ÉVOLUTION SPATIO-TEMPORELLE DE L'ACIDIFICATION DES SOLS FORESTIERS FRANÇAIS AU COURS DU 20ème SIÈCLE À PARTIR DU CARACTÈRE BIO-INDICATEUR DES COMMUNAUTÉS VÉGÉTALES

[ARTICLE 1]

Toward a recovery time: Forest herbs insight related to anthropogenic acidification

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3.1 Abstract

Atmospheric deposition is a global concern contributing to soil acidification and biodiversity changes in forest ecosystems. Although acidifying deposition has decreased in the last decades in Europe, few evidence of ecosystem recovery from acidification has been reported until now. The objective of this study was to reconstruct spatio-temporal changes in soil pH across the entire French forest territory over the last 100-year period through herb species assemblages. Data were collected from floristic databases resulting in a total of 120 216 plots covering French forests and spanning from 1910 to 2010. To define acidity figures, pH values were inferred from herb assemblages for each plot of the prediction dataset based on a WA-PLS model (R^2 = 0.80, SD = 0.59 for the validation dataset). Spatio-temporal trends of mean pH changes were obtained by comparing plots with respect to the period (mean year of the period =1933, 1966, 1984, 1997, 2007) and substrate (acidic and non-acidic forest areas).

Bioindicated pH highlighted a decrease of soil pH in both acidic and non-acidic forest areas. The sharpest and most significant pH decrease occurred before 1984 in acidic areas, reaching 0.34 pH units. Subsequently, no significant changes were observed, with a tendency toward stabilization. By contrast, the pH decrease reached 0.19 pH units in non-acidic areas, only reaching significance between 1984 and 1997. Thereafter we observed a slight and significant pH increase. Spatially, pH trends revealed a regionalized character of acidification regarding the substrate, which could not be related to the extent of deposition modeled by the European Monitoring and Evaluation Programme. Both temporal and spatial trends highlight the lagged responses of non-acidic areas compared to acidic areas. Hence, floristic reconstructed pH

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trends demonstrate a gradual cessation and recovery from acidification of French forests after a period of intense atmospheric pollution.

Keywords: acidic and non-acidic forest areas, bio-indication, France, pH, recovery, spatiotemporal trends of soil acidity, species assemblages.

3.2 Introduction

The acidity of many regions of the world has increased as a consequence of food and energy production to sustain a growing global population (Galloway, 1995). These factors have led to enhanced emissions of air pollutants which in turn lead to increased long-range transport and deposition of sulfur (S) and nitrogen (N). Both are carriers of soil acidity (Dentener *et al.*, 2006a). By approximately the mid-20th century, global S and N emissions from anthropogenic processes overtook emissions from natural processes (Galloway, 2001). The effects of atmospheric pollution are widespread and appear in a number of ways including acidification of freshwater systems (Probst *et al.*, 1999), changes in soil chemistry (De Schrijver *et al.*, 2006), damage to vegetation (Bobbink *et al.*, 2010), and changes in forest ecosystems leading to vegetation changes (Falkengren-Grerup, 1986). Most natural soils are acidic due to both organic matter production and decomposition, but human activities have contributed to making them more acidic (Galloway, 2001).

Atmospheric deposition is a global concern and a current issue. After the peak of S in the 1970s and N in 1980s, and the demonstration of its negative consequences for the environment and human health, measures were implemented to control and reduce air pollution. As a result, S and N deposition within the European area decreased significantly from 1990s to our days, by approximately 60% and 38% , respectively (EMEP, 2011). In France, S and N deposition has followed the European tendency for reduction from their levels in the 1990s (1216 mg $S.m^{-2}$ and 1530 mg $N.m^{-2}$, computed from modeled data), decreasing by approximately 70% and 21 %, respectively, in 2010 (EMEP, 2011). While S deposition has declined sharply, returning to the deposition levels of the early 20th century, N deposition has remained elevated. Although acidifying deposition has substantially decreased in the last decades, few evidence of ecosystem recovery has been reported until now (see Skjelkvåle *et al.* (2005) for freshwater evidence). A likely recovery according to Driscoll *et al.*

(2001) will be a complex, two-phase process in which chemical recovery precedes biological recovery.

The impact of increased acidifying inputs on soils and the species composition of forest ecosystems has been a major concern in North America and Europe (Aber *et al.*, 1998). Since the soils in which forests grow change slowly and forests themselves grow slowly, the effects of acidic deposition on forests may not be manifested for years to decades (Galloway, 2001). As a consequence, detection of the long-term effects on vegetation and environment is needed to better understand how are they interact (Sebesta *et al.*, 2011). Similarly, the detection of such effects over a large scale area is also necessary, because potential drivers and their ecological consequences operate at national and continental scales (Smart *et al.*, 2003). However, such studies are scarce (e.g. Blake *et al.*, 1999; Emmett *et al.*, 2010; Kirk *et al.*, 2010), and the majority of existing works have reported the magnitude of changes over time but covered only small regions or specific plots (De Schrijver *et al.*, 2006; van der Heijden *et al.*, 2011).

Tracking long-term environmental changes is particularly difficult due to the limited historical data with measurements of soil parameters, which in turn is related to the low number of measured sites or monitoring programs (Dengler *et al.*, 2011). Without historical measurements, bioindicator values have become an option to determine the values of environmental parameters and to monitor their change (c.f. Braak et Dame, 1989; Birks *et al.*, 1990). Bio-indication can be defined as making use of specific reactions of organisms to their environment (Diekmann, 2003). Because of the potential ability of plants to indicate the values of environmental variables (Bertrand *et al.*, 2011b), significant insight into soil acidity changes can be identified using the available floristic data for any time period (Wamelink *et al.*, 2005). Forest inventories (mainly started up in the 1980s) and phytosociological studies providing valuable ancient floristic information (e.g. Braun, 1915) represent an important background to long-term research for detecting the impacts of long-range air pollution on vegetation. As most of the plant biodiversity in temperate forest ecosystems is represented by the herb layer, which responds sensitively to disturbances across broad spatial and temporal scales, its dynamics reflect the evolution of forest status (Thimonier *et al.*, 1992; Gilliam, 2007). Previous studies have shown significant shifts in the forest herb layer due to acidification (Diekmann et Dupré, 1997; Baeten *et al.*, 2009; Van Den Berg *et al.*, 2011). In France, while ample evidence from the northeastern region indicates that acidic deposition has deeply altered chemical soil properties, nutrient cycling and vegetation dynamics in forest ecosystems

(e.g. Thimonier *et al.*, 1994; van der Heijden *et al.*, 2011), studies from other French regions are relatively scarce (Landmann, 1995).

Here, we investigated the temporal and spatial changes in soil acidity across the entire French forest territory over the last 100-year period using the bioindicator character of forest herb assemblages. We based our study in a large number of floristic plots (n = 120 216) spread across the whole forest territory of Metropolitan France and spanning from 1910 to 2010. It allowed us to reconstruct spatio-temporal changes in forest soil pH, likely due to the effects of acidifying deposition, with respect to the substrate. We assumed that species assemblages mirrored acidity conditions. If acidification occurred, species assemblages would have reshuffled over time (Keith *et al.*, 2009), possibly in response to increasing atmospheric loads. It means we assumed that species conserve their edaphic niche, but they change their distribution in accordance with changes in soil conditions (Fehlen et Picard, 1994; Thimonier *et al.*, 1994; Hallbäcken et Zhang, 1998; Dulière *et al.*, 1999). We addressed the following questions: (i) In the context of a marked decrease of acidifying S and N deposition, do changes in soil acidity show a cessation of acidification over recent decades? (ii) Are spatio-temporal changes in soil acidity related to the nature of the geological substrate (acidic or non-acidic)? (iii) Is there a regionalized acidification related to the substrate and does it relate to the European Monitoring and Evaluation Programme (EMEP) extent of deposition changes?

3.3 Materials and methods

3.3.1 Study area

A large-scale study was realized spanning the entire French forest territory. This territory occupies a surface of 161 000 km^2 (29.2% of Metropolitan France as determined from the national grid of CORINE Land Cover 2006) covering a pH gradient from very acidic (3 pH units) to base-rich (>8 pH units) conditions. French forest substrate consists of bedrocks of varying base status and soils of different types and depths, which cover the whole acidity gradient. To define the substrate in which forests are located, geological information was extracted from a geological French map at scale 1:1 000 000 (Rabu et Chantraine, 2004) and then classified according to lithological composition into acidic (i.e. those with siliceous character: granite, sandstone, basalt, conglomerates) and non-acidic forest areas (i.e. those with

calcareous character: limestone, marlstone, chalk). These areas occupied 51% and 49% of total forest territory, respectively (**Fig. 3-1a**).

The French forest territory has been affected by S and N deposition over time (Landmann, 1995). A natural low deposition was registered before the industrial era, and a strong anthropogenic deposition occurred during the second half of the 20th century, ranging from 445 mg S m^{-2} and 446 mg N m^{-2} in 1910 to maximal values of 1983 mg S m^{-2} and 1515 mg N m^{-2} in the 1970s and 1980s, respectively (EMEP, 2011).

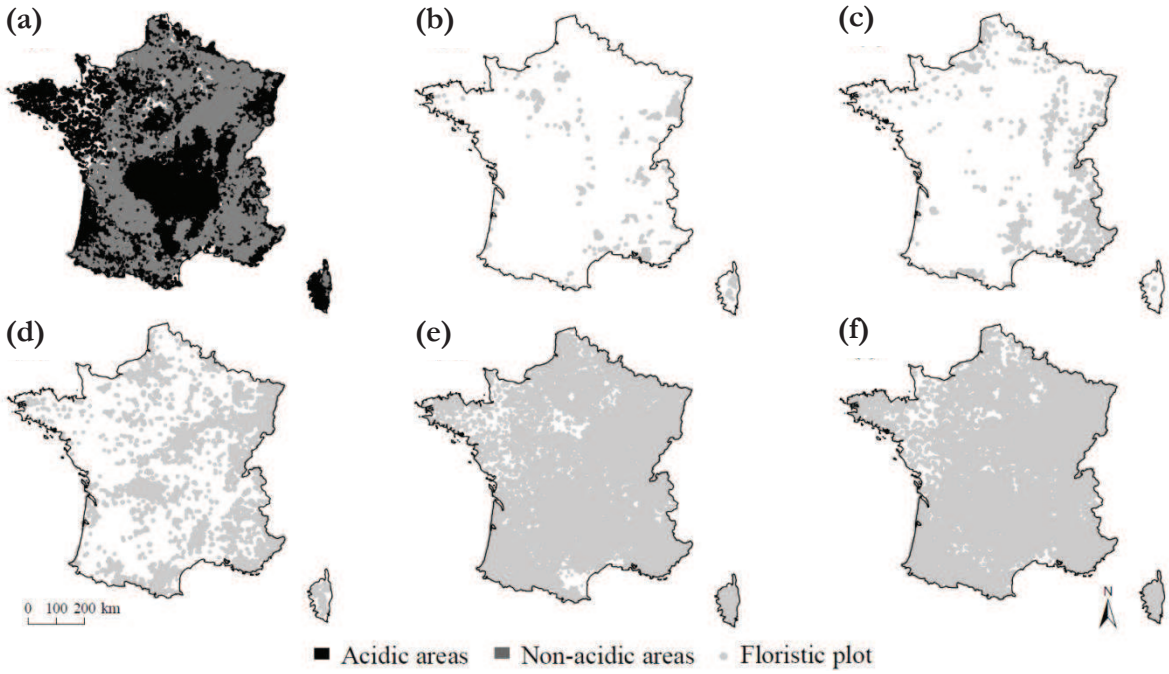

Figure 3-1 Spatial distribution of geological substrates and floristic plots across the French forest territory. **(a)** The spatial distribution of acidic and non-acidic substrates, covering 51% and 49% of forest area, respectively. The spatial distribution of floristic plots over the 20th century: **(b)** first period (1910–1949; mean year of the period= 1933; SD = 9; *n* = 1202), **(c)** second period (1950–1974; mean year of the period = 1966; SD = 6; *n* = 5887), **(d)** third period (1975–1989; mean year of the period = 1984; SD = 4; *n* = 17 161), **(e)** fourth period (1990–2004; mean year of the period = 1997; SD = 4; *n* = 68 925), and **(f)** fifth period (2005–2010; mean year of the period = 2007; SD = 1; *n* = 24 388). Forest cover is based on CORINE Land Cover 2006.

3.3.2 Floristic and environmental data

Three databases of floristic inventories (presence/absence data) were used: EcoPlant(Gégout *et al.*, 2005), Sophy (Brisse *et al.*, 1995), and National Forest Inventory (NFI) (Robert *et al.*, 2010). Together they provided a total of 162 100 floristic plots covering the

entire forested area of France, spanning from 1910 to 2010. They included year and location data with a degree of precision less than 1 km after being spatially georeferenced.

EcoPlant is a phytoecological database including 4913 floristic plots between 1910 and 2010; of which 2854 plots include soil pH measurements. These pH values are pH (H_2O) laboratory values measured from the upper organo-mineral A horizon of sampled soils. Sophy is a phytosociological database that includes 32 330 forest plots from 1915 to 2000. Most sampled plots from these two databases presented an area of 400 m^2, consistent with current phytosociological practice. The NFI database, managed by the French National Forest Inventory, includes 124 857 floristic plots spanning from 1987 to 2009. The NFI sampling method between 1976 to 2004 consisted of covering each French administrative department at a time following a systematic grid (1 x 1 km) and repeating the sampling every 10-12 years (Robert *et al.*, 2010). In 2004, a new sampling method was adopted, covering the whole French forest territory each year. It consists of a systematic grid of 10 x 10 km, which is moved 2 km each year, thereby ensuring 1 km coverage every 10 years (Robert *et al.*, 2010). For the NFI database, all plots consisted of a surface area of 700 m^2. Since each database contains taxonomic and nomenclatural issues, a homogenization procedure was carried out to check and, if necessary, update the names of all plant species. To avoid misidentification issues, we mostly focused on the species level. Due to their short lifespan, high population turnover rate, and likely more reactive response to contemporary global changes (Thimonier *et al.*, 1992; Falkengren-Grerup *et al.*, 2000; Bertrand *et al.*, 2011b), only forest herb species were used to reconstruct spatio-temporal changes in soil acidity.

3.3.3 Training, validation and prediction floristic datasets

The selected floristic plots were divided into 2 datasets: the training, comprising 2854 plots with measured pH values and floristic inventories from EcoPlant database, and the prediction dataset, comprising 159 246 available plots (**Table 3-1**). Floristic plots included in the training dataset were sampled between 1974 and 2010. To minimize the over sampling of some geographic regions and environmental conditions, plots had to be at least 500 m distant from each other. To ensure both a good model fit (which increases with the species frequency; ter Braak, 1995), and the use of a large dataset and pool of species to maximize the spatio-temporal representativeness of our study (which decreases with the species frequency), species with more than five occurrences were selected resulting in a total of 482 forest herb species for calibration.

To define the minimum number of species to consider for inferring the pH value of each floristic plot, an iterative sampling approach was applied over an independent dataset, the 16 x 16 Network or European Network level 1. It was created in 1989 for monitoring forest health, and includes 422 surveys with soil pH measurements and floristic inventories (Badeau et Landmann, 1996). At first, a WA-PLS model was calibrated to link the floristic assemblage of each plot with their corresponding pH measurement. Then, for the iterative approach, a random sampling of species in the plots was defined, with the number of species varying from 1 to 20 species, and iterations repeated 50 times per plot. Finally, for each of these virtual samples bioindicated pH values were predicted from the calibrated WA-PLS model, and R^2 (between predicted and measured pH values) and the root mean square error (RMSE) values were computed. The increase of R^2 and the decrease of RMSE diminish strongly when three or more species were considered, then three species was defined as the minimum number of species per plot to calibrate our predictive model. Thus, the criteria of selection to apply on the different datasets were defined as follows: species with ≥5 occurrences and ≥3 species per plot. When applying them on the training data set 2327 (81.5%) of the initial 2854 plots were selected and used to calibrate the model for inferring pH values from floristic assemblages (**Table 3-1**).

To assess the performance of the model to infer soil pH from the forest herb assemblages in an independent dataset, plots from the16 x 16 Network were used as the validation dataset. Bioindicated pH values were computed from 326 plots (of the initial 422 plots) that met the defined criteria of selection (**Table 3-1**).

The prediction dataset was used to reconstruct pH trends between 1910 and 2010, considering the defined periods (**Fig. 3-1b, c, d, e, f**; **Table 3-1**). To avoid overprediction from our model, plots in the prediction dataset that met the defined criteria of selection were selected, resulting in 117 563 (73.8%) of the initial 159 246 floristic plots. Floristic plots from acidic and non-acidic forest areas were well balanced both in the training (n = 1277 and 1050, respectively) and prediction dataset (n = 54 703 and 62 860, respectively). The defined criteria of selection represent a good compromise between quality of the model and the available data for analyses.

Table 3-1 Description of the training, validation, and prediction datasets.

Dataset	Date of collection	Source	Available data	Application	*n*	*n1*
Training	1974-2010	EcoPlant	− Species presence/absence − Soil pH measurements	− To calibrate the WA-PLS model.	2854	2327
Validation	1989-2004	16 x 16 Network	− Species presence/absence − Soil pH measurements	− To define the minimum number of species to consider for inferring the pH value of each floristic plot. − To validate the WA-PLS model.	422	326
Prediction	1910-2010	EcoPlant Sophy NFI	− Species presence/absence	− To compute bioindicated values from herb assemblages and reconstruct the pH trends between 1910 and 2010.	159 246	117 563

n refers to the total number of plots at the origin.

n1 refers to n after filtering plots according the criteria of selection: species with ≥5 occurrences and ≥3 species per plot.

3.3.4 Weighted Averaging Partial Least Squares (WA-PLS) method to infer soil acidity from floristic assemblages

Among several available techniques using biotic data as a tool for reconstructing past environmental variables, WA-PLS is a powerful inverse approach, i.e. that the adjusted model predicts directly environmental variables as a transfer function from species assemblage with some error (Braak et Juggins, 1993; ter Braak *et al.*, 1993; ter Braak, 1995). WA-PLS is appropriated for calibration when the species-environment relations are unimodal (i.e. one optimum in the ecological niche space), and/or the species data are binary (presence/absence) (ter Braak, 1995). WA-PLS is a combination of both weighted averaging (ter Braak et Barendregt, 1986) and PLS methods (ter Braak, 1995). First, the training dataset is transformed to linearize species-environment relations: (i) Species assemblage dataset is weighted by both the number of species per plot and the species frequencies (hereafter x^*), and (ii) Environmental variable is weighted by the number of species per plot (hereafter y^*). Second, a PLS regression is conducted on the transformed training dataset to fit linear combinations (*f()* or principal components of the PLS) of the predictors (x^*) so as to maximize the prediction of the environmental variable: $y^* = f(x^*) + error$. PLS regression produces an initial component as a set of coefficients, or weighted averages of the species optima with respect to a given environmental variable. The second and further components are selected by optimizing the prediction of the environmental variable (y^*) as for the first component, and use the residual structure in the data to improve the estimates of the species optima (each new component is orthogonal to the previous one) (ter Braak, 1995; Brady *et al.*, 2010). The number of components that gives the best transfer function requires an examination of performance statistics generated by leave-one-out cross-validation (Birks, 1998; Brady *et al.*, 2010), and a confirmation on an independent dataset (ter Braak, 1995). Third, postprocessing transformation of the results of the PLS regression is required to predict values of the environmental variables (for more details read Braak et Juggins, 1993; ter Braak *et al.*, 1993; ter Braak, 1995). WA-PLS is a training procedure that has already been successfully used in pollen and species assemblages analyses to reconstruct past climatic conditions (Pla et Catalan, 2005; Bertrand *et al.*, 2011b).

Here, the WA-PLS approach was used to infer soil pH from the herb assemblages. A WA-PLS model was calibrated linking the floristic assemblage (among a pool of 482 species) of each of the 2327 plots in the training dataset with their corresponding measured pH value

(**Table 3-1**). A 5-component WA-PLS model was selected on the basis of its low standard deviation of the prediction error (SD = 0.59), low bias (mean of prediction error [bioindicated – measured pH] = 0.01 pH units) and high coefficient of determination between observed and predicted values on the validation dataset (R^2 = 0.80; **Fig. 3-2**). Using the prediction dataset, the pH values were inferred from the species assemblages for each floristic plot based on the calibrated 5-component WA-PLS model.

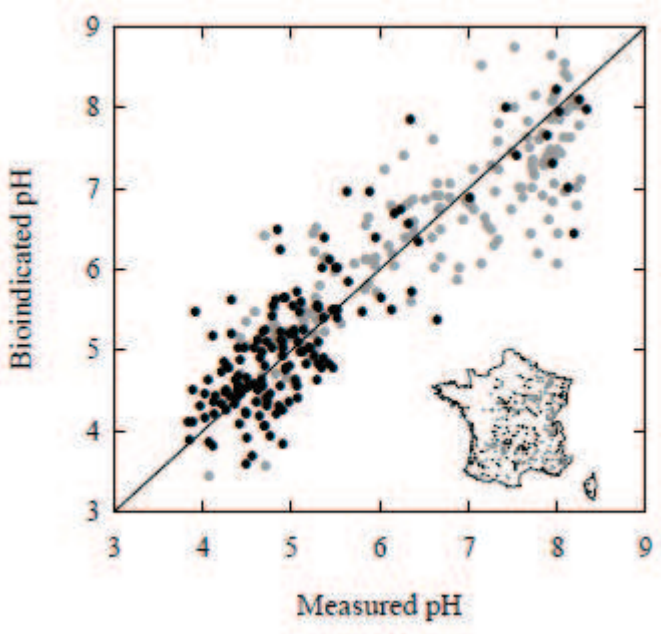

Figure 3-2 Relationship between measured pH values and bioindicated pH values (predicted from the 5-component WA-PLS model) from the validation dataset (R^2 = 0.80; mean difference = 0.01 pH units; SD = 0.59; n = 326 surveys). Black and gray points represent the spatial distribution of surveys in acidic and non-acidic areas, respectively. Solid black line represents the perfect adjustment between measured pH and bioindicated pH ($y = x$). WA-PLS = weighted averaging partial least squares.

3.3.5 Temporal sampling for analysis of acidity trends

To highlight the soil acidity changes over time, the prediction dataset was divided into five periods considering the significance of air pollution between 1910 and 2010 as well as the data availability (**Table 3-2**). The first period (1910–1949; hereafter 1933) was defined as the earliest period, with a mean acidifying deposition of approximately 570 mg S m^{-2} and 562 mg N m^{-2} (EMEP, 2011). Data from EcoPlant and Sophy databases were used (**Fig. 3-1b**). The second period (1950–1974; hereafter 1966) has been described as a period of high air pollution in Europe (on average 1291 mg S m^{-2} and 1051 mg N m^{-2} deposited) (EMEP, 2011) affecting

forest ecosystems. Data from EcoPlant and Sophy databases were used (**Fig. 3-1c**). The third period (1975–1989; hereafter 1984) was defined as a period of control of S deposition, thanks to the implementation of environmental measures (i.e., Convention on Long-range Transboundary Air Pollution, Geneva, 1979); however, atmospheric pollution continues. Data from EcoPlant, Sophy, and NFI databases were used (**Fig. 3-1d**). The fourth period (1990–2004; hereafter 1997) was defined as a period of reduction of N deposition into a lesser extent than that observed for S. Data from EcoPlant, Sophy, and NFI databases were used (**Fig. 3-1e**). The fifth period (2005–2010; hereafter 2007) was defined as a period of continued reduction of S and no change or even a trend toward increase of N deposition. Data from EcoPlant and NFI databases were used (**Fig. 3-1f**).

3.3.6 Sampling for analysis of temporal acidity trends by matching data

To reconstruct long-term changes in soil acidity, and in the absence of permanent surveys, a method that allows the comparison of floristic plots over time was used. First, the floristic plots collected most recently (i.e. 2007 period) were defined as the "reference" data which represents a large number of available floristic plots well distributed across French forest territory (**Fig. 1f**). The 93 175 remaining floristic plots spanning from 1933 to 1997 were defined as "former" data and used to compute soil pH changes regarding the "reference" plots. To control for the potential effect of spatial variability on our assessment of soil pH changes between periods (as described below), 10% of "reference" data were extracted randomly to provide "control" plots (2435 plots). Consequently, 21,953 "reference" plots were used in the matching process (**Table 3-2**).

The method used to compute temporal soil acidity changes consisted of matching each plot from the 1933, 1966, 1984, and 1997 periods (i.e. "former" data) with the nearest plot from the "reference" data, with both plots located on the same substrate. The nearest neighbor was determined by computation of the Euclidean distance (d) between floristic plots. The pH change was computed for each pair ($\Delta pH = pH_{reference} - pH_{former}$) and separate analyses using matched floristic plots were conducted for both acidic and non-acidic forest areas.

Because we aimed to minimize the ΔpH between matched plots due to geographical distance, the "reference" plots were used to explore the spatial autocorrelation between pH values. Then, a threshold of distance was defined to select plots sufficiently closed to each other to allow strong temporal analyses. The spatial autocorrelation between pH values with respect to

the substrate was analyzed using variograms (Fortin et Dale, 2005; Gribov *et al.*, 2006). Considering the variogram outputs, a threshold of distance less than 5 km between "former" and "reference" plots was selected because pH values within this radius were spatially autocorrelated. Considering this comparative distance radius, a total of 77 607 matched plots were obtained, of which 34 524 were situated in acidic areas (median d_{acidic} [1st to 3rd quartile] = 2.6 [2.0–4.5] km) and 43 083 in non-acidic areas (median $d_{non\text{-}acidic}$ [1st to 3rd quartile] = 2.4 [2.0–4.0] km) (**Table 3-2**). The statistical significance of the ΔpH between periods [ΔpH_p *vs.* ΔpH_{p+1}] and the statistical significance of the ΔpH of a period *per se* [ΔpH_p *vs.* $\Delta pH = 0$] (with p defining a period) were both tested using Wilcoxon Rank Sum test ($P < 0.05$).

To assess the validity of our method, distinguishing temporal and geographical variations, the "control" plots were matched to the nearest "reference" plot (as described above). Considering the threshold of distance less than 5 km between "control" and "reference" plots, a total of 2037 matched plots were obtained, of which 896 were situated in acidic areas and 1141 in non-acidic areas (**Table 3-2**). As each pair consisted of two floristic plots belonging to the 2007 period, no differences of pH within "control"-"reference" pairs were expected in the absence of sampling bias in our matching method. The ΔpH did not significantly differ from 0, as shown here, in either acidic (mean ΔpH = -0.001 pH units [SE = 0.03], P = 0.877) or non-acidic areas (mean ΔpH = -0.011 pH units [SE = 0.03], P = 0.631). Further, the median distances between "control"-"reference" pairs was 2.8 km in both acidic and non-acidic forest areas, which is comparable with distances calculated between "former" and "reference" matched plots (see above). Hence, the suitability of our method was validated, indicating no spatial bias.

Table 3-2 Description of the prediction* dataset used in the analysis of temporal pH trends by matching data.

Subdataset	Period of analysis	*n2*	Substrate	*n3*	Subdataset	Period of analysis	Mean year	*n2*
Reference (exclude 10% of control plots)	2005-2010	21 953	Matching "former"-"reference"		Former	1910-1949	1933	1202
			Acidic	34 524		1950-1974	1966	5887
			Nonacidic	43 083		1975-1989	1984	17 161
						1990-2004	1997	68 925
			Matching "control"-"reference"					
			Acidic	896	Control	2005-2010	2007	2435
			Nonacidic	1141				

* Prediction dataset includes only plots meeting the criteria of selection: species with ≥5 occurrences and ≥3 species per plot (i. e. *n1*=117 563; Table 3-1).
n2 refers to the numbers of plots in the subdataset by period.
n3 refers to the number of matched plots, by substrate, and after filtering considering the threshold of distance of ≤5 km between matched plots.

3.3.7 Sampling for analysis of the spatial variation of acidity in forest soils

To visualize where changes in soil acidity had occurred across the French forest territory, the temporal reconstruction of acidity trends was complemented with a spatial reconstruction. The floristic plots of the prediction dataset were mapped, differentiating both acidic and non-acidic areas and considering the 1933, 1966, 1984, 1997, and 2007 periods. Spatial reconstructions were based on the 50 x 50 km EMEP grid (EMEP, 2011) to facilitate the spatial comparison with deposition data (Simpson *et al.*, 2003). A total of 319 grid cells cover Metropolitan France. For each grid cell and period, mean pH values were computed based on at least five plots (threshold determined arbitrarily). Then, the ΔpH between periods was calculated for cells with pH values in both compared periods. Separate analyses were conducted for acidic and non-acidic forest areas. The statistical significance of the pH changes by cell between compared periods [$\Delta pH_{i,p}$ *vs.* $\Delta pH_{i,p+1}$] (with i defining a cell with pH values in both compared periods and p defining a period) was tested using Wilcoxon Rank Sum test ($P < 0.05$).

All models and statistical analyses were performed in the R environment (R Development Core Team, 2011). We used the "pls" package (Mevik et Wehrens, 2007) and personal codes to calibrate and predict pH values. We used ArcGIS and its Geostatistical Analyst extension for spatial and geostatistical analysis (version 9.3.1; ESRI Inc., Redlands, CA, USA).

3.4 Results

3.4.1 Analysis of temporal acidity trends by matching data

The reconstructed trends showed different intensities of ΔpH and lagged responses between acidic and non-acidic forest areas over time. In acidic areas, a significant mean decrease of 0.38 pH units [SE = 0.03] ($P < 0.001$) was observed between the 1933 and 2007 periods. From 1933 to 1984 periods, we observed the highest significant decrease (mean ΔpH = -0.34 pH units [SE = 0.03], $P < 0.001$; **Fig. 3-3a**). Then, a slow decrease of bioindicated pH was observed until the 2007period that was not statistically significant (mean ΔpH = -0.04 pH units [SE = 0.02], $P = 0.465$; **Fig. 3-3a**). In non-acidic areas, a significant mean decrease of 0.19 pH units [SE = 0.02] ($P < 0.001$) was observed between the 1933 and 1997 periods,

which was stronger between 1984 and 1997 (**Fig. 3-3b**). Then, a slight but significant increase was observed until the 2007 period (mean ΔpH = +0.07 pH units [SE = 0.018], P = 0.018; **Fig. 3-3b**) leading to a ΔpH = -0.12 pH units [SE = 0.04], P = 0.029) between the 1933 and 2007 periods.

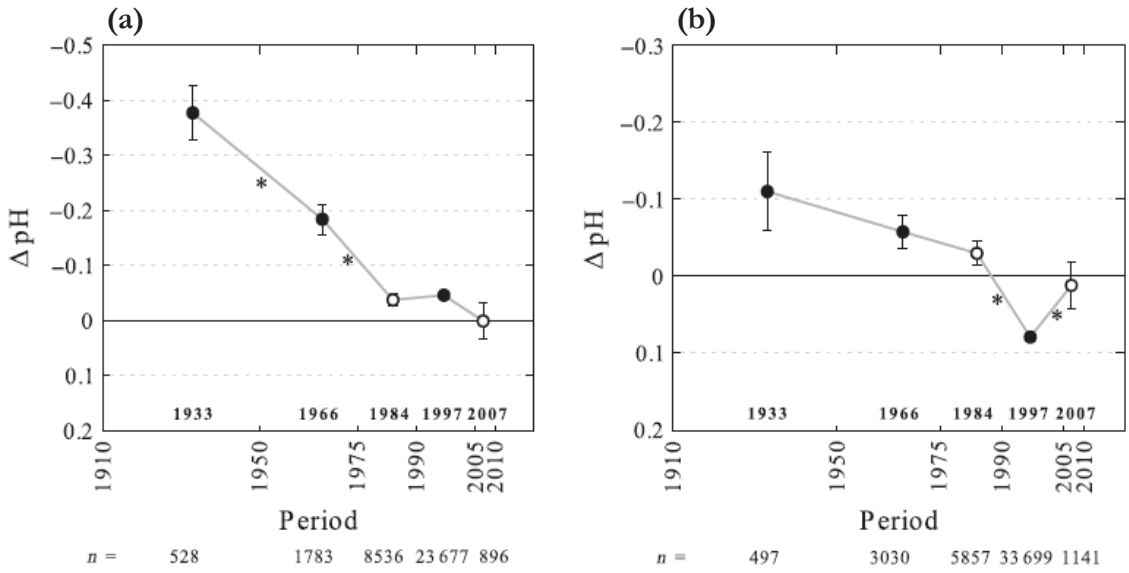

Figure 3-3 Comparison of temporal bioindicated pH changes ($\Delta pH = pH_{reference} - pH_{former}$) in French forest soils between 1910 and 2010. Trends inferred from forest herbs communities **(a)** in acidic areas and **(b)** in non-acidic areas. Mean values of the pH changes are shown (circles) with standard error (error bars) estimated from the samples of each defined period to reconstruct acidity trends. The statistical significance of the pH change of a period *per se* is displayed by closed circles ($P < 0.05$). The statistical significance of the pH changes between periods is displayed by asterisks ($P < 0.05$). The Wilcoxon Rank Sum test was applied. Bold dates above the *x*-axis are the mean year of each defined period. The number of surveys (n) analyzed in each period for acidic and non-acidic areas is displayed below.

3.4.2 Analysis of spatial variation of acidity in forest soils

Spatial patterns of acidification were assessed analyzing pH changes by substrate. No clear geographical trend was distinguished over time in acidic areas. The pH changes were difficult to interpret when comparing 1933 to 1966 (**Fig. 3-4a**) and 1966 to 1984 periods (**Fig. 3-4b**) due to the low number of grid cells available for comparison. However, almost three-fold more cells demonstrated a pH decrease compared to those showing an increase when comparing 1966 and 1984 periods (n = 16 and 6 cells, respectively; **Fig. 3-4b**). Northeastern France (mainly represented by the Vosges Mountains) was highlighted in this comparison as suffering acidification (**Fig. 3-4b**). A well-balanced geographical distribution of ΔpH was

found when comparing 1984 and 1997 periods (n = 30 and 27 cells for decreasing and increasing pH, respectively). Neither a marked trend nor a regional pattern of the effect of acidification on forest plants was observed (**Fig. 3-4c**). The pH changes when comparing 1997 and 2007 periods demonstrated a general trend toward stabilization, as already observed in our temporal trends (**Fig. 3-4d**). A predominant pattern of no change (n = 217 of 259 total cells) was observed, as well as a slow pH decrease in the Central Massif region (n = 11 of 16 total cells exhibiting a minor decrease trend).

By contrast, well-defined geographic patterns were observed over time in non-acidic areas, highlighting a regional pattern of acidification, except when comparing 1933 and 1966 periods due to their low number of grid cells available for comparison (**Fig. 3-4e**). The pH changes between 1966 and 1984 periods indicated a pH decline in the north of France (n = 18 cells), a steady state in the south (n = 7 cells), and a pH increase in the Jura and Alps mountains (n = 9 cells; **Fig. 3-4f**). When comparing the 1984 and 1997 periods, ΔpH demonstrated a generalized acidification in the center and north of France, which tended to become blurred toward southeastern France regarding the previously compared periods. Grid cells showing a pH decrease prevailed in the comparison with respect to cells showing an increase (n = 41 and 17 cells, respectively; **Fig. 3-4g**). Between the 1997 and 2007 periods, a slight pH increase was observed generally in eastern France (n = 25 of 30 total cells exhibiting a minor increase trend). The majority of grid cells demonstrated no significant changes between the 1997 and 2007 periods (n = 161 cells of 208 total cells) in the non-acidic forest areas (**Fig. 3-4h**).

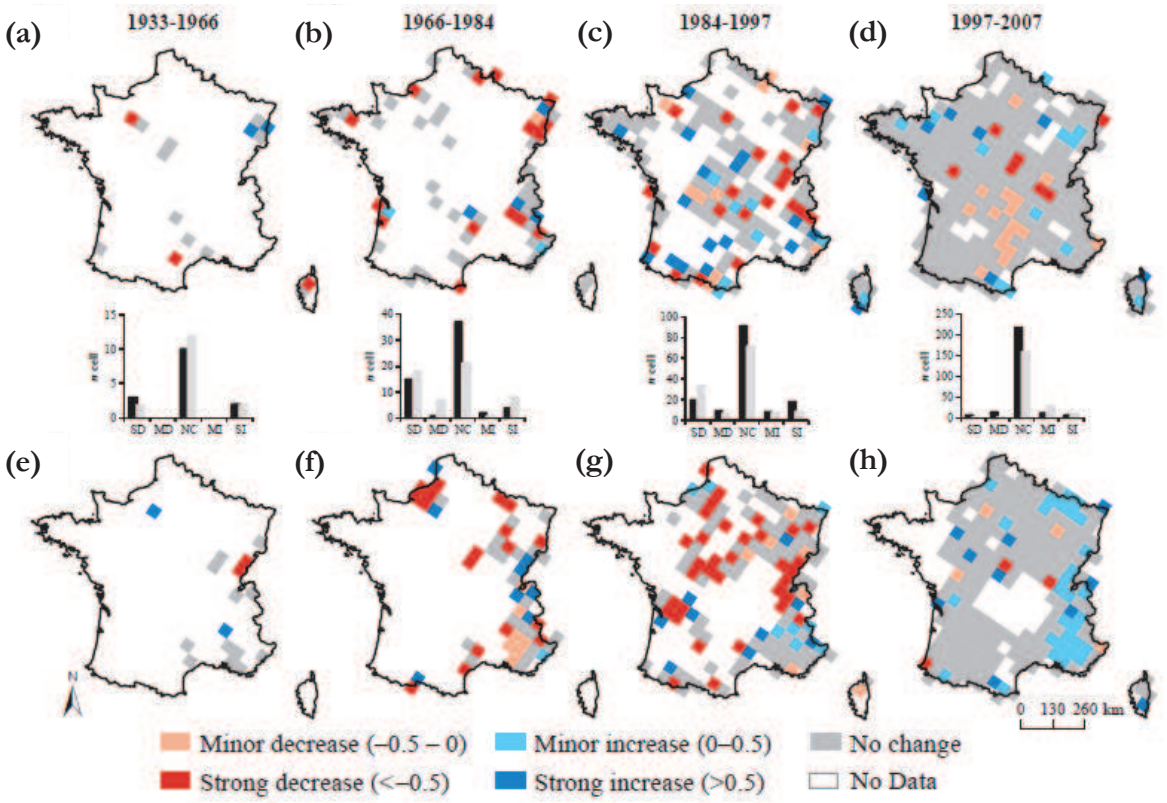

Figure 3-4 Comparison of spatio-temporal bioindicated pH changes ($\Delta pH = pH_{reference} - pH_{former}$) in French forest soils. Mean values of pH changes between compared periods (displayed in the up of the figure) and per cell are shown (**a, b, c, d**) in acidic forest areas and (**e, f, g, h**) in non-acidic forest areas. The statistical significance of the pH changes between periods ($P < 0.05$) is displayed by graduated shades of red for decreasing pH, graduated shades of blue for increasing pH and gray color for non significant changes. Wilcoxon Rank Sum test was applied. White indicates no data. The histograms represent the number of grid cells between compared periods for acidic (black bars) and non-acidic areas (light gray bars) showing a strong decrease (SD), minor decrease (MD), no change (NC), minor increase (MI), and strong increase (SI) of soil pH.

To determine whether the spatial changes of S and N deposition based on the EMEP data matched the changes in the bioindicated pH, their relationships was calculated. In general, no spatial relationship between changes in S and N deposition and changes in pH inferred by bio-indication was observed, neither in acidic nor in non-acidic areas (**Table 3-3**).

Table 3-3 Relationship between S and N deposition changes and bioindicated pH change by compared periods. Coefficient of determination (R^2) and their significance are given. *P* represents the probability values of the relationship. Significant R^2 values are displayed in bold ($P < 0.05$).

	Acidic				Nonacidic			
	S		N		S		N	
	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*
1966-1933	0.010	0.749	0.022	0.628	0.008	0.746	0.003	0.844
1984-1966	0.033	0.186	0.004	0.663	0.046	0.117	**0.072**	0.047
1997-1984	**0.037**	0.027	0.025	0.070	0.001	0.801	0.004	0.496
2007-1997	0.004	0.359	0.002	0.501	0.000	0.796	0.010	0.182

3.5 Discussion

3.5.1 On the method

Our approach to monitoring changes in soil pH mainly depends on the relationship between soil acidity and the plant species that structure forest communities. We argue that this approach is pertinent because forest ecosystems are mainly nutrient constrained (Tamm, 1991), and nutrient resources are an important dimension in the ecological niches of species (e.g. Bertrand *et al.*, 2011a) and on species composition (e.g. Ellenberg *et al.*, 1992). The changes of environmental and nutritional conditions over the time and space have been determined in limited numbers from measurements and over short and recent periods. To track these changes over the long-term, the floristic inventories, which represent a large spatio-temporal database, have been used (Van Landuyt *et al.*, 2008; Verheyen *et al.*, 2012). Ellenberg indicator values have been widely and effectively used to infer soil pH from vegetation (Diekmann et Dupré, 1997; Duprè *et al.*, 2010) and to examine spatial patterns and temporal changes of vegetation in response to acidifying deposition (Diekmann et Dupré, 1997).

Although the Ellenberg approach is conceptually equivalent to the WA-PLS approach used in the present study (ecological niche concept, ter Braak, 1995), it is not in fact strictly comparable. Ellenberg indicator values are discrete values of environmental requirements for plant species based on expert knowledge (Ellenberg *et al.*, 1992). By contrast, the approach used in our work directly focuses on the plant community response to pH gradient and is based on robust statistical methods that allow continuous pH values to be predicted. The

important response of herb species to soil pH was supported by the high R^2 obtained when inferring the pH values from herb assemblages based on a WA-PLS model (R^2 = 0.80, SD = 0.59 for the validation dataset).

The temporal comparison of non-permanent plots is challenged by the required management of spatial variability in the data to avoid confounding results. The large number of both "former" and "reference" plots that we used and the low distance (≤ 5 km) between matched plots are likely to minimize bias in assessing the changes in forest soil acidity from floristic assemblages. Finally, the validation results that we obtained through the "control" plots confirm that the differences observed in our reconstructed pH trends were not biased by geographical variability. This approach, with a large quantity of indirect pH measurements spanning a long period of time, complements the permanent plots approach characterized by the spanning of a short period of time and limited survey locations with accurate direct measurements.

3.5.2 Toward a recovery time from acidification

A key finding of our study is the steady and/or increasing soil pH trends over the last decades in acidic and non-acidic forest areas, respectively. This result is consistent with atmospheric deposition trends, which demonstrated a peak in the 1980s followed by a decrease and stabilization of S and N deposition, respectively (Galloway, 2001). The observed recovery in areas of higher pH and cation exchange capacity in the last decades could be attributed to a quicker replenishment of base cations, as highlighted by O'Sullivan *et al.* (2011). Through experimental data in grasslands these authors reported a rapid recovery response in soil N cycling processes of limestone grassland within five years after the cessation of treatments, implying acidifying N inputs (O'Sullivan *et al.*, 2011). Similarly, Emmett *et al.* (2010) reported results from repeated soil surveys across the United Kingdom with a continuous increase in soil pH of less acidic mineral soils from 1978 to 2007. This recovery observed in less acidic soils and based on measurements of permanent plots as well as the recovery reported by Kirk *et al.* (2010) in England and Wales are consistent with our findings. They differ in the intensity of sampling, the covered area, and the measured variables: directly measured soil pH and bioindicated soil pH. The response of both measured and bioindicated pH was reported in a site in the United Kingdom and also showed a recovery of measured soil pH but not of plant response (McGovern *et al.*, 2011). Using a very large dataset composed of

thousands of floristic plots, the present study has demonstrated that both acidification in acidic and non-acidic soils are possible and have occurred throughout the 20th century, and highlighted the first evidence inferred from forest herb assemblages, to our knowledge, that soil pH may be entering a recovery period in the western part of continental Europe. Even if a return to unpolluted conditions may not be achieved in the foreseeable future (Power *et al.*, 2006), our findings highlight the trend toward a recovery from acidification after a period of intense atmospheric pollution.

In the other hand, the magnitude of our bioindicated pH changes in acidic forest areas is consistent with suggested changes determined through soil chemical analyses in smaller acidic areas and over shorter periods, for example the Flanders region (North Belgium) and the Eastern Carpathians, over 50 and 60 years, respectively (De Schrijver *et al.*, 2006; Sebesta *et al.*, 2011). It is also consistent with the recent stability of measured pH observed in permanent acidic plots in the United Kingdom (Emmett *et al.*, 2010; Kirk *et al.*, 2010).

Spatially, our findings confirm the entrance into a recovery period during the last decades with an increased proportion of areas with no pH change, as well as highlight the high spatial variability of pH change. Within the non-acidic forest areas, the significant recent pH increase in eastern France is consistent with the recent decrease of S and, to a lesser extent, N deposition in this region and the increase of base cation as demonstrated by Croisé *et al.* (2005). In the case of acidic areas, we found a spatial trend toward stabilization when comparing the 1997 and 2007 periods and a slight and regionalized pH decrease. This latter was observed at the Central Massif region (central south of France), where the highest sulfur, nitrate and ammonium deposits were reported in the late 1990s (Croisé *et al.*, 2005) compared with the other French regions. As reported by Kirk *et al.* (2010) in acidic soils there will be a rise in pH following decreases in acid deposition, until the balance between $H+$ consumption in weathering and H^+ production in other processes in the soil is restored.

3.5.3 Lagged response and lower magnitude of pH change in non-acidic forest areas

We found three principal differences between temporal trends of non-acidic and acidic areas: a significant low increase since the 1997 period *vs.* a steady status of soil pH (discussed above), a lower pH decrease (-0.12 *vs.* -0.38 pH units between 1933 and 2007), and a greater lag time for a significant response (1984-1997 *vs.* 1933-1984 periods).

The lower magnitude and lag for a significant response of pH change in non-acidic areas compared to acidic areas are other important insights arising from forest herbs. These patterns may be explained by differences in substrates properties (Ulrich, 1983b; Kirk *et al.*, 2010). In non-acidic areas, the low magnitude (the pH decrease in non-acidic areas was a third of that observed in acidic areas), and late significant pH decrease since the 1984 period could be explained by their natural higher buffer capacity, which delays the response to acidic deposition, and their high base cation status (Ulrich, 1983b). Non-acidic soils are known to be less sensitive to acidification than acidic soils, and limestone is the most important factor counteracting proton input in soils (Reuss et Johnson, 1986; Thimonier *et al.*, 1994). Furthermore, the high acid neutralizing capacity of base-rich areas (Kirk *et al.*, 2010) and the high content of soil carbonates stored in soils (Yang *et al.*, 2012) may limit substantial changes. Even with these favorable characteristics, contrary outcomes have been reported in which limestone grasslands exhibited greater acidification than acidic grasslands (Horswill *et al.*, 2008; Yang *et al.*, 2012). Moreover, the higher nitrification, the higher should be acidification in non-acidic areas as was observed in limestone grasslands in response to N inputs (Horswill *et al.*, 2008). In France, the peak of N deposition has occurred in 1980s (EMEP, 2011). Hence, the coupled effect of high N deposition and high nitrification could explain the lagged acidification in non-acidic areas in comparison with acidic areas.

By contrast, in acidic areas the bioindicated pH decrease demonstrated no lag with respect to the peak of S deposition occurred in 1970s, the point in time when environmental measures were implemented to deal with atmospheric pollution (Convention on Long-range Transboundary Air Pollution, Geneva, 1979), with the end of the significant pH decrease observed in our study coinciding with the reduction of S deposition since 1980s. Even S and N contribute to acidification, acidification by N is not as direct as acidification by S, since the soil will first accumulate N before nitrification sets in (Dise et Wright, 1995; Horswill *et al.*, 2008). The initial faster and more significant decrease on acidic and nitrogen-poor soils can be explained by their initial weak buffer capacity and higher sensitivity to nutrient cation loss following the addition of acid and N (Ulrich, 1983b; Aber *et al.*, 1998).

Another likely interpretation of the lagged response in non-acidic areas could be a slower species reshuffling. However, numerous experimental studies about flora reaction to liming or acidification reported the rapid response of plant species. They have shown that responses of plant assemblages occurred around the five years following the treatment (Fehlen et Picard, 1994; Hallbäcken et Zhang, 1998; Dulière *et al.*, 1999; Spiegelberger *et al.*, 2006). It has been

reported that species respond to soil pH in accordance with their field distribution, which means that experimentation was generally in good agreement with field experience (Falkengren-Grerup et Tyler, 1993). Even so, there is no indication that the rate of response varies depending on the pH level. Regardless the substrate, plant communities are composed by specialist species for which a small variation in pH conditions would lead a reshuffling of plant communities.

3.5.4 Atmospheric deposition changes vs. bioindicated pH changes: no strong spatial relationship

Atmospheric deposition has exerted well-evidenced effects on forests (Blake *et al.*, 1999; De Schrijver *et al.*, 2006). EMEP model data (Simpson *et al.*, 2003) shows a spatial trend for S and N deposition, with minor deposition and changes in the south and the highest observed deposition in the northern half of France, which is not related to our spatial results of pH changes achieved through analyses of herb assemblages. The lack of relationship may be due to the use of bio-indication to infer soil pH, which can differ in time and magnitude from a modeling of soil pH *per se*, and the difference between compared scales (i.e. floristic plots which depict local change in soil conditions *vs.* 50 x 50 km grid). Evaluating more detailed information, the spatial distribution of bulk deposition modeled for France based on the 10 x 10 km grid (Croisé *et al.*, 2005) showed particularities that were also highlighted in our spatial outcomes such as higher altitudes as zones of higher deposition and acidification, which is the case of Central Massif and Cevennes regions.

In conclusion, this study evidences spatio-temporal changes in French forest soil pH highlighting long-term ecosystem acidification and finally a trend toward a stabilization/recovery of soil acidity during the last decades, as revealed by forest herb assemblages. Soils may be recovery slowly, and perhaps incompletely, from the effects of long-term acidification. Due to the absence of measured historical data over a large scale, the use of herb species as indicators of changes in soil conditions and forest status represents an important approach that allows us to go back in time. Because primary data are essential to confirm or deny model outcomes, further efforts should be made to establish and maintain large-scale and long-term worldwide monitoring programs.

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