UNIVERSITÀ CATTOLICA del Sacro Cuore
sede di Brescia

capermed

Comittee on Air Pollution Effects Research on Mediterranean Ecosystems

(E)merging directions on air pollution and climate change research in Mediterranean ecosystems

Tuesday 28th – Thursady 30th June 2016

Dipartimento di Matematica e Fisica "Niccolò Tartaglia"

Università Cattolica del Sacro Cuore

Brescia, Italy

Committee on Air Pollution Effects Research on Mediterranean Ecosystems

CAPERMED aims to bring together people working on air pollution in Mediterranean ecosystems in order to coordinate expertise for quantifying current impacts and identify future challenges.

Mediterranean ecosystems are coping with multiple stresses due to the ongoing climate change processes. The impacts on their stability should be better understood to develop suitable adaptation and mitigation strategies, both for managed and natural ecosystems.
The 2nd CAPERMED meeting will bring together scientists and stakeholders from the Mediterranean basin specifically interested in the effects of air pollution and climate change on Mediterranean ecosystems to share innovative ideas and research methodologies for a more sustainable future.
Main aim of the 2nd CAPERMED meeting is to set the basis of a proposal for a common funded research project

A **SPECIAL ISSUE of Environmental Science and Pollution Research (ESPR),** guest edited by G. Gerosa, R. Ochoa-Hueso, S. Munzi and R. Marzuoli, will host papers of the best CAPERMED contributes, both from oral and poster presentations.

The Scientific Committee:

- María Arróniz-Crespo, Universidad Politécnica de Madrid, Spain
- Giacomo Gerosa, Università Cattolica del S.C., Brescia, Italy
- Riccardo Marzuoli, Università Cattolica del S.C., Brescia, Italy
- Silvana Munzi, Universidade de Lisboa, Portugal
- Raúl Ochoa-Hueso, The University of Western Sydney, Australia

Local organizers:

- Giacomo Gerosa
- Angelo Finco
- Riccardo Marzuoli
- Erica Cabrioli
- Glauco Pentenero

Figure 1 – The OTC research facility of Curno (Bergamo) managed by Catholic University of Brescia

Agenda CAPER*med* 2016

Tuesday 28th June 2016

14:00 – 14:45	Introduction and Meeting aims (The organizing committee CAPER*med* 2016)
14:45 – 15:30	Plenary talk: **Jürg Fuhrer** (Agroscope, Bern, Switzerland) *Ozone and nitrogen – two threats to biodiversity and ecosystem processe*
15:30 – 16:15	Plenary talk: **Lucy Sheppard** (CEH, Edinburgh, UK) *How can we build on our strengths? Observations from CAPER*
16:15 – 16:45	Coffee break
16:45 – 17:05	R. Ochoa-Hueso (Western Sydney University, Sidney, Australia) *Non-linear disruption of ecological interactions in response to nitrogen deposition*
17:05 – 17:25	M. Lo Cascio et al. (University of Sassari, Sassari, Italy) *Nitrogen deposition impacts on microbial abundance and decomposition in three Mediterranean sites: a coordinated study using the NitroMed network*
17:25 – 17:45	J.A. Carreira et al. (University of Jaén, Jaén, Spain) *Combining landscape ecology, isotope chemistry and -omics to assess air pollution and climate change effects on circum-Mediterranean mountain conifer forests*
17:45 – 18:05	L. Cotrozzi et al. (University of Pisa, Pisa, Italy) *Spectroscopy: an efficacious approach for better understanding of stress responses of Mediterranean plants*
18:05 – 19:30	Interactive Poster session

Wednesday 29th June 2016

9:00 – 9:45	Plenary talk: **Lydia González** (Spanish Representative at SC5 - H2020 Programme Committee) *EC priorities under the SC5 and related programmes in H2020*
9:45 – 10:00	Plenary talk: **Elizabeth Borer** (University of Minnesota, USA) *Lessons learned from NutNet*
10:00 – 11:00	Plenary: Brainstorming about innovative ideas and research methodology on the CAPER*med* topics
10:00 – 11:30	Coffee break
11:30 – 13:00	Working group 1: CAPER*med* proposal for networking activities Working group 2: CAPER*med* proposal for a scientific project
13:00 – 14:30	LUNCH
14:30 – 16:30	Working group 1: CAPER*med* proposal for networking activities Working group 2: CAPER*med* proposal for a scientific project
16:30 – 17:00	Coffee break
17:00 – 19:30	Interactive Poster session
20:00	SOCIAL DINNER

Thursday 30th June 2016

9:00 – 10:30	Plenary session: reports from working groups
10:30 – 11:00	Coffee break
11:00 – 12:30	Plenary discussion
12:30 – 13:00	Conclusions and Best Poster Award
13:00 – 14:30	LUNCH
14:30	Optional field trip to Bosco Fontana and visit to Mantua city

Book of abstracts

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ORAL PRESENTATIONS

Oral presentation

Combining landscape ecology, isotope chemistry and -omics to assess air pollution and climate change effects on circum-Mediterranean mountain conifer forests

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Assessing the impact of air pollution and climate change on ecosystems is a challenging task. Field studies are typically confused by the concurrence of multiple stressors that show complex patterns of spatio-temporal variation. As a result, disentangling their partial contributions is obscure since the observed ecosystem response is the outcome of many interactive mechanisms operating across hierarchical levels, from the landscape to the molecular level. Moreover, current responses to those global change components are frequently constrained by legacy effects of past land-use changes. Fortunately, to cope with this challenge, new sensors, computational capacity and methodological approaches from the fields of environmental monitoring, advanced chemistry and molecular biology are now available and ready to be used in ecosystem studies under an integrative and collaborative frame. In this communication, we present the overall design and main observed trends from a research programme in which landscape analysis, forest and soil ecology, isotopic chemistry, dendrological, physiological, metabolomic, genomic and transcriptomic techniques are being integrated, to assess N deposition and climate change effects on western Mediterranean mountain conifer forests. At the landscape level, assessment of back trayectories of air masses, rainfall chemistry, ^{13}C-^{15}N in tree-rings and climatic trends allow to characterize temporal and geographic gradients of stressors. Additionally, natural abundance of ^{13}C-^{15}N in soil profiles further allow for inferences about differential past land-uses among regions (contribution of C3 or C4 plant inputs to the soil organic matter, overall openness of the N cycle). At the plot level, 15-N labelling manipulative experiments and vector analysis of soil eco-enzyme stoichiometric shifts reveals changes in biogeochemical fluxes and nutrient limitations. At the tree level, continuous monitoring of sap-flow and secondary growth, and metabolic profiles of plat tissues allow to draw a picture on the tree physiological responses. Finally, at the molecular level, genomic analysis of soil samples and transcriptomic analysis of plant tissues inform on shifts in soil microbial communities and in the capacity of the trees to cope with stress through differential gene expression.

Acknowledgements to MINECO-Spain, projects CGL2010-18976 and CGL2013-48843-C2-R

Oral presentation

Spectroscopy: an efficacious approach for better understanding of stress responses of Mediterranean plants

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Recent advances in methods utilizing hyperspectral data have made it possible to estimate a variety of plant traits based on foliar optical properties, including nutrients, structure, secondary metabolites and overall physiological status. Plant traits are modelled as a function of reflectance features of leaves in the visible (VIS), near-infrared (NIR) and short-wave infrared (SWIR) spectral regions. The model calibration is accomplished by pairing spectra with reliable trait measurements and then validated by comparing relationships between observed and predicted values of independent samples. Models can then be used to predict the variable of interest in unknown samples on the basis of their spectral signature alone. Importantly, measurements of leaf optical properties are rapid, non-destructive, and relatively inexpensive and allowing for an increase in the number of plants sampled over multiple time periods under different environmental conditions, compared with standard collection techniques.

Here we present the results of a case of study where we used reflectance spectroscopy to characterize variation in leaf water potential (Ψ_{LW}) of *Quercus oleoides* plants from four regions of Central America (Belize, Costa Rica, Honduras and Mexico) exposed to differential water availability, and to test the ability to predict pre-dawn (PD) Ψ_{LW} *a posteriori* (approximately 5 hrs after PD measurements). We built predictive models for Ψ_{LW} using partial least-squares regression and then assessed the phenotypic plasticity (PP) of multiple leaf functional traits and spectral indices related to plant physiological activity.

Reflectance spectroscopy successfully estimated variation in Ψ_{LW} [97% of the models produced a high R^2 (0.50-0.87), RMSE values (0.32-0.70 Mpa), %RMSE 8-20% of the data range, and 85% of the models showed minimal bias (-2.49-1.04)], as well as *a posteriori* predictions of PDΨ_{LW} [95% of the models having a R^2 ranging between 0.50 and 0.79, RMSE values (0.27-0.59 Mpa), %RMSE 9-20% of the data range, and 83% of the models showed minimal bias (-2.45-1.05)]. Spectroscopic predictions of PDΨ_{LW} responded to water availability treatments similarly as reference measurements of PDΨ_{LW}. Mexican plants showed the highest PP value (plasticity index, PI = 0.102), followed by trees from Costa Rica (PI = 0.093), Honduras (PI = 0.075) and Belize (PI = 0.059). PP was generally higher for morphological traits, followed by physiological and biochemical traits in all plants except in Belizeans, where PP was higher for physiological traits. These findings demonstrate a promising approach for evaluation of Mediterranean plant responses to environmental change by providing rapid and cost effective estimates of variation in foliar functional traits.

Acknowledgements

This study was supported by MIUR, Rome, project PRIN 2010-2011. "Planning the green city in the global change era: urban tree functions and suitability for predicted future climates (TreeCity)".

Oral presentation

Nitrogen deposition impacts on microbial abundance and decomposition in three Mediterranean sites: a coordinated study using the NitroMed network

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The establishment of networks of experimental sites is a crucial strategy to answer global ecological questions and correctly address environmental issues. However, Mediterranean and semi-arid ecosystems are underrepresented in global networks, including those focusing on the effects of nitrogen deposition on ecosystem structure and processes. NitroMed is a recent network composed of three nitrogen manipulation experimental sites: Arrábida (Lisbon, Portugal), El Regajal (Madrid, Spain) and Capo Caccia (Alghero, Italy). All three sites present similar characteristics: i) they are located in a Mediterranean biome with spontaneous scrub vegetation; ii) host long term experiments (more than 5 years); and iii) added nitrogen loads simulate future deposition scenarios in the Mediterranean Basin.
The main aim of NitroMed is to provide "field laboratories" to investigate the effects of nitrogen pollution and climate change on the structure and functioning of Mediterranean ecosystems sharing protocols, experimental design and, whenever possible, equipment. In this study, we used structural equation modeling (SEM) to understand the cause-effect mechanisms that determine changes in litter decomposition and stabilization rates using common substrates (the tea bag index) under different nitrogen loads. Our results suggest that nitrogen deposition increases soil nitrogen availability and reduces soil pH, which in turn has an effect on microbial community structure (lower fungi to bacteria ratio) and overall enzymatic activity, responsible for decomposition rates. Our findings also show how site-specific heterogeneity can greatly influence ecosystem processes, highlighting the importance to increase the number of experimental sites in the NitroMed network.

Oral presentation

Non-linear disruption of ecological interactions in response to nitrogen deposition

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Global environmental change (GEC) is affecting species interactions and causing a rapid decline in biodiversity. In this study, I present a new Ecosystem Disruption Index (EDI) to quantify the impacts of simulated nitrogen (N) deposition (0, 10, 20 and 50 kg N ha-1 yr-1 + 6-7 kg N ha-1 yr-1 background) on abiotic and biotic ecological interactions. This comparative index is based on pairwise linear and quadratic regression matrices. These matrices, calculated at the N treatment level, were constructed using a range of abiotic and biotic ecosystem constituents: soil pH, shrub cover, and the first component of several separate principal component analyses using soil fertility data (total carbon and N) and community data (annual plants; microorganisms; biocrusts; edaphic fauna) for a total of seven ecosystem constituents. Four years of N fertilization in a semiarid shrubland completely disrupted (112.4 ± 9.8 %) the network of ecological interactions, with a greater proportional increase in ecosystem disruption at low-N addition levels. Biotic interactions, particularly those involving microbes, shrubs and edaphic fauna, were more prone to be lost in response to N, whereas interactions involving soil properties were more resilient. In contrast, edaphic fauna was the only group directly affected by N addition, with mites and collembolans increasing their abundance with up to 20 kg N ha-1 yr-1 and then decreasing, which supports the idea of higher-trophic level organisms being more sensitive to disturbance due to more complex links with other ecosystem constituents. Future experimental studies evaluating the impacts of N deposition, and possibly other GEC drivers, on biodiversity and biotic and abiotic interactions may be able to explain results more effectively in the context of ecological networks as a key feature of ecosystem sensitivity.

POSTER PRESENTATIONS

Poster

Experimental comparison of two methods of ethylene di-urea application as to their efficacy to protect *Salix udensis* plants against O_3 stress

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Ethylene di-urea (EDU) is an antiozonant used not only in rubber industry but also as a protectant of plants against injury caused by tropospheric O_3 in a plethora of plant species. The experimental use of EDU as protectant of plants against ozone is currently more frequent than it was previously due to elevated O_3 concentrations in most parts of the Northern Hemisphere of Earth. EDU is included in studies dealing with the protection it offers to plants, but also in O_3-biomonitoring studies conducted under field conditions. However, one important question remains unanswered: Soil or foliar application is more appropriate? In order to answer this question, an experiment was established in the free-air-O_3 enrichment system of Sapporo Experimental Forest, in northern Japan. In spring 2014, rooted cuttings of willow (*Salix udensis*) were transplanted in 15 L pots, filled with a mixture (1:1) of commercial well-weathered volcanic ash soil and well-weathered pumice soils. Approximately five weeks later, soil of each pot was irrigated with 200 ml water solution of 0, 200 or 400 mg EDU L^{-1}; the irrigation was repeated every nine days. Eight days after the second EDU application, plants were exposed either to ambient O_3 or to O_3-enriched (targeted at 80 nmol mol^{-1} during daytime) atmospheres; the exposure lasted for approximately two months. The same protocol was followed in 2015 with new cuttings from the same source, but this time EDU was applied as a foliar spray. Comparison of the two methods showed that EDU applied to soil was not effective in protecting the plants against O_3 impact and the variability in the response variables was relatively high. In contrast, EDU applied as the foliar spray was sufficiently effective in protecting plants against O_3 impact, as it was evidenced by prevention of shoots' and roots' biomasses losses, by the prevention of average leaf mass and leaf size reduction and by specific leaf area increase; EDU concentration of 200 mg L^{-1} was enough to protect the plants against O_3.

Acknowledgements to the British Ecological Society for awarding a Training & Travel Grant to the senior author (E.A.) in order to present this study at the second meeting of CAPERmed "(E)merging directions on air pollution and climate change research in the Mediterranean ecosystems".

Poster

Atmospheric nitrogen deposition and ozone interactive effects in Mediterranean ecosystems

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The Mediterranean Basin is one of the Biodiversity Hotspots for conservation priorities. However, very little information is available on the threat that air pollution, and in particular reactive nitrogen (N), and its interaction with other important air pollutants such as ozone (O_3), can pose to biodiversity and ecosystem functioning. Atmospheric N deposition values in Spain are moderate (generally below 10 kg N ha^{-1} yr^{-1} with maximum values up to 25 kg N ha^{-1} yr^{-1}), but some evidences indicate that N enrichment is already occurring in some ecosystems. A first risk assessment has shown that N deposition exceeds empirical N critical loads in some protected areas of the Spanish Natura 2000 network. Furthermore, ozone quality standards for the protection of vegetation are frequently exceeded in Spain.
Broadleaf evergreen forests and "dehesas" are one of the most characteristic landscapes of the Mediterranean countries presenting an understory of annual pastures with high species richness. Atmospheric N deposition has been assessed in Holm oak (*Quercus ilex*) forests in Spain. Canopy uptake occurred during most part of the year. Nitrogen dry deposition represented up to 78% of total N deposition. Loss of NO_3^- in soil water was detected when atmospheric N inputs occurred in periods of low biological activity, highlighting the importance of synchronicity between N deposition and biological demand. Previous experiments with Holm oak seedlings showed that ozone affected plant physiology and biomass production. Also ozone reduced leaf N content. The combination of ozone and drought stress resulted in further decreases of biomass production but not in N content. New experiments were carried out to study the interactive effects of ozone and N fertilization on a simplified annual community. Ozone reduced the fertilization effect of higher N availability increasing N losses of the ecosystem. On the other hand nitrogen could partially counterbalance ozone effects when the levels of ozone were moderate. Significant interactive effects also occurred below ground affecting root nutrient content and root growth in some species. Understanding interactions among air pollutants and how these interactions are modified by climate is one of the main current challenges for Mediterranean ecosystems.

Poster

Throughfall nitrogen deposition and canopy exchange processes in holm-oak forests with a focus on its seasonal dynamics

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Quantifying total atmospheric deposition (wet, dry and occult deposition) to forests is a key issue in forest biogeochemistry. Throughfall measurements (collection of precipitation water that has passed through the canopy) have been frequently used to determine total deposition in forests. However, nitrogen compounds may suffer exchanges and transformations in the canopy so that throughfall measurements underestimate nitrogen deposition if canopy retention and uptake by foliage and transformation by canopy epiphytes are not taken into account. How to address the distinction between dry deposition and canopy exchange in net throughfall fluxes has been the subject of numerous studies in the last decades. To address this issue, the ICP-Forest has adopted a specific protocol to derive a Canopy Budget Model (CBM).
The Mediterranean climate in the Iberian Peninsula is highly seasonal, with a general pattern of wet springs and autumns and dry and hot summers. This marked seasonality may strongly affect these ecosystems, since the maximum potential N demand of plants during the warm season may coincide with dry conditions that may impede nutrient uptake. On the other hand, wet autumn and winters may make N available at a time of lower nutrient needs when N would not be retained, thus being lost from the ecosystem. Previous studies in the Iberian Peninsula forests seem to indicate a seasonal pattern in N net throughfall with negative values in spring-summer coinciding with the development of the new leaves. Here, we will use precipitation and throughfall data from 4 holm oak forests in the Iberian Peninsula to: 1) analyze the seasonal variation of throughfall and net throughfall deposition, and 2) compare canopy uptake and dry deposition estimates from the CBM in the dry *vs*. the wet season.

Acknowledgements to Spanish MINECO funded EDEN Project (CGL2009-13188-C03).

Poster

Biological effects of air pollution in the Italian "triangle of death" assessed using the liverwort *Lunularia cruciata*

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The liverwort *Lunularia cruciata*, known for being a pollution-tolerant species colonizing urban areas, was collected in the town of Acerra (South Italy), to investigate the biological effects of air pollution in one of the three vertexes of the so-called Italian "triangle of death". Acerra is located in one of most polluted areas of the Mediterranean ecosystem and is sadly known for the notable number of deaths by cancer, greatly exceeding the Italian average. The reason for this rise in mortality has to be searched in the high pollution level mostly arising from illegal waste disposal by criminal organizations. The results from Acerra were compared with those of *L. cruciata* collected in the city center of Naples and in a small rural town located far from local sources of air pollution (Riccia, Molise, South Italy). Biological effects of air pollution were investigated in terms of ultrastructural alterations, changes in physiological parameters and induction of phytochelatins, as well as heat shock proteins 70 (Hsp70s). At both Acerra and Naples cell ultrastructure was heavily modified and severe alterations were observed, especially in chloroplasts. Physiological parameters clearly indicated a reduced vitality, and a strong increase in phytochelatins and Hsp70s was detected.

Poster

The effect of nitrogen deposition on rosemary shrubs dynamics.

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The increased N deposition due to pollution is recognized as one of the major drivers of global change. However, the interactions of other nutrients in soil, such as phosphorus, with the N deposition, the ecosystem response has received not enough attention so far. We study the effects of increased N availability in a rosemary (*Rosmarinus officinalis* L.) shrubland, prospecting for the interactions with other nutrients in soils. **Methods**: Our work is conducted in experimental plots in the Natural Park of El Regajal - Mar de Ontígola, near Madrid (Spain), where NH_4NO_3 has been added at four rates (0, 10, 20, and 50 kg $N.ha^{-1}.y^{-1}$) since 2007, and KH_2PO_4 has been added at a rate of 13.73 kg $P.ha^{-1}.y^{-1}$ since 2015, alone or in combination with NH_4NO_3 (20 kg $N.ha^{-1}.y^{-1}$). First, we tracked rosemary cover evolution thorough the eight-year field N fertilization experiment. Second, we measure the annual growth of rosemary plants during the 2015 growing season. Finally, we examine the health condition (percentage of death or defoliated areas) of all rosemary plants in spring 2016. Analysis of soil elements concentrations were also conducted in different periods. Data are analysed by means of linear models, using the treatments as factors and the soil properties as (co-)variables. When necessary, we use AIC to select the best-fitting models. **Results**: Rosemary cover decreases at any level of N treatment but increases in the control level of the factor. We found no significant effect of N fertilization, but a significant effect of other soil elements, on rosemary growth: especially of P concentration, but also of [Ca], [Mn] or [Na] among others. N fertilization treatments harm the health status of plants and may cause plants death. **Conclusions**: N deposition in Madrid surroundings seems to be near its critical load. Current levels of N are no longer limiting, being P and other nutrients determinant for plants growth. Stressful N levels do not affect plant growth but it increases plant mortality and may revert succession.

Aknowledgment: This research was supported by grants financed by the Spanish *Ministerio de Economía y Competitividad* (CGL2013-44661-R), and Madrid Autonomous Community grant S2003/MAE-2719 REMEDINAL 3.

Poster

Testing approaches for calculating stomatal ozone fluxes from passive samplers

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Current ozone (O_3) levels are high enough to negatively affect vegetation and may become worse in the future. Ozone risk assessment has recently shifted from exposure-based to flux-based metrics. Modelling stomatal O_3 fluxes requires of hourly O_3 and meteorological data that are not always available. Large datasets of O_3 concentrations measured with passive samplers exist worldwide, providing usually weekly to monthly means. We tested the suitability of using aggregated data instead of hourly data for O_3 flux calculation, using 3 year time-series of O_3 data from 24 Spanish air quality stations. Four approaches and 3 different parameterizations were tested. Ozone mean values in combination with hourly meteorological data provided the most robust estimates of accumulated O_3 fluxes (Phytotoxic Ozone Dose with no threshold, POD0), with median errors due to aggregation close to 5%. A more complex method consisting of modelling the O_3 daily profile performed also well (median errors about 7%), but the sensitivity to the considered parameterization was higher. Using meteorological data as daily means, for concrete parameterizations, it might be possible to calculate O_3 fluxes taking into account the duration of the day in the calculations. Aggregations from 1 week to 1 month yielded similar errors, which is important in terms of cost-efficiency of the chosen passive sampler exposure periodicity. A major limitation of these approaches is that they are not suitable for high POD thresholds, and that accuracy of the measurements with passive samplers has to be strictly assured, in order to finally obtain acceptable errors. A combination of meteorological data and O_3 passive sampler measurements can be used to estimate O_3 fluxes at remote forest sites as a feasible tool for risk assessment.

Acknowledgements to projects DESESTRES (PROMETEOII/2014/038) and GEISpain (CGL2014-52838-C2-2-R).

Poster

Simulated atmospheric deposition of lead on three common moss species

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Bryophytes are known to be very sensitive to air pollution. However, their direct use to quantify heavy metal deposition in monitoring networks is still under discussion. Usually bryophytes linked to urban environments are considered as tolerant, while species unable to grow on urban environments are assumed to be sensitive, but direct studies on their sensitivity to specific pollutants are very scarce. In this project, we present a simulation of atmospheric lead deposition in three Mediterranean species of bryophytes of the family Pottiaceae: *Syntrichia ruralis*, *Tortula muralis* and *Tortula subulata*. The species were chosen to compare the effect of lead on a species classified as toxitolerant (*T. muralis*) and two others that are considered as sensitive (*S. ruralis* and *T. subulata*).

Different solutions of lead nitrate ($Pb(NO_3)_2$), with concentrations ranging from 0 to 10^{-3} M, have been prepared. Samples of all three species (6 replicas per species and lead dose) have been sprayed with each solution for 5 months, twice a week. We have measured gametophyte damage and growth (number of new shoots, and yellowing of stems and leaves); and recorded the success of sexual reproduction (development and number of sporophytes). We have also explored for these mosses a possible exclusion strategy against lead using histochemical analyses and SEM/EDX for lead detection in the tissues.

The maximum concentration of lead nitrate (10^{-3} M) showed a clear effect on all species, in contrast with the remaining doses, that showed little or no incidence on vegetative growth. It is remarkable the low incidence of damage even at doses as high as 10^{-4} M, close to the saturation level, showing the high tolerance to Pb of the three species. This suggests that the sensitivity of *S. ruralis* and *T. subulata* to urban environments does not depend on their lack of tolerance to lead pollution. On the other hand, the higher toxitolerance of *T. muralis* is also evidenced specifically for Pb.

Our observations are indicative of an exclusion strategy in these mosses. This would hamper the establishment of a linear relationship between concentrations in tissues and atmospheric deposition, and would compromise the use of these species as direct biomarkers. The occurrence of these mechanisms should be checked also in other mosses, especially those commonly used in biomonitoring networks.

Poster

Nitrogen supply modulates the effect of changes in drying-rewetting frequency on soil C and N cycling and greenhouse gas exchange

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Climate change and atmospheric nitrogen (N) deposition are two of the most important drivers of global change. However, the interactions of these drivers have not been well studied. We aimed to assess how the combined effect of soil N additions and more frequent soil drying-rewetting events affects carbon (C) and N cycling, soil-atmosphere greenhouse gas (GHG) exchange, and functional microbial diversity. We manipulated the frequency of soil drying-rewetting events in soils from ambient and N-treated plots in a temperate forest and calculated a resistance index to compare the response of the different treatments. Increases in drying-rewetting cycles led to reductions in soil NO_3^- levels, potential net nitrification rate and soil:atmosphere GHG exchange, but increases in NH_4^+ and total soil inorganic N levels. N-treated soils were more resistant to changes in the frequency of drying-rewetting cycles, and this resistance was stronger for C- than for N-related variables. Both the long-term N addition and the drying-rewetting treatment altered the functionality of the soil microbial population and its functional diversity. Our results suggest that increasing the frequency of drying-rewetting cycles can affect the ability of soil to cycle C and N and soil:atmosphere GHG exchange, and that the response to this increase will be modulated by soil N enrichment.

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Poster

Overview of three years of ozone flux measurements at Bosco Fontana site: flux partition and main meteorological drivers

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Ozone, water, carbon dioxide and energy fluxes were measured since June 2012 at the Bosco Fontana site, a 26 m tall mixed oak-hornbeam forest located in the Marmirolo in the middle of the Po valley in the northern Italy. The tower was 40 m tall and was equipped at the top with fast instrumentation suitable for eddy covariance measurements. Additional meteorological and gas concentration measurements were performed on the top, along and at the bottom of the tower.
The ecosystem behaved as a relevant sink for tropospheric ozone, both in summer and in winter. Total ozone fluxes were divided into a stomatal and a non stomatal part. Stomatal fluxes were estimated by deriving the bulk stomatal conductance and following the big-leaf approach based on the inversion of the Penmann Monteith equation, while the non stomatal deposition was obtained as a residual.
On annual base the most important sink for ozone was non-stomatal deposition, this was due to a relatively high deposition even in the dormant period of the vegetation with a significant ozone deposition (around 4 nmol O_3 m^{-2} s^{-1}) in December and January too. The most intense ozone deposition was observed in 2013 and 2014 summers with average daily maxima around 15 nmol O_3 m^{-2} s^{-1}.
In the active period of the vegetation the stomatal fraction was about half of the total deposition, never exceeding the 55 % of the total deposition and reaching also lower values between 30% and 40%.
Very different meteorological conditions were observed especially in summer, with contrasting hot and droughty periods and milder and rainier ones thus leading to different responses of the total, of the stomatal and of the non-stomatal deposition. The main meteorological drivers of each of these processes were investigated and will be showed as well as a general overview of three years and a half measurements at the Bosco Fontana site.

Poster

Above and below canopy ozone flux measurements at Bosco Fontana site. Implications for modelling and risk assessment

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In the framework of the intensive field campaign of the ECLAIRE project, in June and July 2012, ozone, sensible heat and momentum fluxes were measured at four different heights over a 26 m tall mixed oak-hornbeam forest, at the Bosco Fontana site, in the Po valley (I). At each level (41 m, 32 m, 24 m and 16 m) a sonic anemometer and a fast ozone analyser were mounted while ozone concentrations were measured by an absolute UV analyser.
Ozone concentrations were measured at 0.15 m by means of another UV photometer as well as other meteorological parameters were measured at the top of the tower and along the vertical profile.
Since fast ozone measurements were collected with different instruments, preliminary analysis (despiking, instantaneous rotations, WPL corrections, frequency loss corrections and calculation of the random error) were performed to properly compare the flux measurements.
Ozone deposition was almost constant until midday for the two upper levels while an enhancement of the fluxes was observed at 24 m. This fact was strictly linked with the in-canopy dynamics: a greater heating of the canopy was observed in the afternoon, leading to the formation of an inversion at this level. This inversion divided the in-canopy air volume into two layers: the lower one with a stable stratification and the upper one with a turbulent regime. These results are in contrast with the widely used "big-leaf approach, usually employed for the estimation of the ozone stomatal uptake by the studied ecosystem. This methodology states that the forest is equivalent to a big-leaf laying at d+z0', that is the height at which ozone concentration is supposed to be null and in our case should be around 19 m. Our results showed that at 16 m, the lowest flux level, ozone concentrations were almost equal to the levels above for the first part of the day and even at 0.15 m were substantially different from zero for large part of the day. Moreover the forest stomatal uptake seems to be overestimated by the big leaf model approach if compared with the whole canopy uptake obtained as a difference of flux measurements above and below canopy. This results highlight the need for new methodologies for the estimation of the ozone risk assessment, other approaches for estimating the stomatal ozone uptake will be showed too.

Poster

Effects of nitrogen deposition, drought and their interaction, on functional and structural traits of *Fraxinus ornus* L. and *Quercus ilex* L.

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To understand how ongoing global environmental change will affect Mediterranean forests, multiple stresses response should be taken into account. Water stress is an important driver of vegetation functionality in Mediterranean area affecting plants structural and functional traits. On the other hand, Mediterranean regions are heavily populated being thus characterized by many sources of pollutants due to anthropic activities. In this context increasing attention is given to anthropogenic perturbation of the nitrogen cycle that might jeopardize functionality of Mediterranean forest ecosystems. A controlled experiment was conducted in order to understand how nitrogen (N) addition (30 Kg ha yr^{-1}) bears upon structural and functional traits of species with different leaf habits (*Fraxinus ornus* and *Quercus ilex*) that have an extraordinary ecological role in the Mediterranean basin due to a wide natural distribution in the Mediterranean Basin, as well as a use in urban contexts. Moreover we explore the effect that nitrogen addiction has on the water stress response. The experiment was divided in two stages: stage I, nitrogen addition under well water condition; stage II, nitrogen addition under drought. Functionality of the photosynthetic machinery, growth and biomass partitioning were assessed. The response to N and stress interaction greatly differ between species, highlighting distinct strategy in the allocation pattern of N. In *F. ornus*, N content at leaf level increases, and invests resources on photosynthetic machinery, whereas *Q. ilex* keeps unchanged the N content at leaf level and stores N in non-photosynthetic biomass, increasing relative growth rate and biomass, resulting in different allometric ratio. This effect may had a role in water stress response. Stomatal conductance of *Q. ilex* treated with N and subjected to water stress is lower relative to drought treatment. On the contrary, *F. ornus* takes advantage of N addition that has ameliorative effects on its functionality when drought was imposed. The obtained results, highlighting response mechanisms to multiple stress factors, should help to assess the performance of forest ecosystem under the foreseen environmental changes.

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Poster

Increased nitrogen deposition enhances the negative effect of ozone on biomass of *Carpinus betulus* young trees

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A two-year Open-Top Chamber (OTC) experiment with young trees of *Carpinus betulus* exposed to different levels of ozone (O_3) and nitrogen deposition was performed in Curno (Northern Italy) for the FP7 Project ÉCLAIRE.
Plants were exposed in 12 OTCs to four levels of ozone (-40% of ambient ozone in charcoal-filtered OTCs, -5% in non-filtered OTCs, +30% and +75% in O_3 enriched OTCs) and two levels of nitrogen wet deposition (-N tap water, and +N tap water +70 Kg N ha^{-1} yr^{-1}). Stomatal conductance and A/Ci response curves were measured during the two experimental seasons, while at the end of the experiment plants dry biomass partition between root and stem was assessed.
Ozone alone (in -N treatment) caused a decline of maximum carboxylation rate allowed by Rubisco (V_{cmax}) at the beginning of the second experimental year. However this effect on V_{cmax} was completely lost during the season. In +N conditions on the contrary, the negative effect of ozone was still present (although not statistically significant) in September during the last sampling date.
The biomass data showed that after two years of treatments hornbeam plants resulted to be tolerant to O_3 when no nitrogen was added (-N treatment, +5% of total biomass on average), while a strong positive effect of nitrogen deposition on biomass production was found (+76% of the total biomass, +65% of roots biomass on average).
Phytotoxical ozone dose (POD_1) and the relative effects on biomass were calculated according to methodology provisionally suggested by the Mapping Manual for generic broadleaved deciduous species in Mediterranean Europe using two g_{max} values (for –N and +N plants, respectively) calculated from the g_s measurements performed during the experiment.
Dose-response relationships for root, stem and total biomass loss in –N and +N conditions were defined with linear regressions analysis, considering the mean values calculated for each OTC as single points of the regression.
The dose-response relationship for total dry biomass in –N conditions showed that ozone caused a slight increase in biomass (+3% every 10 mmol O_3 m^{-2}), but the linear regression was not statistically significant, probably due to high variability in the biomass data. On the contrary, in +N conditions the total biomass resulted greatly reduced by ozone with a -8.7% loss every 10 mmol O_3 m^{-2} ($p<0.05$).
These results highlight that increased nitrogen deposition could play a significant modifying role on hornbeam's susceptibility to ozone, triggering the detrimental effects of this pollutant on plants biomass and physiology.

Poster

Integrated eco-physiological response of the moss *Hypnum cupressiforme* Hedw. to increased ammonia concentrations

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Ammonia (NH_3) emissions are considered one of the major environmental problems related to atmospheric N pollution. Agricultural activities, especially animal production and volatilization from livestock excreta, are the main processes involved in the NH_3 release. Even though this gas is known to be linked to negative effects on ecosystems' health (nutritional imbalances, ecosystem eutrophication and acidification, loss of biodiversity, toxic effects, etc.), physiological responses to exposure to NH_3 at species level are still insufficiently quantified. In this sense, bryophytes are among the most vulnerable organisms to increased atmospheric N. The absence of a well-developed cuticle and the lack of a true root system are the main characteristics that make them especially sensitive to N deposition, and thus, good bioindicators of N pollution.

Bearing in mind this context, our study aimed at evaluating the physiological response of the pleurocarpous moss *Hypnum cupressiforme* Hedw. to an NH_3 concentration gradient from a multivariate, comprehensive and temporal perspective.

The experiment was conducted at the vicinity of a swine farm which has been operating for nearly 50 years. Thus, the observed changes derived from this continuous source of NH_3 should reflect the integrated effect of this contaminant at the ecosystem level. A total of 7 sites were seasonally surveyed along a gradient of NH_3 concentration from September 2013 to June 2014. In particular, moss apices were evaluated for 1) tissue chemistry (N, C, P, K, Na, Ca, Mg); 2) activity of N and P metabolic enzymes (Nitrate reductase (NR) and Phosphomonoesterase (PME)); 3) the status of the enzymatic antioxidant system (superoxide dismutase (SOD)); 4) possible deterioration of cell membranes (lipid peroxidation, malondialdehyde (MDA); membrane leakage); 5) photosynthetic and photoprotective pigment composition (Chlorophyll (Chl) a, b and carotenoids); 6) $^{15}N/^{14}N$ and $^{13}C/^{12}C$ fractionation and 7) accumulation of solutes (proteins).

Information obtained of the analysis of these data provided insight into the effects of enhanced NH_3 on moss physiology, showing which variables are the most responsive, and therefore, the most promising for being used as early warning indicators of NH_3 toxicity in ecosystem surveys. Moreover, the evaluation of temporal data demonstrated that not all variables were equally sensitive throughout the year, varying their response seasonally. These data are of primary importance when it comes to developing sampling protocols and conducting biomonitoring surveys based on the analysis of physiological parameters.

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Poster

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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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 Barcelona (Northeastern Spain), one in Madrid (central Spain) and the fourth in Navarra (Northern Spain). In BD the annual volume weighted mean (VWM) concentration of WSON 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, two important seasons for the biological cycle, showing that part of the organic fraction could be directly assimilated by trees. This fact suggests 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. These results may have significant implications when working with the critical load approach, given that the additional input of organic N, which is not include in the risk evaluation, may provide even greater pressures than predicted and may pose a threat to systems where Critical Load does not appear to be exceeded.

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Poster

The use of mosses as indicator of copper environmental contamination. A case study of *Ptychomitrium capillare*, a tolerant species.

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Plants and especially bryophytes have been frequently used in the assessment of environmental contamination. Heavy metals are among the most studied contaminants due their toxicity and persistence in the soil. However, it is unclear how these contaminants affect mosses. Besides, there is some controversy about the reliability of these organisms as heavy metal bioindicators, either direct or indirect.

This work aims to study the effect of heavy metals, copper in particular, on *Ptychostomum capillare*. We have explored its effects on moss populations, the alteration in some growth parameters and the absorption of this metal in the moss tissues in relation to environmental copper. In the mine tailings of an obsolete copper mine in Lozoyuela (Madrid), we have established nine plots (1 x 1 m) along a gradient, from very high to low concentrations of this metal. In each plot, we have recorded the abundance and size of the moss patches, the growth parameters and the observed damage. We used atomic absorption spectrometry to determine copper content in soil and moss samples, and histochemical tests and SEM/EDX for copper detection in the tissues.

High concentrations of total and extractable copper in soil positively affects the size of the populations, epiphytic algae invasion and the percentage of dead cells in the leaves. The elongation of the stems and the propagule production are adversely affected by soil contamination. We have observed stagnation in the protonematal stage in mosses from plots with higher copper concentrations. We discuss the possible exclusion mechanism that could explain the high tolerance to copper of this moss, and that could compromise its use in direct bioindication.

Poster

Pollution Characterization of Atmospheric Deposition for Assessing Air Quality in Albania by Using most Toxic Heavy Metal, Moss as Bioindicator, ICP/AES and ENAA Analysis

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Pollution characterization on atmospheric deposition through the most toxic trace elements (As, Cd, Cr, Co, Cu, Hg, Ni, Pb and Zn) for assesing air quality in whole territory of Albania by using moss biomonitoring survey (Hypnum cupresiforme and Pseudoschleropodim purum sp. collected from 44 sampling sites), ICP/AES and ENAA analysis is presented in this study. Screening ecological risk assessment of these trace metal is performed to atmospheric deposition refered to the methodology given by different authors and by using moss species as bioindicator. The model used for risk assessment was based on contamination factor (CF), pollution loads index (PLI) and the potential ecological index (RI). CF and PLI values show that the whole territory under investigation belongs to a moderately to highly pollution scale. RI values showing high ecological risk and the risk of human exposure to trace metals in areas with the highest concentrations of these metals particularly in central part of the country.

The Box-Cox transformation is used smoothing the variability of the data. Pearson correlation and factor analysis statistical methods are used to the transformed data to identify the most significant association of the elements and their probable sources of origin. The median values of Cr, Hg and Ni in moss samples of Albania are higher than the respective median values of Europe; the median values of Cu and Pb are more or less the same, and those of Cd and Zn in moss samples of Albania are lower than the respective median values of Europe. The values of CFs show the elements are associated with the C3 to C5 contamination scale, and show the moderately to severely contamination scale. Besides it the zone pollution index (PLIzone = 7.2 > 5) shows that the whole territory under investigation is extremely polluted. The ecological risk index values (RI>150) showe a moderately to highly pollution scale. The highest ecological risk and the risk of human exposure to trace metals might be expected in areas with the highest concentrations of these metals in mosses. The RI data showe the central part of Albania as an area at high ecological risk.

Three dominant factors were extracted that represent the atmospheric deposition of these elements associated with mineral and industrial local emission sources particularly for Cr, Ni and Co, and less with Cd, Cu, Pb, Hg and Zn; and the use of pesticides and herbicides in agriculture, particularly in the south part of the country.

Poster

Seed bank and seed germination are affected by Nitrogen deposition and different N forms

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Nitrogen (N) is an essential nutrient for living organisms and usually limits plant growth. Human activities have released high amounts of reactive N to the atmosphere that is deposited on natural and semi-natural ecosystems, altering the N cycle and affecting the vegetation. We aimed at testing if 8 years of realistic N addition affected the composition of the seed bank of a semiarid Mediterranean ecosystem (experiment one). We also evaluated the germination rate of selected species (*Asterolinon-linum-stellatum*, *Brachypodium phoenicoides*, *Diplotaxis siifolia*, *Iberis crenata*, and *Plantago lanceolata*) under different N forms and loads (experiment two). The first experiment was carried out with soils (0-4 cm depth) collected from a field experiment where six replicated plots have been treated with increasing N loads (0, 10, 20 and 50 Kg N ha^{-1} yr^{-1}) for 8 years; samples were incubated in a greenhouse under controlled conditions (temperature and RH) and the emerging seedlings were counted, and when possible, taxonomically determined during a four-month period. In the second experiment, seeds were subjected to: (1) increasing loads of ammonium nitrate (0, 1, 5 and 10 mg N kg $soil^{-1}$); (2) 5 mg N kg $soil^{-1}$ as ammonium nitrate, sodium nitrate or ammonium sulphate. In the experiment one, the number of different species germinated was 21, and the number of germinated plants recorded was 198, giving a mean seed density of 29,783 seeds m^{-2}. Adding 50 kg N/ha/yr resulted in a lower number of germinated seeds compared to control conditions, but this effect was soil fertility-dependent (i.e. organic matter, total soil P) (ANCOVA; $P < 0.05$). In the second experiment, *A. linum-stellatum* and *P. lanceolata* showed the highest germination rates. Responses to N form and load were species-specific, ranging from negative (all species under ammonium sulphate treatment) to neutral (*A. linum-stellatum*, *B. phoenicoides*, and *P.lanceolata*) and positive (*D. siifolia* under the ammonium nitrate treatment), although none of these species germinated under the addition of 10 ppm N or higher. The most negative effects were consistently associated with N as ammonium sulphate. The increasing threat of atmospheric N deposition, particularly in its reduced form, will affect plant germination and the composition of seed bank in natural and semi-natural ecosystems and thus will have consequences in terms of altered plant community composition.

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Poster

Contrasting effects of nitrogen deposition on soil respiration in two Mediterranean ecosystems

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Anthropogenic activities are dramatically altering the global carbon (C) and nitrogen (N) cycles. Increased atmospheric N deposition can alter ecosystem C source-sink dynamics, for example via changes in soil CO_2 fluxes (the second largest C flux in terrestrial ecosystems), and therefore feedback with climate change. A very limited number of experiments has been conducted to assess the effects of N addition in Mediterranean ecosystems and none of them has explored the effects of N addition on soil respiration (Rs). The main objective of this study was to assess the effects of N deposition on Rs dynamics. We hypothesized that Rs would be negatively influenced by N addition and that this effect would be modulated by the high spatial-temporal variability typical of Mediterranean ecosystems. These hypotheses were tested in two Mediterranean sites: Capo Caccia (Italy) and El Regajal (Spain). Capo Caccia is dominated by *Juniperus phoenicea* L. (53% cover on average) and other shrubs (27% cover), while the remaining 20% is soil covered with a well-developed lichen-dominated biological crust (hereafter biocrust). In this site, 8 plots were randomly established and two microsites were selected within each plot, one under *J. phoenicia* canopy and one in the interspace between shrubs. For three years, four plots were treated with 30 kg N ha^{-1} yr^{-1} and the other four were treated as controls. El Regajal is dominated by *Rosmarinus officinalis* L. and *Quercus coccifera* L. In this site, 24 plots were established following a 6-block design. Within each block, three plots were randomly selected and treated with 10, 20 or 50 kg N ha^{-1} y^{-1} for eight years and another plot was used as control. In both sites, Rs showed clear seasonal patterns, mainly driven by soil water content and soil temperature. In Capo Caccia, Rs was significantly increased by N addition in the bare soil microsite, possibly due to the higher sensitivity of the biocrust to N supply. In contrast, soils under the canopy did not respond to the treatment, which we attributed to the buffering effect of shrubs. In El Regajal, N addition significantly reduced Rs, which can be due to cumulative negative N effects. These contrasting results highlight the urgent need for further field studies to better understand how increased N deposition influences Rs in Mediterranean ecosystems.

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Poster

Effects of nitrogen deposition on soil collembolans from a semiarid Mediterranean shrubland

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Global environmental change, including nitrogen (N) deposition, is causing a rapid decline in biodiversity. However, very little is known on the consequences of increased N deposition on soil fauna and how this affects key ecosystem functions that depend on them (e.g., litter breakdown). In this work, we studied the impacts of 4 years of simulated N deposition (0, 10, 20, 50 kg N ha^{-1} $year^{-1}$ + 6-7 kg N ha^{-1} $year^{-1}$ background) on soil faunal populations, with special attention to Collembolans, in a semiarid Mediterranean ecosystem from central Spain (Nature Reserve El Regajal-Mar de Ontígola Natural Park). Each experimental treatment was replicated 6 times.

For soil faunal sampling and determinations, soil surface (0–10 cm) samples were collected from each plot in October 2011 (~250 ml of composite sample) using a garden trowel. Once in the lab, soil samples were stored at 4 °C until processing. Individuals present in 250 ml of soil per plot were then extracted over a 2-week period using Berlese-Tullgren funnels and preserved in 70% ethylic alcohol. Collembolans were identified by light microscopy to the species level.

The abundance of collembolans peaked with 20 kg N ha^{-1} $year^{-1}$ and then decreased; however, using multivariate analyses, we did not detect any change in the structure of the community, which was, in contrast, highly influenced by soil pH. This study suggests that N deposition effects on soil collembolan communities operate at the functional group level, whereas the individual species seem to respond to N in a more stochastic way.

Poster

Ozone dose-response relationships for durum wheat in Mediterranean conditions

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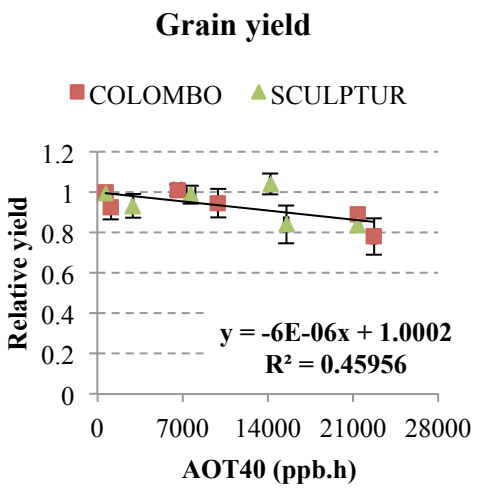
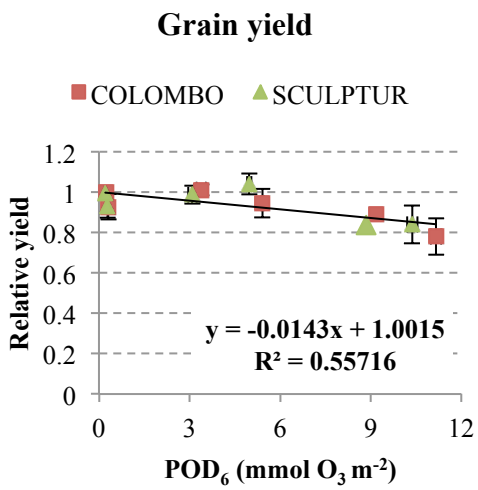
The aim of this study was to identify a critical level of ozone for the protection of *Triticum durum* in Mediterranean conditions. Colombo and Sculptur are two modern durum wheat cultivars that in previous studies proved to be very sensitive to ozone stress at both eco-physiological and agronomical level. Two consecutive experiments on these cultivars were carried out in 2013 and 2014 at the OTC facility of Curno (Northern Italy). Plants of durum wheat were subject to 2 and 4 different levels of ozone in 2013 and 2014, respectively. The seasonal accumulation of phytotoxic stomatal ozone dose (as POD_6) and ozone exposure (as AOT40) were correlated with the reduction of grain yield, total aboveground biomass, stems, number of spikes and hectolitre weight.
Colombo resulted more affected by ozone than Sculptur in both years of the experiments, with a significant decrease in yield and growth parameters. Sculptur showed significant negative effects only in the most ozonated treatments. Regression analysis on grain yield were performed using both the AOT40 and the POD_6, and the relative effects were calculated on the basis of the mean values of plants grown in Charcoal-Filtered OTC (-50% of ambient ozone).
According to this study the POD_6 value causing a 5% of relative grain yield was around 3 mmol O_3 m^{-2} for cv. Colombo and 4 mmol O_3 m^{-2} for cv. Sculptur. Considering the two cultivars together, we can propose a critical level of POD_6 of 3.5 mmol O_3 m^{-2} for a 5% reduction of grain yield to be used in the Mediterranean countries for *Triticum durum*. Analogously the AOT40 critical level could be set to 8'000 ppb.h. The critical level based on POD_6 is 3.5 times higher than that proposed in the Mapping Manual for *Triticum aestivum* (1 mmol O_3 m^{-2}).
Results of this study demonstrate clearly that both relationships based on the ozone exposure and phytotoxic ozone dose proposed in the Mapping Manual could overestimate the ozone effects on durum wheat under Mediterranean conditions.





Poster

Long-term measurements of atmospheric Nitrogen speciation in the Mediterranean: Importance for biogeochemical N cycle

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Water soluble organic nitrogen (WSON) was determined in aerosol samples collected from the Western Mediterranean (Ile Rousse-Corsica) for the period 2002-2012, as well as in wet deposition samples from the Eastern Mediterranean (Finokalia and Heraklion, Crete, 2007-2013). Average concentration of WSON was 4.41 ± 1.62 nmol/m^3 in aerosol samples where as in wet deposition was 33.9 μM and 25.0 μM for Finokalia and Heraklion respectively. In both regions WSON represents a significant fraction of total nitrogen (N), as its contribution reaches up to 30%. The main source of WSON in the atmosphere of both Western and Eastern Mediterranean is emissions related to NH_3.

Poster

N deposition effects on the contribution of the biological crust to the soil CO_2 efflux in a Mediterranean ecosystem

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Lately, the important role of the soil biological crust (hereafter biocrust) in Mediterranean ecosystems is emerging from a multitude of articles. It is becoming apparent that the biocrust has an important role in regulating ecosystem functions and that it interacts with the woody and herbaceous vegetation to a degree depending on the availability of water among other factors. Here we present the first results on the contribution of the biocrust to soil CO_2 efflux, and on how the respiration of the biocrust responds to soil water content, soil temperature, and N deposition. Within a nitrogen (N) deposition experiment in a Mediterranean shrubland ecosystem in Sardinia (Italy) a manipulative experiment was performed to assess the contribution of the biocrust to soil CO_2 efflux and to identify the main environmental drivers of the CO_2 efflux in this ecosystem. For 19 months, in situ soil CO_2 efflux was measured over three different surfaces: soil deprived of biocrust (hereafter Soil), biocrust (hereafter BC) and intact soil (hereafter Soil+BC). Each surface was repliclated three times for weach of the two N level treatments (0 and 30 kg ha^{-1} yr^{-1}). Results showed that N deposition has not altered the soil CO_2 efflux since the beginning of the N deposition experiment (2012). CO_2 effluxes emitted by Soil, BC and Soil+BC were differently driven by soil moisture and temperature: BC respiration was mainly controlled by soil moisture at 5 cm depth, whereas both soil temperature and water content at 20 cm depth determined Soil CO_2 efflux. Soil temperature and water content at 5 cm depth drove Soil+BC respiration. We also found that biocrust can contribute substantially (up to 60%) to the total soil respiration depending on its moisture content. This contribution persists even in periods in which deeper soil layers are inactive, as small water pulses can activate lichens, mosses and cyanobacteria associated to the biocrust as well as the metabolism of carbon in soils, while deeper soil layers remain dormant. The important differences observed in CO_2 efflux between Soil and Soil+BC suggest that projections on carbon budgets may underestimate soil CO_2 efflux in spatially heterogeneous Mediterranean areas. Thus, our results highlight the relevance of accounting for the biocrust contribution to soil respiration and its responses to environmental drivers. Under longer time spans, the N deposition experiment is likely to affect the abundance and composition of the biocrust and thus an effect on soil CO_2 fluxes.

Poster

Do lichens respond to synergistic effects of nitrogen and water stress? A case study from South Portugal

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Lichen physiological parameters and communities' composition reflect the ecological functioning of ecosystems in the short- and the long-term respectively. The rapid response of lichen physiological parameters, e.g. the photosynthetic performance, can provide early warnings of bioclimatic stresses that are specifically referred to the atmospheric compartment, integrating and complementing the information provided by other indicators.
Lichens are known to respond to nitrogen and water stress but so far the synergistic effects of these two drivers has been scarcely investigated.
This experiment, developed within the COST Action ES1104 "Arid Lands Restoration and Combat of Desertification" aims at assessing the effects of nitrogen availability associated to climatic stress in areas under desertification risk.
Thalli of the lichen species *Parmotrema perlatum* (typical of environments with low nitrogen availability, but resistant to period of low water availability) and *Ramalina canariensis* (moderately tolerant to nitrogen, but rather hygrophytic) were transplanted in nine selected sites along a combination of climatic (water availability) and nitrogen (agricultural practices) gradients in a Mediterranean area in Southern Portugal. Lichen photosynthetic performance was investigated prior and after a six months exposure in transplanted thalli and in the most common epiphytic and epigeic lichens collected *in situ*.
Results showed a different response of transplanted *P. perlatum* to nitrogen along the climatic gradient and a seasonal variation in *in situ* lichens.

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Poster

Combining lichen and tree response to evaluate the status of urban forests in Parco Nord Milano (Italy)

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The huge and often unregulated urbanization that has been occurring in the last decades has changed dramatically the environmental conditions of previous rural and natural areas, causing atmospheric and acoustic pollution, loss of biodiversity and climatic alterations, with harmful consequences for ecosystem functioning and human health.
In this context, urban green infrastructures represent a main mitigation measure able to reduce the impact of climate changes and provide ecosystem service, contributing to ameliorate citizen's life quality. However, to evaluate and enhance the effectiveness of such strategies, it's necessary to assess the ecological status of urban forest species in the context of environmental restoration projects.
"Parco Nord Milano" (PNM) is an urban forest plantation of 600 ha located in the northern part of Milan's metropolitan area (Northern Italy) and represents a case study where various methods and approaches were used to compare the status of forest resources at different time scales considering additional variables such as climate conditions, soil quality, and water supply.
This work, developed within the COST Action FP1204 GreenInUrbs, aims at using lichens and trees to provide information on the long-term response of forest resources in PNM to the change of climate conditions and the impact of other biotic/abiotic stressors.
To that, we performed field data collection over three areas within a transect from the outer to inner zones of the park at increasing distance from a heavily polluted urban road. Lichens and tree cores were collected in order to analyze the impact of urban pollution on green areas.
On tree cores we measured annual growths and ^{13}C and ^{14}C carbon isotopes.
On lichens we measured total and functional Lichen Diversity Value, chlorophyll *a* fluorescence parameters, element concentration, and carbon and nitrogen isotopic signatures. Results are presented.

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Poster

The competitive behaviour of trace elements during bioaccumulation in lichen thalli may lead to an underestimation of real deposition

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Lichens have a significant ability to accumulate inorganic and organic atmospheric pollutants and they are effective especially in trapping trace elements from the surrounding environment. Essentially, three uptake mechanisms are important for lichen thalli: extracellular ion exchange, intracellular accumulation and particulate trapping. Mineral uptake and release are reversible processes influenced by thallus morphology and age, physiological status, exposure duration, microclimatic conditions and the presence and type of pollutants in the environment. In general, it is acknowledged that the concentrations of trace elements in lichen thalli are correlated with their environmental levels. The lichen thallus is characterized by cation exchange properties and the uptake of soluble cations may occur by means of passive reversible binding to anionic sites. Noteworthy, the contemporary supply of positively charged elements may displace the original cations from their extracellular exchange sites, depending on chemical affinities and available concentrations. The nature of the elements and their different competitive capacities for exchange binding sites might mask their real environmental load. Therefore, bioaccumulation data might result in an underestimation of some elemental levels measured in biomonitoring studies.

We report the results of experiments carried out to assess the competitive behaviour of divalent heavy metals (Cd, Cr, Pb, Zn) during element uptake in lichen thalli. To this purpose, thalli of the foliose lichen *Xanthoria parietina*, that is commonly used in bioaccumulation studies, were collected from a remote area and incubated with solutions containing 10 or 100 µM $CdCl_2$, $CuCl_2$, $ZnCl_2$ and $Pb(NO_3)_2$, both individually and in combination (Cd+Cu+Pb+Zn). The results showed that owing to the competition between elements, bioaccumulation may lead to an underestimation of 15-30% for Cd and Cu, and of 35-50% for Pb and Zn.

Poster

Monitoring nitrogen air pollution: a rapid method using the biodiversity of epiphytic lichens

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Lichens are among the most valuable biomonitors of atmospheric pollution, especially in urban environments. The diversity of epiphytic lichens is commonly used as a sensitive indicator of the biological effects of air pollution by means of standard procedures. It is quick and inexpensive and provides results on which predictions for human health can be based.
In urban environments vehicular traffic is the main source of particulate matter and airborne metals and a major source of nitrogen compounds, e.g. nitrogen oxides (NO_x) produced by fuel combustion and ammonia (NH_3) formed inside catalytic converters; while domestic heating represents a relevant source of NO_x. Presently the effects of NO_x on lichens are still unclear, while it is know that NH_3 enhances the diffusion of nitrophilous species as indirect effect of the increasing of bark pH.
A rapid method to investigate the lichen biodiversity on pine trees was tested in order to pinpoint the effects of eutrophicating substances in a urban area monitored since 1993 by epiphytic lichens (Montecatini Terme, C Italy). The latest surveys reported a progressive diffusion of nitrophilous species on lime trees. In the present study, the attention focused on pine trees, which are a very interesting substrate, since pines have a naturally acid bark, normally hosting only acidophilous lichens. Therefore, changes in species composition towards nitrophilous species induced by air pollution due to eutrophicating substances, if present, are easily detectable.
To the purpose, 13 sampling sites were selected covering the whole urban area and 10 pine trees were monitored at each site. Lichen biodiversity by (exclusively) nitrophilous species was measured between 100-150 cm from the ground and expressed in terms of percentage coverage. The working hypothesis was that the highest is the diffusion of nitrophilous species, the highest is the effect due to eutrophicating substances. The results showed that nitrophilous species were particularly abundant in areas with traffic peaks and at the hippodrome, and are relevant looking at possible citizen science scenarios.

Poster

Industrial pollution and reproduction: influence of cadmium exposure on *in vivo* and *in vitro* Mediterranean mussel gonad and sperm

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Cadmium emissions to the environment are normally transported continually between the three main environmental compartments, air, water and soils. In ambient air, deriving from industrial sources it is generally deposited onto waters or soils and then transferred to plants and animals, and finally enters the human body through the food chain and poses carcinogenic and other adverse risks to human health due to bioaccumulation over time. We have monitored the effects of cadmium on reproductive health of the bioaccumulator *Mytilus galloprovincialis* also known as Mediterranean mussel, whose sperm chromatin is mainly organized by three protamine-like proteins (PLII, PLIII and PLIV). The mussels (5.2 ± 0.4 cm shell length), obtained from the Consortium of Fishermen aquaculture farm in Goro (Ferrara, Italy) in June 2014, were maintained in aerated seawater in large flow-through holding tanks for two weeks, and then transferred for 30 days in stainless steel cages (50x50x50 cm), deployed by scuba-diving in different sites of Campania and Sicilia respectively close to the disposal metallurgical factory Ilva in Bagnoli and "Augusta-Melilli-Priolo" industrial site, one of the largest and most complex petrochemical sites in Europe. Mussels were also put in tanks for experimental metals treatments. The effects of natural industrial pollution and thank 1,5; 5 and 10µM $CdCl_2$ were evaluated analyzing the gonadal morphology, the immunoreactivity of phase I and phase II biotrasformation enzymes, respectively CYP4Y1 and GST, the metallotionein, MT, the GST expression by real time quantitative PCR; the state of sperm proteins, their DNA binding affinity and oxidative DNA damage by EMSA. The results show a direct correlation between cadmium water concentration or $CdCl_2$ tank amount and the significantly altered gonadal morphology, activity of phase I and phase II enzymes, the MT-immunoreactivity, the increase of gonadal π-gst expression compared to control. At the gonadal detossification and the antioxidant parameter enhancement corresponds also alterations of sperm protamine-like proteins state, their DNA binding affinity and enhancement of DNA oxidative damage. Taken together, our studies indicate not only that cadmium exposure could affect the correct sperm chromatin structure decreasing organisms reproductive fitness but further characterize Cd-induced gonadal and sperm DNA and proteins damage and highlight the potential of protamine-like proteins approach to develop quick and efficient chromatin-based genotoxicity test in pollution biomonitoring programs.

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Poster

Long-term N additions reduce the lability of soil C in temperate forest soils depending on tree species composition

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Increases in atmospheric nitrogen (N) deposition and carbon dioxide (CO_2) concentrations are two of the ongoing global change processes through which humans continue to alter C and N cycles. Understanding how atmospheric N deposition affects C stabilization in forest soils has become an important focus as soils represent the largest terrestrial C reservoir and the primary sink for added N. Recent reviews revealed a positive effect of increased N inputs on C stabilization in soils of temperate forest ecosystems. However, there is still uncertainty about the magnitude of this effect and the role of tree species as possible modulators. We evaluated the response of soil C lability to a 14-years N addition experiment across plots with different dominant tree species (*Acer saccharum*, *Fagus grandifolia*, *Betula alleghaniensis*, *Tsuga canadensis* and *Quercus rubra*). We used a single-species, paired-plot design, and two different approaches to estimate C lability: soil laboratory incubations and density fractionation. Our two principal measures of C lability showed statistically significant interactive effects of N treatment and tree species composition: soils from maple (*Acer*) stands showed the greatest effect of added N on the light fraction mass in the mineral horizon (a 69% increase), and soils from beech (*Fagus*) stands showed the greatest N effect on potentially mineralizable C (a 23% decrease). Decreases in soil decomposition and respiration rates in organic and mineral horizons in response to N addition across all five species suggest a significant suppression of C mineralization, particularly in the first few weeks of the incubation, with the strongest responses in beech and oak (*Quercus*) stands. Our results demonstrate that increased N additions significantly reduce the lability of soil C for some tree species, and indicate that mechanisms other than organo-mineral associations could play an important role in the stabilization of C in these soils. Moreover, our research highlights the need to consider varying responses among different tree species when predicting future consequences of N inputs on soil C storage.

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Poster

Seed germination capacity and seedling viability of annual species from ozone-exposed parental plants

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Dehesas are one of the most characteristic landscapes of the central and SW Iberian Peninsula protected under the 92/43/EEC Habitat Directive. They are composed by open forest with an understory of annual pasture of high species richness, which also constitute a food resource for extensive cattle and wild herbivorous. This ecosystem type is chronically above the ozone (O_3) levels recommended for plant protection, according to the EU Directive of Air Quality (2008/50/EC).

Experimental studies developed during the last decade, based on the response of individual species, have shown the high O_3 sensitivity of annual dehesa species, especially *Trifolium* sps. (Sanz et al., 2014, 2015). A more recent experiment under field conditions, showed losses of yield and quality of the pasture under O_3 exposure, and identified this pollutant as a factor capable of changing the competitive capacity among species, affecting the structure and composition of the pasture (Calvete-Sogo et al, 2014, 2016). Due to the importance of the soil seed bank for annual communities, and based on the former experiment, seed production, germination and seedling viability from O_3-exposed plants of *Trifolium striatum*, *Trifolium cherleri* and *Silene gallica* were analyzed. Two experiments were developed: 1- Seed germination experiment under control ambient in chambers, for analyzing seed germination curves and seed root elongation; 2- Seedlings viability experiment under ambient conditions, for analyzing seedling emergence, survival, plant biomass and morphology (leaf (green+senescent) and radicular biomass, specific leaf area, green/senescent biomass rate, root/shoot rate) at 30 and 90 days. Preliminary results will be presented.

Poster

Physiological and ultrastructural effects of glyphosate in the lichen *Xanthoria parietina* (L.) Th.Fr.

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Glyphosate (N-(phosphonomethyl)glycine) is the most widely used herbicide for the control of weeds in public and private environments. The success of this herbicide is mainly due to its ability to target the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), responsible for the biosynthesis of aromatic aminoacids involved in plant development, leading to the death of weeds in a few days. Recently, glyphosate-based herbicides have raised a global concern, since glyphosate has been classified as "probably carcinogenic to humans" by IARC. In this framework, monitoring the environmental distribution of this herbicide is absolutely necessary. Lichens are considered "sentinels" of air pollution, but there are very few indications about the ability of these organisms to take up and accumulate glyphosate as well as the physiological mechanisms involved in its toxicity. In a first study we investigated the accumulation and the physiological effects of glyphosate treatments in the lichen *Xanthoria parietina*, a species commonly used in biomonitoring studies, notable for its ubiquity and resistance to pollution. Lichen samples were incubated for 24, 48 and 96 hours in different glyphosate solutions, referable to the lowest and highest recommended dose suggested for field treatments (3.6 g/L and 36 g/L, respectively). Soaked samples showed an uptake proportional to the dose provided, paralleled by adverse physiological effects on the photosynthetic apparatus (photobiont) and damages to cell membrane integrity (mycobiont). However, considering that the normal application of glyphosate consists in spraying, the experiment was repeated by spraying the samples. The results showed that toxic effects were still evident at physiological and ultrastructural level, both for the photosynthetic and the fungal partner.
Considering that we have shown that glyphosate remains stable in the lichen *X. parietina* for at least 90 days, the use of this lichen may be of special interest for monitoring the atmospheric distribution of this herbicide.

Poster

Physiological and ultrastructural effects of ozone in the lichen *Xanthoria parietina* (L.)

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Ozone (O_3) leads to the production of reactive oxygen species (ROS) that are harmful to cell membranes of plants and animals. We investigated the physiological and ultrastructural effects induced by O_3 fumigation in the lichen *Xanthoria parietina*. We hypothesized an important protective role of the secondary compound parietin, the main cortical pigment of this species, that has a strong antioxidant power, as well as of the dry state of thalli that have a very reduced metabolism. To the purpose of the study, 4 batches of *X. parietina* samples, originating from the combination of wet, dry, with parietin (P+), without parietin (P-; removed using acetone washing) were fumigated for 1 h with O_3 at a constant concentration of 6.7 ppm. A series of physiological parameters was measured, including photosynthetic efficiency, content of assimilation pigments (chlorophyll a and b, β-carotene), chlorophyll degradation, dehydrogenase activity, soluble proteins, ergosterol content and ROS production. Ultrastructural analysis was carried out using a TEM. The results showed that O_3 fumigation greatly affected all physiological parameters investigated as well as the ultrastructure of the lichen samples, irrespective if wet or dry, P+ or P-. Recovery was estimated incubating the fumigated samples up to 1 week without O_3 and the results suggested a partial recovery of wet P+ samples only for some of the investigated parameters.

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FALLON,
FAORO,
FINCO,
FRONTASYEVA,
FÜHRER,
FUSARO,
FUSCO,
GALLARDO,
GARCÍA MEDINA,
GARCÍA-GÓMEZ ,
GEROSA,
GONZÁLEZ-FERNÁNDEZ,
GOODALE,
GROFFMAN,
GUARNIERI,
GUERRIERO,
HARMENS,
IRIGOYEN ,
IZQUIETA-ROJANO ,
JIMENÉZ ELVIRA,
KINGDON,
KOIKE,
LAFUENTE,
LASHERAS,
LAZO,
LECHA,
LENTINI,
LEO,
LINARES,
LO CASCIO,
LÓPEZ-AIZPÚN,
LOPPI,
LORENZINI,
LOVETT ,
LUCIÁÑEZ,
MANCINI,
MANES,
MANNING ,
MANRIQUE ,
MARRAS,
MARZUOLI,
MEDINA,
MEREU,
MERINO,
MIHALOPOULOS,
MITSOTAKI,
MONACI,
MONGA,
MONTELEONE,
MORILLAS ,
MUNZI ,
NALI,
NOTARIALE,
OCHOA-HUESO,
PAOLETTI ,
PAOLI,
PECO,

PELLEGRINI,
PEREZ-CORONA ,
PILZ,
PINHO,
PISCOPO,
POLLICE,
QARRI,
RÁBAGO,
RABBITO,
RICCIARDIELLO,
ROALES ,
RODRÍGUEZ ,
RODRÍGUEZ-MUÑOZ,
SAITANIS ,
SALVATORI,
SANESI,
SANTAMARÍA,
SANTAMARÍA,
SANZ,
SATOH ,
SCHAUB,
SHEPPARD,
SINGH,
SORBO,
SPANO,
STAFILOV,
SUGAI ,
SULAIMAN,
TANG,
TEMPLER,
TOWNSEND,
TSAGARAKI,
VALIÑO,
VANNINI,
VIÑEGLA ,
WEATHERS

Fig.2 – The eddy covariance flux tower of Catholic University of Brescia in the mixed oak-hornbeam forest at Bosco Fontana (MN)