MEMORIA CIENTÍFICO-TÉCNICA

Sobre las actividades realizadas durante la campaña 2016 en el proyecto:

BIODIVERSIDAD Y FENOLOGÍA DE INSECTA EN EL PARC NATURAL DE LA SERRA DE COLLSEROLA. SEGUIMIENTO DE FAUNA, MONITOREO PERMANENTE DEL CLIMA POR ESTRATOS Y ACTIVIDADES PEDAGÓGICAS.

Presentado a:

Beques i Ajuts Econòmics en el marc del
Programa de Recerca i Conservació del Zoo de Barcelona
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INTRODUCCIÓN

En la presente Memoria Final pretendemos reflejar de manera sintética tanto los resultados de los diversos estudios así como la totalidad de actividades desarrolladas durante la campaña pasada, ya sea aquellas relacionadas directamente con nuestro proyecto en el área así como otras complementarias y que desarrollamos en el Parc Natural de la Serra de Collserola desde enero de 2009 con el apoyo de vuestra institución.

Los resultados de esta Memoria se han estructurado en dos partes: I - Resumen de Actividades y II - Resultados Estudio Fauna y Polución Atmosférica. En la primera parte se enumeran y explican de forma resumida las actividades desarrolladas durante la campaña 2016, mientras que en la segunda se exponen, como anexos, los resultados referentes a los estudios de la fauna y de polución atmosférica realizados en el área, tanto por el Investigador Principal (IP) de este proyecto como en colaboración con otros grupos, en este caso de la UAB.

RESULTADOS

I - Resumen de Actividades

Durante la campaña de 2016 se han desarrollado numerosas actividades, tanto las complementarias como son las visitas programadas a grupos de personas interesadas en conocer nuestro proyecto, como aquellas actividades directamente relacionas con nuestros estudios en el área, los muestreos sistemáticos de fauna, trabajos de mantenimiento y toma de datos climáticos entre otras. El Investigador Principal (IP) de este proyecto participa en todas estas actividades programadas y cuenta además con el apoyo de personas voluntarias permanentes que dan soporte en diversos aspectos.

Las actividades desarrolladas durante la campaña 2016, de forma resumida, se enumeran a continuación:

- Muestreos sistemáticos de la fauna de artrópodos presente en el ecosistema de bosque mixto mediterráneo presente en el área de estudio, el Turó de Balasc en el Parc Natural de la Serra de Collserola (figura 1). Todo el material, capturado con trampas Malaise, se encuentra depositado en parte en la colección del IP del proyecto, en la colección general de artrópodos del Museu de Ciències Naturals de Barcelona y en el Departament de Biología Animal de la facultad de Biología de la Universitat de Barcelona (ver punto tres).

- Obtención de los datos microclimáticos por estratos mediante las dos estaciones inalámbricas Vantage Pro2 ubicadas en el área de estudio. Los datos relativos a las condiciones microclimáticas tanto del dosel como del sotobosque son almacenadas e incorporadas a una base de datos para su posterior análisis y comparación con los resultados de la actividad de la fauna registrada durante el mismo período.

- Inclusión del material entomológico capturado por nuestro proyecto como material de prácticas para estudiantes de 4º año de la Falcutad de Biología de la Universitat de Barcelona. Los estudiantes dispondrán así de un material reciente, y capturado bajo una metodología concreta y sistemática, para el aprendizaje sobre los diversos grupos de artrópodos presentes en este ecosistema mediterráneo concreto, con el cual desarrollar sus trabajos de fin de año. Producto de esta colaboración con la UB se publicarán una serie de trabajos en revistas científicas especializadas en temas concretos de Ecología, Taxonomía y Sistemática.

Figura 1. Arriba: el IP participando durante el BioBlitzBCN 2016 en el Institut Botànic de Barcelona (Jardí Botànic) en calidad de experto. Centro: izquierda durante la celebración del Planta't2016 en las mismas instalaciones, y derecha muestreos sistemáticos de fauna, en este caso durante la reparación de una de las trampas Malaise ubicadas en el área de estudios. Debajo: participación en a Festa de la Ciencia.

- Planta't 2016 (4 de Abril). Se trata del día festivo del Jardín Botánico de Barcelona, de jornada de puertas abiertas y de actividades gratuitas para dar la bienvenida a la primavera. El IP participó en calidad de especialista de artrópodos durante la actividad "A la cerca i captura del bitxo", una actividad abierta para todos los públicos (figura 1).

- BioBlitzBCN 2016 (16-17 de Abril) celebrado en las instalaciones del Institut Botànic de Barcelona (Jardí Botànic de Barcelona). El IP del presente proyecto participa en cada edición de esta actividad naturalista en calidad de experto (figura 1), tanto para los muestreos directos de fauna de ambientes típicos mediterráneos, como apoyo durante la identificación de especies de insectos, principalmente del orden Diptera así como apoyo logístico durante la actividad y como guía durante los recorridos planificados.

- Festa de la Ciencia (19 junio), celebrado en el Parc de Ciutadella, junto a las instalaciones del Laboratori de Natura (Museu de Ciencies Naturals de Barcelona). El IP del presente proyecto participa en cada edición de esta actividad de Ciencia Ciudadana en calidad de experto (figura 1), como apoyo durante la identificación de especies de insectos así como apoyo logístico durante la actividad.

- Salida naturalista a las instalaciones de nuestro proyecto en el Turó de Balasc el 4 de Junio y organizada por la Associació d'Amics del Museu de Ciències Naturals de Barcelona (figura 2). Esta es la cuarta ocasión que la AAMCNB visita el proyecto, siempre con el objetivo de mostrar la riqueza del mundo de los artrópodos en Collserola. Se realizó una salida explicativa dirigida por el IP para adultos y menores, una actividad que se enmarca en uno de los objetivos principales de nuestro proyecto, acercar al público general el patrimonio natural que atesora Collserola, desde la perspectiva única que ofrece la interacción directa con la labor diaria en el terreno de un investigador y de un proyecto real y activo del cual cualquier ciudadano puede formar parte.

Figura 2. Arriba: el IP durante el desarrollo de la salida naturalista organizada por la AAMCNB a las instalaciones de nuestro proyecto en Collserola, y debajo la actividad organizada por el Centre Cívic de Vallvidrera.

Nuestra presencia en las redes sociales permite acercar a la ciudadanía los valores de nuestro patrimonio natural y los resultados de nuestros estudios mediante artículos de carácter divulgativo.

- Salida naturalista a las instalaciones de nuestro proyecto el 2 de Julio, organizada por el Centre Cívic de Vallvidrera Vázquez Montalbán (figura 2). Al igual que con la actividad desarrollada con la AAMCNB, el objetivo de esta salida era la de mostrar la riqueza de los artrópodos presente en Collserola, una salida explicativa dirigida por el IP y cuyo objetivo se enmarca entre las prioridades de nuestro proyecto.

- Inclusión, por tercer año consecutivo, de las áreas de nuestro proyecto por parte de la Universitat Autònoma de Barcelona como plataforma para impartir una clase práctica en el módulo de Máster titulado "Mètodes Experimentals en Ecologia" dirigido a estudiantes de la UAB (figura 3). Esta clase práctica, impartida por el IP del presente proyecto, tiene como objetivo principal que los estudiantes conozcan de primera mano la experiencia y las diversas técnicas de campo en el estudio de los sistemas naturales.

Figura 3. El IP durante la clase práctica que impartió en el área del proyecto en Collserola para módulo de Máster "Mètodes Experimentals en Ecologia" dirigido a estudiantes de la UAB.

Como se comentó en la memoria preliminar, parte de nuestra rutina en el proyecto lo ocupa realizar una valoración del estado de los materiales y herramientas para decidir su potencial sustitución o reparación. Durante 2016, se realizó la revisión de los sistemas de anclaje vinculados directamente a la estabilidad de la plataforma y pasarela sobre el dosel, y que llevan en funcionamiento desde 2011, así como del estado de los peldaños en esta última. Entre las tareas realizadas se encuentra la adición de peldaños nuevos, garantizando tanto la seguridad durante los trabajos que se realizan así como facilitando la circulación de personal por la pasarela.

II – Resultados Estudio Fauna y Polución Atmosférica

El estudio de la biodiversidad presente en un ecosistema concreto conlleva un esfuerzo de trabajo y una complejidad organizativa poco conocidas por parte de la ciudadanía y, en muchas ocasiones, por los propios órganos gestores de los espacios naturales que se pretenden estudiar. En el caso de los artrópodos, como ejemplo cercano a nuestro proyecto, es frecuente la situación de que los investigadores se encuentren ante la imposibilidad de cumplir con todas las demandas de la administración, órganos gestores u otros sponsors del estudio. En la mayoría de las ocasiones, y sin tener en cuenta el tipo de estudio demandado, estas instituciones precisan de resultados en un plazo relativamente corto de tiempo, que suele ser de un año administrativo, mientras que los investigadores necesitan de un período de tiempo mucho más prolongado para culminar todas las fases del estudio. En el caso de un inventario faunístico como el de los artrópodos, el escaso número de especialistas para separar y determinar la ingente cantidad de especímenes y especies obtenidas durante las campañas de muestreos, así como la interpretación de los resultados y redacción de publicaciones y memorias de actividades dificulta el cumplimiento de estos plazos anuales.

A modo de ejemplo, la simple confirmación de una primera cita de una especie concreta para Cataluña o la Península Ibérica puede tardar varios años, en dependencia del estado del conocimiento del grupo al que pertenece la especie, la disponibilidad de especialistas para revisar los especímenes y disponibilidad del material comparativo o bibliográfico necesario para la determinación de la especie en cuestión.

A continuación se anexan los resultados publicados a raíz de los diversos estudios realizados en el área, tanto los faunísticos llevados a cabo por el IP en colaboración con otros especialistas así como los resultados de la colaboración de nuestro proyecto con investigadores de la UAB en referencia al impacto de la polución del aire sobre la masa forestal en nuestra área de estudios.

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NOTA BREU

Dos nuevas citas de Limoniidae de la Serra de Collserola (Cataluña, España) y lista actualizada de especies de Limoniidae y Tipulidae (Diptera) de Collserola

Two new records of Limoniidae from Serra de Collserola (Catalonia, Spain) and check list of Limoniidae and Tipulidae species (Diptera) from Collserola

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Como parte de los muestreos sistemáticos que se realizan a través del proyecto «Biodiversidad Insecta Collserola» en áreas aledañas a la estación biológica del Parc Natural de la Serra de Collserola, se han capturado de forma sistemática especímenes de limónidos y tipúlidos (Diptera) desde 2009. Hasta la fecha, estas prospecciones han ofrecido una lista preliminar que asciende a 5 especies de Limoniidae y 6 de Tipulidae.

El Parc Natural de la Serra de Collserola es un espacio de más de 8.000 ha que forma parte de la Cordillera Litoral Catalana, pero que se encuentra aislada del resto de sierras de la misma. La cobertura vegetal es variada en la sierra, aunque en el área cercana a la estación biológica (Fig. 1), y de donde proceden las muestras, existe una clara dominancia de la encina (*Quercus ilex* L.) y del pino carrasco (*Pinus halepensis* Miller). El estado de conservación de esta área es buena, constituyendo una reserva especial dentro del parque, aunque no se localizan árboles de una madurez notable. Para una mejor descripción del área de estudios consultar Mederos-López & Pujade-Villar (2011).

Incluyendo las dos nuevas citas del presente trabajo y las aportaciones recientes al conocimiento de la familia Limoniidae en España, y Península Ibérica en general (Starý, 2014; Hancock *et al*., 2015; Mederos & Eiroa, 2015) el número de especies de Limoniidae citadas de España se eleva a 157, incluyendo Baleares y Canarias (Oosterbroek, 2016). Las nuevas citas que se presentan en éste estudio se suman a una anterior de la familia Tipulidae (Mederos *et al*., 2014) proveniente también de Collserola, *Dolichopeza (Dolichopeza) hispanica* Mannheims, 1951, evidenciando lo mucho que queda aún por conocer sobre la biodiversidad de este parque natural, a pesar de colindar con una gran ciudad como Barcelona y de sufrir históricamente una considerable presión antrópica tanto en su periferia como en su interior.

Figura 1. Vista general de la vegetación dominante en el área de estudio desde el sotobosque y dosel.

Achyrolimonia decemmaculata (Loew, 1873) (Fig. 2a-c)

Material examinado: Parc Natural de la Serra de Collserola, 230 m s. n. m., 1.V.2016, 1 ♂

Distribución: especie bien distribuida en la región paleártico occidental. Este ejemplar supone la primera cita para España; en la Península Ibérica sólo se conocía del sur de Portugal (Starý (2014).

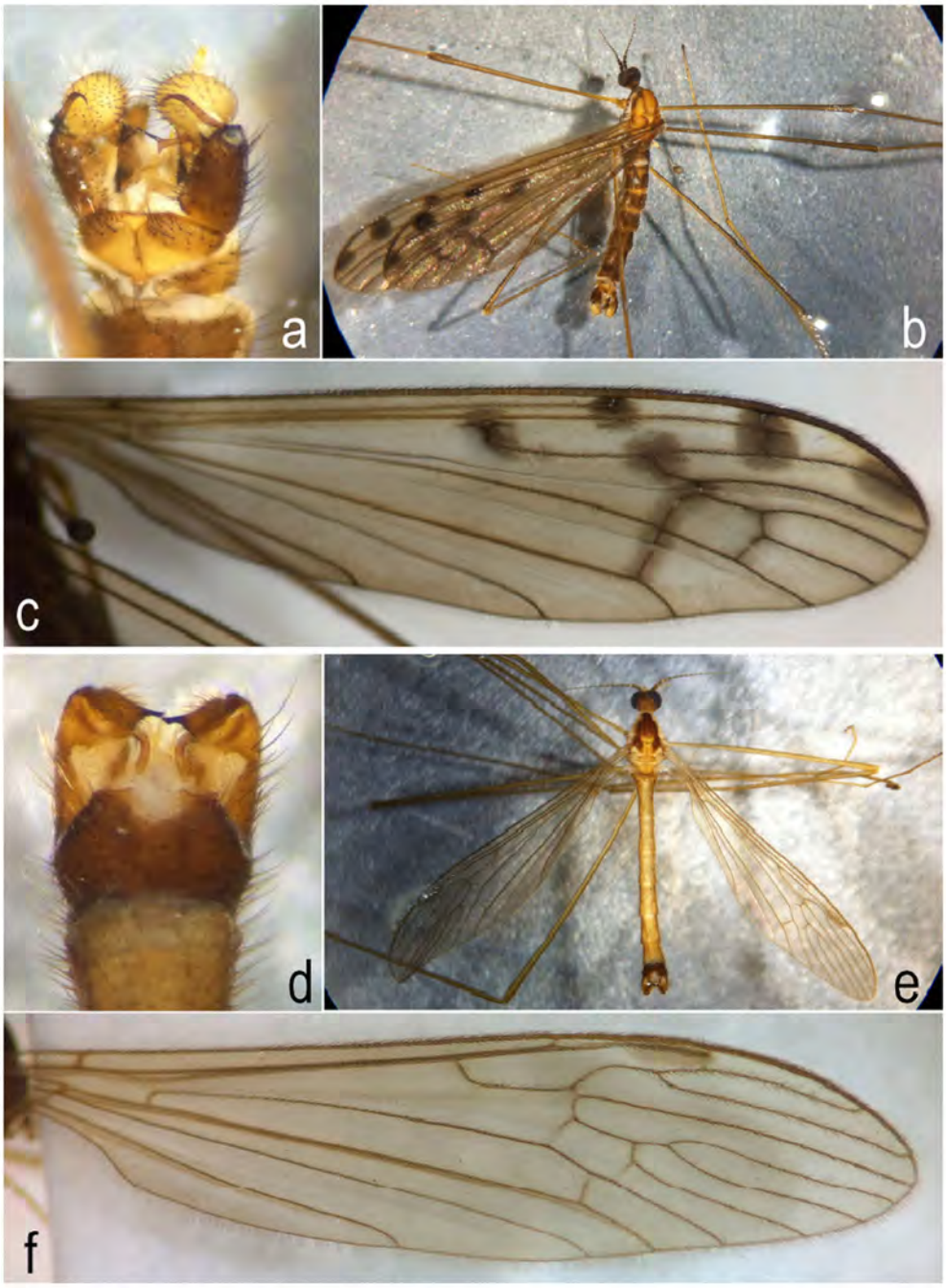

Figura 2. Genitalia masculina (vista dorsal), habitus y venación alar de: a-c) *Achyrolimonia decemmaculata;* d-f) *Austrolimnophila* (*Austrolimnophila*) *latistyla*.

Austrolimnophila (*Austrolimnophila*) *latistyla* Starý, 1977 (Figs. 2d-f)

Material examinado: Collserola, 280 m s. n. m., 21.V.2009, 2 ♂♂, 4.VI.2012, 1 ♂

Distribución: paleártico occidental. Fue descrita a partir de tres machos y una hembra (Starý, 1977), siendo uno de los machos un paratipo procedente de Lanjarón (Granada). Posteriormente Starý (2014) la volvió a citar de Andalucía (Fuengirola y Coín) y de Mallorca (Parc Natural S'Albufera). Primera cita para Cataluña.

Hasta el presente, del entorno cercano a la Estació Biològica del Parc Natural de la Serra de Collserola, así como en algunos otros puntos dentro del parque o limítrofes con él, hemos capturado un modesto número de Limoniidae (5) y de Tipulidae (6), que se enumeran a continuación:

Limoniidae
Limnophilinae
Austrolimnophila (*Austrolimnophila*) *latistyla* Starý, 1977

Limoniinae
Achyrolimonia decemmaculata (Loew, 1873)
Limonia nubeculosa Meigen, 1804
Limonia phragmitidis (Schrank, 1781)
Neolimonia dumetorum (Meigen, 1804)

Tipulidae
Dolichopezinae
Dolichopeza (*Dolichopeza*) *hispanica* Mannheims, 1951

Tipulinae
Nephrotoma flavescens (Linnaeus, 1758)
Nephrotoma flavipalpis (Meigen, 1830)
Tipula (*Lunatipula*) *helvola* Loew, 1873
Tipula (*Lunatipula*) *longidens* Strobl, 1909
Tipula (*Lunatipula*) *lunata* Linnaeus, 1758

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

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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

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. 1999). 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 (García-Gómez et al. 2014).

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-Suarez 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.

Material and methods

Study sites

Three holm-oak (*Q. ilex*) forests were selected in the vicinity of three cities in Spain with increasing population (Fig. 1, Table 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

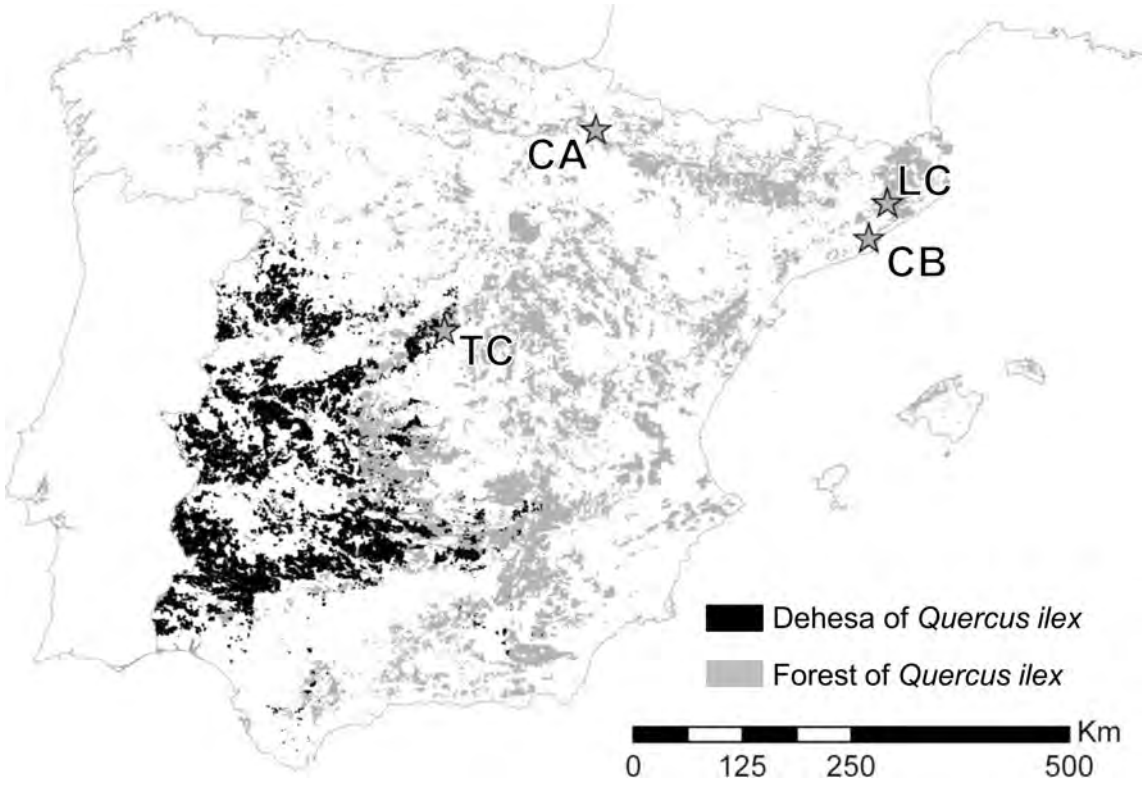

Fig. 1 Distribution of *Q. ilex* habitats in Spain, and location of the study sites. *LC*, La Castanya (Barcelona); *CB*, Can Balasc (Barcelona); *CA*, Carrascal (Navarra); *TC*, Tres Cantos (Madrid)

Table 1 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 site	Peri-urban	Peri-urban	Peri-urban	Rural
Altitude (m)	255	705	592	696
Longitude	2° 04′ 54″ E	3° 43′ 59″ O	1° 38′ 40″ O	2° 21′ 29″ E
Latitude	41° 25′ 47″ N	40° 35′ 17″ N	42° 39′ 13″ N	41° 46′ 47″ N
Mean annual temperature (°C) [1]	15.2	14.6	12.3	13.7
Mean annual rainfall (mm $year^{-1}$)[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 highway (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

[b] Values for 2012 from the Spanish Ministry of Development (http://www.fomento.gob.es/)

[c] From the Corine Land Cover 2006 (http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster-3), using a buffer of 25 km radius around the sampling sites

[d] From the Spanish National Statistic Institute (http://www.ine.es), using a buffer of 25 km radius around the sampling sites

most agricultural-influenced among the three peri-urban forests. The canopy in all the sites is dominated by *Q. ilex*, mixed with *Quercus 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. 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 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. Meteorological variables were monitored in CB, TC and LC sites, and data from the closest meteorological station were collected for the CA site.

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 2 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 2-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 4 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 °C) in darkness until they were analysed in the laboratory.

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 h. 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.

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).

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 2-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 1 January. Statistica software (version 12; StatSoft, Tulsa, OK) was used for statistical analysis. Alpha level was set at 0.05.

Results

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. 2; Table 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 2). The highest 2-week concentration reached 39.3 and 37.1 μg m^{-3} registered in CB and TC, respectively, during the winter 2012 (Supplement, S1). 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. 2).

Atmospheric NH_3 concentration (Table 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 2-week value (5.3 μg m^{-3}) was recorded in CA during late winter (Supplement, S2). 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. 2; Supplement, S2). 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.

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 2). The maximum 2-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 (Supplement, S3). 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. 2).

The annual mean of atmospheric O_3 concentrations (Table 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 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. 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). 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).

Temporal and spatial patterns of particulate matter

The concentration of PM_{10} was higher in CA and TC than in LC (Table 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. 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 24 h 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. 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 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 (Fig. 3c, d). 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

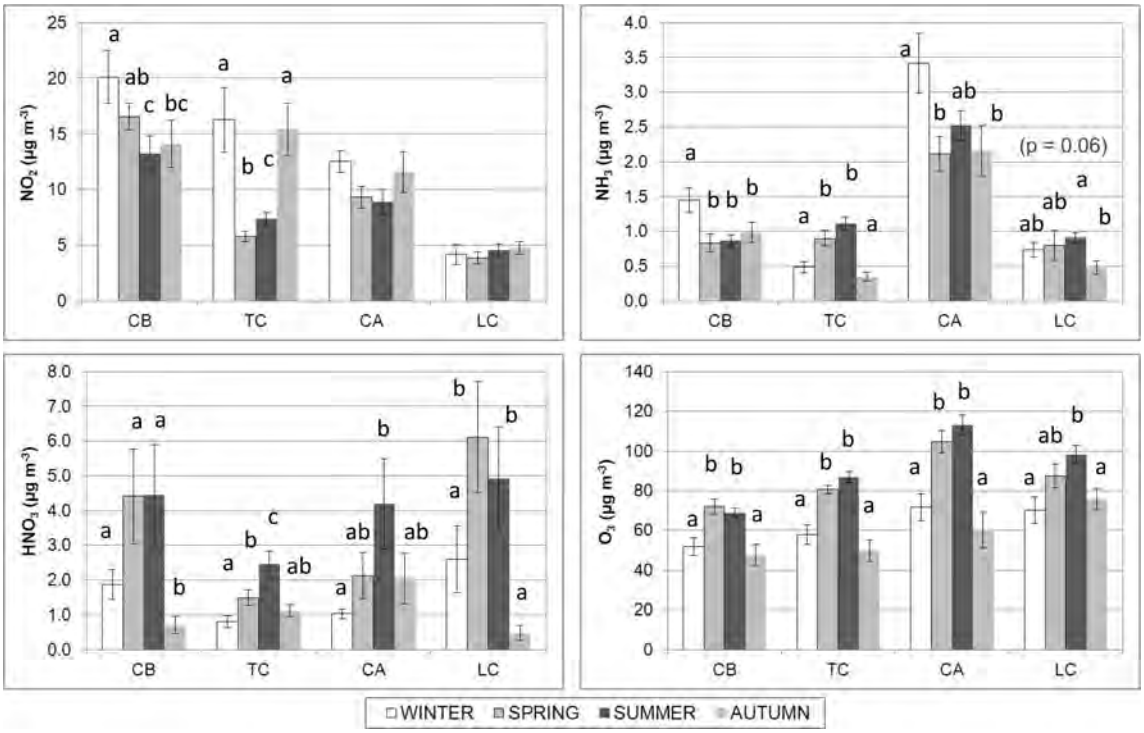

Fig. 2 Seasonal mean concentration of atmospheric pollutants in the open-field (O) plots of the four study sites and standard error of the mean. *Different letters* indicate significant differences among seasons

and summer (Fig. 3e, f). 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 (Fig. 3f).

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. 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).

The reduction of air pollutant concentrations inside the forest showed few evident seasonal patterns. Nitrogen dioxide experienced the highest decrease in concentrations below-canopy (Supplement, S1) 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; Supplement, S2), although smaller during the summer in the three peri-urban forests. Regarding HNO_3 (Supplement, S3), 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; Supplement, S4).

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 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

Table 2 Basic statistics of the monitored pollutant concentrations 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

Different letters indicate significant differences ($p<0.05$) between sites. The absence of letters indicates no significant differences

Mean arithmetic mean±standard error, *min–max* minimum and maximum 2-week values, *CV* coefficient of variation, representing the temporal variability

Table 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

concentrations showed a positive correlation with wind speed in TC and CA, and a negative correlation with precipitation in TC (Table 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 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 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 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).

Discussion

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

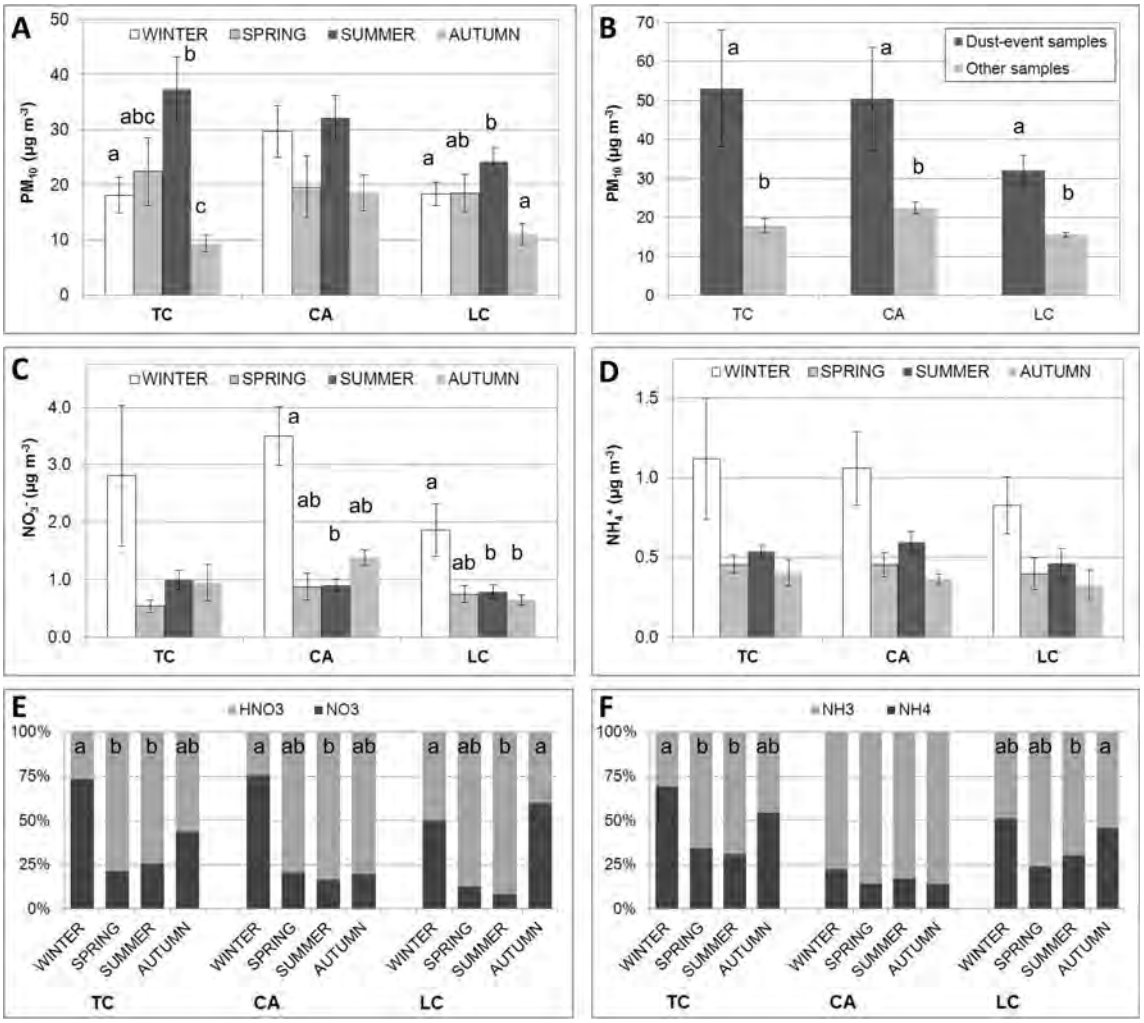

Fig. 3 Seasonal mean concentrations of aerosols and standard errors, and ratios of particulate to gaseous pollutants in the three aerosol monitoring sites. **a** PM_{10} concentration; **b** PM_{10} concentration for measurements during Saharan dust events compared with the rest of the samples; **c** 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**

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.

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 volatilization 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.

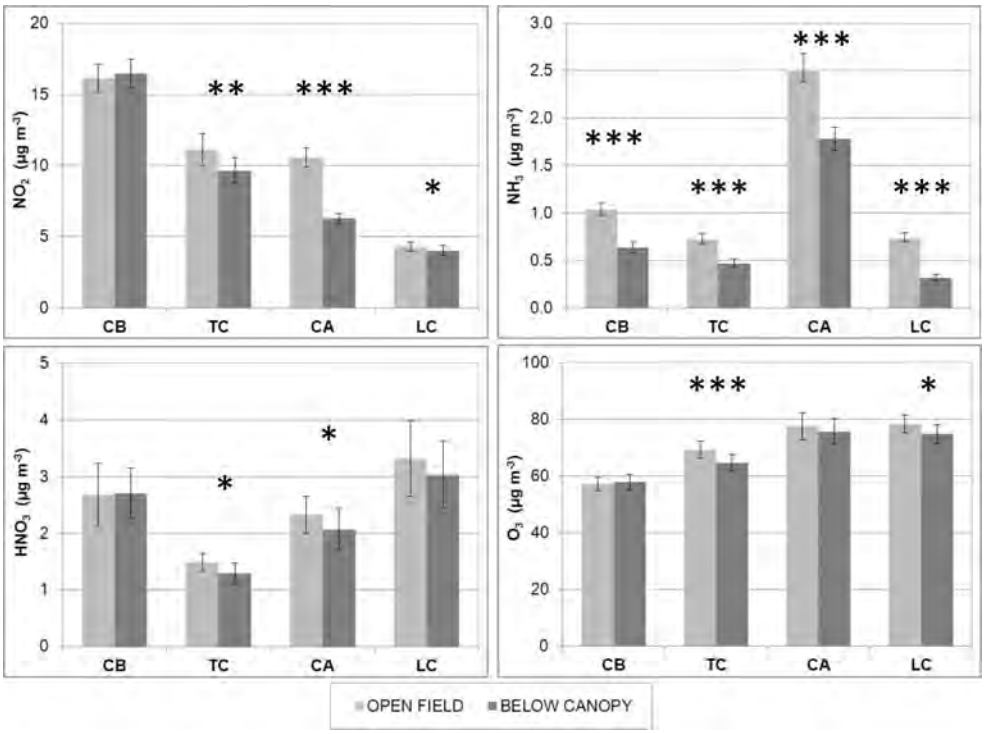

Fig. 4 Mean concentration of pollutants in O plots (open field) and F plots (below canopy), and standard error of the mean. Significance of the Wilcoxon matched pairs test: *$p<0.05$; **$p<0.01$; ***$p<0.001$

The 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. 2009).

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

Table 4 Spearman rank order correlation coefficients for pollutant concentrations in open-field plots, particulate matter (PM) and meteorological variables

	NO_2 ($\mu g\ m^{-3}$)	NH_3 ($\mu g\ m^{-3}$)	HNO_3 ($\mu g\ m^{-3}$)	O_3 ($\mu g\ m^{-3}$)	PM_{10} ($\mu g\ m^{-3}$)	NO_3^- ($\mu g\ m^{-3}$)	NH_4^+ ($\mu g\ m^{-3}$)	SO_4^{2-} ($\mu g\ m^{-3}$)	NO_2 ($\mu g\ m^{-3}$)	NH_3 ($\mu g\ m^{-3}$)	HNO_3 ($\mu g\ m^{-3}$)	O_3 ($\mu g\ m^{-3}$)	PM_{10} ($\mu g\ m^{-3}$)	NO_3^- ($\mu g\ m^{-3}$)	NH_4^+ ($\mu g\ m^{-3}$)	SO_4^{2-} ($\mu g\ m^{-3}$)
CB									TC							
NH_3 ($\mu g\ m^{-3}$)	0.35								−0.36							
HNO_3 ($\mu g\ m^{-3}$)	n.s.	n.s.							n.s.	*0.63*						
O_3 ($\mu g\ m^{-3}$)	n.s.	n.s.	*0.57*						*−0.62*	*0.69*	*0.62*					
PM_{10} ($\mu g\ m^{-3}$)									n.s.	*0.70*	*0.58*	*0.58*				
NO_3^- ($\mu g\ m^{-3}$)									*0.53*	n.s.	n.s.	n.s.	n.s.			
NH_4^+ ($\mu g\ m^{-3}$)									n.s.	n.s.	n.s.	n.s.	0.42	*0.69*		
SO_4^{2-} ($\mu g\ m^{-3}$)									n.s.	n.s.	n.s.	n.s.	*0.71*	n.s.	n.s.	
Temperature (°C)	n.s.	*−0.40*	*0.48*	*0.61*					*−0.50*	*0.85*	*0.68*	*0.82*	*0.62*	n.s.	n.s.	n.s.
Relative humidity (%)	−0.32	*−0.41*	*−0.42*	*−0.42*					*0.42*	*−0.86*	*−0.59*	*−0.86*	*−0.72*	n.s.	n.s.	n.s.
Solar rad. ($W\ m^{-2}$)	n.s.	−0.29	*0.71*	*0.69*					*−0.63*	*0.85*	*0.56*	*0.85*	*0.68*	n.s.	n.s.	n.s.
Wind speed ($m\ s^{-1}$)	n.s.	n.s.	n.s.	n.s.					*−0.71*	*0.39*	0.33	*0.66*	n.s.	n.s.	n.s.	n.s.
Precipitation (mm)	*−0.49*	*−0.49*	n.s.	n.s.					n.s.	*−0.44*	*−0.38*	*−0.39*	*−0.55*	n.s.	n.s.	n.s.
CA									LC							
NH_3 ($\mu g\ m^{-3}$)	n.s.								n.s.							
HNO_3 ($\mu g\ m^{-3}$)	n.s.	n.s.							n.s.	0.34						
O_3 ($\mu g\ m^{-3}$)	n.s.	n.s.	n.s.						n.s.	0.30	*0.41*					
PM_{10} ($\mu g\ m^{-3}$)	n.s.	n.s.	n.s.	n.s.					n.s.	*0.60*	*0.84*	*0.52*				
NO_3^- ($\mu g\ m^{-3}$)	*0.69*	n.s.	*−0.77*	n.s.	n.s.				n.s.	n.s.	n.s.	n.s.	n.s.			
NH_4^+ ($\mu g\ m^{-3}$)	n.s.	n.s.	n.s.	n.s.	n.s.	0.51			n.s.	n.s.	n.s.	n.s.	*0.58*	*0.51*		
SO_4^{2-} ($\mu g\ m^{-3}$)	n.s.	n.s.	n.s.	n.s.	*0.60*	n.s.	*0.70*		n.s.	0.49	*0.71*	*0.65*	*0.85*	n.s.	*0.60*	
Temperature (°C)	n.s.	n.s.	*0.55*	*0.67*	n.s.	*−0.75*	n.s.	n.s.	n.s.	0.36	*0.50*	*0.56*	*0.65*	n.s.	n.s.	*0.69*
Relative humidity (%)	n.s.	n.s.	*−0.45*	*−0.73*	n.s.	0.49	n.s.	n.s.	n.s.	*−0.45*	*−0.43*	−0.33	n.s.	n.s.	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.	n.s.	*0.43*	*0.74*	*0.55*	*0.77*	n.s.	n.s.	*0.64*
Wind speed ($m\ s^{-1}$)	−0.36	n.s.	0.30	0.46	n.s.	−0.48	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*−0.70*	n.s.	*−0.53*	*−0.72*
Precipitation (mm)	n.s.	n.s.	n.s.	n.s.	*−0.62*	n.s.	n.s.	n.s.	*−0.50*	−0.32	n.s.	n.s.	n.s.	−0.43	n.s.	n.s.

Italic numbers are used when $p<0.01$

n.s. none statistically significant correlation ($p<0.05$) was found

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.

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 (Supplement, Fig. S5). 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-Suarez 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. 2010).

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 below-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 is 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.

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 not 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 *Q. 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.

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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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quantitative evaluations of N fluxes (Cornell, 2011). To date, only the work carried out by Walker et al. (2012) can be considered as a real attempt to add ON measurements to the National Trends Network (NADP/NTN). Thus, in spite of the progress achieved over recent years, reviews (Cape et al., 2011; Cornell, 2011; Jickells et al., 2013) have underlined important gaps in our knowledge of budgets, chemical characterization and source identification of the organic fraction. Indeed, we are still far from an understanding of the role that ON may play in human health, ecosystems functioning and interactions in biogeochemical cycles.

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

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

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

2. Material and methods

2.1. Study sites and collection methods

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

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

2.2. Sample treatment, preservation and analysis

In the laboratory, two aliquots of unfiltered sample were reserved for pH and conductivity determinations. A third aliquot was separated in Barcelona for alkalinity estimates. The remaining sample was filtered using 0.45 μm pore filters and distributed in four subsamples for alkalinity (only in TC and CA), NH_4^+, anions and cations, and TDN analysis. In Barcelona, anion and cation determinations were performed in accordance with Izquierdo and Avila (2012). In Navarra and Madrid, ammonium and anion determinations were performed by ion chromatography (IC) (NH_4^+: Dionex 1100, with column CS16 in Navarra and Dionex 2000 with column CG12 in Madrid; SO_4^{2-}, NO_3^-, NO_2^-, Cl^-, PO_4^{3-} anions: Dionex 2000, with column AS19 in Navarra and Madrid), whereas cations were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500a).

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

Accuracy of the TDN analytical protocol was ascertained by analysis of synthetic rain samples from the World Meteorological Organization Global Atmosphere Watch (WMO-GAW) QA-SAC Laboratory Intercomparison Program (http://www.qasac-americas.org/) and certified reference NH_4^+ standard (Sigma–Aldrich). Results agreed (97–101%) with the expected total nitrogen values. Duplicate samples were determined every ten samples in order to assess the precision of the procedure. A relative standard deviation (RSD) below 4% was found for TDN and NH_4^+, and below 3% for NO_3^-. Blank samples were analyzed to ensure that there was no contamination. The 0.05 M HCl solution used to acidify and stabilize the defrosted samples were also analysed to

Table 1
Site descriptions.

Site code	CB	LC	CA	TC
Site name	Can Balasc	La Castanya	Carrascal	Tres Cantos
Province (administrative unit)	Barcelona	Barcelona	Navarra	Madrid
Type of site	Peri-urban; Barcelona city metropolitan area	Rural; Montseny mountains	Rural; agricultural and cattle activities	Peri-urban; historically managed as 'dehesa'
Latitude	41° 25′ N	41° 46′ N	42° 39′ N	40° 35′ N
Longitude	2° 04′ E	2° 21′ E	1° 38′ W	3° 43′ W
Elevation (m)	255	696	592	705
Distance to sea (km)	11	27	80	310
Climate	Oceanic Mediterranean	Oceanic Mediterranean	Mediterranean continental with oceanic influence	Mediterranean continental
Mean annual temperature (°C)	15.1	9.0	12.6	14.4
Mean annual rainfall (mm)	723	840	610	364
Lithology	shales and slates	schist and granodiorites	limestones	arkosic sandstone
Distance to the nearest big city (km)	4 (Barcelona)	40 (Barcelona)	15 (Pamplona)	9 (Madrid)
Population of the nearest big city (million inhabitants)	1.6	1.6	0.2	3.2
Distance to the nearest highway (km)	0.15	16	0.05	1.5
Average daily traffic on the nearest road (thousand vehicles day^{-1})[a]	40–50	20–30	20–30	50–60
Agricultural land uses cover[b]	23%	23%	62%	21%

[a] Values for 2012 from the Spanish Ministry of Development (http://www.fomento.gob.es/).
[b] Data from Corine Land Cover 2006 (European Environment Agency). Summary of 25 km radius buffer around the sampling sites (ArcGIS software, 9.2 version).

check that it did not present an additional source of nitrogen. In addition, the inter-comparability of the TDN analyzer and ion chromatographs from Spain was checked by analysis of ammonium and nitrate standards from each laboratory, in order to check for any systematic error in calibration between centres. No bias could be detected, implying no systematic differences.

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

2.3. Database validation

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

2.4. Air pollution monitoring

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

2.5. Data handling and statistical analysis

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

Given the strong inverse dependence of concentrations on precipitation amounts (small amounts tend to have higher concentrations), within-site correlations were analyzed using deposition data (mg m^{-2} month^{-1}) rather than concentration data, as suggested by Cape et al. (2012), and Spearman's rank correlation coefficient was applied to test for significant correlations over time at a site.

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

3. Results and discussion

3.1. Methodological implications

The analysis of the first batch of samples (from June to November 2012) showed a high percentage of samples with negative DON values. Despite the addition of 100 μL of 0.01 M HCl to control pH, discrepancies were large (values of DON more

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

These findings are of significant importance when working in Mediterranean areas, which are characterized by dry, arid and semi-arid environments, and soil erosion is a frequent phenomenon. If soils are rich in carbonates/bicarbonates, the wind-blown mineral contribution to BD and TF samples might play a vital role in pH regulation, and therefore in N–NH_4^+ preservation, with crucial consequences for underestimating the organic nitrogen fraction.

3.2. Concentrations and deposition

VWM concentrations, annual deposition fluxes and percent contributions for all sites in BD and TF are shown in Table 3. VWM concentrations of all nitrogenous species were higher in TF than in BD, except for N–NH_4^+ at TC (negligible differences) and CA (lower in TF). Deposition fluxes varied similarly, with higher N–NH_4^+ fluxes in TF than in BD at Barcelona sites and lower at CA and TC. DON concentrations and fluxes were higher in TF, except for DON fluxes at CA. The contribution (%) of DON to TDN was also higher in TF samples, except for CB, where differences were minimal between BD and TF.

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

Considering the type of site (Table 1), the plot located in the agricultural region of Carrascal (CA) registered the highest percentage of DON (56%), followed by the peri-urban plots (40% at CB and 38% at TC) and ultimately by LC, the site located in the Montseny mountains which was considered as a background point (34%). This gradient is partially in accordance with data from other spatial networks, which also reported higher contributions of DON to TDN in agricultural areas with elevated N deposition fluxes, especially where organic fertilizer is applied (Zhang et al., 2008, 2012), and lower percentages of DON in urban and sub-urban areas with lower N deposition rates (Pacheco et al., 2004; Zhang et al., 2012). However, those networks also showed that samples from remote areas registered the highest contribution of DON to TDN, with percentages of 70–80% in the Tibetan Plateau (Zhang et al., 2008, 2012) and 92% in Venezuela (Pacheco et al., 2004), although total N deposition was lower in comparison to agricultural and urban areas. These data are opposed to those found at LC, where a low DON to TDN ratio and high fluxes of N deposition were registered. A probable explanation for this discrepancy is that LC is not a true background point far from the influence of anthropogenic emissions, since recent research has found that local sources and long-range transport of pollutants might be influencing rain chemistry at this site (Izquierdo et al., 2012).

DON concentrations in samples from holm oak TF ranged from 0.93 to 1.98 mg l^{-1}, being amongst the highest values reported in

Table 2
Data from analysis of the first batch of samples: from June to November 2012. 'Initial' and 'Final' % of DON as negative values were calculated as follows: Initial or Final number of samples with DON negative values/Total number of analyzed samples* 100. 'Initial % of DON as negative values' includes the negative values from the nitrogen balance (DON = TDN−IN) calculated with the initial HCl additions, whereas 'Final % of DON as negative values' is the percentage of negative values in the final dataset, considering data from samples which remained as negative DON values after re-analysis with the new HCl conditions, plus the initial negative DON values that could not be re-analyzed and thus, corrected. For all % estimates both bulk (BD) and throughfall (TF) data were considered.

	Bulk (BD)			Throughfall (TF)			Initial % of DON as negative values	% of samples with DON negative values which were re-analyzed	% of re-analyzed samples which were improved with the new HCl treatment	Final % of DON as negative values
	pH	Alk.[a,b]	N–NH_4^+[c]	pH	Alk.	N–NH_4^+				
Carrascal (CA)	7.38	196.5	2.30	6.63	1478.9	0.79	24	93	93	3
La Castanya (LC)	5.86	90.7	1.05	5.65	150.9	0.48	19	57	100	8
Can Balasc (CB)	6.18	135.7	0.21	5.86	176.2	0.99	22	83	100	4
Tres Cantos (TC)	5.75	44.02	0.23	5.35	75.9	0.25	2	0		

[a] Alk. => Alkalinity.
[b] VWM in μ eq l^{-1} for that period.
[c] Load in kg ha^{-1} for that period.

Table 3
Annual volume weighted mean concentrations (VWM, mg N l^{-1}), average bulk or throughfall deposition (Bulk Dep., TF Dep.; kg N ha^{-1}) and contributions (%) of measured nitrogen species at the monitoring sites. Minimum and maximum values of VWM concentrations and contributions are shown to illustrate variability of these measurements over the year.

		Can Balasc (CB)			La Castanya (LC)			Carrascal (CA)			Tres Cantos (TC)		
		$N-NH_4^+$	$N-NO_3^-$	DON	$N-NH_4^+$	$N-NO_3^-$	DON	$N-NH_4^+$	$N-NO_3^-$	DON	$N-NH_4^+$	$N-NO_3^-$	DON
Bulk (BD) site	VWM	0.23	0.35	0.38	0.27	0.37	0.33	0.61	0.30	1.14	0.15	0.25	0.25
	Min	0.00	0.04	0.07	0.01	0.21	0.07	0.03	0.10	0.21	0.00	0.06	0.04
	Max	0.43	0.93	1.28	1.51	1.50	1.42	2.88	2.59	9.14	0.62	1.39	1.89
	%	24	36	40	28	38	34	30	14	56	24	38	38
	Min	0.3	3	10	0.7	18.5	12	0.7	6	11	0	18.5	10
	Max	41	61	69	43	58	67	60	81	76	46	69	78
	Bulk Dep.	1.89	2.90	3.17	2.58	3.54	3.11	6.55	3.18	12.27	0.68	1.08	1.08
Throughfall (TF) site	VWM	0.46	1.08	0.93	0.48	1.30	1.75	0.43	0.44	1.38	0.16	0.62	1.98
	Min	0.00	0.07	0.21	0.00	0.07	0.07	0.03	0.17	0.41	0.00	0.03	0.45
	Max	2.64	7.56	3.13	1.65	8.23	3.42	1.11	4.91	9.73	2.38	14.99	58.57
	%	19	43	38	13	37	50	19	20	61	6	22	72
	Min	0.1	2	6	0.2	5	8	0.3	7	21	0	0.5	27.5
	Max	39	77	98	44.5	83	92	51	71	87	17	61.5	97
	TF Dep.	2.62	6.11	5.30	3.25	8.81	11.87	3.70	3.80	11.91	0.44	1.66	5.34

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

3.3. Potential sources and annual variability

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

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

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

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

DON from CB and TC showed positive correlations with NO_3^-, but were negatively correlated to atmospheric NO_2 (BD data, Table 4). This relationship could indicate that organic nitrates may be an important component of the DON at these sites. Organic nitrates are formed as a result of photochemical reactions of hydrocarbons with NO_x ($NO + NO_2$) (Atherton and Penner, 1990; Neff et al., 2002). When these reactions occur, the expected products in precipitation are both organic nitrates and NO_3^- (Keene et al., 2002), while a reduction in NO_2 air concentrations may be predicted. CB is located in the metropolitan area of Barcelona and TC is just 9 km away from

Table 4
Spearman correlation coefficients between fluxes of DON and other species in bulk deposition. Only significant correlations are shown.

		Rainfall	NH_4^+	NO_3^-	TDN	Na^+	Cl^-	nss-K^+	nss-Ca^{+2}	nss-Mg^{+2}	nss-SO_4^{-2}	NO_2
Can Balasc (CB)	BD	0.808**	0.736**	0.753**	0.956**	0.720**	0.670*	0.742**	0.813**	0.852**		- 0.786*
La Castanya (LC)	BD	0.667*			0.817**			0.717*			0.733*	
Carrascal (CA)	BD	0.824**	0.802**	0.857**	0.973**			0.824**		0.571*	0.604*	
Tres Cantos (TC)	BD	0.758**		0.742**	0.863**			0.714**	0.610*	0.687**		- 0.738*

* Significant at $p < 0.05$; ** significant at $p < 0.01$.
TDN => Total dissolved nitrogen.
nss => non sea salt.

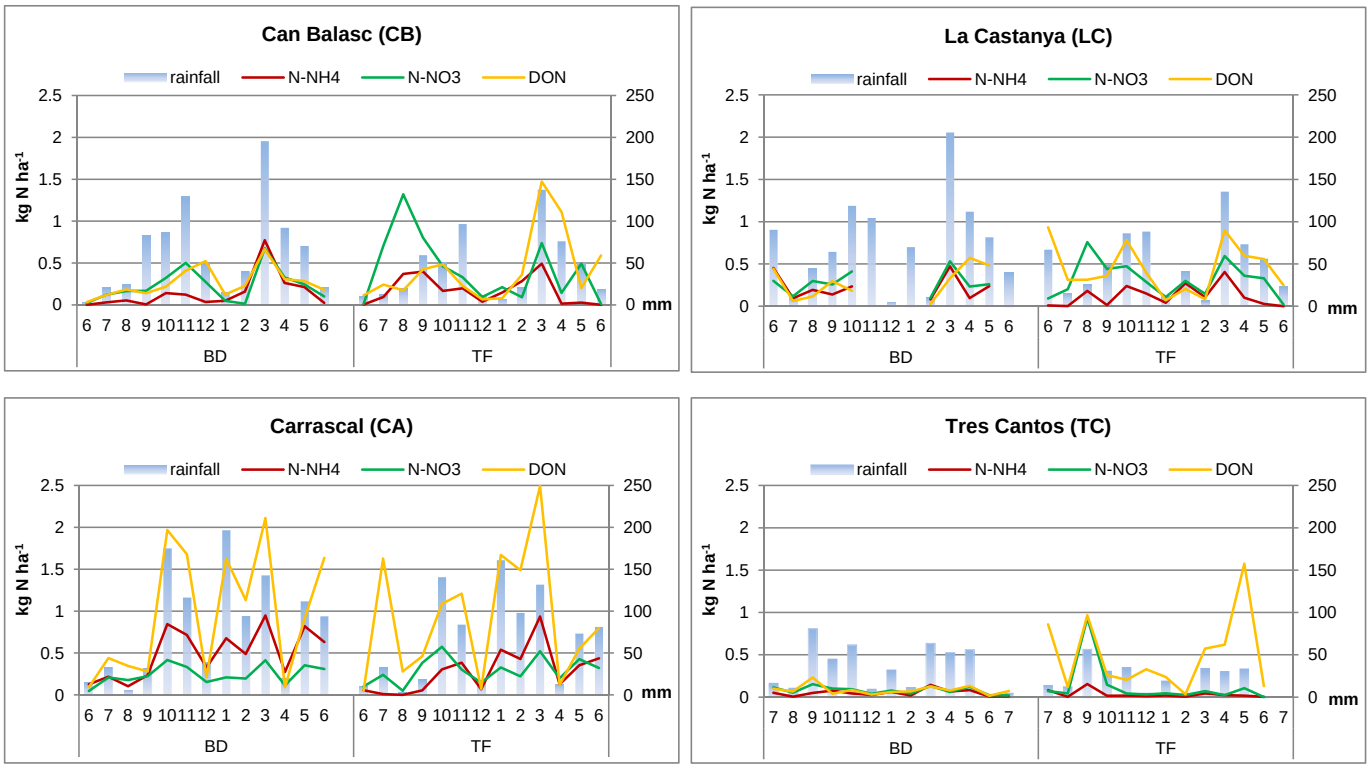

Fig. 1. Monthly bulk (BD) and throughfall (TF) deposition of nitrogenous species (kg N ha^{-1}) and rainfall (mm) at the four monitoring sites in the Iberian Peninsula.

Madrid. These two cities are the biggest in Spain, with more than 3 million inhabitants in their metropolitan areas (www.amb.cat and www.madrid.org). Therefore, polluted air masses derived from combustion and vehicle exhaust might have been a starting point for organic nitrate formation because of their enrichment in NO_x (Salvador et al., 2015; Malik and Tauler, 2015) and VOCs (Perez et al., 2002), precursors of the nitrogen containing organic compounds. The same phenomenon was found by Li et al. (2012) in Guangzhou city.

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

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

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

Correlation data from LC showed no relationship between DON and inorganic N components, being only related to nss-K^+ and nss-SO_4^{2-} (BD data, Table 4). Nss-SO_4^{2-} has widely been used as an anthropogenic tracer, directly linked to pollution emission activities (Violaki et al., 2010; Li et al., 2012). LC site is located about 40 km from Barcelona and it is relatively protected from the influence of the metropolitan area and its industrial activities, being considered as a rural or background plot (Rodrigo et al., 2003). So, it was not expected that anthropogenic emissions would affect this site. However, recent work from Izquierdo et al. (2012) showed that both local sources and long-range transport of SO_4^{2-} generated in Central and Eastern Europe influence rain chemistry at LC, which suggests that DON at this location may be linked to anthropogenic activities rather than to natural processes. Nevertheless, it should be taken into account that only 8 months of DON measurements were available at this site for statistical analysis and this may potentially bias the correlations.

Finally, at all sampling sites DON deposition was strongly correlated with nss-K^+ (BD data, Table 4). Potassium salts are regarded as indicators of plant-derived particles (Pölker et al., 2012). Matsumoto et al. (2014) suggested that correlation between the DON and nss-K^+ would indicate the influence of vegetation sources on the DON budgets. In our study sites, where BD plots are surrounded by holm oak forests (and also crop fields in CA), it seems probable that biogenic processes may be responsible to some extent for the organic budgets at these locations. However,

at CB and TC this significant relationship between DON and nss-K^+ deposition may have another explanation. Pohlker et al. (2012), besides identifying its biogenic origin, observed that potassium salts served as initial seeds for the condensation of VOC oxidation products, being directly related to secondary organic aerosol processes. Therefore, at these peri-urban sites it seems probable that organic nitrogen may be associated with potassium salts after being generated in secondary reactions. This hypothesis would explain why Matsumoto et al. (2014) found significant correlations between DON and nss-K^+ at an urban site and not at a forested one as they expected.

Regarding throughfall patterns, two important conclusions can be reached. On the one hand, data showed that dry deposition also contributes to the total amount of organic nitrogen that arrives at these sites, since DON fluxes were higher in TF than BD at all sites except for CA, where differences were negligible (Table 3). In all TF plots, DON peaks were registered after periods of little rain, both in precipitation events during (CA) or just after the summer season (TC and LC) and also after the drier winter months, in March (CB, LC and TC). This fact suggests that the dry organic nitrogen previously deposited and accumulated in the forest canopy is washed out in subsequent rain events, similarly to that reported in Violaki et al. (2010). Another possible explanation would be that in dry conditions the canopy flora convert dry deposited inorganic N to organic N (Cape et al., 2010). On the other hand, from March to May, high DON rates were registered at the Barcelona and Madrid TF plots. It is likely that this result is due to deposition of pollen, spores and plant debris that are abundant during spring time (Zhang et al., 2008; Violaki et al., 2010). At CA there is a DON maximum in March that does not match with any of the aforementioned hypotheses: previous months were not especially dry and DON fluxes greatly decreased in April, May and June in comparison with the March peak. A possible explanation is that urea fertilizer applied during this month in the surrounding fields also affects the forest canopy, and was recorded in the TF chemistry.

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

3.4. N deposition implications for ecosystems

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

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

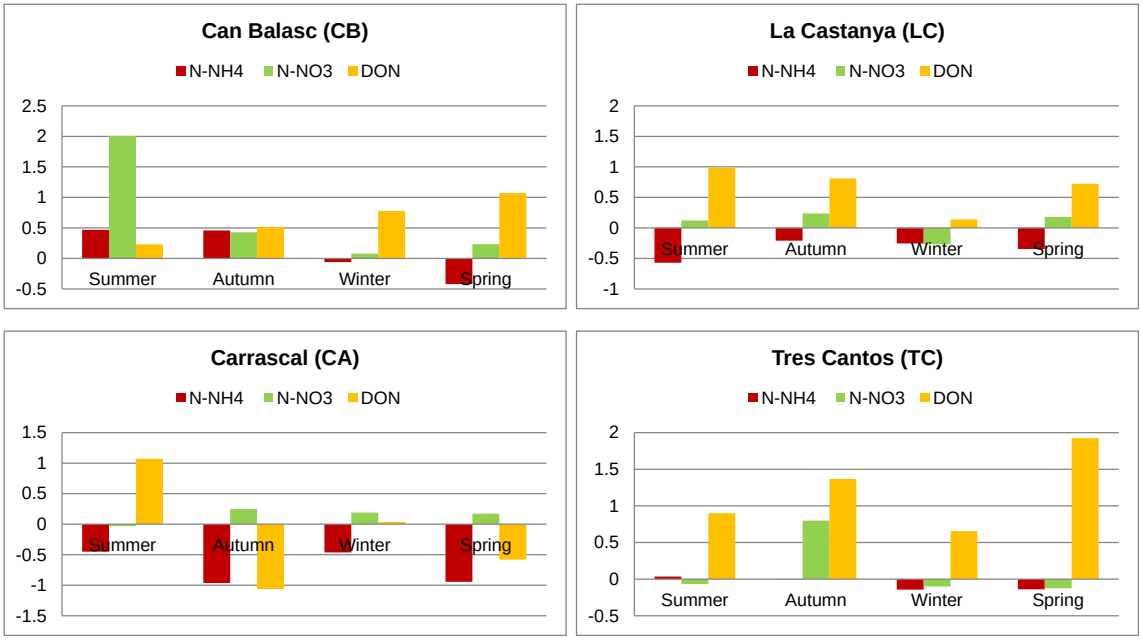

Fig. 2. Seasonal net canopy throughfall (NTF = TF−BD; kg N ha^{-1}) at the four evergreen holm oak forests studied in the Iberian Peninsula. Negative values indicate canopy uptake, whereas positive values indicate release from the canopy.

the formation of directly available DON compounds to Mediterranean forest ecosystems.

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

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

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

4. Conclusions

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

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