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### **High-altitude exposure of three weeks duration increases lung diffusing capacity in humans**

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**On the tracks of Nitrogen deposition effects on temperate forests at their southern  
European range – an observational study from Italy**

Running head: N deposition and forests in southern Europe

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CONECOFOR forest monitoring; N critical loads; soil and foliar nutrients; PLS regression; growth and carbon sequestration

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## Abstract

We studied forest monitoring data collected at permanent plots in Italy over the period 2000-2009 to identify the possible impact of nitrogen (N) deposition on soil chemistry, tree nutrition and growth. Average N throughfall ( $\text{N-NO}_3 + \text{N-NH}_4$ ) ranged between 4 and 29  $\text{kg ha}^{-1} \text{ yr}^{-1}$ , with Critical Loads (CLs) for nutrient N exceeded at several sites. Evidence is consistent in pointing out effects of N deposition on soil and tree nutrition: topsoil exchangeable base cations (BCE) and pH decreased with increasing N deposition, and foliar nutrient N ratios (especially N:P and N:K) increased. Comparison between bulk openfield and throughfall data suggested possible canopy uptake of N, levelling out for bulk deposition  $>4\text{-}6 \text{ kg ha}^{-1} \text{ yr}^{-1}$ . Partial Least Square (PLS) regression revealed that - although stand and meteorological variables explained the largest portion of variance in relative basal area increment ( $BAI_{rel}$  2000-2009) - N-related predictors (topsoil BCE, C:N, pH; foliar N-ratios; N deposition) nearly always improved the  $BAI_{rel}$  model in terms of variance explained (from 78.2 to 93.5%) and error (from 2.98 to 1.50%). N deposition was the strongest predictor even when stand, management and atmosphere-related variables (meteorology and tropospheric ozone) were accounted for. The maximal annual response of  $BAI_{rel}$  was estimated at 0.074-0.085% for every additional kgN. This corresponds to an annual maximal relative increase of 0.13-0.14% of carbon sequestered in the above ground woody biomass for every additional kgN, i.e. a median value of 159 kgC per  $\text{kgN ha}^{-1} \text{ yr}^{-1}$  (range: 50-504 kgC per kgN, depending on the site). Positive growth response occurred also at sites where signals of possible, perhaps recent N saturation were detected. This may suggest a time lag for detrimental N effects, but also that, under continuous high N input, the reported positive growth response may be not sustainable in the long-term.

## Introduction

Human activity has substantially altered the nitrogen (N) cycle and now dominates the creation of reactive N in Europe, America and Asia (Canfield *et al.*, 2010). Such a production has increased globally from ca. 15 TgN yr<sup>-1</sup> (1860) to ca. 156 TgN yr<sup>-1</sup> (early 1990s), with a projected increase up to ca. 270 TgN yr<sup>-1</sup> by 2050 (Galloway *et al.*, 2004). Alongside, total atmospheric emissions of NO<sub>x</sub> and NH<sub>3</sub> increased from 23 TgN yr<sup>-1</sup> in 1860 to 93 (early 1990s) and 189 TgN yr<sup>-1</sup> (projected 2050) (Galloway *et al.*, 2004), with a dramatic increase of N concentration in atmospheric precipitation (Brimblecombe & Stedmann, 1982), and a resulting three- to five-fold increase of deposition in reactive N (Denman *et al.*, 2007). A further deposition increase by a factor of 2.5 is projected at global level by the end of the century (Lamarque *et al.*, 2005).

Concern about the actual and potential effects of high N deposition on forests embraces almost all forest ecosystem's compartments, including vegetation, soil biota, soil, soil water, and run-off (Mc Nulty *et al.*, 2005; Lu *et al.*, 2009; Janssens *et al.*, 2010; Bleeker *et al.*, 2011). Since N availability controls the productivity of many ecosystems (e.g. Zoetl, 1990; Vitousek *et al.*, 2002), considerable emphasis was put on its role in stimulating growth and enhancing carbon (C) uptake (e.g. MacDonald *et al.*, 2011). Although the actual size of such an effect is uncertain (e.g. Nadelhoffer *et al.*, 1999; de Vries *et al.*, 2006, 2008, 2009; Magnani *et al.*, 2007; Sutton *et al.*, 2008; Vicca *et al.*, 2012; see also the review by Erisman *et al.*, 2011), N deposition is considered an essential driver of the residual terrestrial C sink of 2.4±0.8 PgC yr<sup>-1</sup> (Le Quéré *et al.*, 2013). Effects of N deposition on growth and C sequestration are related to three main mechanisms: accelerated photosynthesis (e.g. Fleischer *et al.*, 2013), the so-called allocation shift (decreased C allocation to roots and increased wood formation), and decreased decomposition, which leads to accumulation of surface litter and soil organic matter (Janssens & Luysaert, 2009; Janssens *et al.*, 2010). The nutrient cycle is affected by changes in soil

chemistry (e.g. Moore & Houle, 2013) and N uptake by the foliage (e.g. Adriaenssens *et al.*, 2011; Fenn *et al.*, 2013; Fleischer *et al.*, 2013). These processes may lead to several consequences: under N-limited conditions (which are typical of many temperate forests, Oren *et al.*, 2001), input of additional N may be beneficial, causing enhanced tree growth with no effects on tree health; on the other hand, when N is not a limiting factor, the growth stimulation by additional N deposition may be not supported by other nutrients (e.g. Emmett, 1999) and/or can be counteracted by possible detrimental effects on tree health due to increased sensitivity to pest/pathogens (e.g. Roelofs *et al.*, 1985; Braun *et al.*, 1999) and extreme weather condition, which may in turn have negative effects on growth (e.g. Dobbertin, 2005).

Recent debate about the impact of N deposition on forests ecosystems was ample, but almost entirely based on temperate and boreal forests from mid to high latitudes (e.g. Erisman *et al.*, 2011). With few exceptions (e.g. Blanes *et al.*, 2013), information on occurrence, size and extent of such an impact on the forests of southern Europe (i.e. 37-42° Latitude) is limited (Ochoa-Hueso *et al.*, 2011). This is unfortunate: forests in southern Europe amounts to ca.  $61 \times 10^6$  ha, encompass a full range of ecological condition (from Alpine to Mediterranean), and eutrophication due to N deposition was frequently reported as a potential danger for their health and vitality (FOREST EUROPE, 2011). Here we present an observational study carried out in Italy, where the diversity in species, ecological conditions and N deposition ( $7 - 24 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , Marchetto *et al.*, 2008) offers a good example of those typical of southern Europe. In Italy, the total forest area according to the definition of the United Nations Food and Agriculture Organization (FAO) and Forest Resource Assessment (FRA) 2000 amounts to  $8.8 \times 10^6$  ha with a total volume (stem and branches  $\geq 5 \text{ cm } \varnothing$ ) of  $1269 \times 10^6 \text{ m}^3$  and total annual increment of  $35.9 \times 10^6 \text{ m}^3$  (Gasparini & Tabacchi, 2011). Forest sites in Italy have generally high levels of base saturation (i.e. the proportion of basic exchangeable cations  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,

K<sup>+</sup>, Na<sup>+</sup> to the total cation exchange capacity of the soil) (FOREST EUROPE, 2011; De Vos & Cools, 2013a), and this suggests conditions that can support a positive growth response to the availability of additional N. If such a response would be proven true, given the measured N bulk deposition rates (Marchetto *et al.*, 2008) there could be a considerable N *bonus* (i.e., the potential of N deposition to speed up the sequestration of C in forest trees and soil, Janssens & Luysaert, 2009) for the overall growth and C sequestration of Italian forests even when a conservative estimate of such a *bonus* (i.e., 30-75 kgC per kgN of total deposition, e.g. de Vries *et al.*, 2009) is adopted.

Our study is based on the data collected at the sites of the Italian national long-term forest monitoring program CONECOFOR (acronym of the Italian “Controllo Ecosistemi Forestali”, Forest Ecosystem Monitoring). We considered a total of 26 permanent plots (0.25 hectare each) (Table 1). Over the past two decades, the monitored forests were subjected neither to land-use and land-cover change (LULCC) nor to thinning, deliberate fertilization and irrigation. Therefore, recent growth, productivity and resulting C uptake at these sites can be ascribed entirely on the one hand to the set of environmental drivers, and on the other hand to processes inherent to forest development (e.g., competition, ageing, disturbances) (Erb *et al.*, 2013; Houghton, 2013; Etzold *et al.*, 2014). At these plots, atmospheric deposition has been measured according to harmonized methods since 1996-1998 (Marchetto *et al.*, 2008) together with a suite of other variables, including those related to meteorology, tropospheric ozone (O<sub>3</sub>), soil and foliar nutrients, species diversity, tree health and growth (Ferretti, 2000; Ferretti *et al.*, 2003a, 2006, 2008). To track the potential and actual impact of N deposition, we considered the following questions:

- (i) Are present deposition loads still within protective limits for the investigated plots?
- (ii) Is there evidence for effects of N deposition on nutrients at soil and foliar level?

- (iii) Once stand, management, and atmosphere-related factors (meteorology, tropospheric O<sub>3</sub>) are taken into account, is there evidence for N deposition-related effects on tree growth and C sequestration?

Unlike other studies carried out at much broader geographical scale (e.g. Magnani *et al.*, 2007; Sutton *et al.*, 2008), we used only data arising from actual measurements at the very sites, including in particular N deposition.

## **Materials and methods**

### *Concept and study design*

The study has been conducted considering a ten years time window (2000-2009, coinciding with the start and the end of two subsequent five-years growth measurement periods) in order to (i) smooth the potential noise caused by random events in particular years; and (ii) to have a larger set of data from as many plots as possible for statistical analyses. We deliberately excluded situations where major disturbances have occurred over the study period, i.e. the sessile oak (*Quercus petraea* (Matt.) Liebl.) population at site EMI1 affected by sudden and widespread drought-induced tree mortality (up to 67% of the stems between 2000-2009) (Bertini *et al.*, 2011).

The study consisted of three main phases: in a first phase, Critical Loads (CLs) for nutrient N (e.g., Grennfelt *et al.* 2001; UNECE, 2004) were estimated for the study sites by the Simple Mass Balance (SMB) method (see below) and exceedances calculated with respect to the actual deposition load.

Secondly, predictors and response variables were identified. A careful, expert-based selection was carried out in order to avoid redundancy, to not offset/over emphasize the role of N deposition due to inherent co-linearity among variables, and to rely on the most robust



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response variable. The selection was carried out considering the pathways and relationships described in Fig. 1. Predictor and response variables considered in this study are reported in Table 2 and further described below.

Thirdly, the selected predictors were used to investigate effects of N deposition on soil and foliage chemistry and to model the response growth variable of interest, i.e. the relative Basal Area Increment ( $BAI_{rel}$ , see below). As for the relationship between soil chemistry and deposition, we assumed no major difference exists between N deposition in the period 1995-1996 (when the soil survey was carried out, see below) and 2000-2009 (when most deposition data were actually collected, see below). This assumption is supported by the limited variation of  $NO_3$  and  $NH_4$  emissions registered in Italy over the period 1978-2004 (Marchetto *et al.*, 2008). The same authors reported that, while a consistent, significant decrease of sulphur deposition was obvious at our plots, the deposition of  $NO_3$  and  $NH_4$  showed limited trends. Different sets of predictors were considered to model  $BAI_{rel}$  (Table 2). A model that used predictors related to stand (species, management, tree density, age) and atmosphere (meteorology, ground-level ozone) was used as reference model against which the role of additional predictors was evaluated. Subsequently, variables related to N deposition (soil pH, C:N, BCE; foliar N ratios; N throughfall deposition) and tree health variables (which may be affected by N deposition as well, Roelofs *et al.*, 1985; Mc Nulty *et al.*, 2005; Duarte *et al.*, 2013) were added step-wise to explore their relative importance in improving the reference model.

#### Dataset

Sampling, analyses and measurements at the CONECOFOR plots were all carried out according to the methods described in Ferretti and Fischer (2013) and following Quality

Assurance (QA) and Quality Control (QC) procedures reported therein. Basic information is reported in Table 1.

#### Plot and site information

Plots are distributed across Italy, spanning ca. 1200 km in latitude, 500 km in longitude and 1800 m in elevation. Main tree species (MTS) include deciduous broadleaves (mostly beech, *Fagus sylvatica* L., and oaks, in particular *Quercus cerris* L.), conifers (Norway spruce, *Picea abies* L.) and evergreen oaks (holm oak, *Quercus ilex* L.). Plots were installed within forests having different origin and management regimes. Management operations have ceased since 1995 and, in most cases, since even longer times. Current age (2010) ranges from 40 to 230 years, and tree density from 240 to 4060 trees per hectare. Plot soils span 12 different reference soil groups (IUSS Working Group WRB, 2006).

#### Deposition data and Critical Loads

Openfield and throughfall deposition have been measured at the plots since 1996-1998 according to standard procedures (Hansen *et al.*, 2013; Marchetto *et al.*, 2013). In this paper, N-NO<sub>3</sub> + N-NH<sub>4</sub> throughfall data have been used as a predictor in the regression models and to apply the steady state Simple Mass Balance (SMB) model (Sverdrup *et al.*, 1990, Sverdrup & de Vries, 1994) to calculate the Critical Load (CL) of nutrient N. We considered the throughfall flux because it is the actual flux reaching the forest soil; we concentrated on N-NO<sub>3</sub> + N-NH<sub>4</sub> because they were consistently monitored over time and space.

The SMB model considers forest soil as a whole and its N balance, including all forms of soil N, is summarized by the following equation (Posch, 2004):

$$N_{\text{dep}} + N_{\text{fix}} = N_{\text{ad}} + N_{\text{i}} + N_{\text{de}} + N_{\text{eros}} + N_{\text{fire}} + N_{\text{vol}} + N_{\text{le}} \quad (1)$$

where:

$N_{\text{dep}}$  = total N deposition

$N_{\text{fix}}$  = N input by biological fixation

$N_{\text{ad}}$  = N adsorption

$N_i$  = long-term net immobilization of N in soil organic matter

$N_u$  = net removal of N from the soil due to plant growth

$N_{\text{de}}$  = flux of N to the atmosphere due to denitrification

$N_{\text{eros}}$  = N losses through erosion

$N_{\text{fire}}$  = N losses in smoke due to fires

$N_{\text{vol}}$  = N losses to the atmosphere due to  $\text{NH}_3$  volatilisation

$N_{\text{le}}$  = leaching of N below the root zone

Following Posch *et al.* (1994), to estimate the CLs for nutrient N, we assumed the following processes were negligible: (1) adsorption, as it is N compounds are only stored and released when N deposition changes; (2) fixation, as in Italian plots N-fixing species were not frequent; (3) losses due to fire, erosion and volatilization. The SMB model equation can then be simplified as follows (Posch *et al.* 2004):

$$N_{\text{dep}} = N_i + N_u + N_{\text{de}} + N_{\text{le}} \quad (2)$$

From this equation, a critical load (CL) is obtained by defining an acceptable limit to the leaching of N ( $N_{\text{le,acc}}$ ).

$$\text{CL} = N_i + N_u + N_{\text{de}} + N_{\text{le,acc}} \quad (3)$$

The choice of the  $N_{le, acc}$  is a crucial point for the determination of the critical load.  $N_{le, acc}$  can be obtained by multiplying the precipitation surplus by a constant N concentration in the leaching flux. The latter depends on the target ecosystem to be protected: for example, when dealing with the protection of surface water,  $N_{le, acc}$  can be assumed to be close to zero, while for the protection of groundwater a value so high as the EC target value ( $25 \text{ mg L}^{-1}$ ) can be selected. However, when the target of the CL evaluation is nutrient imbalance in forest trees, Warfvinge et al. (1992) suggest to select a value of  $0.2 \text{ mg L}^{-1}$  for coniferous forests and of between  $0.2$  and  $0.4 \text{ mg L}^{-1}$  for deciduous forests. In this paper, we set the maximum acceptable concentration in the leaching flux to  $0.20 \text{ mg L}^{-1}$  for coniferous forests and  $0.38 \text{ mg L}^{-1}$  for deciduous forests.

Considering the high air temperatures recorded in Italy, N immobilization was set to  $1 \text{ kgN ha}^{-1} \text{ yr}^{-1}$  (Posch, 2004). Denitrification was considered linearly related to the net input of N, following de Vries *et al.* (1994) and the denitrification fraction was set to 0.5 and 0.7 for sandy and clay soils, respectively. The precipitation surplus was calculated on a monthly basis following Thornthwaite (1948). Finally, N uptake was estimated plot by plot using actual increment of standing tree volume over a period of 5 to 13 years, depending on data availability for the concerned plot, and species-specific mean wood N concentration in Italian forests (Bascietto *et al.*, 2003).

## Ozone

Ozone concentration has been measured by passive sampling since 1996 (Mangoni & Buffoni, 2008). Mean April-September concentration over the investigated period was used as an indicator of possible stress due to tropospheric ozone. It is worth noting that - at forest sites - the Accumulated ozone Over Threshold 40 ppb (AOT40), the exposure index used to estimate the possible risk due to ozone, is a linear function of mean  $O_3$  concentration (Ferretti *et al.*,

2012). Only plots with at least five years with complete April-September data in the period 2000-2009 were considered.

## Meteorology

Meteorological stations have been active since 1996, collecting data on hourly basis (Ammoriello & Costantini, 2008). The following meteorological variables and indices were considered in this study:

- the maximum air temperature (mean of monthly maximum temperature,  $T_{max}$ ) as a general indicator of the (possibly changing) temperature regime.
- the ratio between winter index (WI, the sum of daily mean temperatures  $< 0^{\circ}\text{C}$  from December of the year  $t$  to February of year  $t+1$ ) and summer index (SI, the sum of the daily mean temperatures  $> 5^{\circ}\text{C}$  from June to August), indicating the seasonality climate regime (WI/SI).
- the annual precipitation amount (Pr) as a proxy of the average rainfall regime
- the percentage of precipitation observed during the growing season from April to September (PRGI)
- the water surplus (or deficit) based on the ratio of Pr to potential evapotranspiration (ETP),  $P/EPT^{-1}$ , with ETP calculated according to the Hargreaves-Samani standard formula using maximum, minimum and average temperature as well as estimated solar radiation according to the geographical location of the plot.

## Soil chemistry

A number of soil variables was measured for soil organic and mineral layers at the CONECOFOR plots in 1995-1996 (Ammoriello *et al.*, 2000). For the present study, the top mineral layer (0-10 cm) was considered; topsoil layers are likely to show short-term responses

to atmospheric input, while organic layers are in general too thin and variable in most Italian forest soils (Andreetta *et al.*, 2010). The following variables were selected (Högberg *et al.*, 2006; Lu *et al.*, 2009; De Vos & Cools, 2013a,b; Moore & Houle, 2013): pH (CaCl<sub>2</sub>), C:N ratio and the sum of exchangeable base cations (BCE), as extracted by BaCl<sub>2</sub>.

#### Foliar chemistry

The main foliar nutrients (N; calcium - Ca, phosphorous - P, magnesium - Mg, potassium – K, and sulphur – S) have been measured every two years since 1995 in the foliage of five dominant trees of the MTS assumed to be representative on the mean health status of each plot (Matteucci *et al.*, 2000). Sampling and analysis were performed according to Rautio and Fürst (2013). As the absolute nutrient concentrations can often be of limited value to identify possible nutrient imbalances (Dusquenay *et al.*, 2000), we considered nutrient ratios, i.e. N:P, N:Ca, N:Mg, N:K.

#### Tree health

The assessment of a suite of variables on tree health has been carried out annually (Bussotti *et al.*, 2005). In the present study, the frequency of trees with crown transparency (an indicator of the foliage density on the trees as compared to a reference standard) >25% (F>25) and the frequency of trees with observed damage symptoms (i.e., foliage discoloration, dead branchlets) (F\_damage) were used as indicators of tree health and as predictors in the growth model. Only plots with at least eight years of valid data were considered in the present study.

#### Growth and stand

The main variable adopted was basal area (BA), assessed after periodical measurements of trees' circumference carried out in winters 1999-2000, 2004-2005, and 2009-2010 according

to the procedure described by Dobbertin *et al.* (2013). Two main variables calculated after these measurements were formally incorporated in the present study: the adjusted stand density index ( $SDI_{adj}$ , after Zeide, 2005), adopted as predictor variable, and the relative Basal Area Increment ( $BAI_{rel}$ ) (Dobbertin, 2005), adopted as response variable in the statistical modeling.

The  $SDI$  derives from the allometric relationship between the number of trees per unit area and the average stand diameter in undisturbed, even-aged stands.

$$N = a \cdot d_g^b \quad (3)$$

where:

$N$  is the tree number per unit area,

$d_g$  is the quadratic mean diameter,

$a$  and  $b$  define the density of fully stocked stands, also known as normal or equilibrium density, and the slope of the line, respectively.

This relation has often been used to describe the average maximum limits of stand density and, by extension, to provide a relative measure or index of stand density (Reineke, 1933).

Reineke computed the index according to the following equation:

$$SDI = N \cdot (25/d_g)^b \quad (4)$$

where the allometric coefficient  $b$  assumes a value close to -1.605 for all tree species and the index provides the reference to a stand with an average dbh = 25 cm. The allometric coefficient  $b$  explains the species-specific tolerance and the effectiveness in the use of

growing space (Zeide, 1985; Pretzsch & Biber, 2005). Under this assumption and according to Zeide (2005), the actual  $b$  value per plot ( $SDI_{adj}$ ) was then calculated as follows:

$$b = (-\Delta n/N)/(\Delta dg/dg) \quad (5)$$

where  $b$  = ratio between tree number reduction ( $-\Delta n/N$ ) and the increase in average tree diameter ( $\Delta dg/dg$ ) over subsequent time-windows;

$BAI_{rel}$  over the time-window 2000-2009 is the percentage ratio between BAI 2000-2009 and the generating BA at year 2000. Two main benefits are related to this indicator: it is entirely based on quality assured, measured data and it accounts for the actual plot condition at the beginning of the growth period considered.

To allow the interpretation of results in a broader respect, growth in terms of C sequestration was also estimated by the calculated stem volume, the biomass expansion factor (BEF) and basal density, the latter two terms specific for tree species and stand type. The conventional value of 0.5 was used to convert biomass into C content.

### *Statistical methods*

Different regression techniques were adopted to describe the relationships of concern. Relationships between individual deposition, soil, foliage, and growth variables were described by single factor regression, for all the species and for broadleaves separately (Cools *et al.*, 2014).

Partial Least Square (PLS) regression (Wold, 1975; Wold *et al.*, 2001; Ferretti *et al.*, 2014) was used for statistical modeling of  $BAI_{rel}$ . PLS regression generalises and combines features from principal component analysis (PCA) and multiple linear regression (MLR). It is



particularly useful when there is the need to predict a set of dependent variables from a (very) large set of independent variables (i.e., predictors). Let  $I$  observations described by  $K$  dependent variables in a  $I \times K$  matrix denoted  $Y$ , the values of  $J$  predictors collected on these  $I$  observations are in the  $I \times J$  matrix, say  $X$ . The goal of PLS regression is to predict  $Y$  from  $X$  and to describe their common structure. When  $Y$  is a vector and  $X$  is full rank, this goal could be accomplished using ordinary MLR. Otherwise, when the number of predictors is large compared to the number of observations,  $X$  is likely to be singular and the regression approach is no longer feasible (i.e., because of multicollinearity). PLS regression searches for a set of components (called latent variables) that performs a simultaneous decomposition of  $X$  and  $Y$  with the constraint that these components explain as much as possible of the covariance between  $X$  and  $Y$ . If all the latent variables of  $X$  are used, this regression is equivalent to principal component regression. When only a subset of the latent variables is used, the prediction of  $Y$  is optimal for this number of predictors. An obvious question is to identify the number of latent variables needed to obtain the best generalisation for the prediction of new observations. This is, in general, achieved by cross-validation techniques such as bootstrapping or Leave One Out (LOO), the latter being more common in cases in which the number of observation is rather low. In cross-validation,  $n$  different models were created, each time omitting one or more observation, and the Root Mean Square Error (RMSE) in the cross validation (RMSEC) were calculated. PLS is extremely useful when there are more predictors than observations. Conversely to common regression techniques, however, with PLS it is pretty difficult to calculate coefficients confidence interval and other regression figures (say significance for individual predictors). They are replaced by the VIP (Variable Importance in the Projections) scores (Wold *et al.*, 1993). Since the average of the squared VIP scores equals 1, variables with scores  $>1$  were considered to be the most important (Ferretti *et al.*, 2014).

PLS regression for  $BAI_{rel}$  modeling was carried out by the NIPALS (Non-linear estimation by Iterative Partial Least Squares) algorithm (Wold, 1966; Geladi *et al.*, 1986). NIPALS is at the root of PLS regression and can execute PLS even when missing values are >10% by simply leaving those out from the appropriate inner products.

PLS regression was carried out for different set of predictors (see Table 2) and - given that data coverage was different among plots - for two different aggregations of plots: the aggregation that uses all the plots with available data for each given set of predictors (to maximize the degree of freedom for each regression run; missing data imputed by NIPALS), and the aggregation that uses only “common plots”, i.e. the plots with available data for all the different predictors only (to maximize the comparability among models; no missing data).

## Results

*Are present deposition loads still within protective limits for the investigated plots?*

Measured throughfall N ( $N-NO_3+N-NH_4$ ) ranged between 4.5 to 28.8 kgN ha<sup>-1</sup> yr<sup>-1</sup> and were in general very close to the estimated CLs. Exceedance of CLs was infrequent at deposition <10 kgN ha<sup>-1</sup> yr<sup>-1</sup> and almost ubiquitous for deposition >15 kgN ha<sup>-1</sup> yr<sup>-1</sup> (Fig. 2). Highest deposition rates and exceedance were recorded in Northern Italy, at the sites in the Po plain (EMI1, VEN2) and on the nearby reliefs (LOM2, PIE1, VEN1). This is related to the high emissions of NO<sub>x</sub> and NH<sub>4</sub> in this area, where most of the Italian industrial and livestock rearing activities are located. Exceedance of CLs set to protect vegetation were observed at 11 plots, for the most part located in relatively young forests (<70-years old, e.g. EMI1, PIE1) (see Table S2). Lower deposition level and no or slight exceedance were found for plots >150-years old (e.g., TOS3, TRE1, VAL1).

*Is there evidence for effects of N deposition on nutrients at soil and foliar level?*

We considered the three pathways reported in Fig. 1. Pathways 1 and 2 refer to possible impact of N deposition on soil and soil-mediated nutritional status of forest trees. With respect to soil, there was a significant decrease of pH (in broadleaved plots,  $P < 0.05$ ) and BCE (all plots,  $P < 0.05$ ) with increasing N deposition (Fig. 3a, c). Slight or no effect was obvious on C:N (Fig. 3b).

With respect to soil-mediated effect, foliar N ratios (Fig. 4a, b, c, d) correlated negatively, although not always significantly, with soil BCE. This was true especially for N:Ca ( $P < 0.01$ ), N:K ( $P < 0.05$ ), and in the case of broadleaves. When the Norway spruce plots were included, the relationship between BCE and N ratios was always worse, with a reduction of 10-25% in terms of variance explained.

The impact of N deposition on foliar nutrition (pathway 3, Fig. 1) was also investigated by considering its direct relationship with foliar N ratios (Fig. 5), which may indicate – besides uptake via soil – a prompt, direct uptake of N by the canopy via ionic transport across foliar cuticles, stomatal uptake and diffusion through the bark into twigs and branches (Harrison *et al.*, 2000; Sievering *et al.*, 2000, quoted in de Vries *et al.*, 2008). Foliar N ratios increase with increasing N deposition: this pattern is again much more obvious for broadleaves, and for N:P and N:K (Fig. 5a, d,  $P < 0.001$ ). When Norway spruce plots are considered, then there is always a reduction (up to 63% for N:P) of variance explained.

A comparison between N deposition in the openfield and throughfall (Fig. 6) revealed a release of N-NO<sub>3</sub> from the tree canopy for openfield N-NO<sub>3</sub> deposition  $> 4 \text{ kg ha}^{-1} \text{ yr}^{-1}$ ; on the other hand, there is a clear canopy consumption of N-NH<sub>4</sub> for deposition level  $< 6 \text{ kg ha}^{-1} \text{ yr}^{-1}$ . This confirms – at least in part – the direct uptake of N-NH<sub>4</sub> by the canopy. At higher deposition values, and for plots with exceedance of the estimated CL for nutrient N, there is no evidence of possible foliar uptake.

Throughfall N deposition is expected to be higher than openfield N deposition, because the former also accounts for dry deposition of particles on tree canopies. A comparison between N deposition in the openfield and throughfall (Fig. 6), however, revealed a canopy consumption of N in plots receiving low N deposition. In particular, throughfall of both N-NO<sub>3</sub> and N-NH<sub>4</sub> were lower than open field fluxes for deposition <4 and <6 kg ha<sup>-1</sup> yr<sup>-1</sup>, respectively, indicating a possible N uptake from the canopy. At higher deposition values, there is no evidence of possible foliar N uptake.

*Is there evidence for N deposition-related effects on tree growth and C sequestration?*

Mean annual  $BAI_{rel}$  over the 2000-2009 period was 0.92% (min.=-0.2 at plot SIC1; max.=2.6% at plot EMI1), with beech (mean=0.96%; SD=0.375) and Norway spruce (1.12±0.633) plots showing the highest rates. Turkey oak had high  $BAI_{rel}$  values with high between-plot variability (1.06±1.171), while holm oak had consistently much lower rates (0.29±0.365).

Among-plot variability of  $BAI_{rel}$  was studied by means of five PLS regression models. Models performance, importance and coefficients of the predictors for the two aggregations of plots (all plots with available data and common plots) are reported in Tables 3 and 4. Despite huge differences (number and type of plots, range of ecological condition explored) between the two datasets, some important signals were consistent. For both plot aggregations, the largest portion of variance (78 - 87%) was explained by the reference model, i.e. by stand, management and atmosphere-related variables. Topsoil N-related variables did not always improve the reference model. Foliar N ratios always improved the reference model (variance explained augmented by 2-10%), and so did the tree health-related variables (variance explained increased by 2-13%). For both plot aggregations, however, the best performance was obtained when N deposition was added to the reference model. This resulted in the

highest total portion of variance explained (ca. 93 %) and in the lowest error in LOO cross validation (RMSEC: 0.85 - 1.5) (Table 3).

To ensure a more robust evaluation of the modeling outputs, we concentrated on those results that were consistent for the two data aggregations (Table 4). Within the set of the variables ubiquitous in all models (stand and management, atmosphere), some never resulted important (VIP always <1), i.e. Pr EPT<sup>-1</sup> and annual Pr. On the other hand, species type and PRGI were always important (VIP>1) and displayed a consistent pattern (same sign) across all models and for both data aggregations: *BAI<sub>rel</sub>* appeared negatively related to evergreen broadleaves and positively related to PRGI.

When soil variables were added to the reference model, only BCE displayed consistent results between the two data aggregation, and was negatively related to growth. It is worth noting that BCE was also negatively related to N deposition. The inclusion of soil variables in the model led to a reduction of the regression coefficients associated to the species (i.e., evergreen broadleaves). This may be partly due to some effect of the species itself on soil characteristics (e.g. Cools *et al.*, 2014). According to Fig. 3a,c, for our data set this may be actually true in the case of holm oak, whose two plots were in the higher range of pH and BCE values. The same plots, however, were also in the lower N deposition range (7 – 11 kgN ha<sup>-1</sup> yr<sup>-1</sup>) (Fig. 3a,c). When a species with a larger number of plots distributed along a wider N deposition gradient is considered (e.g. beech, seven plots, 10-23 kgN ha<sup>-1</sup> yr<sup>-1</sup>), the effect of deposition on soil appears much clearer, especially on BCE (Fig. 3c).

When foliar N ratios were considered, N:P and N:K showed consistent results for the two data aggregations, with a distinct, positive relation with *BAI<sub>rel</sub>* and with coefficients comparable in size to those obtained by management, species, stand and climate variables (Table 4).

When added to the reference model, N throughfall was found important for both plot aggregations and showed the highest, positive regression coefficients (Table 4). The

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coefficients were comparable or even larger than those related to management, species, stand and climate variables. Coefficients for those variables remained almost unchanged with respect to the reference model, with the only exception of PRGI. This suggests a strong, positive and rather independent role of N deposition on  $BAI_{rel}$  over the period 2000-2009. An overall, significant positive effect of N deposition on growth is evident for all plots ( $R^2 = 0.59$ ; slope: 0.074;  $P < 0.001$ ) and for broadleaved plots in particular ( $R^2 = 0.76$ ; slope: 0.085;  $P < 0.0001$ ) (Fig. 7), with an estimated increase of  $BAI_{rel}$  of 0.074 – 0.085% per  $\text{KgN ha}^{-1} \text{ yr}^{-1}$ . Basal area increment is highly correlated to tree volume, biomass and C content (Pokharel & Dech, 2012). In Fig. 8, growth was re-calculated (see methods) in terms of relative annual C sequestered ( $Cseq_{rel}$ ) over the period 2000-2009 (annual C sequestered in above-ground woody biomass in % of the C stock at year 2000) and plotted against the mean annual N throughfall over the same period. On average, there is a 0.13% increase of C sequestered annually for every additional kg N deposited. When considering that the C stocked in the above-ground woody biomass at the plots in the year 2000 was estimated to range between 40 and 392  $\text{Mg ha}^{-1}$ , the annual C sequestration rate estimated according to the regression in Fig. 8 amounts to an annual median value of 159  $\text{kgC ha}^{-1}$  (min: 51; max: 504) per  $\text{kgN}$ .

## Discussion

### *N deposition and forest ecosystems*

Bobbink & Hettelingh (2011) provided an overview of N deposition effects on broadleaved and temperate coniferous forests. They identified different indicators related to soil processes (acidification, mineralization, nitrification, leaching, N trace-gas emissions, and litter decomposition), trees (nutrition, physiology, phenology, recruitment and susceptibility to pests and pathogens), and biodiversity (macrofungi and mycorrhiza, lichens and green algae, ground vegetation and fauna). On the basis of 82 studies involving those indicators, empirical

N Critical Loads were set in a range from 3-15 kgN ha<sup>-1</sup> yr<sup>-1</sup> for Mediterranean *Pinus* woodland to 15-20 kgN ha<sup>-1</sup> yr<sup>-1</sup> for mesotrophic and eutrophic *Quercus* dominated woodland (Bobbink & Hettelingh, 2011, Table 9.3). Conifers were considered slightly more sensitive than broadleaved forests (e.g. Kristensen *et al.* (2004), although soil nutritional condition can greatly affect the sensitivity, thus rendering any generalization quite difficult. When considering the forest categories investigated by our study, CLs were set at 10-15 kgN ha<sup>-1</sup> yr<sup>-1</sup> (*Picea* and acidophilus *Quercus* dominated woodland) and 10-20 kgN ha<sup>-1</sup> yr<sup>-1</sup> (*Fagus* and mesotrophic and eutrophic *Quercus* dominated woodland) (Bobbink & Hettelingh, 2011). These partly overlapping values implies that it can be very difficult to identify whether a real difference exists in the sensitivity of our sites: actually, higher sensitivity of spruce was not obvious from our data, neither in terms of soil chemistry, nor in terms of foliar nutrition and growth.

It is undisputed, however, that effects of N deposition cover all the above reported indicators, and can lead to a variety of consequences on vegetation (e.g., changes in species coverage and competition pattern, reduction of diversity, Sardans *et al.*, 2012). For example, in a study involving also some of the sites considered in this paper, Dirnböck *et al.* (2013) found that “in European forests oligotrophic species are decreasing in cover, although a decline in species richness is not yet detectable”. Similar results were obtained for lichens by Giordani *et al.* (2014). In this paper, however, we concentrate on N deposition effects on tree nutrition and growth and results will be discussed accordingly.

#### *N deposition, soil and tree nutrition*

N throughfall at the investigated plots approaches – and frequently exceeds - the CLs set to protect vegetation. Inherent to the definitions of CLs, this suggests the potential for an impact. The most frequently reported effects of N deposition are on soil, and include soil acidification

(due to nitrification and leaching of nitrate), leaching of base cations and – in poorly buffered soils - increased toxicity due to Al (Zöttl, 1990; Aber *et al.*, 1998; Bobbink *et al.*, 2003). In general, our plots are well-buffered, with good supplies in base cations: in this context, our data showed a negative relationship between N input, soil BCE and – for broadleaves – soil pH. The impact of leaching of base cations on tree nutrition was supported by the negative relationship between foliar N ratios and soil BCE. The concurrent, positive relationship between N deposition and foliar N ratios is in line with the decrease of foliar base cations – Al ratio, decrease of foliar P and increase of foliar N emerging after observational studies, manipulative experiments and fertilization trials (e.g. Braun *et al.*, 1999; Emmet, 1999; Sardans *et al.*, 2012; Fleischer *et al.* 2013; Lovett *et al.*, 2013). Within our data, these patterns were more pronounced for broadleaves: this was probably related to a combination of internal nutrient cycling (with retranslocation from older needles) and needle longevity (e.g. Wang *et al.*, 2013) that may occur in conifers, and that may help buffering nutrient imbalances.

#### *N deposition and tree growth*

As reported in an earlier study (Ferretti *et al.*, 2003b), growth at our sites was explained for the most part by factors related to site, management and meteorology. The possible role of N-related variables was evaluated starting from this basis. As reported by Oren *et al.* (2001), growth of temperate forests is often limited by nutrient supply, in particular N. Our plots displayed – in general - a rather high supply of base cations, e.g. a situation potentially conducive to a positive response of trees to availability of additional N. We showed that throughfall N had actually a positive effect on growth (with high coefficient in the  $BAI_{rel}$  regression models) and on foliar N:P and N:K, which in turn are also positively related to growth. Although the strong, direct response of  $BAI_{rel}$  to N deposition emerging from Fig. 7 can partly be an artifact due to single-factor regression (see Sutton *et al.*, 2008), it is worth



noting that the role of N deposition remains prominent even when stand and meteorology are taken into account (Table 4). These results confirm previous findings for temperate and boreal forests in central and northern Europe and north America (e.g. Janssens & Luysaert, 2009) by means of manipulative experiments, flux measurements and observational studies.

Tree species and PRGI were also consistently identified as important predictors: in this respect, the N effect reported in Fig. 7 can be perhaps interpreted as the maximal effect, as the single factor regression incorporate, at least in part – also their role. Age, SDI\_adj, management, and ground level ozone were sometimes important, but their estimated role was not always consistent across the various models. In particular, it is worth mentioning that tropospheric ozone was neither always important (and even when important, regression coefficients were low) nor showed a consistent sign among model outputs. Although this is in contrast with results from experiments with juvenile trees (e.g. Wittig *et al.*, 2009) and with other observational studies carried out in Europe (e.g. Braun *et al.*, 1999; Karlsson *et al.*, 2006), it is fully line with previous results obtained in Italy on the same sites (e.g., Bussotti & Ferretti, 2009).

#### *Implications for C sequestration*

Despite recent concerns (Nabuurs *et al.*, 2013), temperate and boreal forests are net C sink (Pan *et al.*, 2011). There is however considerable uncertainty about the actual role of N on C sequestration (Erisman *et al.*, 2011). According to our results, the C sequestered in the above-ground woody biomass in 2000-2009 amounts to an annual median value of 159 kgC ha<sup>-1</sup> (min: 51; max: 504, according to the plot) per kgN. This figure is in the top end of the range for N effect on NPP reported by de Vries *et al.* (2009) and Erisman *et al.* (2011) and in line with the 220 kgC per kgN estimated after Etzold *et al.* (2014, see Fig. 3b therein) for sites with similar species and age. The apparently high figure can be explained by different

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reasons. On the one hand it may actually arise from an overestimation as the single-factor regression in Fig. 8 may incorporate the contribution of the other significant predictors (de Vries *et al.*, 2008; Sutton *et al.*, 2008) reported in Table 4. In addition, we considered deposition of N-NO<sub>3</sub> and N-NH<sub>4</sub> only, rather than total N deposition. Measurements carried out at the plots where total N data were available, revealed that, on average, N-NO<sub>3</sub> and N-NH<sub>4</sub> amounts to 65% (min: 22%; max: 92%) of total N deposition in throughfall and this has an effect on the final estimation of unit N contribution. When these considerations are taken into account, the estimated C sequestration per KgN after Fig. 8 can be interpreted as the maximal value.

On the other hand, some facts are worth mentioning. Firstly, higher deposition rates (>20 kgN ha<sup>-1</sup> yr<sup>-1</sup>) were recorded at younger plots (<70 yrs. old) in transitory crops and stored coppices, and it is acknowledged that, “in young accruing forests, the response of primary productivity to added nitrogen is particularly high” (R. Oren, quoted in Janssens & Luyssaert, 2009).

Secondly, the most responsive plots in terms of both  $BAI_{rel}$  and C sequestration (EMI1, LOM3, PIE1) were actually so also in terms of foliar N:P (Fig. 6), therefore suggesting an actual, substantial response of tree nutrition to N-deposition. Thirdly, besides young plots, our dataset also include mature forests (>150 yrs. old) where management operations ceased since decades. This has led to an accumulation of standing biomass (i.e., up to 784 Mg ha<sup>-1</sup> in the beech high forest TOS3 at year 2000) and – consequently – high absolute values of annual increment (6.4 Mg ha<sup>-1</sup> yr<sup>-1</sup>) even with low relative biomass growth rates (0.8%). This may have contributed to the high C sequestration rate per unit N reported. It is worth noting that mature and old-growth forests have been suggested to maintain their C sequestration potential (Luyssaert *et al.*, 2008).

Depending on the ultimate fate of the deposited N (e.g., leached in the groundwater or stored in the wood), the C uptake per unit N can vary a lot (Janssens & Luyssaert, 2009). Even when

the lower range of our estimates is considered (50 kgC per kgN, consistent with the conservative values reported by de Vries *et al.*, 2008), when scaled up at national level (above-ground estimated phytomass:  $874 \times 10^6$  Mg, Gasparini & Tabacchi, 2011, ca.  $437 \times 10^6$  Mg in terms of C stock) or southern European level (ca. 3100 Mt of C stock in 2010), it implies a considerable N *bonus* (sensu Janssens & Luyssaert, 2009) for European forests even across their southernmost range. Our results confirm the importance of nutritional status (e.g., Oren *et al.*, 2001), and suggest that future C sequestration should be assessed in relation to the limitations imposed by soil fertility and N availability (Reich & Hobbie, 2013). In the same line, the inherent, different response emerged among tree species and plots, suggests that “predictions of forest responses to elevated N deposition should take into account spatial and temporal variation in tree species composition” (Lovett *et al.*, 2013).

Although positive effect of N deposition on productivity cannot be used as an indicator of N saturation (De Shrijver *et al.*, 2008), this can be a risk under long-term, continuous N input. N saturation implies an open N cycle, with possible loss of base cations, reduced P availability and N leaching (Janssens *et al.*, 2010). The issue of N saturation is not the core target of this paper: it is worth noting, however, that some evidence in this respect was already present in our data, with significant reduction in soil BCE and increase of foliar N:P at increasing deposition levels. Nitrogen release from forest soil (a symptom of soil N saturation, e.g. Dise & Wright, 1995) was already detected in previous studies carried out at some of our plots (Mosello *et al.*, 2002; Rogora *et al.*, 2008). A year-round release of  $\text{NO}_3^-$  from the watersheds of five of the plots considered in this paper, and high level of N saturation according to the Stoddard & Traaen (1995) criteria were reported for the plot PIE1. Interestingly, however, both patterns (positive growth response to N and signs of N saturation) exist, for example at PIE1. This may suggest that (i) N saturation was quite recent and (ii) that some time lag exists for effects, a time period in which the investigated forest ecosystems continue to respond

positively in terms of growth to N input while showing symptoms of N saturation. All in all, however, the above may suggest that N saturation is already underway, at least at certain sites: under such a circumstance even the observed, still positive N effect on tree productivity may become no longer sustainable in the future.

### *Synthesis*

Measured N deposition was found to exceed CLs at several of our monitoring sites, to affect soil nutrients (reduction of BCE and pH), to augment foliar N-ratios (particularly N:P and N:K) and to promote growth and C sequestration. Coupled with findings from other studies documenting instances of N saturation at some of our sites (e.g. PIE1), our results provide clear evidence of an impact of N deposition on temperate forests in southern Europe.

Implications may concern ecosystem chemistry (depletion of nutrients, nitrification of soil water, run-off and stream water), diversity (shift in species, changes in species coverage), health (augmented susceptibility to pests and pathogens) and productivity (positive effect at present, but quite uncertain in the long-term). In this perspective, predictions about the future ability of forest to sequester C and thus mitigate climate change are only possible with great caution.

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Table 1. Main forest and site characteristics of the CONECOFOR plots considered in the study and available dataserieS. SM: site and management (species, age, origin and management, SDI\_adj); ME: meteorological variables (total Pr, PRGI; WI/SI index; T\_max; Pr EPT<sup>-1</sup>); O: ozone; SO: soil chemistry (pH, C:N, BCE); F: foliar chemistry (N:P, N:Ca, N:Mg, N:K); H: tree health data (F\_damage, F>25); DEP: N deposition data; G: BAI 2000-2009. See also Table 2. (a) missing soil BCE data; (b) only the *Quercus cerris* population was considered in the study; (c). Basal Area data refer to 2005, no previous measurement was available.

Plot Code	Latitude	Longitude	Elevation (m a.s.l.)	Main tree species	Age (years)	BA (m <sup>2</sup> ha <sup>-1</sup> )	Management	Available dataserieS
ABR1	415051	133523	1500	<i>Fagus sylvatica</i>	115	42.3	High forest	SM, ME, O, SO, F, H, DEP, G
CAL1	382538	161047	1100	<i>Fagus sylvatica</i>	115	40.8	High forest	SM, ME, O, SO, F, H, DEP, G
CAM1 (a)	402558	152610	1175	<i>Fagus sylvatica</i>	105	49.0	High forest	SM, ME, O, SO, F, H, DEP, G
EMI1(b)	444306	101213	200	<i>Q. cerris</i> and <i>Q. petraea</i>	50	10.5	Stored coppice	SM, ME, O, SO, F, H, DEP, G
EMI2	440631	110700	975	<i>Fagus sylvatica</i>	50	36.5	Stored coppice	SM, ME, O, SO, F, H, DEP, G
FRI1	454734	130715	6	<i>Q. robur</i> and <i>Carpinus</i>	50	26.6	Transitory crop	SM, O, F, H, DEP

				<i>betulus</i>				
FRI2	462928	133536	820	<i>Picea abies</i>	105	55.7	High forest	SM, ME, O, SO, F, H, DEP, G
LAZ1	424950	130010	690	<i>Quercus cerris</i>	40	26.5	Stored coppice	SM, ME, O, SO, F, H, DEP, G
LOM1	461416	93316	1190	<i>Picea abies</i>	85	43.6	High forest	SM, ME, O, SO, F, H, DEP, G
MAR1	431738	130424	775	<i>Quercus cerris</i>	40	38.6	Stored coppice	SM, O, SO, F, H, DEP, G
PIE1	454055	80402	1150	<i>Fagus sylvatica</i>	65	30.7	Transitory crop	SM, ME, O, SO, F, H, DEP, G
PUG1	414910	155900	800	<i>Fagus sylvatica</i>	80	45.3	High forest	SM, O, SO, F, H, DEP, G
SAR1	392056	83408	700	<i>Quercus ilex</i>	55	41.4	Stored coppice	SM, ME, O, SO, F, H, DEP, G
SIC1	375432	132415	940	<i>Quercus cerris</i>	55	26.0	Transitory crop	SM, O, SO, H, G
TOS1	433034	102619	150	<i>Quercus ilex</i>	55	30.1	Stored coppice	SM, O, SO, F, H, DEP, G
TRE1	462137	112942	1775	<i>Picea abies</i>	195	55.7	High forest	SM, ME, O, SO, F, H, DEP, G
VAL1	454326	65555	1740	<i>Picea abies</i>	145	52.3	High forest	SM, ME, O, SO, F, H, DEP, G

VEN1	460326	120156	1100	<i>Fagus sylvatica</i>	125	36.2	High forest	SM, ME, O, SO, F, H, DEP, G
LAZ2	415051	133523	190	<i>Quercus ilex</i>	55	39.1	Stored coppice	SM, O, DEP
LOM2	455726	100753	1150	<i>Picea abies</i>	70	56.8	High forest	SM, O, F, H, DEP
LOM3	455441	93017	1250	<i>Fagus sylvatica</i>	50	25.2	Transitory crop	SM, ME, O, F, H, DEP, G
TOS2	425212	104634	30	<i>Quercus ilex</i>	60	35.7	Stored coppice	SM, ME, O, F, H, DEP, G
TOS3	434418	113422	1170	<i>Fagus sylvatica</i>	150	54.2	High forest	SM, ME, O, F, H, DEP, G
BOL1 (c)	463516	112604	1740	<i>Picea abies</i>	175	33.6	High forest	SM, O, F, H, DEP
LIG1 (c)	442410	92730	1290	<i>Fagus sylvatica</i>	105	28.8	High forest	SM, O, F, H, DEP
PIE2 (c)	453129	84234	135	<i>Q. robur,</i> <i>C. betulus</i>	35	23.5	High forest	SM, DEP

Table 2. Response and predictor variables selected for the various PLS regression models.

Variable	N of plots with available measurements	Description	PLS regression model
<b>Response</b>			
Relative Basal Area Increment 2000-2009	20	Ratio (%) between BAI 2000- 2009 and the generating BA at year 2000	All models
<b>Predictors</b>			
<i>Stand and management</i>			
Species	26	Picea abies, Fagus sylvatica, Quercus ilex, Quercus sp. (deciduous)	Reference
Age	26	Age in the year 2000	Reference
Origin and management	26	Transitory crop; stored coppice; high forest	Reference
SDI_adj	26	Species adjusted Reineke's SDI	Reference
<i>Atmosphere</i>			
T_max	15	Mean of monthly maximum temperature	Reference
WI/SI	15	Ratio between winter index (WI, values from December of the year t year to February of year t+1) and summer index (SI, values from June to August of t year)	Reference
Pr	15	Annual precipitation amount	Reference
PRGI	15	Percentage of precipitation observed during the growing season	Reference
P EPT-1	15	Water surplus (or deficit) based on the ratio of Pr to potential evapotranspiration (ETP)	Reference
Ozone	25	Mean April-September ozone concentration	Reference
<i>Soil</i>			
Soil_pH	17	pH (CaCl <sub>2</sub> ) of the mineral top soil	Reference + soil
BCE	15	Exchangeable Basic Cations in mineral top soil	Reference + soil
C:N	17	C:N ratio of mineral top soil	Reference + soil
<i>Tree foliar nutrition</i>			

<i>Tree health</i>	N:P	23	N ratios to P, Ca, Mg, K in the tree foliage (current year for evergreen species)	Reference + foliar
	N:Ca	23		Reference + foliar
	N:Mg	23		Reference + foliar
	N:K	23		Reference + foliar
	F>25	24	Frequency of trees with crown transparency >25%	Reference + health
	F_damage	24	Frequency of trees with symptoms of damage (any type)	Reference + health
<i>N deposition</i>				
	N_throughfall	25	Throughfall deposition of N (N-NO <sub>3</sub> +N-NH <sub>4</sub> )	Reference + N deposition

Table 3. Model performance. Number of plots, number of Latent Variables (LVs), variance explained on the set of predictors (Xvar, %), variance explained on the response (Yvar, %), root mean square error in cross validation (RMSEC).

Models					
	Reference	Reference + soil	Reference + foliar	Reference + tree health	Reference + deposition
<i>All available plots</i>					
Plots, n	20	19	20	20	18
LVs, n	6	7	6	7	7
Xvar, %	91.75	91.69	85.82	88.56	92.74
Yvar, %	78.2	83.88	88.95	91.53	93.53
RMSEC	2.98	2.56	2.12	1.858	1.5
<i>Only common plots</i>					
Plots, n	11	11	11	11	11
LVs, n	4	2	3	4	5
Xvar, %	80.05	46.15	59.06	78.20	87.55
Yvar, %	86.99	73.20	89.03	89.17	92.90
RMSEC	0.96	1.26	1.23	1.16	0.85

Table 4. Regression coefficients for each variable in the five PLS regression models. Coefficients for important variables (VIP>1) within each model and plot aggregation are in bold.

Variable	<i>All plots with available data</i>					<i>Only common plots</i>				
	Reference	+Soil	+Foliar	+Health	+ N deposition	Reference	+Soil	+Foliar	+Health	+ N deposition
	n=20	n=19	n=20	n=20	n=18	n=11	n=11	n=11	n=11	n=11
Age	-0.183	<sup>-</sup> 0.161	-0.261	-0.377	-0.184	<b>-0.198</b>	<sup>-</sup> <b>0.128</b>	<b>-0.191</b>	<b>-0.168</b>	<b>-0.165</b>
Management_HF	0.100	0.181	0.142	0.072	0.128	0.119	0.026	<b>0.130</b>	0.101	0.224
Management_TC	<b>-0.571</b>	<sup>-</sup> <b>0.547</b>	<b>-0.461</b>	-0.561	<b>-0.513</b>	-0.188	<sup>-</sup> 0.093	-0.213	-0.211	-0.213
Management_SC	0.323	0.226	0.196	0.345	0.207	-0.011	0.028	-0.007	0.022	-0.104
Species_Conifers	0.426	0.152	<b>0.494</b>	0.072	0.447	0.066	0.082	<b>0.151</b>	0.049	0.064
Species_Deciduous broad.	-0.081	<sup>-</sup> 0.006	-0.197	0.213	-0.230	0.203	0.099	0.079	0.218	0.113
Species_Evergreen broad.	<b>-0.369</b>	<sup>-</sup> <b>0.161</b>	<b>-0.289</b>	<b>-0.334</b>	<b>-0.247</b>	<b>-0.442</b>	<sup>-</sup> <b>0.293</b>	<b>-0.367</b>	<b>-0.441</b>	<b>-0.288</b>
SDIadj	<b>-0.846</b>	<sup>-</sup> <b>0.795</b>	-0.277	<b>-0.578</b>	<b>-0.566</b>	0.033	<sup>-</sup> 0.015	0.095	0.001	0.100
Tmax	-0.503	<sup>-</sup> <b>0.850</b>	-0.366	-0.566	-0.521	-0.019	0.018	-0.011	-0.020	-0.040
WI:SI	0.516	<b>0.850</b>	0.328	0.224	0.490	<b>0.266</b>	<b>0.124</b>	0.215	<b>0.192</b>	0.313

PR	-0.441	-	-0.293	-0.300	-0.249	-0.247	-	-0.226	-0.249	-0.238
PRGI	<b>0.520</b>	<b>0.348</b>	<b>0.407</b>	<b>0.448</b>	<b>0.215</b>	<b>0.492</b>	<b>0.314</b>	<b>0.363</b>	<b>0.476</b>	<b>0.308</b>
PR-EPT	-0.424	-	-0.257	-0.230	-0.300	-0.265	-	-0.228	-0.252	-0.254
O3	<b>0.026</b>	-	<b>0.003</b>	<b>0.082</b>	<b>0.163</b>	-0.143	-	-0.184	-0.131	-0.077
Soil_pH		-					-			
Soil_C:N		0.235					0.176			
Soil_BCE		-					-			
Foliar_N:P		<b>0.077</b>	<b>0.443</b>				<b>0.203</b>	<b>0.228</b>		
Foliar_N:Ca			0.138					0.033		
Foliar_N:Mg			-0.127					-0.006		
Foliar_N:K			<b>0.328</b>					<b>0.159</b>		
F_damage				<b>-0.466</b>					-0.135	
F>25				<b>0.399</b>					0.176	
N throughfall					<b>0.680</b>					<b>0.436</b>



## Figures, Captions

Figure 1. Study concept, with pathways of possible impact of N deposition and investigations carried out. Pathways of possible impact on soil and tree nutrition (thick lines and numbers) and investigated relationships between different categories of predictors and tree growth (thin lines, letters) once the set other environmental biotic and abiotic factors (species, stand, meteorology and air pollutants - represented by the dashed line enwrapping the tree diagram) are taken into account. [1] Impact on soil chemistry: this was studied by investigating the relationship between deposition and selected soil variables (pH, C:N, BCE). [2] Soil-mediated and [3] direct impact on tree nutrition: they were investigated by studying the relationship between soil-, N-deposition and N-related foliar chemistry. The possible role of N deposition as a source of N for trees was studied by comparing N-NO<sub>3</sub> and N-NH<sub>4</sub> open-field vs. throughfall deposition. The role (size, significance, direction) of N-related variables of soil [a], foliar [b], tree health [c] and deposition [d] and on tree growth was also studied by step-wise addition of the concerned variables to a reference statistical model.

Figure 2. Actual deposition load and estimated Critical Loads at the monitoring sites. Measured average annual throughfall 2000-2009 is plotted against the Critical Load estimated by the Simple Mass Balance. The dotted line is  $x=y$ . The solid line represents the regression. FS: *Fagus sylvatica*; PA: *Picea abies*; QD: deciduous oaks; QI: *Quercus ilex*.

Figure 3. pH (a), C:N (b) and BCE (c) of the mineral topsoil plotted against actual N deposition. Soil data are those obtained after the 1995-1996 survey. Deposition data are mean annual values 2000-2009. Regressions represents always the best fit for the given dataset. Continuous line: all species; dashed line: broadleaves only. FS: *Fagus sylvatica*; PA: *Picea abies*; QD: deciduous oaks; QI: *Quercus ilex*.

Figure 4. Foliar N:P (a), N:Ca (b), N:Mg (c) and N:K (d) plotted against the BCE of the mineral topsoil. Foliar data are mean values after sampling carried out at years 2001, 2003, 2005, 2007, 2009. Soil data are those obtained after the 1995-1996 survey. Continuous line: all species; dashed line: broadleaves only. FS: *Fagus sylvatica*; PA: *Picea abies*; QD: deciduous oaks; QI: *Quercus ilex*.

Figure 5. Foliar N:P (a), N:Ca (b), N:Mg (c) and N:K (d) plotted against measured throughfall deposition. Foliar data are mean values after sampling carried out at years 2001, 2003, 2005, 2007, 2009. Deposition data are mean annual values 2000-2009. Continuous line: all species; dashed line: broadleaves only. FS: *Fagus sylvatica*; PA: *Picea abies*; QD: deciduous oak; QI: *Quercus ilex*.

Figure 6. Plot-wise comparison of openfield and throughfall N-NH<sub>3</sub> (a) and N-NH<sub>4</sub> (b). Mean annual 2000-2009 data. The solid line is  $y=x$ . FS: *Fagus sylvatica*; PA: *Picea abies*; QD: deciduous oaks; QI: *Quercus ilex*.

Figure 7. Annual BAI<sub>rel</sub> (2000-2009) plotted against annual N deposition over the same time window. Solid line: all species; dashed line: broadleaves only. FS: *Fagus sylvatica*; PA: *Picea abies*; QD: deciduous oak (only *Quercus cerris* in this diagram); QI: *Quercus ilex*.

Figure 8. Annual estimated C stocked (2000-2009) plotted against annual N deposition over the same time window. Solid line: all species; dashed line: broadleaves only. FS: *Fagus sylvatica*; PA: *Picea abies*; QD: deciduous oak (only *Quercus cerris* in this diagram); QI: *Quercus ilex*.

















