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Committee on Air Pollution Effects Research on Mediterranean Ecosystems

Wednesday 20th – Thursday 21st June 2018

Facultad de Ciencias. Universidad de Navarra

Proceedings

LICA Laboratorio Integrado de Calidad Ambiental | FACULTAD DE CIENCIAS | Universidad de Navarra

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Committee on Air Pollution Effects Research on Mediterranean Ecosystems

CAPER*med* is an initiative that brings together researchers working on the effects of air pollution in Mediterranean ecosystems, providing a high-level forum for discussion and exchange of information. The main objective of CAPER*med* is to coordinate expertise for quantifying the current impact of air pollution on natural and seminatural ecosystems and identifying future challenges for the Mediterranean area.

Previous CAPER*med* meetings were a great success with around 35-40 researchers showing their work carried out within the Mediterranean region. The first edition of CAPER*med* triggered the creation of new, stable collaborations and networks (e.g., NitroMed) and resulted in the write up of a review paper (dx.doi.org/10.1016/j.envpol.2017.04.062). In the second edition, collaborations among research groups working in the Mediterranean area were reinforced and a special issue entitled "(E)merging directions on air pollution and climate change research in Mediterranean Basin ecosystems" was published with the most remarkable results presented at the meeting (Environmental Science and Pollution Research, Volume 24, Issue 34, December 2017). In the third edition, apart from updating our research we aim to continue encouraging the scientific collaboration among Mediterranean research groups, bringing into focus the effect of air pollution and climate change on biodiversity and the possibility to integrate the research activities carried out within the framework of the infrastructure on biodiversity and ecosystem research LifeWatch ERIC (http://lifewatch.eu/).

Finally, in this edition we will carry out two additional activities:

Activity 1. Mosses as bioindicators of air pollution and climate change: A coordinated study in the Mediterranean Basin.

In this coordinated, crowdfunded bioindication study we aim to take advantage of a Mediterranean scientific platform such as CAPER*med*, devoted to the study of air pollution and climate change effects in Mediterranean Basin ecosystems, to implement the use of *Pleurochaete squarrosa* as a common biomonitor across the Mediterranean Basin.

Activity 2. Questionnaire

This CAPER*med* edition will present the draft of a questionnaire that will be discussed among the participants aimed at identifying the most relevant questions that need to be addressed in the area of "air pollution and climate change effects in Mediterranean ecosystems". We will pay special attention to identifying questions that can be tackled or actions that can be implemented through a coordinated platform such as CAPER*med*. The questionnaire will also target the identification of existing databases, research interests, required data, and existing research locations. Once ready, the questionnaire will be proposed to a broad audience of researchers through a free online survey. The gathered information will be used to write a short Perspectives article.

The Scientific Committee:

• David Elustondo	Universidad de Navarra, Spain
• Esther Lasheras	Universidad de Navarra, Spain
• Silvana Munzi	Universidade de Lisboa, Portugal
• Raúl Ochoa-Hueso	Universidad Autónoma de Madrid, Spain

Local organizers:

- David Elustondo
- Sheila Izquieta-Rojano
- Esther Lasheras
- Carolina Santamaría
- Jesús Miguel Santamaría

Programme

Wednesday 20 June 2018 – AULA 10. Facultad de Ciencias. Universidad de Navarra

09:00 – 09:45 **Registration**
09:45 – 10:00 **Presentation of CAPER*med* 3 by Organizers**

Session 1. Environmental monitoring, indicators and assessment
10:00 – 10:15 ***De Marco A. et al***. *"Importance of soil water content in the modelling: not only a Mediterranean concern"*
10:15 – 10:30 ***Branquinho C. et al***. *"Essential biodiversity change indicators for measuring the effects of Anthropocene in ecosystems"*

10:30 – 11:00 **Coffee break + Posters**

Session 1 (cont). Environmental monitoring, indicators and assessment
11:00 – 11:15 ***Sicard P. et al***. *"MOTTLES: a new-generation long-term monitoring stations across Europe for forest protection against surface ozone"*
11:15 – 11:30 ***Calatayud V. et al***. *"Parameterisations of stomatal ozone flux models for rice and olive tree"*
11:30 – 11:45 ***Munzi S. et al***. *"δ^{15}N in lichens reflects the isotopic signature of ammonia source"*
11:45 – 12:00 ***El Rhzaoui G. et al***. *"Spatial mapping of heavy metals using lichen bioaccumulation capacity to assess air contamination in Morocco"*
12:00 – 12:15 ***García-Gómez H. et al***. *"Atmospheric concentration and deposition of reactive nitrogen in Spanish forests of* Quercus ilex*"*
12:15 – 12:30 ***Zouari M. et al***. *"Physiological evaluation of apricot (*Prunus armeniaca *L.) leaves to air pollution for biomonitoring of atmospheric quality"*
12:30 – 12:45 ***Monteiro J. et al***. *"Measuring the long-term effect of N deposition in highly susceptible ecosystems using riparian-bryophyte functional groups"*
12:45 – 13:00 **Session debate and identification of key questions**

13:00 – 14:30 **Lunch**

14:30 – 15:30 Invited speaker: **Carly J. Stevens** (Lancaster University) *"Impacts of nitrogen deposition on different trophic levels: knowledge gaps and challenges"*
15:30 – 16:30 **Presentation of bioindication study**
16:30 – 17:00 **Coffee break + Posters**
17:00 – 18:30 **Posters session**

20:00 **Social dinner**

Programme

Thursday 21 June 2018 – AULA 10. Facultad de Ciencias. Universidad de Navarra

09:00 – 10:00 Invited speaker: **Fernando T. Maestre** (URJC) "Biotic controls of ecosystem functioning in global drylands in a warmer and more arid world"

Session 2. Experimental manipulation research

10:00 – 10:15 ***Carrara A. et al***. *"Research on carbon and water biogeochemical cycles at Majadas experimental station"*

10:15 – 10:30 ***Carreira J.A. et al***. *"N to P stoichiometric shifts as drivers of change in conifer forests under elevated N deposition: a retrospective from the ecosystem to the molecular levels"*

10:30 – 11:00 **Coffee break + Posters**

Session 2 (cont.). Experimental manipulation research

11:00 – 11:15 ***Morillas L. et al***. *"Interactive effects of N addition and climate change on soil processes in Mediterranean ecosystems: Assessment through the biological soil crust"*

11:15 – 11:30 ***Ochoa-Hueso R. et al***. *"Nitrogen deposition effects on mosses: A global meta-analysis"*

11:30 – 11:45 **Session debate and identification of key questions**

Session 3. Impacts on ecosystem structure, functioning and services

11:45 – 12:00 ***Andreetta A. et al.*** *"Mediterranean atmospheric depositions and their impacts on forest soils: trends and fluxes"*

12:00 – 12:15 ***Giordani P.*** *"Functional redundancy and vulnerability in lichen communities in relation to atmospheric pollution"*

12:15 – 12:30 ***Armas C. et al.*** *"Effects of atmospheric nitrogen and phosphorous deposition on plant and microbe soil communities in alpine systems"*

12:30 – 12:45 ***Serrano H. et al***. *"Plant species performance in an ammonia gradient"*

12:45 – 13:00 ***Pinho P. et al***. *"Nature Based Solutions to optimize the provision of Ecosystem Services to create more liveable and resilient cities"*

13:00 – 14:30 **Lunch**

Session 3 (cont.). Impacts on ecosystem structure, functioning and services

14:30 – 14:45 ***Ben Amor A. et al***. *"Survival strategies of Pomegranate and Date palm trees exposed to air fluoride pollution"*

14:45 – 15:00 ***Paoli L. et al***. *"Issues for conservation of sensitive macrolichens threatened by air pollution and intensive forest management in the Mediterranean area: the case of* Lobaria pulmonaria *(L.) Hoffm."*

15:00 – 16:00 **Proposal of a questionnaire about relevant questions in Mediterranean Ecology and Global Change**

16:00 – 16:30 **Coffee break**

16:30 – 17:00 **Jesús Miguel Santamaría** (UNAV, LIFEWATCH CEO) *Presentation about LIFEWATCH-ERIC, and possible cooperation with CAPER*med

17:00 – 18:00 **Conclusions + Best poster award sponsored by Conor Bikes + Announcements**

Abstracts
Oral presentations

Survival strategies of Pomegranate and Date palm trees exposed to air fluoride pollution

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Air quality biomonitoring using plant leaves has been widely applied to assess the effects of atmospheric pollution. In Gabes city (Tunisia), where the phosphate fertilizer factory constitutes the principal source of fluoride pollution, pomegranate and Date palm trees grow over large areas. This includes areas where high levels of pollution exist such as in agricultural and industrial areas.
In order to reveal strategies adopted by these fruit species to live in restrictive conditions, we investigated the effect of gaseous hydrogen fluoride on the tree leaves of *Punica granatum* and *Phoenix dactylifera* around a phosphate fertilizer-producing factory.

The pigment contents, damaged leaf area, fluoride accumulation, distribution of some elements (Ca, Mg and phosphorus) and some biochemical responses (proline and soluble sugar) in the leaves of *P. granatum* and *P. dactylifera*, were measured. Samples were collected from two locations having different pollution levels in Gabes: one site close to the factory, at 1 km (polluted area), and another experimental site (control site; no polluted area) at 35 km South from the factory. Sampling was done from April to June.

Our results showed that the Date palm and pomegranate trees near the factory accumulated amounts of F in the leaves. The accumulation of proline and soluble sugars contents in leaf tissues were considered to be adaptive reactions to F pollution. In addition, calcium and magnesium played an important role in trapping fluoride and delaying the appearance of necrosis.

These two evergreen tree species highly tolerated fluoride pollutants present in the air, and therefore can be effective at reducing pollutants.

Mediterranean atmospheric depositions and their impacts on forest soils: trends and fluxes

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Forests have been exposed to elevated acidifying atmospheric deposition due to the emission of N and S compounds. Recently, a comprehensive study on the response of soil solution chemistry to decreasing acid deposition across Europe found that a reduction of acidifying atmospheric deposition did not result in clear soil recovery. Soil recovery appears to be prevented by concomitant decreases in base cation (BCE) deposition. The deposition picture for Italy, however, showed different trends, BCE deposition being high and stable. Further, although no changes were found for NO_3^- trends, Italy sees some of the highest N deposition loads, concentrated especially in the North. Within this context, our research aims to explore the specific impacts of peculiar atmospheric deposition, hypothesising a strong influence of aeolian dusts, on forest soil ecosystems in Italy as representative of Mediterranean region. Our main objectives are a) to evaluate the long-term trends in soil solution elemental fluxes in relation to depositions trends; b) to investigate the soil response to pollution change and recovery, individuating whether soil acidification is, or not, an active process; c) to identify sites where the transfer of reactive N from atmospheric N pollution to fresh- or ground-waters is a real concern. We used data describing deposition and elemental concentrations in soil solutions recorded at forest plots of the ICP Forests intensive monitoring network in Italy.

As for other countries in Europe, the concentration of SO_4^{2-}in soil solution significantly decreased concomitantly with decreasing SO_4^{2-} deposition, while, differently from the European trends, an increase in BCE concentrations was found. The observed increase in soil solution pH in northern sites followed an increase of BCE with a decrease of SO_4^{2-} and no change for NO_3^-. In Central-Northern Italy, trends in soil solution pH were not significant; a neutralising effect on acidification was likely due to concomitant increases of NO_3^- and BCE in soil solution. Further, the estimation of the BCE overall budget, comprising canopy and soil fluxes, evidenced a regular accumulation of K^+ and Ca^{2+} in all sites, except for a site where soil base saturation is very high and a depletion of Ca^{2+} was found. For Mg^{2+}, depletion was strongly slowed down by the influence of deposition. These results point out how soil acidification is not an active process in forest sites of Italy. However, high inorganic nitrogen concentrations in soil solutions were found in sites with high N deposition loads, where regular N flux out of the rooting zone can represent a risk of ground- and fresh-water pollution.

Effects of atmospheric nitrogen and phosphorous deposition on plant and microbe soil communities in alpine systems

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Global environmental change threatens biodiversity and natural ecosystems, and one of its main drivers is the atmospheric deposition of nitrogen. If current trends of energy consumption continue, by 2050 the deposition of N will have doubled, so it is very necessary to know its effects in order to predict potential changes and make mitigation proposals. Among the most affected ecosystems, alpine systems are suitable places to conduct global change studies since they have strong altitudinal gradients, are usually refugia of species not existing in other areas and, at the same time, are very sensitive to changes. In this project we propose to study the influence of increasing atmospheric deposition of nitrogen and phosphorus, mainly from Saharan dust, in the plant communities and their interactions with mycorrhizal fungi and soil bacteria, both directly and indirectly, in four Alpine systems, two dry Mediterranean (Sierra Nevada and Guadarrama National Parks) and two Atlantic, one wetter (Picos de Europa National Park), another semiarid (Las Cañadas del Teide National Park), to anticipate impacts of increased nutrients in these ecosystems, both under field and controlled conditions, by simulating deposition scenarios with fertilization and their interaction with other global change drivers.

Essential biodiversity change indicators for measuring the effects of Anthropocene in Ecosystems

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During the Anthropocene Era we expect most ecosystems to be affected by at least one anthropogenic driver (whether it is global or local). Some drivers, such as some pollutants (e.g. DDT) never existed in nature and others existed in much lower amounts or intensity (carbon dioxide or ammonia). Ecosystems are currently affected by drivers, which limit or impact biodiversity with different intensities. In this work, we use the term ‘Driver Intensity’ to convey not only the amount but also the toxicity of the drivers affecting biodiversity. Additionally, driver’s intensity changes over time. In the past, biodiversity was mostly affected by air pollution (e.g. sulphur dioxide and metals), while nowadays nitrogen pollution is the driver with stronger effects on biodiversity, particularly in rural areas. In the future, we expect changes in climate patterns due to climate change to become the most important driver. Sulphur dioxide in the atmosphere increased almost ten-fold during the industrial revolution (with its maximum in the 70´s), whereas ammonia emissions increased more recently and with an intensity of approximately four-fold. Climate change effects on biodiversity are mostly related to changes in the deviation from average climatic variables, such as the case of global surface temperature. Due to this, significant changes were only recently detected, and its intensity is still low in comparison with the magnitude of the previous drivers. In the Era of Anthropocene and under the framework of the Essential Biodiversity Variables, the main aim of this work is to call attention for the need to develop Biodiversity Change Indicators and Surrogates to monitor biodiversity changes. These can only be interpreted and applied after knowing their relationship with the drivers that limit or impact biodiversity. Our conceptual model will be based on the selection of the most cost- effective biodiversity change metric having in mind the nature and intensity of drivers, and the nature of the Essential Biodiversity Variables.

Acknowledgments: NitroPortugal-H2020

Parameterizations of stomatal ozone flux models for rice and olive tree

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The DO3SE model is the reference for O3 risk assessment in Europe as it is embedded in EMEP model, which is instrumental to the development of European air quality policies mainly through their support to LRTAP Convention. For risk assessment, ozone flux metrics as estimated by this model represent an advantage over exposure-based metrics, as the former approach considers the actual stomatal ozone uptake by the plant as well as the influences of meteorological, soil moisture and phenological factors on this uptake. However, important Mediterranean crops such as Mediterranean rice and olive trees have not been parameterized so far for this model. In order to parameterize the model for both crops, different types of measurements were carried out in the field regularly throughout all the growing season of the target plant receptor, including gas exchange (with a LICOR-6400), and leaf water potential (with a SKYE SKPM1400). The responses of stomatal conductance against temperature, Vapour Pressure Deficit, phenology and photosynthetically Active Radiations were studied by boundary line analysis, and different functions for these factors were derived. Finally, the performance of the modelled stomatal conductance estimates has been assessed by comparing them with measured values.

Parameterization of a stomatal conductance model for rice and olive-tree is one of the objectives of GEISPAIN project (CGL2014-52838-C2-2-R, 2015-2018, MINECO, Spain), as a first step to compare modelled O_3 fluxes with measured ones using a fast O_3 analyser (FOS, Sextant) in combination with the eddy covariance technique. In this project, the target Mediterranean ecosystems are paddy rice, olive orchard, holm-oak dehesa and Mediterranean shrub. On the other hand, the recently funded ELEMENTAL project (2018-2020, MINECO) will continue GEISpain objectives but, for the first time, O_3 fluxes will be measured both over the tree canopy and below this canopy at the same time. The target ecosystems will be an Aleppo pine forest (with and without understory vegetation), an olive-tree orchard and a dehesa ecosystem. All the measurements will be carried out at highly instrumented eddy covariance stations, some of them including e.g. sap-flows and lysimeters. Results of both projects are expected to be relevant for improving our knowledge on the models for ozone risk to vegetation under Mediterranean conditions.

Research on carbon and water biogeochemical cycles at Majadas experimental station

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The research activities taking place at Majadas experimental station aim at improve both understanding and modelling of atmosphere-ecosystem exchanges and biogeochemical cycles of carbon and water in dehesa ecosystems, and more generally in Mediterranean tree-grass ecosystems, in the context of global change. An important experimental dispositive was implemented to observe the ecosystem functioning from various complementary perspectives.
The long-term eddy covariance (EC) CO_2 and water flux tower was set up in 2003 in a typical *Quercus Ilex* dehesa, and allow analysing response of overall ecosystem carbon and water fluxes to climatic variations and quantify long-term carbon budgets. Recent studies have shown how human induced N/P imbalances affect essential ecosystem processes, and might be particularly important in water-limited ecosystems. In order to study how ecosystem functioning (e.g. biomass productivity, carbon balance, water use efficiency, albedo, etc.) change as consequence of N and NP fertilization, two additional EC towers sites were set up in 2014 for an ecosystem scale nutrient manipulation experiment and their main EC footprint areas (18 ha) were fertilized, respectively with N and NP, at the beginning of 2015. In order to study the specific water use efficiency of the two vegetation layers (i.e. herbaceous layer and *Quercus Ilex* tree canopy) and their contribution to overall ecosystem carbon and water fluxes, we monitor the spatial and temporal variability of soil + herbaceous layer CO_2 and water fluxes using subcanopy EC systems, tree transpiration using sapflow measurements, and evapotranspiration using lysimeters. As soil processes are crucial to understand overall ecosystem function, a number of soil related information is collected, such as soil respiration data (both automated systems and manual measurements) and mini-rhizotrons to observe fine roots dynamics, in complement to typical soil sampling and lab analysis done at the site.

To interpret the variations in overall ecosystem CO_2 and water fluxes measured with the EC systems, we monitor spatial and temporal variations in phenology, plant traits, and spectral characteristics of vegetation by using optical hyperspectral and multispectral measurements, airborne hyperspectral data, digital repeat photography, and vegetation sampling. The rich proximal remote sensing information collected at various scales at the site is used for a number of works aiming at developing or improving remote sensing products related to biophysical parameters of vegetation and water and carbon fluxes for tree-grass ecosystems at the global scale.

N to P stoichiometric shifts as drivers of change in conifer forests under elevated N deposition: a retrospective from the ecosystem to the molecular levels

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Elevated atmospheric N deposition alters soil N availability, which triggers profound changes in forest biogeochemical cycles and tree nutritional stoichiometry. We here review the critical role played by the induction of secondary P limitations in the development of the so-called N saturation syndrome in forests, focusing on the research done by our research group in the last two decades on conifer forests from the U.K. and S. Spain. Our first observations in the early 90s, on the base of mensurative studies along geographic gradients in the U.K., showed correlations between N deposition levels and P deficiency in temperate conifer forests. This was followed by manipulative experiments (e.g., Glencorse experiment, where acid mist was applied to entire forest canopies) which demonstrated a causal relationship between atmospheric N inputs and changes in the soil P cycle (increased inorganic P fixation, acceleration of the organic P subcycle-phosphomonoesterase activity) linked to P nutritional stress in the trees.
Later on, these patterns were also confirmed for *Abies* forests endemics from the Mediterranean region. These climatic relic forests are physiognomically similar to temperate conifer forests, and develop in areas with comparable annual precipitation and mean temperature values. However, their seasonality is typically Mediterranean-type, which allowed us to indirectly test the hypothesis that forecasted climate change would increase N cycle leakiness and the susceptibility of temperate conifer forest to shift from N to P limitation under chronic N deposition.

More recently, we carried out a field-based manipulative experiment in which compensatory P fertilization was applied to N-saturated forest stands. By ^{15}N labelling of soils and root exclusion treatments we showed that autotrophic (tree-uptake) N retention was re-established following P fertilization, with consequences in shifting back the forest to N limitation, and in alleviating previous N saturation symptoms.

At present we are investigating the transcriptomic and molecular mechanisms in the trees, and associated changes in rhizospheric ectomicorhizal communities, which underlie the forest response to these N:P stoichiometric tensions under N deposition. Ongoing results preliminary show that a lack of differential gene expression to excess N in these oligotrophic species, together with differences among tree species in foliar traits (e.g., leaf longevity), may explain observed differences among forest ecosystems regarding N to P stoichiometric shifts under N deposition.

Importance of soil water content in the modelling: not only a Mediterranean concern

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Plants remove a large amount of atmospheric compounds from the lower troposphere through stomata; however the role of water availability was often neglected in atmospheric chemistry modelling studies as well as in integrated risk assessments Soil moisture and water stress play a pivotal role in regulating stomatal behaviour of plants; however, in the last decade, the role of water availability was often neglected in atmospheric chemistry modelling studies as well as in integrated risk assessments, despite through stomata plants remove a large amount of atmospheric compounds from the lower troposphere.
The main aim of this study is to evaluate the effect of soil water limitation on stomatal conductance and assess the resulting changes in atmospheric chemistry testing various hypotheses of water uptake by plants in the rooting zone; following the main assumption that roots maximize water uptake, i.e. they adsorb water at different soil depths depending on the water availability, we improve the dry deposition scheme within the chemistry transport model CHIMERE.

Results highlight how dry deposition significantly declines when soil moisture is used to regulate the stomatal opening, mainly in the semi-arid environments: in particular, over Europe the amount of ozone removed by dry deposition in one year without considering any soil water limitation to stomatal conductance is about 8.5 TgO_3, while using a dynamic layer that ensures plants to maximize the water uptake from soil, we found a reduction of about 10% in the amount of ozone removed by dry deposition (~7.7 TgO_3). Despite dry deposition occurs from top of canopy to ground level, it affects the concentration of gases remaining into the lower atmosphere with a significant impact on ozone concentration (up to 4 ppb) extending from the surface to the upper troposphere (up to 650 hPa).

Our results shed light on the importance of improving the parameterizations of processes occurring at plant level (i.e. from the soil to the canopy) as they have significant implications on concentration of gases in the lower troposphere

Spatial mapping of heavy metals using lichen bioaccumulation capacity to assess air contamination in Morocco

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Spatial mapping of the distribution of heavy metals was performed with the Inverse Distance Weighting technique (IDW) of Arc Gis 10 information system (GIS). We propose an innovative study based on information system technology and lichen biomonitoring to assess air pollution. The results demonstrated that the contamination of heavy metals (HM) fluctuates with certain some hotspots with high concentration of Cr, Pd, Cu, Cr and Fe. The spatial mapping showed that "Sidi Yayha El Gharb" is the most contaminated area not only lithogenically but also from industries and traffic. Spatial mapping that the environment is highly affected with industrial discharges, remediation activities should be carried out urgently to prevent an ecological disaster.

Key Words: Spatial mapping, Inverse Distance Weighting technique (IDW), air pollution, lichens.

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

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Atmospheric concentration and deposition of N pollutants were monitored in four Spanish holm oak forests with different edaphic and climatic conditions during two years (2011–2013). Passive samplers were used to measure the atmospheric concentrations of the main pollutants (NO_2, NH_3, HNO_3 and O_3,) in open areas and inside the forests. Particulate matter with diameter up to 10 µm (PM_{10}) was monitored using high volume samplers installed in open-field plots of three of the sites. Bulk and throughfall deposition of dissolved inorganic N (NO_3^- and NH_4^+) were monitored by means of conventional bottle collectors, weekly sampled, and by ion exchange resin columns, an easy-to-operate method which does not require so frequent visits to the field. Wet deposition was also sampled using an automatic "wet-only" collector in open areas in three of the sites. Dry deposition was estimated by means of an empirical inferential method (EIM) coupled with the modelling of stomatal conductance, and also by canopy budget model (CBM) and inferential method (IM). Concentration of N in soil water was measured weekly through ceramic suction caps.

These forests showed a significant below-canopy reduction of gaseous concentrations (particularly ammonia, with a mean reduction of 29% – 38 %). Ozone was the only air pollutant expected to have direct phytotoxic effects on vegetation according to current thresholds for the protection of vegetation. The results showed that collection methods based on ion-exchange resins for bulk and throughfall deposition measurements can be recommended for long-term studies, particularly in remote areas. On average for the four forests, total deposition of inorganic N varied with the methodologies for estimating dry deposition: 17.2 ± 4.3 kg N ha^{-1} y^{-1} (EIM), 10.5 ± 2.7 kg N ha^{-1} y^{-1} (CBM) and 9.8 ± 1.9 kg N ha^{-1} y^{-1} (IM). The estimated dry deposition of atmospheric inorganic N represented 64% – 77% of the total deposition (depending on the method employed) and it was dominated by the dry deposition of the oxidized forms (60% – 76% of the dry deposition), which indicated the great importance of measuring ambient nitric acid vapour in Mediterranean forests. Loss of nitrate in soil water was detected when N inputs to the soil occurred in short pulses during periods of low biological activity, in agreement with the Mediterranean asynchrony hypothesis. For the monitoring period, the empirical critical loads proposed for the protection of these ecosystems (10 – 20 kg N ha^{-1} $year^{-1}$) was overreached in three of four forests.

Functional redundancy and vulnerability in lichen communities in relation to atmospheric pollution

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Unlike vascular plants that take nutrients mainly from the soil through the roots, lichens, and in particular epiphytic lichens, take up water, solutes and gases over the entire thallus, thus depending on the atmosphere for nutrition. For this reason, they respond directly to atmospheric pollution and they have been widely used for monitoring air pollution
In spite of advanced process of standardization of sampling protocols of lichen diversity, the aspects related to the interpretation of data collected in relation to various possible factors of anthropogenic disturbance, such as air pollution and forest management, are still subject for discussion.

The complex relationship between lichen diversity, disturbing factors and other natural factors, such as climate, suggests adopting an integrated interpretative approach that takes into account the multiple aspects of diversity.

This work shows examples of the use of some functional diversity indices, rarely used in the field of biomonitoring. These indices complete the information obtained by analyzing the taxonomic diversity, describing the effects of disturbing factors on the functionality of the lichen communities and the ecosystem as a whole.

In this work, the possibilities of applying the indices are discussed through a re-reading of previous biomonitoring data on the effects of atmospheric pollution. Examples are presented from the local to the Continental scale on how atmospheric pollution affects functional diversity, redundancy and vulnerability of lichen communities.

The novelty of the approach relies on a more detailed interpretation of communities in terms of species diversity and functional traits. According to the insurance hypothesis, high species richness is expected to ensure ecosystem stability, because it can provide a sufficient functional redundancy and therefore buffer the possible effects of species loss on function losses. However, this assumption cannot be generalized and many recent studies highlighted how a considerable functional vulnerability could be observed also in species-rich ecosystems, due to the tendency of most species to cluster into a few, over-represented functional entities, leaving many functions under-represented. This phenomenon has been described as ‘functional over-redundancy’ and should be taken into account when evaluating current ecosystem conditions and/or when predicting possible shifts of the communities as a consequence of the effects of global change.

Measuring the long-term effect of N deposition in highly susceptible ecosystems using riparian-bryophyte functional groups

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Nitrogen (N) pollution resulting from intensive anthropogenic activities has been recognized as an important driver that threatens the functioning of ecosystems and their floristic diversity at a global scale. Bryophytes are among the most sensitive components of the vegetation with respect to N pollution, as they obtain most nutrients directly from precipitation and dry deposition with little uptake from the substrate. Some of the indicators used are tissue N content. Biodiversity-based methods have shown shifts in bryophyte communities consistent with increased nutrient availability and ecosystem eutrophication. In this context, Ellenberg N Index Values, which reflect community nutrient status, were found to provide a useful indication of N deposition. The aim of this study was to explore the suitability of a bryophyte-based Ellenberg N Index to indicate N deposition in Portuguese Natura 2000 Sites identified as highly susceptible to N pollution. Sampling was conducted in northwestern Portugal, in headwater streams located within the prioritized Natura 2000 Sites – Alvão/Marão, Corno do Bico, Montemuro, Peneda-Gerês, and Serra de Arga. The abundance of riparian bryophyte species was registered as percentage cover in 139 plots of 0.25m^2. In each plot, land-use cover (Corine Land Cover 2012), the conservation status of streamside natural vegetation, and riparian vegetation structure were assessed in a 50m radius buffer. A cover-weighted Ellenberg N Index value was attributed to each plot. Results suggested that Ellenberg N Index values were positively correlated with heterogeneous agricultural areas, which indicates a compositional shift consistent with increased N pollution. On the other hand, forested riparian areas presented a negative correlation with the Index. N uptake and N retention were shown to be higher in riparian areas with forest cover, highlighting their important role in N dynamics. Moors and heathlands were characterized by oligotrophic bryophyte communities, as reflected in the negative correlation with the Ellenberg N Index. Indeed, studies in these ecosystems have demonstrated a decline in bryophytes in response to increasing N deposition. Moreover, peats, mires, bogs, and similar acidic and oligotrophic habitats within the studied Natura 2000 sites were assessed to have the highest risk of biodiversity change due to N pollution in mainland Portugal. This study emphasized the potential use of the Ellenberg N Index as a cost-effective method to measure the long-term effects of N deposition in highly N-susceptible ecosystem.

Interactive effects of N addition and climate change on soil processes in Mediterranean ecosystems: Assessment through the biological soil crust

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Multiple environmental changes synergistically affect terrestrial ecosystems, generating non-additive effects of which impacts on ecosystem functioning are not predictable from single-factor studies. The Mediterranean Basin is projected to experience an increased nitrogen (N) deposition and important changes in the rainfall dynamics. Despite that, no research has been performed to disentangle the effects of the interactive global change drivers on soil processes in Mediterranean ecosystems to date. To fill this gap, we planned a new research line funded under the Horizon 2020 programme, namely by a Marie Curie individual fellowship, which will: i) investigate the synergistic effects of N status and climate change on soil processes in Mediterranean ecosystems and ii) assess the role of the biological soil crust and its components as key factors modulating the way these global change drivers interact. We hypothesize that the soil biogeochemical processes will be altered by the intensification of climate change (i.e., increased frequency of drying–rewetting cycles) but that this effect will be modulated by soil N supply conditions. Besides, we expect that the biocrust will modulate, and possibly increase, the resistance and resilience of soil processes to the synergistic effects of increased N and climate change. We will also study the role of the three main biological soil crust components (lichens, mosses and cyanobacteria) which are potentially involved in the modulation of soil processes, as well as the species-specific effects of biocrust-forming lichens. Finally, the well-known efficiency of lichens as ecological indicators will be exploited to assess the synergistic effects of multiple environmental changes. These hypotheses will be tested through the NitroMed network, which comprises the unique three sites in the Mediterranean Basin under long-term N-manipulation experiments: Arrábida (Portugal), Capo Caccia (Italy) and El Regajal (Spain). The outcomes of this project are expected to have highly relevant implications on environmental policy and prediction of global change scenarios, with the ultimate societal objective of improving ecosystem management in Mediterranean ecosystems.

δ^{15}N in lichens reflects the isotopic signature of ammonia source

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Although it is generally accepted that δ15N in lichen reflects predominating N isotope sources in the environment, confirmation of the direct correlation between lichen thalli and atmospheric δ15N is still missing, especially under field conditions with most confounding factors controlled. To fill this gap and investigate the response of lichens with different tolerance to atmospheric N deposition, thalli of the sensitive *Evernia prunastri* and the tolerant *Xanthoria parietina* were exposed for ten weeks to different forms and doses of N in a field manipulation experiment where confounding factors were minimized. During this period, several parameters, namely total N, δ^{15}N and chlorophyll a fluorescence, were measured. Under the experimental conditions, δ^{15}N in lichens quantitatively responded to the δ^{15}N of released gaseous ammonia (NH_3). Although a high correlation between the isotopic signatures in lichen tissue and supplied N was found both in tolerant and sensitive species, chlorophyll a fluorescence indicated that the sensitive species very soon lost its photosynthetic functionality with increasing N availability. The most damaging response to the different N chemical forms was observed with dry deposition of NH_3, although wet deposition of ammonium ions had a significant observable physiological impact. Conversely, there was no significant effect of nitrate ions on chlorophyll *a* fluorescence, implying differential sensitivity to dry deposition versus wet deposition and to ammonium versus nitrate in wet deposition. *Evernia prunastri* was most sensitive to NH_3, then NH_4^+, with lowest sensitivity to NO_3^-. Moreover, these results confirm that lichen δ^{15}N can be used to indicate the δ^{15}N of atmospheric ammonia, providing a suitable tool for the interpretation of the spatial distribution of NH_3 sources in relation to their δ^{15}N signal.

Acknowledgments

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Nitrogen deposition effects on mosses: A global meta-analysis

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Mosses are critical components of many ecosystems worldwide, including boreal forests, peatlands, and drylands, where they contribute to C fixation and storage, biogeochemical cycling, and soil stabilization. Mosses are also an important component of food webs in many ecosystems. Mosses are good bioindicators of atmospheric pollution, including N deposition, as they can absorb nutrients (and pollutants) through their entire surface. Moreover, cumulative experimental evidence from N addition studies suggests a short- to mid-term acceleration of moss metabolism and reduced moss abundance and vitality in the long-term due to N toxicity, nutritional imbalances, competition with vascular plants, and faster senescence. However, it is not clear how consistent these responses are globally or under what specific environmental conditions they manifest more strongly. This is due to the current lack of meta-analytical studies on this topic. In this study, we gathered information from 153 manipulative studies and carried out a meta-analysis of N addition experiments. We also obtained information regarding soil properties, bioclimatic variables (WorldClim) and background N deposition values (Dentener model) for each experimental site and used structural equation modelling to evaluate how these variables can modulate the response of mosses to N deposition at the global scale. Preliminary results suggest that N addition consistently reduces variables associated with moss abundance while enhancing physiological variables, possibly indicative of accelerated metabolism. Further analyses will clarify the role of environmental variables in the response of mosses to N addition, which will help to understand how climatic conditions and soil properties may affect our ability to accurately predict atmospheric N deposition based on variables measured from mosses collected under field, unmanipulated conditions.

Issues for conservation of sensitive macrolichens threatened by air pollution and intensive forest management in the Mediterranean area: the case of *Lobaria pulmonaria* (L.) Hoffm

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Atmospheric pollution and intensive forest management may heavily affect epiphytic cryptogams, in particular rare lichen species. The macrolichen *Lobaria pulmonaria* (L.) Hoffm. is such an example: an endangered forest lichen considered as a "flag" indicator species of forest ecosystems with long ecological continuity worthy of conservation, very sensitive to air pollution, red-listed and legally protected in several European countries, but generally not in Mediterranean ones. The species has been declining throughout Europe as a consequence of air pollution and forest management (including habitat fragmentation), whose effects are expected to be further exacerbated by climate change, especially in the Mediterranean area. An ecologically sustainable forest management should take into account the survival of sensitive cryptogams with long generation times, and hence, ensure the overall protection of the ecosystems. Case studies on forest logging for timber within Mediterranean oak forests of central Italy, which damaged large populations of *L. pulmonaria*, offered the opportunity to 1) quantify the impact of logging on the model species within Mediterranean mixed oak forests; 2) test whether logging operations affect the vitality of individuals growing on retained-isolated trees and forest patches; 3) provide issues on species conservation in Mediterranean oak forests and an overview of the status of *L. pulmonaria* (inclusion in red lists and legal protection) in Europe; 4) assess whether transplantations of *L. pulmonaria* thalli to remote or protected areas ensure their effective survival; 5) assess to which extent recent atmospheric pollution still limits the survival of the species. Our research outlines the utmost importance of ensuring the safeguard of forest ecosystems hosting fertile populations of this model species, especially in the case of unprotected forests.

Nature Based Solutions to optimize the provision of Ecosystem Services to create more liveable and resilient cities

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Life in cities in Mediterranean areas is challenged by multiple environmental issues, such as atmospheric pollution and the urban heat-island effect. At the cE3c UrbanL@b and framed by the UN Habitat III New Urban Agenda, we pursue several lines of research that aim ultimately at increasing sustainability in the long term. More specifically, we develop and quantify in multiple urban areas socio-ecological metrics that respond to global change variables, disseminate the knowledge to citizens and support the planning and management of cities blue and green and blue infrastructure.

To create more liveable cities our actions aim at improving human well-being using nature-based solutions and the services provided by them. Ecosystem services are supported by biodiversity present in cities ecosystem, which in turn is affected by the environmental challenges in cities. Thus, we provided evidences how multiple taxa are affected by green areas fragmentation, and how this is related with the density of nearby urbanization. Using ecological indicators, we modelled how green areas size affects the provision of ecosystem services such as air purification and microclimate regulation. This was also quantified using physical measures of pollutants and microclimate. The unequal distribution of green areas in the cities and its importance to human well-being was made evident by people preferences regarding the use of cities green infrastructure, and how this influences their movements across the city to visit green areas. The provision of multiple ecosystem services was then mapped with very high spatial resolution within a large green area, providing clear evidences of the trade-offs that occur and the consequences of management and planning options in the provision of ecosystem services.

Assessment of plant species performance along an ammonia gradient

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Pollutants based on nitrogen (N) are strong impact drivers of ecosystem change. The rate of growth of the release of these pollutants to the environment has already exceeded the safe planetary boundaries for Earth. Air pollution by ammonia (NH3) is one of those strong reactive nitrogen (Nr) drivers with impacts on biodiversity that modify the structure and function of ecosystems.
Ammonia can either cause communities shifts (replacing sensitive species with tolerant ones) or cause an overall species loss. For either case, the effects of NH_3 are dealt at the individuals level. Tolerant individuals will perform a physiological adjustment and maintain a viable population in the community while sensitive organisms will not tolerate and start to fade and disappear from the community. Tolerance versus sensitivity depends on species physiological limits to ammonia. To explore the relation between these physiological limits and species maintenance, we studied the cover and physiological status of plant species along an ammonia concentration gradient, in a manipulative experiment of NH_3 addition.

The ecological niche (realised niche) of the community's dominant species was evaluated along a known artificial gradient of NH_3, by measuring species percent cover in transects perpendicular to the NH_3 gradient. Then, the physiological status of those species in relation to the distance of the ammonia source was evaluated using spectrometric measurements (leaf reflectance) and chlorophyll fluorescence. The scan of leaf reflectance in the visible/near infrared region (310-1100 nm) allows the calculation of a series of physiological indices related to plant chlorophyll content, nitrogen, water, stress, etc., complemented by chlorophyll fluorescence induction kinetics. This assessment of plant performance provides the basis for determining the species' physiological niche (fundamental). By modelling the results of the ecological and physiological performances of the dominant species in the community along the ammonia pollution gradient, we expect to determine some effect thresholds, and also species substitution dynamics related to tolerance and competition. For example, it is hypothesized that a plant may not always be at its best physiological performance where its species has its best ecological performance. Using this information, it could be possible to model the response of species to ammonia and to anticipate the consequence of this pollution to communities' composition in affected areas.

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MOTTLES: a new-generation long-term monitoring stations across Europe for forest protection against surface ozone

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Epidemiology is the study of how often and why diseases, injury and other health-related events occur in a defined population, in order to identify risk factors and targets for prevention and control programs. The impact of surface ozone (O_3) on vegetation is under-investigated at regional scale despite worldwide huge areas are exposed to high surface O_3 levels and its concentration is expected to increase in the next future. Epidemiology of O_3 injury may be very helpful in particular when forests are investigated, as large trees require expensive experimental facilities for realistic O_3 simulation and a few individuals can be usually investigated.
A standard for forest protection is considered biologically relevant when it translates into real-world forest impacts. For this reason, epidemiological investigations where large-scale biological responses are compared with ambient data in the field provide useful information for establishing the best standards and thresholds for forest protection from O_3. The majority of previous epidemiological assessments used ambient O_3 exposure as a metric of injury (e.g. AOT40). However, exposure-based standards for protecting vegetation are not representative of actual field conditions. Ozone effects on vegetation depend on the air concentrations but also on the O_3 uptake through the stomata, i.e. Phytotoxic Ozone Dose above a threshold Y of uptake (PODY).

Unique in the world, the project MOTTLES "*MOnitoring ozone injury for seTTing new critical LEvelS*" (LIFE15 ENV/IT/183) takes place in the main European areas at highest and medium risk of O_3 injury, i.e. Southern and central Europe, and combines field epidemiology with plant responses to O_3. With the effort of implementing permanent new-generation monitoring stations across Europe, capable to return continuous hourly O_3 concentrations from active monitoring as well as real-time meteorological and environmental parameters (e.g. soil water content, solar radiation, air temperature, relative humidity), PODY will be estimated and correlated to plant responses to O_3 (e.g radial growth, crown defoliation, crown discoloration and visible foliar O_3 injury). Based on real-world flux-effect relationships, derivation of suitable epidemiologically-based O_3 critical levels for European tree species represents a considerable progress in the development of a long-term monitoring strategy and methods for quantifying O_3 effects on vegetation at the regional scale.

Keywords: Critical levels, Epidemiology, Ozone, POD, Visible injury

Physiological evaluation of apricot (*Prunus armeniaca* L.) leaves to air pollution for biomonitoring of atmospheric quality

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Air pollution is one of the severe problems facing the world today due to the increasing levels of some gaseous and dust particles in the environment resulting from man's activities such as road transportation and industries. Plants growing in polluted zone are constantly exposed to different pollutants. Plants can be taken pollutants (i) indirectly from soil by absorption via the roots and/or (ii) directly from the air via the leaves. These pollutants were considered as strong phytotoxic pollutants, which has adverse effects on the morphological, biochemical and physiological comportment of plants, such as alterations of nutritional status, inhibition of photosynthetic capacity and water relations. Nowadays, the Sfax region accommodates one of the most important industrial complexes, among which the lead smelter and phosphate fertilizer factory constitute the main pollution source. Fluoride (F) and heavy metals (particularly Pb and Cd) are among the most phytotoxic air pollutants emitted from these industries. Response of plants species to air pollutants is variable. Certain plant species are very sensitive to these pollutants resulting in well visible and measurable symptoms. Morphological damage is generally visible through lesions on the aerial parts while biochemical and physiological changes, which are invisible, can be measured and quantified. This study was designed to investigate the biochemical and physiological biomarkers of apricot (*Prunus armeniaca* L.) grown in polluted site of Sfax and exposed to air pollution. In comparison to unpolluted site, it was observed that lipid peroxidation level was increased in apricot leaf tissues grown in polluted site. Whereas photosynthetic capacity (Net photosynthesis, stomatal conductance, transpiration rate, total chlorophyll and carotenoids) and osmotic regulator (proline and soluble sugars) levels were decreased. These symptoms in *P. armeniaca* leaves can be used as indicators of air pollution stress for its early diagnosis and can be used as markers for a particular physiological disorder.

Abstracts
Poster presentations

Nitrogen transformations in the canopy: Exploring the role of phyllosphere microbes in a Mediterranean holm oak forest

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Forest canopies are very effective at capturing atmospheric pollution owing to their irregular and rough surface. Thus, element fluxes in throughfall (water collected below the canopy) are generally enriched relative to fluxes in the open (wet deposition). For most elements, the difference between them can be taken to represent dry deposition of gases and aerosols to the forest. For nitrogen compounds, however, this simple approach does not hold, since different processes come into play in the canopy (e.g. atmospheric dry deposition, leaf, bark and epiphyte uptake, and microbial transformations).
In this work, we used a chemical approach by measuring the N fluxes in throughfall and wet deposition and the $\delta^{18}O$ and $\delta^{17}O$ in nitrate in both fluxes. We then used real time PCR to quantify functional genes (e.g. the amoA in Archea, associated to nitrification, in the phyllosphere, throughfall and wet deposition) to explore the role of microbial transformations on the tree canopy. The study was conducted in a Mediterranean forest near Barcelona in the transition from dry summer (August) to wet winter (December) conditions.

Overall nitrate fluxes were greater in throughfall than in wet deposition. We found isotopic evidence of biologically derived nitrate in holm oak canopy only in August (about 20%), after a significant drought, while atmospheric deposition was the dominant source of nitrate through September to December. This seasonal partitioning between biologically and atmospherically derived nitrate in throughfall inferred from oxygen isotope data was also reflected in the temporal trend of amoA gene copies. Indeed, Archea nitrification was highest in August and tended to decrease from summer to winter. To our knowledge, this is the first study providing evidence of canopy nitrification for a Mediterranean forest and climate by using two independent, but complementary, approaches.

Current crop breeding drivers in Spain can compromise bread wheat tolerance to tropospheric ozone pollution

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Wheat is considered one of the most sensitive crops to increasing levels of tropospheric ozone (O3). The exposure and dose-response functions developed for this crop are currently used for assessing the risk of O3 effects on agricultural production at the European scale. However, these functions are based on sensitive cultivars from Central and Northern Europe growing under Atlantic and Continental climatic conditions and have never been tested with Mediterranean bread wheat cultivars growing under southern European climatic conditions.
New Open-Top Chamber (OTC) experiments have been performed to determine the sensitivity to O_3 of the most common wheat cultivars in Spain in comparison with old cultivars (used during the XX century), and to evaluate how water availability affects their response to O_3. The relative O_3-sensitivity of 12 Spanish bread wheat varieties, from landraces to modern commercial cultivars, was tested in 2015. Plants were exposed to four O_3 treatments, from lower-than-ambient concentrations to exposures similar to the highest hourly values registered currently in rural areas of Spain. Treatment effects on grain yield and grain protein yield as well as on physiological parameters such as gas exchange parameters, water use efficiency (WUE), chlorophyll content and relative water content (RWC) were measured.

None of the landrace cultivars showed an O_3 induced reduction in grain yield whereas increasing O_3 levels negatively affected the yield of most of the commercial varieties. The grain protein concentration of modern varieties increased under elevated O_3 but the resulting grain protein yield per hectare was lower, compared to the control treatment. Again, no O_3 effect was found on landrace cultivars. RWC and the chlorophyll content were significantly reduced by O_3, especially in modern varieties; similarly, modern varieties exposed to the pollutant reduced their WUE, potentially compromising their availability to cope with water stress.

The study suggests that: i) breeding drivers of modern bread wheat varieties have resulted in plants more susceptible to O_3 damage compared to landraces; ii) drought effects on grain yield under O_3 pollution can be higher than expected due to indirect impacts of O_3 on WUE; iii) future Global Change scenarios with combined water and ozone stresses will further reduce wheat yields derived from breeding activity can be more affected than local landraces in future scenarios of Global Change, where increased levels of tropospheric O_3 are expected.

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Investigating *Racomitrium lanuginosum* tissue nutrient composition in response to changing nutrient supply

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Racomitrium heath is the UK's most extensive near-natural alpine habitat, however, quality and extent of this montane moss heath have been in decline, which has been partially linked to atmospheric nitrogen deposition. We investigated the tissue nutrient composition of the moss *Racomitrium lanuginosum* in response to the development of N deposition in Scotland, UK. Herbarium samples (1989) and fresh samples (2006) collected at the same Scottish mountain sites (ranging in nitrogen deposition from 9.4 – 19.2 kg N ha^{-1} yr^{-1}), were analysed for nitrogen (N), phosphorus (P) and N:P ratio. As a side project, nutrient and vegetation composition analyses were carried out for *Racomitrium* growing on a mountain site influenced by the addition of human ashes. No significant change over the past 17 years in tissue N was detected. N:P decreased but were still within a range indicating P limitation. N values of the moss reflected N deposition patterns at the different sites, indicating its value as a biomonitor. Foliar N increased 0.16 mg g^{-1} for each 1 kg ha^{-1} yr^{-1} increase in N deposition. The lack of change in *Racomitrium* tissue N suggests that although N emissions have decreased slightly, N deposition had not decreased enough over the 17 years to manifest itself in tissue chemistry analysis. N deposition often has persistent effects, especially in habitats sensitive to N which can have slow recovery rates. Using herbarium samples in this study to compare historical and past tissue chemistry proved a reliable method, as re-analysed 1989 sample values for N coincided with values for N given by the original collector. *Racomitrium* growing on a site influenced by the addition of human ashes showed significantly lower N:P values compared to a control, along with higher *Racomitrium* cover. As a result of these findings we suggest that atmospheric N inputs to montane moss heath over decades has caused the system to shift from N to P limitation and that P addition may be a conservation management tool for the most degraded sites of this habitat. This study demonstrates that using a moss as biomonitor can help us to develop hypotheses for experimental studies to understand ecophysiological responses to nutrient imbalances and thereby to interpret and predict external drivers of ecosystem change.

Sensitivity of Iberian mountain-top pasture communities to air pollution and climate change

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Pasture communities of Iberian mountain-tops constitute valuable ecosystems, rich in endemic and endangered species that are often protected natural reserves throughout Spain. These ecosystems are threatened not only by climate-change induced increases in temperature and variations in precipitation, but also by increasing background atmospheric pollution. Risk assessment studies showed that mountain-tops are frequently exposed to tropospheric ozone (O_3) concentrations above air quality standards for the protection of vegetation (Elvira et al., 2016, Environ. Monit. Assess. 188). Modelled atmospheric nitrogen (N) deposition levels are also above empirical critical loads (CLo) defined for comparable ecosystems (García-Gómez et al., 2014, Sci. Tot. Env. 485-486; García-Gómez et al., 2017, Ecosistemas 26(1)). However, the sensitivity of Iberian mountain-top pasture species to air pollution has not been determined experimentally nor the interactive effects between climate change and air pollution that can affect their conservation status.
A new research initiative is being developed to experimentally define the sensitivity of these communities to O_3 and atmospheric N deposition and how air pollution effects can influence climate change impacts in those areas. A set of open-top chamber experiments has been established to screen the sensitivity of representative species to air pollution. Plants will be exposed to above and below ambient O_3 concentrations and to a range of N fertilization treatments in order to establish dose-response relationships that will be used for refined risk assessment exercises. Plants will be germinated from seeds originated in natural populations that will be collected in collaboration with the Germplasm Bank of Universidad Rey Juan Carlos (Madrid), serving the double goal of providing plant material for the experiment and enlarging the Bank collection. Endangered species will be managed following the Nagoya Protocol. The experimental study will be complemented by on-site monitoring of atmospheric pollution and N deposition at Sierra de Guadarrama (at 1850 and 2200 m.a.s.l.) and physiological parameters measured in natural populations, needed to establish the potential sensitivity of natural populations to combined air pollution and climate change stresses.

This information will help to refine current risk assessments of air pollution impacts in these remote areas and to understand how air pollution maybe affecting the resilience of these communities to foreseen climatic changes. The results will contribute to the definition of CLo and critical levels that are being used for developing air pollution policies under the Convention on Long-Range Transboundary Air Pollution, inform managers of protected natural areas about the risks induced by air pollution in combination with climate change for the conservation of ecosystems and to raise awareness in the general population about the impact that their everyday life activity has on the air quality of natural areas far away from emission sources.

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Effect of nitrogen deposition on the abundance and metabolism of lichens: A global meta-analysis

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Lichens are key to nutrient cycling and trophic networks in many terrestrial ecosystems and are very good bioindicators of air pollution, particularly of nitrogen (N) deposition. Experimental studies have shown that N addition can reduce lichen abundance and alter their physiology (i.e., metabolism) but we currently lack information about how widespread this effect is and what are the environmental factors (including climate, soil properties and vegetation type) modulating their response to N at global scale. In this study, we carried out a meta-analysis about the effects of experimental N fertilization on lichen abundance and physiology. We found 33 studies from 30 experimental sites that met our search criteria. These studies show that the addition of N accelerates lichen metabolism in the short term, which causes the senescence of lichens and, consequently, a decrease in their abundance in the medium to long term. Terrestrial chlorolichens from regions with high precipitation and isothermality and with a background deposition of mixed origin (i.e., industrial and agricultural) were the most affected by N, both in terms of abundance and metabolism. Structural equation modelling showed that the rate of N addition was the main factor modulating the response of lichens to N in terms of physiology, whereas isothermality played a very important role modulating the lichen response to N in terms of abundance (i.e., greater negative N effects in locations where isothermality is high). Our study suggests that N deposition effects on lichen abundance and metabolism are widespread and that climatic changes (e.g., changes in seasonal temperature and altered precipitations) will affect the way in which lichens respond to N.

Total N and C contents and stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) in moss tissue at a European scale: a preliminary insight into spatial distribution patterns and feasibility of isotopic signatures as indicators of pollution sources and environmental conditions

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Tissue N accumulation has been proven to be a good marker of increasing N deposition. However, this measurement does not offer additional data about the origin of pollution. In this respect, the analysis of the N isotopic ratios might be a helpful tool in providing supplementary information about the nature of the nitrogenous species in biomonitoring surveys. Furthermore, isotopic signatures have been extensively used in the study of N and C biogeochemical cycles. The main purpose of this study was to determine N and C elemental contents and their stable isotopes in mosses to investigate atmospheric pollution patterns across Europe. We aimed at identifying the main N polluted areas and evaluating the potential use of isotopic signatures in the attribution of pollution sources at a regional scale. With these objectives in mind, more than 1300 samples from 15 countries from Europe, all of them participants of the ICP-Vegetation programme 2005-2006, were analyzed for their C and N content and $\delta^{15}N$ and $\delta^{13}C$. The results were compared to those from EMEP (modeled deposition and emission data) and the most frequent land uses in the sampling sites (CORINE Land Cover 2006). The preliminary evaluation of these data suggested that additional measurements of C and N stable isotopes in mosses might be a promising tool in European surveys, not only in providing useful information for identifying likely pollution sources, but also as biological markers of key environmental processes.

Atmospheric ammonia concentration modulates soil enzyme and microbial activity having a detrimental impact on soil microbial biomass

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The present study was conducted along a NH_3 gradient in a *Q. pubescens* Milld. The aim of the work was to assess the effect of atmospheric ammonia (NH_3) on soil physicochemical properties, soil enzymatic activities (β-glucosidase -β-GLU-, nitrate reductase –NR-, urease –UR-, protease –PRO-, acid phosphatase –PHO-, dehydrogenase –DHA-), soil microbial biomass and soil respiration. N saturation was detected up to 330 m from the farms due to NHy (NHy: NH_3 and NH_4^+) deposition. As a result, soil C:N decreased an soil nitrification processes increased leading to an accumulation of the heavy N isotope (^{15}N) in the soil. N saturation was also reflected in the activity of NR enzyme, which was inhibited. On the other hand, while PRO activity was stimulated by the presence of organic nitrogen compounds and the need of soil organisms to meet the C demand, UR enzyme was inhibited close to the farms possibly due to the high amount of N-NH_4^+ resulting from the hydrolysis of NH_3. Additionally, the relative amount of C and P that organisms need regulated the activity of PHO and β-GLU enzymes. Finally, enhanced NH_3 reduced soil microbial biomass and biomass respiratory efficiency.

Hardly detectable effects of N depositions on the productivity of a Mediterranean ecosystem: what are we missing and where should we look?

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Increasing atmospheric nitrogen (N) depositions are a major concern for biodiversity, ecosystem functioning and services. Past studies have mainly focused on the effects of N deposition in temperate biomes, but lately, research articles in arid or semi-arid ecosystem are increasing, including Mediterranean ecosystems, where N inputs are expected to increase from the 7 kg N ha^{-1} yr^{-1} of mid-1990s to 12 kg N ha^{-1} yr^{-1} in 2050. The NitroMed network has the aim of monitoring general trends of the ecosystem services responding to increased N supply in natural and semi natural systems located in the Mediterranean Basin. NitroMed encompass three experimental sites located in Capo Caccia (Italy), Arrábida (Portugal) and El Regajal (Spain) and has committed to continue the N-manipulation for an undetermined number of years. Long-term experiments are of major importance in semi-arid ecosystems given that detecting the effects of N, especially on the carbon (C) cycle, are extremely challenging because of the low productivity of the sites. In Capo Caccia, after treating with 30 kg N ha^{-1} $year^{-1}$ for 6 years, N fertilization only altered the soil microbial community and decreased soil pH, whereas organic or inorganic C, total N, total P or C/N, and seven soil extracellular enzymatic activities were not affected. Importantly, N addition had no effect on litterfall, shoot elongation or litter decomposition suggesting that the effects of N on the C cycle are not induced by a higher above-ground productivity caused by a higher N availability. However, changes in the C cycle could be mediated by changes in soil biogeochemical process which may require a longer time to affect productivity. In Mediterranean ecosystems, the typically high seasonal and inter-annual climatic variability along with the expected changes in precipitation pattern, determines complex interactions with N availability, producing non-additive effects that are unpredictable from studies focusing only on N deposition. For this reason, we planned a new research line involving the whole NitroMed network, to test if a comprehensive set of soil biogeochemical processes (such as soil physical properties, micronutrients availability, soil C, N and phosphorous cycles, soil microbial activity and community) will be altered by increased frequency of drying–rewetting cycles, and if this effect will be modulated by soil N supply conditions.

Does duration matter? Photosynthesis reactivation in lichens and mosses after drought of different length

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Lichens and mosses are poikilohydric organisms unable to avoid desiccation. Their water content depends directly on the environmental availability of water and lichens and mosses spend their life switching between hydrated and desiccated status.
Their ability to survive with water content close to zero relies on physiological and molecular mechanisms that ensure cellular protection from desiccation-induced damage and cellular recovery and repair during rehydration. Dehydration speed was found to influence the capacity of mosses to recover after dehydration by affecting their proteins profile.
Prevision for 2100 suggest that occurring climate changes will cause an increase in main air temperature and a decrease in precipitation in the Mediterranean Basin region, with a global increase of drought periods. However, the effects of drought duration have been scarcely investigated so far.
In this work, we investigated the reactivation of photosynthetic activity in *Cladonia rangiformis*, *C. foliacea, Hypnum cupressiforme* and *Campylopus introflexus* after periods of drought of different length. This contributes to the knowledge of the effects of climate changes on sensitive ecosystems as arid and semi-arid ecosystems of the Mediterranean basin.

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Ozone risk assessment for forest trees as affected by soil water deficit and nutritional availability

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The level of tropospheric ozone (O_3) concentration has been risen since pre-industrial times in the northern hemisphere, and exceeds the level known to be toxic to forest trees. Ozone risk assessments for forest trees are therefore the crucial issues. The tree sensitivity to O_3 may be affected by other factors such as soil water availability and nutritional condition (excess nitrogen (N), and phosphorus (P)). However, studies on the interacting factorial impacts of O_3 and other stressors such as drought and nutrients on risk assessments are still limited. In this presentation, we would like to introduce our recent developments for the O_3 risk assessments on: 1) three kinds of European oaks (*Quercus ilex*, *Q. pubescens*, *Q. robur*) with different soil water availability, and 2) O_3-sensitive poplar clone (Oxford, *Populus maximoviczii* Henry × berolinensis Dippel) with different soil N and P conditions. We compared the concentration-based approach (i.e., AOT40, Accumulated Ozone exposure over a Threshold of 40 ppb) with the flux-based approach (i.e., PODy, Phytotoxic Ozone Dose). Studies were carried out in a last-generation O_3 Free Air Controlled Exposure (FACE) system.
Regarding 1), soil water availability can significantly affect O_3 risk assessment. In fact, flux-based approach explained better the dose-response relationships than exposure-indices when combining results in different water regimes. In a simplified approach where species were aggregated on the basis of their $_3$3 sensitivity, the best metric was POD0, with a critical level (CL) of 8.8 mmol m^{-2} for the less O_3 sensitive species *Q. ilex* and *Q. pubescens*, and of 5.0 mmol m^{-2} for the more O3 sensitive species *Q. robur*.

Regarding 2), the best O_3 metric for the risk assessment was POD4, suggesting that 4 nmol O_3 m^{-2} PLA s-1 may be an appropriate threshold for sensitive poplar clones to detoxify the O3-induced reactive oxygen species. If all nutritional treatments were pooled together, 4.9 mmol m^{-2} POD4 may be recommended as a CL. The addition of P or N, individually or in combination, increased the CLs for O_3 risk assessment i.e. reduced the sensitivity of poplar to O_3. This is because high nutritional availability enabled new leaf formation, which may partly compensate the productive decline in injured leaves by O_3.

In summary, to explain the O_3 dose-response relationship for forest trees in different soil water availability or nutritional condition, flux-based approach is better than exposure-indices. Although advances of exposure-based approaches still exist for their practicability of use, phytomedically relevant and mechanistic approach such as PODy is recommended for the risk assessment in future climate change.

Low P availability, but not water, limits the positive effects of elevated CO_2 on plant productivity: a glasshouse experiment with seed bank soil from Australian woodland

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Understanding ecosystem responses to rising atmospheric [CO_2] requires integrating the processes that regulate material and energy flows. Elevated levels of CO_2 directly increase leaf-level CO_2 uptake and water use efficiency (WUE), with potential positive feedbacks on plant productivity. However, productivity responses are usually controlled by the supply of belowground resources such as water and mineral nutrients. While water shortage may amplify the effects of CO_2 on productivity due to positive feedbacks from improved WUE, low nutrient supply$_2$ may increase the flux of C to belowground functions of soil forage, triggering soil processes that lead to greater efflux of CO_2 back to the atmosphere. Potential feedbacks of belowground strategies of nutrient and water uptake on net CO_2 exchange rates are unclear, particularly in P-limited soils. In this study, we grew a plant community from a native seed bank using soil collected from a P-limited eucalypt woodland to explore how the supply of P and water controls key functions related to the ecosystem'sability to store C (i.e. plant production, net CO_2 exchange rates and microbial activity in the rhizosphere) under elevated CO_2. We found positive effects of eCO_2 on plant productivity only under P fertilization, while water addition did not show interactive effects with CO_2. Elevated CO_2 did not result in greater investment in belowground functions of soil forage under any level of water and P, as suggested by the lack of interactive effects between CO_2 and water or P on root allocation and rhizosphere enzymatic activity. Interestingly, elevated CO_2 showed a direct effect on the net CO_2 exchange via a reduction of ecosystem level respiration instead of an enhancement on GPP. Our results clearly show a differential control of ecosystem functions by different resources, with P supply controlling plant productivity, water supply directly enhancing rhizosphere enzymatic activity and elevated CO_2 regulating CO_2 fluxes.

Short-term effects of N and P deposition on the soil nutritional status of four Iberian Alpine systems

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Increasing atmospheric nitrogen (N) deposition due to continued alteration of the global N cycle is one of the largest anthropogenic environmental disturbances. Nitrogen deposition is projected to double by 2050, and understanding how this global change driver alters ecosystem functioning has become an essential scientific topic. Nitrogen and phosphorus (P) are essential nutrients for plants and microorganisms that frequently limit primary production. However, in excess, they can also have negative consequences for the ecosystems (e.g. soil acidity and nutritional unbalance). Ecosystem functioning not only depend on the availability of these nutrients but also on their stoichiometry, as both nutrients are tightly linked through important biochemical processes such as ecosystem-atmosphere carbon exchange processes (e.g. photosynthesis and soil respiration). Therefore, N and P availability and relative abundance are among the main controls of terrestrial ecosystem functioning and biodiversity. We aim to study the effect of increasing atmospheric N and P deposition on the nutritional status of Iberian Alpine systems under both field conditions. To do so, we selected four Alpine systems in a latitudinal and aridity gradient (Las Cañadas del Teide, Sierra Nevada, Guadarrama and Picos de Europa) with likely differences in ambient N and P deposition rates. In 2017, we started a fertilization experiment in all of them simulating plausible future deposition scenarios. The fertilization experiment follows a factorial design with three levels of N (0, 10, 20 kg N ha^{-1} yr^{-1}) and two levels of P (0 y 10 kg P ha^{-1} yr^{-1}) fertilization, with 6 plots (replicates) per N and P level (36 plots per site). We collected soil samples before and 1 year after the beginning of the fertilization experiment to assess soil N and P pools, and estimated in-situ inorganic N and P production and availability by using ion exchange membranes (IEMs) in two contrasting microsites (open soil and under shrubs). Preliminary results show a fast fertilization effect on the production and availability of soil inorganic N and P, with significant differences among the different N and P levels barely 1 year after starting the experiment. However, different sites and microsites responded differently to the fertilization treatments, suggesting that an accurate assessment of the effects of atmospheric deposition on N and P cycles will require an explicit consideration of likely differences in previous biotic and/or biotic conditions at different spatial scales.

Effect of lead on four moss species with contrasted ecological affinities shoots and spores

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Mosses obtain most of their nutrients directly from atmospheric sources. As a consequence they are in direct contact with atmospheric pollution, and are regarded as a good proxy of its impact in the ecosystem health. In the past years, we have been studying 1) the effect of experimentally supplied lead on several moss species, 2) the resistance mechanisms they present, and 3) to what extent these mechanisms interfere in their biomonitoring usability.
In this study, we assess the sensitivity to lead in four species of mosses: *Hypnum cupressiforme*, *Homalothecium aureum*, *Ptychostomum capillare* and *Syntrichia ruralis.* All four are common in Central Spain, and representative of phylogenetically distant lineages. The first of them, and in a lesser degree, the second, have been used in several European biomonitoring programs, and are regarded as having medium-low sensitivity to atmospheric pollution. In contrast, *P. capillare* and *S. ruralis* are reported as pollution-resistant.
We tested 4 lead nitrate doses (0-10^{-3} M) in different conditions. First, moss tufts grown in greenhouse were experimentally sprayed with 3.5-4 ml/cm^2 twice a week for 3 months. In these samples we evaluated growth and observable damages. Second, spores and shoot tips were cultivated directly in Petri dishes in liquid MS ¼ with the corresponding lead nitrate dose, and kept in a culture chamber for 3-4 weeks. In the spore germination experiments, we observed 100 sporelings per Petri dish and registered their vitality and developmental stage. In the shoot tips cultures we assessed their survival and damage. In all cases, we established 6 replicates per experiment.

Our results show a high degree of resistance in all four species, as in this experimental period they show but little effect except for the highest dose (10^{-3} M). Even in this dose, we have observed some survival in the tufts of all the species (and sporophyte production in *S. ruralis*), and some survival *in vitro* of the shoot tip in *P. capillare*. Spore germination and protonemal development are observed in doses up to 10^{-4} M. In *S. ruralis*, this dose apparently enhances the protonemal growth.

We assess the effects of lead on the different developmental stages and we discuss both the mechanism of lead resistance in these species and its implications for their use in bioindication.

Impact of nitrogen deposition in rear-edge *Calluna* heathlands

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Ecosystems adapted to low nitrogen (N) conditions such as *Calluna*-heathlands are sensitive to enhanced atmospheric N deposition that affects many aspects of ecosystem structure and functioning. In the Iberian Peninsula, heathlands dominated by *Calluna vulgaris* are restricted to the Cantabrian Mountains (North-Western Spain), where they represent the southern-most distribution limit of this ecosystem type in Europe. These Cantabrian heathlands are considered as a biodiversity hotspot, hosting a wide variety of species, many of them being endemic. In the Cantabrian Mountains, we investigated the effects of five N treatments (0, 10, 20 and 50 kg N ha^{-1} yr^{-1} for 2 years; and 56 kg N ha^{-1} yr^{-1} for 10 years) on plant community composition, and the annual shoot length, flowering, N and P concentrations of *Calluna* plants. The study was carried out in several heathland patches dominated by the dwarf-shrub *Calluna vulgaris* in the southern slope of Cantabrian Mountains (Province of León, NW Spain), at two different heathland life-cycle stages (young/building-phase and mature-phase). Both *Calluna* shoot N and P concentratios significantly increased as a result of N fertilization, particularly under the N56 treatment. In addition, young *Calluna* plants had significantly higher shoot N and P concentrations than mature ones. *Calluna* vital rates (i.e., current year's shoot growth and flowering) were stimulated with increasing N loads, although shifts to decreasing trends were observed under chronic (10-year) N loads in young stands. In general, young stands displayed longer *Calluna* shoots than mature stands due to higher shoot *Calluna* nutrients concentrations and higher plant productivity rates in the building phase. There were no significant N-related changes in plant community composition. Non-vascular species such as bryophytes and lichens showed significantly decreased cover under chronic N inputs. At the same time, bryophytes were significantly more abundant at young heathlands, whereas lichens at mature ones. These results highlighted the impact of accumulated high N loads on the heathland vegetation structure. In order to preserve the biodiversity of montane heathland ecosystems, we proposed traditional management cycles by prescribed burning (20-30 years) to achieve rejuvenated vegetation stands.

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Suitable vegetation cover for Mediterranean green roofs under climate change effects

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In the last 10 years, there are more than 1000 studies about green roofs in urban environments. It is a logical step towards urban sustainability and resilience now and in the future. Their benefits are many and varied such as i) increase rainwater retention time and roof durability; ii) thermal regulation of buildings, mitigating heat loss during the winter; iii) improving the buildings' soundproofing and the urban aesthetic and iv) help biodiversity conservation and v) improving air quality. However, most of these studies were carried out in temperate or humid climates, not in Mediterranean areas. The specific Mediterranean climatic conditions – temperate and rainy winters and dry and hot summers – are not favourable for green roofs due to water limitation in the summer. For this reason, the choice of the green roofs vegetation is crucial for the success of these infrastructures. A priori, plants with high tolerance to drought seem to be suitable for these studies but it is necessary to check how they respond to high temperatures and water scarcity. Therefore, our main aim was to find a suitable vegetation for green roofs in the Mediterranean climate. For that, temperature (ºC) and humidity (m^3 m^{-3}) were measured under a commonly Mediterranean moss species such as *Pleurochaete squarrosa*, two succulent *Sedum sp.* plants and on the substrate of a green roof installed in the Faculdade de Ciências of Universidade de Lisboa. The experiment lasted 4 months (from August to November) and the results showed that the maximum daily temperature was always higher under the moss. This is an expected result since in summer with absence of precipitation, as the moss is drier for a longer period, its colour turns from green to brown and changes its albedo to absorb more solar radiation. Likewise, the daily temperature range measured beneath the moss was higher than in *Sedum* sp. or substrate. On the other hand, humidity was lower under *P. squarrosa*, which also presented the lowest range of variation of all. This means that the small amount of water that reaches the moss is well absorbed and can be released in the form of humidity during drier periods. These findings confirm that the moss *P. squarrosa* are more tolerant to extreme climatic conditions but does not provide climatic regulation services.

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