Nitrogen deposition at Mediterranean holm-oak forests: loads and indicators

Ph.D. Thesis

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A mis padres

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Abstract

With the present PhD we provide new information on N deposition to holm oak (*Quercus ilex*) forests in Spain, in order to contribute to fill the gap about N deposition in the Mediterranean Basin. Three forest sites (CB close to Barcelona, CA near Pamplona and TC close to Madrid) were selected for their proximity to traffic/urban pollution sources. A fourth site was chosen as representative of less polluted environments (LC in the Montseny mountains, 45 km NNE from Barcelona). However, our results showed that the site farthest from pollution had similar anthropogenic pollutant (N and S) deposition loads than two of the more exposed sites. Our results indicated a widespread transport of pollutants from metropolitan areas, maritime traffic and long-range transport to the sampling areas. Dry deposition fluxes were estimated by the analysis of throughfall. Regressions between net throughfall loads and rainfall amounts indicated that K^+ in net throughfall was explained by leaching from the canopy, SO_4^{2-}-S and NO_3^--N (except at one site) were derived from dry deposition and NH_4^+-N was absorbed in the tree canopies. The results from applying a Canopy Budget Model indicated that dry deposition provided most of the total deposition load (74 and 84 % at LC and CB, respectively). Dry plus wet deposition of dissolved inorganic N (DIN, the sum of NH_4^+-N and NO_3^--N) was estimated in 17 and 20 kg N ha^{-1} yr^{-1}at LC and CB, respectively. Since recent studies in these sites indicate that dissolved organic nitrogen (DON) adds around 3 kg N $ha^{-1}y^{-1}$, total N inputs (DIN+DON) to these holm oak forests lies between 20-23 kg N $ha^{-1}y^{-1}$, a value exceeding the critical load value currently used for Mediterranean sclerophyllous forests (15-17.5 kg $ha^{-1}y^{-1}$). On the other hand, retention of N at the canopy level was important for NH_4^+-N (7 and 8 kg ha^{-1} yr^{-1} at LC and CB) and also non-negligible for NO_3^--N (around 4 kg ha^{-1} yr^{-1} at both sites).

The chemistry of bulk deposition at the Montseny mountains during the past 3 decades was analysed in order to understand how the abatement programs implemented by the Convention on Long Range Transboundary Atmospheric Pollution (CLRTAP) from UN/ECE to reduce SO_2, NO_2 and NH_3 emissions have affected atmospheric deposition arriving to this forest site. We found that strong reductions of SO_2 emissions in Spain and neighbouring countries (75-85%) were reflected in reductions of non-sea salt-SO_4^{2-} in precipitation (65% for concentrations and 62% for SO_4^2-S deposition) and dry deposition (29%) at Montseny comparing measurements in 1995-96 and 2011-13. Regarding NO_2 emissions in Spain, they increased from 1980 to 1991, remained constant until 2005, and decreased thereafter, a pattern that was paralleled by NO_3^- in bulk precipitation at Montseny. From 2005 on, electricity generation in Spain changed, favouring renewable energies in front of coal and gas combustion. Since electricity generation has a very high contribution to NOx emissions in Spain, this shift may explain the observed NOx decrease. However, this contrasted with an increase of NO3-N dry deposition from 1995-96 compared to 2011-2012 (from

1.3 to 6.7 kg $ha^{-1}y^{-1}$), that can be attributed to the particular climatic conditions of each period (much drier in the second one). The increase of NH_3 emissions in Spain during the last 30 years (13% from 1980 to 2012) was not reflected in the NH_4^+ bulk deposition trends at Montseny, probably related to a decrease of the formation of ammonium sulphate and nitrate aerosols due to declining SO2 and NOx emissions.

Since NH_3 emissions are still increasing in Spain, we aimed to provide a critical level value as a safety threshold to specifically prevent NH_3 effects on species and ecosystems. For that, we studied the relationships between air NH_3 concentrations and lichen diversity in a distance gradient from an emission point source (cattle farm). Lichen diversity values were separately obtained for lichen functional groups (oligotrophic and nitrophytic). We found an empirical critical level of atmospheric NH_3 < 2.5 µg m^{-3} for this semi-natural Mediterranean holm oak forest. This work confirmed that lichen functional groups can be used to derive critical levels in very different Mediterranean forest types, since the same approach was applied in Portuguese 'montados'.

We found that, besides lichen communities, other parameters (%N, $\delta^{15}N$, C:N, $\delta^{13}C$) measured in different compartments (tree leaves, moss tissue and soil) of the ecosystem may also respond to NH_3 pollution and serve as indicators of pollution. The leaves and moss $\delta^{15}N$ signal variation along the gradient probably reflected the N isotopic signal of the NH_3 source. The %N and $\delta^{15}N$ signal values in moss and leaves were significantly correlated with the relative nitrophytic functional group diversity and air NH_3 concentrations, which highlights the specific response of nitrophytic species to NH_3 pollution. In soils, the $\delta^{15}N$ signal and C:N ratios were significantly related to NH_3 pollution, with soil C:N values (C:N<25) indicative of the onset of N leaching and alerting about the potential N saturation and the risk of groundwater eutrophication at this forest. Finally, the variation in $\delta^{13}C$ content in foliar and moss samples reflected the physiological response of vegetation to enhanced N deposition.

The results of this PhD indicate that total N deposition at various sites in Spain exceeds (about 30%) the proposed critical load for sclerophyllous forests. Dry deposition of N made an important contribution to total DIN loads (75-85%). In Spain, NH_3 emissions have not been reduced. Therefore, studies are needed for early warning of its effects on ecosystems. Here, we propose a critical level value of < 2.5 µg m^{-3} NH_3 air concentrations to protect lichen communities at semi-natural holm oak forest, and propose the some measurements (nitrophytic diversity, $\delta^{15}N$ and %N) in moss and leaves as best indicators of NH_3 point source pollution.

Resumen

En esta tesis se presenta nueva información sobre la deposición de N en los bosques de encinar (*Quercus ilex*) en España y así contribuir a mejorar la falta de estudios acerca de la deposición de N en la cuenca del Mediterráneo. Tres encinares (CB junto a Barcelona, CA cerca de Pamplona y TC de Madrid) fueron seleccionados por su proximidad a fuentes de contaminación urbanas. Un cuarto sitio fue elegido como representativo de ambientes menos contaminados (LC en el Montseny, a 45 km NNE de Barcelona). Se encontró que el sitio más lejano a la contaminación presentaba cargas similares de contaminantes antropogénicos (N y S) a los sitios más expuestos. Nuestros resultados indican un transporte generalizado de contaminantes de las áreas metropolitanas, del tráfico marítimo y del transporte a larga distancia hacia las zonas de muestreo. Los flujos de deposición seca se estimaron a través del estudio de la trascolación. Mediante regresiones entre las cargas de trascolación netas y la cantidad de lluvia se concluyó que el K^+ en el trascol viene explicado por una lixiviación desde el dosel arbóreo, el SO_4^{2-}- S y NO_3^-- N (salvo en un sitio) se originan por deposición seca, mientras que el NH_4^+-N fue absorbido en el copas. Tras aplicar el 'Canopy Budget Model' (CBM) se obtuvo que la deposición seca contribuyó en mayor parte a la carga total de deposito de N (74 y 84% en LC y CB, respectivamente). Al añadir el valor de deposición húmeda de N inorgánico disuelto (DIN, la suma de NH_4^+-N y NO_3^--N) se estimó que el total fue de 17 y 20 kg N ha^{-1} $año^{-1}$ en LC y CB, respectivamente. Dado que estudios recientes en estos sitios indican que el nitrógeno orgánico disuelto (DON) supone alrededor de 3 kg N ha^{-1} $año^{-1}$, la entrada total de N (DIN + DON) a estos encinares se sitúa entre 20 y 23 kg N ha^{-1} $año^{-1}$, valor por encima de las cargas críticas para los bosques esclerófilos mediterráneos (15-17,5 kg ha^{-1} $año^{-1}$). Por último, la retención de N a nivel de dosel fue importante para el NH_4^+-N (7 y 8 kg ha^{-1} $año^{-1}$ en LC y CB) y no despreciable para el NO_3^--N (alrededor de 4 kg ha^{-1} $año^{-1}$ en ambos sitios).

Se ha estudiado la variación de la química de la lluvia en el Montseny durante las últimas 3 décadas con el objetivo de entender cómo los programas de reducción de las emisiones de SO_2, NO_2 y NH_3 implementados por El Convenio sobre la Contaminación Atmosférica Transfronteriza a Larga Distancia (CLRTAP) de la UN/ECE, han afectado a la deposición atmosférica. Las fuertes reducciones en las emisiones de SO_2 en España y en los países vecinos (75-85%) se reflejan en la reducción del nssSO_4^{2-}-S en la lluvia (65% para las concentraciones y el 62% para SO_4^{2-}-S deposición) y en la deposición seca (29%) en el Montseny comparando mediciones en 1995-96 y 2011-13. Las emisiones de NO_2 en España aumentaron de 1980 a 1991, se mantuvieron constantes hasta 2005 y luego disminuyeron, patrón que se refleja de forma paralela en los valores de NO_3^- en la precipitación del Montseny. A partir de 2005 se han favorecido las energías renovables frente a la combustión de carbón y gas, lo que ayuda a explicar los cambios en las emisiones de NO_x en España ya que la generación de electricidad contribuye de manera importante a las emisiones de NO_x. Esto contrasta con los valores más

elevados de NO_3^--N por vía deposición seca en 1995-96 comparado con los valores de 2011-12 (de 1.3 a 6.7 kg $ha^{-1}año^{-1}$), que se puede atribuir a las condiciones climáticas particulares de cada período (más seco en el segundo periodo). El aumento de las emisiones de NH_3 en España durante los últimos 30 años (13% de 1980 a 2012) no se reflejó en la tendencia del contenido de NH_4^+ en la lluvia en el Montseny, probablemente debido a la disminución de la formación de sulfato de amonio y nitrato en aerosoles a causa de la disminución de las emisiones de SO_2 y NO_x.

Dado que las emisiones de NH_3 siguen aumentando en España, hemos querido proporcionar un nivel crítico (CLE) como umbral de seguridad para proteger a las especies y ecosistemas de los efectos perniciosos del NH_3. Para ello, se estudiaron las relaciones entre las concentraciones de NH_3 en el aire y la diversidad de líquenes en un gradiente de distancia a una fuente puntual de emisión (granja de ganado). Los valores de diversidad de líquenes se obtuvieron por separado para los grupos funcionales de líquenes (oligotrófico y nitrofílos). Encontramos un CLE de NH_3 atmosférica < 2.5 mg m^{-3} para este encinar mediterráneo. Este trabajo confirma que los grupos funcionales de líquenes pueden ser utilizados para obtener los niveles críticos de muy diferentes tipos de bosques mediterráneos, ya que se aplicó el mismo enfoque en dehesas portuguesas.

Además de las comunidades de líquenes se han encontrado otros parámetros (% N, $\delta^{15}N$, C: N, $\delta^{13}C$) medidos en diferentes compartimentos del ecosistema (hojas de los árboles, musgos y suelos) que también responden a la contaminación por NH_3 sirviendo como indicadores de la misma. La variación de la señal isotópica $\delta^{15}N$ en hojas y musgos a lo largo del gradiente refleja la señal isotópica de la fuente N. El contenido de N y de $\delta^{15}N$ en musgo y hojas se correlacionó significativamente con la diversidad relativa de líquenes nitrofílos y con las concentraciones de NH_3 en el aire, lo que pone en relieve la respuesta específica de las especies nitrofílas a la contaminación por NH_3. En el suelo, la señal de $\delta^{15}N$ y la ratio C:N se relacionaron significativamente con las concentraciones de NH_3, las ratios C:N (<25) indican el riesgo de saturación de N de la zona estudiada y la consiguiente lixiviación de N y eutrofización de las aguas subterráneas. Por último, la variación en el contenido $\delta^{13}C$ en muestras de hojas y musgos refleja la respuesta fisiológica de la vegetación a una mayor deposición de N.

Los resultados de este estudio indican que la deposición de N total en varios sitios en España supera (aproximadamente en un 30%) las cargas críticas propuestas para los bosques esclerófilos. La deposición seca de N contribuyó en gran medida a las cargas DIN totales (75-85%). En España, las emisiones de NH_3 no se han reducido y por tanto, se necesitan estudios acerca de sus efectos sobre los ecosistemas. En este caso, se propone un CLE de NH_3 en el aire < de 2,5 mg m^{-3}, que protege a las comunidades de líquenes en el bosque semi-natural de encinar, y se proponen nuevos indicadores (δ15N y % N de musgo y hojas) de la contaminación desde una fuente puntual de NH_3.

Chapter 1

General Introduction

Human impacts on the Nitrogen cycle

Human demands for food and energy production have changed the historical limited availability of reactive nitrogen (N_r) in the biosphere. During the last centuries we have passed from a natural world where the dominant N_r source was biological fixation to a current situation where atmospheric deposition constitutes the greatest input (Galloway et al., 2008). Due to anthropic activities, the creation of Nr in a global scale have increased tenfold from 15 TgN yr^{-1} in 1860 to 156 TgN yr^{-1} in the early 1990s, and future scenarios predict increases to *ca.* 270 TgN yr^{-1} by 2050 (Galloway et al., 2004). This rise has affected the N cycle in ecosystems worldwide (Erisman et al., 2013; Fowler et al., 2013)and is currently considered as one of the major global threats to the sustainability of the planet (Rockström et al., 2009). Anthropogenic inputs of N_r comprise reduced forms (NH_x) which are mainly constituted of gaseous NH_3 and particle-bound NH_4^+, and oxidized forms (NO_y), which are mainly constituted of atmospheric gaseous NO_x (NO_2+NO) and HNO_3, and particulate NO_3^-. The main sources responsible of NH_x emissions are the synthesis of fertilizers by the Haber-Bosch process and other activities related to farming, such as animal husbandry and manure application to cultivated fields. However, motor vehicles outfitted with three-way catalytic converters are also significant contributors of NH_3 in urban areas (Cape et al., 2004; Kean et al., 2000). NO_y emissions are generated during energy production processes, industrial activities, road traffic and ship emissions. Most of the long-range transported N occurs as particulate NH_4^+ and NO_3^-, mainly in the size fraction 0.1- 1.0 µm in diameter, which is the size of atmospheric particles with the smallest dry deposition velocity. The gaseous NH_3 form usually is deposited close to the emission sources (Asman et al., 1998; Asman and van Jaarsveld, 1992). All these chemical forms can be transformed and transported in the atmosphere until they eventually are deposited in terrestrial and aquatic surfaces in the form of wet or dry atmospheric deposition.

Considering that N is a major nutrient essential for life, alterations in its natural cycle have an impact on the state and functioning of ecosystems. Several studies have alerted about the negative effects of excessive N to ecosystems. Major issues of

concern are the eutrophication and acidification of soils and freshwaters, alterations in ecosystem functioning and the loss of biodiversity (Bobbink et al., 2010; Dise et al., 2011; Galloway et al., 2003; Manning et al., 2006). Furthermore, nitrogen compounds are precursors of tropospheric ozone and atmospheric particulate material thus contributing to impair the air quality and exacerbate climate change trends. Excessive N_r in air and water also affects human health causing respiratory and cardiac diseases together with several cancers that contribute to increase human mortality (Erisman et al., 2013; Townsend et al., 2003).The principal anthropogenic N_r inputs as well as their main environmental effects are summarized in Figure 1, in which the movement of the N_r molecules from one environmental system to another, the so-called nitrogen cascade (Galloway et al., 2003), is shown.

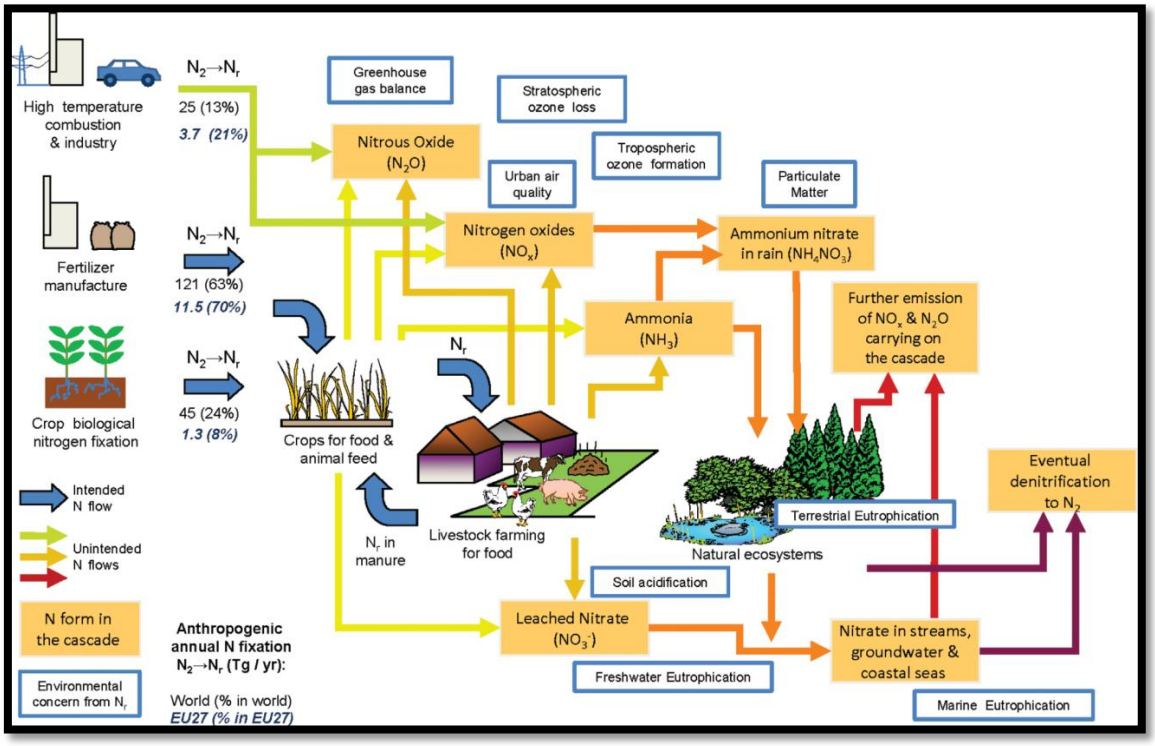


Figure 1. Scheme of the nitrogen cascade. Orange boxes are the main pollutant forms of Nr and boxes outlined with blue are the main environmental concerns. Estimates of N fixation for the world (Tg/yr for 2005, in black; (Galloway et al., 2008)) are compared with estimates for Europe (Tg /yr for 2000, in blue italic; (Leip et al., 2011)). Energy is needed to fix N_2 to Nr, which is gradually dissipated through the cascade with eventual denitrification back to N_2. Blue arrows represent intended anthropogenic Nr flows; all the other arrows are unintended flows. Adaptation from (Sutton et al., 2011).

Even though N deposition is of concern for many ecosystem types, forests are probably the ones receiving larger deposition loads, mainly because their greater aerodynamic roughness enhances the ability to capture 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. Even though short-term inputs of N in forest ecosystems might have a fertilizing effect that favors forest productivity specially at ecosystems limited by N (Ferretti et al., 2014), this effect may disappear in time as N is being incorporated and sequestered in wood and soil organic matter. Some particular effects of excessive N are: (a) direct toxicity to individual sensitive plant species (Kleijn et al., 2008; Krupa, 2003; Munzi et al., 2013; Roelofs et al., 1988), (b) expansion of nitrophilous species that cause losses in biodiversity (Bobbink et al., 1998), (c) soil acidification, base cation depletion and enhanced availability of toxic metals (e.g. Al^{3+}, Fe^{3+}) (De Vries et al., 2003; Horswill et al., 2008; Ulrich, 1983; van Breemen et al., 1983), and (d) Increased susceptibility to secondary stress and disturbance factors (Bobbink et al., 2003; Carroll et al., 1999; Power et al., 1998; Sheppard et al., 2008). Besides, the alteration of inorganic N by human activities might be affecting the N organic formation, whose interaction with the inorganic forms are currently receiving more attention by the scientific community, since they can be directly taken up by vegetation (Uscola et al., 2014).

The nitrogen saturation concept

Nitrogen saturation in forests occurs when N availability exceeds N plant and microbial demand (Aber et al., 1989; 1998). The concept of nitrogen saturation was first proposed by Aber et al., (1989). They proposed that the continuous addition of N to ecosystems may lead to NO_3^- and Al^{3+} mobility in soils, causing soil and stream acidification, nutrient imbalances in trees and finally, forest decline. The response to N deposition was not lineal, showing thresholds with abrupt changes. Further revision of the concept by Aber et al.,(1998) described N saturation as a progressive syndrome of simultaneous responses to chronic N deposition as shown in Figure 2 (Aber et al., 1998). Basically, stage 0 is pre-treatment and assumes strong nitrogen limitations on

growth, stage 1 is characterized by high nitrogen retention and a fertilizer effect of added nitrogen on tree growth; however, ratios of base cations to N (e.g. Ca^{2+}/N, Mg^{2+}/N) in foliage start to decrease, stage 2 includes the induction of nitrification and some nitrate leaching, although growth is still high and finally, at stage 3 tree growth declines, nitrification and nitrate loss continue to increase.

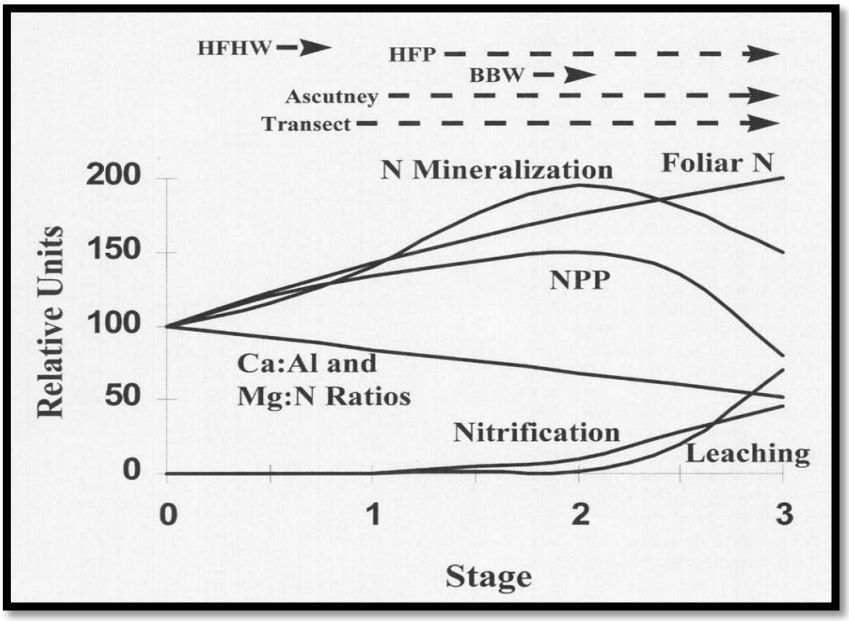


Figure 2. Adapted from Aber et al., 1998. Revised set of hypotheses on the response of temperate forest ecosystems to long-term, chronic nitrogen additions. Changes from initial hypotheses stated in Aber et al., (1989) include the reduction in nitrogen mineralization in stage 3 and the addition of foliar Ca:Al and Mg:N ratios. Abbreviations at the top of the figure denote relative degree of nitrogen saturation in the study sites and the transect stands prior to increased deposition. The position of the arrowheads summarize how far toward saturation each site moved. It is hypothesized that previous land-use history determines the initial position and that deciduous stands move more slowly toward saturation than evergreen stands. HFP, Harvard Forest pine stand; HFHW, Harvard Forest hardwood; BBW, Bear Brook Watershed.

Other authors have defined an ecosystem as N saturated when the physiological N demand of the primary producers is satisfied and at the same time a measurable export of N takes place (Skeffington and Wilson, 1988) or when the export of N surpasses the N input in long-term studies (Ågren and Bosatta, 1988).The latter definition requires a complete N balance,

which not many studies can overtake. To overcome this problem, other authors have defined an ecosystem to be N saturated when atmospheric N input and N mineralization together exceed the retention capacity of the ecosystem and thus there is a permanent leaching of nitrate (Miegroet et al., 1993). According to this, for each given ecosystem there is a defined level of maximum tolerable N input. If this threshold is surpassed, excess nitrate will be exported.

It has also been shown that the responses of ecosystems regarding N saturation are dependent on site characteristics and site management history (Aber et al., 1998; Fenn et al., 1998; Gundersen et al., 1998b). Studies conducted in California Mediterranean-type ecosystems have demonstrated the responses of vegetation and soils in a gradient of increased N deposition from 1 to 70 kg N $ha^{-1}yr^{-1}$ in California (Fenn et al., 2008). Among the most notable effects they found an increase of N concentration in plant tissues, increased rates of nitrification and leaching of NO_3^- into runoff and changes in the composition of the lichen communities (Fenn et al., 2008; Jovan and McCune, 2004). Other effects observed were the increase of invasive species and the loss of native species (Fenn et al., 2010), alterations in mycorrhiza and lichen communities and changes in the chemical composition of lichen tissues (Bobbink and Hettelingh, 2010; Fenn et al., 2010; Jovan and McCune, 2005).

Abatement programs: the establishment of critical loads and critical levels

To counteract the adverse effects of atmospheric pollutant deposition, international amendment programs were launched by the UNECE through the Convention on Long-Range Transboundary Air Pollution (CLRTAP) (UNECE, 2011; www.unece.org/env/clrtap). The Convention was the first multilateral agreement set to protect the environment against ecosystem acidification and eutrophication. It was signed in Geneva in 1979 and entered into force in 1983. Up to now, the Convention has established eight legally binding protocols. The outcome of the implementation of these protocols was a significant decline in S atmospheric deposition, especially observed in Europe and North America beginning in the middle 1980s (Fowler et al.,

2007; Skjelkvåle et al., 2005; Stoddard et al., 1999). In the case of N emissions, the Protocol concerning the Control of Emissions of Nitrogen Oxides or their Transboundary Fluxes was first signed in 1988 in Sofia, and subsequently amended in 1996, 1999 and 2010 (http://www.unece.org/env/lrtap/nitr_h1.html). However, even though strong reductions were implemented in many European countries, the response was not so homogeneous and presented high variability between regions and N forms (Fagerli and Aas, 2008; Konovalov et al., 2008; Lövblad et al., 2004).

A very important tool to guide the abatement policies was grounded on the concepts of critical loads (CLOs) and critical levels (CLEs). CLOs are a quantitative estimate of exposure to an element deposition below which harmful effects on specified sensitive elements of the environment at the ecosystem level do not occur according to present knowledge (Nilsson and Grennfelt, 1988). CLEs are the concentration of atmospheric compounds above which direct adverse effects may occur in receiving systems according to present knowledge (Cape et al., 2009). Receiving systems can be aquatic or terrestrial ecosystems or human health. The critical values for different pollutants and types of receptors are continuously updated according to the progress of scientific knowledge and technical reports published by the Convention (UNECE, 2011). Critical values are used to guide European directives that regulate emissions and air quality and they constitute the basis to develop exceedance maps that indicate where harm to vegetation may be expected.

Empirical critical loads have been defined for specific ecosystems based on observed changes in species composition or N leaching, among other functional or structural variables. Recent revisions of CLOs for N deposition have set values ranging from 3- 30 kg ha^{-1} yr^{-1} for different habitats in Europe (Bobbink and Hettelingh, 2010) and between 1–25 kg ha^{-1} yr^{-1} for North America (Pardo et al., 2015). CLEs have been developed based on observations and experiments addressing different effects on vegetation, such as visible injury symptoms or changes in species composition in semi-natural vegetation. CLEs for NH_3 were initially set to 8 μgm^{-3} NH_3 but a recent revision recommended a new threshold of 1 μgm^{-3}(Cape et al., 2009), a value which has been adopted for the protection of European ecosystems by UNECE (Hallsworth et al., 2010; Pinho et al., 2009).

Nitrogen deposition trends in Spain during the last decades

European policies have brought a substantial reduction of the N emissions in the EEA-33 (33 European members, EEA 2011). NO_x emissions decreased by 44% between 1990 and 2011 with the largest reductions in the road transport sector, while NH_3 emissions were reduced by 25% for the same period (EEA 2011). Even so, many countries are still far from the threshold ceilings proposed by the 1999 Gothenburg Protocol. In the particular case of Spain, emission data for NH_3 indicate a 14% increase from 1990 to 2010, while NO_x emissions decreased by 20% in the same period after a continuous previous increase until 2007, as reported by the Spanish government to EMEP (MAGRAMA, 2013). In fact, data from a wet deposition monitoring network in Catalonia (NE Spain) consisting in 5 stations spread across this autonomous community have shown an increase in rain nitrate concentrations in the period 1996-2010 in some sites (Avila et al., 2010). Average inorganic nitrogen wet deposition in this period ranged between 4 to 7 Kg N $ha^{-1}y^{-1}$. Dry deposition was estimated to contribute in similar amounts, thus total deposition was estimated to range between 10 - 20 Kg N ha^{-1} yr^{-1} (Avila et al., 2010).At the Spanish level, deposition data compiled from 25 measuring stations belonging to the EMEP, ICP-Forests and Catalan networks produced an average inorganic nitrogen wet deposition of 4.4 Kg N $ha^{-1}y^{-1}$ for the period 2005-2008 (García-Gómez et al., 2014). The total N deposition in Spain for year 2008 was estimated with the EMEP and CHIMERE models. Modelled wet deposition was similar to measurements (average of 4.3 and 3.2 Kg N $ha^{-1}y^{-1}$ for the EMEP and CHIMERE models respectively) and total N deposition reached maxima values of 19.5 kg N ha^{-1} yr^{-1}, and 22.9 kg N ha^{-1} yr^{-1}, respectively for each model (García-Gómez et al., 2014). These very high N deposition loads tended to occur in mountain areas with sensitive habitats.

The estimations of NO_x and NH_3 concentrations as well as the N oxidized and reduced deposition derived from the EMEP/MSC-W model during the period from 1995 until 2013 in Spain are shown in Figure 3. This chemical transport model has been performed under the frame of the CLRTAP for the past 30 years and it is based in source-receptor matrices which estimate the contribution of the emissions in any country to the depositions or air concentrations of main pollutants of a receptor country (here Spain).

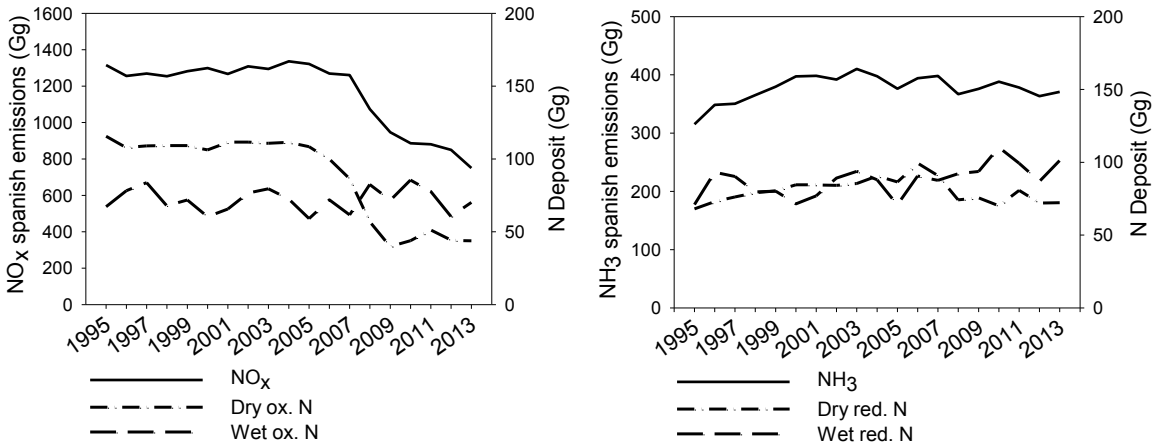


Figure 3. NO_x and NH_3 Spanish emissions, dry and wet N deposition (Gg) for Spain during the period from 1995-2013 estimated with the EMEP/MSC-W model

Despite the emission reductions, Spain was exceeding in 2012 the ceilings for NO_x and NH_3 emissions proposed for that year (847 and 353 Gg respectively).

Atmospheric deposition, measurements and estimations

To establish critical loads and levels, it is necessary to measure the actual deposition of N_r compounds. However, deposition measurements entail some difficulties relative to the estimation of dry deposition. Different approaches and methodologies have been developed to tackle this problem, which are here summarized:

The total atmospheric deposition (TD) delivered to the earth surface involves: (1) wet deposition (WD) which is the flux of atmospheric substances dissolved in rainfall, snow, clouds or fog, and (2) dry deposition (DD) the flux of gases and particles from the atmosphere that does not involve water as a deposition vehicle and which is conveyed by gravitational settling, direct turbulent impaction, and adsorption/ absorption on surfaces (Fig. 4). The deposition involving clouds and fog water droplets is referred as 'occult deposition' (Erisman and Draaijers, 1995) (Fig.4).

In order to understand the effects of atmospheric N deposition on natural ecosystems as well as to determine the effectiveness of emission abatement policies, appropriate methods for measuring deposition are needed. WD is directly collected by wet-only sampling devices placed in the field study sites. These collectors automatically open at the onset of precipitation. However, for economic or logistic reasons, permanently open collectors consisting of a funnel connected to a sampling bucket have been also widely used, the so-called bulk deposition (BD) (Izquierdo and Avila, 2012). In this configuration the collecting surface is permanently open to the atmosphere, thus a fraction of dry gravitational sedimentation is collected besides WD. In some studies, parallel WD and BD measurements are obtained and the WD/BD ratios can be used to estimate WD from BD measured at other sites with similar atmospheric composition (Thimonier et al., 2005). Both WD and BD are frequently referred in the literature as open field precipitation (PD).

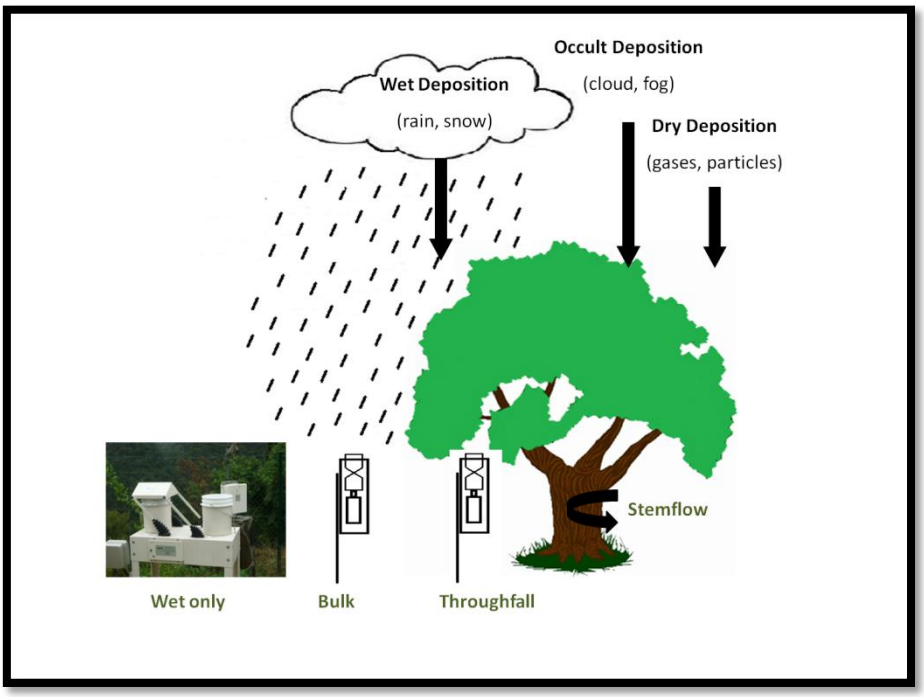


Figure 4. Main deposition fluxes (black) and sampling methods (Green).

Measuring DD is not so straightforward and has been estimated through several approaches, in some cases, used in combination. The main approaches used are: (1) Micrometeorological methods as the eddy covariance and the gradient method (Lenschow and Hicks, 1989; Wesely and Hicks, 2000), (2) Inferential modeling (Flechard

et al., 2011; Zimmermann et al., 2006), (3) Surface accumulation methods, the most extended one being that of throughfall measurements, and (4) Watershed mass balance methods (Likens et al., 1990; Mitchell et al., 2011). Throughfall techniques have the advantage that they can be applied more successfully than micrometeorological approaches to mountainous terrain and in areas with non-homogeneous surfaces. Consequently, it was the natural choice for the study sites comprising this PhD thesis.

- **The throughfall method**

Throughfall (TF) consists in the rainfall collected under vegetation; in our case, under the forest canopy (Fig.4). It has been long recognized that the quantity and quality of precipitation is significantly modified as water cross the canopy. The processes that take place in throughfall have been extensively reviewed (Levia and Frost, 2006; Parker, 1983). In summary, precipitation falling onto the canopy can follow three pathways: 1) direct arrival to the soil without contacting the canopy (free throughfall), 2) interception and evaporation from the canopy (interception loss), and 3) contact on leaves and branches and subsequent drip to the soil, also termed "release throughfall" (Levia and Frost, 2006). Precipitation also flows down from the trunk surfaces, constituting the stemflow (SF) flux. In general, the SF flux is much lower than TF, and because of its small contribution in many cases it is not measured.

The TF chemistry is influenced by two main processes: 1) the wash-off of dry deposited gases and aerosols accumulated on the canopies during dry periods previous to the rain, and 2) the canopy exchange process (CE), which represents the chemical interaction between the rain components and the vegetation surfaces. CE processes include the leaching of elements from internal plant tissues (canopy leaching, CL) and the canopy uptake (CU) of gases or dissolved elements in aqueous films by the foliage, branches and epiphytes in the canopy (Lovett and Lindberg, 1984; Schaefer and Reiners, 1990).

The following equations describe these relationships:

$$TF(+SF) = TD + CE = PD + DD + CE$$
$$TD = PD + DD$$

In case of considering SF as negligible, net throughfall (NTF) is:

$$NTF = TF - PD = DD + CE$$

When aiming to estimate TD with TF measurements, a distinction has to be made between the CE and DD fluxes in NTF. How to address this distinction has been the subject of numerous studies in the last decades (Beier et al., 1992; Draaijers and Erisman, 1995; Lovett and Lindberg, 1984; Mayer and Ulrich, 1978; Stachurski and Zimka, 2000; Staelens et al., 2008). The two most common used approaches are : 1) multiple regression models, a methodology first described by Lovett and Lindberg (1984) that distinguishes NTF into DD and CE based on the duration of previous dry periods (related to DD) and the TF water amounts (related to CE), and 2) canopy budget models (CBM), such as the one first proposed by Ulrich (1983) and subsequently extended by several authors in Europe (Adriaenssens, 2012; Erisman and Draaijers, 1995).The CBM has been adopted as the standard method for TD estimation by the ICP-Forest (ICP-Forest Manual, Appendix 8) and will be used in our work.

The so called “filtering approach" was developed to estimate DD of base cations (Ca^{2+}, Mg^{2+}, K^{+}) by using a trace element which was assumed not to participate in exchange reactions in the canopy (Ulrich, 1983). Although Na^{+} is the most frequently used tracer, SO_4^{2-} (Ignatova and Dambrine, 2000; Ukonmaanaho and Starr, 2002), Cl^{-} (Bouya, 1999) or Ca^{2+} (Balestrini and Tagliaferri, 2001) have also been considered suitable tracers of DD. The CBM follows the next assumptions: (1) the tracer ion is not influenced by canopy exchange processes and (2) aerosols containing the ions of interest have similar deposition behavior than the ion chosen as tracer (Staelens et al., 2008). For full details on the CBM calculations, see chapter 2.

- **Inferential approach**

The inferential method is a combination of measurements and modeling that estimates dry deposition rates on the basis frequent air concentration and meteorological measurements (e.g. hourly, daily data). The method is based on the following steady-state relationship:

$$F = V_d * C$$

Where F stands for the dry deposition flux, V_d is its dry deposition velocity and C the concentration of an airborne pollutant. Meteorological variables are needed to parameterize V_d. This method has been extensively described in the literature mostly for temperate ecosystems (Erisman et al., 1994; Lovett and Lindberg, 1993; Wesely and Hicks, 2000).

Atmospheric N deposition at Mediterranean Forests, the particular case of Spain

The Mediterranean Basin presents an extraordinary biological richness and is considered as one of the 25 Global Biodiversity hotspots for conservation priorities (Myers et al., 2000). Even though Mediterranean ecosystems are particularly vulnerable to increased N inputs, little information is available on the loads and effects of N in this type of ecosystems (Ochoa-Hueso et al., 2011). Most studies concerning the impacts of N deposition in Mediterranean ecosystems have been carried out in California and they have alerted about the important contribution of the DD fraction to the TD at Mediterranean climatic regions, and the importance in developing methods to accurately quantify this fraction (Bytnerowicz et al., 1987; Fenn and Bytnerowicz, 1993). This was lately corroborated in European Mediterranean areas (Avila and Rodà, 2002), as is the case of Spain, where it has been suggested that DD may represent between 50 to 80% of the TD in *Quercus ilex* and *Pinus halepensis* forests (Avila and Rodà, 2012; García-Gómez et al., 2014; Sanz et al., 2002). The important contribution of the DD fraction to the TD of N is mostly explained by the Mediterranean climate particularities. The Mediterranean climate has moderate precipitation inputs, usually

concentrated in spring and autumn, but there is a great variability in monthly and inter-annual precipitation amounts. Dry spells occur frequently between rainy seasons. Characteristically, winter and summer concentrate prolonged dry periods. During these dry spells, dry deposition is accumulated on the canopies and subsequent rains may solubilize the accumulated material and make it available for biological cycling and processing. Therefore, the seasonal distribution of rainfall throughout the year under the Mediterranean climate modulates the availability of water and nutrients to vegetation and forest soils. In some occasions, this availability may occur in winter when the vegetation is at its minimum activity, causing a decoupling between N inputs and water and nutrient demands by vegetation (Ochoa-Hueso et al., 2011).

Vegetation growing under the Mediterranean climate is strongly adapted to water and temperature constraints. Currently, Mediterranean forests are dominated by coniferous and sclerophyllous species (Inventario Forestal Nacional, INF3).One of the most widely distributed tree in the Mediterranean Basin is holm oak (*Quercus ilex* L.), more abundant in the western part of the Basin (Barbero et al., 1992). This is a slow growing species with structural and physiological adaptations to drought that determine its conservative use of water, allowing it to survive under xeric conditions (Terradas and Savé, 1992), being also adapted to extreme temperatures (Costa et al., 1997).*Quercus ilex* forms the most extended evergreen sclerophyllous forests in the Iberian Peninsula covering 3 million hectares in Spain, which is 25% of the national area where trees cover at least 10% of the ground (Terradas, 1999).Historically these holm oak forests have been managed for centuries and different management practices have resulted in different forest types. In the mountainous eastern region of Spain, as in southern France, Italy and Greece, holm oak forests were traditionally exploited for charcoal and firewood production. This management practices were abandoned in the early 1950s and holm oak forests are currently recovering and increasing in biomass (Rodà et al., 1999). On oligotrophic soils in central and western Iberian Peninsula, holm oak open woodlands have been traditionally managed as “dehesas” (named “montados” in Portugal). These are especially diverse ecosystems harboring a high diversity of plants and animals (Díaz et al., 2003; Peco et al., 2001).

There is evidence of N enrichment in natural forest ecosystems in the Mediterranean Basin. An increase of N content in herbarium species of bryophytes collected in the XX century reflects the increase of N loads in Spain (Peñuelas and Filella, 2001). Nitrophilous species have increased in natural areas included in the Spanish Natura 2000 network (Ariño et al., 2000). Recent studies have shown that increasing N loads to natural ecosystems alters soils cation availability that might have implications in terms of plant nutrition and soil microbial communities (Ochoa-Hueso et al., 2014). Despite these evidences, information is still missing in Spain, and generally in the Mediterranean Basin, either about reliable estimates of CLOs and CLEs as well as the quantification of the DD fraction. The information available today is still limited regarding the establishment of the critical load of N as a nutrient (understood as the total atmospheric deposition of N accumulated over a year) for the protection of the Mediterranean terrestrial ecosystems. Tentative values for the protection of evergreen broadleaf forests have been proposed in a revision for most European vegetation types (Bobbink et al., 2010). Therefore, further research is needed at highly biodiverse evergreen woodlands from the Mediterranean Basin that also provide a large number of ecosystem services.

Diagnostic indicators of elevated N deposition

To establish critical thresholds for the protection of ecosystemsas well as to assess the effectiveness of air pollution control strategies, diagnostic indicators at the ecosystem level need to be defined. An ecological indicator is a measurable attribute that describes the state of the environment and provides insights beyond its own measurement (Noon, 2003). Ecological indicators can be used to assess the effect of either natural or anthropogenic disturbances. In the case of N pollution, a broad spectrum of indicators have been considered, such as changes in species diversity, visual foliar injury and the monitoring of N content and N isotope ratios in plant material (Pinho et al., 2011; Pinho et al., 2012; Pitcairn et al., 2003; Sutton et al., 2004).

Lichens are suitable indicator organisms because of their high sensitivity to several types of pollutants. In the case of N, the key issue is that different lichen species have different requirements of N supply: while some species are able to live under high N atmospheric levels, others are intolerant to excessive atmospheric N or affected by direct toxicity of NH_3. Lichen diversity has been consistently found to be the most sensitive ecological indicator for NH_3 air pollution (Frati et al., 2007; Pinho et al., 2011).The study of the lichen diversity response to NH_3 has been used to refine the NH_3 CLEs (Cape et al., 2009). Lichens have also been used to set the CLOs for different type of European forest habitats (Giordani et al., 2014; Pinho et al., 2012).

Many studies have described the close relation between tissue nitrogen content in lichens, mosses and vascular plants with N atmospheric inputs (Pitcairn et al., 2003; Pitcairn et al., 1998; Skinner et al., 2006). It has been shown that total tissue N content of mosses is an excellent indicator of N deposition at sites dominated by dry deposition of NH_3 (Pitcairn et al., 2006). The use of mosses as passive monitors of atmospheric N deposition allowed describing the variation in atmospheric N deposition at a high spatial resolution, including areas where nitrogen deposition monitoring networks were absent. In this sense, it has been suggested that the total nitrogen concentrations in mosses may complement deposition measurements, and will help to identify areas at risk from high nitrogen deposition in Europe (Harmens et al., 2011). Enhanced N cycling lead to changes in N to nutrient ratios in plant tissues and/or soils and it has been suggested that C/N ratios below 25 are indicative of high risk of nitrate leaching (Gundersen et al., 1998a; Van der Salm et al., 2007). Also, the decrease of foliar C/N has been proposed to indicate intermediate states of ecosystem nitrogen saturation (Aber et al., 1998).

On the other hand, anthropogenic induced changes in N cycling may affect the natural abundance of the heavy N isotope ^{15}N ($\delta^{15}N$) in plants and soils (Högberg, 1997). The N isotope abundance is expressed as the relative deviation (in ‰) from the ratio ^{15}N:^{14}N in atmospheric N_2: $\delta^{15}N=1000(R_{sample}-R_{air})/R_{air}$, where R is the ratio ^{15}N:^{14}N of sample and air respectively. The relative abundance of both isotopic forms of N varies among N pools in the ecosystem as one form or the other is differently discriminated. The main processes that affect $\delta^{15}N$ are mineralization, nitrification and denitrification

(Koba et al., 1998). Therefore, it has been suggested that $\delta^{15}N$ can be used as an efficient monitor of ecosystem N dynamics (Fang et al., 2011; Martinelli et al., 1999; Pardo et al., 2006). Moreover, $\delta^{15}N$ can be also used to infer the source of N deposition (Gerdol et al., 2002; Kahmen et al., 2008) since the main anthropogenic N sources have different $\delta^{15}N$ composition. In this sense analyzing $\delta^{15}N$ content in bryophytes is particularly useful because these organisms take up N directly from the atmosphere and thus reflect its composition (Pearson et al., 2000). In general, more negative $\delta^{15}N$ values in moss tissue are associated to NH_y-N deposition sources; while less negative $\delta^{15}N$ values indicate NO_x-N deposition (Liu et al., 2008; Solga et al., 2005).

Objectives and structure of the thesis

The general aim of this PhD is to gain information about the N deposition in Mediterranean holm oak forests. The particular objectives were:

1. Estimate the total deposition of N at four Mediterranean holm oak forests representative of the Iberian Peninsula using bulk and throughfall data.
2. Characterize the temporal evolution of N emissions in Spain in the last three decades and explore their relationships with atmospheric deposition and streamwater export in a Mediterranean holm oak forest in La Castanya (Monsteny).
3. Determine thresholds values (Critical levels, CLEs) for NH_3 deposition through the study of lichen diversity based in functional groups.
4. Compare the response to NH_3 pollution of different parameters (%N, C/N, $\delta^{15}N$, $\delta^{13}C$) in three different ecosystem compartments (moss, holm oak leaves, soil) and that of lichen diversity in order to determine the most sensitive indicators of pollution.

We provide a first chapter as a general introduction in order to contextualize the N atmospheric deposition knowledge, particularly for Mediterranean forest ecosystems.

In **chapter 2** ("Atmospheric deposition to 4 holm oak forests in Spain submitted to a different pollution climate") we aim to understand how the rainfall chemistry is modified in the passage through the tree crowns and the main canopy exchange processes at the canopy level from forests submitted to different environmental conditions (3 peri-urban sites and a rural background site). Here we used the throughfall measurements to estimate the dry deposition fluxes by means of a canopy budget model. For that purpose, we compiled chemical and hydrological data on precipitation and throughfall at the four study sites from June 2011 until June 2013.

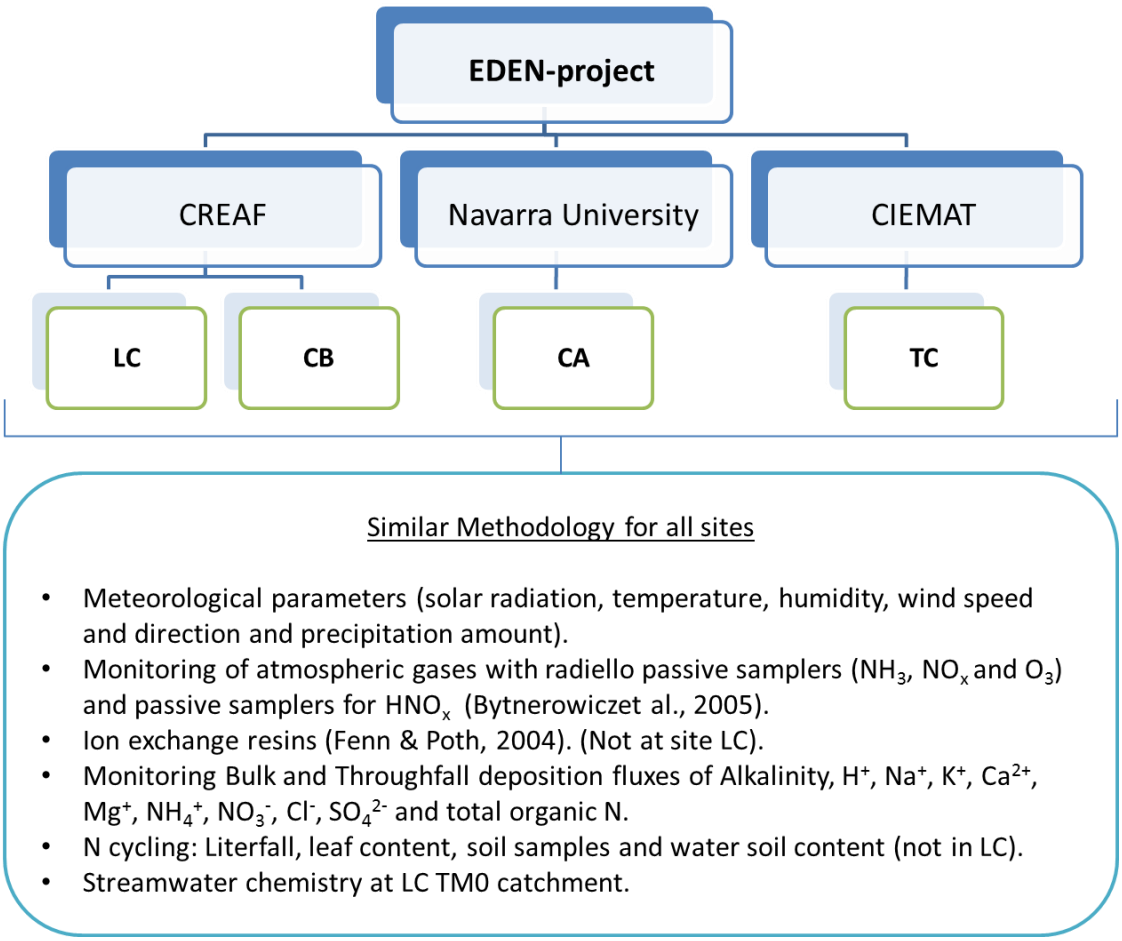
We have examined how changes in S and N emissions from the early 1980s to 2014 are reflected in SO_4^{2-}, NO_3^- and NH_4^+ rain concentrations and deposition at a site in the NE of the Iberian Peninsula. We explore if the abatement measures implemented under

the Convention on Long Range Transboundary Air Pollution (CLRTAP) protocols are reflected in reduced deposition **in chapter 3** ("Long-term effects of changing atmospheric pollution on throughfall, bulk deposition and streamwaters in a Mediterranean forest". This was done by comparing bulk deposition trends at a rural forested site (La Castanya, Montseny) in NE Spain, with trends in emissions in the Iberian Peninsula (Spain + Portugal), France, Italy and the total for the European Union (EU28) for this period. Throughfall measurements in two contrasting periods over this time span (1995-1996 and 2011-2013) were compared in order to provide insights on dry deposition changes. We have also explored whether these changes in atmospheric deposition were reflected in the streamwater chemistry and export fluxes draining an undisturbed forested catchment at this site by analysing a long-term streamwater data series (1990 to 2014).

In **chapter 4** ("The critical levels of atmospheric ammonia in a Mediterranean forest in North-Eastern Spain") we aimed to provide an empirical CLE of atmospheric NH_3 for the first time in a Mediterranean holm-oak (*Quercus ilex* L.) forest in NE Spain. We aim to validate the use of lichen functional groups previously employed in other Mediterranean forest types. For that purpose, we monitored the NH_3 atmospheric concentrations and measured the lichen functional diversity in a gradient of increasing distance from a cattle barn as a point-source of NH_3 emission in a Mediterranean evergreen holm-oak forest in Catalonia (NE Spain).

In line with the results from the last chapter, we have expanded the scope of the observations to understand the ecosystem responses (at the level of soil, holm oak leaves and epiphyte moss) to NH_3 pollution. The purpose of **chapter 5** ("Diagnostic indicators of high atmospheric ammonia pollution in a Mediterranean holm oak (*Quercus ilex* L.) forest") has been to compare lichen diversity (distinguishing between functional groups), and %N content, C:N ratios and $\delta^{15}N$ in *Quercus ilex* leaves, the moss *Hypnum cupressiforme*, and forest soils within a distance gradient from an NH_3 emission source. Besides, in order to gain information about the ecophysiological responses of vegetation under increasing NH_3 pollution we also studied the $\delta^{13}C$ content in the mentioned ecosystem compartments.

Finally we want to mention the EDEN-project in which this PhD is framed, and that emerged to fill the outlined gaps in knowledge about N deposition in Mediterranean forests. The main purpose of the EDEN-project was to determine the N loads at Mediterranean evergreen holm oak forests in Spain, and to gain information on the effects of N deposition at these forests. In order to have information about forests representing different climatic and environmental conditions, four holm-oak forests (*Quercus ilex* L.) were monitored during the period 2011-13. Three sites were exposed to different potential sources of N emissions in the north, center and north-east of the Iberian Peninsula, while an additional site at north-east, a priori protected from atmospheric pollution, was also monitored. The two sites located in the north-eastern Iberian Peninsula were coordinated by CREAF (La Castanya and Can Balasc, LC and CB respectively), the site in Madrid was coordinated by CIEMAT (Tres Cantos, TC) and another site in Navarra was coordinated by Navarra University (Carrascal, CA). These measurements and the EDEN functioning are described in the following scheme.



Furthermore, this PhD also studied the forest ecosystem response in a NH_3 pollution gradient near a farm in Osona (NE Spain). Information on air NH_3 concentrations, epiphytic lichen biodiversity and other parameters of the holm oak forest ecosystem was gathered to find out the best indicators to atmospheric NH_3 pollution in order to contribute to fulfill the objectives of the EDEN project.

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Chapter 2

Atmospheric deposition to 4 holm oak forests in Spain submitted to a different pollution climate

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Abstract

Bulk and throughfall precipitation fluxes of major compounds were measured from June 2011 to June 2013 at four Mediterranean holm oak (*Quercus ilex*) forests in the Iberian Peninsula. The aim was to compare the chemical composition of bulk/throughfall precipitation under the influence of different urban and agricultural pollution sources and to provide an estimation of the total deposition arriving to these sites, with special focus to N deposition. Net throughfall fluxes of NH_4^+ were negative or near cero suggesting retention of this compound at the tree canopies, a fact corroborated by its negative relationship with rainfall amount and by the negative relationship between bulk deposition and net throughfall fluxes. .

The use of a canopy budget model allowed estimating the total atmospheric inorganic N deposition at two forests sites located in northeastern Spain. Estimated values were 17-20 kg N ha^{-1} yr^{-1}. Dry deposition presented the highest contribution (74-84%). A similar contribution has been reported in other studies at Mediterranean holm oak forests estimated with different methodologies.

Introduction

Atmospheric deposition has an impact on forests ecosystem functioning, forest health and biodiversity. However, quantification of atmospheric total deposition (TD) is scarce for Iberian Peninsula forests. In general, the Iberian Peninsula is little affected by acidification, due to the important neutralizing role of carbonate-rich dust deposition, mostly accounted by African events (Avila et al., 1998; Rodà et al., 1993). On the other hand, models indicate that this region is particularly affected by high nitrogen (N) deposition rates (Dentener et al., 2006) which may adversely affect biodiversity (Ochoa-Hueso et al., 2011). In fact, the Iberian Peninsula has been considered as one of the 25 Global Biodiversity Hotspots for conservation priorities due to high N deposition (Myers et al., 2000). Critical loads for N set for the protection of terrestrial habitats under the CLRTAP (Convention on Long-Range Transboundary Air Pollution) seem to be currently exceeded in some habitats of Community interest of the Spanish Natura 2000 network (García-Gómez et al., 2014).

Dry deposition (DD) makes an important contribution to total deposition inputs in Mediterranean forests, where it is estimated to represent between 50-80% of the total deposition in *Quercus ilex* and *Pinus halepensis* forests (Avila and Rodà, 2012; García-Gómez et al., 2014; Rodrigo and Àvila, 2002; Sanz et al., 2002), but more research is needed to refine these estimates.

Quantifying the total atmospheric deposition entails some difficulties. Wet and bulk atmospheric deposition can be directly measured by using wet-only or bulk collectors. However, dry deposition is measured with different methodologies. Throughfall (TF) deposition has been widely used to indicate total deposition to the forest soil (de Schrijver et al., 2007). However, exchanges at the canopy level, either from leaching of intercellular material or from uptake, complicate the quantification of DD. Methods for separating in-canopy sources from external sources in TF deposition rely on two broad type of approach: 1) regression models, such as those described by Lovett and Lindberg (1984) and Lovett et al. (1996), and 2) canopy budget models such as the one first proposed by Ulrich (1983) and subsequently extended by several authors in Europe (Adriaenssens et al., 2012; Balestrini and Tagliaferri, 2001; Draaijers and

Erisman, 1995; Zhang et al., 2006). Canopy budget models (CBM) estimates total deposition of major ions by using a tracer ion based in the following assumptions: (1) the tracer ion is not influenced by canopy exchange processes (leaching or uptake), and (2) aerosols containing the other ions have similar deposition behavior than the ion chosen for reference. The model is based on the balance between fluxes above and below the canopy: TF = PD + DD + CE, where TF stands for throughfall, PD for Precipitation Deposition, DD for Dry Deposition and CE for Canopy Exchange. The PD flux is usually known from measurements with wet-only collectors (WD) or bulk deposition collectors (BD), while DD is estimated from a reference ion, as mentioned. Once dry deposition is known, the canopy exchange (CE) flux can be estimated from subtraction in the above equation. For ions that are absorbed at the canopy level (e.g. N and H^+), DD cannot be estimated with this approach, and the calculation process is reversed: first the canopy uptake flux (CU) is estimated and then DD is obtained from the balance equation (Staelens et al., 2008). Up to now and to our knowledge, no studies have applied canopy budget model approaches in Mediterranean European areas in contrast with the abundant literature for temperate and boreal European forests (Adriaenssens et al., 2012; Kirchner et al., 2014; Staelens et al., 2008).

Holm oak (*Quercus ilex* L.) is a typical sclerophyllous species widely distributed in the Mediterranean Basin, whose traditional management has led to a diversity of forest types differing in structure and maturity.

Atmospheric deposition at a peninsular scale may be influenced by different factors such as 1) climate variation linked to geographical position, 2) atmospheric pollutant load and 3) forest structure. Atmospheric deposition to holm oak forests has been intensively measured in two holm oak forest in N-eastern Spain (Prades & Montseny Mountains), which were chosen as background sites regarding exposure to air pollution (Bellot and Escarré, 1991; Rodà et al., 1990; Rodrigo et al., 2003), but more work is needed for a better knowledge of deposition at more pollution exposed sites. The present work aims to compare the chemical composition of precipitation collected at different sites located throughout the Iberian Peninsula and to provide an estimation of the total deposition arriving to these sites. For that we monitored 3 holm oak forests sites under the influence of urban and agricultural pollution sources and a

background site located far from anthropogenic pollutant emissions. A special focus was directed to N deposition fluxes. The specific objectives were: (1) to characterize the atmospheric deposition loads in 4 Mediterranean holm oak forests; (2) to quantify wet and dry deposition fluxes, this latter estimated through a canopy budget model.

Material and methods

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; Figs. 1-4 Photo Appendix). Two sites were located in Catalonia (La Castanya and Can Balasc, LC and CB respectively), one in Madrid (Tres Cantos, TC) and another site in Navarra (Carrascal, CA). The main characteristics of the sampling sites are shown in Table 1.

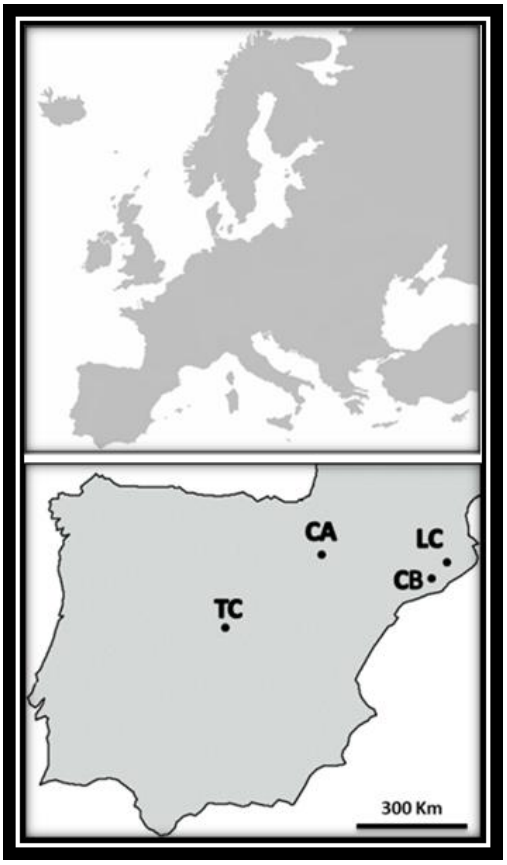

Figure 1. Location of the study sites in the Iberian Peninsula.

Table 1. Study site characteristics, climatic features, forest stand parameters, atmospheric information and potential pollutant sources affecting each study site.

		LC	CB	CA
Study site characteristics	Aspect	SE	NE	SE
	Distance to the sea (km)	27	11	80
Climatic parameters	Climate	Oceanic Mediterranean	Oceanic Mediterranean	Mediterranean continental with oceanic influence
	Mean annual Temperature (ºC)	9.5	15.1	12.6
	Mean annual Rainfall (mm y^{-1})	840	723	609.5
Stand parameters	Number of trees·ha^{-1}	2571	1429	1760
	Mean diameter at breast high (cm)	13	12.6	16.1
Atmospheric data (µg m-3)	NO_2	4.3	16.2	10.6
	NH_3	0.7	1	2.5
	PM_{10}	18	-	26.9
	$PM_{2.5}$	-	-	-
Potential pollution sources		Distant urban agglomerations	Barcelona city	Small urban agglomerations
		Routes within the park	Transportation routes (highways and railways connecting Barcelona to other urban sites)	High traffic intensity highway lying at 100m distance to the study site
		Moderate agricultural activities	Air masses influenced by sea traffic	Agriculture in the fields surrounding the studied site
				Opencast limestone quarry

The site LC (41º46'N, 2º21'E, 760 m.a.s.l.) is located in the Montseny Mountains of the Pre-littoral Catalan Range, 45 km to the NNW of Barcelona city. This site is considered as a rural background station, even though pollution from the Barcelona metropolitan area may be conveyed under certain conditions by sea-land breezes that reach the site by midday (Pérez et al., 2008). Vegetation at LC consists of a 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, and higher temperatures in summer.

The study site of CB (41º25'N, 2º04'Eº, 280 m.a.s.l.) is located in the Collserola Natural Park, a protected park in western Barcelona Metropolitan Area (3.5 million inhabitants). The plot lies at 5.5 km linear distance from Barcelona outskirts. A moderate to heavy traffic highway (C-16) runs about 400m from the study plot. 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 also Mediterranean.

The site CA (42º39'N, 1º38'W, 600 m.a.s.l.) is situated at the foot of the Alaitz-Izco hills, in central Navarra. The nearest larger city, Pamplona (197.604 inhabitants) is 17.5 km to the North. The site lies at ~100 m from a heavy traffic highway (AP-15) and is surrounded by agricultural activities, mainly irrigated crops of cereals. An opencast limestone quarry is located approximately 2 km to the north of the plots. *Quercus ilex* L. woodlands at this site are accompanied by *Quercus faginea* Lam.and *Quercus humilis* Mill. The site lies on calcareous soils mainly composed by washed clays. The climate at CA is Mediterranean continental with oceanic influence from the Atlantic sea.

The site TC (40º35'N, 3º43'W, 700 m.a.s.l.) is located in the outskirts of Madrid (3.2 million inhabitants), 20 km NE from the city center. 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 preserved as a public park. Vegetation was historically managed as a traditional 'dehesa', a savannah-type managed formation with large isolated trees emerging from annual grassland. The low management level during the last decades has allowed the

vegetation to grow as a moderately tree-covered 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 located at 2 km from the monitoring site. The climate is Mediterranean continental, characterized by long dry periods and a more contrasted seasonality than typical oceanic-Mediterranean climate.

Field sampling and chemical analysis

In every location, an open-field and a below-canopy plot to collect bulk (BD) and throughfall (TF) deposition, respectively, were instrumented. Wet-only deposition (WD) was also measured at LC in the open-field plot (Fig 5. Photo Appendix). The same model of sampler was used to collect bulk and throughfall deposition at all sites. It was 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 (Fig. 6 Photo Appendix). A bugsieve was placed into the funnel to prevent leaves and other materials entering into the bottle. The upper edge of the funnel was equipped with an external ring to prevent contamination from bird dropping. The rim of the funnel 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 randomly located inside the forest plot (30*30 m^2) at all sites. Collectors were permanently open to the atmosphere, so they collected dry deposited coarse particles onto the funnel. During no-rain periods the funnel was rinsed with 100 ml of deionized water, in order to collect particles that settle gravitationally, corresponding to coarse dry deposition. The recovered dry deposition (recovered input, RI) during dry periods made a low contribution to the total bulk deposition, but nevertheless it was added to BD and/or TF deposition collected in the next period. Wet-only deposition was sampled at LC with an automatic Andersen sampler (ESM Andersen instruments, G78-1001). All funnels and 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 were periodically obtained and analyzed.

Sampling took place from June 2011 to June 2013 in a weekly/biweekly schedule. All collected samples were kept refrigerated (4ºC) in the darkness until conductivity, pH and alkalinity analysis (within 24–48 h of collection). Alkalinity was measured by a conductivity titration (Golterman et al., 1978) on unfiltered samples at all sites. Samples were then filtered with 0.45 μm size pore membrane filters of cellulose (Millipore) and frozen until the rest of analysis. The ionic measurements from the Catalan sites were performed at CREAF by ionic chromatography (Dionex 100 and Dionex ICS-110). In Navarra and Madrid, ammonium and anions were determined by ion chromatography (NH_4^+: Dionex 1100, with column CS16 in Navarra and Dionex 2000 with column CS12 in Madrid; SO_4^{2-}, NO_3^-, NO_2^-, Cl^-, PO_4^{3-} : Dionex 2000, with column AS19 in Navarra and Madrid), whereas cations were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500a).

Analytical quality was checked with internal control samples of known concentrations and with the ion and conductivity ratios proposed in the ICP-Forest manual (ICP-Forests, 2010) assuming that the main components of deposition are analyzed. For throughfall, there was a systemic excess of cations over the analyzed anions. This difference was attributed to weak acids, similarly to other studies (Balestrini and Tagliaferri, 2001; Hoffman et al., 1980).

Data handling and statistical analysis

Ion concentrations were weighted by water volume (precipitation or throughfall) to calculate volume-weighted means (VWM; expressed as μeq L^{-1}). Bulk and throughfall deposition fluxes were obtained as the product of VWM by the precipitation or throughfall amount. Coarse RI deposition was obtained from the product of concentrations by the fixed rinsing volume (100 mL). All deposition fluxes are expressed as kg ha^{-1} y^{-1}.

All statistical analyses were performed with Statistica (StatSoft 2004) and Sigmaplot 11.0 (Systat Software Inc., San Jose, CA, USA).

The canopy budget model (CBM) recommended in the ICP Forests Manual (ICP-Forests, 2010), was used. The CBM is based on the following balance between fluxes above and below the canopy:

$$TF+SF = PD + DD + CE \quad (1)$$

TF stands for throughfall, SF for stemflow, PD for Precipitation Deposition, DD for Dry Deposition and CE for Canopy Exchange. Stemflow at the LC site contributed only 3% of total rainfall (Rodrigo et al., 2003) and because of its small contribution and to optimize sampling time and efforts, SF fluxes were not here considered. Then,

$$TF-PD = \text{net } TF = DD+CE \quad (2)$$

Even though BD has been widely used as representing PD, WD has been recommended in canopy budget model calculations to avoid the underestimation caused by the inclusion of a fraction of DD (coarse DD) in BD samples (Staelens et al., 2008). In this study we have used WD measurements at LC (measured in parallel to BD), and applied the ratio WD/BD at LC to derive WD fluxes at CB. For TC, WD and BD measurements were available for a 6 month period (January to June 2013), and the ratios WD/BD for this period were applied to the 2011-2013 period . The BD/WD ratios are given in Table 2. WD was not sampled at CA and the ratio WD/BD from the LC or TC sites was considered not to be representative for such environmentally different site; thus, the CBM has not been applied at CA.

Table 2. BD/WD ratios at LC and TC sites.

	H^+	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	NH_4^+	NO_3^-	SO_4^{2-}
LC	0.70	1.39	1.64	1.33	1.63	1.63	1.31	1.60	1.36
TC	0.63	1.18	1.39	2.13	1.47	1.17	1.45	1.51	1.27

For ions such as Na^+, SO_4^{2-} and Cl^- it is often assumed that the exchange between precipitation and plant tissue is negligible and that their NTF fluxes represent dry deposition. In this study we have used Na^+ as tracer ion since previous research in LC forest have shown that Na^+ in throughfall was mostly derived from dry deposition (Rodrigo et al., 2003; Rodrigo and Àvila, 2002) and that the use of this ion has been widely recommended (Staelens et al., 2008).Then, for an ion x, its DD (DD_x) can be obtained by:

$$DD_x=(TF-WD)_{Na}/WD_{Na})*WD_x \quad (3)$$

This approach is quite straightforward for estimating DD for base cations and sulphate aerosols, assuming that the particles containing them are deposited at similar deposition velocities as the Na^+ particles. For ions with an important gaseous component (such as N in its reduced or oxidized forms), the above assumptions do not hold, and other approaches must be implemented. Furthermore, N compounds are known to be retained at the canopy level (and H as well), thus net TF does not represent DD or canopy leaching (Brumme et al., 1992; Ferm, 1993; Geßler et al., 2002). It has been proposed (Balestrini and Tagliaferri, 2001; Staelens et al., 2008) that the NH_3, exchange flux can be estimated by considering that NH_4^+ uptake equals the net leaching of base cations (leaching of Ca^{2+}, Mg^{2+} and K^+) once corrected by weak acid leaching (Zhang et al. 2006):

$$CU_{NH4}^+= CL_{BC} - CL_{WA} \quad (4)$$

Once CU of NH_4^+ is obtained, the NH_4^+ DD flux can be derived from equation 2. However, there might be also NO_3^- canopy uptake, as in our case (see below).Then, a further step is needed to estimate the NO_3^- canopy retention flux. For this, we followed the approach suggested by De Vries et al., (2003) that considers a proportional uptake of NO_3^- related to that of NH_4^+:

$$CU\left(NH4^{+}+NO3^{-}\right)=\left(\frac{xNH4^{+}*(TF)NH4^{+}+(TF)NO3^{-}}{xNH4^{+}*(TF)NH4^{+}}\right)*CU\left(NH4^{+}\right) \quad (5)$$

An efficiency factor of NH_4^+ vs $NO_3^-$of 6 was here considered following results reported in other studies (De Vries et al., 2003; Schmitt et al., 2005; Thimonier et al., 2005; Zhang et al., 2006). Then, to balance the ionic charges NO_3^- uptake is equated to H^+ uptake (Adriaenssens et al., 2012; Staelens et al., 2008).

Results and Discussion

Water fluxes

The annual BP and TF water fluxes are shown in Table 3. Rainfall amount differed markedly between the studied sites: TC was always the driest site, while the wet sites alternated: in 2011-12 the wettest sites were LC and CB, while in 2012-13 it was CA. These differences in rainfall amount are explained by the climatic characteristics of the study regions and are in accordance with the precipitation patterns and interannual variability in Spain (Rodriguez-Puebla et al., 1998). TC, located at the center of the Iberian Peninsula is under a continental Mediterranean climate, dryer and colder than the coastal Mediterranean, while CA receives an important oceanic influence from the Cantabric Ocean. The Catalan sites LC and CB are under a meso-Mediterranean sub-humid climate, with typical summer drought and wet spring and summers. At LC summer drought is attenuated by frequent orographic storms.

The total annual precipitation was nearly double in the second year at CA and TC, while at CB the second year had also higher precipitation. On the contrary, at LC the first year was wetter than the second (Table3). In general, rainfall in the second year was

more homogeneous along the year, while in the first year, some months concentrated most of the precipitation (e.g. November comprised 30% of the annual precipitation at LC and CB, while 32% of the annual precipitation was in April at CA).

Throughfall volumes were always lower than BP volumes due to interception by the canopy, indicating also the negligible role of occult precipitation at these sites (Table 3; Fig. 2). Throughfall water volume represented on average a 66%-77% of the total rainfall with the lowest percentage at TC, which is consistent with the more open canopy structure of this dehesa site.

From equations in Fig. 3 it can be seen that it is necessary an annual precipitation amount of around 100 mm to start producing TF at the close canopy forests (LC, CB, CA), while for the dehesa site (TC) it is necessary an annual rainfall of around 150 mm to start producing TF, though more data are needed to confirm these results.

Table 3. Yearly rainfall amount and average (mm) during the studied period (June 2011-June 2013) at the study sites.

		2011-12	2012-13	Annual average ± standard deviation
LC	BP	996	905	951 ± 46
	WD	883	785	834 ± 49
	TF	741	648	695 ± 47
	%TF	74	72	73 ± 1
CB	BP	643	769	706 ± 63
	TF	437	545	491 ± 54
	%TF	68	71	70 ± 2
CA	BP	496	1075	786 ± 290
	TF	350	863	607 ± 257
	%TF	71	80	76 ± 5
TC	BP	278	415	347 ± 69
	TF	214	245	230 ± 16
	%TF	77	59	68 ± 9

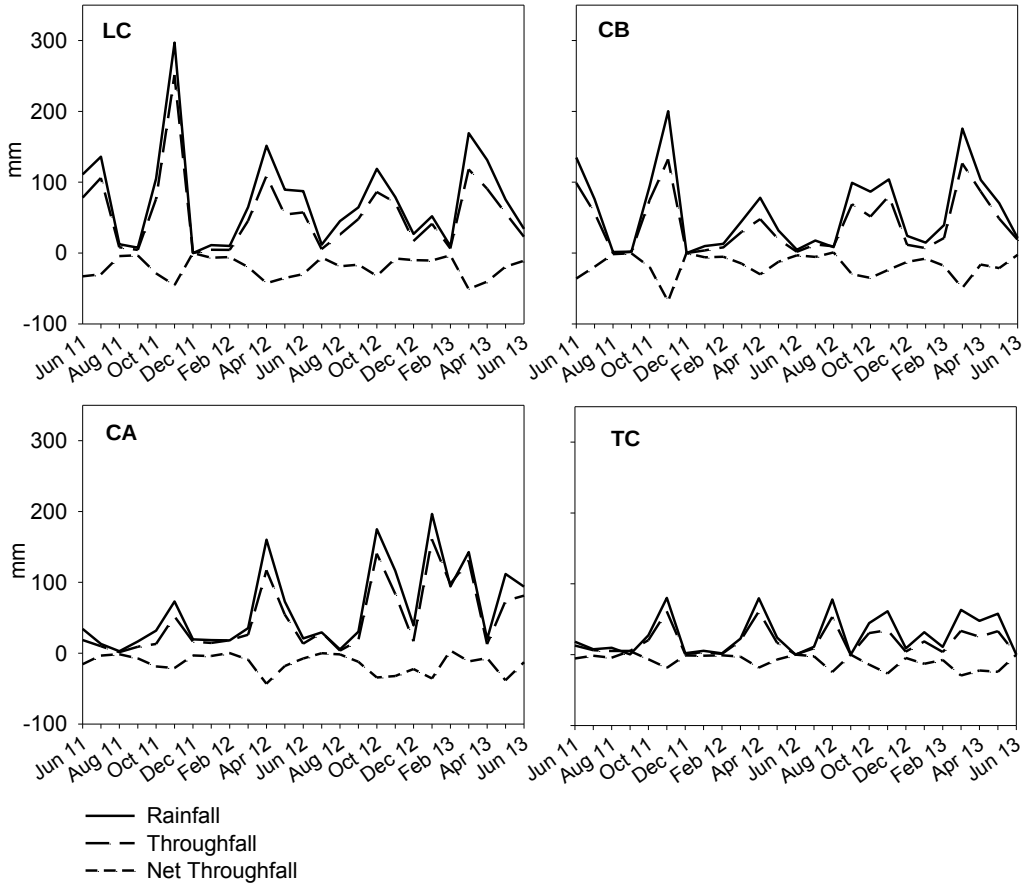

Figure 2. Monthly variation of rainfall, throughfall and net throughfall volumes at the study sites during the period June 2011- June 2013.

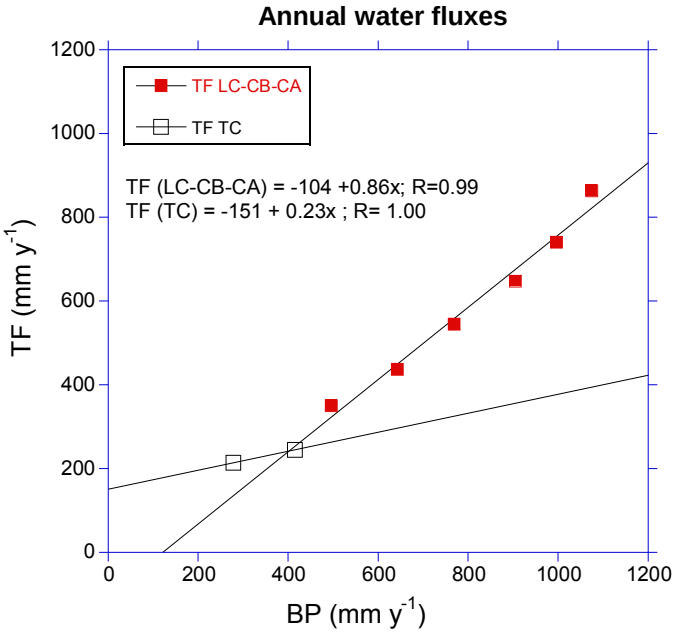

Figure 3. Relationship between bulk and throughfall water fluxes (mm) at each site.

Bulk deposition chemistry and origin of ions

Mean annual concentrations of bulk (BD) deposition for each site are given in Table 4. The rainfall chemistry was conditioned by the distinct sources of ions: 1) from crustal or terrestrial components, 2) from marine sources and 3) from anthropogenic originated compounds. Bulk concentrations at all sites were characterized by presenting Ca^{2+} as the major cation and high alkalinity (here mostly representing bicarbonates) as the major anion counterpart. There was a close relationship between both components ($p<0.001$ at all sites), which is indicated by the close position of both ions in the PCAs for each of the studied sites (Fig. 4). This association is the result of the incorporation of calcium bicarbonates from local and/or distant sources (Saharan dust) into rain droplets, as previously reported for the Iberian Peninsula (Avila et al., 1998) . In this study, the influence of carbonates from the opencast limestone quarry close to CA is seen in the very high concentrations of alkalinity (141 μeqL^{-1}) and Ca^{2+} (164 μeqL^{-1}) at this site (Table 4). On the other hand, at TC, these crustal ions showed very low concentrations (28 and 35 μeqL^{-1} for alkalinity and Ca^{2+} respectively). The Catalan sites showed intermediate concentrations, with the more remote site (LC) presenting lower values than the semi-urban site (CB). At all sites except TC, there were marked differences in ion concentrations between years which were related to differences in rainfall amount (Table 4), as concentrations are usually inversely related to rainfall due to dilution-concentration processes.

Table 4. Bulk deposition (BD), and throughfall (TF) annual volume weighted mean concentrations (μeqL^{-1}) (for 2011-2013, n=60 at LC, n=55 at CB, n=58 at CA and n=53 at TC).

	Year	pH	Alk	Na+	K+	Ca2+	Mg2+	NH4+	NO3-	SO42-	Cl-
LC											
BP	2011-12	6.06	30.0	23.1	3.1	48.2	12.4	26.6	23.8	21.6	24.4
BP	2012-13	5.87	56.2	34.0	5.0	84.8	20.1	19.7	24.0	23.0	36.8
BP	**Mean**	**5.97**	**43.1**	**28.5**	**4.1**	**66.5**	**16.3**	**23.1**	**23.9**	**22.3**	**30.6**
TF	2011-12	5.93	66.4	46.5	66.1	116.2	46.5	11.1	48.4	38.3	71.3
TF	2012-13	5.89	113	46.8	71.4	131.8	51.0	15.9	43.4	33.0	71.1
TF	**Mean**	**5.91**	**89.7**	**46.6**	**68.8**	**124**	**48.8**	**13.5**	**45.9**	**35.7**	**71.2**
CB											
BP	2011-12	6.45	39.0	40.6	3.7	72.3	18.4	21.5	25.1	29.5	44.2
BP	2012-13	6.24	96.2	65.0	4.8	137.1	30.4	17.9	23.0	36.2	71.6
BP	**Mean**	**6.33**	**67.6**	**52.8**	**4.2**	**105**	**24.4**	**19.7**	**24.0**	**32.8**	**57.9**
TF	2011-12	6.12	108	82.1	77.1	209	67.9	27.1	79.9	74.5	122
TF	2012-13	6.12	132	88.5	74.6	202	67.1	28.5	72	73.5	129
TF	**Mean**	**6.12**	**120**	**85.3**	**75.8**	**205**	**67.5**	**27.8**	**75.9**	**74.0**	**125**
CA											
BP	2011-12	7.43	164.3	55.3	12.2	210	13.8	57.1	38.8	35.6	48.9
BP	2012-13	7.27	117.9	35.5	5.7	121	10.1	43.3	21.2	23.2	39.4
BP	**Mean**	**7.34**	**141.1**	**45.4**	**9.0**	**165**	**11.9**	**50.2**	**30.0**	**29.4**	**44.2**
TF	2011-12	7.54	442.2	129.7	86.8	768	60.6	45.4	95.0	80.7	141
TF	2012-13	7.2	282.1	62.8	51.5	360	34.3	32.7	32.8	33.6	72.1
TF	**Mean**	**7.33**	**362.1**	**96.3**	**69.2**	**563.7**	**47.4**	**39.1**	**63.9**	**57.2**	**106**
TC											
BP	2011-12	6.12	31.5	18	4.2	33.0	5.4	20.4	21.1	18.9	11
BP	2012-13	5.92	23.5	15.1	4.2	36.0	5.0	10.7	15.1	19.8	8.5
BP	**Mean**	**6.03**	**27.5**	**16.5**	**4.2**	**34.5**	**5.2**	**15.6**	**18.1**	**19.4**	**9.7**
TF	2011-12	5.77	59.0	45.7	137	131	53.1	12.7	56.3	21.4	35.4
TF	2012-13	5.75	66.1	40.1	147	155	56.8	11.9	46.2	28.5	35.9
TF	**Mean**	**5.76**	**62.6**	**42.9**	**142**	**143**	**54.9**	**12.3**	**51.2**	**25.0**	**35.6**

The marine component was reflected by the close positions of Na^+, Cl^- and Mg^+ in the PCA plots for LC, CB and CA, but not for TC (Fig.4). Sites LC and CB are at a distance of only 27-10 km from the Mediterranean Sea, respectively. The marine influence was more noticeably at CB than at LC (higher Na and Cl concentrations), consistent with the fact that LC is separated from the sea by the pre-litoral Catalan range. Even though CA was 80 km far from the Atlantic coast, the site presented higher Cl^- and Na^+ VWM

concentrations than sites closer to the sea (e. g. LC). This is probably a result of the arrival of air masses from the Atlantic Ocean (Bay of Biscay), since the main wind direction in central Navarra is from the NW (http://meteo.navarra.es/estaciones/mapadeestaciones.cfm). The TC site lies at the center of the Iberian Peninsula (300 km distance to the sea) and consequently it received very little marine influence.

Na^+/Cl^- ratios ranged between 0.91 and 1.00 at the marine-influenced sites, slightly higher than the ratio of the sea-salt (0.86;Keene et al., 1986). In dry climates with NO_x pollution, this result may stem from the interaction of NaCl with HNO_3 to produce $NaNO_3$ and HCl. Evaporation of HCl would produce a Cl^- deficit that would increase the Na^+/Cl^-ratio compared to the sea ratio (Morales-Baquero et al., 2013), as was here the case for the marine influenced sites. At TC, the very high Na^+/Cl^-ratio may indicate crustal sources for Na related to the quarry emissions. Mg^+ can have both a crustal and marine origin, and this is reflected in the PCA plots where Mg^+ stands between the marine and crustal components at all sites except at the continental site TC (Fig. 4).

Ions related to anthropogenic emissions (SO_4^{2-}, NO_3^- and NH_4^+,) were not clearly separated from the crustal fraction in the PCA plots (Fig 4). The highest SO_4^{2-} concentrations were obtained at the semi-urban site of CB followed by the CA site. Sulphate aerosols are formed as secondary products from SO_2 gas emissions that are mostly emitted from point sources, such as coal combustion in power plants. However, in recent years SO_2 emissions from maritime traffic have been found to influence SO_2 pollution and SO_4^{2-}deposition in continental sites (Fowler et al., 2007; Izquierdo et al., 2012; Matthias et al., 2010). Concerning the Catalan sites, previous research indicated the influence of shipping emissions on SO_4^{2-} deposition (Izquierdo et al., 2012), while the results of this study suggest an influence of maritime traffic from the Atlantic routes off the Cantabrian coast on SO_4^{2-}rain concentrations in Navarra. Sulphate presented significant correlations with Ca^{2+} and alkalinity at all sites ($p<0.01$) indicating the interplay of anthropogenic emissions with crustal sources and

the neutralizing role of carbonated dust at a wide spatial range in the Iberian Peninsula.

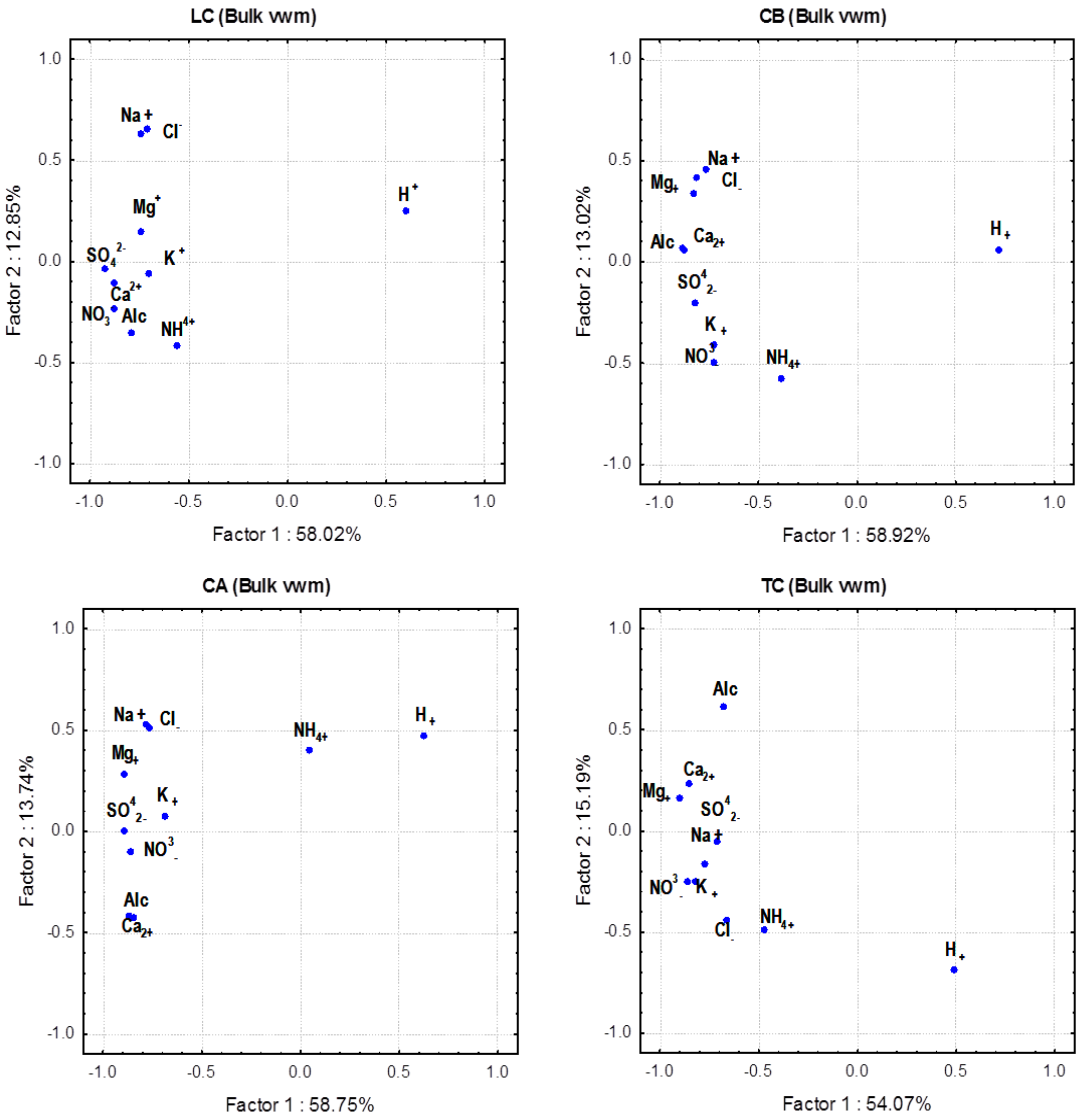

Figure 4. Principal Component Analysis plots for the concentrations (VWM) of the different components in bulk precipitation at the four study sites.

Nitrate mean concentrations were very similar at the Catalan sites (24 µeq L^{-1}) despite their different position relative to traffic emission sources. They ranked intermediate between the lowest concentration at TC (18 µeq L^{-1}) and the highest at CA (30 µeq L^{-1}). Larger differences in NO_3^- concentrations for each year at CA and TC were probably related to differences in rainfall amount. The highest NH_4^+ VWM concentrations were

registered at CA in both studied periods. This may reflect NH_3 emissions from surrounding intense agricultural and farming activities (García-Gomez et al. submitted). Values at the rural site LC were slightly higher than at the semi-urban site CB during both periods. The lowest concentrations were always registered at TC, even though it was the site with lower rainfall and a higher 'concentration effect' was expected.

Canopy changes in rainfall chemistry

The concentrations of solutes delivered to the soil by TF were usually greater than those of BD, a common result in most TF studies (Table 4). The mean TF chemistry at all sites was dominated by Ca^{2+}, with alkalinity as the major anion counterpart (Table 4), a fact that corroborates the non-acidic nature of TF at t the Iberian Peninsula sites in contrast with the more acidic character of TF in Central and North Europe. To better illustrate differences between BD and TF VWM concentrations, TF/BD ratios are represented in Figure 5. In the case of base cations, the TF/BD ratios were particularly large for K^+ followed by Mg^+, especially at the drier site TC. TF/BD ratios for NH_4^+ were below 1 at most sites (except at CB), indicating greater uptake than deposition of this ion. The TF/BD ratios for NO_3^- at the sites close to big cities (CB and TC) were slightly higher than at the other plots, which suggest higher dry deposition at these more polluted-exposed sites, tough this difference was non-significant. On the other hand, CB showed the highest enrichments for NH_4^+, NO_3^- and SO_4^{2-}, ions that result from the secondary aerosol formation.. As suggested by the BD results, CB may be influenced by SO_2 and NO_x shipping emissions from the intense traffic at the Barcelona port and these may contribute through dry deposition to the TF enrichment.

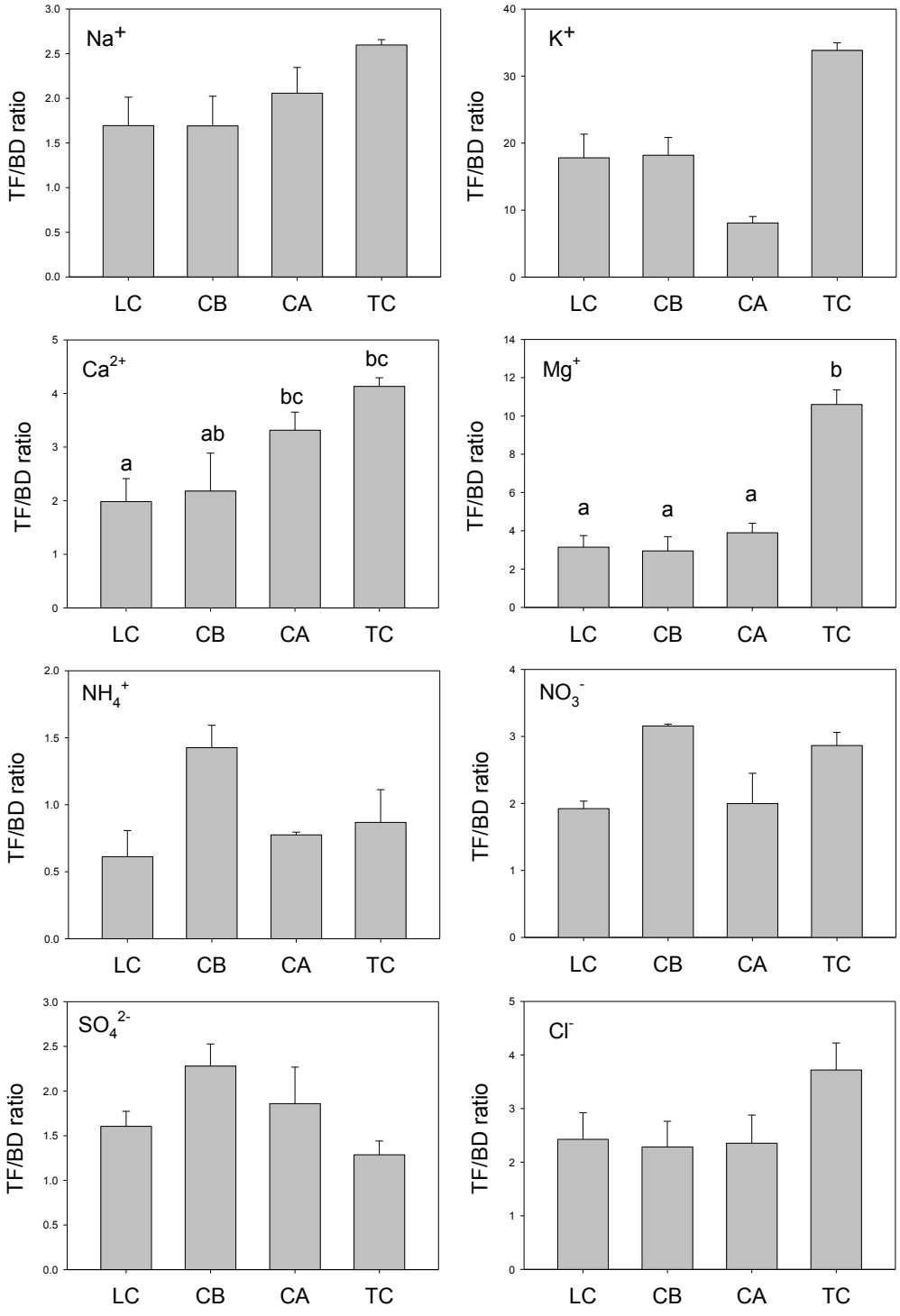

Figure 5. TF/BD ratios of the ionic concentrations (VWM) at each studied site. Superscripts indicate statistical differences in the TF/BD ratios for each ionic form between sites, assessed by one way ANOVA and Tukey test.

Spatial patterns in deposition fluxes and canopy impacts

Since fluxes result from the product of VWM concentrations and volume of water they are strongly linked to rainfall amount. Therefore, lower fluxes of most compounds both in BD and TF were found at the driest site TC compared to the other sites. Mean annual bulk, throughfall and net throughfall deposition fluxes of major ions are shown in Table 5. In Figure 6, Ca results are shown as representing a crustal-derived ion, Na for a marine-derived ion, K for a ion greatly influenced by TF processes, and SO_4^{2-}, NO_3^- and NH_4^+, for anthropogenic pollution- derived ions . Calcium, followed by Cl^- and Na^+, were the most abundant ions transferred to soils through bulk and throughfall deposition (Table 5). Except for NH_4^+-N, TF fluxes were enriched in relation to BD and therefore nTF fluxes were positive (Table 5; Fig 6). The negative nTF of NH_4^+-N indicates retention at the tree crowns.

Similarly to VWMs, TF and nTF fluxes of Ca^{2+} were higher at CA than at the other sites (Fig 6). This was due to dust emissions from the opencast limestone quarries near the site being dry deposited on the tree canopies. Also, at this site, the influence of the agricultural surroundings is reflected in high NH_4^+-N BD and TF deposition.

Bulk and TF deposition of anthropogenic related compounds were not negligible at LC, being similar to fluxes at CB and CA (Table 5; Fig 6). Thus, this site, which a priori was considered as a background station, is receiving similar loads of pollutants than the sites chosen close to traffic and urban pollution, indicating the arrival of air masses influenced by the Barcelona conurbation. The semi-urban site CB registered the highest NO_3^--N and SO_4^{2}-S nTF loads, reflecting an enrichment derived from dry deposition.

Table 5. Mean annual bulk (BD), throughfall (TF) and net throughfall (nTF) deposition fluxes (kg ha^{-1} yr^{-1}) at each studied site ± standard deviation.

	NH_4^+-N	NO_3^--N	SO_4^{2-}-S	Na^+	K^+	Ca^{2+}	Mg^+	Cl^-
BD								
LC	3.1 ± 0.6	3.2 ± 0.15	3.4 ± 0.06	6.3 ± 0.85	1.6 ± 0.2	13 ± 2.72	1.9 ± 0.34	11 ± 1.50
CB	2.1 ± 0.12	2.6 ± 0.07	4.0 ± 0.51	9.3 ± 2.32	1.3 ± 0.17	17 ± 5.13	2.3 ± 0.62	16 ± 4.00
CA	5.2 ± 1.28	3.0 ± 0.25	3.5 ± 0.58	7.6 ± 1.26	2.4 ± 0.02	24 ± 2.72	1.1 ± 0.25	12 ± 3.21
TC	0.8 ± 0.11	1.0 ± 0.00	1.2 ± 0.24	1.5 ± 0.09	0.8 ± 0.01	3.4 ± 0.47	0.3 ± 0.02	1.3 ± 0.02
TF								
LC	1.3 ± 0.15	4.5 ± 0.55	4.0 ± 0.57	7.5 ± 0.49	17 ± 0.55	17 ± 0.09	4.2 ± 0.09	18 ± 1.23
CB	2.0 ± 0.15	5.5 ± 0.01	6.1 ± 0.27	10 ± 0.90	15 ± 0.52	21 ± 0.73	4.3 ± 0.19	23 ± 1.80
CA	3.1 ± 0.85	4.3 ± 0.38	4.6 ± 0.02	12 ± 0.93	16 ± 2.64	57 ± 3.74	3.1 ± 0.49	20 ± 2.15
TC	0.4 ± 0.01	1.6 ± 0.05	0.9 ± 0.19	2.3 ± 0.01	13 ± 1.31	6.6 ± 1.00	1.5 ± 0.16	2.9 ± 0.22
nTF								
LC	-1.8 ± 0.74	1.3 ± 0.41	0.6 ± 0.51	1.3 ± 1.33	17± 0.75	4.5 ± 2.81	2.3 ± 0.43	7.4 ± 2.73
CB	0.0 ± 0.27	2.9 ± 0.07	2.1 ± 0.24	0.9 ± 1.42	14 ± 0.35	4.7 ± 4.40	2.0 ± 0.43	7.3 ± 2.21
CA	-2.1 ± 0.43	1.3 ± 0.63	1.2 ± 0.55	4.0 ± 0.33	12 ± 2.62	35 ± 1.02	2.0 ± 0.24	8.0 ± 1.06
TC	-0.4 ± 0.12	0.6 ± 0.05	-0.3 ± 0.05	0.8 ± 0.09	12 ± 1.30	3.2 ± 0.53	1.3 ± 0.14	1.6 ± 0.20

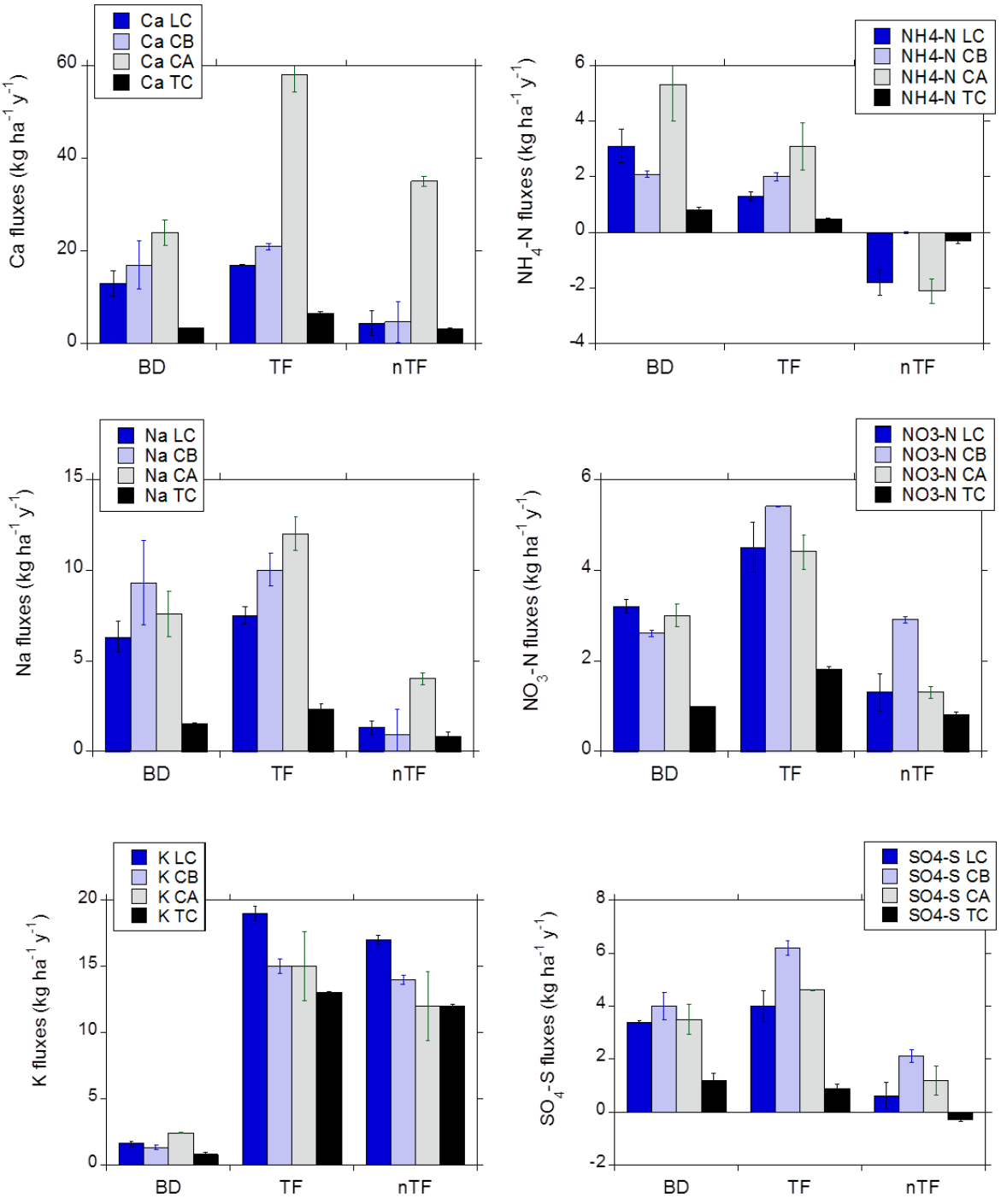

Figure 6. Bulk (BD), throughfall (TF) and net throughfall (nTF) mean annual fluxes at each studied site (in kg ha^{-1} y^{-1}).

As mentioned in the introduction, nTF fluxes result from the interplay of several processes such as dry deposition, canopy leaching or canopy uptake. To distinguish between these processes, the exploration of the correlations between rainfall amount

and net TF fluxes may be useful. It has been proposed that a positive correlation between net throughfall and rainfall amount is related to leaching, a negative correlation to uptake, while the absence of correlation may be attributed to dry deposition (Balestrini and Tagliaferri, 2001; Lovett and Lindberg, 1984; Rodrigo et al., 2003). This approach is based in the assumptions that dry deposition is completely and quickly removed from the canopy by the rain and that foliar leaching is correlated to the depth of rain.

The results of Spearman regressions between rainfall amount and nTF fluxes (Table 6) indicate a consistent behavior at the 4 sites for some elements (K^+, NH_4^+-N, SO_4^{2-}-S) and marked differences for other elements (NO_3^--N, Na^+, Cl^-, Ca^{2+} and Mg^{2+}). Based on the previous assumptions, K^+ in nTF should derive from leaching (positive relationship with $p<0.05$), NH_4^+-N should derive from uptake (negative relationship with $p<0.05$, except at CB where $p<0,1$), and SO_4^{2-}-S would derive from dry deposition (non significant relationships). For NO_3^--N, results suggest uptake at LC and TC (the later with $p<0.1$), but leaching at CA (Table 6).

On the other hand, for Na^+, Cl^-, Ca^{2+} and Mg^{2+} very significant positive relationships were found at CA and TC, while they were non-significant at LC and CB (Table 6). Therefore, results at the Catalan sites indicate dry deposition for these elements, which is consistent with previous research in Montseny (Rodrigo and Avila 2002; Rodrigo et al. 2003). On the other hand, the results at CA and TC suggest leaching of these ions from internal canopy pools, which is a non-expected result.

The above approach has been usually employed to describe canopy processes in temperate forests. However, under the Mediterranean drier climate, the relationships between net throughfall and rainfall may be complicated by the fact that events with low precipitation may not deliver enough water to wash the accumulated dry deposition from previous events, thus producing relationships different from expected. This may be the case at the driest site TC (annual precipitation of only 346 mm during the study period, half of that at CB and CA and one third of that at LC). On the other hand, CA received very high amounts of dust dry deposition (calcium and sodium carbonates from the nearby quarry enterprise) and the amount of weekly rain might

not have been enough to wash all the dry deposited material for part of the weeks. Thus, positive relationships (that are traditionally interpreted as arising from leaching) for ions such as Na^+, Ca^{2+} , NO_3^- (that are usually delivered by dry deposition) may in fact represent mostly dry deposition at these particular sites.

Table 6. Results for Spearman correlations between rainfall amount and net throughfall fluxes at the four studied sites.

	Na^+	K^+	Ca^{2+}	Mg^{2+}	NH_4^+	NO_3^-	SO_4^{2-}	Cl^-
LC								
r-value	0.25	0.65	0.15	0.21	-0.28	-0.28	0.02	0.16
p-value	0.08	0.00	0.30	0.15	0.05	0.05	0.88	0.28
CB								
r-value	0.25	0.84	0.12	0.09	-0.27	-0.10	0.05	0.21
p-value	0.12	0.00	0.45	0.59	0.09	0.52	0.76	0.18
CA								
r-value	0.62	0.89	0.79	0.76	-0.53	0.41	0.20	0.54
p-value	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00
TC								
r-value	0.33	0.67	0.54	0.62	-0.54	-0.18	-0.27	0.34
p-value	0.00	0.00	0.00	0.00	0.00	0.11	0.01	0.00

On the other hand, regression results clearly suggest the uptake of NH_4^+ by the canopies of *Quercus ilex* at all the studied forests. This is also corroborated by the significant negative relationship between NH_4^+ in net TF fluxes and BD deposition fluxes that indicates the retention of the incoming NH_4^+ in rainfall (Fig. 7). Outliers above the x = y line in figure 7 correspond to rainfall events after long dry periods, corroborating the mentioned assumption of dry deposition accumulation related to dry spells of the precipitation at Mediterranean sites.

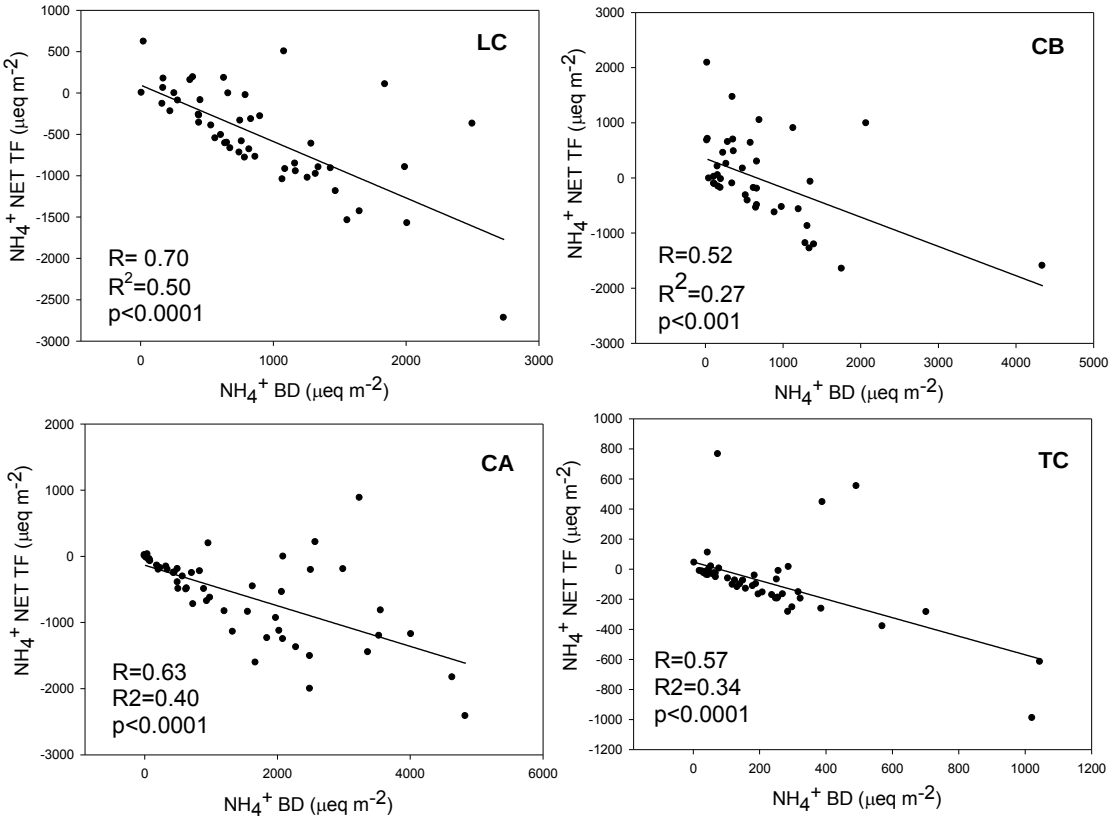

Figure 7. Bulk vs net TF fluxes of NH4+ (µeq m^{-2} yr^{-1}) at each studied site.

Estimating total atmospheric inputs with the canopy budget model

Results for the canopy budget model calculations for the three sites where WD could be obtained (LC, CB and TC) are shown in Table 7. Dry deposition of base cations (Ca^{2+}, Mg^{+} and K^{+}) contributed 40, 34 and 45% of TD at LC, CB and TC respectively. Thus, WD predominated over DD. However, DD was higher at the driest site (TC).

Table 7. Results from the canopy exchange model (kg ha^{-1} yr^{-1}) at sites LC, CB and TC. Canopy uptake (CU) corresponds to negative CU/CL values, while canopy leaching (CL) to positive CU/CL values.

	H^+	NH_4^+-N	NO_3^--N	SO_4^{2-}-S	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-
LC									
WD	0.0	2.4	2.0	2.5	4.5	1.0	9.7	1.2	6.4
DD	0.3	6.0	6.6	1.7	3.0	0.7	6.5	0.8	4.2
CU/CL	0.3	-7.0	-4	-0.1	0.0	17.2	1.2	2.2	7.2
TD	0.3	8.3	8.6	4.2	7.5	1.6	16.2	1.9	10.6
CB									
WD	0.0	1.6	1.6	2.9	6.7	0.8	12.5	1.4	9.7
DD	0.3	8.8	7.6	1.5	3.5	0.4	6.5	0.7	5.0
CU/CL	0.3	-8.3	-3.8	1.7	0.0	14.1	2.3	2.1	8.4
TD	0.3	10.4	9.3	4.5	10.2	1.2	19	2.1	14.7
TC									
WD	0.0	0.6	0.6	1.0	1.2	0.6	1.6	0.2	1.1
DD	0.0	0.6	1.4	0.8	1.0	0.5	1.3	0.2	0.9
CU/CL	0.0	-0.7	-0.5	-0.8	0.0	11.7	3.7	1.2	0.8
TD	0.0	1.1	2.1	1.8	2.3	1.1	2.9	0.4	2.1

Estimations with the CBM indicate that dry deposition of Ca^{2+} was 6.5 kg ha^{-1} y^{-1} at the Catalan sites (Table 7). A previous study in Montseny aimed at deriving DD fluxes by means of repeated washes of metacrylate plates and branches produced a Ca^{2+} DD flux of 2.7 kg ha^{-1} y^{-1} when calculated from plate washes and 6.6 kg ha^{-1} y^{-1} when using foliage washes (Rodrigo and Àvila, 2002). Foliage washes may better represent DD as branches are organized in the three dimensions of the canopy (though some leaching may contribute), while plates are horizontal and may underestimate deposition. Foliage wash results in this experiment produced similar DD estimates than the ones obtained in the present study from CBM model (Table 7).

Total deposition of Ca^{2+} was 16.2, 19.0 and 2.9 at LC, CB and TC respectively. Potassium was the most leached base cation (Table 7), a result which is consistent with the previous correlation analysis, since K^+ nTF fluxes always presented the highest coefficients of correlation (Table 6). Leaching of K^+ is a common result in TF studies (Langusch et al., 2003; Likens et al., 1994; Tukey Jr, 1970; Vitousek and Sanford, 1986), and the loads estimated here are in the range of previous results in other European forest sites (Balestrini and Tagliaferri, 2001; Stachurski and Zimka, 2000; Staelens et al., 2008). In general, the leaching of K^+ is accompanied by weak acid leaching or an exchange with other ionic forms, typically NH_4^+ (Staelens et al., 2008). As indicated by the CBM, NH_4^+-N uptake was important at all study sites varying between 8.3 and 0.7 kg ha^{-1} yr^{-1} (Table 7). For NO_3^--N, the CBM also indicated uptake in the canopy at the study sites: 4 .0, 3.8 and 0.5 kg ha^{-1} yr^{-1} at LC, CB and TC.

The total deposition of N estimated with the CBM was: 16.9, 19.6 and 3.2 kg ha^{-1} yr^{-1} at LC, CB and TC respectively. The contribution of DD to TD was very important at all sites: 74, 84 and 63% at LC, CB and TC. These percentages are in accordance with other studies in Mediterranean forests in the Iberian Peninsula (Table 8; Fig. 8).

Because of the uncertainties associated with the CBM estimations for N compounds (Staelens et al., 2008), a check was carried out by comparing the fluxes obtained with the CBM with fluxes calculated using the inferential method. For inferential calculations we have used the gas and particle atmospheric information from García-Gomez et al., (2015, submitted) and the V_d values selected from literature reports of deposition unto forests (for NH_3 and HNO_3, 2.0 cm s^{-1}; for NO and NO_2, 0.1 cm s^{-1}, from Holland et al., (2005), Kalina et al., (2002), Krupa (2003) and Muller et al., (1993)). The total DD (in kg ha^{-1} yr^{-1}) of N estimated with the CBM was 12.6 at LC, 16.4 at CB and 2 at TC, while the results from the inferential calculations were 10.3 at LC, 10.6 at CB and 8.6 at TC. Even though data for each approach was measured during different periods and despite the uncertainties associated to the use of the inferential approach with the V_d from references instead of measurements in situ, the results for sites LC

and CB, are quite similar. On the contrary, the estimation with the inferential approach at site TC was four times that from CBM calculations (tables 7 and 8).

In fact, we suggest that the use of CBM at sites with low rainfall is probably unreliable: the low rainfall amount may not allow for a proper characterization of the dry deposition fraction (very sensible to the ratio TF/WD of the tracer ion). Since results might be uncertain for the TC site, we will use here results from the LC and CB sites to compare DD and canopy exchange fluxes with those obtained other forest studies in Europe (Fig. 8).

Base cation dry deposition and canopy leaching at the Catalan stations was very similar or always in the range of variability to reported data from other European sites (Fig. 8). In the case of N compounds, the estimated dry deposition was also similar to European estimates while uptake seems higher in the holm oak forests here studied. Finally sulphate dry deposition was smaller here than in Europe. Exchange of SO_4^{2-} was negligible confirming the small role of exchange processes for this ion.

Table 8. Bulk (BD) or wet deposition (WD), dry deposition (DD) and total (TD) deposition estimates (in kg ha^{-1} y^{-1}) of N compounds in Spanish sites estimated with different methods (BRW=Branch washing; IFM=Inferential method; M= measurements in WD and DD collectors). References are: a, Rodà et al., 2002; b Sanz et al., 2002; c, Morales & Baquero, 2006; d data from EMEP.

Ref	site	mm rainfall	WD $N\text{-}NO_3^-$	WD $N\text{-}NH_4^+$	DD $N\text{-}NO_3^-$	DD $N\text{-}NH_4^+$	TWD N	TDD N	TD N	% DD/TD	Approach	Period
a	Catalonia	929 - 1048	2.7 - 3.5	3.0 - 3.8	5.0 - 6.5	4.1 - 8.1	5.7 - 7.4	9.2 - 15	15 -22	62 - 66	BRW	1996
b	Valencia	570 - 184	3.5 - 1.9	3.5 - 0.9	7.0 - 1.5	0.6 - 0.3	6.8 - 3.2	7.4 - 1.8	11 - 5.1	66 - 14	BRW	1999
					Dry ox-N	**Dry red-N**						
c	Andalucía	450					3.07	2.48	5.55	45	IFM	2000
d	Girona				9.32	4.03		13.4			IFM	1999-2008
d	Lleida				19.7	4.81		24.5			IFM	2000-2008
d	Valencia				11.8	4.1		15.9			IFM	1999-2008
d	Granada				8.26	4.22		12.5			IFM	1995-2008
	LC				6.22	4.04		10.3			IFM	2011-2012
	CB				5.3	5.35		10.7			IFM	2011-2012
	TC				4.61	3.95		8.56			IFM	2011-2012

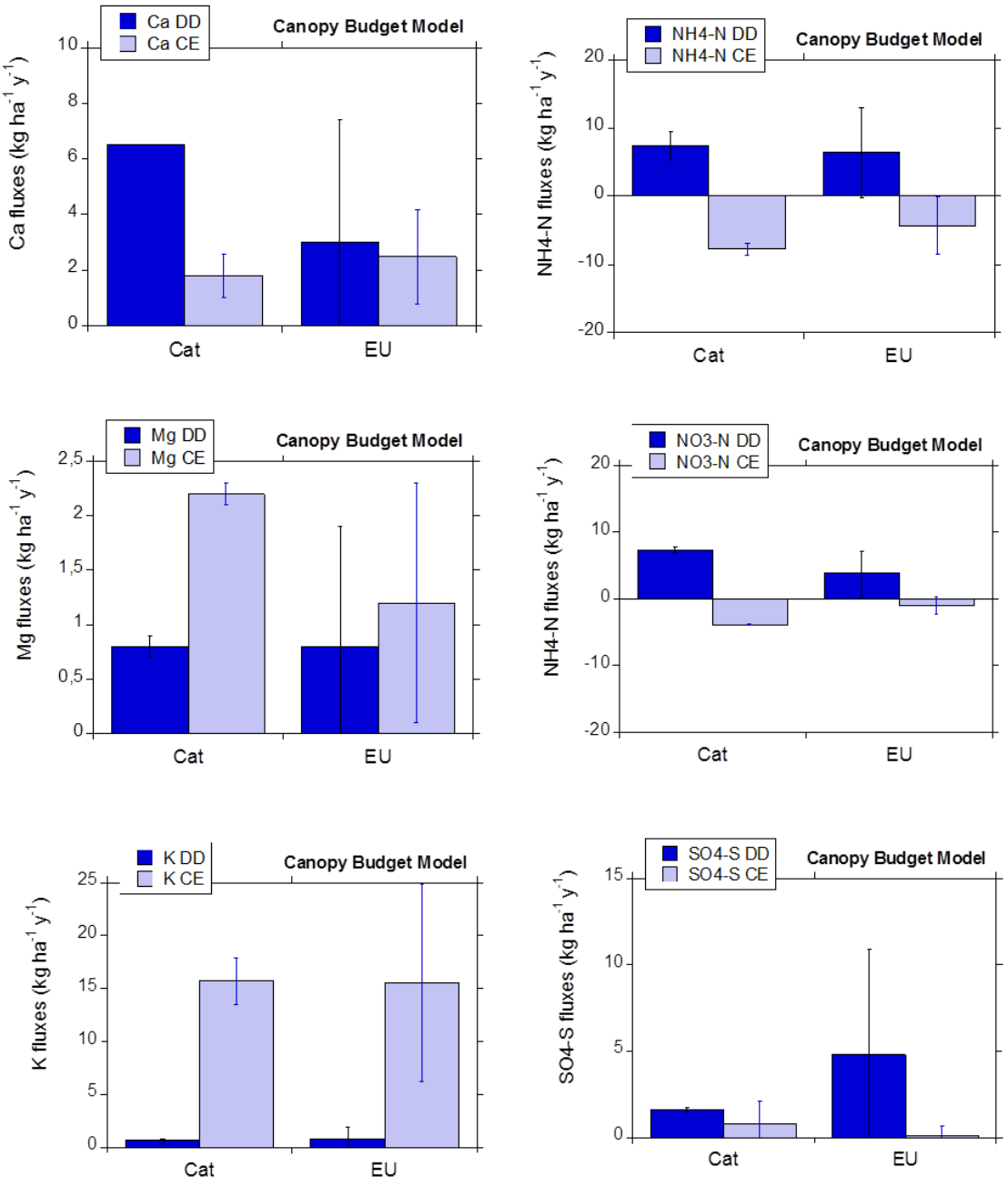

Figure 8. Estimated fluxes with the CBM approach at the catalan sites (LC and CB) compared to the European estimations.

Even though the results in this study are in the range of estimations for other sites in the Iberian Peninsula or Europe (Table 8 and Fig. 8), the CBM approach has some uncertainties that have been recognized elsewhere (Staelens et al. 2008)In our study,

the most relevant uncertainty is related to the fact that the estimates for NO_3^- here have been based on the exchange factor between NH_4^+ and NO_3^- proposed by de Vries et al., (2001) for temperate forests. More research is needed for Mediterranean forests to find out an appropriated efficiency factor of NH_4^+ vs NO_3^- uptake.

While more studies are needed in this regard, the consistency of the DD values using different approaches provides ground for accepting the total N deposition fluxes derived from this study. In this sense, total DIN (sum of oxidized and reduced inorganic deposition) to LC and CB was 16.9-19.6 kg ha^{-1} y^{-1}. Recent studies in these sites indicated that dissolved organic nitrogen (DON) would add around 3 kg N $ha^{-1}y^{-1}$ (Izquieta et al., 2015 submitted). Therefore, the total N input to these holm oak forests can be framed in 20-23 kg $ha^{-1}y^{-1}$. This is an important result that indicates that these forests are receiving a N input that considerably exceeds the CLO values proposed for scleropylous forests (15-17 kg N $ha^{-1}y^{-1}$; Bobbink et al. 2010).

Conclusions

Bulk and throughfall samples were collected at 4 different characteristics holm oak forests in the Iberian Peninsula during a two year period. The results indicate that chemical composition of bulk and throughfall deposition is strongly conditioned by local emissions and by long range transport from distant sources. The effect of the proximity to the sea was clearly marked at the sites near the Catalan coast, while the effects of the emissions from opencast limestone quarries were reflected in the high alkalinity and Ca^{2+} deposition at the site CA, and the rural influence is reflected in the higher levels of NH_4^+. Since net throughfall fluxes of NH_4^+ were negative or near cero retention of this compound is suggested at the canopy level, which is corroborated by the negative correlations between netTF fluxes of NH_4^+ and the mm of rainfall and by the bulk- net throughfall relationships.

At sites LC and CB, the N total deposition estimated was 16.9 and 19.6 kg ha^{-1} yr^{-1} respectively, with a % of contribution of DD to TD of 74 and 84 % respectively. This contribution of DD is very important and is in accordance with previous studies in

Mediterranean forests in the Iberian Peninsula driven from other approaches instead of CBM. Results suggest that CBM model cannot be applied at site with very low rainfall, same as the typical regressions models used for temperate-boreal forest. For all those reasons we suggest to improve the use of inferential methods at sites where dry deposition might not be inferred from the rainfall data.

Acknowledgements

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Chapter 3

Long-term effects of changing atmospheric pollution on throughfall, bulk deposition and streamwaters in a Mediterranean forest

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Abstract

The abatement programs implanted in Europe to reduce SO_2, NO_2 and NH_3emissions are here evaluated by analysing the relationships between emissions in Spain and neighbouring countries and atmospheric deposition in a Mediterranean forest in the Montseny Mountains (NE Spain) for the last 3 decades. A canopy budget model was applied to throughfall data measured during a period of high emissions (1995-1996) and a period of lower emissions (2011-2013) to estimate the changes in dry deposition over this time span.

Emissions of SO_2 in Spain strongly decreased (77%) and that was reflected in reductions for $nssSO_4^{2-}$ in precipitation (65% for concentrations and 62% for SO_4^2-S deposition). A lower decline was found for dry deposition (29%).

Spanish NO_2 emissions increased from 1980 to 1991, remained constant until 2005, and decreased thereafter, a pattern that was paralleled by NO_3^-concentrations in bulk precipitation at Montseny. This pattern seems to be related to a higher share of renewable energies in electricity generation in Spain in recent years. However, dry deposition increased markedly between 1995 and 2012, from 1.3 to 6.7 kg $ha^{-1}y^{-1}$. Differences in meteorology between periods may have had a role, since the recent period was drier, thus probably favouring dry deposition.

Spanish NH_3 emissions increased by 13% between 1980 and 2012 but NH_4^+ concentrations in precipitation and NH_4^+-N deposition showed a decreasing trend (15% reduction) at Montseny, probably linked to the reduction ammonium sulphate and nitrate aerosols to be scavenged by rainfall. NH_4^+-N dry deposition was similar between the compared periods.

The N load at Montseny (15-17 kg ha^{-1} yr^{-1}) was within the critical load range proposed for Mediterranean sclerophyllous forests (15-17.5 kg ha^{-1} yr^{-1}). The onset of N saturation is suggested by the observed increasing N export in streamwaters.

Introduction

High atmospheric deposition of sulphur (S) and nitrogen (N) in the last century has lead to the acidification and eutrophication of many terrestrial ecosystems in Europe and North America, peaking in the 1970-1980 (Aber et al., 1998; EC, 2001; Reuss and Johnson, 1986). To counteract these adverse effects, transboundary amendment programs were launched by the Convention on Long-Range Transboundary Air Pollution (CLRTAP) in the frame of the United Nations Economic Commission for Europe (UNECE, 2011). Upon the implementation of national emission limits, significant declines were observed since the mid 1980s in SO_2 emissions and S in precipitation in Europe (Tørseth et al., 2012). Nitrogen emissions were also significantly reduced in many European countries, although higher variability was found among regions and the changes were different for oxidized or reduced N (Fagerli and Aas, 2008; Konovalov et al., 2008; Lövblad et al., 2004). A summary of the major results following the implementation of pollution control measures can be found in Grennfelt and Hov(2005). Concerning Spain, SO_2 emissions were readily cut. However, the country still exceeded in 2012 the NO_x and NH_3 emissions ceilings for 2010, established at 847 and 353 Gg respectively (EC, 2001). The link between emissions, air concentrations and deposition is complex because of the interplay of the meteorological conditions, the chemical interaction between pollutants in the atmosphere and the spatial scale of the region of influence. In this sense non-linearities have been found between emission reductions and the decline in rainwater concentration (Fowler et al., 2007).

Dry deposition of airborne pollutants makes also a significant contribution to the atmospheric load of most ecosystems. However, long time records of changes in dry deposition for the last decades are scarce. Changes in dry deposition have also been found to change non-linearly with emissions (Fowler et al. 2005). This has been attributed to the fact that the deposition velocity (V_d) of SO_2 is controlled by the NH_3/SO_2 ratio, where SO_2 deposition increases as leaf pH raises in response to NH_3 deposition (Erisman et al., 1998; Fowler et al., 2001). Thus, dry deposition will not only depend on SO_2 concentrations, but also on its relative abundance respect to NH_3. Also, declining air SO_2 concentrations will affect the partitioning of gaseous NH_3 and particulate NH_4^+, which in turn will affect the spatial range affecting deposition, since

NH_3 will tend to be locally deposited while fine-sized NH_4^+-sulfate aerosols have a longer residence time in the atmosphere and will be mostly transported to longer distances (van Jaarsveld et al., 2000).

Throughfall (TF), the water flux collected under the forest canopy, has been widely used to provide an estimation of dry deposition (De Vries et al., 2003; Lindberg and Lovett, 1992). However, throughfall does not truly represent total deposition, since it also is affected by chemical exchanges at the canopy level (Parker, 1983). When using throughfall measurements to derive dry deposition fluxes, a distinction has to be made between dry deposition and canopy exchange processes. These include the leaching of elements from internal plant pools and/or the uptake by the canopy of gases or dissolved solutes (Lovett and Lindberg, 1984; Schaefer and Reiners, 1990). To sort this out, a canopy budget model has been widely used (Draaijers and Erisman, 1995; Staelens et al., 2008) and will be here applied to estimate dry deposition from throughfall measurements.

The Iberian Peninsula, in the south-western corner of the European continent, is influenced by air masses from contrasting provenances. Five main air mass movements have been established based on the frequency of back trajectories: 1) European or continental, 2) from the Atlantic Ocean, 3) from North Africa, 4) from the Mediterranean, 5) from shorter pathways, as recirculating air masses over the Iberian Peninsula (Calvo et al., 2012; Escudero et al., 2007; Izquierdo et al., 2012). A cluster classification of daily back-trajectories for the periods 1984-1993 and 1998-2009 indicated that the most frequent air flows at the Montseny mountains in NE Spain were from the Atlantic Ocean (39 and 31% for the two study periods) and the Peninsular recirculation (27-25%). Thus, the major air pollution influence at the north-east coast of Spain may be from emissions from the Iberian Peninsula itself. However, during winter, a good correlation was observed between air masses from the Mediterranean and NO_3^- deposition (Izquierdo et al., 2014). Also, source receptor models indicated the influence of emissions from eastern provenances on the rain chemical composition in NE Spain (Izquierdo et al., 2012).

The aim of this work is to examine the relationships between S and N emissions in Spain and neighbouring countries from the early 1980s to 2014 and rain concentrations and deposition at a site in the NE of the Iberian Peninsula, in order to check whether the abatement measures implemented by CLRTAP protocols are reflected in reduced deposition. This has been undertaken by comparing the evolution of bulk deposition trends at a rural forested site (La Castanya, Montseny) in NE Spain and the evolution of emissions in the Iberian Peninsula, France, Italy and the totals for the European Union (EU28) for this period. Previous studies have documented a SO_4^{2-} decrease in bulk deposition in NE Spain (Avila, 1996; Avila and Rodà, 2002); here we expand these studies for a longer time series and incorporate the examination of changes in dry deposition. Changes in dry deposition along the last 3 decades cannot be traced in NE Spain because of the lack of a continuous monitoring scheme for dry deposition. However, throughfall measurements in two contrasting periods over this time span (in 1995-1996, a period of high emissions and in 2011-2013, a period of lower emissions) can provide and insight on dry deposition changes, particularly for S.

This paper also explores whether changes in atmospheric deposition are reflected in the chemistry and fluxes of water draining an undisturbed forested catchment close to the deposition measurements as an indicator of the response at the ecosystem level. Many studies have reported changes in surface stream waters in temperate forests of central and north Europe and of North America after reduction of pollutant emissions (Driscoll et al., 1998; Evans et al., 2007; Skjelkvåle et al., 2005). However, the response of Mediterranean ecosystems has been little explored. In this paper we analyze a long-term streamwater data series from 1990 to 2014 to fill gap to provide a case study for a Mediterranean forested catchment.

Material and methods

Study site

The study site was located in La Castanya valley (LC, 41º46'N, 2º21'E, 700 m.a.s.l), within the Montseny mountains which are about 40km to the NNE from Barcelona and

25km from the Mediterranean coast. Dominant vegetation is a closed-canopy forest of holm-oak (*Quercus ilex* L.). Forests at La Castanya valley were exploited in the past for charcoal production, but these activities were abandoned about 60 years ago and the forest is increasing in biomass (Rodà et al., 1999). The upper part of La Castanya valley comprises a belt of beech forest at 1100-1200m, and heathlands and grasslands extend above this altitude up to 1350m. Dominant lithology is metamorphic schist and phyllite. Soils are shallow with an organic layer 0-5cm deep and an average total depth of 60 cm (Hereter and Sánchez, 1999). Soils are classified as Entisols or Inceptisols (Soil Survey Staff 1992).

Climate is meso-Mediterranean sub-humid, with a clear seasonal cycle of higher precipitation in spring and summer. Variability among years is very high (Fig. 2). At the LC station, mean precipitation from 1983 to 2014 was 862mm y^{-1} (range from 518 to 1601 mm y^{-1}; Fig. 2) and mean air temperature was 9.5ºC.

This site is considered as a rural background station that is topographically sheltered to some extent from air pollution from the Barcelona metropolitan area but during the warm half of the year diurnal sea-land breezes carry pollution from the coast and lowland plains to the upper Montseny slopes by midday (Pérez et al., 2008). Besides, long-range pollutant transport also influences atmospheric deposition at this site (Izquierdo et al., 2012).

Field sampling

Open field measurements

Weekly bulk deposition (BD) samples were obtained from August 1983 to August 2014 (interrupted from September 2000 to March 2002). Wet-only deposition (WD; ESM Andersen instruments) was sampled in parallel to bulk deposition during 2008-2013 but because of the longer bulk deposition record, we will deal here with bulk deposition data. Bulk/wet deposition collectors, a rain tipping bucket gauge and a meteorological station (Campbell with CR1000 data logger) were located at a clearing in the forest close to the throughfall plots. From August 1983 to September 2000, the

open field measurements were located near the TM0 outlet (site LC1). Since March 2002, they were located about 850 m distant from LC1, the new site being named LC2. Bulk collectors consisted on 19-cm-diameter funnels connected by a looping tygon tube to a 10-L polyethylene bottle for the period 1983-2010. In 2011, NILU-type (Norwegian Institute for Air Research) funnels were used and a plastic membrane was fitted in the funnels neck which connected to a 2-L polyethylene bottle. Funnels were directly connected to the bottles, which were kept in the dark to avoid the growth of microorganisms. Four (1983-1996) and two (1997-2014) replicate collectors were used.

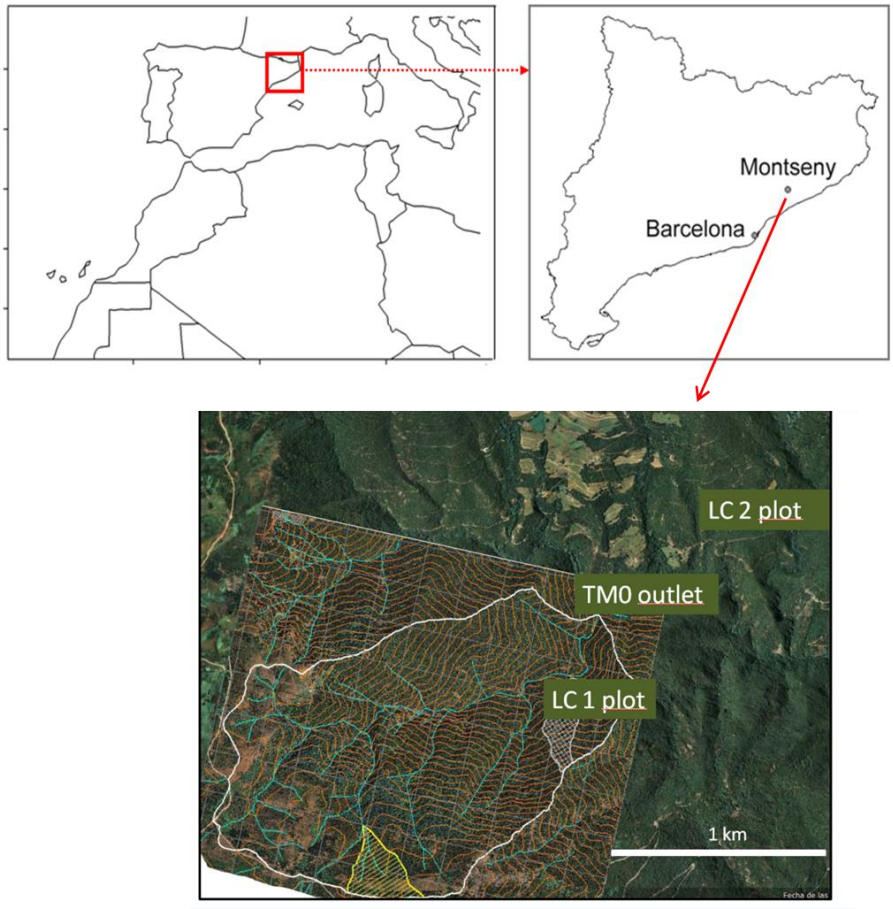

Figure 1. Map of the study site, northeastern Spain.

Throughfall measurements

To characterize the throughfall chemistry in the high emission period we used throughfall data measured from June 1995 to June 1996 in two plots in Montseny. One plot was LC1 located in La Castanya valley. The second plot (RP1) was located 8 km

distant, in the Montseny slopes that face to SW, similarly as the aspect of the LC2 throughfall plot. It was considered that the averaged throughfall composition of RP1 and LC1 better represented the canopy changes on incident precipitation in the early period. Throughfall collectors in this period consisted of a 10-cm funnel connected to a 2-L bottle, kept in the dark (Rodrigo et al., 2003). Throughfall in the period March 2011-March 2013, was sampled in location LC2, using 12 collectors of NILU-funnel type. Details on the forest structure of the 3 throughfall sampling sitesare given in Table 1. Although differences in location will introduce uncontrolled spatial variation in throughfall measurements, the plots had a similar forest structure: they formed a continuous closed monospecific canopy of holm-oak submitted to similar climate, although the difference in aspect may have some effect. A review of the rainfall partitioning by vegetation in Mediterranean conditions has shown that the forest structural parameters that mostly influence throughfall are age, height, DBH and basal area (Llorens and Domingo, 2007). Differences in some of these variables among the plots were small (Table 1) and probably within the error of regressions provided by Llorens and Domingo (2007). Further details on throughfall field sampling can be found in Rodrigo et al.(2003) and Aguillaume et al. (in prep.).

Table 1. Study site characteristics.

	1995-96 LC1	1995-96 RP1	2011-13 LC2
Altitude (m)	731	535	765
Orientation	N	SW	SW
Diameter at breast high (cm)	11.3 ± 4.8	12.0 ± 4.2	13.0 ± 4.1
Trees·ha^{-1}	2127	1753	2571
Basal area (m^{-2})	26.5	22.3	29

For bulk deposition and throughfall, VWM concentrations were calculated based on weekly samples weighted by weekly precipitation volume. The corresponding fluxes were obtained by multiplying annual VWMs by annual precipitation/throughfall volume and are expressed as kg ha^{-1} y^{-1}, except for H^+ and alkalinity, in eq ha^{-1} y^{-1}.

Non sea salt sulphate (nss SO_4^{2-}) concentrations were calculated as:

nss $SO_4^{2-}{}_{BD} = SO_4^{2-}{}_{BD} - (Na^+{}_{BD} * 0.12)$,

where$SO_4^{2-}{}_{seasalt}/Na^+{}_{seasalt} = 0.12$ (Drever, 1982)

Streamwater measurements

Stream discharge was recorded in a stream named Torrent de la Mina (TM0) which is gauged with a 120º V-notch weir and collects drainage from a 200 ha catchment. The precipitation and throughfall sampling plots were within (LC1) or quite close (LC2, RP1) the catchment. The recording period for discharge and streamwater chemistry comprised an initial (1990 to 1999) and a recent period (2010 to 2014).Water level was measured with an OTT water level recorder in the initial period and with a pressure probe (Schlumburger Water Services) in the recent one. Annual discharge ranged between 150 and 888 mm y^{-1} (Fig. 2).

Grab samples of stream water were collected with an approximate weekly schedule from a sampling point upstream from the stilling pond. They were collected in high-density polyethylene 250-mL bottles after a previous triple rinse with stream water.

We calculated annual volume-weighted mean concentrations (VWM) for bulk precipitation and stream waters, expressed in µeq L^{-1} for hydrological water years defined from 1 September. For throughfall, a more restricted data record obliged to consider different annual periods (June 1995 to June 1996 in the initial and March 2011 to March 2013 for the recent period).

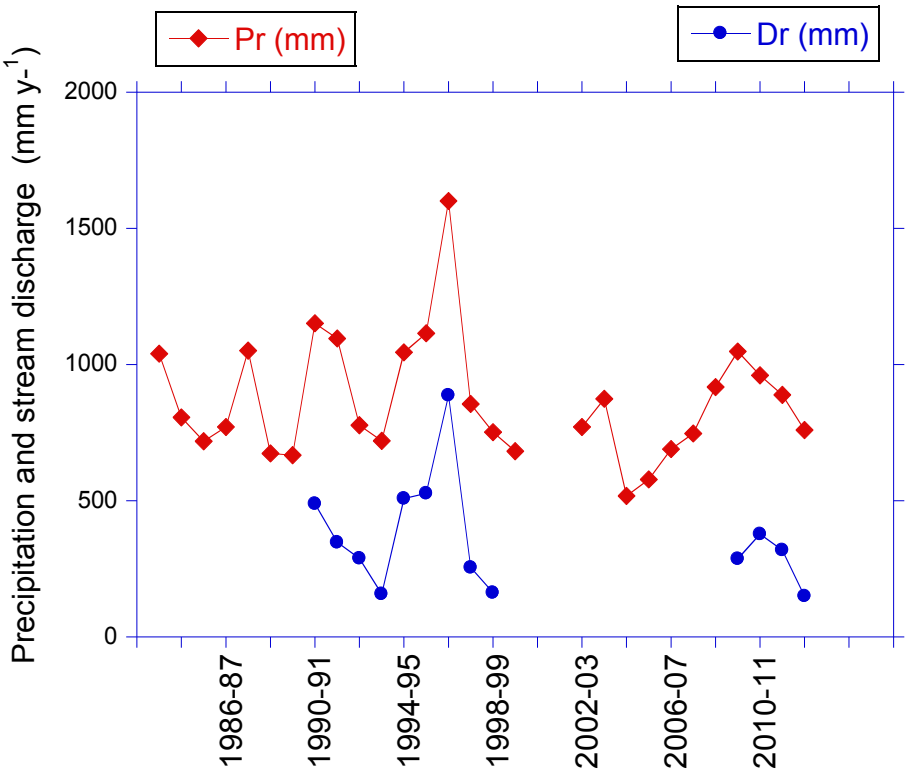

Figure 2. Precipitation (1983-2014) and drainage from the Torrent de la Mina stream. Units are mm y-1. Hydrologic years defined from 1 September.Linear regressions vs time for precipitation and drainage were non-significant.

For the stream waters, we first calculated fluxes, which resulted from multiplying instantaneous solute concentration (x_i, in µeq L^{-1}) by stream discharge between successive time steps corresponding to the sample x_i (D_i, in L). For each time step, D_i was calculated as:

$$D_i = q_i(t_i - t_{i-1}) - \frac{(t_i - t_{i-1})(q_i - q_{i-1})}{2} \qquad (1)$$

Being q instantaneous stream discharge (in L s^{-1}). We summed up the obtained values for the period of interest ($\sum x_i D_i$) to obtain the fluxes. To give the VWM, fluxes were divided by the accumulated stream discharge for the same period ($\sum D_i$).

Several works have attempted to determine the best unbiased estimate of flow-weighted solute concentrations and fluxes, since the appropriateness of the estimate depends on the frequency of sampling related to the duration of stormflows

(Rekolainen et al., 1991; Stone et al., 2000; Swistock et al., 1997). Calculations based on weekly sampling and the water flow corresponding to each one of the samples, the procedure used here, have been recommended as a compromise between the effort of sampling and the accuracy of the estimate. The accuracy of estimates with this procedure has been found to lie within 10% of expected values (Rekolainen et al., 1991; Stone et al., 2000; Swistock et al., 1997).

Chemical analyses

Samples were taken to the laboratory and were analyzed for pH, conductivity and alkalinity within 24–48 h from sampling. Samples were filtered with 0.45μm size pore acetate cellulose membrane filters and 60-mL aliquots were frozen until analysis. Concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+, Cl^-, NO_3^- and SO_4^{2-} were measured by ion chromatography (Dionex, Sunnyvale, USA). Data quality was checked with the inclusion of synthetic samples of known ionic concentrations during analytical runs. The quality of the analytical data was checked by a: 1) conductivity index (ratio of measured conductivity to conductivity calculated from the concentration of all measured ions and their specific conductivities), 2) an ionic index (ratio of the sum of cations to the sum of anions). For BD and WD a 20% variation about the central index value (1.00) was accepted, according to the ICP-Forest manual (ICP-Forest Manual, 1998). Because TF contains weak acids that may not have been included in our alkalinity measurements, we did not impose the 20% restriction about the central value to throughfall ionic index checks. Further analytical checks were undertaken by participating in international comparisons (AQUACON Med-Bas, Mosello et al., 1998), with highly satisfactory results.

Canopy budget method

To separate canopy exchange from dry deposition, a canopy budget model (CBM) has been generally used (Draaijers and Erisman, 1995; Staelens et al., 2008; Adriaenssens

et al., 2012). The CBM is based on the following balance between fluxes above and below the canopy: TF+SF = PD + DD + CE, where TF stands for throughfall, SF for stemflow, PD for Precipitation Deposition, DD for Dry Deposition and CE for Canopy Exchange. Canopy exchange, if positive, indicates the leaching of ions from the canopy (CL), and if negative, the uptake of ions (CU). Stemflow at the LC site contributed only 3% of total rainfall (Rodrigo et al., 2003) and because of its small contribution, SF fluxes have not been considered here. Then,

TF-PD = net TF = DD+CE (2)

In this procedure, dry deposition is estimated based on a tracer ion that is assumed not to be influenced by canopy exchange processes and assuming that aerosols containing the other ions of interest have a similar deposition behavior than the ion chosen as reference (Ulrich, 1983). Here Na^+ was used as an inert tracer as in most studies (Staelens et al., 2008) to derive DD for base cations, SO_4^{2-} and Cl^-. To characterize PD, either WD or BD can be used, but using WD will result in the estimation of fine plus coarse aerosol dry deposition, while BD would mostly indicate dry deposition of fine aerosols (Balestrini et al., 2007); thus WD is used here.

Nitrogen compounds can be taken up in the canopy (Boyce et al., 1996; Ignatova and Dambrine, 2000) and there is a need to estimate this flux in order to derive DD. To estimate nitrogen CU, we considered that NH_4^+ uptake can be equated to the net leaching of base cations and NO_3^- is considered to be retained at a fixed efficiency rate related to NH_4^+. To balance the charges, NO_3^- uptake is then equated to H^+ uptake (Staelens et al., 2008; Adriaenssens et al., 2012).It is well known that NH_4^+ is preferentially retained by leaves relative to NO_3^- and an efficiency factor of 6 for NH_4^+ *vs* NO_3^- uptake has been proposed for forests in Europe (de Vries et al., 2003). This exchange value has been applied in several studies in Europe (Schmitt et al., 2005; Thimonier et al., 2005; Zhang et al., 2006) and will be also applied here:

$$CU(\mathrm{NH4+NO3}) = \left(\frac{xNH4*(TF)\mathrm{NH4}+(TF)\mathrm{NO3}}{xNH4*(TF)\mathrm{NH4}}\right) * CU(\mathrm{NH4}) \quad (3)$$

Statistical analysis

Air pollution emissions per country were obtained from the EMEP database site:http://www.ceip.at/ms/ceip_home1/ceip_home/webdab_emepdatabase/reported_emissiondata/). Local urban NO_x air concentration data was obtained from the Catalan Air Quality network for Barcelona (Gràcia station; Direcció General de Qualitat Ambiental, Generalitat de Catalunya). Data from electricity generation in Spain were downloaded from Red Eléctrica Española (http://ree.es/es/publicaciones).

Mann-Kendall test were used to analyze trends in emissions for the years reported by EMEP (1980-2012), and for bulk deposition measured at Montseny (1983-2014). Stream water concentration and fluxes presented a non-continuous record which prevented using the Mann-Kendall tests, thus differences between an initial (1990-1999) and recent period (2010-2014) were tested with ANOVA tests. Percent changes for emissions and bulk deposition were also recalculated for the same years of streamwater data for a more accurate comparison. Stepwise regressions were used to determine principal contributors to N and S rain concentrations and deposition at Montseny.

Results

Changes in emissions

The evolution of emissions in Spain and its neighboring countries can be seen in Fig. 3. The significance of the trends was checked with the Mann-Kendall test and percent reductions or increases were calculated comparing 5-year averages at the beginning and the end of the reported periods. The significance of the Mann-Kendall Tau, and the % difference between the 5-yr initial and recent period are shown in Table 2.

SO_2 reductions were similar in all countries, decreasing between 75 and 85%. For NO_2, Italy and France accomplished higher reductions (51 and 42% respectively) than Spain and Portugal (34 and 32%). In all countries except Spain, the Mann-Kendall Tau was significant for NO_2 (Table 2). The evolution of emissions in France and Italy showed a

change of trend in the early 1990s with a clear declining evolution thereafter (Fig 2). For Spain, there was an increasing trend until the early 1990s, emissions remained stable at high levels until around 2005, and since 2006 a sharp declining trend was initiated (Fig 3).

For NH_3, Spain and France increased emissions by 13% and 1%, respectively, while NH_3 emission declined in Italy and Portugal (Table 2).

Table 2. Emission trends for Spain and surrounding countries between 1980 and 2012 (Portugal, between 1992 and 2012). Negative percent change indicates reduction and positive percent, increase between the initial and final periods. Man-Kendall p value is also indicated.

		Mann-Kendall p value	5-year initial Gg		5-year final Gg		
Emission			Mean	SD	Mean	SD	%change
SO_2	Portugal	<0.0001	305	31	48	5	-84
	Spain	<0.0001	1870	233	430	26	-77
	France	<0.0001	1004	220	255	29	-75
	Italy	<0.0001	1302	262	195	18	-85
	Iberian P.	<0.0002	2175	249	479	28	-78
NO_2	Portugal	0.003	257	7	170	8	-34
	Spain	0.265	1391	28	942	19	-32
	France	<0.0001	1746	107	1017	44	-42
	Italy	<0.0001	1863	201	904	53	-51
	Iberian P.	0.245	1648	31	1112	26	-33
NH_3	Portugal	<0.0001	61	2	47	1	-22
	Spain	<0.0001	339	26	384	6	13
	France	0.049	688	9	697	22	1
	Italy	<0.0001	455	10	388	14	-15
	Iberian P.	<0.0001	339	25	437	7	8

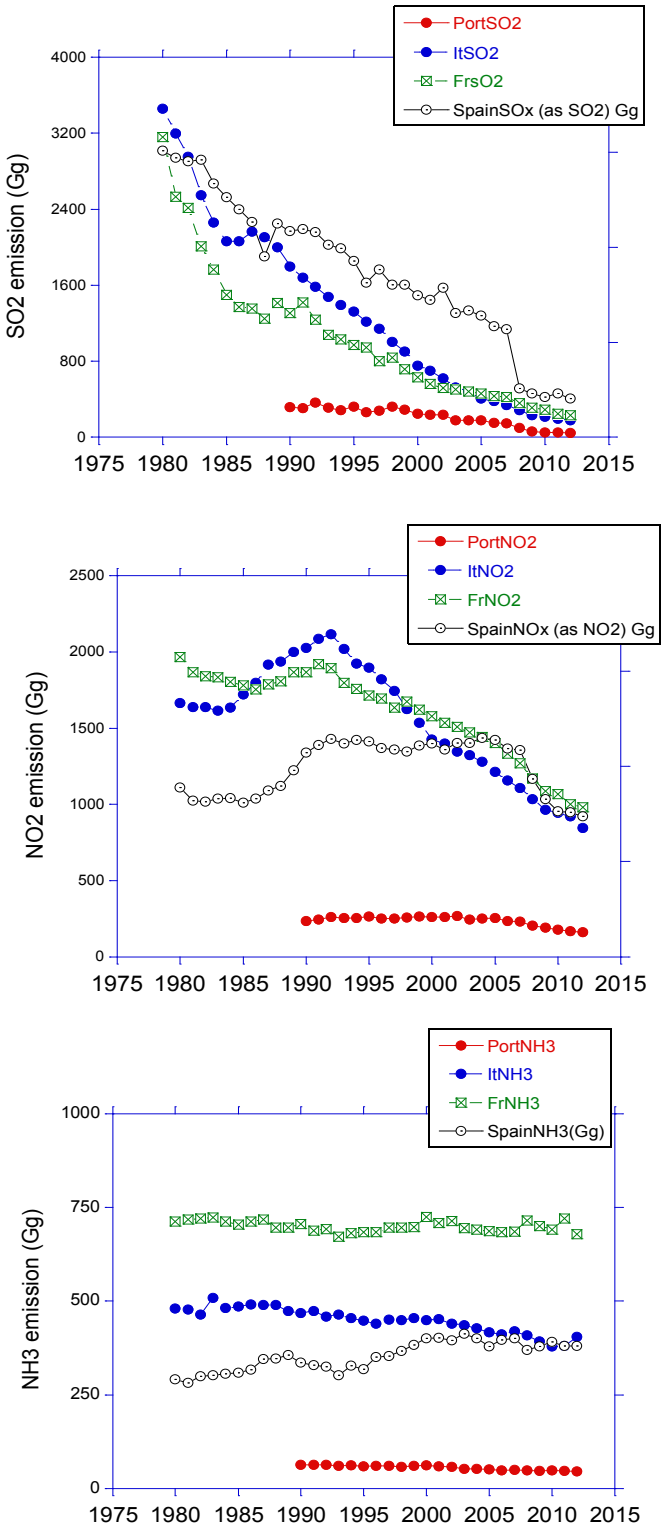

Figure 3. Temporal evolution of emissions reported to EMEP (in Gg) for Spain and neighboring countries

Relationship of rain and throughfall chemistry with emissions

Rain concentrations are strongly influenced by precipitation amounts. The time trend of annual precipitation did not show a significant pattern during the study period (Fig. 2). Hence, trends in element concentrations in precipitation, though partly influenced by the variability of the rainfall amount, were probably more influenced by atmospheric emission and transport processes.

Bulk deposition trends at Montseny are compared to Spanish emissions in Fig. 4. Sulfate VWM concentrations were strongly correlated with the Iberian Peninsula (sum of Portuguese and Spanish) SO_2 emissions (Pearson R=0.85; p<0.0001), with France and Italy emissions (R=0.82 p<0.0001) and with the EU28 emissions as a whole (R=0.65; p=0.0002). SO_4^{2-}-S deposition fluxes at Montseny were also highly correlated with emissions (Pearson R significant to p<0.001 for the Iberian Peninsula, France, Italy and the EU28). Thus, the effective widespread measures undertaken in Europe since the early 1980 to reduce SO_2 emissions have been translated into a reduction in precipitation SO_4^{2-}concentrations and S deposition fluxes. However, while the SO_2 emission reduction between 1990-99 and 2010-14 was around 80% (Table 2), SO_4^{2-} concentrations and S fluxes were only reduced by 56 and 42% respectively.

Spanish NH_3 emissions significantly increased between 1980 and 2012 (Table 2), but NH_4^+ concentrations in bulk deposition did not show a significant temporal trend in this period (p=0.26). From Fig 3b it can be seen that emissions increased steadily until the early 2000s and thereafter remained stable. The correlation between Spanish NH_3 emissions and NH_4^+ concentrations in bulk deposition was marginally significant (R= 0.32; p=0.056), but the correlation increased when considering the Iberian Peninsula emissions (R=0.36; p=0.027). No correlations were found between NH_4^+ VWM concentrations in bulk deposition and Italian and France or the EU 28 NH_3 emissions, nor for NH_4^+-N fluxes and emissions of all the above considered countries.

No significant correlation was found between NO_3^--N fluxes at Montseny and emissions, except for Spanish NO_2 emissions (p=0.014).

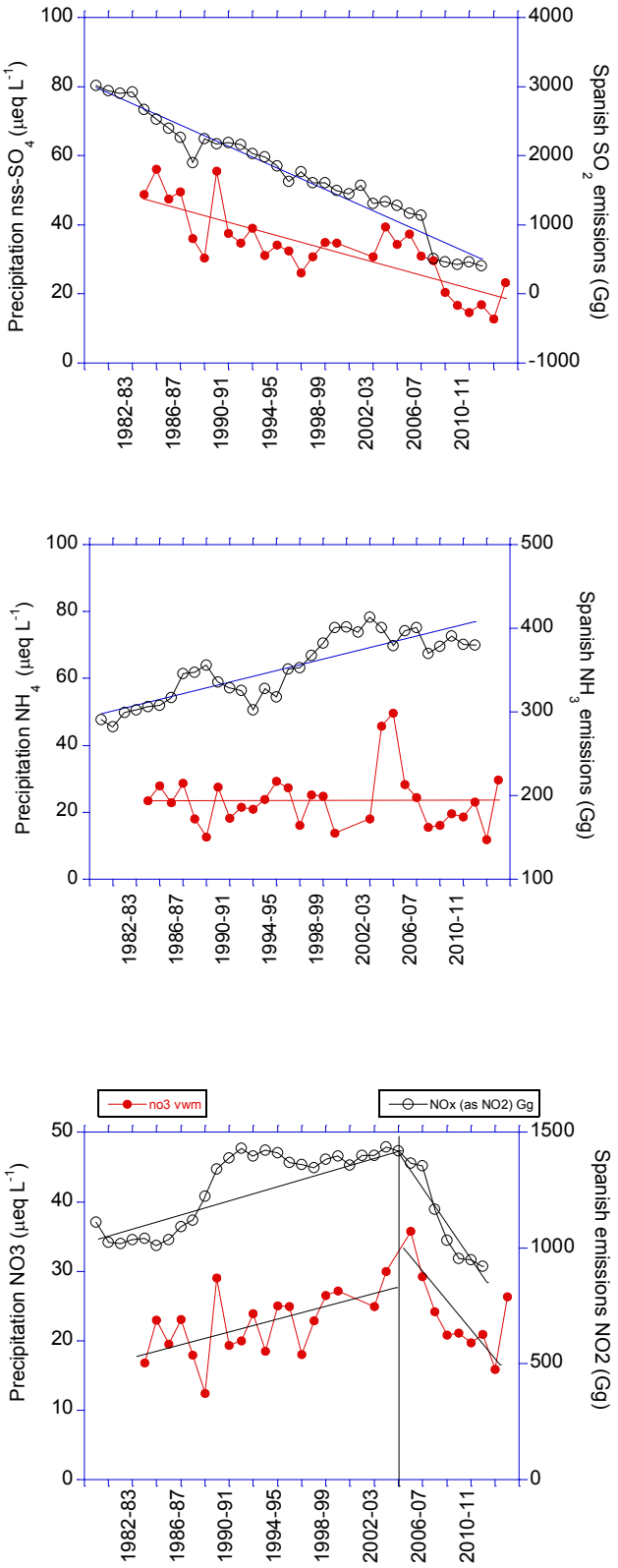

Figure 4. Temporal evolution of Spanish emissions and VWM concentrations in precipitation at la Castanya (Montseny).

The relevance of the different contributions to NO_3^- VWM concentrations was tested with a stepwise regression considering NO_2 emission data from EMEP (France, Italy, the Iberian Peninsula and the EU28) and a proxy of local emissions (near-ground NO_x air concentrations in the center of Barcelona from the Catalan Air Quality network) as independent variables. Precipitation amount was also included due to the marked dilution effect of rain on N concentrations (Prado-Fiedler, 1990). The model included precipitation (B=-0.31; p=0.012); NO_x Barcelona emissions (B= 0.42; p=0.005) and Iberian Peninsula emissions (B=0.43; p=0.011). Correlation coefficient for this model was r=0.92. Thus, both local and national emissions strongly affect NO_3^- deposition in Montseny.

Throughfall was sampled at two moments in this time series: the first (1995-1996) can be taken to represent the period of high N emissions. The more recent one (2011-2013) can be taken to represent the current situation, where SO_2 and NO_x emissions have been reduced but NH_3 emissions still increase. Bulk deposition and throughfall VWM concentrations for these two periods are presented in Table 3. Non sea salt-SO_4^{2-}concentrations in BD and TF declined between the two studied periods (with reductions of 63 and 60% for bulk deposition and throughfall, respectively). Changes in Spanish and the Iberian Peninsula SO_2 emissions during the time span when TF was measured decreased by 72 and 76%, respectively.

Ammonium concentrations in BD and TF also decreased between periods (with reductions of 40 and 56% for bulk deposition and throughfall). However, Spanish NH_3 emissions increased by 8%. Since atmospheric ammonia is principally involved in neutralizing SO_2 to form ammonium sulfate aerosols, the strong decrease of SO_2 may have resulted in a reduced formation of ammonium aerosols and may have facilitated the formation of NH_4NO_3 aerosols and/or NH_3 dry deposition.

Nitrate concentrations decreased in BD between periods but increased by 23% in TF (Table 3). This increase, however, does not directly show the changes in DD, since N is usually exchanged at the canopy (Ignatova and Dambrine, 2000; Staelens et al., 2008). To deduce N dry deposition a canopy budget model was applied.

Table 3. Water volume (mm y^{-1}) and ion VWM concentrations (in ueq L^{-1}) in bulk deposition (BD) and throughfall (TF) and their percent change between an initial (6/6/1995 to 25/6/1996) and final period (1/3/2011 to 1/3/2013) at LC (Montseny). Negative percent change indicates reduction and positive percent, increase between the initial and final periods.

	Water vol.	pH[1]	H	Alk	Na^+	K^+	Ca^{2+}	Mg^{2+}	NH_4^+	NO_3^-	SO_4^{2-}	$nssSO_4^{2-}$	Cl^-
1995-1996													
BD	1161.5	5.28	8.7	19.1	21	3.78	36.9	7.88	32.7	29.5	41.1	38.6	26.6
TF	858.5	6.11	1.25	76.2	32	64.1	79.5	26.2	41.9	33.6	71.2	67.4	55.5
2011-2013													
BD	860	6.06	1.9	41.5	20.3	3.2	53.3	11.1	17.6	18	16.6	14.2	21.2
TF	694	5.93	1.4	75.7	44.4	60.8	108	43	11.8	41.3	32	26.7	63.7
%change						-							
BD	-26.0	14.8	78.2	117.3	-3.3	15.3	44.4	40.9	-46.2	-39.0	-59.6	-63.3	-20.3
%change													
TF	-19.2	-2.9	12.0	-0.7	38.8	-5.1	35.8	64.1	-71.8	22.9	-55.1	-60.4	14.8

[1] Median pH

Dry deposition fluxes derived from a canopy budget model

The methodology used here has been recommended in ICP-Forest manual even thoughsome uncertainties remain for the estimation of NO_3^--N exchanges and dry deposition (Adriaenssens et al., 2012; Staelens et al., 2008). Since the assumptions included in the model were identical for the two study periods, results may be comparable between periods. Bulk deposition and wet deposition were recalculated to coincide with the TF periods (Table 4).

The changes for the element fluxes in WD for the years of throughfall data also indicated strong declines for SO_4^{2-}-S (59%), NH_4^+ (54%) and NO_3^- (42%, Table 4). However, based on the CBM estimations, DD decreased for SO_4^{2-}-S (28%) and NH_4^+-N (5%) while it strongly increased for NO_3^-N from 1.26 to 6.76 NO_3^--N kg ha^{-1} y^{-1} (Table 4). Thus, while in the first period, NH_4^+N accounted for most of total inorganic N DD (94%), in the second it only accounted for 47%. Changes in meteorology between periods can explain part of these differences, as discussed below.

Table 4.Fluxes in wet deposition (WD), net throughfall (nTF) and Total deposition (TD). Dry deposition (DD) and canopy exchange (CE) fluxes have been derived from a throughfall canopy budget model for an initial (1995-1996) and a recent period (2011-2013). Units in kg ha^{-1} y^{-1}.

kg/ha/y	Na^+	K^+	Ca^{2+}	Mg^{2+}	NH_4^+-N	NO_3^--N	sum N	SO_4^{2-}-S	Cl^-
1995-1996									
WD	4.10	1.13	6.29	0.74	5.13	3.51	8.64	6.17	7.23
nTF	2.08	19.5	7.11	1.95	-0.29	0.37	0.08	3.37	9.27
DD	2.08	0.58	3.12	0.37	6.36	1.26	7.62	2.31	3.75
CE	0.00	18.9	3.99	1.58	-6.65	-0.89	-7.54	0.59	5.52
TD	6.18	1.72	9.41	1.11	11.5	4.77	16.3	9.34	11.0
2011-2013									
WD	4.59	0.98	9.78	1.17	2.36	2.03	4.39	2.52	6.44
nTF	3.02	18.4	8.02	3.10	-1.04	2.60	1.56	1.53	11.5
DD	3.02	0.65	6.40	0.77	6.07	6.76	12.8	1.66	4.24
CE	0.00	17.8	1.58	2.33	-7.12	-4.16	-11.3	-0.13	7.23
TD	7.61	1.63	16.2	1.94	8.43	8.79	17.2	4.18	10.7
WD %change	12.0	-13.3	55.5	58.1	-54.0	-42.2	-49.2	-59.2	-10.9
DD %change	45.2	12.1	105.1	108.1	-4.6	436.5	68.4	-28.1	13.1
TD %change	23.1	-5.2	72.4	74.8	-26.7	84.3	5.8	-55.2	-2.7

Changes in streamwater chemistry

We hypothesized that the described changes in N atmospheric deposition will be transferred to the stream water, given the low residence time of water within this

catchment which makes it highly responsive to inputs (Bernal et al., 2013). SO_4^{2-} concentrations decreased (22%) between an initial (1990-99) and a more recent period (2010-2014), though at a lower rate than deposition (44%, Table 5), implying soil sulfate retention. NO_3^- concentrations in streamwater more than doubled (significant to $p<0.1$) but the change in NO_3^--N export was smaller due to the fact that fluxes result from the product of concentrations by water export, which was lower in the second period. In Figure 5, a clear change in the ecosystem response towards N inputs is shown whereby for similar inorganic N (NO3-N + NH4-N) deposition, higher exports are produced in the more recent period, suggesting the onset of N saturation.

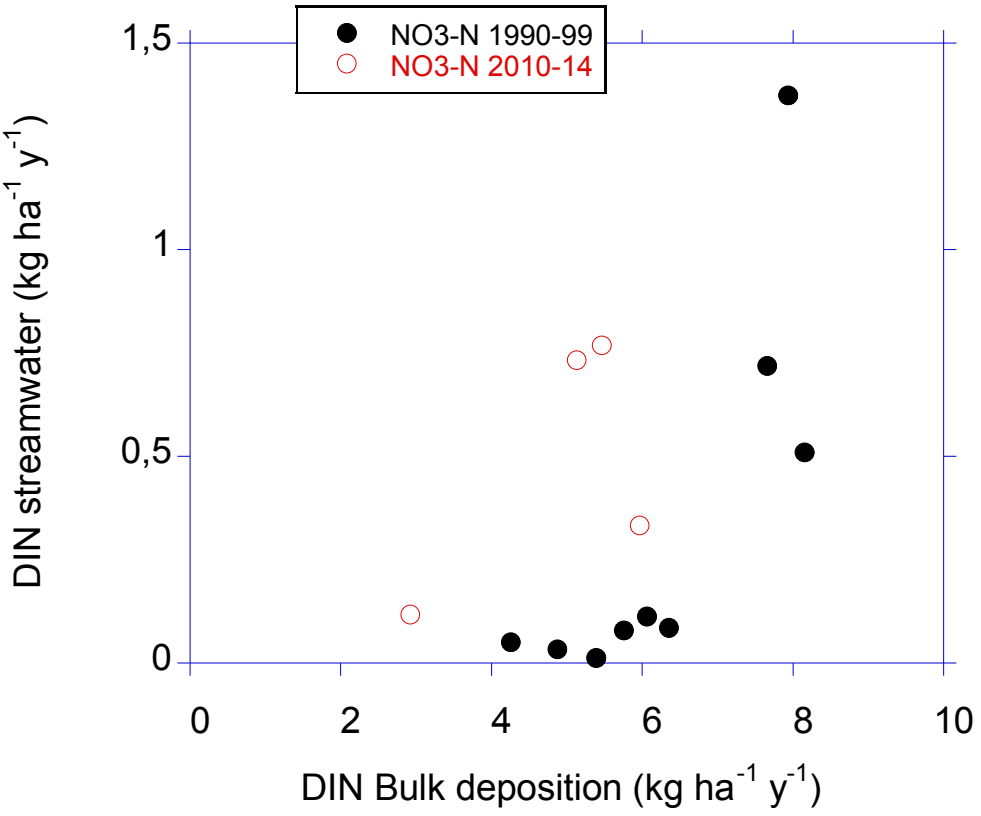

Figure 5. Dissolved inorganic nitrogen (DIN = NO3-N + NH4-N) annual fluxes in the stream waters draining the TM0 catchment and versus annual DIN bulk deposition fluxes at the site, for two recording periods (blak circles, 1990-1999; open circles, 2010-2014).

Discussion

Large changes in the emission of acidifying pollutants have been achieved in Europe following the implementation of abatement measures in the 1980s. These measures are costly and there is an interest in contrasting their long-term effectiveness regarding changes in air quality, precipitation chemistry, atmospheric deposition and the response of ecosystems. However, the link between emissions, concentrations and deposition is complex and non-linearities between emission reductions and precipitation concentrations have been described (Fowler et al., 2005; 2007). Part of the lack of correlation may be due to the influence of meteorological conditions, which may impose a high variability to the rain chemistry and deposition series, especially in the Mediterranean because of its very high precipitation variability. However, long time series may reveal trends robust enough to describe the relationships between emissions and deposition. Here, we compared a simultaneous record of 30 years of emissions and precipitation chemistry and deposition at a site in NE Spain for a period encompassing marked changes in S and N emissions (Figs. 2 and 3).

In this study we observed similar declines in SO_2 emissions in Spain (77%) than in neighboring countries and the EU28 (75-85%, Table 3). However, the decline in nssSO_4^{2-}concentrations in precipitation and SO_4^{2-}-S deposition was lower (65% and 62%, respectively) than emissions. A similar pattern was found in a study of emission and deposition changes for 5 defined source regions in Europe for the period 1980-2000, in which the regions to which Spain belonged presented a 80% reduction in SO_2 emissions and 40-50% reduction in SO_4^{2-}deposition (Fowler et al., 2007).

For the period of common measured wet deposition and throughfall, while S wet deposition decreased by *ca.* 60%, the estimated change in dry deposition between 1995-96 and 2011-13 was only 28% (Table 4), indicating the predominant role of dry deposition over wet deposition as SO_2 emissions are reduced. The small change in dry deposition between periods is consistent with the proposed increase in the SO_2 deposition velocity as ambient SO_2 concentrations are reduced (Fowler et al., 2001). Nevertheless, in our study, meteorological conditions were very different in both

periods (much wetter in 1995-96) that may have also affected the deposition partitioning between wet and dry. Further studies to confirm this trend are warranted.

When considering total deposition amounts (wet+dry), a 50% decline was found. This decline in S deposition can translate through soils to drainage waters. A previous study of small headwater streams in the Montseny mountains indicated a 30% decrease of SO_4^{2-}concentration in streamwaters from 1980 to 2007 (Àvila and Rodà, 2012). The results of the present work consolidate this trend with significant differences for SO_4^{2-} in the TM0 stream, and about half S exports from the catchment (Table 5).

Table 5. Comparison of VWM concentrations (in µeq L^{-1}).water drainage (mm y^{-1}) and ion fluxes in streamwater (in kg ha^{-1} y^{-1}). Differences between periods were tested with ANOVA (initial period=1990-1994. recent period =2010-2014) and significant differences ar highlighted in bold.Percent differences between periods are also indicated.

	Water	cond	Alk	Na^+	K^+	Ca^{2+}	Mg^{2+}	NO_3^-	SO_4^{2-}	Cl^-
VWM										
1990-1999	-	**53.6**	**291**	197	8.2	**203**	192	4.5	**157**	90.7
2010-2014	-	**63.7**	**383**	219	7.2	**253**	145	11.2	**123**	95.4
p-value	-	**0.001**	**0.012**	0.053	0.36	**0.002**	0.064	0.081	**0.005**	0.47
%change		19	32	11	-12	25	-24	250	-22	5
Export flux										
1990-1999	403		1105	17.7	1.29	15.9	6.1	0.33	10.1	12.9
2010-2014	283		1043	14	0.84	14.4	4.9	0.49	5.65	9.6
p-value	0.35		0.83	0.47	0.3	0.68	0.47	0.55	0.15	0.4
%change	-30		-6	-21	-35	-9	-20	48	-44	-26

The emissions of oxidized N declined by 32% in Spain, a higher reduction than model estimates for the regions that include Spain in Fowler et al. (2007) which ranged

between a small 2% reduction and a 6% increase. Overall, from 1983 to 2014, NO_3^- concentrations in precipitation and NO_3^--N deposition slightly increased (3-4%).Nitrate concentrations in precipitation were explained (R^2= 0.92) by a model that included variations in precipitation amount, local NO_x emissions in Barcelona, and emissions at a Peninsular scale. Spanish NO_2 emissions from 1980 to 2013 showed a clear increasing trend until 2005 and a steep decline thereafter and NO_3^- concentrations closely tracked these changes (Fig. 3). Main contributors to NO_x emissions in Spain are road transport (33%) and energy use + electricity supply for transport (60%; EEA 2012). Electricity generation has shifted in recent years in Spain from coal and natural gas (se decrease in Fig. 6) to a higher role of renewable energies. NO_2 Spanish emissions closely tracked this change, suggesting the important role of electricity generation on NO_x emissions and eventually in NO_3^- deposition (Fig. 6).

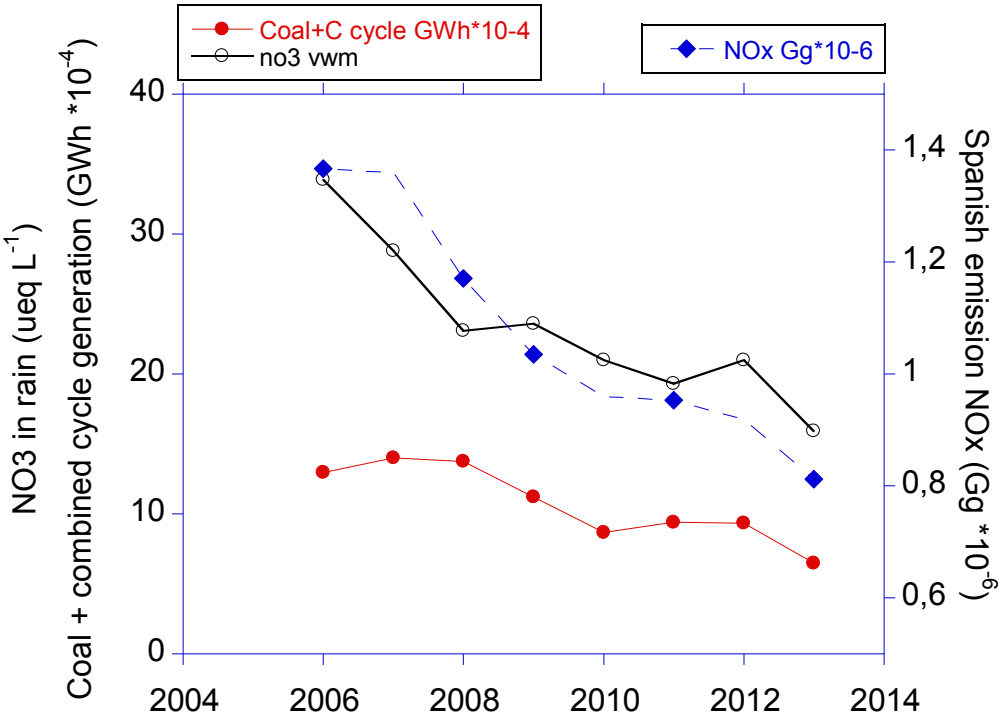

Figure 6. Evolution of NO_3 concentrations in bulk deposition, Spanish NO_x emissions and Spanish electricity generation (from REE) in the period 2006 to 2013.

Ammonia emissions increased by 13% in Spain between 1980 and 2012 (Table 2). However, NH_4^+ in precipitation and NH_4^+-N deposition at the Montseny site showed a

decreasing trend (15% reduction). This can be due to several processes and the interaction between them. Opposite time trends are involved in the formation of NH_4^+ aerosols (increasing NH_3 and decreasing SO_2 and NOx emissions): with declining SO_2 and NO_2 emissions in recent years there is a decreasing formation of ammonium sulphate and nitrate aerosols, thus lower NH_4^+ is available to be scavenged by precipitation and lower NH_4^+ in precipitation is expected. However, due to the reduced formation of aerosols, there are changes in the partitioning between $NH_3/NH_4^+$and this may affect the residence time of NH_4^+ in the atmosphere and thus the spatial scale of NH_3/NH_4^+deposition. Fine NH_4^+ aerosols are long-range transported so that decreasing SO_2 and NO_x emissions will tend to decrease NH_4^+ wet deposition at sites remote from sources, but enhance local dry deposition of NH_3.

Dry deposition changes for N were estimated from TF measures by applying a canopy budget model that indicated increasing NO_3-N dry deposition (Table 4). A reason for this increase may be the very low initial throughfall NO_3-N values int he year 1995-96, a period that was 30% rainier than usual, thus restraining the dry spells for dry deposition. As a further check of the validity of the N dry deposition estimates, we calculated dry deposition with the inferential method using a record of aerosol concentrations and gases (HNO_3, NO_2, NH_3) at the La Castanya plot and taking V_d from reported values for forests (only in the 2012 period because of lack of gas measurements in the 1995 period). This calculation produced a DD estimate of 6.22 kg $ha^{-1}yr^{-1}$ for N-oxidized and 4.04 $kgha^{-1}yr^{-1}$ of N-reduced which compared well with the DD estimate of NO_3^--N calculated with the CBM (6.76 kg ha^{-1} yr^{-1}) but underestimated NH_4^+-N deposition by 50%. However, as a first approach, we can frame the dry deposition in 2011-2013 for NO_3^--N and NH_4^+-N in 6-7 kg $ha^{-1}yr^{-1}$ and 4-6 kg $ha^{-1}yr^{-1}$, respectively. Total N deposition in both periods was similar (16-17 $kgha^{-1}yr^{-1}$, Table 4), but the contribution of the dry and wet modes varied between periods: while in 1995 dry and wet deposition fluxes were similar (around 50%), in the recent period dry deposition dominated (75%).

Chronic addition of N amounts in the range of 15-17 $kgha^{-1}yr^{-1}$ are within the critical loads proposed for Mediterranean sclerophyllous forests (15-17.5 $kgha^{-1}yr^{-1}$; (Bobbink

et al., 2010). Thus, the holm oak forests in the NE Iberian Peninsula may be at the brink of experiencing adverse effects. One way to explore the N status of an ecosystem is to monitor the changes in the drainage waters of unperturbed catchments. At present, a shift is observed towards higher N export.

Conclusions

Emissions of S in Spain, as in other European countries, have strongly decreased (77%) and that was reflected in reductions for $nssSO_4^{2-}$ concentrations in precipitation (65%) and SO_4^2-S deposition (62%) during the period from 1980 to 2012. The observed lower decline (29%) in dry deposition was consistent with reports of lower SO_2 deposition velocity as ambient SO_2 is reduced.

NO_2 emissions in Spain increased from 1980 to 1991, remained constant until 2005, and decreased thereafter, a pattern that was paralleled by NO_3^- in bulk precipitation at Montseny. This pattern seems to be related to a shift in electricity generation from coal and natural gas to a higher role of renewable energies in Spain. However, dry deposition increased markedly in the recent period, from 1.3 to 6.7 kg $ha^{-1}y^{-1}$. Differences between the 2 periods were probably related to differences in rainfall amount, since the latter period was dryer and the contribution from dry deposition was higher.

NH_3 emissions have increased by a 13% between 1980 and 2014 in Spain but NH_4^+ concentrations in precipitation and NH_4^+-N deposition at the Montseny site showed a decreasing trend (15% reduction). We suggest that the reduction of SO_2 and NO_x emissions precludes the formation of ammonium sulphate and nitrate aerosols to be scavenged by rainfall. The estimations with a canopy budget model showed similar NH_4^+-N dry deposition between the compared periods.

The N loads at Montseny were in the range of 15-17 $kgha^{-1}yr^{-1}$, within the critical loads proposed for Mediterranean sclerophyllous forests (15-17.5 $kgha^{-1}yr^{-1}$; (Bobbink et al., 2010). The onset of N saturation at this Mediterranean holm oak forests is suggested

since higher DIN export of during the more recent period sampled was registered under similar DIN loads.

Acknowledgements

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Chapter 4

The critical levels of atmospheric ammonia in a Mediterranean forest in North-Eastern Spain

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Abstract

Atmospheric ammonia (NH_3) emissions have increased during the last decades, being actually considered as a main driver for biodiversity loss and ecosystem change. For that reason itis very important to establish the safety thresholds to prevent NH_3 effects on species and ecosystems, as defined by Critical Levels (CLEs), "the concentration above which direct adverse effects on receptors may occur according to the present knowledge". Lichen functional groups have been used as indicators of NH_3 pollution to establish the lowest CLEs for NH_3 both in Europe and North America, but Mediterranean forest areas lack information about critical levels for ammonia.

To fill this gap, in this work we used the lichen functional diversity changes along a NH3 atmospheric gradient to derive CLEs for the first time in Spain.

For that purpose, we measured NH_3 concentrations for one year in a distance gradient from a cattle barn surrounded by a dense holm-oak forest. The epiphytic lichen functional diversity was evaluated in 59 holm-oak trees along this gradient.

We found an empirical critical level of atmospheric $NH_3 < 2.5$ µg m^{-3}. This study confirms that lichen functional groups can be used to derive CLEs at a wide range of Mediterranean forest types.

Introduction

Ammonia (NH_3) emissions contribute to the general increase of reactive nitrogen cycling in the biosphere, which causes changes on biodiversity, ecosystem functioning and affects the human health (Galloway et al., 2003; Galloway and Cowling, 2002). The anthropic changes of the nitrogen cycle are considered as one of the major global threats to the sustainability of the planet (Rockström et al., 2009). Most NH_3 emissions to the atmosphere are due to intensive livestock farming, animal waste storage and fertilizer application to open fields. However, NH_3 gas has a low residence time in the atmosphere and is either quickly deposited in the vicinity of point sources (Asman et al., 1998) or converted into ammonium nitrate and sulfate aerosols that occur mostly as fine particles that travel for long distances (Finlayson-Pitts and Pitts Jr, 1999).

To protect both ecosystems and human health from the impacts of excessive N, several abatement measures have been proposed under the UN framework (Convention on Long-Range Transboundary Air Pollution, CLRTAP). Instrumental for the definition of the abatement regulations has been the concept of Critical Level (CLE), defined as "the concentrations above which direct adverse effects on receptors, such as plants, ecosystems or materials, may occur according to the present knowledge" (Posthumus, 1988). Regarding CLEs for NH_3, a recent revision proposed the value of 3 μg m^{-3} as threshold to protect vegetation and 1 μg m^{-3} to protect lichens and bryophytes (Cape et al., 2009), after showing that the previous accepted value for CLE in CLRTAP (8 μg m^{-3}) would not protect ecosystem functions. Still, most studies on N effects on ecosystems refer to temperate and boreal areas, while the Mediterranean ones remain underrepresented. Mediterranean ecosystems are regarded as hotspots of biodiversity (Myers et al., 2000), and they are considered particularly vulnerable to the increase of excessive N (Ochoa-Hueso et al., 2011; Phoenix et al., 2006), thus they deserve increased research efforts.

Evidence of long-term changes in lichen and bryophyte communities exposed to different NH_3 concentrations showed that they are one of the most sensitive component of the ecosystem (Cape et al., 2009). Lichens depend on wet and dry atmospheric deposition for their nutritional requirements, including the nitrogen supply. Thus, their growth depends on atmospheric N. However, under increasing NH_3 levels, N-sensitive lichen species cannot thrive and they are replaced by N-tolerant communities. This shift is caused by an increase of N availability which favors N-tolerant species, as well as by the direct adverse toxicity of NH_3/NH_4^+ which impairs the N-sensitive ones (Munzi et al., 2014). Ammonia has also an indirect effect by raising the bark pH thus accelerating the replacement of lichen species (Van Herk, 1999). For these reasons, several works in Europe and North America have been using epiphytic lichens as ecological indicators of atmospheric NH_3 (Geiser et al., 2010; Pinho et al., 2008; Pinho et al., 2009; Sparrius, 2007; Van Dobben and Ter Braak, 1998; Van Herk, 1999). It is commonly accepted that functional groups, rather than total lichen species richness, should be used for the interpretation of NH_3 effects at the ecosystem level (Fenn et al., 2008; Pinho et al., 2012). This approach is based on species specific sensitivities to N. Lichens species can be classified into functional groups according to their tolerance to eutrophication: oligotrophic (sensitive), mesotrophic (intermediate sensitive) and nitrophytic (tolerant). A similar classification has been used by other authors using the terms acidophyte, neutrophyte and nitrophyte lichens (Fenn et al., 2008; Geiser et al., 2010; Geiser and Neitlich, 2007; Jovan, 2008; Sparrius, 2007; Van Herk, 2001).

CLEs thresholds have been proposed for Mediterranean evergreen open woodlands based on functional changes in lichen communities in Portugal (Pinho et al., 2011; Pinho et al., 2014a; Pinho et al., 2014b; Pinho et al., 2012), but more research is warranted in this region to characterize the response of other forest types and in other countries.

NH_3 emissions in Spain are closely linked to agricultural activities. In 2013 values reached the 371 kilotonnes, a value 11% higher than in 1990. NH3 emissions are

currently exceeding the emissions limits marked by the Gothenburg protocol for 2010. Despite this, no research has been carried out to assess the effects of this pollutant in Spanish forest ecosystems. Our aim is to provide an estimation of empirical CLEs of atmospheric NH_3 for a Mediterranean holm-oak (*Quercus ilex* L.) forest in NE Spain and to validate the use of lichen functional groups previously employed in other Mediterranean forests (Pinho et al., 2011; Pinho et al., 2014a; Pinho et al., 2014b). This was done by sampling atmospheric NH_3 and lichen functional diversity in a closed canopy Mediterranean evergreen holm-oak forest in Catalonia (NE Spain), at increasing distances from a cattle barn point-source of ammonia.

Material and methods

Study area

The present study was carried out in a holm-oak forest in NE Spain 65 km away from the city of Barcelona and close to the Montseny Mountains. The forest surrounds a barn of ~130 beef cattle which are permanently housed in an area of 1500m^2. The sampling was performed downwind of the prevailing wind in plots at increasing distances from the barn (Fig. 1). The altitude of the sampling points varied between 618 and 690 m. Vegetation in the region is dominated by holm-oak forests. Climate is humid continental Mediterranean. Meteorological data were not available at the sampling site; thus, they were retrieved from the station of Sant Julia de Vilatorta (http://www.meteovilatorta.cat), located only 3.2 km distant from the sampling area and considered to represent the meteorology of the study site. For the period January 2013 -January 2014, total annual rainfall was 868 mm, mean annual temperature was 11ºC, and predominant winds were from the north-west. Inorganic nitrogen deposition has been estimated to be in the 15-30 kg N ha^{-1} yr^{-1} range at a background rural station located in Montseny (Àvila and Rodà, 2012) at linear distance of 13.5 km from the studied farm.

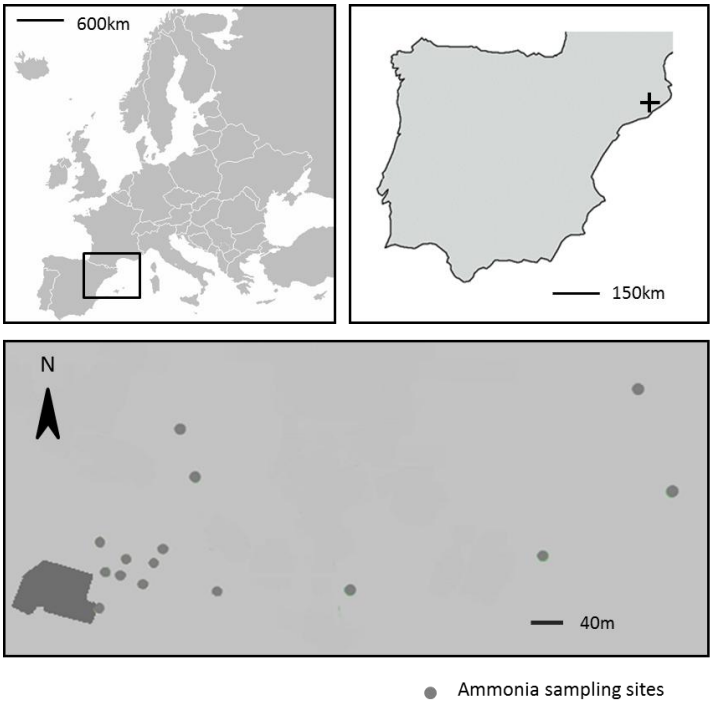

Figure 1. Above: Location of the study area in Europe and Spain (+), Down: Location of the 15 ammonia sampling sites in the gradient of distance to the barn.

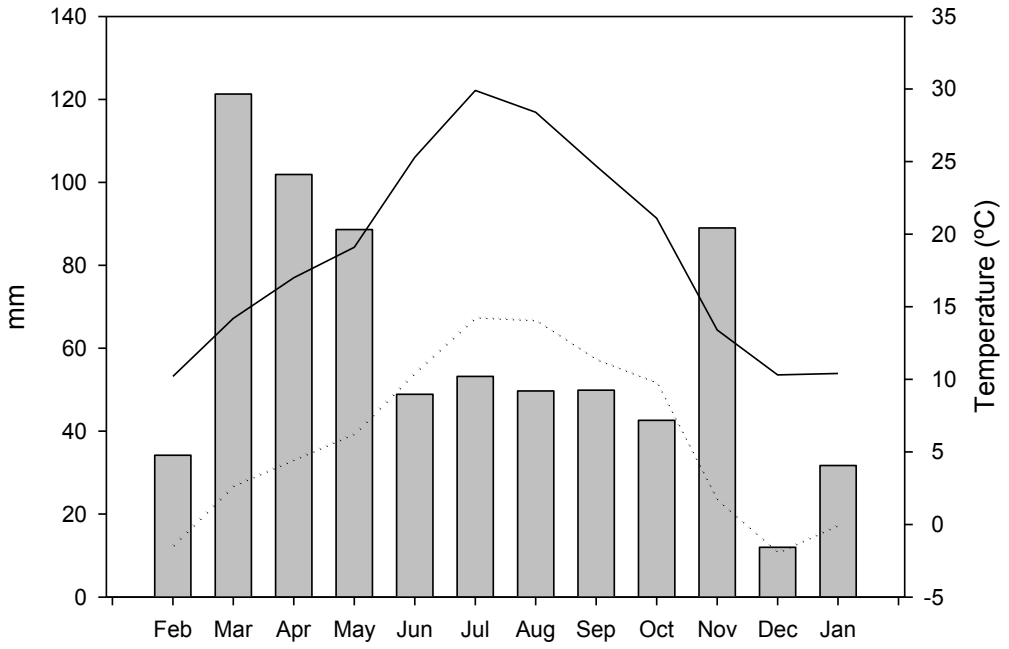

Figure 2. Monthly rainfall amount (mm; gray bars) and maximum (solid line) and minimum (point line) monthly average temperature (ºC). Data from Sant Julia Vilatorta Meteorological Station).

Ammonia measurements

Ammonia concentrations were measured from January 2013 to January 2014 at 15 points downwind from the barn (Fig. 1) using high-sensitivity ALPHA passive diffusion samplers (Tang et al., 2001). An ALPHA sampler is composed of a polyethylene PTFE tube containing an external membrane through which air flows to a collector filter coated with citric acid. Collection filters were prepared at CREAF following the procedure described in (Tang et al., 2001) (Fig. 7 Photo Appendix). ALPHA samplers were placed approximately 2m above the ground attached to a tree. They were collected and replaced by a new kit every 2-3 weeks. A travel blank was used every sampling day to account for possible contaminations during transport. One sampler was displayed per sampling site, but another replicate sampler was deployed at 6 of the sites. For those 6 sites, the reported NH_3 concentration is the average of the 2 samplers.

Passive diffusion tubes (Radiello©) were also disposed at 6 sites in parallel with ALPHA samplers, in order to compare between sampling devices. The Radiello passive samplers are commercially available samplers whose reliability has been tested by the European Reference Laboratory for Air Pollution (ERLAP), while ALPHA passive samplers are user-manufactured following a standard protocol (Tang et al., 2001). In our study, the concentrations measured with the two systems were highly correlated (R^2=0.98; p<0.0001, n=133), thus we considered that concentrations measured with ALPHA samplers were appropriate for deriving NH_3 concentrations. Here we only report ALPHA results.

Both ALPHA and Radiello filters were extracted in water and analyzed by colorimetry for ammonium with a Flow Injection Analysis system (FIA sampler).

Lichen survey, identification and classification

A total of 59 trees were selected for the lichen biodiversity survey close to the air NH_3 sampling sites, excluding the site closest to the main farm where no trees were

available for sampling. An average of 4 trees were sampled in the vicinity of each NH_3 sampling point (trees not shown in the map due to low resolution). The lichen biodiversity measurement followed a standard protocol which was designed to give a lichen diversity value LDV; (Asta et al., 2002). LDV takes into account species richness and species frequencies (Asta et al., 2002). Whenever possible, species were identified in the field, and doubtful species were determined at the laboratory. Specimens only identified at genus level were not used in this study.

The approach used in our work was based on the classification regarding eutrophication tolerance developed for Italy by Nimis & Martellos (2008), which has been successfully applied in Portugal (Pinho et al., 2012). Pinho et al., (2011) have validated Nimis & Martellos (2008) classification for sensitive and tolerant lichen species by ranking the species along a measured long term NH_3 atmospheric concentration gradient, and two lichen species were reclassified. Here we followed Nimis & Martellos (2008) classification, as corrected by Pinho et al., (2011) and applied it for the first time in Spain. Species with values from 4 to 5 were classified as nitrophytic (LDVnitro), species with values of 3 were classified as mesotrophic (LDVmeso) and species with a value up to 2 were classified as oligotrophic (LDVoligo). We considered the highest value given in that classification for each lichen species recorded. Several lichen-variables were calculated per tree. Species richness was calculated as the total number of species. Total lichen diversity value (LDVtotal) corresponds to the sum of the frequencies of all the species in each tree (Asta et al., 2002). LDV was also calculated for eutrophication tolerance functional groups by summing the frequencies of all species of each functional group. Functional group values (LDVoligo, LDVnitro, and LDVmeso) were relativized as percentage of the total diversity value (LDVtotal) for each tree.

Although Nimis & Martellos (2008) database refers to Italian flora, it is also suitable for this work. Firstly, species found in our study are relatively ubiquitous, and occur also in Italy. Secondly, both areas share a Mediterranean climate type and the same database classification has been used successfully in other areas with Mediterranean climate (Pinho et al., 2011). Finally, in order to check for the accuracy of species classification

into functional groups, a principal component analysis was applied on a matrix of the species frequencies from each tree, considering only species appearing in more than three trees. The relative location of species in the first axis of the ordination agreed well with species classification derived from Nimis & Martellos (2008), confirming the given functional group (data not shown) and assuring a correct classification of species into functional groups in our study area.

Statistical analysis

Because we had more sampled trees than Alpha samplers, trees nearest to each Alpha sampler were grouped and their lichen variables values were averaged. Monthly measures of atmospheric NH_3 concentrations were also averaged for calculating the annual concentrations at each sampling site. Atmospheric NH_3 concentrations were then related to lichen functional values (n=15).

CLEs were obtained following the proposal of Cape et al. (2009), which was applied for lichen LDVs in Pinho et al. (2012). The procedure is based in fitting a linear regression (including the 95% confidence limit bands) between NH_3 concentrations and lichen LDV values. The NH_3 concentration at the first point with altered lichen values considering the 95% confidence band of the regression, is taken as the critical level (Cape et al., 2009). In order to ensure a linear fit the NH_3 concentration values were log transformed.

Statistical analyses were performed with Statistica (StatSoft 2004) and Sigmaplot 11.0 (Systat Software Inc., San Jose, CA, USA).

Results

Ammonia concentrations

The monthly atmospheric NH_3 concentrations averaged for the 15 sites downwind from the barn are shown in Figure 3. Monthly mean concentrations between all sites varied between 5.5 and 12.7 µg m^{-3}, and the maximum observed values (corresponding to the measurement point closest to the barn) ranged between 27.0 and 72.2 µg m^{-3} (Fig. 3). The highest NH_3 concentrations were found at the end of summer and beginning of autumn. The annual average concentration of each site (Fig.4) showed an exponential decrease with increasing distance from the barn, reaching an average minimum of 1.8 µg m^{-3}. The monthly concentrations closer to the barn ranged from 27.0 to 88.7 µg m^{-3}, decreasing exponentially with distance to values ranging between 0.7-3.2 µg m^{-3} at ~620 m from the barn.

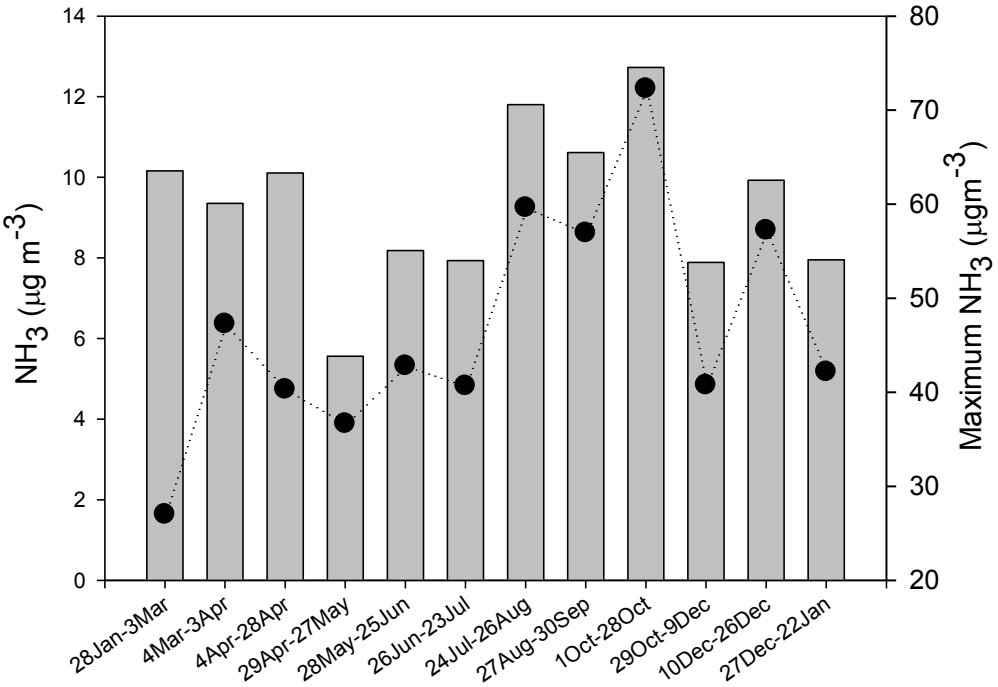

Figure 3. Monthly NH_3 air concentrations (µgm^{-3}) averaged for all sampling sites (bars) and maximum values observed (black dots, note the different scale) for the study area during 2013.

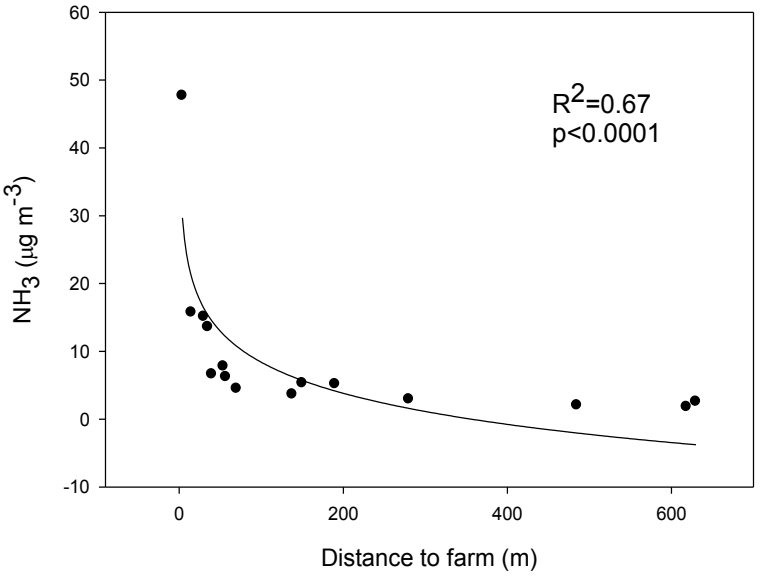

Figure 4 . Regression between NH_3 air concentrations (µgm^{-3}) and distance to the farm (m), (n=15).

Lichen functional diversity

A total of 53 species were recorded in this study (Table 1): 13 were nitrophytic, 18 mesotrophic and 22 oligotrophic. Total LDV ranged from 21 to 83, LDVnitro varied from 3 to 75, LDVmeso ranged from 0 to 18, LDVoligo ranged from 1 to 21. Most of the species observed were crustose lichens (54%), and a few squamulose and leprose species (6%), while the remaining species were foliose (30%) and some fruticose species (10%). The relative average value of LDV for crustose species was 47%, compared to 1% for the fruticose group. The most frequent species (>50% occurrence) were *Candelaria concolor, Flavoparmelia caperata, Hyperphyscia adglutinata, Lecanora chlarotera, Pertusaria amara* and *Phlyctis argena.*

Neither species richness nor total LDV were significantly related with average NH_3 concentrations (Fig. 5). However, the relative values of lichen functional variables were significantly related to average NH_3 concentrations, especially for the nitrophytic functional group (Fig 6). Relative values of LDVoligo and LDVmeso were significantly

and negatively correlated with NH_3 concentration, while LDVnitro showed a significant positive relationship (Fig 6). The relative values ranged from 14-98% (LDVnitro), 0-35% (LDVmeso) and 1-51% (LDVoligo).

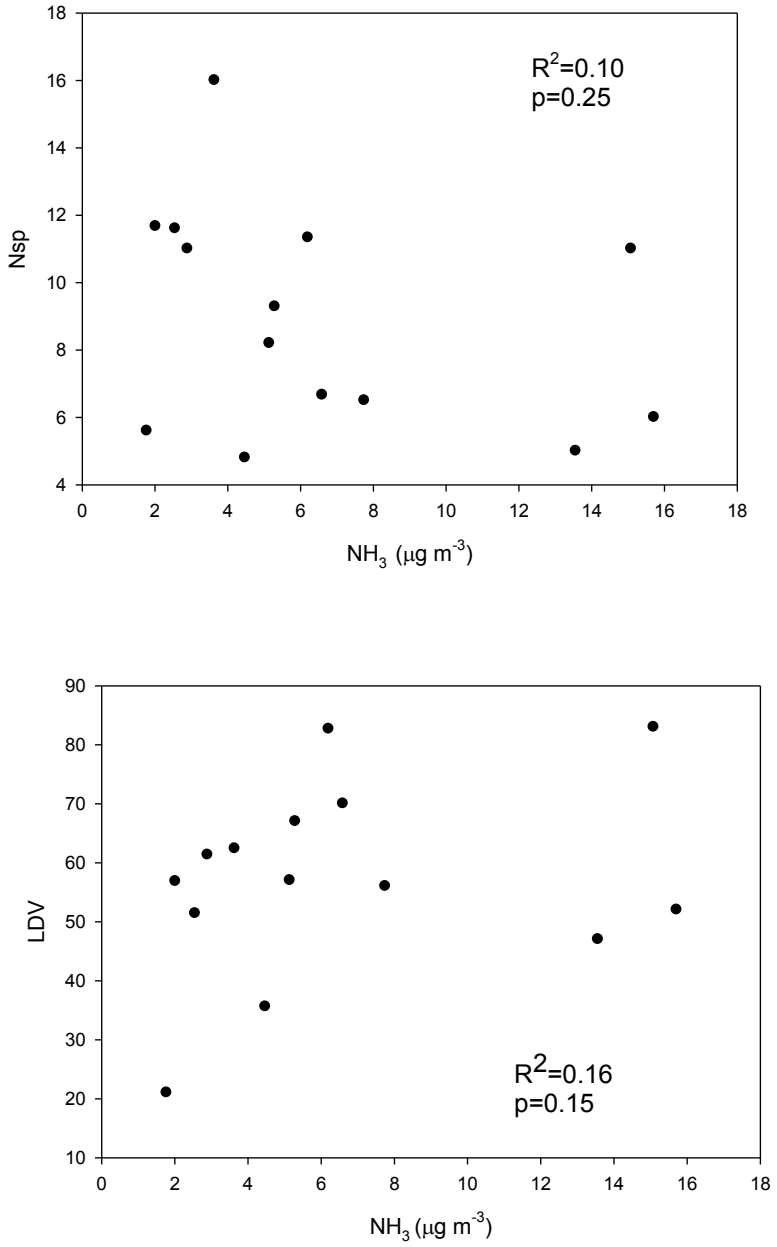

Figure 5. Relationship between NH_3 air concentrations (µgm^{-3}) with number of species (Nsp) and total lichen diversity value (LDV).

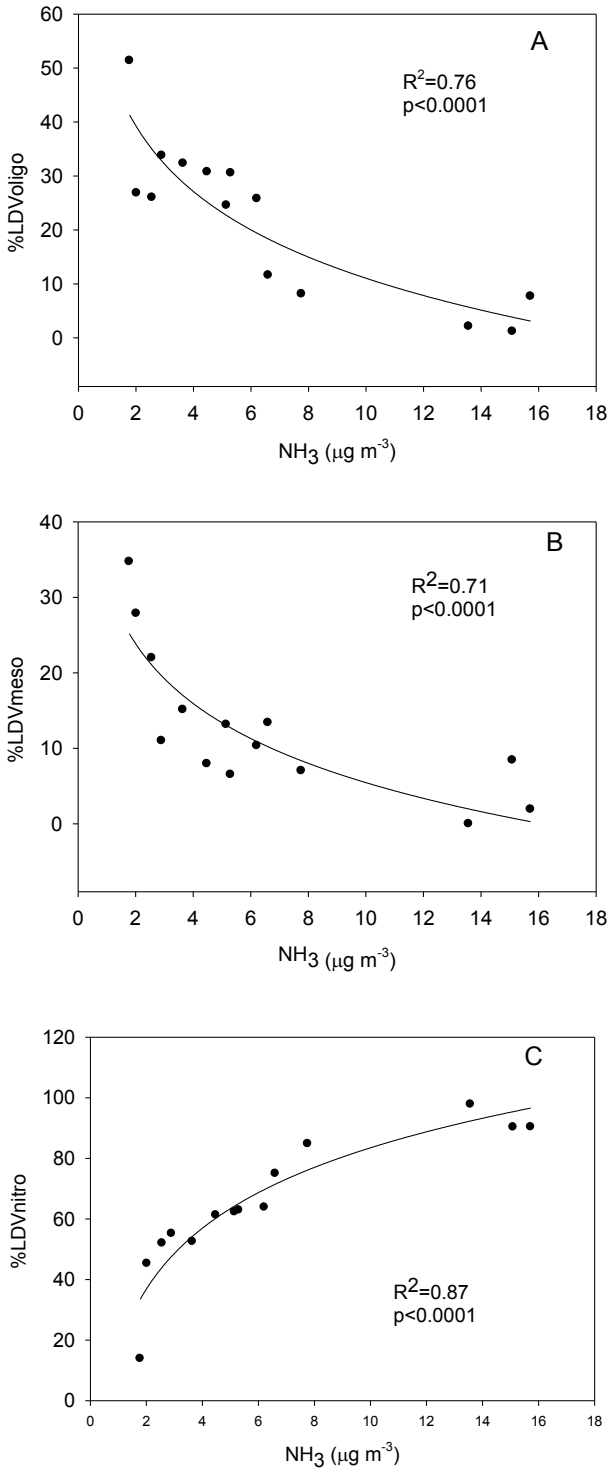

Figure 6. Relationship between NH_3 air concentration (µgm^{-3}) and relative lichen diversity values for oligotrophic (A), mesotrophic (B) and nitrophytic (C) functional groups (n=14).

Critical levels of atmospheric ammonia were calculated taking into consideration the second altered point starting on the lowest concentration of NH_3 (Cape et al., 2009; Pinho et al., 2012). This resulted in the CLE being determined at 3.1 and 2.6 μg m^{-3} respectively for the oligotrophic and nitrophytic functional groups (Fig. 7).

Discussion

For the first time the CLE value of atmospheric NH_3 for a semi-natural Mediterranean holm-oak forest in Spain was estimated, setting it at < 2.6 μg m^{-3}.

Total lichen diversity (LDVtotal and species richness) was not significantly related to atmospheric NH_3, as in previous results (Pinho et al., 2008; Van Dobben and Ter Braak, 1998). Nitrogen is a nutrient that at high concentrations can become toxic for some species, and different species respond differently according to their tolerance. Due to this, although total diversity measures have been satisfactorily used for monitoring atmospheric pollution of compounds that affect similarly all species (Asta et al., 2002; Giordani, 2007; Pinho et al., 2008; Svoboda, 2007), these measures cannot be used to assess the effects of atmospheric NH_3. On the other hand, functional groups of epiphytic lichens based on tolerance to eutrophication have been found to be good indicators of NH_3 deposition (Giordani, 2007; Giordani et al., 2014; Pinho et al., 2011; Pinho et al., 2014a; Pinho et al., 2014b). The proposed CLE was slightly higher than: i) the one accepted at the European level (1 μg m^{-3}); ii) the previous value reported for a Portuguese semi-natural area in an open cork oak woodland (1.9 μg m^{-3}; (Pinho et al., 2011), iii) recent values obtained for remote areas in Portugal (0.6 μg m^{-3}; (Pinho et al., 2014a). However, the value is similar to the 2.5 μg m^{-3} NOEC (No Observable Effect Concentration) suggested in a pig farm in Italy (Frati et al., 2007). It is also noteworthy that the lowest NH_3 concentrations found in this work were above the background levels registered in European remote areas (1 μg m^{-3}; (Cape et al., 2009), or in Portugal (1.4 μg m^{-3}; (Pinho et al., 2012)), an indication of a polluted environment. The relatively higher CLE value than the ones obtained in Portugal may be explained by the

historically high emissions in the region around the sampling plots (la Plana de Vic region), which has a tradition of intense pig and cow farming and extended agricultural activity (Otero et al., 2009). Also, the city of Vic (7 km; 41.627 inhabitants in 2014) may contribute with NO_y emissions from traffic and industrial activity adding to the N pollution load that may affect the surrounding forests and the study site in particular. In this sense, NO_2 emissions from an air quality station located 11 km to the N of the study site amounted to 22 $\mu g\ m^{-3}$ in 2013, one of the highest values recorded in the Catalan Air Quality Network (Xarxa de Prevenció I Vigilància de la Contaminació Atmosfèrica, Generalitat de Catalunya 2014). Therefore, some eutrophication might have occurred in the studied forest in the past that probably eliminated the most N sensitive species.

The proposed CLE based on the oligotrophic and nitrophytic functional groups are to some extent similar. However, because of the uncertainty derived from the probable loss of N-sensitive oligotrophic species in this forest, we considered the nitrophytic lichen functional group as more reliable for the calculation of CLEs.

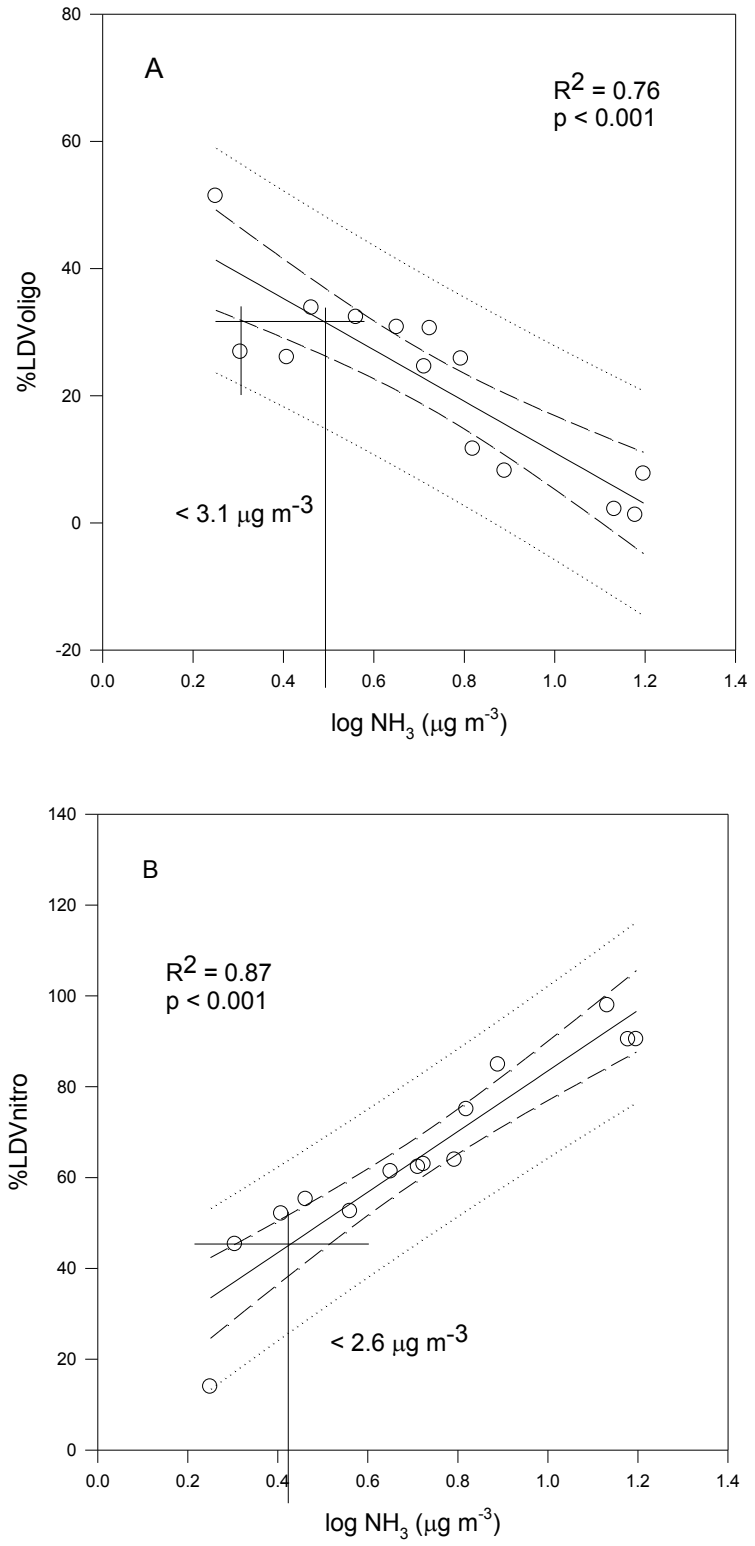

Fig. 7. Calculations of the critical levels of atmospheric NH_3 considering the oligotrophic (A) and nitrophytic (B) functional groups. The annual average NH_3 concentrations (μgm^{-3}) have been log transformed (n=14).

NH_3 air concentrations showed a typical exponential decrease with distance from the barn, reaching values close to background levels in less than 1 km from the emitting point source. This confirms the already reported rapid deposition of NH_3 in the vicinity of point sources and the low residence time of NH_3 in the air (Frati et al., 2007; Pitcairn et al., 1998; Sanz et al., 2007). Here, we did not find any clear seasonal trend for NH_3 atmospheric levels during an annual period. Some studies have reported a NH_3 increase during the warm season due to a temperature raise that favours NH_3 volatilization (Behera et al., 2013; Sommer et al., 1991), as well as during rainy periods, mainly due to higher microbiological activity in wet periods (Kumar et al., 2004), but that pattern was not observed in this study.

Conclusions

In summary, a CLE below 2.6 µg m^{-3} for atmospheric NH_3 was determined for a semi-natural Mediterranean evergreen forest in NE Spain based on lichen functional diversity. Such values are above the currently accepted European CLE of 1 µg m^{-3} (Hallsworth et al., 2010) and higher than previous CLEs reported for open woodlands in the western Iberian Peninsula obtained using the same methodology (Pinho et al., 2014a; Pinho et al., 2012). The values proposed here should protect a vegetation type that has already suffered from an historical N exposure from farming and agriculture. Another important achievement of this work is the demonstration that lichen functional groups can be used to derive CLEs at a wide range of Mediterranean forests, independently of the forest structure type.

Acknowledgements

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Chapter 5

Diagnostic ecological indicators to high atmospheric ammonia pollution in a Mediterranean holm oak (*Quercus ilex* L.) forest

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Abstract

Several changes in forest ecosystems have been driven by the increase of anthropogenic ammonia (NH_3) emissions to the atmosphere. Therefore, there is a need of identifying the sensitive indicators to NH_3 pollution, especially at Mediterranean forests ecosystems, where information is yet very scanty. Here we aim to understand the response of different compartments in a Mediterranean holm oak forests, as indicators of the impact of NH_3 pollution. For that, we have compared lichen functional groups diversity, %N, C:N ratios and $\delta^{15}N$ in *Quercus ilex* leaves, the moss *Hypnum cupressiforme*, and forest soils within a distance gradient from an NH_3 emission source. In order to understand ecophysiological responses of vegetation under increasing NH_3 pollution we studied the $\delta^{13}C$ content in the ecosystem compartments.

We found that the best parameters responding to NH_3 spatial variations along the gradient were the %N and $\delta^{15}N$ signature measured in the different compartments.These parameters were also strongly related to the response of the relative lichen diversity values indicative of the response of the ecosystem to the long term exposure to NH_3 pollution. The $\delta^{15}N$ signal variation under higher NH_3 levels reflected the N isotopic signal of the source, which in this case seems to be enriched in the heavier isotopic form. The $\delta^{15}N$ signal together with C:N ratios were the best indicators in soils, while %N in soils did not reflect the variations in the deposition gradient as the result of the biological and chemical transformations in this compartment. The soil C:N ratios alerted about the high risk of N saturation and potential eutrophication of the underground waters at the studied site. Finally, the variation in $\delta^{13}C$ content represented the physiological response of vegetation to enhanced N deposition.

Introduction

Ammonia gas (NH_3) emissions contribute to the general increase of reactive nitrogen (N_r) deposition which is considered as one of the most important components of global change that threatens biodiversity and the structure and functioning of ecosystems (Bobbink et al., 2010; Rockström et al., 2009; Vitousek et al., 1997). NH_3 is originated mainly from agriculture and farming activities such as synthetic fertilizer application or animal waste management, with minor contributions from transportation or industry (Reis et al., 2009). The NH_3 residence time in the lower atmosphere is only of a few hours or days (Stephen and Aneja, 2008) and therefore it is rapidly deposited in the vicinity of emission sources (Frati et al., 2007; Pitcairn et al., 1998; Sanz et al., 2007).

Air pollution abatement policies led by the UNECE in the frame of the Convention on Long-Range Transboundary Air Pollution (CLRTAP), have relied on the concepts of critical loads (CLOs) and critical levels (CLEs), defined as the deposition amount (CLO) or concentration threshold (CLE) below which no adverse effects on sensitive receptors are detected (Nilsson and Grennfelt, 1988). While CLEs are thresholds related to the direct above-ground effects of a pollutant exposure on forest condition, CLOs are threshold values related to the indirect soil-mediated effects of atmospheric deposition on forests, such as nutrient imbalances caused by soil acidification and N accumulation (De Vries et al., 2000). These thresholds values are continuously updated according to the progress of scientific knowledge and technical reports published by the Convention (UNECE, 2011). The new information is generally based on the response of a particular compartment of the ecosystem to the atmospheric load/concentration of the pollutant. Therefore, managers may highly appreciate information about the specific diagnostic indicators of NH_3 pollution.

Understanding ecosystem responses to NH_3 deposition is of major importance for the conservation of biodiversity in natural and semi natural ecosystems and to guarantee the sustainability of ecosystem services. There is a poor understanding of the impacts of atmospheric NH_3 pollution in Mediterranean ecosystems (Ochoa-Hueso et al., 2011). Typical compartments used in evaluating the impact of N on ecosystems are:

air, vegetation and soils. High atmospheric NH_3concentrations lead to the accumulation of N in plant tissues and soils, and it can cause direct toxicity to some sensitive biota (Krupa, 2003; Pearson and Stewart, 1993; Phoenix et al., 2012). To assess its impact, a wide range of indicators have been evaluated (Pinho et al., 2011; Pitcairn et al., 2006; Pitcairn et al., 1998; Sutton et al., 2004; Van Dobben and Ter Braak, 1998). The study of poikilohidric N sensitive species which only depend on the atmosphere for their nutrition, such as lichen and bryophyte communities, is one of the most extended procedures used. Lichens and mosses lack of a rooting system being strongly affected by atmospheric pollutants, a fact that confers them a good bio-indication capacity. Furthermore, in lichens, different species present different tolerance to eutrophication, which allows for a functional group classification based on their response to atmospheric N. Under increasing NH_3 air concentrations, oligotrophic species are replaced by nitrophytic ones, a fact that has been advantageously used to calculate NH_3 CLEs (Cape et al., 2009; Pinho et al., 2011). Mosses have been specially suggested as biomonitors for sites dominated by dry NH_3 deposition (Pitcairn et al., 2006). These organisms can accumulate N in their tissues and reflect deposition charges in the atmosphere, being considered as useful tools for modelling N deposition loads in the atmosphere (Harmens et al., 2011; Harmens et al., 2014; Poikolainen et al., 2009).

Besides the fact that plant nitrogen (N) levels reflect the status of N both in soils and atmosphere, many studies during the last decades have highlighted the environmental and ecological significance of N isotopes for the understanding of the fate of deposited N (Gerhart and McLauchlan, 2014; Kriszan et al., 2009; Liu et al., 2008a; Skinner et al., 2005; Skinner et al., 2006). The anthropogenic modifications of Nr in the atmosphere induce changes in N cycling that strongly affect the natural abundance of the two stable isotopes of N (^{14}N and ^{15}N) in plants and soils (Högberg, 1997). On one side, the information on the abundance of N isotopes in soils and plants helps to understand the N dynamics in ecosystems because different processes differently discriminate between the two main isotopic forms of N (Koba et al., 1998; Robinson, 2001). For example, processes associated with N loss (nitrification and denitrification)

discriminate against the heavier ^{15}N isotope, and therefore, soils are enriched in ^{15}N (Högberg, 1997). In general, any N transformation in the ecosystem leads to isotope fractionation and therefore, studying isotopic variations among ecosystems is very useful to understand N cycle dynamics under different scenarios. On the other hand, the isotope signature of the N source can be sometimes reflected in N the isotopic signature in vegetation (Stewart and Schmidt, 1999). In fact, the natural abundance of ^{15}N has also been extensively used to trace the influence of different source emissions on ecosystems (Felix et al., 2014; Hastings et al., 2013). For example, more negative $\delta^{15}N$ values in moss tissue have been associated to NH_y-N deposition sources and less negative $\delta^{15}N$ values, to NO_x-N deposition (Gerdol et al., 2002; Liu et al., 2008b; Pearson et al., 2000).In this sense, analyzing the N isotope content in bryophytes has proven to be particularly useful (Pearson et al., 2000).

Furthermore, variations in N availability affect the soil carbon (C) dynamics, since the former has an important fertilizer effect that increases forest soil C stocks, and because N availability also enhances organic matter decomposition rates (Garten et al., 2008). The consequent decrease of the soil C:N ratio under enhanced N deposition has been taken as an early indicator of ecosystem N saturation (Aber et al., 1989; Skeffington and Wilson, 1988). Particularly, it has been suggested that soil C:N ratios below 25 are indicative of risk of nitrate leaching (Dise et al., 2011; Gundersen et al., 1998; Van der Salm et al., 2007).

Ultrastructural changes and ecophysiological responses in vegetation caused by NH_3 pollution have been largely documented in the literature (Britto and Kronzucker, 2002; Krupa, 2003).Particularly, membrane damage and/or inhibition of photosynthesis have been observed in lichens, mosses and vegetation (Munzi et al., 2014; Nimptsch and Pflugmacher, 2007; Vieira et al., 2009). In this sense, recent studies in the Mediterranean basin have shown that NH_3 drives changes in ^{13}C foliar content since N deposition negatively affects the CO_2 fixation in *Quercus* species, by reducing the stomatal and mesophyll conductance, and therefore limiting the normal function of the photosynthetic processes (Pintó-Marijuan et al., 2013). Therefore, information about the natural abundance of ^{13}C in vegetation is a useful tool particularly indicative

of the net CO_2 assimilation and stomatal conductance and its study infoliar content provides information about the physiological stress in vegetation caused by excessive N deposition.

To evaluate the performance of the indicators we will correlate them against both atmospheric ammonia (indicating recent emissions) and lichen functional groups (reflecting the impact on the ecosystem and providing a longer term perspective). The study of lichen functional groups has proven to be strongly related to NH_3, allowing for the estimation of CLEs in the Mediterranean cork oak woodlands in Portugal (Pinho et al., 2009; 2011; 2012) and Spanish holm oak forest (Chapter 4). However, lichen bioindication studies require intensive expert work to identify species; thus, it might be more practical to use easier measurable parameters recording the response of the ecosystem.

There is a need to identify how different compartments of the forest ecosystem react to the modification of the complex N-cycle in order to establish suitable limits for N emissions. Up to now, few studies have compared the joint response of different compartments affected directly or indirectly by atmospheric NH_3 deposition in Mediterranean forests. We aim to provide for the first time in a Mediterranean evergreen forest a description of the responses of several compartments of the ecosystem to NH_3 pollution. To do this, we have monitored the following variables along a gradient of NH_3 pollution: 1) lichen functional group diversity, 2) nutrient content (%C, %N) and isotope $\delta^{15}N$ in the moss *Hypnum cupressiforme*, leaves of holm oak (*Quercus ilex*), and holm oak forest soils, and 3) the C:N ratios in this moss, holm oak leaves and soils. We also aimed to understand the effects of atmospheric NH_3 pollution in vegetation ecophysiology through the study of the $\delta^{13}C$ in moss, holm oak leaves and soil. These measurements were taken in a distance gradient from a NH_3 point emission (cattle farm) in a Mediterranean holm-oak forest at North-Eastern Spain.

Material and methods

Study site

The study site was located 65 km to the North-from Barcelona, within a dense holm-oak forest surrounding a cattle barn. Fourteen sampling plots were established at increasing distances from a barn of ~130 beef cattle which are permanently housed in an area of 1500m^2. Altitude of the plots varied between 618 and 690 m. Vegetation in the region is dominated by holm-oak (*Quercus ilex* L.) forests. Climate is humid continental Mediterranean. Total annual rainfall for the period January 2013 -January 2014 was 868 mm and the mean annual temperature for the same period was 11ºC at Sant Julià de Vilatorta, 3.2 km distant from the sampling area (Fig 1). Predominant winds blew from north-west during that period (http://www.meteovilatorta.cat). The plots stood downwind from this prevailing wind.

Atmospheric NH_3 monitoring

Air NH_3 concentrations were measured at the 14 sites downwind from the barn from January 2013 to January 2014 using high-sensitivity ALPHA passive diffusion samplers (with two replicates at 6 plots). Samplers were prepared at CREAF following the procedure described in the literature (Tang et al., 2001). The ALPHA samplers were collected and replaced by a new kit every 2-3 weeks; they were placed approximately 2m above the ground attached to a tree. ALPHA samplers were extracted in water and analyzed by colorimetry with a Flow Injection Analysis system for ammonium (FIA sampler; FOSS TECATOR).

Lichen survey, identification and classification

Lichen diversity surveys were performed in 59 holm-oak trees in the vicinity of the 14 air NH_3 sampling sites (the site closest to the farm was excluded since it was considered as a lichen desert). Lichen biodiversity was measured following a standard

protocol which has been designed to give a lichen diversity value LDV; (Asta et al., 2002). Lichen species unidentified in the field were collected for further identification at the laboratory. The LDV index takes into account species richness and frequency (Asta et al., 2002). Moreover, species were classified according to functional response groups relative to N deposition. This was performed according to the highest value relative to eutrophication tolerance, classifying species as nitrophytic, mesotrophic and oligotrophic (Nimis and Martellos, 2008). Total lichen diversity value (LDVtotal) corresponds to the sum of the frequencies of all the species in each tree (Asta et al., 2002) and species richness was calculated as the total number of species. LDV was also calculated for eutrophication tolerance functional groups by summing the frequencies of all species in each functional group (LDVoligo, LDVnitro, and LDVmeso). Functional groups data were relativized as percentage of the LDVtotal for each tree.

Sample collection and isotope analysis

During the summer of 2013, samples of the moss *Hypnum cupressiforme* and *Quercus ilex* leaves were collected at the 13 NH_3 sampling points (the site closer to the farm being excluded for consistency with lichen surveys). A composite sample of *Hypnum cupressiforme* was obtained by mixing three sub-samples collected at each same sampling point, and only the green and brown-green parts of the moss were taken for analysis. For *Quercus ilex* leaves, a composite sample was obtained by pooling 5 sub-samples from three different trees close to each sampling point. All samples were cleaned of contaminating particles (soil, dust) in the laboratory and were oven-dried at 60ºC. During the same period 5 cores (5cm diameter) were taken of the first 5-10 cm of the soil and were unified in a composite sample for each NH_3 sampling point. Samples were taken close to each sampled tree. Soil samples were air-dried at room temperature and sieved with a 2mm mesh.

Grinded moss, holm oak leaves and soils samples were separated into capsules for further analysis. Percent C and N content (%) were measured by using an elemental analyser (EURO VECTOR). The isotopic ratios N^{15}/N^{14} ($\delta^{15}N$) and C^{13}/C^{12} ($\delta^{13}C$) were

determined by isotope ratio mass spectrometry (isoprime isotope ratio mass spectrometer - IRMS, Micromass-GV Instruments, UK). Isotope ratios were calibrated against international standards, particularly IAEA CH6 (sucrose) and IAEA CH7 (polyethylene) for carbon isotope ratio and IAEA N1 (ammonium sulphate) for nitrogen isotope ratio. Both $\delta^{13}C$ and $\delta^{15}N$ were expressed as: $\delta X = ([R_{sample}/R_{standard}]-1)*1000[‰]$, where $X=^{13}C$ or ^{15}N, and R is the ratio of $^{13}C:^{12}C$ or $^{15}N:^{14}N$. The $\delta^{13}C$ results were standardized against Vienna Pee Dee Belemnite, as the international standard, and $\delta^{15}N$ reported relative to $\delta^{15}N$ of atmospheric air (Hellmann et al. 2011). The precision of the repeated measurement was 0.06 ‰ for $\delta^{13}C$ and 0.09 ‰ for $\delta^{15}N$.

Statistical analysis

For lichen surveys, four trees were sampled close to each Alpha sampler and the lichen variable values were averaged to provide a single value per sampling point. Monthly measures of atmospheric NH_3 concentrations were also averaged for calculating the annual concentrations at each sampling site. Then, lichen variables were related to atmospheric NH_3 concentrations (n=13).

Spearman correlations between NH_3 concentrations, LDVs for each functional group and the measured parameters were performed with Statistica (StatSoft 2004). Graphs were developed with Sigmaplot 11.0 (SystatSoftware Inc., San Jose, CA, USA).

Results

The annual average concentration of NH_3 at each site showed an exponential decrease with increasing distance from the barn (R^2=0.67; p<0.0001) (see Chapter3; Fig. 4).

The descriptive statistics for the measured parameters in *Quercus ilex, Hypnum cupressiforme* and soil samples are shown in Table 1 and the coefficients of determination for the relationships between them and the NH_3 air concentrations and LDVs are shown in Table 2.

The N content (%) in mosses ranged from 1.7 to 3.4%, while in *Quercus ilex* leaves the range was much narrower (1.4 to 1.9%,Table 1). N percent increased with increasing atmospheric NH_3 levels, both in leaf samples and mosses (Table 2, Fig. 1). On the contrary, N content in soils did not show a significant relationship with NH_3 concentrations (Table 2). C percent ranged between 35.6 to 39.3% in *Hypnum cupressiforme*, 44 to 47% in *Quercus ilex* and 4 to 31.7% in soils (Table 1). These values did not vary significantly in relation to NH_3 through the gradient. On the contrary, the C:N ratio in moss, leaves and soils significantly decreased with increasing NH_3 atmospheric levels near the farm (Table 2; Fig. 2), with values ranging from 11.7, 23.4 and 11.3 near the farm to 22.9, 29.4 and 16.7 at the farthest site respectively (Table 1).

Table 1. Descriptive statistics of the main parameters measured at *Quercus ilex, Hypnum cupressiforme* and soils.

	Foliar			
	d15N	N (%)	C (%)	C:N
Mean	-6	1.6	45.3	28.5
Min	-8.1	1.4	43.9	23.4
Max	-2.8	1.9	46.9	33.5
SD	1.5	0.1	1.1	2.9
	Moss			
	d15N	N (%)	C (%)	C:N
Mean	-6.3	2.6	37.8	15.1
Min	-8.9	1.7	35.6	10.9
Max	-3.1	3.4	39.3	22.9
SD	1.7	0.5	1.1	3.6
	Soil			
	d15N	N (%)	C (%)	C:N
Mean	-0.7	0.6	10.5	15.8
Min	-3.3	0.3	4	11.3
Max	3	1.4	31.7	22.8
SD	1.7	0.3	6.6	2.9

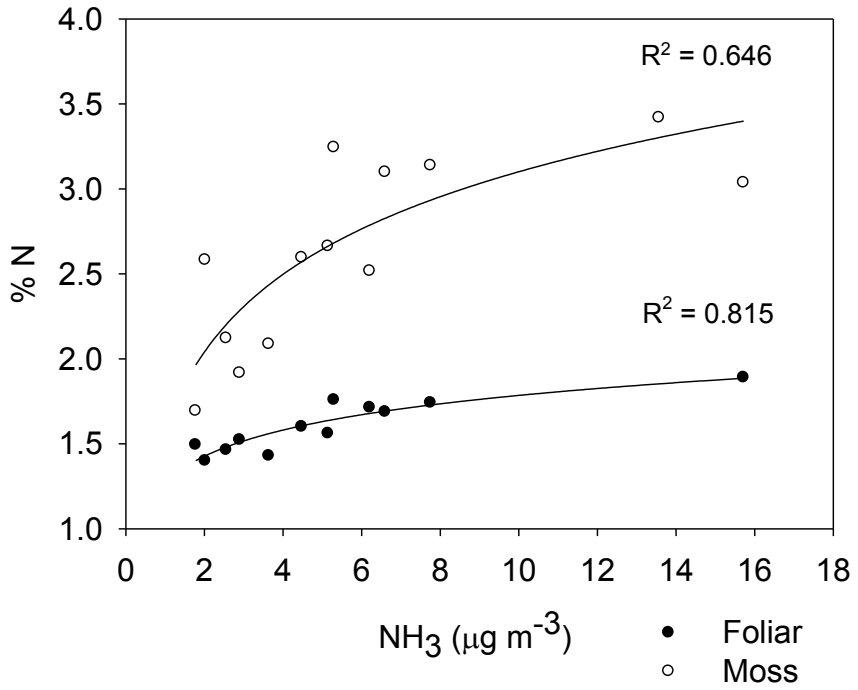

Figure 1. Relationship between N content (%) and atmospheric NH_3 concentrations (µg m^{-3}).

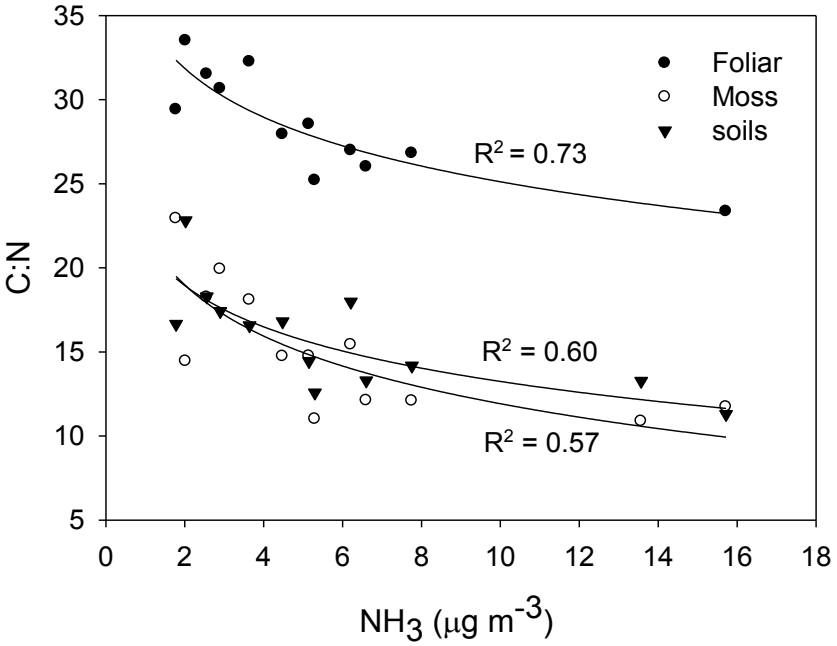

Figure 2. Relationship between C:N ratio and atmospheric NH_3 concentrations (µg m^{-3}).

Soil $\delta^{15}N$ content ranged from positive values near the farm, (+3‰), the points with highest NH_3 atmospheric concentrations, to negative values farther away (-3‰) at the more distant point corresponding to low NH_3 concentrations (Table 1). Foliar and moss $\delta^{15}N$ values were very similar and never attained positive values, ranging from -8.1‰ to -2.8 ‰ and -8.9‰ to -3.1‰ respectively (Table 1) with the most positive values near the farm under higher NH_3 air concentrations. There was a significant positive relationship between $\delta^{15}N$ of soil, leaf and moss and air NH_3 concentrations (Table 2; Fig. 3). Because N percent increased with ambient NH_3 for moss, leaves and soils, there was a positive relationship between N% and $\delta^{15}N$ in leaves and mosses (R^2= 0.85 and R^2= 0.44 respectively, p<0.01) but not in soils (R^2=0.09; p=0.30). Finally, $\delta^{15}N$was significantly and negatively related with the C:N ratio in soils (R^2=0.57, p<0.01).

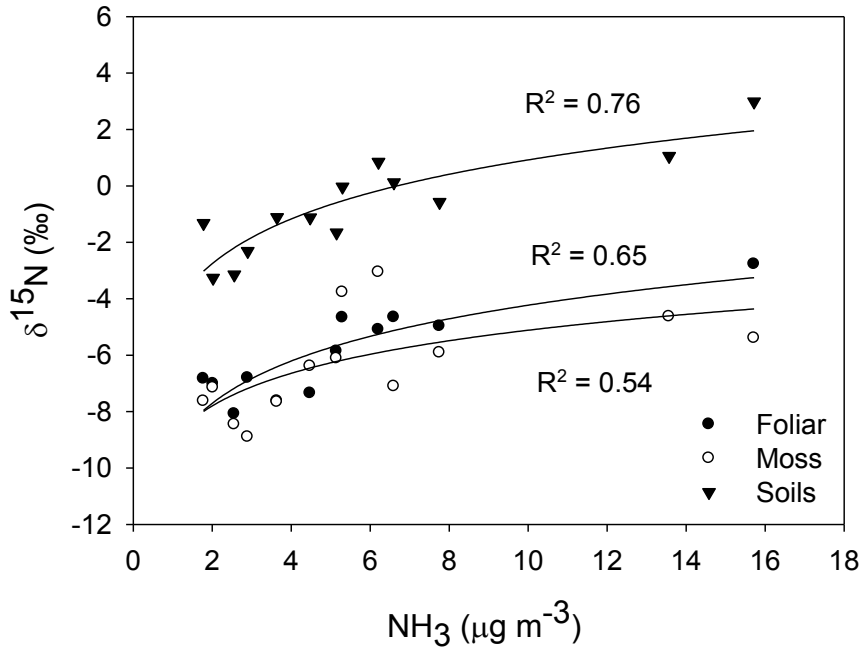

Figure 3. Relationship between $\delta^{15}N$ and atmospheric NH_3 concentrations (μg m^{-3}).

Table 2. Coefficients of determination (R^2) for relationships (Spearman correlations) between NH_3 and relative lichen diversity values for each functional group against the different parameters measured at *Quercus ilex, Hypnum cupressiforme* and soils. (N=12 in *Quercus ilex* while N=13 in soils and *Hypnum cupressiforme*).The asterisks point significance levels (*p<0.05, **p<0.01, ***p< 0.001).

	foliar			moss			soil		
	NH3	%LDV oligo	%LDV nitro	NH3	%LDV oligo	%LDV nitro	NH3	%LDV oligo	%LDV nitro
δ15N	(+) 0.65**	(-) 0.38*	(+) 0.69***	(+) 0.54**	(-) 0.35*	(+) 0.53**	(+) 0.76***	(-) 0.35*	(+) 0.72***
δ13C	(+) 0.68***	(-) 0.28	(+) 0.66**	(+) 0.96***	(-) 0.74***	(+) 0.95***	(+) 0.12	(-) 0.24	(+) 0.11
%N	(+) 0.76***	(-) 0.34*	(+) 0.80***	(+) 0.64**	(-) 0.58**	(+) 0.65***	(+) 0.02	(-) 0.14	(+) 0.01
%C	(-) 0.05	(+) 0.00	(-) 0.04	(-) 0.24	(+) 0.13	(-) 0.21	(-) 0.00	(-) 0.04	(-) 0.00
C:N	(-) 0.73***	(+) 0.34*	(-) 0.75***	(-) 0.60**	(+) 0.52**	(-) 0.60**	(-) 0.57**	(+) 0.25	(-) 0.52**

Similarly, $\delta^{13}C$ values increased from -31.0‰ to -25.7‰ in *Quercus ilex* leaves, -31.5‰ to -27.8‰ in the moss *Hypnum cupressiforme*, and -26.9‰ to -24‰ in soils with decreasing distance from the barn (thus with increasing NH_3 air concentrations). The strongest relation between $\delta^{13}C$ and NH_3 air concentrations was found in mosses followed by leaves (Fig. 4), while no significant relation was found with soils (Table 2).

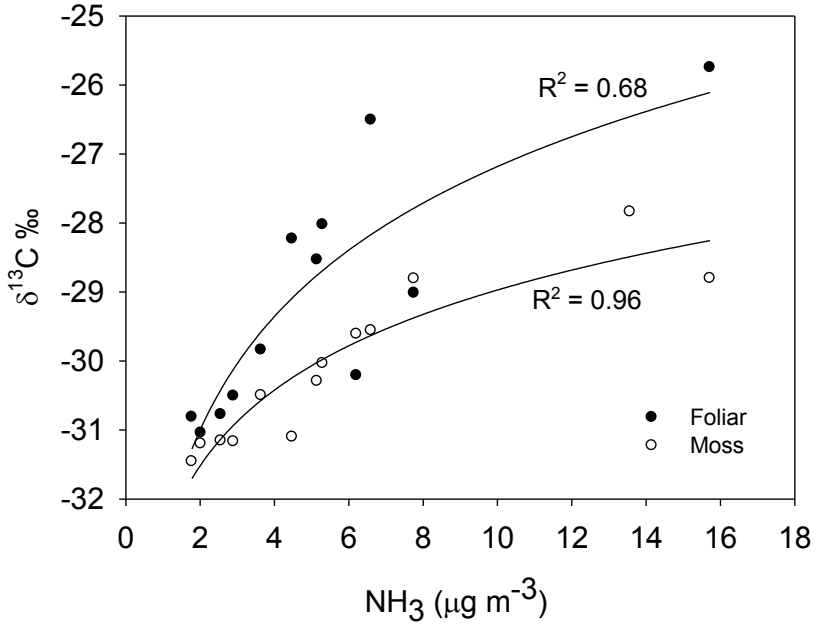

Figure 4. Relationship between $\delta^{13}C$and atmospheric NH_3 concentrations (μg m^{-3}).

Species recorded in this study are shown in chapter 4, table 1; 13 were nitrophytic and 22 oligotrophic while the remaining 18 species were mesotrophic. The most frequent species (>50% occurrence) were *Candelaria concolor, Flavoparmelia caperata, Hyperphyscia adglutinata, Lecanora chlarotera, Pertusaria amara and Phlyctis argena*. The LDVnitro varied from 3 to 75 and LDVoligo ranged from 1 to 21. Relative values of lichen functional groups were significantly related to average NH_3 concentrations, but with an opposite variation: %LDVoligo was negatively and %LDVnitro positively related to NH_3 concentrations (see chapter 4). Strong relations were found between the relative values of LDVs with the nutrient and isotopic contents in the measured receptors (Table 2). Lichen functional groups were significantly related to %N but with different sign: %LDVoligo was negatively related to the N content in mosses and foliar

samples while %LDVnitro was positively related (Table 2; Fig 5), but %LDVoligo and %LDVnitro were no significantly correlated with soil %N. The %LDVs of both oligo and nitrophytic functional groups were significantly related to $\delta^{15}N$ content in all compartments measured (Table 2). When comparing %LDVoligo *vs.* $\delta^{13}C$ content, the relation was negative and significant in moss samples, while for %LDVnitro a significant relation was also found but in this case positive (Table 2).

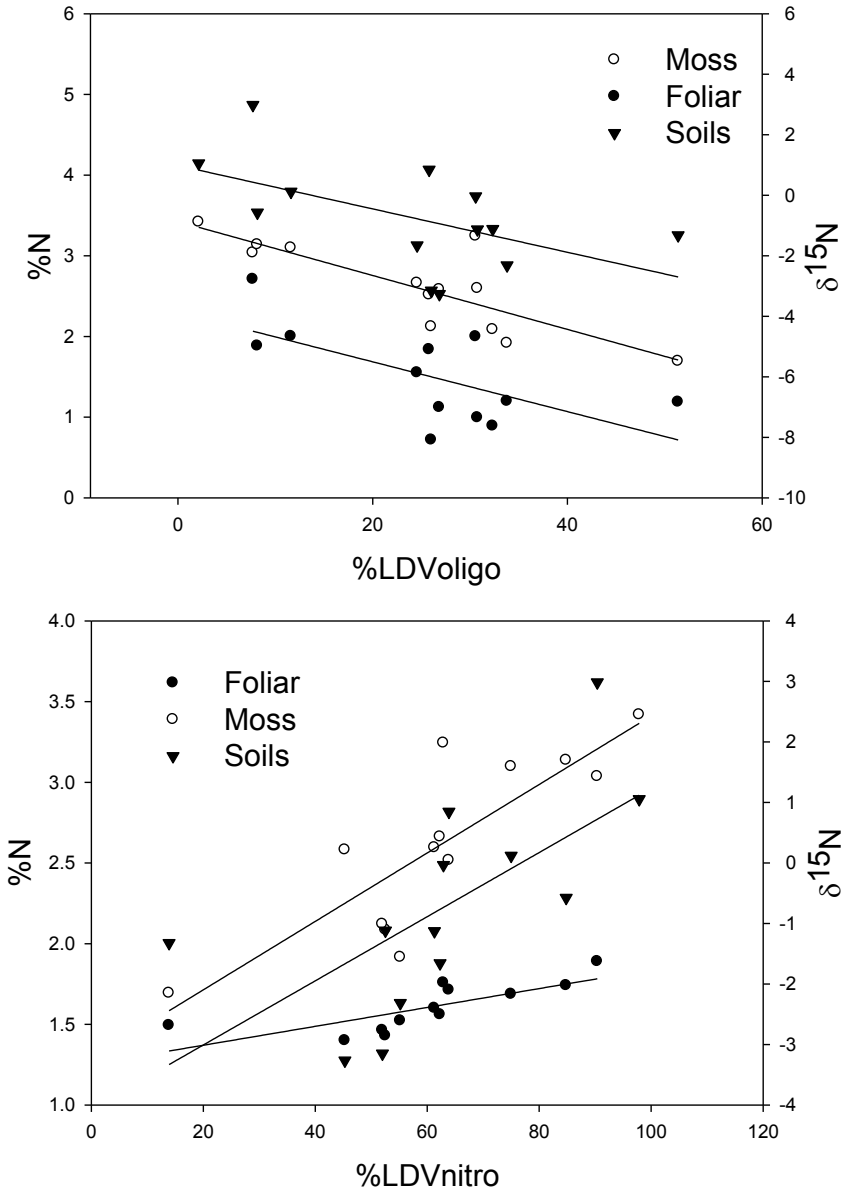

Figure 5. Relationship between the N content (%) in leaves and moss samples (axis y on the left), and the $\delta^{15}N$ in soils (axis y on the right), with the relative LDVs for each functional group.

Discussion

Indicators of NH_3 pollution

Monitoring the N tissue content in mosses has been extensively used to indicate N ambient pollution at a range of spatial scales (Cape et al., 2009; Pesch et al., 2008; Pitcairn et al., 2006). Here we found that the N content in the moss *Hypnum cupressiforme* was related to NH_3 emissions from a farm, with similar %N variation to that reported for intensive livestock farm sites in the UK (1.3 to 5 %N in mosses) (Pitcairn et al., 2003; Pitcairn et al., 2006).

In contrast, the range of variation for holm oak leaves was much smaller, a fact that highlights the effectiveness of mosses for monitoring air concentrations. The %N content measured in leaf samples in this study is in accordance with previous data for *Quercus ilex* in the Mediterranean Basin (Maisto et al., 2013). Also C:N ratios in leaves from the less polluted plots far from the farm were in accordance with the ratios reported in other Mediterranean evergreen forests (Cotrufo et al., 1999; Sardans et al., 2011). Forests in this region act as sinks of N atmospheric deposition (Àvila and Rodà., 2002) and N availability is reflected in the tree leaf content, as shown by a study relating leaf %N and the proximity to urban emissions (Barcelona city)(Sardans et al., 2011). In our study, an increase in N leaf content along the NH_3 gradient is observed.

Mean values and the variation range of $\delta^{15}N$ signature in moss and foliar samples was very similar (Table 2). This finding suggests that both epiphytic mosses and trees are reflecting the N atmospheric signature, dominated by N emissions from the farm. In fact, in mosses, isotopic fractionation during N absorption may be negligible (Bragazza et al., 2005). Nevertheless, leaves may take up NH_3/NH_4^+ directly by stomata, with the consequent discrimination at that level. Our results suggest that no discrimination occurred at the leaf level, since leaf $\delta^{15}N$ values were similar to those of mosses. Understanding the $\delta^{15}N$ results in the measured compartments is difficult since no information on the isotopic form of N is yet available at this site. In general, it is assumed that more negative $\delta^{15}N$ values in vegetation are related to N-NH_x sources

while more positive values are related to N-NO_x pollutant sources (Ariz et al., 2011; Bermejo-Orduna et al., 2014; Gerdol et al., 2002; Liu et al., 2008b; Pearson et al., 2000; Solga et al., 2005). Even though the studied site was dominated by deposition of N-reduced forms, $\delta^{15}N$ values were in the range of results obtained from studies carried out under reduced or oxidized N-deposition (Bermejo-Orduna et al., 2014; Huang et al., 2014).

The significant variation of $\delta^{15}N$ along the NH_3 gradient clearly suggests that the main N-deposited form is responsible of the isotopic signature measured at each compartment. We found that the $\delta^{15}N$ content in all compartments increased (were less negative) when approaching the farm (Table 1; Fig.3). This result is consistent with other references for *Hypnum cupressiforme* (Skinner et al., 2006) and for lichen species under increasing N-reduced deposition (Crittenden et al., 2015). Also, this is consistent with studies that report higher $\delta^{15}N$ foliar values under higher N availability (Ariz et al., 2011; Garten Jr and Miegroet, 1994; Skinner et al., 2005; 2006). It has been reported that the use of animal fertilizers such as manure tends to increase the heavier isotope levels in fertilized vegetation, being assumed that the increase in $\delta^{15}N$ content in manure is paralleled with an increase in the $\delta^{15}N$ value of the fertilized plant (Szpak, 2014). Since the farm site studied here is surrounded by field crops intensively fertilized with manure, we suggest that emissions of NH_3 and enriched in $\delta^{15}N$ from manure also contribute in an important way to the deposition throughout the gradient. Therefore, we suggest that $\delta^{15}N$ is strongly linked to the N deposited form.

Soil $\delta^{15}N$ content is generally higher relative to that of plants (Amundson et al., 2003; Högberg, 1997; Pardo et al., 2006), as here was the case (Tabl1; Fig. 3). This is because during the processes of N transformations in the soil, there is a discrimination against the heavier isotope, mostly during nitrification and denitrification, which favour the loss of N as chemical forms are leached or volatilized from soils. This generates a major loss of the lighter isotope and the consequent increase of the soil $\delta^{15}N$ signal (Högberg, 1997). Along the NH_3 gradient, the $\delta^{15}N$ content increased in soils while no increase was found in the %N content. We suggest that, similarly as in leaves and mosses, the

isotopic signal of the source can be identified in soils; thus, the isotopic signature of the emitted NH_3 can be found in all the studied compartments of the ecosystem.

In contrast with the accumulation of N in *Quercus ilex* along the increasing NH_3 gradient, soils did not accumulate N in the same way. We suggest that the absence of a significant variation of the soil % N along the gradient might be the consequence of the higher loss of N in the vicinity of the farm. In fact, C:N ratios in soils clearly decreased (to <25) at increasing NH_3 values near the farm, which may be indicative of risk of nitrate leaching (Gundersen et al., 1998; MacDonald et al., 2002; Van der Salm et al., 2007). The observation of a decrease in the C:N ratio in soils has been taken as an early indicator of ecosystem N saturation (Aber et al., 1998; Skeffington and Wilson, 1988). Besides, in this study as in previous studies (e.g. Craine et al., 2015), the ratio C:N was also negatively correlated with $\delta^{15}N$, which is consistent with the previous argument of $\delta^{15}N$ enrichment as N content in the soil increases close to the farm.

Measuring NH_3 during one year is generally considered long enough to characterize atmospheric deposition. However, changing meteorological conditions or emissions may not be well characterized in a monthly sampling schedule. In this case, despite the high effort necessary for the lichen sampling in bioindication studies, lichens are very valuable since they give an integrated response of the long term exposure to NH_3 pollution, thus being better indicators of the long time exposure than punctual measurements of NH_3. However, in this study we found that the analyzed chemical variables were also appropriate, since quite similar relationships between the annual NH_3 atmospheric concentrations and both the relative LDVs and the measured parameters in the different ecosystem compartments were observed. Particularly, we found that N and $\delta^{15}N$ in moss and leaves were similarly related to the relative LDVs of the nitrophytic functional group and to the NH_3 measurements (Table 2). The nitrophytic species are specifically sensitive in their response to NH_3, while oligotrophic species are sensitive to a wide range of pollutants and may be also affected by other sources of pollutants or other environmental factors (Pinho et al., 2008).

Physiological response in vegetation

$\delta^{13}C$ values in *Quercus ilex* and *Hypnum cupressiforme* were strongly correlated with atmospheric NH_3 levels in the gradient distance to the farm, indicating that samples were enriched in $\delta^{13}C$ under higher air NH_3 concentrations. Foliar $\delta^{13}C$ has been generally used as an index of water availability for plants and can indicate physiological stress (Werner and Máguas, 2010). Under drought conditions, plants become enriched in the heaviest isotope during CO_2 assimilation through stomata because of reduced discrimination against the heavier isotope. However, it has been shown that the general increase of N favours the photosynthetic activity by enhancing carboxylation rate in plants, also contributing to the increase of $\delta^{13}C$ (Matsushima et al., 2012). Other recent studies have shown that the natural content of $\delta^{13}C$ in *Quercus* species can be modified by the alteration in the normal function of the photosynthetic processes caused by the reduction of the stomatal and mesophyll conductance caused directly by NH_3 rather than by drought stress (Pintó-Marijuan et al., 2013). Our results point to a direct effect of ambient NH_3 on $\delta^{13}C$, since climate was not expected to vary in a 620 m distance with altitude varying only from 618 to 690 m. In any case, bryophytes do not have an epidermis with impermeable cuticle and stomata, thus no changes in the stomata/mesophyll conductance should be expected. Because of these structural differences, a different discrimination against $\delta^{13}C$ should be expected in mosses compared to *Quercus ilex* leaves, which is not the case in this study in which a similar behavior related to a N pollution source is found between *Quercus ilex* and *Hypnum cupressiforme*. We suggest that since the photosynthetic function in mosses is similar to C3 plants (Teeri, 1981), the same model proposed for C isotope discrimination for C3 plants (Farquhar et al., 1989) should be found in *Hypnum cupressiforme*. The increase in N availability is known to favor the carboxylation rate directly by the activation of enzymes and indirectly by the increased demand for C by the growing tissues, which in the end lead to more positive $\delta^{13}C$ (Matsushima et al., 2012).

The relative LDVs for both functional groups (nitro and oligo) was strongly related to $\delta^{13}C$ content measured in *Hypnum cupressiforme* samples and also with *Quercus ilex* trees for the nitrophytic functional group (Table 2). This was particularly strong when considering the nitrophytic species and the moss. Thus, our results reveal how the shift in lichen communities is directly related to nutritional status and physiological stress in vegetation.

Conclusions

In this work we have compared the response of different compartments of the ecosystem to atmospheric NH_3 pollution in a distance gradient to a farm. We found that %N content and $\delta^{15}N$ signature were the best parameters responding to NH_3 spatial variations along the gradient in the studied ecosystem compartments. Besides, these parameters were also strongly related to the response of the relative lichen diversity values for the nitrophytic functional group, which showed a more specific response to NH_3 pollution than the oligotrophic group. The % N in mosses proved to be a particularly useful monitor strongly related to the spatial variations of NH_3 atmospheric concentrations throughout the gradient, and to the lichen diversity values which indicate the response of the ecosystem to the long term exposure to NH_3 pollution. $\delta^{15}N$ average values in *Hypnum cupressiforme* tissues and *Quercus ilex* leaves were similar, and values showed a similar range of variation, suggesting that the $\delta^{15}N$ signal reflects the isotopic signature of the main form of N emitted from the farm. In the soil compartment the best indicators were $\delta^{15}N$ together with C:N ratios, while soil %N did not change in the studied gradient. Low C:N ratios measured in soils alerted about the high deposition of N at sites close to the emission source and their risk of N saturation and potential eutrophication of groundwater.

In this study, the variation in $\delta^{13}C$ suggested a physiological response of vegetation to enhanced N deposition. We suggest the response in moss is related to discrimination

at the carboxylation phase, while in leaves, alterations in the stomatal and mesophyll conductance also contribute.

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Summary discussion and Conclusions

Summary discussion

The alteration of the N biogeochemical cycle constitutes a global threat to terrestrial ecosystems worldwide (Gruber and Galloway 2008). The present PhD provides new information on N deposition to holm oak (*Quercus ilex*) forests in Spain. This is particularly important due to the scarcity of studies in the Mediterranean Basin, compared to temperate forests in Europe.

In **chapter 2**, deposition is presented for three forest sites (CB close to Barcelona, CA near Pamplona and TC close to Madrid) selected for their proximity to traffic/urban pollution sources. A fourth site was considered as representative of less polluted environments (LC in the Montseny mountains, 45 km NNE from Barcelona). However, the results indicated similar anthropogenic pollutant (N and S) deposition loads in bulk deposition at LC and at two of the more urban-related sites (CB and CA), while the Madrid site received very low N and S loads. These results indicate that the LC site cannot be considered pollution-free and indicates the widespread transport of pollutants from metropolitan areas, from maritime traffic and from long-range transport to the sampling areas.

However, local emissions also greatly influenced bulk deposition. For example, the CA site received very high loads of base cations (mainly Ca^{2+}) and alkalinity due to dust emissions from a nearby open cast quarry. This site also received high NH_4^+ deposition related to agricultural and livestock raising activities around the sampling plot.

Under a Mediterranean climate, dry deposition of gases and particles is the dominant form of N deposition (Ochoa-Hueso et al. 2011). In this study, we have attempted to estimate dry deposition fluxes through the analysis of throughfall, one of the most used methods of deriving dry deposition in European forests. Throughfall chemistry provided information on how rainfall was modified during its passage through the canopies. These changes were reflected in net throughfall fluxes (net throughfall = throughfall – bulk deposition). A simple regression model of net throughfall on rainfall amount indicated that alkalinity and K in net throughfall were explained by leaching of these components from the canopy (a well known result in throughfall studies) and

that SO_4^{2-}-S and NO_3^--N (except at one site) derived from dry deposition. On the other hand, NH_4^+-N and H^+ were absorbed in tree canopies. Due to the fact that N compounds are taken in canopies, net throughfall cannot be used as indicator of N dry deposition. To solve this issue, in this PhD the Canopy Budget Model (CBM) used in ICP-Forest (ICP-Forest Manual, Annex 8) was applied. This model requires a series of assumptions that were only accomplished at LC and CB; therefore, CMB-based calculations of dry deposition and total deposition could be obtained only for these two sites.

The CBM estimations indicated a dry deposition flux of 6 and 7 kg ha^{-1} yr^{-1} for NH_4^+-N and NO_3^--N at LC and of 9 and 8 kg ha^{-1} yr^{-1} for NH_4^+-N and NO_3^--N at CB. Summing these compounds resulted in a inorganic N (DIN) dry deposition load of 13 and 16 kg ha^{-1} yr^{-1} at LC and CB, respectively. Summing up the inputs in wet deposition resulted in a total DIN deposition of 17 and 20 kg ha^{-1} yr^{-1}at LC and CB, respectively. Dry deposition provided most of the total deposition load (74 and 84 % at LC and CB). These values are in accordance with previous studies in Mediterranean forests in the Iberian Peninsula (Rodà et al., 2002).

On the other hand, the CBM also allowed to derive the quantities of N that are taken up at the canopy level, which were important for NH_4^+-N (7 and 8 kg ha^{-1} yr^{-1} at LC and CB) but were also non-negligible for NO_3^--N (around 4 kg ha^{-1} yr^{-1} at both sites).

The atmospheric deposition of N and S has been satisfactorily reduced in most European countries as the result of the abatement programs of the Convention on Long Range Transboundary Atmospheric Pollution (CLRTAP) from UN/ECE to reduce SO_2, NO_2 and NH_3 emissions. In **chapter 3** we explored how these emission reductions in Spain and neighbouring countries have affected the deposition patterns in the Montseny mountains. We found that the strong reductions of SO_2 emissions in Spain (77%) were reflected in reductions of non-sea salt-SO_4^{2-} in precipitation (65% for concentrations and 62% for SO_4^2-S deposition) and dry deposition (29%) at the Montseny Mountains from 1995-96 to 2011-13. The lower decline observed in dry deposition (29%) compared to declines in emissions was consistent with the fact that SO_2 deposition velocity increases as ambient SO_2 is reduced (Fowler et al., 2001).

Regarding N compounds, NO_2 emissions in Spain increased from 1980 to 1991, remained constant until 2005, and decreased thereafter, a pattern that was paralleled by NO_3^- in bulk precipitation at Montseny. Since 2005, electricity generation in Spain has changed in favour of renewable energies in front of coal and gas combustion. Since electricity generation has a very high contribution to NO_x emissions in Spain, this shift may explain the observed NO_x decrease. On the other hand, the estimated dry deposition obtained with the CBM at the Montseny site increased markedly in 1995-96 compared to 2011-2012 (from 1.3 to 6.7 kg $ha^{-1}y^{-1}$). While this can be related to the particular climatic conditions of each period (much drier in the second period), there are uncertainties in the CBM regarding the assumption of NO_3^- uptake relative to NH_3 uptake and more work is needed to better define this exchange, particularly for Mediterranean forests.

Ammonia emissions in Spain have increased in the last 30 years (13% from 1980 to 2012). However, this was not reflected in increasing NH_4^+ deposition trends at Montseny. Because NH_3/NH_4^+ readily reacts to form ammonium sulphate aerosols, with declining SO_2 and NO_2 emissions in recent years a decreasing formation of ammonium sulphate and nitrate aerosols would be expected, and then, lower NH_4^+ would be available to be scavenged by precipitation, resulting in lower NH_4^+ deposition.

As mentioned total DIN input at LC and CB was 17-20 kg N $ha^{-1}y^{-1}$. Recent studies in these sites indicate that dissolved organic nitrogen (DON) adds around 3 kg N $ha^{-1}y^{-1}$ (Izquieta et al., 2015 submitted). Therefore, the total N input to these holm oak forests would be between 20-23 kg $ha^{-1}y^{-1}$. The Critical Load value currently used for Mediterranean sclerophyllous forests is 15-17.5 kg $ha^{-1}y^{-1}$ (Bobbink et al. 2010). Our results indicate that the holm oak forests here studied are receiving a N deposition that is exceeding the proposed critical load.

In fact, NH_3 emissions in Spain are exceeding the limits marked by the Gothenburg protocol for 2010 for this country. Therefore, there is a need to establish the safety thresholds to specifically prevent NH_3 effects on species and ecosystems. In **chapter 4** we provide a Critical Level (CLE) for NH_3 at a Mediterranean holm oak forest. This is

particularly relevant since no such a study has been undertaken for holm oak forests in Spain; only results from savannah-like "dehesa"-type forests are until now available in the Iberian Peninsula from studies in Portugal (Pinho et al 2011). To fill this gap, we measured the NH_3 concentrations during one year along a distance gradient from a cattle farm and recorded the epiphytic lichen abundances on holm-oak trees along this gradient. From the relationship between NH_3 and lichen diversity indexes calculated for functional groups (oligotrophic and nitrophytic) an empirical critical level of atmospheric $NH_3 < 2.5$ µg m^{-3} was obtained. This critical level is above the currently accepted European value (1 µg m^{-3} ; Hallsworth et al., 2010) and previous values reported for "dehesas" in the western Iberian Peninsula obtained using the same methodology (Pinho et al., 2009; 2011). One of the main conclusions from this work was that lichen functional groups (nitrophytic and oligotrophic) can be used to derive critical levels in very different Mediterranean forest types.

Since lichen surveys require a high sampling effort, we aimed to test whether other forest ecosystem variables could be also indicative of the effects of pollution. In **chapter 5** we found that some parameters (%N, $\delta^{15}N$, C:N, $\delta^{13}C$) measured in different compartments (tree leaves, moss tissue and soil) of the ecosystem also responded to NH_3 pollution. The %N and $\delta^{15}N$ signature measured in the *Quercus ilex* leaves and *Hypnum cupressiforme* tissues along the gradient were strongly related both to air NH_3 and to the relative lichen diversity values (expressed as % of total diversity). The leaves and moss $\delta^{15}N$ signal variation along the gradient probably reflected the N isotopic signal of the NH_3 source, characterized by a enrichment in the heavier isotopic form, but more work is needed to identify the source signal. The %N and $\delta^{15}N$ signal values in moss and leaves were significantly correlated with the relative nitrophytic functional group diversity and air NH_3, which highlights the specific response of nitrophytic species to NH_3 pollution.

The complexity of the biological and chemical transformations in the soil prevented the use of %N as representing the N transformations along the gradient. However, the soil $\delta^{15}N$ signal and C:N ratios were significantly related to NH_3. In fact, the soil C:N

values were below those indicating the onset of N leaching (C:N<25), thus the forest around the farm would be prone to N saturation and there would be a risk of groundwater eutrophication. The variation in $\delta^{13}C$ content in foliar and moss samples represented the physiological response of vegetation to enhanced N deposition, rather than the usual response to hydrologic stress.

Conclusions

The main conclusions from this thesis are:

- Dry deposition of N was obtained through a canopy budget model which indicated a dry deposition flux for NH4-N and NO3-N of 6 and 7 kg ha^{-1} yr^{-1} at LC and of 9 and 8 kg ha^{-1} yr^{-1} at CB. Therefore the inorganic N dry deposition was 13 and 16 kg ha^{-1} yr^{-1} at LC and CB, respectively.
- Addition of the inputs in wet deposition resulted in a total DIN deposition of 17 and 20 kg ha^{-1} yr^{-1} at LC and CB, respectively. Dry deposition provided most of the total deposition load (74 and 84 % at LC and CB).
- Bulk deposition of non-sea salt-SO_4^{2-} and NO_3^- changes paralleled those of emissions. For SO4, dry deposition (estimated by net throughfall fluxes) also declined, but at a lower rate
- On the other hand, NO_3^- dry deposition increased at Montseny, a fact that can be attribute to drier meteorology in the more recent studied period. However, some uncertainties remain regarding the CBM assumptions for the estimation of NO_3^-.
- Even though NH_3 emission increased in Spain from 1980 to 2014, NH_4^+ concentrations in precipitation and NH_4^+-N deposition at the Montseny site were reduced (15% reduction) which was attributed to lower formation of ammounium aerosols linked to the reduction of SO_2 and NO_x emissions.
- Total DIN input at the LC and CB sites was 17-20 kg N $ha^{-1}y^{-1}$. Recent studies in these sites indicate that dissolved organic nitrogen (DON) adds around 3 kg N $ha^{-1}y^{-1}$ (Izquieta et al., 2015 submitted). Therefore, the total N input to these

holm oak forests can be framed in 20-23 kg $ha^{-1}y^{-1}$, thus exceeding the CLO values proposed for scleropylous forests (15-17 kg N $ha^{-1}y^{-1}$).

- The onset of N saturation at this Mediterranean holm oak forests is suggested at the Montseny forest by higher DIN export related to DIN inputs in recent years.
- A CLE of 2.6 µg m^{-3} for atmospheric NH_3 was determined for a holm oak forest NE Spain based on lichen functional diversity. The proposed value should protect a vegetation type that has already suffered from an historical N exposure from farming and agriculture.
- The lichen functional groups can be used to derive CLEs at a range of Mediterranean forests, independently of the forest structure type.
- The %N content and $\delta^{15}N$ signature in foliar samples and mosses were the best parameters responding to atmospheric NH_3 spatial variations
- The %N content and $\delta^{15}N$ signature in foliar samples and mosses were also strongly related to the response of the lichen diversity values (expressed in %) for the nitrophytic functional group, indicating the usefulness of these parameters as indicators of NH_3 pollution.
- While the $\delta^{15}N$ signal reflected the isotopic signature of the main N form emitted from the source, the %N in mosses mostly tracked the spatial variations of NH_3 atmospheric concentrations throughout the gradient.
- In the soil compartment the best indicators were $\delta^{15}N$ and C:N ratios, while soil %N did not change in the studied gradient. The low C:N ratios measured in soils alerted about the high deposition of N at sites near the emission source and their risk of N saturation and groundwater eutrophication.
- The variation in $\delta^{13}C$ content in *Quercus ilex* and *Hypnum cupressiforme* suggested a physiological response of vegetation to enhanced N deposition. The response in moss would be related to discrimination at the carboxylation phase, while in leaves discrimination related to stomatal and mesophyll conductance would also contribute.

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Photo Appendix

Figure 1. La Castanya sampling site (Montseny Mountains).

Figure 2. Can Balasc sampling site (Collserola Natural Park).

Figure 3. Carrascal sampling site (Navarra).

Figure 4. Tres Cantos sampling site (Madrid).

Figure 5. Dry/Wet only collector.

Figure 6. Bulk/throughfall collector.

Figure 7. Alpha sampler

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