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1 **Evaluating plant lineage losses and gains in temperate forest understories: a phylogenetic**  
 2 **perspective on climate change and nitrogen deposition**

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110 S11.1; Table S11.2;)

111 **Summary**

112

113 • Global change has accelerated local species extinctions and colonizations, often resulting  
114 in losses and gains of evolutionary lineages with unique features. Do these losses and  
115 gains occur randomly across the phylogeny?

116 • We quantified (1) temporal changes in plant phylogenetic diversity and (2) the  
117 phylogenetic relatedness of lost and gained species in 2,672 semi-permanent vegetation  
118 plots in European temperate forest understories resurveyed over an average period of 40  
119 years.

120 • Controlling for differences in species richness, phylogenetic diversity increased slightly  
121 over time and across plots. Moreover, lost species within plots exhibited a higher degree  
122 of phylogenetic relatedness than gained species. This implies that gained species  
123 originated from a more diverse set of evolutionary lineages than lost species. Certain  
124 lineages also lost and gained more species than expected by chance, with Ericaceae,  
125 Fabaceae, and Orchidaceae experiencing losses and Amaranthaceae, Cyperaceae, and  
126 Rosaceae showing gains. Species losses and gains displayed no significant phylogenetic  
127 signal in response to changes in macroclimatic conditions and nitrogen deposition.

128 • As anthropogenic global change intensifies, temperate forest understories experience  
129 losses and gains in specific phylogenetic branches and ecological strategies, while the  
130 overall mean phylogenetic diversity remains relatively stable.

131

132 **Keywords:** biodiversity change; biogeography; forestREplot; global change; phylogeny; plant  
133 functional traits; time lag; vegetation resurvey

## 134 **Introduction**

135  
136 Changes in global environmental conditions are increasingly modifying biodiversity and  
137 associated ecosystem functions (Haddad *et al.*, 2015; IPBES, 2019). Understanding the processes  
138 that underlie spatiotemporal changes in the composition of ecological communities helps us  
139 predict the impacts of global change drivers (Purschke *et al.*, 2013; Letten *et al.*, 2014; Chai *et*  
140 *al.*, 2016). Because ongoing environmental changes tend to favor species with particular traits  
141 that are suited to the new environmental conditions (Keddy, 1992; Díaz *et al.*, 1998), and  
142 because traits partly reflect species' shared evolutionary histories (Losos, 2008), global change  
143 drivers can also shift the phylogenetic diversity and relatedness of ecological communities  
144 (Webb *et al.*, 2002; Gerhold *et al.*, 2015). Understanding how current environmental changes  
145 affect various branches of the tree of life thus helps us identify which phylogenetic lineages,  
146 reflecting distinct evolutionary histories and functional roles, are most likely to expand or go  
147 extinct within local communities.

148         Temporal shifts in community composition reflect local colonizations, extinctions, and  
149 population dynamics (Magurran & Henderson, 2010). The amount of evolutionary history lost  
150 and gained following extinction and colonization events depends on the rate of evolution of  
151 ecological traits (Cavender-Bares *et al.*, 2004). Because traits related to environmental tolerances  
152 are often phylogenetically conserved (Hawkins *et al.*, 2014; De Pauw *et al.*, 2021; Harris *et al.*,  
153 2022a), environmental changes are likely to promote the extinctions of close relatives (Vamosi &  
154 Wilson, 2008; Eiserhardt *et al.*, 2015). In such cases, lost species would represent distinct  
155 phylogenetically clustered subsets of the habitat species pool and be phylogenetically different  
156 from persisting species. Environmental changes and human-mediated biological invasions can  
157 also enhance the colonization of species that are phylogenetically and functionally distant from  
158 the current residents (Mathakutha *et al.*, 2019). This phenomenon can be intensified by  
159 competitive exclusion, which tends to promote the establishment of species displaying  
160 functionally dissimilar characteristics compared to the resident species (Valiente-Banuet &  
161 Verdú, 2013; Li *et al.*, 2015). For instance, during vegetation succession, late successional plant  
162 colonizers represent a broad array of distantly related species with diverse traits (Li *et al.*, 2015).  
163 This diversity could stem from environmental alterations that augment the carrying capacity of  
164 local habitats, thereby facilitating the establishment of species with distinct functional traits

165 (Valiente-Banuet & Verdú, 2013). Although many studies have measured net changes in the  
166 phylogenetic diversity of local plant communities over time (e.g., Purschke *et al.*, 2013; Li *et al.*,  
167 2015; Barber *et al.*, 2016), few of these have evaluated whether environmental changes are  
168 associated with non-random gains and losses of phylogenetic diversity. Importantly, none of  
169 these studies have evaluated gains and losses in plant community phylogenetic diversity at the  
170 continental scale.

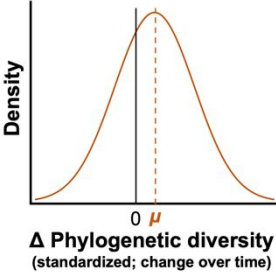
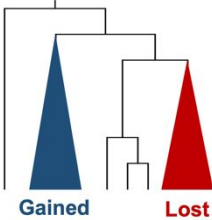
171 The understories of temperate forests support a variety of herbaceous plant species (Loidi  
172 *et al.*, 2021) that compete for the same resources and are susceptible to various global  
173 environmental changes, including climate change, land-use change, habitat fragmentation,  
174 biological invasions and atmospheric nitrogen deposition (Gilliam, 2007). Previous studies at the  
175 European scale found few systematic declines in understory plant species richness (Bernhardt-  
176 Römermann *et al.*, 2015; Perring *et al.*, 2018), consistent with studies of other terrestrial plant  
177 communities (Vellend *et al.*, 2013; Li *et al.*, 2020; Jandt *et al.*, 2022). Nevertheless, temporal  
178 turnover in forests is rarely ecologically random (Kopecký *et al.*, 2012). In European forests,  
179 light-demanding species that tolerate low nitrogen availability and have small geographic ranges  
180 are gradually replaced by shade-tolerant species with higher nutrient requirements and larger  
181 ranges (Kopecký *et al.*, 2012; Staude *et al.*, 2020). Therefore, identifying non-random losses and  
182 gains of specific plant lineages within and across forest understories provides insights into the  
183 ecological strategies favored and disadvantaged by recent environmental changes.

184 Here, we quantify temporal changes in plot-level phylogenetic diversity and the  
185 phylogenetic relatedness of lost and gained plant species in the understory of European  
186 temperate forests resurveyed after an average period of 40 years. Additionally, we test how  
187 phylogenetic diversity and relatedness respond to changes in macroclimate and atmospheric  
188 nitrogen deposition, considering potential confounding factors. We also identify plant lineages  
189 that have experienced more species losses and gains than expected by chance. Finally, we  
190 explore the relationship between species' tendency to be lost or gained over time and their  
191 functional traits. To this end, we test six hypotheses (H1-H6) to understand how species co-  
192 existing in specific habitat types respond to recent environmental changes (Table 1).

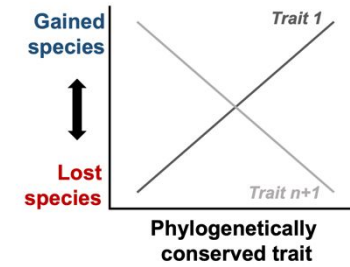
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194 Table 1. Proposed hypotheses with ecological mechanisms, empirical evidence, and graphical representations.  
195

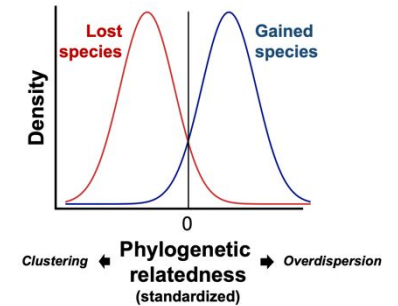
Hypothesis	Ecological mechanism/Empirical evidence	Graphical representation
(H1) Forest understories in temperate Europe have gained more phylogenetic diversity than expected from random species losses and gains	Lost species are phylogenetically clustered subsets of the habitat species pool (Vamosi & Wilson, 2008; Eiserhardt <i>et al.</i> , 2015), while gained species include distantly related species with diverse traits (Li <i>et al.</i> , 2015)	
(H2) Species' losses and gains across communities are not randomly distributed across the phylogeny	Environmental tolerances are often phylogenetically conserved (Cavender-Bares <i>et al.</i> , 2004; De Pauw <i>et al.</i> , 2021; Harris <i>et al.</i> , 2022a)	

(H3) Species' losses and gains across communities are associated with phylogenetically conserved plant traits and their related ecosystem functions (Keddy, 1992; Díaz *et al.*, 1998)



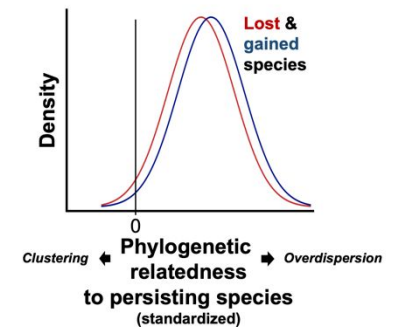
(H4) Lost species are more phylogenetically clustered than gained species within communities

Lost species share conserved traits that make them intrinsically vulnerable to extinction (Vamosi & Wilson, 2008; Eiserhardt *et al.*, 2015). Gained species include distantly related species with diverse traits (Li *et al.*, 2015)



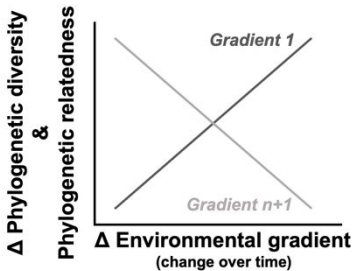
(H5) Lost and gained species are phylogenetically distantly related to persisting species

Abiotic filters exclude subsets of closely related species with ecological strategies distinct from those of persisting species (Eiserhardt *et al.*, 2015). Environmental changes and competitive exclusion create conditions that enable the establishment of species that are functionally dissimilar and distantly related to those already present in a community (Valiente-Banuet & Verdú, 2013; Li *et al.*, 2015)



(H6) Temporal changes in phylogenetic diversity and the phylogenetic relatedness of lost and gained species within communities are associated with changes in macroclimatic conditions and nitrogen deposition over time

Abiotic filters select for phylogenetically conserved ecological strategies (Keddy, 1992; Gerhold *et al.*, 2015)



196

197

or Peer Review

## 198 **Materials and Methods**

199

### 200 *Vegetation surveys*

201

202 We obtained resurvey data from the forestREplot database version 2.3  
203 ([www.forestreplot.ugent.be](http://www.forestreplot.ugent.be); Verheyen *et al.*, 2017). This database contains species occurrence  
204 and cover data from forest resurveys in permanent or semi-permanent plots in natural or semi-  
205 natural temperate forests throughout Europe and North America. Our study focused on European  
206 temperate broadleaved deciduous forests ( $\geq 25\%$  canopy cover of broadleaved trees in the  
207 baseline survey) that have been continuously forested since at least 1850 and have remained  
208 unaffected by any stand-replacing disturbances between the baseline survey and the last  
209 resurvey. Like most forests in Europe, the study sites were largely managed in the past. Plot sizes  
210 varied from 25 to 1,300 m<sup>2</sup> (mean  $\pm$  SD: 264  $\pm$  193 m<sup>2</sup>). We restricted our analyses to the  
211 understory vegetation layer (herbs and woody juveniles).

212 We selected 2,672 pairs of survey/resurvey plots from 64 datasets representing 14  
213 countries (Appendix S1). We standardized plant species names in the plots according to the  
214 World Flora Online taxonomic backbone, using the R package ‘WorldFlora’ (Kindt, 2020). We  
215 restricted this study to angiosperms to prevent inflated overdispersion due to gymnosperms and  
216 pteridophytes linked to deep phylogenetic nodes. Baseline surveys occurred between 1933 and  
217 1999, and resurveys between 1987 and 2020. The time interval between surveys ranged from 12  
218 to 72 years (mean  $\pm$  SD: 40  $\pm$  13 years). These plots contained a total of 1,152 angiosperm  
219 species.

220 To compare lineage losses and gains in plots with similar baseline and interval times, we  
221 restricted analyses related to H1, H2, and H3 to plots resurveyed after 2000 and  $\geq 20$  years after  
222 the baseline survey (N = 2,443 pairs of baseline survey and resurvey plots). By adopting this  
223 approach, we encompassed a significant period during which major environmental changes  
224 occurred in Europe. For example, the European State of the Climate (ESOTC) report for 2022  
225 highlighted that all of the ten warmest years on record for Europe have taken place since 2000  
226 (European Commission, 2022). These selected plots had baseline surveys between 1935 and  
227 1999 and resurveys between 2001 and 2020. This subset of plots contained 1,117 angiosperm  
228 species.

229

230 *Phylogeny*

231

232 We generated a phylogenetic tree for the 1,152 angiosperm species in our dataset with the R  
233 package ‘V.PhyloMaker2’ (Jin & Qian, 2022). This package uses an improved and expanded  
234 version of the mega-phylogeny reported by Smith and Brown (2018) as a backbone to construct  
235 phylogenies. To complete the phylogeny, we added missing species (~23% of the total species)  
236 and genera (~4% of the total genera) to the midpoint of their genus or family branch, respectively  
237 (‘Scenario 3’, Qian & Jin, 2016; Jin & Qian, 2022). A phylogeny created with this approach  
238 produces results comparable to a tree fully resolved at the species level if most genera and all  
239 families are resolved (Qian & Jin, 2021).

240

241 *Plant traits*

242

243 We obtained data on four plant traits known to respond to the biotic or abiotic environment (e.g.,  
244 climatic conditions or resource availability; Lavorel & Garnier, 2002) and to support a strong  
245 phylogenetic signal (Appendix S2). These traits were maximum plant height (H), seed mass  
246 (SM), leaf area (LA), and specific leaf area (SLA). These traits are also linked to various  
247 ecological functions, including dispersal, establishment, reproduction, and resource acquisition  
248 (Westoby, 1998; Díaz *et al.*, 2016; Appendix S2). Because our goal was to determine the  
249 potential traits and ecological strategies of understory plant species that relate to their tendency  
250 to be lost or gained over time, we assumed that the trait values of mature woody plants were  
251 indicative of potential, albeit unrealized, traits in juvenile plants. In other words, we regarded  
252 these traits as latent qualities that may not necessarily manifest in the surveyed individuals at the  
253 time of the study. The four traits were only weakly inter-correlated ( $|Spearman's \rho| \leq 0.45$ ;  
254 Appendix S3).

255 We obtained trait data from the TRY database ([www.try-db.org](http://www.try-db.org); Kattge *et al.*, 2020). We  
256 removed trait records that were  $> 4$  standard deviations from the species’ mean for each trait to  
257 avoid the potential effects of outliers (Bruehlheide *et al.*, 2018). We averaged intraspecific trait  
258 values per species when several trait values occurred for a given trait. Data for individual plant

259 traits (H, SM, LA, or SLA) were available for at least 70% of the species (Appendix S3), but  
260 only 34% of species had data for all four traits.

261

### 262 *Missing trait data imputation*

263

264 We imputed missing trait data with the R package ‘missForest’ (Stekhoven, 2022). This Random  
265 Forest method imputes species trait values using highly accurate phylogenetic information  
266 (Penone *et al.*, 2014) and fast computation times. We incorporated all phylogenetic eigenvectors  
267 (Debastiani *et al.*, 2020) derived from the R package ‘PVR’ (Santos, 2018) as predictor variables  
268 in the ‘missForest’ imputation alongside functional traits.

269

### 270 *Explanatory variables*

271

#### 272 Climate change variables

273

274 We obtained gridded climatic data from the Climatic Research Unit Time Series (CRU TS; v.  
275 4.06) of the University of East Anglia (Harris *et al.*, 2022b). The CRU TS data cover the global  
276 terrestrial surface from 1901 to 2021 and provide monthly climate data at 0.5° (~55 km at the  
277 equator) resolution. For each year between 1901 and 2021, we obtained data for computing three  
278 climatic variables: maximum summer temperature, minimum winter temperature, and annual  
279 precipitation. Selecting these variables accounts for the effects of seasonal extremes and broad  
280 climatic variation.

281 For maximum summer temperature, we averaged monthly maximum daily temperatures  
282 during June, July, and August. For minimum winter temperature, we averaged monthly daily  
283 minimum temperatures during December, January, and February. For annual precipitation, we  
284 summed the monthly precipitation over the year. We then computed the long-term mean values  
285 for these three variables by averaging the annual data for both the ten years before the baseline  
286 survey and the ten years preceding the resurvey (Bernhardt-Römermann *et al.*, 2015). This  
287 approach accounts for likely time lags in vegetation dynamics and how plant species adapt to  
288 long-term environmental changes (De Frenne *et al.*, 2013; Li & Waller, 2017). We then  
289 calculated changes in climatic conditions as the difference between the ten-year average values

290 preceding the baseline survey and the resurvey. We repeated the analyses considering variables  
291 capturing the effects of more extreme climatic conditions and obtained similar results (Appendix  
292 S4).

293

#### 294 Nitrogen deposition

295

296 We obtained cumulative dry and wet annual deposition of oxidized nitrogen (hereafter, N) from  
297 the EMEP database ([https://emep.int/mscw/mscw\\_moddata.html](https://emep.int/mscw/mscw_moddata.html)) at 0.1° (~11 km) resolution.  
298 Annual N deposition was only available for the 1990–2020 period. To obtain annual N  
299 deposition for the years before 1990, we used the correction factors for the different decades  
300 based on the deposition rates in the year 2000, as described in Duprè *et al.* (2010). To measure  
301 cumulative N deposition between surveys, we summed up N deposition data for all years  
302 between the baseline survey and the resurvey.

303

#### 304 Covariates

305

306 Because many variables potentially affect species diversity and dynamics, thus confounding our  
307 ability to detect particular effects, we included ten covariates in our models to account for their  
308 possible effects on community change (Simkin *et al.*, 2016; Perring *et al.*, 2018). These  
309 covariates include plot area; herb-layer species richness and cover from the baseline survey (cf.  
310 baseline plant community conditions); baseline climatic conditions (average maximum summer  
311 temperature, average minimum winter temperature, and annual precipitation over the ten years  
312 before the sampling); management (unmanaged vs. managed); the temporal change in tree  
313 canopy cover to capture changes in microclimate dynamics; current soil pH; and the time (in  
314 years) between surveys. We also considered cumulative N deposition estimated between 1901  
315 and the baseline, but this variable was strongly correlated with the time between surveys  
316 (Spearman's  $\rho = -0.88$ ), so we discarded it in the final models. We obtained raster data for  
317 present-day European soil pH at 15 cm depth (250 × 250 m resolution) from Poggio *et al.*  
318 (2021). We added these covariates to account for potential confounding effects with our main  
319 variables of interest: macroclimate change and atmospheric N deposition. We provide details of

320 the covariates, a full rationale for including them in the models, and their inter-correlations in  
 321 Appendix S5.

322

323 *Calculation of phylogenetic diversity (PD) and phylogenetic relatedness (PR) metrics*

324

325 To measure phylogenetic diversity (PD), we calculated Faith's PD, the sum of all phylogenetic  
 326 branch lengths connecting a set of taxa (Faith, 1992). Faith's PD is a phylogenetic generalization  
 327 of species richness that captures the evolutionary history of a species assemblage (Chao, 2010).

328 We calculated the change in PD over time in the plots as the log-transformed response ratio  
 329 (RR):

330

$$331 \quad RR_{PD} = \frac{\ln \frac{PD_{t+\Delta t}}{PD_t}}{\Delta t}$$

332

333 where  $PD_t$  is the value for PD at the time of the initial survey,  $PD_{t+\Delta t}$  refers to its value at the  
 334 time of the resurvey, and  $\Delta t$  is the number of years between surveys.

335 We calculated two measures of phylogenetic relatedness (PR) within and between species  
 336 assemblages at terminal phylogenetic levels. First, we calculated the Mean Nearest Taxon  
 337 Distance (MNTD; Webb *et al.*, 2002) to quantify the degree of phylogenetic relatedness of lost  
 338 and gained species in the plots. The MNTD is the mean branch length between each taxon and its  
 339 phylogenetically nearest neighbor. Second, we used the  $D_{nn}$  metric (Webb *et al.*, 2008) to  
 340 measure the degree of phylogenetic relatedness between the lost or gained species and those that  
 341 persisted in the plots. We calculated  $D_{nn}$  for each plot as the mean branch length between each  
 342 lost and gained species and its phylogenetically nearest neighbor in the pool of persisting  
 343 species. We also calculated PR at basal phylogenetic levels (i.e., the mean phylogenetic distance,  
 344 MPD, and  $D_{pw}$ ), but we obtained similar results and moved the results of these analyses to  
 345 Appendix S6. A summary table with the PD and PR metrics used in this study and their  
 346 relationship to the hypotheses is provided in Appendix S7. We calculated the PD and PR metrics  
 347 using the R package 'PhyloMeasures' (Tsirogiannis & Sandel, 2017).



348 To determine whether RR\_PD and PR values for each plot were higher or lower than  
349 expected if species were randomly distributed across the phylogeny, we compared the observed  
350 empirical values of these metrics to a null distribution derived from 999 random reshufflings of  
351 the species across the tips of the phylogenetic tree. These reshufflings included all the species  
352 listed within a focal dataset or study site, corresponding to all the species observed across all  
353 individual plots surveyed and resurveyed within the focal study site. This allowed us to estimate  
354 standardized plot-level effect sizes (SES) for each metric (i.e., RR\_PD.ses, MNTD.ses, and  
355  $D_{nn}$ .ses) as the difference between the observed empirical value and the mean of the random  
356 values divided by the standard deviation of the random values. These SES metrics are thus  
357 independent of changes in species richness in the plots. For each plot, positive SES values of  
358 RR\_PD.ses indicate that more phylogenetic diversity was gained than under random expectation,  
359 while negative SES values indicate that more phylogenetic diversity was lost than under random  
360 expectation. Positive SES values of PR metrics indicate that species were more distantly related  
361 than under random expectation (phylogenetic overdispersion), while negative SES values  
362 indicate that species were more closely related than under random expectation (phylogenetic  
363 clustering).

364 We also calculated the lost and gained PD in each plot as the PD of the set of taxa lost or  
365 gained at the site between the resurvey and the baseline survey. However, the standardized effect  
366 sizes of these measures (PD.ses) were highly correlated with MNTD.ses (Spearman's  $\rho > 0.95$ )  
367 and discarded from the main text (Appendix S8).

368

### 369 *Statistical analyses*

370

371 We used R v. 4.2.0 (R Core Team, 2022) for all analyses and set statistical significance at  $\alpha =$   
372 0.05.

373

### 374 Has the phylogenetic diversity of forest understories changed over time? (H1)

375

376 We compared the mean RR\_PD and RR\_PD.ses across plots to zero using Cohen's  $d$  (Cohen,  
377 1998) in the R package 'lsr' (Navarro, 2015). Cohen's  $d$  is a measure of effect size that, unlike  
378 the  $t$ -test statistic, estimates a population parameter and is unaffected by sample size. Effect sizes

379 are considered small around 0.2, medium around 0.5, and large around 0.8 (Cohen, 1998). This  
 380 approach allowed us to compare the magnitude of the difference between the mean values and  
 381 zero rather than testing for statistical differences between the mean values and zero.

382

383 Were particular plant lineages lost and gained over time across plots? (H2)

384

385 For each species ( $N = 1,117$ ), we calculated its tendency ( $U$ ) to be lost or gained in the plots as:

386

387

$$U = \frac{\sum_{i=1}^j S_i}{j}$$

388

389 where  $S$  is the species' response in plots  $i = 1$  to  $j$  (1 if the species was gained, 0 if the species  
 390 persisted, and -1 if the species was lost). A  $U$  value of -1 indicates that the species was lost from  
 391 all the plots where it occurred at the time of the baseline survey, while a  $U$  value of 1 indicates  
 392 that the species was not present at the time of the baseline survey in any of the plots where it  
 393 occurred at the time of the resurvey.

394

395 We evaluated the phylogenetic signal in  $U$  values with Pagel's  $\lambda$  (Pagel, 1999) in the R  
 396 package 'phytools' (Revell, 2012). Values of  $\lambda$  close to zero indicate phylogenetic independence  
 397 of  $U$  values. In contrast, values close to one indicate that  $U$  values co-vary directly with their  
 398 shared evolutionary history. We plotted the  $U$  values of each species in the phylogeny to identify  
 399 lineages with high or low  $U$  values. We also calculated for each node in the phylogeny whether  
 400 the observed mean  $U$  value of species descending from that node was higher or lower than  
 401 expected if species were randomly distributed in the phylogeny. To this end, we reshuffled the  
 402 tips of the phylogeny 999 times and recalculated the mean  $U$  values. Then, we compared the  
 403 observed mean  $U$  value of the node with the distribution of random mean  $U$  values to determine  
 404 a  $P$ -value based on the quantiles' null distribution. Specifically, we calculated  $P$ -values as the  
 405 proportion of random mean  $U$  values lower than the observed mean  $U$  value. Here,  $P$ -values  
 406 below 0.025 or above 0.975 indicated that the observed mean  $U$  value was significantly lower or  
 407 higher, respectively, than expected by chance.

407

408 Plot resurveys may inadvertently overlook certain species during the field sampling,  
 potentially leading to the misidentification of pseudo-colonizations and extinctions. These errors

409 can inflate  $U$  values of rare species (Verheyen *et al.*, 2018). Furthermore, species' absolute  $U$   
410 values exhibited a partial negative correlation with their occurrence frequency across plots  
411 (Pearson's  $r = 0.34$ ). To mitigate these issues and avoid spurious results associated with random  
412 species losses and gains, we recalculated  $U$  values considering species present in at least five ( $N$   
413 = 678 species) and ten ( $N = 534$ ) plots. This sensitivity analysis allowed us to evaluate the effect  
414 of rare species on the results and identify plant lineages that were lost and gained independently  
415 from species frequency across plots.

416

### 417 Are $U$ values associated with plant traits? (H3)

418

419 Species cannot be considered statistically independent in regression models because they are  
420 evolutionarily related (Ives & Zhu, 2006). We therefore applied Phylogenetic Generalized Least  
421 Squares (PGLS) regression in the R package 'caper' (Orme *et al.*, 2018) to test for associations  
422 between  $U$  values and plant traits (H, SM, LA, and SLA). The PGLS approach allows for  
423 flexibility in the underlying evolutionary assumptions and uses generalized least squares to  
424 explicitly include the predicted covariance among species into the model's fit. Models using  
425 ordinary least squares regressions yielded very similar results and are not presented. We log<sub>e</sub>-  
426 transformed all traits to improve normality. To test H2 and H3, we repeated these analyses,  
427 excluding juvenile trees, to evaluate whether tree recruitment associated with mature traits  
428 modified our results.

429

### 430 Does the phylogenetic relatedness of lost and gained species deviate from random expectations? 431 (H4 and H5)

432

433 We used Cohen's  $d$  to compare the MNTD<sub>ses</sub> and D<sub>nn</sub><sub>ses</sub> of lost and gained species across plots  
434 to zero (Cohen, 1998).

435

### 436 Have environmental changes driven alterations in phylogenetic diversity and relatedness? (H6)

437

438 We used linear mixed-effect models in the R package 'nlme' (Pinheiro *et al.*, 2022) to examine  
439 the relationships between plot-level temporal changes in the PD and PR metrics and the set of

440 explanatory variables. We treated the predictor variables of interest (i.e., changes in maximum  
441 summer temperature, minimum winter temperature, annual precipitation, and N deposition) and  
442 the covariates as fixed effects. In the models predicting temporal changes in PD, we did not use  
443 the time between surveys as a covariate because this variable was included in the denominator of  
444 the response and focal predictor variables. In all models, we included ‘dataset’ as a random  
445 intercept term to account for variation in residual variances among plots (Zuur *et al.*, 2007). To  
446 minimize dispersion in model residuals, we log<sub>e</sub>-transformed plot area. We standardized and  
447 centered all explanatory variables before model fitting to obtain comparable coefficients (Zuur *et al.*  
448 *et al.*, 2007). We complemented these analyses with models that separately predicted species’  
449 losses and gains within plots (Appendix S8). The variance inflation factor (VIF), calculated  
450 using the R package ‘usdm’ (Naimi *et al.*, 2014), indicated low multicollinearity among  
451 explanatory variables (VIF < 3).

452

## 453 **Results**

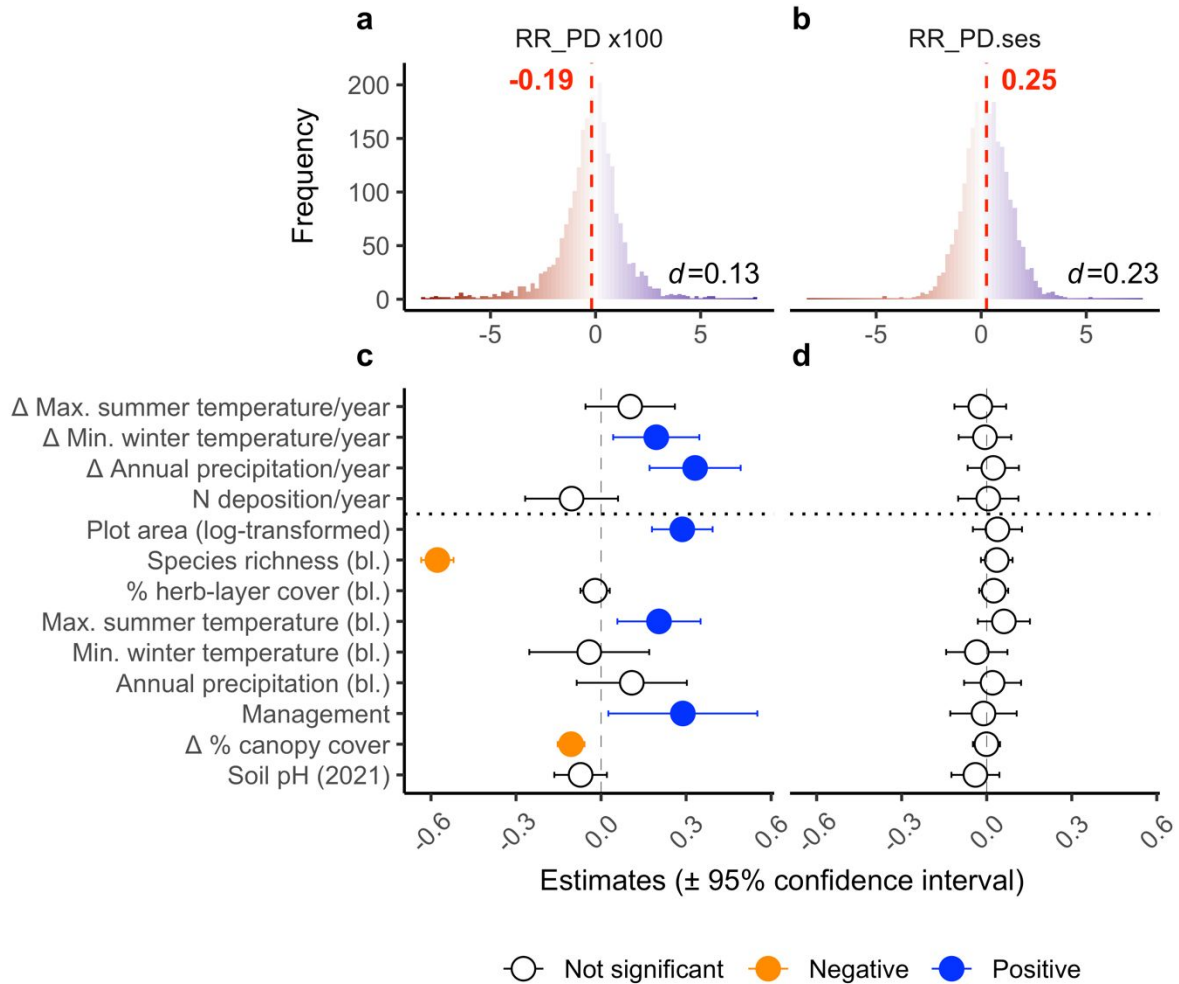
454

### 455 *Temporal changes in the phylogenetic diversity of forest understories (H1)*

456

457 Forest understories experienced a slight decrease in overall phylogenetic diversity (PD; Fig. 1a;  
458 Cohen’s  $d = 0.13$ ). However, after controlling for changes in species richness across plots by  
459 randomizing the evolutionary relationships among species, forest understories had a small  
460 overall increase in PD (Fig. 1b; Cohen’s  $d = 0.23$ ).

461



462

463 **Fig. 1:** Temporal changes in phylogenetic diversity (PD) in temperate forest understories and  
 464 their environmental predictors. **a,b**, Histograms of the change in PD between the baseline survey  
 465 and the resurvey estimated using unstandardized (RR\_PD) (a) and standardized (RR\_PD.ses) (b)  
 466 response ratios. The dashed red lines show mean PD values. Cohen's  $d$  measures the effect size  
 467 of the difference between the mean and zero. **c,d**, Standardized estimated coefficients ( $\pm$ 95%  
 468 confidence intervals) of focal variables and covariates predicting temporal changes in RR\_PD (a)  
 469 and RR\_PD.ses (b) from linear mixed-effect models. The dotted horizontal line separates focal  
 470 predictors (top) from covariates (bottom). Max. = maximum; Min. = minimum; bl. = baseline.

471

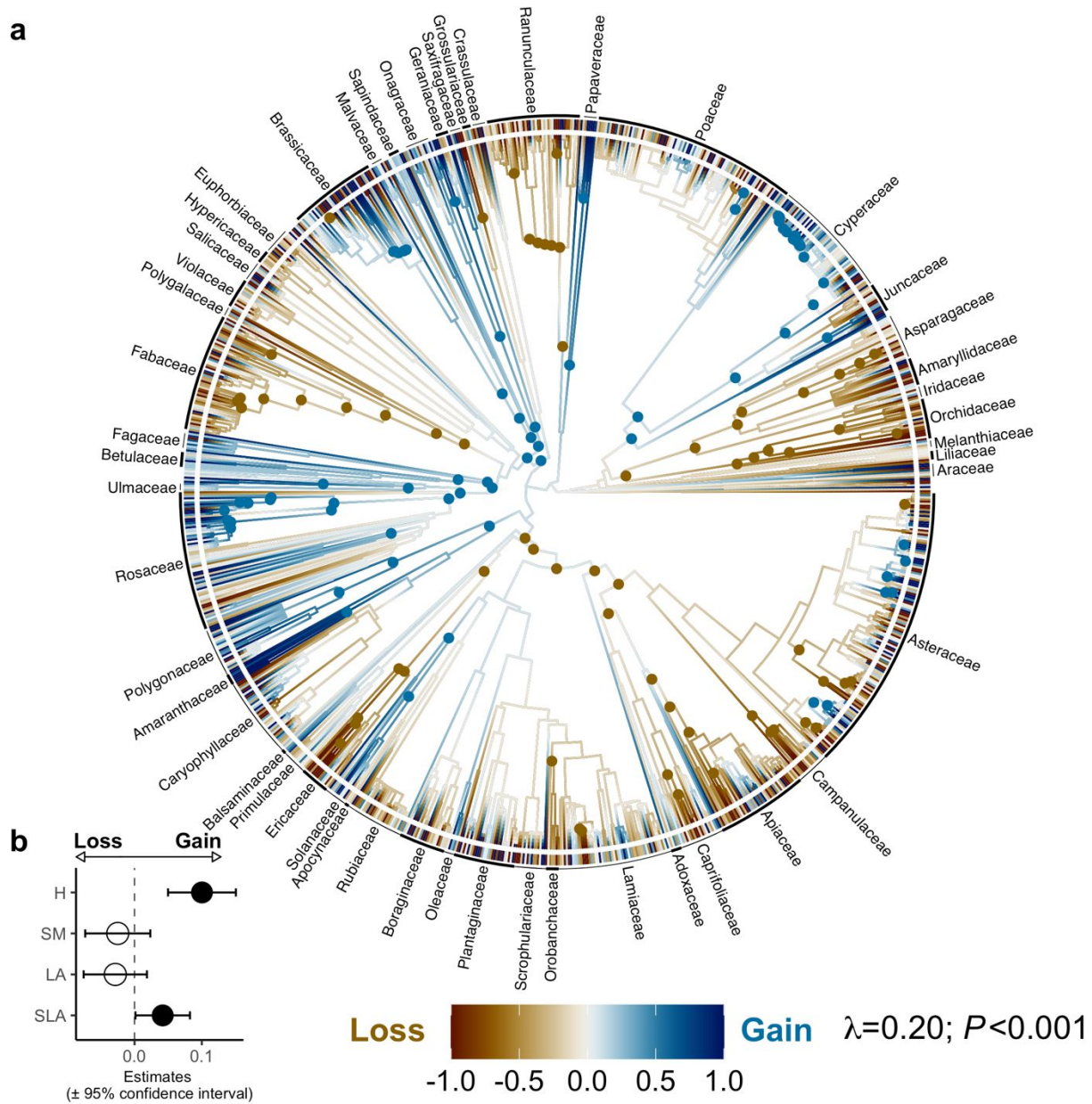
472 *Lost and gained lineages across plots (H2) and their association with plant traits (H3)*

473

474 Species' tendencies to be lost or gained (i.e., their  $U$  values) showed a slight but significant  
475 phylogenetic signal (Pagel's  $\lambda = 0.20$ ;  $P < 0.001$ ). The  $U$  values for individual species varied  
476 considerably (Fig. 2a; Appendix S9). Roughly 14% of the internal nodes in the phylogeny  
477 experienced either more losses (7%) or gains (7%) in descendant species than randomly  
478 expected. For example, species from the Apiaceae, Ericaceae, Fabaceae, Orchidaceae, and  
479 Orobanchaceae families were more likely to be lost, while species in the Amaranthaceae,  
480 Cyperaceae, Rosaceae (subfamily Amygdaloideae), and Urticaceae were more likely to be  
481 gained (Fig. 2a; Appendix S10). The observed trends among these families remained consistent,  
482 regardless of whether all species or only the most common ones were considered (Appendix  
483 S11). Among the tested traits, plant height (H) and specific leaf area (SLA) were positively and  
484 significantly related to species'  $U$  values (Fig. 2b). This effect was still significant after removing  
485 juvenile trees from the analysis (Appendix S12).

486





487

488 **Fig. 2:** Species tendency to be lost or gained in temperate forest understories and associated  
 489 traits. **a**, Distribution of  $U$  values of plant species across the phylogeny. Positive (blue) and  
 490 negative (brown)  $U$  values indicate species and lineages gained or lost over time, respectively.  
 491 Points in the phylogeny indicate nodes with  $U$  values higher (blue) or lower (brown) than  
 492 expected if species were randomly distributed across the phylogeny. Only significant nodes with  
 493 at least three species are shown. The 50 plant families with the largest number of species are  
 494 shown. **b**, Results from a Phylogenetic Generalized Least Squares (PGLS) regression predicting  
 495  $U$  values from plant height (H), seed mass (SM), leaf area (LA), and specific leaf area (SLA).

496 Black and empty circles denote significant and non-significant effects, respectively. Bars  
497 crossing the circles indicate 95% confidence intervals.

498

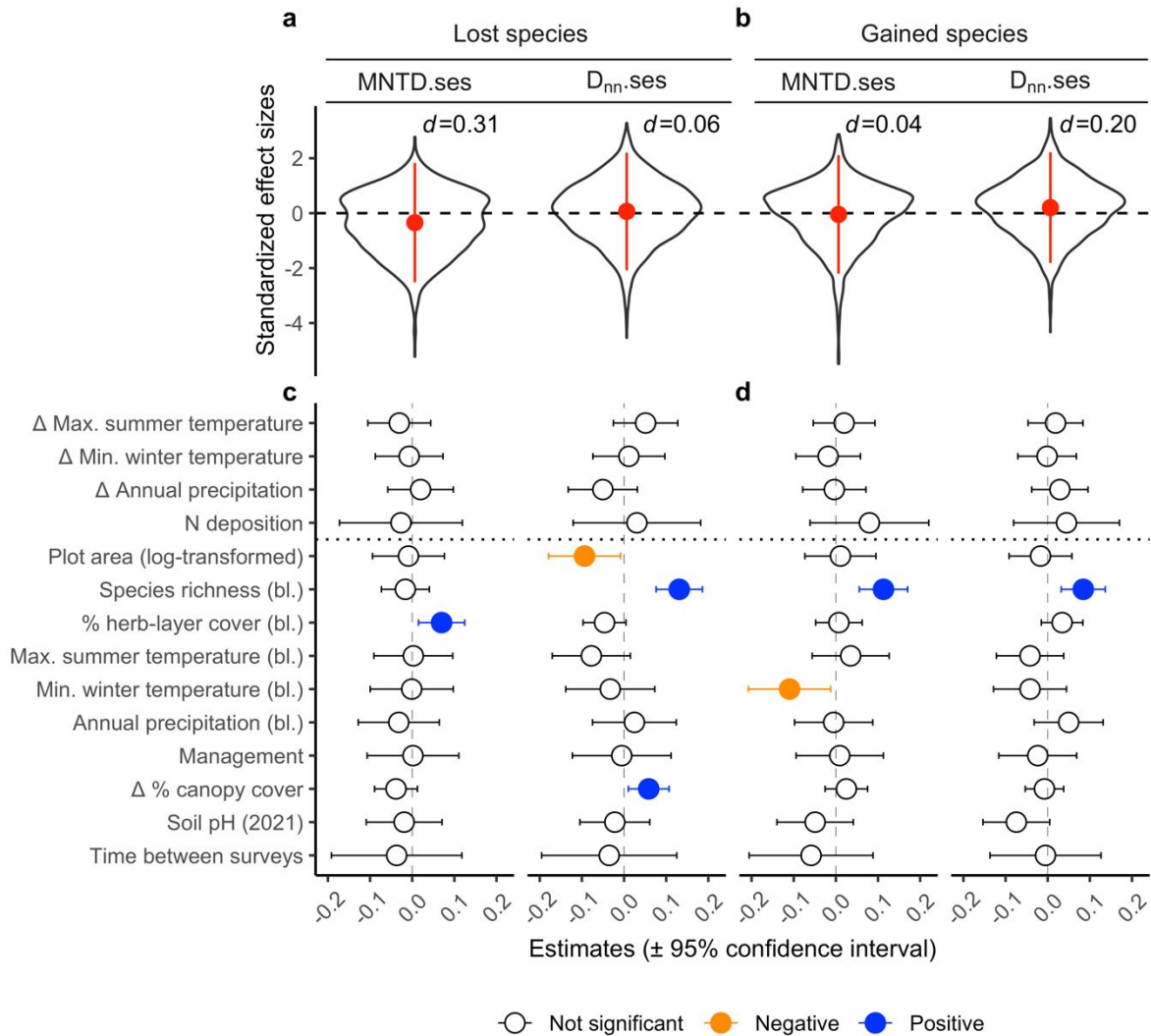
499 *Phylogenetic relatedness of lost and gained species (H4) and between lost or gained species and*  
500 *persisting species (H5)*

501

502 Both the phylogenetic relatedness (PR) of lost and gained species measured at the tips of the  
503 phylogeny (MNTD.ses) and between lost or gained species and persisting species ( $D_{nn.ses}$ ) were  
504 mostly random (Fig. 3a,b). However, lost species were, on average, more phylogenetically  
505 clustered (Cohen's  $d = 0.31$ ) than gained species (Cohen's  $d = 0.04$ ). When lost species were  
506 phylogenetically clustered (7% of plots), Poaceae were often overrepresented (Appendix S13). In  
507 contrast, gained species were, on average, more phylogenetically distantly related to the  
508 persisting species (Cohen's  $d = 0.20$ ) than lost species (Cohen's  $d = 0.06$ ). We found almost  
509 identical results when we measured PR metrics at basal phylogenetic levels (Appendix S6).

510





511  
 512 **Fig. 3:** Phylogenetic relatedness (PR) of (1) lost and gained species and (2) between lost or  
 513 gained species and persisting species in temperate forest understories, and their environmental  
 514 predictors. **a,b**, Violin plots showing PR of lost (a) and gained (b) species (MNTD.ses) and  
 515 between lost or gained species and persisting species ( $D_{nn}.ses$ ) in forest plots. Positive and  
 516 negative values indicate phylogenetic overdispersion and clustering, respectively. Red dots  
 517 represent median values, and bars indicate the 95% confidence interval. Cohen's  $d$  measures the  
 518 effect size of the difference between the mean and zero. **c,d**, Standardized estimated coefficients  
 519 ( $\pm$ 95% confidence intervals) of focal variables and covariates predicting PR metrics of lost (a)  
 520 and gained (b) species from linear mixed-effect models. The dotted horizontal line separates  
 521 focal predictors (top) from covariates (bottom). Max. = maximum; Min. = minimum; bl. =  
 522 baseline.

523

524 *Associations between phylogenetic metrics and environmental change variables (H6)*

525

526 Plots that experienced warmer winters and higher annual precipitation had a significant net  
527 increase in PD associated with an increase in species richness (Fig. 1c). When species' losses  
528 and gains were analyzed separately, plots that experienced increased precipitation lost less PD  
529 and species (Appendix S8). In contrast, plots that experienced warmer summers gained more PD  
530 and species. However, climate change and N deposition had no effect either on the standardized  
531 change in PD (Fig. 1d) or PR metrics for lost (Fig. 3c) and gained (Fig. 3d) species. Among the  
532 covariates, baseline species richness had the highest negative effect on the unstandardized  
533 change in PD, while management and plot size had the highest positive effects.

534

## 535 **Discussion**

536

537 We measured long-term shifts in plot-level phylogenetic diversity (PD) and the phylogenetic  
538 relatedness (PR) of lost and gained species in the understory of 2,672 vegetation plots in  
539 temperate forests in 14 European countries. Over periods that averaged 40 years, species of  
540 certain plant lineages (and their associated ecological strategies) persisted or increased while  
541 others declined. Within plots, species that colonized or went extinct were phylogenetically  
542 random subsets of the forest species pool, suggesting stochastic dynamics. However, lost species  
543 within plots exhibited a higher degree of phylogenetic relatedness than gained species. After  
544 accounting for differences in species richness across plots, changes in PD did not respond to  
545 climate change or nitrogen deposition, confirming that species are lost or gained randomly  
546 throughout the tree of life in response to environmental change.

547

548 *Temporal changes in phylogenetic diversity and its association with environmental change*  
549 *drivers*

550

551 We found that forest understories in temperate Europe have mostly experienced slight declines in  
552 PD due to a general reduction in species richness. This finding matches reports of few systematic  
553 shifts in plot-level plant species richness across continents (Bernhardt-Römermann *et al.*, 2015;

554 Jandt *et al.*, 2022) and globally (Vellend *et al.*, 2013). However, after accounting for differences  
555 in species richness among plots, we observed a slightly greater increase in PD than expected by  
556 chance (H1). This implies that the newly acquired species come from more diverse evolutionary  
557 lineages than the lost species, which were part of more phylogenetically clustered subsets of the  
558 habitat species pool. Accordingly, we also found that the lost species within plots were more  
559 closely related to each other than the gained species (H4). These findings suggest that, assuming  
560 niche conservatism, species that have been gained possess a broader range of ecological  
561 strategies than lost species (see evidence in Appendix S14). Nevertheless, this interpretation  
562 should be treated cautiously, as evolutionary lability may be a common feature across many  
563 ecologically relevant functional traits (Gerhold *et al.*, 2015).

564 Forest sites that experienced warmer winters and higher annual precipitation had greater  
565 increases in PD and species richness (H6). In contrast, neither changes in summer temperature  
566 nor N deposition significantly affected the unstandardized change in PD. Therefore, our findings  
567 indicate that diversity changes may increase at forest sites experiencing greater large-scale  
568 environmental changes. However, drought-induced losses of plant species can become more  
569 common under changing precipitation regimes and varying tree canopy structures (Archaux &  
570 Wolters, 2006). At a finer scale, increased canopy cover in forest plots (reducing light  
571 availability) promoted greater decreases in unstandardized PD over time. This result can explain,  
572 for example, the losses in light-demanding lineages such as Fabaceae and the gains in shade-  
573 tolerant woody lineages. Increased shading may also buffer the effects of a hotter and drier  
574 macroclimate (De Frenne *et al.*, 2013; Suggit *et al.*, 2018) by promoting greater thermal stability  
575 (Zellweger *et al.*, 2020).

576 Our findings also suggest that changes in environmental factors do not influence changes  
577 in PD in forest understories after accounting for differences in species richness (H6). This  
578 implies that losses and gains of specific lineages within local communities and along  
579 environmental gradients occur randomly across the phylogeny. Therefore, if species' ecological  
580 roles are phylogenetically conserved, then losses and gains of specific functions are independent  
581 of recent environmental changes in temperate forests. However, it is possible that filtered traits  
582 are not phylogenetically conserved or that their response to abiotic variation is nonlinear and  
583 may reach tipping points. Furthermore, other biotic and abiotic covariates not included in our  
584 models are also known to affect diversity changes. For example, the interaction between

585 environmental change and forest management practices can favor certain lineages (e.g., clades  
586 within Poaceae or Cyperaceae) through soil compaction (Mohieddinne *et al.*, 2022), canopy  
587 openness (Pilon *et al.*, 2020), and human-assisted dispersal (Closset-Kopp *et al.*, 2019). In  
588 addition, grazing pressure promotes different ecological strategies, particularly along the  
589 eutrophication gradient (Segar *et al.*, 2022).

590

### 591 *Plant lineage losses and gains across plots*

592

593 Despite almost no net change in PD, the likelihood of species being lost or gained between  
594 surveys across all plots depended on their lineage (H2). For example, species from the Fabaceae  
595 family, characterized by strong niche conservatism in their ability to fix atmospheric N, or from  
596 the Ericaceae family, which have adapted to survive in acidic and nutrient-deficient soils through  
597 ericoid mycorrhiza, were more likely to decline. These species groups are probably being  
598 outcompeted by species adapted to using nitrogen supplied by increased atmospheric N  
599 deposition (van Strien *et al.*, 2017; Berendse *et al.*, 2021). Accordingly, we found an increase in  
600 species with acquisitive leaf economics (i.e., higher SLA) across plots (H3). Additional plant  
601 lineages considered of high conservation value, e.g., Orchidaceae (CITES, 2019), or with unique  
602 ecological functions such as the holo- or hemiparasitic Orobanchaceae, have also experienced  
603 consistent declines. These results highlight that regional and global changes in environmental  
604 conditions threaten lineages with particular ecological strategies. The ecological strategies of  
605 plant families can provide insights into community dynamics and ecosystem functioning that are  
606 not immediately apparent when considering individual functional traits alone.

607 In contrast, in recent decades, nitrophilous weedy Amaranthaceae species, moisture-  
608 demanding Cyperaceae species, or woody Rosaceae species have increased across temperate  
609 forest understories. These findings parallel positive associations between species' tendency to be  
610 lost or gained (their  $U$  values) and their potential height and SLA (H3). These associations  
611 remained significant even when juvenile trees were removed from the analysis. Increased  
612 dominance of taller and nitrogen-demanding species in forest understories has already been  
613 documented in Europe (Kopecký *et al.*, 2012; Staude *et al.*, 2020) and may, in part, reflect how  
614 environmental changes interact with management legacies that alter soil resources (Perring *et al.*,  
615 2018). Increased recruitment of taller and more acquisitive species could alter forest vegetation

616 dynamics, ultimately reshaping ecosystem function. These results across plots and the  
617 phylogenetic signal in species' tendencies to be lost or gained underscore the importance of  
618 distinguishing between suppressed and colonizing species when assessing changes in the  
619 evolutionary history of ecological communities at the continental scale.

620 Although plant height and SLA significantly affected species' tendencies to be lost and  
621 gained, seed mass and leaf area did not (H3). These results suggest that relating other  
622 phylogenetically conserved traits to particular drivers of recent species extinctions and  
623 colonizations in temperate forests may be challenging. The overall metric of PD, however, also  
624 captures the functional diversity of hard-to-measure or unmeasured traits such as dispersal mode,  
625 pollination system, and root traits (e.g., mycorrhizal type and N-fixing symbioses), all known to  
626 affect extinction probabilities in other forest types (e.g., Sodhi *et al.*, 2008). The evolutionary  
627 history of a species is, therefore, a useful predictor of extinction risk, with greater risks often  
628 associated with either more diverse evolutionary branches or more recent speciation (Mace *et al.*,  
629 2003). For both extrinsic (i.e., environmental) and intrinsic (e.g., life-history trait) drivers of  
630 extinction, conserving communities with a high PD increases the probability that a broad  
631 combination of traits will persist under uncertain future conditions (Owen *et al.*, 2019).

632

633 *Phylogenetic relatedness of lost and gained species and its association with environmental*  
634 *change drivers*

635

636 Our results support the hypothesis that locally lost species are more phylogenetically clustered  
637 than gained species in forest understories (H4). Nevertheless, phylogenetic relatedness (PR) of  
638 locally lost and gained species in these forest understories was generally random. This finding  
639 complements previous studies showing non-random phylogenetic losses of woody species  
640 between glacial-interglacial cycles (Eiserhardt *et al.*, 2015) and non-random distributions of  
641 global extinction risk among angiosperms (Vamosi & Wilson, 2008). These results also suggest  
642 that traits that confer intrinsic susceptibility to extinction or the ability to colonize new  
643 environments may not necessarily be shared among closely related species in temperate forests.  
644 However, when lost species were phylogenetically clustered (7% of plots), Poaceae were often  
645 overrepresented (Appendix S13). Poaceae are thought to have originated at tropical forest edges  
646 and have since diversified based on their ability to withstand grazing, fire, and drought (Clayton,

647 1981). Hence, the reported loss of closely related grasses in a small subset of temperate forest  
648 understories could be related to a reduction in light availability triggered by canopy closure and  
649 rapid expansion of woody species.

650 The European forest understories that were studied generally exhibited random  
651 phylogenetic relatedness between the lost and gained species and persisting species (H5).  
652 Assuming phylogenetic niche conservatism (Losos, 2008), the functional similarity or  
653 dissimilarity of the lost and gained species to persisting species did not differ from what would  
654 be expected by chance. Notably, the gained species tended to be, on average, more distantly  
655 related to the persisting species than were the lost species. Previous studies of vegetation  
656 succession have demonstrated that late-stage colonizers comprise species from a wider diversity  
657 of lineages with various traits (Li *et al.*, 2015). This pattern may result from environmental  
658 modifications that reduce the strength of environmental filtering, increase the carrying capacity  
659 of local habitats, and facilitate the colonization of species with distinct functional traits  
660 (Valiente-Banuet & Verdú, 2013). Additionally, competition may exclude functionally similar  
661 species that occupy similar niches (i.e., competitive exclusion; Li *et al.*, 2015).

662 We found no evidence that the phylogenetic relatedness of lost and gained species varied  
663 along gradients of environmental change, as initially predicted (H6). The environmental filters  
664 imposed by recent macroclimate changes and N deposition may not be strong enough or have  
665 operated over sufficient time to exclude ecological strategies that increase intrinsic vulnerability  
666 to extinction. Alternatively, these strategies may not be phylogenetically conserved, or stochastic  
667 processes could account for many local extinctions.

668 Our study reveals that despite limited change in mean overall PD in temperate forest  
669 understories, substantial differences exist in the loss or gain of phylogenetic lineages in recent  
670 decades. Each lineage contains unique evolved features that play distinct ecological roles. We  
671 also found that regional shifts in macroclimate only affected changes in PD through changes in  
672 species richness. After controlling for differences in species richness within plots, we observed  
673 random losses and gains of species throughout the phylogeny across environmental gradients.  
674 Moreover, the fact that, within plots, lost species tend to be phylogenetically randomly related  
675 implies that close relatives may not share traits that confer intrinsic susceptibility to local  
676 extinction. Our results enhance our understanding of how shifts in environmental conditions

677 drive both subtle and profound changes in the phylogenetic and functional structure of forest  
678 plant communities.

679

#### 680 **Competing interests**

681

682 The authors have no competing interests to declare.

683

#### 684 **Author contributions**

685

686 JPC conceived the study with input from MC, JL, DL, and FM-C. JPC performed the analyses  
687 and wrote the first draft of the manuscript. All authors contributed substantially to revisions. LB,  
688 MB-R, MC, DC, GD, PDF, MD, TDi, TDu, RH, TH, BJ, MK, MM, FM, TN, AO, PP, RP, KR,  
689 WS, TS, KŚ, BT, KV, OV and MW contributed community composition data.

690

#### 691 **Data availability**

692

693 The data and code that support the findings of this study are available in Zenodo via  
694 <https://doi.org/10.5281/zenodo.8338171>.

695

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697

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