Atmospheric concentration and deposition of reactive nitrogen in Spanish forests of *Quercus ilex*

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Atmospheric concentration and deposition of reactive nitrogen in Spanish forests of *Quercus ilex*

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EL SECRETARIO

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Stern, C. (1965)

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RESUMEN

Resumen

El depósito atmosférico de nitrógeno (N) es considerado la tercera causa más importante de pérdida de biodiversidad, tras los cambios de uso del suelo y el cambio climático. La cuenca mediterránea presenta una extraordinaria riqueza biológica y se reconoce como uno de los 25 puntos de conservación prioritaria de la diversidad biológica mundial. Sin embargo, hay poca información disponible sobre la amenaza que la contaminación atmosférica puede representar para la biodiversidad en esta región. Esta Tesis se ha desarrollado en el marco del proyecto "Efectos del depósito de N en encinares mediterráneos" (EDEN), que surgió tras la publicación de varias evidencias de enriquecimiento en N en algunos ecosistemas españoles, con el objetivo de generar nuevos conocimientos sobre el depósito de N y sus efectos sobre los ecosistemas mediterráneos.

El primer enfoque consistió en realizar una evaluación del riesgo de los efectos nocivos que podría causar el depósito de N atmosférico en las áreas protegidas de la Red Natura 2000, usando para ello datos de modelos de calidad atmosférica de uso extendido. En general, los modelos EMEP y CHIMERE estimaron valores de depósito húmedo de N inorgánico dentro de unos rangos aceptables en comparación con las mediciones disponibles, aunque los resultados deben aplicarse con precaución, sobre todo en escalas pequeñas y en las zonas de orografía compleja. El depósito húmedo de N en España mostró una distribución decreciente a lo largo de un eje NE–SO, mostrando un mayor depósito en las regiones costeras del norte y del este (hasta 16.1 kg de N ha^{-1} $año^{-1}$) que en el interior y las zonas del sur. La distribución del depósito total de N inorgánico (incluyendo depósito húmedo y seco) siguió un patrón similar, mostrando una estimación máxima de 23.0 kg de N ha^{-1} $año^{-1}$.

La evaluación de riesgos se realizó mediante la comparación del depósito total de N inorgánico estimado por modelos EMEP y CHIMERE para 2008 con las cargas críticas (CC) empíricas definidas en el Convenio de Ginebra sobre la Contaminación Atmosférica Transfronteriza a Gran Distancia. Los pastizales naturales (en su mayoría ubicados zonas de alta montaña del norte) fueron los hábitats más sensibles, mostrando un 30–60% de su área evaluada en riesgo. La principal incertidumbre de la amenaza potencial del depósito de N para estos hábitats es que no existen estaciones de monitorización con las que se pueda comprobar el rendimiento de los modelos en la

estimación del depósito de N en estas zonas elevadas. Por otra parte, del 41% al 71% de la superficie dentro de la región biogeográfica alpina en España, junto con algunos brezales, matorrales y bosques en otras zonas montañosas, podrían estar experimentando superaciones de las CC. Por lo tanto, se debería implementar un mayor despliegue de las redes de monitorización de depósito atmosférico en áreas de montaña españolas para controlar la contaminación atmosférica y evaluar el riesgo de los efectos sobre estos ecosistemas tan valiosos. Los resultados de esta evaluación coinciden con anteriores informes de enriquecimiento de N en algunas zonas de España, lo que sugiere que el método aplicado es adecuado para la evaluación del riesgo de aparición de efectos por depósito de N en los hábitats españoles. Sin embargo, se necesita urgentemente más investigación para confirmar la idoneidad de las CC empíricas utilizadas y la evaluación podría mejorarse mediante el uso de valores de depósito seco estimados para cada tipo particular de hábitat.

Los bosques esclerófilos mediterráneos mostraron una gran área amenazada (cerca de 500 km^2). Estos bosques representan un ecosistema y un paisaje característicos de la cuenca mediterránea e incluyen los bosques de *Quercus ilex* L. (encinares), una especie de árbol presente en un amplio rango de ambientes de la cuenca mediterránea. El encinar es el ecosistema objetivo de monitorización intensiva y de estudio del proyecto EDEN. El objetivo principal de este proyecto fue caracterizar las diferentes entradas de N en los bosques de *Quercus ilex* de la Península Ibérica, así como el estudio de los efectos de este depósito en el ciclo biogeoquímico del N en este tipo de bosques. Con este fin, se seleccionaron cuatro encinares situados en tres regiones con diferentes condiciones ambientales en España. Tres de estos sitios estaban cerca de fuentes de contaminantes de origen urbano y por tráfico rodado (considerados aquí como bosques periurbanos) y se situó un cuarto punto en una zona más remota, como referencia no urbana. En la presente Tesis, además del objetivo inicial de evaluación del riesgo a escala nacional anteriormente expuesto, se abordaron otros objetivos principales del proyecto EDEN, tales como la caracterización de las concentraciones atmosféricas y el depósito húmedo y seco de N inorgánico en los encinares, a la vez que se evaluaron los riesgos ambientales derivados de esta contaminación y se buscaron evidencias de una mejora de la calidad del aire atribuible a los servicios de los ecosistemas forestales.

Las concentraciones atmosféricas de dióxido de nitrógeno (NO_2), amoníaco (NH_3), ácido nítrico (HNO_3) y ozono (O_3) se midieron en áreas abiertas y dentro de los bosques

durante dos años; mientras que los aerosoles (PM_{10}) fueron monitorizados sólo en las zonas abiertas durante un año. Los bosques periurbanos estuvieron expuestos a contaminantes atmosféricos procedentes de actividades tanto urbanas como rurales, soportando elevadas concentraciones de contaminantes fotoquímicos secundarios. Los resultados indicaron que el O_3 es el único contaminante atmosférico estudiado del que se pueden esperar efectos fitotóxicos directos en los cuatro bosques. Sin embargo, los compuestos gaseosos nitrogenados podrían estar contribuyendo a través del depósito atmosférico de N a la eutrofización de estos bosques, y su interacción con otros factores de estrés podría estar afectando a su funcionamiento ecosistémico. Es por esto que la monitorización de los compuestos nitrogenados atmosféricos tales como NH_3 y HNO_3 debería incorporarse en las redes de seguimiento de calidad del aire.

Las concentraciones de gases contaminantes bajo el dosel arbóreo fueron, en general, menores que las encontradas en campo abierto. Las reducciones estadísticamente significativas de la concentración de NO_2 en el interior de los encinares fueron comparables, e incluso superiores, a valores presentados en estudios empíricos similares con especies forestales caducifolias. La "reducción bajo dosel" de la concentración de NH_3 fue muy elevada, lo que sugiere que los encinares actúan como sumideros de amoníaco. Esta reducción fue mayor en el bosque más natural (56%) que en los peri-urbanos (29–38%). Aunque se esperaba una alta tasa de depósito de HNO_3, la "reducción bajo dosel" de HNO_3 fue inferior a la de NO_2 y NH_3. Los resultados del estudio proporcionan evidencia científica de la capacidad de estos bosques para mejorar la calidad del aire en las aglomeraciones urbanas, pero todavía se necesitan más investigaciones para cuantificar la relevancia de este servicio ecosistémico y entender los procesos ambientales involucrados. Una buena planificación de programas de vigilancia atmosférica de los bosques urbanos y periurbanos podría lograr este objetivo, a la vez que podría evaluar la amenaza que la contaminación atmosférica puede suponer para la vegetación.

La necesidad de ampliar las redes de monitorización de depósito húmedo requiere e impulsa el uso de métodos cada vez más baratos, más fáciles de operar, y que no requieran frecuentes visitas al campo, como por ejemplo los métodos de colección basados en resina de intercambio iónico (IEC). En la presente Tesis, se muestreó el depósito global y de trascolación de N inorgánico en los tres encinares peri-urbanos del proyecto EDEN durante dos años a través de IEC y un método convencional utilizando

botellas colectoras (CBC). Los resultados indicaron que el método de muestreo de depósito de N basado en IEC puede ser recomendados para estudios a largo plazo en la región mediterránea, ya que, en comparación con el método basado en CBC, mostró un buen rendimiento en la medida del depósito de nitrato (NO_3^-) y un rendimiento aceptable en la medida del depósito de amonio (NH_4^+) y de N inorgánico disuelto (DIN). Las recomendaciones metodológicas para el uso de IEC que surgieron en este estudio deberían tenerse en cuenta en futuros diseños de monitorización en ambientes mediterráneos.

La media anual del depósito global de N varió desde 2.42 hasta 6.83 y de 3.09 a 5.43 kg N ha^{-1} $año^{-1}$ entre los sitios de muestreo de acuerdo a las metodologías CBC e IEC, respectivamente. El depósito de trascolación de DIN varió desde 2.33 hasta 8.20 y de 4.59 a 8.91 kg N ha^{-1} $año^{-1}$. Los datos de trascolación neta indicaron que existe un flujo de N oxidado al suelo forestal procedente del depósito seco, que fue muy elevado en el bosque de mayor influencia urbana. En los bosques de mayor influencia urbana se observaron valores positivos relativamente altos de trascolación neta, que implican entradas espontáneas y relativamente grandes de N en el suelo forestal, con las primeras lluvias tras períodos secos. Estas entradas, junto con el aumento de concentraciones de NO_3^- en el agua del suelo, concuerdan con la "hipótesis de la asincronía" en al menos uno de los sitios de muestreo.

En las zonas mediterráneas, el depósito seco de N puede jugar un papel importante en la entrada total de N en los ecosistemas naturales, particularmente en los forestales. Un enfoque innovador, llamado método inferencial empírico, se utilizó en la presente Tesis para estimar el depósito seco superficial de contaminantes nitrogenados. Además, el modelo DO_3SE (Depósito de Ozono e Intercambio Estomático), empleado en el marco del convenio de Ginebra, se utilizó en la estimación de su depósito estomático. Las tasas de depósito superficial calculados por medio de lavados de ramas fueron muy robustas para las formas reducidas de N, pero muy variables (temporal y regionalmente) para los las formas oxidadas. La estimación del depósito superficial de gases y partículas de N promedió 10.17 ± 3.29 kg N ha^{-1} $año^{-1}$ para los cuatro sitios, mientras que el depósito estomático de gases nitrogenados promedió 3.31 ± 0.83 kg N ha^{-1} $año^{-1}$. El depósito superficial de HNO_3 y partículas de NO_3^- dominó el depósito seco total de N inorgánico y la contribución relativa del depósito de NO_2 al depósito seco total fue el 14.6% en los

bosques periurbanos y el 7.9% en el sitio más natural. El depósito seco de N reducido fue importante sólo en el punto de muestreo más agrario, con 8.16 kg N ha^{-1} $año^{-1}$.

El depósito húmedo anual de N inorgánico, con una media de 3.83 ± 0.71 kg N ha^{-1} $año^{-1}$ para los cuatro sitios, fue ligeramente dominado por el N reducido. El depósito total estimado, con el depósito seco representando el 76.2% ± 2.1%, varió entre los sitios de muestreo en coincidencia con los patrones geográficos previamente encontrados en las estimaciones de los modelos, mostrando un mayor depósito en el norte (30.36 kg N ha^{-1} $año^{-1}$ en el punto de muestreo más septentrional) y en la costa este (17.42 y 12.17 kg N ha^{-1} $año^{-1}$ en los sitios situados en el NE de España) que en el interior (9.29 kg N ha^{-1} $año^{-1}$ en el sitio punto situado en el centro de la península). Para el período de muestreo, la CC empírica propuesta en el marco del Convenio de Ginebra (10–20 kg N ha^{-1} $año^{-1}$) fue superada en todos los puntos de muestreo menos uno, mientras que en todos ellos se superó la CC propuesta para la protección de los líquenes epífitos sensibles en ecosistemas naturales similares de California (5.5 kg N ha^{-1} $año^{-1}$).

ABSTRACT

Abstract

Nitrogen deposition is considered the third most important driver affecting biodiversity after changes in land use and climate change. The Mediterranean Basin presents an extraordinary biological richness and it is recognized as one of the 25 Global Biodiversity Hotspots for conservation priorities. However, little information is available on the threat that air pollution can pose to biodiversity in this region. The present Thesis has been developed in the framework of the project "Effects of N deposition in Mediterranean evergreen forests" (EDEN), that emerged when evidence of N enrichment in Spanish ecosystems was published with the aim of generate new knowledge in N deposition and its effects over Mediterranean ecosystems.

The first approach was to perform a risk assessment of deleterious effects caused by atmospheric N deposition in those areas included in the Natura 2000 networks, using data from commonly-used chemical transport models. The performance of EMEP and CHIMERE models estimating wet deposition of atmospheric inorganic N was in general within acceptable ranges in comparison with the available measurements, although results should be applied with caution, especially at small regional scale and in areas of complex topography. Wet deposition of N in Spain showed a decreasing distribution along a NE–SW axis, with higher deposition in the northern and eastern coastal regions (up to 16.1 kg N ha^{-1} $year^{-1}$) than inland and southern areas. The distribution of total N (including wet and dry deposition) followed a similar pattern and reached a maximum estimate of 23.0 kg N ha^{-1} $year^{-1}$.

The risk assessment was performed by testing the total N deposition estimated with EMEP and CHIMERE models for 2008 against the empirical critical loads (CL) defined under the Convention on Long-range Transboundary Air Pollution (CLRTAP). Natural grasslands (mostly located in northern alpine areas) were the most sensitive habitats, showing a 30–60% of its assessed area at risk. The major uncertainty of the potential threat of N deposition to these habitats is that no monitoring sites are available to test model performance estimating N deposition at this elevation. Moreover, 41–71% of the area within the Spanish Alpine Bio-geographical Region, together with some heathlands, scrublands and forests in other mountainous areas, could be experiencing CL exceedances. Therefore, further deployment of atmospheric deposition monitoring networks should be implemented in Spanish mountain areas to monitor atmospheric

pollution and assess the risk of effects on these particularly rich and valuable ecosystems. The results of this assessment agreed with previous reports of N enrichment in some Spanish areas, which suggest that the methodology applied is suitable for risk assessment of N deposition effects in Spanish habitats. Nevertheless, further investigation is urgently needed to confirm the suitability of the empirical critical loads used and the assessment might be improved by using dry deposition values estimated for each particular habitat type.

Mediterranean sclerophyllous forests showed a large threatened area (ca. 500 km^2). These forests represent a distinctive ecosystem and landscape of the Mediterranean Basin, and include the forests of holm oak (*Quercus ilex* L.), a tree species present over a wide range of environments in the Mediterranean Basin. Holm oak forest was the ecosystem targeted for intensive monitoring and study in the framework of the EDEN project. The main goal of this project was to characterize the different N inputs to *Quercus ilex* forests in the Iberian Peninsula and studying the effects of this deposition in the N biogeochemical cycle through this forest type. To this end, four holm-oak forests placed in three regions with different environmental conditions were selected in Spain. Three of these sites were close to urban and traffic pollutant sources (considered herein as peri-urban forests) and a fourth site was located in a more remote area, as a non-urban reference. The present Thesis deals with the above-exposed initial objective of risk assessment at a country scale, and with other major objectives of the EDEN project, such as the characterization of the atmospheric concentrations and wet and dry deposition of inorganic N in holm oak forests, while assessing environmental risks derived from this pollution and seeking evidence of air quality improvement attributable to forest ecosystem services.

Atmospheric concentrations of nitrogen dioxide (NO_2), ammonia (NH_3), nitric acid (HNO_3) and ozone (O_3) were measured during two years in open areas and inside the forests; and aerosols (PM_{10}) were monitored in open areas during one year. The peri-urban forests were exposed to air pollutants coming from both urban and rural activities, and proved to withstand elevated concentrations of the secondary photochemical pollutants. The results indicated that O_3 is the only air pollutant considered in this work which is expected to have direct phytotoxic effects on vegetation in all the sites. Nevertheless, the gaseous N compounds could be contributing through atmospheric N deposition to the eutrophization of these ecosystems and their interaction with other

stress factors could be affecting the ecosystem functioning. Therefore, monitoring of nitrogen compounds such as NH_3 and HNO_3 should be incorporated into air quality monitoring networks.

Below-canopy concentrations of gaseous pollutants were, in general, smaller than levels found in the open field. Statistically significant reductions of NO_2 concentrations inside holm oak forests were comparable to, and even higher than, values reported in similar empirical studies with deciduous forest species. Below-canopy reduction of NH_3 concentration was very high, suggesting that holm oak forests act as sinks of ammonia. This reduction was higher in the most natural forest (56%) than in the peri-urban ones (29–38%). Although a high deposition rate of HNO_3 was expected, the rates of bellow-canopy HNO_3 reduction were lower than those of NO_2 and NH_3. The study results provide scientific evidence of the ability of these forests to improve air quality in urban agglomerations, but further research is still needed to quantify the relevance of this ecosystem service and understand the environmental processes involved. Well-designed monitoring programs of urban and peri-urban forests could accomplish this objective while assessing the threat that air pollution can pose to vegetation.

The need to expand monitoring networks of atmospheric wet deposition has impelled the use of not-expensive methods, easy to operate, and without requiring frequent visits to the field, like ion-exchange resin collectors (IEC). In the present Thesis, bulk and throughfall deposition of inorganic N were monitored in the three peri-urban holm oak forests of the EDEN project during two years by means of IECs and a conventional method using bottle collectors (CBC). Results indicated that collection methods for N deposition based on IECs can be recommended for long-term studies in the Mediterranean region, since its performance measuring bulk deposition of nitrate (NO_3^-) was good and it was acceptable for ammonium (NH_4^+) and dissolved inorganic N (DIN), in comparison with CBCs. The arising methodological recommendations on the IEC method should be taken into consideration in future monitoring designs in Mediterranean environments.

Mean annual bulk deposition of N ranged 2.42–6.83 and 3.09–5.43 kg N ha^{-1} $year^{-1}$ among the sites according to CBC and IEC methodologies, respectively. The respective throughfall deposition of DIN ranged 2.33–8.20 and 4.59–8.91 kg N ha^{-1} $year^{-1}$. Net throughfall data indicated a net flux of oxidized N to the forest soil coming from dry deposition, which was very high at the most urban-influenced site. Relatively high

positive values of net throughfall, implying ephemeral and relatively large pulses of N into the soil after the first rainfall events, were observed in the most urban-influenced sites. These inputs, together with increased concentrations of NO_3^- in soil-water, were in agreement with the "asynchrony hypothesis" in at least one of the forest sites.

In Mediterranean areas, dry deposition of N compounds may play a major role in the total N input to natural vegetation, particularly to forest ecosystems. An innovative approach, called the empirical inferential method, was used in the present Thesis to estimate surface dry deposition of N pollutants. Besides, the DO_3SE (Deposition of Ozone and Stomatal Exchange) model, employed in the framework of the CLRTAP, was used in the estimation of their stomatal deposition. Surface deposition rates calculated by means of branch-washing experiments were very robust for the reduced forms of N, but very variable (temporarily and across regions) for the oxidized ones. The estimation of surface deposition of gaseous and particulate atmospheric N averaged 10.17 ± 3.29 kg N ha^{-1} $year^{-1}$ for the four sites, while stomatal deposition of N gases averaged 3.31 ± 0.83 kg N ha^{-1} $year^{-1}$. Total dry deposition of atmospheric inorganic N was dominated by the surface deposition of HNO_3 and particulate NO_3^- in all the sites and the relative contribution of NO_2 deposition averaged 14.6% in the peri-urban forests and 7.9% in the most natural site. The dry deposition of reduced N was the most relevant at the most agrarian site, with 8.16 kg N ha^{-1} $year^{-1}$.

The annual wet deposition of inorganic N, with a mean of 3.83 ± 0.71 kg N ha^{-1} $year^{-1}$ for the four sites, was slightly dominated by de reduced form. The estimated total deposition, with dry deposition representing 76.2% ± 2.1%, varied among the sites matching the geographical patterns previously found in the model estimates, showing higher deposition in the northern (30.36 kg N ha^{-1} $year^{-1}$ in the northern study site) and eastern-coast regions (17.42 and 12.17 kg N ha^{-1} $year^{-1}$ in the NE sites) than inland (9.29 kg N ha^{-1} $year^{-1}$ in the central-Spain site). For the monitoring period, only in one site the empirical CL proposed in the framework of the CLRTAP (10–20 kg N ha^{-1} $year^{-1}$) was not overreached, but all sites exceeded the CL proposed for the protection of sensitive epiphytic lichens in similar natural ecosystems in California (5.5 kg N ha^{-1} $year^{-1}$).

ÍNDICE / INDEX

CHAPTER 1

General introduction and objectives

1. General introduction and objectives

1.1. Imbalance of the global nitrogen cycle

Nitrogen (N) is a key element for life, since is a basic component of the molecular structure of proteins and nucleic acids. The greatest pool of this element on Earth (after igneous rocks) is the atmosphere, where molecular nitrogen (N_2) constitutes 78% of the volume (Rothschild and Lister, 2003). It is a nearly inert chemical compound, due to its strong triple bond, which makes nitrogen a very unavailable nutrient element for the living organism. There are other nitrogen forms (inorganic and organic) much more reactive and bio-available, commonly referred as "reactive nitrogen" (N_r). In terrestrial ecosystems the dominant natural source of N_r is biological fixation, a process in which certain microorganisms reduce the atmospheric nitrogen to ammonia (NH_3) in the presence of nitrogenase. Reactive nitrogen is usually scarce in natural and semi-natural ecosystems and its low availability limits the productivity of vegetation (Sutton et al., 2011).

During the last centuries, human demands for food and energy production have changed this limitation of N_r in the biosphere. In the beginnings of 21st Century, human processes such as the manufacture of fertilizer for food production, the cultivation of leguminous crops and the combustion of fossil fuels, fixed around 187 Tg N year^{-1} of N_2 into N_r, which is more than all the combined N fixation from all Earth's terrestrial processes (Rockström et al., 2009), and future scenarios predict increases to ca. 270 Tg N year^{-1} by 2050 (Galloway et al., 2008). Nitrogen fertilizers, produced thanks to the Haber-Bosch process of N fixation, have a tremendously beneficial impact on society, to the extent that the food production for feeding at least half of humanity currently depends on them (Erisman et al., 2008). However, all these processes have altered the N cycle globally, causing many effects on the environment and human health, as a result of N_r pollution. It is widely recognized that the loss of anthropogenic N_r to the environment is causing a cascade of unintended consequences (Fig. 1.1; Sutton et al., 2011) as it circulates across different Earth's compartments (atmosphere, hydrosphere and biosphere), and that it is affecting the N cycle in ecosystems worldwide (Erisman et al., 2013; Fowler et al., 2013). Human activity has altered so deeply the global biogeochemical N cycle that the planetary boundary for human safety has already been overstepped (Fig. 1.2; Rockström et al., 2009).

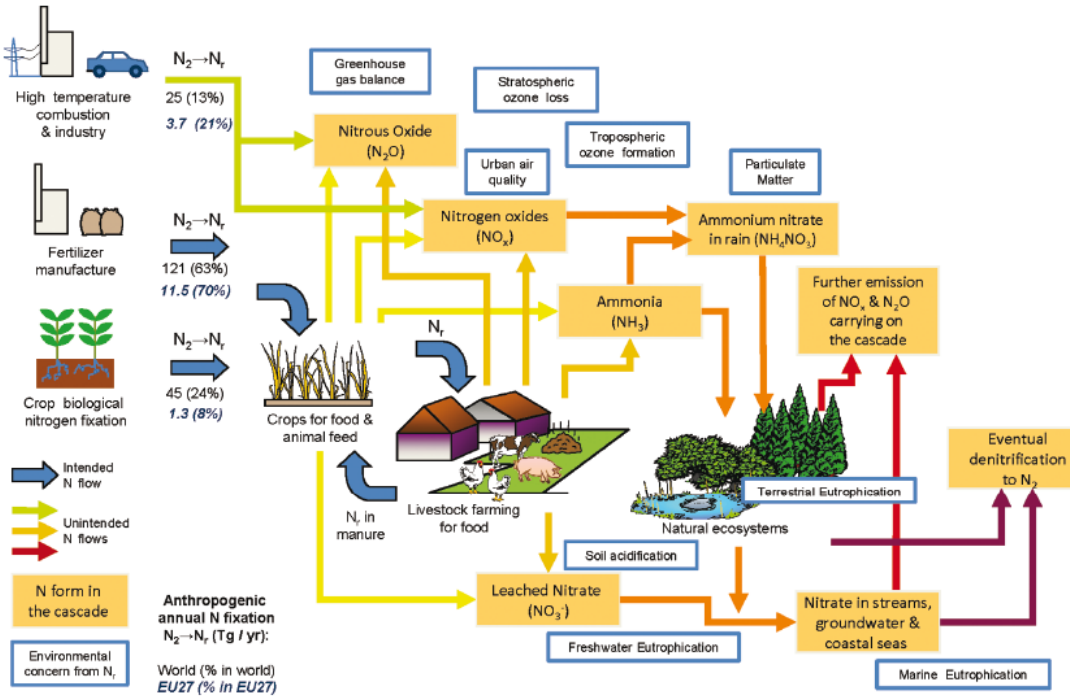


Figure 1.1. Simplified view of the nitrogen cascade, highlighting the major anthropogenic sources of N_r from atmospheric N_2, the main pollutant forms of Nr (orange boxes) and nine main environmental concerns (boxes outlined with blue). Blue arrows represent intended anthropogenic Nr flux; all the other arrows are unintended flux (losses to the environment). Source: Sutton et al., 2011.

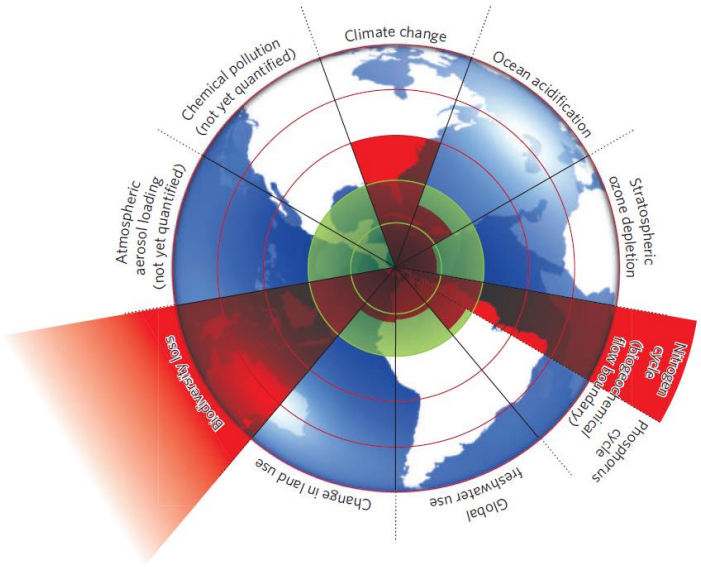


Figure 1.2. The inner green shading represents the proposed safe operating space for nine planetary systems. The red wedges represent an estimate of the current position for each variable. The boundaries in three systems (rate of biodiversity loss, climate change and human interference with the nitrogen cycle) have already been exceeded. Source: Rockström et al., 2009.

The losses of anthropogenic N_r to the atmosphere are dominated by nitrogen oxides (nitric oxide –NO –and nitrogen dioxide –NO_2) and ammonia (NH_3), originated mainly by fossil fuel combustion and intensive agricultural activities, respectively (Galloway and Cowling, 2002; Galloway et al., 2004, 2008). Once emitted to the atmosphere, N_r compounds take part in a series of chemical and physical complex processes until they are eventually deposited to terrestrial and aquatic ecosystems through dry and wet processes (Fig. 1.3; Hertel et al., 2011). Atmospheric deposition of N_r occurs either close to the sources or in remote areas (due to its chemical transformation and transport) located far from human activities, where it is often the dominant source of reactive N in N-limited systems (Bleeker et al., 2011). Some of the potential environmental effects of N deposition are the direct damage to vegetation, ecosystem acidification and eutrophication (Bobbink et al., 2010; Galloway et al., 2008). Particularly, eutrophication of terrestrial ecosystems is a widespread problem that affects most European ecosystems (EEA, 2013) caused by an excess of one or several usually-limited nutrients. Although both nitrogen and phosphorus can be growth-limiting nutrients, the primary productivity of terrestrial ecosystems more often is limited by N than by P, while the opposite occurs in the aquatic ones (Smith et al., 1999). The eutrophication process leads to the alteration of nutrient ratios, changes in soil and vegetation functioning, increasing plant susceptibility to other stressors, which induces changes of community composition, loss of biodiversity and invasions of new species (Dise et al., 2011).

Nitrogen deposition to N-limited forest ecosystems can trigger a particular concatenation of effects, which were first described in forests by Aber et al. (1989, 1998). These authors developed a conceptual model which postulated a hypothetical series of changes called the "N saturation" process that is triggered when available N exceeds plant and microbial demands, causing a gradually increase of nitrate (NO_3^-) export, among other effects such as increased rates of N cycling, soil and surface water acidification, plant nutrient imbalances, and an eventual forest decline (Aber et al., 2003; Fenn et al., 1998; Fig. 1.4). Latter studies showed that the described symptoms may occur earlier in the described progression than initially thought (Emmett, 2007). Recent approaches are focussing on N mass balance rather than the temporal dynamics of N cycling indicators (e.g. Lovett and Goodale, 2011; Fig. 1.4).

Nitrogen deposition is considered the third most important driver affecting biodiversity after changes in land use and climate change (Sala et al., 2000). The Mediterranean Basin presents an extraordinary biological richness and it is recognized as one of the 25 Global Biodiversity Hotspots for conservation priorities (Myers et al., 2000). However, little information is available on the threat that air pollution, and in particular N_r and its interaction with other important air pollutants in the area (i.e. tropospheric ozone), can pose to biodiversity in the Mediterranean region (Ochoa-Hueso et al., 2011).

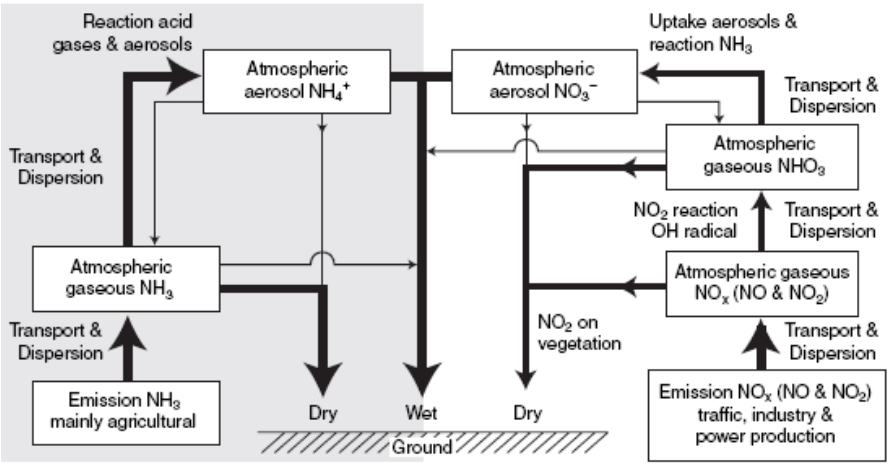


Figure 1.3. General scheme of the pathways of the main forms of atmospheric N_r. Reduced and oxidized forms of N are represented in the left and right sides, respectively. Source: Hertel et al., 2011.

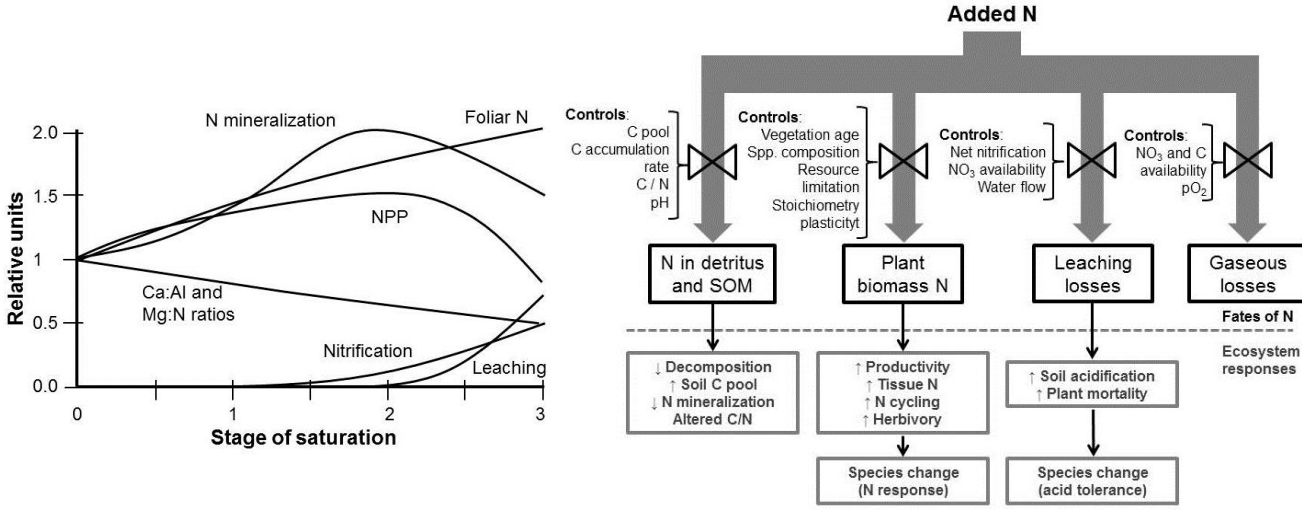


Figure 1.4. Models of N saturation in an ecosystem. Left side: Patterns of response of forest ecosystem properties to continuing N additions, following Aber et al. (1998) hypothesis. Right hand: Conceptual model of N flux to various fates (internal sinks and losses from the ecosystem), controlling factors and ecosystem responses associated with N flow to those fates, following Lovett and Goodale (2011).

1.2. Abatement strategies of N deposition

During the decades of 1960s and 1970s, scientific studies related sulphur emissions to acidification of lakes and it was confirmed that air pollutants can be transported thousands of kilometres before being deposited and causing damage the environment. This triggered the international cooperation in the framework of the United Nations to solve pollution problems such as acidification. The Convention on Long-range Transboundary Air Pollution (CLRTAP; UNECE, 1979) was signed by 34 governments (including Spain) and the European Community, and represented the first international legally binding instrument to deal with problems of air pollution on a broad regional basis. This Convention implies exchanges of information, consultation, research and monitoring among the Parties "to protect man and his environment against air pollution and shall endeavour to limit and, as far as possible, gradually reduce and prevent air pollution including long-range transboundary air pollution by means of policies and strategies" (UNECE, 1979). Currently, the CLRTAP has 51 Parties and eight different Protocols have been developed in its framework.

In particular, the CLRTAP has led the way in delivering a single international agreement dealing with multiple pollutants and multiple effects in the Gothenburg Protocol to Abate Acidification, Eutrophication and Ground-level Ozone (UNECE, 1999). This Protocol sets national emission ceilings for four pollutants: sulphur (SO_2), nitrogen oxides (NO_x), volatile organic compounds (VOCs) and ammonia (NH_3). These ceilings were negotiated on the basis of scientific assessments of pollution effects and abatement options and imply the use of integrated assessment models. Air pollution policies in the European Union, such as the National Emission Ceilings Directive (EC, 2001) or the Directive on Ambient Air Quality and Cleaner Air for Europe (EC, 2008), have similar objectives and methodologies. Together, the Gothenburg Protocol and the related European policies have resulted in substantial reductions of the emissions of N compounds to the atmosphere in the period 1990–2009 in Europe (EEA, 2011).

Critical loads and levels of air pollutants have been established under the CLRTAP in support of effect-based air pollution strategies. Critical loads (CL) are deposition thresholds for the protection of ecosystem function and structure defined as a quantitative estimate of pollutant deposition below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge (CLRTAP, 2011). Different approaches have been adopted in the

Convention to define N critical loads involving either modeling or field evidences. Empirical critical N loads are in almost all cases based on observed changes in the structure and functioning of ecosystems, primarily in species abundance, composition and/or diversity (ecosystem structure), or N leaching, decomposition or mineralisation rate (ecosystem functioning) (Bobbink and Hettelingh, 2011). The exceedance of the empirical critical loads has been used in Europe since 1990s to assess impacts on biodiversity in natural ecosystems. However, the definition and application of N empirical critical loads in Mediterranean areas is still very limited and further research is being required (Bobbink et al., 2010; Ochoa-Hueso et al., 2011).

1.3. Emission and deposition of N in Spain

The above-mentioned reductions of atmospheric emissions of N_r led to a decrease of N deposition during the last decades of the 20th century, which weakened thereafter (Fagerli and Aas, 2008; Waldner et al., 2014). Despite N deposition is still decreasing at numerous monitored forests across Europe, total deposition of inorganic N to forests still exceeds critical loads in some parts of Europe (Lorenz et al., 2008; Waldner et al., 2014). In Spain, emissions of NH_3 have remained stable (being in 2014 a 3% higher than in 1990), while NO_x emissions increased until 2005, and decreased afterwards resulting eventually in 41% lower emissions than in 1990 (Aguillaume et al., 2016; MAGRAMA, 2016) (Table 1.1).

Year	NO_x		NH_3	
1990	386		295	
1995	400	4%	276	-6%
2000	394	2%	343	16%
2005	403	4%	315	7%
2010	233	-40%	305	4%
2014	227	-41%	305	3%

Table 1.1. Total emission of nitrogen oxides (NO_x) and ammonia (NH_3) in Spain. Units: kT N year^{-1} and percentage of variation respect to the emissions in 1990. (MAGRAMA, 2016).

Nitrogen deposition monitoring networks are needed to evaluate the success of this emission decrease in reducing N_r deposition and to relate observable effects with deposition values. Several monitoring networks are currently measuring wet deposition of N across Europe in sites not close to anthropic pollutant sources. It has been recommended to extend wet deposition measurements to provide a good spatial

coverage, particularly within areas of large and variable sources of NH_3 (Erisman et al., 2005). Moreover, dry deposition is not measured by large-scale monitoring networks because direct measurement of dry deposition requires sophisticated instrumentation and methods, and there is not a standardized methodology. Instead, dry deposition is usually modelled by means of inferential methodologies in which the measured or estimated concentration of each gaseous pollutant is multiplied by a correspondent deposition velocity. New empirical approaches mixing empirical and inferential methods are being developed (Bytnerowicz et al., 2015).

Because of these limitations of measured deposition data, chemical transport models constitute a valuable tool to quantify air pollution over broad geographical areas. The EMEP MSC-W model (Simpson et al., 2012) has played a key role in the development of emission control strategies for Europe within the framework of the CLRTAP and the European Union policies. Similarly, the CHIMERE chemical transport model (Menut et al., 2013) has been extensively applied to simulate the evolution and spatial distribution of concentration and deposition of several pollutants over Europe domain (Bessagnet et al., 2004; Vivanco et al., 2008, 2009). Both measured and modeled data show lower deposition values in Spain than those recorded in central Europe (Lorenz and Becher, 2012; Nyíri and Gauss, 2010). Atmospheric N deposition in eastern Spanish forests have been experimentally estimated in 15–30 kg N ha^{-1} $year^{-1}$, with dry deposition ranging from 40% to 80% of the total N deposition, depending on the location, type of forest and year of estimation (Àvila and Rodà, 2012; Rodà et al., 2002; Sanz et al., 2002). Further research is needed to estimate dry deposition, particularly in seasonally dry regions such as the Mediterranean area, to determine the effectiveness of emissions control strategies.

1.4. Effects of deposition of atmospheric N_r in Spanish terrestrial ecosystems

Little information is available on the effects of atmospheric N deposition on the natural ecosystems of Spain, but recent evidence of N enrichment include a continuous increase of nitrophilous species of plants, lichens and mosses in the Iberian Peninsula (Ariño et al., 2011) and an increase in the N content of bryophytes, but not in vascular plants, during the last half of the 20^{th} Century (Peñuelas and Filella, 2001). In a monitoring study of undisturbed headwater catchments in N Spain, it has been described a deposition-driven increase of N exportation from perennial grasslands to aquatic ecosystems (Camarero and Aniz, 2010).

Fertilization experiments have been focused on maximizing biomass production of grasslands for forage use. However, N fertilization reduces the proportion of clover biomass in perennial pastures (3% of Spanish surface), changing pasture structure and fodder quality (Calvete-Sogo et al., 2011). Nitrogen effects on annual grasslands (17% of Spanish surface and understory of broadleaf evergreen forests) have shown a significant interaction with ozone (O_3), the most important air pollutant in the Mediterranean region, in open-top chamber experiments in Spain. Ozone exposure reduced the fertilization effect of enhanced N availability, while N could counteract pernicious O_3 effects on plant and flower biomass production, but only at moderate O_3 levels (Sanz et al. 2011, 2015; Calvete-Sogo et al., 2014). Fertilization experiments in mountain heathlands of the Cantabrian Range have described an increase in the abundance of arthropod herbivores in the short-term (Cuesta et al., 2008) and a significant increase in total plant richness in the long term, due to an increase in the number of perennial herbs without displacing the dominant woody species (Calvo et al,. 2007). In semiarid ecosystems, N fertilization affects soil nutrient cycling and fertility, and alters the functioning of biological soil crusts (Ochoa-Hueso et al., 2013).

Regarding forest ecosystems, holm oak (*Quercus ilex* L.) forests in NE Spain have shown signs of N enrichment such as seasonal increases of NO_3^- concentration in stream waters during peak runoff periods outside the growing season (Àvila and Rodà, 2012). Although most of the deposited N is retained within the ecosystem these increases could be considered as a symptom of the first stages of N saturation (Àvila and Rodà, 2012; Emmett, 2007; Lovett and Goodale, 2011). Nitrogen effects have been also described in *Abies pinsapo* mountain forests along a gradient of atmospheric N deposition in the south of Spain close to the Strait of Gibraltar. Chronic N deposition reduced fine root growth and shifted forests from N limitation to phosphorus limitation. This induced nutritional imbalance has been related to a decrease in photosynthetic nutrient use efficiency (Blanes et al., 2013). Finally, it has been described a tendency of European *Pinus sylvestris* forests to store N in trees and soil in response to N deposition and unveil a trend toward increased nutrient losses in runoff as a consequence of higher N concentrations in soil water (Sardans et al., 2015). Although this study also showed a positive effect of N deposition on *P. sylvestris* growth, the overall effects described pointed to increasing ecosystem nutrient imbalances which may become detrimental for the competitive ability of this species.

1.5. The EDEN project

The present Thesis has been developed in the framework of the project "Effects of N deposition in Mediterranean evergreen forests" (EDEN), funded by the National Program for Scientific Research, Development and Technological Innovation of the Spanish Government (CGL2009-13188-C03-02). This project emerged when evidences of N enrichment in Spanish ecosystems were published, with the aim of create new knowledge in N deposition and its effects over evergreen holm oak forests in Spain which could be useful in the framework of the CLRTAP. For the support of these policies it is important that scientific knowledge be regularly updated with new findings. Holm oak (*Quercus ilex* L.) was selected for this project because is an evergreen broadleaf tree species representative of the Mediterranean Basin and it is present over a wide range of environments in the region, from cold semi-arid to temperate humid bioclimates.

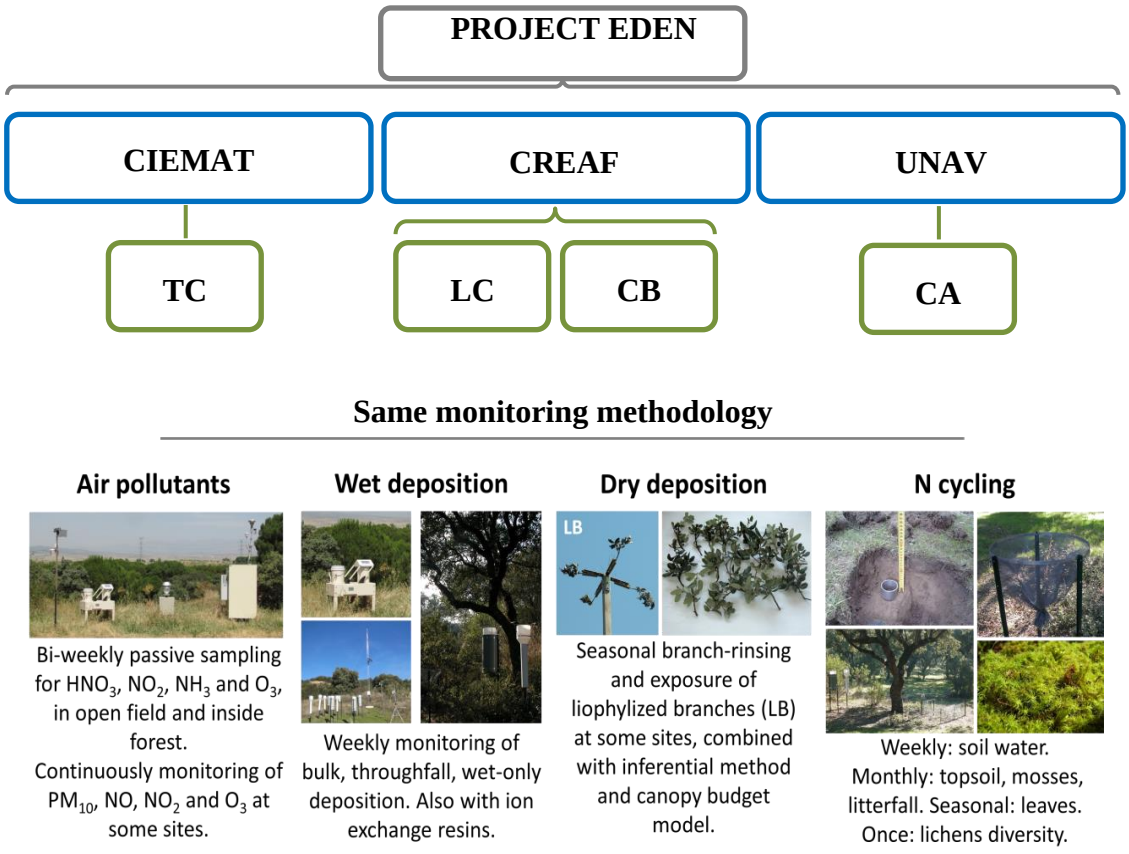


Figure 1.5. Chart showing the research centers (blue boxes), monitoring sites (green boxes) and main monitoring methodology of the Project EDEN (*Effects of N deposition in Mediterranean evergreen forests*). CIEMAT: Research Center for Energy, Environment and Technology; CREAF: Centre for Ecological Research and Forestry Applications; UNAV: University of Navarra.

The Project EDEN is a Coordinated Project in the framework of the Spanish National Research Program involving three Research Centers (Fig. 1.5) whose main goal is characterizing the N total inputs to *Quercus ilex* forests in the Iberian Peninsula and studying the effects of this deposition in the N biogeochemical cycle through this forest type. To this end, four holm-oak forests placed in three regions with different climatic conditions were selected in Spain (Fig. 1.6). Three of these sites were selected because they were close to urban and traffic pollutant sources, with the aim of studying moderately- to high impacted ecosystems that were able to complement the existing monitoring data of low-polluted *Q. ilex* forests in Spain. The extension of the set of monitored holm oak forests and its rank of N_r deposition would facilitate future modelization works and research on N_r deposition effects over these ecosystems. Finally, a fourth holm oak forest was selected in a protected mountainous area as a non-urban reference. The four sites were intensively monitored by means of the same methodology, based on the ICP Forests Level II monitoring sites (UNECE, 2010).

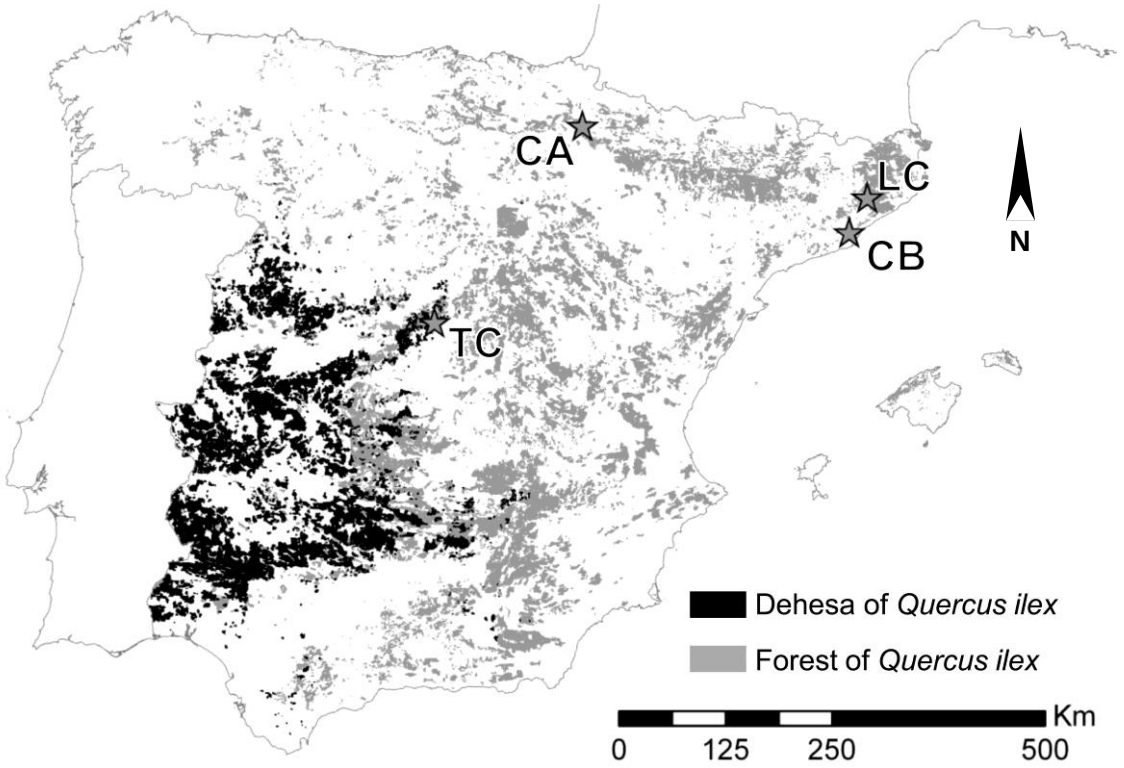


Fig. 1.6. Distribution of *Quercus ilex* habitats in Spain and location of the study sites. CA: Carrascal (Navarra); CB: Can Balasc (Barcelona); LC: La Castanya (Barcelona); TC: Tres Cantos (Madrid).

The Tres Cantos (TC) site is located near from Madrid City (9 km) with Mediterranean semi-arid climate. Only in this case, the historical management of the forest has produced a moderately open forest (72% of tree cover). The Can Balasc (CB) and La Castanya (LC) sites are placed in the Barcelona province, with a sub-humid Mediterranean climate. The former site is close to Barcelona City (4 km), while the last is further from this city (40 km) and relatively sheltered from the surrounding lowland sources of atmospheric pollutants (Hereter and Sánchez, 1999). The Carrascal (CA) site is located in an agricultural area close to Pamplona (15 km), in a region with a Mediterranean humid climate. The canopy in all the sites is dominated by *Quercus ilex*, mixed with *Quercus humilis* in CB. The main characteristics of these sites are shown in Table 1.2 and Annex 1.1.

Table 1.2. Characterization of the study sites.

Site code **Site name**	**CB** **Can Balasc**	**TC** **Tres Cantos**	**CA** **Carrascal**	**LC** **La Castanya**
Province (administrative unit)	Barcelona	Madrid	Navarra	Barcelona
Type of location	Peri-urban	Peri-urban	Peri-urban	Rural
Altitude (m)	255	705	592	696
Longitude	2º 04’ 54” E	3º 43’ 59” O	1º 38’ 40” O	2º 21’ 29” E
Latitude	41º 25’ 47” N	40º 35’ 17” N	42º 39’ 13” N	41º 46’ 47” N
Leaf area index (m^2 m^{-2})	3.3	3.1	5.3	6.1
Tree density (number of trees ha^{-1})	1429	491	1760	2571
Mean diameter at breast height (cm)	13	41[e]	16	13
Mean annual temperature (ºC) [a]	15.2	14.6	12.3	13.7
Mean annual rainfall (mm y^{-1}) [a]	652	348	681	812
Mean annual relative humidity (%) [a]	71.3	54.6	73.7	70.3
Mean annual wind speed (%) [a]	0.8	1.3	6.2	0.9
Distance to the nearest big city (km)	4	9	15	40
Population of the nearest big city (million inhabitants)	1.6	3.2	0.20	1.6
Dist. to the nearest highway (km)	0.15	1.5	0.05	16
Average daily flow in the nearest road (thousand vehicles day^{-1}) [b]	40 – 50	50 – 60	20 – 30	20 – 30
Agricultural land-use cover [c]	23%	21%	62%	23%
Artificial land-use cover [c]	35%	28%	3.1%	7.6%
Livestock density (LU km^{-2}) [d]	14.5	13.7	26.9	88.8

[a]: Mean values calculated for the study period Februay 2011 – February 2013.
[b]: Values for 2012 from the Spanish Ministry of Development (http://www.fomento.gob.es/).
[c,d]: From the Corine Land Cover 2006 (http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster-3) and the Spanish National Statistic Institute (http://www.ine.es), respectively, using a buffer of 25 km radius around the sampling sites.
[e]: Measured in the dominant cohort.

1.6. Specific objectives of the present Thesis

1st objective: Assessment of the risk of atmospheric nitrogen (N) deposition effects on Spanish ecosystems at a country scale.

Although the extraordinary biological richness of the Mediterranean Basin has been widely recognized, very little information is still available for risk assessment of N effects. Quantification and distribution pattern of N deposition in Spain is barely referred in scientific literature. Due to the scarcity data from monitoring networks, deposition data from commonly-used chemical transport models were obtained to fill this gap, and their suitability for estimating wet deposition of N in Spain was previously checked.

Secondly, a risk assessment using empirical CL was performed using those modeled data of N deposition. It is focused in the Spanish Natura 2000 Network since the Habitats Directive is a cornerstone of Europe's nature conservation policy, promoting the maintenance of biodiversity. This study represents a first-level risk assessment by estimating the extent and magnitude of exceedances of empirical critical load within the Natura 2000 Network, in agreement recommendations from experts of CLRTAP

2nd objective: Characterization of the levels, variability and environmental risks of the main atmospheric pollutants in Mediterranean holm oak forests while seeking measurable evidences of air quality improvement inside forests in peri-urban environments.

Concentrations of gaseous and particulate pollutants measured in the framework of the EDEN project were studied and discussed to analyse the main air pollutants that could be affecting holm oak forests and characterize their seasonal changes. Additionally, since three of these forests are located close to big cities, air pollutant concentrations outside and inside the forest were compared to improve the empirical understanding of the influence of vegetation on peri-urban air quality. Urban development is the most rapidly expanding land use change in Europe and the concept of peri-urban environment is arising as a transition zone between the dense urban core and the rural hinterland. Forest in peri-urban areas can provide environmental services, such as air quality improvement. However, there are few studies showing empirical evidences on the potential of forests to mitigate air

pollution, while most of the information comes from large-scale modelling approaches.

3rd objective: Study of bulk and throughfall atmospheric N deposition to holm oak forests by means of conventional an innovative methodologies.

The study of the bulk and throughfall deposition of N in these characteristic Mediterranean forests has not been performed before in wide regional scale in Spain. Besides, the long term monitoring sites in Spanish *Q. ilex* forests (those from the ICP Forests network and the one at La Castanya) are placed in remote areas, in order to study the regional background levels and deposition of pollutants. The results on loads and spatial and temporal variability of N deposition to peri-urban holm oak forests will complement the data from this monitoring network.

Extending the current monitoring networks of atmospheric deposition has been recommended, but new methods inexpensive and easy to operate and without requiring frequent visits to the field are needed. U.S. Forest Service researchers developed a monitoring method by using ion-exchange resins which accomplish these requirements. The method is tested in the EDEN project sites by comparison with conventional methods. The arising methodological recommendations might become a guide document for future research in the Mediterranean area using this methodology.

4th objective: Estimation of dry and total atmospheric deposition of inorganic N to holm oak forests.

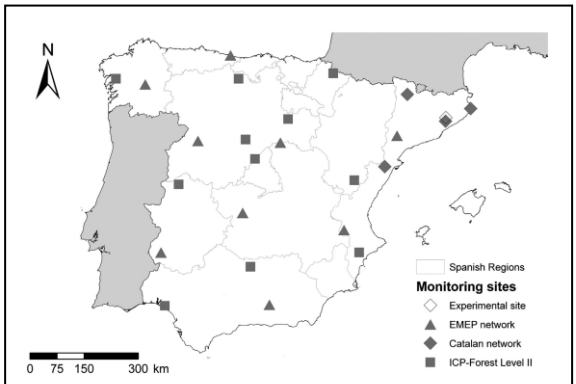
The calculation of total deposition of atmospheric N is a keystone in N effects research in Mediterranean forests. More detailed studies are needed to characterize dry deposition in ecosystems under typically Mediterranean climate conditions, especially since previous results might suggest that the importance of dry deposition could be underestimated by the chemical transport models for the Mediterranean area. Since there is not a standard methodology for measuring dry deposition to forests, different approaches to estimate dry deposition of N are compared among them and with chemical transport model estimates to test the agreement and variability of the approximations. The exceedance of the empirical critical loads in the four EDEN forest sites is also tested.

CHAPTER 2

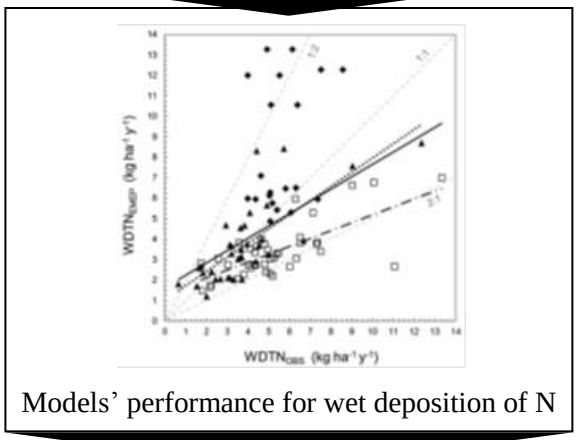
Nitrogen deposition in Spain: modeled patterns and threatened habitats within the Natura 2000 network

ABSTRACT

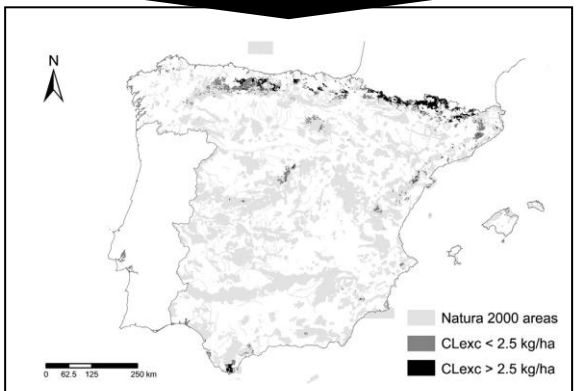
The Mediterranean Basin presents an extraordinary biological richness but very little information is available on the threat that air pollution, and in particular reactive nitrogen (N), can pose to biodiversity and ecosystem functioning. This study represents the first approach to assess the risk of N enrichment effects on Spanish ecosystems. The suitability of EMEP and CHIMERE air quality model systems as tools to identify those areas where effects of atmospheric N deposition could be occurring was tested. For this analysis, wet deposition of NO_3^- and NH_4^+ estimated with EMEP and CHIMERE model systems were compared with measured data for the period 2005-2008 obtained from different monitoring networks in Spain. Wet N deposition was acceptably predicted by both models, showing better results for oxidized than for reduced nitrogen, particularly when using CHIMERE. Both models estimated higher wet deposition values in northern and northeastern Spain, and decreasing along a NE-SW axis. Total (wet + dry) nitrogen deposition in 2008 reached maxima values of 19.4 and 23.0 kg N ha^{-1} y^{-1} using EMEP and CHIMERE models respectively. Total N deposition was used to estimate the exceedance of N empirical critical loads in the Natura 2000 network. Grassland habitats proved to be the most threatened group, particularly in the northern alpine area, pointing out that biodiversity conservation in these protected areas could be endangered by N deposition. Other valuable mountain ecosystems can be also threatened, indicating the need to extend atmospheric deposition monitoring networks to higher altitudes in Spain.



Monitoring sites of wet and bulk N deposition



Models' performance for wet deposition of N



Risk assessment in Natura 2000 network using modeled total deposition

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2. Nitrogen deposition in Spain: modeled patterns and threatened habitats within the Natura 2000 Network

2.1. Introduction

The global biogeochemical cycle of nitrogen (N) has been deeply altered by human activities to the extent that the planetary boundary for human safe operating has long been crossed (Rockström et al., 2009). Anthropogenic reactive nitrogen (N_r) circulates across different compartments (atmosphere, hydrosphere and terrestrial ecosystems) inducing a cascade of environmental effects, such as tropospheric ozone formation, ecosystem acidification and eutrophication (Bobbink et al., 2010; Galloway et al., 2008; Sutton et al., 2011). Eutrophication is a widespread problem that affects most European ecosystems (EEA, 2013). Increased atmospheric N deposition can directly damage vegetation, alter nutrient ratios in soil and vegetation, and increase plant susceptibility to other stressors, resulting in changes of community composition, loss of biodiversity and invasions of new species (Dise et al., 2011).

The Mediterranean Basin presents an extraordinary biological richness recognized as one of the 25 Global Biodiversity Hotspots for conservation priorities (Myers et al., 2000). However, central Europe and circum-Mediterranean countries comprise one of the planet hotspots experiencing high N deposition rates (Dentener et al., 2006). Nonetheless, scarce information is available on the threat that air pollution, and in particular N_r, can pose to biodiversity in the Mediterranean area (Bleeker et al., 2011; Ochoa-Hueso et al., 2011).

The Gothenburg Protocol of the Convention on Long-Range Trans-boundary Air Pollution (CLRTAP) under the UNECE framework and the related European policies have resulted in substantial reductions of the atmospheric emissions of N compounds in the period 1990-2009 in Europe (EEA, 2011). During the same period, Spanish emission of NH_3 increased 12.8% and NO_x emissions eventually decreased 17%, after a continuous increase until 2007 (MAGRAMA, 2013). In this sense, increases in NO_3^- deposition have been detected in the last decades in Catalonia (NE of Spain), while no significant changes were detected in NH_4^+ deposition (Àvila et al., 2010; Àvila and Rodà, 2012; Camarero and Catalán, 2012). The increase in NO_3^- deposition has been related with the increases in NO_x emissions (Àvila et al., 2010). Total annual

atmospheric N deposition loads in eastern Spain have been estimated in 15–30 kg N ha^{-1} yr^{-1}, with dry deposition representing about 40–70% of total N deposition (Rodà et al., 2002; Sanz et al., 2002; Àvila and Rodà, 2012). Atmospheric N deposition in Spain is lower than values recorded in central Europe, both measured (Lorenz and Becher, 2012) and modeled data (Nyíri and Gauss, 2010). However, since changes in species composition occur early in the sequence of N saturation (Emmet, 2007), N deposition effects could be occurring in Spanish natural ecosystems. Some evidences of N enrichment already occurring in Spanish terrestrial ecosystems have been reported. A continuous increase of nitrophilous plant species has been detected in the Iberian Peninsula for the period 1900-2008 using the Global Biodiversity Information facility (GBIF) database (Ariño et al., 2011). Also an increase in the N content in bryophytes, but not in vascular plants, has been observed in herbarium specimens collected in Spain throughout the last century (Peñuelas and Filella, 2001). The reported rises of NO_3^- concentration in headwater streams detected in areas of NE Spain have been considered a sign of the onset of eutrophication (Àvila and Rodà, 2012; Camarero and Aniz, 2010).

Critical loads (CL) are thresholds for N deposition, defined under the CLRTAP for the protection of the ecosystems. CL are defined as a quantitative estimate of pollutant deposition below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge (CLRTAP, 2004). Different approaches have been adopted in the Convention to define N critical loads involving either modeling or field evidences. Empirical critical loads of N have been defined for specific ecosystems (Bobbink et al., 2010; Bobbink and Hettelingh, 2011) based on observed changes in the structure and function of the ecosystem, primarily in species abundance, composition and/or diversity (structure), or N leaching, decomposition or mineralization rate (functioning). Exceedances of critical loads are being used in Europe since 1990s to assess impacts on biodiversity in natural ecosystems. In this sense, the use of empirical CL for nutrient N is recommended to inform whether N deposition should be recorded as a "threat to future prospects" in the framework of the Habitats Directive 92/43/EEC (Henry and Aherne, 2014; Whitfield et al, 2011). The Habitats Directive includes an Annex I with a list of habitat types of Community interest requiring specific conservation measures. Unfortunately, the definition and application of N empirical critical loads in Mediterranean habitats is still

limited and further research is urgently required (Bobbink et al., 2010; Ochoa-Hueso et al., 2011; Pinho et al., 2012).

Suitable N deposition data are needed to identify those areas where effects of N deposition could be occurring in natural ecosystems. Since the availability of air pollutant concentration and deposition data is limited in rural areas, air quality models constitute a valuable tool to quantify air pollution over broad geographical areas. The European Monitoring and Evaluation Program developed the EMEP MSC-W chemical transport model (Simpson et al., 2012), which estimates regional atmospheric dispersion and deposition of acidifying and eutrophying compounds (S, N), ground level ozone and particulate matter all over Europe. This model plays a key role in the development of emission control strategies for Europe within the framework of the CLRTAP/UNECE and the European Union policies. Similarly, the CHIMERE chemical transport model has been extensively applied to simulate the evolution and spatial distribution of concentration of several pollutants such as ozone and its precursors, aerosols, etc. along with estimates of pollutant deposition, in particular over Europe domain (Bessagnet et al. 2004; Vivanco et al. 2008, 2009).

The objectives of this work were 1) to document the performance of the EMEP and CHIMERE model-systems for estimating atmospheric N wet deposition under Mediterranean environmental conditions; 2) to analyze the distribution of atmospheric N deposition in Spain; and 3) to assess the risk of effects of atmospheric N deposition for biodiversity preservation in the Spanish Natura 2000 network. For this analysis, wet deposition of NO_3^- and NH_4^+ modeled by EMEP and CHIMERE models were compared with measured data for the period 2005–2008 obtained from different monitoring networks in Spain: ICP Forests Level II plots, EMEP monitoring network and the Air Quality Network of the Regional Catalan Government. The aim was to evaluate two available and widely used “model-systems”, with their different input data, model setup and the model itself, as tools for ecosystem threat assessment. Modeled N deposition values, including wet and dry deposition, were used to detect those areas with high atmospheric N deposition and to calculate exceedances of empirical critical loads in the Natura 2000 network.

2.2. Material and Methods

2.2.1. Measurements

2.2.1.1. ICP Forests Level II network

The ICP Forests is a biomonitoring program launched in 1985 under the CLRTAP with the aim of providing comprehensive information on forest condition in Europe and the possible relationships to anthropogenic and natural stress factors, in particular air pollution (http://icp-forests.net/). This Program includes Level II plots as intensive monitoring sites offering the possibility of understanding complex ecosystem processes. At these sites, bulk deposition is measured in open areas in the neighbourhood of the forest plots and deposition under canopy is derived from throughfall measurements following standard protocols (http://icp-forests.net/). Measured bulk deposition data of the 13 ICP Forests Level II plots located in Spain (Fig. 2.1) were considered for the period 2005–2008. Fortnightly collected data were used to calculate annual accumulated deposition rates (in some occasions data were collected monthly). Contaminated or unrealistic values were removed from the data set. Only those years and plots with maxima 30 days (2 fortnightly periods) of missing measurements per year were considered. Valid data used for the analysis represented 80% of the total dataset. Missing values were filled in with the monthly mean value for that site estimated from data available of the other years. Annual deposition was calculated adding the product of concentration by precipitation for each measuring period. The Spanish Level II plots are located in a range of 50–1650 m.a.s.l., with 421–1787 mm average annual precipitation and 7.1–17.2 ºC average annual temperature.

2.2.1.2. EMEP measurement network

The EMEP program (www.emep.int) of the CLRTAP includes a network for monitoring air pollutant concentration and deposition following standard methodologies and adequate quality assurance procedures (http://www.nilu.no/projects/ccc/manual/index.html). The EMEP network has focused on measuring air pollutants in rural and background areas. These measurements, in combination with emission inventories and modeled deposition data, allow the assessment of concentration and deposition of air pollutants, the significance of transboundary fluxes and the related exceedances of critical levels and loads. The network needs to ensure an adequate spatial coverage and sufficient temporal resolution

to test the effectiveness of the Convention's protocols. In Spain, this network consists of 10 monitoring stations located from sea level to 1360 m.a.s.l. (http://www.aemet.es/es/idi/medio_ambiente/vigilancia). Daily samples of precipitation were collected with wet-only samplers in 9 of the monitoring stations for the period 2005–2008 (Fig. 2.1). Measured deposition data accumulated throughout the year were estimated following the EMEP protocols (www.emep.int).

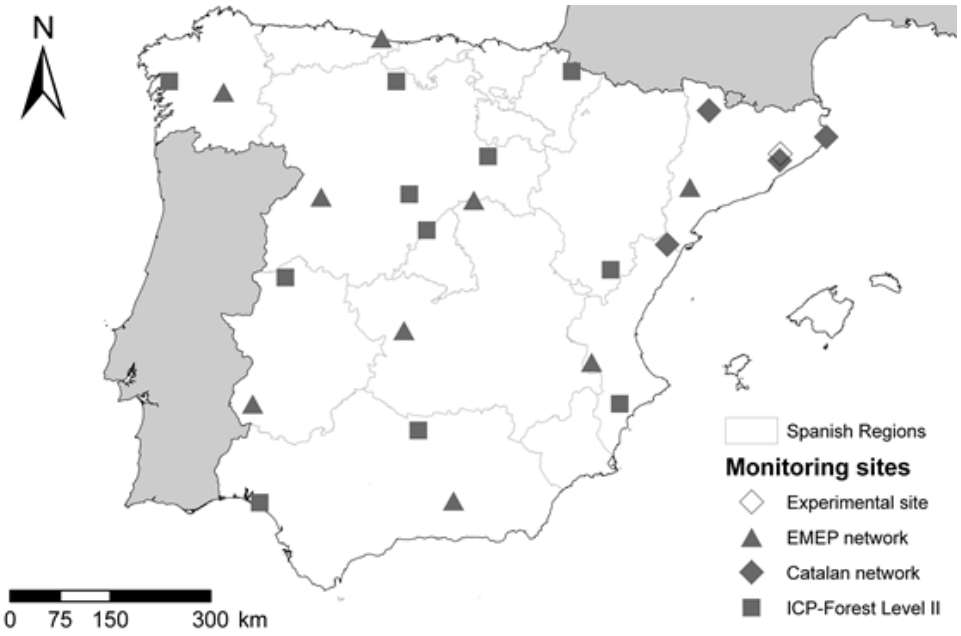

Figure 2.1. Monitoring sites with observed wet deposition of nitrogen included in this study.

2.2.1.3. Catalan Air Quality Network

Precipitation samples were obtained from four stations of the Catalan Air Quality Network (*Xarxa de Vigilància y Prevenció de la Contaminació Atmosfèrica* of the Gereralitat de Catalunya) in NE Spain (Fig. 2.1). Weekly precipitation was sampled with wet-only collectors (MCV®, CPH-004, Spain) at 4 sites ranging 198-685 m.a.s.l. elevation. All the sites were located in the outskirts of small towns with less than 9000 inhabitants (further information of the sites provided in Àvila et al., 2010). Additionally, weekly wet-only precipitation was also collected at La Castanya experimental site in the Montseny Mountains at 720 m.a.s.l. (Fig. 2.1), making a total of 5 sites in the Catalan Region. All samples were analyzed by the CREAF laboratory following protocols published elsewhere (Àvila and Rodà, 2002). Concentrations were weighted by volume to give the annual volume weighted mean (VWM) concentration, and deposition was calculated as the product of annual VWM by annual precipitation.

2.2.2. Air quality models

2.2.2.1. EMEP MSC-W chemical transport model

The EMEP MSC-W model is used within CLRTAP for modelling regional atmospheric dispersion and deposition of air pollutants all over Europe. For standard EMEP calculations, the model employs emission data from the European countries. The performance of the model is regularly evaluated with the measurements of air quality and precipitation data from the EMEP stations. The EMEP rv3.8.1 uses 20 vertical layers and considers about 140 reactions among 70 chemical species. A detailed description of the model is provided in Simpson et al. (2012). For this study, annual atmospheric nitrogen deposition data estimated for the period 2005-2008 with the EMEP model rv3.8.1 over Europe using a grid size of 50×50 km were used (Fagerli et al., 2011). Meteorological data were obtained from ECMWF-IFS Cycle36r1 (http://www.ecmwf.int/research/ifsdocs/) and emissions from the EEA and CEIP Inventory Review of 2011 (Mareckova et al., 2011).

2.2.2.2. CHIMERE regional air quality model

CHIMERE model applications were performed using the regional V200603par-rc1 version for the 2005–2007 simulations and the V2008b version for 2008 simulations. In both cases, 8 vertical levels were used. More information and detailed description of the model can be found in http://www.lmd.polytechnique.fr/chimere/ and Menut et al. (2013). The simulations were performed at a 0.2º-horizontal resolution (approx. 20 km) for the period 2005–2007 (nested to a 0.5º-resolution European-scale simulation) and at a 0.1º-horizontal resolution (approx. 10 km) for 2008 (nested to a 0.2º-resolution European-scale simulation), covering the Iberian Peninsula and Balearic Islands. A further description of the model setup for the 2005–2007 simulations is described in Vivanco et al. (2009). MM5 and WRF models were used to obtain 2005–2007 period and 2008 meteorological fields, respectively. Emissions were derived from the annual totals of the EMEP database on a 50 km grid basis (http://www.ceip.at/webdab-emission-database/emissions-as-used-in-emep-models/). Spatial emission distribution and NMVOC speciation were performed as indicated in Vivanco et al. (2009).

2.2.3. Comparison of measured and modelled data

The location of the monitoring sites of the different networks was matched with the corresponding EMEP and CHIMERE grid cells using ARCGIS version 9.3 (ESRI,

Redlands CA, USA). Unfortunately, none of the monitoring sites included in this analysis were located in the Canary or Balearic Islands, thus the reported values of Spanish N wet deposition represent only the peninsular territory. Annual bulk deposition of NO_3^- and NH_4^+ measured in the ICP Forests plots and annual wet-only deposition measured in the EMEP and Catalan networks were compared with modelled wet deposition data obtained with EMEP and CHIMERE models. A set of metrics commonly used in model evaluation (Chang and Hanna, 2004; Yu et al., 2006), such as index of agreement (IOA), mean normalized bias (MNB), mean normalized absolute error (MNAE) and root mean square error (RMSE, not normalized) were calculated as shown in Table 2.1 for nitrate wet deposition (WDON), ammonium wet deposition (WDRN) and total N wet deposition (WDTN). Also scatterplots, Pearson's correlation coefficient ('r') and linear regression were used to study the relationships between modelled and measured values. All the analysis were performed using Statistica version 11 (StatSoft, Inc. Tule, OK, USA). Significance probability level was set at 0.05.

Statistical metric	Equation
Index of agreement	$IOA = 1 - \frac{\sum (M_i - O_i)^2}{\sum \left(\lvert M_i - \bar{O} \rvert + \lvert O_i - \bar{O} \rvert \right)^2}$
Mean normalized bias	$MNB = \frac{1}{N} \sum \left(\frac{M_i - O_i}{O_i} \right) = \left(\frac{1}{N} \sum \frac{M_i}{O_i} - 1 \right)$
Mean normalized absolute error	$MNAE = \frac{1}{N} \sum \left(\frac{\lvert M_i - O_i \rvert}{O_i} \right)$
Root mean square error	$RMSE = \left[\frac{1}{N} \sum (M_i - O_i)^2 \right]^{\frac{1}{2}}$

Table 2.1. Definition of the metrics used for evaluating model performance. N: pairs of modelled (*Mi*) and observed (*Oi*) deposition. $\bar{O}$ corresponds to the arithmetic mean of observed values. The index *i* is over time series and over all the locations in the domain.

2.2.4. Risk assessment of atmospheric N deposition in the Natura 2000 network

Total atmospheric N deposition, including wet and dry deposition, estimated for 2008 with EMEP and CHIMERE models was used to evaluate the risk of N effects in the Spanish Natura 2000 designated areas. Only the habitat types of Community interest described in the Annex I of the Habitats Directive located within the Natura 2000 network were considered. Natural habitats of Community interest are those habitats

which are in danger of disappearance in their natural range; or have a small natural range following their regression or by reason of their intrinsically restricted area; or present outstanding examples of typical characteristics of their biogeographical regions. These habitats covered 37% of the 188 856.9 km^2 included in the Spanish Natura 2000 network (about 30% of the Spanish territory). The spatial distribution of the habitat types of Community interest within the Natura 2000 network was obtained from the Spanish National Biodiversity Assessment (http://www.magrama.gob.es/es/biodiversidad/servicios/banco-datos-naturaleza/).

The habitat types were matched with the corresponding EUNIS habitat classification used for defining empirical CL (http://eunis.eea.europa.eu/related-reports.jsp). Empirical critical loads (CL) of N deposition recently revised in Bobbink and Hettelingh (2011) were used for estimating N exceedances, calculated as deposition minus CL (positive exceedance is taken to be undesirable). In order to focus on main terrestrial ecosystems, some habitats were excluded of the analysis: coastal and halophytic habitats, freshwater habitats, rocky habitats and caves, and wetlands. Consequently, the area considered for estimating N exceedances occupied an extension of 52 182.9 km^2. The CL assigned to each habitat type was the average of the range of empirical CL reported by Bobbink and Hettelingh (2011) for each habitat type. When a N empirical CL was not defined for a habitat, the CL of similar or equivalent habitats were used (these cases represented about 30% of the total surface assessed). Annex 2.1 of the present work describes the details of the N empirical CL applied to each habitat type included in the analysis. Habitat types of Annex I of the Habitats Directive have been gathered in habitats sub-groups in Table 2.2, showing the minimum and maximum CL used within the sub-group, given that different habitat types are included. The scientific background supporting the empirical CL of N is described in detail in Bobbink and Hettelingh (2011). The exceedance values and the area where the CL is exceeded were estimated for each vegetation type. Additionally, for each habitat sub-group, CL exceedances were estimated weighting exceedances by the corresponding areas. All the analysis were performed using ARCGIS version 9.3 (ESRI, Redlands CA, USA) and MS Access 2010 (Microsoft, Seattle WA, USA).

Table 2.2. Surface area assessed of habitat sub-groups from Annex I of Habitats Directive, nitrogen empirical critical loads (CL) and exceedance of empirical critical loads (CL_{exc}) according to EMEP and CHIMERE estimations of nitrogen deposition.

Sub-groups from Annex I of Habitats Directive	Area assessed (km^2)	CL (kgN ha^{-1} y^{-1}) [a]	EMEP CL_{exc} area (km^2 (%))[b]	CHIMERE CL_{exc} area (km^2 (%))	EMEP CL_{exc} Avg. (kgN/ha)[c]	CHIMERE CL_{exc} Avg. (kgN/ha)
21. Sea dunes of the Atlantic coast	30.3	11.5 – 15.0	n.e.	1.0 (3.4)	n.e.	1.55
22. Sea dunes of the Mediterranean coast	241.6	9.0 – 11.5	2.4 (8.1)	25.0 (10.3)	1.75	1.53
40. Temperate heath and scrub	13 576.4	10.0 – 15.0	102.9 (0.8)	1262.5 (9.3)	2.42	2.19
51. Sub-Med. and temperate sclerophyllous scrub	1661.1	10.0 – 25.0	2.1 (0.1)	176.7 (10.6)	3.24	1.85
52. Mediterranean arborescent matorral	2303.6	25.0	n.e.	n.e.	n.e.	n.e.
53. Thermo-Mediterranean and pre-steppe brush	4077.1	25.0	n.e.	n.e.	n.e.	n.e.
54. Phrygana scrub	2.2	15.0	n.e.	n.e.	n.e.	n.e.
61. Natural grasslands	2054.4	7.5 – 20.0	613.5 (29.9)	1229.9 (59.9)	1.87	4.90
62. Semi-natural dry grasslands and scrubland facies	6250.2	12.5 – 20.0	n.e.	86.6 (1.4)	n.e.	1.77
63. Sclerophillous grazed forests (*dehesas*)	5133.4	20.0	n.e.	n.e.	n.e.	n.e.
64. Semi-natural tall-herb humid meadows	337.4	7.5 – 20.0	34.5 (10.2)	67.6 (20.0)	1.50	3.44
65. Mesophile grasslands	234.9	25.0	n.e.	n.e.	n.e.	n.e.
71. Sphagnum acid bog	37.8	12.5	n.e.	14.2 (37.5)	n.e.	0.65
72. Calcareous fens	31.8	22.5	n.e.	n.e.	n.e.	n.e.
91. Forest of temperate Europe	1931.1	15.0 – 17.5	68.3 (3.5)	129.4 (6.7)	3.79	0.77
92. Med. deciduous forests	3900.5	15.0 – 25.0	23.6 (0.6)	34.0 (0.9)	4.21	2.56
93. Mediterranean sclerophyllous forests	7531.0	15.0 – 17.5	497.4 (6.6)	487.6 (6.5)	3.81	1.98
94. Temperate mountainous coniferous forests	141.2	10.0	54.6 (38.7)	121.9 (86.3)	3.36	3.34
95. Med. and Macaron. mountainous coniferous forests	2706.9	9.0 – 15.0	41.4 (1.5)	148.8 (5.5)	1.33	1.01
TOTAL	**52 182.9**	**7.5 – 25.0**	**1440.8 (2.8)**	**3785.3 (7.3)**	**2.74**	**2.97**

[a]: Range of empirical critical loads, according to Bobbink et al. (2010), used within each habitat sub-group given that different vegetation types are included; [b]: Sum of areas with CL_{exc}, expressed in total area (km^2) and in percentage (%) of the area assessed for each sub-group; [c]: CL_{exc} averaged and weighted for each subgroup; n.e.: None exceedance was found within this sub-group.

2.3. Results and discussion

2.3.1. Comparison of measured and modelled data

Measured values of annual precipitation and N wet deposition were compared with values estimated with the EMEP and CHIMERE air quality models (Table 2.3, Fig. 2.2). In general, the CHIMERE model performed better for estimating WDON than EMEP, since better correlation and IOA and a lower error metrics (RMSE, MNB and MNAE) were obtained (Table 2.3). The scatterplots of model results *vs*. observations showed, in general, similar regression functions for both models. The slope and interception values indicate an underestimation of the high and overestimation the low N deposition values. In the case of WDRN, the CHIMERE model provided less correct estimates than EMEP as deduced from the lower correlation and IOA, higher MNB and MNAE (Table 2.3), and regression functions and scatterplots (Fig. 2.2). CHIMERE clearly underestimated WDRN (with a MNB of -46%). For total wet deposition (WDTN), the correlation coefficients for the CHIMERE results were better, while the EMEP model provided higher IOA. However, CHIMERE clearly underestimated WDTN (with a MNB of -26%), mostly due to the strong underprediction of WDRN. The values of RMSE and MNAE were very similar for both models. The RMSE metrics indicated that the average difference between both model estimations of WDTN was about 0.2 kg N ha^{-1} y^{-1}.

Interestingly, annual precipitation estimates used by both models correlated better with measured values than wet deposition. Some underestimation of high precipitation was shown by both models, but all the evaluation metrics indicated an adequate model performance (high IOA values and relatively low values of MNB and MNAE). The estimates of precipitation for the Spanish ICP Forest plots showed that both EMEP and CHIMERE performed better than values reported when comparing EMEP model with ICP-Forest data across Europe (Simpson et al., 2006a). This result could be explained by the homogeneity of precipitation collectors used in Spain compared to the variety of collectors used by the different countries in previous comparisons (Erisman et al., 2003).

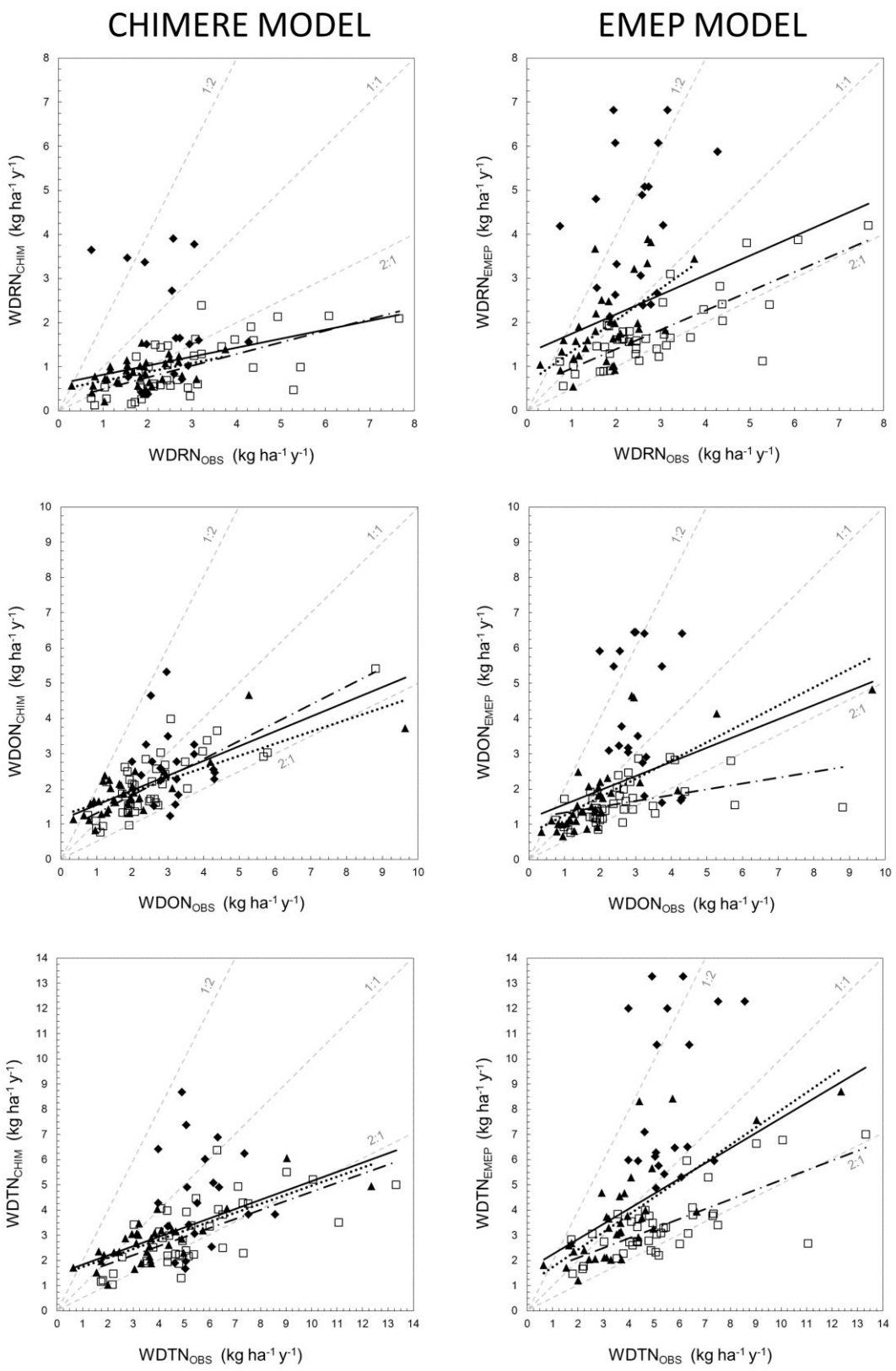

Figure 2.2. Scatterplots and regression lines for modelled *vs*. observed annual values of oxidized (WDON), reduced (WDRN) and total N wet deposition (WDTN) at monitoring sites. ▲·····▲ EMEP monitoring sites; □ —•—•□ ICP-Forests level II monitoring sites; ♦ Catalan monitoring sites. Solid line shows the regression line for all data together.

Table 2.3. Correlation results and comparison metrics of measured and modelled N deposition and precipitation. Values are given for the entire dataset (ALL) and, by network subset.

		CHIMERE MODEL				EMEP MODEL			
METRIC	**NETWORK**	**WDON**	**WDRN**	**WDTN**	**PRECIP**[a]	**WDON**	**WDRN**	**WDTN**	**PRECIP**
n[b]	**ALL**	**97**	**95**	**95**	**97**	**97**	**95**	**95**	**97**
	Catalan	20	20	20	20	20	20	20	20
	EMEP	35	35	35	35	35	35	35	35
	ICP-F	42	40	40	42	42	40	40	42
r[c]	**ALL**	**0.67**	**0.32**	**0.55**	**0.63**	**0.40**	**0.37**	**0.47**	**0.73**
	Catalan	-0.14 [n.s.]	-0.15 [n.s.]	0.04 [n.s.]	0.48	-0.27 [n.s.]	0.32 [n.s.]	0.27 [n.s.]	0.70
	EMEP	0.77	0.49	0.79	0.54	0.76	0.60	0.76	0.66
	ICP-F	0.81	0.64	0.67	0.66	0.43	0.79	0.72	0.90
a[d]	**ALL**	**1.13**	**0.62**	**1.47**	**355.7**	**1.17**	**1.30**	**1.64**	**343.6**
	Catalan	n.s.	n.s.	n.s.	229.6	n.s.	n.s.	n.s.	323.1
	EMEP	1.23	0.48	1.40	315.0	0.74	0.62	1.05	213.3
	ICP-F	0.79	0.22	1.14	385.0	1.17	0.51	1.33	225.0
b[e]	**ALL**	**0.42**	**0.20**	**0.37**	**0.38**	**0.40**	**0.44**	**0.60**	**0.50**
	Catalan	n.s.	n.s.	n.s.	0.71	n.s.	n.s.	n.s.	0.73
	EMEP	0.34	0.20	0.36	0.36	0.52	0.72	0.69	0.86
	ICP-F	0.51	0.27	0.36	0.36	0.17	0.44	0.39	0.53
IOA	**ALL**	**0.74**	**0.48**	**0.60**	**0.72**	**0.62**	**0.60**	**0.66**	**0.82**
	Catalan	0.24	0.31	0.37	0.65	0.19	0.40	0.34	0.71
	EMEP	0.73	0.49	0.68	0.68	0.82	0.75	0.86	0.74
	ICP-F	0.81	0.51	0.58	0.70	0.50	0.69	0.64	0.85
RMSE	**ALL**	**1.14**	**1.78**	**2.47**	**395.1**	**1.63**	**1.52**	**2.66**	**341.2**
	Catalan	1.30	1.69	2.44	245.3	2.25	2.38	4.05	222.4
	EMEP	1.17	1.16	1.83	251.6	1.08	0.74	1.42	320.0
	ICP-F	1.02	2.23	2.92	529.3	1.66	1.47	2.60	402.4
MNB	**ALL**	**0%**	**-46%**	**-26%**	**4%**	**-5%**	**13%**	**0%**	**16%**
	Catalan	-8%	-8%	-17%	16%	39%	95%	53%	36%
	EMEP	19%	-46%	-16%	5%	-1%	17%	5%	30%
	ICP-F	-11%	-65%	-40%	-5%	-29%	-33%	-31%	-7%
MNAE	**ALL**	**32%**	**62%**	**36%**	**35%**	**39%**	**51%**	**39%**	**35%**
	Catalan	35%	75%	37%	31%	64%	98%	56%	37%
	EMEP	38%	51%	31%	35%	28%	41%	32%	42%
	ICP-F	27%	65%	40%	39%	36%	36%	35%	30%

[a]: Annual precipitation; [b]: number of pairs of data compared; [c]: correlation coefficient; [d]: y-axis intercept of the regression equation; [e]: slope of the regression equation; [n.s.]: no significative correlation/regression was found.

Nitrogen wet deposition estimations were also compared with observed values for each measurement network independently. Modelling N wet deposition in EMEP sites obtained the best results, especially when using the EMEP model (Table 2.3), with correlation coefficients in a range (0.60–0.76) similar to those reported for other European areas (Simpson et al., 2006b). In fact, a recent review reported that EMEP model performance for estimating N compounds concentration in precipitation in the Mediterranean area was comparable to the one found in other parts of Europe (Aas et al., 2010).

Model estimates of N wet deposition for the ICP Forests sites were similar or slightly more incorrect than for EMEP sites. In this case, both EMEP and CHIMERE models underestimated wet N deposition values of reduced and oxidized N forms (Table 2.3). These underestimations can be partially explained by the bulk samplers used by the ICP Forests network to collect wet deposition, since some influence of dry deposition onto the funnels cannot be disregarded. The proportion of dry deposition collected in the funnels depends on location, climate, sampler aerodynamic characteristics and chemical component (Erisman et al., 2003). Results from bulk *vs.* wet-only comparison in the experimental Catalan site (La Castanya) indicated an overestimation of about 10% of nitrate concentration in bulk collectors, although ammonium was underestimated by 25% (Izquierdo and Avila, 2012).

Surprisingly, model predictions of N wet deposition were fairly poor when comparing with measurements obtained in the five Catalan monitoring sites. Correlations between modelled and measured deposition were not significant and the IOA metrics were always below 0.5 for both model approaches (Table 2.3). The lack of correlation in the Catalan region was not directly explained by poor predictions of precipitation, which were acceptable with both model systems. The small range of deposition values collected in the area could hinder statistic correlation. Also the complex topography of this region and the influence of local emissions might explain the poor model performance at small regional scale. In these conditions, the EMEP model with a 50×50 km resolution cannot be expected to reproduce small-scale variations in deposition regimes as it is argued in previous studies (e.g. Simpson et al., 2006a). However, CHIMERE model, despite its finer resolution, obtained only slightly better error metrics for the Catalan sites.

The higher resolution used with CHIMERE for 2008 estimations (10×10 km compared to 20×20 km resolution used for 2005–2007) did not improve the overall estimation of wet deposition and all the evaluation metrics considered were within the ranges of values observed for previous years (data not shown). Similarly, increasing EMEP model resolution and a finer placement of emission sources are expected to have significant improvements in polluted areas but a similar performance has been described for wet deposition and concentration in precipitation estimations in rural areas (Cuvelier et al., 2013; Hirst and Storvik, 2003; Nyíri and Gauss, 2010).

In summary, evaluation metrics and scatterplots of modelled *vs*. measured values indicate that both CHIMERE and EMEP models generally underestimate the high and overestimate the low measured atmospheric N deposition values. Nevertheless, estimations of total N wet deposition performance in Spain provided by both models are in general within acceptable ranges (Table 2.3, Fig. 2.2), although results should be applied with caution, especially at small regional scale. Differences on the results obtained with both model systems can be explained by the different input data, setup and model estimations. The models' setup was not harmonized because the analysis performed did not intend to compare both models. A more detailed comparison of both models is currently being developed considering monthly values (Vivanco et al., in preparation).

2.3.2. Atmospheric nitrogen deposition in Spain

Annual values of N wet deposition measured in the monitoring sites for the period 2005–2008 ranged 0.3–7.7 kg N ha^{-1} y^{-1} of WDRN, 0.4–9.6 kg N ha^{-1} y^{-1} of WDON, and 0.7–13.3 kg N ha^{-1} y^{-1} of WDTN. For each monitoring station, interannual variability of measured wet deposition represented about 25–30% of the average value. Similar variability was observed in wet deposition values estimated with CHIMERE in the cells corresponding to those sites, while EMEP model presented lower interannual variability (13–14%). The difference in scale between EMEP and CHIMERE could be the reason for this disparity. Despite this lower interannual variability observed in the EMEP results, both models provided acceptable predictions of wet deposition values as discussed in the previous section. It is interesting that the noticeable 14% reduction of oxidized N emissions reported in 2008 with respect to the previous year (MAGRAMA, 2013) was not reflected in measured nor in modeled WDON (Fig. 2.3). This result is probably related to the higher precipitation rate registered in 2008 compared to 2007,

and highlights the importance of considering precipitation variability when evaluating the effectiveness of control emission strategies on deposition trends. In fact, measured total N wet deposition (WDTN) was significantly correlated with precipitation ($r = 0.61$; $p < 0.05$) for the period considered. Accordingly, maximum wet deposition was mainly located in the Northern area of Spain where the highest precipitation occurs.

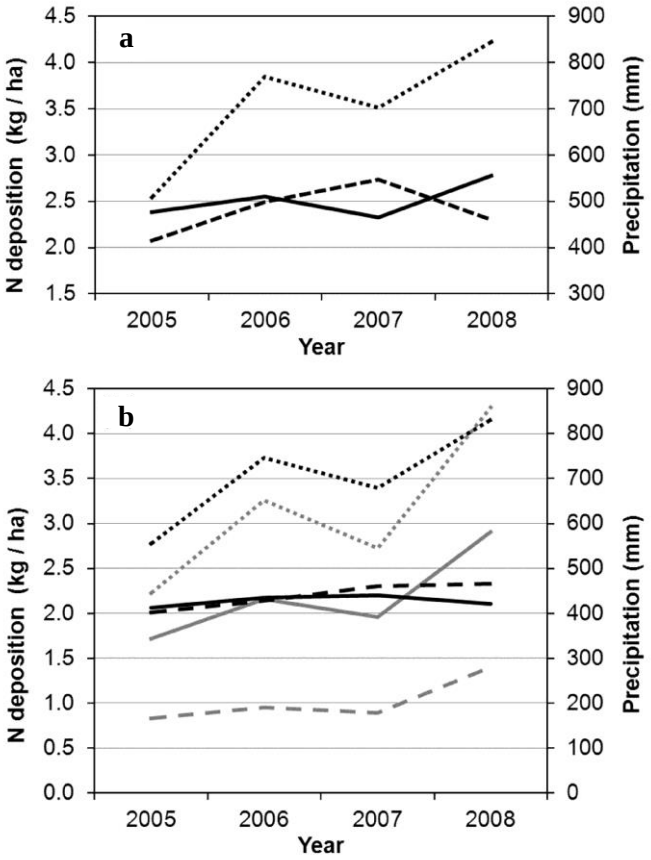

Figure 2.3.

(a) Annual averages of wet deposition of N-nitrate (WDON), N-ammonium (WDRN) and precipitation observed at monitoring sites; and (b) annual averages of wet deposition of WDON and WDRN and precipitation at monitoring sites, predicted by EMEP (black lines) and CHIMERE (grey lines) models.

Solid lines: WDON
Dashed lines: WDRN
Dotted lines: precipitation rate.

Average measured wet deposition of oxidized N (WDON) for the period 2005–2008 was 2.33 kg N ha^{-1} y^{-1}, a 12% higher than the 2.08 kg N ha^{-1} y^{-1} of reduced N (WDRN). However, many of the inland sites located far from the coast and from the main industrial areas showed slightly higher WDRN than WDON. This composition of measured wet deposition seems to reflect total national emissions of reduced and oxidized N, since average values for the period 2005–2008 of oxidized N were 26% higher than emissions of reduced N (400.3 kT of N-NO_x *vs.* 318.3 kT of N-NH_3 respectively; MAGRAMA, 2013). On the other hand, modeled deposition of oxidized and reduced N in these monitoring sites showed an averaged ratio WDON/WDRN slightly lower than expected in the case of EMEP model (0.96) and clearly higher for CHIMERE model (2.67).

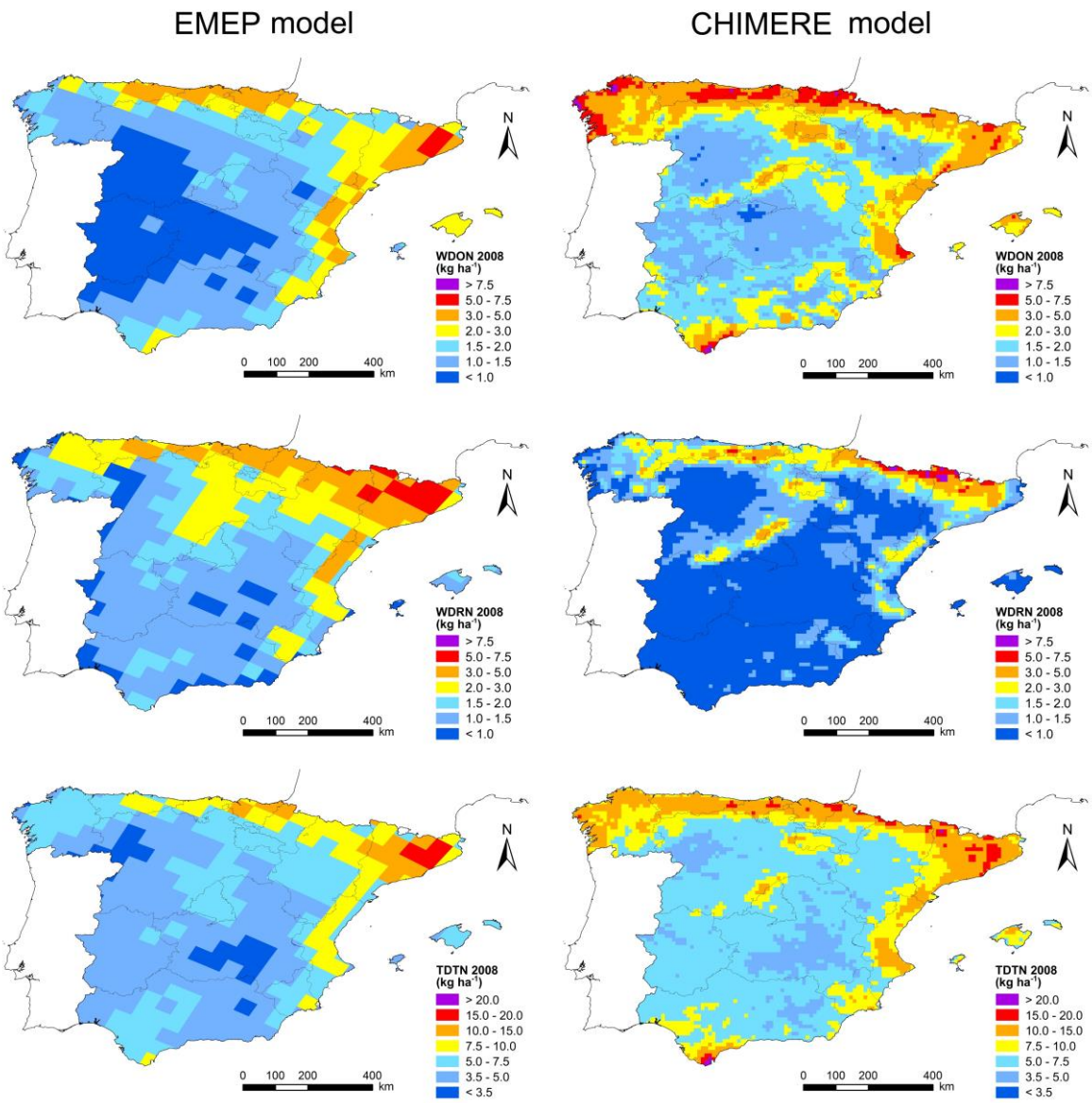

Figure 2.4. Wet deposition of oxidized and reduced nitrogen (WDON and WDRN), and total

Modeled N wet deposition in Spain showed a decreasing distribution along a NE–SW axis, with higher deposition in the northern and eastern coastal regions than inland and southern areas (Fig. 2.4). EMEP model showed similar distribution patterns of oxidized and reduced N deposition, with the highest values in NE of Spain (reaching 6.5 and 7.7 kg N ha^{-1} y^{-1}, respectively). CHIMERE showed higher deposition of reduced N in the Pyrenees along the border with France, with values up to 12.1 kg N ha^{-1} y^{-1}. On the other hand, oxidized N estimated with CHIMERE showed maxima values throughout the northern coast (including the Cantabrian Range) and in the south of Spain close to the Strait of Gibraltar, and also higher WDON and WDTN than EMEP model in Northwestern Spain (Galicia Region) (Fig. 2.4).

This distribution pattern of N wet deposition across the Spanish territory clearly responds to the spatial distribution of the expected three main drivers: regional emissions, precipitation distribution and transboundary contribution. In fact, the areas receiving the highest loads of N wet deposition, mainly located in the north and NE regions, enclose some highly populated and industrialized areas and present high precipitation rates (AEMET, 2011). Transboundary pollution can also represent an important contribution (up to 60–70%) in some of the areas that show high N deposition such as Northern Spain or the vicinity of the Strait of Gibraltar (Nyíri et al., 2010).

Air quality models also provide estimations of N dry deposition. Spanish WDTN estimated for 2008 was within the range 1.5–13.4 kg N ha^{-1} y^{-1} when using EMEP and 0.9–16.1 kg N ha^{-1} y^{-1} when using CHIMERE. Dry deposition estimated with EMEP in 2008 represented 14–59% of total N deposition with an average value of 40%. In the case of CHIMERE, dry deposition represented 11–83% of total N deposition with an average value of 54%. Previous studies performed in Spain calculated that dry deposition represented 62–67% of total N deposition in *Quercus ilex* forests (Rodá et al., 2002) and 40–75% in *Pinus halepensis* forests (Sanz et al., 2002) in NE and eastern Spain respectively. Values estimated by the models on those grid cells where monitoring plots are located in *Q. ilex* or *P. halepensis* forests, showed that 39% with EMEP and 54% with CHIMERE of N total deposition was associated to dry deposition. Although data from different years are compared, these results might suggest that the importance of dry deposition could be underestimated, particularly by the EMEP model, for this Mediterranean area. More detailed studies are needed to characterize dry deposition in ecosystems under typically Mediterranean climate conditions. When considering both dry and wet N deposition, total N deposition in 2008 in Spain reached maxima values of 19.45 kg N ha^{-1} y^{-1} and 22.98 kg N ha^{-1} y^{-1} for EMEP and CHIMERE, respectively. Distribution of total N deposition followed similar patterns to those observed for wet deposition, with higher values in the north and NE of the country and close to the strait of Gibraltar in the south (Fig. 2.4).

2.3.3. Risk assessment of atmospheric N deposition in the Natura 2000 network

Total N deposition (including wet and dry deposition) estimated with EMEP and CHIMERE models for 2008 was used to assess the risk of N enrichment in terrestrial habitats of Community interest included in the Natura 2000 network. Exceedances of empirical N critical load and the area affected were calculated for the different habitat

types (Annex 2.1) and subgroups (Table 2.2). The CHIMERE model predicts an area at risk more than twice as large as the one foreseen with EMEP model (3785.3 and 1440.8 km^2 respectively, Table 2.2). The threatened areas are mainly located in high N deposition regions (Figs. 2.4 and 2.5) and mostly involve habitats with high sensitivity to N deposition, based on their low empirical CL. Sensitive habitats with N empirical CL of 10 kg N ha^{-1} y^{-1} or lower include natural grasslands and humid meadows, mountain forests, and typically Mediterranean heaths (Table 2.2, Annex 2.1).

The most sensitive habitat to atmospheric N deposition based on the low empirical CL and the percentage of area affected is the 'natural grasslands' (subgroup 61). This category presents 30–60% of its area at risk of N enrichment due to atmospheric N deposition (Table 2.2). This sub-group includes the habitat at highest risk within the Spanish Natura 2000 network, the 'siliceous Pyrenean *Festuca eskia* grasslands' (habitat type 6140), with a threatened surface from 79 to 100% of the assessed area depending on the model considered (Annex 2.1). In fact, this habitat type is located in the Pyrenees, where both models predict the highest exceedance occurrence. Most of the empirical CLs used for natural grasslands were ascribed to their specific habitat type, and had good reliability ('#' in Annex 2.1) according to Bobbink and Hettelingh (2011). For this reason, the major uncertainty of the potential threat of N deposition to Pyrenean grasslands, and to other grasslands located in alpine areas, is that no monitoring sites are available to test model performance for estimating N deposition in this alpine level. Moreover, other high-altitude vegetation types like *Pinus uncinata* or *Abies pinsapo* forests, oro-mediterranean heathlands or *Cytisus purgans* formations seem to be highly threatened by N deposition according to the models (Annex 2.1). Therefore, further deployment of atmospheric deposition monitoring networks should be implemented in Spanish mountain areas for monitoring atmospheric pollution and assess the risk of effects on these particularly rich and valuable ecosystems.

Other habitat category which requires special attention is the 'Mediterranean sclerophyllous forests' (subgroup 93). These forests represent a distinctive ecosystem and landscape of the Mediterranean Basin, including forests of Holm oak (*Quercus ilex* L.), the dominant tree species in the Iberian Peninsula. The surface of these forests potentially affected is only 6.5–6.6% (Table 2.2) of the total area assessed in Spain, according to EMEP and CHIMERE models respectively. However, most of the threatened area of this sub-group corresponds to Holm oak forests located in NE Spain,

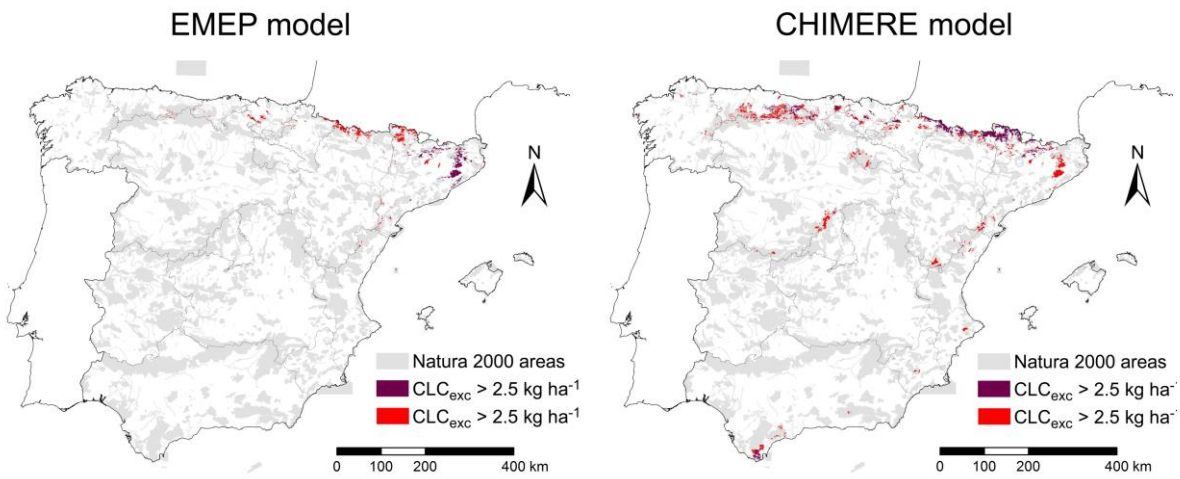

Figure 2.5. Natura 2000 areas experiencing an exceedance of the assigned critical load (CL_{exc}) according to EMEP and CHIMERE models.

close to Barcelona city (Fig. 2.5), where average exceedances up to almost 4 kg N ha^{-1} have been predicted with EMEP. In this sense, high N atmospheric deposition of 15–30 kg N ha^{-1} y^{-1} has been previously reported in this area, together with increases of NO_3^- concentration in streamwater (Rodà et al., 2002; Àvila and Rodà, 2012). The low N concentration found in streamwater suggests that these ecosystems are still far from N saturation since most of the deposited N is still retained within the ecosystem (Àvila and Rodà, 2012; Bernal et al., 2013). Estimated N amounts annually stored in Holm oak above ground biomass are in the range of the N wet deposition occurring in this area (Escarré et al., 1999). However, other effects of N deposition could be already occurring before N saturation (Emmet, 2007) and need further investigation. An empirical CL of 15 kg N ha^{-1} y^{-1} was ascribed to these sclerophyllous forests for preventing nitrate leaching from the ecosystem following expert criteria (Bobbink and Hettelingh, 2011). However, critical loads from 5.5 kg N ha^{-1} y^{-1} to 26 kg N ha^{-1} y^{-1} have been proposed for the protection of epiphytic lichens in similar natural ecosystems (Fenn et al., 2010; Pinho et al., 2012).

Both models highlight that the highest occurrence of threatened areas happen in NE Spain, particularly in the Pyrenees mountain range, where 41% and 71% of the area assessed within the Spanish Alpine Bio-geographical Region could be experiencing CL exceedances, according to EMEP and CHIMERE models, respectively. This high risk is explained by the elevated N deposition and the presence of sensitive habitats such as mountain grasslands, heaths and some forest ecosystems. This result agrees with the

effects of N deposition already reported in the area of Central Pyrenees (*Aigüestortes i Estany de Sant Maurici* National Park, 2236 m.a.s.l.), where increases of nitrate concentration in headwater streams of high altitude catchments have been associated with a N saturation process due to atmospheric N deposition (*Aigüestortes i Estany de Sant Maurici* National Park, 2236 m.a.s.l.; Camarero and Catalán, 2012; Camarero and Aniz, 2010). Other areas, detected with the CHIMERE model, where exceedances of N empirical CL could be occurring are the mountainous regions located north of Madrid City (central Spain), in the Eastern Coast, on the Cantabrian Range (northern Spain) and near the Strait of Gibraltar (southern Spain).

The present analysis represents the first approach to assess the risk of effects of N enrichment for Spanish ecosystems within Natura 2000 network. Exceedances of N critical loads were related with high WDON more often than with high WDRN or dry deposition rates. Although further investigation is urgently needed to confirm the suitability of N empirical critical loads used, this study points out that some natural ecosystems could be receiving atmospheric N deposition above safety thresholds and, consequently, suffering harmful effects. The habitats most at risk are the Pyrenean grasslands, mountain forests of *Pinus uncinata* or *Abies pinsapo*, Mediterranean sclerophyllous forests of Catalonia and the oro-mediterranean heathlands of the Cantabrian Range. Interestingly, some evidences of N effects have been already reported in some of these areas (Àvila and Rodà, 2012; Blanes et al., 2013; Camarero and Catalán, 2012). These agreements suggest that the methodology applied in this analysis results suitable for risk assessment of N deposition effects in Spanish natural and semi-natural habitats.

2.4. Conclusions

EMEP and CHIMERE air quality models constitute suitable tools to provide acceptable estimates of N wet deposition, particularly for oxidized N in Spain. However, estimates should be applied with caution in studies at small regional scale and in regions with complex topography and the influence of local emissions. Measured wet deposition of nitrogen in Spain reached maxima values of WDTN up to 13.3 kg N ha^{-1} y^{-1} in northern Spain for the period 2005–2008. Both models estimated higher wet deposition of N in the north and northeast Spain. Adding dry deposition, total N deposition in 2008 in Spain reached maxima values of 19.5 kg N ha^{-1} y^{-1} and 22.9 kg N ha^{-1} y^{-1} calculated

with EMEP and CHIMERE, respectively. Distribution of total N deposition followed similar patterns observed for wet deposition.

Total atmospheric N deposition exceeded in many areas the empirical critical loads proposed for the protection of terrestrial habitat of Community interest included in the Spanish Natura 2000 network. The habitats presenting the highest risk of N effects are the natural grasslands of mountain areas located in the north (Pyrenees, Cantabrian Range), together with some forests and endemic heaths in the same areas. Biodiversity conservation in these protected areas could be endangered by N deposition. Other habitats showing significant exceedances of N empirical critical loads were located in mountain areas close to high emission sources, such as Mediterranean forests and mountain scrublands close to Barcelona and Madrid cities, in the Eastern Coast, and near the Strait of Gibraltar.

These results highlight that atmospheric N deposition should be considered as a factor that could be affecting the biodiversity and health of the protected natural ecosystems in Spain. Since most of the threatened habitats are located in mountain areas, atmospheric deposition networks should be extended and include some monitoring stations in mountain regions. More detailed investigations should be carried out to quantify current effects, to improve empirical critical loads definition for some Mediterranean ecosystems and to explore possible management practices that might ameliorate these effects.

2.5. Acknowledgements

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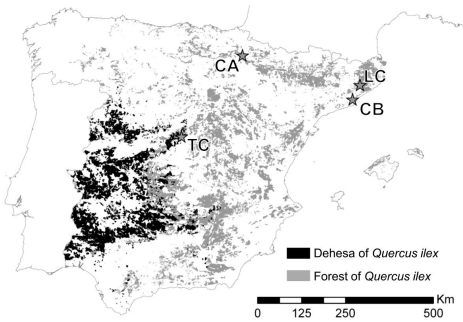
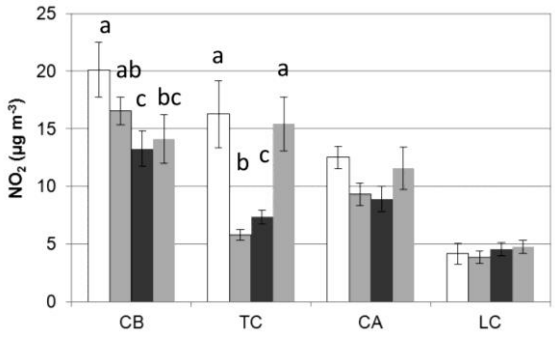
CHAPTER 3
Atmospheric pollutants in peri-urban forests *of Quercus ilex*: evidence of pollution abatement and threats for vegetation

ABSTRACT

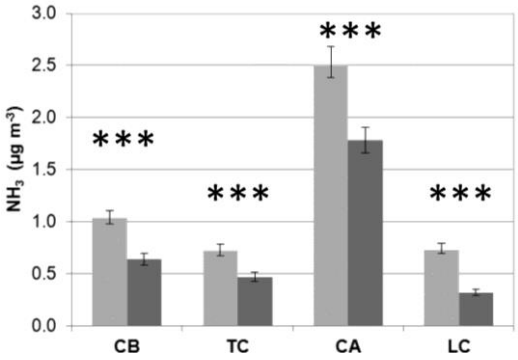
Peri-urban vegetation is generally accepted as a significant remover of atmospheric pollutants, but it could also be threatened by these compounds, with origin in both urban and non-urban areas. To characterize the seasonal and geographical variation of pollutant concentrations and to improve the empirical understanding of the influence of Mediterranean broadleaf evergreen forests on air quality, four forests of *Quercus ilex* (three peri-urban and one remote) were monitored in different areas in Spain. Concentrations of nitrogen dioxide (NO_2), ammonia (NH_3), nitric acid (HNO_3) and ozone (O_3) were measured during two years in open areas and inside the forests and aerosols (PM_{10}) were monitored in open areas during one year. Ozone was the only air pollutant expected to have direct phytotoxic effects on vegetation according to current thresholds for the protection of vegetation. The concentrations of N compounds were not high enough to directly affect vegetation but could be contributing through atmospheric N deposition to the eutrophization of these ecosystems. Peri-urban forests of *Quercus ilex* showed a significant below-canopy reduction of gaseous concentrations (particularly NH_3, with a mean reduction of 29–38%), which indicated the feasibility of these forests to provide an ecosystem service of air quality improvement. Well-designed monitoring programs are needed to further investigate air quality improvement by peri-urban ecosystems while assessing the threat that air pollution can pose to vegetation.



EDEN Project: four monitoring sites of atmospheric pollutant concentrations



Characterization of seasonality in the four sites



Significant below-canopy reduction of pollutant concentrations

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3. Atmospheric pollutants in peri-urban forests of *Quercus ilex*: evidence of pollution abatement and threats for vegetation

3.1. Introduction

The continuous growth of urban population has turned air quality into one of the main environmental concerns worldwide. Current urban development needs to consider designs and strategies that minimize atmospheric pollution to improve well-being and human health. In the last years, particular attention has been paid to investigate the role of urban and peri-urban vegetation in improving air quality. Vegetation can remove air pollutants via dry deposition, through interception in the canopy surfaces, and via absorption of gases through the stomata. In particular, urban and peri-urban vegetation has been proposed as a method to reduce air pollutants such as ozone, nitrogen oxides and particulate matter (Alonso et al., 2011; Kroeger et al., 2014; Nowak et al., 2014; Sgrigna et al., 2015). On the other hand, air pollution can affect these forests, impairing their capacity to provide ecosystem services.

Peri-urban areas are transition zones between the denser urban core and the rural hinterland, where natural habitats can be exposed to intermediate concentrations of pollutants linked to both urban and rural activities. Among the most common gaseous pollutants, nitrogen oxides (NO_2, NO) reach peri-urban areas transported from human agglomerations and highways where they are produced as a result of combustion processes. Nitrogen oxides are in turn precursors for the formation of photochemical oxidants such as ozone (O_3) and nitric acid (HNO_3). Ozone is one of the most important and pervasive air pollutants currently affecting vegetation (Kroeger et al., 2014). This pollutant is particularly important in the Mediterranean region, where the highest concentrations in Europe are registered (EEA, 2013). Ozone levels are usually greater in peri-urban and rural areas than in busy urban centres, due to its rapid destruction by reacting with the NO emitted in the cities (The Royal Society, 2008). Nitric acid is one of the main components of photochemical smog, together with ozone, and with a similar spatial distribution (Bytnerowicz et al., 1999a). In contrast, ammonia (NH_3) is mainly emitted from agricultural and livestock activities in rural areas. Ammonia and nitric acid can quickly react with each other, or with other atmospheric gases, to form secondary inorganic aerosols (SIA), that can represent an important fraction of the particulate matter (PM) concentration measured at regional background stations (EEA, 2013).

Although atmospheric N pollutant levels are usually not high enough to directly damage vegetation, atmospheric N deposition can contribute to both eutrophication and acidification of ecosystems, which is a bigger problem than the direct exposure to these compounds (Dise et al., 2011; EEA, 2013). Atmospheric N deposition can be particularly important in peri-urban areas that are receiving contributions of N compounds from both urban and agricultural activities. In fact, Mediterranean forests and mountain scrublands close to Barcelona and Madrid cities have been reported to be threatened by N deposition (see Chapter 1).

Air pollutant gases and particles are removed from the atmosphere through both wet and dry deposition. In Mediterranean environments, atmospheric deposition can be dominated by dry deposition, which can represent up to 50–95% of the total deposition in Mediterranean forests (Bytnerowicz and Fenn, 1996). In this sense, urban and peri-urban vegetation, through increasing dry deposition, can represent a good strategy to improve air quality, particularly in this region. Dry deposition to vegetation is a function of multiple factors, such as air concentration, chemical properties of the depositing species, atmospheric turbulence, moisture and reactivity of receptor surfaces, and vegetation structure and activity (Fowler et al., 2009).

Measuring pollutant concentrations outside and within peri-urban forests can provide an insight into the role of vegetation in removing air pollutants (Cavanagh et al., 2009; Setälä et al., 2013; Grundström and Pleijel, 2014). Although urban vegetation is accepted as an efficient remover of air pollutants, most of the studies are based on large-scale modelling (e.g. Nowak et al., 2014) or laboratory studies (e.g. Chaparro-Suárez et al., 2011), but there are few empirical evidences of the reduction in pollutant concentrations inside urban forested areas (Cavanagh et al., 2009; Grundström and Pleijel, 2014). Besides, atmospheric pollution represents a risk for the urban and peri-urban vegetation and should be monitored, particularly in forest potentially withstanding other stressful conditions. Interestingly, NH_3 and HNO_3 concentrations are scarcely measured in the main air-quality networks, despite being major drivers of atmospheric N dry deposition to vegetation (Bytnerowicz et al., 2010).

In order to study tropospheric O_3, gaseous N compounds, and suspended PM in peri-urban forests in Spain, three peri-urban forests of holm oak (*Quercus ilex* L.) were selected near to three cities in Spain with increasing population and with different influences of traffic and agricultural pollution sources (based on their distances to

highways, percentage of agricultural land use and presence of livestock). Another holm oak forest site, far from anthropogenic emissions of air pollutants, was established for comparison. Holm oak is an evergreen broadleaf tree species representative of the Mediterranean Basin and it is present over a wide range of environments in the region, from cold semi-arid to temperate humid bioclimates. This study was enclosed in the EDEN project (*Effects of nitrogen deposition in Mediterranean evergreen holm oak forests*), whose main goal was to determine and characterize the nitrogen inputs to holm oak forests in the Iberian Peninsula and the effects in the nitrogen biogeochemical cycle. In the present study, air quality measurements from EDEN project are presented and discussed, with the following objectives: 1) to analyse the main air pollutants that could be affecting holm oak forests close to cities, 2) to characterize air pollutant temporal and geographical variation, and 3) to compare air pollutant concentrations outside and inside the forest to improve the empirical understanding of the influence of vegetation on air quality.

3.2. Material and methods

3.2.1. Study sites

Three holm-oak (*Quercus ilex*) forests were selected in the vicinity of three cities in Spain with increasing population (Fig. 3.1, Table 3.1). The Can Balasc (CB) site is placed in a forest located in a natural protected area 4 km away from Barcelona with acidic soils and Mediterranean sub-humid climate. The Tres Cantos site (TC) is a forest located in a natural protected area at 9 km from Madrid, growing on acidic sandy soil with Mediterranean semi-arid climate. The Carrascal site (CA) is located in an agricultural area close to Pamplona (15 km), with calcareous soil and Mediterranean humid climate, and it is the most agricultural-influenced among the three peri-urban forests. The canopy in all the sites is dominated by *Quercus ilex*, mixed with *Q. humilis* in CB. In the case of TC, vegetation was historically managed as a traditional *dehesa* (a savannah-like agrosilvopastoral system) of *Q. ilex*, but the low management intensity during the last decades has allowed vegetation to grow as a moderately open forest. An additional holm oak forest was selected as a non-urban reference in La Castanya (LC), a long-term biogeochemical study site in a protected mountainous area (Parc Natural del Montseny), situated 40 km away from Barcelona (Fig. 3.1, Table 3.1) and is included in the GAW/ACTRIS monitoring networks ("MSY" station). This site presents moderately

acidic soils and montane Mediterranean climate and it is relatively sheltered from the surrounding lowland sources of atmospheric pollutants (Hereter and Sánchez, 1999). The description of the sites was complemented with land use cover and livestock density data obtained from the Corine Land Cover 2006 of the European Environment Agency (http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster-3) and from the Spanish National Statistic Institute (http://www.ine.es) respectively (Table 3.1). ArcGIS software (version 9.2; Environmental Systems Research Institute Inc., Redlands, CA, USA) was employed to summarize these data using a buffer of 25 km radius around the sampling sites (Annex 1.1). Meteorological variables were monitored in CB, TC and LC sites, and data from the closest meteorological station were collected for the CA site.

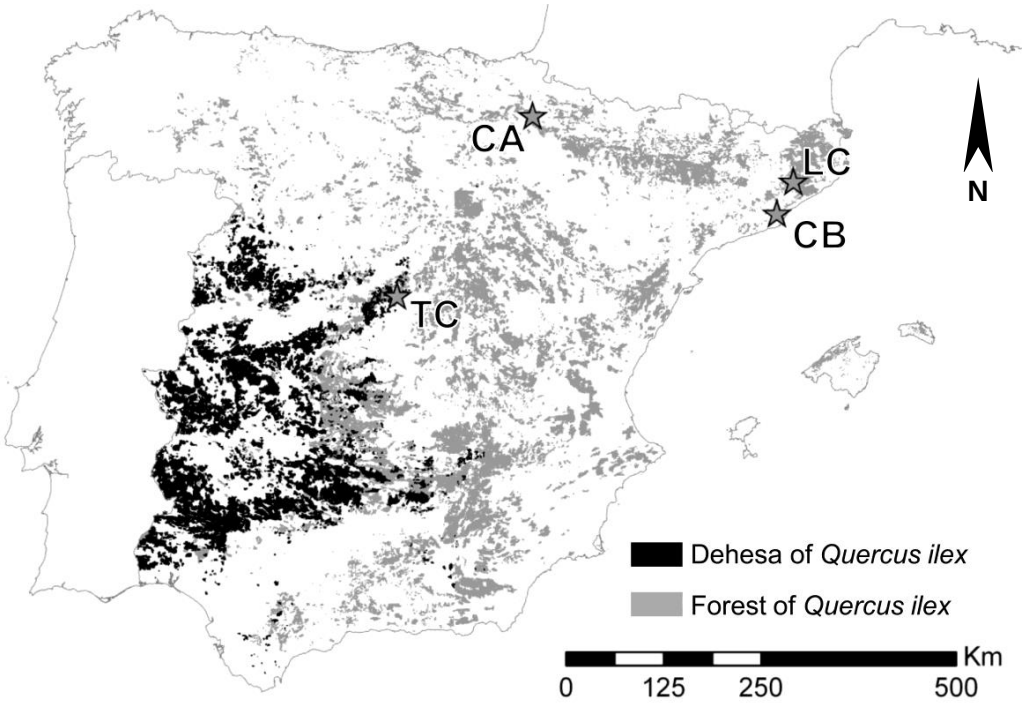

Figure 3.1. Distribution of *Quercus ilex* habitats in Spain and location of the study sites. CA: Carrascal (Navarra); CB: Can Balasc (Barcelona); LC: La Castanya (Barcelona); TC: Tres Cantos (Madrid).

3.2.2. Air pollution monitoring

Atmospheric concentrations of ozone (O_3), ammonia (NH_3), nitrogen dioxide (NO_2) and nitric acid vapour (HNO_3) were monitored during two years using passive samplers. In every location, two plots were installed: an open-field plot (O) and a below-canopy plot (F – forest plot). Open and below-canopy plots were selected in order to maintain the same orientation, exposure and elevation. Two replicate samplers per gaseous species

were exposed at 2 m height in each plot. Gases were measured during two-week-long periods between February 2011 and February 2013; except O_3 in CA, where the sampling survey was only extended until April 2012. Exceptionally, some sampling periods (3% of the total monitoring time) lasted approximately four weeks. In these cases, the same result has been used for the two corresponding regular sampling periods. During every exposure period, unexposed samplers were used as blanks for each site and type of passive sampler. After collection, all samples were kept refrigerated (4 ºC) in darkness until they were analysed in the laboratory.

Table 3.1. Characterization of the study sites.

Site code	**CB**	**TC**	**CA**	**LC**
Site name	**Can Balasc**	**Tres Cantos**	**Carrascal**	**La Castanya**
Province (administrative unit)	Barcelona	Madrid	Navarra	Barcelona
Type of location	Peri-urban	Peri-urban	Peri-urban	Rural
Altitude (m)	255	705	592	696
Mean annual temperature (ºC) [a]	15.2	14.6	12.3	13.7
Mean annual rainfall (mm y^{-1}) [a]	652	348	645	812
Distance to the nearest big city (km)	4	9	15	40
Population of the nearest big city (million inhabitants)	1.6	3.2	0.20	1.6
Distance to the nearest highway (km)	0.15	1.5	0.05	16
Average daily flow in the nearest road (thousand vehicles day^{-1}) [b]	40 – 50	50 – 60	20 – 30	20 – 30
Agricultural land-use cover [c]	23%	21%	62%	23%
Artificial land-use cover [c]	35%	28%	3.1%	7.6%
Livestock density (LU km^{-2}) [d]	14.5	13.7	26.9	88.8

[a]: Mean values calculated for the study period Februay 2011 – February 2013.
[b]: Values for 2012 from the Spanish Ministry of Development (http://www.fomento.gob.es/).
[c, d]: From the Corine Land Cover 2006 (http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster-3) and the Spanish National Statistic Institute (http://www.ine.es), respectively, using a buffer of 25 km radius around the sampling sites.

Tube-type samplers (Radiello®) were used to measure atmospheric concentrations of NH_3, NO_2 and O_3. Laboratory analyses were performed according to Radiello's specifications (Fondazione Salvatore Maugeri, 2006). Atmospheric concentrations of HNO_3 were measured by means of badge-type samplers manufactured following Bytnerowicz et al., (2005). In CA, Passam® passive samplers and methods were employed during the second year for monitoring NO_2 after checking their comparability with Radiello®. For these sampling periods, correction proposed by Plaisance (2011) was applied to avoid biases caused by high wind speeds. The variability of the duplicate passive samplers for each air pollutant averaged from 7% for O_3 to 28% for HNO_3.

Additionally, concentration of O_3 and nitrogen oxides (NO and NO_2) were continuously monitored in open-field locations in LC and TC sites with active monitors (in LC: MCV® 48AV and Thermo Scientific® 42i-TL, respectively; in TC: ML® 9810B and ML® 9841, respectively). Simultaneous measurements with passive samplers and active monitors were used to estimate mean experimental sampling rates, which were applied to calculate atmospheric concentrations. The experimental sampling rates obtained in LC were employed in CB and CA calculations as well, after checking the similarity with concentrations registered at the closest air quality monitoring stations.

Using the data from the active monitors, accumulated O_3 exposure was calculated as AOT40, which is the accumulated amount of hourly O_3 concentrations over the threshold value of 40 nl l^{-1}. Following the Ambient Air Quality Directive 2008/50/EC, AOT40 was calculated for the period May–July with the hourly mean values from 8 to 20 hours. Additionally, following the recommendations from the Convention on Long-Range Transboundary Air Pollution (CLRTAP 2011), AOT40 was calculated for the entire year (the growing season for *Q. ilex*) during daylight hours.

3.2.3. Particulate matter sampling

Particulate matter with diameter up to 10 μm (PM_{10}) was collected with 150 mm quartz micro-fibre filters (2500 QAO-UP, Pall Life Sciences) using high volume samplers installed in open-field plots of TC, CA and LC sites (Digitel® DH80 in LC -MSY monitoring station; MCV® CAV-A/mb in TC and CA). Samples were collected from February 2012 to February 2013 once a week, using a flow of 30 m^3 h^{-1} during 24-h periods. The day of the week for PM_{10} collection changed weekly. The concentration was gravimetrically determined and main secondary inorganic aerosols (SO_4^{2-}, NO_3^- and NH_4^+) were water-extracted and analysed by ion chromatography. For statistical

comparison purposes with gaseous pollutant concentrations, PM_{10} data were grouped and averaged in accordance to passive sampling periods (except for the comparison of the natural dust events with the rest of the samples).

3.2.4. Statistical analysis

Non-parametric statistics was selected for this study because most of the variables did not show a normal distribution according to Shapiro-Wilk test and normal probability plots. Differences among seasons or sites were analysed using the Kruskal-Wallis test; when significant differences were found, differences between pairs of sites were assessed with the Mann-Whitney U test. Correlation between variables was tested with the Spearman rank order correlation coefficient. Differences in pollutant concentration between O and F plots were analysed by applying the Wilcoxon matched pair test to the entire sampling period. The temporal variability is described in this study by the coefficient of variation (CV = standard deviation / mean) of the two-week concentrations for the entire study period. The variability of the duplicate passive samplers for each air pollutant is also described by their respective CV. In this work, seasons were considered as periods of three consecutive months, beginning on 1st January. Statistica software (version 12; StatSoft, Tulsa, OK) was used for statistical analysis. Alfa level was set at 0.05.

3.3. Results

3.3.1. Temporal and spatial patterns of gaseous pollutants

Seasonal and annual pollutant concentrations and differences among sites are described below based on concentrations in the O plots (Fig. 3.2; Table 3.2).

The annual mean of atmospheric NO_2 concentration ranged from 4.3 µg m^{-3} in LC to 16.2 µg m^{-3} in CB (Table 3.2). The highest two-week concentration reached 39.3 and 37.1 µg m^{-3} registered in CB and TC respectively during the winter 2012 (Annex 3.1). On average for the four sites, temporal variability of NO_2 concentration was 53%. Levels of NO_2 tended to peak during the coldest seasons (autumn and winter). Significant seasonal differences were detected in the sites closest to the big cities of Barcelona and Madrid (CB and TC). LC experienced the lowest concentrations and the lowest inter-seasonal variability (Fig. 3.2).

Table 3.2. Basic statistics of the monitored pollutant concentrations in open-field plots for the entire monitoring periods.

	SITE	MEAN	MIN. – MAX.	CV
NO_2 (µg m^{-3})	**CB**	16.2 ± 1.0 a	5.7 – 39.3	42%
	TC	11.1 ± 1.1 b	3.8 – 37.1	71%
	CA	10.6 ± 0.7 b	4.4 – 26.0	45%
	LC	4.3 ± 0.3 c	0.8 – 9.4	52%
NH_3 (µg m^{-3})	**CB**	1.0 ± 1.0 b	0.3 – 2.6	53%
	TC	0.7 ± 0.1 c	0.1 – 1.7	60%
	CA	2.5 ± 0.2 a	0.6 – 5.3	47%
	LC	0.7 ± 0.1 c	0.1 – 1.7	59%
HNO_3 (µg m^{-3})	**CB**	2.7 ± 0.6	0.0 – 14.5	134%
	TC	1.5 ± 0.2	0.0 – 6.4	73%
	CA	2.3 ± 0.3	0.3 – 9.7	98%
	LC	3.3 ± 0.7	0.0 – 13.9	134%
O_3 (µg m^{-3})	**CB**	57.0 ± 2.4 c	10.8 – 86.1	30%
	TC	69.1 ± 2.9 b	28.7 – 101.4	30%
	CA	77.4 ± 4.7 a	25.3 – 122.3	32%
	LC	78.2 ± 3.2 a	34.9 – 117.3	29%
PM_{10} (µg m^{-3})	**TC**	23.0 ± 3.2 ab	5.2 – 61.0	67%
	CA	26.9 ± 2.6 a	6.8 – 49.2	41%
	LC	18.0 ± 1.5 b	4.8 – 32.8	41%
NO_3^- (µg m^{-3})	**TC**	1.3 ± 0.4 b	0.1 – 8.1	129%
	CA	2.2 ± 1.5 a	0.5 – 8.8	99%
	LC	1.1 ± 0.2 b	0.2 – 4.2	80%
NH_4^+ (µg m^{-3})	**TC**	0.6 ± 0.1	0.2 – 2.7	54%
	CA	0.9 ± 0.2	0.3 – 3.7	97%
	LC	0.5 ± 0.1	0.0 – 1.6	71%
SO_4^{2-} (µg m^{-3})	**TC**	1.2 ± 0.2 b	0.1 – 4.2	70%
	CA	1.9 ± 0.2 a	0.8 – 3.7	48%
	LC	1.7 ± 0.2 a	0.4 – 3.3	52%

Mean: arithmetic mean ± standard error. Min.–Max.: Minimum and maximum two-week values. CV: coefficient of variation, representing the temporal variability. Different letters indicate significant differences ($p < 0.05$) between sites. The absence of letters indicates no significant differences.

Atmospheric NH_3 concentration (Table 3.2) was the highest in CA (2.5 µg m^{-3}) and the lowest in TC and LC (0.7 µg m^{-3}). The maximum two-week value (5.3 µg m^{-3}) was recorded in CA during late winter (Annex 3.2). The temporal variability showed a mean of 55% across sites. A consistent seasonal pattern was found in TC, where NH_3 concentration increased during spring and summer and decreased during autumn and winter (Fig. 3.2; Annex 3.2). LC showed a similar seasonal pattern but differences were not statistically significant ($p = 0.06$). On the contrary, in CB and CA, the highest seasonal concentrations occurred in winter.

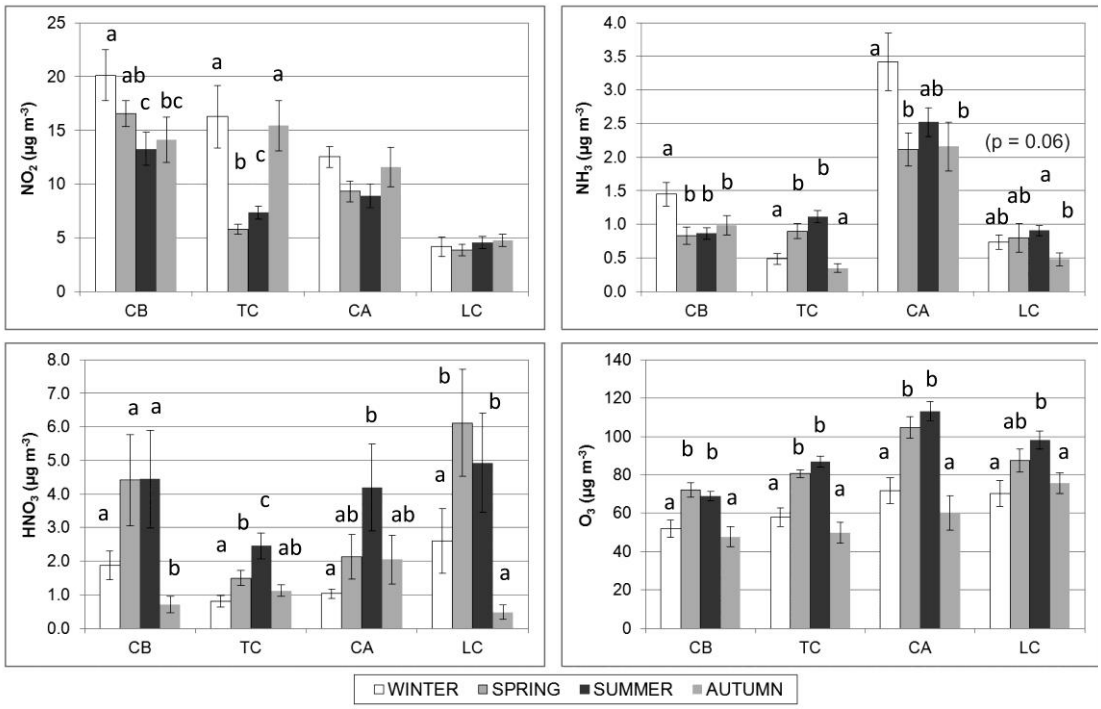

Figure 3.2. Seasonal mean concentration of atmospheric pollutants in the open-field (O) plots of the four study sites and standard error of the mean. Different letters indicate significant differences among seasons.

The concentration of HNO_3 tended to be higher in the sites closest to the Mediterranean coast (CB and LC), but differences among sites were not statistically significant (Table 3.2). The maximum two-week concentrations found in CB and LC (14.5 and 13.9 µg m^{-3} in summer of 2012, respectively) were twice the maximum values found in TC and CA (Annex 3.3). The temporal variability in HNO_3 concentration was higher than the variability found for the other air pollutants, with an average value of 110%. A general seasonal pattern was detected in HNO_3 concentrations, with higher values during spring and summer and lower values in autumn and winter (Fig. 3.2).

The annual mean of atmospheric O_3 concentrations (Table 3.2) were significantly lower in the sites closest to the big cities of Barcelona and Madrid (57.0 µg m^{-3} in CB and 69.1 µg m^{-3} in TC) than in the more rural ones (77.4 µg m^{-3} and 78.2 µg m^{-3} in CA and LC, respectively). Ozone was the air pollutant showing the smallest temporal variability with a mean value of 32%. All sites showed similar seasonal patterns with higher O_3 concentration during spring and summer than in autumn and winter (Fig. 3.2). Ozone exposure accumulated during May–July expressed as AOT40 ranged from 3.9 ppm h in CA in 2011 to 28.3 ppm h in TC in 2012 (Table 3.3). When accumulating O_3 exposure throughout the growing season, AOT40 values ranged from 8.2 ppm h in CA in 2011 to 49.6 ppm h in TC in 2012 (Table 3.3).

Table 3.3. Ozone exposure expressed as AOT40 for years 2011 and 2012, following criteria from the Convention on Long-range Transboundary Air Pollution (CLRTAP) and the Ambient Air Quality Directive 2008/50/EC.

AOT40 (ppm h)				
SITE	**CLRTAP (Jan–Dec)**		**Directive 2008/50/EC (May–July)**	
	2011	**2012**	**2011**	**2012**
CB	8.2	18.8	3.7	9.4
TC	31.8	49.6	17.4	28.3
CA	32.6	32.3	15.5	16.5
LC	27.3	34.9	12.5	18.3

3.3.2. Temporal and spatial patterns of particulate matter

The concentration of PM_{10} was higher in CA and TC than in LC (Table 3.2), although differences were only significant between CA and LC, which showed the lowest annual concentration (18.0 µg m^{-3}). Temporal variability in PM_{10} concentrations was 50% on average for the three sites. Significant seasonal variations were found in TC and LC, with the highest PM_{10} concentrations registered in summer and the lowest in autumn (Fig. 3.3a). Saharan dust events represented 10% of the total amount of samples, and occurred more frequently during the summer season. In the three sites, the highest 24h-concentrations of PM_{10} (up to 126.4 µg m^{-3}) were collected during these natural dust events, generally doubling the levels found in the rest of the samples (Fig. 3.3b).

Regarding SIA composition, no differences among sites were found in particulate ammonium (NH_4^+), while particulate nitrate (NO_3^-) was significantly the highest in CA (Table 3.2). Apparently, Saharan dust intrusions did not affect the NH_4^+ and NO_3^- concentration in PM_{10} (data not shown). The atmospheric concentration of both water-soluble nitrogen aerosols showed a marked seasonality, with higher values detected in winter than in the rest of seasons (Figs. 3.3c and 3.3d). However, only for NO_3^- in CA and LC, these differences were statistically significant. Gaseous nitrogen forms generally predominated over the particulate forms, particularly in spring and summer (Figs. 3.3e and 3.3f). However, NO_3^- clearly predominated over HNO_3 during winter in TC and CA and during autumn in LC, and NH_4^+ predominated over NH_3 during winter in TC. Additionally, no seasonal variations were recorded in ammonium gas/particle ratio in CA (Figs. 3.3f).

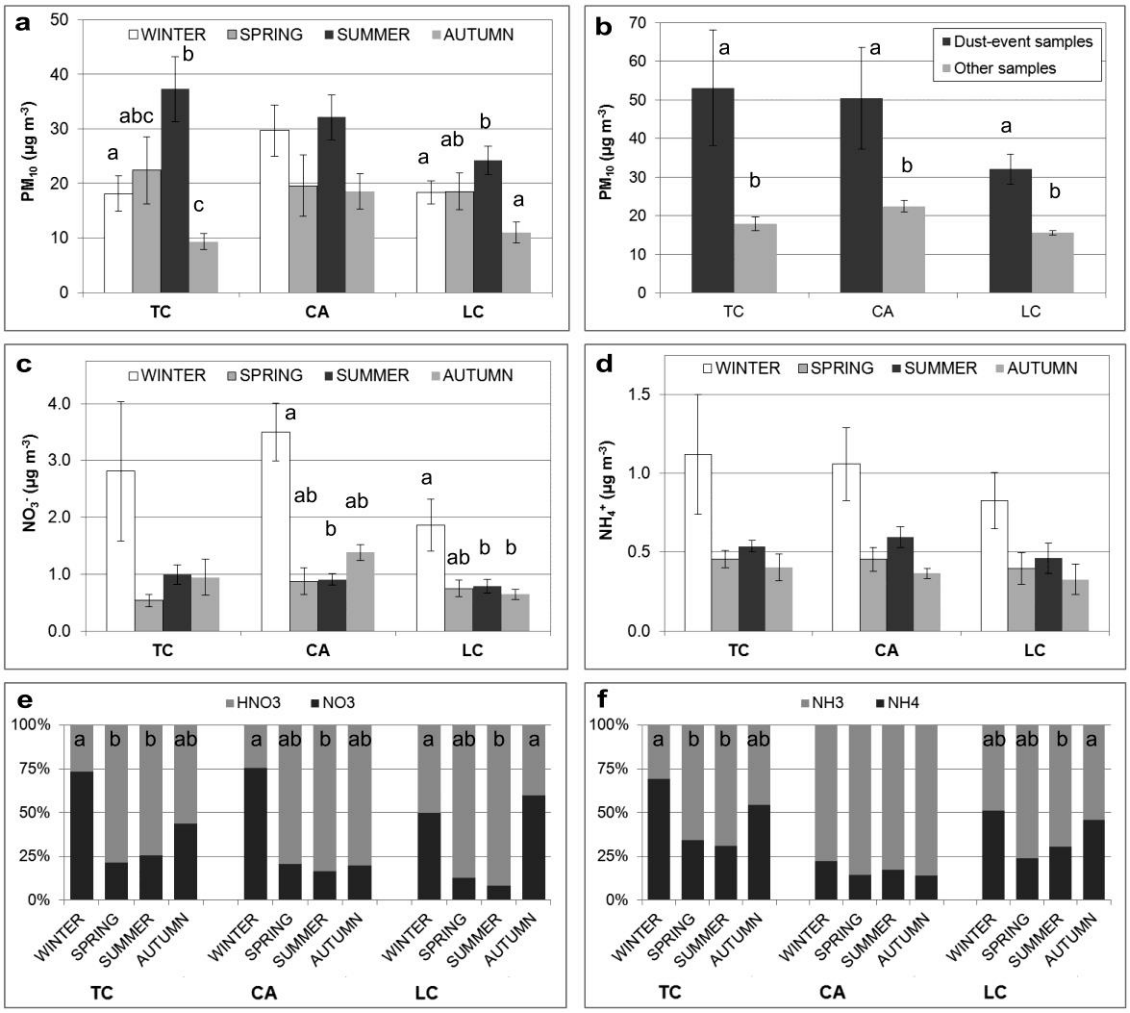

Figure 3.3. Seasonal mean concentrations of aerosols and standard errors, and ratios of particulate to gaseous pollutants in the three aerosol monitoring sites. a) PM_{10} concentration; b) PM_{10} concentration for measurements during Saharan dust events compared with the rest of the samples; c) particulate nitrate concentrations; d) particulate ammonium concentrations; e) concentrations ratios of nitric acid and particulate nitrate, expressed as percentage of the sum of both compounds; f) concentrations ratios of ammonia and particulate ammonium, expressed as percentage of the sum of both compounds. Different letters indicate significant differences between seasons. One outlier value (CA, spring) was removed from the graphs c–f.

3.3.3. Differences in gaseous pollutant concentrations between open-field and below-canopy plots

Below-canopy concentrations of gaseous pollutants were, in general, smaller than levels found in the open-field plots (Fig. 3.4). These differences were more remarkable for NH_3, which showed an annual mean concentration in F plots 40% lower than in the O plots in average for the four sites (56% in LC, and 29–38% in the peri-urban forests). In the case of NO_2, differences were not significant in CB, while the concentrations were significantly lower in the F plots in the rest of sites (41% in CA, 13% in TC and 6% in LC). For HNO_3, the reduction detected inside the forest was significant in TC and CA, showing average concentrations 11–13% lower in the F plot compared to the O plot. Ozone concentrations were significantly lower inside the forests in TC and LC (annual mean difference of 7% and 5%, respectively).

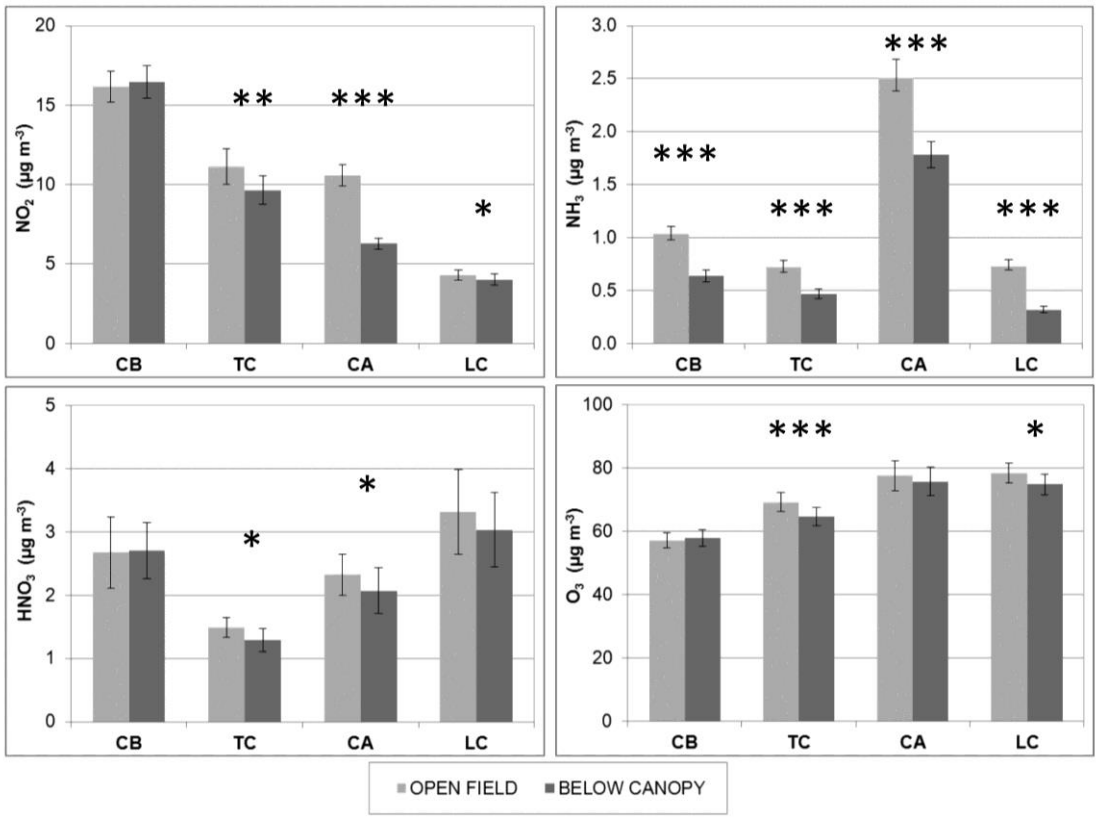

Figure 3.4. Mean concentration of pollutants in O plots (open field) and F plots (below canopy), and standard error of the mean. Significance of the Wilcoxon matched pairs test: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

The reduction of air pollutant concentrations inside the forest showed few evident seasonal patterns. Nitrogen dioxide experienced the highest decrease in concentrations below-canopy (Annex 3.1) during autumn and winter in TC and CA (none and 34% on

average for both seasons, respectively), while in LC this difference was larger in spring (18%). The differences in NH_3 levels were consistent most of the time (31% on average; Annex 3.2), although smaller during the summer in the three peri-urban forests. Regarding HNO_3 (Annex 3.3), differences between forest and open plots were slightly higher during spring and autumn in TC and CA (24% in both sites, averaged for both seasons). The reduction of O_3 concentrations inside the forest resulted slightly larger during summer and autumn (8% in TC and 7% in LC, averaged for both seasons; Annex 3.4).

3.3.4. Correlation analysis of pollutant concentrations and meteorology

Atmospheric concentrations of NO_2 were poorly correlated with meteorological variables, with the exception of TC site, where NO_2 levels were negatively correlated to temperature, daily solar radiation and wind speed, and positively correlated to relative humidity. In the rest of sites, NO_2 concentrations were negatively correlated with precipitation in CB and LC, and with wind speed in CA (Table 3.4). In the case of NH_3 concentrations, no correlation was found in CA. In the other sites, relative humidity was negatively correlated to NH_3 concentration, while temperature and daily solar radiation were positively correlated in TC and LC, and negatively in CB. Concentrations of HNO_3 and O_3 were positively correlated with temperature and daily solar radiation, and negatively with relative humidity in all sites. Besides, HNO_3 and O_3 concentrations showed a positive correlation with wind speed in TC and CA, and a negative correlation with precipitation in TC (Table 3.4).

The concentrations of PM_{10} were negatively correlated with precipitation in TC and CA and positively with solar radiation and temperature in TC and LC. In TC, PM_{10} was also negatively correlated with humidity. Besides, PM_{10} was negatively correlated with wind speed in LC. Particulate nitrate was negatively related to temperature and solar radiation only in CA. NH_4^+ concentrations did not show important correlations with meteorological variables. Particulate SO_4^{2-} was positively correlated to temperature and solar radiation and negatively with wind speed only in LC (Table 3.4).

No significant correlations among gaseous pollutant were found in CA. In the other sites, O_3 and HNO_3 concentrations were positively correlated (Table 3.4). In TC, O_3 was also negatively correlated to NO_2 and NH_3 was positively correlated to O_3 and HNO_3. Particulate NH_4^+ concentration was correlated with particulate NO_3^- in the three sites, and with SO_4^{2-} in CA and LC. However, NH_4^+ was not correlated with NH_3 in any of

the sites. Particulate nitrate was positively related to NO_2 in TC and CA, and negatively correlated with HNO_3 only in CA (Table 3.4). Ammonia and HNO_3 concentrations were positively correlated to PM_{10} in TC and LC. Finally, scarce significant correlations with meteorological variables were found for the below-canopy reductions of atmospheric pollutant concentrations (data not shown).

3.4. Discussion

3.4.1. Air pollution affecting peri-urban forests

The annual mean of atmospheric NO_2 concentrations decreased from CB to LC (from 16.2 to 4.3 μg m^{-3}), indicating an order of influence of urban and traffic emissions (CB > TC ≥ CA > LC). The levels of NO_2 in the three peri-urban forests (CB, TC and CA) were in the range of values recorded in suburban background monitoring stations in 2012 (AirBase v8 dataset; EEA, 2014). Therefore, suburban stations might be considered representative of NO_2 concentration registered in peri-urban forests. Concentrations of NO_2 in the three peri-urban forests followed the expected seasonal pattern of monitoring stations influenced by urban emissions, with highest values recorded during autumn and winter. This seasonal pattern is associated with increasing emissions due to urban combustion for heating purposes and with the lower photochemical intensity during the cold season (Karanasiou et al., 2014). The decrease of NO_2 with wind speed in TC and CA pointed to a higher influence of local sources rather than regional contribution. Similar results have been reported in other Mediterranean urban sites (Karanasiou et al., 2014). An analogous response would be expected at CB, but the higher urban density around the site and the lower wind speed (annual mean of 0.8 m s^{-1}) could be impairing pollutant dispersion. The forest site in LC was more representative of background NO_2 concentrations, since the annual mean was close to the average value of 3.7–3.5 μg m^{-3} recorded in background stations in Spain in 2011 and 2012 respectively (MAGRAMA, 2014). Moreover, NO_2 concentrations in LC did not show clear seasonal variations, demonstrating the lack of influence of urban emissions. After adding the estimated NO concentration (from the active monitors), none of the sites are expected to reach the critical level for the protection of vegetation (30 μg m^{-3}, as annual mean) established in the European Air Quality Directive.

Table 3.4. Spearman rank order correlation coefficients for pollutant concentrations in open-field plots, particulate matter (PM) and meteorological variables.

CB								
	NO_2 (µg m^{-3})	NH_3 (µg m^{-3})	HNO_3 (µg m^{-3})	O_3 (µg m^{-3})	PM_{10} (µg m^{-3})	NO_3^- (µg m^{-3})	NH_4^+ (µg m^{-3})	SO_4^{2-} (µg m^{-3})
NH_3 (µg m^{-3})	0.35							
HNO_3 (µg m^{-3})	n.s.	n.s.						
O_3 (µg m^{-3})	n.s.	n.s.	**0.57**					
PM_{10} (µg m^{-3})								
NO_3^- (µg m^{-3})								
NH_4^+ (µg m^{-3})								
SO_4^{2-} (µg m^{-3})								
Temperature (ºC)	n.s.	**-0.40**	**0.48**	**0.61**				
Relative Humidity (%)	-0.32	**-0.41**	**-0.42**	**-0.42**				
Solar Rad. (W m^{-2})	n.s.	-0.29	**0.71**	**0.69**				
Wind Speed (m s^{-1})	n.s.	n.s.	n.s.	n.s.				
Precipitation (mm)	**-0.49**	**-0.49**	n.s.	n.s.				

TC								
	NO_2 (µg m^{-3})	NH_3 (µg m^{-3})	HNO_3 (µg m^{-3})	O_3 (µg m^{-3})	PM_{10} (µg m^{-3})	NO_3^- (µg m^{-3})	NH_4^+ (µg m^{-3})	SO_4^{2-} (µg m^{-3})
NH_3 (µg m^{-3})	-0.36							
HNO_3 (µg m^{-3})	n.s.	**0.63**						
O_3 (µg m^{-3})	**-0.62**	**0.69**	**0.62**					
PM_{10} (µg m^{-3})	n.s.	**0.70**	**0.58**	**0.58**				
NO_3^- (µg m^{-3})	**0.53**	n.s.	n.s.	n.s.	n.s.			
NH_4^+ (µg m^{-3})	n.s.	n.s.	n.s.	n.s.	0.42	**0.69**		
SO_4^{2-} (µg m^{-3})	n.s.	n.s.	n.s.	n.s.	**0.71**	n.s.	n.s.	
Temperature (ºC)	**-0.50**	**0.85**	**0.68**	**0.82**	**0.62**	n.s.	n.s.	n.s.
Relative Humidity (%)	**0.42**	**-0.86**	**-0.59**	**-0.86**	**-0.72**	n.s.	n.s.	n.s.
Solar Rad. (W m^{-2})	**-0.63**	**0.85**	**0.56**	**0.85**	**0.68**	n.s.	n.s.	n.s.
Wind Speed (m s^{-1})	**-0.71**	**0.39**	0.33	**0.66**	n.s.	n.s.	n.s.	n.s.
Precipitation (mm)	n.s.	**-0.44**	**-0.38**	**-0.39**	**-0.55**	n.s.	n.s.	n.s.

CA								
	NO_2 (µg m^{-3})	NH_3 (µg m^{-3})	HNO_3 (µg m^{-3})	O_3 (µg m^{-3})	PM_{10} (µg m^{-3})	NO_3^- (µg m^{-3})	NH_4^+ (µg m^{-3})	SO_4^{2-} (µg m^{-3})
NH_3 (µg m^{-3})	n.s.							
HNO_3 (µg m^{-3})	n.s.	n.s.						
O_3 (µg m^{-3})	n.s.	n.s.	n.s.					
PM_{10} (µg m^{-3})	n.s.	n.s.	n.s.	n.s.				
NO_3^- (µg m^{-3})	**0.69**	n.s.	**-0.77**	n.s.	n.s.			
NH_4^+ (µg m^{-3})	n.s.	n.s.	n.s.	n.s.	n.s.	0.51		
SO_4^{2-} (µg m^{-3})	n.s.	n.s.	n.s.	n.s.	**0.60**	n.s.	**0.70**	
Temperature (ºC)	n.s.	n.s.	**0.55**	**0.67**	n.s.	**-0.75**	n.s.	n.s.
Relative Humidity (%)	n.s.	n.s.	**-0.45**	**-0.73**	n.s.	0.49	n.s.	n.s.
Solar Rad. (W m^{-2})	n.s.	n.s.	**0.41**	**0.84**	n.s.	**-0.71**	n.s.	n.s.
Wind Speed (m s^{-1})	-0.36	n.s.	0.30	0.46	n.s.	-0.48	n.s.	n.s.
Precipitation (mm)	n.s.	n.s.	n.s.	n.s.	**-0.62**	n.s.	n.s.	n.s.

LC								
	NO_2 (µg m^{-3})	NH_3 (µg m^{-3})	HNO_3 (µg m^{-3})	O_3 (µg m^{-3})	PM_{10} (µg m^{-3})	NO_3^- (µg m^{-3})	NH_4^+ (µg m^{-3})	SO_4^{2-} (µg m^{-3})
NH_3 (µg m^{-3})	n.s.							
HNO_3 (µg m^{-3})	n.s.	0.34						
O_3 (µg m^{-3})	n.s.	0.30	**0.41**					
PM_{10} (µg m^{-3})	n.s.	**0.60**	**0.84**	**0.52**				
NO_3^- (µg m^{-3})	n.s.	n.s.	n.s.	n.s.	n.s.			
NH_4^+ (µg m^{-3})	n.s.	n.s.	n.s.	n.s.	**0.58**	**0.51**		
SO_4^{2-} (µg m^{-3})	n.s.	0.49	**0.71**	**0.65**	**0.85**	n.s.	**0.60**	
Temperature (ºC)	n.s.	0.36	**0.50**	**0.56**	**0.65**	n.s.	n.s.	**0.69**
Relative Humidity (%)	n.s.	**-0.45**	**-0.43**	-0.33	n.s.	n.s.	n.s.	n.s.
Solar Rad. (W m^{-2})	n.s.	**0.43**	**0.74**	**0.55**	**0.77**	n.s.	n.s.	**0.64**
Wind Speed (m s^{-1})	n.s.	n.s.	n.s.	n.s.	**-0.70**	n.s.	**-0.53**	**-0.72**
Precipitation (mm)	**-0.50**	-0.32	n.s.	n.s.	n.s.	-0.43	n.s.	n.s.

n.s.: none statistically significant correlation ($p < 0.05$) was found. Bold numbers are used when $p < 0.01$

The annual mean of NH_3 concentrations in CB, TC and LC were low and similar to the levels recorded in Spanish background stations (0.9 µg m^{-3} in 2012; Hjellbrekke, 2014). These values were lower than concentrations measured in urban backgrounds of their respective closest cities (1.7 µg m^{-3} in Madrid and 7.3 µg m^{-3} in Barcelona; Reche et al., 2014), and far from levels registered in regions with intensive farming or livestock (up to 60 µg m^{-3}; Fowler et al., 1998; Pinho et al., 2012). The higher concentrations found in CA (annual mean of 2.5 µg m^{-3}) probably is related to the presence of livestock in the nearby area. The seasonal pattern of NH_3 concentrations in TC and LC, with higher values during spring and summer, could be explained by an increasing volatilisation and emission of NH_3 from biological sources under warm conditions. In the case of CB, the highest values recorded in autumn and winter might be related to the emissions of NH_3 from an industrial area 6.5 km west of CB. Concentrations of NH_3 at this site were significantly correlated with west winds ($p < 0.01$; data not shown), the most frequent wind in autumn and winter. The winter maxima NH_3 levels in CA were in agreement with the fertilization practices of cereal crops in the region during this season. Since the annual mean of NH_3 concentrations did not exceed the 3 µg m^{-3} critical level proposed for the protection of higher plants in any of the sites, these forests are not expected to experience relevant ammonia pollution effects (CLRTAP, 2011). Moreover, the critical level of 1 µg m^{-3} for the protection of lichens and bryophytes (Cape et al., 2009; CLRTAP, 2011) was only exceeded in CA.

No significant differences in HNO_3 annual concentration were detected among the sites included in this study. The concentrations of HNO_3 in the three peri-urban forests were in the range of values found in other peri-urban areas in the Mediterranean region (summer values of 2.8–4.2 µg m^{-3}; Danalatos and Glavas, 1999) and higher than in urban sites (yearly averaged values of 0.8–1.5 µg m^{-3}; Anatolaki and Tsitouridou, 2007; Tzanis et al., 2009). However, even the highest concentrations were below the values reported in forested areas of San Bernardino Mountains in Southern California, where topography, climate and emissions linked to high population favour HNO_3 formation (Bytnerowicz and Fenn, 1996; Jovan et al., 2012). The typical higher HNO_3 values recorded during spring and summer in the study sites can be explained by the photochemical origin of this pollutant (Bytnerowicz et al., 2010; Tzanis et al., 2009). In this sense, positive correlations between solar radiation and HNO_3 concentration were found for all the sites. The highest levels were found in LC, which must respond to

pollutant-transport mechanisms rather than to an in-situ formation of HNO_3, since this is a rural site with low concentration of NO_2 (chemical precursor of HNO_3). In fact, ageing of air masses over the Iberian Peninsula and recirculation along the Mediterranean coast have been reported as processes increasing levels of oxidants, acidic compounds, aerosols and ozone (Escudero et al., 2014; Millán et al., 2002). Although very little information is available on direct effects of HNO_3 on vegetation, the concentrations found in this study are much lower than the levels reported for epicuticular damage (Padgett et al., 2009a).

The annual mean concentration of O_3 increased from CB to LC, following an opposite order of urban influence to the one found for NO_2 concentration. A similar behaviour has been described in other studies around cities in the Mediterranean area (Domínguez-López et al., 2014; Escudero et al., 2014*)*. CB showed an annual mean similar to values found in 2012 in Spanish suburban areas, while the other sites showed values clearly typical of rural areas (means of 59.0 and 67.8 μg m^{-3}, respectively; EEA, 2014). Ozone concentrations in the peri-urban forests showed the typical seasonal variations with higher levels during spring and summer, responding to the sum of the hemispheric-scale spring maximum, the increased photochemical production and transport processes, as well as the above mentioned ageing of air masses and recirculation (Cristofanelli and Bonasoni, 2009; Millán et al., 2002). In fact, ozone concentrations were significantly correlated with temperature and solar radiation. Besides, the emission of biogenic volatile organic compounds (BVOCs) by vegetation is known to be correlated with temperature, and can exacerbate photochemical reactivity, and thus O_3 formation (Calfapietra et al., 2013). All the calculated AOT40 values were above the concentration-based O_3 critical level proposed by the CLRTAP for protecting forest trees (5 ppm h for the growing season; CLRTAP, 2011). The threshold levels for the protection of vegetation established in the European Directive 2008/50/EC (9 ppm h for the period May–July) were also overreached, with the exception of CB site in 2011. Moreover, experimental values of AOT40 similar to those found in this study have been proved to cause a decrease of growth in seedlings of *Q. ilex* (Alonso et al., 2014; Gerosa et al., 2015).

In the two peri-urban forests with aerosol measurements (TC and CA), the annual mean concentrations of PM_{10} were close to the urban background levels measured in Spanish big cities in 2012 (mean of 26 μg m^{-3}; MAGRAMA, 2014), and well above the values

measured in Spanish background stations (12.9 μg m^{-3}; Hjellbrekke, 2014). On the other hand, concentrations of particulate NO_3^- and NH_4^+ were similar to the national background levels in TC (1.2 μg NO_3^- m^{-3}, and 0.4 μg NH_4^+ m^{-3}; Hjellbrekke, 2014), but almost double in CA. The increased concentration of NO_3^- and NH_4^+ in CA could respond to the elevated NH_3 concentration caused by agricultural activities, which, combined with the low temperatures, facilitates the formation and stability of ammonium nitrate (NH_4NO_3). Moreover, at this site, NO_3^- and HNO_3 showed a negative correlation, suggesting the existence of conversion of one into the other. The seasonality in PM_{10} is in agreement with previous studies that attributed the higher summer concentrations to low precipitation, high resuspension, photochemical oxidation and higher frequency of Saharan dust outbreaks (Escudero et al., 2005; Querol et al., 2008; Rodríguez et al., 2002). Interestingly, the natural events of Saharan dust did not modify NO_3^- and NH_4^+ concentrations. The seasonality observed on particulate N compounds was more related with the thermal instability of NH_4NO_3, pointing out the importance of temperature-dependent processes within the SIA in the Mediterranean region (Querol et al., 2008; Pey et al., 2009). Gaseous HNO_3 and NH_3 predominated over particulate forms most of the year but aerosol fraction was important mainly during winter. This seasonal variation in gas/aerosol ratios may have implications for N dry deposition estimations and, therefore, should be further investigated. Little information is available on direct effects of particles on vegetation and no threshold of aerosol concentration has been defined yet for the protection of vegetation.

According to the established thresholds and the available scientific evidences, the results indicate that O_3 is the only air pollutant considered in this work which is expected to have direct phytotoxic effects on vegetation. The concentrations of N compounds seemed to be not high enough to directly affect vegetation but could be contributing through atmospheric N deposition to the eutrophization of these ecosystems. Moreover, although evergreen broadleaf Mediterranean woody species are assumed to be tolerant to air pollution due to their sclerophyllic adaptations, recent publications suggest that the addition and interaction of different stress factors (O_3, N deposition, drought) can be affecting the growth of the trees (Alonso et al., 2014; Gerosa et al., 2015) and accompanying pastures (Calvete-Sogo et al., 2014). Thus, monitoring of nitrogen compounds such as NH_3 and HNO_3 should be incorporated into air quality monitoring networks.

3.4.2. Below-canopy reduction of atmospheric pollutant concentrations

Air pollutant concentrations measured outside and inside the forest (O and F plots) were compared to analyse the influence of vegetation in air quality. In general, the pollutants considered showed lower concentrations inside the forests. Below-canopy reduction of NO_2 concentration in our study sites ranged from none in CB, to 41% in CA. This high reduction detected in CA could be enhanced by the location of the sampling plots, which were at the same distance, but on the opposite sides of a highway. As a result, the O and F plots were located downwind and upwind from the highway, respectively, in relation to predominant winds (Annex 1.1). Statistically significant reductions of NO_2 concentrations inside holm oak forests were found in TC and LC, with averaged values of 13% and 6%, respectively. These reductions are comparable to (Grundström and Pleijel, 2014) or higher than (Harris and Manning, 2010; Setälä et al., 2013) values reported in similar empirical studies with deciduous forest species. The larger differences in NO_2 levels in LC were detected during spring, the time when holm oak forests usually show higher stomatal conductance (Alonso et al., 2008). Other authors have reported that NO_2 deposition onto forest canopy is governed by plant stomatal aperture (Chaparro-Suárez et al., 2011; Sparks 2009). This behaviour was not observed in TC and CA, where the highest reductions were found during autumn and winter, suggesting that other atmospheric and biogeochemical interactions could be implicated and need further research. In this sense, the lack of below-canopy reduction in CB could not be explained by meteorological variables or different pollutant exposure. Other authors have suggested that NO emissions from forest soil in areas with high O_3 levels, could result in the formation of NO_2 below the canopy (Harris and Manning, 2010; Fowler, 2002), diminishing the difference of NO_2 concentrations between outside and inside the canopy. Since dry deposition of atmospheric pollutants depends on multiple factors such as micrometeorology, spatial heterogeneity, plant structure and physiology, and biochemical interaction, further research is needed to clarify the influence of vegetation on air quality.

Below-canopy concentrations of NH_3 were on average 40% lower than in the open field, suggesting that holm oak forests act as sinks of ammonia. This difference was relatively higher in the most natural forest (56% in LC) than in the peri-urban ones (29–38%). Since NH_3 stomatal fluxes are bi-directional, emission or deposition of NH_3 will occur depending on ecosystem N-status, stomatal conductance, and the ratio between

atmospheric and canopy NH_3 concentration (Behera et al., 2013; Fowler et al., 2009). The below-canopy reductions of NH_3 were consistent throughout most of the year, but smaller during the summer, a period of low plant physiological activity in this type of forest. These results indicate a certain regulation of NH_3 fluxes by stomatal uptake. However, NH_3 canopy retention was not the highest in spring, when plants usually experience maximum stomatal conductance, thus other mechanisms must affect the overall ammonia retention by the canopy in autumn and winter. Among other major drivers of atmospheric NH_3 deposition into the canopy, leaf area density, and leaf surface wetness and acidity can enhance the deposition onto the cuticles and epiphytic communities (Geiser et al., 2010; Massad et al., 2010a).

The differences in HNO_3 concentration between O and F plots were only significantly detected in TC and CA, with reductions of 11–13% on annual average. Among the N gaseous pollutants, HNO_3 is supposed to have the highest surface deposition velocity due to its highly reactive and soluble nature, which should lead to large rates of deposition onto leaf surfaces (Fowler et al., 2009). However, the rates of bellow-canopy HNO_3 reduction are similar to those of NO_2 in TC and LC, and lower than those of NH_3. No clear seasonal patterns were found in the below-canopy reduction of HNO_3 concentrations that could indicate the main processes involved in HNO_3 dry deposition in these forests.

In regards to O_3 concentrations, urban and peri-urban vegetation has been proposed as a strategy to absorb O_3 and diminish atmospheric concentrations (Alonso et al., 2011; Kroeger et al., 2014). In our study, O_3 levels were significantly reduced inside the forests in TC and LC with an average decrease of 5–7%. The largest below-canopy reduction of O_3 concentration occurred in summer and autumn, suggesting that stomatal uptake was not the only process involved in this decline, since stomatal conductance in usually low during the summer in these forests due to drought stress. Actually, non-stomatal O_3 deposition in holm oak forests has been reported to account up to ca. 60 % of the total ozone flux (Fares et al., 2014). Surface wetness of the canopy and other forest surfaces can enhance non-stomatal deposition of O_3 (Altimir et al., 2006). This process could explain the higher reductions of O_3 detected during autumn, the wettest season in all the sites. Besides, increased BVOCs emissions linked to high temperatures during the summer could be favouring the photochemical production of O_3 (Calfapietra et al., 2013). This formation of O_3 should be more apparent in the open-field plots due

to their higher insolation, increasing the difference in O_3 concentrations between O and F plots during this season.

3.5. Conclusions

Peri-urban forests are exposed to air pollutants coming from both urban and rural activities. Ozone concentrations around Spanish cities are high enough to directly impact peri-urban vegetation. The concentrations of N compounds would no directly threat vegetation, but could be contributing, through atmospheric N deposition, to the eutrophization of these ecosystems. Besides, the interaction of different stress factors (O_3, N deposition, drought) could be affecting plant growth and ecosystem functioning. On the other hand, peri-urban forests of *Quercus ilex* have proved to experience a significant below-canopy reduction of pollutant concentrations, particularly of NH_3, but also of NO_2, HNO_3 and O_3. These results provide scientific evidence of the ability of these ecosystems to improve air quality in urban agglomerations, but further research is still needed to quantify the relevance of this ecosystem service. The high variability found in this study across sites and seasons points that processes and environmental factors involved in air pollution removal must be characterized in order to manage these forest for improving air quality. Well-designed monitoring programs of urban and peri-urban forests could accomplish both objectives of further investigate air quality improvement while assessing the threat that air pollution can pose to vegetation.

3.6. Acknowledgements

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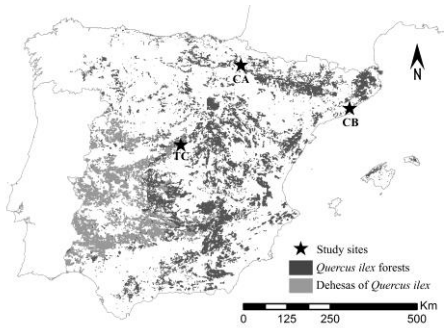
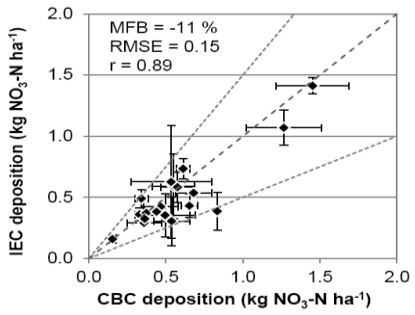
CHAPTER 4
Atmospheric deposition of inorganic nitrogen in Spanish forests of *Quercus ilex* measured with ion-exchange resins and conventional collectors

ABSTRACT

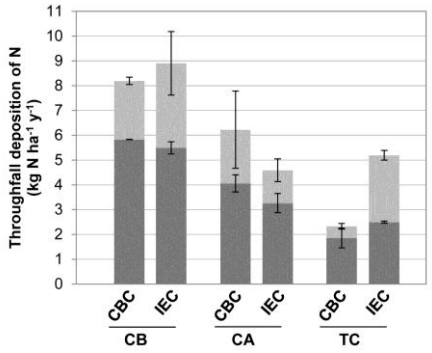
Atmospheric nitrogen deposition is one of the main threats for biodiversity and ecosystem functioning. Measurement techniques less expensive and time-consuming than conventional methods, like ion-exchange resin collectors (IECs), are gaining relevance in the study of atmospheric deposition and are recommended to expand monitoring networks. In the present work, bulk and throughfall deposition of inorganic nitrogen were monitored in three different holm oak forests in Spain during two years. The results obtained with IECs were contrasted with a conventional technique using bottle collectors and with a literature review of similar studies. The performance of IECs in comparison with the conventional method was good for measuring bulk deposition of nitrate and acceptable for ammonium and total dissolved inorganic nitrogen. Mean annual bulk deposition ranged 3.09–5.43 kg N ha^{-1} according to IEC methodology, being on average 0.20 kg NO_3-N ha^{-1} y^{-1} and 0.15 kg NH_4-N ha^{-1} y^{-1} lower than the conventional-method estimates. Intra-annual variability of the net throughfall deposition of nitrogen measured with the conventional method revealed the existence of input pulses of nitrogen into the forest soil after dry periods, presumably originated from the washing of dry deposition accumulated in the canopy. Important methodological recommendations on the IEC method and discussed, compiled and summarized.



EDEN Project: three monitoring sites for comparison of collection techniques



Good to acceptable performance of ion-exchange resin collectors



Annual deposition of N in Mediterranean holm oak forests

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4. Atmospheric deposition of inorganic nitrogen in Spanish forests of *Quercus ilex* measured with ion-exchange resins and conventional collectors

4.1. Introduction

The global alteration of the N cycle has led to an increased deposition of atmospheric nitrogen (N) which could be threatening the extraordinary biological richness of the Mediterranean Basin (Chapter 1; Myers et al., 2000; Rockström et al., 2009). However, limited information is available on atmospheric N deposition and possible effects on the natural ecosystems of Spain. A recent model-based assessment of N deposition threats to habitats within the Spanish Natura 2000 network showed that most of the threatened habitats are located in mountainous and alpine areas in the North (Pyrenees, Cantabrian Range) and mountain areas close to high emission sources, such as the big cities of Madrid and Barcelona, and sclerophyllous forests of *Quercus ilex* in NE Spain (Chapter 1). These high-altitude and orographically-complex areas are difficult to access for monitoring purposes. Besides, current chemical transport models find challenging to simulate small-scale variations in deposition regimes in these areas (Chapter 1; Boutin et al., 2015; Simpson et al., 2006a). Thus, monitoring efforts in such areas would be useful for ecosystem conservation assessments and could be applied for validation and improvement of air quality models.

The use of automatic wet-only samplers, in which the collector is open to the atmosphere only during precipitation events, is widely recommended for monitoring atmospheric wet deposition. But the need to expand monitoring networks has impelled the use of less expensive methods, easy to operate, and without requiring frequent visits to the field (Clow et al., 2015; Erisman et al., 2005). The common alternative is using open samplers (bulk deposition collectors), which have no provision to exclude dry deposition during rainless periods. They basically consist on collection surfaces, typically funnels, ending in containers where the sample is stored until collected for analysis. These conventional collectors are inexpensive and can be easily replicated, but they still require frequent visits to the field. The use of ion-exchange resins (IER) in deposition collectors can dramatically reduce the number of trips to the monitored sites, as well as the number of samples and, therefore, the cost of the analysis (Fenn et al., 2009). The IER collectors are designed and used similarly to conventional collectors,

except that instead of storing the collected sample, the solution is funnelled through an IER column where ions are reversibly adsorbed on the resin. The use of this technique should avoid some of the uncertainties found in deposition measurements with conventional collectors, such as increases or decreases in ammonium concentration ascribed (among others) to biological activity in the solution or adsorption on the bottle walls (Thimonier, 1998) and other changes in sample composition driven by microbiological activity (Dämmgen et al., 2005). Moreover, IER collectors allow long sampling periods (e.g., up to 12 months; Fenn and Poth, 2004), appearing as a suitable tool for monitoring atmospheric deposition in remote locations.

Collectors using IER have been employed to measure atmospheric N input to forest ecosystems since the early 2000s mainly in the USA (e.g. Fenn et al., 2002). To our knowledge, there are not published studies applying this monitoring technique in Mediterranean forests of Europe. In this study, three monitoring sites in three Spanish forests of *Q. ilex* were equipped with conventional and IER collectors to monitor bulk and throughfall deposition of inorganic N (nitrate and ammonium) during two years with the following objectives: (1) to test the performance of IER collectors in relation to the conventional methodology, (2) to provide recommendations for measuring in Mediterranean environments with IER collectors and (3) to study the bulk and throughfall deposition of N in these characteristic Mediterranean forests. In addition, a review of the published works using IER for collecting N deposition was performed and compared with our results.

4.2. Methodology

4.2.1. Study sites

Three holm-oak (*Q. ilex*) forests in Spain, influenced by different pollution sources, soil and climatic conditions were selected for this study (Table 4.1). The Can Balasc (CB) site is located in a forest within a natural protected area 4 km from Barcelona. This site is characterized by acidic soils and a Mediterranean sub-humid climate. This is the site with the highest influence of urban pollutants such as nitrogen dioxide (NO_2) due to its short distance to traffic and urban sources (Table 4.1). The Carrascal site (CA) is located in an agricultural area close to Pamplona (15 km), with calcareous soil and a Mediterranean humid climate. This site is the most agriculturally-influenced among the three forests, with the highest atmospheric concentrations of ammonia (Table 4.1). The

proximity of this site to a highway originates NO_2 values similar to those recorded in suburban background monitoring stations (Chapter 3). The Tres Cantos site (TC) is a forest located in a natural protected area 9 km from Madrid, growing on acidic sandy soil and with a Mediterranean semi-arid climate. The vegetation at TC was historically managed as a traditional dehesa (a savannah-like agrosilvopastoral system) of *Q. ilex*, but the low management intensity during the last decades has allowed vegetation to grow as a moderately open forest (72% of tree cover in the monitored area). Meteorological variables were monitored in the CB and TC sites, and data from the closest meteorological station were collected for the CA site. Further information on the monitoring sites can be found in Table 4.1.

Table 1. Characterization of the study sites.

Site code	**CB**	**TC**	**CA**
Altitude (m)	255	705	592
Longitude	2º 04' 54" E	3º 43' 59" O	1º 38' 40" O
Latitude	41º 25' 47" N	40º 35' 17" N	42º 39' 13" N
Mean annual temperature (ºC) [1]	15.3	14.4	12.3
Mean annual rainfall (mm y^{-1}) [1]	580	386	656
Leaf area index (m^2 m^{-2})	3.3	3.1	5.3
Tree density (number of trees ha^{-1})	1429	491	1760
Distance to the nearest big city (km)	4	9	15
Distance to the nearest highway (m)	150	1500	50
Agricultural land-use cover [2]	23%	21%	62%
Livestock density (LU km^{-2}) [3]	14.5	13.7	26.9
NO_2 concentration (µg m^{-3}) [4]	16.2	11.1	10.6
NH_3 concentration (µg m^{-3}) [4]	1.0	0.7	2.5
HNO_3 concentration (µg m^{-3}) [4]	2.7	1.5	2.3

[1]: Mean values calculated for the study period.
[2, 3]: From the Corine Land Cover 2006 (http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster-3) and the Spanish National Statistic Institute (http://www.ine.es), respectively, using a buffer of 25 km radius around the sampling sites.
[4]: Mean concentration of the main nitrogen gaseous pollutants (Chapter 3).

4.2.2. Sampling and analytical methodologies

Atmospheric deposition of ammonium (NH_4^+) and nitrate (NO_3^-) was monitored in precipitation for two years (spring 2011 – winter 2013) in open-field plots (bulk

deposition) and below the forest canopy (throughfall deposition) by means of IER collectors (IECs). These collectors were manufactured following Fenn and Poth (2004), and consisted of a funnel (20 cm diameter; NILU - Norwegian Institute for Air Research, Kjeller, Norway) attached to a PVC tube with an inner diameter of 15 mm filled with a mixed-bed IER (Amberlite® IRN150) and a valve at the bottom for drainage (Annex 4.1). Generic residue-free synthetic filter wool was inserted in both ends of the resin tube. Each tube was placed in field inside a PVC cylinder to shield it from solar radiation and prevent warming. Replicate IECs were placed in the field with the collection surface at 1.5 m height during periods of 3 to 5 months. For bulk deposition measurements, 4 collectors were deployed in TC and CA, and 2 collectors in CB, in open-field locations; for throughfall deposition measurements, 12 collectors were deployed in TC and CA, and 8 collectors in CB, below the forest canopy. The collectors were deployed randomly in forest with continuous canopy cover. In the case of TC, due to the open structure of the forest, the collectors were randomly placed under dominant trees changing orientations among trees. The entire collector devices were replaced or cleaned in the field at the end of each sampling period, and visually inspected for signs of bird dropping or other potential contaminations of the sample. Once in the laboratory, the columns were pre-rinsed with 100 ml of deionized water and the ions extracted by percolating two 200 ml aliquots of 2*M* solution of KCl (i.e., two consecutive extractions). Extracts were measured for pH as an additional quality control since liquid samples can lose NH_4^+ via NH_3 volatilization as the pH increases (Vlek and Stumpe, 1978). Ammonium and nitrate concentrations in the KCl extracts were determined by automated colorimetric determination using a flow injection analyser (Perkin Elmer, Rodgau, Germany). Background levels of NO_3^- and NH_4^+ in resin columns unexposed to atmospheric deposition and stored at room temperature were also determined and used as blanks. Finally, a set of IECs were spiked with known quantities of NO_3^- and NH_4^+ in laboratory conditions and were extracted and analysed following the same procedure to determine the adsorption and recovery efficiencies of the IECs for this experiment (Table 4.2).

Available published studies using similar IECs (or at least applying similar laboratory tests to them) were collected, and their methodological data (when available) were recorded or estimated from tables or figures. Results from this methodological review are presented in Table 4.2 and Annex 4.2.

Nitrogen deposition was additionally monitored by means of conventional collectors using high-density polyethylene bottles to store the sampled solution (conventional bottle collectors; CBCs) instead of tubes containing IER (see Annex 4.1 for comparison). These bottles were also solar-shielded by PVC tubes. These CBCs were deployed in the study sites paired with the IECs at a distance of 1–1.5 m from each other, applying the same replication, funnel type and height. The CBC samples were collected on a weekly-basis by replacing the collection bottle. During the longest rainless periods, the CBC funnels were rinsed with 100 ml of deionised water every two weeks before replacing the bottle, in order to collect and measure the dry deposition into the funnel. The sampling procedure, storage, analysis by ion chromatography (Dionex, Sunnyvale, USA) and quality control of the analytical results of the CBC samples were performed following the recommendations of the ICP Forests Manual (Clarke et al. 2010), and they are further described in Izquieta-Rojano et al. (2016).

For both measurement methodologies, a filter mesh was placed moderately tight into all the funnels to avoid, as much as possible, the accumulation of litterfall at the bottom of the funnels and a bug-sieve (NILU, Kjeller, Norway), consisting in a perforated plastic disk, was included inside the connection to the bottle. The upper edges of the funnels were equipped with an external metal ring to prevent birds from perching on the tube perimeter (Annex 4.1).

4.2.3. Calculations, comparison metrics and statistical analysis

Nitrogen deposition in each IEC was calculated multiplying the concentration of nitrate-N (NO_3-N) and ammonium-N (NH_4-N) by the volume of the extracting solution and adding the results from the two extractions. Then, the background levels measured in the unexposed blank IER tubes were subtracted, and the result divided by the collection surface. Finally, these results were corrected by the recovery efficiency factor (Table 4.2). For the CBCs, the deposition was calculated for each collection bottle by multiplying the sample concentration by the volume collected (or the rinsing volume for the rainless periods). Deposition values from the replicated samples were then averaged for each period and plot (open-field and below-canopy plots) for both methodologies. Deposition of total dissolved inorganic nitrogen (DIN) was calculated by adding NO_3-N and NH_4-N deposition values. Annual deposition values were calculated as the sum of the sampling period values during the year. When the sum of periods did not exactly match with the duration of a natural year, values were weighted by their added-up

sampling time. Deposition values (means and standard deviations) of the CBC periods were combined to match the IEC periods for comparison purposes, and also grouped and combined on a monthly basis to summarize and describe the intra-annual variability of the N deposition. Net throughfall deposition was calculated subtracting bulk deposition values from throughfall deposition values for every period and methodology.

To compare the two methods, three accuracy metrics commonly used in model evaluation (Boylan and Russell, 2006) were calculated for NO_3-N, NH_4-N and DIN deposition data obtained with both methods: the mean fractional bias (MFB; eq. 1), the root mean square error (RMSE; eq. 2) and the Pearson correlation coefficient (*r*; eq. 3).

$$MFB = \frac{1}{N}\sum\left(\frac{IEC_i - CBC_i}{\frac{IEC_i + CBC_i}{2}}\right) \quad (1)$$

$$RMSE = \left[\frac{1}{N}\sum(IEC_i - CBC_i)^2\right]^{\frac{1}{2}} \quad (2)$$

$$r = \frac{\sum(CBC_i - \overline{CBC})(IEC_i - \overline{IEC})}{\sqrt{\sum(CBC_i - \overline{CBC})^2 \sum(IEC_i - \overline{IEC})^2}} \quad (3)$$

where N is the number of data pairs from the IEC and CBC methods, $\overline{CBC}$ and $\overline{IEC}$ are the mean values for the N data and the index *i* is over the time series, including all the monitoring sites.

Variability of deposition values among collectors (precision of the measurements) was calculated as the coefficient of variation (CV = standard deviation / mean) for every plot and period with two or more sampling data available. Correlations between environmental variables and deposition values were tested using Statistica version 12 (StatSoft, Inc. Tule, OK, USA) with the Pearson correlation coefficient, or with Spearman rank order correlation when the data were not normal. Statistical significance level was set at 0.05.

4.3. Results and Discussion

4.3.1. Laboratory testing of ion-exchange resin collectors

The values of nitrate obtained in blank unexposed IECs (0.001 - 0.006 mg NO_3-N per gram of resin) were similar to previously reported ones (Table 4.2). In the case of ammonium, mean values of 0.048, 0.060 and 0.074 mg NH_4-N per gram of resin for CB, CA and TC, respectively, were obtained. This blank correction was higher than

those shown in Table 4.2, except for one that was somewhat similar: 0.014 mg NH_4-N g^{-1} (Boutin et al., 2015). Resins made of quaternary ammonium compounds, like the one used in this study, can release NH_4^+ (Hansen, 2012; Langlois et al., 2003), which could explain the relatively high NH_4^+ found in the blanks. Although having such high blank values is not expected to cause accuracy problems, it could contribute to a decrease in the precision of the measurement of low deposition values. The adsorption efficiency of the IER tubes was close to 100% for both ions, the same as in most of the reviewed experiments from the literature (Table 4.2). The recovery efficiency for NO_3^- was comparable to that previously reported, whereas it was higher for NH_4^+. In this case, more NH_4^+ was recovered from the spiked resins than the quantity added, giving a recovery factor of 112%. Recovery factors above 100% have been previously described using the same IER (Fenn et al., 2002; Table 4.2). These results highlight the importance of lab tests to explore the performance of the resin used and to provide with suitable correction factors.

4.3.2. Comparison of methods

4.3.2.1. Variability of the measurements

In our study, the IEC extractions from CA exhibited a higher pH than the other sites (mean of 4.9 *vs*. 3.6 and maximum of 13.1 *vs*. 6.3, respectively). Significant negative correlations were found between the pH of the extracts and NH_4^+ concentrations within some periods at CA (data not shown) and a remarkable reduction in NH_4^+ concentration were noticed at pH values higher than 7. Because of this, ammonium values from extracts with a pH value higher than 6.5 (25% of 266 extracted samples from CA) were removed from the dataset before deposition calculations, which reduced the replication of measurements (Table 4.3). The precipitation in CA was much more alkaline (with a mean pH of 7.3 and a mean alkalinity of 141.1 µeq l^{-1}) than in CB or TC (with mean pH of 6.3 and 6.0; and mean alkalinity of 67.6 and 27.5 µeq l^{-1}, respectively) during the study period (Aguillaume, 2015). It may therefore occur that carbonates and bicarbonates present in the rain in this site increased the pH of the extracts provoking the loss of NH_4^+ via volatilization of NH_3 before the analysis (Cape et al., 2012; Izquieta-Rojano et al., 2016). However, such high values may also be originated partially from hydroxide anions which could be leached from the IER; and this possibility and the processes involved merit further study.

Table 2. Compilation of data from different studies reporting blank values, details of bulk deposition sampling and laboratory tests of performance for ion exchange resins.

Study	Ion exchange resin	Number and type of blanks	NO_3^-				NH_4^+			
			Blank correction (mg N g^{-1}) [a]	Intra-site variability (CV)	Adsorption efficiency	Recovery efficiency	Blank correction (mg N g^{-1}) [a]	Intra-site variability (CV)	Adsorption efficiency	Recovery efficiency
Boutin et al., 2015	Mixed-bed IONAC® NM-60	3 in total; field blanks	Non detected	12 % [b]			0.014 ± 0.006	19 % [b]		
Brumbaugh et al., 2012	2-stage columns	9; field blanks	Near detection limits	8–10 %		106 ± 7 %	Near detection limits	5–10 %		101 ± 3 %
Cerón et al., 2015	Mixed bed Amberlite®IRN150	3; field blanks				98.6 %				98.6 %
Clow et al., 2015	Mixed bed Amberlite®IRN150	1 per site; field blank	< 0.003	16 % [b]		100 %	< 0.003	21 % [b]		88 %
Fang et al., 2011	Mixed bed 201x7[717] & 001x7[732]	2 per site; field blank	0.003–0.028	9–34 %	90–99 %	90 %	< 0.001	5–23 %	94–100 %	97 %
Fenn et al., 2002	Mixed bed Amberlite®IRN150	5; lab. blanks	< 0.001			104.4 %	0.001			104.5 %
Fenn and Poth, 2004	Mixed bed Amberlite®IRN150	Lab. blanks	< 0.001	1–16 %			0.001	13–27 %		
Hansen, 2012	Mixed bed Rexin®	1 per plot; field blanks	< 0.001				< 0.001			
Köhler et al., 2012	Mixed bed Amberlite®MB 20	1; lab. blank	0.001			95%				
Sheibley et al., 2014	Mixed bed Amberlite®IRN150	1 field blank (per site) and 3 lab. blanks	0.001–0.003	9–36 % [c]	Approx. 100 %	90–91 %	≤ 0.001	7–89 % [c]	Approx. 100 %	74–96 %
Simkin et al., 2004	Anion-exchange Dowex™ Monosphere 550-A	3; lab. blanks	< DL		100 %	93.9–100.4 %				
Tulloss and Cadenasso, 2015	Mixed bed Amberlite®IRN150					90–95 %				90–95 %
van Dam et al., 1991	Mixed bed Dowex™ 1-X8 and 50W-X8			18 %				8 %		
Wieder et al., 2010	Mixed bed Amberlite®IRN150	3–5 per period; lab. blank								
Yamashita et al., 2014	Mixed bed Amberlite®MB-1	1 per period; field blank		< 10–50 %				< 8–25 %		
Zhan et al., 2015	Mixed bed of #717 anion and #732 cation	2 field blanks			> 99 %	90.3–95.5 %			> 99 %	90.9–100 %
Present work (CB)	Mixed bed Amberlite®IRN150	1–3 per period; lab. blanks	< 0.001–0.002	2–66 %			0.024–0.061	0–141 %		
Present work (CA)	Mixed bed Amberlite®IRN150	2 per period; lab. blanks	< 0.001–0.006	5–67 %			0.039–0.071	25–66 %		
Present work (TC)	Mixed bed Amberlite®IRN150	1 per period; lab. blanks	0.001–0.004	2–20 %	100 %	99 %	0.054–0.103	4–27 %	100 %	112 %

[a]: milligram of N released per gram of resin.

[b]: not CV (coefficient of variation).

[c]: collectors distributed along a larger area.

Variability among IECs for bulk deposition was lower in TC (mean CV of 12% and 11% for NO_3-N and NH_4-N, respectively) than in the other two sites, with mean CVs of 22% and 37% for NO_3-N and 79% and 46% for NH_4-N in CB and CA, respectively (ranges shown in Table 4.2). These high values may be caused by the lower replication in these sites: in CB, only two IECs were installed in open field, while in CA the above-mentioned reduction in the dataset provoked a drastic reduction in the actual replication (Table 4.3). The variability found with IECs was similar to that found using CBCs, with the exception of NH_4-N in CB (79%), which was largely higher (29% using CBCs). It might indicate that a higher replication than two funnels is needed for monitoring N deposition with IER in Mediterranean humid and sub-humid climates. Moreover, a higher replication allows detecting and removing questionable data, as was done for the CA dataset. In the present study, the intra-plot variability of the IEC methodology for bulk deposition measurements was in a similar range to the set of previous experiments shown in Table 4.2, with the exception of NH_4-N in CB.

Table 3. Bulk and throughfall deposition (kg N ha^{-1}) using ion-exchange resin collectors.

Site	Start date	End date	Season [a]	Rain (mm)	Bulk [b]		Throughfall [b]	
					NO_3^-	NH_4^+	NO_3^-	NH_4^+
CB	05/04/11	28/06/11	SPR	181.0	0.54 (2)	0.33 (2)	1.66 (8)	2.01 (8)
	05/07/11	27/09/11	SUM	120.2	0.42 (2)	0.86 (2)	1.22 (8)	0.94 (8)
	04/10/11	10/01/12	AUT	268.1	0.43 (2)	1.03 (2)	1.35 (8)	0.81 (5)
	17/01/12	18/04/12	WIN-SPR	100.0	0.39 (2)	0.01 (2)	1.40 (7)	0.85 (7)
	14/05/12	03/07/12	SPR	50.7	0.49 (2)	0.43 (2)	1.92 (8)	0.59 (8)
	11/07/12	01/10/12	SUM-AUT	112.1	0.29 (2)	0.01 (2)	1.01 (7)	0.30 (7)
	16/10/12	25/02/13	AUT-WIN	213.4	0.30 (2)	0.61 (2)	0.86 (7)	0.65 (7)
CA	25/02/11	31/05/11	SPR	118.3	0.57 (4)	0.41 (1)	1.04 (12)	0.23 (12)
	31/05/11	31/08/11	SUM	66.8	0.59 (4)	0.66 (1)	0.51 (8)	0.23 (12)
	31/08/11	13/12/11	AUT	119.3	0.35 (4)	0.26 (1)	0.80 (7)	0.33 (12)
	13/12/11	13/03/12	WIN	64.6	0.63 (3)	0.40 (2)	0.67 (7)	0.12 (12)
	13/03/12	19/06/12	SPR	267.8	1.07 (3)	2.11 (1)	1.37 (2)	0.76 (11)
	19/06/12	25/09/12	SUM	31.0	0.58 (2)	0.29 (4)	0.92 (11)	0.49 (9)
	25/09/12	06/03/13	AUT-WIN	660.4	1.41 (2)	1.54 (1)	1.29 (2)	0.49 (11)
TC	23/03/11	21/06/11	SPR	173.0	0.73 (3)	1.03 (3)	0.54 (11)	1.55 (11)
	28/06/11	27/09/11	SUM	17.5	0.16 (4)	0.16 (4)	0.80 (11)	0.28 (11)
	11/10/11	21/02/12	AUT-WIN	115.0	0.36 (4)	0.49 (4)	0.77 (11)	0.67 (11)
	06/03/12	21/06/12	WIN-SPR	127.4	0.37 (4)	0.76 (4)	0.64 (12)	0.77 (12)
	26/06/12	23/10/12	SUM-AUT	103.4	0.32 (4)	0.42 (4)	1.69 (11)	1.12 (11)
	30/10/12	25/03/13	AUT-WIN	197.7	0.38 (4)	0.57 (4)	0.25 (7)	0.66 (7)

[a] : SPR: spring; SUM: summer; AUT: autumn; WIN: winter

[b] : The number of collected samples used to calculate the mean deposition value is shown in brackets.

4.3.2.2. Comparison metrics and plots

The comparison metrics and graphics were performed only for bulk deposition values, since throughfall deposition is subjected to a higher variability not directly related to the method used, but related to canopy interactions with atmospheric N, canopy heterogeneity, and biochemical transformations provoked by litterfall or algae in the collectors (Bleeker et al., 2003; Fenn and Poth, 2004). This comparison was performed using the CBC results as the reference for assessing the IEC results, but the former method is likewise not without uncertainty (Bleeker et al. 2003; Dämmgen et al., 2005).

Measurements of bulk deposition of N using the IEC method showed a good agreement with the corresponding CBC values for NO_3-N, and an acceptable one for NH_4-N and DIN, based on the overall values of the calculated metrics (Fig. 4.1). The graphical comparison showed a very good performance of the IEC method in relation to the CBC for NO_3-N measurements, with only one period slightly out of the ±50% lines (Fig. 4.1). Nitrate deposition estimations with IECs compared to CBCs showed a lower error (RMSE = 0.15 kg N ha^{-1}) and a better fit to the 1:1 line than NH_4-N measurements. Measurements with IECs tended to overestimate the NH_4-N low values (which are the majority of the data) and underestimate the high NH_4-N compared to CBC values (Fig. 4.1). The general bias for NH_4-N deposition was low and positive (MFB = 6% for the entire dataset) and the error was about 0.56 kg N ha^{-1}. The values with the highest deviation came from two sampling periods during the second year in CA, with exceptionally low values in the IECs (2.11 and 1.54 kg NH_4-N ha^{-1}) compared to the CBC values (3.15 and 3.29 kg NH_4-N ha^{-1}). These two periods showed the highest mean hourly precipitation of the entire study (0.11 and 0.17 mm h^{-1}) and an elevated maxima hourly precipitation (9.9 and 18.2 mm h^{-1}). The lower deposition values measured with the IEC could therefore be related to a collection problem during those heavy rains. The slower flux of the rainwater through the IEC collector can cause a temporal accumulation of water in the funnel, exposing a relatively large surface of sample to the atmosphere. This could provoke a loss of NH_4^+ via volatilization of NH_3, particularly from rain samples with elevated pH like those found in CA. Indeed, hourly averaged rainfall showed a slight positive correlation with the differences between methods found for NH_4-N deposition ($r = 0.48$; $p = 0.059$), which totally disappeared once the two above-mentioned periods from CA were removed, suggesting that only during these two periods the measurements were affected by heavy rains.

When excluding the most influencing NH_4-N values on Pearson's *r*, this metric became lower (≤ 0.70), while better results were found for RMSE (0.29), and MFB showed and overall overestimation of NH_4-N bulk deposition (27%). Other authors have reported similar overestimations of bulk deposition of NH_4-N when using IECs compared to CBCs (Clow, et al, 2015; Fenn and Poth, 2004; Hansen, 2012; Langlois et. al., 2003). There are three main processes that could account for the discrepancy between methods: release of NH_4^+ from the amine groups of the IER, and nitrification or volatilization losses of NH_3 in the liquid samples of the CBCs (Fenn and Poth, 2004). The high temperatures that are common in the Mediterranean climate could favor any of the three potential processes (Fenn and Poth, 2004). The utilization of field blanks (i.e., sealed tubes containing IER, placed in the field) is recommended to avoid the discrepancies caused by ammonium release from the IER (Fenn and Poth, 2004). However, in the present study, laboratory blanks (i.e., sealed IER tubes not exposed to field condition) were used, which may release less amount of NH_4^+ since they are not exposed to the stronger temperature oscillations experienced in the field. This could account for part of the overestimation of NH_4-N deposition found in IECs when comparing to CBCs that occurred at the lowest deposition range. In any case, the field blanks will never undergo the same conditions as the operational IECs, since they remain sealed in the field without experiencing the drying and rewetting cycles. Regarding the other two processes, nitrification is not expected to occur in the open-field CBCs, since these samples are not expected to be colonized by nitrifier communities (Fenn and Poth, 2004) and the bottles are protected from solar radiation (Thimonier, 1998), but this possibility cannot be totally discarded. Secondly, the volatilization of NH_3 from liquid samples in open field is highly possible in this warm climate, although the narrow passage through the bug-sieve installed in the connection with the bottle is expected to meaningfully reduce the rate of ammonia volatilization, since this process is known to be severely restricted by limiting the movement of air above the water (Vlek and Stumpe, 1978).

Overall, and taking into account all these considerations, the performance of IECs for N deposition measurements in comparison with the CBC method showed an acceptable accuracy, with an error of 0.63 kg DIN ha^{-1} (Fig. 4.1). All these results show that IECs seem a suitable method for monitoring N atmospheric deposition in these environmental conditions with lower cost and effort than CBC methods, once precautions mentioned above are considered.

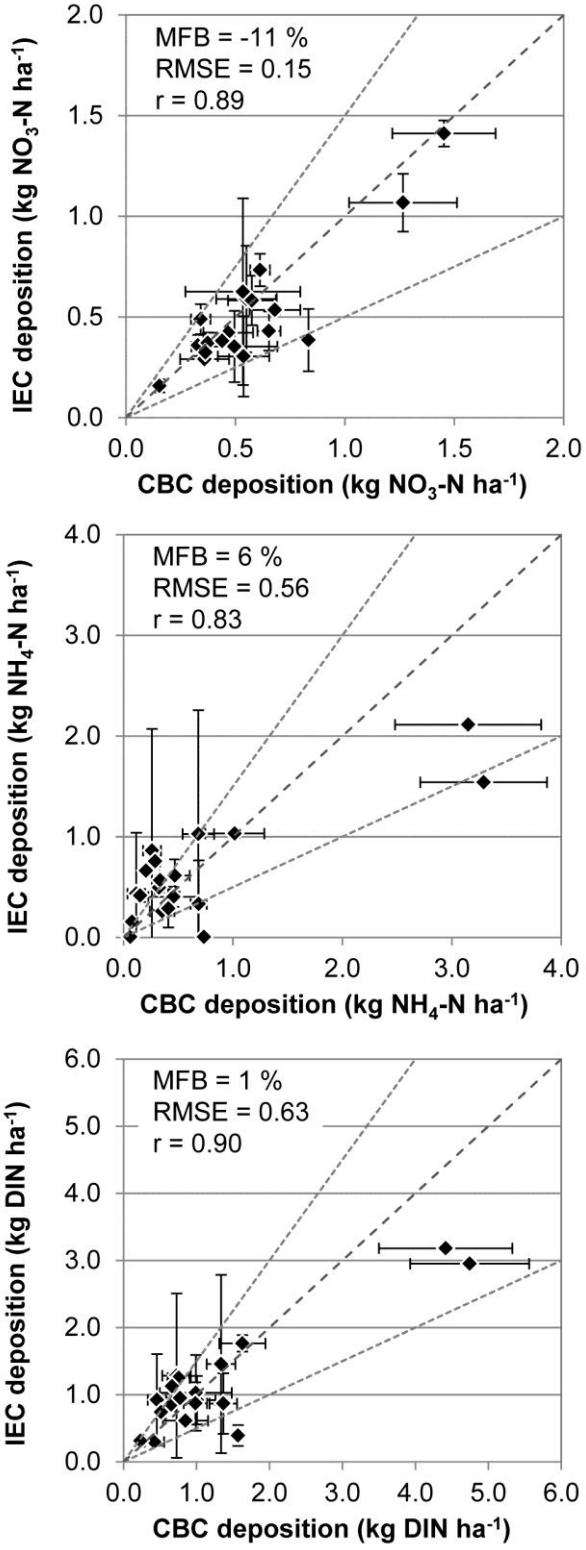

Figure. 4.1. Comparison of bulk deposition values of nitrate, ammonium and dissolved inorganic nitrogen (DIN) measured with conventional bottle collectors (CBC) and ion-exchange resin collectors (IEC). Data from all sites and sampling periods included. Comparison metrics added in the graphic: mean fractional bias (MFB), root mean square error (RMSE) and Pearson correlation coefficient (r). Dashed lines represent the line 1:1 (perfect fit) and lines 1:1.5 and 1:0.5 (±50%). Error bars correspond to standard deviation of the sampling period mean.

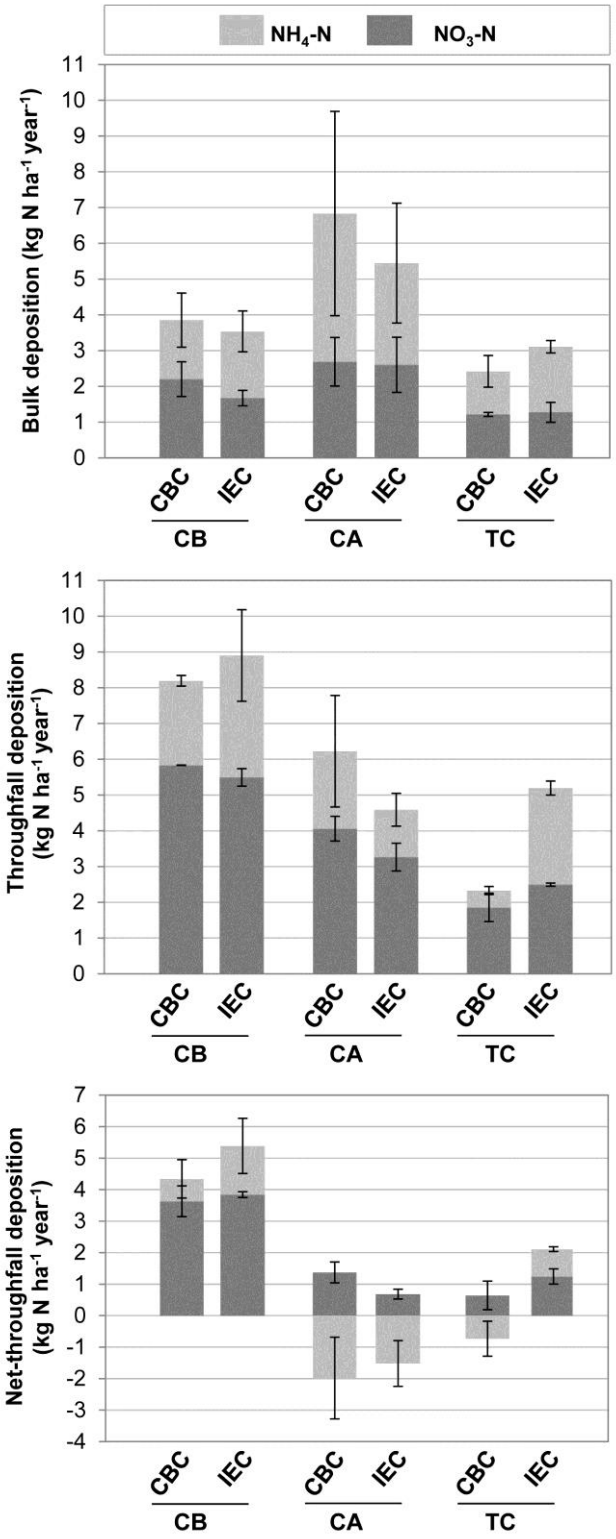

Figure 4.2. Mean of annual bulk, throughfall and net throughfall deposition of inorganic N for the 2-year sampling period. Methodologies: conventional bottle collector (CBC) and ion-exchange resin collector (IEC). Error bars represents the standard error of the mean (n = 2 years).

4.3.3. Nitrogen deposition in holm oak forests

Mean annual deposition of N for the 2-year period of the study estimated with IECs and CBCs is presented in Fig. 4.2. Bulk deposition of DIN ranged 2.42–6.83 and 3.09–5.43 kg N ha^{-1} among the sites according to CBC and IEC methodologies, respectively. Throughfall deposition of DIN ranged 2.33–8.20 and 4.59–8.91 kg N ha^{-1} among the sites with CBC and IEC, respectively. The highest bulk deposition of N occurred in CA and the highest throughfall deposition was recorded in CB. These two sites are located in the North of Spain where high wet deposition has been linked to the high industrialization and population in the region, higher precipitation and transboundary pollution (Chapter 1).

The difference between throughfall and bulk deposition (net throughfall) of N reflects the interaction between the atmosphere and the canopy, including both the wash-off of dry deposited N and the exchange with leaf surfaces. Negative values of net throughfall indicate that at least part of the wet deposited N is being effectively retained in the canopy, while positive values indicate that at least part of the dry deposited N is reaching the forest floor. Annual net throughfall values were positive for NO_3-N with the two measurement methods at all sites (Fig. 4.2). This result indicates a net flux of oxidized N to the forest soil coming from dry deposition, even though some canopy nitrate retention could be occurring. This input of dry deposited NO_3-N is very high at CB, the most urban-influenced site, which experiences the highest concentrations of gaseous oxidized-N compounds (Chapter 3) and high dry deposition of N (Aguillaume, 2015). On the other hand, net throughfall values for NH_4-N were only clearly positive in CB and negative in CA, while in TC depended on the method used and could be considered close to zero. Consequently, the forest canopy at CA seems to retain more NH_4^+ than at CB; especially considering that dry deposition of reduced N is expected to be higher than in CB, since CA is the most agriculture-influenced site with the highest NH_3 concentrations (Table 4.1). Canopy uptake of NH_4^+ has been described to be larger at lower nitrogen foliar content (de Vries et al., 2001), which is coincident with the stoichiometry of these sites, since CA showed the lowest leaf N content (1.44%) and CB the highest one (1.61%). The fact that the forest canopy at CB seems to retain less deposited N than the other two sites (positive and higher net throughfall of both NO_3-N and NH_4-N), agrees with the observation of a lower retention of atmospheric gaseous N (Chapter 3). These results indicate that the *Q. ilex* canopy in this forest cannot retain as

much N as the other sites. Interestingly, other *Q. ilex* forests close to this experimental site and with similar deposition load have been considered at a first stage of eutrophication (Àvila and Rodà, 2012).

In Mediterranean areas, the intra-annual variability of N deposition is expected to be high because of the particularities of the Mediterranean precipitation regime, in which rainfall is not equally distributed over the year. Data from CBCs can be better used for studying intra-annual variability in detail, because deposition can be observed almost on an event basis. In this study, data were monthly aggregated in order to summarize the results (Fig. 4.3). Negative values for net throughfall were most commonly observed with NH_4^+ (Fig. 4.3), indicating that canopy retention of wet-deposited N in these forests occurred more often in the reduced form. Relatively high positive values of net throughfall can be noticed in TC during the months of September and/or October in 2011 and 2012, during the first rains after the summer droughts, and also in March 2012, after a particularly dry winter. These values of positive net throughfall imply relatively large and ephemeral pulses of N into the soil after the first rainfall events, as those previously described in other semi-arid Mediterranean ecosystems (e.g. Meixner and Fenn, 2004; Vourlitis et al., 2009). These pulses can be noticed also in CB, but they rarely occurred in the CA site (Fig. 4.3). These ephemeral inputs, among other effects, can trigger pulses of NO emissions from soil (Homyak and Sickman, 2014) or provoke a flushing of inorganic N to ground- or stream waters if the pulse occurs when plants and soil communities are not able to use this dissolved N (like at the end of the summer, when they are withstanding drought stress). This effect, known as the asynchrony hypothesis (Meixner and Fenn, 2004), is corroborated in TC, where relatively high concentrations of NO_3^- in the soil water (up to 28.15 and 4.90 mg NO_3-N l^{-1} at 20 and 40 cm depth, respectively, using tension lysimeters) have been found after these pulses of N during late-summer and early-autumn, but not during the early spring of 2012, when understory annual pastures were emerged and growing and soil communities were active.

4.3.3.1. Arising issues for measuring N deposition in open forests of holm oak

Measuring N throughfall deposition in the open forest of holm oak in TC highlighted some discrepancies between IEC and CBC methods for NH_4-N throughfall deposition estimations (Fig. 4.2). The IEC method estimated a value 2.25 kg NH_4-N ha^{-1} y^{-1} larger than the CBCs at this site but those differences were not observed in the other sites. This

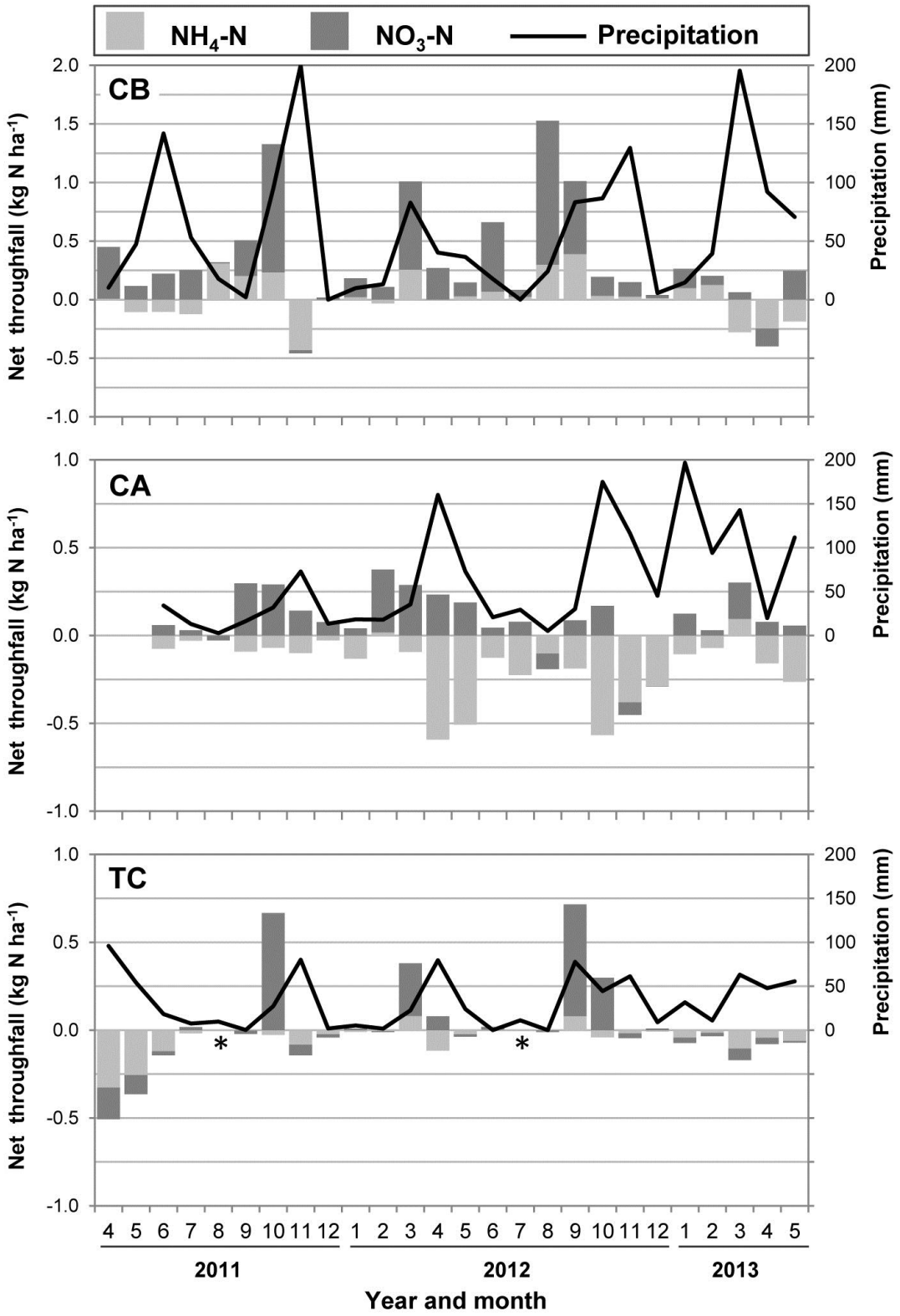

Figure 4.3. Net throughfall deposition of N and collected precipitation on a monthly basis at the three study sites using CBCs. * : bars not shown because of the presence of missing data for this month.

discrepancy could be partially related to the high uncertainty of the CBC method when concentrations are close to or below detection limits (Köhler et al., 2012), as it happened to 30% of the throughfall samples in TC. Also some nitrification processes in CBC samples at TC could be occurring, since some throughfall samples collected 2–3 days after summer rains showed algal growth (while it did not happened in the open-field bulk samples). The addition of a biocide in the collection bottles as a sample preservative (Cape et al., 2010) is therefore recommended under Mediterranean conditions, particularly under semi-arid regimes like at TC site. Bird dropping cannot be disregarded as a cause of the discrepancy either, since visual inspections might not be effective when enough precipitation has occurred to rinse the funnels. Further quality control including analysis of phosphates in the IEC samples could aid to detect bird dropping contamination (Fenn et al., 2015). The largest discrepancy in NH_4-N estimations at TC was found in spring of 2011, the period in which more inflorescences were collected (55.2 g m^{-2}) in the litterfall samplers. Inflorescences of *Q. ilex* are small and easily decomposable while presenting high N content (close to 1.5 %; Bellot et al., 1992). Since the flowers remained longer in the IECs compared to the CBCs, more NH_4^+ could therefore be leached out from older decomposing litter, originating this greater discrepancy. It is therefore recommended to clean or change the funnels of the IECs when big amounts of debris are accumulated in them. The open structure of the canopy in TC might facilitate both, bird dropping and litterfall accumulation, which together with the high temperatures might explain the discrepancies in NH_4-N throughfall estimations observed only at this site.

4.3.4. Summary of methodological considerations

Some methodological recommendations on the IEC method arising from the present study have been already reported in previous works (e.g. Cape et al., 2010; Fenn et al., 2015). Preliminary laboratory tests on adsorption and recovery efficiency need to be done in order to know the performance of the resin. The IEC method poses a potential overestimation of NH_4-N deposition due to the release of NH_4^+ from the amine groups of the IER. To avoid that, field blanks consisting of sealed IECs deployed in the field are recommended over unexposed laboratory blanks in Mediterranean conditions. An overestimation of NH_4-N deposition could also occur when a large amount of leachable debris is accumulated during the long sampling periods of IECs and it might be considered when planning the duration of the different sampling periods along the year.

In *Q. ilex* forests it could particularly occur during the flowering period. Consequently, periodic cleaning of sampling devices is advised during those sensitive periods. On the other hand, NH_4^+ deposition could be underestimated when the flow of the rain across the different parts of the IECs during heavy rains is made difficult (e.g. by small nominative diameter of any part of the collector, or very dense filters or sieves), particularly with alkaline rain. A modification in the design could be studied in those areas withstanding intermittent heavy rains. Our results recommend deploying at least three replicate IECs at every bulk deposition sampling plot. There would not be a recommended replication for throughfall measurements, since it may vary depending on the vegetation cover type, canopy characteristics and its distribution throughout the stand; however, based on the intra-site variability found in this study, at least ten replicates are recommended in this particular kind of forest stands. An additional quality control method for IEC by analysing phosphates in the extracts is also recommended. Finally, it is recommend to measure the pH of the sample IEC extracts, and to acidify the extract if the pH is too high. Regarding the CBC method, it has proven useful for studying the seasonal variability of N deposition and the use of biocides is recommended in these climatic conditions, especially for throughfall measurements.

4.4. Conclusions

The results of the present study showed that collection methods for N deposition based on ion-exchange resins can be recommended for long-term studies in the Mediterranean region, since its performance in measuring NO_3^- deposition was good and acceptable for NH_4^+ and DIN, in comparison with conventional methods. This methodology is particularly recommended in remote areas and when the nitrogen concentration in rain is low. However, the methodological recommendations on the IEC method arising from the present study should be taken into consideration in the monitoring design.

Mean annual bulk deposition of DIN in holm oak forests in Spain ranged 2.42–6.83 and 3.09–5.43 kg N ha^{-1} according to CBC and IEC methodologies, respectively. Intra-annual variability showed significant input pulses of N into the forest soil after dry periods. These pulses are presumably originated from the washing of dry deposition accumulated in the canopy and were particularly noticeable in the forest site with a semiarid climate. The implication of these nutrient pulses for ecosystem functioning, atmospheric chemistry and N leaching should be further investigated.

4.5. Acknowledgements

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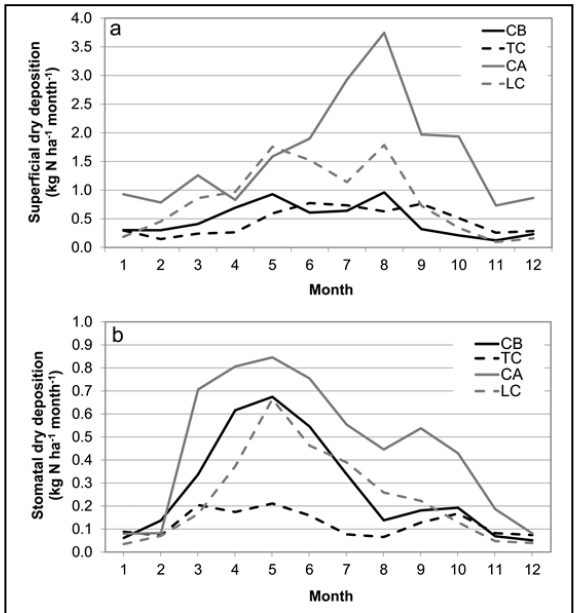
CHAPTER 5

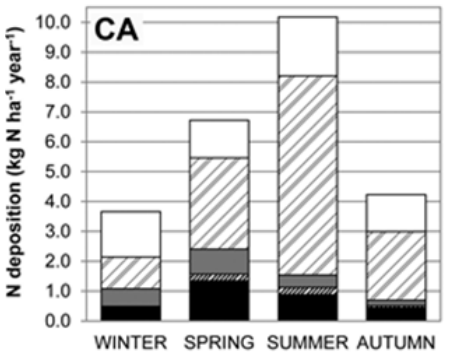
Dry and total deposition of inorganic nitrogen in four Mediterranean evergreen broadleaf forests in Spain

ABSTRACT

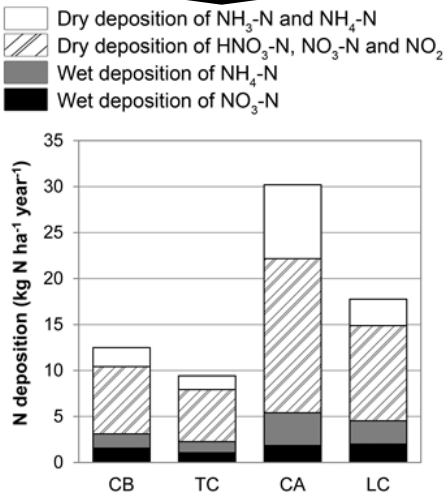
In Mediterranean areas, dry deposition of N compounds may play a major role in the total N input to natural vegetation, particularly to forest ecosystems. An innovative approach, called the empirical inferential method (EIM), together with the modelization of stomatal conductance by means of the DO_3SE (Deposition of Ozone and Stomatal Exchange) model, were used in the present study to estimate dry deposition of N pollutants in four holm oak forests in Spain. The estimation of surface deposition of gaseous and particulate atmospheric N averaged 10.17 ± 3.29 kg N ha^{-1} $year^{-1}$ for the four sites, while stomatal deposition of N gases averaged 3.31 ± 0.83 kg N ha^{-1} $year^{-1}$. Total dry deposition of atmospheric inorganic N was dominated by the surface deposition of HNO_3 and particulate NO_3^- in all the forests and the relative contribution of NO_2 deposition averaged 14.6% in the peri-urban forests and 7.9% in the most natural site. The dry deposition of reduced N was the most relevant at the most agrarian site, with 8.16 kg N ha^{-1} $year^{-1}$. The estimated total deposition, with dry deposition representing 76.2% ± 2.1% of it, varied among the sites matching the geographical patterns previously found in model estimates, showing higher deposition in the northern site (30.36 kg N ha^{-1} $year^{-1}$) and eastern-coast sites (17.42 and 12.17 kg N ha^{-1} $year^{-1}$) than in the central-Spain site (9.29 kg N ha^{-1} $year^{-1}$). For the monitoring period, only in one site the empirical CL proposed in the framework of the CLRTAP (10–20 kg N ha^{-1} $year^{-1}$) was not overreached, but all sites exceeded the CL proposed for the protection of sensitive epiphytic lichens in similar natural ecosystems (5.5 kg N ha^{-1} $year^{-1}$).



Estimation of surface and stomatal deposition



Seasonal dry deposition of inorganic nitrogen



Total deposition of inorganic N

5. Dry and total deposition of inorganic nitrogen in four Mediterranean evergreen broadleaf forests in Spain

5.1. Introduction

Atmospheric deposition of nitrogen (N) in Spain is moderate and lower than in central Europe (Lorenz and Becher, 2012; Nyíri and Gauss, 2010), but it has been identified as a potential threat for some valuable Spanish ecosystems, such as alpine grasslands and shrublands or mountainous forest of holm oak (*Quercus ilex*) in NE Spain (Chapter 2). In Mediterranean areas, dry deposition of atmospheric N compounds may play a major role in the total N deposition to natural vegetation, particularly to forest ecosystems (Fenn et al., 2009). Dry deposition is the direct deposition of gases and aerosols from the surrounding atmosphere into the ecosystems surfaces and it depends on multiple factors, such as their atmospheric concentrations, turbulent transport processes in the boundary layer and the chemical and physical nature of the depositing species and receiving surfaces. Ammonia (NH_3) may dominate the dry deposition processes in agricultural areas, while in more industrial and urban areas nitric acid vapour (HNO_3) and nitrogen dioxide (NO_2) may be more important (Hertel et al., 2011). Particulate N (suspended aerosols containing ammonium and nitrate – NH_4^+ and NO_3^-) is not expected to largely contribute to total deposition across the territory because its deposition rate to vegetation and atmospheric concentration are much lower than those of gaseous compounds (Hanson and Lindberg, 1991; Petroff et al., 2008; Chapter 3). For example, it is estimated to contribute with 0.05% to total N deposition in United Kingdom (Fowler et al., 2009). However, dry deposition of particulate N could be a relevant component of total deposition in forest ecosystems; e.g. it was estimated to contribute up to 39% of the dry deposition of inorganic N in a Mediterranean *Q. ilex* forest in France (Flechard et al., 2011).

Dry deposition of N to vegetation can be summarized to occur in two ways: surface and stomatal deposition. The former is referred to gaseous and particulate N species that eventually are adsorbed by the vegetation surfaces, while the latter is referring to gaseous species that are uptaken through stomatal pores followed by their dissolution in the apoplast. Many factors can potentially regulate the deposition rates onto vegetation surfaces (Fowler et al., 2009), but highly reactive and water soluble species, like HNO_3 and NH_3, are characterized by high deposition velocities and are readily deposited to

leaf surfaces and plant canopies (Hanson and Lindberg, 1991); while in the case of nitric dioxide (NO_2), its flux to vegetation are thought to be mainly controlled by stomatal uptake (Saxe, 1986; Raivonen et al., 2009). Additionally, HNO_3 may be uptaken into the leaf interior via transcuticular transport due to its high reactivity with cuticular waxes (Bytnerowicz et al., 1999b; Cadle et al., 1991; Padgett et al., 2009b). On the other hand, stomatal deposition of gaseous N pollutants is controlled by the degree of stomatal aperture and by gas concentrations in the sub-stomatal cavity (Massad et al., 2010b). This concentration has been proved to be particularly important for NH_3 flux estimation, since when ambient NH_3 concentrations are higher than in the stomatal cavity (stomatal compensation point), plants act as a sink, but when this gradient is reversed, plants function as a source (Massad et al., 2010b; Raivonen et al., 2009). In this way, NH_3 flux depends on the N status of the receiving vegetation (Hertel et al., 2011). In the case of nitric dioxide (NO_2), the compensation point for *Q. ilex* could be considered negligible (Chaparro-Suárez et al., 2011) and its deposition rate to vegetation might be mainly modulated by stomatal aperture. All these processes depend on the different sinks and the interactions with other trace gases at the plant surface, allowing rates of dry deposition to change with time and with surface characteristics (Fowler et al., 2009).

Throughfall analysis is a widely-used technique for estimating dry deposition, which gives a lower-bound estimate of total N deposition to plant canopies (Lovett and Lindberg, 1993). The main disadvantage of this method lies on its inability to estimate stomatal deposition (Draaijers et al., 1997). Besides, in the Mediterranean climate this method has some limitations related to the unpredictable and often insufficient precipitation required for removing the canopy-intercepted N (Bytnerowicz et al., 2015). Micrometeorological methodologies, such as eddy correlation, aerodynamic gradient method, eddy accumulation or mass balance techniques, have been also widely used to estimate dry N deposition to uniform vegetation canopies (Fowler et al., 2009). However, the use of these methods for extended periods or over large areas is impractical because of their complexity and instrumental requirements (Baldocchi et al., 1988). Finally, the inferential method calculates dry-deposition fluxes to the ecosystems based on measurements of atmospheric concentrations of the pollutants of interest and the deposition rates of these pollutants, which are mainly conditioned by meteorological conditions and the physical characteristics of surfaces (Fenn et al., 2009). The most

common approach to obtain the deposition rates is using a multiple resistance analogy approach (deposition process is considered as a series of resistances, by analogy with an electrical circuit; Monteith and Unsworth, 2008), although they can be also obtained from the above-mentioned micrometeorological studies.

Another approach involves using empirical values of surface deposition rates; a method recently proposed by Bytnerowicz et al. (2015), called the empirical inferential method (EIM). In this method the pollutant flux values are determined as the product of the measured concentration by the deposition rate of the N gaseous compounds experimentally estimated at branch level (conductance), instead that at canopy level (deposition velocity). Deposition velocity values for specific N gases are mainly based on complex and expensive micrometeorological studies or are theoretically estimated by the multiple resistance analogy approach, while surface conductance values can be more easily obtained from branch-washing experiments, producing site-specific values. Branch-washing techniques use deionized water to rinse or wash the deposited ions from the vegetation surfaces but cannot distinguish the sources of the surface-deposited NO_3^- (HNO_3 or particulate NO_3^-), nor NH_4^+ (NH_3 or particulate NH_4^+) (Dasch, 1989). Besides, it may underestimate deposition to foliar surfaces due to the transcuticular uptake or translocation processes of the deposited chemical species (Garten and Hanson, 1990). However, despite these deficiencies the branch-rinsing method may be very useful for the development of estimation models for forest with different environmental conditions and pollution exposures.

Atmospheric N deposition in eastern Spanish forests have been experimentally estimated in 15–30 kg N ha^{-1} $year^{-1}$, with dry deposition ranging 40–84% of total N deposition, depending on the location, type of forest and year of estimation (Aguillaume, 2015; Àvila and Rodà, 2012; Rodà et al., 2002; Sanz et al., 2002). More detailed studies are needed to characterize dry deposition in ecosystems under typically Mediterranean climate conditions, especially since previous results might suggest that the importance of dry deposition could be underestimated by chemical transport models for the Mediterranean area (Chapter 2). The objective of this work was to study the dry deposition of atmospheric N in Mediterranean forests by applying EIM estimations in four forests of holm oak with different influences of urban and agricultural pollution sources. The specific objectives of the study were: (1) to estimate the dry deposition of atmospheric N in Spanish holm oak forests by means of the empirical inferential

method, (2) to upgrade the methodology by the performance of branch-washing experiments for more periods apart from summer and by using modelled stomatal conductance values, (3) to compare surface flux and conductance of pollutants to living and lyophilized branches of holm oak, and (4) to estimate and describe the total input of atmospheric inorganic N to these forests.

5.2. Material and methods

5.2.1. Study sites

Four holm-oak (*Q. ilex*) forests placed in three regions with different climatic (and soil) conditions were selected in Spain (Fig. 5.1). The Tres Cantos (TC) site is located near Madrid City (9 km) with Mediterranean semi-arid climate (Med-SA). Only in this case, the historical management of the forest has produced a moderately open forest (72% of tree cover). The Can Balasc and La Castanya (CB and LC) sites are placed in the Barcelona province, with a sub-humid Mediterranean climate (Med-SH). The former site is close to Barcelona City (4 km), while the last is further from this city (40 km) and relatively sheltered from the surrounding lowland sources of atmospheric pollutants (Hereter and Sánchez 1999). The Carrascal (CA) site is located in an agricultural area close to Pamplona (15 km), in a region with Mediterranean humid climate (Med-HU). The canopy in all the sites is dominated by *Q. ilex*, mixed with *Quercus humilis* in CB. In the present work, the sites were assumed to represent their corresponding regional climatic conditions (hence, Med-SA, Med-SH and Med-HU), although it will be considered that they are also influenced by different pollution sources.

5.2.2. Field measurements

Passive samplers (Radiello®) were used to measure atmospheric concentrations of NH_3 and NO_2. Nitric acid vapour (HNO_3) was measured by means of badge-type samplers manufactured following Bytnerowicz et al. (2005). Two replicate samplers per gaseous species were exposed at 2 m height in each plot. Gases were measured during 2-week-long periods between February 2011 and February 2013. During every exposure period, unexposed samplers were used as blanks for each site and type of passive sampler. After collection, all samples were kept refrigerated (4 °C) in darkness until they were analysed in the laboratory. Laboratory analyses were performed according to Radiello's specifications (Fondazione Salvatore Maugeri, 2006) and Bytnerowicz et al. (2005). For further details, see Chapter 3.

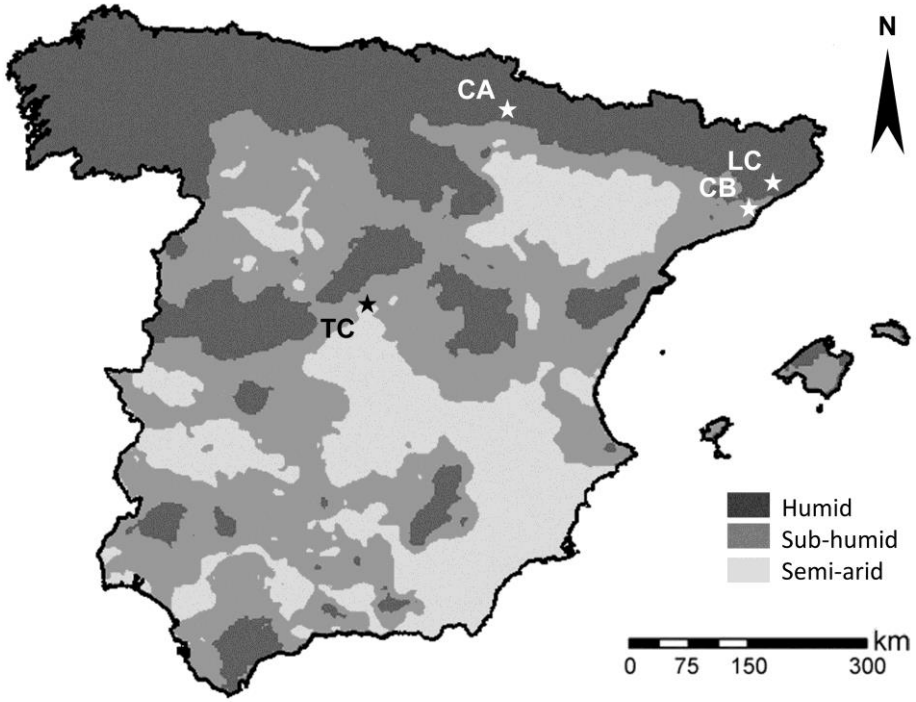


Figure 5.1. Geographical distribution of the monitoring sites. Main climatic regions in Spain are indicated according to the UNESCO humidity index (total precipitation / Pemman evapotranspirative demand). Redrawn from Spanish Ministry of the Environment (2000). Note that LC is placed in the humid region, but it is described as semi-humid by Hereter and Sánchez (1999).

Table 5.1. Characterization of the study sites.

Site code	CB	TC	CA	LC
Type of location	Peri-urban	Peri-urban	Peri-urban	Rural
Altitude (m)	255	705	592	696
Leaf area index ($m^2\ m^{-2}$)	3.3	3.1	5.3	6.1
Tree density (number of trees ha^{-1})	1429	491	1760	2571
Mean diameter at breast height (cm)	13	41[c]	16	13
Mean annual temperature (ºC) [a]	15.2	14.6	12.3	13.7
Mean annual rainfall (mm y^{-1}) [a]	652	348	681	812
Mean annual relative humidity (%) [a]	71.3	54.6	73.7	70.3
Mean annual wind speed (%) [a]	0.8	1.3	6.2	0.9
Distance to the nearest big city (km)	4	9	15	40
Distance to the nearest highway (m)	0.15	1.5	0.05	16
Agricultural land-use cover [b]	23%	21%	62%	23%

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

Four collectors, consisting in a high-density polyethylene funnel (10 cm radius; NILU - Norwegian Institute for Air Research, Kjeller, Norway) attached to a bottle of the same material were installed in every site in open field to collect bulk deposition (BD) of atmospheric N. The bulk deposition samples were collected on a weekly basis by replacing the collection bottle. During the longest periods without rain the funnels were rinsed with 100 ml of deionised water every two weeks before replacing the bottle, in order to collect and measure the dry deposition into the funnel. The sampling procedure, storage, analysis by ion chromatography (Dionex, Sunnyvale, USA) and ulterior quality control of the analytical results of the bulk deposition samples were performed following the recommendations of the ICP Forests Manual (Clarke et al. 2010), and it is furtherly described in Izquieta-Rojano et al. (2016). Simultaneously, wet deposition (WD) of N was measured by mean of wet-only collectors (TE® 78-100) in LC and TC. The WD samples were collected in the same temporal basis as BD samples by collecting a homogenised subsample of around 250 ml after weighting the collector bucket for the estimation of the sample volume. The same storage procedure, analysis and quality control as in BD samples were applied. Meteorological variables were monitored in CB, TC and LC sites, and data from the closest meteorological station were collected for the CA site.

5.2.3. Branch-washing experiment

A branch-washing experiment was performed in ten selected *Q. ilex* trees in every site during several rainless periods (from 3 to 5, depending on the site; Table 5.2). After rainless periods larger than 7 days (mean of 21.5 days), one branch of around 20 cm length was collected from the upper part of the canopy of the ten selected trees. The ten branches represented the different geographic orientations and were enough protruded from the canopy tree to collect atmospheric compounds dry-deposited onto the canopy. After cutting them, their branch edges were sealed with Parafilm® and they were carried to the laboratory in sealed plastic bags, where they were washed for 3 min inside the same plastic bag with 200 ml of distilled water. Care was taken to keep the branch edge outside the bag while washing. Two empty plastic bags were washed to use the results as blank controls. After the wash, they were air-dried and the projected leaf area (PLA) was measured using Li-Cor 3100 area-meter. Stem areas of branches were measured for one period and the resultant stem-/leaf area ratio was extrapolated to all

leaf area measurements to come close to the actual exposure area of the branches. The calculated surfaces areas ranged 175.3–866.3 cm^2 across all sites and periods.

At TC site, an experiment with surrogate surfaces was performed using lyophilized branches (LB) of *Q. ilex* collected from the same ten selected trees and thoroughly washed before the lyophilisation process. Four 10 cm-length LB, with surface areas between 18.1 and 104.2 cm^2, were attached to the top of a 5-meter pole in three different locations inside the forest during different periods. Care was taken to place the poles in open-canopy spots to avoid direct contact with the surrounding trees. They were exposed from 7 to 14 days and collected the dry deposition at the same time as the above-mentioned living branches (herein, natural branches; NB). They were carried to the laboratory in sealed plastic bags, where they were washed for 3 min. inside the same plastic bag with 100 ml of distilled water. Non-exposed LB were kept in sealed flasks after lyophilisation and washed at the same time as the collected ones to use the results as blank controls.

Two sets of three NB and three LB from two different periods in TC were washed three times each, sequentially, to analyse washing efficiency. The third-washing results showed concentrations of inorganic N close to zero and it was considered as the washing-time with a total accumulated removal of surface N. The percentage of recovered N in the first washing with relation to the third one was used as a corrector factor in order to obtain an approximation to the total accumulated in the leaf surface performing only one washing. For NB, the first washing was considered to remove 73% and 89% of the deposited NH_4^+ and NO_3^-, while, for LB, it was considered to remove 79% and 99%, respectively.

The washing solutions were analysed by ion chromatography (Dionex, Sunnyvale, USA) and the results subjected to a quality control following the recommendations of the ICP Forests Manual (Clarke et al., 2010). Nitrogen surface deposition flux (*F*) to branches was calculated for each branch multiplying the concentration of NO_3^- and NH_4^+ by the washing volume and subtracting the background loads measured in the unexposed LB and empty bags. The N load values were divided by the number of days after the last precipitation event (NB exposure period) or the exposure duration (in the case of LB) and by the projected leaf area of the exposed branches to obtain the N surface flux deposition to the branches (μeq N day^{-1} m^{-2}). In the present study, the

leaching of inorganic N from the NB is considered negligible, as seen in previous works with holm oak (Rodrigo and Àvila, 2002).

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

Dry deposition was estimate as the sum of surface deposition and stomatal deposition (leaf uptake). For surface deposition estimates, empirical surface deposition conductance (*K*) for NH_4^+ (resulting from deposition of NH_3 and particulate NH_4^+) and for NO_3^- (resulting from deposition of HNO_3 and particulate NO_3^-) were calculated for every period and site of the branch-washing experiment, according to the formula:

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

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

where:

$F_{NH_3+NH_4^+}$: mean surface flux of NH_3 + NH_4^+ to branches (µeq m^{-2} day^{-1})

C_{NH_3} : mean atmospheric concentration of NH_3 during the exposure period (µeq m^{-3})

$F_{HNO_3+NO_3^-}$: mean surface flux of HNO_3 + NO_3^- to branches (µeq m^{-2} day^{-1})

C_{HNO_3} : mean atmospheric concentration of HNO_3 during the exposure period (µeq m^{-3})

To calculate *K*, the values for *F* and *C* from the branch-washing experiment were matched individually for each exposure period and site. In the case of the two Med-SH sites, the values of both variables were averaged when the exposure periods were coincident. Therefore, 4 to 5 different exposure-period values from each climatic region (Med-SA, Med-SH and Med-HU) were employed in the analysis (Table 5.2). As the passive sampling periods did not always match with the exposure periods, mean concentration of both gaseous species were calculated weighted by the coincident days with the exposure time of the LB and NB branches. Estimates of the surface deposition conductance were calculated separately for LB and NB in TC. A regression between the *F* and *C* values (dependent and explanatory variables, respectively) was performed, since the value of the slope (*F/C*) would be equivalent to the *K* value if the intercept of

Y_{axis} is set to zero. If none significant regression existed between both variables, the regional means of *K* (Table 5.3) were considered as the final *K* values. Surface deposition conductance values for NH_4^+ and NO_3^- were calculated based only on ambient NH_3 and HNO_3. This approach was justified in previous studies in Mediterranean forests (Bytnerowicz et al., 2015) because NH_3 and HNO_3 are expected to dominate the surface deposition of NH_4^+ and NO_3^- over the particulate forms (Bytnerowicz and Fenn, 1996).

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

$$F_{surface} = C \times LAI \times K \quad (3)$$

where:

C: atmospheric concentration of NH_3 or HNO_3 (µg m^{-3})

LAI: leaf area index (m^2 m^{-2})

K: surface deposition conductance for NH_4^+ and NO_3^- (cm s^{-1})

Stomatal uptake ($F_{stomatal}$) of both reduced and oxidized forms of N was calculated for daylight hours according to the formula:

$$F_{stomatal} = C \times LAI \times c_s \quad (4)$$

where:

C: atmospheric concentration of NH_3, NO_2 or HNO_3 (µeq m^{-3})

LAI: leaf area index (m^2 m^{-2})

c_s: stomatal conductance for NH_3, NO_2 or HNO_3 (cm s^{-1})

Stomatal uptake values of reactive N gases for forest trees were calculated based on modelled stomatal conductance for H_2O vapour (g_s) using the DO_3SE (Deposition of Ozone and Stomatal Exchange) model. The European Monitoring and Evaluation Programme, in the framework of the Convention on Long-Range Transboundary Air Pollution (CLRTAP, 1979), is using this ozone (O_3) deposition model to help in defining the areas in which the flux of O_3 into vegetation can pose a threat to the ecosystems (CLRTAP, 2004). This model incorporates a g_s model to calculate O_3 stomatal fluxes for different vegetation types. The model uses the multiplicative

approach defined by Jarvis (1976), modified by Emberson et al. (2001) and parametrized for *Q. ilex* by Alonso et al. (2007, 2008):

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

where g_s for a given species is calculated as a function of the maximum g_s value for that particular species (g_{max}), and modified according to prevailing environmental factors that include photosynthetic active radiation (f_{light}), temperature (f_{temp}) and air vapour pressure deficit (f_{VPD}). Soil water potential (f_{SWP}) is another environmental factor commonly used: however, because of the lack of soil water data in the present work it is set to 1, and considered as partially surrogated by the phenological function (f_{phen}), related to the seasonal growth stage. f_{min} is the relative minimum stomatal conductance that occurs during daylight hours. This model was used at an hourly scale and the results were added up daily.

Following Bytnerowicz et al. (2015), these values were converted to stomatal conductance for NH_3, NO_2 and HNO_3 (c_s) according to Graham's law of molecular diffusion (Massman, 1998) by multiplying g_s by 1.029, 0.626 and 0.534, respectively. Since stomatal fluxes of NH_3 are bi-directional, the $F_{stomatal}$ of NH_3 is not expected to occur below certain ambient concentration of NH_3 (compensation point), which depends on ecosystem N-status, stomatal conductance and the ratio between atmospheric and canopy NH_3 concentration (Behera et al., 2013; Fowler et al., 2009). Following Bytnerowicz et al. (2015), the NH_3 compensation points for all sites were calculated using a method based on daylight air temperature and annual deposition of nitrogen (Massad et al., 2010b). In this study the compensation point was calculated on a daily basis, varying with the changing daily temperature.

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

Table 5.2. Fluxes of deposited nitrogen into natural vegetation from the branch-washing experiment and averaged concentration of ammonia and nitric acid vapour for each exposure period in the three climatic regions studied.

REGION[a]	START DATE	END DATE	NH_4^+ (µeq m^{-2} day^{-1})			NO_3^- (µeq m^{-2} day^{-1})			NH_3 (µeq m^{-3})	HNO_3 (µeq m^{-3})
			N[b]	MEAN	CV	N	MEAN	CV		
Med-SA	07/06/11	28/06/11	10	10.2	47%	10	34.8	36%	0.070	0.024
	16/08/11	18/10/11	10	6.1	40%	10	27.5	42%	0.053	0.034
	10/12/11	11/01/12	10	4.8	55%	10	4.3	64%	0.013	0.009
	20/05/12	05/07/12	10	9.8	60%	10	26.3	60%	0.072	0.045
	04/04/13	24/04/13	9	9.1	37%	9	18.8	29%	0.027	0.007
Med-SH	13/06/11	29/06/11[c]	9-10	4.1	123%	9-10	10.4	49%	0.033	0.032
	25/09/11	05/10/11	7	13.1	55%	7	17.1	45%	0.054	0.023
	01/02/12	22/02/12[c]	10-10	14.1	38%	10-10	8.0	34%	0.056	0.014
	06/08/12	24/08/12[c]	2-6	23.1	120%	9-5	11.4	84%	0.071	0.166
Med-HU	16/09/11	30/09/11	10	2.9	94%	10	48.8	37%	0.129	0.015
	15/02/12	28/02/12	10	25.3	29%	10	47.5	26%	0.132	0.024
	20/06/12	28/06/12	10	37.4	30%	10	20.1	28%	0.184	0.064
	28/08/12	11/09/12	6	39.5	26%	10	50.4	38%	0.192	0.070

TOTAL	N	MEAN	CV[d]	CV[e]	MEAN	CV[d]	CV[e]	MEAN	MEAN
Med-SA	5	8.0	30%	48%	22.3	52%	46%	0.047	0.024
Med-SH	4	13.6	57%	84%	11.7	33%	53%	0.054	0.059
Med-HU	4	26.3	64%	45%	41.7	35%	32%	0.159	0.043
	N	**MEAN**	**CV[f]**	**CV[e]**	**MEAN**	**CV[f]**	**CV[e]**	**MEAN**	**MEAN**
ALL	3	16.0	59%	59%	25.3	60%	44%	0.087	0.042

[a] : Med-SA, Med-SH and Med-HU correspond the Mediterranean semi-arid, sub-humid and humid climatic region, which in turns correspond to TC, CB and LC, and CA sites, respectively; [b] : number of washed branches used in flux calculation; [c] : the results shown are the mean of two simultaneous periods and both N are given**;** [d] : CV of the regional mean, representing temporal variability; [e] : regional mean of the CVs, representing mean intra-site variability; [f] : CV of the all-sites mean, representing inter-regional variability.

5.2.5. Approximation to aerosol deposition conductance

In parallel with the application of the EIM estimations, a novel approach to obtain *K* values for gases and aerosols alike was conducted, based on the same equations. Here, it is theorized that the branch washing method could be also applied for estimating surface conductance of N aerosols, which could be eventually implemented in the EIM calculations. The surface deposition in exposed branches should respond to the concentration and surface conductance of both atmospheric forms of nitrogen, gaseous and particulate:

$$F_{N_{GAS}+N_{PM}} = C_{GAS} \times K_{GAS} + C_{PM} \times K_{PM} \quad (6)$$

This equation is a variation of the eq. 1 in which *F* corresponds to the recovered N from the washing experiments (surface flux of N to the branches) and *C* and *K* are ambient concentrations and surface conductances. The subindexes *GAS* and *PM* correspond to the gaseous and particulate forms of a N pollutant, respectively. From eq. 6 can be deduced:

$$\frac{F_{N_{GAS}+N_{PM}}}{C_{GAS}} = K_{GAS} + K_{PM} \times \frac{C_{PM}}{C_{GAS}} \quad (7)$$

Since *F* and *C* terms are measurable, both *K* terms could be statistically estimated by regression (as coefficients) if a sufficient number of results are achieved in the experiment to accomplish regression assumptions and reach statistically significant results. In the case of the present work, few PM_{10} measurements were coincident with the leaf-washing experiment and, therefore, seasonal values of C_{PM} from Chapter 3 were used instead of using values measured during the exposure periods.

5.2.6. Estimation of total deposition of atmospheric N

Wet deposition from wet-only collectors was estimated by multiplying the N concentration by the collected volume in each period. Where wet-only collector data were not available, concentration and volume from the BD collector was used instead, and the resulting BD value was corrected by the seasonal ratio wet-/bulk deposition (WD/BD) from the nearest monitoring site. The values of the ratios were 1.31 and 1.45 for NH_4^+ and 1.60 and 1.51 for NO_3^- in LC and TC, respectively. In CB the ratio WD/BD from LC was applied, while in CA and the mean of the WD/BD ratios from the LC and TC sites was used. The estimated WD of N in every site was added up

seasonally and annually. Total deposition of N was calculated as the sum of WD and DD in each site in the same seasonal- and annual-basis as WD and DD.

5.2.7. Statistical analysis

Regression analysis and Pearson correlation tests between variables were tested using Statistica version 13 (StatSoft, Inc. Tule, OK, USA), as well as the Student's *t* test for mean comparison. When the data were not normal (according to Shapiro-Wilk test and normal probability plots) the Spearman rank order correlation was used instead of Pearson test and the Mann-Whitney *U* test instead of the Student's *t* test. Alpha level was set at 0.05. The variability of the mean is described in this study whether by the coefficient of variation (CV = standard deviation / mean), or by the standard error (SE = standard deviation / $\sqrt{n}$). Statistica software (version 12; StatSoft, Tulsa, OK) was used for statistical analysis. In this work, seasons were considered as periods of three consecutive months, beginning on 1st January.

5.3. Results

5.3.1. Branch-washing experiment

5.3.1.1. Natural vegetation

The flux of NH_4^+ and NO_3^- deposited to the living branches ($F_{NH_3+NH_4^+}$ and $F_{HNO_3+NO_3^-}$ in eq. 1 and eq. 2) during the exposure periods of the branch-washing experiment showed an overall mean flux of 16.0 µeq NH_4^+ m^{-2} and 25.3 µeq NO_3^- m^{-2} day^{-1} (Table 5.2). The variability of the flux values obtained from the different trees in every site (intra-site variability) was studied, along with the variability across the different periods of the regionally averaged fluxes (temporal variability) and the variability across the three regions of the overall means (inter-regional variability). In general terms, $F_{NH_3+NH_4^+}$ and $F_{HNO_3+NO_3^-}$ showed a slightly larger inter-regional variability (59% and 60%, respectively) than intra-site (mean of 59% and 44%, respectively) or temporal variabilities (mean of 41% and 50%, respectively). The regional mean fluxes were the highest in the Med-HU region (26.3 µeq NH_4^+ m^{-2} day^{-1} and 41.7 µeq NO_3^- m^{-2} day^{-1}), being more than three times larger than the smallest ones. The Med-SH region showed the highest variability between branches (intra-site variability) for both reduced and oxidized forms (84% and 53%, respectively), fairly higher than their respective temporal variability (57% and 33%).

Except for one period in the Med-SH region, NH_3-N was more abundant than HNO_3-N in the atmosphere surrounding the forest sites during all the experiment (Table 5.2). In average, the atmospheric concentration of NH_3 was the highest in the Med-HU region, while the largest concentration of HNO_3 occurred in the Med-SH region. The atmospheric concentrations of NH_3 and HNO_3 for the exposure periods experienced an inter-regional variability of 72% and 42% and a mean temporal variability of 35% and 85%, respectively (data not shown).

The calculated foliar surface conductance values for NH_4^+ and NO_3^- ($K_{NH_4^+}$ and $K_{NO_3^-}$) are shown in Table 5.3. The regression between the flux of ammonium to the branches ($F_{NH_3+NH_4^+}$) and the concentration of NH_3 was significant for the entire dataset. However, one outlier was subtracted from the statistics to improve the regression result (Fig. 5.2). Since the interception coefficient was not statistically different from zero, the regression interception was set to zero in order to obtain a slope coefficient as the only explanatory parameter (equivalent to $K_{NH_4^+}$ value). The surface deposition conductance resulting from this regression was 0.235 cm s^{-1}. None significant regression existed between the flux of nitrate to the branches ($F_{HNO_3+NO_3^-}$) and the concentration of HNO_3 and the *K* values obtained were much more variable among regions for oxidized than for reduced nitrogen. Because of this, and attending to the empirical approximation of the method, the regional values of $K_{NO_3^-}$ were used in the EIM calculations: 1.405 cm s^{-1} for TC site, 0.491 cm s^{-1} for CA site and 1.826 cm s^{-1} for CB and LC sites.

The approximation to an estimation of a surface conductance for particulate and gaseous N according to eq. 7 did not show statistically significant results for NO_3^- and HNO_3. In the case of NH_4^+ and NH_3, a significant regression ($R^2 = 0.35$; $p = 0.033$) gave a K_{PM} of 0.077 ± 0.031 cm s^{-1} and K_{GAS} of 0.185 ± 0.035 cm s^{-1} (coefficient ± SE). However, this regression did not accomplish the basic assumptions of a general lineal model and, therefore, these conductance values will not be taken into consideration for further calculation purposes in the present study.

Significant Spearman correlations of $K_{NH_4^+}$ with temperature and relative humidity (positively and negatively correlated, respectively) occurred for both NB and LB. These correlations were more significant in the case of NB.

Table 5.3. Surface conductance values obtained from the branch-washing experiment for nitric acid vapour ($K_{NO_3^-}$) and ammonia ($K_{NH_4^+}$).

Region	N	$K_{NH_4^+}$ (cm s^{-1})		$K_{NO_3^-}$ (cm s^{-1})	
		Mean	SE	Mean	SE
Med-SA	5	0.258	0.065	<u>1.405</u>	0.478
Med-SH	4	0.272	0.046	<u>0.491</u>	0.168
Med-HU	4	0.181	0.052	<u>1.826</u>	0.775
Mean	3	0.238	0.028	1.241	0.394
Regression	12	<u>0.235</u>	0.013	n.s.	

n.s.: none significant regression was obtained between recovered nitrate and ambient concentration of nitric acid vapour. The values used for dry deposition calculations are underlined.

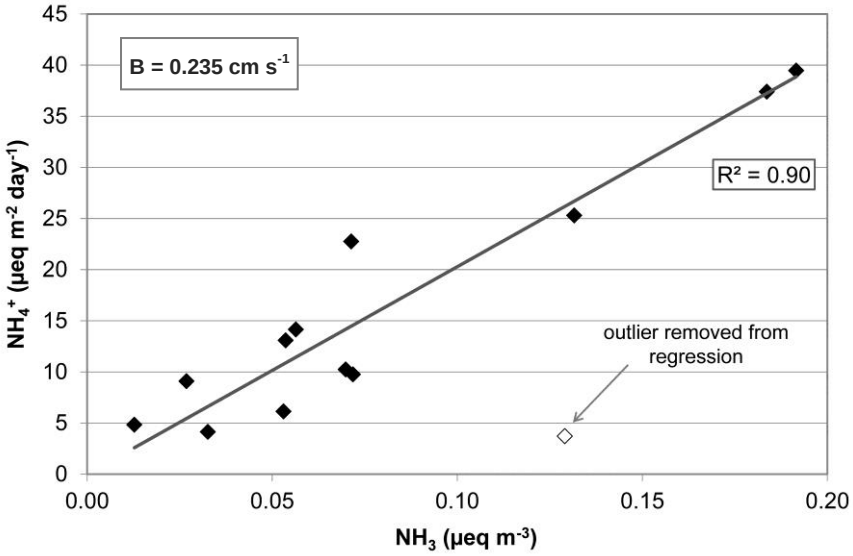


Figure 5.2. Linear regression between the ammonium recovered from leaf-washing experiments (NH_4^+) and the concentration mean of ambient ammonia during exposure periods (NH_3). 'B' corresponds to the slope of the regression (the units were transformed from m day^{-1} to cm s^{-1}).

5.3.1.2. Lyophilized branches

The results of the LB-washing experiment performed in TC site (Med-SA region; n = 5) are shown in Table 5.4. The flux of nitrate ($F_{HNO_3+NO_3^-}$) to LB was slightly higher than to NB (means of 31.84 *vs*. 22.34 µeq NO_3^- m^{-2} day^{-1}). In the case of the flux of ammonium ($F_{NH_3+NH_4^+}$), it was much higher for LB than NB (means of 21.41 *vs*. 8.01 µeq NH_4^+ m^{-2} day^{-1}). The temporal variability of the ammonium flux increased in the

LB experiment with relation to the NB one, while in the case of the nitrate flux, it was the intra-site variability the one which increased noticeably.

None significant regression was found between $F_{NH_3+NH_4^+}$ and NH_3 concentration in TC for neither the LB nor the NB data; and the averaged values of $K_{NH_4^+}$ were, respectively, 0.611 ± 0.125 cm s^{-1} and 0.258 ± 0.065 cm s^{-1} (mean ± SE). Unlike in the NB experiments (both for the entire dataset and for TC data only), a statistically significant regression ($p = 0.042$; $R^2 = 0.80$) between $F_{HNO_3+NO_3^-}$ and HNO_3 concentration was found for LB data. The LB conductance estimated from this regression was 1.491 ± 0.170 cm s^{-1}, while the NB results in TC averaged 1.405 ± 0.478 cm s^{-1}. None significant regression was found when using data from the LB-washing experiment in the approximation of eq. 7.

For the overall dataset, none statistically significant Spearman rank order correlation was found between N fluxes into the branches or calculated conductance values and meteorological variables, apart from a positive one of $F_{HNO_3+NO_3^-}$ and $K_{NO_3^-}$ with wind velocity (R = 0.71 and 0.47, respectively).

Table 5.4. Fluxes of deposited nitrogen into lyophilized vegetation from the branch-washing experiment and averaged concentration of ammonia and nitric acid vapour for each exposure period in TC site (semi-arid Mediterranean region).

REGION	START DATE	END DATE	NH_4^+ (μeq m^{-2} day^{-1})			NO_3^- (μeq m^{-2} day^{-1})			NH_3 (μeq m^{-3})	HNO_3 (μeq m^{-3})
			N [a]	MEAN	CV	N	MEAN	CV		
SA	14/06/2011	28/06/2011	3	20.9	31%	3	46.6	12%	0.074	0.026
	04/10/2011	18/10/2011	3	23.7	8%	3	39.1	24%	0.038	0.032
	04/01/2012	11/01/2012	3	23.6	73%	3	8.5	20%	0.027	0.015
	22/06/2012	06/07/2012	3	20.2	36%	3	52.4	4%	0.066	0.043
	17/04/2013	24/04/2013	3	18.6	72%	3	12.6	35%	0.033	0.007
TOTAL		**N**	**MEAN**	**CV [b]**	**CV [c]**	**MEAN**	**CV [b]**	**CV [c]**	**MEAN**	**MEAN**
		5	21.4	10%	44%	31.8	63%	19%	0.048	0.025

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

5.3.2. Dry deposition estimated with the empirical inferential method

In all the sites, the estimated surface deposition of oxidized N tended to be much larger than the reduced one (means of 7.28 ± 2.15 kg N ha^{-1} $year^{-1}$ *vs*. 2.89 ± 1.14 kg N ha^{-1} $year^{-1}$ for the four sites). The surface deposition of reduced N was more relevant in CA, the most agrarian site, with a two-year mean of 6.24 kg N ha^{-1} $year^{-1}$ (Table 5.5). The intra-annual variability found in the estimates of surface N deposition showed a larger input during late-spring to early-fall in all the sites (Fig 5.3a).

The day-time scaling of the DO_3SE model allowed the estimation of monthly stomatal conductance (g_s; data not shown), which were used for calculate monthly stomatal deposition (Fig. 5.3b). In general, g_s values increased along the year from near-zero values during January and February to maximum monthly-means in May at every site except CA, whose monthly maximum occurred with very similar values from May to August. The decrease from the maximum value to values close to zero again in November-December is slowed down during late-summer and early-fall and this recovery was more noticeable in TC. The site-averaged daytime g_s ranked across the sites as follows: LC > CB > CA > TC; with mean values for a 2011–2013 simulation of 103.5, 98.2, 88.0 and 65.2 mmol H_2O m^{-2} PLA s^{-1}, respectively.

The stomatal deposition of N gases averaged for the four sites 3.31 ± 0.83 kg N ha^{-1} $year^{-1}$, being NO_2 deposition the one contributing the most (2.04 ± 0.45 kg N ha^{-1} $year^{-1}$), although the stomatal uptake of NH_3 was noticeable in CA (Med-HU region), with 1.92 kg N ha^{-1} $year^{-1}$ (Table 5.5). The relative contribution of NO_2 deposition to total dry deposition of N averaged 14.6% in the peri-urban forests, while it represented 7.9% in LC. The use of a stomatal compensation point for NH_3 stomatal exchange in the present study led to an estimated reduction of 59 ± 10% of the stomatal deposition of NH_3 (mean for the four sites) in relation to estimates without compensation point. This resulted in a mean estimated decrease of 4.4% ± 0.5% of the dry deposition of total inorganic N in comparison with the same estimates without using a compensation point for ammonia. The temporal variability found in the estimates of stomatal N deposition (Fig 5.3b) was very similar to the one described above for modeled g_s.

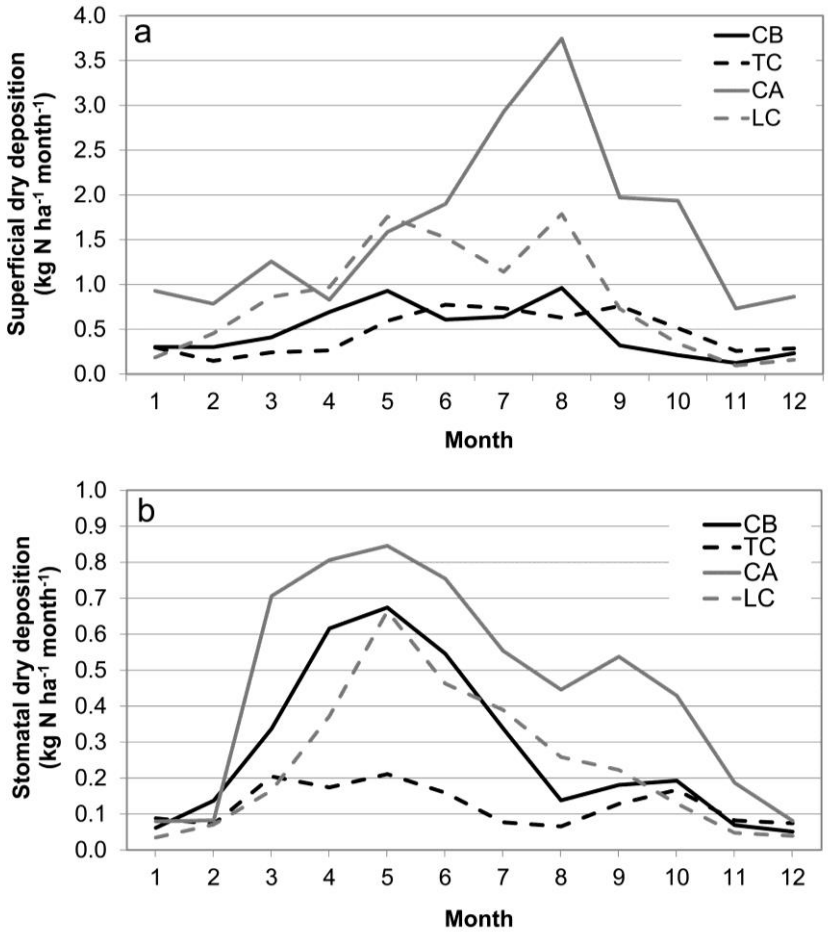


Figure 5.3. Monthly mean (n = 2 years) of (a) surface and (b) stomatal deposition of atmospheric nitrogen estimated for the four *Quercus ilex* forests of the study.

Table 5.5. Estimated and measured N deposition to the four forest sites.

		N deposition (kg N ha^{-1})				N deposition (% of TD)			
		CB	**TC**	**CA**	**LC**	**CB**	**TC**	**CA**	**LC**
Stomatal DD	HNO_3	0.58	0.13	0.54	1.28	5%	1%	2%	7%
	NH_3	0.27	0.17	1.92	0.20	2%	2%	6%	1%
	NO_2	2.52	1.22	3.05	1.38	21%	13%	10%	8%
Surface DD	$HNO_3 + NO_3^-$	3.96	4.25	13.22	7.70	33%	46%	44%	44%
	$NH_3 + NH_4^+$	1.77	1.24	6.24	2.30	15%	13%	21%	13%
Wet deposition	NH_4^+	1.54	1.21	3.56	2.53	13%	13%	12%	15%
	NO_3^-	1.54	1.06	1.83	2.04	13%	11%	6%	12%
	DD N	9.09	7.01	24.97	12.86	75%	76%	82%	74%
	WD N	3.08	2.27	5.39	4.57	25%	24%	18%	26%
	TD N	12.17	9.29	30.36	17.42				

DD: dry deposition; WD: wet deposition; TD: total (wet + dry) deposition

Adding up both stomatal and surface deposition, the dry deposition of total inorganic N ranged from 7.01 kg N ha^{-1} $year^{-1}$ in TC to 24.97 kg N ha^{-1} $year^{-1}$ in CA (Table 5.5), with the surface deposition pathway averaging 74.3 % ± 3.8% of the total dry deposition (Fig. 5.4). In CA, the dry deposition of reduced N represented a 25.0% of the dry deposition of total inorganic N, while in the other of sites it averaged 18.3% ± 0.5%. Dry deposition of N was dominated by the surface dry deposition of oxidized forms of N in all the sites and for most of the seasons (Table 5.5; Fig. 5.4). The highest seasonal estimate of N dry deposition occurred in spring in Med-SH sites and in summer in the other two sites (Fig. 5.4).

5.3.3. Total deposition of N

The estimated annual wet deposition of inorganic N ranged from 2.27 to 5.39 kg N ha^{-1} $year^{-1}$ (in TC and CA sites, respectively), with a mean of 3.83 ± 0.71 kg N ha^{-1} $year^{-1}$ for the four sites (Table 5.5). Contrary to what occurred with dry deposition, the wet deposition of N was slightly dominated by de reduced form (means of 2.21 ± 0.53 kg NH_4-N ha^{-1} $year^{-1}$ *vs*. 1.62 ± 0.21 kg NO_3-N ha^{-1} $year^{-1}$ for the four sites). The predominance of dissolved ammonium over nitrate in wet deposition was more relevant in CA than in the other sites, showing a mean annual deposition of 3.56 kg NH_4-N ha^{-1} $year^{-1}$ (66% of WD in this site).

After adding up wet and dry deposition of N (Fig. 5.5, Table 5.5), the total deposition of TIN varied from 9.29 kg N ha^{-1} $year^{-1}$ in TC to 30.36 kg N ha^{-1} $year^{-1}$ in CA. The dry deposited N represented more than 70% of the annual deposition of TIN in all the sites (mean of 76.2% ± 2.1%). The contribution of the reduced forms to the total deposition was the most important in CA (39%), while it was very similar in the other sites (29% in average). In the same way as dry deposition, total deposition of N was dominated by the surface dry deposition of oxidized forms of N in all the sites (Table 5.5) and for most of the seasons.

5.4. Discussion

5.4.1. Branch-washing experiment

5.4.1.1. Natural vegetation

Fluxes of N to the branches in the Med-SH region showed a relatively high intra-site variability. Besides, it was also higher than the temporal variability for both N fluxes in

this region, while it did not happen in Med-HU region and only for $F_{NH_3+NH_4^+}$ in Med-SA region. However, it was expected that temporal variability were larger than intra-site variability since fluxes of N to the branches are described to vary with the variations in atmospheric concentration of N and meteorological conditions (Andersen and Hovmand, 1999; Fowler et al., 2009; Pratt et al., 1996). The highest intra-site variability for an exposure period found in the Med-SH region occurred in late spring, with CV values of 123% for $F_{NH_3+NH_4^+}$ and 49% for $F_{HNO_3+NO_3^-}$ (Table 5.2), which were similar values to those reported in a previous branch-washing experiment in the LC site (108% for $F_{NH_3+NH_4^+}$ and 49% for $F_{HNO_3+NO_3^-}$, for a similar period of the year; Rodrigo and Àvila, 2002). This high spatial variability can respond to the orography of the terrain in those two sites, since both are placed in a hilly and sloping terrain (> 20º). The

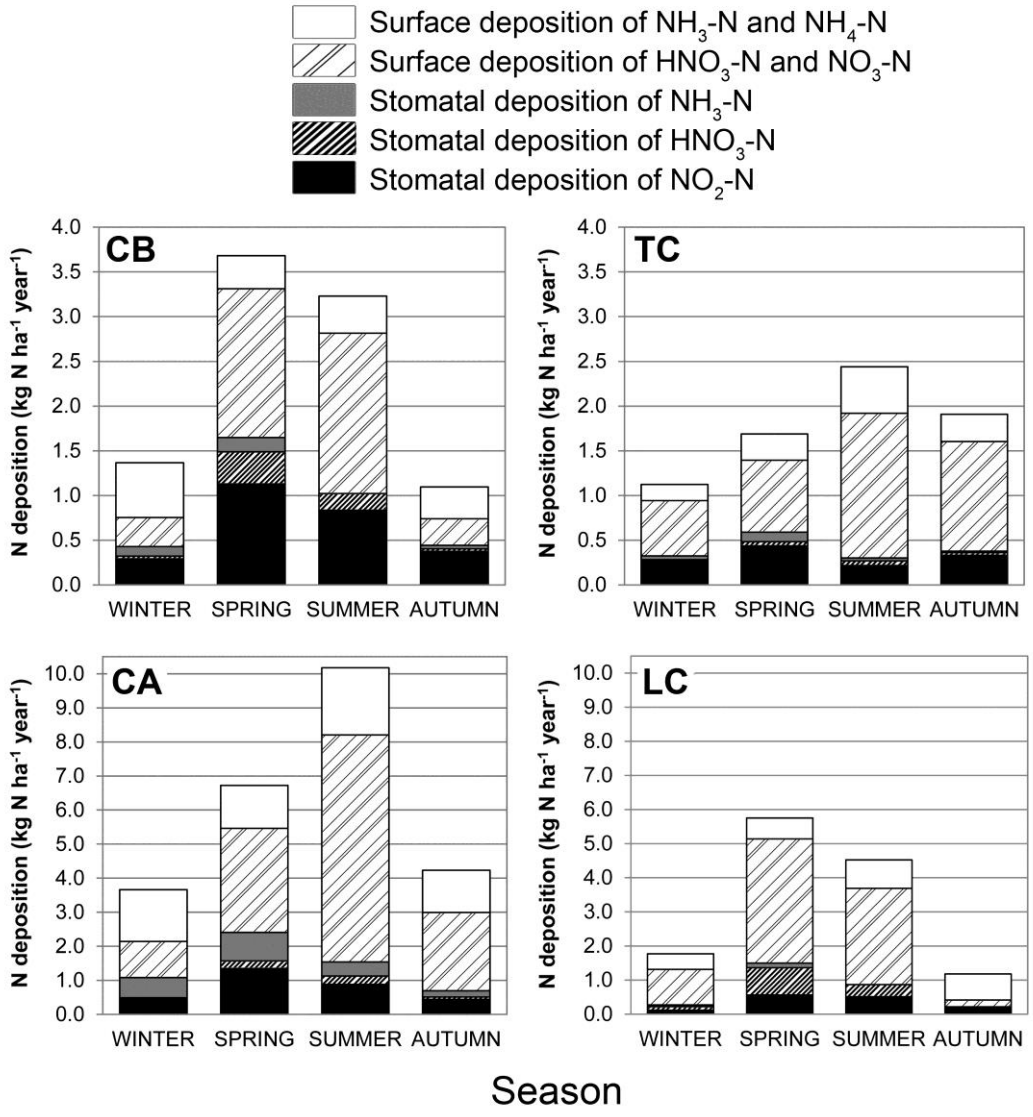


Figure 5.4. Seasonal dry deposition of total inorganic nitrogen into the four *Quercus ilex* forests of the study. Note that the scale for CB and TC is different from the other sites.

representativeness of flux calculations is uncertain in complex terrain, where measurements and calculations have suggested that HNO_3 flux can change by a factor of four over short distances (Clarke et al., 1997). In these conditions, the aerodynamic resistance of the canopy can vary strongly across the monitoring site, making the deposition velocity to the canopy fluctuate as well. The aerodynamic resistance uncertainties are known to induce large uncertainties in deposition velocity particularly for HNO_3, that shows a very small value of surface resistance (Wesley and Hicks, 2000). In the case of $F_{NH_3+NH_4^+}$, the orientation of the branches could enhance this high variability caused by the orography, particularly in the warmest periods of exposure (both late spring and summer showed a CV ≥ 120%). It has been suggested that transpired water causes deposition of hygroscopic particles around stomatal regions, resulting in a partially wetted leaf surface under otherwise dry conditions, and promoting deposition of NH_3 (Wyers and Erisman, 1998). Therefore, sunlight, transpiring leaves are expected to receive more NH_3 deposition than shaded, low-transpiring ones. The complex terrain could be enhancing the differences in the light received by the leaves of the studied forests in the Med-SH region, while the flat terrain and continuous canopy in CA forest (Med-HU region) and the open structure and low LAI of TC forest (Med-SA region) favoured a more equally light incidence.

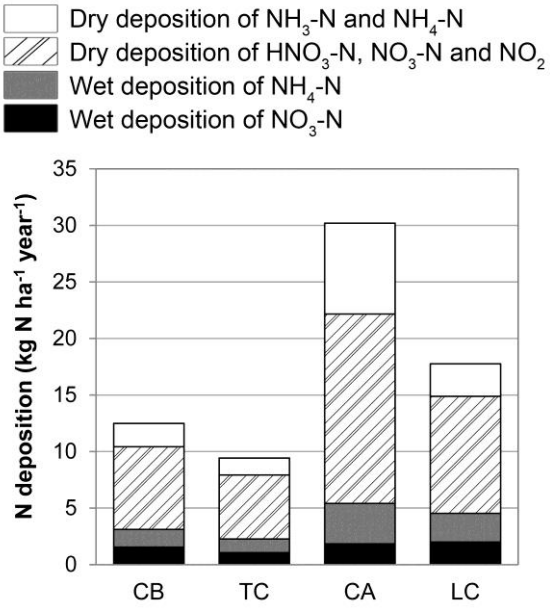


Figure 5.5. Annual deposition of total inorganic nitrogen into the four *Quercus ilex* forests of the study, divided in dry deposition of reduced (NH_3 and particulate NH_4^+) and oxidized (HNO_3, NO_2 and particulate NO_3^-) nitrogen, and wet deposition of reduced (NH_4^+) and oxidized (NO_3^-) nitrogen. Note that the scale for CB and TC is different from the other sites.

With regards to conductance values, the regional means of $K_{NH_4^+}$ were similar among them and similar to the regression-estimated value. The calculated $K_{NH_4^+}$ values varied less than $K_{NO_3^-}$ ones both intra- and inter-regionally (Table 5.3). The good correlation found between $F_{NH_3+NH_4^+}$ and NH_3 (R^2 = 0.95, p < 0.001; Fig. 5.2) indicated that the concentration of NH_3 is a variable that could explain by itself most of the flux of NH_4^+ to the branches, and the low CV values indicated that the deposition surface conductance estimated in this study is robust across time and regions for *Q. ilex* forests. On the other hand, none significant regression was found between $F_{HNO_3+NO_3^-}$ and atmospheric concentrations of HNO_3, and the calculated $K_{NO_3^-}$ showed a high temporal variability, without a clear seasonal pattern, and also a high inter-regional variability, (Table 5.3). These facts altogether made that the estimation of surface deposition of oxidized N were subjected to more uncertainty than the estimation of reduced N deposition. The uncertainty in the deposition conductance of oxidized N (together with LAI values) has been previously described as a substantial component of the total uncertainty of the EIM method and has been described to vary regionally, related to the magnitude of pollutant concentration and deposition values (Bytnerowicz et al., 2015). Since no significant difference was found between the regional mean values of $K_{NO_3^-}$ (data not shown), the overall mean (1.241 cm s^{-1}) could be employed for future estimations of dry deposition by means of branch-washing experiments in the Mediterranean region. Moreover, this value was similar to the value obtained in a previous branch-washing experiments with Mediterranean oaks and used in EIM calculations (1.446 ± 34% cm s^{-1}; Bytnerowicz et al., 2015).

The almost total lack of correlation between meteorological and deposition variables was unexpected, since the deposition rates of pollutants into the forest canopy are known to be very influenced by ambient conditions. The weak correlation between N flux and wind speed were the only exception. Wind-induced turbulences are supposed to enhance deposition rates by increasing atmospheric mixing and surface roughness, particularly for HNO_3, whose deposition rate depends mainly on temperature and wind speed (Pratt et al., 1996). The correlation between $F_{HNO_3+NO_3^-}$ and $K_{NO_3^-}$ indicates that the deposition of HNO_3 is subjected, partially at least, to the spatial and temporal variations of $K_{NO_3^-}$; however, no correlation was found between $K_{NO_3^-}$ and wind speed. It is possible that the heterogeneous duration of the exposure periods (from 8 to 63

days) made the meteorological data less suitable for statistical analysis (in fact, the meteorological variables were not strongly correlated among themselves, as it should be expected).

5.4.1.2. Lyophilized branches

The higher values of $F_{NH_3+NH_4^+}$ obtained using LB in comparison with NB could not be explained by higher ammonia concentrations. The lack of correlation between flux and concentration of reduced N using both NB and LB datasets could be caused by the decreased number of data pairs (n = 5). However, the mean $K_{NH_4^+}$ obtained from NB data was similar to the one estimated from the overall data, while the one obtained from LB data was more than twice this value. Since surface deposition of NH_3 is supposed to follow a main pathway including dissolution in the water film of leaves, a $K_{NH_4^+}$ to LB leaves lower than to NB leaves was expected. A possible explanation would be that the active phylosphere of the NB (living leaves and microorganism), not present in the LB, absorbed part of the surface deposited NH_4^+ during the exposure periods and therefore reduced the $F_{NH_3+NH_4^+}$ observable in the washings.

In the case of $F_{HNO_3+NO_3^-}$, the flux to LB was also higher than the flux to NB, but the difference was smaller than in the $F_{NH_3+NH_4^+}$ case. The intra-site variability for LB experiment was less than a half of the one found in NB washings. This difference could be produced by the most homogeneous exposure of the LB (every pole was installed in an open space of the canopy with similar LB exposed in four different orientations). As it is stated above, the intra-site variability of aerodynamic resistance, caused in this case by the natural heterogeneity in the canopy, is expected to induce large variability in the deposition flux and velocity of HNO_3 across the site (Clarke et al., 1997; Wesley and Hicks, 2000). Although it has been previously observed that the deposition of HNO_3 to coniferous dried branches hanging freely in the space between trees was the same as to those hanging at the outer extremities of the tree (Janson and Granat, 1999), the structure of the canopy of an open holm oak forest is totally different from a Swedish mixed forest of spruce and Scots pine. Apart from the differences in variability, the mean value of the estimated $K_{NO_3^-}$ for LB was very similar to the mean $K_{NO_3^-}$ calculated for NB in the same site and for overall the sites. However, atmospheric concentrations of HNO_3 were significantly correlated with $F_{HNO_3+NO_3^-}$ in the LB experiment but not in the NB one. Similar results were found in a previous study using NB and LB of creosote

bush (Alonso et al., 2005) in which atmospheric concentrations of HNO_3 were positively correlated with NO_3^- fluxes to LB but not with fluxes to NB, and NH_3 concentrations were not correlated with NH_4^+ fluxes, regardless the type of branches used (neither in the present study). In that study, the lack of correlation with NH_3 concentration was explained by a foliar surface deposition of NH_4^+ dominate by particulate deposition of ammonium nitrate (NH_4NO_3).

In the present study, the lack of correlation between fluxes of NO_3^- and NH_4^+ (data not shown) and the low proportion of particulate N during most of the year (Chapter 3) restrained the possibility of an important NH_4NO_3 deposition. However, since aerosol concentration was not taken into account in surface deposition conductance calculations, it should be considered as a possible source of uncertainty; particularly for winter and autumn exposure periods, when the aerosol fraction of atmospheric N became more important (Chapter 3). In fact, the highest mean of $K_{NO_3^-}$ values occurred in CA, the site with the highest concentration of particulate NO_3^- and most favouring conditions for the formation and stability of NH_4NO_3 (Chapter 3), and the mean of $K_{NO_3^-}$ values was higher for winter and autumn than for summer across all the three climatic regions (data not shown). This could mean that particulate NO_3^- deposition was important enough in some exposure periods to bias the $K_{NO_3^-}$ calculations in comparison with the rest of periods. Unfortunately, the approximation exposed in eq. 7 to estimate independent surface deposition conductance for gaseous and particulate forms of N did not show statistically significant results for nitrate flux.

5.4.2. Surface deposition estimates

The resultant intra-annual variability of surface deposition of N (shown here on a monthly basis, Fig. 5.3a) was leaded by the temporal pattern of HNO_3 concentrations (seasonal means are shown in the Chapter 3 of this Thesis, Fig. 3.5). This effect was expectable since $K_{NO_3^-}$ was 2.1–7.8 times higher than $K_{NH_4^+}$, while the atmospheric concentrations of HNO_3-N were only a little smaller than those for NH_3-N (Chapter 3). The inter-sites variability was mainly related to differences in LAI, slightly modulated by site-specific concentrations of HNO_3. It can be noticed how the highest deposition was found in LC (highest LAI and HNO_3 concentrations) and the lowest in TC (lowest LAI and HNO_3 concentrations) (Fig. 5.3a).

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

The monitoring site at TC showed a tree cover of 72%, which was not taken into account in the calculations in order to make possible the comparison among forests at canopy level. Once this correction is applied, the total inorganic N input to the ecosystem due to dry deposition into tree canopies decreased to 6.7 kg N ha^{-1} $year^{-1}$ (ca. 2.5 kg N ha^{-1} $year^{-1}$ lower). Dry deposition of N to understory and grassland vegetation should be added to estimate the total deposition in this particular area of the TC forest. However, since the tree cover is total in other forests in the region, this value is the one which should be used as a standard for comparison or further assessment works.

An estimation of the dry deposition of N to canopy surfaces for LC, CB and TC sites during the same period as the present study were performed by Aguillaume (2015) by means of the canopy budget model (CBM; Staelens et al., 2008). Those estimates showed smaller values than our results for TC site (64% smaller for inorganic N deposition) and larger for CB and LC, particularly for NH_4^+ deposition (276% larger in average for the two sites). Taking into account that NO_3^- deposition estimates in the CBM depends on previous steps of estimation for NH_4^+ deposition, the comparison of these methods is more important for this reduced form of N. In that work, it is indicated that the existing uncertainties in canopy processes and in the parameters employed in this model calculations could be limiting the performance of the model in comparison with other approximations, particularly in TC. The use of annual values in the CBM could have conditioned the results, since dry deposition can be washed by the first rains after dry periods, resulting in important pulses of N inputs in the throughfall (see Fig. 4.3 of the present Thesis) that cannot be studied using annual means.

5.4.3. Stomatal deposition estimates

The site-averaged estimates of leaf uptake of atmospheric gaseous N compounds did not exactly mirrored the inter-site g_s rank, since the largest LAI and NH_3 concentration at CA made the stomatal deposition be twice that in the rest of sites. However, the intra-annual variability mainly followed the variations of the estimated g_s, slightly modulated by the variability of concentration of gaseous N compounds. Particularly, it is noticeable how the forest in CA started to withstand a relatively high stomatal flux of N earlier in the year than the other sites, which was caused by the high winter concentrations of NH_3 derived from agricultural activities in the area (Chapter 3). The inter-site variability responded mainly to a combination of atmospheric concentration of NO_2-N (the predominant gaseous form of N overall the sites), the estimated g_s and the LAI value.

Gaseous N was predominantly uptaken in the form of NO_2-N, despite not having the highest diffusivity (Massman, 1998), because of its highest concentration in air. As expected, the relative contribution of NO_2 deposition to total dry deposition of N was higher at the three peri-urban forests (mean of 14.6%) than in the most remote site (7.9% in LC). Ammonia uptake was relevant (6% of total dry deposition) only in CA, the most agricultural site, because its concentration values were higher than in the rest of sites all along the year (Fig. 3.2). Uptake of N from HNO_3 was even less important because, despite having similar concentrations to NH_3-N, it presents half the diffusivity (Massman, 1998). The natural maxima concentrations of NH_3 and HNO_3 usually occur during summer, when g_s is not the maximum, but in Med-SH region the spring mean concentration of HNO_3 was similar to the summer one (see Fig. 3.2), making the stomatal uptake of HNO_3-N more relevant in these two sites than in the other two (means of 4% *vs*. 2% of total dry deposition, respectively).

5.4.4. Dry and total deposition of N in Spanish *Q. ilex* forest

The estimated dry deposition of atmospheric N was dominated by the surface fluxes of HNO_3 and particulate NO_3^- in all the sites, even when transcuticular deposition of HNO_3 was not taken into consideration. Although many studies have shown the importance of NO_2 and NH_3 contribution to total dry deposition, particularly in sites close to their sources (Flechard et al., 2001; Schmitt et al., 2005), nitric acid deposition is recognized to make a very significant contribution to nitrogen deposition across Europe (Fowler et al., 2009) and other countries (Elliot et al., 2009; Zhang et al., 2005).

The highest contribution of these oxidized forms mainly responded to the high concentration of HNO_3 during spring and summer in relation to the other pollutants. This high levels of HNO_3 can be explained by the photochemical origin of this pollutant (Bytnerowicz et al., 2010; Tzanis et al., 2009), which is enhanced in the sites of the Med-SH region by the ageing of air masses over the Iberian Peninsula and their recirculation along the Mediterranean coast. These seasonal events have been reported in previous studies as processes that increase levels of oxidants, acidic compounds, aerosols and ozone (Escudero et al. 2014; Millán et al. 2002). Nitric acid vapour has been reported to cause epicuticular damage (Padgett et al. 2009a, 2009b) at higher concentrations that those reported herein. The loss of epicuticular integrity could lead to increased susceptibility to other stressors, such as pathogens or ozone (Percy et al., 1992).

The estimated total deposition varied among the sites matching the geographical patterns found using chemical transport models (Chapter 2), which showed a decreasing distribution along a NE–SW axis, with higher deposition in the northern (CA) and eastern coastal regions (LC, CB) than inland (TC). Wet deposition of NH_4^+ measured for the year 2012 in the study sites was slightly higher than the one estimated by CHIMERE model (31% in average) and very similar for NO_3^-. However, modelled values of dry deposition were not larger than 6 kg N ha^{-1} in any of the sites. The main reason would be that the deposition in any cell (10×10 km) is estimated by the model using an averaged LAI based on the land cover of the cell.

Critical loads (CL) are thresholds for N deposition defined as a quantitative estimate of pollutant deposition below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge (CLRTAP, 2004). An empirical CL of 10–20 kg N ha^{-1} $year^{-1}$ was ascribed to Mediterranean *Quercus* forests following expert criteria (Bobbink and Hettelingh, 2011); although a CL of 5.5 kg N ha^{-1} $year^{-1}$ has been also proposed for the protection of epiphytic lichens in similar natural ecosystems in California (Fenn et al., 2010). According to the presented estimations of N deposition, only in TC the threshold proposed in the revision of Bobbink and Hettelingh (2011) was not overreached for the study period, but all sites exceeded the CL proposed for the protection of sensitive epiphytic lichens. Moreover, the interaction with other stressors could be enhancing the harmfull effect of N enrichment in this ecosystem.

5.5. Conclusions

The branch-washing experiment gave surface conductance values similar to values obtained in experiments conducted in other Mediterranean areas. However, the surface conductance values for particulate NO_3^- and HNO_3 varied largely among sites, being lower in the sub-humid region than in the rest of sites. The washing experiment using lyophilized branches gave similar values to the one with living branches for surface conductance values of particulate nitrate and nitric acid vapour, but higher for particulate ammonium and ammonia. This discrepancy could indicate an underestimation of deposited reduced N using the empirical inferential method in the present study.

Concentration of HNO_3 leaded the intra-annual variations of surface N deposition and was also important in the differences detected among the sites, together with LAI. In fact, total deposition of atmospheric N was governed by oxidized forms, which in turn consisted mainly in dry deposition. Further efforts are needed to include reliable HNO_3 measures in broad-scale air quality monitoring networks.

The calculated total deposition of atmospheric N overreached at least one of the proposed empirical critical loads in all the sites. Moreover, the high relevance of HNO_3 deposition can affect cuticular integrity, making trees more vulnerable to other stress factors such as drought and ozone. Although the calculated total deposition varied among the sites following the geographical patterns found using chemical transport models, the dry deposition was underestimated by the model employed in comparison with the EIM. This discrepancy was probably caused by the use of grid-averaged values of LAI by the models. In view of the large importance of dry deposition found in this study, the availability of reliable forest-specific data of measured or estimated LAI is critic for assessment purposes in the Mediterranean region. Besides, the chemical transport models commonly used in risk assessment might include easy-accessible data of dry deposition as a function of LAI.

5.6. Acknowledgments

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CHAPTER 6

GENERAL DISCUSSION

6. General discussion

Nitrogen deposition can be affecting biodiversity in the Mediterranean Basin, which presents an extraordinary biological richness. However, little information is available on the threat that it can pose to biodiversity in the Mediterranean region. Nitrogen deposition and fluxes in holm oak forests have been studied in the framework of the EDEN project because these forests are representative of the Mediterranean Basin. The present Thesis mainly deals with the quantification and characterization of atmospheric N deposition. Besides, the relationships with emission sources and ecosystem effects are also considered in the different chapters.

6.1. Nitrogen deposition in Spain

6.1.1. Wet deposition

Atmospheric deposition of N can pose a threat to ecosystem biodiversity and functioning and suitable N deposition data are needed to identify those natural ecosystems in which effects of N deposition could be occurring. However, monitoring atmospheric deposition is expensive and a limited number of monitoring stations is available. Atmospheric chemical transport models represent a valuable tool to provide these data. CHIMERE and EMEP models are frequently used for obtaining N deposition estimations in European regions and in this Thesis they provided estimations of total N wet deposition in an acceptable agreement with the monitoring data throughout Spain. Their estimates of wet deposition of N in Spain showed a decreasing distribution along a NE–SW axis using both models, with higher values in the northern and eastern coastal regions (up to 16.1 kg N ha^{-1} $year^{-1}$) than inland and southern areas. This distribution pattern clearly responded to the spatial distribution of the expected three main drivers: regional emissions, precipitation distribution and transboundary contribution. In fact, the areas receiving the highest loads of N wet deposition enclose some highly populated and industrialized areas and present high precipitation rates. Transboundary pollution can also represent an important contribution (up to 60–70%) in some of the areas such as Northern Spain or the vicinity of the Strait of Gibraltar (Nyíri et al., 2010). The distribution of total N deposition (including wet and dry deposition) followed similar patterns to those observed for wet deposition, and reached maxima values of 19.4 and 23.0 kg N ha^{-1} $year^{-1}$ according to EMEP and CHIMERE models, respectively.

Generally, both models underestimated the high and overestimated the low measured values of wet N deposition, but each model presented its particularities in their deviations from the measured values. The highest deviation was shown by EMEP model when comparing with values from the Catalan air quality network (RMSE = 4.05 and MNB = 53% for wet deposition of inorganic N). The complex topography of this region and the influence of local emissions might explain the poor model performance. In these conditions, the EMEP model with a 50×50 km resolution cannot be expected to reproduce small-scale variations in deposition regimes as it is argued in previous studies (Simpson et al., 2006a). In fact, CHIMERE model, with a finer resolution (approx. 20×20 km for the period 2005–2007 and approx. 10×10 km for 2008; V200603par-rc1 and V2008b, respectively), obtained better error metrics for the Catalan sites for wet deposition (WD) of inorganic N (RMSE = 2.44 and MNB = -17%), although these results were still worse than for other monitoring networks. Regarding the CHIMERE model, the largest deviations of its estimates with respect to N deposition measurements were found for reduced N, with an overall underestimation of -46% (MNB) and -32% for wet-only deposition (i.e., excluding bulk deposition from ICP Forests monitoring sites). In Chapter 5, the measured and estimated values of wet N deposition for 2012 in the four forest sites of the EDEN project were compared with estimates from the most recent version of CHIMERE model (V2013), showing a better agreement than the previously reported one, with a MNB of -15%. Although results from Chapters 2 and Chapter 5 are not fully comparable, particularly taking into account the number of sites and years employed in each comparison, this could be pointing to an improvement in reduced N deposition estimation by the CHIMERE model. In any case, a recent study comparing the performance of several models in estimating deposition across Europe showed that most of them underestimated the wet deposition of NH_4^+ (Vivanco et al., under review). Finally, another important deviation of modeled values from measurements of wet deposition of inorganic N was detected for the ICP Forests dataset comparison. The general underestimation found with both models (MNB of -40% and -31% for CHIMERE and EMEP estimates, respectively) was partially explained by the bulk samplers used in the ICP Forests network to collect wet deposition, since some influence of dry deposition onto the funnels cannot be disregarded. Results from bulk *vs*. wet-only comparison from previous surveys in LC (Izquierdo and Àvila, 2012) could account only for a small part of this deviation. However, the ratio wet-/bulk deposition (WD/BD) found in LC and TC for NH_4^+ and NO_3^- in the framework of the

EDEN project indicated that the dry deposition of N into the bulk collector funnels can be higher than initially expected and this could be the main reason of the underestimation of ICP Forests deposition data by the models. The proportion of dry deposition collected in the funnels depends on location, climate, sampler aerodynamic characteristics and chemical components (Erisman et al., 2003). Consequently, wet-only collector should be used along with bulk collectors to obtain a WD/BD ratio when long-term measurements of bulk deposition are planned to monitor wet deposition in a particular region. A pre-study period of at least one year with the two types of samplers running in parallel is recommended by the ICP Forests (Clarke et al., 2010).

The scarcity of deposition monitoring sites in high-elevation areas represents a problem for evaluating the performance of air quality models in these areas. In Chapter 2 and in other studies (e.g. Boutin et al., 2015), it has been found some evidence of disagreement between modelled and measured data in mountainous areas. More measurements are needed to improve the evaluation of N deposition estimates in mountainous areas, but also a finer resolution of the emission input and output data would be required. In fact, data from some measurement stations are excluded from the regular evaluations of the EMEP model performance because they are located in mountain areas, and the difference between its elevation and the mean elevation in the respective EMEP model grid cell is larger than 500 m (Fagerli et al., 2015), which highlight the problem of the current resolution of the model for estimating deposition in these mountainous areas. A new resolution for the EMEP model of 0.1×0.1º has been recently developed; however, more significant improvements are expected to be found in sub-urban/urban areas than in rural areas (Fagerli et al., 2015). If atmospheric deposition networks were extended to include more monitoring stations in mountain regions and model resolutions were improved, it would help to assess model deposition performance and to provide reliable deposition data for N enrichment risk assessment in some valuable mountainous ecosystems. For example, thanks to some experimental N deposition monitoring, N deposition has been related to an advanced stage of N saturation in Spanish alpine ecosystems (Camarero and Aniz, 2010).

In air quality monitoring networks, the use of automatic wet-only samplers, in which the collector is open to the atmosphere only during precipitation events, is widely recommended for monitoring atmospheric wet deposition. However, in order to expand monitoring networks to alpine areas it is necessary the use of less expensive methods,

easy to operate, and without requiring frequent visits to the field, like ion-exchange resin collectors (IEC). Results from Chapter 4 indicates that collection methods for N deposition based on IECs can be recommended for long-term studies in the Mediterranean region, since its performance in measuring nitrate (NO_3^-) deposition was good, and it was acceptable for ammonium (NH_4^+) and dissolved inorganic N, in comparison with the conventional bottle collectors (CBC). However, some methodological recommendations on the IEC method, aroused from the present study, should be taken into consideration in any future monitoring design in Mediterranean environments. Preliminary laboratory tests on adsorption and recovery and the use of field blanks are particularly recommended. Moreover, at least one monitoring site, representative of a large alpine area, should be equipped with a wet-only collector to obtain seasonal WD/BD ratios that can be extrapolated to other monitoring sites in the region to obtain wet deposition results, in accordance with what it is stated above.

6.1.2. Dry deposition

In Mediterranean regions, dry deposition largely contributes to the total deposition of N to natural ecosystems and its estimation is still a scientific challenge since none standardized method is available. Estimated by EMEP and CHIMERE models for 2008, dry deposition represented on average 40% and 54% of total deposition of inorganic N, respectively. This estimated dry deposition was similarly distributed in the two modelizations, with the highest values in NE region (Catalonia) and E and SE coastal regions. The relative contribution of dry deposition to total deposition according to both models mainly followed an increasing gradient from NE to SE Spain, but in the case of CHIMERE model, it presented a more detailed distribution in accordance with the annual precipitation across the country. Since the precipitation estimates from both models agreed quite well with the measurements (as it is shown in Chapter 2; Table 2.3), this difference in the spatial pattern could be attributable to the higher spatial resolution of the CHIMERE model.

Atmospheric N deposition in eastern Spanish forests have been experimentally estimated in 15–30 kg N ha^{-1} $year^{-1}$, with dry deposition ranging 40–84% of total N deposition, depending on the location, type of forest and year of estimation (Aguillaume, 2015; Àvila and Rodà, 2012; Rodà et al., 2002; Sanz et al., 2002). When comparing dry deposition values estimated by the models with these previous studies performed in Spanish forests of *Q. ilex* and *P. halepensis*, the results suggested that the

importance of dry deposition could be underestimated, particularly by the EMEP model, for this Mediterranean region. The main reason would be that the deposition is modelled by using a grid-averaged value, based on the land cover of each cell. More detailed studies are needed to characterize measured and modeled dry deposition in forest ecosystems under typically Mediterranean climate conditions. This issue has been addressed in Chapter 5 and is discussed in more detail in section 6.3.2.

6.1.3. Reduced *vs.* oxidized N inputs

The effects caused by the atmospheric deposition of N are proved to differ between the different type of inputs (dry/wet deposition) and forms (oxidized/reduced N) (Hicks et al., 2011; Uscola et al., 2014). It is, therefore, important to consider the different N forms and their relationship with the different pollutant sources and their respective atmospheric transport and chemical processing in order to provide a useful evaluation of N deposition (Hicks et al., 2011).

Annual wet deposition of reduced N for the period 2005–2008 measured in monitoring stations throughout the Spanish territory was lower than wet deposition of oxidized N, showing a ratio of reduced N *vs*. oxidized N (NH_4-N/NO_3-N) of 0.89, which was in agreement with the same ratio for the emissions estimated for the same period (NH_3-N /NO_x-N = 0.86). The model estimates of wet deposition of N showed a mean NH_4-N/NO_3-N ratio of 1.04 for EMEP model and 0.37 for CHIMERE model. This indicated that, even when ammonium deposition estimates of CHIMERE were considered acceptable, their underestimation affected to the relative contribution of reduced N to wet deposition more than it did to the corresponding absolute value of deposition. Considering total deposition (including wet and dry) in 2008, CHIMERE model ratio (0.60) was slightly closer to the emission ratio (0.93) than EMEP model (1.5). This fact, together with the above-exposed underestimation of wet deposition of reduced N by CHIMERE model in comparison with measurements and its higher dry deposition estimates in comparison with EMEP model, points out to an overestimation of dry deposition pathway at the expense of wet deposition one for the reduced forms in CHIMERE model.

Regarding the values for the study period of the EDEN project, annual wet deposition measured in the monitoring sites showed an averaged NH_4-N/NO_3-N ratio of 1.34 for the study period (2011–2013), which was also in agreement with the emission ratio of 1.27 for the same years. Annual bulk deposition showed a ratio of 1.09 and 1.23

according to CBC and IEC methodologies, respectively, not so far from the ratio found for wet deposition. The ratio found for the estimates from CHIMERE model for 2012 was 1.10, in better agreement with the ratio of the emission than in the previous results for 2005–2008 (reflecting a big improvement in this sense with the latest version of the model). In Chapter 2, CHIMERE model applications were performed using the regional V200603par-rc1 version for the 2005–2007 simulations and the V2008b version for 2008 simulations, while the version V2013 was used for 2012 estimations. This latest version seems to give better estimates than the previous ones for reduced N deposition.

With regards to N gaseous compounds for the study period of the EDEN project, NH_3-N was less abundant than the oxidized forms (NO_2-N and HNO_3-N), showing a reduced-/oxidized N ratio of 0.28 on average for the four sites, and being 0.55 in the most agricultural site. This reflects that, even when the emissions of NH_3 are higher at a country level, the concentration of this gas in the air is relevant mainly close to its sources (48% is estimated to come from animal manure and 45% from fertilization; MAGRAMA, 2016), while the oxidized forms are relevant close to the NO_x sources (mainly in the form of NO_2) and also farther due to the secondary formation of HNO_3. On the other hand, the ratio of particulate NH_4-N/NO_3-N showed a mean of 1.52 for the three sites of the EDEN project with PM_{10} samplers. Ammonia is expected to be deposited fast or in turns reacts with acidic gaseous compounds such as sulfuric or nitric acid to form fine particulate matter. Fine-mode secondary aerosols such as ammonium nitrate or ammonium sulphate can have significant long-range transport, up to thousands of kilometres from their site of formation (Behera et al., 2013; Fowler et al., 2009), which could explain the dominance of particulate NH_4^+ over NO_3^- at regional scales.

With respect to dry deposition, the estimations presented in Chapter 5 calculated by means of the empirical inferential method (EIM), reflected a dominant dry deposition of the oxidized N forms over the reduced ones, with a NH_4-N/NO_3-N ratio of 0.33 in average for the four sites. This dominance was leaded by the surface deposition of HNO_3 and particulate NO_3^-, which represented 42% of the total deposition of inorganic N on average for the four sites. Even in the most agricultural-influenced site, dry deposition of reduced N represented only 33% of the dry deposition of inorganic N. On one hand, this result could be expected since the surface deposition rates experimentally estimated for the oxidized forms of atmospheric inorganic N were from 2.1 to 7.8 times

higher than that of the reduced forms, while the atmospheric concentrations of HNO_3-N were only a little smaller, or even similar, than those for NH_3-N. This difference between deposition rates was also shown in other similar studies (Bytnerowicz et al., 2015). On the other hand, below-canopy reduction of gaseous N pollutants found in Chapter 3 was dominated by a general decrease of NH_3 inside the forests, while the below-canopy reduction of HNO_3 was very small and even not statistically significant in half of the sites. Therefore, a dominance of NH_3-N over HNO_3-N deposition could be expected from this point of view. One possible explanation to this discrepancy arouse from the branch-washing experiments using lyophilized branches, in which a higher deposition rate of NH_3 and particulate NH_4^+ (almost twice) was found in comparison to the living branch experiment. The active phylosphere of the living branches (living leaves and microorganism), not present in the lyophilized ones, could have absorbed part of the surface deposited NH_4^+ and NH_3 during the exposure periods, resulting in an apparent lower deposition. This scenario would imply an underestimation of the surface dry deposition of reduced N estimated in this study. Further studies are needed to better estimate and understand the dry deposition rates of reduced and oxidized N in Mediterranean vegetation.

The NH_4-N/NO_3-N ratios for total deposition (dry + wet) estimated in Chapter 5 ranged from 0.40 to 0.62, with a mean of 0.47 ± 0.05 for the four sites. The low ratio found for dry deposition (0.33), due to the high estimated deposition of oxidized forms, forced the ratio of the total N deposition to this low value. Moreover, this ratio for total deposition is not in accordance with the NH_3-N/NO_x-N ratio of the national emissions (1.27). The main reason is probably the early dry deposition of the reduced forms close to the sources, as it was stated above in the discussion about the ratio of the gaseous forms.

As it is stated in Chapter 2, most of the threatened areas showing exceedances of N critical loads (according to the modeled deposition values) received higher oxidized- than reduced N deposition. However, when looking at the surface-weighted means (i.e., the total average of N deposition corrected by the surface of each area assessed), the reduced-/oxidized N ratio in the threatened areas was higher than the same ratio for the entire dataset with both modelizations. The deposition in the threatened areas was obviously higher than in the not-threatened ones, but this difference was more noticeable for the reduced N than for the oxidized forms. This result indicates that the deposition of reduced N is actually more relevant in threatening these protected areas.

Particularly, in those protected areas of the Natura 2000 Network placed in the Alpine Bio-Geographical Region the reduced forms of N clearly dominated the modelled deposition of N, showing ratios of 2.84 and 1.37 according to EMEP and CHIMERE models, respectively.

6.2. Risk assessment of atmospheric N deposition

6.2.1. Assessment of the threats caused by atmospheric N deposition

Total N deposition (including wet and dry deposition) estimated with EMEP and CHIMERE models for 2008 was used to assess the risk of N enrichment in terrestrial habitats of European-Community interest included in the Natura 2000 network. Exceedances of empirical N critical loads were mainly located in high N deposition regions (Figs. 2.4 and 2.5) and mostly involved habitats with high sensitivity to N deposition, based on their low empirical critical load (CL). Sensitive habitats, with N empirical CL of 10 kg N ha^{-1} y^{-1} or lower, included natural grasslands and humid meadows, some mountain forests, and typically Mediterranean heaths (Table 2.2, Annex 2.1).

The most sensitive habitat to atmospheric N deposition based on the low empirical CL and the percentage of area affected was the 'natural grasslands' (subgroup 61), mostly located in the Pyrenees. This category presented 30–60% of its area at risk of N enrichment due to atmospheric N deposition (Table 2.2). Since most of the empirical CL used for natural grasslands were ascribed to their specific habitat type and had good reliability ('#' in Annex 2.1), the major uncertainty of the potential threat of N deposition to Pyrenean grasslands (and to other alpine grasslands) is that no monitoring sites are available to test the model performance for estimating N deposition at this elevation. This constraint is also the major uncertainty in this methodology for assessing the potential threat of N deposition to other habitats located in alpine areas. In fact, other high-altitude vegetation types seemed to be highly threatened by N deposition according to the models (Annex 2.1) and the risk assessment indicated that 41% to 71% of the area assessed within the Spanish Alpine Bio-geographical Region could be experiencing nitrogen CL exceedances. Therefore, further deployment of atmospheric deposition monitoring networks should be implemented in Spanish alpine and mountainous areas to monitor atmospheric pollution, to evaluate models' estimates and to assess the risk of effects on these particularly rich and valuable ecosystems.

This analysis represented the first approach to assess the risk of N enrichment in Spanish ecosystems within Natura 2000 network at a country scale. The results of this assessment agreed with previous works describing some evidence of N effects in particular Spanish ecosystems (e.g., *Quercus ilex* forests of Catalonia, mountain forests of *Abies pinsapo* and Pyrenean grasslands; Àvila and Rodá, 2012; Blanes et al., 2013; Camarero and Aniz, 2010). These concordances suggest that the methodology applied in this analysis is suitable for risk assessment of N deposition effects in Spanish natural and semi-natural habitats. In this sense, other highly valuable natural areas showing CL exceedances should be studied further to characterize possible N effects that could be currently occurring (e.g. Sierra de Guadarrama and Picos de Europa National Park). Besides, further investigation is urgently needed to confirm the suitability of the empirical CL currently used.

Mediterranean sclerophyllous forests showed a large area potentially at risk (ca. 500 km^2). These forests represent a distinctive ecosystem and landscape of the Mediterranean Basin, and include the forests of holm oak (*Quercus ilex* L.), the dominant tree species in the Iberian Peninsula and the target ecosystem within the framework of the EDEN project. Moreover, the comparison of modeled dry deposition values with estimations obtained by other authors showed that the modelled dry deposition could be underestimated for Spanish Mediterranean forests. This underestimation was also suggested in Chapter 5 by the comparison of dry deposition values modelled with CHIMERE and estimated by means of the EIM for the four *Q. ilex* forests of the EDEN project. Therefore, Mediterranean forests in Spain could be withstanding a higher risk derived from N deposition than the one resulting from this first approach and the assessment might be improved by using dry deposition values estimated for each particular habitat type.

6.2.2. Assessment of the threats caused by N gaseous pollutants

Air pollutants cause harmful effects on human health, but can also directly affect natural vegetation. According to the established thresholds and the available scientific evidence, the results presented in Chapter 3 indicated that O_3 is the only air pollutant considered in this work which is expected to have direct phytotoxic effects on vegetation in the studied natural areas. The concentrations of N compounds were not high enough to directly affect vegetation but could be contributing through atmospheric N deposition to the eutrophization of these ecosystems. Morevover, although the annual mean of NH_3

concentrations did not exceed the critical level of 3 μg m^{-3} proposed for the protection of higher plants (CLRTAP, 2011) in any of the sites, the critical level of 1 μg m^{-3} for the protection of lichens and bryophytes (Cape et al., 2009; CLRTAP, 2011) was exceeded in the most agricultural-influenced site (CA) and it was about to be exceeded in the most urban-influenced one (CB). Finally, although none critical level has been proposed for atmospheric HNO_3 concentrations yet, this compound reached high values during spring and summer and this atmospheric compound could cause epicuticular damage (Padgett et al., 2009). The loss of epicuticular integrity could lead to increased susceptibility to other stressors (Percy et al., 1992), such as pathogens, drought or ozone.

Evergreen broadleaf Mediterranean woody species are assumed to be tolerant to air pollution due to their sclerophyllic adaptations. However, recent publications suggest that the addition and interaction of different stress factors (O_3, N deposition, drought) can be affecting the growth of the trees (Alonso et al., 2014; Gerosa et al., 2015) and accompanying pastures (Calvete-Sogo et al., 2014, 2016). Thus, monitoring of nitrogen compounds such as NH_3 and HNO_3 should be incorporated into air quality monitoring networks (in most of them NO_2 and O_3 are already monitored).

6.3. Atmospheric deposition of N in Spanish forests of *Quercus ilex*

The study of N deposition in *Q. ilex* forests has not been performed before over a wide regional scale in Spain. Besides, the long term monitoring sites in these characteristic Mediterranean forests (two sites of the ICP Forests network located in southern and eastern Spain and the experimental site at La Castanya) are placed in remote areas of the Spanish territory, in order to study the regional background concentration levels and deposition of pollutants. The results on loads and spatial and temporal variability of N deposition to peri-urban holm oak forests represent a valuable complement to these monitoring data and will help to establish CL for the protection of this Mediterranean ecosystem.

6.3.1. Wet, bulk and throughfall deposition of inorganic nitrogen

The estimated annual wet deposition of inorganic N ranged from 2.27 to 5.39 kg N ha^{-1} $year^{-1}$ (in TC and CA sites, respectively), with a mean of 3.83 ± 0.71 kg N ha^{-1} $year^{-1}$ for the four sites (Table 5.5). Wet deposition varied among the sites matching the

geographical patterns found using chemical transport models (Fig. 2.4), with higher deposition in the northern (CA) and north-eastern coastal regions (LC, CB) than inland (TC). The wet deposition of N was dominated by de reduced form (2.21 ± 0.53 kg NH_4-N ha^{-1} $year^{-1}$ *vs*. 1.62 ± 0.21 kg NO_3-N ha^{-1} $year^{-1}$ on average for the four sites), which showed the highest relevance in CA with a mean annual deposition of 3.56 kg NH_4-N ha^{-1} $year^{-1}$ (66% of the wet deposition at this site). Despite being measured mostly in peri-urban sites, these wet deposition results are not very different from the wet deposition measured in the Spanish EMEP background monitoring sites. For 2012, the EMEP measurements averaged 3.34 ± 0.75 kg N ha^{-1} $year^{-1}$, while the EDEN measurement averaged 2.46 ± 0.65 kg N ha^{-1} $year^{-1}$. The proportion of reduced and oxidized N in the wet deposition was also similar between both dataset of measurements for this particular year, showing a reduced-/oxidized N ratio around 1.2 in both cases. These results indicate that the wet deposition of N in Spain is not necessarily dominated by the near-source influences, and that the regional processes of atmospheric chemistry and transport are the major drivers.

Mean annual bulk deposition of N for the 2-year period of the study ranged 2.42–6.83 and 3.09–5.43 kg N ha^{-1} $year^{-1}$ among the four sites according to CBC and IEC methodologies, respectively. These values of bulk deposition are similar to those measured in the ICP Forests monitoring network in Spain in the previous period 2005-2008, which showed mean values ranging from 3.53 ± 0.66 kg N ha^{-1} $year^{-1}$ to 10.80 ± 1.30 kg N ha^{-1} $year^{-1}$. The two monitoring sites of this network placed in holm oak habitats ranged from 2.18 kg N ha^{-1} $year^{-1}$ to 6.28 kg N ha^{-1} $year^{-1}$, depending on the site and the year. The deposition data from EDEN project will complement the ICP Forests ones and further research on deposition-effects relationships will be performed with the joined datasets.

Mean annual throughfall deposition of inorganic N ranged 2.33–8.20 and 4.59–8.91 kg N ha^{-1} $year^{-1}$ among the sites, according to CBC and IEC methodologies, respectively. Net throughfall data in the three peri-urban forests indicated a net annual flux of oxidized N to the forest soil coming from dry deposition, even though some canopy NO_3^- retention could be occurring. This input of dry deposited NO_3-N was the highest at the most urban-influenced site (CB), where also a net annual flux of reduced N to the forest soil occurred. By contrast, net throughfall values of TC and CA forests indicated a net retention of deposited NH_4^+ in the canopy (Fig. 4.2). The fact that the forest

canopy at CB seemed to retain less deposited N than the other two sites (because of its positive and higher net throughfall deposition of both NO_3-N and NH_4-N), together with the observation of a lower retention of atmospheric gaseous N (Fig. 3.4), a relatively high leaf N content (1.61%, the highest of the three peri-urban forests) and the evidence of the onset of N saturation in other *Q. ilex* forest in the region (Àvila and Rodà, 2012), suggest that this forest and other peri-urban forest close to Barcelona city could be already experiencing a N saturation process.

Negative values of net throughfall were most commonly observed with NH_4^+ (Fig. 4.3), while positive values were mostly composed of NO_3^-, indicating that canopy retention of wet-deposited N in these forests occurred more often in the reduced form. Moreover, the preferably retention of NH_4^+ over NO_3^- is in concordance with the below-canopy reduction of atmospheric N pollutants, in which the concentrations of the reduced form of N (NH_3-N) decreased more than the concentrations of the oxidized forms (NO_2-N and HNO_3-N). Both results agreed with the hypothesis of reduced N being preferentially assimilated by plants and lichens over and above other forms of N (e.g. Dahlman et al., 2004; Raven, 1988; Ritsuko et al., 2002).

Relatively high positive values of net throughfall, implying relatively large and ephemeral pulses of N into the soil with the first rainfall events after rainless periods, were observed in the most urban-influenced sites (CB and TC). These ephemeral inputs, among other effects, can trigger pulses of NO emissions from soil (Homyak and Sickman, 2014) or provoke a flushing of inorganic N to ground- or stream waters if the pulse occurs when plants and soil communities are not able to use this dissolved N (like at the end of the summer, when they are withstanding drought stress). This effect, known as the asynchrony hypothesis (Meixner and Fenn, 2004), is corroborated in TC, where relatively high NO_3^- concentrations in the soil water (up to 28.15 and 4.90 mg NO_3-N l^{-1} at 20 and 40 cm depth, respectively) have been found after these pulses of N during late-summer and early-autumn. However, none NO_3^- concentration increase in the soil water was observed after an ephemeral input of N during the early spring of 2012, when understory annual pastures were emerged and growing and soil communities were presumably active, avoiding N losses from the ecosystem. Further investigation of these input events is currently ongoing by means of biogeochemical modelization of the forest at TC site with the ForSAFE model.

The ForSAFE model is aimed at the dynamic simulation of changes in soil chemistry, soil organic matter, hydrology, and tree biomass growth in relation to changes in environmental factors (Wallman et al., 2005). Some studies have used ForSAFE to model temperate forests of *Quercus petraea* (Gaudio et al., 2015; Rizzetto et al., 2016), after calibration and validation. The same work is being done for *Q. ilex* by the *Ecotoxicology of Air Pollution* research group of CIEMAT in collaboration with the *Centre for Environmental and Climate Research* of Lund University and *Belyazid Consulting & Communication* AB. Besides, the particularities of Mediterranean meteorology, soil and seasonality of ecological processes make necessary a new calibration and validation of the model using typical Mediterranean values as the input dataset. The objective of the modelizations is verifying that ForSAFE model adapts or can be adapted to simulate the N cycling dynamics in Mediterranean ecosystems, including the ephemeral inputs of N that occurs with the first rains after rainless periods. To achieve this, a previous goal that must be completed is the creation of a reliable input database to fulfil the model input requirements, which includes data on meteorology, ecophysiology of trees, soil characteristics, atmospheric deposition and forest management. The monitoring work conducted in the framework of the EDEN project will fulfil most of these inputs.

6.3.2. Dry deposition of inorganic nitrogen

The good correlation found between NH_4^+ recovered in the branch-washing experiments and the atmospheric concentration of NH_3 (Fig. 5.2), together with the low variability found for the calculated surface conductance values for reduced N ($K_{NH_4^+}$), indicated that the $K_{NH_4^+}$ employed in this study (0.235 cm s^{-1}) is robust across time and regions for *Q. ilex* forests. On the other hand, the calculated $K_{NO_3^-}$ values showed a high temporal variability, without a clear seasonal pattern, and also a high inter-regional variability (0.491–1.826 cm s^{-1}), making the estimation of surface dry deposition of oxidized N subjected to more uncertainty than the estimation of reduced N deposition in *Q. ilex* forests. Some uncertainty could be attributable to the uncertainty in the HNO_3 measurements by means of passive samplers following Bytnerowicz et al. (2005). This method included calculations that were parametrized for Mediterranean conditions, but the authors recommended that careful calibrations against denuder systems or other reference methods were performed in the areas of interest in various seasons, mainly because of potential interferences caused by dust particles and high humidity, and the

changing activity of ambient photochemical processes. A thorough calibration study across different European-Mediterranean climatic regions and seasons should be performed, paying special attention to the commonly co-ocurrence of high concentration of ozone, since ozone has been reported to reduce retention of HNO_3 on nylon filters, and also to sampling periods with extreme humidity, since very high humidity has been shown to increase retention of nitrate on Teflon™ filters (Padget, 2010).

The low correlation found between meteorological and dry deposition variables was unexpected, since the deposition rates of pollutants into the forest canopy are known to be very influenced by ambient conditions. This fact could be related to the heterogeneous duration of the exposure periods (from 8 to 63 days), which made the meteorological data less suitable for statistical analysis. In any case, a correlation between N flux and wind speed was found. Wind-induced turbulences are supposed to enhance deposition rates by increasing atmospheric mixing and surface roughness, particularly for HNO_3, whose deposition rate depends mainly on temperature and wind speed (Pratt et al., 1996). The correlation between $F_{HNO_3+NO_3^-}$ and $K_{NO_3^-}$ indicates that the deposition of HNO_3 is subjected, partially at least, to the spatial and temporal variations of $K_{NO_3^-}$; however, no correlation was found between $K_{NO_3^-}$ and wind speed. Attending to a regional perspective, the annual mean of wind speed in CA was around 5–7 times larger than the other three sites for the 2-year period of the study (Table 1.2). Taking into account that the forests' structure and the averaged wind speeds, the expected rank for turbulence would be CA > TC > CB ≈ LC, which corresponds well with the inter-regional rank found for $K_{NO_3^-}$ (Table 5.2). This correspondence could indicate that there was a regional differentiation of $K_{NO_3^-}$ derived from meteorological and forest structure variables.

The intra-annual variability of the surface deposition of N (Fig. 5.3a) was leaded by the temporal pattern of HNO_3 concentrations (Fig 3.2; Annex 3.3). This effect was expectable since $K_{NO_3^-}$ was 2.1–7.8 times higher than $K_{NH_4^+}$, while the atmospheric concentrations of HNO_3-N were only a little smaller than those for NH_3-N. The variability among sites was mainly related to differences in the leaf area index (LAI), slightly modulated by site-specific concentrations of HNO_3. It can be noticed that the

highest surface deposition was found in LC (highest LAI and HNO_3 concentrations) and the lowest in TC (lowest LAI and HNO_3 concentrations) (Fig. 5.3a).

Estimation of stomatal conductance (g_s) by means of the DO_3SE model followed an expected temporal (intra-annual) variability, which in turns lead the temporal variation of the stomatal deposition of N in the four forests. On the contrary, the values of g_s did not lead the geographical (inter-regional) variability, which was more influenced by the leaf area index (LAI), as it was observed also for surface dry deposition. As expected, gaseous N was predominantly uptaken in the form of NO_2-N because of its highest concentration in air. The relative contribution of NO_2 uptake to total dry deposition of N was higher at the three peri-urban forests (mean of 14.6%) than in the most remote site (7.9% in LC), and NH_3 stomatal uptake was only relevant (6% of total dry deposition) in the most agricultural site (CA), because its concentration values were higher than in the rest of sites all along the year (Fig. 3.2). Some uncertainties are not solved in the present study in relation with the use of the DO_3SE model and they will be addressed in a further research. Firstly, the limiting factor of the g_s in the model related to soil water content (f_{SWC}) was not applied in the present study because of the lack of soil data for some of the sites. Although a phenological limiting factor (f_{phen}) that partially includes the drought stress was employed in the modelization, it has been proved that the best performance is obtained when both f_{phen} and f_{SWP} are included (Alonso et al., 2008). In any case, severe limitations due to water stress should be only expected in the TC site due to its semi-arid conditions. Secondly, the model parametrization employed is intended for continental sites. Although this parameterization was proved to predict reasonably well the actual g_s values recorded under a wide range of environmental conditions, two different parameterizations of the model are recommended, one for marine-influenced sites and another one for continental sites (Alonso et al., 2008). Finally, a correction of the results based on light extinction across the canopy could be proposed (e.g., similar to Grünhage et al. 2000), particularly for *Q. ilex* forests showing a relatively high value of LAI, like in CA and LC sites.

The estimated dry deposition (surface + stomatal) of atmospheric N was dominated by the surface flux of HNO_3 and particulate NO_3^- in all the sites. Due to the lower deposition velocity and smaller concentration of the particulate N, this flux is probably dominated by the gaseous form. In fact, nitric acid deposition is recognized to make a very significant contribution to nitrogen deposition across Europe (Fowler et al., 2009)

and other countries (Elliot et al., 2009; Zhang et al., 2005). The highest contribution of these oxidized forms mainly responded to the high concentration of HNO_3 during spring and summer in relation to the rest of N pollutants. This high levels of HNO_3 can be explained by the photochemical origin of this pollutant (Bytnerowicz et al., 2010; Tzanis et al., 2009), which can be enhanced in the LC and CB sites by the ageing of air masses over the Iberian Peninsula and their recirculation along the Mediterranean coast (Escudero et al., 2014; Millán et al., 2002). These results point out again to the need for including HNO_3 measurements in air pollution monitoring stations in highly valuable natural areas.

6.3.3. Total deposition of inorganic nitrogen

After adding up wet and dry deposition of N (Fig. 5.5, Table 5.5), the estimated total deposition varied among the sites matching the geographical patterns found in the initial modelizations, with higher deposition in the northern (30.36 kg N ha^{-1} $year^{-1}$ in CA) and north-eastern coastal region (17.42 and 12.17 kg N ha^{-1} $year^{-1}$ in LC and CB, respectively) than inland (9.29 kg N ha^{-1} $year^{-1}$ in TC). The dry deposited N represented more than 70% of the annual deposition of inorganic N in all the sites (mean of 76.2% ± 2.1%). Total deposition estimated for CA in relation with the rest of sites was larger than expected, since models estimated higher values in LC and CB than in CA. The higher $K_{NO_3^-}$ calculated for CA, together with the relatively high HNO_3 concentrations and the large LAI value of the forest at this site, are the main cause of the elevated deposition estimated. This kind of particularities cannot be reflected in broad-scale air quality model estimates.

For this study period, the empirical CL proposed for the protection of *Q. ilex* forests in the framework of the CLRTAP (10–20 kg N ha^{-1} $year^{-1}$) was exceeded in all sites except one (TC), but all sites exceeded the CL proposed for the protection of sensitive epiphytic lichens in similar natural ecosystems in California (5.5 kg N ha^{-1} $year^{-1}$; Fenn et al., 2010). Applying the same CL used in Chapter 2 for holm oak forests (15.0 kg N ha^{-1} $year^{-1}$), only the forests at LC and CA would be declared as threatened habitats attending to the study results for the year 2012. Using deposition data estimated with EMEP and CHIMERE models (grid-averaged data, without a forest-specific dry deposition, as they were used in Chapter 2) for the same year, none of the four forests would be declared as threatened using the above-mentioned CL.

6.3.4. Bulk and throughfall deposition of organic N

The measurement of dissolved organic N (DON) is not commonly addressed in deposition monitorization works. A study in the framework of the EDEN project (Izquieta-Rojano et al., 2016) showed that DON may constitute another factor driving the uncertainties in the knowledge of the N cycle in the Region, since it was found to contribute from 34% to 56% to the bulk deposition of dissolved N for the four study sites of the project and from 38% to 72% to the throughfall deposition.

The bulk deposition of DON ranged from 1.08 kg N ha^{-1} $year^{-1}$ in TC to 12.27 kg N ha^{-1} $year^{-1}$ in CA. If it were taking into account as an additional input of N in the deposition estimations the atmospheric N deposition would average 22.37 ± 7.02 08 kg N ha^{-1} $year^{-1}$ for the four sites for the study period, ranging from 10.50 kg N ha^{-1} $year^{-1}$ in TC to 42.47 kg N ha^{-1} $year^{-1}$ in CA. However, the origin of this DON in bulk deposition is difficult to assess and it seems probable that (in-situ) biogenic sources may be responsible to some extent for the organic budgets at these forests, which would mean that not all the DON measured in deposition could be considered as an input into the ecosystems.

The study showed how fuel-combustion sources may form organic nitrates that constitute an important component of the DON deposition in the most urban-influenced sites (CB and TC); while in the most agricultural-influenced site (CA) most of the DON is expected to come from fertilization, which means an important proportion of urea and aminoacids that could eventually release dissolved, reduced forms of N. The possible implication of this hypothetical release in the discrepancies between IEC and CBC found for bulk deposition of NH_4-N in CA merits further research.

The study of DON in throughfall deposition is complicated because a release of DON from the canopy is commonly reported and attributed to the transformation of inorganic N into organic N at the canopy level, among other reasons (Cape et al., 2010; Gaige et al., 2007). The possibility of the existence of similar transformations during dry periods and their implications in the results of the branch-washing experiments and the uncertainties found in the dry deposition estimations could be the aim of further studies in these sites. Finally, negative values of net throughfall of DON were only found of the most agricultural-influenced site (CA) during the period of greatest vegetation activity. It seems to indicate an active uptake by the phyllosphere, which would occur preferably with DON emitted from agricultural sources than from urban and traffic sources.

6.4. Effects of atmospheric deposition of N in *Q. ilex* forests

The present Thesis exposes and discusses the results concerning atmospheric N deposition in *Q. ilex* forests, but the data obtained in the framework of the EDEN project will be exploit in the near future to accomplish other project objectives, including the relationship between N deposition and measurable ecosystem effects and indicators. Both the terrestrial- and the aquatic-based N saturation theories suggest that NO_3^- concentration in streamwater should be the primary indicator of N status and should increase over time with chronic N deposition (Aber et al., 2003). Increases of NO_3^-concentration in streamwater have been already reported in NE Spain forests (Rodà et al., 2002; Àvila and Rodà, 2012). Moreover, there are other indicators of N enrichment in forest ecosystems, such as increasing N content and decreasing C/N ratios in living tissues, soil and litterfall, and nitrate leaching to the groundwater (de Vrieset al., 2014; Fenn et al., 2008). All these variables were sampled and recorded in the framework of the EDEN project, following the methodology proposed in the ICP Forests level II network. Further research will be conducted merging EDEN and ICP Forests datasets in order to seek a CL for the protection of holm oak and other sclerophyllous evergreen forests. As it is pointed out in Chapter 2, there is a need for improving the definition and reliability of the CL used for risk assessment in this kind of Mediterranean ecosystems.

Nonetheless, other early effects from N deposition, such as changes in species composition of sensitive communities (like lichens), can occur (Cape et al., 2009) and have been consequently used to define empirical critical loads and levels. The functional groups of epiphytic lichen have been used as indicators of NH_3 pollution to establish a critical level (the concentration above which direct adverse effects on receptors may occur according to the present knowledge) for NH_3 in NE Spain (Aguillaume, 2015). A critical level of 2.5 µg m^{-3} was proposed for forests that have already suffered from an historical N exposure to farming and agricultural pollutant sources. It was stated that the study of lichen functional groups can be used to derive critical levels under a wide range of Mediterranean conditions. This methodology could also be applied for establishing CL of N deposition in forests influenced by different sources of gaseous N pollutants if measurement- and estimation methodologies exposed in Chapter 4 and Chapter 5 are incorporated to the analysis.

As a result of joining efforts between the EDEN project with other regional, national and European projects, such as AGRISOST (S2009AGR-1630), MONTES (CONSOLIDER-INGENIO CSD2008-00040) and ECLAIRE (EU-FP7-ENV-2011), the effects of N deposition and its interactions with tropospheric ozone (O_3) were studied in annual pastures characteristic of the understory of holm oak forests and *dehesas* (see Annex 6). Two experiments in two consecutive years were carried out with a mixture of representative Mediterranean annual species which were exposed to different O_3 levels and N inputs in open-top chambers. The results showed that O_3 induced visible injury and reduced the yield and gross primary production of the community (Calvete-Sogo et al., 2014; Sanz et al., 2013). Nitrogen could partially counterbalance O_3 effects when the levels of the pollutant were medium. On the other hand, O_3 reduced the fertilization effect of higher N availability, increasing N losses of the ecosystem. Interestingly, nitrogen fixing legumes were more sensitive to O_3 and showed low response to N, while grasses and herbs were more tolerant to O_3 and more responsive to N (Calvete-Sogo et al., 2016). Thus the interactive effects of O_3 and N can alter the structure and species composition of Mediterranean annual pastures via changes in the competitive relationships among species. These results highlight that the interactive effects with tropospheric O_3 concentrations need to be considered when evaluating the impacts of atmospheric N deposition in Mediterranean ecosystems.

Finally, the possible effects of the relatively large and ephemeral pulses of N into the soil showed in Chapter 4 should be studied, particularly in the most urban-influenced sites. These ephemeral inputs, among other effects, can trigger pulses of NO emissions from soil (Homyak and Sickman, 2014) or provoke a flushing of inorganic N to ground- or stream waters in agreement with the asynchrony hypothesis (Meixner and Fenn, 2004), as it was corroborated in TC. Potential changes in soil community and ecosystem functioning related to these pulses need to be further explored.

CHAPTER 7

General conclusions

7. General conclusions

The general conclusions are summarized below following the four main objectives of the Thesis exposed in Chapter 1:

Objective 1. Assessment of the risk of atmospheric nitrogen (N) deposition effects on Spanish ecosystems at a country scale.

- EMEP and CHIMERE models constitute suitable tools to provide acceptable estimates of N wet deposition in Spain.
- Atmospheric N deposition in Spain showed a decreasing distribution along a NE–SW axis, with higher deposition in the northern and eastern coastal regions than inland and southern areas.
- Atmospheric N deposition should be considered as a factor that could be affecting the biodiversity and health of the protected natural ecosystems in Spain, particularly the natural grasslands and endemic heaths in northern Spain (Pyrenees, Cantabrian Range). Other habitats threatened by N deposition were located in mountainous areas close to high emission sources, such as Mediterranean forests and scrublands.
- Further deployment of atmospheric deposition monitoring networks should be implemented in Spanish alpine and mountain areas to monitor atmospheric pollution, to evaluate atmospheric chemical transport model performance and for risk assessment purposes.
- More detailed investigations should be carried out to improve the definition of empirical critical loads for Mediterranean ecosystems.

Objective 2. Characterization of the levels, variability and environmental risks of the main atmospheric pollutants in Mediterranean holm oak forests while seeking measurable evidences of air quality improvement inside forests in peri-urban environments.

- Ozone concentrations around Spanish cities are high enough to directly impact peri-urban forests. Gaseous N compounds could be contributing through atmospheric N deposition to the eutrophization of these ecosystems, and their interaction with other stress factors could be affecting the ecosystem functioning
- Forests of *Quercus ilex* have proved to experience a significant below-canopy reduction of pollutant concentrations, particularly of NH_3, but also of NO_2, HNO_3

and O_3. These results provide scientific evidence of the ability of these ecosystems to improve air quality.

- Although a high deposition rate of HNO_3 was expected, NH_3 and NO_2 experienced the highest relative and absolute reductions (respectively) of below-canopy concentration in comparison with the levels in open field.
- Well-designed monitoring programs of urban and peri-urban forests could accomplish both objectives of further investigate air quality improvement while assessing the threat that air pollution can pose to vegetation.

Objective 3. Study of bulk and throughfall atmospheric N deposition to holm oak forests by means of conventional and innovative methodologies.

- Collection methods based on ion-exchange resins can be recommended for long-term studies of atmospheric N deposition in the Mediterranean region. The methodological recommendations arising from the present study should be taken into consideration in the monitoring design.
- Preliminary laboratory tests on adsorption and recovery and the use of field blanks are particularly recommended.
- Mean annual bulk deposition of inorganic N in the four Spanish holm oak forests ranged 2.42–6.83 and 3.09–5.43 kg N ha^{-1} according to conventional and ion-exchange resin methodologies, respectively.
- Ephemeral input pulses of N into the forest soil after dry periods were found, presumably originated from the washing of dry deposition accumulated in the canopy. The implication of these nutrient pulses for ecosystem functioning, atmospheric chemistry and N leaching should be further investigated.

Objective 4.- Estimation of dry and total atmospheric deposition of inorganic N to holm oak forests.

- The branch-washing experiment gave surface conductance values similar to values obtained for other Mediterranean areas. However, the surface conductance values for oxidized N varied largely among climatic regions.
- Based on a branch-washing experiment performed with lyophilized branches, it was detected a possible underestimation of deposited reduced N using the empirical inferential method in the present study.

- Concentration of HNO_3 modulated the intra-annual variations of surface N deposition, and it was also important to explain the variability among sites. Further efforts are needed to include reliable HNO_3 measurements in broad-scale air quality monitoring networks.
- The estimated deposition of atmospheric inorganic N, with dry deposition representing 76.2% ± 2.1% of it, matched the geographical patterns previously found in model estimates, showing higher deposition in the northern and eastern-coast regions (from 12.17 to 30.36 kg N ha^{-1} $year^{-1}$) than in central Spain (9.29 kg N ha^{-1} $year^{-1}$).
- Total atmospheric N deposition in all the studied sites exceeded some of the currently proposed critical loads. Moreover, the high relevance of HNO_3 deposition could affect the cuticular integrity, making trees more vulnerable to other stress factors such as drought and ozone.
- The improvement of methodologies for estimating dry deposition of N adapted to valuable ecosystems is crucial in the Mediterranean region. Besides, the availability of forest-specific estimates of dry deposition from atmospheric chemical transport models is also essential for assessment purposes.

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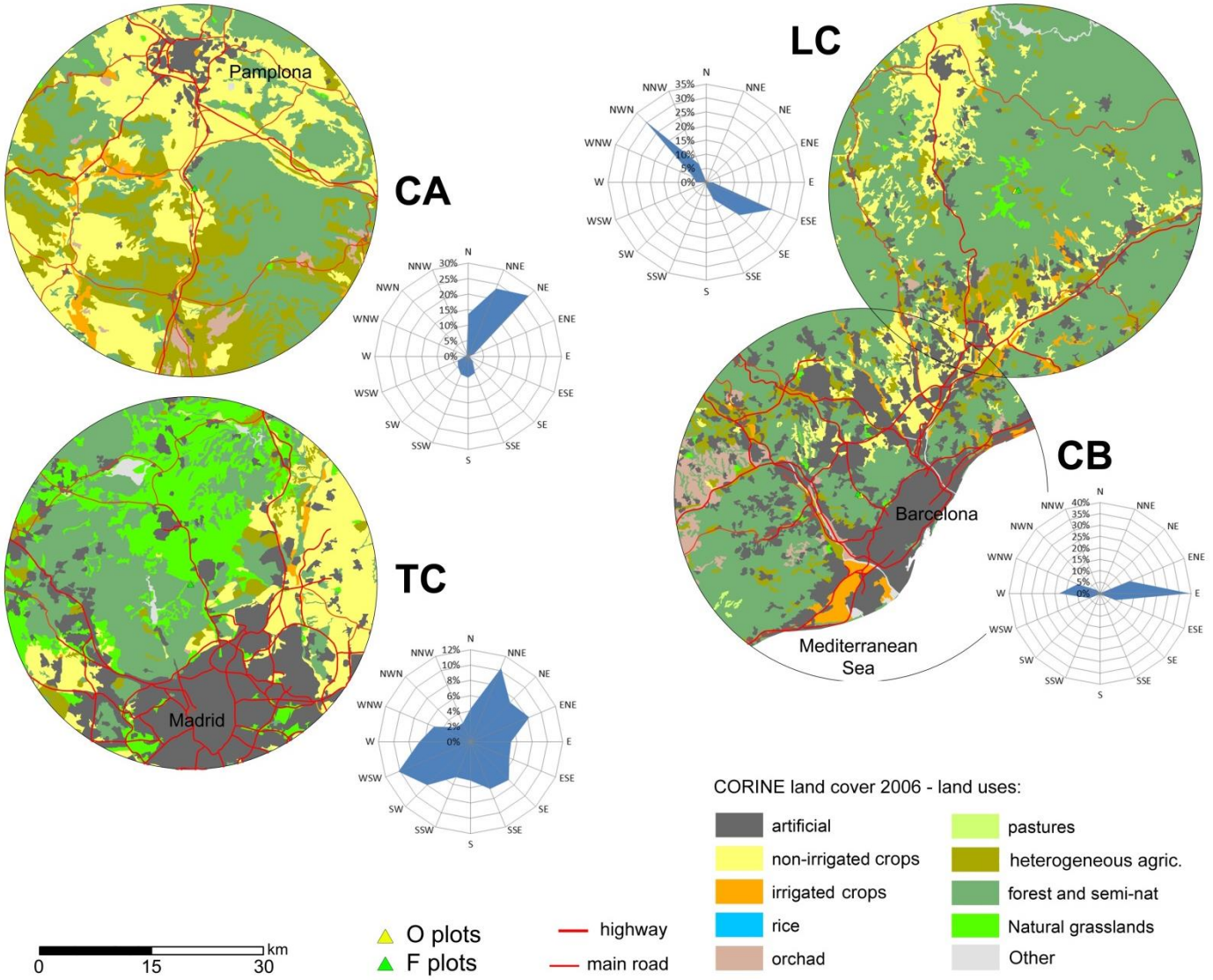
ANNEXES

Annexes

Annex 1.1. Location of the study sites of the EDEN project. Land use cover and roads are mapping in a 25-km radius buffer around the monitoring sites. Wind roses represent the frequency of the wind direction during the 2-year study period.



Annex 2.1. Nitrogen empirical critical loads (CL), surface area assessed and exceedances of empirical critical loads (CL_{exc}) for the different habitat types included in Annex I of Habitats Directive, according to EMEP and CHIMERE estimations of nitrogen deposition. CL values and reliability are obtained from Bobbink et al. (2010).

Annex I name	Codification		Fitting of the CL assigned [a]	EUNIS code of the CL assigned	Reliability of the CL assigned [b]	CL [c]		
	Annex I Habitats Directive	EUNIS				minimum	maximum	applied
Embryonic shifting dunes	2110	B1.31	<	B1.3	(#)	10	20	15,0
Shifting dunes along the shoreline with Ammophila arenaria ('white dunes')	2120	B1.32	<	B1.3	(#)	10	20	15,0
Fixed coastal dunes with herbaceous vegetation ('grey dunes')	2130	B1.4	=	B1.4	#	8	15	11,5
Crucianellion maritimae fixed beach dunes	2210	B1.43	<	B1.4	#	8	15	11,5
Malcolmietalia dune grasslands	2230	B1.48	<	B1.4	#	8	15	11,5
Coastal dunes with Juniperus spp	2250	B1.63	≠	B1.4	#	8	15	11,5
Cisto-Lavenduletalia dune sclerophyllous scrubs	2260	B1.64	≠	B1.4	#	8	15	11,5
Wooded dunes with Pinus pinea and/or Pinus pinaster	2270	G3.7	=	G3.7	(#)	3	15	9,0
Northern Atlantic wet heaths with Erica tetralix	4010	F4.11	=	F4.11	(#)	10	20	15,0
Temperate Atlantic wet heaths with Erica ciliaris and Erica tetralix	4020	F4.12	≠	F4.11	(#)	10	20	15,0
European dry heaths	4030	F4.2	=	F4.2	##	10	20	15,0
Dry Atlantic coastal heaths with Erica vagans	4040	F4.234	<	F4.2	##	10	20	15,0
Alpine and Boreal heaths	4060	F2.2	<	F2	#	5	15	10,0
Endemic oro-Mediterranean heaths with gorse	4090	F7.4	≠	F4.2/F2	##/#	5	15	10,0
Stable xero-thermophilous formations with Buxus sempervirens on rock slopes (Berberidion pp)	5110	F3.12	≠	F5	(#)	20	30	25,0
Mountain Cytisus purgans formations	5120	F3.21	≠	F2	#	5	15	10,0
Arborescent matorral with Juniperus spp	5210	F5.13	<	F5	(#)	20	30	25,0
Arborescent matorral with Zyziphus	5220	F5.171	<	F5	(#)	20	30	25,0
Arborescent matorral with Laurus nobilis	5230	F5.18	<	F5	(#)	20	30	25,0
Low formations of Euphorbia close to cliffs	5320	F5.517	<	F5	(#)	20	30	25,0
Thermo-Mediterranean and pre-desert scrub	5330	F5.5	<	F5	(#)	20	30	25,0
West Mediterranean clifftop phryganas (Astragalo-Plantaginetum subulatae)	5410	F7.11	≠	F4.2	##	10	20	15,0
Rupicolous calcareous or basophilic grasslands of the Alysso-Sedion albi	6110	E1.11	≠	E1.3/E1.26	(#)/##	15	25	20,0
Siliceous Pyrenean Festuca eskia grasslands	6140	E4.314	<	E4.3	#	5	10	7,5
Oro-Iberian Festuca indigesta grasslands	6160	E4.36	<	E4.3	#	5	10	7,5
Alpine and subalpine calcareous grasslands	6170	E4.4	<	E4.4	#	5	10	7,5
Semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco-Brometalia) (* important orchid sites)	6210	E1.2	≠	E1.26	##	15	25	20,0
Pseudo-steppe with grasses and annuals of the Thero-Brachypodietea	6220	E1.3	=	E1.3	(#)	15	25	20,0
Species-rich Nardus grasslands, on silicious substrates in mountain areas (and submountain areas in Continental Europe)	6230	E1.71	=	E1.7	##	10	15	12,5
Dehesas with evergreen Quercus spp	6310	E7.3	≠	E1.3	(#)	15	25	20,0
Molinia meadows on calcareous, peaty or clayey-silt-laden soils (Molinion caeruleae)	6410	E3.51	=	E3.51	(#)	15	25	20,0
Mediterranean tall humid grasslands of the Molinio-Holoschoenion	6420	E3.1	≠	E3.52	#	10	20	15,0
Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels	6430	E5.5	≠	E4.3/E4.4	#/#	5	10	7,5
Lowland hay meadows (Alopecurus pratensis, Sanguisorba officinalis)	6510	E2.2	=	E2.2	(#)	20	30	25,0
Depressions on peat substrates of the Rhynchosporion	7150	D2.3H1	<	D2	#	10	15	12,5
Petrifying springs with tufa formation (Cratoneurion)	7220	C2.121	≠	D4.1	(#)	15	30	22,5
Alkaline fens	7230	D4.1	=	D4.1	(#)	15	30	22,5
Luzulo-Fagetum beech forests	9110	G1.61	<	G1.6	(#)	10	20	15,0
Atlantic acidophilous beech forests with Ilex and sometimes Taxus in the shrublayer (Quercion robori-petraeae or Ilici-Fagenion)	9120	G1.62	<	G1.6	(#)	10	20	15,0
Medio-European limestone beech forests of the Cephalanthero-Fagion	9150	G1.66	<	G1.6	(#)	10	20	15,0
Tilio-Acerion forests of slopes, screes and ravines	9180	G1.A	=	G1.A	(#)	15	20	17,5
Thermophilous Fraxinus angustifolia woods	91B0	G1.7C6	<	G1	##	10	20	15,0
Alluvial forests with Alnus glutinosa and Fraxinus excelsior (Alno-Padion, Alnion incanae, Salicion albae)	91E0	G1.21	<	G1	##	10	20	15,0
Galicio-Portuguese oak woods with Quercus robur and Quercus pyrenaica	9230	G1.7B	<	G1	##	10	20	15,0
Quercus faginea and Quercus canariensis Iberian woods	9240	G1.77	<	G1	##	10	20	15,0
Castanea sativa woods	9260	G1.7D	<	G1	##	10	20	15,0
Salix alba and Populus alba galleries	92A0	G1.3	<	G1	##	10	20	15,0
Riparian formations on intermittent Mediterranean water courses with Rhododendron ponticum, Salix and others	92B0	G1.1	<	G1	##	10	20	15,0
Southern riparian galleries and thickets (Nerio-Tamaricetea and Securinegion tinctoriae)	92D0	F9.3	≠	F5	(#)	20	30	25,0
Olea and Ceratonia forests	9320	G2.4	≠	G2.1	(#)	10	20	15,0
Quercus suber forests	9330	G2.11	<	G2.1	(#)	10	20	15,0
Quercus ilex and Quercus rotundifolia forests	9340	G2.12	<	G2.1	(#)	10	20	15,0
Forests of Ilex aquifolium	9380	G2.6	≠	G1.A	(#)	15	20	17,5
Subalpine and montane Pinus uncinata forests (* if on gypsum or limestone)	9430	G3.3	<	G3	##	5	15	10,0
Abies pinsapo forests	9520	G3.19	≠	G3.7	(#)	3	15	9,0
(Sub-) Mediterranean pine forests with endemic black pines	9530	G3.5	=	G3.5	(#)	15	15	15,0
Mediterranean pine forests with endemic Mesogean pines	9540	G3.7	=	G3.7	(#)	3	15	9,0
Endemic forests with Juniperus spp	9560	G3.9	<	G3	##	5	15	10,0
Tetraclinis articulata forests	9570	G3.96	<	G3	##	5	15	10,0

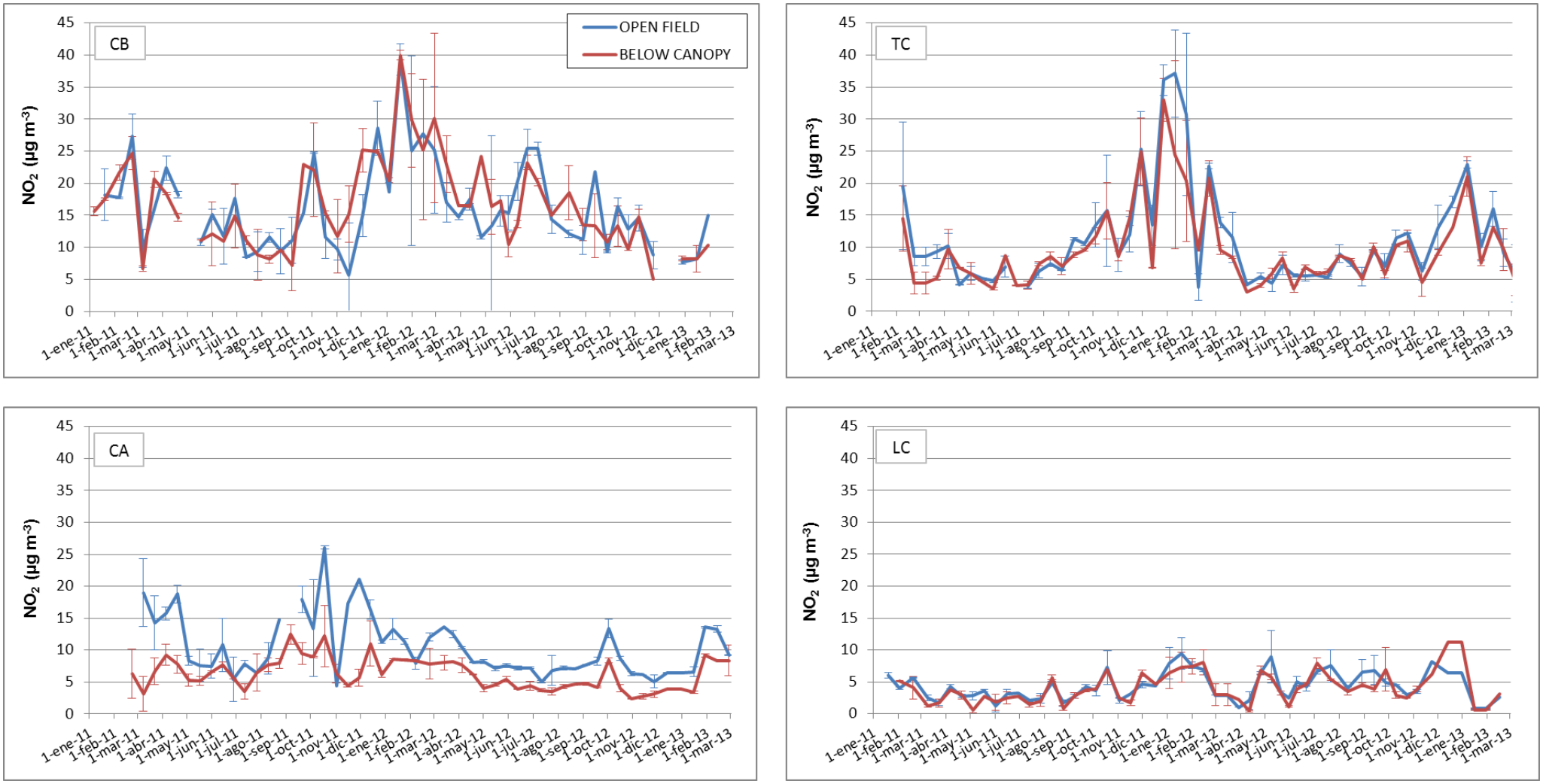
[a] : "<": CL is assigned to a EUNIS category that includes this Natura2000 habitat type, "=": CL assigned to a EUNIS category that matches the Natura2000 classification (exact fitting); "≠": none CL is defined for this particular habitat type; [b] : the reliability is qualitatively indicated by ## reliable, # quite reliable and (#) expert judgment; [c] : Empirical critical loads according to Bobbink et al. (2010)

Annex 2.1. (Cont.) Nitrogen empirical critical loads (CL), surface area assessed and exceedances of empirical critical loads (CL_{exc}) for the different habitat types included in Annex I of Habitats Directive, according to EMEP and CHIMERE estimations of nitrogen deposition. CL values and reliability are obtained from Bobbink et al. (2010).

Annex I name	Codification		Area assessed (km^2)	EMEP assessment			CHIMERE assessment		
	Annex I Habitats Directive	EUNIS		CL_{exc} area (km^2) [d]	CL_{exc} area (%)	CL_{exc} average (kg N / ha) [e]	CL_{exc} area (km^2)	CL_{exc} area (%)	CL_{exc} average (kg N / ha)
Embryonic shifting dunes	2110	B1.31	7,2	n.e.	n.e.	n.e.	<0.1	<0.1	1,12
Shifting dunes along the shoreline with Ammophila arenaria ('white dunes')	2120	B1.32	7,9	n.e.	n.e.	n.e.	0,1	0,7	3,48
Fixed coastal dunes with herbaceous vegetation ('grey dunes')	2130	B1.4	15,2	n.e.	n.e.	n.e.	1,0	6,4	1,43
Crucianellion maritimae fixed beach dunes	2210	B1.43	4,5	<0.1	0,4	3,00	<0.1	0,1	2,60
Malcolmietalia dune grasslands	2230	B1.48	14,0	n.e.	n.e.	n.e.	0,1	0,7	1,89
Coastal dunes with Juniperus spp	2250	B1.63	31,8	n.e.	n.e.	n.e.	2,1	6,7	5,37
Cisto-Lavenduletalia dune sclerophyllous scrubs	2260	B1.64	120,5	n.e.	n.e.	n.e.	0,4	0,3	8,22
Wooded dunes with Pinus pinea and/or Pinus pinaster	2270	G3.7	70,8	2,4	3,4	1,74	22,4	31,6	1,04
Northern Atlantic wet heaths with Erica tetralix	4010	F4.11	0,001	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Temperate Atlantic wet heaths with Erica ciliaris and Erica tetralix	4020	F4.12	409	n.e.	n.e.	n.e.	4,3	1,1	0,72
European dry heaths	4030	F4.2	5559	32,9	0,6	3,95	230,8	4,2	2,26
Dry Atlantic coastal heaths with Erica vagans	4040	F4.234	25	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Alpine and Boreal heaths	4060	F2.2	922	12,5	1,4	2,54	208,3	22,6	3,66
Endemic oro-Mediterranean heaths with gorse	4090	F7.4	6661	57,4	0,9	1,51	819,0	12,3	1,80
Stable xero-thermophilous formations with Buxus sempervirens on rock slopes (Berberidion pp)	5110	F3.12	486,8	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Mountain Cytisus purgans formations	5120	F3.21	1174,4	2,1	0,2	3,24	176,7	15,0	1,85
Arborescent matorral with Juniperus spp	5210	F5.13	2246,9	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Arborescent matorral with Zyziphus	5220	F5.171	55,3	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Arborescent matorral with Laurus nobilis	5230	F5.18	1,4	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Low formations of Euphorbia close to cliffs	5320	F5.517	1,3	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Thermo-Mediterranean and pre-desert scrub	5330	F5.5	4075,8	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
West Mediterranean clifftop phryganas (Astragalo-Plantaginetum subulatae)	5410	F7.11	2,2	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Rupicolous calcareous or basophilic grasslands of the Alysso-Sedion albi	6110	E1.11	75,0	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Siliceous Pyrenean Festuca eskia grasslands	6140	E4.314	477,4	377,1	79,0	1,82	476,8	99,9	7,47
Oro-Iberian Festuca indigesta grasslands	6160	E4.36	312,7	13,2	4,2	1,32	216,2	69,1	1,55
Alpine and subalpine calcareous grasslands	6170	E4.4	1189,2	223,3	18,8	2,00	536,9	45,1	3,96
Semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco-Brometalia) (* important orchid sites)	6210	E1.2	783,9	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Pseudo-steppe with grasses and annuals of the Thero-Brachypodietea	6220	E1.3	5024,3	n.e.	n.e.	n.e.	4,4	0,1	2,21
Species-rich Nardus grasslands, on silicious substrates in mountain areas (and submountain areas in Continental Europe)	6230	E1.71	442,0	n.e.	n.e.	n.e.	82,3	18,6	1,75
Dehesas with evergreen Quercus spp	6310	E7.3	5133,4	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Molinia meadows on calcareous, peaty or clayey-silt-laden soils (Molinion caeruleae)	6410	E3.51	4,8	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Mediterranean tall humid grasslands of the Molinio-Holoschoenion	6420	E3.1	257,5	0,2	0,1	3,57	0,4	0,1	1,23
Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels	6430	E5.5	75,1	34,3	45,7	1,49	67,3	89,6	3,45
Lowland hay meadows (Alopecurus pratensis, Sanguisorba officinalis)	6510	E2.2	234,9	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Depressions on peat substrates of the Rhynchosporion	7150	D2.3H1	37,8	n.e.	n.e.	n.e.	14,2	37,5	0,65
Petrifying springs with tufa formation (Cratoneurion)	7220	C2.121	13,0	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Alkaline fens	7230	D4.1	18,7	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Luzulo-Fagetum beech forests	9110	G1.61	48,4	27,2	56,3	4,37	34,3	71,0	1,47
Atlantic acidophilous beech forests with Ilex and sometimes Taxus in the shrublayer (Quercion robori-petraeae or Ilici-Fagenion)	9120	G1.62	1226,9	n.e.	n.e.	n.e.	35,7	2,9	0,60
Medio-European limestone beech forests of the Cephalanthero-Fagion	9150	G1.66	381,6	35,5	9,3	3,32	50,8	13,3	0,39
Tilio-Acerion forests of slopes, screes and ravines	9180	G1.A	18,4	n.e.	n.e.	n.e.	3,1	16,7	0,88
Thermophilous Fraxinus angustifolia woods	91B0	G1.7C6	91,3	n.e.	n.e.	n.e.	0,7	0,8	0,78
Alluvial forests with Alnus glutinosa and Fraxinus excelsior (Alno-Padion, Alnion incanae, Salicion albae)	91E0	G1.21	164,6	5,6	6,2	3,98	4,7	2,8	1,10
Galicio-Portuguese oak woods with Quercus robur and Quercus pyrenaica	9230	G1.7B	2158,1	n.e.	n.e.	n.e.	0,2	<0.1	0,26
Quercus faginea and Quercus canariensis Iberian woods	9240	G1.77	896,4	2,0	0,2	4,35	11,7	1,3	4,62
Castanea sativa woods	9260	G1.7D	262,1	19,5	7,4	4,29	17,9	6,8	1,22
Salix alba and Populus alba galleries	92A0	G1.3	300,5	2,1	0,7	3,27	1,8	0,6	0,83
Riparian formations on intermittent Mediterranean water courses with Rhododendron ponticum, Salix and others	92B0	G1.1	6,2	n.e.	n.e.	n.e.	2,4	39,2	4,01
Southern riparian galleries and thickets (Nerio-Tamaricetea and Securinegion tinctoriae)	92D0	F9.3	277,2	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Olea and Ceratonia forests	9320	G2.4	200,4	n.e.	n.e.	n.e.	8,6	4,3	4,11
Quercus suber forests	9330	G2.11	1428,9	32,9	2,3	4,42	203,9	14,3	3,38
Quercus ilex and Quercus rotundifolia forests	9340	G2.12	5877,6	464,4	7,9	3,77	275,1	4,7	0,87
Forests of Ilex aquifolium	9380	G2.6	24,1	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
Subalpine and montane Pinus uncinata forests (* if on gypsum or limestone)	9430	G3.3	141,2	54,6	38,7	3,36	121,9	86,3	3,34
Abies pinsapo forests	9520	G3.19	10,9	n.e.	n.e.	n.e.	6,2	57,4	0,77
(Sub-) Mediterranean pine forests with endemic black pines	9530	G3.5	1494,7	37,0	2,5	1,24	20,8	1,4	0,22
Mediterranean pine forests with endemic Mesogean pines	9540	G3.7	92,0	<0.1	<0.1	1,51	64,9	70,5	1,18
Endemic forests with Juniperus spp	9560	G3.9	1109,0	4,4	0,4	2,09	56,8	5,1	1,15
Tetraclinis articulata forests	9570	G3.96	0,3	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.

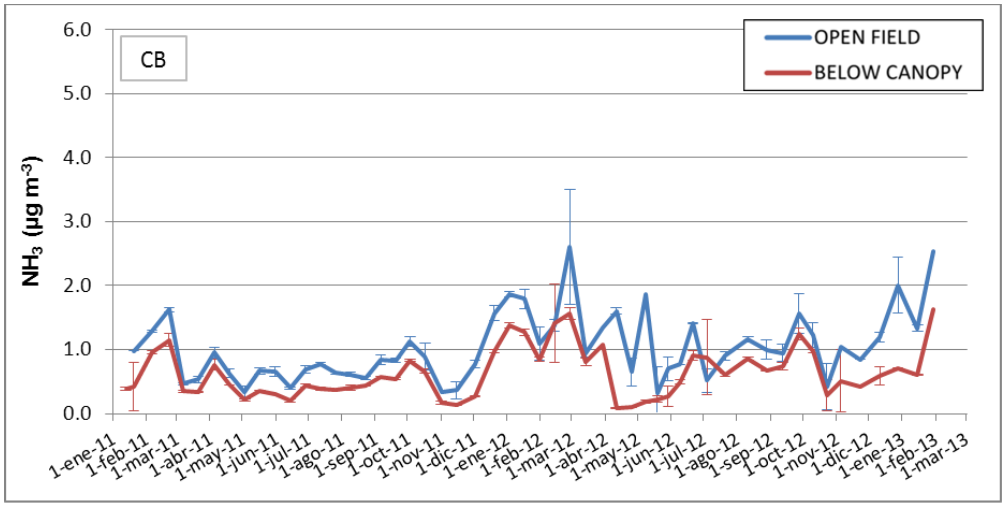
[d] : Sum of areas showing an exceedance of CL; [e] : CL_{exc} averaged and weighted for each habitat type; n.e.: None exceedance was found in this habitat type.

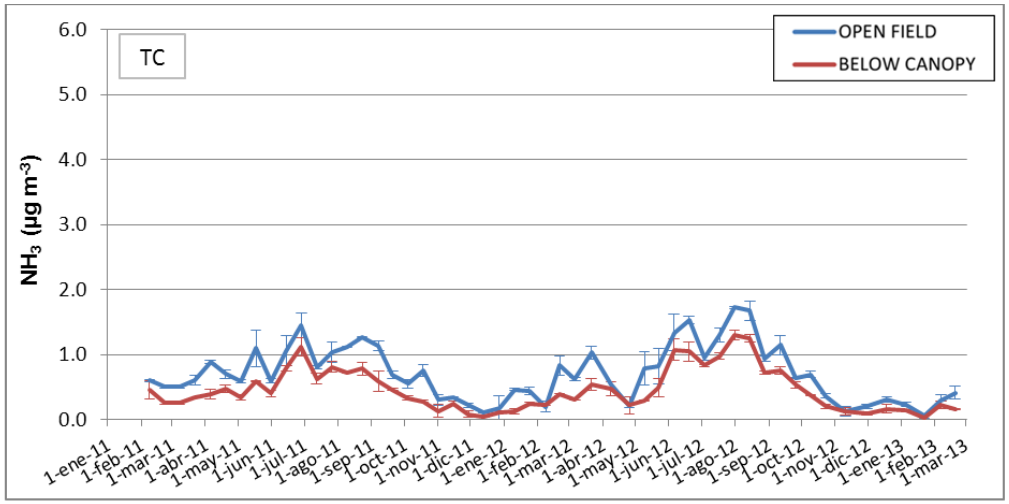
Annex 3.1. Bi-weekly concentrations of nitrogen dioxide in the four EDEN-project sites.

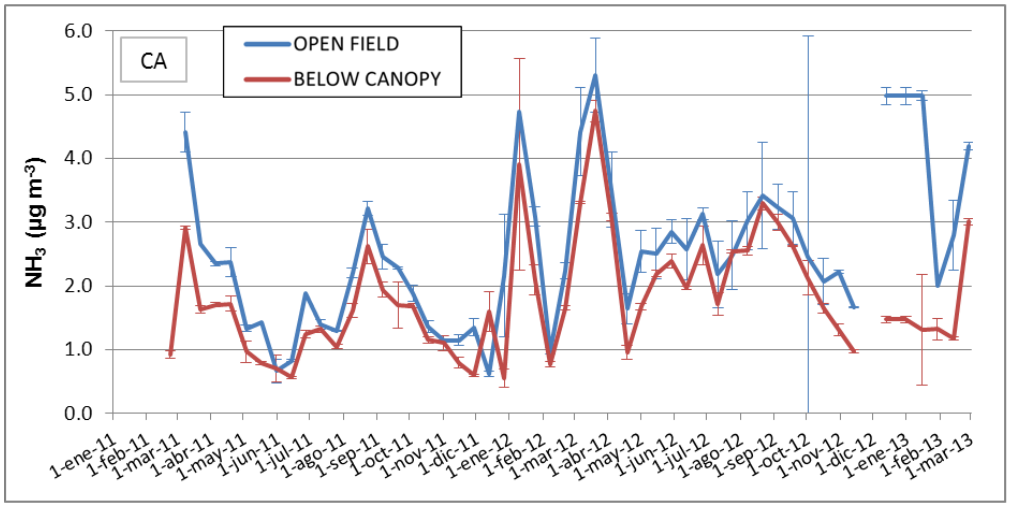


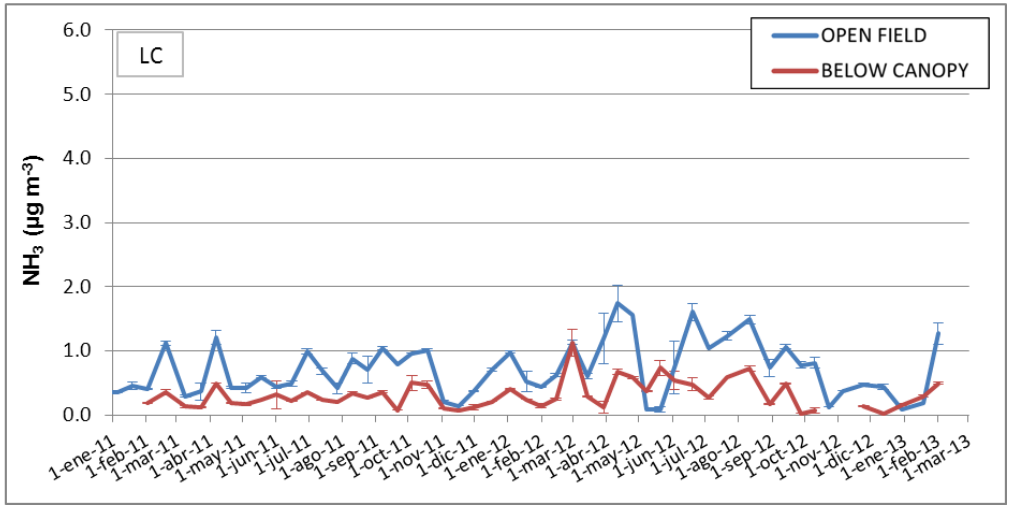
Error bars correspond to the standard error of the mean calculated for the two sampling duplicates.

Annex 3.2. Bi-weekly concentrations of ammonia in the four EDEN-project sites.



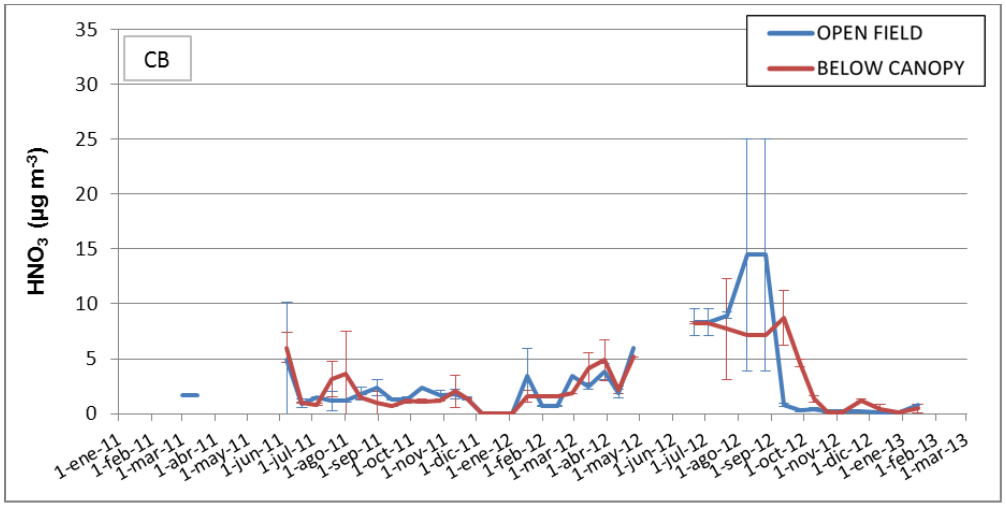


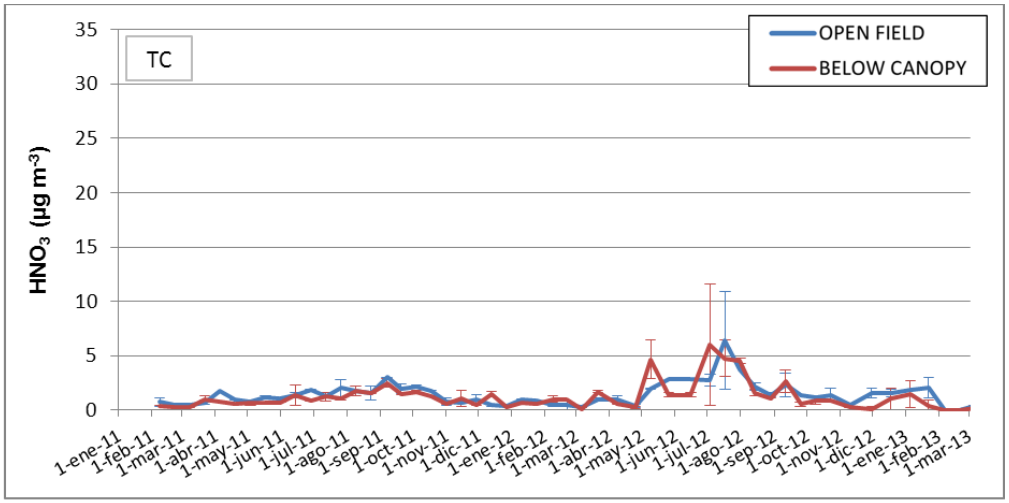


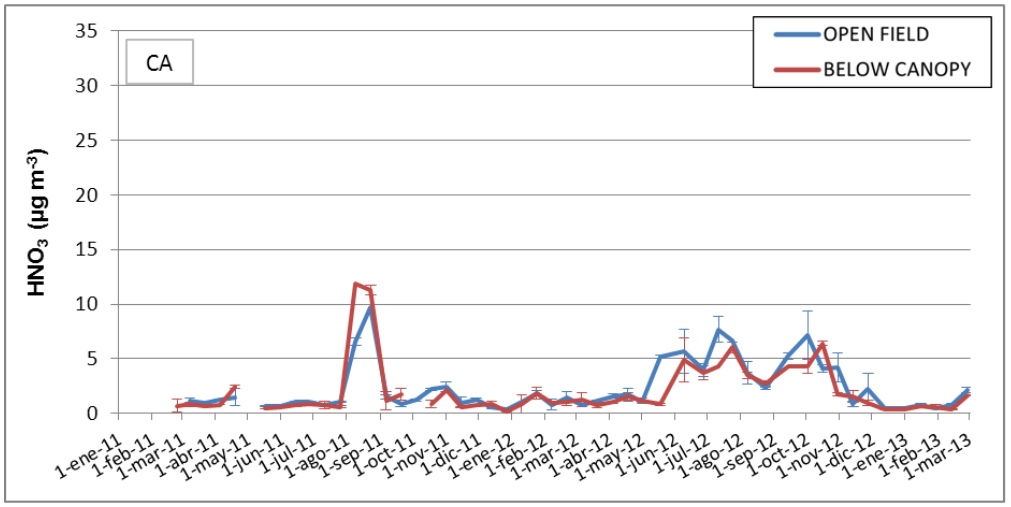


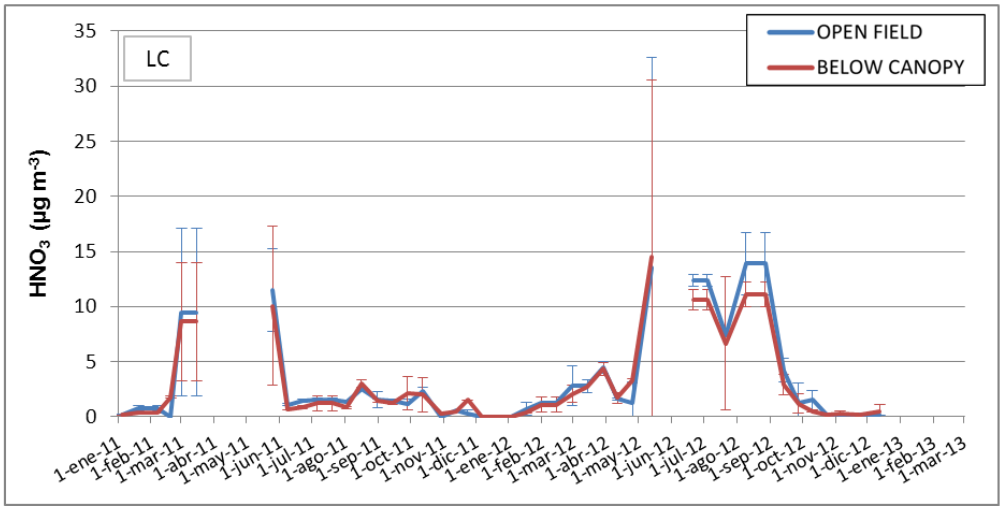
Error bars correspond to the standard error of the mean calculated for the two sampling duplicates.

Annex 3.3. Bi-weekly concentrations of nitric acid vapour in the four EDEN-project sites.



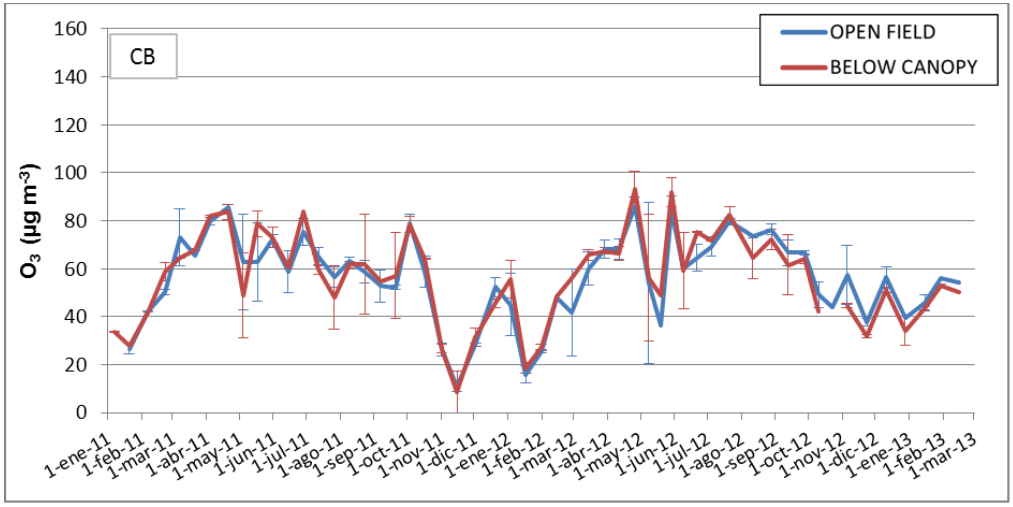


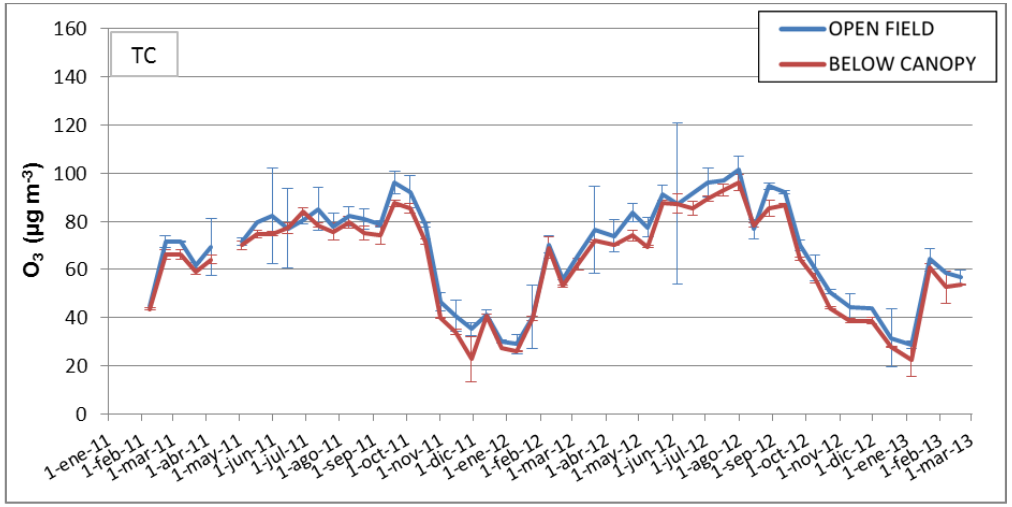


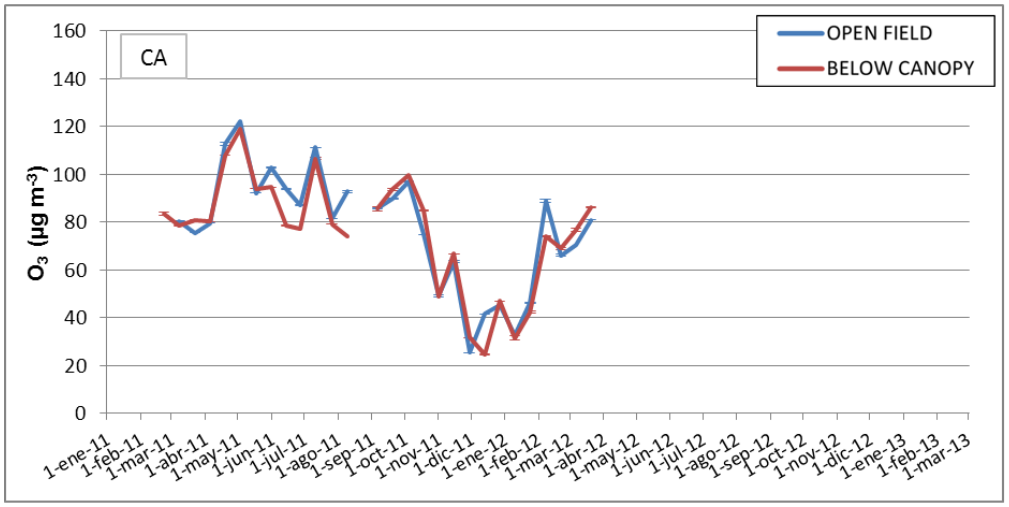


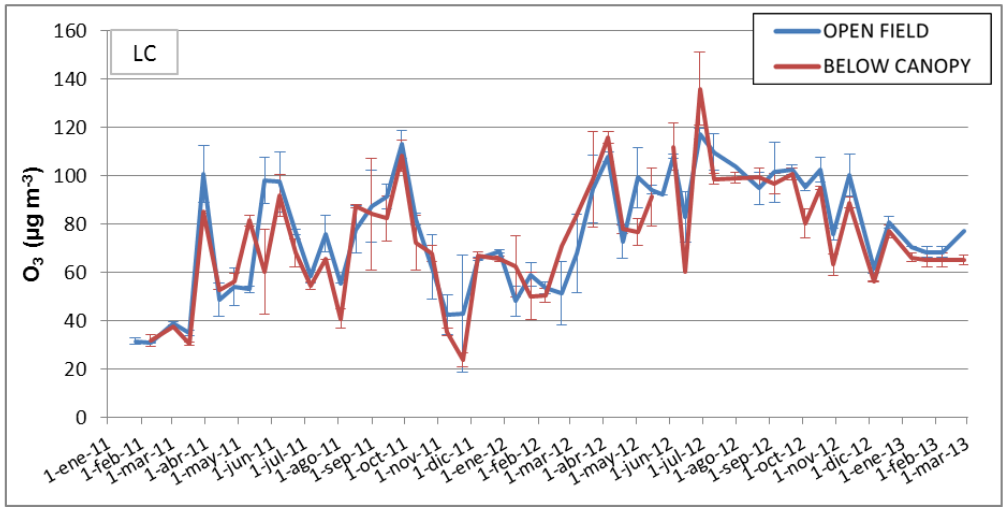
Error bars correspond to the standard error of the mean calculated for the two sampling duplicates.

Annex 3.4. Bi-weekly concentrations of ozone in the four EDEN-project sites.



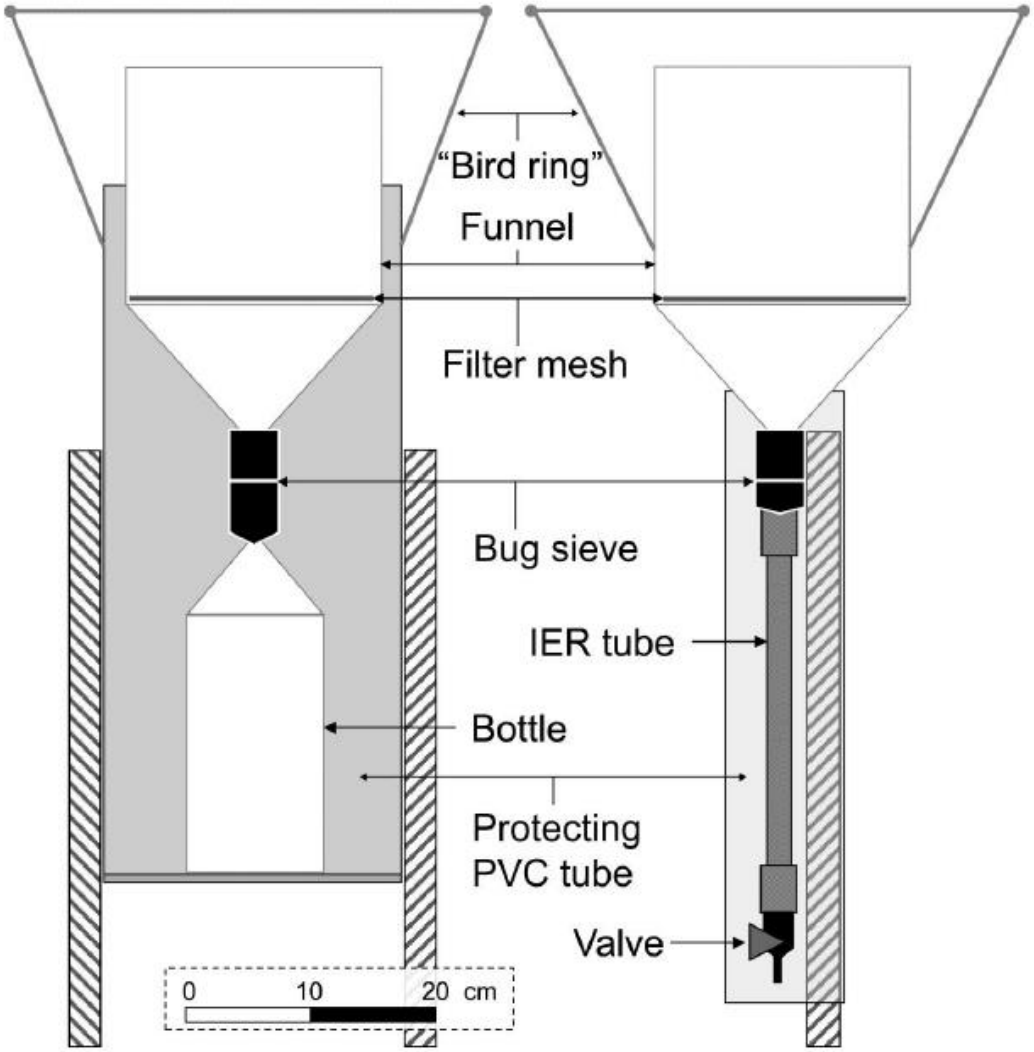






Error bars correspond to the standard error of the mean calculated for the two sampling duplicates.

Annex 4.1. Diagrams of the collectors deployed in the study sites: conventional bottale collector (CBC) on the left, ion-exchange resin collector (IEC) on the right.



Annex 4.2. Methodological and environmental information from other studies using ion-exchange resin.

Study	Location	Climate	T (ºC)	P (mm/y)	Deposition of N	Extractant	Number of extractions	Sampling period lenght	Number collectors per open-field plot	Area of collection per plot (cm^2)
Boutin et al., 2015	Pyrenees (France)	Subalpine	9.6	1245	4.5–6.6 kg ha^{-1} y^{-1}	2 M KCl (200 ml)	2	1 month; during 5 months	2	628
Brumbaugh et al., 2012	Alaska	Boreal	-4.4	1821	< 1 kg ha^{-1} y^{-1}	1 M KI		6 months	3	236
Cerón et al., 2015	SE-Center Mexico	Sub-humid warm / humid sub-warm	27,0 / 19.1	1300 / 2238	only throughfall < 1 kg ha^{-1} y^{-1}	2 M KCl (200 ml)	3	2 months	only throughfall	only throughfall
Clow et al., 2015	Rocky Mountain National Park (USA)	Alpine / subalpine			approx. 2 kg ha^{-1} y^{-1}	1 M KI (200 ml)	2	summer (> 3 months) and winter (> 8 months)	2	470
Fang et al., 2011	Southern China	Subtropical	19.5–21.9	1380 –1927	16.2–38.2 kg ha^{-1} y^{-1}	2M KCl (50 ml for 6.4 ml of resin)	2	4 months	5	565
Fenn et al., 2002	San Bernardino Mountains (CA, USA)	Mediterranean semi-arid		155–249	1.9–5.1 kg ha^{-1} y^{-1}	2 M KCl (200 ml)	3	Two periods of 3 and 4 months, respectively	4	314
Fenn and Poth, 2004	San Bernardino Mountains (CA, USA)	Mediterranean semi-arid		340–840	4.0–15.6 kg ha^{-1} y^{-1}	2 M KCl (200 ml)	2	3-, 6-, 9- and 12-month periods simultaneously during one year	4	314
Hansen, 2012	Grand Teton National Park (USA)	Alpine semi-arid	8.5	155	0.2–0.3 kg ha^{-1} (summer)	2 M KCl (100 ml)	1 (shaken for one hour)	70–90 days during the alpine growing season (Jul-Oct)	4	2922
Köhler et al., 2012	Lore Lindu National Park, Central Sulawesi (Indonesia)	Tropical	24.5	2500	1.4–2.7 kg $N\text{-}NO_3$ ha^{-1} y^{-1}	1 M NaCl (for 10 g of resin)	2	15–30 days during 1-2 years	3	851
Sheibley et al., 2014	Mount Rainier, North Cascades, & Olympic Nat. Parks (WA, USA)	Alpine			0.1–0.5 kg ha^{-1} (summer)	1 N KI	2	59–86 days; during summer 2008	5	1571
Tuloss and Cadenasso, 2015	California	Mediterranean	14–17	500–940		2 M KCl (100 ml)	3	6 weeks; during 8 months	5	2454
Zhan et al., 2015	North– South Transect of Eastern China		-5.6–22.1	526–1771	2.7–33.0 kg ha^{-1} y^{-1}	0.2 M KCl (100 ml)	3	1 month; during 3 years	5	192
Simkin et al., 2004	Only laboratory test are considered here					1 M KI (50ml)	3 (shaken 30 min at 120 rpm)	4-6 weeks	3 per concentration	
van Dam et al., 1991	Netherland					1M KCl (500 ml) and 1M HCl (500 ml)	1	2 weeks		860
Wieder et al., 2010	Athabasca Oil Sands Region, Alberta (Canada)	Boreal - Subartic	1	387	< 1 kg ha^{-1} y^{-1}	2 M KCl (40 +10 ml)	2: bath + rinsing	5–7 months (during 3,5 years)	3–5	236–1418
Yamashita et al., 2014	Borneo island (Malaysia)	Equatorial with modest annual seasonality		2778	2.2 kg ha^{-1} y^{-1}	1 M KCl	2	5–7 months (during 2 years)	3	339
Present work (CB)	Barcelona region, Northeastern Spain	Mediterranean subhumid	15.2	652	3.5 kg ha^{-1} y^{-1}	2 M KCl (200 ml)	2	2–4 months (during 2 years)	2	628
Present work (CA)	Navarra region, Northern Spain	Mediterranean humid	12.3	645	5.4 kg ha^{-1} y^{-1}	2 M KCl (200 ml)	2	3–5 months (during 2 years)	4	1257
Present work (TC)	Madrid region, Center Spain	Mediterranean semiarid	14.6	348	3.1 kg ha^{-1} y^{-1}	2 M KCl (200 ml)	2	3–5 months (during 2 years)	4	1257

Annex 6. Publications in the framework of the EDEN project in which the candidate has participated.

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Nitrogen deposition in Spain: Modeled patterns and threatened habitats within the Natura 2000 network

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HIGHLIGHTS

- CHIMERE and EMEP models acceptably estimate atmospheric N wet deposition in Spain.
- Total (wet + dry) atmospheric N deposition in Spain in 2008 was up to 19–23 kg N ha^{-1}.
- Natural grasslands are the habitats most threatened by N deposition.
- Biodiversity conservation in 3–7% of the assessed area could be threatened by N deposition.
- Habitats in mountain areas are particularly threatened by N deposition.

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ABSTRACT

The Mediterranean Basin presents an extraordinary biological richness but very little information is available on the threat that air pollution, and in particular reactive nitrogen (N), can pose to biodiversity and ecosystem functioning. This study represents the first approach to assess the risk of N enrichment effects on Spanish ecosystems. The suitability of EMEP and CHIMERE air quality model systems as tools to identify those areas where effects of atmospheric N deposition could be occurring was tested. For this analysis, wet deposition of NO_3^- and NH_4^+ estimated with EMEP and CHIMERE model systems were compared with measured data for the period 2005–2008 obtained from different monitoring networks in Spain. Wet N deposition was acceptably predicted by both models, showing better results for oxidized than for reduced nitrogen, particularly when using CHIMERE. Both models estimated higher wet deposition values in northern and northeastern Spain, and decreasing along a NE–SW axis. Total (wet + dry) nitrogen deposition in 2008 reached maxima values of 19.4 and 23.0 kg N ha^{-1} $year^{-1}$ using EMEP and CHIMERE models respectively. Total N deposition was used to estimate the exceedance of N empirical critical loads in the Natura 2000 network. Grassland habitats proved to be the most threatened group, particularly in the northern alpine area, pointing out that biodiversity conservation in these protected areas could be endangered by N deposition. Other valuable mountain ecosystems can be also threatened, indicating the need to extend atmospheric deposition monitoring networks to higher altitudes in Spain.

1. Introduction

The global biogeochemical cycle of nitrogen (N) has been deeply altered by human activities to the extent that the planetary boundary for human safe operating has long been crossed (Rockström et al., 2009). Anthropogenic reactive nitrogen (N_r) circulates across different compartments (atmosphere, hydrosphere and terrestrial ecosystems) inducing a cascade of environmental effects, such as tropospheric ozone

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RESEARCH PAPER

Drought stress does not protect *Quercus ilex* L. from ozone effects: results from a comparative study of two subspecies differing in ozone sensitivity

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Keywords
Evergreen broadleaf Mediterranean vegetation; gas exchange; growth; Holm oak; ozone response functions.

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ABSTRACT

Long-term effects of ozone (O_3) exposure and drought stress were assessed on two subspecies of *Quercus ilex:* ssp. *ilex* and ssp. *ballota*. Two-year-old seedlings were continuously exposed for 26 months in open-top chambers to three O_3 treatments: charcoal filtered air, non-filtered air and non-filtered air supplemented with 40 nl·l^{-1} O_3. Additionally, two irrigation regimes were adopted: half of the plants were well-watered and the others received half of the water supplied to control plants. Growth, shoot water potential and gas exchange rates were assessed seasonally, and biomass accumulation was determined at the end of the experiment. Drought stress caused higher reductions of gas exchange, growth and biomass accumulation than O_3 exposure in both subspecies. The combination of O_3 and drought stress caused further decreases of accumulated aboveground biomass but no additive effects were observed on gas exchange rates or root biomass. Thus, drought stress did not protect *Q. ilex* from O_3 effects on biomass when the response of the whole plant was considered. *Q. ilex* ssp. *ballota* was more sensitive to O_3 and ssp. *ilex* was more affected by drought stress. The different O_3 sensitivity was not only related to pollutant uptake but also to the ability of plants for resource acquisition and allocation. Based on biomass dose–response functions, *Q. ilex* is more resistant to O_3 than other European evergreen tree species, however, O_3 represents an additional stress factor that might be impairing plant ability to withstand current and future climate change.

INTRODUCTION

Ozone (O_3) is the most important and pervasive air pollutant currently affecting vegetation, not only in Europe and North America, but also in many developing countries (Ashmore 2005). Ozone pollution is especially relevant for the Mediterranean region where climatic conditions favour O_3 photochemical formation (Millán *et al.* 1996; Cristofanelli & Bonasoni 2009). Ozone concentrations in this area frequently exceed the concentration-based limits established to protect vegetation (Paoletti 2006; Ferretti *et al.* 2007; EEA 2010). However, field observations do not report the expected evidence of plant injury caused by O_3 (Paoletti 2006; Ferretti *et al.* 2007; MARM 2011). Several reasons have been proposed to explain this discrepancy, such as the inadequacy of the established limits or the inherent higher O_3 tolerance of Mediterranean vegetation. A flux-based approach might provide a more realistic estimate of the O_3 risk for vegetation in this area. Flux-based approaches are currently being developed to establish more accurate O_3 critical levels for vegetation in Europe, although insufficient data are available yet to derive a critical level specific to trees in the Mediterranean area (LRTAP Convention 2010).

Evergreen broadleaf Mediterranean woody species are assumed to be tolerant to air pollution due to their sclerophyllic adaptations (Manes *et al.* 1998; Bussotti & Gerosa 2002; Nali *et al.* 2004; Paoletti 2006). Leaf morphology and anatomy, with packed mesophyll cells and sunken and protected stomata well adapted to reduce water loss, diminish at the same time gas exchange and pollutant uptake. Also, the high foliar concentration of antioxidant compounds normally present in many Mediterranean evergreen plants, and/or the ability to increase antioxidant status when needed, might protect plant tissues from the oxidative damage caused by O_3 (Nali *et al.* 2004; Paoletti 2006; Calatayud *et al.* 2010). Despite these characteristics, both foliar visible symptoms and effects on growth and plant physiology caused by O_3 have been described in some evergreen broadleaf species under experimental conditions (Bussotti & Gerosa 2002; Elvira *et al.* 2004; Nali *et al.* 2004; Ribas *et al.* 2005a,b; Vitale *et al.* 2008; Calatayud *et al.* 2010, 2011; Mereu *et al.* 2011).

Ozone tolerance of Mediterranean vegetation is expected to further increase under field conditions, when summer drought stress limits gas exchange and hence O_3 flux into the leaves. However, the way in which O_3 exposure interacts with drought stress is very complex and contrasting results have been found: a protective role of drought stress against O_3-induced effects by means of reducing pollutant uptake (Panek & Goldstein 2001; Karlsson *et al.* 2002); additive effects mainly through an O_3-induced loss of stomatal regulation favouring drought stress (Grulke 1999; Maier-Maercker 1999; Alonso *et al.* 2003; Paoletti & Grulke 2010); or no significant interaction between the two stressors (Le Thiec *et al.*

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Current ozone levels threaten gross primary production and yield of Mediterranean annual pastures and nitrogen modulates the response

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HIGHLIGHTS

- O_3 and N effects on yield and gas exchange of an annual pasture were analyzed.
- Ozone induced visible injury and reduced the yield and gross primary production.
- Nitrogen compensated O_3 effects on yield when O_3 concentrations were moderate.
- Ozone limited the fertilization effect of higher soil N availability.
- Current O_3 levels represent a threat for Mediterranean annual pastures.

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ABSTRACT

Pastures are among the most important ecosystems in Europe considering their biodiversity and distribution area. However, their response to increasing tropospheric ozone (O_3) and nitrogen (N) deposition, two of the main drivers of global change, is still uncertain. A new Open-Top Chamber (OTC) experiment was performed in central Spain, aiming to study annual pasture response to O_3 and N in close to natural growing conditions. A mixture of six species of three representative families was sowed in the field. Plants were exposed for 40 days to four O_3 treatments: filtered air, non-filtered air (NFA) reproducing ambient levels and NFA supplemented with 20 and 40 nl l^{-1} O_3. Three N treatments were considered to reach the N integrated doses of "background", +20 or +40 kg N ha^{-1}. Ozone significantly reduced green and total aboveground biomass (maximum reduction 25%) and increased the senescent biomass (maximum increase 40%). Accordingly, O_3 decreased community Gross Primary Production due to both a global reduction of ecosystem CO_2 exchange and an increase of ecosystem respiration. Nitrogen could partially counterbalance O_3 effects on aboveground biomass when the levels of O_3 were moderate, but at the same time O_3 exposure reduced the fertilization effect of higher N availability. Therefore, O_3 must be considered as a stress factor for annual pastures in the Mediterranean areas.

1. Introduction

Tropospheric ozone (O_3) and atmospheric nitrogen (N) deposition are two of the main air pollutants affecting natural and semi-natural areas and causing harmful ecological effects (EEA, 2011; Sutton et al., 2011). In the Mediterranean area, high solar radiation, temperature and prevailing stable atmospheric conditions favor photochemical O_3 formation (Millán et al., 2000; Cristofanelli and Bonasoni, 2009), resulting in some of the highest surface O_3 concentrations in Europe (EEA, 2011). Ozone concentrations in Spain frequently exceed current thresholds established for plant protection according to the EU Air Quality Directive 2008/50/EC or the Convention on Long-Range Transboundary Air Pollution of the UN/ECE (CLRTAP) (Fernández-Fernández et al., 2011). Moreover, O_3-induced effects have been reported in crops and natural

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RESEARCH ARTICLE

Atmospheric pollutants in peri-urban forests of *Quercus ilex*: evidence of pollution abatement and threats for vegetation

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Abstract Peri-urban vegetation is generally accepted as a significant remover of atmospheric pollutants, but it could also be threatened by these compounds, with origin in both urban and non-urban areas. To characterize the seasonal and geographical variation of pollutant concentrations and to improve the empirical understanding of the influence of Mediterranean broadleaf evergreen forests on air quality, four forests of *Quercus ilex* (three peri-urban and one remote) were monitored in different areas in Spain. Concentrations of nitrogen dioxide (NO_2), ammonia (NH_3), nitric acid (HNO_3) and ozone (O_3) were measured during 2 years in open areas and inside the forests and aerosols (PM_{10}) were monitored in open areas during 1 year. Ozone was the only air pollutant expected to have direct phytotoxic effects on vegetation according to current thresholds for the protection of vegetation. The concentrations of N compounds were not high enough to directly affect vegetation but could be contributing through atmospheric N deposition to the eutrophization of these ecosystems. Peri-urban forests of *Q. ilex* showed a significant below-canopy reduction of gaseous concentrations (particularly NH_3, with a mean reduction of 29–38 %), which indicated the feasibility of these forests to provide an ecosystem service of air quality improvement. Well-designed monitoring programs are needed to further investigate air quality improvement by peri-urban ecosystems while assessing the threat that air pollution can pose to vegetation.

Keywords Atmospheric pollution · Nitrogen · Ozone · Aerosols · Ecosystem services · Mediterranean vegetation

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Introduction

The continuous growth of urban population has turned air quality into one of the main environmental concerns worldwide. Current urban development needs to consider designs and strategies that minimize atmospheric pollution to improve well-being and human health. In the last years, particular attention has been paid to investigate the role of urban and peri-urban vegetation in improving air quality. Vegetation can remove air pollutants via dry deposition, through interception in the canopy surfaces and via absorption of gases through the stomata. In particular, urban and peri-urban vegetation has been proposed as a method to reduce air pollutants such as ozone, nitrogen oxides and particulate matter (Alonso et al. 2011; Kroeger et al. 2014; Nowak et al. 2014; Sgrigna et al. 2015). On the other hand, air pollution can affect these forests, impairing their capacity to provide ecosystem services.

Peri-urban areas are transition zones between the denser urban core and the rural hinterland, where natural habitats

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

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ABSTRACT

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

1. Introduction

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

In the 1970s two important monitoring programs, the US National Atmospheric Deposition Program (NADP) and the European Monitoring and Evaluation Program (EMEP), began to work on the study of nitrogen deposition, but in both cases addressing only inorganic N (Cape et al., 2011). The first serious discussions and analyses of organic nitrogen (ON) emerged with the work carried out by Cape et al. (2001) and the reviews published by Neff et al. (2002) and Cornell et al. (2003), who greatly contributed to the promotion of the subsequent surveys developed in this field. In fact, since then, many surveys have been carried out taking into account various aspects of ON: contributions to wet (Keene et al., 2002; Cape et al., 2012) and dry deposition (Mace et al., 2003; Matsumoto et al., 2014); elemental and functional characterization (Altieri et al., 2012; El Haddad et al., 2013); interactions with vegetation (Hinko-Najera and Wanek, 2010), soil microorganisms (Jones et al., 2004; Farrell et al., 2014) and climate (Du et al., 2014); and modelling and prediction studies (Kanakidou et al., 2012; Im et al., 2013). These surveys have highlighted the important contribution of the organic form to total N deposition, ranging on average from 10 to 40% depending on the study area. However, even if organic N has long been known to be a quantitatively significant component of atmospheric nitrogen deposition, it is still not routinely assessed, nor are best-estimates factored into

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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Heterogeneous responses to ozone and nitrogen alter the species composition of Mediterranean annual pastures

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Abstract Air pollution represents a threat to biodiversity throughout the world and particularly in the Mediterranean area, where high tropospheric ozone (O_3) concentrations and atmospheric nitrogen (N) deposition are frequently recorded. Mediterranean annual pastures are among the most important ecosystems in southern Europe due to their high biodiversity and extension. Aiming to study the responses of these communities to the main atmospheric pollutants in the Mediterranean region, an experimental study was performed in an open-top chamber (OTC) facility. A mixture of six species representative of annual pastures was grown under field conditions inside the OTC. Plants were exposed for 39 days to four O_3 treatments and three doses of N. The species responded heterogeneously to both factors. Legumes did not react to N but were very sensitive to O_3: *Trifolium* species responded negatively, while *Ornithopus* responded positively, taking advantage of the greater sensitivity of clovers to O_3. The grasses and the herb were more tolerant of O_3 and grasses were the most responsive to N. Significant interactions between factors indicated a loss of effectiveness of N in O_3-polluted atmospheres and an ability of O_3 to counterbalance the damage induced by N input, but both effects were dependent on O_3 and N levels. The inclusion of plant competition in the experimental design was necessary to reveal results that would otherwise be missed, such as the positive growth responses under elevated O_3 levels. Surprisingly, competition within the legume family played the most important role in the overall response of the annual community to O_3. Both tropospheric O_3 and N deposition should be considered important drivers of the structure and biodiversity of Mediterranean annual pastures.

Keywords Dehesa · Annual grasslands · Tropospheric ozone effects · Atmospheric pollutants · Species competition

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Introduction

Changes in community structure or species abundance caused by global change drivers are difficult to detect and reverse, despite their direct repercussions on ecosystem properties, conservation status, and ecosystem services (Zimov et al. 1995; Hooper et al. 2012). The study of regional biodiversity loss has mainly focused on land-use

Grass and Forage Science
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Foliar senescence is the most sensitive response to ozone in *Bromus hordeaceus* and is modulated by nitrogen input

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Abstract

A study was conducted on the effect of tropospheric ozone (O_3) on soft brome (*Bromus hordeaceus*) and the modulation of its response by nitrogen (N). Two assays were conducted using open-top chambers (OTCs). Three O_3 treatments were considered: filtered air, with concentrations below background levels (charcoal-filtered air), non-filtered air (NFA) that simulates ambient O_3 concentrations, and unfiltered air to which 40 nL L^{-1} O_3 above-ambient concentrations was added (NFA+), simulating elevated values recorded in natural areas of annual pastures in the Iberian Peninsula. Three N rates were used, simulating the increase in soil N through atmospheric deposition and excreta from livestock grazing. Ozone caused an augmentation in foliar senescence, whereas green biomass was not altered; consequently, an increased senescent/green biomass ratio was produced. A stronger O_3 effect was detected in the second assay compared with the first. This was related to the estimated absorbed O_3 fluxes, which were double the value calculated in the former. Increasing N input enhanced biomass production, but its effectiveness was greater in the first assay, under less-favourable weather conditions and lower plant growth. In the first assay, the O_3 response was modulated by N availability, which mitigated the effects of O_3 to medium concentration values. In the first assay, O_3 reduced the aerial/subterranean biomass ratio, caused by a positive-trend effect on roots. Foliar concentration of lignin was increased by O_3, and *in vitro* digestibility of aerial biomass and the plant cell wall fraction tended to decrease with increasing O_3.

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Introduction

Chemical composition of the atmosphere, particularly the abundance of tropospheric ozone (O_3) and its precursors, has experienced significant changes since the beginning of the industrial revolution in the mid-eighteenth century (Hauglustaine and Brasseur, 2001). Ozone is formed primarily from photochemical reactions between its precursors, mainly nitrogen oxides (NO_x), hydrocarbons and volatile organic compounds (VOCs) generated by anthropogenic and natural activities. Its formation also requires favourable climatic conditions such as atmospheric stability with elevated solar radiation and temperature (Crutzen *et al.*, 1999). Tropospheric O_3 is considered a greenhouse gas (Mickley *et al.*, 2001) that may contribute to global warming and climate change, but the singular aspect that has led to greatly increased scientific interest during the past 30 years is its high toxicity. It is now considered the most phytotoxic air pollutant due to the large number of plant species sensitive to its high oxidative capacity (Ashmore, 2005; Hayes *et al.*, 2007). Its effects range from loss of production and quality in crop harvest (Mills *et al.*, 2007; Booker *et al.*, 2009) to reduction in forest and herbaceous vegetation growth (Davison and Barnes, 1998; Skärby *et al.*, 1998), and alterations in the structure and diversity of natural ecosystems (Davison and Barnes, 1998; Pfleeger *et al.*, 2010).

The Iberian Peninsula has experienced a progressive increase in emissions of O_3 precursors due to extensive industrial development and transport since the 1950s. This trend continues today (MARM, 2009; Fernandez-Fernandez *et al.*, 2011), despite several EU laws having been enacted in recent decades that aim

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Atmospheric deposition of inorganic nitrogen in Spanish forests of *Quercus ilex* measured with ion-exchange resins and conventional collectors☆

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ABSTRACT

Atmospheric nitrogen deposition is one of the main threats for biodiversity and ecosystem functioning. Measurement techniques like ion-exchange resin collectors (IECs), which are less expensive and time-consuming than conventional methods, are gaining relevance in the study of atmospheric deposition and are recommended to expand monitoring networks. In the present work, bulk and throughfall deposition of inorganic nitrogen were monitored in three different holm oak forests in Spain during two years. The results obtained with IECs were contrasted with a conventional technique using bottle collectors and with a literature review of similar studies. The performance of IECs in comparison with the conventional method was good for measuring bulk deposition of nitrate and acceptable for ammonium and total dissolved inorganic nitrogen. Mean annual bulk deposition of inorganic nitrogen ranged 3.09 −5.43 kg N ha^{-1} according to IEC methodology, and 2.42–6.83 kg N ha^{-1} y^{-1} using the conventional method. Intra-annual variability of the net throughfall deposition of nitrogen measured with the conventional method revealed the existence of input pulses of nitrogen into the forest soil after dry periods, presumably originated from the washing of dry deposition accumulated in the canopy. Important methodological recommendations on the IEC method and discussed, compiled and summarized.

1. Introduction

The global alteration of the N cycle has led to an increased deposition of atmospheric nitrogen (N) which could be threatening the extraordinary biological richness of the Mediterranean Basin (García-Gómez et al., 2014; Myers et al., 2000; Rockström et al., 2009). However, limited information is available on atmospheric N deposition and possible effects on the natural ecosystems of Spain. A recent model-based assessment of N deposition threats to habitats within the Spanish Natura 2000 network showed that most of the threatened habitats are located in mountainous and alpine areas in the North (Pyrenees, Cantabrian Range) and mountain areas close to high emission sources, such as the big cities of Madrid and Barcelona, and sclerophyllous forests of *Quercus ilex* in NE Spain (García-Gómez et al., 2014). These high-altitude and orographically-complex areas are difficult to access for monitoring purposes. Besides, current chemical transport models find challenging to simulate small-scale variations in deposition regimes in these areas (Boutin et al., 2015; García-Gómez et al., 2014; Simpson et al., 2006). Thus, monitoring efforts in such areas would be useful for ecosystem conservation assessments and could be applied for validation and improvement of air quality models.

The use of automatic wet-only samplers, in which the collector is open to the atmosphere only during precipitation events, is widely recommended for monitoring atmospheric wet deposition. But the need to expand monitoring networks has impelled the use

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