

1 **Title**

2 **Combining multiple investigative approaches to unravel functional**
3 **responses to global change in the understory of temperate forests**

4 **Running title**

5 Understorey responses to global change

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42 **Abstract**

43 Plant communities are being exposed to changing environmental conditions all around the globe, leading to
44 alterations in plant diversity, community composition, and ecosystem functioning. For herbaceous
45 understorey communities in temperate forests, responses to global change are postulated to be complex,
46 due to the presence of a tree layer that modulates understorey responses to external pressures such as
47 climate change and changes in atmospheric nitrogen deposition rates. Multiple investigative approaches
48 have been put forward as tools to detect, quantify and predict understorey responses to these global-change
49 drivers, including, amongst others, distributed resurvey studies and manipulative experiments. These
50 investigative approaches are generally designed and reported upon in isolation, while integration across
51 investigative approaches is rarely considered. In this study, we integrate three investigative approaches (two
52 complementary resurvey approaches and one experimental approach) to investigate how climate warming
53 and changes in nitrogen deposition affect the functional composition of the understorey and how functional
54 responses in the understorey are modulated by canopy disturbance, i.e. changes in overstorey canopy
55 openness over time. Our resurvey data reveal that most changes in understorey functional characteristics
56 represent responses to changes in canopy openness with shifts in macroclimate temperature and aerial
57 nitrogen deposition playing secondary roles. Contrary to expectations, we found little evidence that these
58 drivers interact. In addition, experimental findings deviated from the observational findings, suggesting that
59 the forces driving understorey change at the regional scale differ from those driving change at the forest floor
60 (i.e. the experimental treatments). Our study demonstrates that different approaches need to be integrated
61 to acquire a full picture of how understorey communities respond to global change.

62 **Key words**

63 Herbaceous layer, plant height, SLA, mesocosm experiment, resurvey study, forestREplot, climate change,
64 nitrogen deposition, forest management

65

66 **1. Introduction**

67 Climate warming and eutrophying atmospheric deposition are having profound consequences for individual
68 plants, species, communities and ecosystems around the globe (Malhi et al., 2020; Maskell et al., 2010;
69 Parmesan & Yohe, 2003). As the number of scientific articles in the domain of global change ecology has
70 increased tremendously over the past decade, we have added nuance to our understanding of global change
71 impacts across systems (e.g. Bjorkman et al., 2018; van de Waal & Litchman, 2020). For systems
72 encompassing multiple structural layers such as temperate forest ecosystems, this nuance has led to the
73 recognition of a modulating effect of the overstorey on global change-induced community responses in the
74 understorey, i.e. the herbaceous vegetation growing on the forest floor (Landuyt et al., 2020; Segar et al.,
75 2022; Verheyen et al., 2012; Zellweger et al., 2020). These understorey plant communities are pivotal to
76 temperate forest plant biodiversity (Gilliam, 2007; Spicer et al., 2020), and, depending on their functional
77 signature (determined by their cover, leaf traits and height) also affect nutrient and water cycling processes,
78 and tree regeneration rates in these forest systems (De Lombaerde et al., 2021; Landuyt et al., 2019).

79 Past studies on the understorey's response to global-change drivers such as atmospheric nitrogen (N)
80 deposition and climate warming mainly detected shifts in species composition: excess N deposition was
81 found to increase the dominance of nitrophilous, acid-tolerant or generalist species (Gilliam, 2006; Segar et
82 al., 2022), while climate warming was found to promote warmth-demanding species (De Frenne et al., 2013;
83 Stevens et al., 2015). In contrast, responses of the understorey's functional signature (often expressed in
84 terms of total herbaceous cover and community weighted mean (CWM) functional trait values such as plant
85 height and specific leaf area (SLA)) have been found to be less pronounced or highly context-dependent
86 (Depauw et al., 2020; Perring et al., 2018). A high canopy cover - that might buffer the response of the
87 understorey to these global change drivers - has been put forward as a potential reason for weak or absent
88 understorey responses (Hedwall et al., 2021; Richard et al., 2021; Verheyen et al., 2012; Zellweger et al.,
89 2020). Plant community theory, indeed, states that a limiting resource (such as light) can restrict a
90 community's response to other perturbations in resource availability and growing conditions, as those
91 induced by global-change drivers (von Liebig, 1855). Hence, elevated levels of N availability, as induced by N
92 deposition, might not lead to growth responses and, eventually, shifts in the functional characteristics of the
93 understorey if light availability is still limiting growth (Segar et al., 2022; Strengbom et al., 2004; Verheyen et
94 al., 2012). Following the same reasoning, also functional responses in the understorey to warming can be
95 expected to amplify when canopies open up, which has already been shown in experimental conditions (De
96 Frenne et al., 2015).

97 Most of the studies reported upon above rely on long-term resurvey datasets that aim to describe plot-level
98 community changes over time by repeating historical plant community surveys up to several decades after
99 initial surveys took place. These resurvey studies can reveal the rather slow responses of the understorey to
100 various global-change drivers. When multiple of those resurvey studies are combined into a larger database,

101 they can span broad environmental gradients in space and time and can reveal understory responses to a
102 variety of global-change drivers in a representative way (Verheyen et al., 2017). However, within these
103 databases, resurvey studies are generally distributed at random across space, and often lack uniform, plot-
104 level auxiliary data (e.g. soil resources, light availability) to be able to accurately quantify local drivers of
105 change or to be able to control for potentially confounding drivers, leading to low signal-to-noise ratios in
106 general. Moreover, sampling methods and plot selection strategies often depend on the specific objectives
107 of individual resurvey studies. As a result, these so-called *opportunistic resurvey databases* are often plagued
108 by data heterogeneity. To increase the signal-to-noise ratio in understory research while preserving a
109 regional perspective covering broad environmental gradients, one can carry out new, more targeted,
110 distributed resurvey studies, with an orthogonal (i.e. maximizing independence among the global-change
111 drivers of interest) plot network design and field measurement protocol shaped by a number of clear
112 hypotheses (e.g. Baeten et al., 2013; Depauw et al., 2020; Fischer et al., 2010). Although these *orthogonally-*
113 *distributed resurvey studies* will increase our understanding of understory responses to global change, they
114 will still fail to distinguish correlation from causation or disentangle the consequences of correlated drivers.
115 For true mechanistic understanding, *manipulative global-change experiments* are needed because they allow
116 canceling out impacts of confounding drivers and enable detailed measurements of several mechanisms of
117 change (Rustad, 2008). The downsides of those experiments are their often short-term nature, artefacts
118 linked to the experimental settings (e.g. artificial belowground processes in pot experiments, unwanted side
119 effects of treatments) and the difficulty to generalize findings towards other species or communities than
120 those included within the experiment. Typically, these different investigative approaches are designed in
121 isolation, making direct comparison among approaches impossible or extremely challenging. As a result, the
122 scientific literature often reflects on those adopted approaches and their findings in isolation and therefore
123 fails to fully exploit the complementarity among approaches (Luo et al., 2011).

124 In this study, we integrate three complementary investigative approaches, as introduced above, to reveal
125 and understand general patterns of functional understory responses to N deposition and climate warming,
126 and how changes in light availability induced by moderate canopy disturbances modulate these responses.
127 We focus specifically on functional signature responses, as a proxy for changes in understory functioning
128 over time. To do so, we compiled data from the forestREplot database, an opportunistic resurvey database
129 focusing on understory communities in temperate forests, set up an orthogonally-distributed resurvey
130 study and designed a large manipulative global-change experiment. We analysed the different datasets using
131 a uniform statistical approach targeted towards the main hypotheses being tested:

132 (1) Light availability dynamics are the dominant driver of functional changes in the herbaceous
133 understory of temperate forests

134 (2) Functional understory responses to N deposition and climate warming are generally weak, but
135 become more pronounced when canopies open up over time.

136 We do not expect different approaches to yield identical results, but rather expect that different approaches
137 will supply different pieces of information that will enhance our mechanistic understanding of observed
138 community changes.

139 **2. Methods**

140 2.1. Overview of the data

141 We report upon data from three studies that were jointly designed, as part of a larger scientific project
142 'PASTFORWARD', to understand responses of the understorey to key global-change drivers, and especially
143 interactions among those drivers, including climate warming, N deposition and changes in (past) forest
144 management (for an overview of the full study design see <http://pastforward.ugent.be>). The first dataset we
145 use is based on the forestREplot database, a European-scale compilation of understorey resurvey studies
146 (<http://forestreplot.ugent.be>), which we refer to as an *opportunistic resurvey database* in this study. The
147 forestREplot database contains understorey community change data collected in a large number of
148 unmanaged or only extensively managed temperate forests across Europe, covering broad environmental
149 gradients in terms of N deposition and climate (Fig. 1a, Fig. 2a,b). As included forest types are diverse and
150 detailed plot-level data on resource availability (e.g. light and nutrients) is often lacking, the dataset is typified
151 by a large amount of variability. In conjunction, we designed an *orthogonally-distributed resurvey study*
152 targeted towards the current study's hypothesis by selecting plots along predefined gradients of the
153 considered global-change drivers (climate and N deposition), while making sure that plots were comparable
154 in terms of site characteristics and forest type (mesophytic deciduous forests and lowland beech forests). For
155 this study, we selected plots in 19 regions, covering the geographical extent of the database (see Fig. 2a),
156 that vary in terms of past climatic warming and N deposition rates and combinations thereof (Fig. 2b). Within
157 each region, we selected multiple plots with contrasting past management leading to a variety of canopy
158 conditions (mostly in terms of canopy density/openness, see also Fig. 2b). To lower amounts of noise and
159 unexplained variability in the data, we complemented vegetation resurveys with detailed plot-level
160 measurements of light and nutrient availability. Finally, we also set up a *manipulative global-change*
161 *experiment* in a deciduous forest in Belgium where we exposed artificial understorey communities to the
162 considered global-change drivers in a full-factorial design (Fig. 1b). To compile these artificial communities,
163 we imported genuine forest soils from several regions spread across the European temperate forest biome,
164 and selected species with contrasting ecological strategies from the database study's species pool.

165 In the following sections, we outline methodological details of each investigative approach to allow
166 understanding of our synthesis, highlighting key aspects to allow integration across methodologies. Further
167 details on each separate approach can be consulted in earlier publications on these individual studies: see
168 Perring, Bernhardt-Römermann et al. (2018) for the opportunistic resurvey database, Depauw et al. (2020)
169 for the orthogonally-distributed resurvey study and Blondeel et al. (2020) for the manipulative global-change
170 experiment. The full dataset can be consulted at figshare (Landuyt et al., 2023).

171 *Opportunistic resurvey database*

172 The database contained resurvey data from 1700 forest plots (subset of the forestREplot database as
173 analysed in Perring, Bernhardt-Römermann et al. (2018), but retaining only plots with overstorey cover data
174 at both survey dates), scattered across 39 regions spanning the European temperate forest biome (Fig. 2a).
175 In each of these plots, understorey and overstorey cover and composition has been recorded at two points
176 in time, with a period of 38 years on average between both survey dates. All plots were situated in ancient
177 forests (continuous forest cover since 1850, as we omitted data from more recent, post-agricultural forests
178 (as described in Perring, Bernhardt-Römermann, et al., 2018). As variation in soil properties were not
179 monitored, we relied on (1) community-weighted Ellenberg Indicator Values (EIV, weighted by cover) for soil
180 fertility (EIV-N) and acidity (EIV-R) (based on Ellenberg et al., 2001) and (2) a scoring of the overstorey's litter
181 quality as proxies for forest floor resource availability and growing conditions. These proxies were all based
182 on community composition data at the resurvey. The considered EIVs, on an ordinal scale from 1 to 9, reflect
183 a species' ecological niche in terms of resource availability and/or growing conditions, while litter quality
184 scores reflect tree species-specific litter decomposition rates (based on Baeten et al., 2009; Hermy, 1985;
185 Van Calster et al., 2008; Verheyen et al., 2012).

186 *Orthogonally-distributed resurvey*

187 For the orthogonally-distributed resurvey, we selected 192 historical understorey surveys spread across 19
188 European regions (Fig. 2a), along gradients of N deposition and climate (Fig. 2b), and conducted a resurvey
189 of those plots in the late Spring of 2015 or 2016 (42 years on average between survey dates). Replicating
190 initial survey methods in each region, as detailed in Depauw et al. (2020), the cover of all understorey species
191 that occurred within these plots was re-estimated. Simultaneously, representative samples of soil were
192 collected for lab analyses, allowing direct characterization of soil acidity (pH) and nutrient content (C:N, Olsen
193 P) (See Maes et al., 2019 for more details), variables that could co-determine community trajectories of
194 change. In addition, data on overstorey composition and canopy openness were recorded by visual cover
195 estimates and densiometer measurements (Lemmon, 1956), respectively. For the initial survey, only
196 composition and cover data were available for the overstorey. Our measurements together with the
197 historical overstorey data allowed a more precise estimation of light availability changes between surveys in
198 comparison with the database study (see S3). Since historical data on overstorey cover was absent for 20
199 plots, we here only analyse a subset of 172 plots.

200 *Manipulative global change experiment*

201 For the experimental study, we planted 384 mesocosm understorey communities in well-drained plastic
202 containers. Per mesocosm, we planted 5 species with contrasting ecological strategies, selected from a
203 common species pool of 15 species (See S2). Mesocosms were dug into the soil of a deciduous forest in
204 Northern Belgium. Next, mesocosms were exposed to warming (+0.7°C increase of MAT, using open top

205 chambers (OTC)), N fertilization (+50 kg N/ha.y, through liquid ammonium nitrate (NH₄NO₃) fertilization,
206 minimizing effects on soil pH) and light addition (+24 μmol/m².s, mimicking a 5% increase of PAR
207 transmittance, using fluorescent tubes, as shown in Fig. 1b). Ambient temperature (MAT of around 10°C at
208 15 cm above the forest soil), light conditions (around 10% PAR transmitted to the understory) and N
209 deposition levels (between 20 and 25 kgN/ha.y measured in throughfall water) at the experimental site were
210 used as control. These treatments were applied each time to blocks consisting of 4 mesocosms (referred to
211 as 'plot' in our analyses). As the original study also focused on soil effects (bearing the imprints of past land
212 use) and how they interact with the considered global change treatments, all mesocosms were planted in
213 genuine forest topsoils, collected in ancient (forested since the time of the earliest written records) and
214 recent (afforested after 1960) mature forest plots from 8 European regions spread along gradients of N
215 deposition and climate. All collected soil samples were analyzed in the lab to determine their nutrient content
216 (C:N, Olsen P) and acidity (pH). In this synthesis, we aim to control for these soil effects, while testing our
217 main hypothesis which does not focus on those effects specifically. The experiment was set up in 2016 after
218 which the cover of all planted species has been estimated during spring. In May 2021, after 5 years of
219 treatment application, all communities were resurveyed to evaluate community responses.

220 2.2. Statistical analysis

221 *Response variables*

222 As response variables, we considered (i) changes in community-weighted mean (CWM) trait values and (ii)
223 changes in total herbaceous cover, both assessed in terms of log-response ratios (LRR, i.e. the natural
224 logarithm of the ratio between CWM trait values (or cover values) at the resurvey divided by the CWM trait
225 values (or cover values) at the initial survey). We, hence, analyze changes over time and not across space as
226 done in space-for-time analyses. For LRR cover calculations, we added 1% to all cover estimates to avoid
227 division by zero. We focused the analysis on changes in CWM plant height (H) and specific leaf area (SLA).
228 These community traits, together with total herbaceous cover, relate to the functional importance of the
229 understory in temperate forests (Landuyt et al., 2019). CWM trait values were calculated using relative plant
230 cover as weights and using species-specific trait values extracted from a literature trait database according
231 to Perring, Bernhardt-Römermann et al. (2018). To calculate total cover and CWM trait values, we only took
232 into account herb layer species *sensu stricto*. Hence, we omitted seedlings, climbers and shrubs from all
233 vegetation datasets. In the experiment, however, we included *Hedera helix* as it here behaves as a ground-
234 covering herb.

235 *Predictor variables*

236 In the analysis, two types of predictor variables were considered, including focal predictor variables that
237 relate to the hypotheses being tested and covariates we aim to control for. Focal predictor variables included
238 (i) absolute changes in light availability (expressed in terms of light transmittance %, ΔLT, derived from

239 overstorey data (tree and shrub cover); see S13 for details on the calculations for the different datasets), (ii)
240 absolute changes in N deposition rate between survey dates (ΔN_{dep}), and (iii) absolute changes in mean
241 annual temperature (ΔMAT) between survey years (See S1 for calculation methods and distribution of these
242 predictor variables across the investigative approaches). For the experiment, we included all global-change
243 drivers as binary predictors in accordance with the binary treatments in the experiment. As we do not aim to
244 compare the magnitude of our slope estimates among investigative approaches but only the signs, this
245 methodological choice will not have a bearing on our results.

246 Covariates included (i) time between the initial survey and the resurvey (years, i.e. fixed for the experiment,
247 and therefore not considered for this approach), and covariates characterizing soil characteristics, including
248 (ii) soil acidity (pH H₂O), (iii) phosphorous availability (Olsen P) and (iv) the carbon-to-nitrogen ratio (C:N). For
249 the database study, we relied on EIV R, EIV N and an overstorey litter quality score as direct measurements
250 of soil characteristics were not available. These indirect measurements were based on community
251 composition at the resurvey to ensure comparability with the orthogonally-distributed resurvey where we
252 account for soil characteristics that were measured at the resurvey only. Finally, we also considered baseline
253 community characteristics (being the CWM SLA, plant height or total herbaceous cover, depending on the
254 modelled response) at the initial survey, as an additional predictor variable to account for the regression to
255 the mean phenomenon when modelling change dynamics (Mazalla & Diekmann, 2022).

256 *Mixed-effects modelling*

257 We tested our main hypothesis, i.e. whether understorey responses to climate warming and N deposition
258 are modulated by changes in canopy openness, using linear mixed-effects models with all covariates and
259 focal predictor variables as fixed main effects. In addition, we included two interaction terms: i) the
260 interaction between changes in light availability and changes in N deposition rates and ii) between changes
261 in light availability and changes in MAT (See Table 1 for full R syntax). These interaction terms were included
262 to test whether changes in canopy openness modify understorey responses to changes in MAT and N
263 deposition. Random terms were specified based on dataset structures that differed depending on the
264 investigative approach (see Table 1). Prior to model fitting, all variables were standardized (mean=0, SD=1)
265 and tested for independence based on variance inflation factors. All analyses were performed in R version
266 4.2.1.(R Core Team, 2022), using packages lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017) and
267 sjPlot (Lüdtke, 2022). In total, 9 separate models were fitted, modelling three response variables, each
268 based on three investigative approaches.

269 **3. Results**

270 3.1. Observed functional composition changes across investigative approaches

271 The distribution of the observed community changes was centered around no change across all investigative
272 approaches (Fig. 3). Only in the orthogonally-distributed resurvey study did CWM plant height increase and

273 herb cover decrease on average between the two survey dates. Across all investigative approaches, the
274 variability in community changes was higher in terms of cover and CWM plant height when compared to
275 community changes in terms of CWM SLA.

276 3.2. The main drivers of functional community changes

277 All fitted mixed-effects models achieved a moderate model fit, denoted by marginal R^2 values between 0.18
278 and 0.45 (Table 2). These high R^2 values were, however, mainly driven by the inclusion of the baseline values
279 (CWM SLA, plant height and cover at the initial survey) as additional predictors in the models (for model
280 outputs without the baseline included, see S4). Given that marginal R^2 values are comparable among
281 investigative approaches, the analysis also suggests that none of the investigative approaches outcompetes
282 the others in terms of explainable variability in the data. Considering the focal predictor variables, being
283 changes in light availability, changes in N deposition rates and changes in MAT, we found that the different
284 investigative approaches yielded contrasting results (Fig. 4).

285 First of all, we found limited evidence underpinning our main hypothesis, that changes in canopy openness
286 modify how the functional composition of understorey communities responds to N deposition changes and
287 climate warming over time. Only one significant interaction was detected, in experimental settings (Fig. 4a).
288 However, we did detect several main effects of the considered global-change drivers (Fig. 4b,c,f,g,h,i). Based
289 on the database study, an increase of light availability was found to increase herb cover ($p < 0.01$) and plant
290 height ($p < 0.05$) (Fig. 4f,i). A similar trend, although only significant for herb cover changes ($p < 0.05$), was
291 observed in the resurvey study (Fig. 4h). In contrast, light addition alone did not affect the functional
292 composition of the experimental understorey communities (Fig. 4a,d,g). Only in combination with a warming
293 treatment, illumination was found to decrease CWM SLA in the experiment ($p < 0.05$) (Figure 4a). Effects of
294 climate warming (at the macroscale) were not detected in the orthogonally-distributed resurvey (Fig. 4b,e,h),
295 while negative effects on herb cover, CWM plant height and CWM SLA were detected in the analysis based
296 on the opportunistic resurvey database (Fig. 4c,f,i). In contrast, in the experiment, microclimate warming was
297 found to increase total herb cover ($p < 0.05$) (Fig. 4g). Also the effects of changes in N deposition rates were
298 found to differ among approaches. Increases in N deposition rates were found to decrease total herb cover
299 ($p < 0.05$, based on the opportunistic resurvey database, Fig. 4i) and increase CWM SLA ($p < 0.05$, based on the
300 orthogonally-distributed resurvey, Fig. 4b).

301 The precision of the estimates was found to differ depending on the investigative approach. The precision of
302 the coefficient estimates was found to increase with the number of datapoints, with low precision in the
303 experiment and orthogonally-distributed resurvey and higher precision in the database study.

304 4. Discussion

305 By comparing results from three different investigative approaches, we indeed found that changes in light
306 availability are an important driver of functional changes in the understorey, but found limited evidence for

307 our second hypothesis, being that overstorey trees can buffer functional responses in the understorey to
308 global change. While we did detect several main effects of the considered global-change drivers, the number
309 of significant interaction effects between changes in light availability, on the one hand, and changes in N
310 deposition rates and warming on the other hand was limited to one significant interaction between light
311 addition and warming on CWM SLA in experimental settings. Below we discuss all detected main and
312 interaction effects for each global-change driver independently and end the discussion with a reflection on
313 the added value of combining different investigative approaches as done in our study.

314 *4.1. Canopy effects*

315 The importance of canopy dynamics for steering changes in understorey community composition and
316 structure has already been put forward by multiple studies in the past (Axmanová et al., 2011; Depauw et al.,
317 2020; Landuyt et al., 2020). Most of these studies based their conclusions upon relationships between
318 understorey community composition and overstorey canopy cover or openness. We found similar
319 relationships emerging in the opportunistic resurvey database and orthogonally-distributed resurvey study.
320 An increase in light availability over time (as a result of canopy opening due to natural disturbances, tree
321 mortality or management) gave rise to a taller understorey community, with a higher cover. These findings
322 are in line with the literature. Biomass production, which is closely related to plant height and cover
323 (Heinrichs et al., 2010), is promoted when the availability of light (generally the most limiting resource for
324 temperate forest understoreys) increases (Landuyt et al., 2020). Surprisingly, we did not detect a direct
325 response to light addition in the experiment. We can think of two potential explanations for this discrepancy
326 between our observational and experimental findings. First of all, light addition levels in the experiment are
327 subtle compared to light dynamics following canopy changes observed in the field. Being able to detect
328 understorey responses to subtle changes in light availability probably requires more replicates and/or more
329 precise measurements. We expect that more elevated light addition levels will induce more pronounced
330 understorey responses, also in experimental settings (see e.g. De Pauw et al., 2022). Second, discrepancies
331 between observational and experimental findings can also be explained by the fact that light availability
332 changes in the field are strongly confounded with other changes in resource availability and growing
333 conditions at the forest floor, which was not the case in the experiment. Canopy opening in the field leads to
334 increases in light availability, but also increases in microclimate temperature, vapour pressure deficit,
335 throughfall water, etc. (Von Arx et al., 2013; Zhang, Landuyt, et al., 2022; Zhang, Verheyen, et al., 2022). Our
336 findings across investigative approaches might therefore also suggest that the response of understorey
337 communities to canopy opening cannot be reduced to a simple light response, but should be interpreted as
338 a response to a combination of micro-environmental changes induced by canopy opening.

339 *4.2. Warming effects and interactions with changes in light availability*

340 The absence of any interaction effects between macroclimate warming and changes in light availability
341 suggests that the effect of macroclimate warming does not increase when the canopy opens up over time.
342 This finding contradicts our expectation but can potentially be explained by water availability dynamics
343 (which we could not quantify) following changes in canopy openness. Microclimate warming following
344 canopy opening might introduce drought stress due to increases in vapour pressure deficit, which may
345 counteract plant growth-enhancing effects of temperature increases. In contrast, the open-top-chambers,
346 that were used to mimic microclimate warming in the experiment, do not induce drought stress (in contrast,
347 a small but significant increase in soil water availability and a decrease in vapour pressure deficit inside OTCs
348 has been reported by De Frenne and colleagues (2010)). As a result, clear warming effects as detected in the
349 experiment (for herbaceous cover, specifically) might not be that clear or even reverse in the field, when
350 water availability effects might be at play as well. Indeed, based on the database study, macroclimate
351 warming was found to have a negative effect on CWM SLA, plant height and total herbaceous cover, which
352 seems to contradict previous findings (Bjorkman et al., 2018; Chelli et al., 2019; Vanneste et al., 2019). A
353 second potential explanation for undetected interaction effects in the two resurvey studies might be the
354 relatively small variability in Δ MAT among sites (especially when compared to the variability in N deposition
355 rate changes and canopy openness changes). This low variability lowers the statistical power to detect
356 responses to warming (which were only detected in the opportunistically-distributed resurvey database,
357 characterized by a large sample size) as well as interactions between warming and canopy opening (not
358 detected).

359 *4.3. Nitrogen deposition effects and interactions with changes in light availability*

360 Interaction effects from a combination of changes in light availability and changes in N deposition rates were
361 again found to be insignificant across methodological approaches. Absence of interaction effects might be
362 related to the fact that changes in N deposition on top of the forest canopy do not necessarily reflect changes
363 in N availability at the forest floor. The relationship between N deposition on top of the canopy and
364 throughfall N differs depending on the degree of canopy opening. While small gaps often increase canopy
365 roughness and, hence, dry deposition of N and throughfall N, large gaps may decrease dry deposition due to
366 a decrease of the canopy's total leaf area and, thus, contact surface (Erisman & Draaijers, 2003). These
367 aspects likely complicate the identification of interactions between N deposition and light availability (driven
368 by canopy openness) in our analysis. However, in the experimental study, where we eliminated potential
369 confounding with changes in the overstorey, we did not detect clear effects of N addition either. For this
370 specific dataset, high levels of background N deposition (> 20 kg N/ha.y, see also Fig. 2) may have biased our
371 findings as both treated and control plots probably experienced high levels of N deposition.

372 Although we did not detect interaction effects, we did detect a few main effects of N deposition changes
373 over time. CWM SLA was found to increase with increasing N deposition (based on the orthogonally-
374 distributed resurvey study), while cover was found to decrease (based on the opportunistic resurvey

375 database). Previous studies mainly reported increases of nitrophilous species at the expense of N-efficient
376 species, leading to declines in species richness and biotic homogenization at the stand or landscape scale
377 (Bobbink et al., 2010; Gilliam et al., 2016; Staude et al., 2020). Our detected increase of CWM SLA might be
378 related to that because nitrophilous species are often competitive species, characterized by a higher SLA
379 (Westoby, 1998). In addition, declines in understorey cover, density or biomass have been reported in
380 experiments in North America (Gilliam et al., 2016) and China (Lai et al., 2018). These negative effects on
381 plant performance (generally following a short period of positive growth responses due to enhanced nutrient
382 availability) have been hypothesized to be related to soil acidification (mainly because high N deposition
383 coincided with high acidifying sulphur deposition in the past) and increased N and base cation leaching
384 following N saturation in temperate forest ecosystems (Bobbink et al., 2010; Gilliam, 2006).

385 4.4. Novel insights from integrating methodological approaches

386 The most striking observation of this synthesis study is that findings differ depending on the investigative
387 approach. Although this might sound discouraging, it actually stresses the need to consider approaches in
388 concert to acquire a full picture of processes underlying plant responses to global change.

389 First of all, our analysis clearly shows that findings from experimental studies can complement findings based
390 on observational field work. The warming effects detected in the experimental study (on understorey cover
391 and CWM SLA) did not reflect macroclimate warming effects based on observational data. This is likely
392 because changes in microclimate temperature in the field covary, not only with macroclimatic changes, but
393 also (and probably more strongly) with changes in canopy cover (Zellweger et al., 2019). In other words,
394 experiments are helpful to detect drivers that act at the local scale and that directly steer understorey
395 community development, ruling out numerous indirect effects of global-change drivers, as occurring in the
396 field. Field observation, on the other hand, are more robust to report and predict actual changes in the
397 understorey, which is not the case for experiments which are often only maintained for a short period, and
398 where growing conditions and especially the imposed treatments are artificial.

399 Second, our analysis also shows striking differences between the two observational approaches
400 (opportunistic database and orthogonally-distributed resurvey) that actually only differ in terms of data
401 collection protocol and plot network design. The more targeted and detailed measurements, as carried out
402 in the orthogonally-distributed resurvey, should have reduced the amount of noise in the data and definitely
403 reduced confounding among global-change drivers, in comparison to the database study. Our analysis,
404 however, suggests that this investment in data quality does not always compensate for the loss in terms of
405 data quantity. Indeed, many of the trends that emerged from the opportunistic database could not be
406 detected based on the data from the orthogonally-distributed resurvey. The latter approach is probably more
407 suitable to investigate hypotheses that require very specific plot network designs and measurement
408 protocols.

409 **Conclusion**

410 Our findings suggest that small-scale forest disturbances or management activities (e.g. single tree mortality
411 or harvest) will probably not intensify functional understorey responses to global change in contrast to what
412 has been found in previous studies focusing on compositional responses. Irrespective of global change,
413 however, these small-scale disturbances will affect the functional composition of the understorey, by altering
414 resources and growing conditions at the forest floor. Whether our findings also hold for more severe canopy
415 disturbances remains to be studied. By combining evidence from multiple investigative approaches, we found
416 that large datasets are needed to detect understorey responses to regional-scale drivers of change and that
417 increasing data quality at the expense of data quantity does not necessarily increase our ability to detect
418 potential drivers of change. Given that experimental findings deviated from observational findings, our
419 analysis also demonstrates that understorey responses to regional-scale warming and changes in N
420 deposition (as detected by resurvey studies) may be different from understorey responses to these drivers
421 at the forest floor (as detected by experiments).

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435 **Author contribution statement**

436 DL, MPP and KV conceived the idea of this study; HB, LD, SLM, EL and MPP coordinated the experimental
437 setup and data collection campaigns, DL lead the statistical analysis and writing with significant contributions
438 from MPP, HB, LD, EDL, EL, SLM and KV. All other authors contributed historical understorey vegetation data
439 and/or supported field data collection campaigns. All authors commented on earlier drafts of the manuscript
440 and gave final approval for submission.

441 **Data availability**

442 The data that support the findings of this study are openly available in figshare at
443 <http://doi.org/10.6084/m9.figshare.24598458> (Landuyt et al., 2023)

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648 **Tables**

649 *Table 1*

650 Overview of the datasets and dataset-specific terms included in the mixed-effects models, using lme4 R-
 651 syntax (Bates et al., 2015). Abbreviations refer to soil phosphorous (P), soil carbon (C), soil nitrogen (N),
 652 Ellenberg Indicator Values (EIV N and EIV R), litter quality (LQ), light transmittance (LT), nitrogen deposition
 653 (Ndep), and mean annual temperature (MAT). Baseline refers to the CWM SLA, CWM plant height or herb
 654 cover at the initial survey date or at the start of the experiment. 'Δ' refers to absolute changes in the
 655 respective drivers between survey dates, '+' signs in between random intercepts indicate crossed random
 656 effects.

Dataset	Main effects	Interaction effects	Covariates	Random structure
<i>Manipulative global change experiment</i>	Light + Nitrogen + Warming	+ Light:Nitrogen + Light:Warming	+ Baseline + pH + Olsen P + C:N	+ 1 Region + 1 Community + 1 Plot
<i>Orthogonally-distributed resurvey</i>	ΔLT + ΔNdep + ΔMAT	+ ΔLT:ΔNdep + ΔLT:ΔMAT	+ Baseline + Time interval + pH + Olsen P + C:N	+ 1 Region
<i>Opportunistic resurvey database</i>	ΔLT + ΔNdep + ΔMAT	+ ΔLT:ΔNdep + ΔLT:ΔMAT	+ Baseline + Time interval + EIV R + EIV N + LQ	+ 1 Region

657

658 *Table 2*

659 R-squared values for all fitted models. LRR refers to log response ratio, CWM to community weighted mean
 660 trait values, SLA to specific leaf area, and R²m and R²c to marginal and conditional R² values respectively,
 661 calculated based on Nakagawa and Schielzeth's r-squared calculation for mixed models.

Response	Manipulative global change experiment		Orthogonally-distributed resurvey		Opportunistic resurvey database	
	R ² m	R ² c	R ² m	R ² c	R ² m	R ² c
LRR CWM SLA	0.39	0.55	0.43	0.63	0.45	0.54
LRR CWM plant height	0.23	0.51	0.34	0.66	0.30	0.45
LRR total herb cover	0.35	0.49	0.18	0.38	0.23	0.54

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663

664 **Figures**

665 *Figure 1*

666 Illustration of the applied approaches to investigate understorey responses to global change: understorey
667 resurvey studies (a) and manipulative mesocosm experiments (b). The photos in panel (a) illustrate a classical
668 resurvey method, where plant species composition is recorded in the same plot at two points in time, here
669 shown as typical views in southern Sweden in 1938 and 1989 (courtesy of Jörg Brunet). Picture (b) shows an
670 experimental understorey plot in the Aelmoeseneie forest, near Ghent, Belgium where light is added to
671 mimic changes in canopy cover (courtesy of Haben Blondeel).

672 *Figure 2*

673 Spatial distribution of all studied observational and experimental plots within the West-European temperate
674 forest biome (a) and studied gradients of global-change drivers (b) based on own below-canopy
675 measurements (for the experiment) and on data extracted from CRU TS V4.05 (mean annual temperature
676 data) and EMEP RV4.42 (nitrogen deposition data) for the year 2016 (for the two resurvey approaches). Light
677 availability (transmittance) was estimated based on PAR measurements or estimated based on overstorey
678 canopy cover and/or composition records (see S3). Nitrogen deposition was extracted from EMEP as the total
679 sum of wet and dry deposition of inorganic nitrogen. Mean annual temperature was calculated for the period
680 2007-2016. The colour legend at the bottom-left also applies for the environmental gradient panels at the
681 right. Map lines delineate study areas and do not necessarily depict accepted national boundaries.

682 *Figure 3*

683 Distribution of all response variables: log response ratios of community weighted mean specific leaf area (a),
684 community weighted mean plant height (b) and total herb cover (c) in the three considered datasets. Log
685 response ratios were calculated as the natural logarithm of the community characteristic (CWM SLA, CWM
686 plant height or total herb cover) at the resurvey divided by the community characteristic at the initial survey
687 (i.e. the vegetation survey directly after planting for the experiment). Positive log response ratios refer to
688 increases of the considered community characteristic, while negative values refer to a decrease over time.
689 Note differences in x-axis scales.

690 *Figure 4*

691 Coefficients of the fitted mixed-effects models, separately fitted to standardized data from the manipulative
692 global-change experiment (1st column), the orthogonally-distributed resurvey (2nd column) and the
693 opportunistic resurvey database (3rd column). n denotes the number of experimental units in the
694 experimental study, and the number of vegetation plots for the other two investigative approaches. Open
695 circles denote insignificant slope estimates, while closed circles ($p < 0.05$), triangles ($p < 0.01$) and squares
696 ($p < 0.001$) denote significant slope estimates. Although we included baseline community characteristics

697 (CWM plant height, SLA and herb cover at the initial survey) and a set of covariates as predictors, we excluded
698 the respective slope estimates from the graph. LT refers to light transmittance, MAT to mean annual
699 temperature, Ndep to nitrogen deposition, and Δ refers to the change of these drivers between the initial
700 survey and the resurvey.