24th New Phytologist Symposium

Plant respiration and climate change: scaling from mitochondria to the globe

St Hugh's College, University of Oxford, UK
11–14 April 2010

Programme, abstracts and participants

New Phytologist

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St Hugh's College, University of Oxford, UK

Organizing committee

Owen Atkin *(ANU, Canberra, Australia)*

Harvey Millar *(UWA, Perth, Australia)*

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Speaker Abstracts

Session 1: Respiratory carbon release over large spatial & temporal scales
Chair: Owen Atkin

1.1 Coupling between canopy photosynthesis, below ground respiration and overall ecosystem carbon fluxes

NINA BUCHMANN
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Understanding the coupling of below- and aboveground processes is still a major issue in ecosystem ecology. Although it has been shown that belowground respiration is strongly affected by canopy photosynthesis, the magnitude, the timing and the drivers of such a coupling are not always clear. While respiration typically increases with temperature across scales (from tissue to ecosystem), other drivers such as water availability or phenology might interact, and time-lagged responses might be seen. For example, temperature sensitivities of roots and microorganisms in the field differed significantly, not only between the two actors, but also across the seasons and phenological phases. Ecosystem respiration measured with eddy-covariance technique is coupled to soil respiration, but also affected by stem growth and species-specific behavior. Using different approaches, i.e., experimental manipulations (e.g. girdling or trenching experiments), observations (measurements of net ecosystem or soil CO_2 exchange), tracer experiments (using $^{13}CO_2$) or combinations thereof, helps to gain mechanistic understanding of the processes involved and the organisms contributing to ecosystem C fluxes. Using ^{13}C tracer under controlled conditions, we found that drought reduced the coupling between canopy photosynthesis and belowground processes significantly. Residence times of recently assimilated photosynthates in beech leaves increased, absolute allocation belowground decreased, and turnover from plants to microorganisms slowed down tremendously under drought conditions. In wheat, these time-lagged responses to environmental change scaled linearly with stomatal conductance. Thus, partitioning of ecosystem C fluxes as well as their attribution to the responsible organisms, for example in ecosystem models, require integrated approaches, taking into account different environmental settings and complex interactions.

1.2 Fine root respiration: importance for ecosystem carbon fluxes

K. S. PREGITZER
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Much of the CO_2 efflux from the soil is derived from the activity of plant roots. An increasing amount of evidence suggests that position of an individual root on the branching root system is the most important determinate of root respiration. This work has revealed that in many ways, there is symmetry in the physiology of the canopy and the root system. Like branches, roots exhibiting secondary growth are largely conductive tissue with low metabolism and they play little role in uptake. Like leaves, the most distal lateral roots are ‘hot spots’ for metabolic activity. The physiological traits of these roots are also similar to leaves, with an ephemeral life span and high surface area. The high metabolic capacity of roots and leaves leads to two other shared traits—high tissue nitrogen concentration and high rates of respiration. Unlike leaves, the activity and turnover of distal lateral roots creates a supply of highly labile and metabolically active compounds —sugars, amino acids, and proteins—that immediately stimulate biological activity in the soil surrounding them. Consequently, the metabolic activity of roots directly influences a large proportion of the heterotrophic respiration in the soil. If the plant-microbe system in the soil is well coupled (as in most closed-canopy temperate and tropical forests), changes in the activity and abundance of these early order roots creates a proportional change in heterotrophic respiration in the rhizosphere. When the root – microbial continuum in the rhizosphere is uncoupled as in seasonally dry or cold soil, or disturbed ecosystems, soil respiration depends more fundamentally on substrates accumulated in the soil when plant metabolism was active. We still lack a clear understanding of how dominant plant taxa control root and total soil respiration.

1.3 Radiocarbon measurements and the age of plant-respired CO_2

<u>S. TRUMBORE</u>, C. CZIMCZIK
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Perennial plants allocate a significant fraction of their annual carbon budget to non-structural carbon pools (low-molecular weight sugars, starch or lipids). These pools are used to fuel a number of plant processes and sustain metabolism when photosynthesis is not active, e.g. during annual dormant seasons. While isotope-labeling experiments clearly demonstrate that a portion of plant respiration is derived from recently fixed photosynthetic products, the radiocarbon signature of respired CO_2 or in newly grown tissues sometimes indicate the use of C substrates fixed from the atmosphere up to several years previously. This talk will describe the radiocarbon methods and report initial results from our investigations of radiocarbon in newly grown material, non-structural C pools and CO_2 respired by plants in the context of constraining the age and dynamics of non-structural carbon pools.

1.4 Using stable isotopes to determine the contribution of respiration to the carbon balance of terrestrial ecosystems

L. WINGATE[1], J. OGEE[2]
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Since the 1940's, environmental concerns and improvements in analytical capabilities have expanded the use of stable isotopes in diverse biogeochemical studies. For instance, the study of CO_2 isotopologue ($C^{16}O_2$, $^{13}CO_2$ and $C^{18}O^{16}O$) exchange within and above forests has been proposed as a potential tool to quantify respiration rates at larger scales and partition the contributions of different components within an ecosystem. Understanding better the processes that regulate plant and soil respiration and how they impact the growing concentration of CO_2 in the atmosphere is indeed a major challenge in global carbon research. Here we will present the principles of flux rate partitioning in forests using the stable isotopes of carbon and oxygen and, from recent stable isotope studies conducted at the whole plant and ecosystem scales, we will demonstrate the potential of stable isotope approaches to partition the contribution of respiratory components within ecosystems and at larger scales.

1.5 Incorporating plant respiration into predictive dynamic vegetation and global climate models

STEPHEN SITCH[1], PETER M COX[2], ROSIE FISHER[3], DAVID GALBRAITH[4], CHRIS HUNTINGFORD[5], CHRIS D JONES[6], JON LLOYD[1, 7], LINA MERCADO[5]
[1]School of Geography, University of Leeds, Leeds, UK; [2]School of Engineering, Computing and Mathematics University of Exeter, Exeter, UK; [3]Los Alamos National Laboratory, Los Alamos, New Mexico, USA; [4]School of Geography, University of Oxford, Oxford, UK; [5]Centre for Ecology and Hydrology Wallingford, UK; [6]Met Office Hadley Centre, Exeter, UK; [7]The University of Queensland, Brisbane, Queensland, Australia

First we review methods to represent recent plant respiration in Dynamic Global Vegetation Models (DGVMs) and assess the consequences of widely used alternative formulations on predictions of future land-atmosphere carbon exchange. We then present more recent results whereby acclimation of assimilation and respiration to elevated temperature has been included in the Joint UK Land Environment Simulator (JULES).

Session 2:	**Mitochondrial composition and respiratory function** **Chair: Harvey Millar**

2.1 Mitochondrial supercomplexes and their role in respiratory function

<u>HANS-PETER BRAUN</u>
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The functional context of mitochondrial respiration differs in autotrophic and heterotrophic organisms. As a consequence, the Oxidative Phosphorylation (OXPHOS) system has several special features in plants: (i) the OXPHOS complexes comprise plant-specific subunits, some of which introduce side activities into the complexes not directly related to respiration (e.g. carbonic anhydrases within complex I); (ii) the OXPHOS system includes quite a number of additional oxidoreductases, like the 'alternative oxidase' and three to four different 'Rotenone-insensitive NAD(P)H dehydrogenases'; (iii) the OXPHOS complexes associate forming supramolecular structures, which have special compositions in plants. While the structures of several OXPHOS supercomplexes meanwhile have been well characterized by electron microscopy and other experimental procedures, their functional roles are still a matter of debate. For instance, it is speculated that formation of the $I+III_2$ supercomplex indirectly affects alternative respiration because it limits access of 'alternative oxidase' to its substrate ubiquinol. However, recent results on the OXPHOS system in highly thermogenic tissue of *Arum maculatum* seem not to support this hypothesis. Other possible functions of OXPHOS supercomplexes in plants will be presented and discussed.

<u>Recent publications:</u>

Klodmann J, Sunderhaus S, Nimtz M, Jänsch L, Braun HP. 2010. Internal architecture of mitochondrial complex I from *Arabidopsis thaliana*. *The Plant Cell, in press.*
Sunderhaus S, Klodmann J, Lenz C, Braun HP. 2010. Supramolecular structure of the OXPHOS system in highly thermogenic tissue of *Arum maculatum*. *Plant Physiol. Biochem., in press. [doi:10.1016/j.physletb.2003.10.071]*
Dudkina NV, Kouřil R, Peters K, Braun HP, Boekema EJ. 2010. Structure and function of mitochondrial supercomplexes. *Biochim. Biophys. Acta, in press. [doi:10.1016/j.bbabio.2009.12.013]*

2.2 Partitioning of electrons between the cytochrome and alternative pathways

M. RIBAS-CARBO, J. A. BERRY, J. FLEXAS, I. FLOREZ-SARASA, L. GILES, H. LAMBERS, S. A. ROBINSON
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Plant respiration presents a major feature with the presence of the cyanide-resistant pathway which is essentially uncoupled of ATP synthesis. This ‘energetically inefficient’ respiratory pathway competes directly with the ‘energetically efficient’ cytochrome pathway and it represents an important fraction of total respiration. The role of the alternative pathway has been debated for many years now.
The activity of the two respiratory pathways (cytochrome and alternative oxidases) has been measured with the oxygen isotope fractionation technique, using isotope ratio mass spectrometry, both in isolated mitochondria and intact tissues. Some of the most recent technical advances will also be presented.
This lecture will comprise an overview of more than 15 years of research compiling data obtained of the ‘in vivo’ activities of the two respiratory pathways with large emphasis on the effects of several stresses as well as the relationship between gene expression, protein amounts and activity of the alternative pathway.
Finally, the response of the electron partitioning between the two respiratory pathways will be put in perspective of climate change, such as changes in temperature and other climate conditions. The goal of finding more ‘respiratory efficient’ plants will be raised.

2.3 Regulation of respiratory metabolism in germinating seeds

D. MACHEREL, A. BENAMAR, M-H. AVELANGE-MACHEREL
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Germination and early seedling growth are critical periods in the life cycle of plants. Starting from a quiescent dry state in the seed, embryos and seedlings need to maintain an efficient heterotrophic metabolism and cope with often stressful conditions, in order to rapidly reach autotrophy and start competing for nutriments and space. These processes are almost entirely dependent on mitochondrial respiration, which provides cellular energy as well as a metabolic platform involved in the conversion of seed reserves into building blocks for growth metabolism. It is therefore no surprise that seed mitochondria exhibit unusual properties in respect of desiccation and temperature tolerance. Stress proteins such as LEA (late embryogenesis abundant) proteins and sHSPs (small heat shock proteins) are involved in the protection of mitochondria in the dry state, and likely contribute to their thermal tolerance during germination. In many cases, fast germination increases the chances of successful emergence and establishment of seedlings, and this requires an efficient energy metabolism. Oxygen availability for respiration can be a challenge because of limiting oxygen diffusion rates in large seeds and/or within soils. It appears that, at least in legume seeds, mitochondria are able to self-adjust their oxygen consumption with the support of nitric oxide (NO) metabolism. This allows maximal energy production to be achieved under hypoxic conditions, without subjecting tissues to deleterious anoxia.
In the context of ongoing and future climate change, it is of general importance to understand how mitochondrial functions have evolved to maintain energy homeostasis in organisms and tissues exposed to extreme environmental conditions such as desiccation. Such traits could offer interesting targets for plant adaptation and improvement.

2.4 The response of respiratory carbon fluxes in Arabidopsis cells to altered environment as revealed by metabolic network models

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Carbon supplied to heterotrophic cells enters a complex metabolic network that results in the synthesis of a variety of biopolymers that support growth and are laid down as storage reserves. The main entry point of carbon into this heterotrophic metabolism is glycolysis and the TCA cycle where transformation into a range of biosynthetic precursors occurs. In addition, these oxidative pathways provide the driving force for ATP synthesis (respiration). Both the biosynthetic reaction network and respiration contain a number of decarboxylation steps that result in loss of carbon as CO_2. The overall carbon-use efficiency of metabolism thus depends on the relative flux of these decarboxylative reactions. To gain a more detailed view of carbon fluxes through the network and to assess factors affecting carbon-use efficiency, we have used both modelling and experimental approaches to quantify flux distributions. The analyses revealed that different environmental conditions have profoundly different impacts on the flux distribution and carbon use efficiency.

Session 3: Regulation of respiration in plants and fungal partners
Chair: Andrew Leakey

3.1 Controlling carbon flow at the plant-fungus interface in mycorrhizae

ALASTAIR FITTER[1], ANGELA HODGE[1], OWEN ATKIN[2]
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Most roots exist as symbioses with mycorrhizal fungi, principally the arbuscular mycorrhizas (AM) formed with fungi in the Phylum Glomeromycota. The fungi acquire their entire carbon supply from the plant and can be responsible for a substantial fraction of ion uptake, notably of phosphate; they may therefore represent a significant carbon sink. We will examine the evidence for the quantities of C moving through this pathway, the impact of colonisation by the fungus on root respiration rate and the mechanisms by which the flux from plant to fungus is regulated. The biomass of AM fungi may represent a large fraction of soil biomass, suggesting that their contribution to carbon cycling has been overlooked.

3.2 Regulation of respiration in plants: the role of cytosolic pyruvate kinase

SANDRA N. OLIVER, JOHN E. LUNN, EWA URBANCZYK-WOCHNIAK, ANNA LYTOVCHENKO, JOOST T. VAN DONGEN, BENJAMIN FAIX, ELMAR SCHMAELZLIN, ALISDAIR R. FERNIE, PETER GEIGENBERGER
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Plant respiratory metabolism involves three main pathways: glycolysis, the tricarboxylic acid cycle, and the mitochondrial electron transport chain. While the structural components of these pathways have been well defined, much less is known about their regulation and the interaction between the different pathways. Pyruvate kinase (PK) is a key enzyme in respiratory metabolism, and catalyses the final step of the glycolytic pathway. Plants contain several forms of PK that exist in different cellular compartments and in different tissues. Despite their importance for respiration, the impact of altered activity of these different PK isozymes on plant metabolism has not been extensively studied. The aim of our work was to investigate the effect of decreased cytosolic PK (PKc) activity on potato tuber metabolism. Transgenic potato plants with reduced levels of PKc were generated and tubers were analysed. Metabolic profiling and flux analysis revealed that decreased PKc activity caused reduced levels of pyruvate and some organic acids in the tricarboxylic acid cycle, as well as changes in carbon partitioning. Total respiration rates were unchanged by decreased PKc activity, but transgenic tubers showed a decrease in the levels of the mitochondrial alternative oxidase (AOX) protein as well as lower capacity of the alternative pathway of respiration. Pyruvate feeding experiments showed that pyruvate activated the alternative pathway, suggesting that pyruvate may regulate AOX activity. Our results indicate that PKc regulates the levels of pyruvate and AOX in heterotrophic plant tissue, and that pyruvate and AOX levels are correlated *in planta*. Our study provides further evidence of functional interaction between the cytosolic and mitochondrial components of plant respiration.

3.3 Day respiratory metabolism of illuminated leaves and its interactions with N assimilation

<u>GUILLAUME TCHERKEZ</u>[1], PAUL GAUTHIER[1], ALINE MAHÉ[1], RICHARD BLIGNY[2], ELIZABETH GOUT[2], GABRIEL CORNIC[3], MICHAEL HODGES[1]

[1]Institute of Plant Biology, University Paris-Sud 11, 91405 Orsay cedex, France; [2]Laboratory of Plant Cellular Physiology, CEA-Grenoble, 17 rue des Martyrs, 38009 Grenoble cedex, France; [3]Ecology Systematics & Evolution, University Paris-Sud 11, 91405 Orsay cedex, France

Illuminated plant leaves simultaneously assimilate CO_2 through photosynthesis and produce CO_2 through photorespiration and day respiration (or 'mitochondrial' respiration, defined as the non-photorespiratory process by which leaves produce CO_2 in the light). While the response of leaf or canopy photosynthesis and leaf or ecosystem respiration to environmental conditions has already been studied to some extent, several factors remain poorly known, even at the leaf level. It is the case of mitochondrial metabolism. In addition to CO_2 balance, day respiration is a key-process for N assimilation by plants, because the associated metabolic pathway provides the intermediate molecules that are the primary NH_2- acceptors. Furthermore, the impact of CO_2 increase (driven by global change) on plant growth is modest and a limitation of nitrogen assimilation is assumed to contribute to this effect: for example, at ordinary N levels, C_3 herbage grass shows a yield increase of 1% only under $2\times CO_2$ conditions. Therefore, the connection between carbon and nitrogen input seems as essential as input rates themselves. Mitochondrial metabolism is precisely at the heart of this connection and so, more knowledge of day respiratory metabolism, under varying CO_2 levels, is more important than ever. Here, our recent results on day respiratory metabolism and its relationships with nitrogen assimilation and photorespiration in illuminated leaves will be reviewed.

3.4 Mitochondrial metabolomics and the regulation of respiration

<u>ALISDAIR FERNIE</u>, ADRIANO NUNES NESI
Max Planck Institute for Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam-Golm Germany

In recent years we have taken a saturation, transgenesis approach in order to understand the role(s) of the component enzymes of the tricarboxylic acid (TCA) cycle in tomato (*Lycopersicum esculentum*). Work began with the observation that the spontaneous mutant *Aco*1 of *L. pennellii* which was deficient in aconitase expression displayed elevated photosynthetic performance. We have subsequently generated plants deficient in the expression of each enzyme of the cycle and characterised them broadly both physiologically, molecularly and metabolically. In this talk I will focus on our attempts to associate the various physiological phenotypes observed to changes in the metabolic signatures of leaf mesophyll and apoplast and alternatively of roots and fruits. I will additionally discuss this research in terms of circumstance dependent modularity of the cycle.

3.5 Scaling respiration using stable carbon isotopes: rapid changes in leaf respiratory biochemistry at sunset are evident at the ecosystem scale

MARGARET BARBOUR
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The stable carbon isotope composition of leaf respiration is well known to be enriched compared to respiratory substrates due to fragmentation fractionation. It has been demonstrated that the CO_2 released by leaves during the light-enhanced dark respiration peak (LEDR, i.e. immediately after darkening) is strongly enriched in ^{13}C, likely as a result of organic acid respiratory substrates. The CO_2 released during the LEDR peak can be 10 per mil more enriched in ^{13}C than leaf sucrose, followed by a slow decline in ^{13}C over a number of hours. Recently we found that the slope of the initial rapid decline in ^{13}C is related to the light environment prior to that start of the dark period. Using high-frequency measurements of CO_2 concentration and $\delta^{13}C$ profiles within and above a pasture ecosystem to construct Keeling plots every six minutes, we found evidence of the influence of LEDR on the ecosystem $^{13}CO_2$ isoflux during the first hours after sunset. Further, the rate of ^{13}C depletion of ecosystem respiration was positively related to the total daily irradiance of the preceding day, as would be expected based on leaf-level incubations. Concurrent chamber-based measurements of soil-respired CO_2 in the field indicate little diurnal variation in $\delta^{13}C$ of soil-respired CO_2 in this ecosystem. We conclude that the dynamics of leaf respiratory biochemistry are evident at the ecosystem scale.

Session 4: **Heterogeneity of respiration in contrasting cells and tissues**
Chair: Matthew Turnbull

4.1 Proteomics of mitochondria in contrasting cell types

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ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, 35 Stirling Hwy, Crawley, 6009, Western Australia, Australia

Respiratory function in plant cells is constrained by a number of factors, notably including the capacity of enzymatic machinery of organic acid oxidation, respiratory electron transport and the coupling to ATP synthesis. Understanding heterogeneity of this constraint in different tissues types, under different environmental conditions and even during daily rhythms provides a foundation for building a whole plant model of respiratory and photorespiratory capacity. We are developing this principle in Arabidopsis by isolating and quantitatively comparing mitochondrial protein abundances in root, shoot, flower, stem and silique with the abundances in rapidly growing cell cultures. This shows the constitutive and the variable components of the respiratory machinery, gives insight into potential mechanisms of tissue specific phenotypes of mitochondrial mutants and will ultimately allow the quantitative evaluation of a whole plant respiratory model at the level of individual enzymatic steps. We have also accessed the changes in the mitochondrial proteome during day and night cycles in Arabidopsis leaves, revealing a daily rhythm in the capacity of key pathways in respiration and photorespiration. We are now assessing the impact of the knockout of key mitochondrial components on the rest of the mitochondrial proteome and its function.

4.2 Xylem transport: An unaccounted flux path for respired CO_2 from roots and stems

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A substantial portion of the CO_2 released from respiring cells in roots and stems can dissolve in xylem sap and move upward in the xylem stream with the transpirational demand for water. Recent measurements in trees indicate that this internal transport accounts for 30 to 70% of the total CO_2 released from root and stem respiration. Barriers to diffusion promote the build up of CO_2 in stems and roots to high concentrations, often in the range of 3 to 10% and sometimes exceeding 20%, substantially higher than that of the atmosphere (~0.04%). Some CO_2 released by respiring cells diffuses to the atmosphere near its source, but the amount is often obscured by the simultaneous diffusion of xylem-transported CO_2 that originated lower in the tree. These fluxes cannot be separated easily because the amount of CO_2 transported in the xylem varies diurnally and seasonally, and is influenced by many factors, including sap [CO_2], pH, rate of sap flow, temperature, barriers to diffusion in the xylem and bark, and the number and activity of live cells in tissues along the pathway. Internal transport of CO_2 appears to be part of a CO_2 recycling mechanism in which respired CO_2 is re-assimilated. In measurements of *Platanus occidentalis* branches, ~35% of xylem-transported CO_2 was assimilated by photosynthesis in woody tissues and leaves. A new approach for measuring respiration of root and stem tissues will be discussed that accounts for both external and internal fluxes of respired CO_2. Measurements using this approach indicated that when the internal flux was not considered, root respiration was substantially underestimated, and stem respiration was both under- and over-estimated in different instances. Although the internal transport of respired CO_2 has only recently been recognized and measured, it has important implications for our understanding of carbon dynamics at both plant and ecosystem levels.

4.3 Predicting respiration of leaves, stems, and roots in a warming world

MARK G. TJOELKER
Department of Ecosystem Science and Management, Texas A&M University, College Station, TX 77843-2138, USA

Quantifying respiration rates and temperature responses in plants is fundamental to predicting carbon fluxes in response to climatic change. The patterns and mechanisms of short-term temperature acclimation and long-term climatic adaptation in respiratory traits of leaves, stems, and roots among diverse plant taxa remain poorly understood, but potentially important in constraining respiratory carbon fluxes with climate warming. Laboratory and field studies reveal that temperature acclimation, characterized by a reversible decrease in rates with warming and increase in rates upon cooling, is manifest through changes in base rates of respiration and temperature sensitivity (Q_{10}) of the short-term temperature-response functions. Modeling demonstrates that temperature acclimation reduces respiratory carbon fluxes with warming at larger spatial and temporal scales. Analysis of global datasets reveals scaling relations of respiration with nitrogen for leaves, stems, and roots of diverse plant taxa and contrasting climates. However, respiration rates at any common nitrogen concentration are lower in leaves than stems or roots, providing a basis for organ-specific scaling relationships. Temperature acclimation of respiration is often, but not always, associated with changes in tissue nitrogen concentration. Importantly, respiration-nitrogen relationships may be influenced by concurrent changes in soluble carbohydrate concentrations, supporting a joint enzyme and substrate-based model of respiratory temperature acclimation. These findings suggest that separate leaf, stem, and root scaling relationships should be used in models that simulate respiration rates based on tissue nitrogen concentrations. Examining respiration in terms of short-term temperature response functions, nitrogen, and carbohydrate concentrations provides a framework to discern mechanisms of temperature acclimation and inform global carbon models.

Session 5: Respiratory responses to environmental gradients
Chair: Richard Norby

5.1 Defining the regulatory context of nuclear genes encoding mitochondrial proteins

JAMES WHELAN
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Research in the last 50 years has defined the various biochemical processes of mitochondria and current proteomic studies are providing a more detailed view of the total protein complement. The majority of the protein complement of mitochondria is encoded by nuclear located proteins. Thus regulation of expression takes place in the nucleus and can be divided into Anterograde (nuclear to organelle) and Retrograde (organelle to nuclear) regulatory pathways. Although many studies have documented changes in transcript abundance of genes encoding mitochondrial proteins, little is known about the *cis*-acting regulatory elements (CAREs) and the transcription factors that bind these elements that control expression of nuclear genes encoding mitochondrial proteins.
We have undertaken a research program to define the *cis* and *trans* factors that regulate the expression of nuclear genes encoding mitochondrial proteins. Two approaches have been undertaken; i) we have defined the mitochondrial stress response, as transcripts of genes encoding mitochondrial proteins that change significantly in abundance under a variety of stresses and are dissecting the signalling and regulatory factors that control the mitochondrial stress response. ii) Many genes encoding mitochondrial proteins remain largely unchanged under a variety of conditions. We have selected a variety of these genes, encoding diverse biochemical functions, and defined *cis* and *trans* regulatory factors.
The regulatory pathways and factors defined above will be presented and the interactions with other regulatory networks in the cell highlighted. It will be shown that perturbation of these regulatory networks has implications beyond the immediate biochemical function in mitochondria and has implications for plant growth and development under normal and stress conditions.

5.2 Light inhibition of leaf respiration and its response to environmental gradients as a mechanism for woody shrub invasion of arctic ecosystems

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Understanding the processes regulating plant carbon balance and how these respond to current and predicted future global environmental change is essential. Here we report on a unique and important aspect of arctic plant ecophysiology, the inhibition of respiration by the constant daylight conditions of the short arctic growing season. Because measurements of net photosynthesis (A) are confounded by respiration in the light (R_L), and the ratio of R_L to A is clearly influenced by environmental conditions such as ambient temperatures, a mechanistic predictive understanding of ecosystem carbon exchange requires accurate estimates of R_L in this vast ecosystem exposed to continuous light and a rapidly warming climate. This work was conducted at the Arctic LTER site at Toolik Lake, Alaska, and takes advantage of established treatment plots simulating potential global environmental changes and landscape scale measurement of ecosystem carbon flux *via* eddy covariance. We compare plants from different functional groups (evergreen, woody deciduous, forbs and gramminoids) growing in a variety of treatment plots (warming & nutrient additions). In addition we test the specific hypothesis that light inhibition of respiration would result in a higher ratio of net carbon to gross carbon gain in dwarf birch (*Betula nana*) than in cottongrass (*Eriophorum vaginatum*), particularly in samples grown in conditions mimicking warming.

5.3 Responses of plant respiration to drought

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At present, drought is the main environmental factor limiting plant growth and yield worldwide. Global change will likely make water scarcity an even greater limitation to plant productivity across an increasing land extension. Drought-induced plant growth limitation is mainly due to reductions of plant carbon balance, which is dependent on the balance between photosynthesis and respiration. Although both processes are intimately linked, photosynthesis responses to drought have been widely studied, while responses of respiration remain poorly evaluated. Here we review the current knowledge on plant respiration responses to drought, and highlight the importance of respiration in reducing plant carbon balance and water use efficiency under drought conditions. Root respiration responses are revealed as a serious gap of knowledge. A hypothesis is presented where reducing the capacity of alternative oxidase (AOX) may potentially result in an increased plant carbon balance at moderate drought, although perhaps at the expense of photoinhibition-mediated reduction in plant carbon balance at more severe drought. Preliminary data on the response of several respiratory mutants – including AOX-transformants – to drought are presented and discussed.

5.4 Respiration and the axes of variation in a climate of change

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No, this is not the name of a new musical group. Instead through work with numerous colleagues we seek to identify and understand patterns of respiration as it varies along important axes such as environment (e.g., temperature, light), organ (leaf, root, stem), size (e.g., tree size), geography (e.g., climate origin), microbial associates (e.g. mycorrhizal symbionts), co-variation in other leaf traits (e.g., photosynthetic capacity, nitrogen) and others. We ask whether there are repeated patterns or relationships that provide a framework for generalizing about causes and consequences of variation in respiration rates. Data are derived from observational studies across natural gradients (local to global) as well as from manipulated experiments, including open-air climate warming studies, and in some cases extended to ecosystem scale via a biogeochemical cycling model. The roles of adaptation and acclimation will be emphasized, with focus on implications in a warming world.

Poster Abstracts

Listed alphabetically by first author, presenting author is underlined.

1. Incorporating acclimation of respiration into predictive coupled global climate-vegetation models using leaf-trait scaling relationships

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Whilst plant respiration (*R*) is an important contributor to global atmospheric CO_2 levels, most dynamic vegetation models assume that *R* increases exponentially with temperature and do not incorporate thermal acclimation of *R*. Here we present data from 19 species grown at four temperatures (7, 14, 21 and 28^{o}C), which were used to assess whether long-term changes in growth temperature systematically alter the scaling relationships between leaf *R* and leaf mass per unit leaf area (LMA) and leaf nitrogen (N) concentration. The impact of thermal history on these *R*-LMA-N generalized scaling relationships was highly predictable and could therefore be quantitatively incorporated into a coupled global climate-vegetation model. Accounting for acclimation of *R* within the model had negligible impact on predicted annual rates of global *R*, net primary productivity (NPP) or on future atmospheric CO_2 concentrations. However, accounting for acclimation decreased modeled plant respiration by up to 20% in high temperature biomes with these changes balanced at a global scale by increases in predicted *R* in cold ecosystems. We conclude that thermal acclimation of *R* needs to be taken into account when predicting potential regional level responses of terrestrial carbon exchange to climatic change.

2. The inhibition of succinate dehydrogenase results in elevated photosynthesis and plant growth in tomato via an effect on stomatal aperture

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Transgenic tomato (*Solanum lycopersicum*) plants expressing a fragment of the *Sl*IC1-SDH gene encoding the iron sulphur subunit of the succinate dehydrogenase protein complex in the antisense orientation and exhibiting considerable reductions in the activity of this enzyme exhibit an enhanced rate of photosynthesis. These changes were associated to considerable changes in the levels of metabolites associated with the tricarboxylic acid (TCA) cycle. Furthermore, in comparison to wild type plants, carbon dioxide assimilation was up to 25% increased in the transgenics under atmospheric conditions and mature plants were characterised by an increased biomass at the whole plant level. Analysis of additional photosynthetic parameters revealed that, whilst there were only relatively minor differences in pigment content in the transformants, the rate of transpiration and stomatal conductance were markedly elevated. In addition, the rate of carbon dioxide assimilation as a function of the external carbon dioxide concentration and the kinetics of guard cell opening were assessed. These experiments revealed that the transformants displayed both a strongly enhanced assimilation rate under sub-optimal environmental conditions and an elevated maximal stomatal aperture. Altogether our results indicate that the photosynthesis is enhanced in these transgenic plants by a mechanism that promotes carbon dioxide uptake via an organic acid-mediated effect on stomatal aperture.

3. Long-term patterns of root-derived CO_2 efflux via xylem stream and soil CO_2 efflux

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Ecosystem respiration consumes a majority of annual gross primary productivity in forest ecosystems and is dominated by belowground autotrophic and heterotrophic respiratory processes. Recent evidence suggests that, on a daily basis, the amount of root-respired CO_2 that remains within tree root systems and is transported aboveground via the xylem stream (F_T) can be of similar magnitude to the amount of CO_2 which diffuses from the soil surface to the atmosphere. Here, we provide further evidence of this alternative flux pathway in nine 10-year-old *Populus deltoids* trees over an entire growing season. We calculated F_T as the product of sap flow and dissolved CO_2 concentration ($[CO_2]$) in the xylem at the base of the stem and measured soil CO_2 efflux using the $[CO_2]$ gradient approach. We found that F_T accounts for a large portion of total belowground respiration during the growing season and differences between the magnitudes of F_T and soil CO_2 efflux were primarily driven by temporal patterns in soil CO_2 efflux as opposed to temporal patterns in xylem dissolved $[CO_2]$. Our observations indicate that F_T should be measured concurrently with soil CO_2 efflux to understand root metabolism and carbon economies of trees and forests.

4. Asynchronous impacts of drought on leaf respiration in darkness and in the light

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In addition to rising atmospheric CO_2 concentrations, global climate change is also likely to result in average temperatures rising and droughts becoming more frequent. In seeking to understand how such factors impact on respiratory metabolism of a fast-growing evergreen tree (*Eucalyptus saligna*), we quantified the effect of three atmospheric [CO_2] (280, 400 and 640 ppm), two growth temperatures (ambient and ambient plus 4°C) and two watering regimes (well watered and sustained drought) on leaf respiration (*R*) and associated rates of photosynthetic CO_2 assimilation (*A*). Plants were grown in pots in climate-controlled glasshouses. Leaf *R* was measured in darkness (R_{dark}) and in the light (R_{light}). We found that light inhibited leaf *R* in all cases (i.e. $R_{light} < R_{dark}$). Growth [CO_2] had little impact on area-based rates of R_{dark} or R_{light}, with rates at a common temperature being lowest in warm-grown plants. Sustained drought resulted in reduced rates of R_{dark}, R_{light} and *A;* however, the inhibitory effect of drought on *A* and R_{light} was greater than on R_{dark}. Collectively, our data suggests that there is: (1) an asynchronous response of leaf carbon metabolism to drought; and, (2) a tighter coupling between R_{light} and *A* than between R_{dark} and *A.*

5. A deficiency of mitochondrial malate dehydrogenase activity results in increased respiration and slow growth in *Arabidopsis*

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In the dark, the reaction catalyzed by the mitochondrial NAD-dependent malate dehydrogenase (mMDH) is part of the TCA cycle, providing reducing equivalents to the electron transfer chain for ATP synthesis through oxidation of malate to OAA. In the light, mMDH is thought to use NADH generated by glycine decarboxylase to reduce OAA to malate, which is exported to reduce nitrate in the cytosol or hydroxypyruvate in the peroxisomes. Recent results indicate a stimulation of photosynthetic performance and growth in transgenic tomato plants using antisense silencing of mMDH expression. We report the study of *Arabidopsis* knock-out T-DNA insertion mutants for mMDH1 and mMDH2, or for both isoforms (double KO) of the enzyme. The absence of the respective mMDH isoforms in the KO lines has been confirmed at the transcript, protein and enzyme level. The double KO line showed a rate of germination and growth lower than the wild type. The reduced growth of the double KO plants can be partly explained by a lower net CO_2 assimilation rate, due to higher release of CO_2 by mitochondrial respiration. Additionally, gas exchange measurements and GC-MS metabolite analysis give corroborating evidence suggesting that the photorespiratory pathway is impaired in the double KO line.

6. Respiration is more sensitive than photosynthesis in tree seedlings at high altitude and latitude

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Photosynthesis is the mechanism of carbon fixation in plants, although variation in net carbon gain may be more influenced by changes in respiration than photosynthesis. The aim of our project was to assess how carbon balance of tree seedlings differed between contrasting environmental conditions within high altitude and high latitude ecosystems. We observed larger effects on respiration compared to photosynthesis in 1) first-year conifer seedlings growing at the lower compared to upper edges of a timberline ecotone in the Rocky Mountains, USA, 2006 and 2) in second-year broadleaved seedlings growing in a clear-cut compared to forested condition in a boreal forest of northern Sweden, 2009. For the conifers near tree line, photosynthetic rates did not significantly change with elevation, whereas respiration rates decreased by 20%. For the broadleaves growing in the clear-cut condition, a 13% decrease in net photosynthesis was partially attributable to a 37% increase in respiration. In both the high altitude and high latitude settings, respiration was a principal driver of variation in net carbon balance of establishing tree seedlings (albeit in opposite directions), suggesting that respiration is a more sensitive process than photosynthesis to variation in environmental conditions in cool climates.

7. Investigating substrate channelling in the TCA cycle

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The tricarboxylic acid (TCA) cycle is a central pathway in plant respiration, but is also part of a larger network of metabolic reactions. These reactions can be thought of as competing for intermediates with the more conventional cyclic flux in respiration. Currently little is known about how the regulation of these competing demands on the TCA cycle is achieved.
Substrate channelling, where substrates are passed between enzymes without diffusion into the bulk aqueous phase, may be important for such regulation. An advantage of this process is that it enables metabolic pathways to be organised into discrete physical units with defined inputs and outputs. This allows independent regulation of pathways which share intermediates.
The extent to which this phenomenon occurs in plants is being investigated in isolated mitochondria. To do this two experimental approaches are being employed. Firstly, *in vivo* NMR is being used to assess whether the labelling pattern in symmetrical molecules is conserved through several reactions of the cycle. Secondly, GC-MS is being used to analyse the effect of isotope dilution on the labelling of organic acids in isolated mitochondria. Together these approaches will show the extent to which substrate channelling occurs between enzymes of the cycle in plants.

8. Influence of springtime phenology on the ratio of soil respiration to total ecosystem respiration in a mixed temperate forest

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Total ecosystem (Reco) and soil (Rs) respiration are important CO_2 fluxes in the carbon balance of forests. Typically Rs accounts for between 30–80% of Reco, although variation in this ratio has been shown to occur, particularly at seasonal time scales. The objective of this study was to relate changes in Rs/Reco ratio to changing springtime phenological conditions in forest ecosystems. We used one year (2003) of automated and twelve years (1995-2006) of manual chamber-based measurements of Rs. Reco was determined using tower-based eddy covariance measurements for an oak-dominated mixed temperate forest at Harvard Forest, Petersham, MA, USA. Phenological data were obtained from field observations and the JRC fAPAR remote sensing product. The automated and eddy covariance data showed that springtime phenological events do influence the ratio of soil to total ecosystem respiration. During canopy development, Reco rose strongly, mainly the aboveground component, due to the formation of an increasing amount of respiring leaf tissue. An increase in Rs was observed after most of the canopy development, which is probably the consequence of a shift in allocation of photosynthate products from above- to belowground. This hypothesized allocation shift was also confirmed by the results of the twelve year manual chamber-based measurements.

9. Daytime ecosystem respiration

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We explored a method for estimating daytime ecosystem respiration (ER_d) using eddy-covariance measurements. This method is an alternative to the common extrapolation of nocturnal temperature relationship of net ecosystem exchange (NEE) into daytime.
We applied a modification of the Kok-method, which at leaf level is used to estimate apparent light inhibition of respiration by analyses of the net CO_2 exchange/irradiance relationship close to the light compensation point. Data are from a beech forest and collected August 2001. A linear regression to the irradiance dependence of –NEE between 150 and 550 µmol photons m^{-2} s^{-1} is extrapolated to zero irradiance. Night-time canopy-*R* was estimated from a measured leaf-R/temperature relationship and scaled by leaf area index.
Using this new method, we estimated ER_d to be only half of that estimated from extrapolation of nocturnal temperature relationship of NEE into daytime. This major discrepancy appeared to be the result of canopy-*R* accounting for two-thirds of nocturnal E*R* together with a high degree (82%) of apparent light inhibition of canopy-*R*. If potential apparent light inhibition of ER is taken into account then the cumulated gross primary production for the average day is only 76% than without taken light inhibition into account.

10. Mining *Arabidopsis* late embryogenesis abundant (LEA) proteome for mitochondrial candidates

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Late Embryogenesis Abundant (LEA) proteins are highly hydrophilic proteins that accumulate to high level during seed development. They are also found in other anhydrobiotic organisms, suggesting an important role with respect to desiccation and water stress tolerance.
Two mitochondrial LEA proteins were previously characterized in pea seeds and in the brine shrimp *Artemia franciscana*. The mitochondrial LEA proteins were shown to contribute to stress tolerance at the cellular and organelle levels. We are interested in characterizing mitochondrial LEA proteins in the model plant *Arabidopsis thaliana*, to further explore their functions with genetic approaches.
Since the *Arabidopsis* genome harbours 51 LEA protein genes, we have undertaken a detailed bioinformatic analysis to basically predict the subcellular localization and structural features of the whole set of *LEA* gene products. In addition to the putative orthologs of the pea mitochondrial LEA protein, several other LEA proteins were predicted with a possible mitochondrial localization.
To further investigate the location of the LEA proteins, we will perform transient expression of GFP translational fusions in leaf protoplasts. This should provide additional clues to identify mitochondrial LEA proteins in *Arabidopsis*, and contribute to the subcellular annotation of the *LEA* gene family.

11. Soil CO_2 efflux in oak savanna: Resolving the effects of species composition, warming, and rainfall redistribution

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Projected climate change may alter soil CO_2 efflux from terrestrial ecosystems; yet disentangling species effects from climate drivers remains a key challenge. We explored the effects of the dominant plant species, warming, and drought on soil CO_2 efflux in southern oak savanna. Oak savanna in the south-central US are dominated by three contrasting plant functional types: *Schizachyrium scoparium* (a C4 grass), *Quercus stellata* (a C3 deciduous tree), and *Juniperus virginiana* (an invasive C3 evergreen tree). We warmed monocultures and tree-grass plots using infrared heaters and manipulated rainfall events to intensify summer drought and augment cool season rainfall. Soil CO_2 efflux was measured monthly from May 2005 to September 2009. Initially, soil CO_2 efflux was higher in plots populated with *S. scoparium* however, as the trees matured efflux was higher in plots populated with *J. virginiana*. Warming treatments had no consistent effect on soil CO_2 efflux. Soil CO_2 efflux was decreased by intensified summer drought and increased spring rainfall. Overall, the effect of species combination was greater than that of either treatment. These findings suggest that soil CO_2 efflux in oak savanna will likely respond more to changes in species composition than to direct effects of climate drivers.

12. The influence of drought and growth rate on leaf dark respiration of *Eucalyptus globulus* trees

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A better knowledge of factors underlying rates of autotrophic respiration in forest ecosystems is necessary to improve estimates of carbon sequestration. We compared two monospecific stands of *Eucalyptus globulus*, differing in age and growth rates. Leaf dark respiration was measured for consecutive years, in spring and summer, corresponding to the peak of growth rate and water stress, respectively. After normalization of respiration rate to 20º C (R_{20}), a strong pattern of seasonal variation emerged. In both stands, we observed a strong decline in R_{20} during the summer, concurrent with the decrease in leaf water potential and soluble sugars. There was a significant correlation between R_{20} and pre-dawn leaf water potential in both stands, underlying the role of drought in determining carbon balance in these ecosystems. Leaf concentration of glucose, fructose and sucrose were similar in the two stands but the ratio of the sum of glucose and fructose to sucrose was found higher in the older stand with lower growth rate. The concentration of soluble sugars was found significantly correlated with R_{20} only in the older stand. Together these results suggest a different relationship between carbon metabolism and carbon efflux to the atmosphere in the two stands.

13. Drought accentuates acclimation of leaf respiration to summer heat: a comparison of whole trees growing under ambient and elevated atmospheric CO_2

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Understanding the impacts of seasonal changes in climate on leaf respiration (*R*), both now and in the future, is critical to predicting rates of plant growth and improving global climate models. We quantified the impacts of ambient and elevated atmospheric [CO_2] (+240 ppm) and summer drought on seasonal shifts in the daily temperature response curves of *R* and related functional traits of *Eucalyptus saligna* growing in whole tree chambers in SE Australia. Seasonal acclimation of *R* was evident, as illustrated by the: (1) 59% lower *R* (measured at 20^{o}C, R_{20}) in summer compared to the previous spring; and (2) downward shift in temperature response curves in summer (relative to spring). Acclimation occurred in both [CO_2], irrespective of whether trees experienced summer drought or not. R_{20} was near 35% higher under elevated [CO_2] across both watered and droughted trees. Moreover, summer drought further reduced R_{20} by 19% in both [CO_2] treatments, with summer drought accentuating the seasonal downward shift in temperature response curves of *R.* Our findings highlight the need for the combined effects of seasonal changes in temperature and water availability to be accounted for when predicting future rates of net CO_2 exchange at local, regional and global scales.

14. Diurnal and seasonal dynamics in temperature normalized stem CO_2 efflux of Norway spruce

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Stem respiration is an important part of forest ecosystem CO_2 flux. We measured stem CO_2 efflux continuously by an automatic system in a Norway spruce forest planted in 1981. The respiration chambers were placed on eight trees in the height of 1.3 m and on two of them also at the heights of 3.0 and 4.5 m in the growing seasons 2006–2009. We measured also incident radiation, precipitations, transpiration flux, stem increment rate and stem temperature. Seasonal course of stem CO_2 efflux normalized to 10°C (R10) followed the pattern of the stem growth. They increased at the beginning of the season up to a maximum in July and then decreased. However, this dependence was disturbed by external factors especially by alternating of water stress and rain events. Rain events after a dry period caused increase in R10 up to 60%. We observed diurnal course of R10 with maxima during night hours. Oscillation of R10 had amplitude up to 50% of mean daily (24 hours) value of R10.

15. The effect of acute ozone treatment on carbon metabolism enzymes of *Arabidopsis thaliana* mutant

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In this study we analyzed the changes in activities of several carbon metabolism enzymes from the ozone-sensitive mutant rcd1 (*rcd1*) and the ozone-tolerant wild type Columbia (col-0) of *A. thaliana* in response to an acute ozone treatment. Plants were fumigated with 350 ppb ozone for 6 hours and the whole rosettes were collected immediately at the end of the exposure (6h), then 24 hours and 48 hours respectively during the recovery period. The ozone treatment resulted in a decrease in *ribulose-1,5-bisphosphate carboxylase oxygenase* (rubisCO) activity for rcd1 and col-0. A similar response was also obtained for plants exposed to chronic ozone treatment (Dizengremel, 2001). However, concerning *phosphoenolpuryvate carboxylase* (PEPc) activity, known to be stimulated in response to chronic ozone treatment for C3 plants, a different effect was noticed between rcd1 and col-0 in our conditions. At the end of the recovery period, the activity of this enzyme was slightly stimulated for rcd1, while it was inhibited for col-0. The acute ozone treatment yielded an increased activity of ME *malic enzyme* and *glyceraldehyde-6-dehydrogenase* (G6PH) in both rcd1 and col-0. For col-0, the enzyme stimulation already reached a high level at the end of the exposure period (6h). On the other hand, the stimulation was more progressive for *rcd1*, with the highest value measured 48 hours during the recovery period. The activation of these NADP-dependent enzymes may highlight their role in reducing power supply and should perhaps be defined as one of the major events in the plant detoxification process.

16. Responses of the cytochrome and alternative pathways to drought and subsequent re-watering in *Nicotiana sylvestris*

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The frequency and intensity of drought events is increasing as a result of climate change. Drought is considered one of the most important factors limiting plant productivity and growth worldwide. The limitation of plant growth imposed by drought is mainly due to reductions in plant carbon allocation/aquisition, which depends on the balance between photosynthesis and respiration. In contrast to the large number of studies assessing drought impacts on photosynthesis, little is known about the response of plant mitochondrial respiration during drought in the field and even less during the recovery phase after re-watering.
Changes in respiration and photosynthetic activities have been studied during severe drought and after consecutive re-watering in *Nicotiana sylvestris* WT and CMSII mutant plants grown under field and growth chamber conditions. Drought was imposed to the plants by withholding water for 5-7 days and after their stomatal conductance for water vapour dropped below 50 mmol H_2O m^{-2} s^{-1}. With regard to the different responses of WT and CMSII to the treatments, the role of cythocrome and alternative pathways under drought and subsequent recovery is discussed in relation to changes in photosynthesis and osmotic adjustments.

17. Kinetic characterisation of respiratory carbon pools in a grassland ecosystem

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For the kinetic characterisation of the main sources supplying ecosystem respiration we present a new apparatus for continuous $^{13}C/^{12}C$ labelling. Four open-top chambers (OTCs) were flushed with a mix of CO_2-free air with ^{13}C-depleted CO_2. Two different methods (open dynamic and closed static chamber mode) were applied for observation of the tracer during night-time respiration in the field. Mechanisms underlying discrepancies between the two chamber modes were investigated with a soil CO_2 transport model.
The concentration (367±6.5 µmol mol^{-1}) and $\delta^{13}C$ (-46.9±0.4‰) of CO_2 in the OTCs was stable during photosynthesis. The labelling kinetics of respiratory CO_2 measured in the open dynamic mode in the field agreed with that of excised soil+vegetation blocks measured in a laboratory-based reference system. The kinetics fitted a two-source system, with a rapidly labelled source ($T_{1/2}$ 2.6 d) supplying 48% of respiration, and the other source (52%) releasing no tracer during 14 days of labelling. On the other hand, measurements in the closed static mode yielded a ~1.5-fold tracer content in ecosystem respiration. This discrepancy was largely explained by labelling CO_2 stored in the soil gas and water pores during the preceeding labelling period and re-diffusing into the chamber headspace during the closed mode measurements.

18. *In folio* isotopic tracing demonstrates that nitrogen assimilation into glutamate is mostly independent from current CO_2 assimilation in illuminated leaves of *Brassica napus*

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For N assimilation and reduction by plants, a sustainable provision of primary NH_2-acceptors is necessary. The respiratory metabolic pathway is able to provide intermediates (e.g. α-ketoglutarate) for N reduction and day respiration appears to be the key process for N assimilation by plants. Plants produce CO_2 through day respiration and photorespiration and assimilate CO_2 through photosynthesis in light. As such, the connection between nitrogen and carbon seems as essential as the input rates themselves. However whether the supply in carbon skeletons by current assimilation through respiration in the light is the origin of carbon atoms that are used for N fixation remains unclear. As an aid in clarifying such C/N interactions, labeling experiments were carried out with $^{13}CO_2$ (99% ^{13}C) and ^{15}N-ammonium nitrate ($^{15}NH_4{}^{15}NO_3$ 99% ^{15}N) on rapeseed detached leaves and ^{13}C-NMR and ^{15}N-NMR analyses were performed. Our results indicate that the remobilization of night-stored molecules plays a major role to feed 2-oxoglutarate synthesis, the precursor of glutamate synthesis and N assimilation in light. In other words, the natural day/night cycle is critical for nitrogen assimilation as intermediates produced in the dark may be used for the subsequent light period during which N is reduced and assimilated in leaves. Here we confirmed the importance of dark heterotrophic metabolism to improve N contents in plants. In other words, our results explain why the improvement of plant growth does not strictly correlate with an improved photosynthesis but rather, with an accurate balance between respiration and CO_2 assimilation.

19. Carbon isotope discrimination during dark respiration by autotrophic and heterotrophic organs and potential impact on ecosystem studies

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Until recently changes in the ^{13}C signal of ecosystem respired CO_2 have been attributed to changes in the photosynthetic discrimination due to changes in environmental conditions. However, the generally accepted hypothesis in such studies is that no discrimination occurs downstream of photosynthetic fixation is now questioned. We recently showed by compiling data from the literature that C3-leaves are in general ^{13}C-depleted compared to other organs. Post-photosynthetic discriminations do likely occur, leading to this ^{13}C-difference between autotrophic and heterotrophic tissues/organs. We measured the dark-respired $^{13}CO_2$ on intact leaves and roots using a closed gas-exchange system coupled to IRMS, and sucrose ^{13}C after purification by HPLC. We demonstrated an opposite respiratory fractionation in leaves compared to roots; leaf-respired CO_2 being ^{13}C-enriched compared to sucrose varying among species and environmental conditions, while root-respired CO_2 being ^{13}C-depleted compared to root material. We also showed that leaf- and root-respired $^{13}CO_2$ diverges when leaves become green (leaf-respired CO_2 becomes ^{13}C-enriched, while root-respired CO_2 becomes ^{13}C-depleted), the differences in the ^{13}C-signal of organic matter between organs appear at the same time. Mass balance at the whole-plant level clearly showed that when the plant is heterotrophic, the overall respired CO_2 is ^{13}C-depleted, while plant organic matter is ^{13}C-enriched. When the plant becomes autotrophic, the tendency reversed. We also demonstrated the metabolic origin of this leaf-root difference. These results are relevant for ecosystem studies and should be taken into consideration for disentangling photosynthetic and respiratory fluxes of net ecosystem exchange.

20. Metabolic origin of $\delta^{13}C$ of dark-respired CO_2: Comparison between leaves and roots

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The generally accepted hypothesis in ecosystem studies that no discrimination occurs downstream of photosynthetic fixation is now questioned. Indeed, leaf-respired CO_2 in the dark is ^{13}C-enriched compared to organic matter, while root-respired CO_2 is ^{13}C-depleted. We have previously shown that the $\delta^{13}C$ of leaf-respired CO_2 linearly decreased with a decrease in respiratory quotient. This strongly suggested that the variation in $\delta^{13}CO_2$ is a direct consequence of a switch from carbohydrate oxidation producing ^{13}C-enriched CO_2 to β-oxidation of fatty acids producing ^{13}C-depleted CO_2. This is consistent with the assumption that the leaf dark-respired $^{13}CO_2$ is determined by the relative contribution of the major decarboxylation processes: PDH and Krebs cycle. To address this issue in roots, we conducted ^{13}C-analysis on CO_2 and metabolites under natural abundance and following labelling with positionally ^{13}C-enriched glucose or pyruvate using IRMS and NMR techniques. Surprisingly, it was found that the $\delta^{13}C$ of root-respired CO_2 remained constant under continuous darkness, despite the decrease in the respiration rate and respiratory quotient. In typical conditions, we calculated an important contribution of the pentose phosphate pathway to respiration (22%) and fluxes that appeared quite similar along glycolysis and the Krebs cycle. Continuous darkness mainly affected the Krebs cycle which seemed to become notably reduced, the ongoing synthesis of glutamate being sustained by the anaplerotic action of PEPc. It is concluded that the invariance in the root-respired $^{13}CO_2$ under continuous darkness is driven by compensations between both the different fractionating steps and the composition of the respiratory substrate mix.

21. Seasonal changes in soil respiration in olive orchards

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Olea europaea is cultivated on over 10 Mha worldwide. The objective of this work was to determine the seasonal courses of soil respiration rates under different conditions of soil humidity (RH) in two olive orchards in Tuscany. Respiration was measured in sandy-clay (tilled) or sandy-loam (grass covered) soils at monthly intervals, using a gas exchange closed system. Three or four sampling points beneath the canopy of either 8- or 4-year old trees and one point between the rows were used. The highest rates were measured in the summer under irrigated conditions, when RH ranged from 17 to 34% in volume. Rates in the rain-fed treatment were high (0.6–1.0g CO_2 m^{-2} h^{-1}) when soil temperature and RH were about 17–20°C and 25%, respectively, but dropped to 0.3 and 0.1g CO_2 m^{-2} h^{-1} (beneath the canopy and between rows, respectively) when RH was less than 10%. In grass-covered soil rates were high (0.86–1.79g CO_2 m^{-2} h^{-1}) when temperature and RH were 18–22°C and above 14% in volume. In orchard 1, the estimated C respired yearly was 7.842 and 7.324 t ha^{-1} under irrigated and rain-fed conditions, respectively, whereas in orchard 2 it was 11.79 t ha^{-1} $year^{-1}$.

22. Environmental and plant controls on ecosystem respiration in a beech forest in Central Italy

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The amount of carbon that is absorbed or emitted from a forest ecosystem (NEE) is the result of the difference between the gross primary production (GPP) and the total ecosystem respiration (TER). Most of the variability on NEE among the different ecosystems has been attributed to the variability of the TER rates. With the objective to analyse the responses of TER and its components to environmental 'drivers', we measured ecosystem carbon fluxes by the eddy-covariance technique and major components such as soil (R_S), stem (R_W) and leaf (R_L) respiration, by dynamic chambers. Over the study period the variability of TER was explained for a 63% by changes on R_S, according to the variation of soil temperature and soil water content. The rates of R_W differed significantly among trees according to the 'social classes' and it was strongly related with stem temperature and daily NEE. Under common temperature R_L changed during the day depending on the availability of the total non-structural carbohydrates. Our results show how ecosystem respiration and thus its components can be controlled by environmental variables, forest structure and photosynthetic activity.

23. Ammonium-dependent respiratory induction is dependent on cytochrome pathway in *Arabidopsis thaliana* shoots

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Concentrated ammonium as the sole N source often induces O_2 uptake rates compared with nitrate. Several explanations for this respiratory induction have been suggested, but the underlying mechanisms are still unclear. To reveal important factors of the respiratory induction, we measured O_2 uptake rates, the activities and transcript levels of respiratory components and the concentrations of adenylates and reducing equivalents using *Arabidopsis thaliana* shoots grown in medium containing various N sources. O_2 uptake rates were induced with the ammonium accumulation in shoots. This induction was not accompanied by the deficiency of ATP or accumulation of reducing equivalents, and was not related to the ammonium assimilation. The capacity of the ATP-coupling cytochrome pathway and its related genes were up-regulated with concentrated ammonium as the sole N source, whereas the deficiency of ATP-uncoupling alternative oxidase did not influence the induction of O_2 uptake rate. We indicate that the ammonium-dependent induction of O_2 uptake rates is related to the ATP consumption via H^+-ATPase.

24. Will trees die from carbon starvation in CO_2-enriched world?

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Climate change scenarios predict increasing global temperatures and more frequent occurrences of extreme droughts. During drought, trees close their stomata to prevent water loss but thereby also inhibit CO_2 diffusion into leaves and thus carbon assimilation. Maintenance respiration in heterotrophic tissues and decreased carbon assimilation can yield a negative carbon balance during prolonged drought. Carbon starvation during drought could be the causal mechanism of regional vegetation die-offs that have been observed around the globe. Manipulative experiments seem to confirm this hypothesis but several issues (e.g., storage pool depletion, long-distance carbon transport) and alternative hypotheses (i.e. catastrophic xylem dysfunction) have not yet been addressed.
Here we present our work-in-progress of an experimental study that aims at testing either hypothesis (carbon starvation vs. xylem dysfunction). Two tree species with different cavitation vulnerabilities and carbon storage capacities are exposed to an intense and prolonged drought. We measure growth rates, carbon assimilation and (leave, stem, root) respiration rates and carbon isotope ratios of respired CO_2 after labelling as well as carbon storage loading and transport (xylem and phloem) fluxes. These measures will elucidate whether carbon starvation or catastrophic xylem dysfunction, or an interaction of both is the causal mechanism in drought-induced tree mortality.

25. The carbon balance of Scots pine, Norway spruce and silver birch in changing climate: the effects of temperature and ectomycorrhizal fungal communities

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Climate change will change boreal forest soil temperatures and cause changes in biological activities and microbial communities in soils. Ectomycorrhizal fungi (ECM) are mostly dependent on plant-derived carbon and photosynthesis and contribute significantly to soil respiration. Therefore, the relation of ECM to the carbon balance of a tree and its temperature sensitivity is of major importance, if boreal forest soil CO_2 balance will be estimated. We conducted a series of experiments using Scots pine, Norway spruce and Silver birch seedlings in temperature controlled custom-made microcosms. CO_2 gas exchange measurements, $^{14}CO_2$-pulse-chase labeling and archaeal as well as ECM identification by DGGE or morphotyping and DNA sequencing were performed for determining the allocation pattern of assimilated carbon between tree biomass, above- and belowground respiration and soil, as well as the associated microbial populations. The different tree species had differences in their carbon economy (photosynthetic activity, carbon allocation pattern and turnover rate) and some members of ECM community affected the carbon allocation patterns in Scots pine seedlings. Soil temperatures had effects on CO_2 exchange as well as on mycorrhizal community parameters. The diversity of *Euryarchaeaota* tended to increase along with the increasing temperature while the case was opposite for *Crenarchaeota*.

26. Interpreting the variations in soil and canopy respiration observed in a spruce forest in Denmark

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The carbon balance of forests is largely determined by respiration. Autotrophic and heterotrophic soil respiration, as well as stem and canopy respiration, are controlled by a variety of environmental factors that operate at different time scales. A new field study in a spruce plantation in Denmark tries to disentangle some of the most important control mechanisms of forest respiration by use of continuous measurements of the total atmospheric exchange of carbon dioxide (CO_2) above the forest, the CO_2 concentrations in the air at different heights and the emission of CO_2 from the soil as observed with automated chambers. The first data, collected in the summer and autumn 2009, show that soil respiration accounted for about three quarters of the total respiration during this period. The observed variations in soil respiration could largely be explained by changes in soil temperature and soil moisture content, with the temperature response being modified by both very dry and very wet soil conditions. The sum of canopy and stem respiration, calculated as the difference between night-time ecosystem respiration and soil respiration, showed a clear seasonal trend that masked the responses to meteorological variables. Possible implications of these observations for predictive respiration models will be discussed.

27. Mitochondrial response to fertilization and warming in two dominant tundra species

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The vegetation and soils of the Arctic store large amounts of the world's carbon and global climate change is altering the balance of this system. To better predict the future effects on carbon storage in northern latitudes, there is a need for a greater understanding of plant responses to environmental change. Examining plant respiration, the process responsible for carbon efflux, will provide necessary information for refining predictions about tundra carbon fluxes. Using two dominant tundra species of different functional types, *Eriophorum vaginatum* and *Betula nana,* respiration variables were made under control, fertilization, warming, and fertilization with warming treatments. Oxygen consumption rates varied both across species and treatments, with fertilization increasing rates more significantly than warming in both species. Mitochondrial density was significantly increased under fertilization across species, but warming effects were species specific. The correlation of mitochondrial density and oxygen consumption across species indicates a general structural-functional relationship, though is more accurately defined as a taxon-specific characteristic. These changes may reflect a shift in plant metabolism and energy balance that may help to explain future the carbon balance at the species and community level under future climate change scenarios.

28. Are Amazon palm swamps a methane source?

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Large areas of Western Amazonia are covered by Mauritia palm swamps. They contain much carbon, which accumulated during the Holocene. Current remote sensing and atmospheric studies show high atmospheric methane concentrations in this region, indicating a significant terrestrial source of this greenhouse gas. We suspect palm swamps to dominate this source. We measured the assimilation and respiration rates of the dominant palm species and the carbon concentrations over palm swamp soils. Data were collected from three locations from the limit of inundation to the center of a palm swamp. The sites varied significantly in palm density, standing biomass, and depth of soil organic layer. The organic layer had a maximum depth that exceeded, sometimes, 8m. Maximum assimilation rates correlated with higher standing biomass. Dark leaf respiration rates varied between 0.31 and 0.89 µmol CO_2 m^{-2} s^{-1} and between 0.35 and 1.75 µmol CO_2 m^{-2} s^{-1} for common understory species. The highest release of CO_2 and especially of CH_4 was observed in the area with high standing biomass and productivity. Thus, palm swamps appear to be mainly a source of methane.

29. Temperature divergence and its Interrelationship between physiological and biochemical processes of *Artemisia monosperma* in Saudi Arabia

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Increases in temperature raise the rate of many physiological processes such as photosynthesis in plants, to an upper limit. Extreme temperatures can be harmful when beyond the physiological limits of a plant. Variation in temperature is ecologically and physiologically significant due to their effect on different biochemical parameters in plants. Short-term effects of temperature on plant photosynthesis and respiration were demonstrated to be dependent on the actual measurement temperature. The biochemical and physiological mechanisms responsible for these effects are discussed in *Artemisia monosperma* which is grown in Saudi Arabia**.** *Artemisia monosperma* adapted to such changes, which resulted in stimulation of plant growth as a result of increased photosynthesis especially during the hot period. Changes in sugar metabolism were also associated with significant increase in concentrations of N- content in protein. Osmotic potentials ($\Psi\pi$) in leaves of *Artemisia monosperma* varied from -1.3 MPa to -2.5 MPa in response to temperature variation. Osmotic potential ($\Psi\pi$) under cold conditions was varied and remained depressed by 0.4 MPa in comparison with plants under hot conditions, indicating that solute concentration per unit water content had changed in response to temperature divergence. Plant research, is required to assess the consequences of putative changes on such complex systems.

30. Exploring the impact of salt stress on respiration and mitochondrial function in wheat varieties

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The simultaneous application of multiple abiotic stresses is damaging to crops. It is predicted that climate change will increase the frequency and severity of extreme weather events, such as droughts and heatwaves. Such stresses will exacerbate crop losses caused by pre-existing agricultural problems, such as soil salinity, by placing additive stresses on top of the initial one. To develop crop plants which tolerate multiple stresses simultaneously, researchers will need to identify mechanisms of stress toxicity and stress tolerance in crops. Furthermore, effects specific to one stress will need to be discriminated from the general stress response.
Wheat varieties exhibit different degrees of salt tolerance, and there is evidence suggesting that respiratory properties may contribute to salt tolerance. For instance, salt-sensitive wheat varieties display a higher respiration rate under salt treatment, while salt-tolerant varieties maintains similar a respiration rate under both control and salt treatments. However, the molecular basis of such results remains unexplored. Here we present data from a quantitative proteomic comparison of mitochondria isolated from two Australian commercial wheat genotypes, Wyalkatchem and Janz. While there is remarkable similarity between the mitochondrial proteomes, Wyalkatchem displays higher expression of a specific superoxide dismutase (Mn-SOD), a protein which is vital for defence against reactive oxygen species (ROS). We have used mass spectrometry to characterise this difference at the molecular level. This is part of an ongoing research project which hypothesises that there is a correlative link between salinity tolerance, respiration rates, and mitochondrial proteomes across different wheat varieties, and that respiration could be altered to influence salt tolerance.

31. Involvement of reducing power in the degree of ozone sensitivity of wheat cultivars

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NADPH is an important molecule in the redox balance of the cell. It appears as an indispensable substrate for detoxification processes required for protection against oxidative damages (Dizengremel *et al*., 2009). To validate this hypothesis, we have considered two wheat cultivars presenting ozone sensitivity differences and analyzed physiological and biochemical parameters as the pyridine nucleotide levels. In response to ozone exposure (40 and 120 ppb), the stomatal conductance decreased in both cultivars. Photosynthetic parameters (net CO_2 assimilation, RubisCo *in vitro* activity) were also negatively affected in both cultivars but the decrease was faster in the sensitive cultivar than in the tolerant one. The pool of pyridine nucleotides (oxidised and reduced forms) did not show important modifications all along the treatment. However, relative to the cumulative ozone uptake (CUO), higher amount of NADPH were obtained for the sensitive cultivar. It was also for this cultivar that the phospoenolpyruvate carboxylase (PEPc) activity was stimulated (+80% relative to control plants). Overall, these results suggest that NADPH-generating enzymes could be involved in the response of sensitive plants to ozone.

32. Water status dominantly controls belowground respiratory patterns in the sub-tropical and tropical ecosystems

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Here we compared our studies on the belowground respiration measurements in the sub-tropical (southern Australia) and tropical ecosystems (Sumatera and Java) with average temperature of 15^{o}C vs 26^{o}C and annual precipitation 600–800 vs 1200–2400 mm respectively. Despite the variations of forests, soil types and climates condition included in this study, there was strong generic water status control on the respiratory patterns. We also found smaller controls of the vegetation (autotrophic) as revealed by its leaf area, temperature, short-term weather conditions which constituted smaller control of the spatial/temporal-variability of the respiratory patterns.

33. Mitochondrial implications during heavy metal toxicity in *Arabidopsis thaliana*

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Contamination with heavy metals is of growing concern due to their known toxicity regarding the environment and human health. Cadmium (Cd), a non-essential element, has shown to evoke cellular and molecular responses in *Arabidopsis thaliana*. Oxidative stress is one of the central cellular responses to Cd exposure, leading to an imbalance between pro- and antioxidants in favour of the former (Smeets *et al.* 2009). Accumulation of reactive oxygen species (ROS) can result in cellular damage, but can also activate signalling pathways potentially influencing the cellular redox state. Mitochondria are suggested to play an important role in the redox state and signalling. In environmental stress conditions, such as Cd exposure, mitochondrial signals have a clear impact on nuclear gene expression (Rhoads and Subbaiah, 2007). The goal of our study is to determine mitochondrial responses to Cd-induced stress. For that purpose, *Arabidopsis thaliana* plants will be exposed to Cd during different time spans. Growth parameters are surveyed, next to biochemical analyses in relation to oxidative stress. Also, the gene expression of nuclear genes coding for mitochondrial proteins involved in respiration and possibly retrograde signalling, as well as genes related to oxidative stress, will be measured using real-time PCR after exposure to Cd.

34. Allometric scaling of foliar respiration to photosynthetic rates and nitrogen contents in tropical tree leaves

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Foliar respiration is an important determinant of the daily carbon balance in plants. How do respiration rates of different plant species change in relation to decline of photosynthetic capacity with leaf aging? We addressed this question by quantifying dark respiration rates (R_d), light-saturated photosynthetic rates (A_{max}) and nitrogen contents (N_{area}) per unit leaf area for 3-yr old saplings of 10 Neotropical tree species raised in common gardens in tree fall gaps in Panama. CO_2 exchange rates were measured on intact leaves of known age from monthly leaf demography censuses. Median leaf span differed from 140 to 800 days among species, and was negatively correlated with the initial A_{max}. In all species, R_d, A_{max} and N_{area} declined linearly with leaf age, but at different rates among species. In all species, R_d was positively related to A_{max} and N_{area} in log-log plot, without significant difference in scaling exponent among species. The allometric exponents < 1 reflected imperfect downregulation of R_d when A_{max} and N_{area} decreased with leaf age. This scaling relationship of foliar respiration to photosynthetic capacity should be useful for modeling efforts to scale net primary productivity in time and space, especially in species-rich tropical forests.

35. Physiological role of mitochondrial gamma type carbonic anhydrases in the CO_2 metabolism of plant cells

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During photosynthesis, the CO_2 concentration in chloroplasts of leaf cells often is limiting for carbon fixation. At the same time there is an excess of CO_2 in mitochondria due to the decarboxylation of organic acids (TCA cycle) and glycine (photorespiration). This inner cellular imbalance in CO_2 distribution is especially drastic if plants grow at dry or hot locations due to stomata closure. The role of respiratory CO_2 is rather complementary in plant and animal cells: in animal cells, it more or less is 'waste', whereas in plant cells it represents an important substrate of photosynthesis. How is plant mitochondrial CO_2 metabolism adapted to this situation? Recently, gamma-type carbonic anhydrases (γCAs) were discovered in plant mitochondria, which were suggested to be involved in an active CO_2 transfer system between mitochondria and chloroplasts. This system resembles the well studied 'carbon concentration mechanism' (CCM) of cyanobacteria. The γCAs discovered in plants are associated with the NADH-dehydrogenase complex (complex I) of the mitochondrial respiratory chain and form a plant specific extra domain on its matrix exposed side. The physiological role of these enzymes in relation to the postulated inner cellular CO_2 transfer mechanism is currently investigated by our laboratory using Arabidopsis knock out plants.

36. Soil nutrient status increases alternative oxidase engagement in the field

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Small shifts in the relationship between plant photosynthesis and respiration could result in dramatic shifts in the amount of carbon released into the atmosphere, with possible consequences for global climate change. Previous research has shown that increased engagement of the respiratory protein alternative oxidase (AOX) *vs*. cytochrome *c* oxidase (COX) can shift the balance towards increased CO_2 release. Laboratory studies have shown that deficiencies of nutrients such as N and P may increase the relative engagement of AOX and could thus potentially increase a plant's carbon output. Global climate change may lead to just such deficiencies and we therefore chose to investigate whether AOX engagement would, under field conditions, increase in plants exposed to long term nutrient deficiencies. *In vivo* AOX/COX engagement is best measured using isotope fractionation methods. We have developed a method for capturing respiration samples in the field for later analysis in an isotope-ratio mass spectrometer (IRMS). Using this technique, we measured respiration on several canopy species along a soil chronosequence in New Zealand and in a long-term nutrient manipulation site in the Alaskan tundra. Preliminary results suggest that relative AOX engagement *increases* with increasing nutrient availability, contrary to our hypothesis. While the increase in *in vivo* AOX engagement appears to correlate with foliar N, it does not correlate with other leaf traits such as carbohydrate status, AOX/COX protein abundance, respiration rate, or Fe and Cu levels.

37. Ecosystem respiration dependency on photosynthesis

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We measured net ecosystem CO_2 flux (F_n, ambient light) and ecosystem respiration (R_E, darkened chamber), and estimated gross ecosystem photosynthesis (P_g) by difference, for two years in a temperate heath ecosystem using a chamber method. Model fit of R_E of a classic, first-order exponential equation related to temperature (second year; $R^2 = 0.65$) was improved when incorporating a linear relationship between R_E and P_g (second year; $R^2 = 0.79$), suggesting that daytime RE increased with increasing photosynthesis. Furthermore, the modified model showed a more realistic Q_{10} of 2.5 in both years compared to 3.3-3.9 by the classic equation. The model introduces R_{photo} as the fraction of instant ecosystem respiratory activity, which is directly associated with instant photosynthetic production. It increases the reference value of R_E by 5% per unit assimilated carbon dioxide flux at 0ºC and by 35% at 20 ºC implying a high sensitivity of ecosystem respiration to photosynthesis during summer. Annually, R_{photo} accounted for 24 % of R_E. The simple model provides an easily applied, non-intrusive tool for investigating seasonal trends in the relationship between ecosystem carbon sequestration and respiration.

38. Protein-protein interactions in the glycolytic metabolon

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Glycolysis is a central metabolic pathway not only providing pyruvate for the respiratory pathway but being linked to sugar, amino acid and lipid metabolism and both to the oxidative and non-oxidative branch of the pentose phosphate pathway. Additionally, glycolytic enzymes function in sugar and oxidative stress signalling which is associated with a translocation of these enzymes into the nucleus. Consequently, glycolysis must be tightly regulated to ensure meeting the demand of the cell for individual metabolites. One way of regulating metabolic pathways is realised by forming metabolons in which intermediates can be directly passed from one enzyme to the active site of the consecutive enzyme (channelling). This minimises diffusion of intermediates into the bulk phase and, therefore, their availability for competing pathways. In plants glycolytic enzymes are organised in a metabolon which localises to the outer mitochondrial membrane (OMM) in dependence on respiratory demand. The underlying mechanisms of metabolon formation and attachment to the OMM have not been studied yet. Thus, the major perspective is establishing a platform to study protein-protein interactions. To achieve this, yeast mitochondria, complemented with each of the four *Arabidopsis* voltage-dependent anion channel (VDAC) isoforms, were chosen as basis for interaction experiments with heterologously expressed proteins.

39. Transcriptional reprogramming of leaf respiratory metabolism in plants grown at elevated [CO_2]

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Plant respiration is a major flux in the global carbon cycle. At the tissue and plant scale, respiration is a key determinant of growth and yield. Predictions of future ecosystem services, including food supply, are uncertain due to our poor mechanistic understanding of respiration responses to elevated [CO_2]. Molecular, biochemical and physiological changes in the carbon metabolism of soybean in a free-air CO_2 enrichment experiment were investigated over two growing seasons. Growth of soybean at elevated [CO_2] (550 μmol mol^{-1}) under field conditions stimulated the rate of night-time respiration by 37%. Microarray analysis revealed that greater respiratory capacity was driven by greater abundance of transcripts encoding enzymes throughout the respiratory pathway. Greater foliar respiration at elevated [CO_2], will reduce carbon balance, but could facilitate greater growth and yield through enhanced energy production for photoassimilate export to sink tissues. Transcript abundance of 25 transcription factors was also greater at elevated [CO_2]. Three knock-out lines of *Arabidopsis*, each lacking one of these transcription factor genes, showed normal growth under ambient [CO_2] but no stimulation of growth by elevated [CO_2]. These three genes are being investigated as putative regulators of respiratory transcriptional reprogramming that is essential to stimulation of growth by elevated [CO_2].

40. Investigation of cadmium treatment on gas exchange parameters in *Zygophyllum fabago*

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The succulent perennial *Zygophyllum fabago* displays resistance to high concentrations of cadmium and zinc but a high intrapopulational variability occurs in response to heavy metal treatments. The present study focused on the impact of 10 µM $CdCl_2$ applied in nutrient solution on the photosynthetic activity and some gas exchange parameters in selected individuals differing in their tolerance to Cd.

A strong heterogeneity in Cd accumulation and tolerance was observed between individuals and some plants could exhibit high Cd accumulation without any biomass reduction. The Cd concentration surprisingly increased faster in plants showing the highest tolerance. Sensitive plants showed a slight decrease in photosynthetic activity and strongly decreased their leaf stomatal conductivity and transpiration rate after 4 weeks in response to Cd. A decrease in C isotope discrimination indicated a limited diffusion of CO_2 in both tolerant and sensitive individuals. A quantitative proteomic analysis of *Z. fabago* leaves showed that among proteins which abundance was lowered in stressed plants, some proteins related to photosynthetic apparatus and energy metabolism were found. Altogether these results suggest that in both tolerant and sensitive individuals, photosynthesis and respiration were affected, but at a lower degree in the most tolerant plants despite a stronger Cd accumulation.

41. The quantitative significance of carbon stores for shoot and root respiration of perennial ryegrass

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This work investigates the system supplying substrates to respiratory processes in perennial ryegrass plants (*Lolium perenne* L., a fructan-storing species) and its response to the same amount of daily photosynthetically active radiation provided either as 275 µmol m^{-2} s^{-1} during 24 h of continuous illumination (continuous light) or as 425 µmol m^{-2} s^{-1} in a 16/8 h light/dark regime (discontinuous light). Plants were grown in controlled environments and labelled with $^{13}CO_2/^{12}CO_2$ for intervals ranging from 1 h to 1 month, followed by measurements of the rates and $^{13}C/^{12}C$ ratios of CO_2 respired by shoots and roots in the dark. Compartmental analysis of tracer kinetics revealed that shoot and root respiration in both treatments was fed by current assimilates and stores. Within a treatment, the turnover of stores was near-identical for shoot and root respiration.

Specific growth rates were similar in continuous and discontinuous light treatments, but continuous light slowed the turnover (+200%) and increased the size (+70%) of the respiratory storage pool. In continuous light, the fractional contribution of stores to respiration was only marginally lower than in discontinuous light. This result indicates a difference to starch-storing species, which are known to adjust storage deposition/mobilization fluxes to day length, and suggests that the involvement of stores in supplying respiration may depend on the form of storage.

42. Effect of moderately high temperatures on the oxygen consumption rates of *Vigna unguiculata* mitochondria

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Temperature-mediated changes in plant respiration are now accepted as an important component of the biosphere response to global climate change. However, little is known about mitochondrial respiration responses induced by increasing temperatures. The aim of this work was to evaluate the mitochondrial respiration of *Vigna unguiculata* seedlings under a stress with moderated high temperature. The seeds were germinated on filter paper embedded in distillate water for three days on the dark at 25ºC. After this time part of the plants were transferred to a chamber at 40ºC. On the seventieth day physiological parameters measures were taken and the hypocotyls used for mitochondrial isolation. The seedlings submitted to grow under 40ºC for 4 days showed a lowering in biomass (48%) and size (42%) when compared to control ones grown at 25ºC. The stressed seedlings had their development impaired, especially the epicotyl. In this case, mitochondria from seedlings under stress showed respiratory control and ADP/O ratio comparable to respective control independent of the used substrates. However, there was a decrease in the oxygen consumption rate of 50%. In both conditions, malate dehydrogenase activity was predominant and AOX seemed to be inactivated. Our results suggest that increasing of temperature promotes a down regulation concerning oxygen uptake in mitochondria.

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43. The effects of GNC/GNL transcription factors on the photosynthetic and respiratory metabolism of *A. thaliana* grown under elevated [CO_2] and differing nitrogen availabilities

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GNC and GNL are paralogous proteins in the GATA transcription factor family shown to be nitrate inducible and recently implicated in partially regulating sugar sensing in *A. thaliana*. Transcript abundance of GNC was 32% greater in soybean grown at elevated [CO_2] under field conditions (Leakey *et al.* 2009 PNAS). Preliminary data on a GNC loss of function mutant (*gnc*) in *A. thaliana* showed no growth stimulation when grown under elevated [CO_2] (1000ppm) compared to ambient [CO_2] (400ppm). This is in contrast to a ~37% growth stimulation of wild type (col-0) under elevated [CO_2] compared to ambient growth [CO_2]. We describe the results of an experiment assessing the changes in photosynthetic carbon assimilation, carbohydrate storage, respiratory flux and gene expression profiling in response to a transition from growth at 400 ppm to 1000 ppm CO_2 in GNC/GNL double knockout lines versus wild type plants. High precision measurements of respiratory flux in the dark from attached single leaves were achieved using a custom built gas exchange chamber for *A. thaliana* connected to a closed gas exchange system.

44. Interannual variation of belowground carbon process inferred from combined eddy covariance and biometric net ecosystem productivity

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Two methods of estimating net ecosystem carbon balance, eddy covariance (NEE) and biometric (NEP), rely on different sets of assumptions that can often lead to great errors. Fortunately, the temporal information in the Net Ecosystem Exchange (NEE) and the component and total magnitudes of Net Ecosystem Production (NEP) are still very useful and relatively unbiased. Therefore, we paired a 7 year record of both the temporal variability of NEE and the biometric components of NEP to determine trends of belowground process that are unaccounted for using standard static NEP methods. The difference of NEE and NEP variability was assumed to represent inter-annual variation of belowground processes that were previously held static among years. This residual anomaly correlated to spring air temperatures and growing season soil water content indicating that static assumptions of belowground carbon processes are false for conditions when decomposition rates were expected to be high. This trend could represent the inter-annual variability of RA/RH ratios, fine root production, fine root turnover, or a combination of any of these processes; all of which are difficult to quantify reliably and are rarely done so among multiple years.

45. Modeling diel soil respiration: Accounting for temporal transience of carbon dioxide production and the possibility of resolving trends independent of temperature

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A record of hourly soil respiration was spatial and temporally deconstructed in attempts to determine CO_2 production depths. We developed a simple heat transport and diffusion model to estimate the depth at which a surface CO_2 flux measurement was produced. We found that thermal transfer was relatively constant during snow free periods while diffusion rates varied considerably among seasons, largely a function of soil water content. Diel cycles of soil fluxes varied seasonally, with the peak daily flux rates occurring later in the day as soil water content decreased. Simple modeling of the soil environments at fixed production depths indicated that changing diffusion rates would not wholly account for the patterning. Spatial variation of the temporal trends was high and may correlate with rooting depth; however, temperature responses at a given depth did not explain the large diel range of soil CO_2 fluxes. This may provide some evidence of the dependence of root derived CO_2 production on recent canopy activity. Our results imply that hourly measurements of soil CO_2 flux contain much spatial and temporal variation at many scales, and failure to account for the simple drivers of the variation can lead investigators to erroneous assumptions about temporal and spatially cumulative soil CO_2 fluxes.

46. Contribution of alternative oxidase isoforms of Arabidopsis leaves to respiration under progressive drought-stress

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Different responses have been reported concerning the impact of water deficit on respiration, which might be related to the species being analysed and the duration of the stress imposed. Besides the cytochrome c oxidase plants have an alternative oxidase (AOX), which accepts electrons directly from ubiquinol, lowering ATP yield. In Arabidopsis AOX is encoded by five genes and *AOX1a* is the predominant isoform in leaves. We have investigated the contribution of AOX to respiration in plants expressing *AOX1a* in antisense (AS) and WT plants. Watering was withheld in soil-grown plants and samples were collected at different time-points. An up-regulation of the cyanide-resistant respiration, measured in leaf discs with an oxygen electrode, was observed in both plant lines, suggesting that multiple AOX isoforms are involved in the drought response. Results from RT-PCR experiments agree with this hypothesis. Interestingly, *AOX2* transcripts, absent in control plants, are detected concomitantly with a substantial decrease in the soil water content. It is noteworthy that the levels of AOX respiration in AS plants remain always lower than those found in the WT confirming the major contribution of AOX1a. Our results highlight the complexity of the response to water deficit of the multigenic AOX family of Arabidopsis.

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47. Taxonomic distribution and characteristics of alternative oxidase in non-angiosperm members of the Viridiplantae

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Alternative oxidase (AOX) is an ubiquinol oxidase in the respiratory chain of all angiosperms. AOX distribution in other members of the Viridiplantae is less clear. Our goal was to assess the taxonomic distribution of AOX and to determine whether AOX multigene families exist in non-angiosperms using bioinformatics. Multiple sequence alignments were used to identify AOX1 and AOX2 protein subtypes, and to examine amino acid residues involved in AOX catalytic function and post-translational regulation. Novel AOX sequences were found in both Chlorophytes and Streptophytes and we conclude that AOX is widespread in the Viridiplantae. AOX multigene families are common in non-angiosperm plants and the appearance AOX1 and AOX2 subtypes pre-dates the divergence of the Coniferophyta and Magnoliophyta. The glutamate and histidine residues involved in AOX catalytic function are highly conserved between Chlorophytes and Streptophytes, while AOX post-translational regulation likely differs in these two lineages due to the presence or absence of a key regulatory cysteine residue. Our findings indicate that AOX will exert an influence on plant respiration and carbon cycling in non-angiosperms such as green algae, bryophytes, liverworts, lycopods, ferns, and gymnosperms and that this fact must be accounted for in any climate change modelling efforts.

48. Origin and fate of CO_2 in tree stems: a conceptual model

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A conceptual model was developed that graphically depicts the production, flux and ultimate fate of CO_2 in woody tissues of trees. Most CO_2 in tree stems is sourced from respiration in roots and above-ground woody plant parts, though a small amount may enter roots dissolved in soil water. CO_2 produced by respiration can remain temporarily in the root or stem, building up to very high concentrations, or flux radially to the soil or atmosphere, or become entrained in the transpiration stream and move upward by mass flow in the xylem. Transported CO_2 may flux to the atmosphere remote from its site of production or be re-fixed by photosynthetic green cells in woody tissues or in leaves. Rates of CO_2 production, external flux, entrainment, internal transport, and re-fixation are influenced by many factors including barriers to CO_2 diffusion, radial CO_2 concentration gradients, air and tissue temperature, tissue water status and aeration, carbohydrate supply, transpiration rate, sap pH, and stem illumination. The conceptual model provides a detailed illustration of the effects of these rate-modifying factors on the physiological and physical processes that produce, dissipate and consume CO_2 in woody tissues.

49. Improving the representation of fine roots in ecosystem models

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Trees allocate a considerable but poorly quantified portion of carbon, fixed through photosynthesis to fine roots. Changes in growing conditions for an individual tree will affect the carbon allocated to fine roots for growth and respiration. When scaled to the stand level, small changes in environmental conditions therefore have the potential to lead to major changes in the carbon balance of a forest. To address the question of how fine root growth and respiration is coupled to above-ground forest processes, the Soil-Plant-Atmosphere model has been parameterised with meteorological measurements, leaf level fluxes, and measurements of the release of carbon from soil. At an evergreen pine forest in Yorkshire and a deciduous oak forest in Surrey, continuous soil respiration measurements have been collected with a mycorrhizal mesh collar design, partitioning root, mycorrhizal and heterotrophic soil CO_2 flux components. Ingrowth cores and rhizotrons are also monitoring in situ root production at a high temporal resolution at these sites. The collection of below-ground carbon turnover data, alongside a diverse set of above ground forest measurements, enable the validation and improvement of ecosystem carbon models.

50. Impaired ATP production in the respiration mutant *ndufs4* alters the control of metabolism at night

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In aerobic organisms, respiratory oxidative phosphorylation plays an essential role in cellular metabolism as it is providing energy for the whole cell. The efficiency of this process is assumed to be optimized for maximum ATP production. In plants, the presence of dynamically regulated nonphosphorylating bypasses implies that plants can alter phosphorylation efficiency and can benefit from lowered energy generation during respiration under certain conditions. In order to understand the consequences of altered respiration, we characterized an *Arabidopsis thaliana* mutant, *ndufs4*, lacking complex I of the respiratory chain. We measured similar total respiration but reduced mitochondrial ATP synthesis in *ndufs4* compared to wild-type plants, indicating that complex I contribution (ca. 1/3) to the proton gradient used for ATP synthesis is absent. This reduced capacity to produce ATP through respiration is slowing down *ndufs4* growth. Using leaf metabolomics, we observed increased inorganic acid and amino acid pools in the mutant, especially at night, concomitant with alteration of the adenylate content. Our data show that the cellular metabolism adapts to reduced phosphorylation efficiency caused by the absence of complex I and suggest that the adenylate control plays an important role in the adaptation of cellular metabolism.

51. Effect of elevated concentration of CO_2 on various physiological and biochemicals parameters of Wheat *(Triticum aestivum)*

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Global climate change is elevating the CO_2 concentration of the atmosphere. The elevated concentration of CO_2 is known to interfere with several metabolic processes of tree species. However, the information on the interaction of CO_2 with crops is limited. Therefore the present study deals with the elevated concentration of CO_2 on various metabolic processes of common Indian crop wheat (*Triticum aestivum*). The test plant was grown at normal (350 micromolCO_2/mol) and elevated (750 micromolCO_2/mol) concentration of CO_2 and rate of respiration, lipid peroxidation, level of carbohydrate, ascorbic acid and photosynthetic pigments, and antioxidant enzymatic activities were determined. The elevated CO_2 stimulated the respiration by 35%, carbohydrate (soluble sugars) by 20%, ascorbic acid by 25% and total chlorophyll by 15%. Whereas lipid peroxidation measured in terms of MDA content produced, was also increased but it was insignificant in comparison to control. The antioxidant enzymes activities of wheat also responded differently, the SOD activity was found to decrease by 30%, whereas ascorbate peroxidase activity was increased by 10%, but catalase activity remained unchanged.

52. Summer drought reduces the heterotrophic, but not the rhizosphere component of soil respiration

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We investigated the effects of prolonged summer drought on soil respiration (SR) in a mountainous Norway spruce forest in south Germany. Drought was induced on three manipulation plots by excluding summer throughfall in 2006 and 2007. We measured SR fluxes in comparison to three control plots. Using radiocarbon measurements we quantified the contribution of rhizoshpere (RR) and heterotrophic respiration (HR) to total SR. Mean annual CO_2 emissions from the throughfall exclusion (TE) plots were smaller than from the control plots in both years (in 2006: 5.7 vs.6.7 t C ha^{-1}; in 2007: 5.9 vs. 7.0 t C ha^{-1}). Under control conditions, CO_2 originated mainly from HR (60–95 % of SR). Prolonged drought reduced HR, whereas RR was not affected or even increased slightly. Reduction of CO_2 emissions on the TE plots was found up to 6 weeks after differences in matric potential conditions disappeared, possibly either because water repellency inhibited homogeneous rewetting of the organic horizons or because of severe damage to the microbial population. Continuous measurements in 2008 (no manipulation) did not reveal increased CO_2 emissions on the TE plots that could compensate for the reduction during the years 2006/2007.

53. Impacts of diel temperature range on ecosystem carbon balance: an experimental test in grassland mesocosms

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Although extensive research has determined ecosystem responses to equal increases in day and night temperatures, current temperature increases have generally been asymmetrical, with increases in minimum temperature (T_{min}) exceeding increases in maximum temperature (T_{max}). We conducted an ecosystem warming experiment in a perennial grassland to determine the effects of asymmetrically elevated diel temperature profiles using precision climate-controlled sunlit environmental chambers. Asymmetrically warmed chambers (+5/+2ºC, T_{min}/T_{max}) were compared with symmetrically warmed (+3.5ºC continuously) and control chambers (ambient). We tested three alternative hypotheses comparing the carbon balance under symmetric (SYM) and asymmetric (ASYM) warming: H1) SYM<ASYM due to higher respiratory costs from higher T_{max}; H2) SYM>ASYM, because warmer nights in the ASYM treatment increase respiration more than photosynthesis; H3) SYM=ASYM, due to a combination of effects. Results from the third growing season support H3, that C balance is the same under the two diel temperature profiles. Asymmetric warming resulted in higher night-time respiratory losses than symmetric warming, but these greater loses were compensated by increased early morning photosynthesis. Because photosynthesis and respiration were tightly coupled, respiration was not greatest in the treatment with highest daily temperatures, as would be expected from Arrhenius temperature relationships.

54. Supercomplex organization of plant respiratory complexes changes with physiological conditions

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Supercomplexes (SC) are formed by the aggregation of two or more respiratory complexes. To learn more about the function of the formation of SCs, we isolated SCs from mitochondria and analyzed their activity afterwards by using blue native PAGE and *in gel* activity staining for complex I and complex IV. We searched for conditions that affected specific SCs. AOX overexpressing *Arabidopsis* plants increased the activity of SCs containing the complex I, III and IV, suggesting an electron channeling function to by-pass AOX. During hypoxia and the concomitant acidification of the cell, the largest SCs from potato tubers partially degraded. The large SC also dissociated after treating isolated mitochondria with succinate at slight acidic levels. Dissociation of the SC during hypoxia might be related to an increased involvement of the external NADH dehydrogenases, which are also activated by lowering the pH, whereas complex I is inhibited when the pH goes down. Our data suggest a conditional role of SC to direct electrons between the various respiratory pathways.

55. What causes high respiration fluxes over the dry season in semi-arid ecosystems?

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Semiarid ecosystems cover nearly 43% of the surface of the Earth and yet very few estimates of their net carbon exchange exist so far. We measured the carbon exchange of a semiarid steppe in the SE of Spain by eddy covariance over two years and estimated that these ecosystems are a net source of CO_2 to the atmosphere of about 51.9–108.4 g C m^{-2} depending on annual precipitation (211 and 251 mm, respectively). The ecosystem was a small sink during the few winter months in which vegetation was active. Large CO_2 pulses were observed during the dry season that were not related to temperature or rainfall. Two major non biological processes may be involved: (1) carbonaceous rock dissolution or weathering processes and (2) photodegradation. Here we explore the contribution of these two processes as important processes of CO_2 production in these ecosystems.

56. Sensitivity of leaf dark respiration to temperature: the effect of leaf age, canopy position, and water availability

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The response of leaf respiration to the reduction in rainfall and the increase in temperature that are expected to occur in the Mediterranean basin through this century will play a role in the survival of trees and hence in the structure and composition of forests. To see how abiotic factors modulate leaf respiration and the seasonal sensitivity of respiration to temperature, we measured the dark respiratory rates of top and mid-canopy current-year leaves of *Quercus ilex* L. trees that had been subjected to either six years of rainfall partial exclusion or normal rainfall. In addition, at the canopy top, previous-year leaves of trees subjected to normal rainfall were selected for comparison with younger leaves. Respiration was lower in the mid canopy than at the canopy top; it was similar in trees receiving normal and reduced rain; and it was lower in older leaves. At a common measuring temperature, respiration decreased from winter to summer in relation with increasing monthly temperatures. Because this response was similar in all treatments, it is concluded that leaf age, canopy position, and drought severity have little impact on the capacity of *Q. ilex* leaves to thermally acclimate.

57. Seed-specific upregulation of hemoglobins affects endogenous nitric oxide, mitochondrial respiration and more

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The low prevailing level of oxygen in the seeds of crop plants imposes a major limitation for respiration and biosynthetic processes (Borisjuk & Rolletschek, 2009). We recently proposed that seeds use nitric oxide (NO) to adjust glycolysis and mitochondrial respiration according to oxygen availability and the metabolic demands. Thereby, nitric oxide might affect storage activity, stress response and seed yield /fitness in general. To test this hypothesis, we generated transgenic *Arabidopsis* plants over-expressing non-symbiotic hemoglobin 1 (*AHb1*) to modulate NO levels. Embryos from transgenic plants with seed-specific *AHb1*-overexpression showed significantly reduced levels of endogenous NO upon hypoxic stress. Transcript profiling using ATH1-chips (Affymetrix) revealed that this was accompanied by changes in energy metabolism, nitrate assimilation and stress responses. Several genes of the mitochondrial electron transport chain (e.g. ATP synthase, NADH dehydrogenase, cytochrome c oxidase) were up-regulated, especially when plants were exposed to hypoxic stress. Under hypoxia, seeds of transgenic plants were able to maintain a higher adenylate energy charge, indicating a shift in respiratory activity *in vivo*. The transgenic model offers a tool for studying seed-specific respiratiory adjustments under hypoxic stress.

58. Quantifying the respiratory carbon flux of a tropical rainforest drought

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To model future carbon–climate interactions in the Amazon rain forest, it is vital to quantify the potential responses of both photosynthesis and respiration to changing meteorology. Research has, however, focused largely on photosynthesis, and respiration is poorly simulated in most ecosystem models. This poster outlines a research design to quantify flows of carbon at a throughfall exclusion experiment (TFE, i.e. a drought simulation) on a tropical rain forest site in Brazil. The fieldwork element of this research design will focus on how leaf respiration in the light changes between the TFE plot and a corresponding control plot. Quantifying light respiration in tropical biomes, and furthermore how it is altered by drought, will be a new and novel measurement. This has the potential to be of significant value for improving quantification of present and future carbon-climate interactions in Amazonian tropical forest. Alongside pre-existing ecological measurements, the observations from this study will be used as part of a data assimilation technique to model drought response using the Soil-Plant-Atmosphere model. Modelling the TFE will enable greater exploration of the processes underlying the drought response, and give the potential to scale these responses from leaf to ecosystem.

59. EU-infrastructure project INCREASE funds scientist visiting six climate manipulation experiments in Europe

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The impact of climate changes has been studied over the last 10 years in six large-scale field experiments across Europe with non-intrusive manipulations of temperature and precipitation and at one site also combined with enhanced atmospheric CO_2 concentrations in a free air CO_2 enrichment (FACE) setup. The experiments are placed in semi-natural shrubland ecosystems along natural gradients between sites of temperature and precipitation. With the new EU-infrastructure project INCREASE (2009–2013) the research facilities are now made accessible for the wider European scientific community where visiting scientists can test their scientific hypotheses at field scale. Upon acceptance of a science proposal visiting scientists will receive a refund of their travel expenses. The poster will present the experimental approach of the experiments in the infrastructure, a summary of the climate change effects observed so far on ecosystem processes and function, and information on how to apply for access to the experimental sites of the INCREASE infrastructure.

60. Seasonal acclimation of respiration in New Zealand alpine grasses

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The response of plant respiration (R) to temperature is dynamic on long and short timescales, and the mechanisms driving this response remain uncertain. Here, we examine the thermal acclimation of R in *Chionochloa pallens* and *C. rubra*, two species of native perennial tussock grass growing on Mt. Hutt, New Zealand, to both seasonal and short term (several day) temperature fluctuations in the field and under laboratory conditions. In particular, using a novel gas sampling technique, we investigate whether *in vivo* engagement of alternative oxidase (AOX), which catalyzes the so-called ‘energy wasteful’ CN-resistant respiratory pathway in plants, plays a role in regulating acclimation of *Chionochloa spp*. We find that R in both *C. pallens* and *C. rubra* acclimates to seasonal changes in temperature in the field, but does not show significant acclimation on shorter timescales. Results are supported by growth cabinet experiments. Seasonal changes of R in the field are correlated with changes in AOX engagement in *C. rubra* and with changes in the relative abundance of AOX protein in *C. pallens*. We conclude that R in *Chionochloa spp.* acclimates primarily to seasonal temperature changes, and that this acclimation is underpinned by changes in AOX.

61. The effects of cattle impact and vegetation on CO_2 fluxes from soil

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Cattle overwintering areas common in central Europe may represent significant point sources of the important greenhouse gases including carbon dioxide (CO_2). A two-year field flux measurements were performed along a gradient of animal impact, characterized also by different extent of vegetation damage, to test the hypothesis that emissions of CO_2 are positively related to the degree of cattle impact. CO_2 fluxes were determined by using non-vented manual closed chambers. The emissions of CO_2 showed a strong seasonal pattern, being correlated with soil temperature: the highest emissions thus occurred in June–July, while very low fluxes were found in winter. Emission values ranged from zero to 700 mg C-CO_2 m^{-2} h^{-1}. In contrary to the hypothesis, the highest CO_2 fluxes were mostly recorded at the least impacted location, despite the fact that microbial biomass and activity were significantly increased in the cattle-impacted soil, which corresponded to higher inputs of organic carbon and nitrogen in excrements. This suggests that respiration of plants was a significant component of the overall CO_2 production; therefore less impacted and unimpacted control sites with less disturbed or undisturbed vegetation produced significantly more CO_2 than site without vegetation.

62. Divergent impacts of shade and drought on the temperature sensitivity of leaf dark respiration in *Geum urbanum*

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The respiratory response of plants to temperature is a critical biotic feedback in the study of global climate change. Few studies, however, have investigated the effects of environmental stresses on the short-term temperature response of leaf dark respiration (R_{dark}). We investigated the effect of shade and transient drought on the temperature sensitivity (Q_{10}; the proportional increase in respiration per 10°C temperature increase) of R_{dark} of *Geum urbanum* in controlled experiments. Following sustained, near-darkness, R_{dark} and the Q_{10} of R_{dark} were both reduced. By contrast, R_{dark} and the Q_{10} of R_{dark} both increased in response to severe drought. Drought was associated with a rapid decline in photosynthesis (P_{sat}) and stomatal conductance (g_s). The concentration of soluble sugars in leaves did not decline during drought or shading, but during drought the starch concentration dropped, suggesting that starch to sugar conversion helped maintain sugar homeostasis. Thus, the changes in R_{dark} were unlikely to be due to stress-induced changes in substrate supply. If widespread, such changes in the Q_{10} of R_{dark} could have important implications for predicted rates of ecosystem carbon exchange in the future, particularly in areas that experience more frequent droughts.

63. The fate of recently assimilated carbon in Arctic bryophytes

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Significant temperature rise in the terrestrial Arctic will influence the carbon (C) cycle, through effects on plant growth and soil decomposition. If respiratory losses of C exceed uptake by plant growth, a dangerous feedback on global climate warming could result. The ABACUS project aims to further our ability to predict the size and direction of shifts in Arctic C balance through developing ecosystem models of C exchange between atmosphere, vegetation and soils. A key component is quantifying the fraction of Gross Primary Productivity (GPP) that is immediately respired by plants, versus that incorporated and stored longer term in tissues. Current vegetation models are based on an understanding of GPP partitioning in ecosystems dominated by vascular plants. Bryophytes however, are a significant component of arctic tundra vegetation, often accounting for more than 30% of standing plant biomass. They have a fundamentally different physiology to higher plants; lacking stomata, true roots or storage organs. It is likely therefore that bryophyte respiratory energy demand differs from that of vascular plants.
We use ^{13}C isotope labelling to determine the partitioning of GPP into growth versus respiration in Fennoscandian sub-Arctic moss and vascular plant communities. We track assimilated ^{13}C in three contrasting moss species, *Sphagnum fuscum, Polytrichum piliferum*, and *Pleurozium schreberi*, and in *Empetrum nigrum*, a common evergreen shrub, following fumigation under 95% atom enriched $^{13}CO_2$. We present data on the isotopic enrichment in moss tissues as well as in respired CO_2 over time following pulse labelling. Our results show clear differences between species in the relative amounts of assimilated C returned to the atmosphere through respiration versus that stored as biomass through growth. These data emphasise not only the importance of including bryophytes in ecosystem models of the Arctic carbon cycle, but provide an insight into lower plant respiration.

64. Pathways of C and evidence of priming linked to plant C allocation belowground

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A large proportion of assimilated carbon (C) allocated belowground is respired by both plant organs and associated organisms in the rhizosphere. The dynamics of this respiratory pathway, its relation to plant productivity, and consequences on heterotrophic processes in the rhizosphere are only poorly understood, yet changes in environmental conditions are likely to have major impacts on the rate of C cycling between plants, soil and the atmosphere. We used tree girdling in a mature Western Hemlock (*Tsuga heterophylla*) stand to separate belowground autotrophic respiration and soil heterotrophic respiration. Results show a rapid decrease in total soil CO_2 efflux (R_S) by about 40% in girdled plots. Rhizopspheric CO_2 flux was a significant yet variable proportion of R_S throughout the experimental period, and radiocarbon result confirm the apportioning of R_S from flux measurements. Mesh in-growth cores indicate that about 50% of this respiratory flux is via mycorrhical hyphal networks. Litter bag incubations show a significant enhancement of decomposition in the presence of an active rhizosphere, indicating that the belowground allocation of recently assimilated C primes the decomposition of organic matter in soil. Models predicting the response of ecosystems to environmental change have to account for this interaction between plants and soil.

65. The remodeling of mitochondrial metabolism in response to thermal variation

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Fluctuations in temperature affect the metabolic processes of photosynthesis and respiration and can have dramatic implications on biosynthesis, cellular maintenance and biomass allocation. Plants can acclimate to the extremes of temperature following a pre-exposure to a lower sub-lethal increase/decrease in temperature that allows them to adjust their metabolism and to survive. The respiratory and photosynthetic rates of these plants remain similar to plants grown at optimal temperatures and plants that have a greater thermal tolerance tend to more quickly adjust their metabolism and restore respiration to pre-thermal change levels. Using both Arabidopsis whole plants and cell culture I have produced data that suggests a remodelling of proteins in the mitochondrial electron transfer chain allows respiratory homeostasis to be achieved during acclimation. Also changes in other mitochondrial membrane and soluble proteins have been measured quantitatively at the proteome level following chilling. As plant biomass accumulation is governed by the equation of assimilation of CO_2 minus respiratory CO_2 loss, the rate, degree and timing of respiratory acclimation is a critical component in plant growth and provides an insight to the dynamic nature of the mitochondrial proteome.

66. Ectomycorrhizal identity determines respiration and concentrations of nitrogen and nonstructural carbohydrates in root tips: a test using *Pinus sylvestris* and *Quercus robur* saplings

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Fine roots play a significant role in plant and ecosystem respiration (RS), therefore understanding factors controlling that process is important both to understanding and potentially in modeling carbon budgets. However, very little is known about the extent to which ectomycorrhizal (ECM) fungal species may influence RS or the underlying chemistry that may determine those rates. In order to test these relationships we examined RS, nitrogen, carbon and nonstructural carbohydrate concentrations of ECM root tips of *Pinus sylvestris* L. and *Quercus robur* L saplings. Roots of *P. sylvestris* were colonized by *Rhizopogon roseolus*, *Tuber* sp. 1, and an unknown species of *Pezizales*. Fungal species colonizing *Q*. *robur* roots were *Hebeloma* sp., *Tuber* sp. 2 and one unidentified ECM fungus described as *Tuber*-like. Ectomycorrhizal RS for different host species were significantly different and more than 97% of the variation in RS within a host species was explained by ECM root tip nitrogen concentrations. This may indicate that some of the variability in fine root RS-N relationships observed between and within different host species or their functional groups may be related to intraspecific host species differences in root tip N concentration among ectomycorrhizal fungal associates.

67. Respiration under hypoxic conditions

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Plants have to deal continuously with changes in the availability of oxygen. Oxygen diffusion to roots can become strongly limited due to soil compaction or waterlogging. However, even when oxygen availability from the environment is not restricted, inner parts of bulky plant tissue like stem, root, seed or tubers can easily become hypoxic. Due to the resistance for oxygen diffusion through the various cell layers, plant internal oxygen concentrations can drop to less than 10% of ambient even under optimal growth conditions. Evidence is provided that respiration rates decrease already at oxygen concentrations that are clearly higher than those that would be rate limiting at the substrate level. This is interpreted as an adaptive response of respiration to prevent complete anoxia of the tissue. Here, I present various adaptive metabolic responses and regulatory mechanisms on how plant respiration changes as a function of the oxygen availability. A system was developed to manipulate the *in vivo* production of nitric oxide, and the impact of NO on respiration and the plant internal oxygen concentration is described. Furthermore, a metabolic pathway is revealed that explains the role of alanine accumulation during hypoxia.

68. Effects of *AOX1a* deficiency under low nitrogen stress in *Arabidopsis thaliana* plants

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Expression of the alternative oxidase (AOX) and CN-resistant respiration are highly enhanced in plants exposed to low nitrogen (N) stress. Here, we examined the effects of AOX deficiency on plant growth, expression of respiratory components and antioxidant enzymes, and metabolic profiles under low N stress, using an *aox1a* knockout line (*aox1a*) of *Arabidopsis thaliana*. We exposed wild-type (WT) and *aox1a* plants to low N stress for seven days and analyzed their shoots and roots. In WT plants, the *AOX1a* mRNA levels and AOX capacity increased corresponding to low N stress. Gene expression of other respiratory components and some antioxidant enzymes were enhanced in *aox1a* plants. Metabolome analyses revealed that AOX deficiency altered the response of some metabolites to low N stress. However, there were no dramatic differences in plant growth, total respiratory rates, and carbon (C)/N ratios between WT and *aox1a*. Our results indicated that the N-limited stress induced AOX expression, and the AOX deficiency leads to some changes in gene expressions and metabolites under low N stress. Meanwhile, the induced AOX may not play indispensable roles under only N-limited stress, and the C/N balance under low N stress may be tightly regulated by systems other than AOX.

69. New insights into spatial and temporal patterns of plant dark-respired $^{13}CO_2$

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We present a species comparison of spatio-temporal variations of dark-respired $\delta^{13}CO_2$ ($\delta^{13}C_{res}$) and its putative substrate (water-soluble organic matter, WSOM) of leaves and roots along the plant axis and over the diurnal course. Pronounced spatial differences in $\delta^{13}C_{res}$ (up to 10.2‰) between top-most leaves and roots tips were found. Additionally, a species-specific diurnal enrichment of leaf $\delta^{13}C_{res}$ above WSOM up to 15.9‰ was found. The amount of diurnal $\delta^{13}C_{res}$ enrichment was highly correlated (R^2=0.98) with the difference in $\delta^{13}C_{WSOM}$ between leaves and roots. This result indicate that fractionation during dark respiration is a major cause for the well-known ^{13}C-depletion of leaves compared to heterotrophic tissues. Furthermore, we found very rapid post-illumination changes in $\delta^{13}C_{res}$ (up to 10‰ within 30 min). Interestingly, the magnitude of these short-term changes exhibited a diurnal cycle.
Positional labelling experiments with ^{13}C-pyruvate showed that the observed diurnal increase in $\delta^{13}C_{res}$ is due to an increase in C-flux through pyruvate dehydrogenase during the light period relative to a constant Krebs cycle activity. In contrast to foliage respiration none of the investigated species displayed distinct diurnal pattern in $\delta^{13}C_{res}$ of roots. These results indicate important organ-specific differences in post-photosynthetic fractionation during respiration, probably tracing carbon allocation mechanisms.

70. Diurnal dynamics of isotopic fractionation during dark respiration: pattern and processes

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Recently, increasing information on diurnal variation in the isotopic composition of dark-respired CO_2 ($\delta^{13}C_{res}$) has been gained in leaves, stems, and roots of several plant species, as well as in ecosystem respiration. The origin of enriched $\delta^{13}C_{res}$ is an ongoing matter of debate, which may increase by >10%
above the putative respiratory substrate $\delta^{13}C$ along the day. The diurnal variation of leaf $\delta^{13}C_{res}$ differed significantly between plant functional groups, which may be attributed to post-photosynthetic fractionation in the respiratory pathways and differences in carbon allocation (deviation of acetyl-CoA) into secondary metabolism. Positional ^{13}C-pyruvate labelling experiments revealed an increasing decarboxylation rate of pyruvate dehydrogenase (PDH) during the light period relative to a constant Krebs cycle (KC) activity in species with a marked increase in $\delta^{13}C_{res}$. In contrast fast growing herbs without significant temporal $\delta^{13}C_{res}$ variations did not exhibit significant changes in PDH or KC activity. Further we present mathematical evidence that changes in the decarboxylation rate of PDH and KC can potentially account for large changes in $\delta^{13}C_{res}$ and will discuss possible mechanisms which may be involved in short-term dynamics in $\delta^{13}C_{res}$.

71. Distinct responses of the mitochondrial respiratory chain to long- and short-term high-light environments in *Arabidopsis thaliana*

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The mitochondrial respiratory system is dramatically modulated in response to various environmental stresses. We previously demonstrated that the non-phosphorylating alternative oxidase (AOX) is up-regulated under high-light (HL) condition and plays key role in maintaining cellular redox homeostasis. In the present study, we examined responses of the respiratory chain to long- and short-term HL environments, and effects of the AOX deficiency. Plants grown under HL condition (HL-plant) possessed larger ubiquinone (UQ) pool and higher amount of cytochrome *c* oxidase than plants grown under low-light condition (LL-plant). The AOX amounts were not differed between HL- and LL-plants. When LL-plant was transferred to HL condition, AOX was rapidly induced at transcript and protein levels. The UQ reduction level was elevated after the transfer to HL. In wild-type, the light-dependent elevation of UQ reduction level was alleviated concomitant with the AOX up-regulation. On the other hand, the UQ reduction level of AOX1a-deficient plant (*aox1a*) remained to be high. Photosynthetic analysis revealed that the efficient photosynthetic electron transport was impaired in *aox1a* under HL conditions. These results suggest that AOX plays important role for the acclimation of the respiratory chain to short-term HL environment, which is essential for optimization of photosynthesis.

72. Climate dependent variations in foliar respiration of three dominant Siberian boreal forest species

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Our research focused on climate-dependent changes in foliar respiration (*R*) in a dominant boreal forest ecosystem in NE Siberia. This ecosystem is characterised by its low annual net primary productivity (NPP), with trees experiencing extremely cold winters and hot/dry summers. To understand the factors responsible for low NPP at this site, we measured diurnal and seasonal variations in foliar CO_2 exchange of deciduous and evergreen conifers (*Larix cajanderii* and *Pinus sylvestris*) and a deciduous broadleaved species (*Betula platyphylla*) in 2008. For each species, we conducted two sets of measurements: (1) diurnal variation in rates of foliar *R* taking place in darkness (R_{dark}) and photosynthesis (P_{sat}) and (2) measurements of rates of foliar *R* taking place in the light (R_{light}). R_{dark} declined in response to sustained drought in the two conifer species (*Larix* and *Pinus*), with R_{dark} rising sharply in response to rainfall. By contrast, little temporal variation in rates R_{dark} occurred in *Betula*. Irrespective of soil moisture, R_{light} was always lower than R_{dark}, with light inhibiting *R* by up to 40%, with, R_{light} also decreasing in drought stress trees. These findings have important implications for our understanding of how climate change may impact on the carbon economy of boreal forests

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