit these assumptions were log-transformed prior to analysis.

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236 Count data were modelled using a Poisson error structure. For proportional and percentage 237 data, a small non-zero value was added to avoid infinite logit transformed values<sup>22</sup>. AICc 238 values were calculated using the maximum likelihood value of the model<sup>23</sup>. AICc values were 239 determined using the MuMIn R package<sup>24</sup> and used to define the most parsimonious model, 240 following an information theoretic approach<sup>23</sup>. Performance of models was evaluated by 241 calculating the marginal  $r^2$  <sup>25</sup>.

#### Supplementary Methods: SM2

#### *Ground-dwelling arthropods collection*

 Pitfall trapping was carried out in five out of the 12 sites. In each site eight pitfall traps were placed on the perimeter of the 10m x 10m sub-plot; one in each corner and one midway along each edge. A soil auger was used to create holes in which plastic cups (8 cm in diameter and 11 cm tall) were placed. Approximately 3 cm of propylene glycol, a cost effective preservative, was poured into each cup. Water was allowed to escape through the use of drainage holes in the top of the cups; this also prevented the trap flooding. A galvanised steel square which was supported by turned-down corners was placed over each trap. Forestry Commission staff collected the contents of each pitfall trap weekly from late May to late July 252 2014, totalling eight collections and 56 trapping days. The arthropod material from the eight pitfall traps in each plot were pooled into a single labelled and sterilised 1 litre sample bottle 254 and then stored in  $-5 \,^{\circ}\text{C}$  to preserve the specimens for metabarcoding.

#### *Ground-dwelling arthropods analysis*

 DNA metabarcoding was employed for invertebrate identification using a methodology 258 tailored from the approach described in Yu *et al.*<sup>26</sup>. Samples were stored in absolute ethanol at 4°C, followed by the extraction of DNA using the Qiagen blood and tissue extraction kit. Polymerase Chain Reactions (PCR) were performed targeting the 658 base pair C terminal region in the gene encoding the mitochondrial cytochrome oxidase subunit I (COI); primers used for the COI region of interest were: Forward: LCO1490 (5'- GGTCAACAAATCATAAAGATATTGG-3') and Reverse: mlCOIintGLR (5'-GGNGGR TANANNGTYCANCCNGYNCC-3'). Three separate PCRs were carried out for each sample. An aliquot was checked on a 1.4% agarose gel and then the PCRs pooled before library construction. A multiplex identifier (MID) tag was attached to the forward primer in addition to the relevant adaptor for the sequencing platform. The MID tag was specific to each sample and allowed multiple samples to be pooled for sequencing and then separated out bioinformatically afterwards. A touch-down thermocycling profile was used, followed by a low number of cycles with an intermediate annealing temperature. Indexing barcodes were added to the amplicons following the Illumina TruSeq Nano protocol from the 'Clean-up Fragmented DNA' stage. In a deviation from this protocol fragments were size-selected using blue Pippin size selection of the 300-670bp region to remove larger fragments. The barcoded samples were pooled into a single pool and 250bp paired end reads were generated on one

 lane of the Illumina MiSeq platform. The pool was demultiplexed into the individual samples using the Illumina bcl2fastq (v 1.8.4bin) software. The samples were clustered into OTUs (operational taxonomic units) using the approach described in Yu *et al.*<sup>27</sup> starting with demultiplexed samples in step 1. Instead of the described step 6 of the pipeline we used the BOLD database and website for taxonomic assignment and confidence assessment. Accepted matches had to have at least 97% sequence similarity at a given taxonomic level. For this we queried the website by using a custom script that created the urls and parsed the output for each OTU. In a final step the taxonomic assignment, OTU and the number of reads of each sample mapping to the OTUs was collated into a single table. The final species lists were checked against previous records of species occurrence in Britain using primarily the 285 National Biodiversity Networks Gateway<sup>27</sup> but also Fauna Europaea<sup>28</sup>, Antweb<sup>29</sup>, the British 286 • Arachnological Society<sup>30,</sup> and Araneae: Spiders of Europe<sup>31</sup>. Where no previous record was found to species level, occurrence in Britain to Genus level was checked. 

289 *Table S2: Summary of variables measured and units used.*







290 *Table S3: Generalised linear mixed models used to determine whether a threshold was exhibited in all the response variables and associated* 

291 *measures of parsimony (AICc), support (ΔAICc, AICc weight) and goodness of fit (Marginal r2). Mod\_cont\_NL specifies that the model* 

292 *contained a linear and quadratic term of BA loss indicating a non-linear response; Mod\_cont specifies that the model only contained a linear* 

293 *term of BA loss indicating a linear response; and Modnull1 specifies that the model indicated little or no change over the gradient of BA loss.*



































Response variable	Name	Model structure	df		Log likelihood	AICc	<b>AAICC</b>	AICc weight	Marginal $r^2$	Threshold?
	Mod cont NL	<b>BA</b> decline $+$ BA decline <sup>2</sup>			4 -1364.378	2737.483	0.000	1.000	0.116	
Abundance of holly seedlings	Mod_cont	<b>BA</b> decline		$\overline{3}$	$-1849.403$	3705.234	967.751	0.000	0.033	No
	Modnull1	Null model		$\mathbf{2}^{\prime}$	$-1895.355$	3794.921	1057.438	0.000	0.000	
	Mod cont NL	<b>BA</b> decline $+$ BA decline <sup>2</sup>			4 -279.394	567.515	0.000	1.000	0.217	
Abundance of beech seedlings	Mod_cont	<b>BA</b> decline		$\overline{3}$	$-302.158$	610.744	43.229	0.000	0.170	Yes
	Modnull1	Null model			2 -331.657	667.524	100.009	0.000	0.000	
	Mod_cont_NL	BA decline+ BA decline <sup>2</sup>		4	$-50.284$	109.295	0.000	0.999	0.444	
<b>Abundance of oak</b> seedlings	Mod_cont	<b>BA</b> decline		3 <sup>1</sup>	$-58.639$	123.706	14.412	0.001	0.147	Yes
	Modnull1	Null model			2 -65.866	135.942	26.648	0.000	0.000	
	Mod cont NL	BA decline+ BA decline <sup>2</sup>		4	$-1403.461$	2815.650	0.000	1.000	0.134	
<b>Abundance of tree</b> seedlings	Mod_cont	<b>BA</b> decline		3.	$-1907.548$	3821.524	1005.874	0.000	0.046	No
	Modnull1	Null model			2 -1970.624	3945.459	1129.809	0.000	0.000	
Abundance of palatable	Mod_cont_NL	<b>BA</b> decline $+$ BA decline <sup>2</sup>		4	$-267.337$	543.401	0.000	1.000	0.293	Yes

295 *Table S4: Updated version of Table S3 with only linear and quadratic term of BA included as fixed effects.* 









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300 *properties across the stages of dieback.*

## 299 *Table S5: Statistics of the soil properties. Mean, standard deviation (SD), standard error (SE), and confidence interval (CI) of several soil*



Supplementary Methods: SM3. Graphs to support the space-for-time

# assumption



 *Fig S3: Mean values (n = 12) of a) clay soil content; b) depth of the organic soil layer; c) pH of the soil across the gradient of dieback; and d) diameter at breast height (DBH) of the living beech trees across the gradient of dieback. The black bars indicate the standard error of the mean.*

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*Fig S4: Mean values of a) the total herbivore dung count, and b) percentage of holly shoots browsed by* 

*herbivores across the gradient of dieback. The black bars indicate the standard error of the mean.*

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