Throughfall and bulk deposition of dissolved organic nitrogen to holm oak forests in the Iberian Peninsula: flux estimation and identification of potential sources

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Abstract

Deposition of dissolved organic nitrogen (DON) in both bulk precipitation (BD) and canopy throughfall (TF) has been measured for the first time in the western Mediterranean. The study was carried out over a year from 2012 to 2013 at four evergreen holm oak forests located in the Iberian Peninsula: two sites in the Province of Barcelona (Northeastern Spain), one in the Province of Madrid (central Spain) and the fourth in the Province of Navarra (Northern Spain). In BD the annual volume weighted mean (VWM) concentration of DON ranged from 0.25 mg l^{-1} in Madrid to 1.14 mg l^{-1} in Navarra, whereas in TF it ranged from 0.93 mg l^{-1} in Barcelona to 1.98 mg l^{-1} in Madrid. The contribution of DON to total nitrogen deposition varied from 34% to 56% in BD in Barcelona and Navarra respectively, and from 38% in Barcelona to 72% in Madrid in TF. Agricultural activities and pollutants generated in metropolitan areas were identified as potential anthropogenic sources of DON at the study sites. Moreover, canopy uptake of DON in Navarra was found in spring and autumn, showing that organic nitrogen may be a supplementary nutrient for Mediterranean forests, assuming that a portion of the nitrogen taken up is assimilated during biologically active periods.

Capsule: Contribution of DON to total nitrogen deposition is very important in Mediterranean forests because of anthropogenic activities.

Keywords: Dissolved organic nitrogen, canopy throughfall, bulk deposition, anthropogenic nitrogen, Mediterranean ecosystems.

1. Introduction

From 1950 to 2010, global reactive nitrogen (Nr) production on a per capita basis rose from approximately 12 kg N y^{-1} to 30 kg N y^{-1}, generating three-fold more Nr than natural terrestrial processes do (Galloway et al., 2014). This massive alteration of the nitrogen cycle has resulted in changes in atmospheric composition, with detectable consequences for the climate system, food and energy security, human health and ecosystem services (Erisman et al., 2011).

In the 1970s two important monitoring programs, the US National Atmospheric Deposition Program (NADP) and the European Monitoring and Evaluation Program (EMEP), began to work on the study of nitrogen deposition, but in both cases addressing only inorganic N (Cape et al. 2011). The first serious discussions and analyses of organic nitrogen (ON) emerged with the work carried out by Cape et al. (2001) and the reviews published by Neff et al. (2002) and Cornell et al. (2003), who greatly contributed to the promotion of the subsequent surveys developed in this field. In fact, since then, many surveys have been carried out taking into account various aspects of ON: contributions to wet (Keene et al., 2002; Cape et al., 2012) and dry deposition (Mace et al., 2003; Matsumoto et al., 2014); elemental and functional characterization (Altieri et al., 2012; El Haddad et al., 2013); interactions with vegetation (Hinko-Najera and Wanek 2010), soil microorganisms (Jones et al. 2004; Farrell et al. 2014) and climate (Du et al., 2014); and modelling and prediction studies (Kanakidou et al., 2012; Im et al., 2013). These surveys have highlighted the important contribution of the organic form to total N deposition, ranging on average from 10 to 40% depending on the study area. However, even if organic N has long been known to be a quantitatively significant component of atmospheric nitrogen deposition, it is still not routinely assessed, nor are best-estimates factored into quantitative evaluations of N fluxes (Cornell 2011). To date, only the work carried out by Walker et al. (2012) can be considered as a real attempt to add ON measurements to the National Trends Network (NADP/NTN). Thus, in spite of the progress achieved over recent years, reviews (Cape et al., 2011; Cornell 2011; Jickells et al., 2013) have underlined important gaps in our knowledge of budgets, chemical characterization and source identification of the organic fraction. Indeed, we are still far from an understanding of the role that ON may play in human health, ecosystems functioning and interactions in biogeochemical cycles.

Considering Mediterranean-type ecosystems, the information gap is even greater. Although atmospheric nitrogen deposition is well known to cause nutritional imbalances

with negative consequences (Erisman et al., 2013; Shibata et al., 2015), ecosystems from the Mediterranean Basin have been systematically neglected and are strongly under-represented (Dias et al., 2011, Pinho et al., 2012) compared with central and northern Europe and America (Fenn et al., 2003; Bobbink et al., 2010). At present, little is known about the effects of anthropogenic nitrogen inputs in these valuable regions (Bobbink et al., 2010; Ochoa-Hueso et al., 2011), and the scarcity of data related to air pollution characterization and effects is presented as one of the biggest concerns and challenges in the Mediterranean area. In the Iberian Peninsula, nitrogen fluxes have been estimated using a variety of approaches (Àvila and Rodà 2012; García-Gómez et al., 2014; Vet et al., 2014). However, in spite of these efforts, there are still many unresolved matters, specifically related to dry deposition, which is recognized as the main form of atmospheric input of N in Mediterranean systems (30 – 70%), and up to 90% in certain areas (Sanz et al., 2002; Àvila and Rodà 2012), but is not usually assessed due to the difficulty of measurement.

The lack of ON data constitutes another factor that greatly increases the uncertainties and hinders our knowledge of the nitrogen cycle in this region. A search of the literature revealed few studies which addressed organic nitrogen deposition in the Mediterranean Basin, and most of them were focused on the eastern Mediterranean (Mace et al., 2003; Violaki et al., 2010; Violaki and Mihalopoulos 2010). In the western Mediterranean ON deposition has been measured in some coastal environments (Markaki et al., 2010), and in model-based surveys (Im et al., 2013), but no evidence has been found that considered inland deposition.

The aim of the present study is to determine the dissolved organic nitrogen (DON) fraction in canopy throughfall (TF) and bulk precipitation deposition (BD) samples of four evergreen holm oak forests located in Spain, and to give a more comprehensive characterization of the nitrogen fluxes in these Mediterranean ecosystems. To the authors' knowledge, this is the first effort to quantify the contribution of atmospheric deposition of DON in total dissolved nitrogen (TDN) in forests and open field sites of the western Mediterranean. This work was part of the EDEN project (*Effects of nitrogen deposition in Mediterranean evergreen holm oak forests*), which was developed with the purpose of determining the total nitrogen inputs to evergreen holm oak forests in the Iberian Peninsula and studying the effects of this deposition in the nitrogen biogeochemical cycle in this forest type.

2. Material and Methods

2.1. Study sites and collection methods

The present work was carried out in four evergreen holm oak forests (*Quercus ilex* L.) of the Iberian Peninsula: Barcelona (Can Balasc, CB, and La Castanya, LC), Madrid (Tres Cantos, TC) and Navarra (Carrascal, CA). Although the vegetation type was common to all locations, growing factors (climatic and edaphic conditions), landscapes, management and anthropogenic activities affected each site differently, providing a good opportunity to study ON deposition in forests developed and affected by different situations, and thus to evaluate potential sources of ON. The main characteristics of the sites and brief descriptions of the surroundings are shown in Table 1. Meteorological variables were monitored onsite at CB, LC and TC, and data from the closest meteorological station were collected for the CA site.

At each location two monitoring plots were set up with the purpose of collecting both throughfall (TF) and bulk deposition (BD) samples. The instruments selected for sampling of precipitation were those developed by the Norwegian Institute for Air Research (Norsk institutt for luftforskning, NILU). Four replicates of NILU-type rain gauges (20 cm diameter) were installed in the open-field plots and twelve replicates were placed under the canopy. Criteria suggested by the UNECE-CLRTAP ICP Forests manual, Part XIV (ICP Forests manual, 2010) were followed to avoid contamination and to preserve samples. Sampling frequency was weekly or fortnightly in wet periods, whilst in CA and TC, during dry or less frequent rain periods samples were collected after each rain event. The sampling campaign was extended for a whole year, from June 2012 to June 2013.

2.2. Sample treatment, preservation and analysis

In the laboratory, two aliquots of unfiltered sample were reserved for pH and conductivity determinations. A third aliquot was separated in Barcelona for alkalinity estimates. The remaining sample was filtered using 0.45μm pore filters and distributed in four subsamples for alkalinity (only in TC and CA), NH_4^+, anions and cations, and TDN analysis. In Barcelona, anion and cation determinations were performed in accordance with Izquierdo and Avila (2012). In Navarra and Madrid, ammonium and anion determinations were performed by ion chromatography (IC) (NH_4^+: Dionex 1100, with column CS16 in Navarra and Dionex 2000 with column CG12 in Madrid; SO_4^{2-}, NO_3^-, NO_2^-, Cl^-, PO_4^{3-} anions: Dionex 2000, with column AS19 in Navarra and Madrid),

whereas cations were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500a).

Subsamples reserved for TDN were initially frozen at -20ºC and stored for 3-4 months. The samples were then thawed at room temperature and carefully prepared for an optimum preservation during the shipment to the Centre for Ecology & Hydrology (CEH) in Edinburgh, UK where they were analyzed. Defrosted samples (1.5 ml aliquot) were placed in 2 ml chromatography vials previously filled with 200 μg of thymol biocide. Moreover, hydrochloric acid (300 μL 0.05 M in Barcelona and Navarra, and 100 μL 0.01 M in Madrid) was added to lower the pH and avoid NH_3 losses from the vial. Criteria for selection of HCl volume and concentration for each site are explained in the first section of 'Results and discussion'. TDN measurements were made using high-temperature chemiluminescence in flow injection mode (ANTEK 8060M) as described in Cape et al. (2012).

Accuracy of the TDN analytical protocol was ascertained by analysis of synthetic rain samples from the World Meteorological Organization Global Atmosphere Watch (WMO-GAW) QA-SAC Laboratory Intercomparison Program (http://www.qasac-americas.org/) and certified reference NH_4^+ standard (Sigma-Aldrich). Results agreed (97-101%) with the expected total nitrogen values. Duplicate samples were determined every ten samples in order to assess the precision of the procedure. A relative standard deviation (RSD) below 4% was found for TDN and NH_4^+, and below 3% for NO_3^-. Blank samples were analyzed to ensure that there was no contamination. The 0.05 M HCl solution used to acidify and stabilize the defrosted samples were also analysed to check that it did not present an additional source of nitrogen. In addition, the inter-comparability of the TDN analyzer and ion chromatographs from Spain was checked by analysis of ammonium and nitrate standards from each laboratory, in order to check for any systematic error in calibration between centres. No bias could be detected, implying no systematic differences.

DON was calculated as the difference between total N and the sum of dissolved inorganic N (DIN; ammonium and nitrate): DON = TDN – DIN. This method may result in some small negative DON values as a result of uncertainties in the total N and inorganic N determinations, where concentrations are low or near method detection limits. In the present study, these apparent negative values have been included in the final dataset to avoid the bias caused by ignoring or treating them as zero. In the present work, the total sum of analytical uncertainties was estimated to be near 10%. Thus, data from samples with values of DON less negative than 10% of the measured

TDN were considered in the final dataset, whereas data from samples with values of DON more negative than 10% of the measured TDN were discarded, since other problems apart from analytical uncertainties might be altering the sample and causing the negative results.

2.3. Database validation

The criteria used to identify valid precipitation samples were, on the one hand, those described in the UNECE ICP Forests manual, Part XVI (ICP Forests manual, 2010): i) ion balance; ii) measured conductivity vs estimated conductivity; iii) Na/Cl ratio; and iv) nitrogen balance (TDN ≥ DIN). Additional criteria proposed by Cape et al. (2015) were also applied, i) invalid sample due to evidence of contamination in BD (PO_4^{3-} > 10 μeq l^{-1}; NH_4^+ > 100 μeq l^{-1} and K^+ > 8 μeq l^{-1}) and ii) missing data (precipitation less than 2.1 mm).

2.4. Air pollution monitoring

Atmospheric concentrations of ozone (O_3), ammonia (NH_3) and nitrogen dioxide (NO_2) were monitored at the sampling sites using tube-type passive samplers (Radiello®). Two replicate samplers per gaseous species were exposed at 2 m height in each plot at fortnightly periods over two years (2011 – 2013). Laboratory analyses were performed according to Radiello's specifications (Fondazione Salvatore Maugeri, 2006).

2.5. Data handling and statistical analysis

Annual volume weighted mean (VWM) concentrations were calculated as described in Araujo et al. (2015). Yearly deposition fluxes were obtained as the product of these VWM concentrations and the corresponding annual bulk/throughfall water volume. Seasonal, monthly or per sampling period deposition fluxes were obtained following the same procedure: VWM concentrations were calculated for each period and multiplied by the corresponding BD/TF water volumes. In the validated data set, in order to fill the gaps due to missing values, VWM concentrations were calculated with the available samples, but the precipitation volume of excluded samples was included in calculating annual precipitation (Cape et al., 2012; ICP Forests manual, 2010). Non sea-salt (nss) concentrations were calculated according to Avila (1996).

Given the strong inverse dependence of concentrations on precipitation amounts (small amounts tend to have higher concentrations), within-site correlations were analyzed

using deposition data (mg m^{-2} $month^{-1}$) rather than concentration data, as suggested by Cape et al. (2012), and Spearman's rank correlation coefficient was applied to test for significant correlations over time at a site.

All statistical analyses were performed employing the SPSS v. 15.0 program.

3. Results and discussion

3.1. Methodological implications

The analysis of the first batch of samples (from June to November 2012) showed a high percentage of samples with negative DON values. Despite the addition of 100 μL of 0.01M HCl to control pH, discrepancies were large (values of DON more negative than -10%) and could not be explained by uncertainties from DIN and TDN measurements (see section 2.2.). The highest number of negative DON values was found in Navarra, followed by Barcelona (both CB and LC), whereas in Madrid this problem was not found. There was clear evidence that the higher the pH, alkalinity and $N\text{-}NH_4^+$ load, the higher the losses (Table 2). Cape et al. (2012) suggested that in some places, inclusion of sub-micron particles of minerals in the filtered sample could have increased the pH during transit and led to losses of NH_3 in the vials. Our findings are in agreement with this hypothesis. It seems probable that carbonates and bicarbonates present in the rain samples as mineral particles were dissolving, increasing the pH and releasing NH_3 in the sealed vials. Since then, in both Barcelona and Navarra samples, HCl volume and concentration were adjusted to 300 μL and 0.05 M respectively, while in Madrid the initial HCl proportions were maintained. All available samples from the first batch were re-analyzed with the new higher HCl concentrations, considerably reducing the number of negative results. At CA, where 93% of initially negative samples could be re-analyzed, the percentage of negative DON values was reduced from 24% to 3.4%. At CB the same improvements were shown when applying the HCl adjustment to the available samples (83%), reducing the percentage of negative DON values from 22% to 4%; but at LC, only 57% of the initial negative DON samples could be re-analyzed with the correct acid addition because of insufficient samples volumes, resulting in higher data loss (8%) than at the other sites.

These findings are of significant importance when working in Mediterranean areas, which are characterized by dry, arid and semi-arid environments, and soil erosion is a frequent phenomenon. If soils are rich in carbonates/bicarbonates, the wind-blown mineral contribution to BD and TF samples might play a vital role in pH regulation, and

245 therefore in $N\text{-}NH_4^+$ preservation, with crucial consequences for underestimating the
246 organic nitrogen fraction.
247

248 3.2. Concentrations and deposition

249 VWM concentrations, annual deposition fluxes and percent contributions for all sites in
250 BD and TF are shown in Table 3. VWM concentrations of all nitrogenous species were
251 higher in TF than in BD, except for $N\text{-}NH_4^+$ at TC (negligible differences) and CA (lower
252 in TF). Deposition fluxes varied similarly, with higher $N\text{-}NH_4^+$ fluxes in TF than in BD at
253 Barcelona sites and lower at CA and TC. DON concentrations and fluxes were higher
254 in TF, except for DON fluxes at CA. The contribution (%) of DON to TDN was also
255 higher in TF samples, except for CB, where differences were minimal between BD and
256 TF.

257 Concentrations of the inorganic N component in BD were within the range of those
258 measured at other sites across Europe ($N\text{-}NH_4^+$: 0.07-0.99 mg l^{-1}; $N\text{-}NO_3^-$: 0.11-0.50 mg
259 l^{-1}; Cape et al., 2012), whereas DON concentrations were higher than at any sites from
260 that survey (0.02-0.18 mg l^{-1}; Cape et al., 2012). However, DON concentrations from
261 our sites agreed with those found in the eastern Mediterranean (0.21 mg l^{-1}, Mace et al.
262 2003; 0.32 mg l^{-1}, Violaki et al., 2010), except at CA, where the concentration was
263 considerably higher and similar to the averaged value of 1.08 mg l^{-1} measured in China
264 by Zhang et al. (2012). Indeed, the DON flux in BD at CA was 12 kg ha^{-1} y^{-1}, 4-fold
265 higher than sites in Barcelona and 10-fold higher that in Madrid, and exceeding
266 deposition rates recorded in other places around the world (3.1 kg ha^{-1} yr^{-1}, mean value
267 from 41 data sets, Neff et al., 2002; 8.4 kg ha^{-1} yr^{-1}, Guangzhou city in China, Li et al.,
268 2012). In terms of proportion, DON percentages ranged from 34% at LC to 56% at CA.
269 These values were in general higher than those found in Europe (2-38%, Cape et al.,
270 2012), USA (3-8%, Keene et al., 2002) and the eastern Mediterranean (17%, Mace et
271 al., 2003; 23%, Violaki and Mihalopoulos 2010), but agreed with those reported by
272 Vanguelova et al. (2010) for the UK (20-50%), and Markaki et al. (2010) for coastal
273 locations in the Mediterranean Basin (26-38%).

274 Considering the type of site (table 1), the plot located in the agricultural region of
275 Carrascal (CA) registered the highest percentage of DON (56%), followed by the peri-
276 urban plots (40% at CB and 38% at TC) and ultimately by LC, the site located in the
277 Montseny mountains which was considered as a background point (34%). This
278 gradient is partially in accordance with data from other spatial networks, which also
279 reported higher contributions of DON to TDN in agricultural areas with elevated N

deposition fluxes, especially where organic fertilizer is applied (Zhang et al., 2008 and 2012), and lower percentages of DON in urban and sub-urban areas with lower N deposition rates (Pacheco et al., 2004; Zhang et al., 2012). However, those networks also showed that samples from remote areas registered the highest contribution of DON to TDN, with percentages of 70-80% in the Tibetan Plateau (Zhang et al., 2008 and 2012) and 92% in Venezuela (Pacheco et al., 2004), although total N deposition was lower in comparison to agricultural and urban areas. These data are opposed to those found at LC, where a low DON to TDN ratio and high fluxes of N deposition were registered. A probable explanation for this discrepancy is that LC is not a true background point far from the influence of anthropogenic emissions, since recent research has found that local sources and long-range transport of pollutants might be influencing rain chemistry at this site (Izquierdo et al., 2012).

DON concentrations in samples from holm oak TF ranged from 0.93 to 1.98 mg l^{-1}, being amongst the highest values reported in throughfall surveys: 0.35 mg l^{-1} in boreal forest (Piirainen et al. 1998), 0.27 mg l^{-1} in tropical wet forest (authors' estimates from deposition and precipitation data from Schwendenmann and Veldkamp, 2005), or 0.25-1.11 mg l^{-1} in temperate forest (Michalzik et al., 2001). No TF references for comparison were found in the Mediterranean area or other semi-arid environments. Annual deposition and percent contribution were within the range of those reported in the literature for non-water limited forests (56%, Piirainen et al., 1998; 1.2-11.5 kg ha^{-1} yr^{-1}, Michalzik et al., 2001; 80%, Gaige et al., 2007; 31-48%, Mustajarvi et al., 2008).

3.3. Potential sources and annual variability

The DON inferred approach has the advantage of estimating the total amount of the organic fraction, but the origin and identity of individual components of that fraction have not been characterized. To identify the potential origins of DON, correlation analysis between DON and other ions in solution (monthly deposition data), meteorological variables and atmospheric concentrations of NO_2, NH_3 and O_3 was performed (table 4). Only BD correlation data were taken into account, since TF data may be influenced by canopy interactions and may lead to misinterpretations. Moreover, monthly deposition patterns of the studied nitrogenous species for both BD and TF plots were depicted in order to better understand variability over the year and recognize temporal trends that helped us to support hypotheses about likely sources of DON (figure 1).

In our study, precipitation amount was identified as an important meteorological factor affecting the amount and annual distribution of both inorganic and organic nitrogen deposition at all locations (table 4). Other surveys also found a strong dependence of deposition with rainfall patterns (Violaki et al., 2010; Li et al., 2012).

Positive relationships were found between DON and nss-Mg^{2+} and nss-Ca^{2+} at CB and TC, and between DON and nss-Mg^{2+} at CA (BD data, table 4). These ions have been identified as dust indicators in previous surveys (Avila et al., 1998; Mace et al., 2003; Lesworth et al., 2010). However, their association with DON does not show if they have the same origin or whether organic N from other sources has been adsorbed on the mineral aerosol.

In CA (agricultural area), BD deposition data showed significant positive relationships of DON with N-NH_4^+ and N-NO_3^- (table 4). DON peaked in October, January, March and June (figure 1), coinciding with peaks in ammonium and nitrate (except in June). Although rainfall amounts may affect these peaks, it is likely that other factors are involved, since variations in the size of the peaks are not proportional to the precipitation amount. In fact, DON deposition peaks in October and March are higher than the one in January, the month with the highest rainfall amount. Events in agricultural practices appear to be correlated with peaks in inorganic N: sowing time in October with ammonium-nitrate fertilization; from January to March additional fertilizer is applied (generally twice, one in January with inorganic fertilizer and a second one in March, usually with urea); late June or early July is the harvest season. These findings are in agreement with those from Zhang et al. (2008) and Zhang et al. (2012), who saw a clear influence of agricultural activities in the organic N budgets.

DON from CB and TC showed positive correlations with NO_3^-, but were negatively correlated to atmospheric NO_2 (BD data, table 4). This relationship could indicate that organic nitrates may be an important component of the DON at these sites. Organic nitrates are formed as a result of photochemical reactions of hydrocarbons with NO_x ($NO+NO_2$) (Atherton and Penner 1990; Neff et al., 2002). When these reactions occur, the expected products in precipitation are both organic nitrates and NO_3^- (Keene et al., 2002), while a reduction in NO_2 air concentrations may be predicted. CB is located in the metropolitan area of Barcelona and TC is just 9 km away from Madrid. These two cities are the biggest in Spain, with more than 3 million inhabitants in their metropolitan areas (www.amb.cat and www.madrid.org). Therefore, polluted air masses derived from combustion and vehicle exhaust might have been a starting point for organic nitrate formation because of their enrichment in NO_x (Salvador et al., 2015; Malik and

Tauler 2015) and VOCs (Perez et al., 2002), precursors of the nitrogen containing organic compounds. The same phenomenon was found by Li et al. (2012) in Guangzhou city.

Moreover, the peaks of the dissolved nitrogenous species at certain periods seem to corroborate this hypothesis (figure 1). At TC, DON and N-NO_3^- peaked together in September, whereas N-NH_4^+ peaked in March. TF data also showed large co-occurring DON and N-NO_3^- peaks at this site in September, while in May another DON peak was found along with a smaller N-NO_3^- one. Thus, both BD and TF data at TC showed a common trend for nitrate and DON which differed from the ammonium one. At CB, maximum values of DON in December followed a N-NO_3^- peak in November, which could imply the transformation of inorganic nitrate forms into organic ones (Roberts et al. 1990; Atkinson et al. 1990). However, BD graphical data also depicts a common temporal trend of DON with both inorganic N ions, peaking together in March. TF data also recorded this peak in spring. Therefore, it seems likely that not only nitrate but also ammonium participated in the DON formation at site CB.

Indeed, according to visual observations of temporal patterns at CB, DON deposition was also significantly related to N-NH_4^+ (BD data, table 4). This may be attributable to emissions from three-way catalytic converters on motor vehicles, which are a source of ammonia emissions (Kean et al., 2009). CB is sited only 150 m from the nearest highway, which has an average daily traffic flow of 40 – 50 thousand vehicles per day. Therefore, the relationship between DON and N-NH_4^+ may corroborate the hypothesis that DON at this location is mainly generated in secondary processes linked to road traffic emissions. At TC this association was not seen, probably because of the longer distance between the monitoring site and the highway (1.5 km).

In addition, another correlation was found at CB. This plot, 11 km from the sea, was the only site that showed significant correlations between DON and Na^+ and Cl^- (BD data, table 4), suggesting that part of the organic fraction at this site has a marine origin (Violaki and Mihalopoulos 2010), or at least is associated with marine aerosol.

Correlation data from LC showed no relationship between DON and inorganic N components, being only related to nss-K^+ and nss-SO_4^{2-} (BD data, table 4). Nss-SO_4^{2-} has widely been used as an anthropogenic tracer, directly linked to pollution emission activities (Violaki et al., 2010; Li et al., 2012). LC site is located about 40 km from Barcelona and it is relatively protected from the influence of the metropolitan area and its industrial activities, being considered as a rural or background plot (Rodrigo et al., 2003). So, it was not expected that anthropogenic emissions would affect this site.

384 However, recent work from Izquierdo et al. (2012) showed that both local sources and
385 long-range transport of SO_4^{2-} generated in Central and Eastern Europe influence rain
386 chemistry at LC, which suggests that DON at this location may be linked to
387 anthropogenic activities rather than to natural processes. Nevertheless, it should be
388 taken into account that only 8 months of DON measurements were available at this site
389 for statistical analysis and this may potentially bias the correlations.

390 Finally, at all sampling sites DON deposition was strongly correlated with nss-K^+ (BD
391 data, table 4). Potassium salts are regarded as indicators of plant-derived particles
392 (Pölker et al., 2012). Matsumoto et al. (2014) suggested that correlation between the
393 DON and nss-K^+ would indicate the influence of vegetation sources on the DON
394 budgets. In our study sites, where BD plots are surrounded by holm oak forests (and
395 also crop fields in CA), it seems probable that biogenic processes may be responsible
396 to some extent for the organic budgets at these locations. However, at CB and TC this
397 significant relationship between DON and nss-K^+ deposition may have another
398 explanation. Pohlker et al. (2012), besides identifying its biogenic origin, observed that
399 potassium salts served as initial seeds for the condensation of VOC oxidation products,
400 being directly related to secondary organic aerosol processes. Therefore, at these peri-
401 urban sites it seems probable that organic nitrogen may be associated with potassium
402 salts after being generated in secondary reactions. This hypothesis would explain why
403 Matsumoto et al. (2014) found significant correlations between DON and nss-K^+ at an
404 urban site and not at a forested one as they expected.

405 Regarding throughfall patterns, two important conclusions can be reached. On the one
406 hand, data showed that dry deposition also contributes to the total amount of organic
407 nitrogen that arrives at these sites, since DON fluxes were higher in TF than BD at all
408 sites except for CA, where differences were negligible (table 3). In all TF plots, DON
409 peaks were registered after periods of little rain, both in precipitation events during (CA)
410 or just after the summer season (TC and LC) and also after the drier winter months, in
411 March (CB, LC and TC). This fact suggests that the dry organic nitrogen previously
412 deposited and accumulated in the forest canopy is washed out in subsequent rain
413 events, similarly to that reported in Violaki et al. (2010). Another possible explanation
414 would be that in dry conditions the canopy flora convert dry deposited inorganic N to
415 organic N (Cape et al. 2010). On the other hand, from March to May, high DON rates
416 were registered at the Barcelona and Madrid TF plots. It is likely that this result is due
417 to deposition of pollen, spores and plant debris that are abundant during spring time
418 (Zhang et al., 2008; Violaki et al., 2010). At CA there is a DON maximum in March that
419 does not match with any of the aforementioned hypotheses: previous months were not

especially dry and DON fluxes greatly decreased in April, May and June in comparison with the March peak. A possible explanation is that urea fertilizer applied during this month in the surrounding fields also affects the forest canopy, and was recorded in the TF chemistry.

All these findings show the multiple sources and compounds that may participate in the organic nitrogen budgets in the western Mediterranean, and highlight the limitations in estimating the amount and mechanisms that contribute to those budgets.

3.4. N deposition implications for ecosystems

The interactions of the tree canopy with N fluxes were evaluated as the net canopy throughfall (NTF = TF – BD; figure 2). DON fluxes increased (BD < TF) at all sites during all seasons as rainfall filtered through the forest canopy, except at CA, where DON uptake was observed in autumn and spring.

The release of DON from the canopy is the most common situation reported by other authors, who suggest transformations in the canopy of the inorganic fraction into the organic one (Gaige et al., 2007; Mustajarvi et al., 2008; Cape et al., 2010), or changes in the nutrient status of trees through soil N enrichment (Crockford and Khanna, 1997) as possible causes of higher fluxes of DON in TF. However, the DON uptake detected at CA is less frequently reported in the literature (Piirainen et al. 1998). It has been demonstrated that the type of nitrogen compound is a determining factor for its assimilation in forests and other ecosystems. Previous surveys carried out by Hinko-Najera and Wanek (2010) in forest, Li et al. (2013) with mosses and Yuan et al. (2012) with phytoplankton all agreed in showing that $N\text{-}NH_4^+$ and organic N are preferred to $N\text{-}NO_3^-$. Considering the huge variety of organic nitrogen compounds (Altieri et al. 2012), it seems also probable that differences in uptake between them exist. At CA, agricultural activities seem to be the main source of DON, and may generate more labile and bioavailable compounds such as amino acids or urea. These compounds would be easily assimilated by vegetation in contrast to the less soluble organic nitrates that may be formed at CB and TC. Differences in solubility, bioavailability and toxicity among the organic compounds reaching each plot would explain why DON leaks from the canopy at CB and TC instead of being captured as occurs at CA. This finding is of primary importance if one considers that approximately 25 million of hectares in Spain (around 47% of surface) are dedicated to agricultural activities (Censo agrario 2009, National Statistics Institute), which can be emitting or enhancing the formation of directly available DON compounds to Mediterranean forest ecosystems.

On the other hand, it is noteworthy that DON uptake at CA occurred in autumn and spring. These are the periods when the greatest vegetation activity was expected. Firstly, seasonal changes in the N behaviour of ecosystems are driven by seasonal fluctuations of physical drivers (i.e. weather conditions) and biological factors (Shibata et al. 2015). In the Mediterranean area, nitrogen dry deposition accumulates in soil and on plant surfaces during dry periods, becoming available as high N concentration pulses with rainfall events (Meixner and Fenn 2004; Ochoa-Hueso et al. 2011). In our study, those rainfall events were registered in autumn, allowing the uptake of the nitrogen deposited during the summer. Secondly, spring is the main growing season, and therefore a period of maximum biological demand. Thus, assuming a portion of the nitrogen taken up is assimilated by vegetation, our finding would imply that certain DON compounds constitute an additional nutrient supply in Mediterranean ecosystems during biologically active periods.

These results may have significant implications when working with the critical load approach, given that the additional input of organic N, which is not included in the risk evaluation, may provide even greater pressures than predicted, and may pose a threat to systems where the Critical Load does not appear to be exceeded (Cape et al. 2011, Cornell 2011). In aquatic ecosystems it has already been shown that DON is an important source of nutrients that can stimulate the productivity of these environments (Seitzinger and Sanders, 1999; Violaki et al. 2010). However, in terrestrial ecosystems DON effects have been poorly studied and little is known about the possible damage that deposition of the organic fraction may pose for them. Moreover, recent findings have revealed that C sequestration and other processes of the C cycle in soils might be dependent on the IN to ON ratio, highlighting the importance of the organic fraction in controlling the ecological effect of N deposition (Du et al., 2014).

Hence, quantification of the organic fraction is important to more fully represent the nitrogen cycle in forest ecosystems and to evaluate unequivocally the possible consequences of its alteration.

4. Conclusions

Mediterranean regions have been overlooked in the study of nitrogen deposition and its possible effects. The present survey has shown that DON may constitute another factor that increases uncertainties in the knowledge of the nitrogen cycle in this area, since it has not been routinely assessed and yet was found to contribute from 34% to 56% to TDN in BD. Specific methodological improvements were established in order to avoid NH_3 losses during sample preservation for TN determination that would otherwise result in an underestimation of DON. The methodology developed here may be useful for preservation of samples in other locations with similar characteristics. Depending on the study site, different anthropogenic activities were identified as potential sources of DON (agricultural practices and pollution derived from combustion processes, among others), showing that the organic component is extremely complex and currently poorly understood. Finally, DON uptake was observed at CA during autumn and spring, two important seasons for the biological cycle, suggesting that at least part of the organic fraction could be directly assimilated by Mediterranean forests, which may have significant ecological implications.

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