# Detoxification capacity of poplar exposed to ozone: intraspecific variability of three Populus x euramericana genotypes

Yves Jolivet, Didier Le Thiec, Ata Allah Dghim, Jennifer Dumont, Marie-Noëlle Vaultier, Marie-Paule Hazenfratz-Sauder, Fabien Spicher, Elina Oksanen, Pierre P. Dizengremel

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# PROGRAMME

## JUNE 14

8:00-9:00 **Registration**

***Opening session*** (Chair: Pavel Cudlin)

9:00-9:15 **Welcoming addresses by P. Cudlin and E. Paoletti**

9:15-9:40 **S. Cieslik: Ozone, a peculiar atmospheric gas with good and bad effects. A review**

9:40-10:05 **F. Loreto: Biosphere-atmosphere interactions in an ozone-enriched world**

10:05-10:30 **J. S. King : Responses of forest carbon and water cycling to elevated atmospheric $CO_2$ and $O_3$ at Aspen FACE: A summary of ten years of research**

10:30-11:15 **Coffee Break**

11:15-11:40 **R. Matyssek, L. Grünhage, M. Baumgarten, G. Wieser: Lessons from Kranzberg Forest - Ozone research quo vadis?**

11:40-12:05 **W. J. Manning, J. M. Albertine, T. B. Harris: Global warming and increasing background concentrations of ozone: problems or opportunities for plants?**

12:05-14:00 **Lunch**

***Session 1A: Ozone fluxes and effects*** (Chair: Juha-Pekka Tuovinen)

14:00-14:15 **S. Fares, J-H. Park, R. Weber, D. Gentner, J. Karlik, A. H. Goldstein: Fluxes of ozone from an orange orchard: partitioning in stomatal and non-stomatal deposition**

14:15-14:30 **T. N. Mikkelsen, H. Ro-Poulsen, K. Pilegaard: Ozone uptake at leaf and ecosystem level by Norway spruce in relation to environmental conditions**

14:30-14:45 **J. Neirynck, B. Gielen, I. A. Janssens, R. Ceulemans: Long-term measurements of ozone fluxes over a mixed temperate forest**

14:45-15:00 **M. Nosal, A. H. Legge, M. C. Hansen: Statistical uncertainty of ambient air quality measurements and the potential consequences for ozone flux characterization**

15:00-15:15 **Z. Večeřa, P. Mikuška, M. Vojtěšek, M. Zapletal, P. Chroust, P. Cudlín: Ozone and nitrogen dioxide vertical profiles in forest canopies measured using passive samplers**

15:15-16:00 **Coffee Break**

16:00-16:15 **P. Büker, L.D. Emberson, T. Morrissey, D. Simpson, J.P. Tuovinen, et al.: Modelling of soil moisture using $DO_3SE$ to determine ozone fluxes to European forest trees**

16:15-16:30 **Y. Hoshika, K. Omasa, E. Paoletti: Stomatal closure is sluggish in poplar leaves with ozone visible injury**

16:30-16:45 **Y. Jolivet, D. Le Thiec , A. A. Dghim , J. Dumont , M-N. Vaultier , M-P. Hasenfratz-Sauder , F. Spicher , E. Oksanen , P. Dizengremel: Detoxification capacity of poplar exposed to ozone: intraspecific variability of three *Populus x euramericana* genotypes**

16:45-17:00 **M. Zapletal, P. Cudlín, P. Chroust, O. Urban, R. Pokorný, M. Edwards-Jonášová, R. Czerný, D. Janouš, K. Taufarová, Z. Večeřa, P. Mikuška: Net ecosystem production of mountain spruce forest under increased stomatal uptake of ozone**

***17:15-18:00 Poster session*** *(5-min talk in front of each poster)* (Chair: Elena Paoletti)

1) **M. Díaz-de-Quijano, À. Ribas , J. Peñuelas: Increasing trends of ozone mixing ratios in the Catalan Pyrenees over the last 16 years**

2) **M. Díaz-de-Quijano, J. Peñuelas, T. Menard, P. Vollenweider: Visible and microscopical ozone injury in mountain pine (*Pinus mugo* subsp.*uncinata*) foliage from the Catalan Pyrenees**

3) **P. Vollenweider, B. B. Moura, E. S. Alves: Validation of specific ozone symptoms in rain forest trees of southern Brazil**

4) **I. Hůnová, L. Matoušková: Ozone visible foliar injury and stomatal flux in native young trees of *Fagus sylvatica* L.: a field study from the Jizerske hory Mts., the Czech Republic**

5) **Z. Večeřa, P. Mikuška, K. Křůmal, M. Zapletal, P. Chroust: New chemiluminescence reagent detector for measurement of eddy covariance fluxes of ozone**

6) **R. Kerner, E. Delgado-Eckert, J.W. Dupuy, J.B. Winkler, M. Jürgensen, Ch. Lindermayr, D. Ernst, G. Müller-Starck: Responses of European beech saplings following four vegetation periods of ozone exposure: an integrative study**

7) **V. Calatayud, A. Palomares, J. Cerveró, E. Calvo: Measuring and estimating ozone levels an the Valencian Community**

## JUNE 15

***Session 1B: Monitoring of ozone effects*** (Chair: Nicholas Clarke)

9:00-9:15 **M. Díaz-de-Quijano, P. Vollenweider, R. Ogaya, J. Peñuelas: Ozone concentrations and dieback of *Pinus mugo* subsp. *uncinata* stands in the Catalan Pyrenees**

9:15-9:30 **H. Andreae, D. Reinhold: Results from ozone monitoring at Level-II-plots in Saxony**

9:30-9:45 **A. Augustaitis, A.Kliucius, R.Girgzdiene, V.Marozas, M. Pilkauskas, E. Beniusis: Sensitivity of beech trees to surface ozone at most northern latitude of their occurrence**

9:45-10:00 **P. Parvanova, N. Tzvetkova, S. Bratanova-Doncheva, N. Chipev, R. Fikova, E. Donev: Responses of *Fraxinus excelsior*seedlings to ambient ozone in urban and mountain areas based on some physiological characteristics and antioxidant activity**

10:00-10:15 **O. Badea, D. Silaghi, Neagu, S. Tamas: Monitoring of ozone and its phytotoxic effect on forest ecosystems in Romania**

10:15-10:30 **V. Šrámek, R. Novotný, I. Hůnová, E. Bednářová, M. Vejpustková, V. Fadrhonsová: Monitoring of ozone effects on the vitality and increment of Norway spruce and European beech in the Czech Republic**

10:30-11:15 **Coffee Break**

***Session 2: Ozone standards for forests*** (Chair: Teis Mikkelsen)

11:15-11:30 **S. Braun, C. Schindler, P. Büke: From AOT40 to ozone flux for forest trees – progress and uncertainties**

11:30-11:45 **E. Paoletti, A. De Marco: Testing the DO3SE model on a broadleaf Mediterranean forest species: is the model working?**

11:45-12:00 **R. Alonso, G. Gerosa, Á. Ribas, V. Calatayud , M. Díaz de Quijano, S. Elvira, E. Calvo, R. Marzuoli, J. Peñuelas, F. Bussotti, M. Pollastrini, S. Mereu, L. Fusaro, I. González-Fernández: Ozone critical levels for Mediterranean forests**

12:00-12:15 **G. Gerosa, A. Finco, M. Ferretti, E. Gottardini: Errors in ozone risk assessment using standard conditions for the conversion of ozone concentrations from passive samplers in mountain regions**

12:15-12:30 **L. Grünhage, R. Matyssek , K.-H. Häberle , U. Metzger , M. Leuchner , A. Menzel , J. Dieler , H. Pretzsch , W. Grimmeisen , S. Raspe: Evaluation of the ozone-related risk for beech forests**

12:30-14:30 **Lunch**

14:30-14:45 **I. Hůnová, M. Coňková: Ambient ozone phytotoxic potential for Czech mountain forests**

14:45-15:00 **E. Oksanen, V. Pandey, S. Kontunen-Soppela, S. Keski-Saari: Ozone sensitivity of the most important Indian crop and tree species**

15:00-15:15 **S. Barth, H. Pleijel, M.E. Kubiske, J. King, L. Rhea, J. Nagy, W. Jones, J. Uddling: Ozone flux-response relationships for aspen and aspen-birch forests in the Aspen FACE experiment**

15:15-16:00 **Coffee Break**

*16:00-17:00* ***Poster session*** *(5-min talk in front of each poster)* (Chair: Elena Paoletti)

8) **A. Augustaitis, I. Augustaitiene, E. Plausinyte, D. Mozgeris: Effect of surface ozone on Scots pine and Norway spruce health and productivity under changing climate and air pollution in Lithuania**

9) **A. Kasurinen, C. Biasi, T. Holopainen, E. Oksanen: Integrated effects of elevated ozone and temperature on growth and carbon partitioning in silver birch (*Betula pendula*)**

10) **M. Zapletal, J. Pretel, P. Chroust, I. Hůnová, P. Cudlín, O. Urban, R. Pokorný, R. Czerný, Z. Večeřa: The influence of climate change on stomatal ozone flux to forest ecosystems**

11) **G. Gerosa, A. Finco, S. Oliveri, J. Zueger, E. Gebetsroither, W. Winiwarter: MANFRED Project. Management strategies to adapt Alpine Space forests to climate change risks**

12) **I. Štraus, M. Bajc, P. Železnik, H. Kraigher: Temperature and elevated $CO_2$ concentrations effects on fine root turnover and ectomycorrhiza of beech seedlings**

13) **B. Turk, K. Eler, F. Batič: Overview of the Slovenian bean experiment in the last years**

18:00-22:30 **Visit to Prague and dinner**

## JUNE 16

***Session 3: Interaction of ozone effects with climate change*** (Chair: Rainer Matyssek)

9:00-9:15 **W. de Vries, M. Posch, D. Simpson, A. Nyiri, L. Bonten1, G. J. Reinds: Modelling past and future impacts of changes in climate, nitrogen deposition, ozone and $CO_2$ exposure on carbon sequestration in European forests**

9:15-9:30 **L. Emberson, Kitwiroon, N. Beevers, S. Büker, P. Cambridge, H. Cinderby: Understanding climates influence on ozone deposition and implications for human health and ecosystem impacts: a case study for the UK**

9:30-9:45 **G. Soja, E. Scheicher, S. Pietsch: Trend analysis of ground-level ozone exposure and ozone uptake of oaks in Eastern Austria for the period 1990-2010**

9:45-10:00 **G. Gerosa, A. Finco, S. Oliveri, R. Marzuoli, J. Zueger, E. Gebetsroither, W. Winiwarter: Assessing ozone hazards to natural forests in the Alpine area. Comparison of a wide scale mapping technique with local passive sampler measurements**

10:00-10:15 **A. Ballarin-Denti, P. Angelini, L. Cetara: The impact of climate change, ozone and natural risks on Alpine forests: adaptation versus mitigation policies**

10:15-10:30 **M. Kivimäenpää, J. Riikonen, V. Ahonen, A. Tervahauta , T. Holopainen: Emission of volatile organic compounds (VOCs), expression of VOC-related genes, gas exchange and growth of Norway spruce saplings exposed to elevated ozone and elevated temperature**

10:30-11:15 **Coffee Break**

11:15-11:30 **H. Harmens, F. Hayes, J. Williamson, G. Mills: Species-specific responses of tree saplings to a combination of increasing background ozone and drought**

11:30-11:45 **A. De Marco, V. Marcello, M. Di Traglia, A. Screpanti, C.Proietti, F.Attorre: How is tree primary productivity related to climate and atmospheric pollution in Italy?**

11:45-12:00 **B. Maňkovská, J. Oszlányi: Influence of greenhouse gases on epicuticular waxes of *Populus tremuloides* Michx.**

12:00-12:15 **A. Kühn, M. Baumgarten, H.-P. Dietrich, R. Matyssek: The potential of beech (*Fagus sylvatica* L.) for coping with drought and ozone impact as components of climate change**

12:15-14:30 **Lunch**

## *Session 4: Ozone effects on below-ground part of forest ecosystems* (Chair: Hojka Kraigher)

14:30-14:50 **K. Pritsch: Reactions to elevated ozone in the plant-mycorrhiza-soil system of forest trees**

14:50-15:10 **S. Toet, V. Oliver, D. Sherlock, S. McLoughlin, T. Helgason, S. Peacock, J. Barnes, P. Ineson, M. Ashmore: Long-term effects of ozone on carbon fluxes in peatlands**

15:10-15:30 **C. Cruz, M. Delgado, H. Kraigher: Belowground hormonal interactions in plants & ozone**

15:30-15:45 **S. Manninen, T. Kanerva, H. Aaltonen, A. Palojärvi: Ozone-related changes in the biomass and structure of the microbial community in meadow soil**

15:45-16:30 **Coffee Break**

## ***Session 5: ICP Vegetation: insights from crop science*** (Chair: Harry Harmens)

16:30-16:45 **G. Rana, M. Mastrorilli, N. Katerji: A daily model of actual evapotranspiration for soybean growing inside OTC under contrasting conditions of ozone air concentration and plant water stress**

16:45-17:00 **M. Anaya-Romero, M. Muñoz-Rojas, D. de la Rosa: MicroLEIS DSS, a sustainable land use and management decision support system for maximizing carbon sequestration**

17:00-17:15 **H. Harmens, G. Mills, F. Hayes, D. Norris, D. Simpson, H. Harmens: Quantifying the impacts of ozone on food security**

17:15 **Conclusions**

# KEYNOTE PRESENTATIONS

# GLOBAL WARMING AND INCREASING BACKGROUND CONCENTRATIONS OF OZONE: PROBLEMS OR OPPORTUNITIES FOR PLANTS?

***J. M. Albertine, T. B. Harris, W. J. Manning****

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Legislation and fuel-efficient vehicles have reduced pollution levels of ozone ($O_3$) resulting in lower levels of $O_3$ during the growing season for plants. Normal background levels of $O_3$, however, are slowly increasing. Several models predict that as global warming progresses, background levels of $O_3$ could reach or exceed 70 ppb, depending on location. Using greenhouse exposure chambers, we grew $O_3$-sensitive (S156) and $O_3$- tolerant (R331) bean plants (Phaseolus vulgaris) to determine their growth responses to 25 ppb, 50 ppb, and 75 ppb $O_3$ during a five-week period. Plants were harvested weekly and among the several criteria used for effects evaluation were total plant dry weight, total leaf area/plant and total leaf weight/plant. Beginning with week 3, S156 plants grown in 50 ppb $O_3$ had the highest total plant dry weights, and highest total leaf area and total leaf dry weights, higher than any values obtained from plants grown at 25 ppb $O_3$. Growth was reduced and foliar injury occurred on plants in 75 ppb $O_3$. Similar results were obtained for R331 plants, but the differences between results by exposure were less than for S156. The rationale for this hormetic effect of 50 ppb $O_3$ on bean plant growth is not known. Further experiments with other plants may provide more information on the extent and cause of growth stimulation in plants due to exposure to 50 ppb $O_3$.

# OZONE, A PECULIAR ATMOSPHERIC GAS WITH GOOD AND BAD EFFECTS

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Ozone is both a natural atmospheric constituent and an air pollutant. Its cycle in the atmosphere results from highly non-linear, mostly photochemical processes. A well documented record going back to more than a century proves that the tropospheric ozone concentration increased considerably from preindustrial (10ppb) to the current mean levels (40 to 50 ppb). Its unstable molecular structure is responsible for its strong chemical reactivity, resulting in high complexity of the photochemical mechanisms governing its sources and sinks. This causes difficulties in defining abatement policies, which did not yet manage to reduce the ozone levels, despite the reduction of precursors' emissions.

Ozone has adverse effects on human health and damages vegetation, e.g. by causing slower plant growth, counteracting in turn carbon sequestration, with consequences on global climate. On the other hand, the presence of ozone at higher altitudes in the stratosphere is beneficial to life on earth as it filters harmful ultraviolet radiation. A great concern arose from the observation, predicted as well by modeling, of the depletion of the stratospheric ozone layer, which seems to be currently slowly recovering thanks to the implementation of the Montreal Protocol which includes a ban on ozone-destroying substances.

In conclusion, ozone plays an important role in the environment; it has both beneficial and adverse effects and is rather difficult to study because of the complexity of the mechanisms controlling ozone concentration.

# FOREST CARBON AND WATER CYCLING RESPONSES TO ELEVATED ATMOSPHERIC $CO_2$ AND $O_3$ AT ASPEN FACE: A SUMMARY OF TEN YEARS OF RESEARCH

*J. S. King**

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The Aspen FACE Project in Rhinelander, Wisconsin, USA, was one of the most elegant and productive ecological experiments in the world, and has been a watershed study for understanding complex ecosystem responses to the interaction of multiple global change factors. The experimental design consisted of a factorial arrangement of whole-plot treatments of elevated atmospheric CO2 (eCO2; 550 ppmv) and O3 (eO3; 60 ppbv) applied during the growing season from 1998 until 2008. The split-plot factor was community type, consisting of pure aspen, mixed aspen-birch, and mixed aspen-maple, allowing the study of competition as influenced by air pollution. We found that net primary production (NPP) was consistently and prominently affected by $eCO_2$ (+35 %) and $eO_3$ (-18%), but the combined treatment (eCO2+eO3) differed little from the control. Treatment effects on NPP propagated through the ecosystem, affecting C and water cycling and storage. Larger trees under eCO2 produced bigger canopies and root systems, resulting in greater litter inputs to soil, however specific rates of litter decomposition did not change. This suggests increased C storage in plant biomass and soils. Elevated O3 had the opposite effect, demonstrating its capacity to compromise gains in ecosystem C storage expected from eCO2. In terms of water, leaf area responses consistent with the eCO2 and eO3 treatments predominated in affecting stand level transpiration, rather than changes in unit leaf area stomatal conductance or water use efficiency. Together with increased interception losses under eCO2, this implies less water available to recharge groundwater and surface aquifers. As with C cycling, eO3 tended to counteract these hydrologic responses to eCO2. Aspen FACE has resulted in significant changes to U.S. national air quality policy and better scientific understanding of forest ecosystem responses to eCO2 and eO3 that will allow more accurate modeling and better prediction of forest adjustments to global change.

# BIOSPHERE-ATMOSPHERE INTERACTIONS IN AN OZONE-ENRICHED WORLD

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Biogenic Volatile Organic Compounds (BVOC) are emitted at very high rates by forest plant species. BVOC play a critical role in biosphere-atmosphere interactions, and are key constraints of the physical and chemical properties of the atmosphere, potentially influencing the climate and the quality of air, especially in areas exposed to in situ release or long-range transport of anthropogenically polluted air masses. Under these conditions reactive BVOC may contribute to ozone and particle production. The very large amount of BVOC emitted by vegetation, estimated today between 500 to 1000 Tg C annually, is dominated by isoprenoids and methanol. Such a high rate of emission implies a large metabolic cost and hence likely indicates very important plant functional roles for these compounds. BVOC can be emitted by plants constitutively, or the emission may be induced in response to biotic and abiotic factors. Both constitutive and induced BVOC sometimes act as defensive compounds and are often crucial for plant protection in stressful environments. The importance of volatile isoprenoids as molecules protecting against environmental constraints has been widely discussed, but compelling evidences obtained using genetically modified forest ad natural plants now support this important physiological role for isoprene and monoterpenes. The idea emerges that BVOC act as antoxidants in planta, whereas they contribute to the oxidation potential of the atmosphere.

# LESSONS FROM KRANZBERG FOREST – OZONE RESEARCH *QUO VADIS*?

***R. Matyssek*, L. Grünhage, M. Baumgarten, G. Wieser***

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Ground-level ozone ($O_3$) has gained awareness as an agent of climate change on both hemispheres. Nevertheless, uncertainty prevails on $O_3$-caused mitigation of the C sink strength of forest ecosystems worldwide, except for non-validated modelling predictions, under increasing atmospheric $CO_2$ and changing environments. The eight-year free-air $O_3$ canopy fumigation experiment at Kranzberg Forest (Freising/Germany) was the first to examine adult trees of climax species and contrasting foliage habit (evergreen Norway spruce vs. deciduous European beech) under prevalent site conditions. What did we learn from this case study, and which are the new scientific questions and needs arising from the outcome for consolidating $O_3$ risk assessment?

Key findings will be wrapped up on $O_3$ effects on the tree-soil system as a starting point for defining new challenges for empirical research and modelling, and for elucidating conceptual and methodological advancement. Amongst challenges is the perception of $O_3$ action as part of multi-factorial scenarios (including biotic above and belowground interactions), $O_3$ dose-related risk assessment (covering tree sensitivity per unit influx and stand-level $O_3$ fluxes), and the clarification of non-linearity in tree response and of transition ranges rather than thresholds towards injury. A both mechanistic and holistic view on tree performance and tree-soil resource fluxes is required. Particular challenge is on ecosystem-level $O_3$ research (making use of novel methodologies) and up-scaling to regional and hemispheric scopes (overcoming research deficits outside the temperate climate zone).

Novel intrinsically linked process-based empirical and modelling approaches are required. The vision of integrative research "super-sites" will be introduced. Transfer of $O_3$ flux-modelling concepts from agricultural to forest systems will be exemplified. A pan-European research concept will be outlined that may unify mechanistic clarification, geographically differentiated up-scaling and model development for C pool and related risk assessment. Will integrative $O_3$ studies remain a "non-topic" in research on global change?

# VOLUNTARY PRESENTATIONS

# OZONE CRITICAL LEVELS FOR MEDITERRANEAN FORESTS

***R. Alonso[1*], G. Gerosa[2], Á. Ribas[3], V. Calatayud[4] , M. D. De Quijano[5], S. Elvira[1], E. Calvo[4], R. Marzuoli[2], J. Peñuelas[5], F. Bussotti[6], M. Pollastrini[6], S. Mereu[7], L. Fusaro[7], I. González-Fernández[1]***

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Tropospheric ozone ($O_3$) is considered one of the most important air pollutants inducing adverse effects on a wide range of ecosystems and vegetation types. In Europe, an effects-based approach has been used to establish $O_3$ critical levels for the protection of vegetation under the framework of the Convention on Long-Range Transboundary Air Pollution (CLRTAP). The $O_3$ critical levels for different vegetation types have recently been revised to include the latest scientific information. The traditional critical levels based on $O_3$ exposure (expressed as AOT40) have been complemented with new flux-based critical levels proposed for some vegetation types. These new critical levels based on the amount of pollutant entering the plant through the stomatal pores allow incorporating some modifying factors of plant $O_3$ sensitivity such as species type, phenology and environmental conditions.

Many studies have reported $O_3$-induced effects on the physiology and growth of numerous Mediterranean forest species. However, some discrepancies exist between the predicted $O_3$ effects on Southern European forests based on the proposed $O_3$ critical levels and the smaller effects observed in the field. A review of $O_3$ exposure experiments performed with Mediterranean tree species has been carried out. Effects of $O_3$ on tree biomass or growth were used to derive flux-based response functions. Ozone critical levels are proposed for specifically assessing the risk of $O_3$ damage to Mediterranean forests.

# MICROLEIS DSS, A SUSTAINABLE LAND USE AND MANAGEMENT DECISION SUPPORT SYSTEM FOR MAXIMIZING CARBON SEQUESTRATION

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In order to predict the effects of land use change on soil carbon sequestration it is necessary to know the current land carbon stocks. The vegetation carbon stock accounts for an estimated 5 percent or less of total land carbon. On the contrary, the largest land carbon stock lies below ground as soil carbon stock. In this sense, there are some examples for the estimations on soil carbon stocks in UK, 9,800 Mt (millions of tones or Tg); Spain, 3,778 Mt; or Andalusia, 604 Mt. Although the vegetation carbon stock is relatively small, plant matter is the single most important source of carbon inputs to the soil. Each particular soil possesses a limited carbon storage capacity which is a function of the vegetation type, climate, hydrology, topography and the intrinsic characteristics of the soil. Soil organic carbon takes from decades to centuries to accumulate, but carbon losses that result from land use changes that accelerate biotic and abiotic carbon cycling can occur rapidly, within years.

Land use planning can consider strategies to prevent or reduce soil carbon loss as a result of land use and management changes. Also, it is necessary to consider soil carbon stocks and sequestration in the context of climate change and land degradation and their interactions.

MicroLEIS DSS is an agro-ecological decision support system for soil-specific planning of land use changes and soil management programs. The application of this technology aims to optimize biomass production and minimize environmental risks, and presently it is also priority to maximize carbon sequestration. In this sense, preliminary results are obtained for Andalusia region based on standard data base such as CORINE Land Cover, (CLC) and SEIS.net soil data bank as input data in order to be used through Europe.

# RESULTS FROM OZONE MONITORING AT LEVEL-II-PLOTS IN SAXONY

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Since 2001, the public enterprise Sachsenforst is measuring ozone, ammonia, nitrogen dioxide and sulphur dioxide with passive samplers at six ICP Forests Level-II-plots for the national data submission. Two regional monitoring plots have been included in these activities since 2003. The measurements are situated in altitudes between 170 and 840 m. a. s. l. in the open field near the forest monitoring plots.

In general regularly recurring increases of ozone concentration were observed in spring, maximum values in high summer and declines till late autumn/winter. The investigated plots can be grouped into two classes in terms of the ozone concentration in the summer months June to August: The measured values at the plots in the low range mountains are more than 30% higher than at the plots in the lowlands.

Ozone concentrations of more than 90µg/m³ were exceeded in 2003, 2006 and 2009 at the Level-II-plots Klingenthal, Olbernhau and Bautzen in the Ore Mountains and upper Lusatia.

In the lower regions mean concentrations above the critical level were not registered. The bi-weekly double-measurements from March to September 2009 and 2010 at two Level-II-plots located in Laußnitz and Olbernhau in the project "FutMon" show no significant differences between the exposed samplers.

Up to now neither in the higher and ridge-top altitudes of the Saxon low-range mountains nor in lowlands of Saxony optically visible damages on trees not found within the annual crown condition assessment (i. e. beech (*Fagus sylvatica) or* sessile oak (*Quercus petraea*)) and biannual harvesting of needles and leaves.

# THE IMPACT OF CLIMATE CHANGE, OZONE AND NATURAL RISKS ON ALPINE FORESTS: ADAPTATION VERSUS MITIGATION POLICIES

***P. Angelini*[1], *A. Ballarin-Denti* [2*], *L. Cetara*[3]**

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The Alpine forests are subjected to climate changes more pronounced than in the rest of Europe. The impact of climate-dependent natural risks is amplified by their intrinsic vulnerability and the weakness of territorial resilience factors. At the same time, Alpine forests can provide a relevant carbon sink and stock capacity, able to compensate a significant share of the local GHG emissions. Their sink potential is however subjected to the threat of elevated ozone levels, which are heavily dependent on precursores emission sources active in surrounding industrial areas and in the local road and highway systems.

Climate change mitigation policies, based on GHG emissions reduction, can hardly be effective in the Alpine territory, owing to its very limited contribution to the overall required reduction and to the related high marginal costs. On the contrary, adaptation policies represent a more promising approach to minimise climate change impacts, since they can be managed at local level, aimed at specific vulnerabilities and based on intrinsic resilience potential. A further advantage of climate change adaptation policies in based on their easier coupling to other sets of local policies addressed to decrease ozone precursor emissions deriving from road traffic and specific industrial sectors.

To be more cost-effective and co-beneficial, a comprehensive policy strategy targeted both to climate change adaption and ozone abatement, should rely on sound integrated risk assessment based on field investigations and appropriate modelling. A particular attention should be paid to risk threshold values, in filling existing knowledge gaps and in developing early warning systems aimed at integrated risk prevention and management. Natural risk impacts are better evaluated in relation to forest growth and land use changes, air pollution and other environmental stressors and good practices aimed at counteracting the effects of extreme meteorological events.

# SENSITIVITY OF BEECH TREES TO SURFACE OZONE AT MOST NORTHERN LATITUDE OF THEIR OCCURRENCE

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For more than a few decades ozone has been considered to be one of the key phytotoxic air pollutants affecting forest health in North America and South and Middle Europe, which since the beginning of the 21st century has become a pollutant of great concern.

European beech is among the most important and sensitive to ozone exposure forest species response of which to climate change including ozone is well documented. However, little is known about the effect of local factors on beech invasion into native forest stands on the most northern latitude of their possible occurrence. The present study focuses on beech stands planted in western part of Lithuania, where climatic factors are most favourable for beech. Dendrochronological method was used to establish correlations between radial growth variability in beech stands and ambient ozone exposure and meteorological factors. Data on ozone concentrations since 1982 was obtained from Preila EMEP station which is located about 40 km from the experimental sites. Over this period annual mean ozone concentrations showed a trend towards increasing (0.42 ppb per year, $p<0.001$). Obtained data indicated that surface ozone, mean monthly concentrations of which often were below phytotoxic levels is among key factors resulting in beech stand productivity at northern latitude of their occurrence.

# MONITORING OF OZONE AND ITS PHYTOTOXIC EFFECT ON FOREST ECOSYSTEMS IN ROMANIA

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Since 1997, ambient ozone ($O_3$) concentrations in the Romanian forest ecosystems were measured on passive sampler networks. At the beginning (1997-1999), 6 out of 26 Carpathian network's ozone monitoring sites have been located in Romania. Furthermore, in the Romanian forest areas, additional 3 ozone and other phytotoxic pollutants monitoring networks were installed in Retezat (11 locations) and Bucegi – Piatra Craiului (22 locations) ILTER sites. Since 2007 in four intensive monitoring plots (core plots) measurements of ozone concentrations were developed. All these monitoring sites have a good spatial distribution throughout the study areas.

During the growing season, ozone concentrations were measured using Ogawa® passive samplers, both monthly (in Retezat and Bucegi – Piatra Craiului) and biweekly (in intensive monitoring network).

The seasonal and monthly (or biweekly) means of ozone concentrations were generally situated under 50 ppb. The spatial and temporal ozone distributions in the monitored areas were relatively stable (the coefficients of variation ranged between 17% and 22%). The lowest mean values were generally registered in Retezat National Park (< 40 ppb) and the highest in Bucegi Natural Park (~ 55 ppb). Monthly ozone concentrations means are positively and significantly correlated with altitude, following linear or polynomial trends.

In the year 2010, AOT40 was higher than the critical level for forest trees at Stefanesti (15 ppm h) core plot according to ICP Modeling and Mapping methodology (reference period April 1$^{st}$ –September 30$^{th}$), but smaller (6.4 ppm h) than the critical threshold established for vegetation according to the European Commission AOT40 Directive (reference period May 1$^{st}$ – July 31$^{st}$).

At monitoring network level, the ambient ozone had no significant influence on share of damaged trees. In addition, in Bucegi Mountain's forests, ozone concentrations had no effect on annual volume growth losses due to actions of different stress factors. In fact, the ambient ozone concentrations in Romanian forested areas are below the levels considered to be toxic for main tree species.

# OZONE FLUX-RESPONSE RELATIONSHIPS FOR ASPEN AND ASPEN-BIRCH FORESTS

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The negative impact of tropospheric ozone ($O_3$) on plant productivity is mediated by the phototoxic $O_3$ dose, i.e. the accumulated flux of $O_3$ through stomata. Based on sap flux and aboveground productivity data, we derived stand-level $O_3$ dose-response relationships for pure aspen and mixed aspen-birch communities in the factorial free-air $CO_2$ x $O_3$ experiment near Rhinelander, Wisconsin (Aspen FACE). Our three main objectives were to determine whether or not effects on $O_3$ dose caused by (1) community type, (2) $CO_2$ treatment and (3) among-year variation in weather translated into similar effects on annual productivity. Dose-response relationships derived were also compared with relationships obtained in meta-analyses of open-top chamber experiments with European tree species.

# FROM AOT40 TO OZONE FLUX FOR FOREST TREES – PROGRESS AND UNCERTAINTIES

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The critical level for ozone has been previously based on the external ozone concentration, expressed as the AOT40 (ozone concentrations accumulated over a threshold of 40 ppb during daylight-hours). However, it is widely recognised that ozone uptake through the stomates expressed as the phytotoxic ozone dose (POD) would be a more biologically meaningful and as such a better predictive tool to characterise the threat ozone poses on vegetation. To be able to identify a critical ozone flux, flux models for stomatal uptake are required to establish species-specific dose-response relationships. During the last years, progress has been made in the development of uptake functions and dose-response relationships for the most important forest trees using the $DO_3SE$ (Deposition of Ozone for Stomatal Exchange) model. Within the framework of the ICP vegetation, the dose-response relationships used for the derivation of the AOT40 critical levels for forest trees were reanalysed using these new flux-based uptake functions, and critical flux levels were defined at an ICP vegetation workshop in 2010. The new flux-based dose-response relationships show a clear improvement compared to the previously used concentration-based relationships for birch and beech, i.e. the relation to the observed effects was more evident. In the case of Norway spruce, however, only a slight improvement could be detected using the flux-based approach. Analysis of sap flow data suggest that the function used in $DO_3SE$ to define the effect of vapour pressure deficit (VPD) on the stomatal uptake of ozone by Norway spruce trees may need a revision. This improvement is important to support the effect based air pollution abatement policy of UNECE and EU.

# MODELLING OF SOIL MOISTURE USING $DO_3SE$ TO DETERMINE OZONE FLUXES TO EUROPEAN FOREST TREES

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The $DO_3SE$ (Deposition of $O_3$ for Stomatal Exchange) model is an established tool for estimating ozone ($O_3$) deposition, stomatal flux and impacts to various vegetation types across Europe. $DO_3SE$ has been embedded within the EMEP photochemical model to relate the risk of vegetation damage to $O_3$ precursor emissions for use in policy formulation. A limitation of flux-based risk assessments has been the assumption that soil water deficits are not limiting $O_3$ flux due to the unavailability of evaluated methods of modelling soil water deficits and their influence on stomatal conductance ($g_{sto}$). This has posed limitations on the estimate of $O_3$ flux to forest trees.

This paper describes the development and evaluation of a method to estimate soil moisture status and its influence on $g_{sto}$ for various forest tree species. The soil moisture module uses the energy balance method according to Penman-Monteith to drive water cycling through the soil-plant-atmosphere system and empirical data describing $g_{sto}$ relationships with predawn leaf water status to estimate the biological control of transpiration.

Four different methods to estimate the control of transpiration are evaluated against field data for Norway spruce, Scots pine, beech and Holm oak collected from 11 sites across Europe and North America. Modelled estimates of soil moisture, $g_{sto}$ and transpiration show a reasonable agreement with observed data, with the timing and magnitude of soil drying events and the subsequent reduction in transpiration generally being captured well. However, sensitivity analysis showed that model performance was dependent upon local parameterisation of key model drivers such as $g_{max}$, LAI, root depth and soil texture.

These results suggest that less complex modelling methods that relate $g_{sto}$ directly to soil water content and potentials are capable of providing reasonable estimates of soil moisture and subsequent influences on $g_{sto}$ and hence $O_3$ flux for forest trees across Europe.

# BELOWGROUND HORMONAL INTERACTIONS IN PLANTS & OZONE

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Plant hormones are essential factors for plant development and are important signals for the interaction of plants with microbes. Mycorrhiza formation and root elongation result from a permanent tuning between resource allocation by the plant and the production of growth substances by the fungi. The role of different classes of hormones in the mycorrhizal symbiosis is just beginning to unravel. Auxins might be necessary for root growth, while cytokinins might be involved in recognition as well as in establishment of functional mycorrhiza, but for most hormones functional analysis is missing.

Stress related hormones such as abscisic (ABA) and salycilic acids (SA) increase while jasmonic acid (JA) decreased in EM compared to nonEM-roots. Similar effects have been observed in AM plants, although the magnitude of the response seems to be dependent on the plant and fungal species involved in the symbioses and on the stress factor (water, nutrients, ozone). This suggests that EM and AM recruit signaling pathways to influence plant stress responses. Salt exposed EM roots showed stronger accumulation of myo inositol, ABA and SA than non-EM roots. EMs activated stress related genes and signaling pathways apparently leading to priming of pathways conferring abiotic stress tolerance. Activation of plant defence mechanisms by production of hormonal-induced UV-B-absorbing compounds has been reported for AM and EM. As a general rule these changes in the leaf chemical composition and physiology are associated with higher partition of carbon to the roots and with a change in the root mycorrhizal symbionts.

Since it is now accepted that changes in the plant hormonal balance is part of the mycorrhizal mechanisms involved in improved tolerance of mycorrhizal plants to stress conditions, the aim of this work is to review data on the influence of mycorrhizal symbiosis (endo and ecto) on plant hormonal balance with potential implications on plant tolerance to increased ozone levels. Finally some thoughts about below- to aboveground communication in response to ozone levels will be given.

# HOW IS TREE PRIMARY PRODUCTIVITY RELATED TO CLIMATE AND ATMOSPHERIC POLLUTION IN ITALY?

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Recent estimates suggested that human activities have at least doubled the rate of annual input of inorganic nitrogen (N) into the terrestrial N cycle, and increases in N inputs are expected to continue to grow during the next few decades as a result of human population growth, increasing fossil fuel consumption, and increasing reliance on industrially produced fertilizers (Galloway et al., 2004 and Throop et al., 2004). The same trend is expected for tropospheric ozone pollution, expecially in Medietrranean area. Forest productivity has been hypothesised to be affected by nitrogen deposition (Nadelhoffer et al., 1999), ozone pollution (Paoletti, 2006) and temperature (Myneni et al., 1997). Measures to reduce nitrogen emission have begun to take effect, and deposition has a downward trend at least in some areas. The same consideration is not realistic for ozone pollution.

Several statistical techniques have been applied to evaluate different roles of climatic data and pollutant depositions affecting to the primary productivities of tree populations of *Fagus sylvatica*, *Quercus cerris* and *Q. ilex*, growing in Italy. It is known that relationships between physiological parameters and photosynthesis are non linear; as a consequence, here we have adopted statistical non linear techniques such as Generalised non linear Models (GLZ) and Generalised regression Models (GRM). Results demonstrated that combined factors were more affecting primary productivity than single ones. Furthermore, it was evident that each plant species was specifically sensitive to nitrogen deposition or ozone concentration in relation to the growing locations (Northern to Southern Italy) and to the oxidised/reduced forms of nitrogen deposition. These aspects could be most important for defining statistical models to predict primary productivity by taking into account for pollutants and climatic scenarios also.

# OZONE CONCENTRATIONS AND DIEBACK OF PINUS MUGO SUBSP. UNCINATA STANDS IN THE CATALAN PYRENEES

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Ozone mixing ratios have usually exceeded semi-natural vegetation and forest protection thresholds in the Catalan Pyrenees during the last years. Although mountain pine (*Pinus mugo* subsp. *uncinata*) stands become the dominant forest in this mountainous range from 1600 to 2400m a.s.l., and dieback in some stands has been reported by foresters, the effect of ozone on this species has never been studied so far. Our aim was to assess the health status of mountain pine stands in the Central Catalan Pyrenees and determine any possible relationship with ozone mixing ratios. Three sites were studied along an altitudinal gradient in the county of La Cerdanya at 1500m, 1900m and 2200m a.s.l. We measured defoliation, occurrence of ozone visible symptoms, mortality and population structure. Tree water status was measured twice a year and ozone mixing ratios every fortnight in 2008 and 2009. Defoliation, percentage of trees showing ozone visible symptoms, mortality and ozone mixing ratios increased with altitude as well as soil moisture and relative water content in needles. Tree density was lower at the highest site which excludes dieback and mortality due to competition. These preliminary results point to ozone affectation of mountain pine. In fact, the ozone visible symptoms found in our study sites have also been found in mountain pine saplings fumigated with ozone during two growing seasons in the Swiss Alps. Nonetheless, the results do not exclude that other factors might be also responsible of the general mountain pine dieback observed at high altitude in the Pyrenees. Hence, further research should be conducted to detect whether there are other implied factors.

# MODELLING PAST AND FUTURE IMPACTS OF CHANGES IN CLIMATE, NITROGEN DEPOSITION, OZONE AND $CO_2$ EXPOSURE ON CARBON SEQUESTRATION IN EUROPEAN FORESTS

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Carbon sequestration in forests and forest soils is influenced by various drivers including changes in climate (temperature and water availability), nutrient (nitrogen, base cations, phosphorous) availability, carbon dioxide ($CO_2$) exposure and ozone ($O_3$) exposure. We modelled the combined effects of past and expected future changes in those drivers on carbon sequestration in European forests for the period 1900–2050. Forest inventory data around 1980 (EFI database) were used to assess reference forest growth rates, which were then modified for other years by factors accounting for deviations in climate and air quality, including the phytotoxic ozone dose, compared to 1980. The impacts were evaluated using various assumptions with respect to interactions between drivers. Impacts of soil macro-nutrient availability (P, Ca, Mg, K) were also accounted for. The forest growth model was coupled to a soil model predicting nutrient availability and soil carbon sequestration.

Historical meteorological data were taken from a high resolution European data base that contains monthly values of temperature, precipitation and cloudiness for the years 1901–2000. Oxidised and reduced N deposition was calculated with the EMEP model. In addition, the phytotoxic ozone dose (POD) was calculated by the EMEP model, incorporating the DO3SE deposition module, which parameterises ozone uptake as functions of phenology, light, temperature, humidity, and soil moisture. Historic $NO_x$, $NH_3$ and VOC emissions were taken from Lamarque et al., 2010. For the future (2010-2050) we used two scenarios for deposition (current legislation and maximum technically feasible reductions) and two climate scenarios (no change and SRES A1 scenario). Results of the simulations will be presented during the conference.

# UNDERSTANDING CLIMATES INFLUENCE ON OZONE DEPOSITION AND IMPLICATIONS FOR HUMAN HEALTH AND ECOSYSTEM IMPACTS: A CASE STUDY FOR THE UK

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The extremely strong connection between $O_3$ deposition to vegetated surfaces (which represents the primary sink for atmospheric $O_3$) and ambient $O_3$ concentrations has not been fully investigated at the UK, or in fact any, regional scale. The connection between $O_3$ deposition and $O_3$ concentration has been demonstrated in the UK during the recent hot summers of 2003 and 2006 when drought effectively "turned off" the $O_3$ vegetation sink leading to an increase in atmospheric $O_3$ concentrations. Under these situations, changes in $O_3$ deposition will have a substantial effect on human exposure to $O_3$ with consequences for human health. This paper brings together the $DO_3SE$ $O_3$ deposition model with an $O_3$ photochemical model (CMAQ; hosted by Kings College in London) to combine tools that have been developed respectively for ecosystem and human health risk assessment. The outputs achieved using this new tool (CMAQ-$DO_3SE$) which is capable of assessing the combined effects on ozone on human health and ecosystems, are investigated with a view to answering the following questions: i) how much of the $O_3$ damage caused within the UK is a result of domestic emissions; ii) to what extent does climate influence $O_3$ formation and $O_3$ deposition and hence the mass balance of $O_3$ in the atmosphere and; iii) how does this climatic influence translate into effects on human health and ecosystems. As such, the development of this new "CMAQ-$DO_3SE$" tool allows, for the first time, integrated assessments of $O_3$ risk to ecosystems and human health in a manner consistent with approaches currently used by the UNECE LRTAP, but tailored for UK conditions. Analysis of the results obtained from application of this tool are discussed in relation to understanding the role of emission reduction policies within the UK, but also in terms of understanding the contribution to domestic $O_3$ concentrations from $O_3$ and precursor emissions from outside the UK. The development and application of this tool is also considered in relation to benefits for the LRTAP process through an assessment of the importance of recent improvements made to the $DO_3SE$ model (e.g. the new $DO_3SE$ soil moisture module) that have relevance at the European scale.

# FLUXES OF OZONE FROM AN ORANGE ORCHARD: PARTITIONING IN STOMATAL AND NON-STOMATAL DEPOSITION

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Orange plants are widely cultivated in many countries experiencing Mediterranean climates, where they are often exposed to high levels of tropospheric ozone ($O_3$) due to their exposure to polluted airsheds. *Citrus* take up $O_3$ through their stomata and emit biogenic volatile organic compounds (BVOC), which, together with NO, can contribute to non-stomatal $O_3$ removal through fast gas-phase reactions with $O_3$. The study was performed in a Valencia orange orchard in Exeter, California. From fall 2009 to winter 2010, $CO_2$, water, ozone & BVOC fluxes, were measured continuously *in situ* with specific sensors (e.g. fast ozone analyzer and Proton Transfer Reaction Mass Spectrometer) using the eddy covariance techniques. Meteorological parameters (PAR, temperature and relative humidiy of the air, soil, and leaves) necessary to model other important sinks of ozone deposition (NO, cuticles, soil) were also recorded continuously. The orchard represented a sink for ozone, with uptake rates on the order of 10 nmol $m^{-2}$ $s^{-1}$ during the central hours of the day. We found that stomatal uptake was a minor fraction of the total ozone deposition in the orchard, in favor of non-stomatal $O_3$ deposition phenomena. We show how these $O_3$ deposition sinks changed in magnitude during the year in response to environmental parameters. Our research may help building more robust metrics for ozone-risk assessment, as well as help understanding the role of *Citrus* in the complex oxidation mechanisms taking place in the polluted atmosphere of Mediterranean countries where this crop is cultivated.

# ASSESSING OZONE HAZARDS TO NATURAL FORESTS IN THE ALPINE AREA. COMPARISON OF A WIDE SCALE MAPPING TECHNIQUE WITH LOCAL PASSIVE SAMPLER MEASUREMENTS

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MANFRED is an Interreg IIIb project evaluating the best management policies to adapt Alpine forest to climate change. Ozone exposure under an alternate climate regime is one hazard to be taken into account. In order to estimate ozone fluxes we need to assess the diurnal cycle of ozone concentrations at a spatial resolution matching the complexity of the terrain. In this paper we use ozone monitoring data from the whole Alpine Space as compiled by the EEA (AirBase) for spatial interpolation of hourly ozone concentrations. Mapping uses geostatistical techniques for each hour in the growing season for the years 2003-2007 at a 1x1 $km^2$ resolution. The interpolation scheme considers the effects of relative altitudes on the diurnal ozone cycle as previously described by Loibl et al. (1994). For the year 2010 this exercise was repeated for a sub-region based on data from Italian networks (Lombardy region and Trentino province).

In order to better understand the impact of spatial diversity, ozone measurements have been performed at a finer scale in 11 sites in Valle Camonica (Italy). Both passive samplers and a mobile lab were operated between 1st April 2010 and 8th October 2010, as well as a flux tower was installed over a larch forest at the upper timberline in July 2010. Twenty-six weekly mean concentrations measured every week at 11 Valle Camonica sites by passive samplers have been used to map local ozone distributions.

Comparing the local map estimations based on passive sampler results to the spatial interpolations allows an evaluation of the need to include small scale information and helps validate the regional data as a basis for assessing AOT40 values and ozone flux data. This is a precondition to further interpret model results on potential ozone hazards under an altered climate based on regional climate models.

# ERRORS IN OZONE RISK ASSESSMENT USING STANDARD CONDITIONS FOR THE CONVERSION OF OZONE CONCENTRATIONS FROM PASSIVE SAMPLERS IN MOUNTAIN REGIONS

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Passive samplers are often employed to collect ozone concentration data in remote areas such as mountain forests (e.g. Sanz et al., 2007); such data can be subsequently used to assess the potential ozone risk for vegetation by estimating the AOT40 exposure index (e.g. Gerosa et al 2007).

Lab sheets typically report ozone concentrations in mass unit per cubic meter (e.g. $\mu g \cdot m^{-3}$) and these values are then converted to ppb since this unit let to appreciate the relative abundance of the pollutant molecules with respect to the other molecules in ambient air. Moreover the AOT40 index to assess the vegetation exposure to ozone is based on this unit.

Conversion from mass unit to ppb is usually carried out applying a *standard* coefficient of 1.96, referred to SATP conditions (Standard Ambient Temperature and Pressure = 25°C and 100 kPa). However, such conditions can greatly vary with the elevation and the average temperatures of the measuring periods at each site. Moreover the temperature at a certain elevation site depends on the temperature gradient which is not always known. As a consequence, the blanket application of a standard coefficient may lead to substantial errors in reporting and mapping ozone concentration and in the assessment of potential risk in terms of AOT40, particularly in mountain regions.

In this paper, examples from two mapping exercises are presented, with regards to the ozone concentrations and the AOT40 estimation errors in the Italian Alps. Adiabatic lapse rates for temperature and pressure were applied to derive P and T at each given elevated station, starting from the T and P values recorded at some station located in the valley bottom. The error magnitude of concentration estimations ranged from 0 to 12% within the forest vertical distribution gradient, but much greater errors were recorded for AOT40 estimation during daylight hours.

# EVALUATION OF THE OZONE-RELATED RISK FOR BEECH FORESTS

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Tropospheric ozone poses a critical hazard and problem to present and future forest ecosystem services such as fiber and timber production or carbon storage capacity. In the recent years a stomatal flux based risk evaluation methodology at leaf level was established in the context of the Convention on Long-Range Transboundary Air Pollution for crops, forest trees and grasslands (LRTAP Convention, 2010). According to the Directive 2008/50/EC the assessment of the ozone-related risk at local scale for relevant ozone-sensitive biological receptors have to be based on parameters routinely measured by the air quality monitoring networks and, where necessary, on micrometeorological parameters routinely measured by the national weather services. Furthermore, the ICP Forests level II monitoring site concept requires a methodology for the evaluation of ozone-related risk at forest stand level.

The LRTAP Convention's stomatal flux parameterisation is based on ozone concentrations at canopy top, which are not measured by the air quality monitoring networks or at the ICP Forests level II sites, normally. Therefore, the ozone concentrations measured at a nearby monitoring station in a reference height above ground must be transformed to that at the top of the forest canopy applying an appropriate deposition (SVAT, soil-vegetation-atmosphere-transfer) model.

The ozone fumigation experiment "Kranzberger Forst" near Freising in southern Bavaria, Germany, on mature trees of beech in a mixed stand with Norway spruce over 8 years (2000-2007) offers the unique opportunity to improve and validate the LRTAP Convention's parameterisation of stomatal uptake for sunlit beech leaves, the stomatal flux-based response function as well as the SVAT model $FO_3$REST developed for the evaluation of ozone-related risk for mature beech stands.

The presentation gives an overview of the proposed ozone risk assessment approach for beech stands at local scale. First results of the validation study will be reported.

LRTAP Convention, 2010. Mapping Manual 2004. Manual on methodologies and criteria for modelling and mapping critical loads & levels and air pollution effects, risk and trends. Chapter 3. Mapping critical levels for vegetation. 2010 revision. (available at http://icpvegetation.ceh.ac.uk)

# SPECIES-SPECIFIC RESPONSES OF TREE SAPLINGS TO A COMBINATION OF INCREASING BACKGROUND OZONE AND DROUGHT

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Saplings of alder, ash, beech, birch, hazel and oak were exposed to an episodic ozone regime in hemi-spherical glasshouses (solardomes) for 12 weeks. Ozone exposure over the 12 week period ranged from a mean of ca. 16 to 74 ppb. All trees were well-watered for the first five weeks. Thereafter, half of the trees remained well-watered, whereas the other half were subjected to a moderate drought and received approximately 45% of the amount of water compared to the well-watered treatment.

Species-specific responses to ozone, drought and the combination of ozone and drought were observed. Due to differences in resource partitioning under stress conditions, ranking of sensitivity of these species to ozone and drought was dependant on the response measured. Several of the species maintained their leaf biomass at the expense of stem and/or root biomass. Large reductions in root biomass with elevated ozone were found for some species, which might affect the ability of these species to survive subsequent drought conditions. The observed biomass effects might affect potential carbon sequestration in forests in a future climate with higher ozone concentrations and increased frequency of drought.

Although alder only showed a slight decrease (10%; not significant) in root weight with increasing ozone for both well-watered and droughted trees, there was a large impact of both ozone and drought on the biomass of root nodules. The biomass of root nodules was reduced with elevated ozone exposure and in addition, there was a large reduction in biomass of root nodules in droughted compared to well-watered trees. The reduced nodulation of alder with increased ozone and drought might reduce the ecosystem service of nitrogen cycling for soil formation provided by this pioneer species.

# STOMATAL CLOSURE IS SLUGGISH IN POPLAR LEAVES WITH OZONE VISIBLE INJURY

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Leaf gas exchange was measured to characterize a relationship between stomatal response to a severe water stress induced by severing the leaf and degree of ozone visible injury. Measurements were carried out on an ozone sensitive poplar clone (Oxford, *Populus maximoviczii* Henry × *berolinensis* Dippel) under field conditions after exposure to 25ppm AOT40 h. The reduction of stomatal conductance after leaf severing was significantly slower in injured leaves than in healthy (control) leaves, because both the time to respond to the closing signal and the rate of closing after the signal was received were sluggish. A non-linearity of the response was observed with increasing ozone visible injury. The slower stomatal response in injured leaves is suggested to be a symptom of accelerated leaf senescence. The stomatal sluggishness may increase transpiration and ozone uptake under water stress conditions.

# AMBIENT OZONE PHYTOTOXIC POTENTIAL FOR CZECH MOUNTAIN FORESTS

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The contribution estimates ozone phytotoxic potential for forests in the Czech Republic using the AOT40 exposure index. The trends for selected rural sites during 1994–2008, and maps showing the AOT40 spatial distribution are presented. The maps are based on real-time monitoring ozone data from 24 sites, the mapping method uses the linear regression model with subsequent IDW interpolation of its residuals. The high ozone levels recorded in 1994 and 1995 decreased substantially after the decrease of ozone precursor emissions (NOx and VOC) in Europe during the 1990s. The high ozone levels recorded later (2003, 2006) resulted from meteorological conditions extremely conducive for formation of ozone and its accumulation in atmosphere. Our results indicate high phytotoxic potential for most of the CR. The interannual variability is considerable. The highest AOT40 values were recorded at the Sous site (Jizerske hory) in 1994 (39 ppm.h), at the Prebuz (Krusne hory) and Rychory (Krkonose) sites in 2003 (38 ppm.h) and at the Bily Kriz (Beskydy) site in 1995 (37.7 ppm.h). The curve of AOT40 increase for individual vegetation seasons differs significantly regarding the meteorology. Critical level for forests is usually exceeded rather early, in the beginning of the vegetation season, generally in May. In vegetation periods with meteorological conditions conducive for ozone formation the critical level is exceeded more folds as compared to the vegetation periods with non-conducive conditions; nevertheless it is exceeded at all stations always since the beginning of the ozone monitoring in the CR in 1993. In extremely hot and dry 2003 vegetation season, the critical level for forests was exceeded over the 31 % of the Czech forested area even more than 6 times. In spite of ozone levels measured over the Czech territory, no significant forest injury, ascribed explicitly to elevated ozone exposure, has been reported so far.

# DETOXIFICATION CAPACITY OF POPLAR EXPOSED TO OZONE: INTRASPECIFIC VARIABILITY OF THREE POPULUS X EURAMERICANA GENOTYPES

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It is well admitted that to apprehend the critical level of ozone susceptible to damage vegetation, the effective ozone flux, corresponding to the non-detoxified ozone molecules, needs to be determined (Musselman *et al.*, 2006; Wieser and Matyssek, 2007; Dizengremel *et al.* 2008). This concept firstly integrates the stomatal conductance to appreciate the amount of ozone entering in the leaf. Additionally it needs to consider the capacity of the cell to neutralise ROS which could be estimated by the levels of antioxidant molecules but also by the activity of enzymes generating NADPH, useful to regenerate ascorbate and glutathione (Dizengremel *et al.*, 2009). In a preliminary experiment, we have selected 3 *Populus x euramericana* genotypes (Carpaccio, Cima and Robusta) for their distinct response to chronic ozone exposure (extent of necrosis and leaf loss). Subsequently several metabolites (ascorbate, glutathione, carotenoids, polyphenols) as well as the activity of NADPH delivering enzymes (malic enzyme, isocitrate dehydrogenase, glucose-6-P-dehydrogenase, glyceraldehyde-3-P-dehydrogenase) have been measured in poplar genotypes exposed to 120 ppb ozone for 21 days. The results which were expressed relative to the instantaneous ozone flux through stomata, underlined intraspecific variability of these poplar genotypes in their capacity to detoxify.

# EMISSIONS OF VOLATILE ORGANIC COMPOUNDS (VOCS), EXPRESSION OF VOC-RELATED GENES, GAS EXCHANGE AND GROWTH OF NORWAY SPRUCE SAPLINGS EXPOSED TO ELEVATED OZONE AND ELEVATED TEMPERATURE

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Volatile organic compounds (VOCs) emitted by plants participate physical and chemical atmospheric processes, and have significant feedback to climate change. VOCs, especially terpenoids, can protect plants from stress effects by acting as cellular antioxidants or reacting in the air with e.g. ozone. Soil-grown Norway spruce (*Picea abies*) saplings were exposed to the elevated ozone (1.4 x ambient ozone concentration) and elevated temperature (0.9 ºC + ambient daytime, 1.6 ºC + ambient at night) alone and in combination in open-field conditions 9 June - 30 September 2009. AOT40 was 4409 ppbh in elevated ozone and 119 ppbh in ambient ozone. Isoprene, monoterpenes and sesquiterpenes were collected with the dynamic headspace collection technique. In July, when ozone concentrations and temperature were high, ozone doubled the total terpenoid emissions by increasing the emissions of many monoterpenes and sesquiterpenes. Elevated temperature changed the VOC profile by increasing the emissions of oxygenated monoterpenes, but did not influence on total emissions. In August, when ozone concentrations were low and temperature normal to the season, control treatment had the highest terpenenoid emissions. Accordingly, elevated ozone and temperature reduced the expression of the genes involved in early steps of plastidial monoterpene synthesis: elevated ozone reduced the expression of *DXR* (1-deoxy-D-xylulose 5-phosphate reductoisomerase) and elevated ozone, elevated temperature and their combination reduced that of *DXS2b* (1-deoxy-D-xylulose 5-phosphate synthase type II). Elevated temperature reduced the stem growth, net photosynthesis and stomatal conductance. Results suggest that 1) elevated temperature and ozone differently alter the VOC composition of Norway spruce, 2) emissions are dependent on severity of the stress, 3) elevated temperature and ozone regulate VOC emissions by affecting the genes *DXR* and *DXS2b*. Compared to previous studies, temperature-responses of Norway spruce were opposite to many other species. Decreased gas exchange may have reduced the carbon available for VOC synthesis and growth.

# THE POTENTIAL OF BEECH (*FAGUS SYLVATICA* L.) FOR COPING WITH DROUGHT AND OZONE IMPACT AS COMPONENTS OF CLIMATE CHANGE

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The objective of the introduced research is to evaluate the capacity of beech (*Fagus sylvatica*) for coping with predicted climate change conditions across a broad ecological range of forest sites in Bavaria/Germany. Focus is on water availability (expected to become limited through frequent and severe drought incidences) in combination with ozone impact (predicted to stay high), as affecting tree growth.

Eight of the ten study sites are part of the Level II network, two are intensive research and monitoring sites (Kranzberg Forest and Forellenbach UNECE Monitoring), being located in the important forest regions of Bavaria and well documented, concerning stand history and growth conditions. Since 2010, xylem sap flow is measured at each of the study sites along with the relevant climatic and edaphic factors. Transpiration data provide tree and stand-level water demand and the basis for assessing the stand water balance, and in parallel, stomatal ozone uptake is derived to determine the phytotoxically relevant ozone dose. Datasets are related to annual stem growth.

The relationship of stem growth and its water-use efficiency to water availability and ozone uptake characterizes the stress tolerance of beech as a basis for concluding about the future potential of this tree species in forestry. Preliminary results from 2010 reflect the inherent coupling between the trees' transpiration and ozone uptake in dependence on the climatic and edaphic site conditions, stressing incipient water limitation as a determinant of the factorial interrelationships. The overall aim of the study is the identification of site-overarching functionalities in tree and stand performance under drought and $O_3$ stress scenarios, enabling for derivations of indicators in view of silvicultural risks concerning beech in an exacerbating environment.

# INFLUENCE OF GREENHOUSE GASES ON EPICUTICULAR WAXES OF POPULUS TREMULOIDES MICHX. : RESULTS FROM AN OPEN-AIR EXPOSURE AND A NATURAL $O_3$ GRADIENT

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Epicuticular waxes of three trembling aspen (*Populus tremuloides* Michx.) clones differing in $O_3$ tolerance were examined over six growing seasons (1998–2003) at three localities (Rhinelander, WI -clean and control site Kalamazoo, MI -moderate pollution loading and Kenosha, WI -high pollution loading) in the Lake States regions of the USA and at the Aspen FACE site in Rhinelander, WI. Differences in epicuticular wax structure were determined by scanning electron microscopy and quantified by a coefficient of occlusion. Statistically significant increases in stomatal occlusion occurred for the three $O_3$ bioindicator sites as we predicted with the higher $O_3$ sites having the most affected stomata for all three clones as well as for all treatments including elevated $CO_2$, elevated $O_3$, and elevated $CO_2$ + $O_3$. The results suggest that $O_3$ pollution of the Kenosha and Kalamazoo sites show significant negative impact on epicuticular waxes of aspen and these impacts are the most severe on the most $O_3$ sensitive clones. We recorded statistically significant differences between aspen clones, between sampling period (spring, summer, fall) and localities between Rhinelander, Kalamazoo and Kenosha. However, we found no statistically significant differences in stomatal occlusion between treatments at Aspen FACE.

# OZONE-RELATED CHANGES IN THE BIOMASS AND STRUCTURE OF THE MICROBIAL COMMUNITY IN MEADOW SOIL

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Elevated ozone ($O_3$) concentrations often reduce plant root biomass due to reduced below-ground carbon (C) allocation. As $O_3$ may also affect above-ground plant litter quantity and quality, it can have an indirect negative effect on the biomass and structure of the soil microbial community. We studied below-ground $O_3$ responses in two open-top chamber (OTC) experiments in which forbs, grasses and legumes typical of northern European hay meadows were grown in a soil low in nitrogen (N) and exposed to realistic $O_3$ levels of 40-50 ppb for three growing seasons. $O_3$ exposure decreased total, actinobacterial, bacterial, mycorrhizal (arbuscular mycorrhizal fungi) and/or fungal biomasses, as analysed by the PLFA (phospholipid fatty acid) method, as well as fungal: bacterial PLFA biomass ratio in a mesocosm bulk soil and in the bulk soil beneath a pot-grown monocultured $N_2$-fixing legume, *Lathyrus pratensis*. No such changes were observed beneath the monocultured grass, *Agrostis capillaris*, though (positive) correlations between above- and below-ground plant biomasses and microbial PLFA biomasses were only found in the grass monoculture. The observed $O_3$-related changes in the mesocosm soil may thus mainly be explained by the relatively high abundance of legumes (*Trifolium medium* and *Vicia cracca*) in the community. The negative correlations between PLFA variables and bulk soil C:N ratio beneath *L. pratensis* further suggest that the below-ground effects were mediated by changes in plant litter quality and subsequently in soil C:N ratio and competition for N between plants and microbes. $O_3$ may also affect soil sulphur (S) and phosphorus (P) cycling. The decrease in the relative abundance of PLFA 17:1, which is associated with the genus *Desulfobulbus*, suggests decreased bacterial S reducer activity. The decreased concentration of readily soluble P suggests, in turn, a decrease of bacteria with phosphatase activity. The interactions between above- and below-ground factors will be further discussed in the meeting.

# OZONE UPTAKE AT LEAF AND ECOSYSTEM LEVEL BY NORWAY SPRUCE IN RELATION TO ENVIRONMENTAL CONDITIONS

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In a 35 years old Norway spruce dominated forest in Denmark ozone uptake at leaf level and ozone flux at ecosystem level was measured simultaneously during autumn. At ecosystem level ozone flux was measured with a profile system and at leaf level ozone uptake was measured with 12 climatized branch cuvettes installed in the top of the canopy on current years needles. The cuvettes allowed branch exposure to an ambient or enhanced ozone concentration, while tree crowns were exposed to ambient conditions. Ozone uptake at leaf level and ozone deposition at ecosystem level follow the same response patterns in relation environmental parameters, but reactions are more pronounced at leaf level. Net photosynthesis, at leaf level, is inhibited by ozone increased concentrations of ozone (10-30 ppb) and high solar radiation seems to amplify ozone effects.

# QUANTIFYING THE IMPACTS OF OZONE ON FOOD SECURITY

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The ICP Vegetation conducted a review of the current knowledge on impacts of ozone on food security in Europe and SE Asia, including effects on yield quantity and quality, food and feed safety, and effects in a changing climate (drought, $CO_2$, temperature interactions). The full report is available at http://icpvegetation.ceh.ac.uk. We report here on an updated crop sensitivity index and quantification of impacts in Europe using the flux-based methodology for wheat and tomato including economic costs of losses for 2000 and 2020.

A crop sensitivity index was developed based on dose-response functions for 7 hour mean ozone concentrations. The threshold 7h mean ozone concentration, above which ozone effects on yield become significant at $p \leq 0.05$ was determined using the confidence intervals and the regression line. The lowest thresholds were for soybean (8 ppb), wheat (9 ppb) and rice (11ppb), with several other crops having threshold concentrations below 40 ppb, including peas and beans (16 ppb), maize (25 ppb), alfalfa (26 ppb) and potato (29 ppb). We will also describe how a flux-based sensitivity index has been developed for those crops for which flux-effect relationships exist taking into account the effect of varying maximum stomatal conductance for each crop.

As part of this study, an economic impact assessment for Europe was conducted for wheat and tomato applying the ozone flux-based response functions and using their distribution data, ozone stomatal flux data per 50km x 50km EMEP grid, production statistics and costs. The impacts of ozone were quantified and mapped for 2000 and estimated for 2020 and 2030, based on the emission scenarios being used for the revision of the Gothenburg Protocol of the Long-range Transboundary Air Pollution Convention. The outcome of the economic impact assessment and the review will be discussed, including policy recommendations.

# LONG-TERM MEASUREMENTS OF OZONE FLUXES OVER A MIXED TEMPERATE FOREST

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Long-term fluxes of ozone ($O_3$) have been measured over a mixed temperate forest using the aerodynamic gradient method. The long term average ozone flux ($F$) was $-350 \mu g m^{-2} s^{-1}$ for the period 2000-2009 corresponding to an average $O_3$ concentration of 48 $\mu g\ m^{-3}$ and a deposition velocity $v_d$ of 9mm $s^{-1}$. Average nocturnal ozone deposition amounted to -180 $\mu g\ m^{-2}\ s^{-1}$, which was about one third of the daytime flux. Also during winter period substantial ozone deposition was measured. In addition, total ozone fluxes were found to differ significantly among canopy wetness categories.

Flux partitioning calculations revealed that the stomatal flux ($F_{st}$) contributed for 20 % to the total ozone flux. The concentration-based index AOT40 (accumulated dose over a threshold of 40 ppb) and the cumulated stomatal ozone fluxes were weakly correlated. The ozone deposition was found to be largely controlled by non-stomatal sinks, whose strength was enhanced by high friction velocities ($u_*$), optimizing the mechanical mixing of ozone into the canopy and the trunk space. The long-term annual geometrical mean of the non-stomatal resistance ($R_{ns}$) was 140 $s\ m^{-1}$ but lower $R_{ns}$ were encountered during the winter half-year due to higher $u_*$. The $R_{ns}$ was also subjected to a marked diurnal variability, with low $R_{ns}$ in the morning hours, when turbulence took off. We speculate that non-stomatal deposition was largely driven by quenching of ozone by VOCs or NO emitted from the crown or the forest floor. The presence of NO-emission at the nitrogen saturated site was indicated by upward fluxes of $NO_x$, which ranged between 15-30 ppb m $s^{-1}$.

# STATISTICAL UNCERTAINTY OF AMBIENT AIR QUALITY MEASUREMENTS AND THE POTENTIAL CONSEQUENCES FOR OZONE FLUX CHARACTERIZATION

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It is important to understand the overall nature of uncertainties associated with any type of measurement. One's ability to accurately interpret the meaning of any set of measurements is closely tied to understanding the associated measurement uncertainty. This is true with ambient air quality monitoring measurements. The assessment of uncertainty is often legally required. In the Province of Alberta, Canada, the Alberta Environment (AE) *Air Monitoring Directive (AMD) 2006* requires that uncertainty be addressed as part of the air monitoring and reporting process. Furthermore, AE requires that the procedures developed should be in line with the recent *ISO Guideline 20988.* We will present these concerns in relation to *Wood Buffalo Environmental Association* (WBEA) monitoring network within the *Athabasca Oil Sands Region* (AOSR) of Northeastern Alberta, Canada.

The measurements of air pollutants collected by the WBEA monitoring network contain spurious values which are the result of instrument uncertainty. Often such values are at or below the field effective Lower Detection Limit (LDL), which is usually different from the manufacturer's published LDL. Such measurements are recorded as negative values. Due to the remote location of some monitoring sites, the frequency of negative values often exceeds 50% of all measurements. A specific example of frequency distributions of NO and O3 concentrations at air monitoring station AMS1 in January 2010 are presented in the paper. We further present a statistical algorithm for treatment of spurious values due to instrument measurement uncertainty based on Weibull probability distribution.

The above described method works well for homogeneous pollutant concentrations with unimodal distribution. WBEA data often results in polymodal distributions and then the above algorithm fails. The polymodality of air quality data is caused by emission sources located along the very distinct corridors of prevailing winds. To identify the major homogeneous wind direction sectors, we use a polar graph known as a "***pollutant frequency median colored wind rose***" **.** Such procedure characterizes the *horizontal ozone flux*. The corresponding *vertical ozone flux*

is similarly assessed from similar graphs based on wind directions at different monitoring heights. After segregating the pollutant concentration data into sectors with similar air quality and meteorological characteristics, the data from these sectors often have a unimodal distribution which can be well approximated by a simple Weibull distribution for QA/QC of the data within that specific sector using methods described in the beginning of this paper.

# OZONE SENSITIVITY OF THE MOST IMPORTANT INDIAN CROP AND TREE SPECIES

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Economic growth and increasing global population will drive the processes that lead to increasing emissions of ozone precursors especially in rapidly developing Asia, Africa and Latin America. It has indicated that surface ozone levels are much above critial levels at most places in the Indian region. Ozone caused losses in gross primary production in India is expected to be roughly 20-30% between 1901 and 2100. However, species-level information about ozone sensitivity is very scarce.

The main purpose of our new developmet project is to screen the general ozone sensitivity of the most important Indian crop and tree species (rice, wheat, pea, prosopis, teak), genetic variation within the selected species, and to investigate the related tolerance mechanisms. The focus of this presentation will be in Indian tree species prosopis (*Prosopis julifera*) and teak (*Tectona grandis*) that are valuable for fuel and timber, respectively. In parallel, we collect ozone concentration data from the main agricultural and forested regions of India with passive ozone samplers. The results and datasets from India will be collected for ozone risk assessment. For ozone exposures and validation of results we utilize different and complementary methodological approaches: laboratory experiments in Finland and free-air approaches and natural field sites in India. The plants will be studied for growth, gas exchange, ozone uptake, chlorophyll fluorescence, nitrogen concentration, anatomical properties, phenomics, changes in metabolome and protein profiles, and activities of related key genes and enzymes.

# TESTING THE DO3SE MODEL ON A BROADLEAF MEDITERRANEAN FOREST SPECIES: IS THE MODEL WORKING?

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Ozone can cause injury to plants that may have both ecological and economic impacts, resulting in the need for standards to protect plants. Different criteria for protecting vegetation from ozone have been developed. Exposure-based critical levels do not include environmental conditions that affect plant responses to ozone, while the stomatal ozone flux approach provides an estimate of the critical amount of ozone entering the stomata and has the capacity of accounting for environmental influences on ozone uptake through the modelling of stomatal aperture. The stomatal flux approach is mostly important in situations where either high ozone concentrations are associated with environmental conditions that are unfavourable to uptake or low concentrations are associated with mild and wet condition. A main limiting factor in a successful application of the DO3SE model to Mediterranean forests is the poor availability of data about soil moisture.

The aim of this presentation is to suggest a new function for soil moisture effects on stomatal conductance of Mediterranean forests, by cross-comparing data from different sources.

Ozone flux was estimated in the broadleaf Mediterranean forest site of Castelporziano, in central Italy over several years, and was compared to NEE (net ecosystem exchange between vegetation and atmosphere) measurements that were carried out by eddy correlation. Because the photosynthetic process is mainly related to the variation of stomatal conductance, NEE can provide an independent way to assess the conductance simulated by the DO3SE model and is an index of plant physiological activity Data of NEE, soil moisture and other environmental variables were provided by the EUROFLUX network (Papale et al., 2006. Biogeosciences 3, 571; Reichstein et al., 2005. Global Change Biology 11, 1424). Different parameterizations were tested to select the most performing.

# RESPONSES OF *FRAXINUS EXELSIORS* SEEDLINGS TO AMBIENT OZONE IN URBAN AND MOUNTAIN AREAS BASED ON SOME PHYSIOLOGICAL CHARACTERISTICS AND ANTIOXIDANT ACTIVITY

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The dynamics of photosynthesis, transpiration, stomatal conductance and activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in three-year old seedlings of *Fraxinus exelsiors* L. were studied during a four-month period (June-August) of 2009. Seedlings were exposed to ambient ozone in urban (the Central City Park of Sofia - Bulgaria) and mountain (the Plana Mountain) areas. The experimental sites were located near to monitoring stations, providing data for ozone concentrations and meteorological parameters. During the experimental period ozone exposure at the mountain site was more than two times higher compared to those at the urban site. The changes of photosynthesis and transpiration in the seedlings, exposed in the city and the mountain locations, are complex. The main factor, leading to decline of photosynthesis in the city, was high concentration of ozone ($R^2$ = - 0,872*), while in the mountain a strong impact on the rate of photosynthesis had the environmental factors. In our experiments the seedlings of *Fraxinus excelsior* L. showed cautiously low stomatal conductance during the periods with high ozone concentration both in the urban and mountain areas. Stomatal conductance was negatively effected by ozone exposure ($R^2$ = -0,644*) at the station Plana. At the mountain site catalase activity was induced mainly by air temperature ($R^2$ = 0,651*), while relative humidity had a negative effect ($R^2$ = -0,863*). At both experimental sites correlation between the activity of SOD, ozone and climatic parameters were not established. The plants at the city site showed almost constant level of catalase activity. At the mountain site catalase activity was induced mainly by environmental factors. The parameters examined could be used in future studies with different trees species.

# REACTIONS TO ELEVATED OZONE IN THE PLANT-MYCORRHIZA-SOIL SYSTEM OF FOREST TREES

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Ozone interferes with plant carbon metabolism and changes carbon allocation below ground. As ectomycorrhizal fungi of forest trees receive about one third of assimilated carbon, they may react sensitively to reduced carbon allocated below ground. Ectomycorrhizal roots are the absorbing organs for nutrients and water which are taken up through their mycelia. The diversity of ectomycorrhizae is usually high and functions were shown to be redundant among ectomycorrhizal species. Our question was if and to which extent ozone changes ectomycorrhizal functioning, including microbial communities in the soil surrounding mycorrhizae (= mycorrhizosphere).

In the Kranzberg Forest experiment and in controlled experimental setups, the effect of elevated ozone on structural and functional changes in the mycorrhizosphere of Norway spruce and European beech were studied. Mycorrhizal and soil microbial communities showed shifts in their composition under ozone treatment. As parameters addressing functions of ectomycorrhizae in ecosystems, we measured potential enzyme activities on individual mycorrhizal tips. The measured enzyme activities (cellulases, chitinase, phosphatase, laccase) address functions related to nutrient release from organic sources.

We found changes in the composition and functioning of ectomycorrhizal communities that indicated an influence of ozone on nutrient cycles in forest soils.

# A DAILY MODEL OF ACTUAL EVAPOTRANSPIRATION FOR SOYBEAN GROWING INSIDE OTC UNDER CONTRASTING CONDITIONS OF OZONE AIR CONCENTRATION AND PLANT WATER STRESS

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The present study describes an analytical model, based on the Penman-Monteith equation, for estimating the daily evapotranspiration (ET) inside Open Top Chambers (OTCs). It includes two functions, making it possible to separately take into account the effects of water stress and of concentrations of air ozone. The input variables of the model are: (i) the diurnal values of global radiation and temperature, usually measured routinely in a standard agro-meteorological station; (ii) the daily values of the AOT40 index accumulated (accumulated ozone over a threshold of 40 ppb during daylight hours, when global radiation exceeds 50 $Wm^{-2}$); and, (iii) the periodic measurements of the predawn leaf water potential. These latter two variables were determined inside the OTC. The validation of the model was carried out in Mediterranean region during three successive years on 10 crop growth cycles of soybean cultivated in contrasted conditions of ozone concentrations (filtered, high level and low level) and water stress at the plant level. Tests were carried out in several chambers for each year and take into account the intra- and inter- year variability of ET measured inside the OTCs. The presented model had good performances, as demonstrated by the comparison between measured by water balance and calculated ET. On the daily scale, the slope of the linear regression between the measured and calculated ET under different water conditions are 0.94 and 1.05 for the filtered and unfiltered or enriched (high and low level) OTCs. On the seasonal scale, the mean difference between measured and calculated ET is equal to +3% and +10 % for the filtered and unfiltered OTCs respectively. This study also permits to evaluate the modification of ET for crops grown inside OTCs, as compared to ones cultivated in the open field.

# TREND ANALYSIS OF GROUND-LEVEL OZONE EXPOSURE AND OZONE UPTAKE OF OAKS IN EASTERN AUSTRIA FOR THE PERIOD 1990-2010

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The north-eastern part of Austria is a largely agricultural region with about 15 % forests. *Quercus petraea* and *Qu. robur* are the dominant species for silvicultural use. These oak forests have experienced serious stress episodes in the previous decades, leading to dieback symptoms of mature trees. Therefore a further reduction of the low proportion of forest cover is at risk. This study analyses the question to which extent surface-level ozone might be a stress co-factor for these oak forests.

This study had the objective to find or disprove evidence for long-term trends in ozone concentrations and in meteorological conditions determining the uptake of ozone into oak leaves. Therefore the ozone and weather data of two monitoring stations in rural environments close to the forest area were recovered for the period 1990-2010, quality-checked, partly homogenised and analysed for statistical trends. The data from April to October of each year were analysed for the whole period or in monthly resolution for the existence of significant time trends by linear regression analyses. Ozone data were corrected for atmospheric conductance and measurement height. Ozone exposure was calculated as AOT40 or 7 h-mean value. Ozone uptake calculations used the multiplicative Emberson-model [$g_{sto} = g_{max} * f_{phen} * f_{light} * \max \{f_{min}, (f_{temp} * f_{VPD} * f_{SWP})\}$] with parameters optimised for temperate oak species from published literature or the DO3SE-model by Büker and Emberson.

The evaluations did not confirm an increasing trend of ozone exposure for 7 h-means of ozone and for AOT40 from mid-April to mid-October. However, stomatal uptake by the oak leaves increased significantly over the 21 years (sun leaves: p=0,0002; +0,23 mmol.m$^{-2}$ per year $CUO_{1,6}$). There was no significant trend for precipitation (neither for the whole year nor for the summer months), wind speed or for vapour pressure deficit (April-October). However, temperature increased significantly (p=0,022) as well as global radiation (p=0,00002). Especially the decreased cloudiness has contributed to the long-term increase of ozone uptake.

# MONITORING OF OZONE EFFECTS ON THE VITALITY AND INCREMENT OF NORWAY SPRUCE AND EUROPEAN BEECH IN THE CZECH REPUBLIC

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Ozone is supposed to represent a significant risk for the health of forest ecosystems in Central Europe. So far, however, its real impact on forests growing under natural conditions has not been clearly assessed. The project focused on the monitoring of ozone effect on forest vitality and forest growth was conducted on 64 plots of Norway spruce and European beech stands in the Czech Republic during 2005 – 2008. Defoliation of forest stands, the malondialdehyde (MDA) concentration in the foliage, the amount of epicuticular waxes as well as the radial increment was assessed yearly and compared with the modeled and measured ozone concentration. Results show, that the ozone influence on the forest health is less pronounced than expected from the exceedance of the limit threshold based on the AOT40 index. Defoliation, which is broadly used as a general parameter of forest health, does not exhibit any enhancement according to the elevated ozone concentration. The visual symptoms of ozone damage, however, have been observed regularly with the increasing intensity in the higher altitudes. Also MDA concentration and amount of epicuticular waxes are related mainly to the altitude; the significant relation to the ozone concentration was proved only in individual years, their regional differences within the country were negligible. Statistical evaluation shows decrease of radial increment of European beech trees with the elevated ozone concentration – surprisingly only in lower and middle altitudes. It could be assumed, that the ozone concentration is also an important factor in mountain areas, where, however, its influence on European beech is covered by other dominant factors as summer temperatures or winter damages.

# LONG-TERM EFFECTS OF OZONE ON CARBON FLUXES IN PEATLANDS

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Tropospheric ozone poses a significant threat to crop yield and forest productivity of sensitive species, while northern hemisphere background ozone concentrations are expected to increase further during the next decades. Knowledge to date on the effects of ozone on peatlands, and ecosystem functioning in general is limited, while peatlands are a significant store of carbon and an important source of methane ($CH_4$). The aims of this study are to assess effects of ozone on $CH_4$ and $CO_2$ fluxes in peatlands, and to identify the underlying plant, soil and microbial processes.

Mesocosms from a wet heath (Scotland) with vegetation dominated by the sedge *Schoenus nigricans* and the peat moss *Sphagnum papillosum* were exposed for 2.5 years to control and elevated ozone levels in open-top chambers. The control treatment received non-filtered air (NFA), whereas the elevated ozone treatments consisted of non-filtered air plus 10 (24h/day), 25 (24h/day) and 40/10 ppb (8h in daytime in summer/winter).

Methane emissions were reduced by elevated ozone from the end of the first growing season onwards. Methane oxidation potentials measured in the surface *Sphagnum* layer and $CH_4$ production potentials deeper down the peat profile were affected by ozone, but could not fully elucidate the processes underlying the observed $CH_4$ emission response to ozone. Results of a $^{13}CO_2$ labelling experiment assessing the effect of elevated ozone on the contribution of recent photosynthates to $CH_4$ emission will also be presented. Ecosystem respiration was enhanced by elevated ozone from the second growing season. Gross photosynthesis was increased by elevated ozone from the onset of the experiment, which was largely caused by enhanced respiration of the sedges. Above and belowground sedge growth was not affected by ozone, while *Sphagnum* biomass production was higher at elevated ozone. Overall effects of ozone on the C budget of this peatland will be discussed.

# OZONE AND NITROGEN DIOXIDE VERTICAL PROFILES IN FOREST CANOPIES MEASURED USING PASSIVE SAMPLERS

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Ozone and nitrogen dioxide were measured at three heights at forested localities Bílý Kříž and Načetín (the Czech Republic). Cumulative measurements of ozone and nitrogen dioxide concentrations were performed in vegetation seasons of 2007-2010. Forests at Bílý Kříž and Načetín are characterized by the prevailing presence of Norway spruce. In addition, the vertical profiles of ozone and nitrogen dioxide in European beech forest in parallel to the measurement in spruce forest have been observed at Načetín.

The passive samplers were calibrated under real ambient conditions on the base of 10-day measurements using a commercial UV ozone analyzer and a chemiluminescence nitrogen dioxide analyzer at Bílý Kříž during summer once every year.

Daily average ozone and nitrogen dioxide concentrations were mostly higher at Načetín forested area in comparison to the data obtained at Bílý Kříž measurement station at the same period of appropriate year.

The shape of vertical profiles at Načetín were similar without consideration if the profiles were measured at Norway spruce forest and/or at European beech forest, and moreover, it did not matter if ozone or nitrogen dioxide profiles were concerned. The vertical profiles of ozone and nitrogen dioxide observed at Bílý Kříž were substantially different from those found at Načetín.

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# NET ECOSYSTEM PRODUCTION OF MOUNTAIN SPRUCE FOREST UNDER INCREASED STOMATAL UPTAKE OF OZONE

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Daily ozone deposition and stomatal ozone flux to mountain spruce (Picea abies) forest was measured using the gradient method and consequently modelled for the selected summer perion (2008) as well as whole growing season (2009). The experiments were done at Bílý Kříž experimental site (Beskydy Mts., Czech Republic). Ozone flux and deposition velocity peaked around noon, while ozone concentrations reached the highest values in the afternoon. The average deposition velocity estimated by the gradient method and by the deposition model was very similar during both growing seasons. Stomatal flux shared by 30 % in total deposition O3 flux during growing seasons (2009). The O3 exposition exceeded almost two-fold the critical level 5000 ppb h-1 in both growing seasons. Similarly, the stomatal ozone uptake exceeded the critical flux level of 8 mmol m-2, suggested by International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops (2010) for the protection of Norway spruce.

In addition, net ecosystem production (NEP) was measured by Eddy Covariance technique and correlated with O3 concentrations at 15 m a.g.l., total O3 deposition and stomatal $O_3$ uptake. The highest percentage of explained variability in NEP data was obtained using the stomatal flux values, where the influence of radiation and stomatal flux on NEP was tested in the form of polynomial of the 2$^{nd}$ degree. A slightly lower percentage of variability was explained by the model based on $O_3$ deposition calculated from the gradient method. However, the influence of ozone on NEP was statistically significant only under higher values of temperature (> 20 °C) and radiation (> 800 W m$^{-2}$). Based on the obtained results, it is possible to hypothesize that NEP can be reduced by 10-15% due to stomatal ozone uptake during the growing season.

This study was funded by the project MZP VaV SP/1b7/189/07) and Project CzechGlobe CZ.1.05/1.1.00/02.0073. The paper was written with the support of the COST Action FP0903 and MSMT project OC10022.

# POSTER PRESENTATIONS

## June 14

***17:15-18:00 Poster session*** *(5-min talk in front of each poster)* (Chair: Elena Paoletti)

1) **M. Díaz-de-Quijano, À. Ribas , J. Peñuelas: Increasing trends of ozone mixing ratios in the Catalan Pyrenees over the last 16 years**

2) **M. Díaz-de-Quijano, J. Peñuelas, T. Menard, P. Vollenweider: Visible and microscopical ozone injury in mountain pine (*Pinus mugo* subsp.*uncinata*) foliage from the Catalan Pyrenees**

3) **P. Vollenweider, B. B. Moura, E. S. Alves: Validation of specific ozone symptoms in rain forest trees of southern Brazil**

4) **I. Hůnová, L. Matoušková: Ozone visible foliar injury and stomatal flux in native young trees of *Fagus sylvatica* L.: a field study from the Jizerske hory Mts., the Czech Republic**

5) **Z. Večeřa, P. Mikuška, K. Křůmal, M. Zapletal, P. Chroust: New chemiluminescence reagent detector for measurement of eddy covariance fluxes of ozone**

6) **R. Kerner, E. Delgado-Eckert, J.W. Dupuy, J.B. Winkler, M. Jürgensen, Ch. Lindermayr, D. Ernst, G. Müller-Starck: Responses of European beech saplings following four vegetation periods of ozone exposure: an integrative study**

7) **V. Calatayud, A. Palomares, J. Cerveró, E. Calvo: Measuring and estimating ozone levels an the Valencian Community**

## June 15

*16:00-17:00* ***Poster session*** *(5-min talk in front of each poster)* (Chair: Elena Paoletti)

8) **A. Augustaitis, I. Augustaitiene, E. Plausinyte, D. Mozgeris: Effect of surface ozone on Scots pine and Norway spruce health and productivity under changing climate and air pollution in Lithuania**

9) **A. Kasurinen, C. Biasi, T. Holopainen, E. Oksanen: Integrated effects of elevated ozone and temperature on growth and carbon partitioning in silver birch (*Betula pendula*)**

10) **M. Zapletal, J. Pretel, P. Chroust, I. Hůnová, P. Cudlín, O. Urban, R. Pokorný, R. Czerný, Z. Večeřa: The influence of climate change on stomatal ozone flux to forest ecosystems**

11) **G. Gerosa, A. Finco, S. Oliveri, J. Zueger, E. Gebetsroither, W. Winiwarter: MANFRED Project. Management strategies to adapt Alpine Space forests to climate change risks**

12) **I. Štraus, M. Bajc, P. Železnik, H. Kraigher: Temperature and elevated $CO_2$ concentrations effects on fine root turnover and ectomycorrhiza of beech seedlings**

13) **B. Turk, K. Eler, F. Batič: Overview of the Slovenian bean experiment in the last years**

# EFFECT OF SURFACE OZONE ON SCOTS PINE AND NORWAY SPRUCE HEALTH AND PRODUCTIVITY UNDER CHANGING CLIMATE AND AIR POLLUTION IN LITHUANIA

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The study aimed to explore if changes in crown defoliation and stem growth of Scots pines (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* Karst.) could be related to changes in ambient ozone ($O_3$) concentration in the eastern part of Lithuania. To meet this objective the study was performed at Aukstaitija Integrated Monitoring Station from which data on meteorology and pollution (acidifying species and ozone) from 1994 to 2010 were provided. During this period crown defoliation and radial increment of pine trees changed synchronically. From 1994 to 2004 increment increased and defoliation decreased, afterwards from 2004 to 2010, adverse results were recorded – defoliation increased and increment decreased. Spruce increment demonstrated downwards trend over the entire considered period, meanwhile changes in defoliation were similar to those of pine. Contribution of peak $O_3$ concentrations to the integrated impact of acidifying compounds and meteorological parameters on stem growth was found to be more significant than its contribution to the integrated impact of acidifying compounds and meteorological parameters on defoliation of the considered tree species.

# MEASURING AND ESTIMATING OZONE LEVELS IN THE VALENCIAN COMMUNITY

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A combination of active and passive samplers was used to describe the temporal and spatial patterns of ozone in the Valencia Community (eastern Spain) for the period April-September 2007. Mean ozone concentrations showed a good correlation with altitude, increasing from the coast to inner mountain areas. A geostatistical approach was applied to represent the spatial patterns of this pollutant in all this territory.

Complementarily, the daily ozone curve for the period April-September has been modelled using the relative altitude of the plots, and AOT40 values were estimated from passive samplers. For many of these plots, the AOT40 value of 5000 ppb h was by far exceeded.

The feasibility of estimating ozone fluxes for these plots based on a combination of active and passive sampler measurements was also tested.

# INCREASING TRENDS OF OZONE MIXING RATIOS IN THE CATALAN PYRENEES OVER THE LAST 16 YEARS

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Ozone mixing ratios were monitored at 1-hour intervals in the Western, Central and Eastern Catalan Pyrenees during the last 16 years. In the Central Catalan Pyrenees, $NO_x$ and meteorological variables were also monitored over a period of 5 years (2004–2008). In this part of the Pyrenees, ozone mixing ratios and $NO_x$ were measured with passive samplers along two altitudinal transects ranging from 1040 to 2300 m a.s.l. The AOT40 in the three stations along the Pyrenees greatly exceeded the critical level, the target value and the long-term objective for forest and semi-natural vegetation protection. Furthermore, the AOT40 also showed an increasing trend over time and with increasing altitude. At the highest site of the altitudinal gradient (2300m), annual means of ozone mixing ratios were 1.6 times higher than at the valley-bottom site (1040m), ranged between 38 and 74 $ppb_v$ during the warm period and increased at a rate of 5.1 $ppb_v$ $year^{-1}$. The analysis of meteorological variables and $NO_x$ values suggests that the ozone mainly originated from urban areas and was transported to high-mountain sites, remaining aloft in absence of NO. These results indicate that plant life can be affected by ozone, especially at high altitude sites, where ozone mixing ratios are very high. They also suggest that long-term thresholds for ozone seem to be difficult to achieve in the forthcoming years. Nevertheless, more effort is warranted to determine the ozone uptake by vegetation in this mountainous range. An ozone flux-based index that takes into account the local-specific environmental conditions, plant phenology and possible ozone uptake during the night would provide a more accurate ozone risk assessment for the particular vegetation in each area.

# VISIBLE AND MICROSCOPICAL OZONE INJURY IN MOUNTAIN PINE (*PINUS MUGO* SUBSP. *UNCINATA*) FOLIAGE FROM THE CATALAN PYRENEES

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The Central Catalan Pyrenees have been experiencing high levels of troposheric ozone with AOT 40 (Apr-Sept) ranging from 9.6 to 23 ppm·h increasing by more than 0.5 ppm·h per year over the last two decades. At the same time, foresters have expressed concerns about the vitality of Mountain pine, a dominant tree species at high elevation in forests of this region and with a sensitivity to ozone so far unknown. Aims in this study were to survey mountain pine stands for visible injury and to validate the injury diagnosis with microscopy. Visible injury was recorded in 12 plots along two altitudinal and ozone monitoring transects in the spring of 2009. Using a representative plot subsample, needles from 4 trees were collected with a view to detailed morphological and structural observations. Microscopical analyses in light, fluorescence and electron microscopy were performed using serial sections centred on selected mottles identifying structural markers of the physiological responses elicited by ozone stress. Typical visible ozone injury - in the form of tiny and diffuse greenish mottles centred on stomata lines of the needle light-exposed side - were found in mountain pines. The symptoms were observed on 2 year or older needles and showed some frequency but few morphological differences between plots. They were similar to those induced by ozone in another experiment using a FACE facility. Structurally, mottles were underlined by numerous changes in the cell wall structure and cell content - especially the chloroplast structure - peaking in the outer mesophyll layers and in cells below stomata. Overall, structural changes in mottles indicated increased oxidative stress and hypersensitive reactions, both typical of ozone stress effects. Hence besides Aleppo pine, Mountain pine is the second European pine species to be found with specific ozone injury as a reaction to ambient ozone.

# MANFRED PROJECT. MANAGEMENT STRATEGIES TO ADAPT ALPINE SPACE FORESTS TO CLIMATE CHANGE RISKS

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With the expected Climate Change (CC), the ecological conditions for forests in the Alpine Space will be fundamentally changing – with unknown effects on the forests' essential protective, ecological, economical and social functions. Under different climate and land-use change scenarios only an adaptive management can provide the conservation of the natural heritage and the multiple functions.

MANFRED is an Interreg IIIb project evaluating the best management policies to adapt Alpine forest to climate change. Ozone exposure under an alternate climate regime is one hazard to be taken into account. MANFRED bridges the gap between research and practical forest management and seeks to a) collect knowledge with regard to CC effects on 4 main topics: forest growth and land use changes, hazards & stressors (among with ozone, drought, fires and windthrows), best practices to face extreme events, protection forests; b) identify hot spots with concrete need for action on the regional & local level; c) develop management strategies able to adapt to changing environmental conditions; d) contribute to the implementation of suggested adapted management strategies in cooperation with political decision makers in four transnational case study regions.

This poster illustrates the Project structure and the main results available at this midterm stage.

# OZONE VISIBLE FOLIAR INJURY AND STOMATAL FLUX IN NATIVE YOUNG TREES OF FAGUS SYLVATICA L.: A FIELD STUDY FROM THE JIZERSKE HORY MTS., THE CZECH REPUBLIC

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The Jizerske hory Mts. are situated in the northern part of the CR. High $O_3$ levels monitored in this area represent the threat for recovering forest ecosystems damaged by acid rain in the past. Regarding the optimal species composition of the Czech forests, the assessment of $O_3$ impact on species essential for the ecological stability as common beech (*Fagus sylvatica* L.) is, should be considered a priority.

$O_3$ visible symptoms on beech were assessed during three vegetation seasons (2006–2008).

In 2006 and 2007, symptoms on beech (brown stippling) were assessed at one site. The symptoms appeared both years in September. After the confirmation of $O_3$ influence

(a hypersensitive-like reaction) by the Ozone Validation Centre for Central Europe, the study has been extended. Symptoms and $O_3$ stomatal flux have been assessed according to the UNECE (2004a, 2004b)[1] at five sites in 2008.

Symptoms appeared at all sites during September again. The critical level (CL) of accumulated stomatal $O_3$ uptake ($AF_{st\ >1.6}\ O_3$) was exceeded at all sites. $O_3$ uptake values exceeded the CL of 4 mmol $O_3$ $m^{-2}$ from *ca* 10% to 180%. The first exceedance of CL of $AF_{st\ >1.6}\ O_3$ was recorded on 23. June, the last exceedance was recorded in mid August. The dependence of the visible injury expressed as amount of symptomatic sample squares on uptake was significant ($R^2 = 0.87$). No significant limitation of stomatal flux due to environmental conditions has been determined. Influence of ambient $O_3$ on beech in the typical Czech mountain is evident regarding actual critical levels for accumulated $O_3$ stomatal uptake.

[1] UN/ECE (2004a). Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests. Part X/B. Assessment of $O_3$ Injury.
[2]UN/ECE (2004b). Mapping Manual Revision, UNECE convention on long-range transboundary air pollution, Manual on the Methodologies and Criteria for Modelling and Mapping Critical Loads and Levels and Air Pollution Effects, Risks and Trends.

# INTEGRATED EFFECTS OF ELEVATED OZONE AND TEMPERATURE ON GROWTH AND CARBON PARTITIONING IN SILVER BIRCH (*BETULA PENDULA*)

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There is substantial and growing evidence that northern ecosystems are already experiencing a climate change. In the near future, anthropogenic influence on climate and thereby on northern forests is expected to accelerate even further. According to the climate change scenarios, the mean global surface temperatures will continue to rise during this century, and simultaneously forests will be exposed to damaging ozone stress. Both increasing ozone and temperature alone exert multiple effects on forest trees, but the combined effects of these climate change factors cannot be predicted on the basis of single exposures.

In summer 2007 and 2008, four silver birch genotypes (gt12, gt14, gt15 and gt25) growing in submerged pots were exposed to elevated ozone and elevated temperature alone and in combination in an open-air exposure field in Kuopio, central Finland. After the second exposure season 2008, tree growth and soil respiration showed variable responses to the treatments. Trees grew taller and had a greater total biomass in elevated temperature treatments relative to those grown in ambient temperature, whereas ozone-caused reductions in growth were mainly observed in the shoot biomass of the fastest growing genotypes (gt14 and gt25). Elevated temperature increased soil respiration rates beneath the trees, but tree genotype and prevailing ozone level also modified these responses. Hence, gt14 showed a clear temperature-induced increase in soil respiration regardless of ozone levels, but in gt12 this increase in soil respiration was also partly reduced due to $O_3$ in the combination treatment. In other genotypes significant temperature-induced increases in soil respiration were either observed only under ambient (gt25) or elevated $O_3$ (gt15). Carbon partitioning among the foliage and roots and return of recently fixed carbon to air via soil respiration was also studied in a labelling ($^{13}C$-$CO_2$) study with gt15. Results of this labelling experiment will be discussed in the presentation.

# RESPONSES OF EUROPEAN BEECH SAPLINGS FOLLOWING FOUR VEGETATION PERIODS OF OZONE EXPOSURE: AN INTEGRATIVE STUDY

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Tropospheric ozone, one of the most phytotoxic air pollutants, may specially impose in long-lived forest trees substantial reductions in productivity and biomass. Changes in the proteome of European beech saplings upon elevated ozone concentration were monitored following four vegetation periods of exposure. A proteome study based on highly sensitive two-dimensional fluorescence difference gel electrophoresis (2-D DIGE) was performed to identify protein changes in European beech, the most important deciduous tree species in Central Europe. Main emphasis was put on identifying differentially expressed proteins after long-time period of ozone exposure under natural conditions rather than short-term responses or reactions under controlled conditions. Our results clearly demonstrate a differential response of European beech saplings to long-term elevated ozone fumigation at the protein level. We indicate changes in the protein abundance of 237 protein spots showing absolute ratios of expression higher than 30% compared to the controls. From this spots, a total of 76 resembled the preparative silver stained spot pattern. These spots were subjected to mass spectrometry identification (LC-MS/MS) followed by a homology driven search. Some of the identified proteins have been previously described in the context of short-term ozone responses in plants. These findings indicate the congruence, at least for certain cellular functions, of plant reactions following short- and long-term ozone exposure. Differentially displayed proteins participate in key biological processes including 1) carbon assimilation; 2) photosynthetic electron transport chain; 3) catabolic/metabolic pathways; 4) detoxification mechanisms; 5) defense and stress related responses; 6) protein folding and degradation; and 7) senescing mechanisms. Furthermore the present proteomic time line analyses have been described with responses at the transcript, metabolite and morphological level previous reported for the same experimental setup. Our analyses highlight the importance of interdisciplinary studies to better understand plants responses to stress environments.

# VALIDATION OF SPECIFIC OZONE SYMPTOMS IN RAIN FOREST TREES OF SOUTHERN BRAZIL

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Toxic effects of current tropospheric ozone concentrations on the natural vegetation have been reported primarily for ecosystems in the northern hemisphere with a mediterranean, temperate or boreal climate. In the southern hemisphere and tropical ecosystems, the effects of ozone still remain little known whilst the air pollution is increasing as a consequence of the sustained growth within emerging economies. The city of Paulinia in the region of Sao Paulo, Brazil, experiences high levels of tropospheric ozone with monthly averages increasing during the onset of the rainy season from around 1000 ppb to more than 2000 ppb and peaking above 3000 ppb in October (2000-2009 averages). Aims in this ongoing study are to investigate the effects of current ozone concentrations in three pioneer tree species (*Astronium graveolens*, Anacardiaceae; *Croton floribundus*, Euphorbiaceae; *Piptadenia gonoacantha*, Fabaceae) from the Brazilian coastal rain forest and to compare macro and micro-morphological changes to findings in other continents and ecosystems. Eight month-old seedlings were exposed to O3-enriched air using indoor chambers with ozone concentrations from 70 to 250 ppb. After a week, the fumigated foliage showed stippling and bronzing symptoms, varying according to species. Injuries were underlain by apparent alteration in chlorophyll primary fluorescence, accumulation of vacuolar tannins and cell wall phenolics and cell death and collapse primarily in the upper palisade parenchyma layer. So far, similar visible and microscopical symptoms were also found in samples from *A. graveolens* collected in the field. Hence, these preliminary findings already show that some rain forest species in southern Brazil can be affected by ambient ozone concentrations and respond in a way similar to those found in other parts of the world. New fumigation experiments and field surveys are being performed with a view to further microscopical validation.

# TEMPERATURE AND ELEVATED $CO_2$ CONCENTRATIONS EFFECTS ON FINE ROOT TURNOVER AND ECTOMYCORRHIZA OF BEECH SEEDLINGS

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Ectomycorrhizal fungi (ECMF) are the key biotic link between the sources of food and symbiotic tree partners in forest ecosystems. Changes in the environment caused by natural or human induced disturbances are reflected in ECM. Tracking changes in ECMF composition is essential for understanding of ecosystem and single tree functioning, providing the information on carbon allocation belowground and between different partners of the singal fungal symbiont. We have therefore monitored the occurrence of ECMF on beech seedlings growing in rhizotrons at four different temperature regimes (15-25° C, 15-25° C and cooling of roots, 30-50° C, outside air temperature in Ljubljana) and at an ambient and elevated $CO_2$ atmosphere. Fine root turnover was studied wising WinRhizoTronMF, types of ectomycorrhiza were identified by anatomy, sequencing of the ITS1-5.8S rDNK-ITS2 ribosomal region and construction of phylogenetic trees, and substrate respiration was measured using a construction of chambers and LiCOR system. Temperature was found to influence fine root growth and respiration, and also a different ectomycorrhizal community was identified on roots of seedlings grown in different conditions. However, the correlations including elevated $CO_2$ regime and substrate respiration yet need to be analysed.

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# OVERVIEW OF THE SLOVENIAN BEAN EXPERIMENT IN THE LAST YEARS

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The ICP-Vegetation programme study "Yield response and ozone injury on *Phaseolus vulgaris*" has been running for three subsequent years in Ljubljana, Slovenia. In the study we are using ozone resistant (R123) and ozone sensitive (S156) genotypes of bush bean selected in North Carolina, USA. Each year 10 plants of R type and 10 plants of S type were potted alternately in two rows of self watered pots. The setup of experiment, assessment of ozone injuries and final harvest procedure were performed according to ICP-Vegetation protocol.

Some comparison between measurements and observations of ozone concentration and impact on bean plants in the years 2008, 2009 and 2010 are presented. The plants were exposed on 3$^{rd}$ of July 2008, 15$^{th}$ of June 2009 and on 16$^{th}$ of June 2010. AOT40 values were relatively low in all three years, exceeding 250 ppbh only on few occasions each year. The AOT40 values were higher in the first half of the exposure period, especially so in year 2010.

Higher weekly cumulative AOT40 values were gathered in the first 5 weeks in 2008 and 2010 and between the 4$^{th}$ and 6$^{th}$ week in 2009, with highest values around 1200 ppbh. The "Injury index", calculated as a function of the number of leaves in each injury class, showed some inconsistency between years. In 2008 the indices of both genotypes were highest in the 8$^{th}$ week. The differences between genotypes were small. In 2009 the injuries started to show later in the experiment (in accordance with maximal AOT40 values) and were almost twice as high in the sensitive genotype, compared to the resistant one. In 2010 the senescence of sensitive genotype was obvious, and in the 8$^{th}$ week plants were completely leafless. The results suggest difficult direct comparison of the injuries between years and complex relations between visible condition of plants and ozone concentrations.

Very variable results were found counting the seedless pods and pods with seeds. In 2008 the average number of all pods on resistant plants was 38 (32 with seeds, 6 without) and on sensitive plants 26 (20 with seeds, 6 without). In 2009 the average total number of pods was much higher in sensitive plants with 56 (29 with seeds, 27 without) than in resistant with 35 (29 with seeds, 6 without). In 2010 average total number of pods in resistant type was 26 (12 with seeds, 14 without) and in sensitive clone 28 (13 with seeds, 15 without). As one can see from the mentioned numbers, the ratio between fertile and sterile pods can vary greatly.

More consistent were the results on the number and weight of seeds. In all three years the average number and average mass were approximately twice as high in resistant plants as in sensitive ones. Due to some unresolved reason, the number of seeds in 6 resistant plants in year 2010 was extremely low (0, 6, 9, 9, 9 and 19 seeds).

The quick conclusions of this three year overview would be that the visual condition of plants, number and weight of seeds, number of fertile and sterile pods and their ratio, and the senescence of plants can vary greatly, between the years and between the individuals, so the ozone effects can remain hidden in the bias.

# NEW CHEMILUMINESCENCE REAGENT DETECTOR FOR MEASUREMENT OF EDDY COVARIANCE FLUXES OF OZONE

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A new chemiluminescence (CL) analyzer for the continuous monitoring of ambient ozone has been developed in the course of project „Decreasing of forest ecosystem services due to influence of ground ozone under conditions of climatic change".

The ozone is continuously detected on the base of the chemiluminescent reaction performed during passing of gaseous ozone (at the air flow rate of 1 l/min) over liquid chemiluminescence reagent in front of a photomultiplier tube. The optimized chemiluminescence reagent is composed of alkaline solution of eosine Y, gallic acid, $Na_2SO_3$, diethylene glycol and ethanol. The re-circulating CL reagent (at the flow rate of 400 μl/min) and sufficient robustness of the analyzer allows its 1 month unattended operation. To avoid unreliability of results, the analyzer was calibrated every 6 hours by means of UV ozone calibrator. The detection limit of ozone is 0.7 ppb (v/v) for criterion 3S/N>1. Calibration curve is linear over two orders up to 196 ppb (v/v) of ozone.

The CL analyzer provides a good alternative to commercial UV analyzers that under certain conditions suffer from incorrect results. In addition, the CL analyzer is proper for the measurement of eddy covariance fluxes of ozone.

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# THE INFLUENCE OF CLIMATE CHANGE ON STOMATAL OZONE FLUX TO FOREST ECOSYSTEMS

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Results of study for Norway spruce indicate that in a future climate (around 2030) the exceedance of the flux-based critical level (Phytotoxic Ozone Dose for Norway spruce) of $O_3$ might be lower in the Bílý Kříž locality, even when taking into account an increase in tropospheric background $O_3$ concentration. In contrast, the exceedance of the concentration-based critical level (AOT40) of $O_3$ will increase with the projected increase in tropospheric background $O_3$ concentration. The influence of climate change should be considered when predicting the future effects of $O_3$ on conifer forest.

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