Post-print of: Ávila, A. et al. "Quantitative study on nitrogen deposition and canopy retention in Mediterranean evergreen forests" in Environmental science and pollution research, vol. 24, issue 34 (Dec. 2017), p. 26213-26226. The final versión is available at DOI 10.1007/s11356-017-8861-4

Quantitative study on nitrogen deposition and canopy retention in Mediterranean evergreen forests

Anna Avila[1*], Laura Aguillaume[1], Sheila Izquieta-Rojano[2], Héctor García-Gómez[3], David Elustondo[2], Jesús Miguel Santamaría[2], Rocío Alonso[3]

[1] CREAF, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
[2]LICA, Universidad de Navarra, 31009 Pamplona, Spain
[3]Ecotoxicology of Air Pollution, CIEMAT, 28040 Madrid, Spain

*Corresponding author: Anna Avila (anna.avila@uab.cat)

Keywords: Wet deposition, throughfall, canopy uptake, Mediterranean, nitrogen, critical loads

Abstract

To assess the impact of N pollutants on forest ecosystems, the role of the interactions in the canopy needs to be understood. A great number of studies have addressed this issue in heavily N polluted regions in north and central Europe. **Much less information is available for the Iberian Peninsula, and yet this region is home to mountain forests and alpine grasslands that may be at risk due to excessive N deposition. To establish the basis for ecology-based policies, there is a need to better understand the forest response to this atmospheric impact.**

To fill this gap, in this study we measured N deposition (as bulk, wet and throughfall fluxes of dissolved inorganic nitrogen) and air N gas concentrations from 2011 to 2013 at four Spanish holm-oak (*Quercus ilex*) forests located in different pollution environments. One site was in an area of intensive agriculture, two sites were influenced by big cities (Madrid and Barcelona, respectively) and one site was in a rural mountain environment 40 km north of Barcelona.

Wet deposition ranged between 0.54 and 3.8 kg N $ha^{-1}y^{-1}$ for NH_4^+-N and between 0.65 and 2.1 kg N $ha^{-1}y^{-1}$ for NO_3^--N, with the lowest deposition at the Madrid site for both components. Dry deposition was evaluated with three different approaches: 1) a canopy budget model based in throughfall measurements, 2) a branch washing method, and 3) inferential calculations. Taking the average dry deposition from these methods, dry deposition represented 51-67% (reduced N) and 72-75% (oxidized N) of total N deposition.

Canopies retained both NH_4^+-N and NO_3-N, with a higher retention at the agricultural and rural sites (50-60%) than at sites located close to big cities (20-35%, though more uncertainty was found for the site near Madrid), thereby highlighting the role of the forest canopy in processing N pollutant emissions.

Introduction

Quantifying nitrogen (N) atmospheric deposition to forests is a key issue to understand nutrient availability for forest growth and to assess the forests status regarding excess N deposition (Johnson and Lindberg 2013). Even though N deposition is of concern for many ecosystem types, forests probably receive larger deposition loads, mainly due to their greater aerodynamic roughness that favors the capture of gases and fine particles (Gallagher et al. 1997). Therefore, high N deposition affects forest ecosystem compartments comprising vegetation, soil, soil water and the animal, fungi and microbial biota (Sutton et al. 2011).

Wet deposition (WD) or bulk deposition (BD), which includes part of coarse particle fallout, can be quite straightforwardly quantified with wet or bulk collectors. However, for dry deposition (DD) no standard method exists, and various approaches have been used for its determination (Hanson and Lindberg 1991). Dry deposition to foliar surfaces refers to the transfer of gas and particulate species between the atmosphere and vegetation surfaces in the absence of precipitation. Several processes control dry deposition, such as ambient gas and aerosol concentrations, physicochemical characteristics of the species of interest, canopy characteristics and the site prevailing meteorology (Hanson and Lindberg 1991). For N species, the deposition behavior of N gases can be separated in two main groups: 1) highly reactive and water soluble gases (HNO_3 and NH_3) that are readily deposited on leaf surfaces , and 2) less soluble gases that diffuse through the stomata (NO, NO_2 and partly NH_3, Hosker and Lindberg 1982). Also, HNO_3 can be transported via cuticular uptake (Padgett et al. 2009). Gaseous HNO_3 and NH_3 can be incorporated into atmospheric particles, mostly through reactions with sulfate and nitrates to form fine particles, but they can also react with soil dust and sea salts to form coarse nitrate particles (Querol et al. 1998; Pey et al. 2009). These N-containing particles (pNO_3^- and pNH_4^+) may be incorporated into cloud water and be deposited via wet deposition, and also they can deposit via dry deposition to the leaf surfaces (Hanson and Lindberg 1991).

To estimate dry N deposition to forests, micrometeorological methodologies, such as eddy correlation and the aerodynamic gradient method have been widely used. These methods are economically costly and cannot be applied to sites with complex topography (Hicks et al. 1991). To overcome these drawbacks, approaches based on recovering accumulated deposition on deposition surfaces have been developed, e.g the throughfall and branch washing methods. Throughfall measurements (collection of precipitation water that has passed through the canopy) have been frequently used and net throughfall fluxes have been used as indicators of dry deposition (De Schriever et al. 2007). However, nitrogen compounds can experience

exchanges and transformations in the canopy that need to be taken into account when determining N dry deposition (Parker 1983; Hanson and Lindberg 1991). This is particularly true for sites in low pollution environments with moderate N deposition loads where canopy N retention and transformation by canopy epiphytes and microorganisms may have a higher relative contribution (Guerrieri et al. 2014). Branch rinsing techniques have also been widely used to recover deposited N compounds from foliar surfaces (Bytnerowicz et al. 1987, 2015; García-Gómez 2016).

Models indicate that western Europe may be particularly affected by high N deposition in 2030 under current legislation scenarios (Dentener et al. 2006). In fact, empirical N critical loads set for the protection of terrestrial habitats under the Convention on Long-Range Transboundary Air Pollution (CLRTAP) are being currently exceeded in some habitats of Community interest of the Spanish Natura 2000 network (García-Gómez et al. 2014). **N deposition estimated with the EMEP and CHIMERE models indicated that a surface of 3785 km^2 (modeled with the EMEP model) and 1441 km^2 (modeled with CHIMERE) corresponding to habitats of the Annex 1 of the Habitats Directive received N deposition that exceeded the habitat critical loads (García-Gómez et al. 2014)**.

Other studies indicate N enrichment in forest ecosystems in Spain, such as the observed increase of N content in herbarium bryophytes collected in the 20th century (Peñuelas and Filella 2001), the increase of nitrophilous species in natural areas from the Spanish Natura 2000 network (Ariño et al. 2000) and the increased streamwater nitrate concentrations in headwater streams (Avila and Rodà 2012). On the other hand, N deposition has been related to acidification, with implications on plant nutrition and soil microbial community structure in pine forests in central Spain (Ochoa-Hueso et al. 2014).

Data on N deposition and the contribution of DD to total N deposition is rather scarce in Spain. In a study of five rural localities in NE Spain, wet N deposition ranged between 4 and 7 kg N ha^{-1}y^{-1} and total N deposition was in the range of 12-19 kg N ha^{-1}y^{-1} with dry deposition accounting between 50 to 70% of total N deposition (Avila et al. 2010). In central-western Spain (Salamanca region), wet deposition ranged between 3- 5 kg N ha^{-1}y^{-1}, but dry deposition (estimated with the regression method of Lovett and Lindberg 1984) was only 0.8 and 1.5 kg N ha^{-1}y^{-1} and made a lower contribution to total N deposition amounts (25-45%; Moreno et al. 2001).

Recently, research has been carried out in Spain to describe N deposition in holm oak forests and major advances have been done in the quantification of dissolved organic nitrogen (DON) deposition (Izquieta-Rojano et al. 2016), in testing methods for wet and throughfall deposition

sampling (García-Gómez et al. 2016b), in analysing the effect of forests to improve air quality (García-Gómez et al. 2016a) and modelling N deposition at a Spanish scale (García-Gómez et al. 2014).

In this paper, we will provide information on total N deposition fluxes and assess the role of dry deposition and canopy uptake based on wet deposition, throughfall and ambient gas measurements from 4 holm oak forests under different pollution environments in the Iberian Peninsula. Dry deposition is evaluated with three different approaches (a canopy budget model based in throughfall, branch washings and inferential calculations). The range of the obtained dry deposition values is used to provide a tentative total deposition estimate and to evaluate the scope of canopy uptake in these forests.

2. Material and Methods

2.1 Locations and experimental sites

The study was conducted at 4 holm-oak forests (*Quercus ilex* L.) in the north, center and north-east of the Iberian Peninsula (Fig. 1). Two sites were located in Catalonia in NE Spain near Barcelona (La Castanya and Can Balasc, LC and CB respectively), one in Madrid (Tres Cantos, TC) and another site in Navarra, North Spain (Carrascal, CA). The main characteristics of the sampling sites are shown in Table 1.

The LC site (41º46'N, 2º21'E, 696 m.a.s.l.) is located in the Montseny Mountains, 40 km to the NNE of Barcelona. This site is considered as a rural background station with some influence of pollution from the metropolitan area of Barcelona. Vegetation at LC consists of a dense and closed canopy forest dominated by holm-oak (*Quercus ilex* L.) trees. Lithology at this area is composed by schists and granodiorites. Climate is Mediterranean, with a clear seasonal cycle with lower precipitation in summer and winter.

The CB site (41º25'N, 2º04'Eº, 255 m.a.s.l.) is located in the Collserola Natural Park, a protected area lying to the west of the Barcelona Metropolitan Area (3.5 million inhabitants). The plot lies at 4 km linear distance from Barcelona outskirts. A moderate to heavy traffic highway (C-16) runs about 150 m from the study plot, and it is affected by industrial emissions from the Baix Llobregat area (García-Gómez et al. 2016a). Vegetation at CB is characterized by a continuous cover of holm-oak (*Quercus ilex* L.) mixed with *Quercus humilis* Mill. Lithology consists of shales and slates with granitic outcrops. Climate is Mediterranean.

The CA site (42º39'N, 1º38'W, 645 m.a.s.l.) is situated at the foot of the Alaitz-Izco hills, in central Navarra. The nearest larger city, Pamplona (~200 000 inhabitants) is 15 km to the North. The site is about 50 m distant from a moderate to heavy traffic highway (AP-15) and is surrounded by fields of irrigated and fertilized cereal that have been found to influence N organic and inorganic inputs to this site (Izquieta-Rojano et al. 2016). An opencast limestone quarry is located approximately 2 km to the north. The forest comprises mostly *Quercus ilex* L. trees with scattered *Quercus faginea* Lam. and *Quercus humilis* Mill. individuals. The site lies on calcareous soils. The climate at CA is Mediterranean continental with oceanic influence from the Atlantic sea.

The TC site (40º35'N, 3º43'W, 705 m.a.s.l.) is located 9 km NE from Madrid outskirts (3.2 million inhabitants). The site lies in the north-eastern border of the holm-oak forest of El Pardo, which extends over an area of 170 km^2 and is a protected area. Vegetation was historically managed as a traditional 'dehesa', a savannah-type managed formation of low density isolated trees. The low level of management during the last decades has allowed the vegetation to grow as an open low density forest with an understory of shrubs and grasslands. Lithology is composed by sandy arkoses sediments from granites and gneisses. A moderate to high traffic intensity highway (M-607) is ~ 2 km distant from the monitoring site. The climate is continental Mediterranean, characterized by long dry periods and a more contrasted seasonality than the typical Mediterranean climate.

2.2 Field sampling and bulk deposition and throughfall chemical analysis

In every location, an open-field (for bulk deposition, BD) and a below-canopy plot (for throughfall, TF) were instrumented. The same model of sampler was used for bulk and throughfall deposition collection at all sites, composed of an ISO-standardized funnel (Norwegian Institute for Air Research, NILU) with a 314 cm^2 horizontal interception surface, connected to a polypropylene 2 L bottle. A bug sieve was placed at the funnel neck to prevent leaves and other materials from entering into the bottle. The upper edge of the funnel was equipped with an external ring to prevent contamination from bird droppings. The rim of all funnels stood approximately at 1.5 m above ground level. For bulk sampling, two collectors were used per site at LC and CB, and 4 at CA and TC. For throughfall sampling, 12 collectors were used at all sites; they were randomly located in a forest plot of 30*30 m at LC, CB and CA. At the dehesa-like forest of TC, the collectors were randomly placed in different orientations under dominant trees. Wet deposition (WD) was also measured at LC and TC in the open-field plot, by means of an automatic Andersen sampler (ESM Andersen instruments, G78-1001) consisting on

a wet and a dry bucket covered with a moving lid that covers the wet collector in dry periods and moves to open WD the collector at the onset of rain. All funnels and WD buckets were thoroughly cleaned in the field with deionized water after each sampling. Bulk and throughfall sampling bottles were retrieved and replaced by clean ones at each site. Field blanks (recovered distilled water after rinsing the funnels and buckets in the field) were periodically obtained and analyzed.

Sampling took place from June 2011 to June 2013 in a weekly schedule or biweekly in case of rainless weeks. All collected samples were filtered with 0.45 μm size pore membrane filters of cellulose (Millipore) and frozen until analysis. Ammonium (NH_4^+) and nitrate (NO_3^-) were determined by ion chromatography at all sites. Analytical accuracy was checked with internal control samples of known concentrations, with differences being lower than 10%. In addition, all major anions and cations in the precipitation and throughfall samples were analyzed by ion chromatography (Dionex, Sunnyvale, USA) and an accuracy check for analytical quality was applied based in recommendations of the ICP-Forests manual (2010). The balance of the sum of cations and anions, and the calculated conductivity related to the measured one was also scrutinized and outliers (>10%) were discarded (Izquieta-Rojano et al. 2016). Detection limit for NO_3^- and NH_4^+ was 1.5 μeq L^{-1}.

Precipitation amount has been found to vary depending on the device employed for measurement (Erisman et al. 1994). In this study, precipitation and throughfall amounts were obtained from the water volume collected in bulk collectors, divided by the collector exposed surface, and expressed as Lm^{-2}. To ascertain the accuracy of these measurements, we compared the water depths recorded by 4 different sampling devices deployed in parallel from August 2011 to June 2013 at the LC site: 1) a wet Andersen collector, 2) two replicated bulk collector buckets, 3) a Hellmann standard rain gauge and 4) a Campbell tipping bucket rain gauge. An ANOVA analysis performed on log-transformed weekly data indicated non-significant differences between these measurement methods (p=0. 76).

2.3 Gas and particulates sampling and analysis

Atmospheric concentrations of ammonia (NH_3), nitrogen dioxide (NO_2) and nitric acid vapor (HNO_3) were monitored from February 2011 to February 2013 using passive samplers. A full description of the sampling is given in García-Gómez et al. (2016a). Although an open-field and a below-canopy plot were installed in each plot, here we will only consider open-field

measurements. The open plots were >500m distant from the forest edges to achieve a proper exposure to ambient concentrations.

Two replicate passive samplers per gaseous species were exposed during two-week periods at 2 m height in each plot. In parallel, unexposed samplers were used as blanks for each site, period and type of sampler. After collection, all samples were kept refrigerated (4º C) in darkness until analysis. Tube-type samplers (Radiello®) were used to measure atmospheric concentrations of NH_3 and NO_2. Tubes were extracted according to Radiello's specifications (Fondazione Salvatore Maugeri, 2006). Atmospheric concentrations of HNO_3 were measured by means of badge-type samplers manufactured following Bytnerowicz et al. (2005). In CA, Passam® passive samplers and methods were employed during the second year for monitoring NO_2 after checking their comparability with Radiello®.

Particulate matter with diameter up to 10 µm (PM_{10}) was collected with 150 mm quartz micro-fibre filters (2500 QAO-UP, Pall Life Sciences) using high volume samplers installed in open-field plots of TC, CA and LC sites (Digitel® DH80 in LC -MSY monitoring station; MCV® CAV-A/mb in TC and CA). Samples were collected from February 2012 to February 2013 once a week, using a flow of 30 m^3 h^{-1} during 24-h periods. The day of the week for PM_{10} collection changed weekly. The concentration was gravimetrically determined and NO_3^- and NH_4^+ were water-extracted and analyzed by ion chromatography. For statistical comparison with gaseous pollutant concentrations, PM_{10} data were grouped and averaged in accordance to passive sampling.

2.4 Data handling and statistical analysis

Annual BD and TF mean concentrations were calculated as volume-weighted means (VWM, expressed as µeq L^{-1}). Annual BD and TF fluxes were obtained as the product of their respective VWM by the annual precipitation or throughfall volume and are expressed as kg N ha^{-1} y^{-1}.

The Kruskall-Wallis was applied to explore differences in rainfall amount or N compounds and the Wilcoxon signed-rank test was used to determine differences between site pairs.

2.5 Dry deposition estimation

In this work, an estimation of dry deposition fluxes is proposed based on three model approaches: 1) canopy budget model (CBM), 2) branch surface washings (BW), and 3) inferential model with V_ds obtained from references in forest studies (IM).

2.5.1 Canopy budget model

A complete description of this model is given elsewhere (Drraijers and Erisman 1995, Balestrini and Tagliaferri 2001, Staelens et al. 2008, ICP-Forest Manual 2010, Adriaenssens et al. 2012, Drapelova 2013) and here we will give a brief summary. The model is based on the balance:

$$nTF = TF - WD = DD + CE \quad \text{(eq. 1)}$$

where nTF stands for net throughfall, TF for throughfall, WD for Wet Deposition, DD for Dry Deposition and CE for Canopy Exchange. Canopy exchange can be positive and then it is attributed to leaching of ions from the leaf pool (canopy leaching, CL) or be negative and then it is attributed to the uptake/transformation of the deposited ions (canopy uptake, CU).

The aim of the CBM is to distinguish and make an apportionment of DD and CE fluxes. To this purpose the filtering approach proposed by Ulrich (1983) is generally used. This considers that some ions do not interact with the canopy and then their enrichment in nTF is solely due to DD. Here we have used Na as reference ion. Other ions in aerosols (e.g base cations, SO_4^{-2}, Cl^-) are considered to behave as the Na-containing aerosols and, therefore, are considered to deposit at similar rates as the reference ion. Nitrogen compounds, which in our sites are mostly deposited as gases (García-Gómez et al. 2016a) do not comply with the above assumptions and another approach has to be taken: N exchange is determined first and then DD is derived from equation 1. It has been proposed (Balestrini and Tagliaferri 2001; Staelens et al. 2008) that the NH_4^+ canopy uptake (CU) flux can be estimated by considering that its canopy uptake equals the canopy leaching of base cations (the sum of leaching of Ca^{2+}, Mg^{2+} and K^+) once corrected by the sum of all leached anions (Staelens et al. 2008; Zhang et al. 2006). Several studies have only taken into account weak acid leaching (Adrieanssens et al. 2012; Balestrini and Tagliaferri 2001; Thimonier et al. 2005), but since our data also suggested Cl^- leaching (Aguillaume et al. 2017), it was also included in the sum of leached anions. Besides, experimental and field work has shown that NO_3^- can also be retained by the canopies (Harrison et al. 2000; Stachurski and Zimka 2002; Fenn et al. 2013). It has been proposed that the CU of $NH_4^+ + NO_3^-$ can be calculated based on the TF fluxes of both, distributing their relative CU weight by using an efficiency factor of NH_4^+ *vs.* NO_3^- uptake (xNH_4= moles of NH_4^+ taken up for each NO_3^- mol) (De Vries et al. 2003; Staelens et al. 2008).

In the CBM method, the use of WD provides more accurate DD estimate than BD, since the later includes a fraction of DD (coarse particle DD). Since WD was not sampled at CB and CA their BD value was corrected by the ratio WD/BD from LC and TC (0.76 and 0.69 for NH_4^+ and 0.65 and

0.67 for NO_3^- at LC and TC respectively). Since the ratio differences between sites were small, we used the averaged WD/BD of the two sites (0.72 and 0.65).

In the CBM, calculations are made on an equivalent basis but we express results in kg N $ha^{-1}y^{-1}$ for comparison with the other methods.

The NH_4^+ and NO_3^- dry deposition values estimated with this method depend on the DDs of base cations which are estimated with the filtering method. The accuracy of the model is affected by analytical errors in base cations and anions. To minimize biases, the analytical accuracy was scrutinized in all WD and TF samples with the protocol of the ICP-Forest Manual (2010), and values differing by >10% of the charge and the conductivity balances were discarded. Another source of uncertainty in the CBM is the efficiency factor of NH_4^+ *vs.* NO_3^- uptake. We have used here a value of xNH_4=6, which is backed up by experimental work in holm oak saplings (Uscola et al. 2014) and also is the one proposed at an European scale (de Vries et al. 2003).

2.5.2 Branch washings

At each site and for rain-free periods of > 7 days distributed along the year in the period June 2011 to June 2013 (Table 2), deposition measurements were made by washing selected holm oak branches: one branch of 20 cm length was cut from the top of 10 selected trees at each site. The branch tips were sealed with Parafilm and carried to the laboratory in sealed plastic bags where they were washed for 3 min with 200 mL distilled water. Bag blanks were also obtained.

The branch exposure was considered to begin at the end of the previous rain producing throughfall. Linear regressions between precipitation and throughfall have been generally used to describe the canopy storage capacity (Zinke 1967). These regressions were explored for the study sites and indicated a storage capacity (in mm) of 2.8 for LC, 1.5 for CB and CA, and 0.9 for TC (with correlation coefficients of 0.98-0.99). Therefore, we considered that rainfalls greater than these quantities were an adequate starting point. In general, previous rainfall was well above the storage amounts (except for two occasions at TC of 1-1.5 mm), as shown in Table 2. Thus, we considered these previous rain amounts to be sufficient to wash previous dry deposition, though we are conscious that deposition obtained from branch washings is probably an overestimation due to the fact that evaporation of intercepted rainfall would leave an ionic residue from the previous rain event. To overcome this pitfall, branches would need to have been washed with distilled water at the onset of each sampling period, but this was not possible at our study sites for technical, logistic and economic reasons. We

are confident that the exposure periods were long enough at all sites to minimise the contribution of the residual previous deposition. In fact, at TC, the exposure time was longer than one month for the periods of low antecedent precipitation (Table 2).

After washing, branches were air-dried and the leaf surface of each branch was obtained from Li-Cor 3100 area-meter measurements. The washing solutions were analysed by ion chromatography (Dionex, Sunnyvale, USA) with the same quality controls as reported above. The N deposition flux to branches was calculated as the product of the NH_4^+ or NO_3^- concentrations in the washing solutions (corrected for blanks) by the volume used (200mL) and divided by the exposure duration (in days) and the projected leaf area (in cm^2) to obtain the daily surface flux deposition to branches. To extrapolate to fluxes to canopy and year, the daily flux to branches was multiplied by each site's LAI and days for the year, and is expressed as kg N ha^{-1} y^{-1}.

2.5.3 Inferential model

The inferential method is based on the assumed steady-state relationship:

$$F_a = V_d * C_a \quad \text{(eq. 2)}$$

where the dry deposition flux (F_a) is a product of the dry deposition velocity (V_d) and the concentration (C_a) of the considered air pollutant (a). It involves the measurements of pollutant air concentrations and modeled V_ds. In our sites, N gas and particle atmospheric measurements were available for the period of the study (only 2012-2013 for particle measurements). However, models for V_d need data of meteorological variables taken at high frequency (Wesely and Hicks 2000), which were not available at the sites. We provide here a preliminary analysis of DD fluxes based on a compilation of V_d values from literature reports from forests studies that have applied the inferential method (Tables 3 and 4).

The different methods applied for the DD estimation are based in different approaches, and each of them has its own particularities and drawbacks, which are briefly examined here. The CBM considers ion exchanges of the N compounds at the leaf surfaces and is based on the equilibrium of charges between all ions reaching the canopy. This method was developed to overcome the difficulties in interpreting TF results derived from the fact that TF includes both deposition and exchanged ions from the canopy. The exchange processes comprise ion diffusion or exchange between the water layer covering the leaves and the apoplast (Bytnerowicz et al. 2015; Padgett et al. 2009). Stomatal and cuticular uptake of some N gases (e.g. HNO_3, NH_3, NO_2)

348 can also occur and modify TF fluxes if they are dissolved in the leaf surface or within stomata
349 (Draaijers et al. 1997; Gessler et al. 2002; Bytnerowicz et al. 2015). On the other hand, the CBM
350 method needs to take into account an efficiency factor of NH_4^+ *vs.* NO_3^- uptake (xNH_4= moles of
351 NH_4^+ taken up for each NO_3^- mol). To account for this, we took advantage of an experimental
352 work of N uptake with holm oaks to better attune this value to the studied species (Uscola et al.
353 2014). Interestingly, the value obtained (xNH_4 =6) was the same to that proposed for European
354 forests (de Vries et al. 2003). Although the CBM approach has the important drawback that
355 analytical errors will propagate through the enchained calculations, it is a method widely in use
356 (Thimmonier et al. 2005; Balestrini et al. 2007; Staelens et al. 2008; Adriaenssens et al. 2012;
357 Drapelova 2013) and it is more appropriate to describe the ongoing canopy processes than the
358 assumption that nTF is equivalent to DD for N compounds.
359
360 The branch washing method is also a direct method for measurement of dry deposited material
361 to leaf surfaces. By excluding wet episodes, this approach expects to reduce cuticular exchanges,
362 which are favored by the dissolution of compounds to water films, though some uptake or
363 transformation of the deposited chemical species may also occur (Hanson and Lindberg 1991).
364 This method is similar to TF measurements, the most important difference being its more
365 episodic sampling nature (4-5 periods during the year vs. weekly/biweekly sampling for
366 throughfall) and the avoidance of wet deposition.
367
368 The inferential method applied here relied on measured air concentrations and V_d from the
369 bibliography. While differences between sites in V_d are expected due to variation of
370 meteorological and canopy structure factors, the V_ds of the different constituents were quite
371 consistent over the range of forests surveyed (Table 3), so we considered the average V_d from
372 the compiled data in Table 3 for flux calculations presented in Table 4. The inferential method is
373 the sole method of those examined that allows to differentiate gas and particle deposition since
374 both TF and BW give the sum of N oxidized and N reduced compounds in the form of NH_4^+-N
375 and NO_3^--N fluxes. For comparison with TF and BW, inferential calculations have considered the
376 sums of HNO_3 + pNO_3^- (N oxidized), NH_3 + pNH_4^+ (N reduced), and as such are presented in Table
377 4.
378

379 **3 Results and discussion**

380 3.1 Water fluxes

Rainfall amount differed markedly between sites, with TC being the driest location (Fig. 2). Precipitation was very variable between years, particularly at CA and TC where the second year experienced a wetter spring and winter. All sites, except CA, showed a seasonal pattern with spring and autumn receiving significantly higher precipitation than winter and summer (Fig. 2). At CA, higher precipitation in the 2012-2013 winter resulted in the average winter precipitation not significantly differing from that of spring and autumn.

Differences in seasonal and total rainfall in the study sites are explained by the climatic characteristics of the Iberian Peninsula and are in accordance with the precipitation pattern of the Mediterranean climate in this region characterized by wet springs and autumns (Rodriguez-Puebla et al. 1998). TC, located at the center of the Iberian Peninsula is under a continental Mediterranean climate, drier and colder than at the coastal Mediterranean region. The northern CA site is affected by the passage of low pressure fronts from the north-northwest that brings precipitation from the Atlantic. The frequency of these fronts is higher in winter and spring, thus this site differs in the seasonal precipitation from the other sites which present dry winters (Fig. 2).

Throughfall was highly correlated with rainfall (r^2= 0.97; 0.98, 0.96 and 0.73 for LC, CB, CA and TC respectively). Similarly to rainfall, significant differences with higher throughfall in the wet spring and autumn seasons were found at all sites, except at CA.

The difference between precipitation in the open (BD collectors) and throughfall (TF) indicates the water quantity intercepted by the canopies (In). The lowest interception was at TC (Table 5), and this is attributed to the open structure of this site (Table 1) that will allow for direct passage of rainfall to the soil thus avoiding evaporation on the canopy. In a revision of rainfall partitioning in Mediterranean forests and shrubs, lower interception was found for forests with lower leaf area index, basal area, and height (Llorens and Domingo 2007), though no relationship with tree density was observed.

3.2 Nitrogen fluxes in wet deposition and throughfall

Annual wet deposition and throughfall fluxes for NH_4^+-N and NO_3^--N and the sum of inorganic N (DIN) are shown in Table 6, where the percent contribution of NO_3^--N to DIN is also indicated. Wet deposition ranged between 0.54 and 3.8 kg N $ha^{-1}y^{-1}$ for NH_4^+-N and between 0.65 and 2.1 kg N $ha^{-1}y^{-1}$ for NO_3^--N , with the lowest deposition being at TC for both components, and the highest at CA and LC for NH_4^+-N and NO_3^--N, respectively. The high NH_4^+-N deposition at CA can be attributed to the intensive agriculture activities in surrounding fields where ammonium

nitrate and urea fertilizers are regularly applied in winter and spring (Izquieta-Rojano et al. 2016). Wet deposition at LC can be compared to previous wet deposition measurements in 2002-2003 and 2009-2010 from the work of Izquierdo and Avila (2012). In the period of the present study, NH_4^+-N was 33% and 40% lower respectively than these previous periods and NO_3^--N was 13% and 25% lower. An analysis of the trends in atmospheric deposition at the LC site for the last 30 years only found a significant declining trend for NO_3^- concentrations, not for N fluxes in both forms (Aguillaume et al. 2016). This indicates that the study period had a particularly low wet N deposition, probably a result of low NH_4^+ and NO_3^- concentrations and lower precipitation than the compared periods. Of the two sites located in NE Spain, and contrary to expectations, the semi-urban site close to Barcelona (CB) had lower wet deposition of N compounds than the more remote site LC. Part of this difference may stem from lower precipitation at CB (Table 5), since the difference in VWM concentration in WD between these sites was small (WVM of 17 and 14 $\mu eq\ L^{-1}$ for NH_4^+ and 16.1 and 15.9 $\mu eq\ L^{-1}$ for NO_3^- at LC and CB respectively). This result indicates that the LC site, which has been taken as a rural background station, was also affected by urban and industrial pollution from the Barcelona metropolitan area, as also found for aerosols (Pey et al. 2008; Pérez et al. 2009) and HNO_3 gases (García-Gómez et al. 2016a). In fact, Aguillaume et al. (2016) showed that NO_3^- concentrations in bulk deposition at this site was mainly explained (r^2=0.85) by NO_x air concentrations in Barcelona city center, national NO_2 Spanish emissions and the amount of precipitation.

The site in central Spain had the lowest wet deposition inputs owing to the combination of low precipitation at this site and lower rain concentrations due the predominant air mass fluxes coming from low polluted areas in the west and the Atlantic Ocean (Salvador et al. 2011).

DIN wet deposition ranged between 1.2 kg N $ha^{-1}y^{-1}$ at TC and 5.8 kg N $ha^{-1}y^{-1}$ at CA, and the northeastern sites had an intermediate value of 3-4 kg N $ha^{-1}y^{-1}$ (Table 6). NH_4^+-N and NO_3^--N showed a similar contribution to DIN, except at the agriculture-affected CA site, where NH_4^+-N was dominant (Table 6).

Throughfall NO_3^--N deposition was higher (range 1.8 and 5.4 kg N $ha^{-1}y^{-1}$) than NH_4^+-N (range 0.5 and 3.1 kg N $ha^{-1}y^{-1}$, Table 6). Similarly to WD, the lowest TF deposition for both N components was found at TC, while CA and CB showed the highest NH_4-N TF and NO_3-N TF fluxes, respectively. The DIN flux that reached the soil varied between 2 and 7.4 kg N $ha^{-1}y^{-1}$ in the studied sites, with the lowest N input in TC. In a study of ICP-Forest plots at a European scale, elevated nitrate concentrations in seepage water were found over a threshold of 7 kg N $ha^{-1}y^{-1}$ in DIN TF input (De Schrijver et al. 2007). In our study, DIN TF inputs at the site with high

446 agricultural influence (CA) and the one close to Barcelona (CB) were above the proposed
447 threshold value, and might be the more susceptible to soil solution N enrichment. Consistently
448 with the above study, the LC site which receives a TF DIN input lower than the proposed
449 threshold retains N the catchment scale, although the ratio N export/N input was found to
450 increase in recent years (Aguillaume et al. 2016).

451 Forest canopies play a significant role in altering deposition of N compounds, either because of
452 its filtering effect to capture dry deposition or because of their capacity to retain, take up or
453 transform N species (Sparks 2009). Net throughfall fluxes (nTF), the difference between TF minus
454 BD, indicate the net contribution of the canopy to below canopy fluxes. For inorganic N,
455 lixiviation may be negligible (Rodrigo and Avila 2002), therefore positive nTF fluxes indicate that
456 dry deposition is higher than canopy retention, while negative values indicate that the canopy
457 retains more than the dry deposited amounts.

458 Net throughfall was positive for NO_3^--N but negative for NH_4^+-N at LC and CA (Table 6), suggesting
459 that reduced N was more efficiently retained in the canopy than the oxidized forms of N, as it
460 has been shown with ^{15}N labeled rain experiments (Boyce et al. 1996), by surrogate surface
461 washings (Ignatova and Dambrine 2000), and as is suggested by differences in N gas
462 concentrations in the open and below the forest at the study sites (García-Gómez et al. 2016a).
463 A negative NH_4^+-N nTF flux was found at the agriculture site receiving the highest NH_4^+-N wet
464 deposition, thereby suggesting a strong ability of this holm oak forest to retain high N inputs. A
465 similar nTF value was found at LC, though this site received 40% less NH_4^+-N inputs (Table 6).

466 3.3 Dry deposition estimation

467 In this work, three approaches have been used to derive DD: 1) canopy budget model (CBM), 2)
468 branch washing (BW), and 3) inferential model with V_ds obtained from forest studies (IM, Table
469 4). The resulting estimates from the three methods are shown in Table 7.

470 The different approaches show fairly consistent estimates given the various assumptions in the
471 different methods. Relative differences between methods were more pronounced in TC, the site
472 with lower DD: differences between the lowest and highest values were approximately 70-80%.
473 However, in absolute terms, these differences were of 1 kg N $ha^{-1}y^{-1}$ for NH_4^+-N and 3 kg N $ha^{-1}y^{-1}$ for NO_3^--N, similar to differences at the other sites (except for NO_3^--N at CA). Estimated DD
475 values with the three approaches matched better for N reduced than for N oxidized deposition
476 (Table 7). For N oxidized deposition, a good match was observed between CBM and IM methods,
477 but BW estimates were about double (CA, TC) or 40% greater (LC) than the other estimates.

When considering DIN dry deposition from the applied methods, the range of estimates was of 6 to 11 kg N $ha^{-1}y^{-1}$ for the NE Spain sites, 8-14 kg N $ha^{-1}y^{-1}$ for CA and 1.5 to 6 kg N $ha^{-1}y^{-1}$ for TC (Table 7; Fig. 3). The range of variation of these estimates (1.5 to 4) is similar to that reported in a study that compared 4 inferential models in a network of 55 monitoring sites in Europe, in which between-model differences were of a factor 2–3 (Flechard et al. 2011).

Considering the averages between minimum and maximum estimates (Table 8), the contribution of DD to TD was of 51 to 67% for reduced-N and 72 to 75% of oxidized-N compounds. DIN dry deposition contributed between 65 to 71% to total DIN deposition, indicating the importance of taking into account the dry deposition flux when tackling with the effects of N deposition to ecosystems.

In various locations along the Levantine coast of Spain, dry deposition percentages were similar to the values in this study: 58% for N reduced and 60% for N oxidized forms (Avila and Rodà 2012). However, in oak (*Quercus pyrenaica*) forests in subhumid western Spain, the DD contribution was lower (10-20% and 30-40% for reduced and oxidized-N respectively (Moreno et al. 2001); though differences in procedure also may have a role since DD was estimated with the regression method of Lovett and Lindberg (1984).

In the USA, a recent study based in 37 localities has reported a substantial decline in oxidized-N emissions that leads to an ammonium-dominated atmospheric composition. Under these conditions, dry deposition of NH_3 has been found to play a key role in N deposition, contributing 19-65% of total deposition (Li et al. 2016). In agricultural and rural locations in northern China, reduced-N contributed similarly (28-60%) while oxidized-N represented only 13-30% of total N deposition (Pan et al. 2012). In contrast, in our sites DD of oxidized compounds was the dominating deposition flux (Table 8). This agrees with the fact that NO_x emissions in Spain are about triple of NH_3 emissions and only started to decline since 2005 (Aguillaume et al. 2016).

3.4 Canopy uptake

The ranges of canopy uptake (calculated as TD minus TF) for the different N compounds are shown in Table 9 and Fig. 3. It is seen that N is retained either in the oxidized or reduced forms: the values were similar in both N forms at LC, but were higher in the NH_4^+-N form at CB and CA, and in the NO_3^--N form at TC. Many findings derived from labeled ^{15}N experiments have shown retention and stomatal uptake and transformation of dissolved and gaseous N species on foliage (Garten and Hanson 1990; Gaige et al. 2007). Microbial transformations of N deposition can also

511 alter the N forms, transforming inorganic N to organic forms (Cape et al. 2001, Neff et al. 2002)
512 that may explain part of the inorganic N reduction. On the other hand, nitrification in the canopy
513 has been shown to be of significance in beech forests (Guerrieri et al. 2015), a process that may
514 also account for part of the NH_4^+ "retention" in the canopy
515
516 Canopy uptake was highly correlated with wet deposition (r^2=0.992; p<0.001) and total DIN
517 inputs (Fig. 4), indicating that these forest canopies have not reached a limit in their capacity to
518 take up N from the atmosphere in the wet form and the sum of wet and dry (assuming negligible
519 changes due to DON formation). The agricultural site, receiving the highest deposition fluxes
520 (14-20 kg N $ha^{-1}y^{-1}$) also presented the highest NCU values (7-12 kg N $ha^{-1}y^{-1}$ Table 9, Fig. 4),
521 most of this uptake (60%) being in the reduced N form.
522
523 The results of this study indicate that the holm oaks canopies can retain an important part of
524 the incoming N deposition, thus reducing the direct impact of N deposition to soils. The total N
525 inputs to these forests (assuming a range of dry deposition estimates obtained with three
526 different approaches) were between 3 and 20 kg N $ha^{-1}y^{-1}$. Recent studies in these sites indicate
527 that DON would add around 3 kg N $ha^{-1}y^{-1}$ in bulk deposition (Izquieta-Rojano et al 2016).
528 Therefore, the total N input to these holm oak forests can be framed in 20-23 kg N $ha^{-1}y^{-1}$
529 exceeding the critical loads values proposed for sclerophylous forests (15-17 kg N $ha^{-1}y^{-1}$,
530 Bobbink et al. 2010) except at a lower impacted site in central Spain. The long term effects of
531 these continued N inputs and their evolution as N emissions change in recent years has not been
532 yet fully addressed and may deserve attention given its potential impact on soil chemistry, water
533 quality, forest functioning and plant biodiversity.

534

535 Conclusions

536 Atmospheric N deposition to 4 sites in Spain (one affected by an agricultural environment, two
537 by big cities and one as rural background) was determined, distinguishing the wet and dry
538 deposition pathways. To estimate DD, three different methods were applied and compared: a
539 canopy budget model, a branch washing method and the inferential method with V_ds obtained
540 from bibliographical references of forest studies. Higher consistency between methods was
541 found for reduced N tan for oxidized N. The branch washing method tended to produce the
542 highest estimates. The site receiving the lowest dry deposition presented the highest relative
543 differences between minimum and maximum estimates, but in absolute terms, differences were

similar to the other sites. Taking the average DD from the various methods, DD represented 51-67% (reduced N) and 72-75% (oxidized N) of total reduced and oxidized N deposition. The canopies retained both NH_4^+-N and NO_3^--N, with the agricultural site and the urban site close to Barcelona retaining more in the reduced than the oxidized form. A very good correlation (r=0.92 and 0.99) between N deposition and canopy uptake indicated that holm oak forests in Spain retain N deposition inputs up to 17.5 kg N $ha^{-1}y^{-1}$. **The uptake efficiency (N taken up in the canopy related to N deposition) was higher at the agricultural and rural sites (50-60%) compared to the site close to Barcelona (20-35%), while for Madrid, great differences in DD estimation precluded this analysis. This result points to a decreasing N removal capacity in the canopies of peri-urban forests that may lead to higher N impacts to the soil and soil waters in the future.**

Acknowledgements

The financial support from the Spanish Government projects EDEN (CGL2009-13188-C03-01/02/03) is fully acknowledged. This research was also funded by the project from Autonomous Government of Madrid AGRISOST-CM (P2013/ABI-2717) and by the European Projects ECLAIRE (FP7-ENV-2011/282910) and Life RESPIRA (LIFE13 ENV/ES/000417). CIEMAT work in this study was partially supported by an agreement between the Spanish Ministry of Agriculture, Food and Environment and CIEMAT on Critical loads and levels. The utilization of Tres Cantos monitoring site was possible thanks to an agreement between CIEMAT and Ayuntamiento de Madrid.

565 **References**

566 Adon M, Galy-Lacaux C, Delon C et al. (2013) Dry deposition of nitroben compounds (NO2,
567 HNO3, NH3), sulfur dioxide and ozone in west and central African ecosystems using the
568 inferential method. Atmospheric Chemistry and Physics 13: 11351-11374.

569 Adriaenssens S, Staelens J, Wuyts K et al. (2011) Foliar nitrogen uptake from wet deposition and
570 the relation with leaf wettability and water storage capacity. Water, Air, and Soil Pollution 219:
571 43-57.

572 Adriaenssens S, Hansen K, Staelens J et al. (2012) Throughfall deposition and canopy exchange
573 processes along a vertical gradient within the canopy of beech (*Fagus sylvatica* L.) and Norway
574 spruce (*Picea abies* (L.) Karst). The Science of the Total Environment 420: 168-182.

575 Aguillaume L, Izquieta-Rojano S, García-Gómez H, Elustondo D, Santamaría J.M, Alonso R, Avila
576 A (2017) Dry deposition and canopy uptake in Mediterranean holm-oak forest estimated with a
577 canopy budget: a focus on N estimations. Atmospheric Environment 152: 191-200.
578
579 Aguillaume L, Rodrigo A, Avila A (2016) Long-term effects of changing atmospheric pollution on
580 throughfall, bulk deposition and streamwaters in a Mediterranean forest. The Science of the
581 Total Environment 544: 919-928.
582
583 Ariño A, Gimeno B, de Zabalza AP, Ibáñez R, Ederra A, Santamaría J (2000) Influence of nitrogen
584 deposition on plant biodiversity at Natura 2000 sites in Spain. Nitrogen Deposition and Natura
585 2000: pp140.

586 Avila A, Molowny-Horas R, Gimeno BS, Peñuelas J (2010) Analysis of Decadal Time Series in Wet
587 N Concentrations at Five Rural Sites in NE Spain. Water, Air and Soil Pollution 207: 123-138.

588 Avila A, Rodà F (2012) Changes in atmospheric deposition and streamwater chemistry over 25
589 years in undisturbed catchments in a Mediterranean mountain environment. The Science of the
590 Total Environment 434: 18-27.

591 Balestrini R, Tagliaferri A (2001) Atmospheric deposition and canopy exchange processes in
592 alpine forest ecosystems (northern Italy). Atmospheric Environment 35: 6421-6433.

593 Balestrini R, Arisci S, Brizzio MC et al. (2007) Dry deposition of particles and canopy exchange:
594 comparison of wet, bulk and throughfall deposition at five forest sites in Italy. Atmospheric
595 Environment 41: 745-756.

596 Bobbink R, Hicks K, Galloway J, Spranger T et al. (2010) Global assessment of nitrogen deposition
597 effects on terrestrial plant diversity: a synthesis. Ecological Applications 2: 30-59.

598 Boyce RL, Friedland AJ, Chamberlain CP, Poulson SR (1996) Direct canopy nitrogen uptake from
599 N15 labeled wet deposition by mature red spruce. Canadian Journal Forest Research 26: 1539-
600 1547.

601 Bytnerowicz A, Johnson RF, Zhang L et al. (2015) An empirical inferential method of estimating
602 nitrogen deposition to Mediterranean-type ecosystems: the San Bernardino Mountains case
603 study. Environmental Pollution 203: 69-88.

604

605 Bytnerowicz A, Miller P, Olszyk DM, Dawson PJ, Fox CA (1987) Gaseous and particulate air
606 pollution in the San Gabriel Mountains of southern California. Atmospheric Environment 21,
607 1805-1814.

608 Bytnerowicz A, Sanz MJ, Arbaugh MJ, Paddgett PE, Jones DP, Davila A (2005) Passive sampler for
609 monitorin ambient nitric acid (HNO3) and nitrous acid (HNO2) concentrations. Atmospheric
610 Environment 39: 2655-2660.

611 Cape JN, van Dijk N, Tang YS (2009) Measurements of dry deposition to bulk precipitation
612 collectors using a novel flushing sampler. Journal Environmental Monitoring 11: 353-358.

613 De Schrijver A, Geudens G, Augusto L, Staelens J, Mertens J, Wuyts K, Gielis L, Verheyen K (2007)
614 The effect of forest type on throughfall deposition and seepage flux: a review. Oecologia 153:
615 663-674.

616 De Vries W, Vel E, Reinds G et al. (2003) Intensive monitoring of forest ecosystems in Europe:
617 1. Objectives, set-up and evaluation strategy. Forest Ecology and Management 174: 77-95.

618 Dentener F et al. (+16 co-authors) (2006) Emissions of primary aerosol and precursor gases in
619 the years 2000 and 1750 prescribed data-sets for AeroCom. Atmospheric Chemistry and Physics
620 6: 4321-4344.

621 Draaijers G, Erisman JW (1995) A canopy budget model to assess atmospheric deposition from
622 throughfall measurements. Water, Air and Soil Pollution 85: 2253-2258.

623 Draaijers G, Erisman JW, van Leeuwen NFM et al. (1997) The impact of canopy exchange on
624 differences observed between atmospheric deposition and throughfall fluxes. Atmospheric
625 Environment 31: 387-397.

626 Drapelova I (2013) Evaluation of deposition fluxes in two mountain Norway spruce stands with
627 different densities using the extended Canopy Budget Model. Journal of Forest Science 59: 72-
628 86.

629 Duyzer JH, Dorsey JR, Gallagher MW, Pilegaard K, Walton S (2004) Oxidized nitrogen and ozone
630 interaction with forests. II: Multi-layer process-oriented modelling results and a sensitivity
631 study for Douglas fir. Quarterly Journal of the Royal Meteorological Society, 130: 1957-1971.
632
633 Enders G, Teichmann U (1986) GASDEP—Gaseous Deposition Measurements of SO2, NOx, and
634 O3 to a spruce stand: Conception, instrumentation, and first results of an experimental
635 project. In: Georgii HW (Ed.) Atmospheric pollutants in forest areas. Springer, Netherlands.
636
637 Endo T, Yagoh H, Sato K, Matsuda K, Hayashi K, Noguchi I, Sawada K (2011) Regional
638 characteristics of dry deposition of sulfur and nitrogen compounds at EANET sites in Japan from
639 2003 to 2008. Atmospheric Environment 45: 129-1267.

640 Erisman J W, Beier C, Draaijers G, Lindberg S (1994). Review of deposition monitoring methods.
641 Tellus 46: 79-93.

642 Erisman JW, Mols H, Fonteijn P et al. (2003) Field intercomparison of precipitation
643 measurements performed within the framework of the pan European intensive monitoring
644 program of EU/ICP forest.Environmental Pollution 1215: 139-155.
645

Fenn ME, Ross CS, Schilling SL et al. (2013) Atmospheric deposition of nitrogen and sulfur and preferential consumption of nitrate in forests of the Pacific Northwest, USA. Forest Ecology and Management 302: 240-253.

Flechard CR, Nemitz E, Smith RI et al. (2011) Dry deposition of reactive nitrogen to European ecosystems: a comparison of inferential models across the NitroEurope network. Atmospheric Chemistry and Physics 11: 2703-2728.

Gaige E, Dail D, Hollinger D et al. (2007) Changes in canopy processes following whole-forest canopy nitrogen fertilization of a mature spruce-hemlock forest. Ecosystems 10: 1133-1147.

Gallagher M, Fontan J, Wyers P, Ruijgrok W et al. (1997) Atmospheric particles and their interactions with natural surfaces: In: Slanina S (Ed.) Biosphere-Atmosphere Exchange of Pollutants and Trace Substances. Springer, pp 45-92.

García-Gómez H (2016). Atmospheric concentration and deposition of reactive nitrogen in Spanish forests of *Quercus ilex*. PhD dissertation. Escuela Técnica Superior de Ingenieros Agrónomos. Universidad Politécnica de Madrid.

García-Gómez H, Garrido J, Vivanco M et al. (2014) Nitrogen deposition in Spain: Modeled patterns and threatened habitats within the Natura 2000 network. The Science of the Total Environment 485: 450-460

García-Gómez H, Aguillaume L, Izquieta-Rojano S et al. (2016a) Atmospheric pollutants in peri-urban forests of *Quercus ilex*: evidence of pollution abatement and threats for vegetation. Environmental Science and Pollution Research 23: 6400-6413.

García-Gómez H, Izquieta-Rojano S, Aguillaume L et al. (2016b) Atmospheric deposition of inorganic nitrogen in Spanish forests of *Quercus ilex* measured with ion-exchange resins and conventional collectors. Environmental Pollution, dx.doi.org/10.1016/j.envpol.2016.06.027.

Garten CT jr, Hanson PJ (1990) Foliar retention of 15N.nitrate and 15-N ammonium by red maple (Acer rubrum) and white oak (*Quercus alba*) leaves from simulated rain. Environmental Experimental Botany 30: 333-342.

Guerrieri R, Vanguelova E , Michalski G, Heaton TH, Mencuccini M (2015) Isotopic evidence for the occurrence of biological nitrification and nitrogen deposition processing in forest canopies. Global Change Biology 21: 4613-4626.

Geßler A, Rienks M, Rennenberg H (2002) Stomatal uptake and cuticular adsorption contribute to dry deposition of NH3 and NO2 to needles of adult spruce (*Picea abies*) trees. New Phytologist 156: 179-194.

Harrison AF, Schulze ED, Gebauer G, Bruckner G (2010) Canopy uptake and utilization of atmospheric pollutant nitrogen. In: Schulze ED (Ed.) Carbon and nitrogen cycling in European forest ecosystems. Ecological Studies, 142. Springer, Berlin.

Hicks BB, Hosker RP, Meyers TP, Womack JD (1991) Dry deposition inferential measurement techniques- I. Design and tests of a prototype meteorological and chemical system ofr determining dry deposition. Atmospheric Environment 25: 2345-2359.

Holland EA, Braswell BH, Sulzman J, Lamarque JF (2005) Nitrogen deposition onto the United States and Western Europe: synthesis of observations and models. Ecological Applications 15: 38–57.

691 Hosker RP, Lindberg SE (1982) Review: Atmospheric deposition and plant assimilation of gases
692 and particles. Atmospheric Environment 5: 889-910.

693 Horváth L (2003) Dry deposition velocity of PM2.5 ammonium sulfate particles to a Norway
694 spruce forest on the basis of S and N balance estimations. Atmospheric Environment 37: 4419-
695 4424.

696 ICP-Forests Manual (2010) Manual on methods and criteria for harmonized sampling,
697 assessment, monitoring and analysis of the effects of air pollution on forests. UNECE ICP Forests
698 Programme Coordinating Centre, Hamburg.

699 Ignatova N, Dambrine E (2000) Canopy uptake of N deposition in spruce (*Picea abies* L. Karst)
700 stands. Annals of Forest Science 57: 113-120.

701 Izquierdo R, Avila A (2012) Comparison of collection methods to determine atmospheric
702 deposition in a rural Mediterranean site (NE Spain). Journal of Atmospheric Chemistry 69: 351-
703 368.

704 Izquieta-Rojano S, García-Gomez H, Aguillaume L et al. (2016) Throughfall and bulk deposition
705 of dissolved organic nitrogen to holm oak forests in the Iberian Peninsula: Flux estimation and
706 identification of potential sources. Environmental Pollution 210: 104-112.

707 Johnson DW, Lindberg SE (1992) Atmospheric deposition and forest nutrient cycling: a
708 synthesis of the integrated forest study. Vol. 91. Springer, Berlin.
709
710 Kalina MF, Stopper S, Zambo E, Puxbaum H (2002) Altitude-dependent wet, dry and occult
711 nitrogen deposition in an Alpine region. Environmental Science Pollution Research 2: 16–22.

712 Kelly VR, Weathers KC, Lovett GM, Likens GE (2012) A comparison of two collectors for
713 monitoring precipitation chemistry. Water Air and Soil Pollution 223: 951-954.

714
715 Llorens P, Domingo F (2007) Rainfall partitioning by vegetation under Mediterranean
716 conditions. A review of studies in Europe. Journal of Hydrology 335: 37-54.
717
718 Li Y, Schichtel BA, Walker JT et al. (2016). Increasing importance of deposition of reduced
719 nitrogen in the United States. Proceedings of the National Academy Sciences USA ,
720 doi: 10.1073/pnas.1525736113
721
722 Lindberg SE, Lovett GM, Meiwes KJ (1987) Deposition and forest canopy interactions of airborne
723 nitrate. In: Hutchinson TC, Meema KM (Eds.) Effects of atmospheric pollutants on forests,
724 wetlands and agricultural ecosystems. Springer, Berlin.

725 Lovett G, Lindberg S (1984) Dry deposition and canopy exchange in a mixed oak forest as
726 determined by analysis of throughfall. Journal of Applied Ecology 21: 1013-1027.

727 Lovett G, Lindberg S (1986) Dry deposition of nitrate to a deciduous forest. Biogeochemsitry 2:
728 137-148.

729 MeyersTP, Hicks BB, Hosker RP, Womack JD, Satterfield LC (1991) Dry deposition inferential
730 measurement techniques – II Seasonal and annual deposition rates of sulfur and nitrate.
731 Atmospheric Environment 25A: 2361-2370.

732 Meyers TP, Huebert BJ, Hicks BB (1989) HNO3 deposition to a deciduous forest. Boundary-
733 Layer Meteorology 49: 395-410.

734

735 Moreno G, Gallardo JF, Bussotti F (2001) Canopy modification of atmospheric deposition in
736 oligotrophic *Quercus pyrenaica* forests of an unpolluted region (central-western Spain). Forest
737 Ecology and Management 149: 47-60.

738 Müller H, Kramm G, Meixner F, Dollard G.J, Fowler D (1993) Determination of HNO3 dry
739 deposition by modified Bowen ratio and aerodynamic profile techniques. Tellus 45: 346–67.

740 Myers N, Mittermeier RA, Mittermeier C.G, Da Fonseca G.A, Kent J (2000) Biodiversity hotspots
741 for conservation priorities. Nature 403: 853-858.

742 Ochoa-Hueso R, Arróniz-Crespo M, Bowker MA et al. (2014) Biogeochemical indicators of
743 elevated nitrogen deposition in semiarid Mediterranean ecosystems. Environmental Monitoring
744 and Assessment 186: 5831-5842.

745 Padgett PE, Cook H, Bytnerowicz A, Heath RL (2009) Foliar loading and metabolic assimilation of
746 dry deposited nitric acid air pollutants by trees. Journal Environmental Monitoring 11: 75-84.

747 Pan YP, Wang YS, Tang GQ, Wu D (2012) Wet and dry deposition of atmospheric nitrogen at ten
748 sites in Northern China. Atmospheric Chemistry and Physics 12: 6515-6535.

749 Parker G (1983) Throughfall and stemflow in the forest nutrient cycle. Advances in Ecological
750 Research 13: 57-133.
751
752 Peñuelas J, Filella I (2001) Herbaria century record of increasing eutrophication in Spanish
753 terrestrial ecosystems. Global Change Biology 7: 427-433.

754 Pérez N, Pey J, Castillo S, Viana M, Alastuey A, Querol X (2008) Interpretation of the variability
755 of levels of regional background aerosols in the Western Mediterranean. Science of the Total
756 Environment 407: 527-540.

757 Pey J, Pérez N, Castillo S et al. (2009) Geochemistry of regional background aerosols in the
758 Western Mediterranean. Atmospheric Research 94: 422-435.

759 Puxbaum H, Gregori M (1998). Seasonal and annual deposition rates of sulphur, nitrogen and
760 chloride species to an oak forest in north-eastern Austria (Wolkersdorf, 240 m ASL).
761 Atmospheric Environment 32: 3557-3568.
762
763 Querol X, Alastuey A, López-Soler A et al. (1998) Seasonal evolution of atmospheric suspended
764 particles around a coal-fired power station: chemical characterization 32: 719-731.
765
766 Rodrigo A, Avila A (2002) Dry deposition to the forest canopy and surrogate surfaces in two
767 Mediterranean holm oak forests in Montseny (NE Spain). Water, Air and Soil Pollution 136: 269-
768 288.

769 Rodriguez-Puebla C, Encinas A, Nieto S, Garmendia J (1998) Spatial and temporal patterns of
770 annual precipitation variability over the Iberian Peninsula. International Journal of Climatology
771 18: 299-316.

772 Sanz M, Carratalá A, Gimeno C, Millán M (2002) Atmospheric nitrogen deposition on the east
773 coast of Spain: relevance of dry deposition in semi-arid Mediterranean regions. Environmental
774 Pollution 118: 259-272.

775 Salvador P, Artiñano B, Viana MM et al. (2011) Spatial and temporal variations in PM10 and
776 PM2.5 across Madrid metropolitan area in 1999-2008. Urban Environmental Pollution 4: 198-
777 208.

778 Schmitt M, Thöni L, Waldner P, Thimonier A (2005) Total deposition of nitrogen on Swiss long-
779 term forest ecosystem research (LWF) plots: comparison of the throughfall and the inferential
780 method. Atmospheric Environment 39: 1079-1091.

781 Sparks JP (2009). Ecological ramifications of the direct foliar uptake of nitrogen. Oecologia 159:
782 1-13.

783 Staelens J, Houle D, De Schrijver A, Neirynck J, Verheyen K (2008) Calculating dry deposition and
784 canopy exchange with the canopy budget model: review of assumptions and application to two
785 deciduous forests. Water, Air and Soil Pollution 191: 149-169.

786 Stachurski A, Zimka JR (2002) Atmospheric deposition and ionic interactions within a beech
787 canopy in the Karkonosze Mountains. Environmental Pollution 1118: 75-87.

788 Sutton MA, Howard CM, Erisman JW, et al. (2011) The European Nitrogen Assessment: Sources,
789 Effects and Policy Perspectives, Cambridge University Press.

790 Thimonier A (1998) Measurement of atmospheric deposition under forest canopies: some
791 recommendations for equipment and sampling design. Environmenta Monitoring Assessment
792 52: 353-387.

793 Thimonier A, Schmitt M, Waldner P, Rihm B (2005) Atmospheric deposition on Swiss long-term
794 forest ecosystem research (LWF) plots. Environmental Monitoring and Assessment 104: 81-118.

795 Ulrich B (1983) Interaction of forest canopies with atmospheric constituents: SO2, alkali and
796 earth alkali cations and chloride. In: Ulrich B and Pankrath J (Eds.) Effects of Accumulation of
797 Air Pollutants in Forest Ecosystems. Springer pp. 33-45.

798 Uscola M, Villar-Salvador P, Oliet J, Warren CR (2014) Foliar absorption and root translocation
799 of nitrogen from different chemical forms in seedlings of two Mediterranean trees.
800 Environmental and Experimental Botany 104: 34-43.

801 Wesely ML, Hicks BB (2000) A review of the current status of knowledge on dry deposition.
802 Atmospheric Environment 34: 2261-2282.
803
804 Zhang L, Cet R, O'Brien JM, Mihele C, Liang Z, Wiebe A (2009) Dry deposition of individual
805 nitrogen species at eight Canadian rural sites. Journal Geophysical Research 114 doi:
806 10.1029/2008JD010640.
807
808 Zhang G, Zeng GM, Jiang YM, et al. (2006) Effects of weak acids on canopy leaching and uptake
809 processes in a coniferous-deciduous mixed evergreen forest in central-south China. Water, Air
810 and Soil Pollution 172: 39-55.

811 Zinke PJ (1967) Forest interpretation studies in the United States. In: Sopper WE and Lull HE
812 (eds.) International Symposium on Forest Hydrology. Pergamon Press, Oxford, pp 137-161.

813

814

Table 1. Study site characteristics, climatic features, forest stand parameters, atmospheric information and air quality at the study sites. Climate and pollutant data are mean values for the study period.

		LC	CB	CA	TC
Study site characteristics	Aspect	SE	NE	SE	S
	Distance to the sea (km)	27	11	80	310
Climatic parameters	Climate	Mediterranean	Mediterranean	Mediterranean continental with oceanic influence	Mediterranean continental
	Mean annual Temperature (ºC)	9.0	15.1	12.6	14.4
	Mean annual Rainfall (mm y^{-1})	938	723	786	343
Stand parameters	Leaf area index ($m^2 \cdot m^{-2}$)	6.1	4.7	5.3	3.0
	Number of trees·ha^{-1}	2571	1429	1760	491
	Mean diameter at breast high (cm)	13.0	12.6	16.1	41
Air Quality	HNO_3 (μg m^{-3})	3.3	2.7	2.3	1.5
	NO_2 (μg m^{-3})	4.3	16.2	10.6	11.1
	NH_3 (μg m^{-3})	0.7	1.0	2.5	0.7
	PM_{10} (μg m^{-3})	18.0	-	26.9	23.0

825 **Table 2**. Exposure period for the branch washing experiment at the study sites. Final date
826 corresponds to the date of branch washing. Precipitation during 1 to 2 days previous to the
827 onset of the experiment is also indicated.

828

Flux	Initial date	Final date	Pr. (mm)
LC	13 Jun 2011	29 Jun 2011	34.7
	25 Sept 2011	5 Oct 2011	5.2
	1 Feb 2012	22 Feb 2012	4.6
	6 Aug 2012	24 Aug 2012	18.5
CB	13 Jun 2011	29 Jun 2011	21.2
	25 Sept 2011	5 Oct 2011	4.5
	1 Feb 2012	22 Feb 2012	8.3
	6 Aug 2012	24 Aug 2012	9.1
CA	16 Sept 2011	30 Sept 2011	2.0
	15 Feb 2012	28 Feb 2012	4.3
	20 Jun 2012	28 Jun 2012	13.5
	28 Aug 2012	11 Sept 2012	6.7
TC	7 Jun 2011	28 Jun 2011	18.3
	16 Aug 2011	18 Oct 2011	8.4
	10 Dec 2011	11 Jan 2012	1.0
	20 May 2012	5 Jul 2012	1.5
	4 Apr 2013	24 Apr 2013	24.8

829

830

Table 3. Compilaton of deposition velocities (V_ds in cm sec^{-1}) from dry deposition studies in forests, with specification of forest type, method and study period.

Reference	HNO3	NO2	pNO3	NH3	pNH4	Forest type	Site/Country	period	Method
Meyers et al. (1989)	3.40					Broadleaved	Oak Ridge. Ten.. USA	growing	Flux gradient
Holland et al. (2005)	2.00						USA and West Europe	annual	model
Enders and Teichmann (1986)		2.40				Conifer			Flux gradient
Granat and Johnson (1983)		0.09				Conifer			
Duyzer et al. (2004)		0.15				Conifer			
Puxbaum and Gregori (1998)	2.39	0.26	0.17	0.81	0.17	Broadleaved	NE Austria	annual	big-leaf inferential model
Lovett and Lindberg (1986)	2.00		0.30			Broadleaved	Walker Branch Ten.. USA	growing	regression
Horváth (2003)					0.84	Conifer	NW Hungary	annual	Flux gradient and canopy balance
Zhang et al. (2009)	1.04	0.12	0.13	0.35	0.13	Broadleaved	Canadà	annual	big-leaf inferential model
"	1.40	0.18	0.14	0.44	0.12	Mixed	"	"	
"	1.00	0.16	0.13	0.36	0.10	Conifer	"	"	
Endo et al. (2011)	5.44		0.30	0.70	1.04	forests (from LUC)	EANET sites. Japan	annual	inferential model
Adon et al.(2013)	2.10	0.18		0.80		forests	Africa	annual	inferential model
min	1.00	0.09	0.13	0.35	0.10				
max	5.44	2.4	0.3	0.81	1.04				
mean	2.31	0.44	0.20	0.58	0.40				
st.dev	1.39	0.79	0.08	0.22	0.42				
n	9	8	6	6	6				

Table 4. Annual dry deposition fluxes for N gaseous compounds (in kg $ha^{-1}y^{-1}$), calculated by the inferential method considering the average V_d values in Table 3 for the different N gases.

	LC			CB			CA			TC		
	mean	min	max	mean	min	max	mean	min	max	mean	min	max
HNO3	5.4	1.4	12.6	4.5	1.2	10.3	3.8	1.0	8.8	2.4	0.6	5.6
pNO3	0.1	0.1	0.2	0.1	0.1	0.2	0.3	0.2	0.5	0.2	0.1	0.3
sum Nox	5.6	1.5	12.8	4.6	1.3	10.5	4.1	1.2	9.3	2.6	0.8	5.9
NH3	1.1	1.0	1.6	1.6	1.3	2.3	3.9	3.3	5.6	1.1	0.9	1.6
pNH4	0.5	0.1	1.2	0.5	0.1	1.2	0.8	2.0	0.2	0.6	0.2	1.6
sum Nred	1.6	1.1	2.8	2.0	1.5	3.5	4.7	5.3	5.8	1.7	1.1	3.1
sum DIN	7.2	2.6	15.6	6.6	2.7	14.0	8.8	6.5	15.0	4.3	1.8	9.0

Table 5. Spatial patterns: Basic statistics of water amount in bulk precipitation (BP). throughfall (TF) and Interception (In= BP-TF) in L m^{-2} per period. Number of observations = 49 for LC. 41 for CB 58 for CA and 50 for TC during the period June 2011 to June 2013. Kruskal –Wallis test indicated significant differences (P<0.001) for all the variables. Differences between site pairs by means of a Mann-Whitney test are indicated with letters.

Flux		Mean	Std. Dev.	C.V. (%)
BP	LC	38.3 a	31.1	81.3
	CB	35.3 a	32.3	91.6
	CA	27.1 b	28.1	103.9
	TC	14.0 b	14.5	103.9
TF	LC	28.2 a	25.8	91.3
	CB	24.9 a	24.5	98.3
	CA	22.0 a	24.0	108.8
	TC	11.1 b	11.9	107.4
In	LC	10.1 a	7.09	68.5
	CB	10.4 a	11.1	110.5
	CA	5.1 b	5.08	99.8
	TC	2.9 b	3.0	103.7

Table 6. Annual wet deposition and throughfall fluxes (in kg $ha^{-1}y^{-1}$) at the study sites (period June 2011 to June 2013). Percent contribution of oxidized N to DIN is also indicated. WD = wet deposition; TF = throughfall; nTF= net throughfall.

	LC	CB	CA	TC
WD NH4-N	2.24	1.47	3.80	0.54
WD NO3-N	2.10	1.56	1.98	0.65
WD sum DIN	4.34	3.02	5.78	1.20
% WD Nox to DIN	48	52	33	55
TF NH4-N	1.31	1.96	3.10	0.45
TF NO3-N	4.49	5.35	4.32	1.78
TF sum DINN	5.79	7.31	7.42	2.23
% TF Nox to DIN	77	73	58	80
nTF NH4-N	-0.93	0.49	-0.70	-0.09
nTF NO3-N	2.38	3.80	2.34	1.13
nTF sum DIN	1.45	4.29	1.64	1.04
% nTF Nox to DIN	164	89	143	109

Table 7. Estimated dry deposition with the different methods (in kg $ha^{-1}y^{-1}$). CBM= Canopy budget model, BW= Branch washing, IM = Inferential model at the 4 studied sites.

DD method	NH4-N				NO3-N				DIN			
	LC	CB	CA	TC	LC	CB	CA	TC	LC	CB	CA	TC
CBM	3.1	4.0	5.0	0.3	4.9	5.4	3.8	1.2	8.0	9.4	8.8	1.5
BW	2.7	3.3	5.1	1.3	6.8	4.7	8.8	4.3	9.5	8.0	13.9	5.6
IM	1.7	2.2	4.8	1.6	4.8	4.0	3.5	2.2	6.5	6.2	8.3	3.8

Table 8. Average and range of percent contribution of dry deposition to total deposition for reduced N (Nred), oxidized N (Nox) and inorganic N (DIN).

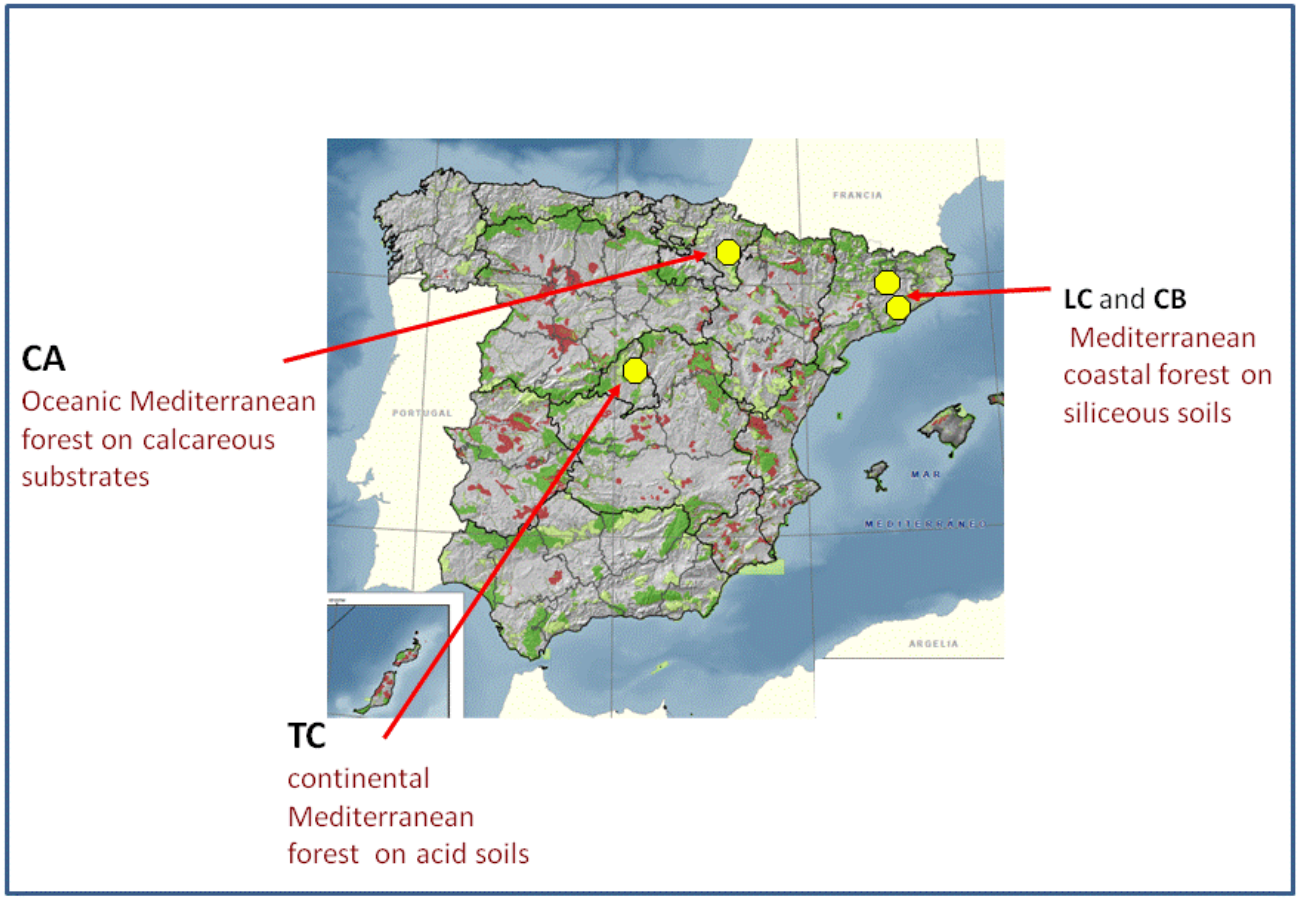
	LC			CB			CA			TC		
	mean	min	max	mean	min	max	mean	min	max	mean	min	max
%DD Nred	51	43	58	59	60	73	67	56	57	64	36	75
%DD Nox	73	70	76	74	72	78	75	66	82	72	65	87
%DD DIN	65	60	70	68	67	76	71	60	70	68	56	83

Table 9. Annual wet (WD), dry (DD), total deposition (TD), throughfall (TF) and nutrient canopy uptake (NCU) N estimated fluxes (in kg ha^{-1} y^{-1}) at 4 holm sites in Spain.

	LC	CB	CA	TC
WD NH4-N	2.24	1.47	3.80	0.54
WD NO3-N	2.10	1.56	1.98	0.65
WD DIN	4.34	3.02	5.78	1.20
DD Nred	1.3-3.1	1.8-4.0	4.5-5.1	0.3-1.3
DD Nox	4.5-6.8	4.7-6.7	3.8-8.8	1.2-4.4
DD DIN	5.8-9.9	6.5-10.7	8.3-13.9	1.5-5.7
TD NH4-N	3.5-5.3	3.3-5.5	8.3-8.9	0.84-1.8
TD NO3-N	6.6-8.9	6.3-8.3	5.8-10.8	1.9-5.1
TD DIN	10.1-14.2	9.6-13.8	14.1-19.7	2.7-6.9
TF NH4-N	1.31	1.96	3.10	0.45
TF NO3-N	4.49	5.35	4.32	1.78
TF DIN	5.79	7.31	7.42	2.23
NCU NH4-N	2.6-4.0	1.7-3.5	5.5-5.7	0.4-1.7
NCU NO3-N	2.4-4.4	0.2-1.6	1.5-6.5	0.7-3.2
NCU DIN	5.0-8.4	2.0-5.1	7.0-12.2	0.5-4.9

63

64 Fig 1. Location of the study sites



66

67

68

Fig 2. Seasonal precipitation amounts at the study sites.

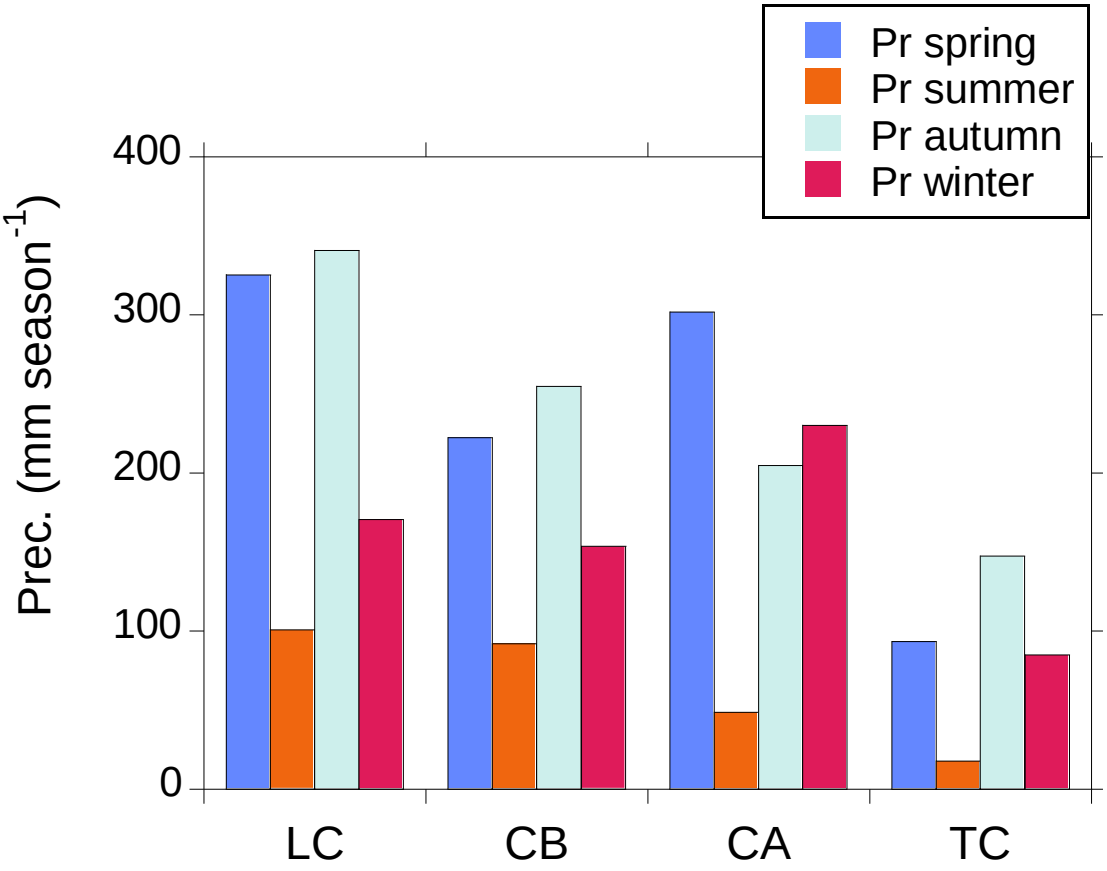


Fig. 3. Canopy inorganic nitrogen (DIN) budget for the study sites (all fluxes in kg ha^{-1} y^{-1}). Blue arrow = wet deposition; orange arrow=dry deposition, pale green arrow = throughfall, inset = nitrogen canopy uptake.

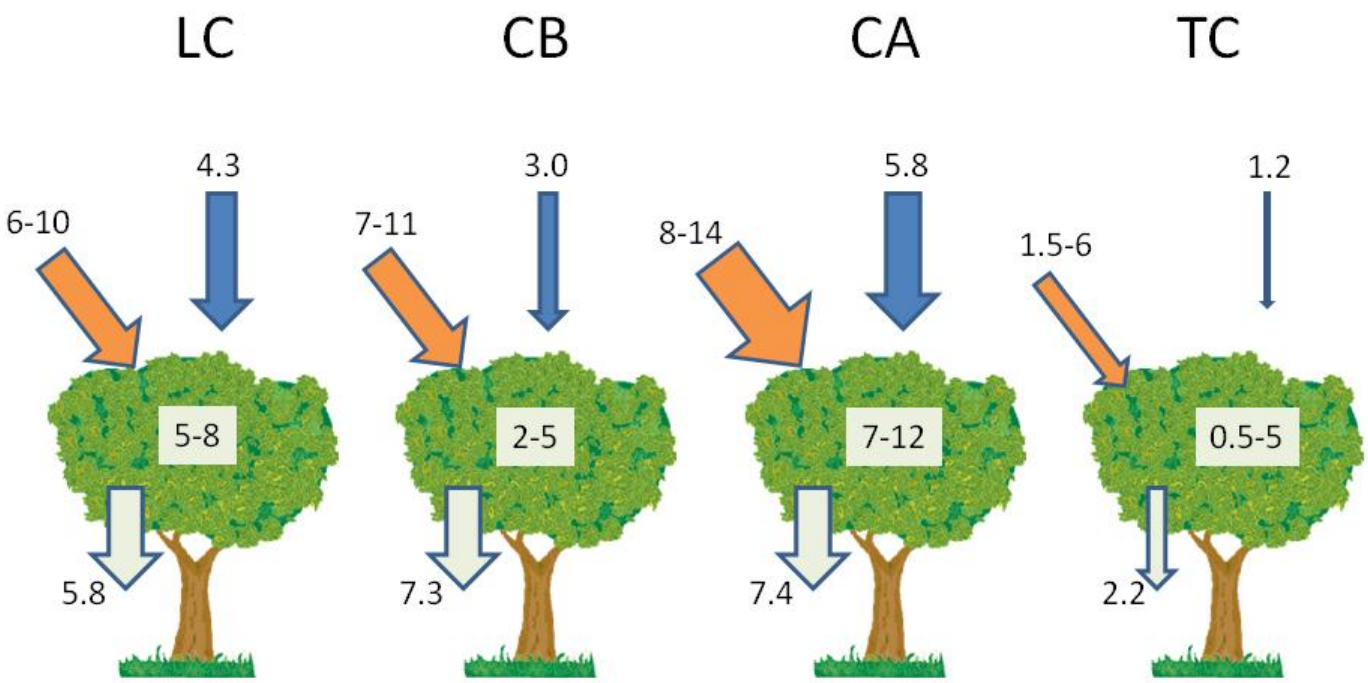


Fig 4. Relationship between nitrogen canopy uptake (NCU) and total deposition of reduced N. oxidized N and DIN. Linear regressions are indicated for significant correlations (P<0.01)