Larger direct than indirect effects of multiple environmental changes on leaf nitrogen of forest herbs

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Abstract

- **Aims.** Disentangling direct and indirect effects of global change drivers on plant nitrogen (N) uptake in leaves is important for understanding species and community responses in a changing world.
- **Methods.** We created understorey herb communities on forest soils with and without recent agricultural history. We traced pulse additions of $^{15}\text{NH}_4^{15}\text{NO}_3$ within these mesocosms while applying two-level factorial treatments of N enrichment, warming and illumination. We modelled direct and indirect effects of treatments on leaf N content and ^{15}N uptake in leaves.
- **Results.** Warming and illumination had three times larger direct negative effects on leaf N content per dry mass and percentage leaf N derived from label (Ndfl%) than their indirect negative effects via an increasing community cover. These results imply a tissue dilution of N with increasing growth, in response to environmental change directly and indirectly exacerbated by community cover. We additionally found that interspecific differences in Ndfl% correlated with a species' colonisation capacity and resource acquisition strategy.
- **Conclusions.** Global change can directly affect allocation of N into foliage, with simultaneous indirect effects via altered community properties that influence individual plant responses. Predicting the future of plant communities in a changing world requires accounting for such understudied pathways.

Key words

^{15}N label, isotopic tracer, herbaceous understorey, ancient forest, post-agricultural forest

Abbreviations

- at.‰: atomic percentage
- C: carbon
- CCI: colonisation capacity index

- CI: confidence interval (95%)
- CWM: community weighted mean
- Leaf N content (%): leaf nitrogen content per unit of leaf dry mass ($\text{gram gram}^{-1} * 100$)
- N: nitrogen
- NH_4NO_3 : ammonium nitrate
- Ndf1%: percentage of leaf nitrogen content derived from label
- P: Phosphorus
- PAR: Photosynthetically active radiation
- SLA: specific leaf area
- SE: standard error
- SEM: structural equation model

Introduction

The extent of abandoned arable land has been increasing across the globe since the latter half of the 20th century (Cramer et al. 2008; Waters et al. 2016). Legacies of prior agriculture, particularly nutrient enrichment in the soil, can persist there for decades to centuries (Falkengren-Grerup et al. 2006; Grossmann and Mladenoff 2008; Leuschner et al. 2014; Yesilonis et al. 2016). These land-use legacies can, combined with other global-change drivers, steer important ecosystem patterns and processes such as plant community composition and nitrogen (N) cycling (Perring et al. 2016). In temperate forests, herbaceous communities have a key role in the cycling of N via their foliage and back into the soil organic matter (Gilliam 2007). Despite this layer only representing about half of the foliar biomass as compared to overstorey trees (Landuyt et al. (in press); Gilliam 2007), herbaceous species have higher N assimilation rates than canopy trees (Buchmann et al. 1996) and root in shallower nutrient rich topsoil layers (Jobbágy and Jackson 2001). This means the leaf litter of herbaceous species is of better quality compared to foliage of woody species, due to leaf traits that promote N uptake (Cornwell et al. 2008; Díaz et al. 2016). Changing N uptake in herbaceous communities due to legacies of prior agriculture and other global change drivers could thus affect forest ecosystem functioning as a whole, and may depend on ecological strategies of species (Gilliam 2007).

Functional leaf traits determine N uptake of forest herbs and correlate with a species' colonisation capacity and life-history strategy (Verheyen et al. 2003). Leaf traits of species follow a fast-slow resource acquisition continuum (Wright et al. 2004). These leaf traits, such as the leaf area to mass ratio (specific leaf area, SLA) and the leaf N content per dry mass, either promote large N uptake for fast carbon accumulation (resource acquisition), or resource conservation with slow carbon accumulation (Wright et al. 2004; Reich 2014; Díaz et al. 2016). Species that initially colonise abandoned arable land (post-agricultural forest) occur at the fast end of the resource acquisition continuum and have a "fast" life-history, i.e. high relative growth rates and short lifespans (Díaz et al. 2016; Beckman et al. 2018). Such fast colonisers have leaves

that are characterised by a high SLA and high leaf N content per dry mass (Verheyen et al. 2003) to efficiently capture light in bright and nutrient enriched conditions (Poorter and Evans 1998; Evans and Poorter 2001).

Contrastingly, on the slow and resource conservative end of the spectrum, we observe species that slowly colonise into post-agricultural forest and are typical of ancient forest (Verheyen et al. 2003). Slow colonisers have resource conservative leaf traits (Díaz et al. 2016) that, between species, are characterised by a low SLA and low leaf N content per dry mass (Verheyen et al. 2003). These resource conservative leaf traits arise from two contrasting life-history strategies that allow competition for light in shaded conditions (Verheyen et al. 2003; Valladares et al. 2016). First, there are “vernal” geophytes with relatively thick leaves that emerge under fully lighted conditions in early spring (before tree canopy flush) and senesce when the forest canopy closes in late spring (Mabry et al. 2008). Second, there are shade tolerant species, which keep photosynthetic tissues throughout the growing season under a closed canopy in dark conditions. Intraspecific variation of leaf traits in these species can occur as they are particularly sensitive to sudden changes in light conditions (Valladares and Niinemets 2008; Liu et al. 2016).

Multiple global change drivers such as climate change, N enrichment from atmospheric deposition and land-use legacies additionally affect foliar N in the herbaceous understorey (Gilliam 2007). Environmental changes can have direct physiological effects or indirect community effects on foliar N (Manning et al. 2006). N content per dry mass in herbaceous leaves can directly respond to light and warming, and “dilute” (i.e. decrease) due to increases in plant size and carbon acquisition rates (Jarrell and Beverly 1981; Takeuchi et al. 2001; Yin 2002; Sardans et al. 2017). Contrastingly, a direct increase in leaf N content per dry mass can occur in response to N enrichment from deposition (Fraterrigo et al. 2009; Farrer and Suding 2016) or legacies of prior agriculture (Baeten et al. 2011; Siefert and Ritchie 2016). At the community level, indirect effects can occur as nutrient enrichment in warm and bright conditions could steer community trait distributions towards dominance of species with innate high tissue N and

SLA (Gilliam et al. 2016). On the other hand, these environmental drivers could enhance biomass production of the whole community (Kardol et al. 2010) and increase vegetation cover (Muukkonen et al. 2006). Larger vegetation cover raises asymmetric competition for light between individuals, which pushes plants to invest more in vertical growth (Freckleton and Watkinson 2001; DeMalach et al. 2016, 2017). Environmental change could thus indirectly exacerbate a decline in leaf N content per dry mass of plants via the community biomass production (Craine et al. 2018; Stevens 2019). Biomass production in forest understoreys is, however, seasonally variable and depends on life-history strategies of species (Jagodziński et al. 2016).

To understand the effects of environmental change on forest herb N uptake, on both a species and community level, we need to determine N uptake independently of species' preference for either ancient or post-agricultural forest. We therefore mixed forest herbs with different colonisation abilities and life-history strategies to document herb N uptake under multiple environmental changes and on soils with different land-use history. We monitored direct *in situ* added inorganic N uptake in forest herbaceous communities via $^{15}\text{NH}_4^{15}\text{NO}_3$ pulse labelling. We hypothesize that:

1. Warming, light availability, N enrichment and agricultural legacies can affect N allocation to leaves in plants. Changes in plant community dynamics can alter these effects. Enhanced growth of plants could potentially lead to a dilution of tissue N within plant species.
2. The ability of forest herbs to take up the inorganic ^{15}N tracer in leaves correlates with a species' colonisation capacity and its resource acquisition strategy. Fast colonising resource acquisitive species, as characterised by high SLA and leaf N content per dry mass, retrieve more ^{15}N tracer than slow colonising resource conservative species, which are characterised by a low SLA and leaf N content per dry mass.

Materials and Methods

We performed an *in situ* forest mesocosm experiment where we mixed herbaceous species with different colonisation abilities and planted these on soils from both ancient forest (forested before at least 1850) and post-agricultural forest (forested since 1950). We selected 64 mesocosms (from a larger field experiment) with closely matching soil texture and pH to inject with the ^{15}N tracer. This was to limit confounding of plant N uptake with inherent soil fertility and to isolate effects that agricultural history had on the chemistry of the soil samples (See Supplementary Table 1 and Blondeel et al. 2018).

We have two soils from two north-western European regions, with two ancient and two post-agricultural forest soils of each region (treatment “LU” with two replicates), using two-level treatments of N enrichment (treatment “N”), warming (treatment “T”) and light addition (treatment “L”). The 64 mesocosms thus comprise a full-factorial design, i.e. 2 regions x 2 replicates x 2 LU x 2N x 2T x 2 L = 64. In the following sections, we describe the specificities of the forest site, the soil collection and plant community creation (set-up of mesocosms), the environmental treatments, the use of ^{15}N tracer, the measurement of plant traits and community variables, and data analysis.

Site description

The study was conducted in the long-term ecological research site (LTER) Aelmoeseneie forest (50°58'30" N, 3°48'16" E, 20 m a.s.l), a temperate mixed deciduous forest in Northern Belgium (DEIMS-SDR Database 2019). It has a temperate Atlantic maritime climate with a mean annual temperature (MAT) of 10.6 °C, mean annual precipitation (MAP) of 768 mm and annual N deposition (2016) of 15.5 kg N ha⁻¹ (DEIMS-SDR Database 2019, ICP forest). The tree canopy (90% cover) is dominated by *Fagus sylvatica*, *Quercus robur*, *Acer pseudoplatanus*, *Fraxinus excelsior* and *Larix decidua*. The *in situ* manipulations on the mesocosms occur in a 1.8 ha fenced area within the forest.

Set-up of mesocosms: planting soils with herbaceous communities

All soils were collected from ancient forest and post-agricultural forest across regional gradients of soil fertility and N deposition (Blondeel et al. 2019). The soil provenances of the 64 mesocosms are Flanders, northern Belgium (50°58'30" N, 3°48'16" E, MAT = 10.1 °C, MAP = 772 mm and N deposition = 22.5 kg N ha⁻¹) and Skåne, southern Sweden (55° 32' 47" N, 13° 14' 28" E, MAT = 7.7 °C, MAP = 765 mm, N deposition = 12.2 kg N ha⁻¹). Within each of these two regions, we use soil from two adjacent pairs of ancient forest (forested since at least 1850) and post-agricultural forests (forested since 1950) as substrates for the plant communities (treatment land-use history, "LU"). The legacy of prior agriculture in the particular soils was most evident in the higher soil pH (+0.61 pH units ±0.052 standard error (SE)), lower soil C:N ratio (-1.77±0.09 SE) and a higher soil Olsen-P concentration (+45 mg kg⁻¹ soil ±2.64 SE) of post-agricultural forest in comparison with ancient forest (supplementary Table 1). We placed the sampled soil (13L per mesocosm) in pre-drilled trays (46.5 × 31.5 cm, depth of 19.5 cm) on top of 9 L of white sand to ensure proper drainage. We applied a root fabric liner in the trays to ensure that plants would not root in the site's local soil. The mesocosms were buried into the soil such that the top of the mesocosms was level with the soil surface.

We randomly introduced slow and fast colonising plant species to these soils. We compiled a pool of fifteen species commonly found in temperate European forests (Supplementary Table 2) and divided this species pool according to three emergent groups that are linked with a species' colonisation capacity (Verheyen et al. 2003; De Frenne et al. 2011). We used the colonisation capacity index (CCI) reported by Verheyen et al. (2003) to determine a species ability to successfully colonise into post-agricultural forest. Verheyen et al. (2003) observed differences in functional and life-history traits between 216 herbaceous species that have colonised and/or established in post-agricultural forests to varying degrees, or either remain confined in ancient forest. These recorded establishments allow the correlation of traits that lead to a successful colonisation (as in assessing dispersal syndromes, see Stevens et al. 2013). The colonisation

capacity index (CCI) is a scale that captures this variation in colonisation success between species and is related to functional and life-history traits (Verheyen et al. 2003). The CCI ranges from -100 to +100, where more negative values indicate a higher capacity to successfully colonise into post-agricultural forests (Verheyen et al. 2003). All but one species (*Hedera helix*) was reported by Verheyen et al. (2003), for this species we assume a CCI of 50 based on the fact that it can be dispersed by birds but that it has difficulty in colonising isolated forest patches (Butaye, Jacquemyn, & Hermy, 2001).

The three emergent groups comprise species that differ in their capacity to colonise post-agricultural forest. The first group are six poor colonisers with resource conservative traits that are typical of ancient forest (Group A: *Anemone nemerosa* L., *Galium odoratum* (L.) Scop., *Vinca minor* L., *Hyacinthoides non-scripta* (L.) Chouard ex Rothm., *Polygonatum multiflorum* (L.) All. and *Carex sylvatica* Huds). The second group are six common colonizers of post-agricultural forest with intermediate colonisation capacity and resource acquisition strategies (Group B: *Ajuga reptans* L., *Glechoma hederacea* L., *Geranium robertianum* L., *Hedera helix* L., *Poa nemoralis* L., *Ficaria verna* Huds.). The final group are three resource acquisitive nitrophilic species with a higher affinity towards post-agricultural forest (Group C: *Aegopodium podagraria* L., *Poa trivialis* L., *Urtica dioica* L.). Each of the twelve randomly assembled communities hosts five species and was randomly assigned two poor colonisers, two common colonisers of post-agricultural forest and one nitrophilic species (Supplementary Table 3). These five species were planted four times per tray at equal distances (a grid of $7 \times 8.5 \text{ cm}^2$), resulting in 20 plants per mesocosm. All mesocosms were installed in random groups of four (referred to as a “plot”) to apply manipulative experimental treatments, as explained in the next section and following methods of De Frenne et al. (2015) and Ma et al. (2018).

Manipulating the environment: N enrichment, warming and enhanced light availability

We have applied three full-factorial environmental changes with two levels per treatment to all mesocosms. Treatments started in April 2016 and consist of (i) N enrichment; (ii) experimental warming and (iii) enhanced light availability. A detailed overview of the mesocosms and their assigned treatments are given in Supplementary Figure 1 and Supplementary Table 4.

The first treatment is N enrichment (treatment “N”) where we add the equivalent of $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ to our mesocosms in addition to the ambient amount of N deposition ($15.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in 2016). This amount of N addition is high on a regional level, but possible near localised sources of N deposition e.g. near livestock farms (Bobbink et al. 2015). We added 0.25 L of a 2.01 g L^{-1} solution of NH_4NO_3 per mesocosm ($50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ eq.) and sprayed the plants with 0.25 L of demineralised water. We performed this treatment four times per year at the start of each season. The control mesocosms received 0.5 L of demineralised water at the same time as the N addition.

The second treatment consisted of experimental warming (treatment “T”) via the use of open top chambers with a 75 cm open top (De Frenne et al. 2010). Control plots were left to ambient temperatures. We measured air temperature (15 cm above forest floor), surface temperature (0 cm) and soil temperature (5 cm depth) in eight plots, one for each factorial combination of light x warming x nitrogen, using 24 thermocouples (Type T miniature, TC Direct, The Netherlands) which log every 30 seconds (CR1000, Campbell Scientific, USA). At the time of the tracer experiment (6 March 2017 - 20 May 2017), we measured a significant increase ($p < 0.05$) in daily mean air temperature (at 15 cm height) of $1.13 \pm 0.36 \text{ }^\circ\text{C}$, and insignificant increases (0.23 ± 0.36) in the surface temperature (at 0 cm) and soil temperature ($0.39 \pm 0.36 \text{ }^\circ\text{C}$) at 5 cm depth (Supplementary Figure 2).

The third treatment, enhanced light availability (treatment “L”), adds $24 \pm 4 \mu\text{mol m}^{-2}\text{s}^{-1}$ PAR to the ambient light conditions by use of two 18 W fluorescent tubes suspended 75 cm above

ground level of each plot (Supplementary Figure 3). These lights are programmed to follow the natural photoperiod throughout the year (De Frenne et al. 2015). Control plots receive ambient light under the tree cover of 90% ($8 \pm 0.7 \mu\text{mol m}^{-2}\text{s}^{-1}$) and have a dummy lamppost suspended over the plot.

Addition, sampling and analysis of labelled $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$

The uptake of mineral N was followed via four pulse additions of $^{15}\text{NH}_4^{15}\text{NO}_3$ (with ^{15}N at 98 atom % excess). A grid with 20 sections of $8.5 \times 7 \text{ cm}^2$ each was placed over the mesocosms with each section receiving four injections (of 0.5 mL) in order to obtain a homogenous distribution of the ^{15}N tracer over the whole mesocosm. The total amount of ^{15}N tracer added, equalled 8 % of the average total soil N concentration or 29.9 mg N per mesocosm. We decided to add 8 % of the average total soil N concentration as tracer, to minimise a fertilisation effect but still ensuring sufficient recovery of ^{15}N . The injection was performed with a syringe that could penetrate the soil at 1 cm depth. The injections were repeated four times: once per week from the 19th of April until the 10th of May 2017. This is the time when community peak biomass develops with considerable species turnover in understorey communities (Jagodziński et al. 2016). We acknowledge that varying phenology between species can be the basis of differences in uptake of inorganic tracer N. Early-flowering species would have allocated N to leaves prior to the second half of April from either nutrient-storing organs (Klimešová et al. 2016), and inorganic or organic N from the soil (Miller et al. 2007). By applying the tracer in this time-window, we aim to infer the fate of the inorganic ^{15}N pulses when all species in the community are present and the community as a whole reaches peak biomass under multiple global change treatments.

We harvested at least two leaves per individual on 17th May 2017, and only leaves which emerged during that growing season. We pooled leaf samples of the same species within each mesocosm prior to ^{15}N analysis. *Glechoma hederacea* and *Geranium robertianum* were excluded from the analysis since an insufficient amount of leaf samples (<5) were collected. Leaf samples

were dried at 65 °C for 48 h and milled for 5 min at 200 rpm (Mixer Mill MM 200, Retsch, Germany). Grass tissue samples were milled for 10 min to obtain the same fragmentation degree as the other plant species. Total leaf N content per leaf dry mass (% , gram gram⁻¹ ×100) and ¹⁵N abundance (at. %) were measured using an elemental analyzer (ANCA-SL, SerCon, UK) coupled to an isotope ratio mass spectrometer (20-22, SerCon, UK). The ¹⁵N natural abundance of all species was around 0.37 % in the original planting material, so we used this value throughout the analysis (Supplementary Table 5). We expressed the enrichment in ¹⁵N as a percentage of N derived from label (Ndfl%) (International Atomic Energy Agency 1983), which gives the percentage of N in the leaves that are added via the tracer, with 0% being no tracer recovered (Equation 1).

$$Ndfl\% = \left(\frac{{}^{15}N_{leaf\ sample} - 0.37}{{}^{15}N_{excess\ NH_4NO_3}} \right) * 100$$

Equation 1: Calculation of the percentage N derived from label (referred to as "N derived from fertiliser" (Ndff) by International Atomic Energy Agency, 1983). The numerator consists of the measured ¹⁵N abundance in a given leaf sample (at. %) subtracted with the natural abundance of ¹⁵N (0.37 at. %). The denominator notes the abundance of ¹⁵N in excess in the labelled NH₄NO₃, which was 98 at. %.

Measuring plant and community variables

We measured leaf N content per dry mass and specific leaf area (SLA) as the functional leaf traits in our analysis (for terminology, see Garnier et al. 2016). Leaf N content per dry mass (%) was measured during the analysis of ¹⁵N isotopes and is described in the section above. We measured specific leaf area (SLA) from fresh leaves (excluding petioles) at the time of peak biomass (supplementary information Table 2). We took digital RGB images at 30 cm height (13 MP camera) of the leaves on a white background with a red square reference area (4 cm²). We calculated leaf area using the digital analysis tool "Easy Leaf Area" (Easlon and Bloom 2014), which calibrates the area of green pixels in the image in relation to the red reference area. After measuring leaf area, samples were dried for 48 h at 65°C to ensure a constant dry weight.

Specific leaf area was calculated as the ratio of the leaf area to dry mass and expressed in $\text{cm}^2\text{mg}^{-1}$.

Total vegetation cover is a community property that is linked with biomass production, vegetation density, and asymmetric competition for light (Muukkonen et al. 2006; DeMalach et al. 2017). Total vegetation cover could thus influence N uptake, via a larger community biomass production and larger asymmetric competition for light, which could dilute leaf N. Total vegetation cover was measured three times during the experiment: before the first ^{15}N pulse (17th of April), at the third ^{15}N pulse (2nd of May) and just before the harvest of leaves for ^{15}N analysis (17th of May). We took digital RGB images at 75 cm height of the mesocosms perpendicular to the soil surface and used the “Canopy Area” tool which measures green pixels of vegetation and recalculates this into a total cover percentage (Easlon and Bloom 2014). The vegetation cover ranged between 40% and 80% (see Figure 2B). These plant communities are consequently not overly dense, while individual leaves of plants can still overlap but remain recognisable. Individual species cover was later assessed by visually checking the digital images for the share of each species in the total vegetation cover of each mesocosm, because the “Canopy Area” tool cannot discriminate cover between species. We used this species cover value to weight the intraspecific SLA in each mesocosm in order to calculate a community weighted mean value of SLA (CWM SLA).

Data analysis

All hypothesis tests and data analyses were performed using R (R Core Team 2019). We consistently applied linear mixed effects models (package nlme,; Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2018) with “Region” as a random intercept.

In hypothesis 1, we test direct and indirect effects of environmental changes on species’ leaf N content per dry mass and Ndf%. As this is a complex question, we used the package “piecewiseSEM” to build a structural equation model (SEM) (Lefcheck 2016). The main idea of this type of SEM is to perform SEM analyses on hierarchically structured data. A certain

response variable can be added as a predictor variable in a next model, with regression coefficients and p-values for each model calculated directly when fitting the SEM. To check whether the overall hypothesised relationships are consistent with the data, a Fischer C test is computed for which p has to be greater than alpha (0.05).

The hypothesised relationships allow checking for direct and indirect effects of the four environmental treatments (T, L, N, LU as main effects) on four partial responses: CWM SLA (a community trait characteristic, cm² leaf area per mg of leaf dry mass), total vegetation cover (%), leaf N content per dry mass (%) and the retrieved ¹⁵N as Ndf % (Figure 1a, Table 1). The first relationship shows the CWM SLA as a community response to the four environmental treatments, as we have assembled twelve communities with different species and leaf trait combinations. This relationship tests whether communities veer towards dominance of individuals and species with high SLA under bright, warm and nutrient enriched conditions. The second is a response of total vegetation cover to the CWM SLA and the four environmental treatments. This relationship tests whether vegetation cover increases due to a changing community or to environmental treatments directly. The third is the response of leaf N content per dry mass (accounting for species identity) to the vegetation cover and the environmental treatments. This relationship tests whether enhanced community growth could cause a dilution of tissue N. We adopted leaf N content per dry mass in the SEM in favour of SLA because we expect that these two variables are highly correlated (see hypothesis 2) and that leaf N would potentially better reflect the uptake of N (see Firn et al. 2019). The final relationship tests the ¹⁵N retrieval (Ndf%) to variation in species' leaf N content per dry mass (species identity effect + leaf N) together with direct effects of the four environmental treatments. In this model, two opposing trends in foliar N are tested: species can show interspecific differences in leaf N, but environmental changes can directly cause growth dilution of tissue N across species. Prior to running the SEM, we tested these relationships for normality assumptions using a Shapiro-Wilk test and checking residuals with a histogram and qq-plot. We log-transformed the response variable if necessary to attain normality in residuals, which only applied to Ndf%.

After computing the SEM, we compared the effect sizes of direct vs indirect effects of experimental treatments on both leaf N content per dry mass and Ndf%. Quantifying the indirect effect size of an environmental treatment is obtained by multiplying the coefficients of significant pathways that start from a given experimental treatment and indirectly lead to one of either focal response variables. We calculated net effects of each treatment by adding the significant direct and indirect effects, and computed a ratio of Direct:Indirect effects to compare effect sizes. The pathway of indirect effects for leaf N content per dry mass can potentially occur via CWM SLA and/or Cover, while the pathways for indirect effects of a treatment on Ndf% can potentially occur via the net effect of that treatment on leaf N.

In hypothesis 2, we test whether the labelled ^{15}N will be retrieved most by fast-colonising nitrophilic species with high leaf N content per dry mass (%) and high specific leaf area (SLA, $\text{cm}^2\text{mg}^{-1}$), i.e. resource acquisitive leaves (Díaz et al. 2016). We calculated Pearson's correlation coefficients (Pearson's r , $\alpha = 0.05$) between the percentage N derived from label (Ndf%) in leaves and leaf N content per dry mass, SLA and a species' colonisation capacity index (CCI). To check for the relationships between the leaf traits and colonisation ability independently of Ndf%, we computed Pearson's r between leaf N content per dry mass and SLA, leaf N content per dry mass and CCI, and SLA and CCI.

Results

In hypothesis 1, we tested direct and indirect effects of N enrichment, warming, enhanced light availability and agricultural land-use history on leaf N content per dry mass (%) and ^{15}N uptake (Ndf%), mediated by the community vegetation cover (%) and community weighted mean of specific leaf area (CWM SLA) in a structural equation model (Figure 1b). The overall SEM had a Fischer C of 3.16 with a p-value of 0.977, which indicates that the retained relationships are a valid description of the system (for the full model description with all coefficients, see Supplementary Table 6). The N enrichment treatment and the CWM SLA did not significantly affect any response variable. We found significant positive and direct main effects of past

agricultural history ($+9.26 \pm 2.3$, $p < 0.0001$, unit: %), enhanced light availability ($+17.1 \pm 2.3$, $p < 0.0001$, unit: %) and warming ($+12.4 \pm 2.3$, $p < 0.0001$, unit: %) on total vegetation cover (Figure 2a). The increasing total vegetation cover in response to environmental change in turn led to a lower species' leaf N content per dry mass (-0.006 ± 0.003 , $p = 0.03$, Figure 2b) which constitutes as an indirect effect. Direct negative effects of warming (-0.25 ± 0.09 , $p = 0.0095$) and illumination (-0.31 ± 0.10 , $p = 0.0013$) on leaf N content per dry mass across species occurred as well. Finally, the log-transformed ^{15}N uptake by species ($\log(\text{Ndf}\%)$) had a direct negative response to warming (-0.23 ± 0.11 , $p = 0.03$), but a positive response ($+0.43 \pm 0.09$, $p < 0.0001$) to species' leaf N content per dry mass (%).

When comparing the effect sizes of the direct and indirect effects of the environmental treatments on both leaf N content per dry mass and $\text{Ndf}\%$ (Table 2), we observed that significant direct effects were between 1.7 and 3.3 times larger than significant indirect effects of the same treatment. However, more significant indirect effects occurred for both leaf N content per dry mass (2 direct, 3 indirect) and $\text{Ndf}\%$ (1 direct, 3 indirect). All significant direct and indirect effects of environmental treatments on both responses were negative, hence net effects of environmental treatments lowered both leaf N content per dry mass and $\text{Ndf}\%$. When summing significant effects of experimental treatments, we found that the direct effects of warming (T) and illumination (L) on leaf N (%) surpassed the indirect effects (from T,L,LU) via vegetation cover by 2.4 times. Notice that indirect effects on leaf N % occur via Cover, and on $\log(\text{Ndf}\%)$ via leaf N. For $\text{Ndf}\%$, the direct effect of warming (T) was 0.7 times smaller than the indirect effect of combined treatments via leaf N content per dry mass (originating from T,L,LU via vegetation cover). But leaf N % also directly affects $\text{Ndf}\%$, and the summed indirect effects of the treatments on $\log(\text{Ndf}\%)$ via leaf N (-0.341) did not cancel out the positive direct effect of leaf N % ($+0.43$, see Figure 1B).

In hypothesis 2, we tested whether ^{15}N retrieval in foliage is greatest in fast colonising nitrophilic species with resource acquisitive leaf traits. When looking at the correlations

between the leaf traits and CCI, we found that leaf N content per dry mass and SLA were positively correlated (Pearson's $r = 0.54$, $p < 0.0001$), and that leaf N content per dry mass (Pearson's $r = -0.45$, $p < 0.0001$) and SLA (Pearson's $r = -0.38$, $p < 0.0001$) were both negatively correlated with CCI. More importantly, we found that the percentage N derived from label (Ndf%) in leaves was negatively correlated with a larger affinity of plant species for ancient forest (Colonisation Capacity Index (CCI), Pearson's $r = -0.38$, $p < 0.0001$, Figure 3a) and positively correlated with leaf N content per dry mass (unit: %, Pearson's $r = 0.45$, $p < 0.0001$, Figure 3b) and SLA (unit: $\text{cm}^2 \text{mg}^{-1}$, Pearson's $r = 0.64$, $p < 0.0001$, Figure 3c). Looking at the three best colonisers (*Urtica dioica*, *Poa trivialis* and *Aegopodium podagraria*), these had an Ndf% of over 6 %, with the highly competitive nitrophile *Urtica dioica* (Parzych et al. 2017) even obtaining 9 % of its foliar N from the pulse additions. Contrastingly, the most N conservative species (Ndf < 1%) that we found were *Hyacinthoides non-scripta*, *Anemone nemorosa*, *Polygonatum multiflorum*, *Hedera helix* and *Vinca minor*, all understorey species typical of ancient forest.

Discussion

We documented plant N uptake of forest herbs under the influence of environmental change treatments and agricultural legacies, by analysing direct and indirect of these changes on foliar N. Applying a ^{15}N tracer at the height of spring allows us to infer the fate of inorganic N pulses in leaves at a time when all species in the community are present and the community as a whole reaches peak biomass under multiple global change treatments. The structural equation model confirmed our first hypothesis that environmental change treatments and agricultural legacies can have direct effects on leaf N content per dry mass and percentage leaf N derived from label (Ndf%) with indirect effects via community properties. Of these treatments, warming and illumination were the most important, and their direct effects were between 1.7 and 3.4 times larger than their indirect effects. Additionally, we confirmed our second hypothesis that large interspecific differences in Ndf% would correlate with a species' colonisation capacity and resource acquisition strategy. The results thus show that environmental changes directly and

indirectly affect forest herb N uptake, and that the magnitude of this effect depends on the position of a species along the resource acquisitive – conservative continuum (Wright et al. 2004; Reich 2014; Díaz et al. 2016).

Direct and indirect effects of environmental changes on plant N uptake

Using structural equation modelling, we were able to disentangle direct and indirect effects of agricultural land-use history and environmental changes on leaf N content per dry mass and the inorganic ^{15}N uptake in leaves (Ndf%) within species. Contrary to our expectations, we found neither direct nor indirect effects of N enrichment and community weighted mean of SLA (CWM SLA) on any response variable. The N enrichment treatment may produce interactions with other drivers to influence community responses (Dirnböck et al. 2017; Perring et al. 2018; Schmitz et al. 2019), with a potential absence of any main effect. We deliberately excluded interactions from the analysis as it would overcomplicate and convolute the SEM, and we mostly expected strong main effects. Due to the seasonal frequency of the N addition, main effects of N enrichment could also have been overshadowed by the rather high ambient N deposition in the test site ($15.5 \text{ kg N ha}^{-1}$ in 2016) which falls in the range of the critical load for deciduous forest (Bobbink et al. 2015). The lack of environmental treatments affecting CWM SLA suggests that there were no major shifts in community composition towards selection of species with high SLA under nutrient enriched, bright and warm conditions since the time of planting (2 years). The community cover did however increase in those conditions, but independently of CWM SLA.

Leaf N content per dry mass responded directly and indirectly to the treatments. We found direct negative effects of enhanced light availability and warming on leaf N content per dry mass. This effect could indicate that more foliar N is beneficial in low light environments within species, which could originate from a compensatory mechanism where plants invest more in foliar N under suboptimal conditions for photosynthesis (Niinemets 1997). On the other hand, warm and bright conditions (canopy gaps) can increase photosynthetic rates (Paul-Limoges et al. 2017), which raises carbon acquisition and enhances plant growth, but with a higher tissue

C:N ratio which reflects a tissue dilution of N (Jarrell and Beverly 1981; Takeuchi et al. 2001; Yin 2002; Sardans et al. 2017). This tissue dilution of N can have important effects on N cycling in the food web, as it is a mechanism that decreases the quality of leaf litter (Cornwell et al., 2008; Hobbie, 2015). We observed that this dilution mechanism could be exacerbated by enhanced community growth via an indirect effect of environmental treatments. Warming, illumination and agricultural history together nearly doubled vegetation cover, which for one correlates with community biomass production (Muukkonen et al. 2006; Heinrichs et al. 2010). This increase in vegetation cover explained additional variation of decreasing leaf N content per dry mass across species. This additional dilution effect might originate from asymmetric competition for light between individuals, which pushes plants to invest more in vertical growth (Freckleton and Watkinson 2001; DeMalach et al. 2016, 2017). The effect size of this indirect effect via vegetation cover is, however, three times smaller than the direct lowering effects of illumination and warming on leaf N content per dry mass. In addition, the slope of this vegetation cover effect is subtle compared to the large interspecific differences in leaf N content per dry mass that we observed.

The percentage leaf N derived from label (Ndf_l%) responded to the leaf N content per dry mass of species and the warming treatment. The direct positive effect of leaf N content per dry mass showed that there are large interspecific differences in Ndf_l%, and these differences can be predicted by a species' average leaf N content (%). This positive direct effect on Ndf_l% did not cancel out when accounting for the indirect negative effects of combined environmental treatments (T,L,LU) on leaf N content per dry mass. In addition, warming was the only environmental treatment that directly affected Ndf_l%, and reflected a lower Ndf_l% in warmer conditions. A similar negative effect was found in leaf N content per dry mass, and may also result from a tissue dilution in N due to enhanced growth (Jarrell and Beverly 1981). Overall, we found that species' innate leaf N content remains the main predictor for foliar N uptake (as measured by Ndf_l%) as its effect size is nearly double than that of the warming treatment.

N uptake by forest herbs follows the fast – slow continuum of plant forms

At the time of community peak biomass and across environmental treatments, fast colonising forest herbs with acquisitive leaf traits had more leaf N derived from label (Ndfl%) than slow colonising and resource conservative species. These results are intuitive and expected (Tilman 1990), but this is to our knowledge the first time where this has been tested using a N tracing experiment in temperate forest herbs and across multiple environmental manipulations (for other ecosystems, see Andersen and Turner 2013; de Vries and Bardgett 2016). Our N tracing experiment confirms that forest herbs follow the global fast – slow continuum of functional plant traits and strategies (Reich 2014). This fast-slow continuum of plant forms arises from resource acquisition and leaf construction (Wright et al. 2004; Díaz et al. 2016), its connection with fast and slow life-history strategies (Adler et al. 2014; Reich 2014) and dispersal ability (Beckman et al. 2018). We found that species that naturally grow in post-agricultural forests retrieved more of the inorganic tracer N in their leaves. These species, e.g. *Urtica dioica*, have higher relative growth rates and accumulate biomass until the end of the growing season (Fichtner and Schulze 1992), unlike spring flowering (vernal) species who quickly stall in growth once they reach peak biomass (Paul-Limoges et al. 2017).

Caution in inference of the experimental findings

We identified four potential limitations to our study, which need to be addressed to properly infer the findings from our experimental study. First, the phenology and stalled growth of vernal species towards May likely contributed to a lower Ndfl% in their leaves in comparison with the fast colonisers, which tend to flower later in the growing season. However, our finding of a lower leaf N content per dry mass in slow colonisers still suggests that these early flowering species are N conservatives (as in Hofmeister et al. 2012). Second, species that had already reached peak aboveground biomass before our tracing period (half of April) would have built up N from other sources (including organic and inorganic soil N) and could have allocated the tracer N to roots or other nutrient storing organs. We did not sample any below ground traits to avoid

damage to the communities, and instead were interested in responses of N uptake in leaves to environmental change treatments. Such allocation patterns of N to roots in favour of leaves can however differ under environmental changes, depending on plant functional types (Sugiura and Tateno 2011; Freschet et al. 2015; Sardans et al. 2017) and the preferred N source of species (Miller et al. 2007). Whether organic N uptake could alter in forest herbs due to environmental change is speculative but worthy of further research.

A third issue is a potential inhibiting effect of labile Al on the uptake of inorganic N in plants, which could vary due to the forest land-use history (Burnham et al. 2017). While labile Al can inhibit N uptake in many species, it is likely that the forest herbs of our species pool are less affected by the adverse effects of Al. Many woodland species prefer to utilise NH_4^+ , the more common inorganic N form in slightly ($\text{pH} < 5$) acidic forest soils (Falkengren-Grerup 1995). Instead, soluble Al can strongly decrease the availability of NO_3^- indirectly, via an inhibition on nitrifying microbes (Yang et al. 2018), which is of less importance in our experimental setup because we added inorganic NH_4NO_3 to our mesocosms directly. Furthermore, the availability in labile soil Al varied more strongly between regions than it did between pairs (i.e. due to land-use history, see Supplementary Table 1), so that any variation in Al is largely taken into account with the hierarchical modelling.

A final issue is the possibility that a limited N supply (N limitation) was a driver for dilution in tissue N, rather than changing N allocation patterns in response to environmental change. In a supplementary analysis, we compared the maximal N demand of aboveground biomass in the mesocosms to a conservative estimate of the N supply (from available inorganic soil N pool and N deposition) to check whether an N deficit could occur. If N demand calculated at maximum possible observed leaf N content per dry mass is always lower than the minimum N supply, then N limitation is an unlikely explanation for any altered N allocation to the leaves under changing environmental conditions. To be unequivocal in ruling out N limitation, it would be necessary to also include the N demand for belowground biomass (i.e. roots), but also the N that is supplied

via mineralisation of organic matter. We chose not to perform any destructive measures to our plant communities, so that we instead approach these estimates conservatively by accounting for aboveground biomass as N demand, and only N deposition and the available inorganic soil N as N supply. Our estimates show that maximal N demand from aboveground biomass was lower than the minimal supply in all instances (in most cases, substantially lower), meaning that N limitation is not a likely driver for changing leaf N. Furthermore, we found that current estimates of N stocks in aboveground biomass still increase due to larger productivity under environmental change despite a dilution of tissue N. Given that N stocks in aboveground biomass increased strongly with larger total vegetation cover, and no mesocosms surpassed their N supply, our results imply that N is a non-limiting resource. A lack of N limitation may be further demonstrated by the absence of an effect of the N fertilization treatment in our model analyses (Figure 1B, Supplementary Table 6).

Forest herbs and N uptake: implications for conservation in a changing world

The results of our N tracing experiment could elucidate the poor performance of typical forest herbs in post-agricultural forests in the natural world. We observed a differentiated inorganic N uptake between slow and fast colonisers during the growing season's peak of community turnover. This differentiated N uptake could exemplify common hurdles for biological conservation in ancient forest (Foster et al. 2003) and has potential effects for ecological restoration of post-agricultural forest (Honnay et al. 2002). The most N conservative species (NdfI <1%) that we found were *Hyacinthoides non-scripta*, *Anemone nemorosa*, *Polygonatum multiflorum*, *Hedera helix* and *Vinca minor*, all understorey plants of conservation concern in Western Europe (Hermy et al. 1999). Threats to these flora in ancient forest are well documented (Gilliam 2007; Baeten et al. 2010), as communities shift towards higher abundance of competitive and resource acquisitive species in response to global changes (Bernhardt-Römermann et al. 2011; Gilliam et al. 2016). Contrastingly, the fate of slow colonising and resource conservative species in post-agricultural forests is less well-known as they naturally

hardly occur there (Honnay et al. 2002). Development of forest understoreys can take decades up to centuries even if abiotic conditions are suitable for these species (De Frenne et al. 2011; Holmes and Matlack 2018). Individual slow colonising species can, however, gradually increase in abundance over time towards a community assembly akin to that of ancient forest (Naaf and Kolk 2015; Kolk et al. 2016). However, the gradual assembly of slow colonising species into post-agricultural forest could create novel species interactions with the resident fast N species (Alexander et al. 2015) and lead to a potential competitive exclusion of slow colonisers in the long run (Baeten and Verheyen 2017). Successful biological introductions in these forests would therefore be difficult to accomplish, with global environmental change inducing an additional level of uncertainty.

Conclusions

Our work assessed forest herb performance under environmental change from a distinct mechanistic angle by measuring *in situ* plant N uptake in leaves. We found that environmental changes can directly alter allocation of N to leaves in plants, with simultaneous but smaller indirect effects by affecting community properties which influence individual plant responses. We demonstrated that spectra in resource acquisition and plant strategies (Wright et al. 2004; Adler et al. 2014; Reich 2014; Díaz et al. 2016; Beckman et al. 2018) can additionally be used to predict such plant responses to multiple environmental changes. Future directions which focus on the role of forest herb communities in the cycling of N from multiple sources would be worthy of further research. Experiments able to disentangle direct and indirect effects of multiple environmental drivers are still scarce, but have the strength to assess mechanisms and test ideas that are difficult to infer in the natural world. The insights that we have gained are relevant for conservation and restoration of herbaceous communities in temperate forest.

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Authors' contributions

- All authors contributed to conceiving ideas and designing methodology. HB, PDF, DL, MPP and KV designed the multi-factor experiment. ER, SB and PB led the isotopic section of the experimental design.
- HB, ER and SB collected the data.
- HB and ER analysed the data.
- ER wrote an initial outline of the manuscript, after which HB led the writing. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

Data will be made public via GitHub and www.pastforward.ugent.be

Tables

Table 1: Four separate linear mixed effects models feed into the piecewise structural equation model, where the response of a former model becomes a predictor in the next, alongside the environmental treatments of land-use history (LU), warming (T), light addition (L) and Nitrogen enrichment (N). Two variables are measured on the community level: CWM SLA (community weighted mean of specific leaf area) and total vegetation cover (Cover). Leaf N content per dry mass (Leaf N) and the retrieval of ^{15}N (Nitrogen derived from label, Ndf%) are measured within species, hence the addition of the species identity (ID) as a predictor.

Level	Response	Model
Community	CWM SLA	T + L + N + LU + 1 Region
Community	Cover	CWM SLA + T + L + N + LU + 1 Region
Species	leaf N	Identity + Cover + T + L + N + LU + 1 Region
Species	Log(Ndf%)	Identity + leaf N + T + L + N + LU + 1 Region

Table 2: Partitioning of significant ($p < 0.05$) direct and indirect effects of treatments warming (T), light addition (L), N addition (N) and agricultural land-use history (LU) on leaf N content per dry mass (leaf N %) (a) and leaf nitrogen derived from label (Ndf%) (b). Coefficients for direct and indirect effects are obtained by multiplying the coefficients that were calculated by piecewise structural equation modelling (see Figure 1b) and are in unit of the response variable per treatment level. The net effect of a treatment is the sum of both the direct and indirect effect. When a treatment has both direct and indirect effects on a response variable, a ratio of Direct (Dir) over indirect (Ind) effect is calculated for comparing the effect size. Insignificant effects are greyed out, see supplementary Table 6 for all model coefficients (including $p > 0.05$). Notice that indirect effects on leaf N % occur via Cover, and on $\log(\text{Ndf}\%)$ via leaf N%. The summed indirect significant effects of the treatments on $\log(\text{Ndf}\%)$ via leaf N (-0.341) do not cancel out the positive direct effect of leaf N % (+0.43, see Figure 1b).

a)	Response	Treatment	Direct	Indirect (via Cover)	Net effect	Ratio Dir:Ind
	Leaf N (%)	T	-0.250	-0.074	-0.324	3.4
		L	-0.310	-0.103	-0.413	3.0
		N				
		LU		-0.056	-0.056	
		Sum	-0.560	-0.232	-0.792	2.4
b)	Response	Treatment	Direct	Indirect (via Leaf N)	Net effect	Ratio Dir:Ind
	$\log(\text{Ndf}\%)$	T	-0.230	-0.139	-0.369	1.7
		L		-0.177	-0.177	
		N				
		LU		-0.024	-0.024	
		Sum	-0.23	-0.341	-0.571	0.7

Figures

Figure 1: **Structural equation model showing direct and indirect effects of environmental treatments on leaf N (%) and Ndf1%.** Panel A. Graphical representation of the hypothesised

structural equation. All four treatment variables are included to predict each of the four response variables: community weighted mean of SLA (CWM SLA, cm^2/mg), Total vegetation cover (Cover, %), leaf N content per dry mass (Leaf N %) and leaf N derived from label (NdfI%).

Panel B. The result of the analysis using piecewiseSEM, with the retained paths being in accordance with the data (Fischer C = 3.16, $p = 0.97$). Only the significant ($p < 0.05$) paths are shown here, for all coefficients see supplementary table 6. The asterisks relate to the significance levels of the coefficients (* < 0.05 , ** < 0.01 , *** < 0.0001). Marginal R^2 (fixed factors alone) are given here; the conditional R^2 (with random effects included) did not differ from these, meaning that no extra variation was explained by the random effect terms. The model estimates with standard errors are given in the units of the response variable per unit of change in the predictor variable for continuous variables. For categorical variables, these are relative to control conditions (for T, L) and relative to ancient forest (for LU). The estimates of the species-effects on leaf N and NdfI% are given in Supplementary Table 6. Treatment “N” and community variable “CWM SLA” are greyed out for not having significant effects on a response variable.

Figure 2: **Environmental treatments increased community vegetation cover, which caused a dilution in leaf N content per dry mass (%) across species.** **Panel A:** Significant positive main effects of past agricultural history ($+9.26 \pm 2.30$, $p < 0.0001$), illumination ($+17.1 \pm 2.32$, $p < 0.0001$) and warming ($+12.4 \pm 2.31$, $p < 0.0001$) on total vegetation cover (%). The averages (95 % CI) within the replicates are shown here. **Panel B:** The bold dashed line (95% CI) represents the significant decrease (-0.006 ± 0.003 , $p = 0.03$) of leaf N content per dry mass in response to total vegetation cover across species. The coloured lines gives the trend for each species. Species in the legend are ordered on colonisation capacity index (CCI), with the fastest colonisers on top.

Figure 3: The labelled ^{15}N (Ndf%) was retrieved most by resource acquisitive species with a fast colonisation into post-agricultural forest, a high leaf N content per dry mass (%) and high specific leaf area (SLA). **Panel A.** CCI correlated negatively (Pearson's $r=-0.38$, $p<0.0001$) with Ndf% and is thus larger within fast colonisers. The number of leaf samples of each species are given alongside the species code. **Panel B.** Plants with a high leaf N content per dry mass had a higher Ndf% (Pearson's $r=0.48$, $p<0.0001$). **Panel C:** SLA correlated positively with Ndf% (Pearson's $r = 0.64$, $p<0.0001$). All confidence intervals are on the 95% confidence level.

References

- Adler PB, Salguero-Gomez R, Compagnoni A, et al (2014) Functional traits explain variation in plant life history strategies. *Proc Natl Acad Sci* 111:740–745. doi: 10.1073/pnas.1315179111
- Alexander JM, Diez JM, Levine JM (2015) Novel competitors shape species' responses to climate change. *Nature* 525:515–518. doi: 10.1038/nature14952
- Andersen KM, Turner BL (2013) Preferences or plasticity in nitrogen acquisition by understorey palms in a tropical montane forest. *J Ecol* 101:819–825. doi: 10.1111/1365-2745.12070
- Baeten L, De Frenne P, Verheyen K, et al (2010) Forest herbs in the face of global change: a single-species-multiple-threats approach for *Anemone nemorosa*. *Plant Ecol Evol* 143:19–30. doi: 10.5091/plecevo.2010.414
- Baeten L, Verheyen K (2017) Changes in the nature of environmental limitation in two forest herbs during two decades of forest succession. *J Veg Sci* 28:883–892. doi: 10.1111/jvs.12545
- Baeten L, Verstraeten G, De Frenne P, et al (2011) Former land use affects the nitrogen and phosphorus concentrations and biomass of forest herbs. *Plant Ecol* 212:901–909. doi: 10.1007/s11258-010-9876-9
- Beckman NG, Bullock JM, Salguero-Gómez R (2018) High dispersal ability is related to fast life-history strategies. *J Ecol* 106:1349–1362. doi: 10.1111/1365-2745.12989
- Bernhardt-Römermann M, Gray A, Vanbergen AJ, et al (2011) Functional traits and local environment predict vegetation responses to disturbance: A pan-European multi-site experiment. *J Ecol* 99:777–787. doi: 10.1111/j.1365-2745.2011.01794.x
- Blondeel H, Perring MP, Bergès L, et al (2019) Context-dependency of agricultural legacies in temperate forest soils. *Ecosystems* 22:781–795. doi: 10.1007/s10021-018-0302-9
- Bobbink R, Tomassen H, Weijters M, et al (2015) Chapter 4: Effects and empirical critical loads of Nitrogen for Europe. In: *Critical Loads and Dynamic Risk Assessments*. pp 297–326
- Buchmann N, Gebauer G, Schulze ED (1996) Partitioning of N-15-labeled ammonium and nitrate among soil, litter, below- and above-ground biomass of trees and understory in a 15-year-old *Picea abies* plantation. *Biogeochemistry* 33:1–23. doi: 10.1007/BF00000967
- Burnham MB, Cumming JR, Adams MB, Peterjohn WT (2017) Soluble soil aluminum alters the relative uptake of mineral nitrogen forms by six mature temperate broadleaf tree species: possible implications for watershed nitrate retention. *Oecologia* 185:327–337. doi: 10.1007/s00442-017-3955-8
- Butaye J, Jacquemyn H, Hermy M (2001) Differential colonization causing non-random forest plant community structure in a fragmented agricultural landscape. *Ecography (Cop)* 24:369–380. doi: 10.1034/j.1600-0587.2001.d01-193.x
- Cornwell WK, Cornelissen JHC, Amatangelo K, et al (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecol Lett* 11:1065–1071. doi: 10.1111/j.1461-0248.2008.01219.x
- Craine JM, Elmore AJ, Wang L, et al (2018) Isotopic evidence for oligotrophication of

- terrestrial ecosystems. *Nat Ecol Evol* 2:1735–1744. doi: 10.1038/s41559-018-0694-0
- Cramer VA, Hobbs RJ, Standish RJ (2008) What's new about old fields? Land abandonment and ecosystem assembly. *Trends Ecol Evol* 23:104–112. doi: 10.1016/j.tree.2007.10.005
- De Frenne P, Baeten L, Graae BJ, et al (2011) Interregional variation in the floristic recovery of post-agricultural forests. *J Ecol* 99:600–609. doi: 10.1111/j.1365-2745.2010.01768.x
- De Frenne P, De Schrijver A, Graae BJ, et al (2010) The use of open-top chambers in forests for evaluating warming effects on herbaceous understorey plants. *Ecol Res* 25:163–171. doi: 10.1007/s11284-009-0640-3
- De Frenne P, Rodríguez-Sánchez F, De Schrijver A, et al (2015) Light accelerates plant responses to warming. *Nat Plants* 1:. doi: 10.1038/nplants.2015.110
- de Vries FT, Bardgett RD (2016) Plant community controls on short-term ecosystem nitrogen retention. *New Phytol* 210:861–874. doi: 10.1111/nph.13832
- DEIMS-SDR Database (2019) DEIMS-SDR: Dynamic Ecological Information Management System - Site and dataset registry. <https://deims.org/4a469a86-868b-4160-b72e-10a1b4e09356>
- DeMalach N, Zaady E, Kadmon R (2017) Light asymmetry explains the effect of nutrient enrichment on grassland diversity. *Ecol Lett* 20:60–69. doi: 10.1111/ele.12706
- DeMalach N, Zaady E, Weiner J, Kadmon R (2016) Size asymmetry of resource competition and the structure of plant communities. *J Ecol* 104:899–910. doi: 10.1111/1365-2745.12557
- Díaz S, Kattge J, Cornelissen JHC, et al (2016) The global spectrum of plant form and function. *Nature* 529:167–171. doi: 10.1038/nature16489
- Dirnböck T, Foldal C, Djukic I, et al (2017) Historic nitrogen deposition determines future climate change effects on nitrogen retention in temperate forests. *Clim Change* 144:221–235. doi: 10.1007/s10584-017-2024-y
- Easlon HM, Bloom AJ (2014) Easy Leaf Area: Automated digital image analysis for rapid and accurate measurement of leaf area. *Appl Plant Sci* 2:1400033. doi: 10.3732/apps.1400033
- Evans JR, Poorter H (2001) Photosynthetic acclimation of plants to growth irradiance: The relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell Environ* 24:755–767. doi: 10.1046/j.1365-3040.2001.00724.x
- Falkengren-Grerup U (1995) Interspecies differences in the preference of ammonium and nitrate in vascular plants. *Oecologia* 102:305–311. doi: 10.1007/BF00329797
- Falkengren-Grerup U, ten Brink D-J, Brunet J (2006) Land use effects on soil N , P , C and pH persist over 40 – 80 years of forest growth on agricultural soils. *For Ecol Manage* 225:74–81. doi: 10.1016/j.foreco.2005.12.027
- Farrer EC, Suding KN (2016) Teasing apart plant community responses to N enrichment: the roles of resource limitation, competition and soil microbes. *Ecol. Lett.* 19:1287–1296
- Fichtner K, Schulze E-D (1992) The effect of nitrogen nutrition on growth and biomass partitioning of annual plants originating from habitats of different nitrogen availability. *Oecologia* 92:236–241. doi: 10.1007/BF00317370
- Firn J, Mcgree JM, Harvey E, et al (2019) Leaf nutrients, not specific leaf area, are consistent indicators of elevated nutrient inputs. *Nat Ecol Evol*. doi: 10.1038/s41559-018-0790-1

- Foster DR, Swanson F, Aber J, et al (2003) The importance of land-use legacies to ecology and conservation. *Bioscience* 53:77. doi: 10.1641/0006-3568(2003)053[0077:tiolul]2.0.co;2
- Fraterrigo JM, Pearson SM, Turner MG (2009) The response of understory herbaceous plants to nitrogen fertilization in forests of different land-use history. *For Ecol Manage* 257:2182–2188. doi: 10.1016/j.foreco.2009.02.036
- Freckleton RP, Watkinson AR (2001) Asymmetric competition between plant species. *Funct Ecol* 15:615–623. doi: 10.1046/j.0269-8463.2001.00558.x
- Freschet GT, Swart EM, Cornelissen JHC (2015) Integrated plant phenotypic responses to contrasting above- and below-ground resources: Key roles of specific leaf area and root mass fraction. *New Phytol* 206:1247–1260. doi: 10.1111/nph.13352
- Garnier E, Stahl U, Laporte M-A, et al (2016) Towards a thesaurus of plant characteristics: an ecological contribution. *J Ecol* 298–309. doi: 10.1111/1365-2745.12698
- Gilliam FS (2007) The ecological significance of the herbaceous layer in temperate forest ecosystems. *Bioscience* 57:845–858
- Gilliam FS, Welch NT, Phillips AH, et al (2016) Twenty-five-year response of the herbaceous layer of a temperate hardwood forest to elevated nitrogen deposition. *Ecosphere* 7:1–16. doi: 10.1002/ecs2.1250
- Grossmann EB, Mladenoff DJ (2008) Farms, fires, and forestry: Disturbance legacies in the soils of the Northwest Wisconsin (USA) sand plain. *For Ecol Manage* 256:827–836. doi: 10.1016/j.foreco.2008.05.048
- Heinrichs S, Bernhardt-Römermann M, Schmidt W (2010) The estimation of aboveground biomass and nutrient pools of understorey plants in closed Norway spruce forests and on clearcuts. *Eur J For Res* 129:613–624. doi: 10.1007/s10342-010-0362-7
- Hermý M, Honnay O, Firbank L, et al (1999) An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. *Biol Conserv* 91:9–22. doi: 10.1016/S0006-3207(99)00045-2
- Hofmeister J, Hošek J, Bůžek F, et al (2012) Foliar N concentration and $\delta^{15}\text{N}$ signature reflect the herb layer species diversity and composition in oak-dominated forests. *Appl Veg Sci* 15:318–328
- Holmes MA, Matlack GR (2018) Assembling the forest herb community after abandonment from agriculture: long-term successional dynamics differ with land-use history. *J Ecol* 0–2. doi: 10.1111/1365-2745.12970
- Honnay O, Bossuyt B, Verheyen K, et al (2002) Ecological perspectives for the restoration of plant community in European temperate forests. *Biodivers Conserv* 11:213–242. doi: 10.1023/A:1014531011060
- International Atomic Energy Agency (1983) A guide to the use of nitrogen-15 and radioisotopes in studies of plant nutrition: calculations and interpretation of data. Vienna, Austria
- Jagodziński AM, Dyderski MK, Rawlik K, Katna B (2016) Seasonal variability of biomass, total leaf area and specific leaf area of forest understory herbs reflects their life strategies. *For Ecol Manage* 374:71–81. doi: 10.1016/j.foreco.2016.04.050
- Jarrell WM, Beverly R (1981) The dilution effect in plant nutrition studies. *Adv Agron* 34:

- Jobbágy EG, Jackson RB (2001) The distribution of soil nutrients with depth : global patterns and the imprint of plants. *Biogeochemistry* 53:51–77. doi: 10.1023/A:1010760720215
- Kardol P, Campany CE, Souza L, et al (2010) Climate change effects on plant biomass alter dominance patterns and community evenness in an experimental old-field ecosystem. *Glob Chang Biol* 16:2676–2687. doi: 10.1111/j.1365-2486.2010.02162.x
- Klimešová J, Tackenberg O, Herben T (2016) Herbs are different : clonal and bud bank traits can matter more than leaf – height – seed traits. *New Phytol* 210:13–17
- Kolk J, Naaf T, Wulf M (2016) Paying the colonization credit: converging plant species richness in ancient and post-agricultural forests in NE Germany over five decades. *Biodivers Conserv* 1–21. doi: 10.1007/s10531-016-1271-y
- Landuyt D, De Lombaerde E, Perring MP, et al The functional role of temperate forest understorey vegetation in a changing world. *Glob Chang Biol*
- Lefcheck JS (2016) piecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods Ecol Evol* 7:573–579. doi: 10.1111/2041-210X.12512
- Leuschner C, Wulf M, Bäuchler P, Hertel D (2014) Forest continuity as a key determinant of soil carbon and nutrient storage in beech forests on sandy soils in Northern Germany. *Ecosystems* 17:497–511. doi: 10.1007/s10021-013-9738-0
- Liu Y, Dawson W, Prati D, et al (2016) Does greater specific leaf area plasticity help plants to maintain a high performance when shaded? *Ann Bot* 118:1329–1336. doi: 10.1093/aob/mcw180
- Ma S, Verheyen K, Props R, et al (2018) Plant and soil microbe responses to light, warming and nitrogen addition in a temperate forest. *Funct Ecol* 32:1293–1303. doi: 10.1111/1365-2435.13061
- Mabry CM, Gerken ME, Thompson JR (2008) Seasonal storage of nutrients by perennial herbaceous species in undisturbed and disturbed deciduous hardwood forests. *Appl Veg Sci* 11:37–44. doi: 10.3170/2007-7-18302
- Manning P, Newington JE, Robson HR, et al (2006) Decoupling the direct and indirect effects of nitrogen deposition on ecosystem function. *Ecol Lett* 9:1015–1024. doi: 10.1111/j.1461-0248.2006.00959.x
- Miller AE, Bowman WD, Suding KN (2007) Plant uptake of inorganic and organic nitrogen: neighbor identity matters. *Ecology* 88:1832–1840. doi: 10.1890/06-0946.1
- Muukkonen P, Mäkipää R, Laiho R, et al (2006) Relationship between biomass and percentage cover in understorey vegetation of boreal coniferous forests. *Silva Fenn* 40:231–245. doi: 10.14214/sf.340
- Naaf T, Kolk J (2015) Colonization credit of post-agricultural forest patches in NE Germany remains 130 – 230 years after reforestation. *Biol Conserv* 182:155–163. doi: 10.1016/j.biocon.2014.12.002
- Niinemets Ü (1997) Role of foliar nitrogen in light harvesting and shade tolerance of four temperate deciduous woody species. *Funct Ecol* 11:518–531. doi: 10.1046/j.1365-2435.1997.00109.x
- Parzych A, Jonczak J, Sobisz Z (2017) Bioaccumulation of macronutrients in the herbaceous plants of mid-forest spring niches. *Balt For* 23:384–393

- Paul-Limoges E, Wolf S, Eugster W, et al (2017) Below-canopy contributions to ecosystem CO₂ fluxes in a temperate mixed forest in Switzerland. *Agric For Meteorol* 247:582–596. doi: 10.1016/j.agrformet.2017.08.011
- Perring MP, De Frenne P, Baeten L, et al (2016) Global environmental change effects on ecosystems: the importance of land-use legacies. *Glob Chang Biol* 22:1361–1371. doi: 10.1111/gcb.13146
- Perring MP, Diekmann M, Midolo G, et al (2018) Understanding context dependency in the response of forest understorey plant communities to nitrogen deposition. *Environ Pollut* 242:1787–1799. doi: 10.1016/j.envpol.2018.07.089
- Pinheiro J, Bates D, DebRoy S, et al (2018) nlme: Linear and Nonlinear Mixed Effects Models. R Packag version 3.1-137
- Poorter H, Evans JR (1998) Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. *Oecologia* 116:26–37. doi: 10.1007/s004420050560
- R Core Team (2019) R: A language and environment for statistical computing. URL <https://www.r-project.org/>
- Reich PB (2014) The world-wide ‘fast-slow’ plant economics spectrum: a traits manifesto. *J Ecol* 102:275–301. doi: 10.1111/1365-2745.12211
- Sardans J, Grau O, Chen HYH, et al (2017) Changes in nutrient concentrations of leaves and roots in response to global change factors. *Glob Chang Biol* 23:3849–3856. doi: 10.1111/gcb.13721
- Schmitz A, Sanders TGM, Bolte A, et al (2019) Responses of forest ecosystems in Europe to decreasing nitrogen deposition. *Environ Pollut* 244:980–994. doi: 10.1016/j.envpol.2018.09.101
- Siefert A, Ritchie ME (2016) Intraspecific trait variation drives functional responses of old-field plant communities to nutrient enrichment. *Oecologia* 181:245–255. doi: 10.1007/s00442-016-3563-z
- Stevens CJ (2019) Nitrogen in the environment. *Science* (80-) 363:578–580
- Stevens VM, Trochet A, Blanchet S, et al (2013) Dispersal syndromes and the use of life-histories to predict dispersal. *Evol Appl* 6:630–642. doi: 10.1111/eva.12049
- Sugiura D, Tateno M (2011) Optimal leaf-to-root ratio and leaf nitrogen content determined by light and nitrogen availabilities. *PLoS One* 6:. doi: 10.1371/journal.pone.0022236
- Takeuchi Y, Kubiske ME, Isebrands JG, et al (2001) Photosynthesis, light and nitrogen relationships in a young deciduous forest canopy under open-air CO₂ enrichment. *Plant, Cell Environ* 24:1257–1268. doi: 10.1046/j.0016-8025.2001.00787.x
- Tilman D (1990) Constraints and tradeoffs : Toward a predictive theory of competition and succession. *Oikos* 58:3–15
- Valladares F, Laanisto L, Niinemets Ü, Zavala MA (2016) Shedding light on shade: ecological perspectives of understorey plant life. *Plant Ecol Divers* 9:237–251. doi: 10.1080/17550874.2016.1210262
- Valladares F, Niinemets Ü (2008) Shade tolerance, a key plant feature of complex nature and consequences. *Annu Rev Ecol Evol Syst* 39:237–257. doi: 10.1146/annurev.ecolsys.39.110707.173506
- Verheyen K, Honnay O, Motzkin G, et al (2003) Response of forest plant species to land-use

- change: a life-history trait-based approach. *J Ecol* 91:563–577. doi: 10.1046/j.1365-2745.2003.00789.x
- Waters CN, Zalasiewicz J, Summerhayes C, et al (2016) The Anthropocene is functionally and stratigraphically distinct from the Holocene. *Science* (80-) 351:aad2622–aad2622. doi: 10.1126/science.aad2622
- Wright IJ, Westoby M, Reich PB, et al (2004) The worldwide leaf economics spectrum. *Nature* 428:821–827. doi: 10.1038/nature02403
- Yang JL, Fan W, Ryan P (2018) Aluminum-Nitrogen interactions in the soil-plant system. *Front Plant Sci* 9:35–49. doi: 10.3389/978-2-88945-909-4
- Yesilonis I, Szlavecz K, Pouyat R, et al (2016) Historical land use and stand age effects on forest soil properties in the Mid-Atlantic US. *For Ecol Manage* 370:83–92. doi: 10.1016/j.foreco.2016.03.046
- Yin X (2002) Responses of leaf nitrogen concentration and specific leaf area to atmospheric CO₂ enrichment: A retrospective synthesis across 62 species. *Glob Chang Biol* 8:631–642. doi: 10.1046/j.1365-2486.2002.00497.x