ÉVOLUTION DE LA VÉGÉTATION ET DES SOLS FORESTIERS À L'ÉCHELLE LOCALE : LE CAS DE LA FORÊT DE VILLEY-LE-SEC DANS LE NORD-EST DE LA FRANCE

[ARTICLE 3]

Forest eutrophication does not slow down: Evidence from Northeastern France

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5.1 Abstract

Questions: Could a reshuffling of forest communities have occurred over the last 40 years and be identified? If so, is this reshuffling related to changes in nitrogen availability? Could other factors, such as forest management and dynamics and/or the climate, also be related to floristic changes? Could the three available surveys allow us to identify when floristic and environmental changes have mainly occurred and their rates of change over time?

Location: Villey forest, Northeastern France.

Methods: Floristic inventories from 1972, 1991, and 2011 were available for 40 forest plots located in northeastern France. Detrended correspondence analysis (DCA) and variation partitioning were used to determine the relative importance of different drivers on the species composition of the studied forest. Changes in environmental conditions were inferred through changes in Ellenberg figures.

Results: Species composition has reshuffled, with nitrophilic species increasing between 1972 and 2011. Soil pH mostly explained DCA axis1 variation (*partial R^2* R_E=0.647), and soil nitrogen mostly explained DCA axis2 variation (*partial R^2* N_E=0.152) over time. The acidification signal was non significant while eutrophication trend was significantly greater after 1991 (ΔN_E=+0.2). Flora bioindication revealed changes in light, and temperature and soil moisture. N_E and L_E are the figures changing more among the three surveys.

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Conclusions: The three surveys highlight differences in the rate/dynamics of changes. Over the 40-year period in the study area, eutrophication has not slowed down whereas other environmental conditions, reflecting forest management and climate, exhibit significant changes.

Key words: bio-indication; environmental changes and dynamics; local scale; repeated surveys; reshuffling of species composition.

5.2   Introduction

Global biodiversity is changing at an unprecedented rate as a complex response to several human-induced changes in the global environment (Vitousek *et al.*, 1997b). By about the mid-20th century, global sulfur (S) and nitrogen (N) emissions from anthropogenic processes surpassed emissions from natural processes (Galloway, 2001), widely affecting terrestrial ecosystems (Aber *et al.*, 1998), freshwaters systems (Schöpp *et al.*, 2003), and soils chemistry (Falkengren-Grerup et Eriksson, 1990). Disturbance in forests appears as direct toxicity due to increased ammonium concentration (Van Den Berg *et al.*, 2005), damage to vegetation (Bobbink *et al.*, 2010), taxonomic homogenization (Keith *et al.*, 2009), decline in species richness (McClean *et al.*, 2011), and species reshuffling (Bernhardt-Römermann *et al.*, 2007). At a European scale, a sharp decrease in S deposition has been observed since the 1980s, and some studies have already pointed out a recovery from anthropogenic acidification (Kirk *et al.*, 2010; Riofrío-Dillon *et al.*, 2012). However, N deposition has remained at a more or less constantly high level (EMEP, 2011). In France, inventories of atmospheric pollutants have reported that changes in emissions between 1980 and 2010 have shown a downward trend, with a strong, an important, and a significant reduction in SO_x, NO_x, and NH_3, respectively (CITEPA, 2012). Under such conditions, recovery from the effects of eutrophying N compounds should be expected. Forest management systems have also changed, transitioning from coppice-with-standards to high forest management, thus affecting the species composition across European forests (Baeten *et al.*, 2009). In France, forest conversion began before the 20th century, around 1860 (Degron, 1998). Consequently, changes in stand structure due to forest management system and stand maturation might confound the signal of other global changes. Forest dynamics have been reported as the cause of an apparent shift in species composition within forest ecosystems (Bodin *et al.*, 2012).

Furthermore, the reshuffling of plant communities in response to contemporary climate warming has been pointed out, with the pattern of biotic responses varying greatly among species and geographic environmental conditions (Lenoir *et al.*, 2010; Bertrand *et al.*, 2011b; Crimmins *et al.*, 2011). In addition, a greater lag has been reported in the response of lowland plant communities compared to that observed in highland forests facing climatic change, which has been explained by greater species tolerance and a lower opportunity for short-distance escape to track climate changes (Bertrand *et al.*, 2011b). Hence, a combination of eutrophication with acidification, forest management, stand dynamics, and climate change are assumed to be major drivers of long-term vegetation changes and plant loss (Sala *et al.*, 2000; Van Calster *et al.*, 2007; Bertrand *et al.*, 2011b).

Tracking such long-term human-induced changes is particularly difficult due to the limited historical data. Consequently, their effects in reshuffling of species composition have been studied by experimental (Nordin *et al.*, 2005; Clark *et al.*, 2009) or observational approaches. The latter includes studies along geographical gradients, i.e., spatial comparison (Smart *et al.*, 2003; Duprè *et al.*, 2010; Stevens *et al.*, 2011a), or by resurvey of permanent or semi-permanent plots, i.e., temporal comparison (Diekmann *et al.*, 1999; Hédl, 2004; Lenoir *et al.*, 2010). Experimental and observational approaches have certain shortcomings due to the simultaneous occurrence of other determinant environmental factors (Verheyen *et al.*, 2012). Despite this, it has been reported that changes induced by atmospheric pollution, local forest dynamics and management (Decocq *et al.*, 2005; Baeten *et al.*, 2009; Lenoir *et al.*, 2010), and/or climate warming (Bertrand *et al.*, 2011b) could be detected (Verheyen *et al.*, 2012). In turn, these factors may influence light intensity, soil temperature and moisture, and nitrogen availability (Diekmann *et al.*, 1999),

The resurvey of permanent plots is a historical analysis that compares vegetation inventories in initially non-permanent plots. Most of the previous studies have compared two survey years to detect changes in species composition and the environmental factors explaining them over the time (cf. Baeten *et al.*, 2009; Lenoir *et al.*, 2010; Sebesta *et al.*, 2011). In France, the resurvey of floristic plots has allowed species reshuffling and associated environmental conditions to be assessed, in addition to gathering information about how they change over the time. Some studies have reported that significant and simultaneous forest acidification and eutrophication combined with forest management and dynamics, and climate change were responsible for vegetation changes and species decline during the 20th century (Thimonier *et al.*, 1992; Thimonier *et al.*, 1994; Decocq *et al.*, 2005; Van Calster *et al.*, 2008; Lenoir *et al.*, 2010). It has

been suggested that, depending on the spatial scale, different factors have a dominant control over species distribution. At a local scale (small spatial extent) determinant factors include soil type, land use, and topography. In contrast, climate is the main factor at a global scale (large spatial extent) (Pearson et Dawson, 2003). In general, the previous studies cited here were carried out at a local scale pointing out the relative importance of local-acting factors. Furthermore, the effects of climate warming over smaller extents have already been reported (Lenoir *et al.*, 2010), which means that it could potentially be identified at different scales, ranging from local to global.

Here, we evaluated changes in species composition in 40 semi-permanent plots in Villey forest, Northeastern France, for which floristic inventories are available from previous surveys carried out in 1972 (Becker, 1979) and 1991 (Thimonier *et al.*, 1994). In 2011, we relocated and surveyed the 40 vegetation plots for a third time, spanning a period of almost 40 years from the first survey. The assessment of vegetation data allows us to determine whether a reshuffling of species composition has occurred among the three surveys and, if so, to determine the rate of change and to identify the temporal trends of environmental factors to which it might be related. Because measured environmental variables were not available for our plots, we relied on Ellenberg figures as surrogates for light (L_E), soil moisture (F_E), soil nitrogen (N_E), soil pH (R_E), and air temperature (T_E). Between 1972 and 2011, there has been much variation in deposition trends, with high levels of atmospheric deposition occurring around the 1970s and 1980s for S and N, respectively. Due to the implementation of policies to control atmospheric pollutants in the 1980s, an important decrease in S and less marked decrease in N deposition has been reported since 1990s (EMEP, 2011). Moreover, changes in the forest management system over the last 140 years have occurred, and the effects of climate warming in the reshuffling of plant communities have been demonstrated. Therefore, we assumed that it is possible for species composition to have reshuffled over time in response to atmospheric loads, forest management, local forest dynamics (forest aging) and climatic change. We addressed the following questions: (i) Could a reshuffling of forest communities have occurred over the last 40 years and be identified? (ii) If so, is this reshuffling related to changes in nitrogen availability? (iii) Could other factors, such as forest management and dynamics and/or the climate, also be related to floristic changes? (iv) Could the three available surveys allow us to identify when floristic and environmental changes have mainly occurred and their rates of change over time?

5.3   Methods

5.3.1   Floristic data from the 1972, 1991, and 2011 surveys

The initial systematic sampling which was carried out in late July 1972, and encompassed the entire Haye forest (including Villey forest, which is located to the west of the main Haye forest) in Northeastern France (Becker, 1979). The sampling was based on a grid of 250 m × 250 m, with a floristic inventory being conducted at every intersection on the grid. The inventory consisted of a comprehensive record of the plant species present in a circular area of 400 m². An abundance-dominance coefficient using the Braun-Blanquet scale was assigned to each species. Of all the plots surveyed, a total of 57 plots were located in Villey forest, our study area.

In May 1991, of the 57 plots in Villey forest originally studied in 1972, 47 were repeat surveyed (termed "resurveyed" here). These plots were clearly marked and could be validated by paint marks remaining on trees (Thimonier *et al.*, 1994). The vegetation sampling followed the same methodology used in the 1972 survey. In addition, samples of the upper soil layer were collected from each plot, and soil pH (H_2O) was measured in the laboratory.

Of the 47 plots in Villey forest that were resurveyed in 1991, those that had been undisturbed by anthropogenic transformation (i.e., clearing cuttings, walking trails) and/or the 1999 storms named Lothar and Martin, were surveyed a third time in late June and early July 2011, resulting in a total of 40 forest plots (**Fig. 5-1**). The plots were relocated in the field using a Global Positioning System supported by geographical coordinates derived from the original map used in the 1991 survey. After locating the geographical position of the plots, paint marks on trees were searched for. Tree marks were found in only 24% of the 40 relocated plots. Different technical teams participated in the three surveys; however, the author of the 1972 survey the participated in the 1991 survey, and oversaw the careful intercalibration of the sampling methods, preventing systematic errors during data collection (Thimonier *et al.*, 1994). Furthermore, two participants from the 1991 survey also assisted in the 2011 survey, again allowing us to calibrate the sampling methods with those used in the previous survey, and hence minimizing the likelihood of any systematic errors occurring during data collection. Floristic inventories were again conducted during the 2011 field work, which followed the same sampling methodology used in the 1972 and 1991 surveys. In addition, general topographical information was collected (slope, aspect, position), as well as information about

silvicultural management and soil characteristics (i.e., humus, soil type) for each plot. Soil samples from the 0-5 cm A horizon were collected at three different random points within each 400 m^2 plot, which were then analyzed in the laboratory to obtain, among others, a single measurement of soil pH (H_2O) per plot. Species nomenclature follows *Base de Données Nomenclaturale de la Flore de France* (BDNFF version 4).
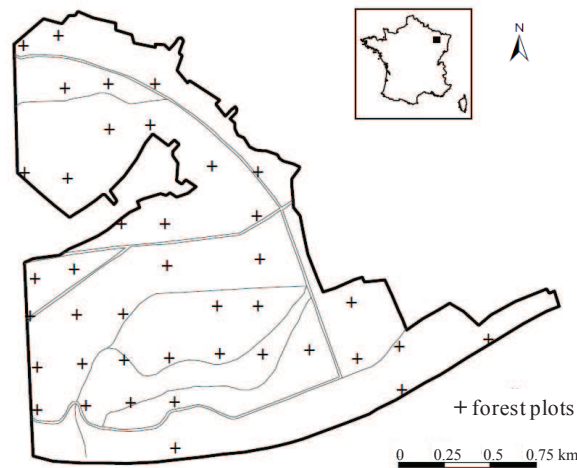

Figure 5-1 Study area (Villey forest) located in Northeastern France. The geographical positions of plots across the forest area are displayed.

5.3.2 Data analysis

Floristic data from the 1972, 1991, and 2011 surveys were analyzed. Abundance-dominance coefficients were transformed into presence/absence data to avoid any bias, due to the different participants estimating these coefficients during each of the three surveys.

5.3.2.1 Analysis of vegetation dynamics

To identify any bias caused by the different sampling dates, changes in species number in each plot between the surveys were correlated against the number of calendar days between the 1972 and 1991 surveys, and the 1991 and 2011 surveys (Keith *et al.*, 2009). To determine changes in species occurrence (i.e., the number of plots where species was observed), the species frequency for 1972, 1991, and 2011 were compared. A total of 205 species, including tree ($n = 29$), shrub ($n = 33$), and herb ($n = 143$) species, were recorded between 1972 and 2011, and included in the analysis. Changes in species occurrence were tested using Fisher's exact test for count data ($P < 0.05$).

Differences in species composition among surveys were examined through analysis of similarity (ANOSIM), based on a matrix of Bray-Curtis (also known as Sørensen) dissimilarity indices to calculate the statistic R, which reveals the observed differences among compared surveys. The significance of R was assessed by running 1000 permutations (Oksanen *et al.*, 2012). ANOSIM was complemented by a multivariate DCA of floristic plots (ter Braak, 1985; Jongman *et al.*, 1987). The 40 plots from each of the three surveys were used to produce the ordination diagram, for which the default options were followed. Variation in the plot scores of the first two axes, which explained most of the variability within the data, was examined over the 1972-2011 survey timeframe, and was then analyzed with respect to environmental conditions. Herb and shrub species with more than or equal to five occurrences (threshold arbitrarily selected) over the 1972-2011 period were selected. Then, 99 species were integrated as floristic variables in DCA ordination coded as presence/absence. Taking into account that seasonal phenological phases differed among surveys, species flowering between March and July were distinguished among the 99 species, and only three species were identified as vernal (i.e., flowering in spring): *Adoxa moschatellina, Arum maculatum,* and *Ranunculus ficaria*. Vernal species were not excluded from analyses, due to the low number of species identified. To determine the plotting structure on the ordination diagram, standard deviational ellipses were created for DCA1 and DCA2 at the 95% confidence interval for the plots from each survey. The change in plot scores between the 1972 and 1991 surveys, and the 1991 and 2011 surveys relative to DCA1 and DCA2 were computed ($\Delta DCAx = DCAx_{p+1} - DCA_p$, with x representing the first or second DCA axis, and p defining a survey year), and used as indicators of changes in species composition. The statistical significance of the difference between the median plot scores for the compared surveys was tested using the Wilcoxon paired signed rank test ($P < 0.05$).

5.3.2.2   *Analyses of underlying environmental conditions*

Due to the absence of measurements for the environmental variables (except for soil pH, measured in the latter two surveys), Ellenberg figures for light (L_E), soil moisture (F_E), soil N (N_E), soil pH (R_E), and temperature (T_E) were used as surrogates (Ellenberg *et al.*, 1992). Only shrub and herb species were used to compute the Ellenberg figures, because they have a sensitive response to disturbances across broad spatial and temporal scales (i.e., they have a higher species turnover than tree species), and could reflect the evolution of forest status through their dynamics (Diekmann et Dupré, 1997; Gilliam, 2007). We used R_E as surrogate

of soil acidity conditions because (i) measured soil pH and R_E were significantly correlated (r_s = 0.782, $P < 0.001$, $n = 80$; with r_s = Spearman's rank correlation), (ii) an R_E value could be computed from floristic inventories for each of the three surveys, and (iii) the different soil sampling method between the 1991 (upper soil horizon) and 2011 (0-5 cm A horizon) surveys to obtain the $pH(H_2O)$ measurements.

Ellenberg figures were used to assess the environmental significance of DCA axes. The relationship between the plot scores of DCA1 and DCA2 with the Ellenberg values were inspected through a multiple linear regression: $DCAx = f (L_E + F_E + N_E + R_E + T_E)$; with x representing the first or second DCA axis. Variation in the response variable explained by the linear combination of the explanatory variables was measured through the coefficient of multiple determination R^2 (Legendre et Legendre, 1998). To determine the contribution of each environmental factor in the axis definition (i.e., the fraction of variation explained by an environmental variable k), the *partial* R^2 was then computed using variation partitioning (Legendre et Legendre, 1998). Thus, the *partial* R^2 is the determination coefficient of the relationship: *residuals* $DCAx_{-k} = z(X_k^{\perp})$, where $DCAx_{-k}$ represents the residuals of the linear model explaining $DCAx$ in function of all variables except k, and $X_k^{\perp}$ represents the effect of k uncorrelated from the effect of the other explanatory variables (i.e., the residuals of the linear model explaining k in function of the other explanatory variables). The level of confusion represents the fraction of variation explained by the collinearity between explanatory variables, and corresponds to R_k^2 – *partial* R_k^2, where R_k^2 is the coefficient of determination of the simple linear relationship $DCAx = g(k)$ (Legendre et Legendre, 1998; Bertrand, 2012).

5.3.2.3   *Analysis of environmental dynamics*

To determine the environmental dynamics between the 1972 and 1991 surveys, the 1991 and 2011 surveys, and the 1972 and 2011 surveys, a mean value of each environmental variable per survey was computed, in addition to determining the changes between the compared surveys ($\Delta k = k_{p+1} - k_p$, with p defining a survey year). The statistical significance of the environmental differences between surveys was tested using the Wilcoxon paired signed rank test ($P < 0.05$).

All statistical analyses were performed in the R environment, version 2.10.1 (R Development Core Team). We used the "vegan" package for ANOSIM, DCA, and plotting structure (Oksanen *et al.*, 2012).

5.4 Results

5.4.1 Vegetation dynamics

No significant correlation was found between changes in the number of species among plots and the number of calendar days between the compared surveys ($r_s = 0.237$, $P = 0.14$ for the comparison 1972-1991, $r_s = -0.039$, $P = 0.81$ for the comparison 1991-2011, and $r_s = -0.249$, $P = 0.121$ for the comparison 1972-2011), which indicated that the species composition of communities was not biased by any differences in survey year (**Fig. 5-2**). With respect to species frequency, from the 205 total species recorded across the 40 plots in Villey forest over the 40-year period, 21 species showed significant changes (increasing or decreasing) in frequency (**Table 5-1**). Nitrophilic and mesophilous species showed an increased frequency in Villey forest (*Paris quadrifolia, Geum urbanum, Bromus ramosus*) compared to some meso-xerophilic species occurring in semi-shaded/open conditions (*Melica nutans, Carex montana, Brachypodium sylvaticum*). The vernal species *Anemone nemorosa* exhibited a high percentage of change (63%), as did *Arum maculatum* to a lesser extent (18%) (**Table 5-1**). However, conclusions about changes in vernal species could not be drawn because their increased frequency probably reflected the effect of the seasonal differences between compared surveys.
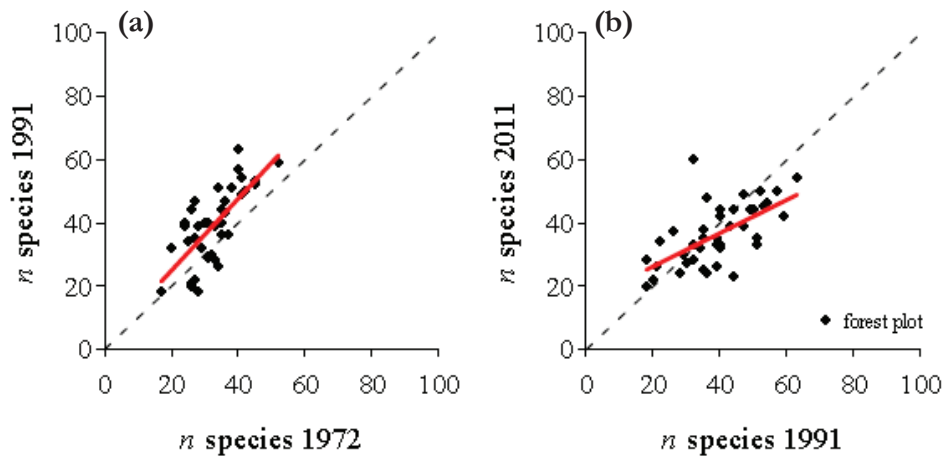
Figure 5-2 Species number at the different survey years: **(a)** between the 1972 and 1991 surveys, and **(b)** between the 1991 and 2011 surveys. The dashed line represents no change in the number of species within a community among the compared surveys ($y = x$). The solid red line represents the fitted relationship. When comparing the 1972 and 1991 surveys, the fitted relationship indicates that species number tended to increase over time ($R^2 = 0.525$, $P < 0.001$). When comparing the 1991 and 2011 surveys, the fitted relationship indicates that sites with a high species number in 1991 tended to decrease over time, while sites with low species number tended to increase over time ($R^2 = 0.388$, $P < 0.001$).

Table 5-1 Species frequency in 1972, 1991, and 2011 surveys, and percentage change with respect to all 40 plots. Onlyspecies with significant changes are listed. Fisher's exact test for count data ($P < 0.05$) was applied over the three surveys. Vernal species are underlined.

Species	1972	1991	2011	Change 2011-1972 (%)
Increase in frequency				
Anemone nemorosa	12	40	37	63
Tilia platyphyllos	2	1	27	63
Fraxinus excelsior	6	27	30	60
Carex sylvatica	13	19	34	53
Acer pseudoplatanus	0	4	18	45
Geum urbanum	3	11	19	40
Bromus ramosus	0	3	14	35
Fagus sylvatica	27	36	38	28
Phyteuma spicatum	9	29	19	25
Paris quadrifolia	0	6	9	23
Rosa canina	0	0	8	20
Dryopteris filix-mas	2	3	9	18
Arum maculatum	0	16	7	18
Angelica sylvestris	0	3	6	15
Dryopteris carthusiana	0	1	6	15
Decrease in frequency				
Melica nutans	6	3	0	-15
Carex pairae	7	0	0	-18
Cornus sanguinea	12	3	3	-23
Tilia cordata	20	23	8	-30
Carex montana	24	25	9	-38
Brachypodium sylvaticum	24	16	7	-43

Significant differences in species composition among plots were identified through ANOSIM, with $R = 0.21$ ($P < 0.001$) when comparing the 1972, 1991, and 2011 surveys. When comparing the 1972 and 1991 surveys, $R = 0.16$ ($P < 0.001$), and when comparing the 1991 and 2011surveys, $R = 0.20$ ($P < 0.001$). Differences in species composition were also supported by the DCA and plotting structure among surveys. The eigenvalues were 0.27 and 0.13 (which corresponded to 66.2% of the sum of eigenvalues of the first four DCA axes) for DCA1 and DCA2, respectively. For DCA1, the plot scores differed significantly when comparing the 1972 and 1991 surveys leaning toward more negative scores ($\Delta DCA1 = -0.248$, $P < 0.001$). In contrast, when comparing the 1991 and 2011 surveys, plot scores leaned toward

the positive axis ($\Delta DCA1 = +0.183$, $P = 0.506$). For DCA2, differences in plot scores leaned toward the positive axis when comparing both the 1972 and 1991 surveys ($\Delta DCA2 = +0.277$, $P < 0.001$), and 1991 and 2011 surveys ($\Delta DCA2 = +0.306$, $P < 0.001$). Standard deviational ellipses at a confidence interval of 95% for each survey strongly overlapped, but also showed a shift in plot scores toward the upper right part of the ordination diagram between the 1972 and 1991 surveys and toward the upper left part of the ordination diagram between the 1991 and 2011 surveys (**Fig. 5-3**).
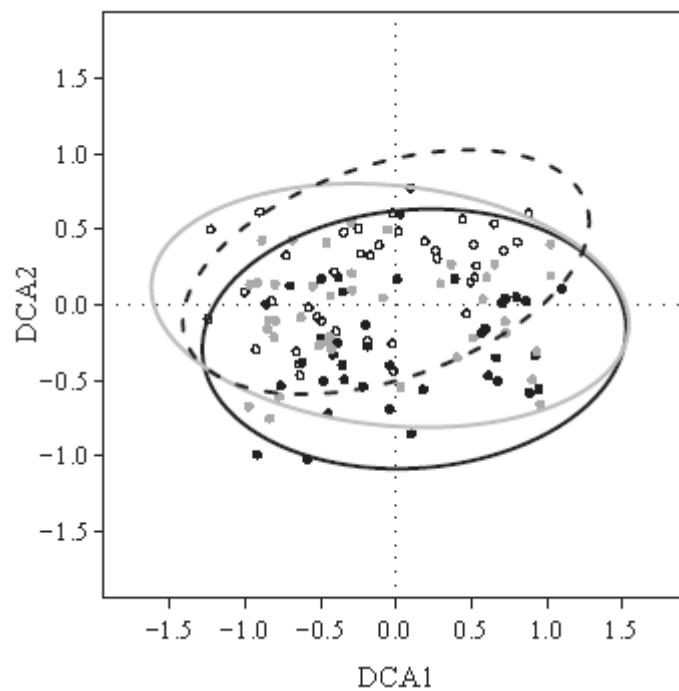

Figure 5-3 Changes in species composition across the 1972, 1991, and 2011 surveys represented on a DCA ordination diagram based on the 40 forest plots from each survey. Standard deviational ellipses showed the plotting structure at a confidence interval of 95% for each survey. Plots from 1972, 1991, and 2011 surveys are displayed by black closed circles, gray closed circles and open circles, respectively. Ellipses for 1972, 1991, and 2011 surveys are displayed by solid black lines, solid gray lines, and dashed black lines, respectively. DCA1 and DCA2 = the first and second axis of the detrended correspondence analysis, respectively.

5.4.2   Environmental significance and dynamics

The standardized coefficients of the regression models and the significance of variables obtained when regressing DCA plot scores on environmental variables are presented in **Table 5-2**. The environmental variables that explained the most variation in species composition along DCA1 and DCA2 were R_E and N_E, respectively, as indicated for the

computed *partial* R^2 (**Table 5-2**). R_E and F_E explained 65% and 11% of the variation in DCA1, respectively. Light, nitrogen, and temperature conditions did not contribute significantly to axis definition. In the case of DCA2, N_E explained 15% of the variation in species composition along this axis, while L_E and T_E explained 10% and 7% of the variation, respectively. Changes in moisture and acidity conditions did not contribute significantly to the DCA2 definition.

Table 5-2 Relationship between the plot scores of DCA axes 1 and 2 and environmental variables substituted with Ellenberg figures for light (L_E), soil moisture (F_E), nitrogen (N_E), soil pH (R_E), and temperature (T_E). The fraction of variability of the DCA axis explained by each environmental variable is indicated through the *partial* R^2. The variable explaining most of the variability of each DCA axis is indicated in bold. Regression standardized coefficients are provided. Significant regression coefficients are indicated by *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, and ns = not significant. DCA1, DCA2 = first and second axis of the detrended correspondence analysis, respectively.

Variable	DCA1			DCA2		
	Partial R^2	Coefficient		*Partial* R^2	Coefficient	
L_E	0.000	-0.009	ns	0.105	-0.324	***
F_E	0.111	-0.333	***	0.008	-0.091	ns
N_E	0.008	-0.089	ns	**0.152**	0.390	***
R_E	**0.647**	0.804	***	0.004	-0.064	ns
T_E	0.002	-0.046	ns	0.069	0.263	**

The *partial* R^2 measures the contribution of each environmental factor in DCA axis definition. It means the fraction of DCA axis variation explained by an environmental variable.

Significant changes in L_E, F_E, N_E, and T_E between the 1972 and 1991 and the 1991 and 2011 surveys were found (**Table 5-3**), reflecting the environmental dynamics in Villey forest over the time. For R_E, a decrease (ΔR_E = -0.05, P = 0.342) followed by an increase (ΔR_E = +0.09, P = 0.183) was observed when comparing the 1972 and 1991 surveys, and the 1991 and 2011 surveys, respectively. The overall comparison between the 1972 and 2011 surveys showed an increase in R_E (ΔR_E = +0.04, P = 0.627). N_E exhibited a non significant increase when comparing the 1972 and 1991 surveys (ΔN_E = +0.06, P = 0.113), whereas a more marked significant increase was detected between 1991 and 2011 (ΔN_E = +0.20, P = 0.001). The overall comparison between the 1972 and 2011 surveys showed a significant increase in N_E

(ΔN_E = +0.26, P < 0.001). With respect to the light variable, significant decreases were found when comparing the 1972 and 1991 surveys (ΔL_E = -0.08, P = 0.031) and the 1991 and 2011 surveys (ΔL_E = -0.17, P < 0.001). Comparison of 1972 and 2011 showed a general decrease (ΔL_E = -0.25, P < 0.001) in light variable. The variable reflecting soil moisture conditions exhibited a significant increase between 1972 and 1991 (ΔF_E = +0.09, P < 0.001), and a smaller significant increase between 1991 and 2011 (ΔF_E = +0.04, P = 0.048). In general, over the 1972-2011 period, a significant increase in F_E was detected (ΔF_E = +0.13, P < 0.001). Finally, a non significant increase was observed for T_E between 1972 and 1991 (ΔT_E = +0.01, P = 0.581), whereas a significant increase was observed between 1991 and 2011 (ΔT_E = +0.05, P = 0.011). The overall comparison between the 1972 and 2011 surveys showed a significant increase in T_E (ΔT_E = +0.06, P = 0.013) (**Table 5-3**).

Table 5-3 Mean Ellenberg figures for the 1972, 1991, and 2011 surveys. Probability values (P) for changes to each Ellenberg figure between 1972 and 1991, 1991 and 2011, and 1972 and 2011 are provided. Significant values are displayed in bold. Wilcoxon paired signed rank test was applied (P < 0.05). L_E = light, F_E = soil moisture, N_E = nitrogen, R_E = pH, T_E = temperature.

	Survey	L_E	F_E	N_E	R_E	T_E
	1972	4.94	4.91	4.97	6.54	5.32
	1991	4.86	4.99	5.03	6.50	5.33
	2011	4.69	5.04	5.23	6.58	5.38
Compared surveys	1972-1991 *(P)*	**0.031**	**<0.001**	0.113	0.342	0.581
	1991-2011 *(P)*	**<0.001**	**0.048**	**0.001**	0.183	**0.011**
	1972-2011 *(P)*	**<0.001**	**<0.001**	**<0.001**	0.627	**0.013**

5.5   Discussion

Temporal comparisons are usually limited by the absence of permanent plots, or knowledge about the exact location of original plots. Some studies are less subject to this limitation due to the geographical isolation of study areas, which facilitates the conservation of signs (i.e., paint or scoring) to recognize original plots (e.g. primeval forest; Sebesta *et al.*, 2011). However, this is not necessarily the case in many studies, for which ancient maps, sites

and vegetation descriptions are available, and/or remaining paint marks allowed the successful relocation of plots (Falkengren-Grerup, 1986; Thimonier *et al.*, 1994; Baeten *et al.*, 2009; Keith *et al.*, 2009; Lenoir *et al.*, 2010; McGovern *et al.*, 2011; Van Den Berg *et al.*, 2011; Kapfer *et al.*, 2012). Another obvious limitation is the absence of environmental measurements; however, the use of Ellenberg figures as surrogates have been proved to be effective in the context of historical comparisons (Diekmann, 2003).

The originality of our study was the availability of data from three comparable surveys spanning a period of 40 years, used to assess the reshuffling of species composition and determine the trends of underlying environmental factors. This information provided a foundation on which we could discriminate different rates of change in environmental conditions from vegetation bio-indication (i.e., the period of time [1972-1991 or 1991-2011] over which changes became more or less marked and significant). The majority of studies that focus on temporal comparisons to assess changes in species composition only compared two survey years (Falkengren-Grerup et Eriksson, 1990; Thimonier *et al.*, 1992; Hédl, 2004; Van Landuyt *et al.*, 2008; Baeten *et al.*, 2009; Keith *et al.*, 2009; Lenoir *et al.*, 2010; McGovern *et al.*, 2011; Sebesta *et al.*, 2011; Kapfer *et al.*, 2012), from which overall vegetation changes may be reported over the respective analyzed periods. However, it is not possible to detect the rate of changes from such datasets.

Our study provides clear evidence of a reshuffling in species composition in Villey forest based on ANOSIM and DCA between the 1972 and 2011 surveys. The initial *R* from ANOSIM for the 1972 and 1991 surveys was smaller than the *R* associated for the 1991 and 2011 surveys, confirming that the rate of change in species composition among surveys was greater during the 1991-2011 period compared to the 1972-1991 period. A previous study has already reported floristic changes, in terms of changes in species frequency, in Villey forest between 1972 and 1991 (Thimonier *et al.*, 1994). Here, we confirmed that the reshuffling of species composition is still in progress, and is actually increasing.

Among the environmental factors underlying the observed changes in species compositions, changes in acidity (through R_E) and nitrogen availability (through N_E) primarily explained the variation along DCA1 and DCA2, respectively. The explanation of changes along the DCA axes of each factor increased with time, highlighting their importance in the reshuffling of species composition and their determinism, depending on the analyzed period of time. Our signal for acidification showed there was no overall acidification in Villey forest. A shift in pH

toward acidification was detected between 1972 and 1991, followed by a recovery between 1991 and 2011; however, these changes were not significant. Changes over the last 20 years reflect the trends observed in recent studies for decreased acidification (Riofrío-Dillon *et al.*, 2012).

The changes in DCA plot scores in the current study confirmed those reported by Thimonier *et al.* (1994), who reported a general eutrophication between 1972 and 1991 in Villey forest. After this period, between 1991 and 2011, our signal for eutrophication revealed a significantly marked increase in nitrogen. Signals from eutrophication were in accordance with reported trends of N atmospheric deposition. N slightly declined after the 1990s, but remains quite elevated. Moreover, our results were consistent with studies that reported changes in nitrogen availability over time, with analyzed periods varying from around 20 to 70 years (Thimonier *et al.*, 1994; Duprè *et al.*, 2010; Van Den Berg *et al.*, 2011; Bahr *et al.*, 2012). However, these previous studies did not show the dynamic of change within the survey period, particularly during the key period that environmental policies were implemented. In contrast, our results highlighted whether the rate of change/dynamics in environmental conditions speeded up or slowed down over time, with insight over the period that environmental policies were implemented.

While L_E tended to decline between 1972 and 2011, F_E and T_E showed a low significant increasing trend. The observed L_E trend might be an effect of forest aging related to stand dynamics and forest management (cf. Thimonier *et al.*, 1994). It has been demonstrated that changes in ground layer vegetation might be attributed to changes in light penetration linked to aging, management, or natural disturbance (Lenoir *et al.*, 2010). The F_E trend might be the effect of changes in precipitation. According to data from a nearby meteorological station (Tomblaine), precipitation in our studied region has generally increased over time (mean total annual precipitation = 718 mm yr^{-1} between 1954 and 1972, 768 mm yr^{-1} between 1973 and 1991, and 770 mm yr^{-1} between 1992 and 2010). Moreover, the negative relationship between canopy closure (L_E) and soil moisture (F_E) (r_s = -0.483, P < 0.001) may also explain the observed increase in F_E, due to a limitation of potential evapotranspiration, which in turn favors mesophilous species. The observed T_E trend might reflect the onset of the effects of climatic warming in Villey forest over the 1991-2011 period. On the basis of data from Tomblaine meteorological station, mean temperature increased during the second half of the 20th century (mean annual temperature = 9.4°C between 1954 and 1972, 9.7°C between 1973 and 1991, and 10.7°C between 1992 and 2010, data were gathered from the French National

Climatic Network Météo-France). Over a similar timeframe, Lenoir *et al.* (2010) reported that forest plant composition is facing broad directional changes, which are probably driven by climate change and local stand dynamics.

In conclusion, our study highlights the rate of change/dynamics of environmental factors based on inferences from floristic data, which have been induced by anthropogenic changes over the last 40 years. This interpretation was made possible by the availability of data from three surveys conducted at the same plots and using the same methodology. Moreover, our study highlights the importance of pH and nitrogen availability in the reshuffling of forest species composition. Eutrophication, in combination with acidification, forest management, and climate change, has probably been modulating the reshuffling of species composition in Villey forest over the last 40 years. The observed changes in nitrogen availability, which highlight that eutrophication has not slowed down in Villey forest, were unexpected. This finding could imply that a revision of environmental policies regarding nitrogen is required, because current levels of deposition remain too high to attain a decreasing signal of eutrophication, despite being lower than that recorded in the 1980s.

5.6 Acknowledgements

5.7 References

Aber J, McDowell W, Nadelhoffer K, Magill A, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez IJ. 1998. Nitrogen saturation in temperate forest ecosystems. BioScience 48(11): 921-934.

Baeten L, Bauwens B, De Schrijver A, De Keersmaeker L, Van Calster H, Vandekerkhove K, Roelandt B, Beeckman H, Verheyen K. 2009. Herb layer changes (1954-2000) related to the conversion of coppice-with-standards forest and soil acidification. Applied Vegetation Science 12(2): 187-197.

Bahr A, Ellström M, Schnoor TK, Påhlsson L, Olsson PA. 2012. Long-term changes in vegetation and soil chemistry in a calcareous and sandy semi-natural grassland. Flora - Morphology, Distribution, Functional Ecology of Plants 207(5): 379-387.

Becker M. 1979. Une étude phyto-écologique sur les plateaux calcaires du Nord-Est (Massif de Haye-54). Utilisation de l'analyse des correspondances dans la typologie des stations. Relations avec la productivité et la qualité du hêtre et du chêne. Annals of Science Forest 36(2): 93-124.

Bernhardt-Römermann M, Kudernatsch T, Pfadenhauer J, Kirchner M, Jakobi G, Fischer A. 2007. Long-term effects of nitrogen deposition on vegetation in a deciduous forest near Munich, Germany. Applied Vegetation Science 10(3): 399-406.

Bertrand R. 2012. Réponse spatio-temporelle de la végétation forestière au réchauffement climatique. Evaluation du remaniement de la végétation et caractérisation de l'effet des facteurs écologiques et géographiques le modulant à l'échelle de l'espèce et des communautés. PhD thesis, AgroParisTech, Nancy, 305 pp.

Bertrand R, Lenoir J, Piedallu C, Riofrío-Dillon G, de Ruffray P, Vidal C, Pierrat J-C, Gégout J-C. 2011. Changes in plant community composition lag behind climate warming in lowland forests. Nature 479(7374): 517-520.

Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M, Cinderby S, Davidson E, Dentener F, Emmett B, Erisman JW, Fenn M, Gilliam F, Nordin A, Pardo L, De Vries W. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20(1): 30-59.

Bodin J, Badeau V, Bruno E, Cluzeau C, Moisselin J-M, Walther G-R, Dupouey J-L. 2012. Shifts of forest species along an elevational gradient in Southeast France: climate change or stand maturation? Journal of Vegetation Science: DOI: 10.1111/j.1654-1103.2012.01456.x.

CITEPA. 2012. Inventaire des émissions de polluants atmosphériques en France au titre de la Convention sur la Pollution Atmosphérique Transfrontalière à Longue Distance et de la Directive Européenne relative aux plafonds d'émissions nationaux (NEC). CITEPA 956nfr. Paris, France: Centre Interprofessionnel Technique d'Etudes de la Pollution Atmosphérique - CEE-NU.

Clark CM, Hobbie SE, Venterea R, Tilman D. 2009. Long-lasting effects on nitrogen cycling 12 years after treatments cease despite minimal long-term nitrogen retention. Global Change Biology 15(7): 1755-1766.

Crimmins SM, Dobrowski SZ, Greenberg JA, Abatzoglou JT, Mynsberge AR. 2011. Changes in Climatic Water Balance Drive Downhill Shifts in Plant Species' Optimum Elevations. Science 331(6015): 324-327.

Decocq G, Aubert M, Dupont F, Bardat J, Wattez-Franger A, Saguez R, de Foucault B, Alard D, Delelis-Dusollier A. 2005. Silviculture-driven vegetation change in a European temperate deciduous forest. Annals of Forest Science 62(4): 313-323.

Degron R. 1998. La conversion des forêts domaniales feuillues françaises (1860-1888) : Une grande vague brisée. Revue Forestière Française 50(1): 71-84.

Diekmann M. 2003. Species indicator values as an important tool in applied plant ecology – a review. Basic and Applied Ecology 4(6): 493-506.

Diekmann M, Brunet J, Ruhling A, Falkengren-Grerup U. 1999. Effects of Nitrogen Deposition: Results of a Temporal-Spatial Analysis of Deciduous Forests in South Sweden. Plant Biology 1: 471-481.

Diekmann M, Dupré C. 1997. Acidification and eutrophication of deciduous forests in northwestern Germany demonstrated by indicator species analysis. Journal of Vegetation Science 8: 855-864.

Duprè C, Stevens CJ, Ranke T, Bleeker A, Peppler-Lisbach C, Gowing DJG, Dise NB, Dorland EDU, Bobbink R, Diekmann M. 2010. Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. Global Change Biology 16(1): 344-357.

Ellenberg H, Weber HE, Düll R, Wirth V, Werner W, Paulissen D. 1992. Zeigerwerte von Pfl anzen in Mitteleuropa. Scripta Geobotanica 18: 1-248.

EMEP. 2011. EMEP/MSC-W modelled air concentrations and depositions. Available at: http://webdab.emep.int/Unified_Model_Results/ (accessed: 04/11/2011).

Falkengren-Grerup U. 1986. Soil acidification and vegetation changes in deciduos forest in southern Sweden. Oecologia 70: 339-347.

Falkengren-Grerup U, Eriksson H. 1990. Changes in soil, vegetation and forest yield between 1947 and 1988 in beech and oak sites of southern Sweden. Forest Ecology and Management 38(1-2): 37-53.

Galloway J. 2001. Acidification of the world: Natural and anthropogenic. Water, Air, & Soil Pollution 130(1): 17-24.

Gilliam FS. 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. BioScience 57(10): 845-858.

Hédl R. 2004. Vegetation of beech forests in the Rychlebské Mountains, Czech Republic, re-inspected after 60 years with assessment of environmental changes. Plant Ecology 170(2): 243-265.

Jongman RHG, ter Braak CJF, van Tongeren OFR. 1987. Data analysis in community and landscape ecology. Wageningen, The Netherlands: Pudoc.

Kapfer J, Virtanen R, Grytnes J-A. 2012. Changes in arctic vegetation on Jan Mayen Island over 19 and 80 years. Journal of Vegetation Science 23(4): 771-781.

Keith SA, Newton AC, Morecroft MD, Bealey CE, Bullock JM. 2009. Taxonomic homogenization of woodland plant communities over 70 years. Proceedings of the Royal Society B: Biological Sciences 276(1672): 3539-3544.

Kirk GJD, Bellamy PH, Lark RM. 2010. Changes in soil pH across England and Wales in response to decreased acid deposition. Global Change Biology 16(11): 3111-3119.

Legendre P, Legendre L. 1998. Numerical Ecology. Second english edition. Amsterdam, The Netherlands: Elsevier Science B.V.

Lenoir J, Gégout JC, Dupouey JL, Bert D, Svenning JC. 2010. Forest plant community changes during 1989-2007 in response to climate warming in the Jura Mountains (France and Switzerland). Journal of Vegetation Science 21(5): 949-964.

McClean CJ, van den Berg LJL, Ashmore MR, Preston CD. 2011. Atmospheric nitrogen deposition explains patterns of plant species loss. Global Change Biology 17(9): 2882-2892.

McGovern S, Evans CD, Dennis P, Walmsley C, McDonald MA. 2011. Identifying drivers of species compositional change in a semi-natural upland grassland over a 40-year period. Journal of Vegetation Science 22(2): 346-356.

Nordin A, Strengbom J, Witzell J, Näsholm T, Ericson L. 2005. Nitrogen Deposition and the Biodiversity of Boreal Forests: Implications for the Nitrogen Critical Load. AMBIO: A Journal of the Human Environment 34(1): 20-24.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Michin PR, O'Hara B, Simpson GL, Solymos P, Henry M, Stevens H, Wagner H. 2012. Vegan: Community Ecology Package. In: http://vegan.r-forge.r-project.org/: http://cran.r-project.org/.

Pearson RG, Dawson TP. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Global Ecology and Biogeography 12(5): 361-371.

R Development Core Team. 2011. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Riofrío-Dillon G, Bertrand R, Gégout J-C. 2012. Toward a recovery time: forest herbs insight related to anthropogenic acidification. Global Change Biology 18(11): 3383-3394.

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld Mn, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH. 2000. Global Biodiversity Scenarios for the Year 2100. Science 287(5459): 1770-1774.

Schöpp W, Posch M, Mylona S, Johansson M. 2003. Long-term development of acid deposition (1880-2030) in sensitive freshwater regions in Europe. Hydrology and Earth Systems Science 7(4): 436-446.

Sebesta J, Samonil P, Lacina J, Oulehle F, Houska J, Bucek A. 2011. Acidification of primeval forests in the Ukraine Carpathians: Vegetation and soil changes over six decades. Forest Ecology and Management 262(7): 1265-1279.

Smart S, Robertson JC, Shield EJ, Van de Poll HM. 2003. Locating eutrophication effects across British vegetation between 1990 and 1998. Global Change Biology 9(12): 1763-1774.

Stevens C, Duprè C, Gaudnik C, Dorland E, Dise N, Gowing D, Bleeker A, Alard D, Bobbink R, Fowler D, Vandvik V, Corcket E, Mountford JO, Aarrestad PA, Muller S, Diekmann M. 2011. Changes in species composition of European acid grasslands observed along a gradient of nitrogen deposition. Journal of Vegetation Science 22(2): 207-215.

ter Braak CJF. 1985. Correspondence Analysis of Incidence and Abundance Data: Properties in Terms of a Unimodal Response Model. Biometrics 41(4): 859-873.

Thimonier A, Dupouey J-L, Bost F, Becker M. 1994. Simultaneous eutrophication and acidification of a forest ecosystem in North-East France. New Phytologist 126: 533-539.

Thimonier A, Dupouey JL, Timbal J. 1992. Floristic changes in the herb-layer vegetation of a deciduous forest in the Lorraine Plain under the influence of atmospheric deposition. Forest Ecology and Management 55(1-4): 149-167.

Van Calster H, Baeten L, De Schrijver A, De Keersmaeker L, Rogister JE, Verheyen K, Hermy M. 2007. Management driven changes (1967–2005) in soil acidity and the understorey plant community following conversion of a coppice-with-standards forest. Forest Ecology and Management 241(1-3): 258-271.

Van Calster H, Vandenberghe R, Ruysen M, Verheyen K, Hermy M, Decocq G. 2008. Unexpectedly high 20th century floristic losses in a rural landscape in northern France. Journal of Ecology 96(5): 927-936.

Van Den Berg LJL, Dorland E, Vergeer P, Hart MAC, Bobbink R, Roelofs JGM. 2005. Decline of acid-sensitive plant species in heathland can be attributed to ammonium toxicity in combination with low pH. New Phytologist 166(2): 551-564.

Van Den Berg LJL, Vergeer P, Rich TCG, Smart SM, Guest DAN, Ashmore MR. 2011. Direct and indirect effects of nitrogen deposition on species composition change in calcareous grasslands. Global Change Biology 17(5): 1871-1883.

Van Landuyt W, Vanhecke L, Hoste I, Hendrickx F, Bauwens D. 2008. Changes in the distribution area of vascular plants in Flanders (northern Belgium): eutrophication as a major driving force. Biodiversity and Conservation 17(12): 3045-3060.

Verheyen K, Baeten L, De Frenne P, Bernhardt-Römermann M, Brunet J, Cornelis J, Decocq G, Dierschke H, Eriksson O, Hédl R, Heinken T, Hermy M, Hommel P, Kirby K, Naaf T, Peterken G, Petřík P, Pfadenhauer J, Van Calster H, Walther G-R, Wulf M, Verstraeten G. 2012. Driving factors behind the eutrophication signal in understorey plant communities of deciduous temperate forests. Journal of Ecology 100(2): 352-365.

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM. 1997. Human Domination of Earth's Ecosystems. Science 277(5325): 494-499.