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# Decreasing before increasing: Evolution of nitrogen availability conditions in French forest ecosystems over the last century according to forest herbs

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## Abstract

Based on a large number of floristic plots ($n$ = 82 581), the French forest status related to soil nitrogen (N) availability was reconstructed from herb species bio-indication spanning from1910 to 2010. The temporal changes highlighted a decrease in available-N until 1990s in coniferous and broadleaved forests (mean $\Delta C{:}N$=+0.79 and +0.74 C:N units, respectively). It was followed by an increase in available-N until 2010, in both coniferous and broadleaved forests (mean $\Delta C{:}N$=-0.10 and-0.16 C:N units, respectively). Spatially, our results confirm the temporal trends, and highlight the center-to-south of France as mainly impacted by the increased N availability in both forest types. The observed decrease in available-N may be related to acidification impacting on soil processes while the recent increase is consistent with the current cessation of acidification. The past N deposition is still impacting both forest soils and vegetation.

**Keywords**: bio-indication, C:N ratio, coniferous and broadleaved forests, eutrophication, long term, large scale, spatiotemporal trends, change in plant communities.

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## Introduction

Humans activities have transformed the global nitrogen (N) cycle at a record pace (Galloway *et al.*, 2008). Over the last half-century, anthropogenic emissions of nitrogen compounds to the atmosphere have overtook emissions from natural processes (Galloway, 2001), and therefore N deposition has increased worldwide (Aardenne *et al.*, 2001; Bobbink *et al.*, 2010). N is a key element in ecosystems functioning (Vitousek *et al.*, 1997a), and its observed increase over time have widely influenced on terrestrial, freshwaters and marine ecosystems (Aber *et al.*, 1998; Carpenter *et al.*, 1998; Schöpp *et al.*, 2003; Rowe *et al.*, 2006). The nutrient enrichment caused by N deposition has given rise to changes in soil chemistry (Kristensen *et al.*, 2004) which in turn influence on species yield and ground layer vegetation (Falkengren-Grerup et Eriksson, 1990; Falkengren-Grerup *et al.*, 2000). In forest ecosystems, N increase led to changes in N cycling processes (McNulty *et al.*, 1991; O'Sullivan *et al.*, 2011), soil eutrophication and/or acidification (Thimonier *et al.*, 1994; Riofrío-Dillon *et al.*, 2012), tree growth enhancement, higher forest productivity (Solberg *et al.*, 2009; Bontemps *et al.*, 2011), reshuffling of species composition (Smart *et al.*, 2003), species decline and/or loss (Stevens *et al.*, 2004; De Schrijver *et al.*, 2011), and increased plant susceptibility to other biotic or abiotic stress factors (Matson *et al.*, 2002).

After the peak of N deposition in 1980s, and the strengthening of implemented policies to control and reduce emissions of nitrogen oxides in 1988 (http://www.unece.org/env/lrtap/welcome.html), a deposition decrease of approximately 29% et 39% in reduced ($NH_y$) and oxidized nitrogen ($NO_x$), respectively, was reported for the period 1990-2010 (EMEP, 2011). In France, N deposition followed the European trend with a reduction of about 13% in $NH_y$ and 34% in $NO_x$ during the period 1990 and 2010 (EMEP, 2011). However, deposition levels of N remains more or less constant on a continuously high level (EMEP, 2011). The effects of the ongoing input of N deposition on ecosystems can still be observed (Bobbink *et al.*, 2010; Thimonier *et al.*, 2010; De Schrijver *et al.*, 2011).

It was suggested that not all ecosystems respond to N deposition similarly. Some of them are more susceptible to excess N (Aber *et al.*, 1989; Matson *et al.*, 2002). Elevated N input over time may lead to increased N concentrations in plants and soils, and thus to a release of protons in the soil solution through ammonium ($NH_4^+$) uptake or nitrification (Thimonier *et al.*, 2010). Factors as species composition, forest type, soil, climate, N retention capacity, litter quality, land use history contribute to variation in ecosystem responses to N deposition (Fenn *et al.*, 1998; Matson *et al.*, 2002; Emmett, 2007). In Europe, there is large variation in nutritional conditions across forest, which is not strongly

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related to current or historic N deposition (Dise *et al.*, 1998). In general, broadleaved forests are more abundant on fertile and N-rich soils, whereas coniferous dominate on less fertile, N-poor soils (Kristensen *et al.*, 2004).

The fact that species respond to environmental conditions (Bobbink *et al.*, 2010) and the limited historical data with measurements of soil parameters highlight the potential usefulness of species composition to bioindicate the environmental status and monitoring its changes over the long-term (Braak et Juggins, 1993; Riofrío-Dillon *et al.*, 2012). Then, because of the powerful ability of plants to indicate the values of environmental variables (Bertrand *et al.*, 2011b; Riofrío-Dillon *et al.*, 2012), significant insight into changes in soil nitrogen availability can be identified using the available floristic data, from forest inventories and/or ancient phytosociological studies, for any time period (cf. Thimonier *et al.*, 1992; Smart *et al.*, 2003). Detecting the effects of N atmospheric deposition on vegetation and soil conditions over the long-term and at large-scale is important to better understand how they are interacting over time and across a large spatial extent (Galloway *et al.*, 2008; Sebesta *et al.*, 2011). It was suggested that the study of temporal changes in species composition over the short-term could provide unclear trends of changes (Thimonier *et al.*, 2012).

Most of the plant biodiversity is represented by the herb layer vegetation in temperate forest ecosystems. It has been showed that herb species have a high sensitiveness to disturbances and therefore a more rapidly and specifically response to them across broad spatial and temporal scales (Falkengren-Grerup *et al.*, 2000; Gilliam, 2007). Then, its dynamics could reflect the evolution of forest status over time (Thimonier *et al.*, 1992; Riofrío-Dillon, 2013). Previous studies have shown significant shifts in the forest herb layer due to direct (Maskell *et al.*, 2010; McClean *et al.*, 2011; Bahr *et al.*, 2012) or indirect (through soil mediated effects) effect of N deposition (Stevens *et al.*, 2011a). In France, N deposition has deeply influenced changes in environmental conditions over time. These changes have been inspected through vegetation analyzes from which a simultaneous forest acidification and eutrophication over time have been pointed out (Thimonier *et al.*, 1992; Thimonier *et al.*, 1994). Moreover, it was recently indicated that, at current N deposition levels, forest eutrophication did not slow down and even accelerated since 1991 (Riofrío-Dillon, 2013).

Here, we aimed to reconstruct the spatiotemporal changes in soil nitrogen availability, likely due to the effects of N atmospheric deposition, across the entire French forest territory between 1910 and 2010, with respect to the forest type if coniferous or broadleaved forest. Our study was based on forest herb assemblages and its bioindicator character regarding N availability, evaluated here through the C:N ratio. Our analysis were based in a large number of floristic plots ($n$ = 82 581) well-distributed

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across the forest territory in Metropolitan France and spanning a 100-year period. If increases in available-N have occurred due to elevated N atmospheric deposition, species assemblages should have reshuffled over time. Then, we assumed that species follow their nutritional requirements changing their geographical distribution in accordance with changes in nutrient soil conditions to conserve in that way their edaphic niche (Bobbink *et al.*, 1998; Turkington *et al.*, 1998; Zechmeister *et al.*, 2007; Horswill *et al.*, 2008; Johansson *et al.*, 2012). The questions we address are: (i) What is the long-term N availability trend in French forests? (ii) At the current level of N atmospheric deposition and considering that they show a decline compared to levels in the 1980s, could changes in soil N availability reflect a decrease in N deposition? (iii) Are spatiotemporal changes in soil N availability related to forest type, whether coniferous or broadleaved? (iv) Is there a regionalized pattern of N availability independent of the forest type?

## Materials and methods

### *Study area*

Our study area is the French forest territory which covers 29.2% of Metropolitan France, i.e. a surface of 161 000 km$^2$ as determined from the national grid of CORINE Land Cover 2006. French forests are covered by different tree species, which allows the discrimination between pure coniferous and broadleaved forests, and mixed-species forests. Coniferous dominate on less fertile, N-poor soils and are mainly located in mountain range. Broadleaved forests cover more than twice the area of coniferous forests and are mainly located on sites characterized by fertile, N-rich soils (**Fig. 1**). Because coniferous and broadleaved forests respond differently to N deposition, these two forest types need to be analyzed separately (Kristensen *et al.*, 2004). The forest cover information was extracted from CORINE land cover grid 1990 and 2006 to define the forest type according the year of sampling of floristic plots. Then, the plots sampled before 1990 were classified according CORINE land cover grid 1990, and forest plots sampled after 1990 were classified according CORINE land cover grid 2006.

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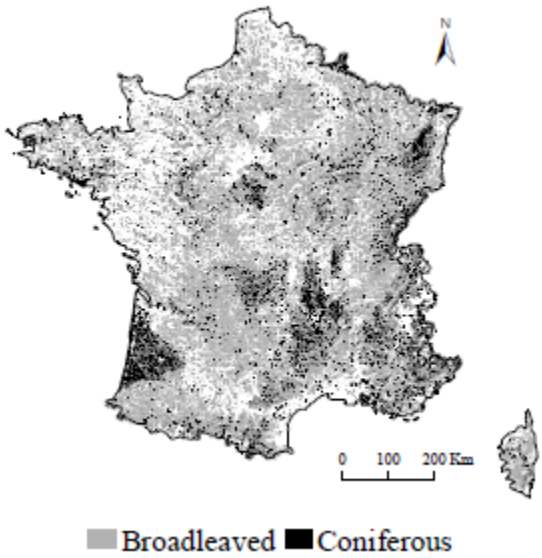


**Figure 1: Spatial distribution of coniferous and broadleaved forests across Metropolitan France.** Coniferous forests cover 22% of forest territory, and broadleaved forests cover 56% of forest territory. Mixed forests (nor represented) cover the remaining 22% of forest territory. Forest cover is based on CORINE Land Cover 2006.

Atmospheric deposition has affected the French forest territory over the 20$^{th}$ century (Landmann, 1995). A low N deposition occurred during the first half of the 20$^{th}$ century, and a strong anthropogenic deposition occurred since 1950, ranging from 343 mg $NH_y$ $m^{-2}$ and 108 mg $NO_x$ $m^{-2}$ in 1910 to maximal values of 730 mg $NH_y$ $m^{-2}$ and763 mg $NO_x$ $m^{-2}$ in 1980s (EMEP, 2011).

*Floristic and environmental data*

Three databases of floristic inventories with presence/absence data were used: EcoPlant (Gégout *et al.*, 2005), Sophy (Brisse *et al.*, 1995), and National Forest Inventory (Robert *et al.*, 2010). Together they provided a total of 113 406 floristic plots covering the coniferous or broadleaved forest area in 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 4544 floristic plots between 1910 and 2010; of which 2483 plots include soil C:N measurements. C and N were measured in the laboratory from the upper organo-mineral A horizon of sampled soils. Total nitrogen and organic carbon were determined by Kjeldahl and Anne methods, respectively (Kjeldahl, 1883; Anne, 1945). Sophy is a phytosociological database that includes 24 850 coniferous or broadleaved forest plots from 1915 to

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2000. Most sampled plots from these two databases presented an area of 400 $m^2$, consistent with current phytosociological practice. The NFI database includes 84 012 floristic plots spanning from 1987 to 2009 (Robert *et al.*, 2010), which consisted of a surface area of 700 $m^2$. Given that each database contains taxonomic and nomenclatural issues, a homogenization procedure was carried out to check and 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 (Falkengren-Grerup *et al.*, 2000; Bertrand *et al.*, 2011b; Riofrío-Dillon *et al.*, 2012), only forest herb species were used to reconstruct spatiotemporal changes in N availability.

*Training, validation and prediction floristic datasets*

The selected floristic plots were divided into 2 datasets: the training, comprising 2483 plots with measured C:N values and floristic inventories from EcoPlant database, and the prediction dataset, comprising 110 923 available plots only with floristic inventories (**Fig. 2a**). Floristic plots included in the training dataset were sampled between 1965 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 spatiotemporal representativeness of our study, species with more than five occurrences were selected resulting in a total of 451 forest herb species for calibration (**Fig. 3**).

Following Riofrío-Dillon *et al.* (2012), we used a minimum number of three species per plot with at least 5 occurrences into the database for calibrating our predictive model and inferring the C:N value of each floristic plot. These criteria led to a selection of 1506 (60.7%) of the initial 2483 plots of the training dataset that were used to calibrate the model for inferring C:N values from floristic assemblages (**Fig. 2a**). To assess the performance of the model to infer soil C:N from the forest herb assemblages with an independent dataset, French plots from the16 x 16 km Network (Badeau et Landmann, 1996) were used as the validation dataset. Bioindicated C:N values were computed from 232 plots (of the initial 400 plots) that met the defined criteria of selection (**Fig. 2a**). The prediction dataset was used to reconstruct nitrogen availability trends between 1910 and 2010, considering the defined periods (see below, subsection "*Temporal sampling for analysis of nitrogen availability trend*"). To avoid overprediction from our model, plots in the prediction dataset that met the defined criteria of selection were selected, resulting in 80 843 (72.9%) of the initial 110 923 floristic plots (**Fig. 2a**).

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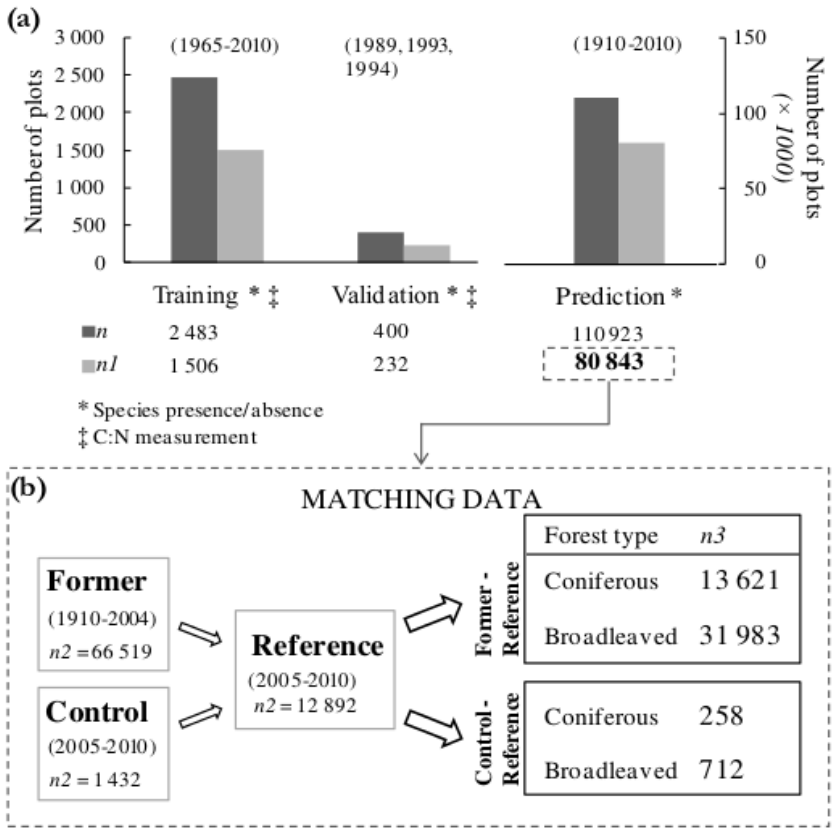


**Figure 2: Synthetic representation of the different datasets. (a)** Available information and initial and final number of plots in the training, validation and prediction datasets. **(b)** Scheme of the matching data process in the analysis of temporal C:N trends based on the prediction dataset. *n* = refers to the total initial number of plots, *n1* = refers to *n* after filtering plots according to the criteria of selection: species with ≥5 occurrences and ≥3 species per plot, *n2* = refers to the number of plots in the prediction subdatasets, *n3* = refers to the number of matched plots analyzed according to the forest type, and after filtering considering the threshold of distance of ≥3.5 km between matched plots.

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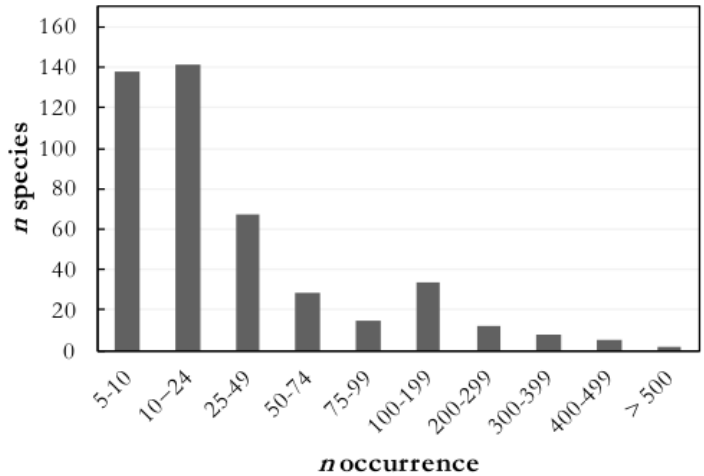


**Figure 3: Histogram of occurrence of the 451 forest herb species (with ≥5 occurrences in the training dataset) used for calibrating a WA-PLS model.**

*Weighted Averaging Partial Least Squares (WA-PLS) method to infer soil N availability 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 (ter Braak & 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 & Barendregt, 1986) and PLS methods (ter Braak, 1995). It can be summarized as follows:

(i) The training dataset is first transformed to linearize species-environment relationship: species assemblage dataset is weighted by both the number of species per plot and the species frequencies ($x^*$), and the environmental variable is weighted by the number of species per plot ($y^*$).

(ii) 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,

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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).

(iii) Postprocessing transformation of the results of the PLS regression is required to predict values of the environmental variables (for more details read ter Braak & 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 (Pla & Catalan, 2005; Bertrand *et al.*, 2011b) and edaphic conditions (Riofrío-Dillon *et al.*, 2012).

Here, the WA-PLS approach was used to infer soil available-N from herb assemblages. A WA-PLS model was calibrated linking the floristic assemblage (among a pool of 451 species) of each of the 1506 plots in the training dataset with their corresponding measured C:N value. A 5-component WA-PLS model was selected on the basis of its standard deviation of the prediction error (SD = 3.13), low bias (mean of prediction error [bioindicated – measured C:N] = -0.07 C:N units) and coefficient of determination between observed and predicted values on the validation dataset ($r^2$ = 0.31; **Fig. 4**). Using the prediction dataset, the C:N values were inferred from the species assemblages for each floristic plot based on the calibrated 5-component WA-PLS model.

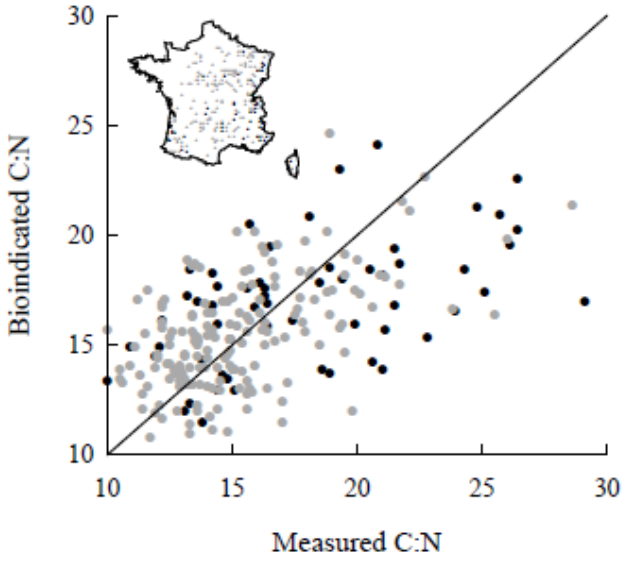


**Figure 4: Relationship between measured C:N values and bioindicated C:N values (predicted from the 5-component WA-PLS model) from the validation dataset ($r^2$ = 0.31; mean difference = -0.07 C:N units; SD = 3.13; *n* = 232 surveys).** Black and gray points represent the spatial distribution of surveys in coniferous and broadleaved forests, respectively. Solid black line represents $y = x$. WA-PLS = Weighted Averaging Partial Least Squares.

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*Temporal sampling for analysis of N availability trend*

To determine changes in the soil N availability 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. The first period (1910–1949, $n$ = 1043 plots; hereafter 1933) was defined as the earliest period, with a mean deposition of approximately 379 mg $NH_y$ $m^{-2}$ and 179 mg $NO_x$ $m^{-2}$ (EMEP, 2011). Data from EcoPlant and Sophy databases were used. The second period (1950–1974, $n$ = 5186 plots; hereafter 1966) has been described as a period of increasing N pollution (on average 521 mg $NH_y$ $m^{-2}$ and 458 mg $NO_x$ $m^{-2}$ deposited) (EMEP, 2011). Data from EcoPlant and Sophy databases were used. The third period (1975–1989, $n$ = 14 324 plots; hereafter 1984) was defined as a period of the highest N atmospheric deposition. Moreover, at that time environmental policies were implemented to control air pollution (i.e., Convention on Long-range Transboundary Air Pollution, Geneva, 1979). Data from EcoPlant, Sophy, and the national forest inventory databases were used. The fourth period (1990–2004, $n$ = 45 966 plots; hereafter 1997) was defined as a period of slight reduction of N deposition. Data from EcoPlant, Sophy, and the national forest inventory databases were used. The fifth period (2005–2010, $n$ = 14 324 plots; hereafter 2007) was defined as a period of no change in N deposition, remaining however high. Data from EcoPlant and the national forest inventory databases were used.

*Sampling for analysis of temporal N availability trends by matching data*

To reconstruct long-term changes in soil available-N, and in the absence of permanent surveys, a method that allows the comparison of floristic plots over time was used (cf. Riofrío-Dillon *et al.*, 2012). 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. The 66 519 remaining floristic plots spanning from 1910 to 2004 (i.e. from 1933 to 1997 periods) were defined as "former" data and used to compute soil C:N changes regarding the "reference" plots. To control for the potential effect of spatial variability on our assessment of soil C:N changes between periods (as described below), 10% of "reference" data were extracted randomly to provide "control" plots (1432 plots). Consequently, 12 892 "reference" plots were used in the matching process (**Fig. 2b**).

The method used to compute temporal changes in soil available-N 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 forest type. The nearest neighbor was determined by computation of the Euclidean distance (d) between floristic plots. The C:N change was computed

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for each pair ($\Delta C{:}N = C{:}N_{reference} - C{:}N_{former}$) and separate analyses using matched floristic plots were conducted for both coniferous and broadleaved forests.

Because we aimed to minimize the $\Delta C{:}N$ between matched plots due to geographical distance, the "reference" plots were used to explore the spatial autocorrelation between C:N 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 C:N values with respect to the forest type was analyzed using variograms (**Fig. 5ab**) (Fortin et Dale, 2005; Gribov *et al.*, 2006). Considering the variogram outputs, a threshold of distance less than 3.5 km between "former" and "reference" plots was selected because C:N values within this radius were spatially autocorrelated. Considering this comparative distance radius, a total of 45 604 matched plots were obtained, of which 13 621 were situated in coniferous forests (median $d_{coniferous}$ [1st to 3rd quartile] = 1.8 [1.1–2.5] km) and 31 983 in broadleaved stands (median $d_{broadleaved}$ [1st to 3rd quartile] = 1.8 [1.0–2.4] km) (**Fig. 2b**). The statistical significance of the $\Delta C{:}N$ between periods [$\Delta C{:}N_t$ vs. $\Delta C{:}N_{t+1}$] and the statistical significance of the $\Delta C{:}N$ of a period per se [$\Delta C{:}N_t$ vs. $\Delta C{:}N = 0$] (with $t$ 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 3.5 km between "control" and "reference" plots, a total of 970 matched plots were obtained, of which 258 were located in coniferous forests and 712 in broadleaved forests (**Fig. 2b**). As each pair consisted of two floristic plots belonging to the 2007 period, no differences of C:N within "control"-"reference" pairs were expected in the absence of sampling bias in our matching method. In according with this expectation, the $\Delta C{:}N$ did not significantly differ from 0, in either coniferous (mean $\Delta C{:}N = 0.13$ C:N units [SE = 0.22], $p = 0.446$) or broadleaved forests (mean $\Delta C{:}N = 0.02$ C:N units [SE = 0.11], $p = 0.727$). Further, the median distances between "control"-"reference" pairs was 2.0 km in both coniferous and broadleaved forests, which is comparable with distances reported between "former" and "reference" matched plots. Hence, the suitability of our method was validated, indicating no spatial bias.

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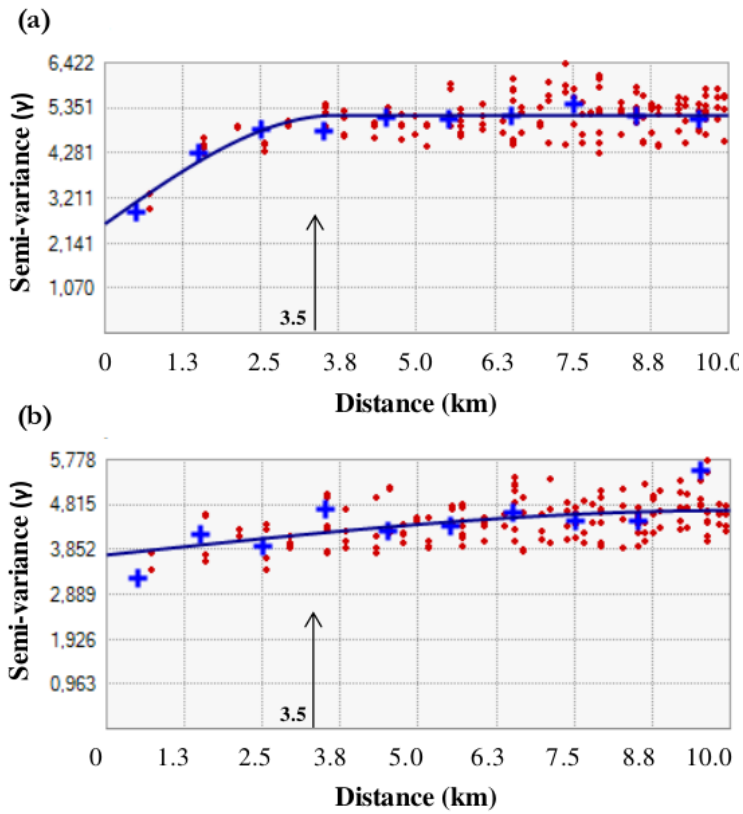


**Figure 5: Variogram for the "reference" floristic plots in (a) coniferous and (b) braodleaved forests in France.** The solid blue line describes a fitted spherical model. The threshold of distance selected because C:N values within this radius were spatially autocorrelated is indicated by arrows (i.e., 3.5 km).

*Sampling for analysis of the spatial variation of N availability in forest soils*

To visualize where changes in soil available-N had occurred across the French forest territory, the temporal reconstruction of N availability trends was complemented with a spatial reconstruction. The floristic plots of the prediction dataset were mapped, differentiating both coniferous and broadleaved forests and considering the 1933, 1966, 1984, 1997, and 2007 periods. Spatial reconstructions were based on the 50 x 50 km EMEP grid (EMEP, 2011). A total of 319 grid cells cover Metropolitan France. For each grid cell and period, mean C:N values were computed based on at least five plots (threshold determined arbitrarily). Then, the $\Delta C{:}N$ between periods was calculated for cells with C:N values in both compared periods. Separate analyses were conducted for coniferous and broadleaved forests. The statistical significance of the C:N changes by cell between compared periods [$\Delta C{:}N_{i,t}$ *vs.* $\Delta C{:}N_{i,t+1}$] (with $i$ defining a cell with C:N values in both compared periods, and $t$ defining a period) was tested using Wilcoxon Rank Sum test ($p < 0.05$).

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All models and statistical analyses were performed in the R environment (R Development Core Team). We used the "pls" package (Mevik & Wehrens, 2007) and personal codes to calibrate and predict C:N bioindicated values. We used ArcGIS and its Geostatistical Analyst extension for spatial and geostatistical analysis (version 10.1; ESRI Inc., Redlands, CA, USA).

## Results

### *Analysis of temporal N availability trends by matching data*

The reconstructed trends of $\Delta C{:}N$ showed similar magnitudes of change between coniferous and broadleaved forests over time. In coniferous forests, a significant mean increase of +0.79 C:N units [SE = 0.12] ($p < 0.01$) was observed between the 1933 and 1997 periods. The highest significant increase was observed from 1966 to 1984 periods (mean $\Delta C{:}N$ = +0.38 C:N units [SE = 0.08], $p < 0.01$; **Fig. 6a**). Between 1997 and 2007 periods, a slow decrease in bioindicated C:N ratio was observed that was not statistically significant (mean $\Delta C{:}N$ = -0.10 C:N units [SE = 0.13], $p = 0.643$; **Fig. 6a**). In broadleaved forests, a significant mean C:N increase of +0.74 C:N units [SE = 0.07] ($p < 0.001$) was observed between the 1933 and 1997 periods (**Fig. 6b**). The highest significant increase was observed between 1933 and 1966 periods (mean $\Delta C{:}N$ = +0.49 C:N units [SE = 0.11], $p < 0.001$). Then, a slight non significant decrease was observed until the 2007 period (mean $\Delta C{:}N$ = -0.16 C:N units [SE = 0.06], $p = 0.182$; **Fig. 6b**).

### *Analysis of spatial variation in N availability in forest soils*

Spatial patterns of eutrophication were assessed analyzing C:N changes by forest type. No clear geographical trend was distinguished over time in coniferous forests. The C:N changes were difficult to interpret when comparing 1933 to 1966 period due to the low number of grid cells available for comparison (**Fig. 7a**). A well-balanced geographical distribution of $\Delta C{:}N$ was found when comparing 1966 to 1984 period ($n$ = 8 and 7 cells for decreasing and increasing C:N, respectively). Eastern France (Jura and Alps mountains mainly) was highlighted in this comparison as suffering an increase in available-N while a pre-Alps area in the South of Grenoble city exhibited a decrease in available-N (**Fig. 7b**). When comparing 1984 to 1997 period a regional C:N increase was observed in Southeastern France. Contrary, an available-N increase was identified in western coastal areas and in the Central Massif region (**Fig. 7c**). Between the 1997 and 2007 periods, a C:N decrease was mainly observed in center-eastern France ($n$ = 16 of 24 total cells exhibiting a decrease trend), and also in Pyrenees

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Mountains. The majority of grid cells demonstrated no significant changes between the 1997 and 2007 periods ($n$ = 107 cells of 136 total cells) in the coniferous forests (**Fig. 7d**).

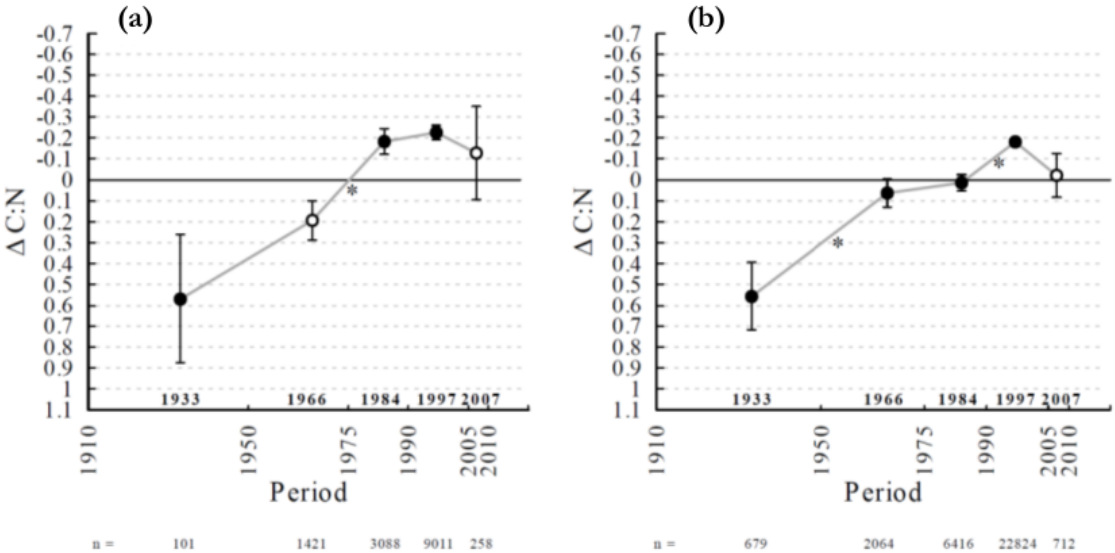


**Figure 6: Temporal bioindicated C:N changes ($\Delta C{:}N = C{:}N_{reference} - C{:}N_{former}$) between 1910 and 2010 in (a) coniferous and (b) broadleaved forests.** Mean values of the C:N changes are shown (circles) with standard error (error bars) estimated from the samples of each defined period to reconstruct N availability trends. The statistical significance of the C:N change of a period *per se* is displayed by closed circles ($p < 0.05$). The statistical significance of the C:N 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 matched records ($n$) analyzed in each period for coniferous and broadleaved forests is displayed below.

With respect to broadleaved forests, regionalized geographic patterns of available-N changes were observed over time. A pattern of available-N increase was mainly detected in the center and south of France while a pattern of available-N decrease was mainly detected in the center and north of the country over the 20$^{th}$ century. When comparing 1933 to 1966 period, and 1966 to 1984 period, C:N changes were difficult to interpret due to their low number of grid cells available for comparison (**Fig. 7ef**, respectively). However, the C:N changes between 1966 and 1984 periods indicated a C:N increase in the north of France ($n$ = 12 of 17 total cells exhibiting a change in C:N in the North of France, **Fig. 7f**). When comparing 1984 to 1997 periods, $\Delta C{:}N$ demonstrated a generalized eutrophication in the center and south of France. A contrary trend was observed in the center and north, i.e., a trend toward a decrease in available-N (**Fig. 7g**). Between the 1997 and 2007 periods, a predominant pattern of no change ($n$ = 215 of 249 total cells) was observed in broadleaved forests. In addition, an increase in

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available-N in center-southern areas (west of Central Massif, Pyrenees Mountains and southeast of France), and a decrease in available-N in the north were still identified (**Fig. 7h**).

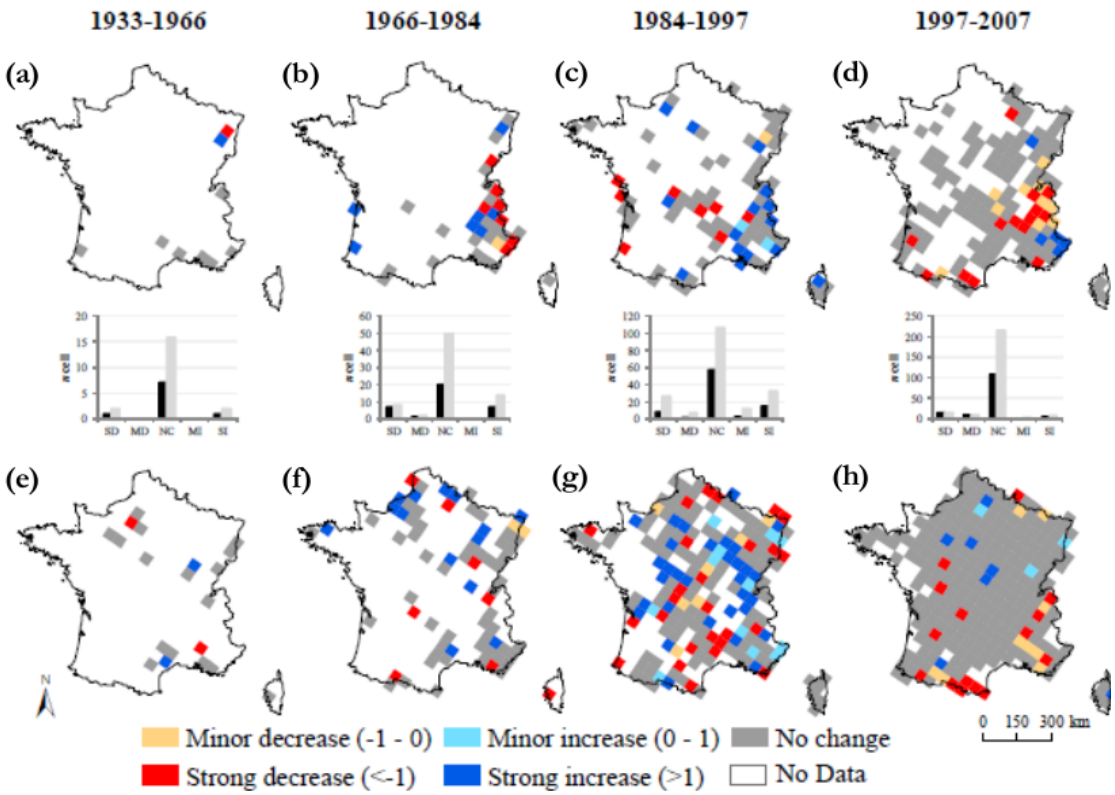


**Figure 7: Comparison of spatiotemporal bioindicated C:N changes ($\Delta C{:}N = C{:}N_{reference} - C{:}N_{former}$) in (a, b, c, d) coniferous and (e, f, g, h) broadleaved forests.** Mean values of C:N changes between compared periods (displayed in the up of the figure) and per cell are shown. The statistical significance of the C:N changes between periods ($p < 0.05$) is displayed by graduated shades of red for decreasing C:N, graduated shades of blue for increasing C:N 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 coniferous (black bars) and broadleaved forests (gray bars) showing a strong decrease (SD), minor decrease (MD), no change (NC), minor increase (MI), and strong increase (SI) of soil C:N.

## Discussion

*On the indicator of N availability and the method*

Nutrient resources, including N which is constraining element in forest ecosystems (Tamm, 1991), represent an important dimension in the ecological niche of species (Bertrand *et al.*, 2011a), and on species composition (Ellenberg *et al.*, 1992). The carbon-to-nitrogen ratio is thought to be an indicator

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of the amount of inorganic N in soils which is available for plant uptake and leaching (Gundersen *et al.*, 1998; Emmett, 2007; Nave *et al.*, 2009), a key diagnostic factor of ecosystem response to N addition (Adams *et al.*, 2004), a variable that provides information on the relative change in C and N storage in soils (Nave *et al.*, 2009; Emmett *et al.*, 2010), and an important parameter in soil chemistry models (Emmett, 2007). Soil C:N has also been used to understand the relationship between the N retention capacity of the soil and the potential for $NO_3^-$ leaching from forest systems (Dise *et al.*, 1998; Gundersen *et al.*, 1998; MacDonald *et al.*, 2002). Furthermore, soil C:N ratio, in combination with soil pH, has been suggested to be strongly related to plant community composition (Andrianarisoa *et al.*, 2009).

In the other hand, bulk soil properties as C:N ratio has been reported to be a poor predictor of changes in plant species composition (Emmett *et al.*, 2010), increased N availability which drives changes in species richness (Stevens *et al.*, 2004), and deposition rate (Stevens *et al.*, 2011b). It could be due to shifts in the form of N used by plants, delayed changes in bulk soil C:N as N deposition increases (Emmett, 2007), variability in organic matter content in soils (Stevens *et al.*, 2004), and/or increases in soil C relative to soil N (Stevens *et al.*, 2011b). If soil C:N ratio is used as an indicator for N status or nitrate leaching risk, it should be related to decomposed material accumulated above the mineral soil as suggested by Gundersen *et al.* (1998).

With respect to the method, our approach for monitoring changes in N availability is based on the relationship between organo-mineral soil C:N and plant species. Given the high responsiveness of plants to nutrient-richer conditions (Bobbink *et al.*, 1998; Turkington *et al.*, 1998; Zechmeister *et al.*, 2007; Horswill *et al.*, 2008; Johansson *et al.*, 2012), we argue that our approach is pertinent. However, the observed response of herb species to N availability is hardly supported by the $r^2$ obtained when inferring C:N values from herb assemblages ($r^2$ = 0.31, SD = 3.13 for the validation dataset). This low adjustment between measured and bioindicated values could be most probably related to imprecise C:N measurements in the training and/or validation datasets. It could also be explained by differences in sampling soil horizon whether the organic or mineral horizon (cf. Kristensen *et al.*, 2004; Nave *et al.*, 2009). If different definitions of the upper soil horizons have been used in the samplings of the different datasets composing the training dataset, according to depths or horizons, a bias in measured C:N ratio may have occurred (cf. Rowe *et al.*, 2006), creating a noise in the measured-bioindicated C:N relationship.

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*Decreasing N availability trend across French forest territory*

An interesting insight arising from forest herbs related to soil C:N is the trend to decrease in available-N in both coniferous and broadleaved forests until the 1980s and 1990s, respectively. Paradoxically, N deposition widely increased until 1980s. This observed decrease in available-N may be due to a large stock of soil organic C without at the same time building up the soil N content (Nagel *et al.*, 2011; Stevens *et al.*, 2011b). It is possibly related to increased productivity leading to increased litter and/or changes in the decomposition rate of litter (Stevens *et al.*, 2011b). Soils buffer N pollution, particularly when C:N ratios are high and there are large stocks of labile C (Emmett *et al.*, 2010). The decrease in available-N could also be related to soil acidity conditions and nutrient imbalances (Nagel *et al.*, 2011; Stevens *et al.*, 2011b; Blanco *et al.*, 2012). Increased soil acidity, which was identified in French forests until 1990s (Riofrío-Dillon *et al.*, 2012), can influence on changes in nutrient cycling (Johnston *et al.*, 1986; Blanco *et al.*, 2012) and inhibition of microbial and microfaunal decomposer communities, these latter dependent on balanced nutrient soil composition (Baath *et al.*, 1980; Blagodatskaya et Anderson, 1998). Then, changes in microbial communities, as a consequence of soil acidification, have led to reduced litter consumption and decomposition, and to litter accumulation (Baath *et al.*, 1980; Nagel *et al.*, 2011). The reduction in litter consumption and the increased accumulation of organic C may results in reduced mineralization rates, less N released to the soil solution, and therefore reduced N availability (Evans *et al.*, 2006; Nagel *et al.*, 2011; Blanco *et al.*, 2012). Moreover, soil acidification decreases cation exchange capacity and base saturation, and increases the leaching of base cations. These alterations then reduce the amount of available-N for plants uptake (Blanco *et al.*, 2012). Soil C:N exhibit a negative relationship with soil pH (**Fig. 8**) which may indicates that the above mentioned changes can be related to acidification impacting on soil processes (cf. Stevens *et al.*, 2011b).

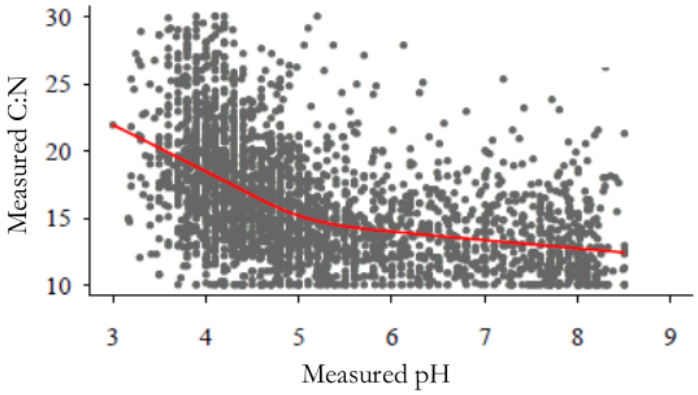


**Figure 8: Relationship between measured pH and C:N values for the training dataset.** Measured

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values were obtained from EcoPlant database (Gégout *et al.*, 2005).

Spatially, our findings suggest a high spatial variability in changes in N availability in both coniferous and broadleaved forests. They also confirm the available-N decreasing trend until the 1990s in French forest ecosystem. Coniferous forests in the southeast of France and broadleaved forests in the center and north of France have been highlighted as suffering a decrease in N availability. As we argued above, acidification could be related to this observation. In fact, these forest areas were identified as impacted by soil acidification until the 1990s (Riofrío-Dillon *et al.*, 2012).

*Signals of eutrophication in coniferous and broadleaved forests*

An increasing N availability was observed in coniferous and broadleaved forests from 1990s. In coniferous stands, the first eutrophication signal was even observed since 1980s with the cessation of N availability decrease. It means that soil eutrophication signal in forest herb species is highly time lagged compared to the N deposition trends. Several studies, based on experimentation, modeling or measured data, have reported an increase in available-N (i.e., a soil C:N decrease or increases in soil %N) due to elevated N inputs (Power *et al.*, 1998; Evans *et al.*, 2006; Power *et al.*, 2006; Nave *et al.*, 2009). However, it was demonstrated that, depending on the level and duration of inputs as well as on the conditions of the site prior to deposition (e.g. soil nutrient status, buffering capacity, microclimate), species composition and diversity may be altered (Matson *et al.*, 2002; Stevens *et al.*, 2004; Stevens *et al.*, 2011a). Moreover, the amount of N retained in soil and vegetation can affect the duration and strength of the influence of N as a driver of change in ecosystem functioning (Phoenix *et al.*, 2012). Furthermore, in a former study, we observed the cessation of forest acidification from 1990s (Riofrío-Dillon *et al.*, 2012). It could lead to an enhanced litter decomposition and promote a restart in affected soil processes and consequently to an increase in available-N to plants. An increase in N availability in ecosystems suggests that it is moving through the stages of or to a nitrogen saturation as a result of continued high inorganic N deposition (Nagel *et al.*, 2011).

Spatially, our findings confirm the increasing N availability observed in the temporal trends in both coniferous and broadleaved forests. In coniferous forests, the eutrophication signal was identified in the Central Massif and North Alps regions, and the Pyrenees Mountains. In broadleaved forests, it was identified in the center and south of France, i.e., the Central Massif region, southeastern France, the Pyrenees Mountains, and in the Northeastern national limit. The highest nitrate and ammonium deposition levels were reported for these regions in the late 1990s (Croisé *et al.*, 2005), and until mid-

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2010s they have hardly changed (Croisé et al., not published data).

## Conclusion

Long-term and elevated N deposition have likely influenced soil C:N ratio in coniferous and broadleaved forests during the last time. While some soil conditions are likely entering a recovery time (e.g. soil pH) at the current levels of atmospheric deposition, the eutrophication signal has just been identified in French forests. The observed decrease in available-N may be related to acidification impacting on soil processes while the recent increase, which involves a time lag for eutrophication, is consistent with the current cessation of acidification. Therefore, the past N deposition is still impacting both forest soil and vegetation. It could have important implications in current environmental policies and their current permissible levels.

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