Individualistic responses of forest herb traits to environmental change

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Abstract

- Intraspecific trait variation (ITV; i.e. variability in the mean and/or distribution of plant attribute values within species), can occur in response to multiple drivers. Environmental change and land-use legacies could alter trait values within species directly, but also indirectly by changing the vegetation cover. Greater variability in environmental conditions could lead to more ITV but responses could differ among species. Disentangling these drivers on ITV is necessary to accurately predict plant community responses to global change.

- We planted herb communities into forest soils with and without recent agricultural history. Soils were collected across temperate European regions, while the fifteen selected herb species had different colonising abilities and affinities to forest habitat. These mesocosms (384) were exposed to two-level full-factorial treatments of warming, nitrogen addition and illumination. We measured plant height and specific leaf area (SLA).

- For the majority of species, mean plant height values increased as vegetation cover increased in response to light addition, warming and agricultural legacies. The coefficient of variation (CV) for plant height was larger in fast colonising species. Mean SLA for vernal species increased with warming, while light addition mainly decreased mean SLA for shade-tolerant species. Interactions between treatments were unimportant predictors.

- Environmental change treatments influenced ITV, either via an increasing vegetation cover or by affecting trait values directly. Species' ITV was individualistic, i.e. species responded to different single resource and condition manipulations that benefited their growth in the short term. These individualistic responses could be important for altered community organisation after a prolonged period.

1 Introduction

2 Functional traits are morphological, physiological or phenological traits which affect growth,

3 reproduction, and survival of plants (Violle *et al.* 2007; Funk *et al.* 2017). Variation in functional

4 traits related to leaf economics ("leaf"), growth ("height") and reproduction ("seed") has been

5 associated with trade-offs that plant species face to compete for resources across environmental

6 gradients (Westoby 1998; Borer *et al.* 2014; Reich 2014; Garnier *et al.* 2016; Díaz *et al.* 2016;

7 Beckman *et al.* 2018). While many have focused on trait variation between species and how different

8 environments influence this (Wright *et al.* 2017; Bruelheide *et al.* 2018), trait variation also occurs

9 within species (Siefert *et al.* 2015; Fajardo & Siefert 2018). This intraspecific trait variation (ITV)

10 can occur in response to environmental drivers (Albert *et al.* 2010; Jung *et al.* 2010), and is maintained

11 by processes of phenotypic plasticity and genetic differentiation (Valladares *et al.* 2006). Phenotypic

12 plasticity is an individual's plastic response to its environment, and its effect can be larger than that

13 of genetic adaptation in altering trait values of species at small spatiotemporal scales (Lajoie &

14 Vellend 2015, 2018; Turcotte & Levine 2016). Changes in mean trait values of species in response

15 to altered environments can ultimately affect variability in traits across populations, and thus

16 contribute to ITV (Moran *et al.* 2016). ITV greatly influences community patterns and processes

17 (Bolnick *et al.* 2011; Escudero & Valladares 2016), and accounting for these effects improves

18 predictions of plant communities in a changing world (Laughlin *et al.* 2012; Cadotte *et al.* 2015).

19 Evidence exists that competition for light enhances ITV in height and leaf trait values (Le Bagousse-

20 Pinguet *et al.* 2015; Bennett *et al.* 2016; Henneron *et al.* 2017). This is most evident in asymmetric

21 competition for light, where light as a resource is disproportionally removed by individuals that

22 manage to grow taller than their direct neighbours (Freckleton & Watkinson 2001). In dense plant

23 communities with high vegetation cover, larger growth of individuals can be achieved as long as

24 additional mineral nutrition and water are provided to sustain the increased growth (Watkinson 1983;

25 Begon *et al.* 1996; Freckleton & Watkinson 2001; Craine & Dybzinski 2013). Asymmetric

1 competition for light in such dense communities shapes the structure of canopies (Nagashima &

2 Hikosaka 2011) and the plant community as a whole (DeMalach *et al.* 2016). Plants that manage to

3 survive under the shade of the taller neighbours can alter leaf morphology to deal with photosynthesis

4 when light availability is low (Valladares & Niinemets 2008). A common plastic response of

5 individual plants is to increase the leaf area to leaf mass ratio (specific leaf area, SLA) to optimize

6 light capture for growth maintenance (Milla & Reich 2007; Valladares & Niinemets 2008; Liu *et al.*

7 2016; Smart *et al.* 2017). Plants can also compete for light that is inherently scarce, such as in

8 herbaceous communities at the forest floor under the shade of large canopy trees (Valladares *et al.*

9 2016). Sudden gaps in the tree canopy could then enhance light availability and photosynthesis in the

10 understorey at very short notice (Paul-Limoges *et al.* 2017). This change in light availability could

11 lead to quick plastic responses in plant height and SLA (Valladares *et al.* 2002) in synchrony with

12 shifts in community composition (De Frenne *et al.* 2015) and an increased community biomass

13 production (Jagodziński *et al.* 2016).

14 Apart from a varying light availability (Navas & Garnier 2002; Rozendaal *et al.* 2006; Lemke *et al.*

15 2015; Garnier *et al.* 2016; Burton *et al.* 2017), three main global change drivers are affecting

16 temperate forest herb ecology (Gilliam, 2007) and thus potentially ITV. First, climate warming can

17 alter growth and leaf trait values within herbaceous plants to resemble those of warmth-adapted

18 communities (Hoeppner & Dukes 2012; De Frenne *et al.* 2015; Helsen *et al.* 2017; Bjorkman *et al.*

19 2018; Henn *et al.* 2018). Second, nitrogen (N) deposition (Bobbink *et al.* 2010) can cause increasing

20 mean values of plant height and SLA by enhancing growth and increasing N content per dry mass of

21 plant tissues (Hejcman *et al.* 2012; Mao *et al.* 2017). Finally, elevated soil N and phosphorus (P)

22 concentrations stemming from agricultural land-use history can cause increases in productivity and

23 variability in functional leaf traits (Fraterrigo *et al.* 2009; Baeten *et al.* 2010, 2011; Siefert & Ritchie

24 2016). These drivers can interact to affect trait values in plant communities (Perring *et al.* 2016).

1   Plant functional traits can differ between species depending on how species capture resources (Poorter

2   *et al.* 2009; Garnier *et al.* 2016; Jagodziński *et al.* 2016; Mao *et al.* 2017). In forest herbs, there is

3   evidence that life-history traits and plant height are correlated to forest colonisation and specialisation

4   in forests habitat use (Verheyen *et al.* 2003). Herbaceous species that are confined to ancient forests

5   are typically small-statured slow colonisers that can photosynthesise in mature forests, either as

6   spring-flowering geophytes (Rothstein 2000; Tessier & Raynal 2003; Mabry *et al.* 2008) or as shade

7   tolerant plants that grow under fully-developed tree canopies (Valladares & Niinemets 2008). These

8   perennial ancient forest herbs consequently invest more nutrients into roots, bulbs, suckers or large

9   seeds for proliferation (Verheyen *et al.* 2003), but at a cost of slow dispersal (Klimešová *et al.* 2016).

10 Contrastingly, the ability of forest herbs to colonise post-agricultural forests correlates positively with

11 large relative growth rates and aging quickly (Verheyen *et al.* 2003), both plant characteristics

12 indicative of a "fast life-history" (Adler *et al.* 2014; Beckman *et al.* 2018). Such fast colonisers are

13 tall with light seed which allows further dispersal than species that are typical of ancient forest

14 (Verheyen *et al.* 2003; Thomson *et al.* 2011; Beckman *et al.* 2018). Fast colonising forest herbs

15 additionally have a high SLA and high leaf N content (Verheyen *et al.* 2003) which is a leaf

16 construction that favours resource acquisition over resource conservation (Wright *et al.* 2004; Adler

17 *et al.* 2014; Reich 2014; Díaz *et al.* 2016). Fast colonisers could thus benefit more from variation in

18 height and leaf traits than slow colonisers to quickly capitalise on resources in rapidly changing

19 environments (Alpert & Simms 2002).

20 Here we assess within-species responses of plant height and SLA to environmental change and land-

21 use legacies directly, but also indirectly by looking at change in vegetation cover. Furthermore,

22 responses could also differ between species and potentially link to colonisation capacity. We

23 performed a manipulative experiment with temperate herbaceous communities in forests comprising

24 species with varying colonisation capacities and affinities to forests habitat. This experiment allows

25 us to disentangle effects of enhanced light availability, warming, N addition and agricultural land-use

26 history on intraspecific variation in plant height and SLA. We specifically hypothesize that:

1. Within species, high vegetation cover increases mean plant height and SLA, likely due to asymmetric competition for light. Higher vegetation cover could stem from community growth due to nutrient enrichment (from either N addition or agricultural land-use history) in warmer and brighter conditions.

2. Within-species differences in mean plant height and SLA could result from variability in growing conditions induced by environmental changes and agricultural land-use history. The effect of these drivers can depend on each other. Concretely, we examine two-way interactions between enhanced light availability, warming, N addition and agricultural land-use history on changes of mean values in plant height and SLA.

3. Species with a good ability to colonise post-agricultural forests can exhibit a greater variability in trait values for both plant height and SLA. Since these species colonise novel environments, they need to cope with higher environmental unpredictability, and could potentially benefit from larger trait variation than species confined to ancient forests.

Material and Methods

We performed a full-factorial experiment with 384 experimental units (mesocosms) to disentangle interactive two-level effects of forest agricultural land-use history, enhanced light availability, nitrogen addition and warming on fifteen common European herbaceous forest species that differ in colonisation capacity and affinity to forest habitat (Figure 1). We measured plant height on 3445 individual plants and characterised SLA using 1125 leaf samples with measurements taken during the growing season of 2017, from the first week of March until the first week of September.

Land-use legacies: ancient and post-agricultural forest soils across environmental gradients

We collected soil (0.1 m³ in each forest) from eight temperate European regions across gradients of inherent soil fertility, regional phosphorus balance and nitrogen deposition (further details in Blondeel

1   *et al.* 2019). Regions spanned from Southwest Estonia (N 58 ° 8' 45.10", E 24° 47' 04.83") to Central

2   France (N 47° 50' 10.04",E 2° 45' 39.80") and included Southern Sweden (N 55° 32' 58.67", E 13°

3   14' 28.11"), Northwest Germany (N 53° 20' 25.12", E 9° 25' 17.34"), Northeast Germany (N 53° 13'

4   41.41", E 12° 07' 30.06"), Northern Belgium (N 51° 00' 00.30", E 4° 20' 17.13"), Southern Belgium

5   (N 50° 03' 56.05", E 4° 22' 01.54") and Northern France (N 49° 53' 42.65", E 2° 18' 41.36"). The

6   collected soils were classified in three groups using cluster analysis according to inherent soil fertility

7   (texture and calcareous properties). These soil types were either i) "Eutrophic" which are rich in clay

8   and carbonates with high pH (6.6-7.1), ii) "Oligotrophic which are high in sand and low in pH (4 –

9   5) or iii) "Mesotrophic" with intermediate fertility, siltier textures and intermediate pH (5-5.6) (see

10   Blondeel *et al.,* 2019). These soil groups are relative terms to categorise inherent soil fertility within

11   our samples, and should not be used to compare outside of our population. We use this categorical

12   soil type variable as a covariate when testing variation in mean plant height and mean SLA in response

13   to interactive environmental changes (see section "Data analysis").

14   To enable a comparison by agricultural land-use history, we searched for three adjacent pairs of

15   ancient and post-agricultural broadleaved forest with similar canopy composition within each region,

16   leading to 48 forest patches included in the study (Blondeel *et al.* 2019). We searched for pairs of

17   forest patches where one patch was in existence before 1850 (ancient forest) and the other established

18   in mid-20th century (post-agricultural forest). The agricultural legacies in the post-agricultural forest

19   soils varied along gradients of inherent soil characteristics, regional P balance and N deposition

20   (Blondeel *et al.* 2019). Overall, the soils from post-agricultural forest had higher pH (+0.4 ± 0.16

21   standard error (se)), lower C:N (-1.49 ± 0.24 se) , higher total P concentration (+118 mg kg^{-1}± 60 se)

22   and higher Olsen P concentration (+13 mg kg^{-1} ± 5.5 se) compared to ancient forest (Blondeel *et al.*

23   2019).

24   We collected a large volume of soil (ca. 0.1 m³) in each forest patch from a pit with a depth of 15 cm

25   and a surface of 70 cm x 100 cm. We transported all 48 collected soil samples to the long-term

1   ecological research site (LTER) in the Aelmoeseneie forest in Belgium during November 2015-

2   February 2016 (50°58′30" N, 3°48′16" E, mean annual temperature (MAT) = 10.6 °C, mean annual

3   precipitation (MAP) = 768 mm, altitude = 20 m, N deposition (2016) = 15.5 kg N ha^{-1}) (DEIMS-SDR

4   Database). We sieved the 48 bulk soil samples through a 5 mm mesh sieve. The sampled soil from

5   each forest patch was divided over eight mesocosms, one for each two-level factorial combination of

6   light x warming x temperature. Each mesocosm consisted of 13L sampled soil, placed in trays with

7   12 drainage holes (46.5 x 31.5 cm, depth of 19.5 cm) on top of 9 L of white sand to ensure proper

8   drainage. The bottom of the trays were first lined with a root fabric to ensure that plants would not

9   root in the site's soil upon burying the mesocosms with their tops level to the ground.

Plant community assembly: slow and fast colonisers grow together

11   We compiled a pool of fifteen species commonly found in temperate European forests (Table 1) and

12   divided this species pool according to three emergent groups that are linked with a species'

13   colonisation capacity and affinity to forests habitat (Verheyen *et al.* 2003; De Frenne, Baeten, *et al.*

14   2011; Heinken *et al.* 2019).Verheyen *et al.* (2003) quantified differences in functional and life-history

15   traits among 216 herbaceous species that colonised and established in post-agricultural forests to

16   varying degrees or remain confined to ancient forests, allowing the determination of traits that lead

17   to a successful colonisation. The varying success between species to colonise post-agricultural forest

18   was captured in the colonisation capacity index (CCI), where more negative values indicate a higher

19   capacity to successfully colonise into post-agricultural forests (Verheyen *et al.* 2003). The continuous

20   CCI scale exists from -100 to +100, where -100 means that the species only occurred in post-

21   agricultural forest, while +100 means that the species was only found in ancient forest. The CCI scale

22   thus allows to estimate a species' affinity to post-agricultural forest vs. ancient forest. We classified

23   these species in three groups based on our expert knowledge, guided by the CCI (Verheyen *et al.*

24   2003; De Frenne, Baeten, *et al.* 2011) and whether a species can be specified as a forest habitat

25   specialist (Heinken *et al.* 2019). The first group (A) are six poor colonisers and forest specialists,

typical of ancient forest (Table 1). The second group (B) are six intermediate colonizers of post-agricultural forest and not strictly forest specialists (Table 1). The final group (C) are three fast colonising and generalist nitrophilic species with a higher affinity towards post-agricultural forest and a large negative CCI (Table 1). We took both colonisation of species and forest habitat specificity into account to classify these species. For those reasons, *Polygonatum multiflorum* is in group A as it is a typical forest species even though it has a relatively low CCI. Likewise, group B incorporates species with a wider range in habitat use that are not strictly forest specialists. *Ajuga reptans* and *Poa nemoralis* can occur in grassy habitats and grasslands, while *Hedera helix* is often found in shaded urban and rural habitats. The groups supported the planting process (see next paragraph). We used the CCI directly to characterise species in the analysis (see "Data analysis"), because of its established correlations between several life-history traits (Verheyen *et al.* 2003).

We created twelve species combinations (Supplementary Table 1) in which we randomly assigned two poor colonisers (group A), two common colonisers of post-agricultural forest (group B) and one fast-colonising nitrophilic species (group C). These twelve species combinations serve as representative species assemblages in temperate mesotrophic deciduous forests in Europe, and are solely used as random effect term in the analysis. The twelve species combinations are repeated four times over the 48 soil samples in each two level factorial combination of light x warming x nitrogen addition (48 soil samples x 8 factorial combinations = 384 mesocosms). We planted the 384 mesocosms in the first week of March 2016. Per mesocosm, we planted four individuals of each of the five species in a grid (7x8.5 cm) in a randomised order using the *sample* function in R. This planting scheme results in 20 plants per mesocosm. We planted these species from vegetative plant material (Table 1) and obtained our plants from either the local population in the Aelmoeseneie forest or Brakelbos (Belgium), or a local plant nursery that deals in wild plants for horticulture (ECOFLORA, Halle, Belgium). During the first growing season (May, June, September 2016), we applied three rounds of weeding, where we eradicated species that had emerged spontaneously from the mesocosm soil. We buried all mesocosms with their tops at surface level, in a fenced area in the

1 Aelmoeseneie Forest (Belgium) with a tree canopy (95% cover) dominated by *Fagus sylvatica*,

2 *Quercus robur, Acer pseudoplatanus, Fraxinus excelsior* and *Larix decidua.* We buried mesocosms

3 in 96 random groups ("Plots"), of four mesocosms per plot, to apply manipulative experimental

4 treatments (see Fig. 1), as explained in the next section and following methods of De Frenne *et al.*

5 (2015). In total, we planted 7680 (384 mesocosms x 20) individuals of fifteen species (512 individuals

6 per species).

Manipulating the environment: N addition, experimental warming and enhanced light availability

9 We have applied three two-level environmental treatments in a full-factorial design since April 2016.

10 Ongoing treatments consist of (i) nitrogen addition; (ii) experimental warming and (iii) enhanced

11 light availability (Supplementary Table 2 and 3). The first treatment is N addition (further referred to

12 as treatment "N") by adding 0.25 L of a 2.01 g L^{-1} solution of NH_4NO_3 (50 kg N ha^{-1} yr^{-1} eq.) per

13 mesocosm. We then rinsed the leaves with 0.25 L of demineralised water to ensure that the applied

14 N did not remain adhered to plant tissues. We performed this treatment four times per year at the start

15 of each season, with the control mesocosms receiving 0.5 L of demineralised water. The second

16 treatment consisted of experimental warming (referred to as treatment "T") with 75 cm-wide open

17 top chambers (De Frenne *et al.* 2010). We measured air temperature (15 cm above forest floor),

18 surface temperature (0 cm) and soil temperature (5 cm depth) in eight plots, one for each factorial

19 combination of light x warming x nitrogen, using 24 thermocouples which log every 30 seconds

20 (Type T miniature, TC Direct, The Netherlands and datalogger type CR1000, Campbell Scientific,

21 USA). In the first half of our sampling period (from March 1 until May 31), the forest canopy

22 developed and light could easily reach the open top chambers in order to warm the chamber. During

23 this period, we measured a significant increase ($p<0.05$) in daily mean air temperature of 1.04±0.47

24 °C, and statistically insignificant increases in surface temperature (0.46±0.26) and soil temperature

25 (0.13±0.30 °C) at 5 cm depth (Supplementary Fig. 1). In the latter half of our sampling period (June

1   1 until August 31), the forest canopy closed which resulted in less effective warming, with no

2   significant differences (p>0.05) between the warming and control treatments at any depth. The third

3   treatment, light addition (referred to as "L"), adds 23.98±4.40 µmol m^{-2}s^{-1} PAR to the ambient light

4   conditions (7.79±0.68 µmol m^{-2}s^{-1} under fully closed canopy) by use of two 18 W fluorescent tubes

5   suspended 75 cm above ground level of each plot (Supplementary Fig. 2). This increment in light

6   availability can be associated to a small forest gap, and could increase carbon assimilation rates in

7   typical forest understorey plants by three times (Rothstein & Zak 2001). The lights are programmed

8   to follow the natural photoperiod throughout the year and did not significantly affect air temperatures

9   (De Frenne *et al.* 2015). Control plots receive ambient light and have a dummy lamppost suspended

10 over the plot to account for undesired side effects of the lamp installation other than light addition

11 (e.g. precipitation, interception, see Fig. 1).

12 Plant trait and community measurements

13 We measured plant height and SLA in the second growing season of the experiment, from March

14 2017 to September 2017, at the time of each species' estimated biomass peak (Table 1). We chose

15 not to measure reproductive traits (i.e. seed mass) as proposed in the common leaf-height-seed

16 scheme (Westoby 1998; Laughlin *et al.* 2010). Seed mass and plant height are strongly positively

17 correlated in the global plant trait spectrum (Thomson *et al.* 2011; Díaz *et al.* 2016; Beckman *et al.*

18 2018), but also in forest herb species (Verheyen *et al.* 2003). Furthermore, several herbaceous species

19 in our pool favour clonal reproduction, so that seeding is often limited in these species (Klimešová *et*

20 *al.* 2016). Finally, these reproductive traits can be intensive to correctly measure and quantify given

21 our large numbers of test plants (Pérez-Harguindeguy *et al.* 2013).

22 We were able to measure plant height on 3445 survivors of the 7680 initially planted individuals

23 (45% survival). The number of plant height measurements (i.e. individual survivors) did not depend

24 on treatment effects (Supplementary Fig. 3). Differences in survival among species existed (Table 1,

25 Supplementary Table 4). Vegetative plant height was measured as the shortest distance between

1 ground level and the upper boundary of main photosynthetic tissues. This means that we solely

2 measured foliage height, not inflorescence or seeding height (Pérez-Harguindeguy *et al.* 2013). We

3 measured plant height using a common folding meter in centimetres up to one decimal place.

4 We measured average specific leaf area (SLA) as the one-sided area of multiple leaves from a single

5 species in each mesocosm, divided by the oven-dry mass and expressed in mm² mg^{-1} (Pérez-

6 Harguindeguy *et al.* 2013). We collected maximally eight healthy leaves of each species and no more

7 than two per individual to avoid excessive damage to any individual plant. We only sampled outer

8 leaves (excluding petioles) because SLA can be strongly affected by light intensity (Pérez-

9 Harguindeguy *et al.* 2013). The amount of SLA measurements did not vary due to treatments within

10 any species (Supplementary Fig. 4), while there were larger differences in measured SLA values

11 between species (Table 1, Supplementary Table 4). The projected area of the leaves was measured

12 with the *Easy leaf area free* mobile application (Easlon & Bloom 2014) within two hours of picking

13 the leaves, after taking a picture with a mobile phone (13 MP camera). This open-source software

14 package can estimate green leaf area in a RGB photograph by counting the total amount of green

15 pixels and converting this into an area measurement by counting red pixels from a 4 cm² calibration

16 square provided in the image. After the area measurement (mm²), each leaf sample was dried in an

17 oven at 65°C for 48 hours to ensure constant mass upon weighing (in mg).

18 We measured total vegetation cover (%), which is tightly linked with productivity, leaf biomass and

19 competition for light (Muukkonen *et al.* 2006). We measured vegetation cover as the one-sided

20 projection of vegetation in the tray. We measured vegetation cover two times during the experiment:

21 the first week of May (4th of May) and the second week of August (11th August). We used digital

22 RGB photographs of the mesocosms taken perpendicular to the ground surface and the *Canopy Area*

23 tool that measures green pixels of vegetation and recalculates this into a cover percentage (Easlon &

24 Bloom 2014).

25 Data analysis

1   All data analysis was performed in R (R Core Team 2019). We performed three analyses to assess

2   whether trait variation in plant height and SLA within species is a function of 1) vegetation cover, 2)

3   agricultural land-use history interacting with multiple environmental drivers and 3) a species'

4   colonisation ability. We consequently addressed within-species trait variation in two ways. First, by

5   assessing change in mean trait values due to vegetation cover and treatments (hypothesis 1 and 2

6   respectively). Second, by looking at variability in traits across all measured values via the coefficient

7   of variation (CV, hypothesis 3). Prior to the analyses, we excluded *Glechoma hederacea* because this

8   species had fewer than 30 measurements (see Table 1). During the analysis on variation in mean plant

9   height and SLA (hypothesis 1 and 2), we excluded *Geranium robertianum* because there were too

10 few measurements to fit the model structure robustly.

11 In the first analysis, we tested whether vegetation cover explains differences in mean plant height and

12 SLA in each species (hypothesis 1). We calculated separate hierarchical linear mixed – effects models

13 (package: nlme, Pinheiro *et al.* 2018) for each species to estimate the mean trait value in response to

14 vegetation cover. The original Region of the soil (8 levels), Community (12 levels) and Plot (96

15 levels) are separate random effect terms in this structure, i.e. (1|Region) + (1|Community) + (1|Plot).

16 Adding the "Plot" random effect term was not possible for SLA because of limited degrees of freedom

17 (SLA is an averaged measure for each species in each mesocosm). The predictor variable is the

18 average vegetation cover (%) of each mesocosm measured between May 2017 and August 2017. We

19 checked normality in residuals with a Shapiro-Wilk test (function shapiro.test in stats package, R

20 Core Team 2019) on each model, and log-transformed the response variable when necessary to

21 achieve normality in model residuals. Log transforming the response was needed for six species in

22 plant height and five species in SLA (see Supplementary Table 5). We additionally tested whether

23 the covariate (Soil type), and two-way interactions (including main effects) of agricultural land-use

24 history (LU), enhanced light availability (L), nitrogen addition (N) and warming (T) directly

25 explained variation in total vegetation cover. We used the same hierarchical model structure as

26 explained above.

1   We tested the second hypothesis by estimating mean plant height and SLA of species in response to

2   two-way interactions of agricultural land-use history (LU), enhanced light availability (L), nitrogen

3   addition (N) and warming (T). Concretely, we calculated separate hierarchical linear mixed effects

4   models (function "lme" in package nlme, Pinheiro *et al.* 2018) for each species with six double

5   interactions including all four main effects (LU, L, N, T, LU:L, LU:N, LU:T, L:N, L:T, N:T) and an

6   extra covariate "Soil type". We included the covariate "Soil type" (three levels: "eutrophic",

7   "mesotrophic", "oligotrophic") in the models because inherent soil fertility can be a major source of

8   trait variation, even though it is not one of our focal environmental change predictors. The random

9   effects structure of these models is the same as in hypothesis 1, with "Region" (8 levels), "Community

10   combination" (12 levels) and "Plot" (96 levels) included. Prior to computing the tests, we checked

11   normality assumptions using a Shapiro-Wilk test (function shapiro.test in stats package, R Core Team

12   2019) on each model accompanied by a qq-plot and histogram on model residuals. We log-

13   transformed when necessary to achieve normality in residuals, which was the case for seven species

14   for plant height and six species for SLA (see Supplementary Table 6). We performed ANOVA (F-

15   test, function "anova" in stats package, R Core Team 2019) on one linear model for each of the

16   thirteen species per trait, which contain 11 terms per model (four main effects, six double interactions

17   and one covariate main effect). We applied an adjusted alpha ($p < 0.007$) so that the expected value of

18   a false positive effect is less than 1 out of the 143 tested terms (alpha = 1/143 = 0.007) for a given

19   trait. If we had used the common alpha of $p < 0.05$, the expected value of interpreting false positive

20   effects would have been 7 terms (143*0.05 = 7.15) for each trait.

21   We tested the third hypothesis by calculating the coefficient of variation (CV) for each species across

22   all measurements of plant height and SLA. We performed linear regression of the CV as function of

23   the species' capacity to colonise post-agricultural forest. We used the colonisation capacity index

24   (CCI) to determine a species ability to successfully colonise post-agricultural forest (Verheyen *et al.*

25   2003), where more negative values denote a larger capacity to colonise post-agricultural forest. We

26   calculated the CV in plant height and SLA for the whole range of species' trait values (so across all

1 treatments) as the ratio of the standard deviation to the estimate of the population mean. The

2 coefficient of variation is a simple measure for intraspecific trait variation and phenotypic plasticity

3 in the wide sense, i.e. when traits are not measured on individuals of the same genotype but of the

4 same species (Valladares *et al.* 2006). As we have computed one CV for each species for the whole

5 data set, we performed a non-hierarchical linear regression (function "lm" in stats package, R Core

6 Team 2019) of the species' coefficient of variation in response to the species' CCI for both traits

7 (alpha =0.05).

Results

9 Response of mean traits to vegetation cover

10 In the first analysis, we found that increasing vegetation cover related to changes in mean values of

11 both plant height and SLA. Vegetation cover (Table 2) was significantly higher on post-agricultural

12 forest soils (+6.06, se = 1.68), with light addition (+15.88, se = 1.79) and with warming (+8.41, se =

13 1.83). There was no evidence for interactions between environmental treatments in affecting

14 vegetation cover. In turn, we found that vegetation cover affected both species' mean plant height

15 and mean SLA, while mean plant height was more responsive to vegetation cover than mean SLA

16 (Figure 2). Ten of the thirteen species had increasing mean plant height estimates with increasing

17 vegetation cover, all on p <0.001 (Supplementary Table 5). The largest absolute increase in plant

18 height occurred in *Urtica dioica,* also the fastest coloniser into post-agricultural forest (most negative

19 CCI). Its predicted mean plant height was 15.8 cm (95% confidence interval (CI): 8.4– 29.7 cm) at

20 the lowest community cover (36%) and 41.6 cm (95% CI: 22.2 - 78.0 cm) at the highest community

21 cover (96%). This is, over the range of total cover values that it occurred in, a relative increase in

22 mean plant height of 160%. However, the largest relative increase was a five-fold increase in mean

23 plant height that occurred in *Ficaria verna*. The model results show that this species increased its

24 mean plant height from 1.9 cm (95% CI: 1.05 – 2.83 cm) at 9 % total cover to 10.6 cm (95% CI: 8.7

25 – 12.5 cm) when the vegetation cover was 97 %.

For SLA, we found both significant increases and decreases in response to community vegetation cover depending on the species. Species mean SLA decreased significantly in four species (*Aegopodium podagraria, Poa nemoralis, Vinca minor, Carex sylvatica*) and increased in two (*Ficaria verna, Hyacinthoides non-scripta*) in response to vegetation cover. However, the slopes were not as pronounced as for the plant height response (Figure 2) with 30 % as a maximal decrease of SLA for both *Aegopodium podagraria* and *Carex sylvatica.* The significant increases occurred within a similar magnitude of 31% for *Ficaria verna* and 30% for *Hyacinthoides non-scripta* (Supplementary Table 5).

Response of mean traits to environmental treatments

Plant height increased in response to the environmental treatments and agricultural land-use history across several species ($p < 0.007$, see Supplementary Table 6 for p-values). The pattern in significant responses (Fig. 3) shows that a total of six species had significant plant height responses (excluding the Soil type covariate), which mostly occurred in slow-colonising species (5 species), compared to fast-colonising species (1 species). However, the covariate "Soil type" significantly affected plant height in three species, all relatively fast colonisers based on CCI. The most important significant ($p < 0.007$) main effect in the plant height response was that of light addition which increased plant height for four species (Fig. 4A, Supplementary Table 7). The largest relative increase in mean plant height to light was found in *Urtica dioica* (+65%) followed by *Carex sylvatica* (+40%), *Anemone nemorosa* (+28%) and *Hyacinthoides non-scripta* (+19%). Nitrogen addition did not affect any species. Agricultural land-use history and warming each affected mean plant height of one species. *Galium odoratum* responded positively to agricultural land-use history with a relative increase in mean plant height of 15% (Fig. 4B and Supplementary Table 7). *Vinca minor* responded positively to warming as a main effect with a relative increase of 2% (Fig. 4C, Supplementary Table 7). However, the warming effect depended on light addition; when added together, its height increased

16

by 33%. This was the only interaction that we found in the analysis across all considered species (Fig. 4D, Supplementary Table 7).

Specific leaf area (SLA) had less significant responses to the environmental treatments and agricultural land-use history than plant height (Fig. 3). The most common significant ($p<0.007$) main effect was of warming. Warming increased SLA by 11% for *Hyacinthoides non-scripta,* by 9 % for *Anemone nemorosa* and by 10% for *Ficaria verna*. Warming negatively affected SLA of *Carex sylvatica* with a decrease of 6% (Fig. 4E, Supplementary Table 7). Light addition had a significant negative main effect for two species: *Carex sylvatica* with a 15% decrease and *Polygonatum multiflorum* with a 10% decrease (Figure 4F, Supplementary Table 7). SLA within species did not respond to N addition and the forest land-use history and there were no significant interactions. In summary, SLA increased in response to warming in the early flowering species *Hyacinthoides non-scripta*, *Anemone nemorosa* and *Ficaria verna*. Conversely, SLA decreased in late-flowering species, in response to both light and warming (separately) for Carex sylvatica.

ITV in relation to a species' colonisation capacity

In the final analysis (Fig. 5), we found that the coefficient of variation (CV) for plant height was highest ($p = 0.001$, $R^2 = 0.61$) for species that exhibit fast colonisation into post-agricultural forest (negative values in the colonisation capacity index, CCI). CV for plant height ranged from 0.29 for the slow colonising *Galium odoratum* to 0.71 for the fast colonising *Poa trivialis*. CV for SLA ranged from 0.12 for the slow colonising *Anemone nemorosa* to 0.33 for the fast-colonising *Aegopodium podagraria,* but this response of SLA to the species' colonisation capacity index was statistically not significant ($p = 0.50$, $R^2 = 0.038$). Fast colonisers into post-agricultural forest thus show more intraspecific variation in plant height as measured by CV than slow colonisers across the whole trait range, but not in SLA.

Discussion

We found that variation in plant height and SLA of forest herbs under environmental change related to the trait's function. A general observation is that trait values of most species did not respond to more than one main effect of a treatment. Interactions between the multiple environmental treatments were hardly important in explaining variation in mean trait values of species. However, generalizable patterns in plant height and SLA responses arose across species. Mean plant height values increased, as vegetation cover increased in response to light addition, warming and agricultural land-use history. The coefficient of variation (CV) for plant height was larger in fast colonising species. Contrastingly, changes in mean SLA only occurred within selected species to improve light acquisition in shaded conditions. These individualistic, functional trait responses to single factors benefit a particular species' growth in the short term (Garnier *et al.* 2016). Our results suggest that 1) variation in plant height relates to a greater vegetation cover in brighter conditions and largely benefits fast colonisers, and 2) changing SLA is beneficial for species that have acclimatised to shaded conditions in forests, i.e. vernal geophytes and shade-tolerant species.

Intraspecific variation in plant height

The variability in mean plant height could benefit growth, light acquisition and dispersal (Moles *et al.* 2009). Plant size differences between individuals in communities can be an outcome of asymmetric competition for light (DeMalach *et al.* 2016) as individuals invest in height to disproportionately capture light from neighbours when vegetation becomes denser (Freckleton & Watkinson 2001). We observed that mean vegetation cover of the community increased from 40% to 75 % due to combined effects of primarily light addition, followed by warming and an agricultural land-use history. As the vegetation became denser and increased to maximal cover, individuals of 10 out of 13 species nearly doubled in mean plant height. This plant height response to vegetation cover was stronger than the plant height response of species to environmental change directly. This observation could indicate that competition mediated by larger vegetation cover better explains plant

1 height change than altered environmental conditions per se. A large meta-analysis on functional trait

2 variation in forest herbs also found that changing vegetation cover, due to environmental change or

3 otherwise, was a strong predictor for larger plant height values in forests herbs (Burton *et al.* 2017).

4 Light was the main driver for mean plant height increases and affected four out of thirteen species.

5 Lemke *et al.* (2015) also found that plant height increased in response to light availability, but only

6 in a graminoid and not in a forb. Warming was of minor importance and affected one out of thirteen

7 species. We had expected that more plant species would respond positively to warming, given that

8 individuals of forest herb species are taller in warmer areas of their range (Kollmann & Bañuelos

9 2004; De Frenne, Graae, *et al.* 2011; De Frenne *et al.* 2014; Lemke *et al.* 2015). Agricultural land-

10 use history and N addition barely affected plant height values. This suggests that, in general, nutrient

11 availability was less limiting than light availability. Adding the equivalent of ca. 50 kg N ha^{-1}yr^{-1} (the

12 equivalent of N deposition near a point source, e.g. a pig farm) yielded limited individualistic plant

13 responses. This is likely due to a high ambient N deposition (15.5 kg N ha^{-1}yr^{-1}) which already falls

14 within the critical load range (10 – 20 kg N ha^{-1}yr^{-1}) for temperate broadleaved forest (Bobbink *et al.*

15 2015). However, including the inherent soil fertility as a covariate in the analysis did explain mean

16 plant height differences in three species. This suggests that pH – influenced nutrient availability can

17 be an important driver for trait variation as well (Siefert 2012). Other studies suggest that nutrient

18 enrichment from N addition or agricultural land-use history can increase height of forest herb

19 individuals, with a taller plant community as a result (Baeten *et al.* 2010; Tatarko & Knops 2018).

20 However, many of these studies were performed on individual species growing in pots, rather than in

21 a field community such as in our study. The relatively few responses of plant height to environmental

22 change directly could thus exemplify that community properties (i.e. vegetation cover) determine an

23 individual's short-term response to environmental change (Bennett *et al.* 2016).

24 We also found that the variability in plant height values (CV) was larger within species with a good

25 colonisation capacity into post-agricultural forest than within slow colonisers. For fast-colonising

1   wind-dispersers, plasticity in height is important to disperse seed further than neighbouring plants

2   (Thomson *et al.* 2011) apart from the benefits it yields in capturing light (Freckleton & Watkinson

3   2001). Slow colonising forest herbs would need to invest less in above-ground biomass to disperse,

4   as bud banks are more common for multiplication (Klimešová *et al.* 2016; Ott *et al.* 2019). Overall,

5   these findings suggest that variation in plant height largely benefits fast colonisers when vegetation

6   cover is high.

7   Intraspecific variation in specific leaf area (SLA)

8   SLA is a functional trait that is related to trade-offs in photosynthetic machinery and the associated

9   leaf economics spectrum (Wright *et al.* 2004). We observed responses of SLA in selected species that

10 typically perform photosynthesis in shaded temperate forest, while we did not find a clear relation

11 between colonisation capacity and the CV of SLA in a species. SLA responded only to light and

12 warming, and solely in shade-avoiding geophytes or shade-tolerant plants that grow under the closed

13 canopy (Verheyen *et al.* 2003; De Frenne, Baeten, *et al.* 2011). Interestingly, within-species SLA can

14 both increase or decrease in response to a gradient going from shady to sunny conditions (Garnier *et*

15 *al.* 2016). We observed both directions in our study, given contrasting species responses to warming

16 and light addition respectively.

17 Growing temperatures can positively affect SLA of individuals (Atkin *et al.* 2006). This relationship

18 for forest herbs has been established along latitudinal (De Frenne, Graae, *et al.* 2011) and elevation

19 gradients (Midolo *et al.* 2019) where lower SLA is expected at colder, higher latitudes and altitudes.

20 The increasing mean SLA in response to warming of the early flowering geophytes *Hyacinthoides*

21 *non-scripta*, *Anemone nemorosa* and *Ficaria verna* would thus be advantageous for these spring-

22 flowering geophytes. These are the three earliest species in our species pool to reach their peak

23 biomass during the growing season, and need to withstand frosty nights in early spring to sustain

24 growth. A common response to low air temperatures in plants is to have thicker leaves, which raises

25 leaf mass (Pérez-Harguindeguy *et al.* 2013) but invokes an important mass-investment trade-off

considering the short life-span of their leaves (Wright *et al.* 2004; Jagodziński *et al.* 2016). Our three vernal species likely benefitted from the 1°C increase in air temperature because they decreased leaf thickness, and thus mass investments, which yields a higher photosynthetic surface area per unit of mass investment (SLA). Conversely, we found that SLA decreased in response to light for *Carex sylvatica* and *Polygonatum multiflorum*, shade-tolerant species that flower under closed canopies in ancient forests (Wulf 1997; Bossuyt *et al.* 1999). Shade tolerant species are generally not plastic in leaf physiology, but can be in leaf morphology (Wright *et al.* 2004; Milla & Reich 2007; Valladares & Niinemets 2008). Individual plants usually develop a higher SLA when grown under low light conditions (Reich *et al.* 2003; Rozendaal *et al.* 2006; Feng & Van Kleunen 2014; Liu *et al.* 2016) to optimize light capture and carbon gain in those environments (Evans & Poorter 2001). A lower SLA of the shade-tolerant species *Carex sylvatica* and *Polygonatum multiflorum* within the light treatment followed this pattern.

We found limited effects of N addition and agricultural land-use history on SLA of species. Other studies suggest that intraspecific SLA either has a limited or positive response to enhanced nutrient availability due to agricultural land-use history (Siefert 2012; Siefert & Ritchie 2016) or N addition (Zhou *et al.* 2018; Firn *et al.* 2019). In any case, leaf nutrient content and leaf dry matter are more consistent in predicting leaf trait variation in response to larger nutrient availability (Smart *et al.* 2017; Firn *et al.* 2019). SLA of multiple forests herbs have been found to be highly sensitive to a changing light availability, which makes SLA a relatively unreliable predictor of altered nutrient availability (Burton *et al.* 2017).

Perspectives on trait variation in a changing world

Our experimental design allows disentangling of multiple environmental change treatments on trait expressions of typical forest herb species. A major outcome of this experiment is the lack of treatment interactions on intraspecific trait values. Instead, simple univariate treatment responses of height and SLA were detected. Patterns in plant species growth responses to warming (Hollister *et*

al. 2005), light availability (Rozendaal *et al.* 2006) and high nutrient availability (Fynn & O'Connor 2005) have long been shown to be dependent on the species, suggesting different limiting factors for plant species that naturally occur in the same communities (Chapin & Shaver 1985). Such selective plant responses to our considered environmental factors imply a "Gleasonian" perspective regarding plant responses to global change and its effect on community organisation: one where individualistic trait expressions to simple environmental factors determines the interactions between species (Gleason 1926; Nicolson & Mcintosh 2002; Götzenberger *et al.* 2012). This individualistic perspective could however pose a gross underestimation of the complex multidimensional interactions between energy and nutrients, consumers and competitors, and time, in affecting plasticity of traits in observed communities (Westneat *et al.* 2019). Our experimental approach did allow us to unravel the importance of drivers that are entangled in the natural world. Important entangled factors are light availability and warming in forest gap conditions, and species composition of understorey communities due to agricultural land-use history. This disentangled view showed that vegetation cover responded to environmental change and in turn affected trait values of species. Individualistic species responses further promoted ITV due to changing resources and conditions.

Apart from competition in the community affecting ITV (Bennett *et al.* 2016), species richness effects on ITV could occur as well as a result of resource partitioning (Lipowsky *et al.* 2015). We did not look into these effects by keeping a constant species richness across communities at the time of planting (5 species); this was not to inflate the experimental design. We also did not look into genetic variation within species, as the component of genetic variation and local adaptation in ITV generally becomes more prominent at larger geographical scales (Albert *et al.* 2011; Lajoie & Vellend 2015; Moran *et al.* 2016; Veresoglou & Penuelas 2019). However, it is likely that local adaptation in species across a large geographical gradient could alter various trade-offs between functional traits within those species (Oldfather 2019). Such genetic variation between populations of species could consequently influence the magnitude of ITV in response to environmental change (Kahl *et al.* 2019).

1   Against our expectations, this short-term mesocosm experiment barely revealed any interactive

2   effects between the multiple treatments on plant trait variation. This lack of interaction on the short-

3   term does not imply that such interactive effects are not important for functional trait responses to

4   global change. It rather shows the complementary of experimental research to long-term vegetation

5   resurveys (Verstraeten *et al.* 2013; Perring, Diekmann, *et al.* 2018; Perring, Bernhardt-Römermann,

6   *et al.* 2018) or mechanistic modelling approaches (Dirnböck *et al.* 2017; Landuyt *et al.* 2018). Long-

7   term experiments, vegetation resurveys and modelling are perhaps better suited to unravel such long-

8   term interactive effects between global change drivers on understorey trait variation (Luo *et al.* 2011;

9   Verheyen *et al.* 2017). Most probably, interactions between the treatments in this experiment could

10 emerge after prolonged time when resources and conditions other than light become more limiting

11 (Oliver & Morecroft 2014).

12 Conclusions and future directions

13 It is known that estimating ITV in response to land-use legacies (Siefert & Ritchie 2016) and

14 environmental change (Bolnick *et al.* 2011; Albert *et al.* 2011) can be important for predicting the

15 future of plant communities. Here we assessed ITV in plant height and SLA of forest herbs with

16 different colonisation abilities and affinity to forest habitat, in response to community growth and

17 multiple environmental changes, and on a distinct local scale via a manipulative experiment. Contrary

18 to our expectations, we found that interactions between environmental drivers were not important in

19 explaining variation in traits. A lack of interactive treatment effects could suggest that traits respond

20 individualistically to single factors that benefit a species' growth. We showed that increases in within-

21 species mean plant height relate to a greater vegetation cover in brighter conditions, and largely

22 benefitted fast colonisers. Trait variation in SLA promoted species that are acclimated to shaded

23 conditions in forests, as within-species mean SLA distinguished between shade-avoiding vernal

24 species, given their response to warming, and shade-tolerant species, given their response to light

25 addition.

The responses in plant height and SLA occurred after one growing season following herb layer introduction in the experimental communities. Understorey plant responses to environmental change remained individualistic for now, but with decades of time after the initial trait response (Gross *et al.* 2009), these patterns could reveal how community assembly veers towards fast-growing and fast-colonising species in disturbed forests when light availability is high. When typical forest herbs would be introduced in such disturbed forests, these could be excluded from community assembly in the long run by more generalist species (see Baeten and Verheyen, 2017). Patterns in ITV at initial stages of plant species establishment could thus potentially reflect the trajectory in community assembly after prolonged time.

Author contributions

- HB, PDF, EDL, LD, SLM, MPP and KV designed the study. SG and PV added conceptual views in a later stage of the study.
- HB and EDL collected the data.
- HB performed statistical analyses, with significant inputs from MPP, DL and KV.
- HB led the writing of the manuscript, with significant inputs from MPP and KV. All authors provided comments on the manuscript at several stages in its development.

Data archiving

Data will be made available via http://www.pastforward.ugent.be/data.html

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Tables

Table 1: Pool of 15 species used in the experiment and their most important attributes. Scientific names are drawn from the plantlist.org. Number of measurements (N) for height (maximum 512) and SLA (maximum 128) are given. The colonisation capacity index (CCI, see Verheyen *et al.,* 2003) indicates the affinity of a species with ancient forest (positive values) and ranged from 100 (slow colonisation into post-agricultural forest) to -100 (fast colonisation). We classified these species in three groups based on our expert knowledge, guided by the CCI and whether a species can be specified as a forest habitat specialist (Heinken *et al.* 2019). Group A are poor colonisers and forest specialists, Group B are intermediate colonizers of post-agricultural forest and not strictly forest specialists, and group C are fast colonising and generalist nitrophilic species with a large negative CCI. Plant material used at the time of planting is indicated as well (March 2016), which all came from adults of perennial species to ensure that the trait values we measured would come from individuals at the same life stage. Symbols denote the origin of the plant material, and is either from an organic horticultural nursery (°, ECOFLORA, Belgium) or the native population in Brakelbos (*) and the Aelmoeseneie forest (**) in Belgium.

Scientific name	Group	% survivors	N (Height)	N (SLA)	Life form	Peak biomass	CCI	Forest specialist?	Plant material
Hyacinthoides non-scripta (L.) Chouard ex Rothm	A	51%	263	100	Geophyte	May	100	Yes	Bulb°
Galium odoratum (L.) Scop.	A	76%	387	102	Hemicryptophyte	June	95	Yes	Whole plant°
Anemone nemorosa L.	A	28%	142	63	Geophyte	April	77	Yes	Rhizome°
Carex sylvatica Huds	A	82%	422	114	Therophyte	July-August	74	Yes	Whole plant°
Vinca minor L.	A	72%	369	109	Chamaephyte	May-June	33	Yes	Whole plant°
Polygonatum multiflorum (L.) All.	A	75%	383	122	Geophyte	May-June	15	Yes	Rhizome°
Ajuga reptans L.	B	19%	99	37	Hemicryptophyte	June	60	No	Whole plant°
Hedera helix L.	B	29%	147	67	Chamaephyte	July-August	50	No	Whole plant°
Poa nemoralis L.	B	59%	301	96	Hemicryptophyte	May-June	29	No	Whole plant*
Ficaria verna Huds.	B	85%	434	108	Geophyte	March	11	No	Root nodule**
Glechoma hederacea L.	B	2%	12	4	Hemicryptophyte	June	-5	No	Whole plant°
Geranium robertianum L.	B	10%	50	21	Hemicryptophyte	June-July	-9	No	Whole plant°
Aegopodium podagraria L.	C	34%	175	71	Hemicryptophyte	August-September	-45	No	Rhizome**
Poa trivialis L.	C	35%	179	64	Hemicryptophyte	May-June	-67	No	Whole plant°
Urtica dioica L.	C	16%	82	47	Hemicryptophyte	August-September	-71	No	Rhizome**
Total			**3445**	**1125**					

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Table 2: Model fit of the significant (p<0.05) treatments that influenced mean vegetation cover (%). Estimates with standard error (S.E.), denominator degrees of freedom (D.F.), F-value and p-value are given for the model terms. The three fixed effect terms were the two-level terms Land-use history (LU), Light treatment (L) and Temperature treatment (T) which together explained less than 20% of variation ($R^2m = 0.17$). 65% of the variation is accounted for when including the random effect terms (R^2c), i.e. Region of soil, Plot and community combination. The intercept term is the average intercept across random effect terms and is the estimate for an Ancient forest soil with control treatments for light and warming.

term	Estimate	S.E.	D.F.	F-value	p-value
Intercept	43.15	3.40	276	376	<0.0001
Post-agricultural	+6.06	1.68	9	12	0.0074
Light addition	+15.88	1.79	276	79	<0.0001
Warming	+8.41	1.83	276	21	<0.0001

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Figure legends

Figure 1: **Images of the multifactor experiment, showing mesocosms in groups of four combined as a "plot". Panel A.** Overall view of the fenced area in the Aelmoeseneie forest (Belgium). **Panel B.** A plot with a factorial combination of Warming and Light addition. **Panel C** Singular treatment of Light addition, with a temperature sensor to measure air temperature. **Panel D.** Control treatment where the plot receives ambient conditions. Plots that receive the N enrichment treatment are not discernible in the field.

Figure 2. **Plant height (panel A) is more responsive to total vegetation cover than SLA (panel B) across species.** Results of the within species linear mixed effects models testing plant height and SLA in response to total vegetation cover in a mesocosm. Significance levels are 0.05*, 0.01** and 0.001***. The lines are the average predicted values of the linear mixed effects model for each species (either normal or log-transformed response). Marginal R^2 (R^2m) are given, which denote the model fit on the only fixed effect (vegetation cover). Species are ordered on colonisation capacity index (CCI) with the fastest colonisers into post agricultural forest on top and hues ranging from purple-blue-yellow from fast to slow colonisers.

Figure 3: **Species selectively respond to environmental change in both plant height (panel A) and specific leaf area (SLA) (panel B).** Results of the ANOVA (p<0.007) testing two-way interactions of the forest's land-use history (LU), light availability (L), nitrogen addition (N) and warming (T). We applied an adjusted alpha (p<0.007) so that the expected value of a false positive effect is less than 1 out of the 143 tested terms (alpha = 1/143 = 0.007). Species are ordered on colonisation capacity index (CCI) with the fastest colonisers into post-agricultural forest on top.

Figure 4: **Significant effects (p<0.007) of the experimental treatments on within-species plant height (A-D) and SLA (E-F).** Panels are illustrations to the significant effects shown in Figure 3. Species are ordered on colonisation capacity index (CCI) with faster colonisers into post-agricultural forest on the left.

Figure 5: **Faster colonisers into post-agricultural forest exhibit significantly higher ITV, for plant height (panel A) but not SLA (panel B).** Coefficient of variation for plant height (panel A) and specific leaf area (panel B) for each species in regression (95% CI) to that species' colonisation capacity index (CCI). CCI values closer to 100 indicate higher affinity for ancient forest; values closer to -100 indicate a faster colonisation into post-agricultural forest (Verheyen *et al.,* 2003).
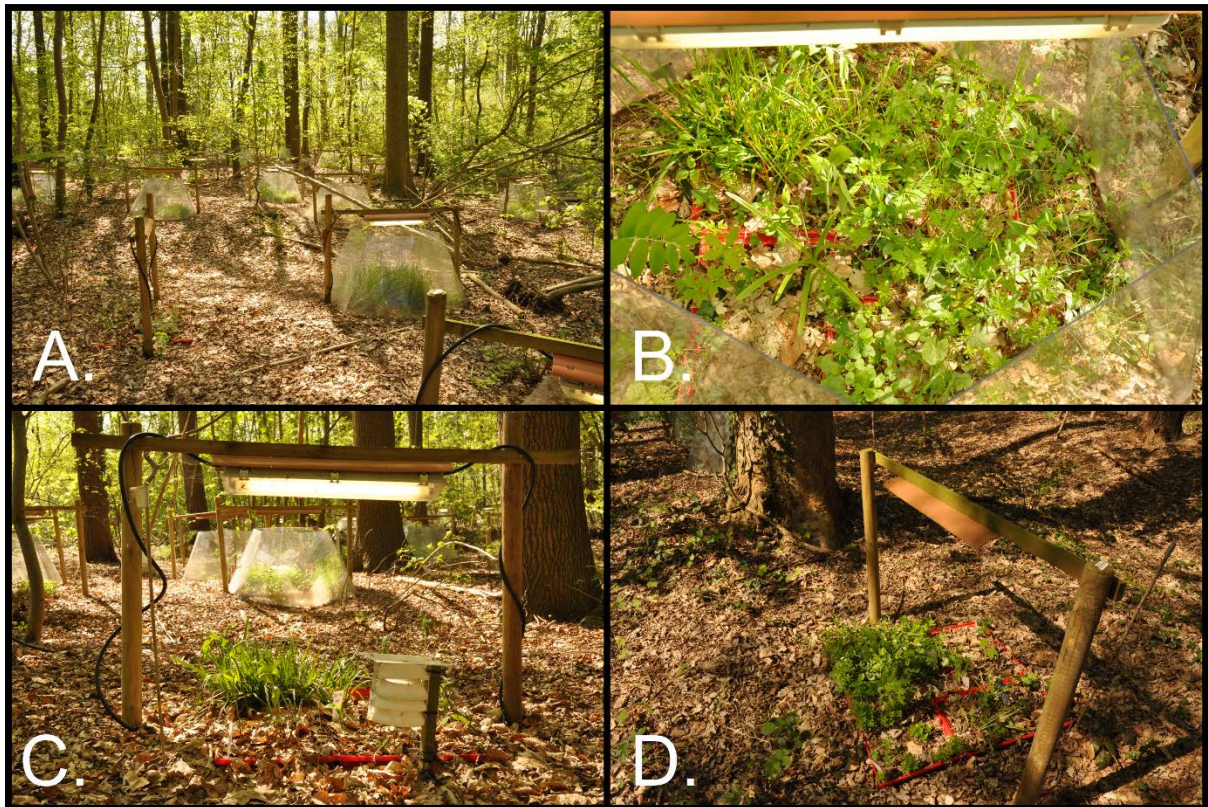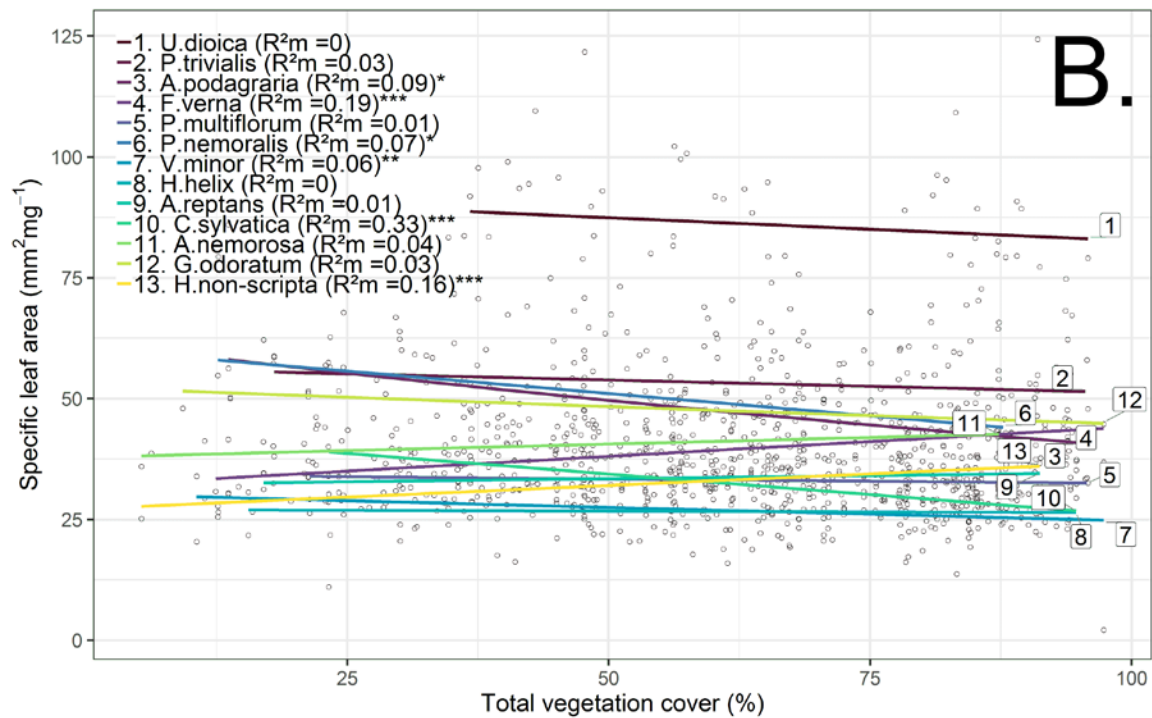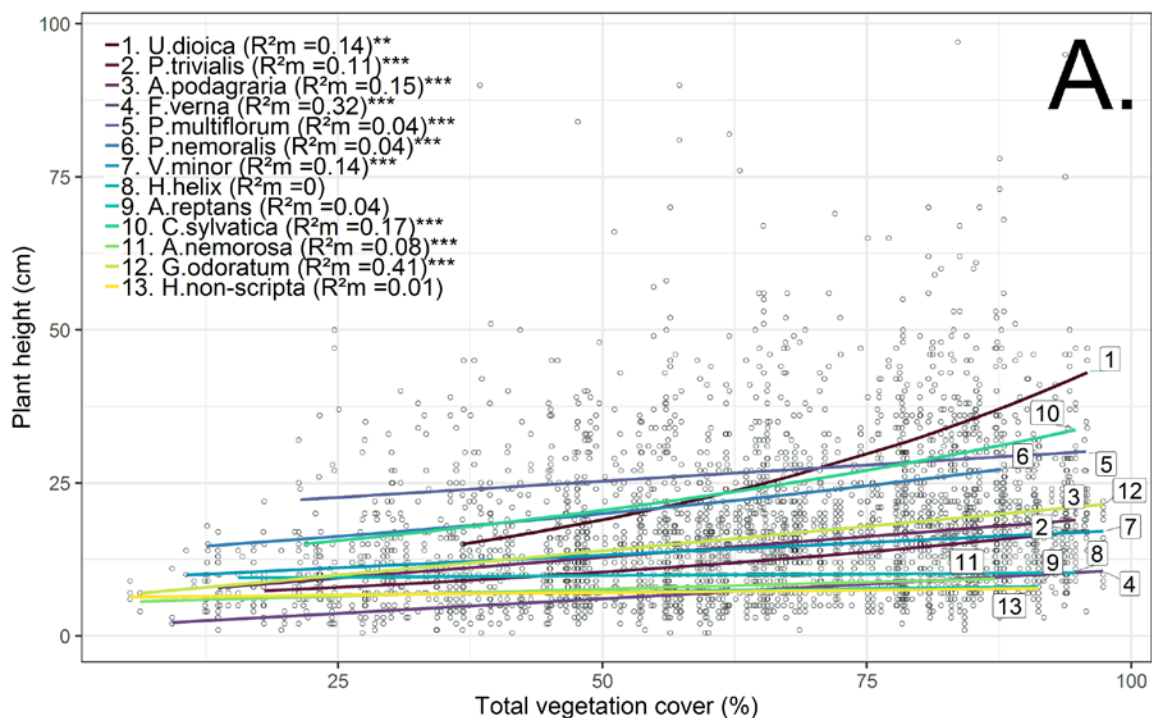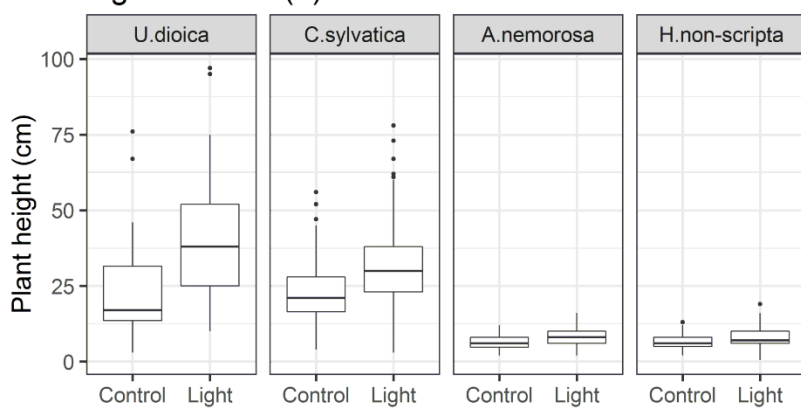
Figures

Fig. 1

Fig. 2

A.

1. U.dioica (R²m =0.14)**
2. P.trivialis (R²m =0.11)***
3. A.podagraria (R²m =0.15)***
4. F.verna (R²m =0.32)***
5. P.multiflorum (R²m =0.04)***
6. P.nemoralis (R²m =0.04)***
7. V.minor (R²m =0.14)***
8. H.helix (R²m =0)
9. A.reptans (R²m =0.04)
10. C.sylvatica (R²m =0.17)***
11. A.nemorosa (R²m =0.08)***
12. G.odoratum (R²m =0.41)***
13. H.non-scripta (R²m =0.01)

B.

1. U.dioica (R²m =0)
2. P.trivialis (R²m =0.03)
3. A.podagraria (R²m =0.09)*
4. F.verna (R²m =0.19)***
5. P.multiflorum (R²m =0.01)
6. P.nemoralis (R²m =0.07)*
7. V.minor (R²m =0.06)**
8. H.helix (R²m =0)
9. A.reptans (R²m =0.01)
10. C.sylvatica (R²m =0.33) ***
11. A.nemorosa (R²m =0.04)
12. G.odoratum (R²m =0.03)
13. H.non-scripta (R²m =0.16)***

Fig. 3

Plant height (cm)

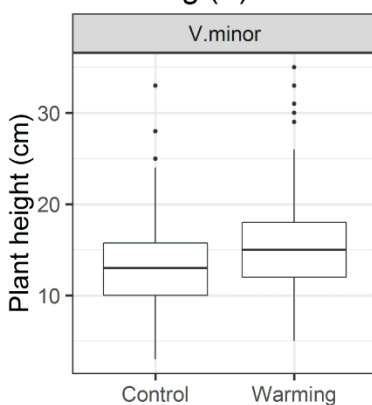SLA (mm^{2}mg^{-1})

Fig. 4

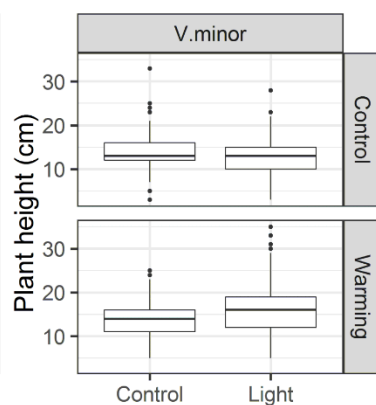# Significant Plant height effects
A. Light addition (L)
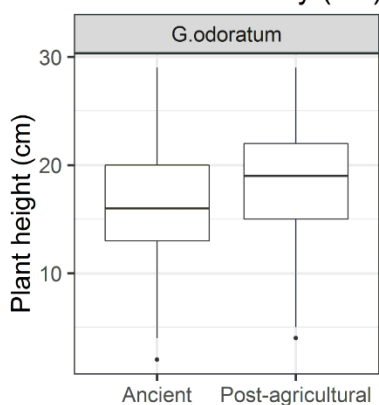
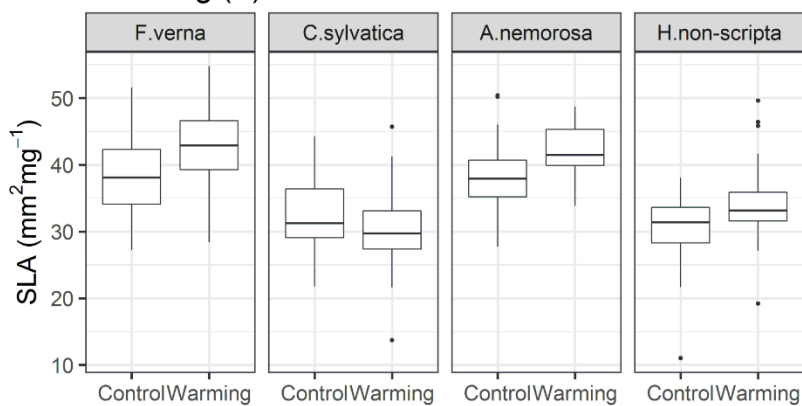## B. Land-use History (LU)
C. Warming (T)
D. Interaction of L*T

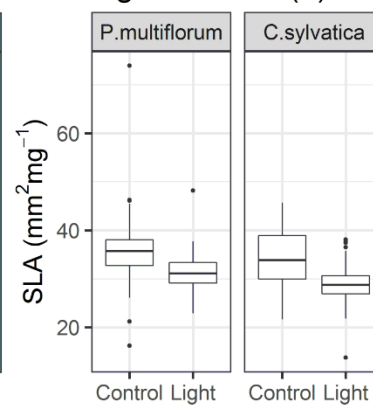# Significant SLA effects
E. Warming (T)
F. Light addition (L)

Fig. 5