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# Joining empirical and modelling approaches to estimate dry deposition of nitrogen in Mediterranean forests☆

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ABSTRACT

In Mediterranean areas, dry deposition is a major component of the total atmospheric N input to natural habitats, particularly to forest ecosystems. An innovative approach, combining the empirical inferential method (EIM) for surface deposition of $NO_3^-$ and $NH_4^+$ with stomatal uptake of $NH_3$, $HNO_3$ and $NO_2$ derived from the $DO_3SE$ (Deposition of Ozone and Stomatal Exchange) model, was used to estimate total dry deposition of inorganic N air pollutants in four holm oak forests under Mediterranean conditions in Spain. The estimated total deposition varied among the sites and matched the geographical patterns previously found in model estimates: higher deposition was determined at the northern site (28.9 kg N $ha^{-1}$ $year^{-1}$) and at the northeastern sites (17.8 and 12.5 kg N $ha^{-1}$ $year^{-1}$) than at the central-Spain site (9.4 kg N $ha^{-1}$ $year^{-1}$). On average, the estimated dry deposition of atmospheric N represented 77% ± 2% of the total deposition of N, of which surface deposition of gaseous and particulate atmospheric N averaged 10.0 ± 2.9 kg N $ha^{-1}$ $year^{-1}$ for the four sites (58% of the total deposition), and stomatal deposition of N gases averaged 3.3 ± 0.8 kg N $ha^{-1}$ $year^{-1}$ (19% of the total deposition). Deposition of atmospheric inorganic N was dominated by the surface deposition of oxidized N in all the forests (means of 54% and 42% of the dry and total deposition, respectively). The relative contribution of $NO_2$ to dry deposition averaged from 19% in the peri-urban forests to 11% in the most natural site. During the monitoring period, the empirical critical loads provisionally proposed for ecosystem protection (10 −20 kg N $ha^{-1}$ $year^{-1}$) was exceeded in three of the four studied forests.



## 1. Introduction

Atmospheric deposition of nitrogen (N) in the European Mediterranean region, and particularly in Spain, is lower than in central Europe (Lorenz and Becher, 2012; Nyíri and Gauss, 2010). However, N deposition has been identified as a potential threat for some valuable Spanish ecosystems, such as alpine grasslands and heathlands or mountainous forest of holm oak (*Quercus ilex*) in NE Spain (García-Gómez et al., 2014, 2017). In Mediterranean areas, dry deposition of atmospheric N compounds may play a major role in the total N deposition to natural vegetation, particularly to forest ecosystems (Fenn et al., 2009).

Dry deposition of N on vegetation can occur either via surface or stomatal deposition. Surface deposition is referred to gaseous and particulate N species that are adsorbed by the vegetation surfaces, while stomatal deposition represents the gaseous species absorbed through stomatal pores followed by their dissolution in the apoplast. Many factors can potentially regulate the deposition rates onto vegetation surfaces (Fowler et al., 2009), with highly reactive and water soluble species, like $HNO_3$ and $NH_3$, being readily

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deposited to leaf surfaces and plant canopies (Hanson and Lindberg, 1991). On the other hand, the flux of nitrogen dioxide ($NO_2$) to vegetation is mainly controlled by stomatal uptake (Saxe, 1986; Raivonen et al., 2009). Stomatal deposition of gaseous N pollutants is controlled by the degree of stomatal aperture and by gas concentrations in the sub-stomatal cavity (Massad et al., 2010). This concentration is particularly important for $NH_3$ flux estimation, since plants can act as either a sink or as a source of $NH_3$, depending on the relative concentration gradient between the near atmosphere and the stomatal cavity, defining a stomatal compensation point (Massad et al., 2010; Raivonen et al., 2009).

Since dry deposition cannot be readily measured, several approximations exist to estimate this input into the ecosystems. The inferential methods are often considered the best for assessing long term dry deposition on wide scales, despite the presence of some uncertainties (Vet et al., 2014; Wesely and Hicks, 2000). These methods calculates dry-deposition fluxes to the ecosystems based on measurements of atmospheric concentrations of the pollutants of interest and their velocity deposition rates, which are mainly driven by meteorological conditions and the physical characteristics of surfaces (Fenn et al., 2009). These deposition rates are commonly estimated by using a multiple resistance analogy approach in which the deposition process is considered as a series of resistances, by analogy with an electrical circuit (Monteith and Unsworth, 2008). Deposition rates can be also obtained from micrometeorological studies (Fowler et al., 2009), which are complex and expensive systems, and, therefore, difficult to replicate in different sites.

Mixed methodologies using modelled and empirical approximations might improve the estimation of the dry atmospheric input, particularly in arid and semi-arid ecosystems (Cook et al., 2018). The empirical inferential method (EIM), inserting empirical results from branch-washing experiments into inferential modelling methodology, was recently proposed by Bytnerowicz et al. (2015) for evaluation of N deposition in Mediterranean-type ecosystems. It involves the use of values of surface deposition rates experimentally estimated at branch level (conductance), instead that at canopy level (deposition velocity). Surface conductance values can be more easily obtained from branch-washing experiments than from micrometeorological studies, and produce site-specific values. Branch-washing techniques use deionized water to rinse the dry-deposited ions from the vegetation surfaces, although cannot distinguish the sources of the surface-deposited $NO_3^-$ ($HNO_3$ or particulate $NO_3^-$), nor $NH_4^+$ ($NH_3$ or particulate $NH_4^+$) (Dasch, 1989). Besides, it may underestimate deposition to foliar surfaces due to the transcuticular uptake, translocation processes of the deposited chemical species and volatilization of the deposited compounds (Garten and Hanson, 1990). However, the branch-rinsing method may be very useful for the development of estimation models for forest with different environmental conditions and pollution exposures. Regarding stomatal deposition of reactive N gases, in the EIM approximation it has been estimated so far from measurements of stomatal conductance values for $H_2O$ vapour of several species, temporally and spatially extrapolated regardless the environmental conditions. In this work, we propose an improvement through estimating stomatal conductance values depending on the environmental conditions using a stomatal conductance model (CLRTAP, 2004) parameterized based on measurements performed in the studied species, i.e. holm oak (Alonso et al., 2008).

The main objectives of the study were: (1) to estimate the dry deposition of atmospheric N in Spanish holm oak forests by means of the empirical inferential method, (2) to complement the EIM methodology with the modelling of stomatal conductance values using a standard modelling approach, (3) to compare surface flux and conductance values of pollutants to living and lyophilized branches of holm oak, and (4) to estimate and describe the total input of atmospheric inorganic N to these forests.

## 2. Material and methods

### 2.1. Study sites

Four monitoring plots were located in four holm oak (*Q. ilex*) forests located in three areas of the Iberian Peninsula characterized by different climatic and soil conditions, as well as canopy structure and distance to main sources of pollutants (Table 1). Tres Cantos (TC) site is located in the central region of the Peninsula, near Madrid city (9 km), with a Mediterranean semi-arid climate. The historical management of this forest has produced a moderately open woodland (72% of tree cover). Can Balasc (CB) and La Castanya (LC) sites are located in the North-eastern Spain, with a sub-humid Mediterranean climate. Whereas CB is close to Barcelona city (4 km), LC is far from this city (40 km) and relatively sheltered from

**Table 1**
Characterization of the study sites.

| Site code | CB | TC | CA | LC |
|---|---|---|---|---|
| Type of location | Peri-urban | Peri-urban | Peri-urban | Rural |
| Longitude | 2° 04′ 54″ E | 3° 43′ 59″ O | 1° 38′ 40″ O | 2° 21′ 29″ E |
| Latitude | 41° 25′ 47″ N | 40° 35′ 17″ N | 42° 39′ 13″ N | 41° 46′ 47″ N |
| Altitude (m) | 255 | 705 | 592 | 696 |
| Leaf area index ($m^2\ m^{-2}$) | 3.3 | 3.1 | 5.3 | 6.1 |
| Tree density (number of trees $ha^{-1}$) | 1429 | 491 | 1760 | 2571 |
| Mean diameter at breast height (cm) | 13 | 41[d] | 16 | 13 |
| Mean annual temperature (°C) [a] | 15.2 | 14.6 | 12.3 | 13.7 |
| Mean annual rainfall (mm $y^{-1}$) [a] | 652 | 348 | 681 | 812 |
| Mean annual relative humidity (%) [a] | 71.3 | 54.6 | 73.7 | 70.3 |
| Mean annual wind speed (%) [a] | 0.8 | 1.3 | 6.2 | 0.9 |
| Distance to the nearest big city (km) | 4 | 9 | 15 | 40 |
| Population of the nearest big city [b] | 1.6 | 3.2 | 0.2 | 1.6 |
| Agricultural land-use cover [c] | 23% | 21% | 62% | 23% |

[a] Mean values calculated for the study period February 2011–February 2013;
[b] Million inhabitants;
[c] From the CORINE Land Cover 2006 (http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster-3) using a buffer of 25 km radius around the sampling sites;
[d] Measured in the dominant cohort.

the surrounding lowland sources of atmospheric pollutants (Hereter and Sánchez, 1999). The Carrascal (CA) site is located in the North, in an agricultural area near Pamplona city (15 km), in a region with Mediterranean humid climate.

### 2.2. Field measurements

Duplicated passive samplers were used to measure two-week averages of atmospheric concentrations of $NH_3$ and $NO_2$ (Radiello®) and $HNO_3$ (badge-type samplers; Bytnerowicz et al., 2005) during 2011–2013. Collection and laboratory analyses were performed according to the Radiello's specifications (Fondazione Salvatore Maugeri, 2006) and Bytnerowicz et al. (2005). For further details, see García-Gómez et al. (2016).

Bulk deposition (BD) was collected on a weekly basis by 2–4 high-density polyethylene funnels (NILU, Kjeller, Norway) attached to collection bottles. The sampling procedure, storage, analysis by ion chromatography (Dionex, Sunnyvale, USA) and ulterior quality control of the analytical results of these samples were performed following the recommendations of the ICP Forests Manual (Clarke et al., 2010), described in detail by Izquieta-Rojano et al. (2016). Simultaneously, N wet deposition (WD) was measured by wet-only collectors (TE® 78–100) in LC and TC. The WD samples were collected on the same temporal basis as BD and applying the same storage procedure, analysis and quality control. Meteorological variables were monitored in CB, TC and LC sites, and data from the closest meteorological station were collected for the CA site.

### 2.3. Branch-washing experiment

Washing of branches from 10 selected *Q. ilex* trees for every site were performed after rainless periods (from 3 to 5 periods, depending on the site; Table 2) longer than 7 days (mean of 21.5 days). One branch of about 20 cm length was collected from the upper part of the canopy of each selected tree. All branches in each site were selected to represent the different geographic orientations and were protruding enough from the canopy tree to collect atmospheric compounds dry-deposited onto the canopy. After cutting, branch edges were sealed with Parafilm® M (Neenah, Wisconsin, USA) and were transported to the laboratory in sealed plastic bags, where they were washed for 3 min inside the same plastic bag filled with 200 ml of distilled water. Care was taken to keep the branch edge outside the bag while washing. Two empty plastic bags were washed for blank controls. Afterwards, projected leaf area (PLA) of the dried branches was measured using either LI-3100 area-meter (Li-Cor Inc., Lincoln, NE, USA) or image analysis of digital photography. Stem areas were measured for one period and the resultant stem-/leaf area ratio was extrapolated to all measurements to come close to the actual exposure area of the branches. The calculated surfaces areas ranged 175.3–866.3 $cm^2$ (mean of 314.5 $cm^2$) across all sites and periods.

Additionally, in the TC site, an experiment with lyophilized branches was performed following Alonso et al. (2005). For every experimental period, branches from the same ten selected trees were collected and thoroughly washed before lyophilisation. Four 10 cm-length lyophilized branches (LB), with surface areas between 18.1 and 104.2 $cm^2$, were attached to the top of a 5-m pole in three different locations inside the forest from 7 to 14 days during the exposure periods of the above-mentioned living branches (hereinafter, natural branches; NB). Care was taken to place the poles in open-canopy spots to avoid direct contact of LB with the surrounding trees. After exposure, the LB were transported to the laboratory in sealed plastic bags, where they were washed for 3 min inside the same plastic bag with 100 ml of distilled water. Non-exposed LB were kept in sealed flasks after lyophilisation and washed at the same time as the collected ones for control purposes.

Washing efficiency was tested performing three sequential washes in two sets of three NB and three LB from two different

**Table 2**
Fluxes of deposited nitrogen into natural vegetation (NB) from the branch-washing experiment and averaged concentration of ammonia and nitric acid vapour for each exposure period in the three studied regions (sites in brackets).

| Region [a] | Start Date | End Date | $F_{NH_3+NH_4^+}$ (μmol $m^{-2}$ $day^{-1}$) | | | $F_{HNO_3+NO_3^-}$ (μmol $m^{-2}$ $day^{-1}$) | | | $NH_3$ (μmol $m^{-3}$) | $HNO_3$ (μmol $m^{-3}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | N [b] | MEAN | CV | N | MEAN | CV | | |
| **Central Spain** (TC) | 07/06/11 | 28/06/11 | 10 | 10.2 | 47% | 10 | 34.8 | 36% | 0.07 | 0.02 |
| | 16/08/11 | 18/10/11 | 10 | 6.1 | 40% | 10 | 27.5 | 42% | 0.05 | 0.03 |
| | 10/12/11 | 11/01/12 | 10 | 4.8 | 55% | 10 | 4.3 | 64% | 0.01 | 0.01 |
| | 20/05/12 | 05/07/12 | 10 | 9.8 | 60% | 10 | 26.3 | 60% | 0.07 | 0.05 |
| | 04/04/13 | 24/04/13 | 9 | 9.1 | 37% | 9 | 18.8 | 29% | 0.03 | 0.01 |
| **Northeast** (CB, LC) | 13/06/11 | 29/06/11 [c] | 9–10 | 4.1 | 123% | 9–10 | 10.4 | 49% | 0.03 | 0.03 |
| | 25/09/11 | 05/10/11 | 7 | 13.1 | 55% | 7 | 17.1 | 45% | 0.05 | 0.02 |
| | 01/02/12 | 22/02/12 [c] | 10–10 | 14.1 | 38% | 10–10 | 8.0 | 34% | 0.06 | 0.01 |
| | 06/08/12 | 24/08/12 [c] | 2–6 | 23.1 | 120% | 9–5 | 11.4 | 84% | 0.07 | 0.17 |
| **Northern Spain** (CA) | 16/09/11 | 30/09/11 | 10 | 2.9 | 94% | 10 | 48.8 | 37% | 0.13 | 0.02 |
| | 15/02/12 | 28/02/12 | 10 | 25.3 | 29% | 10 | 47.5 | 26% | 0.13 | 0.02 |
| | 20/06/12 | 28/06/12 | 10 | 37.4 | 30% | 10 | 20.1 | 28% | 0.18 | 0.06 |
| | 28/08/12 | 11/09/12 | 6 | 39.5 | 26% | 10 | 50.4 | 38% | 0.19 | 0.07 |
| **TOTAL** | | **N** | **MEAN** | **CV** [d] | **CV** [e] | **MEAN** | **CV** [d] | **CV** [e] | **MEAN** | **MEAN** |
| **Central Spain** (TC) | | 5 | 8.0 | 30% | 48% | 22.3 | 52% | 46% | 0.05 | 0.02 |
| **NE** (CB, LC) | | 4 | 13.6 | 57% | 84% | 11.7 | 33% | 53% | 0.05 | 0.06 |
| **Northern Spain** (CA) | | 4 | 26.3 | 64% | 45% | 41.7 | 35% | 32% | 0.16 | 0.04 |
| | | **N** | **MEAN** | **CV** [f] | **CV** [e] | **MEAN** | **CV** [f] | **CV** [e] | **MEAN** | **MEAN** |
| **ALL** | | 3 | 16.0 | 59% | 59% | 25.3 | 60% | 44% | 0.09 | 0.04 |

[a] Site/s location;
[b] Number of washed branches used in flux calculation;
[c] The results shown are the mean of two simultaneous periods and both N are given;
[d] CV of the regional mean, representing temporal variability;
[e] Regional mean of the CVs, representing mean intra-site variability;
[f] CV of the all-sites mean, representing inter-regional variability.

periods in TC. The third-washing produced concentrations of inorganic N close to detection limits indicating a total accumulated removal of surface N. The percentage of the recovered N in the first washing with relation to N accumulated in three consecutive washing was used as a correction factor for $NH_4^+$ and $NO_3^-$ recovery: for NB those were 73% and 89%, respectively; for LB those were 79% and 99%. The washing solutions were analysed by ion chromatography (Dionex, Sunnyvale, CA, USA).

Nitrogen surface deposition flux ($F$) to branches was calculated as follow. For each branch, the concentration of $NO_3^-$ and $NH_4^+$ was multiplied by the washing volume and the background loads measured in the empty bags and unexposed LB were subtracted. These N load values were divided by the number of days of the exposure periods (days after the last precipitation event in the case of NB) and by the projected leaf area of the exposed branches to obtain $F$ (μmol N $m^{-2}$ $day^{-1}$). In the present study, the leaching of inorganic N from the NB was considered negligible, as seen in previous works with holm oak (Rodrigo and Àvila, 2002).

### 2.4. Estimation of dry deposition of atmospheric N by the empirical inferential method (EIM)

Dry deposition was estimated as the sum of surface deposition and stomatal uptake. Following Bytnerowicz et al. (2015), for surface deposition estimates, empirical surface deposition conductance ($K$, cm $s^{-1}$) for $NH_4^+$ (resulting from deposition of $NH_3$ and particulate $NH_4^+$) and for $NO_3^-$ (resulting from deposition of $HNO_3$ and particulate $NO_3^-$) were calculated for every period and site of the branch-washing experiment, according to the formula:

$$K_{NH_4^+} = F_{NH_3+NH_4^+} \Big/ C_{NH_3} \quad (1)$$

$$K_{NO_3^-} = F_{HNO_3+NO_3^-} \Big/ C_{HNO_3} \quad (2)$$

where:

$F_{NH_3+NH_4^+}$ : mean surface flux of $NH_3 + NH_4^+$ to branches (μmol $m^{-2}$ $day^{-1}$)
$C_{NH_3}$ : mean atmospheric concentration of $NH_3$ during the exposure period (μmol $m^{-3}$)
$F_{HNO_3+NO_3^-}$ : mean surface flux of $HNO_3 + NO_3^-$ to branches (μmol $m^{-2}$ $day^{-1}$)
$C_{HNO_3}$ : mean atmospheric concentration of $HNO_3$ during the exposure period (μmol $m^{-3}$)

Surface deposition conductance values for $NH_4^+$ and $NO_3^-$ were calculated based only on ambient $NH_3$ and $HNO_3$ following by Bytnerowicz et al. (2015). This approach was justified in previous studies in Mediterranean forests because $NH_3$ and $HNO_3$ are expected to dominate the surface deposition of $NH_4^+$ and $NO_3^-$ over the particulate forms (Bytnerowicz and Fenn, 1996). To calculate $K$, the values for $F$ and $C$ from the branch-washing experiment were matched individually for each exposure period and site. In the case of LC and CB in NE Spain the values of both variables were averaged when the exposure periods were coincident, following regional representativeness purposes. Therefore, 4 to 5 different exposure-period values for each region (northern, central and north-eastern) were used in the analysis (Table 2). Estimates of the surface deposition conductance were calculated separately for LB and NB in TC. Apart from calculating the mean regional $K$, a regression between the $F$ and $C$ values (dependent and explanatory variables, respectively) was performed, since the value of the slope ($F/C$) would be equivalent to the $K$ value if the intercept of $Y_{axis}$ is set to zero.

Surface dry deposition ($DD_{surf}$) of both reduced and oxidized forms of N was calculated for the rainless days (in this study, days with rain <0.5 mm) according to the formula:

$$DD_{surf} = C \times LAI \times K \quad (3)$$

where:

C: atmospheric concentration of $NH_3$ or $HNO_3$ (μg $m^{-3}$)
LAI: leaf area index ($m^2$ $m^{-2}$)
K: surface deposition conductance for $NH_4^+$ and $NO_3^-$ (m $day^{-1}$)

Stomatal uptake ($DD_{stom}$) of both reduced and oxidized forms of N gases was calculated according to the formula:

$$DD_{stom} = C \times LAI \times c_s \quad (4)$$

where:

C: atmospheric concentration of $NH_3$, $NO_2$ or $HNO_3$ (μmol $m^{-3}$)
LAI: leaf area index ($m^2$ $m^{-2}$)
$c_s$: stomatal conductance for $NH_3$, $NO_2$ or $HNO_3$ (m $day^{-1}$)

Stomatal conductance values of reactive N gases for forest trees were calculated based on the modelled stomatal conductance to $H_2O$ vapour ($g_s$) using the $DO_3SE$ (Deposition of Ozone and Stomatal Exchange) model (CLRTAP, 2004). The model uses the multiplicative approach defined by Jarvis (1976), modified by Emberson et al. (2000) and parametrized for *Q. ilex* by Alonso et al. (2008):

$$g_s = g_{max} \times f_{light} \times f_{phen} \times \max\{ f_{min}, (f_{temp} \times f_{VPD} \times f_{SWP}) \} \quad (5)$$

Where $g_s$ for a given species is calculated as a function of the maximum $g_s$ value for that particular species ($g_{max}$), modified according to the phenological stage ($f_{phen}$) and prevailing environmental factors that include photosynthetic active radiation ($f_{light}$), temperature ($f_{temp}$), air vapour pressure deficit ($f_{VPD}$) and soil water availability ($f_{SWP}$). $f_{min}$ is the relative minimum stomatal conductance that occurs during daylight hours. In the present work, because of the lack of soil water data, $f_{SWP}$ was set to 1, and considered as partially surrogated by the phenological function ($f_{phen}$). This model was used at an hourly scale and the results were added up daily. Quasilaminar resistance and leaf surface resistance were taken into account in the calculation of the fluxes, according to the Mapping Manual (CLRTAP, 2004). Following Bytnerowicz et al. (2015), the values of stomatal conductance for $H_2O$ vapour ($g_s$) were converted to stomatal conductance for $NH_3$, $NO_2$ and $HNO_3$ ($c_s$) according to Graham's law of molecular diffusion (Massman, 1998) by multiplying $g_s$ by 1.029, 0.626 and 0.534, respectively. Since stomatal fluxes of $NH_3$ are bi-directional, the $DD_{stom}$ i.e. uptake of $NH_3$ is not expected to occur below certain ambient concentration of $NH_3$ (compensation point). The compensation point was estimated on a daily basis using a method based on daylight air temperature and annual deposition of nitrogen (Massad et al., 2010). Absence of stomatal uptake of ammonia was assigned to the days with concentrations lower than the respective daily compensation point following Bytnerowicz et al. (2015).

Measurements of LAI were conducted in every site and their seasonal variation was estimated on a daily basis following the methodology from CLRTAP (2004). The daily concentration of each pollutant was equal to the mean concentration of the corresponding passive-sampling period. These values were used in the calculations of both daily $DD_{surf}$ and $DD_{stom}$, and both values were added up to obtain the total dry deposition (DD) of inorganic N.

**Table 3**
Surface conductance values obtained from the branch-washing experiment using natural branches (NB) for oxidized ($K_{NO_3^-}$) and reduced ($K_{NH_4^+}$) nitrogen.

| Region | N | $K_{NH_4^+}$ (cm s$^{-1}$) | | $K_{NO_3^-}$ (cm s$^{-1}$) | |
|---|---|---|---|---|---|
| | | Mean | SE | Mean | SE |
| **Central Spain** (TC) | 5 | 0.26 | 0.07 | 1.41 | 0.48 |
| **Northeastern** (CB, LC) | 4 | 0.27 | 0.05 | 0.49 | 0.17 |
| **Northern Spain** (CA) | 4 | 0.18 | 0.05 | 1.83 | 0.78 |
| **Mean** | 3 | 0.24 | 0.03 | 1.24 | 0.39 |
| **Regression** | 12 | 0.24 | 0.01 | n.s. | |

n.s.: no significant regression was obtained between recovered nitrate and ambient concentration of nitric acid vapour.

### 2.5. Estimation of total deposition of atmospheric N

Wet deposition from wet-only collectors was estimated by multiplying the N concentration by the collected volume in each period. Where the wet-only collector data were not available, concentration and volume from the BD collector was used instead, and the resulting BD value was corrected by the seasonal ratio wet/bulk deposition (WD/BD) from the nearest monitoring site. The values of the ratios were 1.31 and 1.45 for $NH_4^+$ and 1.60 and 1.51 for $NO_3^-$ in LC and TC, respectively. In CB the ratio WD/BD from LC was applied, while in CA the mean of the WD/BD ratios from the LC and TC sites was used. The estimated WD of N in every site was added up seasonally and annually. Further details of the methodology and results have been published in García-Gómez et al. (2016) and Aguillaume et al. (2017). Total deposition of N was calculated as the sum of WD and DD in each site in the same seasonal- and annual-basis.

### 2.6. Statistical analysis

Regression analysis and Pearson correlation tests between variables, as well as the Student's *t*-test for mean comparison, were performed by using Statistica version 13 (StatSoft, Inc. Tule, OK, USA). When the data were not normal (according to Shapiro-Wilk test and normal probability plots) the Spearman rank order correlation was used instead of Pearson test and the Mann-Whitney *U* test instead of the Student's *t*-test. Alpha level was set at 0.05. The variability of the mean is described in this study either by the coefficient of variation (CV = standard deviation/mean; expressed as percentage) or by the standard error (SE = standard deviation/ $\sqrt{n}$). In this work, seasons were considered as periods of three consecutive months, beginning on 1st January.

## 3. Results

### 3.1. Branch-washing experiment

#### 3.1.1. Natural branches

The flux of N deposited to the living branches ($F_{NH_3+NH_4^+}$ and $F_{HNO_3+NO_3^-}$ in eq. (1) and eq. (2)) during the exposure periods showed an overall mean flux of 16.0 ± 5.4 μmol $NH_4^+$ m$^{-2}$ day$^{-1}$ and 25.3 ± 8.8 μmol $NO_3^-$ m$^{-2}$ day$^{-1}$ (Table 2). In general terms, $F_{NH_3+NH_4^+}$ and $F_{HNO_3+NO_3^-}$ showed a slightly larger inter-regional variability (59% and 60%, respectively) than intra-site (mean of 59% and 44%, respectively) or temporal variabilities (mean of 41% and 50%, respectively). The mean fluxes were the highest in CA (26.3 ± 8.4 μmol $NH_4^+$ m$^{-2}$ day$^{-1}$ and 41.7 ± 7.2 μmol $NO_3^-$ m$^{-2}$ day$^{-1}$). CB and LC sites showed the highest intra-site variability for both reduced and oxidized forms (84% and 53%, respectively), fairly higher than their respective temporal variability (57% and 33%).

The calculated foliar surface conductance values ($K_{NH_4^+}$ and $K_{NO_3^-}$) ranged 0.18–0.27 cm s$^{-1}$ (mean of 0.24 cm s$^{-1}$) for $K_{NH_4^+}$ and 0.49–1.83 cm s$^{-1}$ (mean of 1.24 cm s$^{-1}$) for $K_{NO_3^-}$ (Table 3). The regression between the flux of ammonium to the branches ($F_{NH_3+NH_4^+}$) and the ambient concentration of $NH_3$ was significant for the entire dataset. Since the interception coefficient was not statistically different from zero, it was set to zero in order to obtain a slope coefficient equivalent to the $K_{NH_4^+}$. This value was 0.235 cm s$^{-1}$, very similar to the averaged $K_{NH_4^+}$ value of 0.24 cm s$^{-1}$. No significant regression existed between the flux of nitrate to the branches ($F_{HNO_3+NO_3^-}$) and the concentration of $HNO_3$. Moreover, the *K* values obtained were much more variable among the studied regions for oxidized than for reduced nitrogen. Because of this difference in the results between N forms, and attending to the empirical approximation of the method, the regional averaged values of $K_{NH_4^+}$ and $K_{NO_3^-}$ presented in Table 3 were used for the $DD_{surf}$ calculations.

Significant Spearman correlations of $K_{NH_4^+}$ with temperature and relative humidity (positively and negatively correlated, respectively) occurred for both NB and LB. These correlations were more significant in the case of NB. No significant relationships between $K_{NO_3^-}$ and meteorological variables were found.

#### 3.1.2. Lyophilized branches

In TC site, the flux of oxidized N ($F_{HNO_3+NO_3^-}$) to LB was slightly higher than to NB (means of 31.8 ± 1.7 *vs.* 22.3 ± 5.2 μmol $NO_3^-$ m$^{-2}$ day$^{-1}$), while $F_{NH_3+NH_4^+}$ was much higher for LB than NB (means of

**Table 4**
Fluxes of deposited nitrogen into lyophilized vegetation from the branch-washing experiment and averaged concentration of ammonia and nitric acid vapour for each exposure period in TC site (Central Spain).

| Start date | End date | $F_{NH_3+NH_4^+}$ (μmol m$^{-2}$ day$^{-1}$) | | | $F_{HNO_3+NO_3^-}$ (μmol m$^{-2}$ day$^{-1}$) | | | $NH_3$ (μmol m$^{-3}$) | $HNO_3$ (μmol m$^{-3}$) |
|---|---|---|---|---|---|---|---|---|---|
| | | N [a] | Mean | CV | N [a] | Mean | CV | | |
| 14-06-11 | 28-06-11 | 3 | 20.9 | 31% | 3 | 46.6 | 12% | 0.07 | 0.03 |
| 04-10-11 | 18-10-11 | 3 | 23.7 | 8% | 3 | 39.1 | 24% | 0.04 | 0.03 |
| 04-01-12 | 11-01-12 | 3 | 23.6 | 73% | 3 | 8.5 | 20% | 0.03 | 0.02 |
| 22-06-12 | 06-07-12 | 3 | 20.2 | 36% | 3 | 52.4 | 4% | 0.07 | 0.04 |
| 17-04-13 | 24-04-13 | 3 | 18.6 | 72% | 3 | 12.6 | 35% | 0.03 | 0.01 |
| | **N** | **Mean** | **CV** [b] | **CV** [c] | **Mean** | **CV** [b] | **CV** [c] | **Mean** | |
| | 5 | 21.4 | 10% | 44% | 31.8 | 63% | 19% | 0.05 | 0.03 |

[a] Number of plots; each plot value corresponds to the mean of 3–4 branches exposed in the same pole;
[b] CV of the mean, representing temporal variability;
[c] Mean of the CVs, representing mean intra-site variability.

**Table 5**
Estimated and measured annual N deposition to the four forest sites.

| | N deposition (kg N $ha^{-1}$) | | | | N deposition (% of TD) | | | |
|---|---|---|---|---|---|---|---|---|
| | CB | TC | CA | LC | CB | TC | CA | LC |
| $DD_{stom}$ | | | | | | | | |
| $HNO_3$ | 0.6 | 0.1 | 0.5 | 1.3 | 5% | 1% | 2% | 7% |
| $NH_3$ | 0.3 | 0.2 | 1.9 | 0.2 | 2% | 2% | 7% | 1% |
| $NO_2$ | 2.5 | 1.2 | 3.1 | 1.4 | 20% | 13% | 11% | 8% |
| $DD_{surf}$ | | | | | | | | |
| $HNO_3 + NO_3^-$ | 4.0 | 4.3 | 13.2 | 7.7 | 32% | 45% | 46% | 43% |
| $NH_3 + NH_4^+$ | 2.0 | 1.4 | 4.8 | 2.7 | 16% | 15% | 17% | 15% |
| WD | | | | | | | | |
| $NH_4^+$ | 1.5 | 1.2 | 3.6 | 2.5 | 12% | 13% | 12% | 14% |
| $NO_3^-$ | 1.5 | 1.1 | 1.8 | 2.0 | 12% | 11% | 6% | 11% |
| DD N | 9.4 | 7.1 | 23.5 | 13.2 | 75% | 76% | 81% | 74% |
| WD N | 3.1 | 2.3 | 5.4 | 4.6 | 25% | 24% | 19% | 26% |
| TD N | 12.5 | 9.4 | 28.9 | 17.8 | | | | |

$DD_{stom}$: stomatal dry deposition; $DD_{surf}$: surface dry deposition; DD: dry ($DD_{stom} + DD_{surf}$) deposition; WD: wet deposition; TD: total (wet + dry) deposition.

$21.4 \pm 1.0$ *vs.* $8.0 \pm 1.1$ μmol $NH_4^+$ $m^{-2}$ $day^{-1}$). The temporal variability of $F_{NH_3+NH_4^+}$ and the intra-site variability of $F_{HNO_3+NO_3^-}$ were higher in the LB experiment (Tables 2 and 4).

The averaged values of $K_{NH_4^+}$ for NB and LB were $0.26 \pm 0.06$ cm $s^{-1}$ and $0.61 \pm 0.12$ cm $s^{-1}$, respectively (data not shown). No significant regression was found between $F_{NH_3+NH_4^+}$ and $NH_3$ concentration at TC for neither the LB nor the NB datasets. This result could be explained by the small number of data pairs ($n = 5$) and the small range of $NH_3$ concentrations (Table 4). The averaged values of $K_{NO_3^-}$ for NB and LB were $1.41 \pm 0.48$ cm $s^{-1}$ and $1.41 \pm 1.07$ cm $s^{-1}$, respectively (data not shown). A statistically significant regression ($p = 0.042$; $R^2 = 0.80$) between $F_{HNO_3+NO_3^-}$ and $HNO_3$ concentration was found for LB but not for NB data. The LB conductance estimated from this regression was $1.49 \pm 0.17$ cm $s^{-1}$.

For the overall dataset, no statistically significant Spearman rank order correlation was found between N fluxes into the branches or calculated conductance values and meteorological variables, apart from a positive correlation of $F_{HNO_3+NO_3^-}$ and $K_{NO_3^-}$ with wind velocity ($r = 0.71$ and 0.47, respectively).

### 3.2. Dry deposition estimation

For all the sites, the estimated surface deposition of oxidized N tended to be much larger than the reduced one (means of $7.3 \pm 2.2$ kg N $ha^{-1}$ $year^{-1}$ *vs.* $2.7 \pm 0.7$ kg N $ha^{-1}$ $year^{-1}$ for the four sites). As an average of all sites, surface deposition represents a mean of $54\% \pm 4\%$ of the dry deposition of atmospheric inorganic N. The surface deposition of reduced N was particularly relevant in CA, the most agrarian site, with a two-year mean of 4.8 kg N $ha^{-1}$ $year^{-1}$ (Table 5). The intra-annual variability found in the estimates of surface N deposition showed a larger input during late-spring and summer in all the sites (Fig. 1a).

In regards to stomatal conductance, $g_s$ values increased as the year progressed, reaching maximum monthly-means in May. The site-averaged daytime $g_s$ for 2011–2013 ranked across the sites as follows: LC > CB > CA > TC; with mean values of 103.5, 98.2, 88.0 and 65.2 mmol $H_2O$ $m^{-2}$ PLA $s^{-1}$, respectively. The stomatal deposition of N gases averaged for the four sites $3.3 \pm 0.8$ kg N $ha^{-1}$ $year^{-1}$, with $NO_2$ contributing the most ($2.0 \pm 0.4$ kg N $ha^{-1}$ $year^{-1}$). Stomatal uptake of $NH_3$ was noticeable in CA, with 1.9 kg N $ha^{-1}$ $year^{-1}$ (Table 5). The relative contribution of $NO_2$ deposition to dry deposition of N averaged 19% in the peri-urban

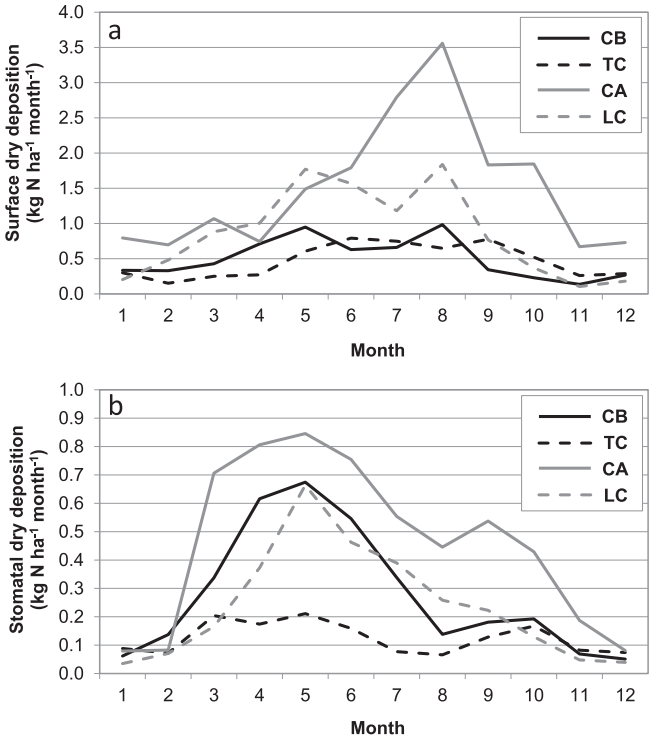


**Fig. 1.** Monthly mean ($n = 2$ years) of (a) surface and (b) stomatal deposition of atmospheric nitrogen estimated for the four *Quercus ilex* forests of the study.

forests (CB, TC and CA), while it represented 10% in LC (Fig. 2). The temporal variability found in the estimates of stomatal N deposition (Fig. 1b) was very similar to the one described above for the modelled $g_s$.

Dry deposition (stomatal uptake + surface deposition) of inorganic N ranged from 7.1 kg N $ha^{-1}$ $year^{-1}$ in TC to 23.5 kg N $ha^{-1}$ $year^{-1}$ in CA (Table 5), with the surface deposition pathway averaging $74\% \pm 3\%$ of the dry deposition (Fig. 2). Dry deposition of N was dominated by the surface dry deposition of oxidized forms of N in all the sites and for most of the seasons (Table 5; Fig. 2). In CA, reduced N accounted for a 29% of the dry deposition of inorganic N, while it represented 23% in the other sites. The highest seasonal estimate of N dry deposition occurred in spring in CB and LC sites and in summer at the other two sites (Fig. 2).

### 3.3. Total deposition of N

The estimated annual wet deposition of inorganic N ranged from 2.3 to 5.4 kg N $ha^{-1}$ $year^{-1}$ (in TC and CA sites, respectively), and it was slightly dominated by the reduced form (mean of $2.2 \pm 0.5$ kg $NH_4$–N $ha^{-1}$ $year^{-1}$ for the four sites), particularly in CA (Table 5). Total deposition of inorganic N (dry + wet) varied from 9.4 kg N $ha^{-1}$ $year^{-1}$ in TC to 28.9 kg N $ha^{-1}$ $year^{-1}$ in CA. The dry deposited N represented more than 70% of the total annual deposition of inorganic N at all the sites (mean of $77\% \pm 2\%$). The contribution of the reduced forms (both wet and dry altogether) to the total deposition was the most important in CA (36%), while it was very similar in the remaining sites (30% on average). Surface dry deposition of oxidized forms of N was the most contributing load to total deposition at all the sites ($41\% \pm 3\%$; Table 5) and for most of the seasons (≥27%, except winter in CA and CB, and autumn in CB and LC; data not shown).

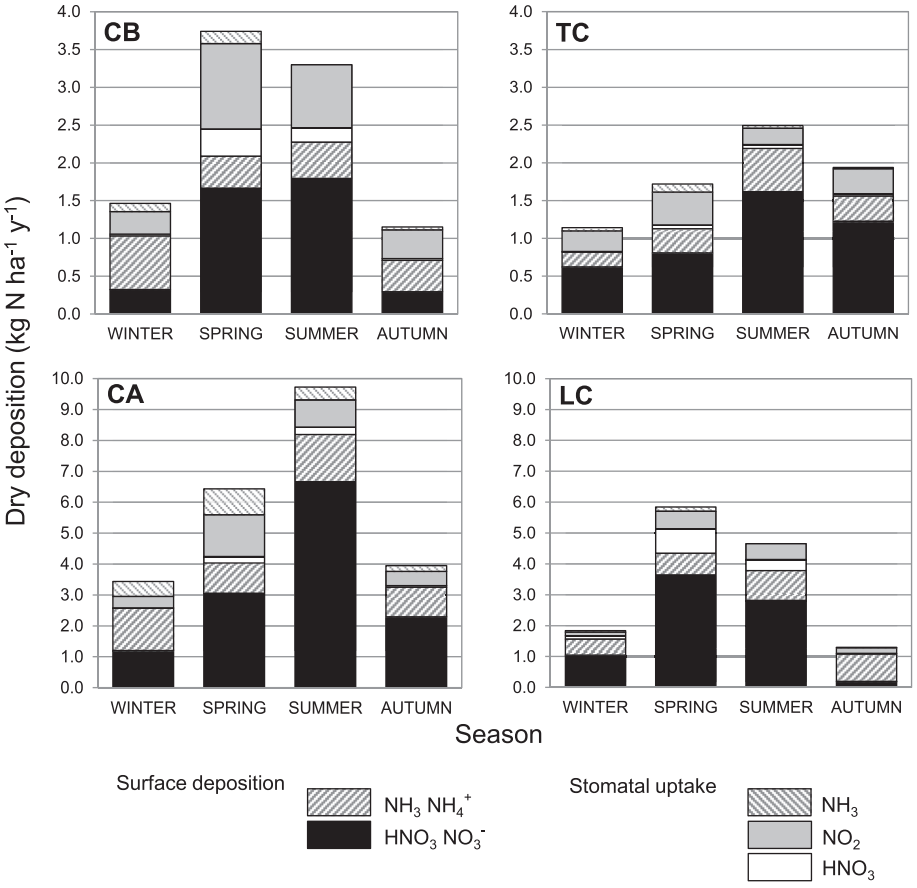


**Fig. 2.** Seasonal dry deposition of total inorganic nitrogen into the four *Quercus ilex* forests of the study. Note that the scale for CB and TC is different from the other sites.

## 4. Discussion

### 4.1. Branch-washing experiment

#### 4.1.1. Natural branches

Fluxes of N to the branches ($F_{HNO_3+NO_3^-}$ and $F_{NH_3+NH_4^+}$) in the NE region showed an intra-site variability higher than the temporal variability for both N fluxes in this region, while it did not happen in CA and only for $F_{NH_3+NH_4^+}$ in TC. It could be expected that temporal variability would be larger than intra-site variability since fluxes of N to the branches are described to change with the variations in atmospheric concentration of N and meteorological conditions (Andersen and Hovmand, 1999; Fowler et al., 2009; Pratt et al., 1996). The highest intra-site variability for an exposure period found in CB and LC occurred in late spring, with CV values of 123% for $F_{NH_3+NH_4^+}$ and 49% for $F_{HNO_3+NO_3^-}$ (Table 2), similar to values reported in a previous branch-washing experiment in LC (108% for $F_{NH_3+NH_4^+}$ and 49% for $F_{HNO_3+NO_3^-}$, for a similar period of the year; Rodrigo and Àvila, 2002). This high spatial variability can respond to the orography of the terrain at those two sites, since both are placed in a hilly and sloping terrain. In these conditions, the aerodynamic resistance of the canopy can vary strongly across the monitoring site, making the deposition velocity to the canopy to fluctuate as well.

With regards to surface conductance values, the good correlation found between $F_{NH_3+NH_4^+}$ and $NH_3$ indicates that the concentration of $NH_3$ is a variable that explains most of the flux of $NH_4^+$ to the branches robustly across time and regions for *Q. ilex* forests in this study. On the other hand, no significant correlation was found between $F_{HNO_3+NO_3^-}$ and atmospheric concentrations of $HNO_3$, and the calculated $K_{NO_3^-}$ showed a high temporal variability, without a clear seasonal pattern. In this sense, the estimation of surface deposition of oxidized N presented higher uncertainty than the estimation of reduced N, pointing out that further research is needed to understand the processes involved in the dry deposition of oxidized N.

The almost complete lack of correlation between meteorological and deposition variables was unexpected, since the deposition rates of pollutants into forest canopies are strongly influenced by ambient conditions (Fenn et al., 2009; Fowler et al., 2009). One explanation could be the heterogeneous duration of the exposure periods (from 8 to 63 days) that made the meteorological data less suitable for statistical analysis (in fact, the meteorological variables were not strongly correlated among themselves, as it should be expected). Another source of uncertainty could be related to the fact that aerosol concentration was not taken into account in the calculation of surface deposition conductance. This issue could be relevant for the winter and autumn exposure periods, when the aerosol fraction of atmospheric N is more important (García-Gómez et al., 2016). In fact, the highest $K_{NO_3^-}$ values occurred in CA, the site with the highest concentration of particulate $NO_3^-$ and most favouring conditions (high $NH_3$ concentrations and low temperatures) for the formation and stability of ammonium nitrate ($NH_4NO_3$) (García-Gómez et al., 2016). Besides, the $K_{NO_3^-}$ values across all the three climatic regions were higher for winter and autumn than for summer exposure periods (data not shown). This

could mean that particulate $NO_3^-$ deposition was important enough in some exposure periods to bias the $K_{NO_3^-}$ calculations in comparison with the rest of periods. However, no significant correlation was found between the fluxes of $F_{HNO_3+NO_3^-}$ and $F_{NH_3+NH_4^+}$ (data not shown), restraining the possibility of a potentially important deposition of $NH_4NO_3$. In any case, a better quantification of atmospheric aerosol and its inclusion in the calculations derived from branch washing experiments could increase the reliability and robustness of the EIM estimates.

Apart from the above-discussed sources of uncertainty in dry deposition estimate (orography, length of exposure, aerosol concentration), there is also another potential one. Since living branches could not be washed with distilled water at the onset of each sampling period, we considered exposure periods to begin at the end of the previous rain. This caused the heterogeneous exposure periods but also implied an uncertainty in the initial state of cleanness of the branches at each period. However, the precipitation storing capacity (Zinke, 1967) of the canopies indicated that rains above 2.8, 1.5 and 0.9 mm for LC, CB and CA, and TC, respectively, could be considered sufficient for a clean start of dry deposition periods (Àvila et al., 2017). All the periods showed previous precipitations above these values and, moreover, the exposure periods were long enough at all sites to minimise the contribution of the residual previous deposition.

#### 4.1.2. Lyophilized branches

The use of lyophilized compared to natural branches offered the possibility to disentangle some of the canopy processes linked to biological activity that could modify atmospheric deposition estimations. The higher values of $F_{NH_3+NH_4^+}$ obtained using LB in comparison with NB could be related to the active phyllosphere present in the NB but absent in the LB. The phyllosphere might absorb or transform part of the surface deposited $NH_4^+$ during the exposure periods and therefore reduce the $F_{NH_3+NH_4^+}$ observable in the washings. Recent studies of throughfall deposition suggest that tree canopies play an active role in the N cycling within forest ecosystems (Guerrieri et al., 2015). Further experiments comparing NB and LB washing, together with throughfall and bulk deposition monitoring, could help in clarifying in-canopy processing of dry deposition.

In the case of $F_{HNO_3+NO_3^-}$, the flux was also higher to LB than to NB, but the difference was smaller than in the $F_{NH_3+NH_4^+}$ case. The intra-site variability of $F_{HNO_3+NO_3^-}$ for LB experiment was less than half of the one found in NB washings, which could be caused by the more homogeneous exposure of the LB (every pole was installed in an open space of the canopy with similar LB exposed in four different orientations). Aside from differences in variability, the mean value of the estimated $K_{NO_3^-}$ for LB was very similar to the mean $K_{NO_3^-}$ calculated for NB in the same site. These results could indicate that the estimation of dry deposition of N to vegetation surfaces is more affected by biological processes for the reduced N than the oxidized forms.

### 4.2. Dry deposition

Surface dry deposition ($DD_{surf}$) for each region was estimated using the surface deposition conductances obtained with NB washing experiments. Surface deposition was dominated by N oxidized forms. In fact, the temporal variability of $DD_{surf}$ of N was driven by the temporal pattern of $HNO_3$ concentrations showing higher values during spring and summer (García-Gómez et al., 2016). The inter-sites variability was mainly related to differences in site-specific concentrations of $HNO_3$ and in LAI.

Estimation of the dry deposition of N to canopy surfaces by means of the canopy budget model (CBM; Staelens et al., 2008), which does not include stomatal deposition estimation, for the same sites and period as the present study have been previously presented (Aguillaume et al., 2017; Àvila et al., 2017). Those studies showed in general lower values (from 23% to 73% lower) of surface deposition, except for CB (57% higher). Àvila et al. (2017) indicated that the existing uncertainties in canopy processes and in the parameters employed in CBM calculations, particularly in dry environments, could be limiting the performance of that method in comparison with other approximations. It is therefore important to fully understand these in-canopy processes (nitrification, plant and lichen uptake, microbial immobilization, among other possible) to be able to further improve the methodologies for estimating dry deposition to vegetation.

In regards to the stomatal deposition ($DD_{stom}$), gaseous N was predominantly taken up in the form of $NO_2$–N because of its highest concentration in air (the predominant gaseous form of N at all the sites), despite not having the highest diffusivity (Massman, 1998). As expected, the relative contribution of $NO_2$ deposition to total dry deposition of N was higher at the three peri-urban forests than in the most remote site (19.0% and 10.4%, respectively). Therefore, $NO_2$ deposition should be always taken into consideration for estimating dry deposition of N, particularly in sites close to the pollution sources, as it has been pointed out by other authors (Zhang et al., 2005). Ammonia uptake was relevant (7% of total dry deposition) only in CA, the most agricultural site, because of its higher concentration values (Table 2; García-Gómez et al., 2016).

The inter-site variability of leaf uptake of atmospheric gaseous N compounds did not exactly mirror the inter-site $g_s$ rank, since other factors such as LAI and gas concentration were more relevant. In this sense, the largest LAI and $NH_3$ concentration at CA caused the highest stomatal deposition of all the sites. However, the seasonal variability mainly followed the variations of the modelled $g_s$ in all the locations, slightly modulated by the variability of concentration of gaseous N compounds.

The noticeable importance of LAI in deposition estimation suggests that a more detailed research on how N is deposited to the surface of tree branches is needed. In a branch-washing experiment performed in a coniferous forest by Janson and Granat (1999), the flux of $HNO_3$ to the branches was revealed to decrease up to 70% from the exposed upper parts of the crown to the inner and less exposed parts. This decrease is not predictable to occur in *Q. ilex* forests in that degree, since approximately 60% of the total leaf area is expected found in the uppermost meter of the canopy and more than 81% in the upper 2 m (Sabaté et al., 1999). Therefore, although a correction in this way could be developed, it would not be expected to produce a big reduction in the results for these particular forests.

The estimated total dry deposition of atmospheric N in the four *Q. ilex* forests was dominated by the surface deposition of $HNO_3$ and particulate $NO_3^-$ at all the sites, even when transcuticular deposition of $HNO_3$ was not taken into consideration. Although many studies have highlighted the importance of $NO_2$ and $NH_3$ contribution to total dry deposition, particularly in locations close to their sources (Flechard et al., 2011; Schmitt et al., 2005), $HNO_3$ deposition is also recognized to make a significant contribution to nitrogen deposition across Europe (Fowler et al., 2009; Garten et al., 1998) and other countries (Elliott et al., 2009; Zhang et al., 2005). In our study, the highest contribution of these oxidized forms responded mainly to the high concentration of $HNO_3$ during spring and summer, caused by photochemical formation of $HNO_3$, which is a well-documented Mediterranean particularity (Bytnerowicz and Fenn, 1996; Millán et al., 2002; Tzanis et al., 2009). A high contribution

of $HNO_3$ to the atmospheric N dry deposition has been reported in other forests in Mediterranean areas (Bytnerowicz et al., 1999, 2015). Therefore, further efforts are needed to include reliable $HNO_3$ measurements in broad-scale atmospheric deposition monitoring networks, particularly during summer.

### 4.3. Total deposition of N

Dry deposition (surface deposition + stomatal uptake) represented the highest contribution (77% ± 2%) to the total N deposition in the *Q. ilex* forest studied. Total deposition was dominated by the surface dry deposition of oxidized N forms at all the sites and for most of the seasons. The estimated total deposition varied among the sites matching the geographical rank pattern previously found in model estimates (García-Gómez et al., 2014): higher deposition was determined at the northern site (28.9 kg N $ha^{-1}$ $year^{-1}$) and at the north-eastern sites (17.8 and 12.5 kg N $ha^{-1}$ $year^{-1}$) than at the central-Spain site (9.4 kg N $ha^{-1}$ $year^{-1}$). An additional bulk deposition of organic N compounds has been reported as well, representing up to 56% of the total dissolved N deposition (Izquieta-Rojano et al., 2016). Further research is needed to disentangle the origin of these organic N compounds and their role in the ecosystem N cycling.

Values of total inorganic N deposition were compared to the proposed N critical loads (CL) for conservation of *Q. ilex* forests. A critical load is a threshold for N deposition established within the Convention on Long-Range Tranboundary Air Pollution framework (CLRTAP). The CL is defined as the quantitative estimate of pollutant deposition below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge (CLRTAP, 2004). An empirical CL of 10–20 kg N $ha^{-1}$ $year^{-1}$ has been ascribed to Mediterranean *Quercus* forests following expert criteria (Bobbink and Hettelingh, 2011). A smaller CL of 3–6 kg N $ha^{-1}$ $year^{-1}$ has been also proposed for the protection against shifts in epiphytic lichen communities in Mediterranean chaparral ecosystems of California (Pardo et al., 2011). According to the presented estimates of N deposition, only in TC the threshold proposed in the revision of Bobbink and Hettelingh (2011) for the protection of Mediterranean evergreen *Quercus* woodlands was not reached for the study period, and all sites exceeded the CL proposed for the protection of epiphytic lichens of similar habitats in California (Pardo et al., 2011). These results point out the need to monitor atmospheric N pollutant and to estimate total (wet + dry) deposition as a factor that could be altering the functioning and biodiversity of *Q. ilex* forests in Spain.

## 5. Conclusions

The empirical inferential method (EIM) has been combined with an empirical stomatal conductance model to estimate the atmospheric dry deposition in *Q. ilex* forests in Spain. The surface conductance values for N compounds obtained using branch washing techniques were similar to values reported in experiments conducted in other Mediterranean areas. The washing experiment using lyophilized branches gave similar surface conductance values compared to the ones with living branches for particulate nitrate and nitric acid vapour, but higher for particulate ammonium and ammonia. This discrepancy could indicate a preferential uptake or transformation of the reduced dry-deposited N in the phyllosphere. Further studies are needed to characterize and quantify the fluxes of N in the phyllosphere. The comparison of LB and NB experiments have shown helpful for studying the in-canopy processes of the forest N cycle and can help to improve the estimation of N deposition with EIM.

Dry deposition in *Q. ilex* forests in Spain ranged 7.1–23.5 kg N $ha^{-1}$ $year^{-1}$, which implies an important contribution (77% ± 2%) to the total inorganic N deposition in these forests. Total deposition was dominated by the dry deposition of oxidized N forms at all the sites and for most of the seasons. Particularly, concentrations of $HNO_3$ led to the pronounced seasonal variations of surface N deposition. The inter-site variability in dry deposition responded to a combination of factors, with special relevance of $HNO_3$ concentration and LAI values. Further efforts are needed to include reliable $HNO_3$ measurements in broad-scale atmospheric deposition monitoring networks (at least in summer), together with $NO_2$ measurements (especially in peri-urban forests, due to the important contribution to total deposition), and to increase the availability of reliable forest-specific LAI data.

The total atmospheric N deposition in the *Q. ilex* forest studied, resulting from adding dry deposition estimations with wet deposition, reached values above the critical loads of N proposed for the conservation of Mediterranean broadleaf evergreen forests. Thus, atmospheric N deposition needs to be considered in monitoring programs of ecosystems and biodiversity conservation. In this sense, the proposed improved empirical inferential method offers an affordable and simple methodology to obtain site-specific estimations of dry deposition of atmospheric nitrogen.

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