Characteristics of main research directions investigated at the institute and the achievements 2010−2014

Institute	Global Change Research Centre of the CAS, v. v. i.

The Global Change Research Centre CAS (GCRC) was established in 2010/2011 and it builds on the previous successful scientific activities of the Institute of Systems Biology and Ecology CAS. The main reason for this new Centre establishment was the successful obtaining of the support for realisation of the project of European Centre of Excellence CzechGlobe – Centre for the Study of Global Climate Change Impacts within the EU Operational Programme Research and Development for Innovations, involving the major part of the Institute. Thus in the period 2011-2014, the main effort was put in building of top research infrastructure and top expert teams enabling interdisciplinary research on the issue of global change.

The main structure of the Global Change Research Centre (GCRC) consists of two relatively autonomous institutes:

- CzechGlobe (seated in Brno)
- The Institute of Nanobiology and Structural Biology (INSB, seated in Nové Hrady)

This division is reflected in submitted evaluation documents of the whole institution (GCRC) and seven evaluated GCRC teams.

After successful negotiations with the central bodies of the Czech Academy of Sciences, the INSB is currently (in the beginning of the year 2015) prepared to shift to the Institute of Microbiology of ASCR, and partially also to the Biology Centre ASCR, respectively. This transition is expected to finish de lege on 1st January 2016. After this date, GCRC will only consist of CzechGlobe.

Below in this chapter nb. 1 is characterised CzechGlobe part of GCRC. The details of the two evaluated INSB teams (team nb. 5. Center of Nanobiology and Structural Biology and team nb. 6 Department of Nanobiotechnology) are in described in the team reports.

The numbering of the evaluated teams is based on the automatic numbering of the electronic CAS submission system. The two ISBE teams are represented by team nb. 5. Center of Nanobiology and Structural Biology and team nb. 6 Department of Nanobiotechnology, the other teams (number 1-4 and number 7) belong to CzechGlobe.

The concrete GCRC scientific achievements are in detail described in the individual reports of the teams and on the list of the scientific results that were handed over during the first evaluation phase. Below is just overview of the achievements from the CzechGlobe perspective.

The main research directions investigated in CzechGlobe and their achievements

Thanks to long-term research success related to the global change (e.g. ecosystem research) of the preceding Institute of Systems Biology and Ecology (e.g. active participation in the projects of EU FP5, FP6, FP7; institute is founding member of ESFRI ICOS - European Strategy Forum on Research Infrastructure Integrated Carbon Observation System and active member of ESFRI EUFAR - EUropean Facility for Airborne Research in environmental and geo-sciences) the Centre CzechGlobe was established in 2010.

CzechGlobe is the European Centre of Excellence focusing on complex global change interdisciplinary research that was constructed within the EU Operational Programme Research and Development for Innovation (OP RDI). The CzechGlobe project application was based mainly on the achievements of previous research work and high quality scientific project had been successfully evaluated among other competing proposals during the years 2009 and 2010 by international assessment commission and evaluation of the OP RDI managing authority - the Ministry of Education, Youth and Sports (MEYS). The construction phase (with budget of 35 mil. EUR) of the CzechGlobe was realised in period December 2010 – December 2014. During this phase unique state-of-the-art scientific infrastructure has been constructed (e. g. network of ecosystem, atmospheric and unique experimental stations, flying laboratory of imaging systems, hi-tech laboratories…) and scientific capacities and worldwide unique scientific expertise were developed. The scientific staff of the CzechGlobe Centre has been significantly extended (see details in the statistics provided in the 1st phase of the evaluation and below) in all career levels (Ph.D. students up to senior experts).The international collaboration and participation in the projects (e.g. FP7, Horizon 2020, ESA, COST, cross-border and bilateral projects) and infrastructures (mainly ESFRI ICOS, ESFRI AnaEE, ESFRI ISBE, ESFRI EUFAR, ACTRIS) have been significantly developed or newly established.

Although a great deal effort had to be devoted to building the Centre CzechGlobe, the Centre reached the following significant achievements:

- Successful realisation of the OP RDI project CzechGlobe fulfilling all the planned indicators (e.g. excellent scientific publications, use of infrastructure, scientific mobility, cooperation with research application sphere – companies and public service), milestones and tasks. Already now (since the beginning of the year 2015) the CzechGlobe state-of-the-art infrastructure is fully used by CzechGlobe experts and by the third parties via Open Access and other forms of cooperation. The success of the project was crowned by very positive international evaluation of the evaluation international expert committee organised by the MEYS in the end of the project CzechGlobe realisation (2014/2015):
    - *"The centre CzechGlobe – and thus, the host institution GCRC – has seen a quick growth in the last five years due to OP funding. This funding has been successfully used as a catalyst both to strengthen and integrate various research lines dealing with climate change issues and to create a dedicated centre for this topic. Through the OP funding, an originally medium sized, good research centre with a mixed portfolio could build up resp. renew an impressive array of infrastructures, grow significantly, attract talented people and increase the outputs and focus*

on climate change as a mission / a Grand Challenge. The resources available were well used."

- *"The centre has attracted very talented people, designed a comprehensive research programme and is strong in scientific and other outputs."*
- *"The infrastructure investments are well chosen and the procurement / installation procedures equally well accomplished. The centre now has a quite unique infrastructure in Central Europe and a number of investments put the centre in a very favourable position on the European stage."*
- *"CzechGlobe has extensive and close partnership networks with a number of universities for PhD education, infrastructure use and joint research. The centre management has therefore done a good job."*

- High interest in scientific cooperation with CzechGlobe and use of its infrastructure and expertise. More than 50 cooperation agreements have been signed with research centres, universities, private partners and public service. The amount of cooperation is expected to increase in the future thanks to prepared national and international projects. The increasing number of users will be generated primarily by the Open Access in the frame of ESFRI projects (e.g. AnaEE, ICOS, EUFAR) and other European programs (e.g. HALOA ITN). In period 2010-2014, The Centre cooperated within Open Access infrastructure regime with >40 foreign institutions: e.g. Universita Autonoma de Barcelona, Spain (annual number of use or projects: (4); University of California Berkeley, USA (1), National Ecological Observatory Network, USA (2), National Oceanic and Atmospheric Administration NOAA USA (1), University of Tuscia, Italy (3); National Research Council, Italy (4); National Drought Mitigation Centre, USA (1), University of Nebraska, USA (1); University of Helsinki, Finland (5); University of Tokyo, Japan (1); University of Cork, Ireland (1); University of Vienna, Austria (2); University of Edinburgh, UK (3); University of Lodz, Poland (1); University of Poznan, Poland (4); University of Pécs, Hungary (2); University of Panama, Panama (2); Vietnam Academy of Science and Technology, Institute of Tropical Biology, Vietnam (4); Patna University, India (1); TU Dresden, Germany (2); Kasetsart University, Thailand (1), Deutscher Wetterdienst, Germany (1), University of Wageningen, Netherlands (1), etc. There was also cooperation with >25 national institutions: e.g. Mendel University in Brno (annual number of users or projects: 15); Charles University in Prague (6); Institute of Physiology AS CR, v. v. i., Prague (2), Brno University of Technology (2); University of Ostrava (5); University of South Bohemia in Č. Budějovice (4); Institute of Inorganic Chemistry AS CR, v. v. i., Řež (2); Silesian University in Opava (1); University of Hradec Králové (1); Institute of Atmospheric Physics AS CR, Prague (1); Masaryk University, Brno (3); Czech Geological Survey, Prague (3); University of Veterinary and Pharmaceutical Sciences Brno (2); Research Institute of Brewing and Malting, Prague (1); Central Institute for Supervising and Testing in Agriculture (1) etc. In addition, there were >25 private companies cooperating and using the infrastructure: e.g. Photon System Instruments s.r.o., Brno (annual users or number of projects 3); GRYF HB s.r.o. (2), Agrotest fyto, s.r.o. (3); MJM Litovel, a.s. (2); Brněnské vodárny a kanalizace, a.s. (2); Mondi, a.s., Štětí (1), EXCON a.s.(2).

- Success in applied research activities and deepening the cooperation with private partners (see above and in the annex 3.2. of the II. Evaluation Phase). The results of CzechGlobe research are used e.g. in fields of XXX), forestry (Lesy ČR, forest management; XXX, risks of ecosystem stability under global change), agriculture (Czech Agrarian Chamber, drought monitoring and early warming), precision agriculture (XXX, development of optical sensors and vegetation indices; XXX, XXX, development of diagnostic methods of vegetation health statute and vegetation structure), water management (XXX; protection of water quality associated with an intensive agricultural production) and algal biotechnology (XXX, waste water treatment; XXX, glycerine production). In addition, the metabolomic and biochemical laboratories of CzechGlobe were used for analyses of contaminants in the pulp and raw paper material XXX.
- Deepening or establishing of new international research cooperation with >50 research partners important for CzechGlobe research e.g. USA (NEON - the National Ecological Observatory Network, NOAA – National Oceanic and Atmosphere Administration), relevant EU and non-EU research centres and universities) including e.g. Vietnam – Vietnamese Academy of Sciences and Technology, Panama – University of Panama. The outcomes of these cooperation are joint research and publications, joint projects, effective use and share of the research infrastructure and expertise, researchers mobility, joint projects.
- High number (51 in 2014) of national and international Ph.D. students using the CzechGlobe infrastructure and closely cooperating with CzechGlobe.
- High interest of the wide public in Centre´s scientific popularisation activities and events e.g. the Centre open days (annually thousands of visitors), Week of Science and Technology (more than 500 guests in 2014), Quo vadis, scientia?, Prague 2012 (2500 guests), European Helicopter Show, Hradec Králové 2013 and 2014 (presentation of the air carrier of hyperspectral sensors; 20 000 guests each year), or Centre of Excellence in the research of GZ impact (within the Olympic Games Park, London 2012).
- Worthwhile achievement of Open Access scientific results and data management. Many external international and national users utilize CzechGlobe data (including NASA, IPCC UN) e.g. data of CO2/energy fluxes and meteorological data achieved by CzechGlobe and downloaded to the international databases (e.g. FLUXNET, ESFRI databases, World Meteorological Organisation), see more in other evaluation documents.
- Successful evaluation of the GCRC research infrastructure in the national evaluation of the Research Infrastructures organised by MEYS in 2014. In the competition of about 100 other proposals, all three assessed research infrastructures (CzeCOS, ACTRIS, C4SYS) nominated by GCRC, covering majority of the GCRC infrastructure, that were evaluated by this complex evaluation process, were assessed by the highest ranking. Based on this successful evaluation, MEYS proposed all GCRC infrastructures to the Czech Government for the National Roadmap of Research Infrastructures for the Period 2016-2022. E.g. Scientific Board Consensus Report Research Infrastructures Evaluation: *"The CzeCOS-II research infrastructure is mature and unique within the Czech research infrastructure landscape. CzeCOS-II is well structured to have a significant impact in the Czech, but primarily also in the European, landscape of research infrastructures. Within the Czech Republic CzeCOS-II is*

the only research infrastructure that is dealing with global climate change issues (in the broadest sense of the term). The research infrastructure contains a multitude of high-quality technology equipment that is unique in the Czech Republic.“

- Regular (annual) assessment of the Scientific Advisory Board of CzechGlobe (body of ten international distinguished experts in the main CzechGlobe scientific activities and representing excellent research centres, universities and international scientific infrastructures) underlines successful implementation of the project CzechGlobe and its scientific activities.
- In the end of 2014 National Sustainability Programme project CzechGlobe 2020 of the whole Centre CzechGlobe has been successfully assessed (based on international scientific evaluation) by MEYS. The project CzechGlobe is including plan of scientific activities of the Centre CzechGlobe for the five years period 2015-2019 (see more below in the subchapter 4). The project is fully based on the CzechGlobe activities and using the successful CzechGlobe outcomes from the period 2010-2014.
- Thanks to the Centre´s success in various research programmes and projects scientific and financial sustainability and vitality is secured. For example the total CzechGlobe expected income for 2015 (without investments) is approx. 7.1 Mil. EUR (including 1.9 Mil. - National Sustainability Programme – project CzechGlobe 2020, 1.25 Mil. - institutional funding by CAS, 1.8 Mil. - national projects, 1.12 Mil. - international projects, 0.35 Mil. - contracted research, 0.62 Mil - other resources e.g. ESF projects).
- In the end of 2014, European Commission (in EC JRC: *Synergies between EU R&I Funding Programmes. Policy Suggestions from the Launching Event of the Stairway to Excellence Project.* Luxembourg, 2014) points out CzechGlobe as the best example how to participate in EU research programmes: ”*This Policy Brief addresses the concept of synergies arising from the two major EU funding sources (The European Structural and Investment Funds and Horizon 2020) in the context of the new Stairway to Excellence Project. This project is centred on the provision of assistance to Member States who joined in 2004, 2007 and 2013 in using innovation funding under ESIFs via the early and effective implementation of RIS3 with the aim of closing the innovation gap and promote scientific and technological excellence. This Policy Brief summarises the discussion and case studies presented at the launching conference of the Stairway to Excellence Project held in Prague in October 2014. This event offered a first opportunity to identify the key elements for building successful synergies and gave a useful insight into how synergies could be achieved in practice. A diverse set of experiences from five EU countries (Cyprus, Czech Republic, France, Spain, and the UK) and an international organisation were presented. In turn, this could be a source of inspiration for other regional and national managing authorities and the research community.”*
- In the year 2014, the Centre CzechGlobe became a model Centre of Excellence for research ministries and governments in Slovenia and Portugal and they asked CzechGlobe, MEYS and ESFRI ICOS for consultation.

The CzechGlobe mission statement formulated by the Centre´s leaders and in cooperation with the Scientific Advisory Board of CzechGlobe is following:

CzechGlobe aims to be the main research centre in the Czech Republic for the integrated study of global changes. CzechGlobe examines interactions between the atmosphere, biosphere and geosphere from climatological, biological and societal view point including socioeconomic and other adaptation and mitigation change.

CzechGlobe consist of 5 divisions and this structure is followed according to the team nominations for the evaluation:

1. Division of Climate Analysis and Modelling
2. Division of Ecosystem Analysis
3. Division of Impact Studies and Physiological Analyses
4. Division of the Human Dimensions of Global Change Impacts
7. Division of Innovation and Adaptation Techniques

1. Division of Climate Analysis and Modelling

Team individual mission:

Atmosphere is regarded as the principal deponium and source of greenhouse gasses (GHG). The development, trends and composition of the atmosphere as well as its variability are connected to changes in GHG emissions and amount of their takeover by biosphere. Climate progress is immensely complicated and any improvement in accuracy of the forecast scenario is extremely problematical and methodologically very exacting. An analysis of climatic and hydro-meteorological extremes brings principal answers regarding the trends of climate changes and their consequences for socio-economic sphere. Thus, investigation and understanding of climate changes trends is of great importance. To be able to prepare some scientifically-based forecasts, the utilization of a sophisticated and acceptable regional climate model is invited. Thus, the application, validation and use of the climate model ALADIN is an important part of research activities in these areas. All climate changes and their development as well as their variability are immediately reflected by ecosystems' functions, vitality and stability. They are particularly managed ecosystems, mainly agriculture and forestry systems, that seem to be extremely vulnerable to climate change impacts.

The main research areas related to team:

- Analysis of historical time sets used for the identification of climate extremes events frequency,
- Climate modelling,
- Climate development, changes and extreme events and managed ecosystems,

Main achievements of the Division from the Centre perspective:

The team has successfully coordinated various projects. The multidisciplinary project InterSucho (Building up a multidisciplinary scientific team focused on drought; 2012-2015) interconnected various research CzechGlobe teams and external stakeholders (e.g. farmers, agricultural partners) in the drought research, drought forecasts, agricultural applications and drought mitigations. The research results were also published in high IF journals and highly appreciated by agricultural community (e.g.

Agricultural Chamber). The project has been extended by the project Czechadapt – System for Exchange of Information on Climate Change Impacts, Vulnerability and Adaptation Measures on the Territory of the Czech Republic (2015-2016).

Another scientific achievement of the team is the successful realisation of the cross-border Czech-German INTERKLIM project (Climatological cooperation for Czech - Saxon border area (2013-2014). Apart achieving the project goals and publications in highly IF journals the project results were appreciated by regional governments, widely published by Czech and German mass media and outcomes of the research are used by other CzechGlobe teams for further research. INTERKLIM project is also showcased by German and Czech prime ministers and relevant ministries (environment, research) as a good example of bilateral German / Czech research cooperation.

The scientific outputs (e.g. regional climate modelling) is not only used by scientific CzechGlobe teams and CzechGlobe partners but they are also used in applied collaboration with private partners (e. g. energy power companies, electric grids, high precision farming).

2. Division of Ecosystem Analysis

Carbon cycle, carbon sequestration into biomass and soil are strongly coupled to the Global Change. Thus, the investigation of its all aspects (including the carbon budget of selected landscape units) by the use of a wide mosaic of methodological approaches is of great importance. The understanding of the mass and energy exchange between ecosystem and its environment plays the main role in this investigation. These exchanges are carried out in different environmental, spatial and temporal scale. It is advantageous to be able to conduct the observation on the level of hydro-geochemical cycles, atmosphere-plant canopy exchange and biodiversity. These analyses must be carried out in the dynamic mode, to able to detect changes on the spatial and temporal level. At present, the application of truly current and modern methods is inevitable. Thus, only modern and elaborated eddy-flux observation, application of remote-sensing based on process imaging, small-scale-automatically operated catchments and mathematical models based on biodiversity investigation are acceptable. This division also contributes to the world research through the creation and operation of the national point of atmospheric GHG observation which is incorporated in the European network of GHG observation. This monitoring point contributes to GHG budget and long-range transport studies in the atmosphere, which eventually affect terrestrial ecosystems.

The main research areas related to the team:

- A mosaic of various ecosystem types based on a network of eddy-covariance towers used for permanent observations,
- Meso- and macro-scale remote sensing observation of carbon sinks,
- Long-term observation of geochemical and hydrological cycles on the scale of forest catchments,
- Dynamic modelling of biodiversity research
- Monitoring of aerosol properties related to climate change
- Long-term monitoring of GHG transport
- Carbon budget of selected landscape units and relation to their economic structure

Main achievements of the Division from the Centre perspective:
The team significantly contributes to Centre activities by creation, operation and research use of the state-of-the-art research infrastructure that is involved in pan-European research consortiums as a single Czech national infrastructural point: ESFRI ICOS and ACTRIS (Aerosols, Clouds, and Trace gases Research Infrastructure Network). The team is characterized by a good balance of success in national (GAČR – Czech Science Foundation, TAČR - Technology Agency of the Czech Republic, NAZV - National Agency for Agricultural Research) and international (H2020) projects and consortia. The team, despite the effort spent in building an ambitious research infrastructure in the last years, maintained its high level on publications in impacted peer review journals. The infrastructure operated by the team is used in synergies by other CzechGlobe teams as well and by the third parties in Open Access mode and various national and international projects and programmes.

3. Division of Impact Studies and Physiological Analyses

Comprehensive investigation of ecosystems response to the changing world is closely related to the deeper understanding of processes forming a whole chain of carbon assimilation and related processes. Primarily the investigation of ecophysiology of carbon assimilation is a crucial point of the comprehensive understanding of biosphere response to the changing atmospheric carbon dioxide and thus to the changing world. Eco physiological investigation requires deeper studies of the mentioned processes. Environmental metabolomics seems to be a modern and big challenge for the understanding of internal metabolic response to the changing environment. The application of carbon isotopes supports deeper investigation of the carbon “destiny” within plant tissues and makes it possible to identify the metabolic pathways of assimilated carbon. To be able to understand the process under field conditions a certain level of controlled impact studies is necessary and a system of modern impact studies is needed.

The main research areas related to the team:

- Ecophysiology of carbon assimilation related to the changing environment,
- Construction of a “*library*” of basic metabolites which are typical for an individual tree and plant species,
- Identification of “*targe*t” metabolites as a consequence of the impact of selected factors of plants environment,
- Modern controlled field impact studies,

Main achievements of the Division from the Centre perspective:
The team developed and established world class unique research infrastructure that is involved (CzechGlobe as a founding member and single Czech research participant) in ESFRI AnaEE (Analysis and Experimentation on Ecosystems). The infrastructure operated by the team is used in synergies by other CzechGlobe teams (e.g. for ecosystems research) as well and by the third parties in Open Access mode and various national and international projects and programmes including applied research (mainly in cooperation with agricultural companies). The team has coordinated whole CzechGlobe project ENVIMET (2013-2015) focusing on the scientific activities linking environmental metabolomics and ecophysiology and its integration into international networks.

4. Division of the Human Dimensions of Global Change Impacts

Global Change impacts significantly affect the socio-economic systems, i.e. the level of human welfare. At present time clear evidences show the extent of a relation between the global changes and local critical extreme events. Thus, a comprehensive elaborated system of indicators of society changes and integrated evaluation of the effect of environmental impact on the structure and function of local – global society and economic systems are of great importance. The concept of "ecosystem services" as an evaluator of ecosystems' ability to mitigate or adapt to the global change must be widely accepted and better elaborated.

The main research areas related to the team:

- Assessment and modelling of ecosystem services
- Environmental security, risk and vulnerability of social-ecological system to global change
- Climate Change Adaptation
- Sustainable governance of ecosystems and policy support

Main achievements of the Division from the Centre perspective:

Even though the newly established team (founded in the end of the year 2010) in very beginning was quite small, it developed in resilient team important in synergy for the other CzechGlobe teams. It has successfully coordinated and participated in various projects and programmes of FP7 and Horizon 2020, Technology Agency of the Czech Republic, Ministry of Interior, COST etc. The team research results published in important scientific journals and included in national governmental and EU strategies is not only based on the research of other CzechGlobe teams (e.g. integrated assessment of ecosystems services) but it contributes vice versa bringing new inputs to other teams (e.g. environmental security issues like e.g. drought issues). Even though the team is oriented in socio-economic research field it is closely collaborating with applied partners such are ministries, municipalities, companies or NGOs.

7. Division of Innovation and Adaptation Techniques

The mitigation and mainly the adaptation to the Global Change impact provoke searching for acceptable techniques which would make it possible to utilize the surplus of carbon dioxide in industrial wastes. Due to the fact, that Global Changes are strongly connected to the carbon dioxide atmospheric concentration, it is evident that the use of autotrophic organisms in carbon dioxide sequestration is of great importance. The use of autotrophic organisms in the carbon sequestration is based on the utilization of an atrophic micro-organism. CzechGlobe has had long-term tradition in the investigation of photosynthetic specific properties of algae and cyanobacteria. Thus, the orientation on the targeted selection of proper organisms is a very promising way how to elaborate a form of "biotechnology-based" carbon sequestration. Moreover, as a by-product, it is possible to produce special valuable agents with a potential use in pharmacy and cosmetics. This is a promising part of CzechGlobe innovation.

The other important component of this team is innovative methodology based on state of the art unique Centre´s research infrastructure – mainly aircraft (application of remote-sensing based on process imaging), unique photobioreactor for research and cultivation of autotrophic organisms and Bílý Kříž experimental research station (biomass increment and water budget).

The main research areas related to the team:

- development and running of a special experimental pilot large-scale bioreactor used in long-term cultivation of massive colony of microorganisms ,
- targeted cell sorting used in the identification of requested properties,
- cell manipulation targeted to the production of bio-components used in the production of next generation biofuels and other valuable bio-compounds
- meso- and macro-scale remote sensing observation of carbon sinks
- biomass increment and water budget under the changing world conditions.

Main achievements of the Division from the Centre perspective:

The team developed and established world class unique research infrastructure that is involved (CzechGlobe as a founding member and single Czech research participant) in ESFRI EUFAR and ISBE (Infrastructure for Systems Biology in Europe). The infrastructure operated by the team is used in synergies by other CzechGlobe teams as well (e.g. remote sensing research, system biology) as well and by the third parties in Open Access mode and various national and international projects and programmes including applied research (mainly in cooperation with companies working agriculture, forestry, GIS, pharmacy, hi-tech instruments). The team has developed specific instruments (e.g. photobioreactor, the pocket size instrument for estimation of a leaf area index) in cooperation with other CzechGlobe teams that are commercially utilised. The team also coordinates important international consortium for computational modelling of a cyanobacterial cell (2012-2015) that uses the scientific outputs and expertise from other CzechGlobe teams as well.

Research Report of the team in the period 2010–2014

Institute	Global Change Research Centre of the CAS, v. v. i.

Scientific team	Division of climate analysis and modelling

The research program of the evaluated Team (**Section of Climate Analyse and Modelling**) consists of 4 main research lines that are organized in 3 research groups. During the 2010-2014 all three groups have been organized, equipped and consolidated into the effective working teams. In the same time the great effort was made to achieve self-sustained financing primarily through research grants and applied research contracts. This was accompanied with significant workload associated with overseeing the procurement, instalment and fielding of new instruments, buildings and laboratories. However whole Team produced series of internationally acknowledged results as well as left their mark in number of awarded projects, organized workshops, summer schools and meetings with the private and public sector result users.

The Team has focused on past, contemporary, and future climate projections, model output validation and correction as well as on the use of modelling (and experimental tools in collaboration with GCRC evaluated Teams 2 and 3) for climate change impact assessment and adaptation analysis.

The Team participated in projects INTERDROUGHT, INTERKLIM and other funded by GA ČR (Czech Science Foundation). It is acting as modelled climate data provider for other research groups and teams (primarily in Team 1) but includes most of CzechGlobe teams and many researchers in the agronomy, forestry and socio-economic sector in CR outside CzechGlobe. The project team has been involved in … XXX … developing and implementing high precision forecasting systems for photovoltaic power-plants and wind farm and participating in the projects of TA ČR. Further it is involved in running a project of basic research granted by GA ČR and collaborates on 3 OPVK projects (in two of them as main coordinators), on the Joint Programming Initiative project, the National Agency for Agricultural Research project, the Austrian Climate Research Programme project (as co-applicant), the KONTAKT project US-Czech collaboration (North Carolina State University) and 2 COST projects with follow-up national financial support grants.

Achievement no. 1: Building up interdisciplinary and inter-connected research program consisting of three focused research groups

Climate Variability and Climate Change Analysis
Climate Modelling
Climate Change Impacts on Agroecosystems

This goal has been reached through the clear support of the Team from the CzechGlobe management but also through sustained effort focused on obtaining sustainable financial support from projects. As a result all three scientific groups are

interconnected through series of projects cutting across their field of expertise and there is high level of formal but especially informal collaboration at all levels starting with the team leaders all the way to the PhDs. Through the 2010-2014 the research Team was equipped and can now rely on unique infrastructure including new measurement systems, new experiments but also on the entirely new field laboratory (group of Climate Change Impacts on Agrosystems). Through the support of the projects obtained by the Team, the Team has invested considerable funds into the databases in which data for close to 10 mil. CZK are being concentrated (as of April 2015). The data are crucial for providing climate research services to other teams.

Achievement no. 2: Scientific results in the area of modelling of the climate extremes and trends in climate elements, creation of local emission scenarios, regional projections of climate change and modelling of the climate change impacts on controlled ecosystems can be demonstrated by high number of scientific publications between 2010-2014 which were among the highest within the teams of the GCRC. It is particularly important to note that many of these research papers included highly international authorship teams and covered Central European or European scale. Number of the research papers reached highest standards with their respective fields including publications in journals as Nature Climate Change, Global Change Biology or Agricultural and Forest Meteorology. Given the success rate in such of these journals the ability to produce results at this level in the time of research program build-up phase holds a clear promise for the near future. It should be also mentioned that the research teams (and individual researchers) of the Team had the highest number of citations in the last Intergovernmental Panel for Climate Change Assessment Report of any research organization from the Czech Republic. The excellence in the conducting and presenting the results of the scientific research has been targeted through the Team workshops and summer schools where scientists with top publishing record (Nature, Science, PNAS etc.) have been lecturing to the all Team members including some hands-on training. Examples of some papers with highlighted names of the Team investigators are shown below:

Miroslav Trnka, Reimund P. Rötter, Margarita Ruiz-Ramos, Kurt Christian Kersebaum, Jørgen E. Olesen, **Zdeněk Žalud** & Mikhail A. Semenov. **(2014)** Adverse weather conditions for European wheat production will become more frequent with climate change Nature Climate Change 4, 637–643 (2014) doi:10.1038/nclimate2242 **IF 14,472**

Trnka, M., Olesen, J. E., Kersebaum, K. C., Skjelvåg, A. O., **Eitzinger, J.,** Seguin, B., Peltonen-Sainio, P., Rötter, R., Iglesias, A., Orlandini, S., **Dubrovský, M., Hlavinka, P., Balek, J.,** Eckersten, H., Cloppet, E., Calanca, P., Gobin, A., Vučetić, V., Nejedlik, P., Kumar, S., Lalic, B., Mestre, A., Rossi, F., Kozyra, J., Alexandrov, V., **Semerádová, D.** And **Žalud, Z**. (2011), Agroclimatic conditions in Europe under climate change. Global Change Biology, 17: 2298–2318. doi: 10.1111/j.1365-2486.2011.02396.x **IF 8,224**

Büntgen, U.; Egli, S.; Camarero, J. J.; Fischer, E. M.; Stobbe, U.; Kauserud, H.; Tegel, W.; Sproll, L.; Stenseth, N. C. Drought-induced decline in Mediterranean truffle

harvest. *Nature Climate Change*. 2012, volume 2, issue 12, p. 827-829. ISSN 1758-678X. **IF 14,472**

Bunde, A.; **Büntgen, U.**; Ludescher, J.; Luterbacher, J.; Von Storch, H. Is there memory in precipitation? *Nature Climate Change*. 2013, volume 3, issue 3, p. 174-175. ISSN 1758-678X. **IF 14,472**

Büntgen, U.; Kyncl, T.; Ginzler, Ch.; Jacks, D. S.; Esper, J.; Tegel, W.; Heussner, K. U.; Kyncl, J. Filling the Eastern European gap in millennium-long temperature reconstructions. *Proceedings of the National Academy of Sciences of the United States of America*. 2013, volume 110, issue 5, p. 1-6. ISSN 0027-8424. **IF 9,737**

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Achievement no. 3: included software tools for projections of local climate change and its parameters as well as its impacts. These included e.g. stochastic climate change scenario generator, stochastic meteorological series generator M&Rfi., statistical downscaling models as well as models SoilClim for determination of soil climate – temperature and hydrological regimes of soils and AgriClim for processing of measured climate data) are under development and the end users of the models were identified, yet. These models are being used by number of teams within CzechGlobe and many teams across the Czech Republic but also through international consortia as e.g. JPI or COST ES1106 projects. In addition the software developed by the Team (SoilClim, M&Rfi, AgriClim) is being used e.g. in preparing Overall Plan of the Water Management in the Landscape of the Czech Republic or at operational drought monitoring. The AgriClim software was chosen by the Leads University for two of their research projects while SoilClim project is now used by consortia in Austria, Germany, Slovakia and USA. Number of interested users from academic community and private companies is increasing every month. The ability to handle complex climate-impact model analysis has led to number of “invitations” resulting in growing segment of Climate-services type of contracts. The results of the software is also serving for the decision making process of the State Administration of the CR (ministries and State Administration authorities), The Agrarian Chamber of the Czech Republic, etc. in the areas of security, health, economic and agricultural policies especially in connection with the climate change. Within the applied research, the Team through the CzechGlobe signed contract with the E.ON company (power and gas supply) on the project „Models of photovoltaic production in South Bohemian and South Moravian Energy Distribution districts”.

Achievement no. 4: Through the sustained work of the Team not only the expert groups were build, papers published and software developed but also the Team expertise has been recognized by the public and private stakeholders. For example thanks to the drought monitoring system (www.intersucho.cz) which has been developed by the whole Team, the researchers from the Team have been tasked not only to lead national effort on water management in the landscape but also to serve as advisors in case of drought insurance and preparation of the drought plans. The work of the Team on the field of drought research has been presented through invited

presentation to the upper chamber of the Czech Parliament (lower chamber meeting is planned for latter 2015) and to the ministers of Agriculture and Environment. The regional government in the South Moravian Region selected the drought monitoring efforts of Team for its regional project support and the Academy of Sciences itself decided to support the activity. Similar trends are being seen in case of renewable resources and climate services.

Through the fulfilling of the above presented achievements the Team is contributing to the overall mission of the Centre.

Research Report of the team in the period 2010–2014

Institute	Global Change Research Centre of the CAS, v. v. i.

Scientific team	Division of ecosystem analysis

General overview

The scientific activities of the **Division of Ecosystem Analysis** are built upon modern and large research and monitoring infrastructure elements, which are in some aspects unique on an international and even worldwide level. Some parts of the Division infrastructure network were newly built in the evaluated period of 2010-2014 and some were modernized. The involvement of these infrastructures into national and international monitoring and research networks supports collaboration activities of the Division at various levels including ESFRI. Increasing collaboration is to a significant degree based on providing top quality and large datasets to national and international databases.

Although the Division was to a considerable degree occupied by building of the new infrastructure elements and also team setup, scientific results in form of numerous papers published in prestigious international impacted journals, national journals as well as books were achieved. The publishing activities are based on long-term monitoring data provided by the infrastructure and also on theoretical, experimental and field approaches. For data interpretation, also advanced statistics and modelling is used. Achieved scientific results were in the evaluated period presented at numerous national and international conferences.

The multidisciplinary and demanding research needs and opportunities provided by the infrastructure as well as non-infrastructure based scientific studies led to the establishment of new research teams. Staff was hired both from the national as well as international human resources pool. The involvement of international colleagues in the team supports also prestigious international collaboration activities, e.g. advice on building new infrastructures or terrain studies abroad. Further, the GCRC infrastructure and measured data is via open access offered to interested national and international research organizations. This strategy contributed to the Divisions increasing success of joining interesting consortia and projects. Individual scientists made use of mobility possibilities (both from and to GCRC), achieved significant progress in their career (e.g. graduating as PhDs and associate professor) and were rewarded by prizes (e.g. Antonín Frič Prize, see form 3-10).

Scientific activities conducted by the Division researchers or based on the knowledge and infrastructure provided and run by the Division were not only of basic, but also applied character. This is to a significant degree supported by open access to the Division data and infrastructure. It once again leads to interesting partnerships with national as well as international applied research or private business subjects.

It can be summarized, that 2010-2014 was a period of infrastructure and team building which is step by step resulting in excellent scientific results and collaborations. Scientific activities will fully flourish in the next period 2015-2019 as a strong basement in terms of infrastructure, data, interdisciplinary scientific methods and approaches and human resources is now provided. In the following text, the individual parts building the complex and interdisciplinary research activities of the Division of Ecosystem Analysis are presented, explained and discussed in detail.

Research infrastructure

The research infrastructure run by the Division of Ecosystem Analysis on its own or in cooperation with other subjects contains several observational research infrastructure elements (monitoring towers and sites) and an analytical infrastructure (laboratory). These offer a unique base for the realization of complex sophisticated research on global change topics such as (a) greenhouse gas (GHGs) fluxes

between the atmosphere and selected terrestrial ecosystems, (b) atmospheric composition up to 250 m height above ground and (c) biogeochemical cycles of especially carbon and nitrate. In the Czech Republic there are no comparable observational infrastructures focused on topics (a) and (b). Some aspects of the observational infrastructure are unique also in the European research area (e.g. some air composition sampling designs, ecosystem stations in flooded areas). The Division´s observational research infrastructure forms a national complement to the existing ESFRI infrastructure ICOS (Integrated Carbon Observation System). The Division is responsible for the operation of the following observatory facilities equipped with the most modern measuring systems:

1. Ecosystem Stations (ESs) are owned by GCRC and focused on the observational research and quantification of greenhouse gas fluxes in different ecosystem types representative for the Czech Republic and central Europe (young Norway spruce forest at Bílý Kříž, mature Norway spruce forest at Rájec, mature beech forest at Štítná, agroecosystem at Křešín u Pacova, grassland at Bílý Kříž, wetland at Třeboň, floodplain forest at Lanžhot, urban ecosystem in Brno). The aim is to quantify carbon sinks and to estimate their dynamics in space and time as a response to environmental drivers. The ESs are equipped with state-of-art instruments for monitoring of GHGs (CO_2, CH_4, N_2O, O_3) and water fluxes according to the latest standards of ICOS. The ESs consist of measurement towers (5 to 44 m tall) equipped with eddy covariance systems for measurements of GHGs and energy fluxes based on the latest models of infrared analysers and ultrasonic anemometers. Fluxes of CH_4 and N_2O at the wetland and flooded forest are measured by chamber systems developed at GCRC and based on laser analysers. Meteorological sensors (temperature, humidity, wind speed, radiation) are placed in the vertical canopy profile. Automated systems for CO_2 fluxes from soil and woody tissues and for CO_2 concentration in vertical gradient were developed. The ESs are equipped by a set of precise soil water probes, rain gauges, and water table sensors. These facilities allow enhanced research of water regime in ecosystems, which is one of the crucial issues of global change research, and to join top-ranked consortia dealing with global change (ICOS). The infrastructure allows for monitoring of supporting growth variables (radiation quantity/quality, temperature of different ecosystem parts etc.), soil properties, physiological traits (sap flow, leaf area, biomass increment, respiration of ecosystem components, photosynthesis etc.) and hyperspectral reflectance from towers.

2. The Atmospheric Station (AS) Křešín u Pacova is owned by GCRC and equipped in order to cover the list of mandatory measurements demanded for an ICOS Level 1 station. The current list of measurements contains: (a) concentrations of CO_2, CO, CH_4, N_2O (continuously in 10, 50, 125 and 250 m height above ground), (b) elemental and organic carbon (aerosol optical properties – only such measurements in the Czech Republic, elemental and organic carbon in aerosols, all continuously at ground), (c) tropospheric ozone (continuously in 10, 125 and 230 m, rarely measured worldwide), gaseous elemental mercury (continuously at ground and in 240 m height, probably the only such sampling design worldwide), (d) meteorological parameters (air temperature and pressure, relative humidity, wind speed and direction) in all sampling heights and (e) the planetary boundary layer height. The measurements at the AS are representative for the central European background. In contrast to many European and worldwide tall towers, the AS is an infrastructure built primarly for scientific purposes and not limited by commercial activities (e.g. broadcasting) conducted at the tower itself.

Further, there are two more infrastructures run in cooperation with other subjects. The network of 14 small forested catchments GEOMON shared with the Czech Geological Survey is used as a research tool to measure relevant data, mainly hydrology and chemistry of precipitation, soil solution and running water. The network is operated since 1994 and measured data provide a basis for analyses of long-term trends, processes in ecosystems and biogeochemical and hydrological modelling. In the evaluated period of 2010 to 2014, the catchment instrumentation was improved by the installation of automatic water level recording with online data transfer and renovation of precipitation collectors and lysimeters for soil water.

The setup of the biogeochemical laboratory run jointly with Geotest Brno was finished in 2013. GCRC equipped the biogeochemical laboratory with an analyzer (varioTOCcube) of total carbon, inorganic and organic (difference) carbon and total nitrogen in water (groundwater, drinking water and waste water), N, C, S analyzer NCS Flash 2000 for analysis of nitrogem carbon and sulfur in samples of soils, needles, leaves, root or grain, Ion Chromatography System Dionex ICS-5000 for analysis of basic anions (nitrare,

sulfate, chloride, phosphate) and cations (calcium, potassium, magnesium, sodium or ammonia) in groundwater and drinking water or growth media for cyanobacteria and other, UV-VIS Spectrofotometer Specord 250PLUS for measuring absorbance on various wavelengths and Automatic Titrator 907 Titrando with pH electrode for potentiometric measurements. For sample preparation drying oven, siever, ball mill, centrifuge, decomposing furnace (microwave) and microbalance are used. For analysis of heavy metals and forms of nirogen in extracts the ICP-OES analyzer belonging to the inorganics part of the laboratory run by Geotest Brno is used.

The above described research infrastructure was newly established in the evaluated period or re-built and innovated between 2010 and 2014. This was possible thanks to the CzechGlobe project under the Operational Programme Research and Development for Innovations (OP R&DI, European Centres of Excellence) obtained by GCRC. The construction of the AS Křešín u Pacova was finished in 2013, the construction of the Lanžhot, Brno and Křešín u Pacova ESs between 2012 and 2014. The setup of the biogeochemical laboratory was finished in 2013.

The scientific strategy of the observational infrastructure elements (i.e. ESs and AS) is focused on (a) reaching a comparable level with world leading global change research infrastructures and ensuring the standardization of methods, procedures and equipment to achieve full comparability of data with similar infrastructures in Europe and (b) development and implementation of new state-of-the-art methods enabling a deeper understanding of the processes within the biogeochemical cycles and processes of acclimation and adaptation to changing environmental conditions. The role of the biogeochemical laboratory within the Division of Ecosystem Analysis but also for other Divisions of the GCRC is more of a necessary service significantly supporting these teams in conducting current and future top level research.

Monitoring and research projects, programmes and environmental conventions

The crucial advantage of the above described observation research infrastructures is the interconnection of facilities, experts and research approaches under the key ESFRI infrastructure ICOS covering ecosystem and atmospheric research. Sufficiently robust description and prediction of various ongoing changes in e.g. terrestrial ecosystems function, carbon sequestration, atmospheric composition and biogeochemical and hydrological cycles (which are all part of the global change phenomenon), is only possible if conducted in the context of large spatially distributed networks.

The Division of Ecosystem Analysis is by means of its research infrastructure and scientific activities contributing within GCRC to the Czech international and EU contributions and obligations in the area of climate and environmental policy (e.g. UN Framework Convention on Climate Change, Kyoto Protocol to the United Nations Framework Convention on Climate Change). The Division significantly contributes to the global change research and monitoring efforts due to the cooperation with the following international and national networks and systems: ICOS, InGOS - Integrated non-CO_2 Greenhouse Gas Observing Systems, GMOS - Global Mercury Observation System, GAW - Global Atmosphere Watch, NEON - US National Ecological Observatory Network, ACTRIS - Aerosols, Clouds, and Trace gases Research Infrastructure Network, EMEP - European Monitoring and Evaluation Programme under the CLRTAP (Convention on Long-Range Transboundary Air Pollution directed by the UNECE), ICP Waters - Integrated Cooperative Programme on Waters (by UN ECE), and ISKO - Czech Information System on Air Quality (run by the Czech Hydrometeorological Institute). Two biodiversity scientists are members of important scientific bodies of IUCN World Commission on protected Areas, Species Survival Commission, Orchid Specialist Group, In Situ Conservation Committee and of the Society for Conservation Biology (Policy Committee). One is a member of the Government Council for Sustainable Development, Committee for Landscape, Water and Biodiversity, where he works on the European Landscape Convention. Several scientists of the Division are members of the Czech National Committee of Long-term Ecological Research and one is a member of the IOBC Working Group Biological Control of aphids and coccids.

The AS Křešín u Pacova is an infrastructure able to fulfill the interconnecting obligations of the Czech Republic in international programs and networks such as ICOS, InGOS, ACTRIS (in collaboration with the Czech Hydrometeorological Institute, Institute of Chemical Process Fundamentals AS CR and RECETOX, Masaryk University) and GMOS (in collaboration with the Czech Hydrometeorological Institute). The AS is located adjacent to the Košetice Observatory run by the Czech Hydrometeorological

Institute which is also connected to numerous national and especially international programmes and networks. Both facilities form the Collocated Station Košetice-Křešín u Pacova. The high level of networking and interconnected monitoring and research activities prelude the Collocated Station to become one of the European atmospheric research supersites which are envisioned in the comming years.

The scientists of the Division of Ecosystem Analysis not involved in infrastructure activities frame their research also under other numerous national and international project and grant agencies. These include COST - European Cooperation in Science and Technology (e.g. the project Resilience Capacity and Perspective of Mountain Spruce Forests under Environmental Change, 2014-2016), ECOP – Education for Competitiveness Operation Programme (e.g. the project Platform for Study and Inventarization of Forest Ecosystems, 2012-2014), GACR - Czech Science Foundation (e.g. the project Role of Plants in the Greenhouse Gas Budget of a Sedge Fen, 2011-2014) and TACR - Technology Agency of the Czech Republic (e.g. the project Extension of the Instrument Base by Gazometric Modules as Components of Smart Growth Chambers, and by Automatic Gazometric System for Measurements of Gas Exchange in situ, 2012-2015). The full list of project activities can be found in evaluation form nb. 3.1. An interesting example leading to inspiring cooperations is also the project of Norwegian funds „CzechAdapt – system for information exchange on climate change impacts, vulnerability and adaptation measures in the Czech Republic“, connecting practically all GCRC divisions. Its aim is to create an open and updated on-line database of current and predicted data, integrating information on climate change impacts, vulnerability and adaptation measures in the whole territory of the Czech Republic. Interesting projects from the field of biogeochemical cycles are e.g. the project BIOMDOC (Acid base character of dissolved organic carbon in different bioms) comparing dissolved organic carbon from U.S. far north at Alaska with other worldwide bioms.

Open Access and Data Policy

The policy of the Division of Ecosystem Analysis as well as whole GCRC is to establish Open Access to the research infrastructure and data to the maximal possible extent. The data from the ESs (greenhouse gas fluxes, meteorological variables etc.) were provided in the frame of Open Access to many international users (e.g. 39 applicants in 2013). The Open Access regime for the AS was established in 2013. Since then two international (Germany, France) and three national research and business subjects asked for measured data or access to the infrastructure itself in order to install their instruments. The other example of the Open Access use of the teram research infrastructure is the Biogeochemical laboratory use by various external partners including e.g. public and private partners.

The ESs and AS are fully in line with the prepared ICOS ERIC data policy. The ICOS Data policy final draft has been adopted by the ICOS member states and European Commission and should be implemented after the establishment of ICOS ERIC in 2015. Data provided by the AS are subject to data policies of the ACTRIS, GMOS, EMEP, GAW and ISKO databases. The ESs share data within the international project FLUXNET (comprises more than 500 ESs all over the world). All of these databases encourage the wide use of contained data by the scientific community. Most of these databases are also accessible to the general public and data can be downloaded for free under specified conditions. Data from the GEOMON catchment network are available on request for all institutes involved in the research using the catchment´s infrastructure.

Some of the ESs are infrastructure elements already established in the last decades. Their outcomes comprises top quality and large datasets, some of them also important in terms of a long measurement period (ES Bílý Kříž measuring since 1994). For example, data-sharing of results from different ecosystems and different geographical/climatic regions in the professional databases (ICOS, FLUXNET) lead to an increased interpretability and robustness of predicted future changes. Use of these data in cooperation with user community is related to the following areas: (a) comparisons, calibration, and validation (e.g. of satellite data and models), (b) monitoring, reporting and verification at both national and international levels, (c) assessing carbon fluxes and budgets, (d) information and educational purposes, and (e) synthesis (including maps and GIS) and predictions. The ESs data are used for analysing and modeling of biogeochemical cycles (especially carbon cycle) in terms of management practices, effects of water regime, nutrition etc.

Research characteristics and outputs

The Division follows the newest trends in terrestrial ecosystem, biogeochemical and atmospheric research. The research activities provide a comprehensive platform for a wide spectrum of scientific disciplines (analytical chemistry, atmospheric sciences, plant physiology, molecular biology, forestry, agriculture, geochemistry, hydrology, ecology, statistics and mathematical modelling etc.). This high level of multidisciplinarity is both a challenge as well as major advantage, because global change is a phenomenon affecting and being affected by the natural environment in its whole complexity. A significant portion of research is conducted in cooperation with other GCRC divisions who are using either data provided by the Division of Ecosystem Analysis, make use of conclusions obtained by their interpretation or participate in common field studies. The Division of Ecosystem Analysis significantly contributes to the concentration of knowledge potential of the whole GCRC, leading to its use also in strategic decision-making and supporting technologies for mitigation and adaptation to global change impact, especially in the forest-agriculture landscape.

The Division provides, thanks to its active participation in an ESFRI (ICOS) and other consortia and projects, to users in the European Research Area mainly access to a set of data and products including standards, calibration, protocols, instrumentation, software, information on essential climate and ecosystem variables, and support to environmental policies. The data are often used for synthesis works and meta-analyses and development or validation of mathematical models of ecosystems and atmospheric processes. A comprehensive, interdisciplinary approach at different temporal–spatial scales conducted together with other GCRC divisions enables the achievement of breakthrough discoveries and brings solutions for applied research. Users of the ES research infrastructure are represented by national and international scientists involved in joint data analysis and evaluation, and the results of such open access are usually joint publications in scientific journals (Stoy et al., 2014, Photosynthesis Research; Sulkava et al., 2011, Journal of Geophysical Research; Smith et al., 2010, Agriculture, Ecosystems & Environment). The data from the ESs are also used for the quantification of ecosystem function performance, evaluation of ecosystem services and for the estimation of carbon sequestration. The AS infrastructure construction was finished in 2013, thus large measurement datasets start to be produced just now.

During the period 2010–2014 115 articles in impacted journals and 5 scientific books were published by the researchers of the Divison of Ecosystem Analysis (for the whole list of publications see the list provided during the first evaluation phase). This number of publication outputs is influenced by the fact that many researchers were intensively preoccupied with setting up the Divisions infrastructure in the evaluated period. However, thanks to the challenges solved during the infrastructure setup, several functional samples were published (e.g. No. 24073 from 2013. Dušek J., Stellner S., Pavelka M.: Equipment for the measurement of gas emissions emitted from vegetation or soil, especially in locations with fluctuating water levels). Despite the time consuming involvement into infrastructure setup, various papers in the field of ecosystem fluxes were published (e.g. Acosta et al., 2013, Geoderma; Sedlák et al., 2010, Agriculture and Forest Meteorology) including also the results on the influence of ozone fluxes in ES Bílý Kříž on net ecosystem production (Zapletal et al. 2011, Environmental Pollution).

Researchers not involved in infrastructure building kept their high level of research and publication activities, especially in the field of biodiversity research. The main biodiversity research topics include: (a) study of the impact of global change on population dynamics of insect pests of agricultural crops (mainly aphids) and their predators (e.g. such model groups as ladybugs and ground beetles) while applying different scenarios of landscape use, global change, and intensification of agricultural activities in the landscape; (b) evaluation of the impact of global change on ecosystem functions, particularly at the level of relations between an insect pest and its natural enemy under various schemes of agroecosystem management; (c) analysis of the impact of changes in temperature (possible consequence of global change) on the population dynamics of some rare species; (d) evaluation of the importance of biocorridors in facilitating species migration necessary for their survival under global change and their importance as shelters and overwintering sites for the monitored organisms; (e) evaluation of the dependence of changes in biodiversity (especially species loss) and ecosystem services caused by the effects of global change on landscape connectivity; (f) analysis of changes in basic laws governing the dynamics of ecosystems and the interactions between species with changing temperature. The sum of IF papers per biodiversity scientist (recalculated to 100% FTE, students excluded) was 5.9 in 2013-2014. 52 papers and 5 books (2 of them in Springer) on biodiversity research were published in impacted journals during 2010-2014. A series of papers on Nepalese orchids,

especially those used for medicinal purposes, vastly enriched not only the knowledge of these endangered plants, but also the abilities to use extracts of these plants for curing diseases (Luitel et al., 2014, Journal of Ethnobiology and Ethnomedicine; Rokaya et al., 2014, Journal of Ethnopharmacology). A series of papers on population dynamics of aphids and their predators under conditions of agriculture intensification enabled to make predictions of the effects of various conservation measures, like grassy strips, for protection of aphid enemies in order to improve biological control of aphids – important insect pests (Hassan et al., 2012, European Journal of Environmental Sciences; Hassan et al., 2013, Agricultural and Forest Entomology;).

In the field of biogeochemical and hydrological cycles papers on the following topics were published in top ranked impacted journals: (a) analyses of long-term chemistry changes of glacier lakes in Šumava Mts. to distangle effects of recovery from acidification and ongoing bark beetle infestation (Oulehle et al., 2013, Biogeochemistry), (b) participation in whole European study of streamwater acidification recovery (Hruška et al., 2014, Water Air and Soil Pollution; Heliwell et al., 2014, Environmental Science and Technology), (c) analyses of long-term hydrological patterns of GEOMON catchment network showing no visible trend of runoff and precipitation (Lamačová et al., 2014, Soil and Water Research), (d) model evaluation of different pathways, sources and concentrations of dissolved organic carbon on historical acid/base status of soil and water (Garmo et al., 2014, Water Air and Soil Pollution) and (e) the evaluation of age and sources of dissolved organic carbons in different bioms (Evans et al., 2014, Global Biogeochemical Cycles).

Good examples of multidisciplinary scientific cooperation within the Division of Ecosystem Analysis are well impacted papers concerning the influence of ozone fluxes a the ES Bílý Kříž on net ecosystem production (Zapletal et al. 2011, 2012, Environmental Pollution), and Norway spruce forest ecosystem recovery after strong air pollution impacts in the Ore Mts. (Oulehle et al. 2011, Global Change Biology).

National collaborations

The cooperation with Czech universities in the field of student education and teaching courses is in detail described in Form 3-10. Here the national collaborations connected with the above described research infrastructures, resulting from solving selected interesting projects and running joint organisations are described.

1. Ecosystem stations are owned by the GCRC and run by the Division of Ecosystem Analysis. Measurement data are mutually exchanged with the Czech Hydrometeorological Institute, which runs several meteorological monitoring sites in the vicinity of the ESs. GCRC cooperates in the field of ES scientific activities also with the Mendel University in Brno, University of South Bohemia in České Budějovice and Institute of Atmospheric Physics AS CR.

2. The Atmospheric Station Křešín u Pacova is owned by the GCRC and run by the Division of Ecosystem Analysis. Due to the large research potential of such an infrastructure and broad range of covered scientific disciplines, collaborations with other institutes are welcome and necessary. The aerosol measurements are conducted in cooperation with the Institute of Chemical Process Fundamentals (Czech Academy of Sciences). The AS is located adjacent to the Košetice Observatory run by the Czech Hydrometeorological Institute. Both infrastructures form a collocated station, thus the cooperation with the Czech Hydrometeorological Institute is of very close nature. One of its major features is the free exchange of meteorological and air quality data between the Košetice Observatory and the AS. The AS is also part of the ACTRIS Czech Republic consortium together with both mentioned collaborating organizations and also RECETOX (Masaryk University). ACTRIS Czech Republic was positively evaluated by the Ministry of Education, Youth and Sports of the Czech Republic as a national research infrastructure in December 2014.

3. The biogeochemical laboratory is run jointly by GCRC and Geotest Brno. Both subjects equipped the laboratory with a sufficient spectrum of analytical instrumentation which enables the laboratory to cover the incomming demands for sample analyses.

4. The GEOMON network of small forested catchments is opearated jointly by GCRC and the Czech Geological Survey. Several other institutes like Czech Hydrometeorological Institute,

Research Institute of Forestry and Game Management, Geological Institute (Czech Academy of Sciences) and the Institute of Hydromechanics also contributes to the management of individual chatchments. Most biogeochemical research is done by GCRC and Czech Geological Survey.

5. The Centre for Tropical Biology was established under a grant from the Czech National Science Foundation together with the Institute of Environmental Studies, Charles University in Prague and the Biological Centre (Czech Academy of Sciences) in České Budějovice

6. A good example of the scientific cooperation of the Division of Ecosystem Analysis with Czech universities is the creation of two joint laboratories with the Forestry and Wood Technology Faculty of Mendel University in Brno – Dendroecological Laboratory and Rhizological and Molecular Biological Laboratory. Their aim is to study the health status of Norway spruce monocultures, individual carbon allocation in below-ground biomass, compartments of soil respiration, and identification of mycorrhizal symbionts by molecular-biological methods.

International collaborations

Major international collaborations of the Division of Ecosystem Analysis are realised under the ICOS ESFRI and together with the ICOS members (15 partner institutions). For more effective joint cooperation ICOS plans to become ICOS ERIC in 2015. The GCRC is a founding member of ICOS and represented by the ESs and the AS. Moreover, the ES Bílý Kříž is one of four ICOS demonstration ecosystem sites. The ES Lanžhot is proposed as ICOS Site Level 1, ES Bílý Kříž as ICOS Site Level 2 site, ES Třeboň as ICOS associated site and the AS Křešín u Pacova as ICOS Level 1 atmospheric station. One Division researcher is vice-president of the Ecosystem Monitoring Stations Assembly of ICOS since 2013, two researchers lead the Chamber Measurement Working Group of ICOS since 2013. Thanks to the ESs and AS run by the Division of Ecosystem Analysis, the GCRC represents the Czech Republic as one of the first countries fulfilling the ICOS national scientific infrastructural obligations. The AS participates also in the EU infrastructural consortium ACTRIS, which plans to become an ESFRI infrastructure in the coming years and an ACTRIS ERIC probably in 2018. Other international project based collaborations of the AS are described above.

The Division of Ecosystem Analysis established cooperation with non-European partners to extend the infrastructure expertise and use with partners from different geographical areas. The Division researchers cooperate with the Institute of Tropical Biology of the Vietnamese Academy of Technology and Sciences and the University of Panama. The main goal is the transfer of knowledge and technologies for ecosystem greenhouse gas and energy flux monitoring and to help the establishment of two ecosystem stations in tropical forests. The National Oceanic and Atmospheric Administration (NOAA, USA) expressed interest in data from the AS Křesín u Pacova (greenhouse gases, ozone). The ESs infrastructure has signed cooperation agreements with leading international research institutions, universities and networks (e.g. NEON - National Ecological Observatory Network, USA).

Intensive ecological and biodiversity field research is conducted in collaboration with institutes in France (University Rennes 1, Rennes), Japan (Yamagata University), Nepal (Tribhuvan University, Kathmandu), Bolivia (Universidad Mayor de San Andres, La Paz) and Papua New Guinea (New Guinea Binatang Research Centre, Madang). This international cooperation has led to dozens of publications of the Division.

Cooperation on carbon biogeochemistry and on modelling of catchment´s nitrogen cycle is conducted with Centre of Ecology and Hydrology, United Kingdom. Cooperation on biogeochemical modelling, especially on MAGIC model development and applications is organized with the Norwegian Institute for Water Research. Cooperation on dissolved organic carbon and dissolved organic nitrogen origin and sources in catchments is established with University of New Hampshire, USA.

Close cooperation on the interaction between ozone and volatile organic compounds and their impacts on ecophysiological processes of forest trees between the Division of Ecosystem Analysis and the Institute for Plant Protection CNR, Florence, and Research Centre for the Soil-Plant System, Rome, Italy was established recently. In the two field campaigns, carried out in Castelporziano near Rome and on the ES Bílý Kříž in 2014, several PhD students took part. Further cooperation was contracted with

the Leibniz Institute of Ecological Urban and Regional Development (Dresden, Germany) with the aim to prepare and submit several mutual projects (Horizon 2020, Trans-border cooperation) on problems of biodiversity, carbon allocation and ecosystem functioning in the forestry-agriculture landscape.

The staff of the Dendroecological laboratory (run jointly with the Mendel University in Brno) cooperates also with many scientific institutes in Europe, e.g. Group Dendroecology (Swiss Federal Institute for Forest, Snow and Landscape Research, Switzerland), Institute of Wood Science and Technology (BOKU, Vienna, Austria), Department of Wood Science and Technology (University of Ljubljana, Slovenia), Chair of Forest Growth (University of Freiburg, Germany), Department of Ecology (University of Barcelona, Spain), Norwegian Forest and Landscape Institute (As, Norway), National Forest Centre (Zvolen, Slovakia) and Deutsches Archäologisches Institut (Berlin, Germany). They are developing new methods and approaches for best information mining from recent and fossil tree ring increments, explaining the past climate development.

Collaborations with other divisions of the GCRC

The following collaborations with other Divisions of the institute took place in the evaluated period 2010 – 2014:

1. The research on matter and energy fluxes is conducted in cooperation with the Division of Innovation and Adaptation Techniques and the Division of Impact Studies and Physiological Analyses in development of new pocket size instrument for measurement of the leaf area index, in the area of research of fluxes of energy, water and CO_2 between terrestrial ecosystems and the atmosphere. Automatic measurements of fluxes using eddy covariance system is accompanied by measurement of photosynthesis and respiration on leaf level area and sap-flow in timbers to improve our knowledge in the issue of matters and energy fluxes.

2. The AS research infrastructure started to be used in 2014 by the Division of Impact Studies and Physiological Analyses which installed two types of passive air samplers on the tall tower. The effectiveness of these devices in sampling selected atmospheric pollutants is investigated in a 6 months pilot sampling campaign.

3. The biogeochemical laboratory is run by the Division of Ecosystem Analysis and used also by researchers from the Division of Impact Studies and Physiological Analyses, Division of Climate Analysis and Modelling and the Division of Innovation and Adaptation Techniques

4. The research focused on carbon storage in the landscape is conducted in cooperation with the Division of Climate Analysis and Modelling, the Division of Innovation and Adaptation Techniques and the Division of Human Dimensions of Global Change Impacts. The Division of Ecosystem Analysis plays an important unifying role in this cooperation. Its employees obtain detailed regional climate scenarios from the Division of Climate Analysis and Modelling and using special space data (e.g. on biomass distribution in the landscape) from the Division of Innovation and Adaptation Techniques they provided to the Division of the Human Dimensions of the Global Change Impacts data about e.g. production function (carbon amount) of individual habitat types for the valuation of ecosystem service provision.

Conferences, summer schools

The staff of the Division of Ecosystem Analysis took part in more than 50 international and more than 60 national seminars and conferences including big international congresses. Important international conferences actively visited by the employees of Division of Ecosystem Analysis were e.g. WorldDendro (Rovaniemi, Finland, 2010); TRACE (Orleans, France, 2011); Historical Wood Utilization (Stuebing, Austria, 2011); Archaeological and Historical Wood Utilization (Egmond aan Zee, the Netherlands, 2011); EGU General Assembly (Vienna, Austria, 2013, 2014), TRACE (Aviemore, Scotland, 2014), 13th Quadrennial iCACGP Symposium and 13th IGAC Science Conference on Atmospheric Chemistry (Natal, Brazil, 2014), 1st International Meeting on Population Ecology of Tropical Orchids: Advanced Tools for Conservation (Guanahacabibes, Cuba, 2010); 24th Annual Meeting of the Society for Conservation Biology (Edmonton, Canada, 2010); Ecology of Aphidophaga 11 (Perugia, Italy, 2010); 20th World

Orchid conference (Singapore, 2011); 4th International Symposium on Biological Control of Arthropods (Christchurch, New Zealand, 2011); 3rd European Congress Conservation Biology (Glasgow, UK, 2012); 24th International Congress of Entomology (Daegu, Korea, 2012); Towards a Roadmap for Biodiversity and Ecosystem Research Infrastructures in Europe, European Commission (Brussels, Belgium, 2013); Midterm EuroVol Conference (Firenze, Ityaly, 2013); 9th International Symposium on Aphids (Beijing, China, 2013); Ecology of Aphidophaga 12 (Belgrade, Serbia, 2013); Meeting of the Policy Committee, Society for Conservation Biology (Eberswalde, Germany, 2013) and IUCN World Parks Congress (Sydney, Australoa, 2014). The AS Křešín u Pacova infrastructure was presented in a solicited oral presentation at the annual meeting of the European Meteorological Society in Prague (2014). A new tool for CO_2 flux partitioning with soil chamber flux implementation as a solution for site in topographically complex terrain was presented as an oral presentation on EGU General Assembly in Vienna, 2014.

The Division organized the annual Task Force Meeting of the Cooperative Programme on Waters (ICP Waters) in October 2013 in Český Krumlov, together with the Division of Human Dimensions of Global Change Impacts the Czech-Iceland workshop on ecosystem services and sustainable development (November, 2014) and together with the Czech Hydrometeorological Institute, Institute of Chemical Process Fundamentals (Czech Academy of Sciences) and RECETOX (Masaryk Unviersity) the international seminar Towards the Atmospheric Research Supersite (Humpolec, June 2013). An important event was also the organization of own technical sessions and introductional lectures at the World Forestry IUFRO Congresses (Soul, South Korea, 2010 and Salt Lake City, USA, 2014). Further conference organization activities include the International Orchid Conservation Congress IV (main organizer, Hluboká, 2011), Benefits and Risks of Exotic Biological Control Agents (main organizer, Hluboká, 2011), Population dynamics of terrestrial orchids (member of the scientific board, Rende, Italy, 2013) and International Orchid Conservation Congress V (member of the scientific board, Reunion, 2013).

Applied research

Only a part of the research conducted within the Division of Ecosystem Analysis can be considered applied research. This is due to the character of the scientific disciplines covered by the Divison themselves. These are predominantly of basic research character. However, the Division does significantly contribute to fulfilling the obligations of the Czech Republic under numerous international environmental programmes and conventions (see above). Further, the Divisions research had the following application potential during 2010 - 2014:

1. prediction of stability of ecosystems (together with other Divisions in GCRC),
2. identification of potential risks and suggestion of adaptation measures (together with other Divisions in GCRC),
3. datasets of greenhouse gas and pollutant fluxes and concentrations and energy fluxes for the development and validation of production models for a strategic decision-making (politics, industry),
4. interdisciplinarity regarding instrumentation, data-sharing, and interpretation of data at all hierarchical levels,
5. dynamics and statics of tall slender constructions (thanks to the AS 250 tall tower, open access request by the Czech XXX construction company),
6. research connected to renewable energies, i.e. solar and wind power (data open access request by Deutscher Wetterdienst, Germany and Laboratoire des Sciences du Climat et de l'Environnement, France).
7. protection of biodiversity, nature conservation, and biological control of pests also in the context of global change,
8. a series of papers on conservation strategies of the Šumava National Park elucidated the underlying mechanisms governing population dynamics of endangered species in the largest National Park in Central Europe. The results were used by the Czech Ministry of Environment for preparation of the new Management Plan of the Šumava NP,
9. analysis of long-term trends in the composition of aphidophagous coccinellid communities in Central Europe enabled the prediction of global change consequences to the abundances of these economically important insect predators,
10. coperation with the Office of the Krkonoše National Park on the retrieval of last genome of original Norway spruce trees using vegetative reproduction methods,

11. land use change observation and modelling enables the staff of the Division of Ecosystem Analysis to predict biodiversity hot spots and weak places of ecosystem functioning in the forestry-agricultural cultural landscape. The Division of the Human Dimensions of the Global Change Impacts can involve these data in the creation of mitigation and adaptation strategy against climate change impacts in the frame of participative approach with local stakeholders.

Within ICOS, recommendations of mitigation strategies of global change impacts are formulated for policy and decision makers. The outcomes of the Division are relevant for the development of strategic documents for the society, policy makers, industry etc. and are being used to reduce the negative impacts of global change on environment, sustainability of energy and food production and water management. This is achieved in cooperation with other Divisions of GCRC. Activities conducted by the Division are also aimed at evaluating the role of biodiversity in ecosystem functioning and selected ecosystem service provision with an emphasis on landscape carbon deposition.

Team successes and structure

Except numerous defended PhD titles (see form 3-10), one defended associated professor title was achieved in 2010 in the Division of Ecosystem Analysis. The Division has a strong international accent, which is reflected by the nationalities of team members. In the recent years, two PhD titles were awarded to students from Nepal and one to a student from Portugal. The team members were in the evaluated period from the following countries (except the Czech Republic): Bolivia, Panama, Vietnam, Hungary, Nepal, UK, Poland, Italy and India.

Research Report of the team in the period 2010–2014

Institute	Global Change Research Centre of the CAS, v. v. i.

Scientific team	Division of impact studies and physiological analyses

Main achievements

Publications

The researchers within the **Section of Impact Studies and Physiological Analyses** published 77 papers in impacted scientific journals and more than 23 papers in peer-review journals. In addition, popularization publications are described within the Appendix 3.10. The most important outcomes are:

1. an investigation of sky conditions (diffuse radiation, temperature, vapour pressure deficit etc.) on the processes of carbon uptake. We have elucidated increased light use efficiency of ecosystem carbon uptake under cloudy sky conditions with prevailing diffuse radiation and how forests maintain a positive C balance, despite having an apparently high self-shading degree. Penetration of diffuse radiation into lower canopy depths has direct effects on the productivity and structure of vegetation and is crucial for the modelling of global C cycle. We have also shown that a stimulation of photosynthesis rate by an elevated atmospheric CO2 concentration (EC), in contrary to clear sky, is negligible or even reduced under cloudy sky conditions. Such reduction is associated mainly with EC-stimulated thermal dissipation of absorbed light energy and insufficient openness of stomata. This result indicates that an expected increase in cloud cover associated with climate warming may reduce the stimulatory effect of EC on plants C uptake and growth. Our research was highlighted by Science Daily and European Commission web sites.
   i. Urban O., Klem K., Ač A., Havránková K., Holišová P., Navrátil M., Zitová M., Kozlová K., Pokorný R., Šprtová M., Tomášková I., Špunda V., Grace J.(2012) Impact of clear and cloudy sky conditions on the vertical distribution of photosynthetic CO2 uptake within a spruce canopy. Functional Ecology 26, 46–55.
   ii. Urban O., Klem K., Holišová P., Šigut L., ŠprtováM., Teslová P., Zitová M., Špunda V., Marek M.V., Grace J. (2014) Impact of elevated CO2 concentration on dynamics of leaf photosynthesis in *Fagus sylvatica* is modulated by sky conditions. Environmental Pollution 185, 271–280.

2. an investigation of leaf-level hyperspectral indices enabling to track key physiological processes associated with carbon assimilation by plants like net ecosystem (forest, grassland) exchange, light use efficiency and/or deepoxidation of xanthophyll pigments. In particular, we explored the behaviour of new hyperspectral index (ANMB) for the application on spruce trees grown under complex natural environment. We showed that integral ANMB index is able to track diurnal and seasonal variability in deepoxided state of xanthophylls more efficiently that conventional PRI index. Result of the experiment moved concept of the ANMB closer to the application on Remote Sensing data acquired on field experimental sites. The most relevant publications are:
   iii. Ač A., Malenovský Z., Urban O., Hanuš J., Zitová M., Navrátil M., Vráblová M., Olejníčková J., Špunda V., Marek M. (2012) Relation of chlorophyll fluorescence sensitive reflectance ratios to carbon flux measurements of montane grassland and

Norway spruce forest ecosystems in the temperate zone. Scientific World Journal, Article Number 705872.

iv. Kováč D., Navrátil M., Malenovský Z., Štroch M., Špunda V., Urban O. (2012) Reflectance continuum removal spectral index tracking the xanthophyll cycle photoprotective reactions in Norway spruce needles. Functional Plant Biology 39, 987–998.

v. Kováč D., Malenovský Z., Urban O., Špunda V., Kalina J., Ač A., Kaplan V., Hanuš J. (2013) Response of green reflectance continuum removal index to the xanthophyll de-epoxidation cycle in Norway spruce needles. Journal of Experimental Botany 64: 1817–1827.

3. a target and non-target investigation of changes in metabolic profiles in plants exposed to different abiotioc and biotic stresses. The key findings are summarized. We showed that (1) shoots and roots have different metabolomes and elemental stoichiometry, (2) the shoot metabolome is more variable among species and seasons than is the root metabolome, and (3) the metabolic response of shoots to drought contrasts with that of roots.
   In addition, we studied how the drought-induced changes in metabolome of oak affects folivory activity. These shifts in metabolism (accumulation of potassium, total sugars, and phenolics) produced an indirect relationship between drought and folivory activity. The study represents a breakthrough application of metabolomics in plant ecology.
   Finally, we found cold acclimation induced abundance of gluconapin and flavon-3-ol glycosides, respectively, in the cold-sensitive and the cold-tolerant Arabidopsis accessions. Also we demonstrated that metabolomics fingerprinting coupled to chemometric methods can be an effective tools in screening plant cold tolerance.

   vi. Gargallo-Garriga A., Sardans J., Pérez-Trujillo M., Rivas-Ubach A., Oravec M., Večeřová K., Urban O., Jentsch A., Kreyling J., Beierkuhnlein C., Parella T., Penuelas J. (2014) Opposite metabolic responses of shoots and roots to drought. Scientific Reports 4, Art.no. 6829.

   vii. Rivas-Ubach A., Gargallo-Garriga A., Sardans J., Oravec M., Mateu-Castell L., Pérez-Trujillo M., Parella T., Ogaya R., Urban O., Penuelas J. (2014) Drought enhances folivory by shifting foliar metabolomes in *Quercus ilex* trees. New Phytologist 202, 874–885.

   viii. Václavík L., Mishra A., Mishra K.B., Hajšlová J. (2013) Mass spectrometry-based metabolomic fingerprinting for screening cold tolerance in *Arabidopsis thaliana* accessions. Analytical and Bioanalytical Chemistry 405, 2671–2683.

4. an investigation of effects of elevated atmospheric CO2 concentration (EC) of growth, physiology and biochemistry of forest tree species. Among other things, we have shown that EC enhances the light-saturated CO2 assimilation rate and reduces dark respiration, however, it has no effects on mesophyll structural parameters and accumulation and localization of phenolics and lignin in both sun and shade needles. Most importantly, we discovered molecular mechanisms of Rubisco acclimation (a key photosynthetic enzyme) to EC. Long-term exposure to EC reduces Rubisco activity due to a decrease in the activation state and a reduction in the total content of Rubisco protein. However, the specific activity was increased implying the presence of enzymatically active forms. Our data thus show that Rubisco may not be fully active in leaves and inactive Rubisco may serve as nitrogen storage. For the first time we studied effects of natural fluctuations in light intensity on CO2 assimilation in plants exposed to EC conditions. We found significant stimulation of photosynthetic induction but only a minor effect of EC on the rate of induction loss. The rate constants determined are valuable for the parameterization of carbon uptake models.

Based on a meta-analysis we showed that EC induces a C allocation shift towards below-ground biomass. However, soil C content was not affected by EC due to increased heterotrophic respiration.

ix. Dieleman W.I.J., Luyssaert S., Rey A. et al. (2010) Soil [N] modulates soil C cycling in CO2-fumigated tree stands: a meta-analysis. Plant, Cell and Environment 33, 2001–2011.

x. Holišová P., Zitová M., Klem K., Urban O. (2012) Effect of elevated carbon dioxide concentration on carbon assimilation under fluctuating light. Journal of Environmental Quality 41, 1931–1938.

xi. Lhotáková Z., Urban O., Dubanková M., Cvikrová M., Tomášková I., Kubínová L., Zvara K., Marek M.V., Albrechtová J. (2012) The impact of long-term CO2 enrichment on sun and shade needles of Norway spruce (*Picea abies*): Photosynthetic performance, needle anatomy and phenolics accumulation. Plant Science 188, 60–70.

xii. Urban O., Hrstka M., Zitová M., Holišová P., Šprtová M., Klem K., Calfapietra C., De Angelis P., Marek M.V. (2012) Effect of season, needle age and elevated CO2concentration on photosynthesis and Rubisco acclimation in *Picea abies*. Plant Physiology and Biochemistry 58, 135–141.

5. an investigation of mineral supply (N,P) and drought on the biomass production in grassland ecosystems and fern communities typical for deforested areas. For example, the effects of high nitrogen inputs on growth of biomass and competitiveness of three grass species *Calamagrostis*, *Bromus* and *Brachypodium* were examinated. Results show clear evidence of competitive superiority of *Calamagrostis* to *Bromus* in N enhanced substrate. Thus, a higher N deposition associated with human activity may play a critical role in the expansion and persistence of studied tall grasses in the landscape. The most relevant publications to this topic are:

xiii. Holub P., Tůma I. (2010) The effect of enhanced nitrogen on aboveground biomass allocation and nutrient resorption in the fern *Athyrium distentifolium*. Plant Ecology 207, 373–380.

xiv. Fiala K., Tůma I., Holub P. (2011) Effect of nitrogen addition and drought on above-ground biomass of expanding tall grasses *Calamagrostis epigejos* and *Arrhenatherum elatius*. Biologia 66, 275–281.

xv. Holub P., Tůma I., Fiala K. (2011) Effect of fertilization on root growth in the wet submontane meadow. Plant, Soil and Environment 59, 342–347.

xvi. Holub P., Fabšičová M., Tůma I., Záhora J., Fiala K. (2013) Effects of artificially varying amounts of rainfall on two semi-natural grassland types. Journal of Vegetation Science 24, 518–529.

xvii. Holub P., Tůma I., Fiala K. (2012) The effect of nitrogen addition on biomass production and competition in three expansive tall grasses. Environmental Pollution 170, 211–216.

6. an investigation of interactive effects of photosynthetically active radiation (PAR) on plants' acclimation to ultraviolet (UV) radiation in two barley varieties differing in sensitivity to oxidative stress. We discovered that higher PAR treatment triggered photoprotective mechanisms which partially ameliorated the UV effects on photochemistry and carbon assimilation. Higher PAR intensity treatment induced accumulation of flavonols, mainly in young leaves, whereas in old leaves UV-induced accumulation was more pronounced. New spectral reflectance index for detection of epidermal flavonols was also proposed and validated. Furthermore, the trade-off mechanisms between accumulation of flavonols and morphological parameters were studied. An inverse proportion was found between flavonol

content and specific leaf area. Enhanced UV radiation reduced the final leaf length, particularly in plants under reduced PAR, in young leaves and in sensitive variety Barke. However, higher intensity of PAR mitigated the morphological effects induced by higher UV radiation, particularly changes in SLA.

xviii. Klem K., Ač A., Holub P., Kováč D., Špunda V., Robson T. M., Urban O. (2012) Interactive effects of PAR and UV radiation on the physiology, morphology and leaf optical properties of two barley varieties. Environmental and Experimental Botany 75, 52–64.

xix. Robson T.M., Klem K., Urban O., Jansen, M.A. (2015) Re-interpreting plant morphological responses to UV-B radiation. Plant, Cell & Environment 38, 856–866.

xx. Klem K., Holub P., Štroch M., Nezval J., Špunda V., Tříska J., Jansen M.A.K., Robson T.M., Urban O. (2015) Ultraviolet and photosynthetically active radiation can both induce photoprotective capacity allowing barley to overcome high radiation stress. Plant Physiology and Biochemistry http://dx.doi.org/10.1016/j.plaphy.2015.01.001.

7. an investigation of biologically active compounds distribution in selected plants (*Vitis vinifera, Picea abies*) under the influence of changing external global conditions. In grapevine, the response of the plants to the attack of fungal disease due to *Plasmopara viticola* has been studied. New phenanthrene derivative with the strong fluorescence arising from trans-resveratrol was found for the first time in the grapevine leaves as a result of *Plasmopara viticola* attack and environmental condition, especially UV radiation. Based on the results of basic research the findings were applied for the irradiation of grapes with the goal to increase the content of biologically active substances.

xxi. Tříska J., Vrchotová N., Olejníčková J., Jílek R., Sotolář R. (2012) Separation and identification of highly fluorescent compounds derived from *trans*-resveratrol in the leaves of *Vitis vinifera* infected by *Plasmopara viticola*. Molecules 17, 2773–2783.

xxii. Šebela D., Olejníčková J., Sotolář R., Vrchotová N., Tříska J. (2014) Towards optical detection of *Plasmopara viticola* infection in the field. Journal of Plant Pathology 96, 309–320.

xxiii. Tříska J., Houška M.: Physical methods of resveratrol induction in grapes and grape products – A review. Czech Journal of Food Sciences 30, 489–502.

8. the composition of biologically active substances of the invasive plants (Knotweed, *Impatiens* sp.) was studied together with the relation to other plants in order to understand the mechanisms and effects of their invasiveness. A related research was focused also on the study of the biological effects of different plant substances on pests in agriculture. Growing conditions were studied (influence of nitrogen) to pollen formation in *Festuca rubra*. Based on the results of the basic research the technological solution and conditions were proposed for the preparation of beverages with an increased content of biologically active substances.

xxiv. Tříska J., Vrchotová N., Sýkora J., Moos M. (2013) Separation and identification of 1,2,4-trihydroxynaphthalene-1-O-glucoside in impatiens *Glandulifera royle*. Molecules 18, 8429–8439.

xxv. Vrchotová N., Šerá B., Krejčová J. (2011) Allelopathic activity of extracts from *Impatiens* species. Plant, Soil and Environment 57, 57–60.

xxvi. Hromádková Z., Košťálová Z., Vrchotová N., Ebringerová A. (2014) Non-cellulosic polysaccharides from the leaves of small balsam (*Impatiens parviflora* DC.). Carbohydrate Research 389, 147–153.

xxvii. Pavela R., Žabka M., Vrchotová N., Tříska J., Kazda J. (2013) Selective effects of the extract from *Angelica archangelica* L. against *Harmonia axyridis* (Pallas) – An important predator of aphids. Industrial Crops and Products 51, 87–92.

xxviii. Pavela R., Vrchotová N. (2013) Insecticidal effect of furanocoumarins from fruits of *Angelica archangelica* L. against larvae *Spodoptera littoralis* Boisd. Industrial Crops and Products 43, 33–39

xxix. Ratajová A., Tříska J., Vrchotová N., Kolář L., Kužel S. (2013) Grass pollen pollution from biofuels farming. Paediatric Allergy, Immunology and Pulmonology 26, 199–203.

xxx. Totušek J., Tříska J., Lefnerová D., Strohalm J., Vrchotová N., Zendulka O., Průchová J., Chaloupková J., Houška M. (2011) Content of sulforaphane, total isothiocyanates, antimutagenic activity, and inhibition of clastogenicity in pulp juices from Cruciferous plants. Czech Journal of Food Sciences 29, 548–556.

9. biologically active phenolic compounds of buckwheat which are responsible for its allelopatic properties were studied. Penetration of caprolactam into buckwheat sprouted achenes in the connection with the changing environmental conditions and the increasing concentration of anthropogenic substances was studied. Furthermore, the influence of crop management (conventional and organic farming) on the content of selected metabolites of buckwheat was studied. It was found in both cases a strong influence of plant metabolism (decrease of certain secondary metabolites).

xxxi. Kalinová J., Tříska J., Vrchotová N. (2011) Occurrence of eugenol, coniferyl alcohol and 3,4,5-trimethoxyphenol in common buckwheat (*Fagopyrum esculentum* Moench) and their biological activity. Acta Physiologiae Plantarum 33, 1679–1685.

xxxii. Kalinová J.P., Tříska J., Vrchotová N., Moos M. (2014) Verification of presence of caprolactam in sprouted achenes of *Fagopyrum esculentum* Moench and its influence on plant phenolic compound content. Food Chemistry 157, 380–1384.

xxxiii. Kalinová J., Vrchotová N. (2011) The influence of organic and conventional crop management variety and year on the yield and flavonoid level in common buckwheat groats. Food Chemistry 127, 602–1608.

10. the adaptation/acclimatization of micro-algae *Chlorella mirabilis* to low temperatures and production of specific biotechnologically important compounds was studied. The innovative analytical methods using Raman micro-spectroscopy focused on lipids of algae *Trachydiscus minutus* was developed.

xxxiv. Shukla S.P., Kvíderová J., Tříska J., Elster J. (2013) *Chlorella mirabilis* as a potential species for biomass production in low-temperature environment. Frontiers in Microbiology 4, 1–12.

xxxv. Samek O., Jonáš A., Pilát Z., Zemánek P., Nedbal L., Tříska J., Kotas P., Trtílek M. (2010) Raman microspectroscopy of individual algal cells: Sensing unsaturation of storage lipids *in vivo*. Sensors 10, 8635–18651.

11. an applied research focused on the development of novel spectral reflectance sensors utilizable in precision agriculture for simultaneous evaluation of nutritional status and canopy structure. As part of this research new spectral reflectance index based on red-edge spectral band was developed. This index, together with the commonly used index NDVI enables the separation of the effects of nitrogen nutrition and biomass production from canopy reflectance curves. Based on validation of these indices in multi-annual experiments the prototype of sensor for simultaneous evaluation of nitrogen status and canopy structure was developed. Furthermore, the decision algorithms for agronomical recommendations

such as application of fertilizers or growth regulators were developed as an integral part of instrument. These algorithms together with detailed procedure of measurement with developed sensor were published in methodology certified by the Ministry of Agriculture and are available for advisory services and farmers.

xxxvi. Míša P., Klem K., Navrátil V., Míša, M. (2013) Mobile instument for simultaneous measurement of stand nutient status and density. ***Prototype***

xxxvii. Klem K., Míša P., Míša M., Křen J. (2014) Use measuring of spectral reflectance and derived specialized vegetation indices in the crop management practices of spring barley. ***Certified methodology***

12. an applied research was performed in the field of biologically active compounds contained in the spruce trees (phytosterols and lignans).
The goal of the first applied research was focused on the development of novel procedure for extraction and purification of phytosterols (β-sitosterol, campestrol and stigmasterol) from waste tall soap from paper mills using vibration extraction column. The whole procedure was patented (see xxxviii).
The second field of the applied research was the extraction of lignans from spruce knots and the use of extracts for the nutrition purposes, e.g. as food supplements. The results are Patent Pending (xxxix).

xxxviii. Heyberger A., Tříska J., Rousková M., Krtička M.: Způsob a zařízení k získávání fytosterolů. (Czech) Process and Equipment for Phytosterols Recovering. Pat. No. 301 716. Applied: 08.12.30., Approved 22.4.2010, Uveřejněno ve Věstníku Úřadu průmyslového vlastnictví 2.6.2010

xxxix. Híc P., Balík J., Kulichová J., Tříska J., Strohalm J., Vrchotová N., Houška M., Způsob extrakce lignanů ze suků jehličnanů a využití extraktu pro potravinářské účely, (patentový zástupce Ing. Jaroslav Novotný), PV 2014 - 870, přihlášeno 5. 12. 2014

Extension, modernization and construction of a new research infrastructure

1. Central physiological, isotopic and metabolomic laboratory for studying the processes of carbon assimilation, molecular mechanisms of plants acclimation and adaptation to environmental perturbations and climate change was constructed within the OP RDI (CZ.1.05⁄1.1.00⁄02.0073) during 2010–2013.
Physiological Laboratory is mainly based on a combination of gasometric and fluorescence systems for the study of photosynthesis, transpiration and respiration in conjunction with the methods of determining the spectral optical properties of vegetation and basic biomolecules detection by Raman spectroscopy. Basis metabolomic laboratory systems represent gas and liquid chromatography with mass spectrometry detection (TSQ Quantum XLS triple quadrupole and HRMS LTQ Orbitrap XL), complemented by a number of necessary laboratory equipment (decomposition and extraction systems, precision scales, spectrometers) and complementary techniques (e.g. Infrared spectrometer with Fourier transformation) for targeted and non-targeted metabolome screening of primary and secondary metabolites. Isotopic mass spectrometer for detection of stable isotopes of H, C, O, N and S in connection with the elemental analyser, pre-concentration unit and systems of the gas and liquid chromatography is used for the study of isotopic composition of atmospheric and biological samples, including the isotopic composition of selected primary and secondary metabolites for the understanding of metabolic changes plants when exposed to stress (UV irradiation, altitude, draught, nanoparticles, herbivores etc.). During

the years 2013–2015, a number of laboratory and field experiments/measurements were realized. The most important study limitations include photosynthesis in trees growing in different altitude (results published in the prestigious journal: Rajsnerová P. et al. Tree Physiology 35 (1), 47–60, 2015). Metabolomic laboratories are used, in the form of open-access, also by employees of other institutions (University of Ostrava, Brno Technical University, IBAF-CNR Italy, CREAF Barcelona Spain etc.). An important outcome is e.g. broad analysis of metabolic profile in oak (*Quercus ilex*) and its change during drought and subsequent folivory activity (Ubach-Rivas, A., et al., New Phytologist 202, 874–885, 2014).

2. Open-top chamber experimental facility in Domaninek near Bystrice nad Pernstejnem for simulation future climate conditions, especially the effects of elevated CO_2 concentration, drought stress, the influence of UV radiation, nitrogen nutrition and increased temperatures in agricultural ecosystems. This infrastructure was built in 2012 within the OP RDI (CZ.1.05/1.1.00/02.0073) and started full operation in 2013. The basic part of the experimental station consists of 24 chambers of hexagonal base covered by roof with rotating lamellas that allow to control chamber ventilation and manipulate incident rainfall. The number of chambers enable to study all combinations of three environmental factors (e.g. elevated CO_2, drought and UV) in three replications. Inside chamber the split plots of barley/wheat and unfertilized/N fertilized are conducted. The operation is fully automatic based on feedback regulation using CO_2 analysers, air temperature sensors inside chamber, soil moisture sensors and rain-gauge. The station is equipped with a number of instruments for physiological and morphological measurements: Gas exchange systems, fluorometers, spectroradiometers, water potential, leaf area meters etc. The facility is focused not only on crop productivity and physiology, but the final grain quality for malting and baking industry is also of interest. Detailed studies focused on mechanistic understanding of physiological and matabolic processes are running every year e.g. the role of accumulation of carbohydrates on down-regulation of photosynthesis under elevated CO_2 or effects of elevated CO_2 and UV induction on accumulation of flavonoids and their protective role.

3. The growth chamber facility in Brno was constructed within the OP RDI (CZ.1.05/1.1.00/02.0073) and started full operation in 2013. The facility consists of 10 large (step-in) and 2 small state-of- the art growth chambers. All the chambers allow regulation of the relative air humidity, temperature and intensity of PAR. In addition 6 of them enable to control CO_2 concentration, spectral reflectance of incident light in UV-A, R, G, B, FR spectral bands and to reduce temperature up to -15°C. The experimental facility of growth chambers is focused on the study of global change impacts on plants, particularly the effect of elevated CO_2 in combination with other environmental drivers such as temperature (heat, cold), drought stress, spectral composition of incident light. During first two years of operation the facility was used to perform several experimental studies and testing specific scientific hypotheses e.g.: i) interactive effects between elevated CO_2 concentration, VPD and drought stress - the stomatal responses and impact on plant productivity; ii) downregulation of photosynthesis under elevated CO_2 concentration - role accumulation of non-structural carbohydrates; iii) cold stress tolerance in Arabidopsis natural accessions iv) effect of spectral composition of incident light on stomata opening in combination with elevated CO_2 concentration ; v) photoprotective mechanisms under high light conditions - utilization of spectral reflectance methods for detection of photoprotective mechanisms; vi) effect of heat stress during winter wheat anthesis etc.

Organisation of conferences, training courses and workshops

1. International conference: "Global Climate Change – Challenge to Human Society of 21st Century", Olomouc, 9.–11.11. 2010. 80 attendees (25 from abroad)
2. International conference: „COST Action FA0906: 2nd Annual Network Meeting.", Mikulov, 14–16.4. 2013. 110+ attendees (100 from abroad)
3. International conference (co-organisers): "S-TROM Open Forum – discussion forum", Brno, 27.–29.5. 2013. 80 attendees (15 from abroad)
4. International workshop of ClimMani and INTERFACE networks. Mikulov, 4.-7.6.2013. 45 attendees (36 from abroad)
5. EPPN Workshop – Methods for measurement of water use and water use efficiency. Prague, 15-16.7.2014. 19 attendees (17 from abroad)
6. Training course: EKOTECH (Operational Programme Education for Competitiveness), Bílý Kříž, summer semester, 31.5–4.6. 2010, 9 attendees
7. Training course: EKOTECH (Operational Programme Education for Competitiveness), Bílý Kříž, summer semester, 30.5–3.6. 2011, 10 attendees
8. Training course: EKOTECH (Operational Programme Education for Competitiveness), Bílý Kříž, summer semester, 4.6–7.6 2012, 11 attendees
9. Training course: "Scientific presentation and photography in science", led by prof. Eva Rosenquist (University of Copenhagen, Denmark) and prof. Carl Otto-Ottosen (Aarhus University, Denmark), Mikulov, 17.–18.4. 2013. 22 attendees (17 from abroad)
10. Training school: "Emissions of Biogenic Volatile Organic Compounds: A Link between Plant Physiology and Metabolomics", led by dr. Ilaria Lusini a dr. Gabriele Guidolotti (CNR, Italy), Brno/Bílý Kříž, 20.–26.8. 2013. 20 attendees (5 from abroad)
11. Training course: "Plant ecological physiology, climate change and green infrastructure. Introduction and challenges." led by dr. Carlo Calfapietra (Italy), Brno, 16.–17.4. 2014. 20 attendees (5 from abroad)
12. Training school: "Principles and Practices on Analysing Metabolomics Data", led by dr. Xiaoliang Sun, Brno, 3.–4.7. 2014. 20 attendees (3 from abroad)
13. Training school: "Plant Stress Biology: Introduction and Challenges", led by Dr. Marcel A.K. Jansen (University of Cork, Ireland), targeted for PhD students with a focus on the effects of stress on biological systems, Brno, 10.-12.11.2014. 36 attendees (4 from abroad)
14. Workshop: "Imaging techniques from the cell to the canopy level: From 2D to 3D composite imaging.", led by prof. Kenji Omasa (University of Tokio, Japan), Brno, 9.10. 2012. 40 attendees (7 from abroad).
15. Workshop: "Coupling Metabolomics and Stoichiometry in Ecology", led by dr. Albert Rivas-Ubach (CREAF – Centre for Ecological Research and Forestry Applications, Universitat Autonoma de Barcelona), Brno, 4.12. 2012. 21 attendees (5 from abroad)
16. Workshop: "EcoMetabolomics", led by dr. Albert Gargallo (CREAF - Centre for Ecological Research and Forestry Applications, Universitat Autonoma de Barcelona), Brno, 30.5. 2014.

Important grant projects

The researchers within the Section of Impact Studies and Physiological Analyses participated on 43 grant projects in total. The most important projects in the categories following are:

European Commission

i. EPPN – European Plant Phenotyping Network (co-investigator), 2012-2015, 282.7ths EUR
ii. AnaEE – Infrastructure for Analysis and Experimentation on Ecosystems (co-investigator), 2012-2016, 35.2ths EUR

National Science Foundation (GA ČR)

iii. Impact of CO2 enrichment and irradiance on structure and performance of forest tree ... (co-investigator), 2010–2014, 1 518ths CZK (total budget of CVGZ for the whole period of the investigation),
iv. Impact of temperature and photosynthetically active radiation on dynamics of regulation of photosystem II function ... (co-investigator), 2013–2016, 4 191ths CZK

Academy of Sciences (a programme for internal support of international collaboration)
v. Application of environmental metabolomics approaches ....(investigator), 2013–2015, 1 728ths CZK; Close cooperation with the laboratory of prof. Josep Peñuelas (CREAF, Spain) focused on application of metabolomic approaches in plant ecological physiology

National Agency for Agricultural Research (Ministry of Agriculture)
vi. Grape juices from given sort of vine with health benefit (co-investigator), 2009–2013, 2 765ths CZK,
vii. Extractions of lignans from wood biomass and their use as a food supplement with significant biological response (co-investigator), 2012–2016, 3 757ths CZK

KONTAKT – programme for support of international (bilateral) cooperation in research with countries outside EU (Ministry of Education, Youth and Sports)
viii. Investigation of steroids and phytosterols in water environment, 2013-2015, 1 294ths CZK; cooperation with Dr. W.T Foreman (USGS, Colorado, USA) focused on determination of broad range of pharmaceuticals in water environment,

Participation in COST Activities
ix. FA0906 – UV-B radiation: A specific regulator of plant growth and food quality in a changing climate; FP1204 – Green Infrastructure approach: linking environmental with social aspects; TD1102 – Photosynthetic proteins for technological applications: biosensors and biochips; TD1203 – Food waste valorization for sustainable chemicals, material & fuels; ES1308 - Climate change manipulation experiments in terrestrial ecosystems – Networking and outreach (ClimMani); FP1106 – Studying Tree Responses to extreme Events: a SynthesiS (STReESS). Most of these activities were supported by Ministry of Education, Youth and Sports.

Important personal achievements

2010
- Martina Košvancová-Zitová got a PhD degree, Mendel University in Brno
- Alexander Ač received a PhD degree, University of South Bohemia in České Budějovice

2011
- Otmar Urban was working as a national representative and member of Management Committee in COST Action FA0906 (UV4Growth; 2011–2014)

2012
- Jan Tříska received an "Professor" degree in Environmental Chemistry, Masaryk University in Brno,
- Otmar Urban was working as a national representative and member of Management Committee in COST Action FP1204 „Green Infrastructure approach: linking environmental with social aspects in studying and managing urban forests" (2011–2015)

2013

- Professor Michal Marek was appointed a doctor honoris causa at Technical University in Zvolen
- Otmar Urban received an "Associated professor" degree, Palacky University in Olomouc, Biophysics
- Petra Holišová received a PhD degree, Mendel University in Brno
- Daniel Kováč received a PhD degree, Mendel University in Brno
- Otmar Urban was invited as a Guest Editor for the special issue of Plant, Cell and Environment journal (UV radiation)

2014

- Professor Michal Marek was appointed a member of the Royal Swedish Academy of Agriculture and Forestry
- Alexander Ač chairs Czech national committee Geosphere-Biosphere (IGBP)
- Kateřina Macháčová was awarded by the ExpeER project fellowship and she realised two measuring campaigns in Hyytiälä, Finland. Her results show that pine trees can emit both N2O and CH4 from stems and shoots under non-stressed field conditions. The results were presented at some scientific conferences.
- Petra Holišová and Daniel Kováč were awarded by the postdoctoral funding from the Academy of Sciences
- Iveta Marešová received a PhD degree, University of South Bohemia in České Budějovice

Research Report of the team in the period 2010–2014

Institute	Global Change Research Centre of the CAS, v. v. i.

Scientific team	Division of the human dimensions of global change impacts

The Division of Human Dimensions of Global Change represents a newly established and relatively small but expanding interdisciplinary team, which started its work within the establishment of CzechGlobe (Global Change Research Centre AS CR). However, the team gained a momentum and quickly became established not only on the Czech research map but also in the international context, which is documented by involvement in 2 EU (FP7 and H2020) projects.

The scientific team has achieved significant results in several areas of its focus, especially ecosystem services analysis, adaptation to climate change, risk assessment, social-ecological research and governance of ecosystems. The major outputs include a broad spectrum of results, including not only scientific papers, but also results of applied research such as certified methodologies, maps and summary research reports.

1. Assessment and modelling of ecosystem services

Outputs in the area of ecosystem service analysis include integrated approaches to the assessment and valuation of ecosystem services and modelling of trade-offs of ecosystem service supply and use by a society.

Within the project Integrated assessment of ecosystem services in the Czech Republic, funded by the Technological Agency of the Czech Republic in the years 2012 – 2013, we developed several decision-support tools, including an integrated database of ecosystem service values, methodological framework for the national assessment of ecosystem services and consolidated mapping layer of ecosystems in the Czech Republic. Based on a spatially-explicit benefit transfer of economic values of ecosystem services, we showed that ecosystems in the Czech Republic provide an annual value reaching the equivalent of 1.5 of national GDP, or 237 billion EUR annually.

The innovative project focusing on ecosystem services and benefits to people aimed to identify and assess interactions between social and natural systems at the national level by applying the integrated approach to ecosystem assessment. The project used the concept of ecosystem services, their mapping, quantification and economic valuation for the purpose of decision-making processes at the national level. The implementation of results has been also started by the end users (for example, Ministry of Environment and Czech Nature Conservation Agency), which will help to streamline existing management, particularly in the field of spatial planning, sustainable land use and natural resource management. Within the project, we also developed certified methodology for the integrated assessment of ecosystem services. Specific outputs include:

1.1. *Consolidated layer of Ecosystems of the Czech Republic*. As the existing spatial data sources are not directly applicable for the national ecosystem assessment, we have developed the Consolidated Layer of Ecosystems of the Czech Republic (CLES) which is going to be widely used as a baseline source for geographic mapping of ecosystem services. The consolidated layer has been produced in cooperation with the Nature Conservation Agency of the Czech Republic and utilized all the major sources of land cover/land use data in the Czech Republic. The consolidate layer is comprised of 40 categories of ecosystems, classified at four hierarchical levels and presents a novel unique approach to the assessment and monitoring of ecosystems in the Czech Republic and services they provide.

1.2. *Ecosystem service value database for Europe*. For the purpose of integrated national assessment, we have assembled a database of ecosystems service values, based on the search of up-to-date literature and combining the corresponding data from the ESP Ecosystem Services Valuation Database (ESVD). We have applied a strict review strategy using pre-defined keywords and a specific geographic and socio-economic filters ensuring correspondence of ecosystem service values between studies and our national case. For integrated ecological-economic accounting, we have considered the values reported as a value per area of the ecosystem (for example, EUR ha^{-1}) only. In total, we were able to build a database of more than 200 records based on 58 source studies. Currently, our valuation database contains values on 17 ecosystem services and presents a unique source for benefit transfers on ecosystem service social values in Europe.

1.3. *Modelling of ecosystem services trade-offs and synergies*. To be relevant and support decision-making, the information on the effects of policy alternatives and possible future outcomes of these policies is required. Therefore, we have been focusing on the trade-off and synergies analysis of effects of future land use on ecosystem services within the context of landscape exploitation and climate change adaptation. We tested different approaches to ecosystem service modelling in Třeboňsko Landscape Protected Area and Biosphere Reserve and Šumava National park and Biosphere Reserve. We applied a scenario approach to trade-off modelling, utilizing European land-use and climate change scenarios as well as participatory approaches (see section 4.2). We performed a modelling of ecosystem services within the InVEST (Integrated Valuation of Environmental Service and Trade-offs) modelling suite, which enables participatory inputs, biophysical modelling as well as economic valuation of ecosystem services.

Publications relevant for ecosystem service integrated assessment and trade-off modelling:

Frélichová, J., Vačkář, D., Pártl, A., Loučková, B., Harmáčková, Z. V., Lorencová, E., 2014. Integrated Assessment of Ecosystem Services in the Czech Republic. ***Ecosystem Services*** 8: 110-117.

Harmáčková, Z.V., Vačkář, D., 2015. Modelling regulating ecosystem services trade-offs across landscape scenarios in Třeboňsko Wetlands Biosphere Reserve, Czech Republic. ***Ecological Modelling*** 295: 207-215.

Results and outputs of applied research relevant to the area of ecosystem service research and analysis:
Vačkář, D., Frélichová, J., Lorencová, E., Pártl, A., Harmáčková, Z., Loučková, B. (2014). Metodologický rámec integrovaného hodnocení ekosystémových služeb v České republice. [Methodological framework for integrated assessment of ecosystem services in the Czech Republic.] Certified methodology by the Ministry of Environment, 63394/ENV/14, 35 pp.
Consolidated layer of ecosystem of the Czech Republic. Mapping source for the analysis of ecosystem in the Czech Republic developed within the project TD010066 - Integrated Assessment of Ecosystem Services in the Czech Republic, Technology Agency of the Czech Republic. Currently distributed by the Czech Nature Conservation Agency.

2. Environmental security, risk and vulnerability of social-ecological system to global change

Results achieved within the risk and vulnerability of social-ecological systems and ecosystem services show possible threats to human society resulting from the changing environment. We developed classification of risks, mapping of multiple hazards and developing policy-support tool for decision on disaster risk reduction.

2.1. *Spatial database of multiple hazards*. Within a large project focusing on environmental security – Integrated assessment of global change impacts on environmental security of the Czech Republic, funded by the Ministry of Interior within the Security Research programme, we have been developing an integrated database of environmental risks and hazards which has been integrated into the complex vulnerability assessment of human society and ecosystem services. We developed interactive mapping application which is now being made accessible to final users and results of the project has been made accessible at the national geoportal, maintained by one of the project partners, Czech Environmental Information Agency. The results will be further used by the Ministry of Environment and related organizations.

2.2. *Vulnerability and risk assessment of ecosystem services*. By integrating multiple hazard database within the vulnerability and risk assessment of ecosystem services, we were able to map and evaluate risk hotspots and most fragile areas and ecosystems, i.e. areas which are most vulnerable to multiple hazard forcing. The risk analysis has been developed as a component of environmental security framework and aims to support the revision of the national Environmental security strategy of the Czech Republic. By spatially integrating and aggregating multiple hazards, such as floods, drought, atmospheric, soil and water pollution, invasive species, soil erosion and loss, we were able to highlight vulnerability hotspots with regard to human population and ecosystem services.

Publications relevant to the vulnerability and environmental security research:
Janoušková, S., Hák, T., Lorencová, E., Vačkář, D. (2013). Environmentální bezpečnost: Návrh koncepčního rámce pro aplikace v České republice. (Environmental Security: A conceptual framework proposal for applications in the Czech Republic). ***Obrana a strategie*** (***Defence and Strategy***). 2013, roč. 13, č. 2, s. 25-40. ISSN 1802-7199. DOI : 10.3849/1802-7199.13.2013.02.025-040

Results and outputs of applied research relevant to the area of vulnerability of ecosystem services:
EnviSec vulnerability and total risk maps, National Geoportal CENIA, http://geoportal.gov.cz/web/guest/map/.

3. Climate Change Adaptation

One of our major activities in the field of climate change adaptation is, inter alia, within the participation in the FP7 project BASE (Bottom-up Adaptation Strategies towards a Sustainable Europe), http://base-project.eu/ . Our results comprise outputs of several activities, including application of land-use and climate scenarios for the assessment of ecosystem service use in adaptation, participatory climate change adaptation studies and cost-benefit analysis of adaptation options for systems such as the Šumava national park or the City of Prague. These activities are further developed within the Czech-Norway grants, where we are focusing on adaptation strategies planning and implementation in large cities (Prague, Brno and Pilsen), utilizing ecosystem-based approaches. The results of the project are being used in cities governance and strategical planning.

3.1. *Land use and climate change scenarios for climate change adaptation*. Climatic and land use change are amongst the greatest global environmental pressures resulting from anthropogenic activities. Both significantly influence the provision of crucial ecosystem services, such as carbon sequestration, water flow regulation, and food and fibre production, at a variety of scales. The aim of this study was to provide spatially explicit information at a national level on climate and land use change impacts in order to assess changes in the provision of ecosystem services. This work provided a qualitative and quantitative analysis of the impacts on selected ecosystem services (carbon sequestration, food production and soil erosion) in the agricultural sector of the Czech Republic. This assessment shows that, historical land use trends and land use under projected climate scenarios display some shared spatial patterns. Specifically, these factors both lead to a significant decrease of arable land in the border fringes of the Czech Republic, which is to some extent replaced by grasslands, in turn affecting the provision of ecosystem services. Moreover, this assessment contributes to a useful method for integrating spatially explicit land use and climate change analysis that can be applied to other sectors or transition countries elsewhere.

3.2. *Ecosystem-based approaches to climate change adaptation*. In the context of ecosystem service research and applications, we have been developing a policy appraisal analysis in the area of climate change adaptation. Here, we have been focusing on several sectors and areas, such as floods, drought (in cooperation with the team of prof. M. Trnka), and adaptation in different sectors such as the agricultural sector or adaptation in cities. We are applying tools such as cost-benefit analysis and participatory scenario building and modelling.

Publications related to climate change adaptation:
Lorencová, E., Frélichová, J., Nelson, E., Vačkář, D., 2013. Past and future impacts of land use and climate change on agricultural ecosystem services in the Czech Republic.

Land Use Policy 33: 183-194, ISSN: 0264-8377, doi: 10.1016/j.landusepol.2012.12.012.
Loučková, B. (2014). *Eastern European Perspective on the Environmental Aspects in Current Flood Risk Management: The Example of the Czech Republic*. Chapter 13 in Bhaduri, A., Bogardi, J., Leentvaar, J., Marx, S. (Eds.), The Global Water System in the Anthropocene: Challenges for Science and Governance, Springer, pp. 183-196.

4. Sustainable governance of ecosystems and policy support

Within the sustainable governance and policy support, we are providing information on possible implications of global change for sustainability and governance social-ecological systems. Our outputs in this field include for example international intercomparison of the demand of social-ecological system on natural resources or development of methodology for the long-term social-ecological research and monitoring in biosphere reserves. Members of our team are established in international networks related to governance of ecosystem services. David Vačkář was a co-signatory of initiative calling for an inclusive conservation, which appeared in Nature (515, 2014).

4.1. *Comparing indicators of human pressures and state of ecosystems and biodiversity*. Our results support findings that human economic activity and environmental pressures are related to threats to biodiversity. The ecological footprint and biocapacity as indicators of human demand on ecosystems have been compared with several commonly used environmental indices measuring various aspects of ecological sustainability and biodiversity. We found that the ecological footprint and biocapacity are closely related especially to the human appropriation of net primary production (HANPP). On the other hand, the ecological demand is negatively related to other measures of ecosystem health and biodiversity such as the ecosystem wellbeing index (EWI). We explored patterns of correlation between the ecological footprint and ecosystem and biodiversity measures, including threatened species numbers. The analysis provides evidence that the ecological footprint is a meaningful ecological indicator of human demand on ecosystem resources which can be compared to equivalent measures of the appropriation of ecosystem productive capacity and land use pressures.

4.2. *Governance of social-ecological systems*. Within the policy support for governance of social-ecological systems, we developed a methodology for long-term social ecological research in biosphere reserves. The methodology sets out the basic assumptions and practices of long-term socio-ecological research (LTSER) in biosphere reserves in the Czech Republic. The aim of the methodology is to provide a basic framework for the research of socio-ecological systems in the Czech Republic and integrate the social dimension into long-term ecological research (LTER). Capturing the impacts of global change requires long-term monitoring of the development of basic indicators affecting the dynamics of socio-ecological systems. Given the crucial role of society in shaping the natural environment leads to greater integration of social and environmental sciences. The methodology provides basic approaches, indicators and methods for long-term socio-ecological research. The methodology establishes basic procedures that should help the long-term socio-ecological research in biosphere reserves.

4.3. *Strategic policy planning and evaluation at the national level*. Our team has been involved in providing policy support and appraisal of national strategies in the areas of biodiversity, ecosystem services, environmental security and adaptation to climate change. Within the contracted research, we have been contributing to the revision of National Adaptation Strategy, evaluation of National Biodiversity Strategy and Action Plan (NBSAP) and analysis of the implications of Aichi Biodiversity 2020 biodiversity targets. The NBSAP has been thoroughly evaluated according to a set of criteria. For the evaluation of the Strategy, we used best information available, including published reports, grey literature, endorsed policies and programmes, or personal interviews. Moreover, we have been involved in the revision of the National Strategy of Environmental Security.

Publications related to sustainable governance and policy support:

Vačkář, D., 2012. Ecological Footprint, environmental performance and biodiversity: a cross-national comparison. ***Ecological Indicators*** 16: 40-46, ISSN 1470-160X, doi:10.1016/j.ecolind.2011.08.008.

Tallis, H., Lubchenco, J., et al. (with D. Vačkář as a co-signatory author) (2014). A call for inclusive conservation. Comment in ***Nature*** 515: 27–28; see Supplementary information for a full list of co-authors.

Other results and outputs related to sustainable governance of social-ecological systems:

Harmáčková, Z., Rynda, I., Vačkář, D. (2015). Metodika rozvoje dlouhodobého sociálně-ekologického monitoringu (LTSER) v biosférických rezervacích České republiky. (*Methodology for developing long-term social-ecological monitoring (LTSER) in biosphere reserves in the Czech Republic*). Centrum výzkumu globální změny AV ČR a Fakulta humanitních studií UK, Český národní komitét programu MAB, 16 pp.

Vačkář, D. (editor), Bujalský, L., Harmáčková, Z.V., Loučková, B., Frélichová, J., Lorencová, E. (2012). Analýza rozhodnutí COP10 - X/2 „Strategický plán CBD na období 2011-2020“ a zhodnocení plnění cílů současné Strategie ochrany biologické rozmanitosti jako podklad pro příští národní zprávu o implementaci CBD (*Analysis of decision COP10 - X/2 "CBD Strategic Plan 2011-2020" and evaluation of the implementation of the objectives of the current National Biodiversity Strategy as the basis for the next national report on the implementation of the CBD*). Assigned by the Ministry of the Environment of the Czech Republic. 147 pp.

Vačkář D (ed.), Harmáčková Z, Lorencová E, Frélichová J, Loučková B, Hubatová M, Pártl A (2013) Analýza plnění Strategie ochrany biologické rozmanitosti České republiky 2005 – 2015 (*Analysis of the implementation of the National Biodiversity Strategy of the Czech Republic 2005 – 2015*). Assigned by the Ministry of the Environment of the Czech Republic, 390 pp.

Organisation of conferences, training courses and workshops

Czech-Iceland workshop on ecosystem services. Ecosystem services and sustainable development: integrating experiences from different regions and scales. 20. – 21. 11. 2014, Sweerts-Špork's Palace, Hybernská 3, Prague 1.
Ecosystem based solutions for adaptation and disaster risk reduction when considering multiple risks, 9 September 2014, 13.30-16.45, Ecosystem Services Partnership Annual Conference, San José, Costa Rica. Session organized by CzechGlobe, United Nations University and IUCN.
Ecosystem services: adaptive EU policies for future EU regions. CE SPECTRA - Slovak Academy of Sciences -Institute of Forest Ecology, Slovak University of Technology, and CzechGlobe -Global Change Research Centre, Academy of Sciences of the Czech Republic. International Colloquium: September 30- October 1, 2013 Bratislava.
Training Workshop BASE Participatory methodologies, September 4th – 6th 2013, Prague, Průhonice conference centre. Workshop organized within FP7 BASE project.
Ecosystem Services, Human Values and Global Change, 24-27 April 2012, scientific conference, workshop and training. Karolinum, Prague. Organized by CzechGlobe within MOSUR project (Interdisciplinary Sustainable Development Network).

Selected projects

EU FP7 and H2020
BASE - Bottom-up Climate Adaptation Strategies towards a Sustainable Europe (FP7, ENV.2012.6.1-3 Strategies, costs and impacts of adaptation to climate change)
ESMERALDA (Enhancing ecoSysteM sERvices mApping for poLicy and Decision mAking) (Horizon 2020 – the Framework Programme for Research and Innovation 2014-2020).

National projects
VG20122015091 - Integrated assessment of global change impacts on environmental security of the Czech Republic (2012-2015, Ministry of the Interior/Security Research)
TD020064 Analysing the Services of Urban Ecosystems and their Impact on Resident Well-being in the Czech Republic (2014-2015, Technology Agency of the Czech Republic/OMEGA)
TD010066 - Integrated Assessment of Ecosystem Services in the Czech Republic (2012-2013, Technology Agency of the Czech Republic/OMEGA)
TD010026 - Developing long-term socio-ecological monitoring in the Czech Republic (2012-2013, Technology Agency of the Czech Republic/OMEGA)
LD13033 - Challenges of Urban Agriculture Challenge in Europe (2013-2015, MSM/LD)
LD13032 - Climate Change and Migration as Adaptation (2013-2015, MSM/LD)

Important personal achievements

2013 Eliška Krkoška Lorencová earned doctoral degree (Ph.D.) at the Faculty of Humanities, Charles University in Prague, with the thesis topic “Assessment of climate change impacts on selected ecosystem services in the Czech Republic: Application of land use scenarios”, (Supervisor D. Vačkář).

2014 Jana Frélichová earned doctoral degree (Ph.D.) at the Faculty of Science, Charles University in Prague, with the thesis topic “Land Use Change Impacts on Ecosystem Services Availability in Czechia” (Supervisor: doc. RNDr. Ivan Bičík, CSc.).

2014 David Vačkář has been elected as a member of the Steering Committee of the Ecosystem Services Partnership (ESP).

Research Report of the team in the period 2010–2014

Institute	Global Change Research Centre of the CAS, v. v. i.

Scientific team	Center of Nanobiology and Structural Biology

The herein described team is part of the Global Change research center of the Academy of Sciences of the Czech Republic, and represents the part of the **Institute of Nanobiology and Structural Biology (INSB)** which is located in Nové Hrady. As the Nove Hrady as a team is shifting in the course of this year from the Center for global change research-Czechglobe to the Institute of Microbiology, the whole Nove Hrady team, including its subteams (the individual labs), is evaluated as one unit.
Since 2002, when the Academy of Sciences became the owner of the chateau in Nové Hrady and started to invest in large-scale reconstructions, a modern research and educational campus was built up in this small town close to the Austrian border. The campus in Nove Hrady was, from the very beginning, meant to serve not only as a research but also as a scientific training facility. INSB closely collaborates with the Faculty of Sciences in the study programs in biophysics and biological chemistry, and organizes a reasonable large number of courses, workshops, conferences and symposia. Despite the short time of its existence, the INSB team already achieved excellent scientific results published in highly visible journals such as Nature, Nature Structural & Molecular Biology, Nature Methods, Nature Chemical Biology, Angewandte Chemie, PLOS etc. In the evaluated period the team described here published 54 impacted publications. Thanks to that, scientists from Nove Hrady are regularly invited to speak at international conferences and symposia, get invitations to contribute review papers, book chapters and invited papers. INSB is regularly visited by foreign scientists and participants of international internship programs, for example each year the institute hosts up to 10 students from Princeton University and other American universities.

Scientists in INSB use methods from bioinformatics, molecular biology, microscopy, molecular modeling and structure determination. They work in a field called structural systems biology on a molecular, cell, and tissue level and provides knowledge on the molecular structure of structural system elements, their principal metabolic and control pathways, identifies links between these elements and thus describes the structure of biological systems.

The research team has its roots in the year 2002, when the laboratory of high performance computing was established in Nove Hrady (R. Ettrich, M. Kuty) and started to cooperate with the also newly established laboratory of crystallogenesis (I. Kuta Smatanova). The research group grew quickly and the demands for molecular biology on a high level lead to engagement of E. Csefalvay in 2005. In the following years the strength of the research team formed by the two groups was in the close collaboration of the two laboratories that allow a very complex research approach using a broad range of methods with a synthesis of theoretical and experimental protein research. The research topics focused mainly at the elucidation of the relationships between structure and function of proteins, dynamic changes related to functional processes on the level of proteins and the mutual interaction of cofactors and subunits in protein complexes. Also, processes and structures on the level of large molecular complexes, cells and tissues are investigated. The collected empirical evidence provides insights into the molecular structural elements of the systems, their metabolic and regulative pathways, their interactions, and serves to describe the structure-function relationships of the biological systems on the aforementioned scales of complexity. Today the research approach is very complex using various methods of protein research with a synthesis of theoretical and experimental methods. Molecular dynamics calculations, quantum chemical and semiempiric calculations of optical spectra, charge localisation or energy transfer and molecular modelling methods are combined with mostly spectroscopic, microscopic and crystallographic methods of protein structure determination. In 2008, the two groups were joined by Josef Lazar, who established a laboratory of cell biology and focused on the development of two-photon polariation microscopy. In March 2011 David Reha from Essex University joined the team to establish a knowledge base for QM/MM hybrid methods in computational biology. In January 2014, Jost Ludwig moved his lab from Bonn university to Nove Hrady and joint the team, bringing in his expertise on functional analysis of ion channels and transporters, cation transport in yeast (*Saccharomyces cerevisiae*), cation homeostasis in yeast (more specifically: Localisation of cation transport proteins and regulatory proteins, cation flux measurements using ion selective electrodes), and his research in multiple drug resistance with the analysis of promoters involved in expression of MDR relevant genes. Additionally, he is also active in the development of yeast and bacterial expression systems for ion channel genes, the development of Screening systems for

compounds inhibiting transcriptional networks (MDR) and the development of production systems for pharmaceutically active peptides/proteins. Recent research was connected to SYSMO (Systems Biology on Microorganisms) within the EU-project TRANSLUCENT: Gene interaction networks and models of cation homeostasis in *Saccharomyces cerevisiae*.

Thus from 2014 on we consider the portfolio within the research team as optimal, making maximum use of the synergies of the individual researchers. The research team is a founding member of the Czech Infrastructure for Systems Biology C4SYS, and currently coordinating this infrastructure as the future node of the European ESFRI infrastructure in systems biology, ISBE. Several of the subteams are contributing services to C4SYS and are involved in its implementation. The move to the Institute of Microbiology is a consequent decision of this development over the last five years, and is expected to stabilize the research unit and give the opportunity for further development. Generally spoken, the research team as it stands now has highly ambitious aims with regard to the development of new methods in molecular systems biology and its application to systems of a common interest and/or hot topic systems.

Currently six laboratories are closely collaborating within the research team in Nove Hrady:

1. Structure and Function of Proteins (Prof. Rudiger H. Ettrich)
2. Crystallogenesis and Biomolecular Crystallography (assoc. Prof. Ivana Kuta Smatanova)
3. Cell Biology (Dr. Josef Lazar)
4. Computational Biology (Dr. David Reha)
5. Molecular Liquids (Dr. Babak Minofar)
6. Membrane Physiology and Bioenergetics (Dr. Jost Ludwig)

Hereunder we report the scientific activities of the individual labs within INSB:

1. Structure and Function of Proteins (Prof. Rudiger H. Ettrich)

The Ettrich lab is interested mainly in the relationship between structure and function of proteins, dynamic changes related to functional processes on the level of proteins and the mutual interaction of cofactors and subunits in protein complexes. The research approach is complex using various methods of protein research with a synthesis of theoretical and experimental methods. Quantum chemical and semiempiric calculations and molecular modeling methods are combined with mostly spectroscopic, thermodynamic and crystallographic methods and methods of protein structure determination.

A. One focus in the evaluted period, based on the long experiences with computational modeling and molecular dynamics simulations of ion channel (see earlier work on TrpV1, and TrpA1), was on cation translocation systems and the lab recently published several studies on ion channel simulations in membranes (Vasilina Zayats, Abdul Samad, Babak Minofar, Katherine Roelofs, Thomas Stockner, Rudiger Ettrich (2013) Regulation of the transient receptor potential channel TRPA1 by its N-terminal ankyrin repeat domain Journal of Molecular Modeling, DOI:10.1007/s00894-012-1505-1; Azadeh Nikouee, Morteza Khabiri, Stephan Grissmer, Rüdiger Ettrich (2012) Charybdotoxin and margatoxin acting on the human voltage-gated potassium channel hKv1.3 and its H399N mutant: An experimental and computational comparison Journal of Physical Chemistry B 116 (17): 5132-5140; Morteza Khabiri, Azadeh Nikouee, Lukasz Cwiklik, Stephan Grissmer, Rüdiger Ettrich (2011) Charybdotoxin unbinding from the mKv1.3 potassium channel: A combined computational and experimental study Journal of Physical Chemistry B 115: 39. 11490–11500.). In the last two years this research direction was strengthened by two new projects, modeling of the human ORAI channel in collaboration with the Romanin lab in Linz, and modeling of yeast TrK in collaboration with the Ludwig lab in house.

a) human ORAI channel:

Calcium release-activated calcium channel protein 1 belongs to an extremely intersesting class of channels, as they differ from the typical tetrameric channels that are well studied in their fold as well as they have a hexameric arrangement. Two years ago was published the first crystal structure of such a channel from drosophila. Homology is high enough with the human channel to build a reliable human model, which we used for the study of several dynamical aspects of the channel. There are several subprojects (role of interaction between loop1-loop3, gating mechanism in the pore, role of cholesterol binding to the channel). Herby we studied the channel using homology modeling, molecular dynamics, cholesterol docking. In this project all experimental work is done in the group of Christoph Romanin at Linz University (molecular biology, electrophysiology) and we managed to get to the final round with a Human Frontier Science project that included the Linz group, Muhammad Trebak from Albany, and Dirk Trauner from LMU Munich. Our research in that direction is so far focused on

i) Determination of the unique, extracellular Ca2+ sink of Orai channels

ii) Regulation of Ca2+ sink by basic residues of the extracellular loop3

iii) Exploration a unique selectivity filter that binds two Ca2+ ions
iv) Requirement of Ca2+ sink and Ca2+ selectivity filter for Orai1/CRAC permeation and selectivity
Currently there are two papers submitted, one to Science Signaling with the title and topic "A calcium accumulating region, CAR, in the Orai1 channel regulates Ca2+- permeation and gene transcription" on which Vasilina Zayats shares first authorship with two experimentalists, and Rudi Ettrich is the senior author and a second to Nature Commulications that is dealing with the molecular mechanism of Orai1-cholesterol interaction.
b) yeast TRK1:
In this case it is not clear if we have "only a channel" or if it is a transporter, there are some evidences that it actually is a transporter. The structurally interesting feature here is that is has the basic fold as the tetrameric potassium channels but it is one long chain that folds into that "tetramer" which we know from KcsA or others. This gives the opportunity to make mutations on one "monomer" only. Also it arranges into dimers (even some tetramers are proposed), which means that we have two or even four of the "tetramers".
Only two bacterial crystal structures are known and a lot of the work is therefore homology modeling and a going forth and back between experiment and model adaption. A first paper was published in early 2015 in BBA-biomembranes in collabration with Jost Ludwig who was responsible for fluorescence spectrocopy, electrophysiology, potassium uptake, point mutations, molecular biology

B. A project that finished by last year was focused on Thiazoline inhibitors of β-N-acetylhexosaminidase based on new, non-natural substrates. This project was in collaboration with Vladimir Kren from the Microbiology institute, and waqs backed up by a common GACR grant which fully employed one post-doc, Natalia Kulik, in the lab.n The aim of this project was the design and preparation of potent and more selective β-HexNAc-thiazoline inhibitors of β-N-acetylhexosaminidase mimicking their oxazoline transition state, with a therapeutic potential. New inhibitor structures are based on recently discovered non-natural modified β-N-acetylhexosaminidase substrates (e.g. 4-deoxy, 6-phosphate, 6-sulfate, 6-oxo and 6-acyl). Methods applied in our lab were homology modeling, MD, docking, while the collaborating group was responsible for the experimental work. Seven impacted publications resulted from this project.

C. A long time research focus is on the E. coli protein WrbA is an FMN-dependent NAD(P)H:quinone oxidoreductase that has been implicated in oxidative defense. Three subunits of the tetrameric enzyme contribute to each of four identical, cavernous active sites that appear to accommodate NAD(P)H or various quinones. Kinetics results reveal that WrbA conforms to a ping-pong mechanism with respect to the constancy of the apparent Vmax to Km ratio with substrate concentration. Docking and energy calculations find that electron-transfer-competent binding sites for NADH and benzoquinone present severe steric overlap, consistent with the ping-pong mechanism. Plots of initial velocity as a function of either NADH or benzoquinone concentration present one or two Michaelis-Menten phases depending on the temperature at which the enzyme is held prior to assay. The effect of temperature is reversible, suggesting an intramolecular conformational process. WrbA shares these and other details of its kinetic behavior with mammalian DT-diaphorase, an FAD- dependent NAD(P)H:quinone oxidoreductase. Sedimentation velocity analysis of WrbA indicates a large shift in size of the multimer with temperature, suggesting that subunit assembly coupled to substrate binding may underlie the two- plateau behavior. FAD does not support WrbA redox activity, our results clearly demonstrate that traces of FMN contaminating the FAD stocks were likely to be responsible for the apparent activity with FAD reported in earlier assays. Our molecular dynamics simulation, the first reported for WrbA, elucidated the origins of the two major differences between apo- and holoWrbA crystal structures, an alteration of inter-subunit distance and a rotational shift within the tetrameric assembly. A large series of ITC experiments and the resulting binding affinities together with the parallel series of AUC experiments of apo and holo proteins of WrbA and DT-diaphorase enabled to compare the dimer-tetramer, resp. monomer-dimer equilibrium in both cases and the results were matched with the H/D exchange data, and computational calculations to interpret solvent accessibility and structural changes during multimerization. We gained a high-resolution X-ray crystal structure at 1.2A that shows a twist of the FMN that allowed QM/MM calculations for the various redox states. All possible redox forms of FMN (oxidized flavin mononucleotide; anionic semiquinone; neutral semiquinone; anionic hydro-quinones, with the proton on N1 or N5, respectively; hydroquinone), were calculated using DFT method with the B3LYP functional and the 6-31G* basis set was selected as the QM method and the OPLS2005 force field was selected as the MM method. Oxidised FMN and the anionic semiquinone forms display comparably small r.m.s.d. values and a slight, and very similar, propeller twist in the QM/MM results like that observed in the WrbA crystal. The finding that even oxidized FMN, which was previously thought to be planar, is predicted to adopt a twisted conformation in the protein environment suggests a strong influence of the protein. This high-resolution X-ray crystal additionally revealed a methionine sulfoxide residue with full occupancy in the FMN- binding site, a finding that was confirmed by mass spectrometry. We also report the first structure of WrbA from E. coli with benzoquinone stacked in the active site between FMN isoalloxazine ring and Trp97 in the position ideal for electron transfer. The active site is fully occupied by benzoquinone ring and cannot accommodate the second substrate NADH. This finding is consisted with ping-pong

mechanism of WrbA from E. coli suggested previously. Integration of ITC, AUC, limited proteolysis, H/D exchange, MD and chemical cross-linking lead to a full understanding of the dimer-tetramer equilibrium and the allostery observed in WrbA. The biochemical and biophysical results obtained illuminate structure and function in the novel WrbA family of stress-defense proteins. The molecular explanation of the observed two-plateaus in in the Michaelis-Menten plots by the dimer-tetramer equilibrium seems to be valid in general for allosteric regulation of multimeric, two plateau enzymes, which implies that these enzymes are finely tuned to the physiological concentrations of their substrates. The results lead to new understanding of structure and function relationships in two very well-studied proteins, the flavodoxins and the NADH:quinine oxidoreductases, to which WrbA is related on two sides of its family tree. The gained findings represent a major contribution to the understanding of structure and function in the novel WrbA family of proteins. Results were published so far in 5 impacted papers and one PhD-thesis was successfully defended (I Kishko: Kinetic behavior of the NAD(P)H: Quinone oxidoreductase WrbA from Escherichia coli.), one further paper has been just submitted (O. Degtjarik, J. Brynda, J. Carey, O. Ettrichova, I. Kuta Smatanova and R. Ettrich, Crystal structure of WrbA from E. coli in complex with benzoquinone, submitted to ActaCryst) and one last major publication (On the dimer-tetramer equilibrium of the allosteric oxidoreductase WrbA, prepared for Structure), is currently finalized for submission. In this project all protein purification, kinetic measurements, ITC and computational studies were performed in the Ettrich lab, the Kuta Smatanova lab contributed protein crystallization and mass spec data were gained by the Novak lab in Prague. J. Carey from Princeton University is a visiting scientist at INSB and spends 2 month/year in Nove Hrady, and most of her contribution came from her and her students appointment and work in Nove Hrady. The significance of the work derives from its aim to create a comparative framework for understanding structure and function in these proteins. The aims are related to our long-term interests in the molecular origins of intramolecular structural organization, stability, and dynamics, and intermolecular affinity, specificity, and cooperativity.

D. Another long term focus is on allostery. A controversial prediction of the famous allostery model of Monod, Wyman, and Changeux is that constraints imposed on protein subunits by multimerization are relaxed by ligand binding, but with conservation of symmetry in partially-liganded states. Interpretation of thermodynamic ligand-binding data through the lens of molecular dynamics simulation has led to structural and energetic description of such a state for the hexameric Escherichia coli arginine repressor, which displays strong negative cooperativity of L-arginine binding. The results indicate that partially-liganded states can be structurally symmetric despite their conceptual asymmetry. The symmetric relaxed state is visualized as a multimer with all subunits anchored near the center, and with motions transferred to the periphery of the assembly. Thus, even during sequential filling of binding sites, symmetry can be maintained by exploiting the dynamics of the assembly and the distributed nature of its cohesive free energy.
This elegantly simple and probably ancient molecular mechanism of allostery is described for the Escherichia coli arginine repressor ArgR, the master feedback regulator of transcription in L-arginine metabolism. Molecular dynamics simulations with ArgRC, the hexameric domain that binds L-arginine with negative cooperativity, reveal that conserved arginine and aspartate residues in each ligand-binding pocket promote rotational oscillation of apoArgRC trimers by engagement and release of hydrogen-bonded salt bridges. Binding of exogenous L-arginine displaces resident arginine residues and arrests oscillation, shifting the equilibrium quaternary ensemble and promoting motions that maintain the configurational entropy of the system. A single L-arg ligand is necessary and sufficient to arrest oscillation, and enables formation of a cooperative hydrogen-bond network at the subunit interface. The results were used to construct a free-energy reaction coordinate that accounts for the negative cooperativity and distinctive thermodynamic signature of L-arginine binding detected by calorimetry. The symmetry of the hexamer is maintained as each ligand binds, despite the conceptual asymmetry of partially-liganded states. The results thus offer the first opportunity to describe in structural and thermodynamic terms the symmetric relaxed state predicted by the concerted allostery model of Monod, Wyman, and Changeux, revealing that this state is achieved by exploiting the dynamics of the assembly and the distributed nature of its cohesive free energy. The ArgR example reveals that symmetry can be maintained even when binding sites fill sequentially due to negative cooperativity, which was not anticipated by the Monod, Wyman, and Changeux model. The molecular mechanism identified neither specifies nor requires a pathway for transmission of the allosteric signal through the protein, and it suggests the possibility that binding of free amino acids was an early innovation in the evolution of allostery.

E. Another key project is focused on cleavage and translocation by Type I restriction-modification complexes. This project started with resolving the crystal structure of the motor subunit of EcoRI that we published in Nature Structural&Molecular Bilogy in 2009, and is aimed at providing fundamental knowledge about restriction enzymes and translocases that can help to describe the function and evolution of cellular DNA defense and repair systems. Furthermore, understanding the cooperation of translocase and nuclease activities could provide a tool to regulate the nuclease activities as a potential therapeutic target. The Type I RM enzymes have been suggested as potential tools for assembly of nano-machines because they provide a molecular motor that would not cut its own DNA

track during translocation (Youell & Firman, 2007). This rearch is still ongoing, although two additional papers have been published already. For solving this project a large set of experimental techniques had to be established in the Ettrich lab as are cleavage assyas, in vivo restriction tests, ATPase activty measurements, translocation assyas based on triplex replacement, advanced crystallization techniques, second site mutagenesis etc...

Additionaly there were couple of small projects/collaborations :
-Coarse graining of lipid bilayers (together with Zofie Sovova (now Olomouc university)
-Oxygen-evolving complex of photosystem II (together with Ivanas group (mainly Jarka Kohoutova, and Jiri Heller) and Norberts Muellers group in Linz (solving NMR structure of PsbP and PsbQ),
-Organic solvent influence on protein struture and enzyme function with Jiri Damborsky, MU Brno.
-Modeling of TrpA1 together with the Vlachova group from the Institute of Physiology ASCR, Prague.

Altogether the Ettrich lab published 32 impacted publications since 2010 including PLOS Computational Biology, Angewandte Chemie, Acta Crystallographica Sction D etc.

2. Crystallogenesis and Biomolecular Crystallography (assoc. Prof. Ivana Kuta Smatanova)

The main aim of the Kuta Smatanova lab is in structural studies of membrane and soluble biological macromolecular complexes using methods of X-ray diffraction. X-ray crystallography is the major technique to get the structure of biological macromolecules at atomic resolution. These protein structures are central to understand the detailed mechanisms of biological processes and to discover novel therapeutics using a structure-based approach. Several non-membrane membrane protein complexes have been crystallized in the lab.

The main projects in the evaluation period focused on:

a) Structure-functional Relationships of Haloalkane Dehalogenases

Haloalkane dehalogenases (EC 3.8.1.5) are microbial enzymes with catalytic activity for hydrolytic conversion of xenobiotic and toxic halogenated aliphatic compounds to corresponding alcohols. Structures of several novel enzymes crystallized are solved and deposited to Protein Data Bank within the framework of this project. Additionally, recently cloned and biochemically characterized enzymes are crystallized and structuraly characterized. Although several molecular structures of haloalkane dehalogenases were known, some details of the reaction mechanism are not understood yet. Therefore, neutron diffraction is used in parallel to X-ray diffraction to describe the role of hydrogens in water and protonated residues in the active site and access tunnels connecting the active site cavity. Structural data is linked to the functional biochemical data leading to better understanding of structure-function relationships of haloalkane dehalogenases and related alpha/beta-hydrolases.

b) *Crystallization and structural studies of higher plants membrane protein complex*

The process of photosynthesis cannot be understood without detailed knowledge of the structure of PSII and its single subunits. In order to designate molecular details of water oxidation catalyzed by the oxygen-evolving center (OEC) assembly, degradation, structure, function, and dynamic regulation of large protein-cofactor complex it is very important to crystallize and describe 3D structure of PS II from higher plants. No crystal structure of PS II from higher plants is available until now therefore our research was focused on structural studies of the membrane complex PSII. To develop a reproducible purification protocol controlling all chemical and physical parameters represents a complex task, however it is a crucial step toward reproducible crystallization experiments. Once this protocol is developed and optimized, we believe the pathway towards PS IICC crystallization and structure determination will be successful.

Key results of the above described projects in brief:

Four novel haloalkane dehalogenases DpcA from Psychrobacter cryohalolentis K5, DmxA from Marinobacter sp. ELB17, DspA from Strongylocentrotus purpuratus and DbeA from Bradyrhizobium elkanii USDA94 were biochemically characterized and used for crystallization experiments. Three out of four novel enzymes were successfully crystallized and their three dimensional structures were determined. Simultaneously, several variants of haloalkane dehalogenase DhaA with enhanced catalytic efficiency, improved thermal stability, enhanced resistance towards organic co-solvents and balanced activity-stability trade-off were constructed and biochemically characterized. Crystal structure of selected DhaA variants and their complexes with the

substrates/ligands were solved. Set of variants of haloalkane dehalogenases LinB from Sphingobium japonicum UT26 with closed and de novo opened tunnels were designed, constructed, biochemically and structurally characterized. The effects of selected organic co-solvents, inorganic ions and deep eutectic solvents on structure, stability, activity and enantioselectivity of three haloalkane dehalogenases were also tested with a goal to understand protein-solvent interactions at the molecular level. Screening of new haloalkane dehalogenases, their detailed characterization and mutagenesis resulted in 16 international publications (including Nature Chemical Biology and Angewandte Chemie) and 9 structures deposited to RCSB protein data bank (PDB ID 4E46, 4F5Z, 4F60, 3SK0, 3RK4, 4FWB, 4HZG, 4K2A and 4WCV) during the last three years.

Another project focused on the complex process of crystallization of proteins. In parallel to modern high-throughput approaches used in the protein crystallization, in recent years we performed basic research on physico-chemical properties and molecular interactions influencing crystal growth and wrote a book chapter that is focused on advantages and disadvantages of each described crystallization technique. The main chapter describing the alternative crystallization techniques is taking advantage of discussing the importance of additives in protein crystallization, as well as, the novel approach to macromolecular crystallization and reporting the effects of Cross Influence Procedure CIP, a method developed in our lab, on crystallization of two different proteins. In addition, for the first time the detailed protocol of CIP is given within this chapter to help readers to perform their own cross-crystallization experiment by using selective additives. Until now there has been no monograph devoted exclusively to the role of cross-influence of additives and their general usage guideline in protein crystallization. The present text is an attempt to fill this gap. Thus, this book chapter stands as a valuable guide to the alternative protein crystallization. (Ivana Nemcovicova and Ivana Kuta Smatanova: Chapter 11: Alternative Crystallization Technique: Cross Influence Procedure (CIP). In the Crystallization and Materials Science of Modern Artificial and Natural Crystals, Pages 249-276, Edited by: Elena Borisenko, ISBN 978-953-307-608-9, Publisher: InTech (January 2012))

3. Cell Biology (Dr. Josef Lazar)

The main achievement of the team was development of the technique of two-photon polarization microscopy (2PPM) and its applications. The results achieved during the 2010-2014 period were the fruits of a long previous period of work, during which virtually no publications were produced, in order not to hamper patenting of the 2PPM method and a publication of a large impact article in a major journal. Although this approach carried its risks and difficulties, it ultimately paid off in the form of a large article in Nature Methods (IF 20.9), Czech and US patents, and several follow-up publications.

The 2PPM technique is a novel tool of optical microscopy, allowing sensitive observations and monitoring of changes in protein conformation (such as during functional activity of an enzyme) in living cells and animals. Apart from allowing observations of protein function, the technique should also allow gaining quantitative insights into molecular structure of protein molecules, directly in living cells. 2PPM takes advantage of the fact that excitation properties of molecules of fluorescent proteins (FPs) are anisotropic. Our initial mathematical modelling work indicated that when orientation of FP molecules is restricted (such as by attachment to a membrane protein) anisotropic properties of FP molecules should be observable, even if the restriction of molecular orientation is only partial, and even at the relatively low counts of FP molecules that are present in living cells. Our understanding of optics allowed us to recognize the advantages of observing anisotropic properties of fluorophore excitation, rather than anisotropic properties of fluorescence emission, although the two are commonly (but wrongly) considered interchangeable. After obtaining initial encouraging experimental results confirming the validity of our mathematical model, we worked with a company (BME Bergmann, Germany) to developed a polarization modulator system allowing rapid polarization modulation in synchrony with the acquisition of individual image pixels by the laser scanning microscope. This system then allowed acquisition of large amounts of imaging data in agreement with our mathematical model.

Our data confirmed that 2PPM is indeed capable of observing even subtle biases in orientational distributions of fluorescent proteins, as well as changes in orientation due to protein function. We demonstrated the capabilities of 2PPM by directly observing activation of heterotrimeric G-proteins with sensitivity better than previously achieved by other methods, changes in membrane protein conformation affected by changes in intracellular calcium concentration, and by interpreting (albeit only qualitatively) our 2PPM imaging data in terms of protein structure. The results (Lazar & al., Nature Methods 2011; Czech patent #302233, US patent #8,643,177) were, with the exception of parts of the mathematical modelling work, obtained fully by the team in Nove Hrady. Correspondingly, the intellectual property rights belong to the Global Change Research Center AS CR (67%) and University of South Bohemia (33%). The work has led to formation of two spin-off companies, Innovative Bioimaging, LLC (based in USA) and Innovative Bioimaging, s.r.o. (based in Czech Republic) trying to commercialize the technique and its applications.

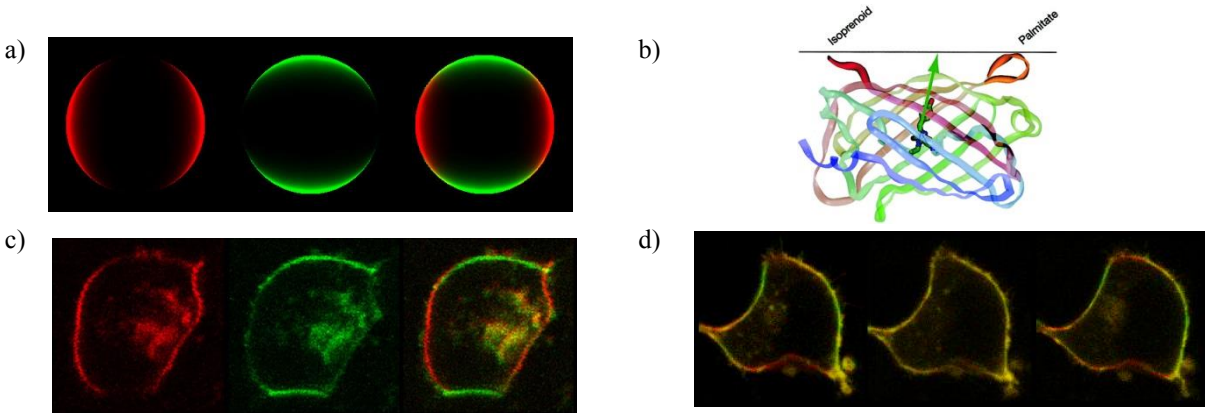

Fig. 1: 2PPM microscopy. a) Computer-simulated 2PPM images of a model spherical cell with a fluorophore perpendicular to the cell membrane. Fluorescence excited by light polarized horizontally (colored red), vertically (colored green), and the two images superimposed. The red-green pattern is indicative of fluorophore orientation with respect to the membrane. b) A construct (dleGFP) consisting of eGFP and two lipidic tags anchoring the FP moiety to the cell membrane in a well-defined orientation, with the fluorophore oriented close to perpendicular to the membrane. c) A 2PPM image of a live HEK-293 cell expressing dleGFP, colored as in a); agreement with the mathematical modeling results shown in a) is apparent; d) 2PPM imaging of G-protein activation and inactivation (shown as changes in red/green pattern of the cell outline). Similar changes can be also observed in proteins undergoing changes in conformation (not shown).

Since developing the technique, we have applied it (Bondar and Lazar, J.Biol.Chem., 2014) to addressing mechanistic questions of G protein signalling (illuminating interactions between G protein subunits, G proteins and G protein coupled receptors and various effectors). We have also applied 2PPM towards development of genetically encoded sensors of cell membrane voltage, a project carried out in collaboration with the laboratories of V. Pieribone and L. Cohen at Yale University (Han & al., PLoS One, 2014).

We have also been striving to further develop the capabilities of the technique, particularly the ability to yield information about structure of membrane proteins in living cells. We have carried out 2PPM experiments on simple model systems (lipid vesicles and fluorescent dyes), showing that 2PPM can be used for accurate determination of molecular orientation of the dyes within the lipid membrane (Timr & al., J.Phys.Chem, 2014). This work is being carried out in close collaboration with the laboratory of P. Jungwirth (Inst. of Organic Chemistry and Biochemistry, Prague), whose members perform molecular dynamics simulations and quantum mechanics calculations aiming to verify our 2PPM results through independent means. We are now trying to extend the results obtained on artificial lipid membranes and fluorescent dyes to fluorescent proteins attached to lipid vesicles and FPs attached to membranes of living cells. In order to be able to make the step up to FPs, we need to determine the directional optical properties of FP molecules. Therefore, we are now purifying and crystallizing representative FPs, observing the optical properties of the FP crystals and deducing the optical properties of the individual FP molecules. This work is being done in collaboration with A. Royant of the European Synchrotron Radiation Facility in Grenoble, France.

4. Computational Biology (Dr. David Reha)

David Reha was working on development of methods and tools for the study of biological systems with main focus on the QM/MM methods. Computational methods are important tools for the study of biomolecules. They are much cheaper then the experimental methods and can provide important insights to structure and dynamics of complex biomolecular system. The vast majority of the computational studies on biological systems are mostly based on methods that facilitate the classical molecular mechanics (MM). These methods can successfully describe structure and dynamics of the complex, however due to the nature of the empirical forecfields, they are deficient for the detailed description of the intermolecular interactions, as are for example interactions between protein and ligand (substrate, inhibitor) within the binding pocket. They also cannot be used for the modeling of intermediate states, transition structures and generally for any chemical reaction (systems where a covalent bond is forming or breaking). These are the situations where methods based on quantum mechanics (QM) can be successfully applied. QM calculations describes system on the electronic level, therefore it can be used for the study of the systems

which cannot be accurately described by MM (like transition and intermediate states) as well as for the study of chemical reactions. Since QM calculations are computationally very demanding and the description of the large biomolecules by purely QM methods is very limiting, hybrid QM/MM methods represent a good approach. QM/MM methods combine advantages of both QM methods for accurate calculation of the small region of the interest (active site) and MM methods for the fast calculations of the remaining part (Fig.1). These methods combined with MD simulation and molecular docking can be successfully used for the study of the mechanisms of enzymatic reactions (calculation of the energy barriers), positions of the binding site and selectivity of the binding for particular substrates, inhibitors or drugs.

One of the most important contributions to intermolecular energy is electrostatic energy. T he vast majority of MM empirical forcefields treats it as the interaction between permanent atomic charges (forcefield parameters) thus effectively neglecting polarization. This can be justified by the fact that the polarization of the charge distribution is a more subtle then the electrostatic interactions between charges. However this approach can lead to inaccuracies for certain situations, like modeling highly charged ions (e.g. phosphate in ATP), polar molecules in low-dielectric media (e.g. ligands binding to receptors) and interactions of ions with π-electron systems. Polarization is particularly important in QM/MM methods. Due to the complexity and non-triviality of the implementation of MM polarization, the vast majority of QM/MM software employs electrostatic embedding only, where the QM part (described by QM methods) is naturally polarized but MM part described by the fixed atomic charges does not include explicit polarization. It creates unbalanced approach since polarization is included in the QM entity and so should be included at least in the immediate environment to generate a balanced approach. Polarization energy can also make a significant contribution to transition state stabilization in enzymes, since it is comparable in magnitude to the difference in energy between alternative mechanisms. It is therefore desirable to introduce methods to enable explicit polarization of the MM environment to be incorporated in a way that is fully consistent with QM.

D. Reha has has developed (during his recent postdoctoral position in University of Essex 2009-2011) the way to introduce the QM/MM polarized embedding, using the method of ‘induced charges’ that is very easy to implement to various QM/MM programs. This approach is based on the more common method of induced dipoles, where the induced dipole on particular atom (calculated from atomic polarizability) is approximated by the set of induced charges on the neighboring atoms. This brings a considerable simplification and allows the use of standard programs for molecular docking, as they already evaluate electrostatic interactions using atomic charges.

Then he had employed those modified QM/MM programs to investigate various systems with real biological relevance, that was studied by other groups at INSB as wal as our collaborators from other institutes.

There are other applications of polarization 'induced charges' method then direct implementation to the QM/MM programs. The one example studied by D.Reha is implementation of polarization for molecular docking. The molecular docking is widely used for finding of the best ligand and its position (orientation) within binding pocket of the protein (ligand receptor). The Docking programs standardly use the interaction energy between the ligand and protein as one of the main factors to evaluate suitability of the ligand (pose). The electrostatic interactions are treated classical by fixed atomic charges. The way to introduce polarization is via polarized charges of ligand calculated by QM/MM methods. D.Reha has utilized the methods of 'induced charge' to include polarization also to the MM region of the protein. The methods based on polarized molecular docking provides more accurate results especially of the weekly bound ligands.

In the following years (2011-2014) D.Reha has applied QM/MM methods and methods of explicit polarization to the various biological systems:

1. The method was applied to study the mechanisms of NADH:quinone oxidation reduction reaction in flavoprotein WrbA. During the enzymatic reaction, the NADH is oxidized to NAD+ and quinone is reduced to hydroquionone. The reaction proceeds via FMN acting as an enzyme cofactor. We have used molecular docking improved by QM/MM calculation with MM polarization to estimate the positions and relative binding energies of the substrates of the WrbA protein. The results of calculations supports the experimental evidence of the hopping mechanisms, where in the first step, the NADH is oxidized to NAD+ by FMN and in the second step (after the NAD+ leaves to active site), the quinone is reduced to hydro-quinone by FMNH2. Furthermore, our calculations helps to explain the unusual position of NAD in crystal structure of Andrade *at al* . The crystal structure represents the product (NAD+) leaving the active site after the reaction.

2.The method was also applied to study the binding affinity of progesterone, propranolol and warfarin to human α1-acid glycoprotein. The ligands were docked to the protein using fully polarized molecular docking (based on QM/MM). The relative binding strength of the ligands corresponds to the experimental evidence.

3. The method was applied to study the activity of various enantiomers of hydroxyglutamates and hydroxyaspartates as inhibitors for Serin Racemise (including cofactor PLP) be means of polarized molecular docking and QM MM calculation of binding energies. Results nicely correspond to the experimental evidence.

4. Finally, the method was applied for the calculation of the conformational changes connected to the coupling of translocation and endonuclease activity in the restriction-modification system EcoR1241. We have calculated the

binding energies of ATP with the aminoacid residues in the active site. The results showed some significance of LYS220 interaction with ATP helping to explain its role to the system during conformational changes.

The diversity of biological systems studied would demonstrate the broad applicability of improved QM/MM methods.

5. Molecular Liquids (Dr. Babak Minofar)

The main research during last five years in the Minofar lab was related to the solvation and dynamics of ions and biomolecules in non-aqueous media such as organic solvents and ionic liquids (ILs) as room-temperature ionic liquids have remarkable industrial applications. Their remarkable properties such as negligible vapor pressure, low flammability, high thermal and electrochemical stability made them very useful for many applications. One of the remarkable application of ILs is application in lithium secondary batteries. In our experimental and theoretical study which was performed by high-energy X-ray diffraction technique (HEXRD) and classical molecular dynamics (MD) simulations we elucidate the lithium ion conduction mechanism in bulk solution which is used to understand the electrolyte solution/electrode interface where the electron transfer is accompanied by the Li+ ion solvation/desolvation. The results of the study also was supported by Raman spectroscopic studies. The results of this work made a breakthroughs on understanding the solvation of Li+ ion in ILs and was published in high impact factor journal in the field of chemistry, the journal of physical chemistry B in 2011 which is cited 35 times with the title of "Liquid Structure of and Li+ Ion Solvation in Bis(trifluoromethanesulfonyl)amide Based Ionic Liquids Composed of 1-Ethyl-3-methylimidazolium and N-Methyl-N-propylpyrrolidinium Cations"

Another study concerning the structure and dynamics of protic ionic liquids which was performed by our group was studying the liquid structure and ion–ion interactions in a series of primary alkylammonium nitrate ionic liquids. This work were studied by means of high-energy X-ray diffraction (HEXRD) experiments and classical molecular dynamics (MD) simulations. The results of our work showed for first time special liquid structure structural heterogeneity and unique distorted hydrogen bonding in primary ammonium nitrate ionic liquids.
It was revealed that by increasing the length of alkyl chain of ionic liquids the scattering vector in low region increases which shows distorted hydrogen bonding in the liquid state in which structural heterogeneity in protic ILs is dominant. The findings of this work suggests that the terminal methyl group in the cation can competitively interact with both the terminal methyl group in the other cation and the oxygen atom in nitrate anions, and that nitrate ions no longer exist around the terminal methyl group when lengthening the alkyl chain. Hence, the anions around the nonpolar group are excluded by the alkyl chain aggregation with lengthening the alkyl chains.
This work brought new insights in the field of liquids state chemistry of ionic liquids thus it was published in journal of physical chemistry B in 2012 and it is cited already 26 times.
In another study on solvation of protic ionic liquids we proposed new mechanism for proton transfer in solution which was breakthroughs in the field of ionic liquids. In this study we performed both experiment and theory to understand the proton transfer in mixture of acetic acid and imidazol. Raman spectroscopy showed no ions in the solution but electrically neutral molecular species predominantly exist in solution of N-methylimidazole and acetic acid equimolar mixture. Now the question was that how such solution can have strong ionic conductivity? Figure 2 shows "good ionic" or "superionic" behavior of the solution. But how it is possible to have no ionic forms in the solution but having strong ionic conductivity.
The only way was to introduce new specific proton conduction mechanism such as a proton relay mechanism in which fast proton transfer and reorientation of the surroundings might lead to specific proton conduction. A possible proton conduction mechanism is shown in figure 2.

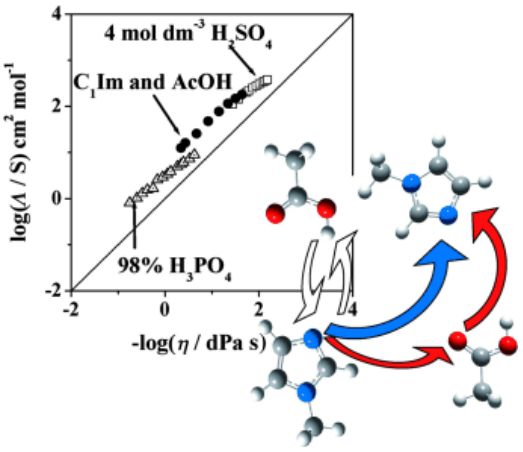

Figure 2. Possible proton conduction mechanism for N-methylimidazole and acetic acid ionic liquid.

Recently enzymes in organic solvents significantly expands their technological applications as many limitations happen in aqueous solutions. Organic solvents enhance the solubility of hydrophobic substrates, prevent undesirable water-induced side reactions, and can rise the enzymatic activity.
We have studied Haloalkane dehalogenases enzymes in organic co-solvents such as acetone, formamide, and isopropanol. Molecular-dynamics simulations, time-resolved fluorescence spectroscopy, and steady-state kinetic measurements were employed to gain an insight into the mechanisms governing the enzyme–solvent interactions at the molecular level.
A broadly applicable computational method, involving molecular-dynamics simulations and quantitative analysis of co-solvent occupancies inside the access tunnels and active sites, was developed to aid the selection of an appropriate organic co-solvent. In this way, predicted changes in the accessibilities of the active site were confirmed by time-resolved and steady-state fluorescence spectroscopy and molecular dynamics simulations.
This work revealed that how organic solvents can influence the rate of enzymatic reactions. The outcome of this study has very important impact on applications of enzymes on waste management and was published in journal of CHEMBIOCHEM in 2013 with impact factor of 3.060. This work was also breakthroughs in the field of enzyme engineering as it revealed the mechanism of activation of enzyme in organic solvents.

6. Membrane Physiology and Bioenergetics (Dr. Jost Ludwig)

The yeast *Saccharomyces cerevisiae* is not only a versatile eukaryotic model organism but has also many biotechnological applications. Maintenance of cation homeostasis is an essential process (not only) in yeast that affects different physiological parameters such as membrane potential, intracellular pH, cell volume and that also directly influences nutrient uptake and growth. Most of the elements mediating uptake and efflux of the major alkali cations (potassium and sodium) have been discovered and genetically and physiologically characterized during the last decades. However, there are still many uncertainties concerning the contribution of each component, their regulation under basal and stress conditions, and the interaction between cation fluxes. Even the exact mechanisms and the energy source of cation transport are not fully elucidated in all cases yet. Within the framework of two subsequent multinational SysMO (System Biology of Microorganisms) projects (Translucent and Translucent-2) the mechanisms of cation homeostasis of *S. cerevisiae* were studied by consortia composed of laboratories from in total six European countries. The approach consisted of an iterative interaction between "wet labs" using various "classical" and newly developed techniques to generate data that was implemented into models by the biomathematical "theoretically working" groups. Within the consortium Jost Ludwig, while being still in Bonn, concentrated mainly on the development and application of methods allowing highly time resolved measurements of (i) cation (including proton-) fluxes across the plasma membrane ("Flux estimation using ion selective electrodes", FLISE) and (ii) changes of pH upon the application of external stimuli. Jost Ludwig, who also was the only lab member until the end of the reporting period, moved from Bonn to Nove Hrady in 2014. Thus, significant time was spent in re-establishing the laboratory and now both, the FLISE setup as well as the available MIFE system for non-invasive ion flux measurements, a powerful tool for *in vivo* studies of membrane-transport processes in living organisms, is fully functional. Nonetheless, besides reestablishing the lab, a project in which structure/function relationships of a fungal K^+ translocation (uptake) system, the *Saccharomyces cerevisiae* (*S. c.*) Trk1 were examined in a combined experimental (molecular biology, physiology, fluorescence microscopy, biophysics) and modeling approach (contributed by Vasilina Zayats and other members of the Ettrich lab) significantly progressed. Results obtained so far were submitted for publication within the reporting period and was published in its final version in February 2015 (Vasilina Zayats, Thomas Stockner, Saurabh K Pandey,

Katharina Wörz, Rüdiger Ettrich, Jost Ludwig (2015) A refined atomic scale model of the Saccharomyces cerevisiae K+-translocation protein Trk1p combined with experimental evidence confirms the role of selectivity filter glycines and other key residues. *BBA-Biomembranes* 1848 (5): 1183-1195.)

Research Report of the team in the period 2010–2014

Institute	Global Change Research Centre of the CAS, v. v. i.

Scientific team	Department of Nanobiotechnology

Research performed at the **Department of Nanobiotechnology** is mainly focused on the development of biocompatible magnetically responsive materials and their applications in various areas of biosciences, biotechnology and environmental technology.
In the period 2010-2014 the attention was mainly focused on the following topics:

- Preparation of magnetically responsive materials from non-magnetic
- precursors
- Magnetic modification of microbial and algae cells
- Magnetic materials for xenobiotics removal
- Magnetic materials for enzymes immobilization
- Magnetic particles–based biosensor for biogenic amines
- Magnetic materials for the separation of biologically active compounds
- Study of magnetoferritin
- Magnetic review papers
- Non-magnetic research

Preparation of magnetically responsive materials from non-magnetic precursors

Huge amounts of non-magnetic materials with interesting properties (e.g., adsorbents, catalysts, carriers, etc.) exist. Recently, waste and secondary biological materials from e.g. agriculture and food industries are of high interest because of their easy availability and low price. Application potential of these materials can be improved after their magnetic modification. Magnetically responsive materials exhibit several responses to external magnetic field. The most important property is the possibility of their selective separation from complex and difficult-to-handle samples using appropriate magnetic separator.

A simple procedure was developed for the conversion of non-magnetic powder materials into their magnetic derivatives. This technique is based on the mixing of the material to be modified with water based magnetic fluid. After drying, aggregates of maghemite were deposited on the treated material, enabling its magnetic separation [22].

An alternative, one-pot, microwave-assisted synthesis of various types of magnetically responsive materials from non-magnetic precursors was also developed. The preparation is based on the microwave irradiation of the suspension of the treated material with iron hydroxides prepared by alkalization of iron(II) sulfate. Submicrometer magnetic particles formed during the microwave treatment deposited on the surface of the treated materials in the form of individual particles and their aggregates [25]. The developed magnetization procedure was patented [42].

A really generally applicable, rapid and simple procedure for the magnetic modification of non-magnetic materials was designed from the above mentioned method. The postmagnetization procedure employs magnetic iron oxide nano- and microparticles (prepared using a microwave assisted procedure from ferrous sulfate) which are thoroughly mixed with the powder material to be modified. After drying, stable magnetically responsive materials are formed. The procedure can easily be scaled up, and magnetic response of the modified materials can be finely tuned [33]. Mechanochemical synthesis of various types of magnetically responsive materials from non-magnetic powdered precursors was developed. The preparation is based on the mechanochemical conversion of ferrous and ferric ions at the presence of alkaline hydroxide into magnetic iron oxides nanoparticles (maghemite identified by XRD measurements). The presence of powdered nonmagnetic materials during the mechanochemical process led to the efficient deposition of magnetic nanoparticles on the surface of the treated materials in the form of individual nanoparticles and their aggregates [36]. The described procedure was patented [43].

The applicability of magnetically responsive materials is presented in the following paragraphs.

Magnetic modification of microbial and algae cells

The majority of prokaryotic and eukaryotic cells can interact with a wide range of nano- and microparticles and films. The modified cells usually maintain their viability, but the presence of foreign material on their surfaces, in protoplasm or in intracellular organelles can provide additional functionalities. Cells modified using different procedures can be employed as whole-cell biosensors, whole-cell biocatalysts, applied in toxicity microscreening devices and also as efficient adsorbents of different types of organic and inorganic xenobiotics. Cells are usually nonmagnetic and their modification with magnetic nano- and microparticles is exceptionally important. Magnetically modified cells have been used in many applications.

Microbial and algae cells (*Kluyveromyces fragilis* and *Chlorella vulgaris*) were modified with maghemite nanoparticles stabilized as low-pH ionic magnetic fluid, leading to the formation of magnetically labeled cells. This simple procedure allows using the prepared materials as new, cheap and easily obtainable magnetic affinity adsorbents to the removal of water-soluble dyes from polluted water sources by magnetic separation techniques. In cooperation with colleagues from the Institute of Physics (Warsaw, Poland) magnetically modified cells were investigated by means of electron spin resonance spectroscopy and conventional magnetic methods over the temperature range 4–300 K. The magnetic behavior of these materials was dominated by the superparamagnetic relaxation of isolated single domain maghemite particles, although a little amount of agglomerates was also present on the cell surface. The ferrofluid-modified microbial cells represent new interesting magnetic affinity adsorbents, which could be utilized in large-scale magnetic separation processes [2].

Brewer's yeast (bottom yeast, *Saccharomyces cerevisiae* subsp. *uvarum*) cells were magnetically modified by means of water-based, perchloric acid stabilized magnetic fluid. In collaboration with colleagues from Hacettepe University (Ankara, Turkey) the magnetically modified yeast cells were used for the biosorption of copper

ions. Biosorption increased with increasing pH and then reached almost a plateau value around pH 4.0. The yeast biomass was easily regenerated by 0.1M HNO_3 [11].

Alternatively, baker's yeast (*Saccharomyces cerevisiae*) cells were magnetically modified with magnetic iron oxide particles prepared by microwave irradiation of iron(II) sulfate at high pH. The modification procedure was very simple and fast. Both non-cross-linked and glutaraldehyde cross-linked magnetic cells enabled efficient sucrose conversion into glucose and fructose, due to the presence of active intracellular invertase. The prepared magnetic whole-cell biocatalyst was stable; almost the same catalytic activity was observed after 1-month storage at 4°C. Simple magnetic separation and stability of the developed biocatalyst enabled its reusability without significant loss of enzyme activity [28].

Magnetic modification of algae cells can be successfully used to simplify their separation from the culture media [21]. A suspension of inexpensive iron oxide magnetic microparticles prepared by microwave treatment was presented as a new agent for separating and harvesting *Chlorella vulgaris* from a highly diluted suspension. Separation efficiencies were tested (in collaboration with colleagues from the University of Chemistry and Technology, Prague) under various conditions (model environment, cultivation media, different pH), revealing not only a dependency on the pH value and amount of magnetic particles, but also the influence of the ions present in the culture medium. Phosphorus ions were identified as the medium component interfering with algae–magnetic particles interactions that are essential for magnetic cell separations in the culture medium. Phosphorus limited *C. vulgaris* cells were magnetically separated from the medium with very high efficiency. A rapid and complete demagnetization of harvested algae was achieved by acidic treatment at 40 °C under the influence of ultrasound [26]. The mechanisms leading to cell-magnetic particles attachment/detachment were studied using real experiments and predictions were made by colloidal adhesion (XDLVO) model [31].

Many diatoms are able to reduce gold and silver salts into corresponding nanoparticles that are bound to the diatoms surface. Subsequent magnetization with magnetic fluid led to the formation of magnetically responsive catalysts and antimicrobial agents. This approach was patented (collaboration with Technical University, Ostrava) [44].

A comprehensive book chapter describing large amount of procedures for magnetic decoration and labeling of prokaryotic and eukaryotic cells was written [39].

Magnetic materials for xenobiotics removal

Agricultural products, by-products and wastes, as well as selected inorganic materials (e.g., clays) have been widely investigated as possible low-cost adsorbents for removal of heavy metal ions and organic xenobiotics from water and wastewater. In order to improve their application potential, magnetically responsive derivatives have been prepared, which can be easily separated from water solutions and suspensions using an appropriate magnetic separator. In our department, magnetically modified adsorbents were used to remove water soluble organic dyes, heavy metal ions and radionuclides.

Water soluble organic dyes were adsorbed on several magnetic (bio)sorbents prepared from peanut husks [8], spruce sawdust [25], spent grain [9], spent coffee grounds [16] and montmorillonite [36]. In all cases adsorption of majority of tested dyes followed the Langmuir adsorption pattern and maximum adsorption capacities reached relatively high values.

Selected heavy metal ions were removed by magnetically modified adsorbents derived from yeast cells [11], spent tea leaves [35] or clays [45].

Magnetically modified biological materials were efficiently employed also for radionuclides separation and removal. In collaboration with National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention (Beijing, China) the biosorption of strontium ions on magnetically modified yeast cells was studied [4], while magnetically modified wheat bran was used for the adsorption of uranium [41].

The application of magnetic biosorbents for xenobiotics removal was reviewed several times [1, 3, 10].

Magnetic materials for enzymes immobilization

Magnetic carriers have been often used for the immobilization of enzymes and other important biologically active compounds. The main advantage of this system is the possible selective magnetic separation and recovery of magnetic biocatalyst form difficult-to-handle samples.

Ferrofluid-modified spent grain was utilized as a low-cost, biocompatible and magnetically responsive carrier for the immobilization of *Candida rugosa* lipase. Several immobilization procedures were tested using both native and poly(ethyleneimine)-modified magnetic spent grain. Activity of immobilized lipase per unit mass of carrier, operational stability, time stability and Michaelis constant were compared. In general, magnetic spent grain modified with poly(ethyleneimine) bound a smaller amount of active lipase than unmodified magnetic spent grain, but the operational and storage stabilities of enzyme immobilized on poly(ethyleneimine)-modified carrier were very high [24].

Magnetically responsive composite materials prepared by direct microwave treatment were used as carriers for enzymes immobilization. Lipase was immobilized on four postmagnetized materials, namely magnetic microcrystalline cellulose, powdered peanut husks, spent tea leaves and spent grain because they all contain hydroxyl groups in the surface structure. Two types of immobilization methods were used for the attachment of lipase to these magnetic carriers, namely periodate oxidation of hydroxyl groups and adsorption of enzyme on the surface of a carrier followed by glutaraldehyde cross-linking. Immobilization of enzyme in its active state was confirmed by measuring enzyme activity bound on a mass unit of a magnetic composite carrier [25].

An extremely simple, one pot microwave assisted procedure was developed for the preparation of magnetic chitosan-based carrier for lipase and β-galactosidase immobilization. Immobilized enzymes showed long-term stability without leaching of enzyme from the support and enabled their repeated use without significant loss of activity [29].

Other experiments were focused on the immobilization of recombinant CGTase JCGT8-5 on magnetically-modified silicates and biological supports (cooperation with the University of Food Technologies, Plovdiv, Bulgaria) [37], immobilization of lipase and β-galactosidase on montmorillonite modified with water based magnetic fluid [22] and on the immobilization of lipase on cellulose microparticles magnetically modified using a mechanochemical process [36].

A comprehensive review covering the topic of immobilization of biologically active compounds on magnetic carriers and their subsequent applications in biosciences and biotechnology was written [13].

Magnetic particles–based biosensor for biogenic amines

In collaboration with the Institute of Chemical Processes CAS, Prague, a fibre optic biosensor with incorporated magnetic microparticles for the determination of biogenic amines was developed. The enzyme diamine oxidase from *Pisum sativum* was immobilized either on chitosan-coated magnetic microparticles or on commercial microbeads modified with a ferrofluid. Both the immobilized enzyme and the metal organic ruthenium complex were incorporated into a UV-cured inorganic–organic polymer composite and deposited on a lens that was connected by optical fibres to an electrooptical detector. The enzyme catalyzed the oxidation of amines under consumption of oxygen. The latter was determined by measuring the quenched fluorescence lifetime of the ruthenium complex [27].

Magnetic materials for the separation of biologically active compounds

Magnetically responsive materials have often been used for the isolation and purification of biologically compounds.

Magnetic chitosan particles were successfully employed for large scale magnetic affinity separation of *Solanum tuberosum* tuber lectin from potato starch waste water. The procedure is very simple because chitosan directly interacts with the lectins having N-acetylglucosamine specificity [6].

Magnetic porous corn starch was prepared as an affinity and highly selective adsorbent for the efficient and simple scale-up procedure enabling one-step purification of cyclodextrin glucanotransferase (CGTase) from *Bacillus circulans*. Magnetic affinity separation enabled isolation of CGTase from cultivation media with high recovery after elution with alkaline buffers containing soluble starch. The majority of ballast proteins was removed during the purification process, the enzyme purification factor was 19 – 25 in different batches (cooperation with the University of Food Technologies, Plovdiv, Bulgaria) [17].

A review paper describing magnetic affinity separation of recombinant fusion proteins was written [5].

Study of magnetoferritin

In collaboration with the Institute of Experimental Physics SAV (Kosice, Slovakia) peroxidase-like activity of magnetoferritin was studied. Magnetoferritin is a spherical biomacromolecule with a diameter of about 12 nm. It consists of a protein shell composed of apoferritin that is surrounding magnetic nanoparticles of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3). Magnetoferritins with various iron content (loading

factor) were synthetically prepared and their peroxidase-like activities studied via the oxidation of the chromogenic substrate N,N-diethyl-p-phenylenediamine sulfate by hydrogen peroxide. Magnetoferritin with higher loading factor exhibited a higher peroxidase-like activity [34].

Review papers

Magnetic (nano)materials and related techniques have already found many applications. However, there are still many scientists with no or very low experience with these progressive materials, processes and technologies. That's why members of the Department wrote many (mostly invited) review papers and book chapters covering different parts of the biomagnetic research, namely:

- magnetically modified prokaryotic and eukaryotic cells [39]
- magnetic materials for environmental technology [3, 10]
- magnetic affinity separation of recombinant fusion proteins [5]
- immobilization of biologically active compounds on magnetic carriers [13]
- preparation and application of magnetic composite materials [1, 3, 18, 19, 38]
- magnetic materials for biomedicine [14, 15]
- magnetic techniques for the detection and determination of xenobiotics and cells [20]
- applications of biosynthesized metallic nanoparticles [40]
- poultry and dairy related application of magnetic particles [7, 12, 23]

Other research

In collaboration with the Okayama University, Japan, thermal treatment of biogenic manganese oxide (BMO) precipitates produced by manganese oxidizing microorganisms was studied. Thermally-processed BMO at 100 − 1000 °C was analyzed by XRD, XANES, SEM, TEM and nitrogen adsorption method to reveal the effects of heat-treatment on its crystal structure, Mn valence state, globule morphology, microstructure, pore size distribution and specific surface area. Morphology of BMO was maintained below 800 °C, while nanosheets composing BMO were changed into nanoparticles at 600 °C. The crystal structure was transformed from birnessite into hausmannite (Mn_3O_4) at 600 °C. The specific surface area was increased by heat-treatment between 300 and 500 °C. In cases of artificial manganese oxides, such changes of crystal structure and specific surface area were not observed, and combustion of organic materials produced by microorganisms in BMO probably has a large effect on them [30].

Due to their biochemical background, members of the Department of Nanobiotechnology cooperated with the Mendel University in Brno in order to study the influence of different levels of extruded full-fat soybean (EFFSB) in the diet on growth performance, apparent ileal amino acids digestibility, intestinal morphology, and trypsin activity in digests of broilers. The study showed that EFFSB at the level of 120 g/kg in grower broiler diet had no adverse effect on performance [32].

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Research Report of the team in the period 2010–2014

Institute	Global Change Research Centre of the CAS, v. v. i.

Scientific team	Division of innovation and adaptation techniques

The team covers broad research area including investigation of aquatic ecosystems in simulated environment on both lab-scale and industrial level, water balance and biomass allocation of forest ecosystems in a field as well as artificial conditions, and airborne imaging spectroscopy of selected types of ecosystems at different spatial and temporal scales

During the initial period of the Centre establishment the team mainly focused on building of the new technical infrastructure and a development of novel methods that it uses. The team infrastructure creation involved development of the platform for characterization and optimization of photosynthetic microorganism closely linked to breeding and molecular engineering platform, establishment of Flying Laboratory of Imaging Systems for airborne imaging spectroscopy and also the scientific group for plant water balance a biomass production in forest ecosystems as a verification for the eddy-covariance measurements and remote sensing.

Local team and international collaborations

In parallel, constitution of multidisciplinary, gender-balanced international team that can fully use research potential of the infrastructure was established. On 31st December 2014, the team had 30 members (11 senior, junior and postdoc scientists, 13 PhD students and 6 technicians or research assistants) and the team included scientists from 9 countries.

Strengthening and further extension of international collaborations led to establishment of strong international network consisting of more than 40 collaborating units and we put great deal of our effort to active involvement of the team members in international projects. As a result, the team has been included in several international projects:

a) CyanoNetwork consortium – the team acts as the coordinator of whole consortium and supports the activities of consortium members both financially and scientifically within “CyanoTeam-Local Team and International Consortium for Computational Modelling of a Cyanobacterial Cell” project budget.

b) EUFAR2 – (FP7), where the team took role in Joint Research Activity called HYLIGHT focussing of fusion of Light Detection and Ranging (LiDAR) and hyperspectral data.

c) The ESA projects related to satellite SENTINEL programmes. In the first one we contribute to the “RedEdge positioning” for estimation of vegetation chlorophyll content and Leaf Area Index, SENTINEL-2 mission.

d) FLEX – FLuorescence EXplorer. After two ESA “campaign oriented” projects run at CzechGlobe permanent ecosystems sites in 2012-14 (the first results will be published in Geophysical Research Letters), the team coordinates the HYPER (HyPlant Processing Experiment) project. The main objective of HYPER is to establish a processing chain for the newly developed set of hyperspectral scanners, where the

first one performs in the fluorescence region and the other two cover the full range of the reflectance spectral region.

Partial list of international collaborators:

- University of Vienna, Institute of Physical Chemistry, Austria
- University of Vienna, Institute of Theoretical Chemistry, Austria
- University of Copenhagen, Department of Plant and Environmental Sci., Denmark
- ABO Akademi University, Finland
- University Turku, Finland
- Hebrew University, Israel
- Alfred Wegener Institute, Germany
- Forschnungszentrum Jülich, Germany
- Humboldt University zu Berlin , Institute for Theoretical Biology, Germany
- University Freiburg, Institute of Biology, Germany
- University of Rostock, Germany
- TU Dresden, Germany
- University Zagreb, Croatia
- University Novi Sad, Serbia
- Slovenian Forestry Institut, Slovenia
- TU Zvolen, Slovakia
- Amsterdam University, Netherlands
- Univeriteit Maastricht, Netherlands
- Wageningen University, Netherlands
- K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences,Russia
- Grand Canyon University, College of Arts and Sciences, USA
- Idaho State University, USA
- Oklahoma State University, College of Arts and Sciences, USA
- Purdue University, USA
- Saint Luis University School Of Medicine, USA
- Washington University St. Luise, USA
- University of Colorado at Boulder, USA

and more …

The team published during the period 2010-2014 39 impacted peer-reviewed publications, 4 book chapters and books, and 41 other scientific publications.

Research

The team research was partially focused on development of platform for characterization and optimization of photosynthetic microorganisms in simulated (aquatic) environment of photobioreactors that allows to explore perspective of selected microorganisms for use in biotechnologies, investigation of their primary production and carbon sequestration capacity, investigation of stress and adaptation mechanisms, and other physiological and biochemical properties. The platform consists of series of high-tech flat panel photobioreactors with real time control and monitoring capabilities and high throughput imaging and sorting flow cytometry. Associated with development of new platform was also development of novel methods for characterization and breeding that extends and improves previously published methods with precise control of cultivation parameters and significant reduction of

experimental time required for comprehensive biosystem characterization. Description of this platform has been published in “Engineering in Life Sciences” journal. To show potential of the platform we also investigated strongly dynamic behaviour of model cyanobacterium *Cyanothece* ATCC 51142 that has potential for use in biotechnological applications. Basic characterization of this strain was first published in the “Journal of Biotechnology” and later rhythmic behaviour of the same strain was characterized in detail and published in one of the most prestigious and highly cited multidisciplinary research journals “Proceedings of the National Academy of Sciences of the United States of America”. The characterization led to discovery of new evidence of important rhythmic component that reflects the true metabolic state of investigated model microorganism and clarifies the interrelationship to circadian rhythms that are believed to have dominant role in metabolic state control.

Both the lead and corresponding author are long-term researchers of the team and the work is continuation of their research first published in 2009. The team authors designed and performed research, analysed the data and wrote the paper. Dr. Lou Sherman as the external co-author of this paper contributed to writing as an expert on the organism. Strong international collaboration resulted from this publication including establishment of core for international consortia CyanoNetwork.

We also successfully characterized and optimized growth requirements of another model cyanobacterium *Synechocystis* sp. PCC 6803. This study was performed by the team researchers with one external co-author (former member of the team) who contributed mainly as a consulting expert for experiments design and results interpretation.

Building on detail knowledge of growth characteristics of model cyanobacterium *Synechocystis* sp. PCC 6803, we further studied heat stress response in this organism. We revealed important role of signal protein Hik34 as well as of several heat-shock proteins in long-term heat stress tolerance in this model cyanobacterium. The study was published in journal “Life” and it was result of equal collaboration between the team researchers and Laboratory of intracellular regulation, Timiryasev Institute of Plant Physiology, RAS.

Essential part of the platform is fluorescence activated cell sorting that extends the capacity of the platform towards controlled evolution/breeding of various microorganisms. Methods and protocols for fluorescence staining specific for light sensitive photosynthetic microorganisms were designed with respect to staining limitations (extracellular polysaccharides formation, membrane/ cell wall permeability, etc.). The methods and protocols allow routine analyses such as quantification of cell morphological properties, quantification of physiological parameters like cell viability and DNA topology, and identification of lipid droplets formation. Routine method for quantification of bacterial contamination was also established that provides important information for consistent and reproducible culturing, missing in most published experimental works. All data generated using these methods contain single cells level information from statistically significant sample of thousands cells per sample. Successful establishment of sorting technique and organism specific cytoflometric parameters for non-destructive sorting resulted already in first breeded subpopulation with requested, “on-demand”, set of properties of cultivated model algae *Chlamydomonas reinhardtii.* These results will be further used for targeted engineering of new hyper productive strains that will increase the efficiency of interesting compounds production by selected photosynthetic microorganisms.

To address challenges of production and aquatic systems monitoring on large scale/industrial level we focused also on characterization and optimization of

cultivations in experimental large scale photobioreactor. The experimental large scale photobioreactor consists of two inoculation units (flat panel photobioreactor-volume 25L and 120 L) and three types/different designs of large scale units (tubular, spiral and 3D photobioreactors). Photobioreactors are operated together with fully automatic media mixing and harvesting units. Long-term operation of photobioreactor was tested during second half of year 2014.
Additional research activity linked to aquatic systems research was evaluation of microalgae potential for use in wastewater treatment applications. Selected algal species with predicted potential of nutrient remediation in wastewater treatment processes were isolated from different natural locations. Several microalgal and cyanobacterial species were immobilized to form algal biofilm. The algal biofilm was cultivated on different artificial and natural substrates to choose and design optimal surface for algal biofilm cultivation. Developed algal biofilm was tested and optimized for selected nutrients removal capacity. Results of this study are submitted in the field highly ranked journal “Water Research”. The system of biofilm cultivation will be used for pilot municipal waste water treatment system.
As a part of above mentioned research activities we established collection of characterized photosynthetic microorganisms for biotechnological applications, suitable for both basic and applied research. This collections includes for example model cyanobacteria strain *Synechocystis PCC 6803 (*5 strains including Ethylene producing mutants), *Cyanothece ATCC 51142* strain with strong biological rhythms and potential to produce hydrogen. Further included are model algal strains *Chlamydomonas reinhardti* (3 strains including lipids producing mutants) and three photoautotrophic as well as mixotrophic and heterotrophic suspension cell cultures (*Arabidopsis thaliana, Tomato and Chenopodium rubrum*).
To extend our understanding of dynamic behaviour of complex biological systems we developed web-based platform for modeling of photosynthetic processes (www.e-photosynthesis.org and www.e-cyanobacterium.org) in collaboration with Systems Biology Laboratory, Masaryk University in Brno headed by Dr. David Safranek. The platform provides an easy and intuitive navigation through the structure of photosynthetic system, storage and presentation of wet-lab experiments, and unified representation of related biological networks. The platform serves as both educational and research oriented tool. Scientific results generated during development of this platform were published in “BioSystems” journal and two times in “Electronic Notes in Theoretical Computer Science” journal. The team established base for development of the web-based modeling platform, first published by the team members as a book chapter, already in 2009. The co-authors from local research community (FI MUNI Brno; UPOL Olomouc) contributed on informatics and computational parts and the team contributed to biology and mathematics related parts of the study.

Under the research focused on tree/ stand water balance and biomass production, the seasonal variation of both characteristics and their components were investigated during growing seasons in forest ecosystems of different structures and in the experimental facilities with simulated future climatic conditions (i.e. air elevated CO2 conditions and drought). Different silvicultural treatments were tested there as well. The stand structures and biomass data were inventoried in a field conditions including position of each tree in a stand by the FieldMap. For an accurate estimation of tree/stand biomass the set of allometric equations was assessed after harvest analysis of sampled trees only in chosen stands. For phenolgical observations of buds and leaf area development a set of cameras was established in the four different forest

ecosystems. There, also stem diameter increment, tree height and leaf area index dynamic were monitored during the growing seasons. For leaf area index estimation a new instrument for forest practice was developed under collaboration with the company Photon Systems Instrument spol. s. r. o. This instrument LaiPen LP100 is fully comparable with the world standards like the LAI-2000 and 2200 (Li-Cor, USA) etc. The two software, one for communication and row data recalculation and one for final calculation of many other parameters and data visualisation throughout a google map were developed at the same time. Certified methodology for practical and easy LAI estimation in a non-mixed canopies was also published.
The mentioned practically oriented research was also utilized in education activities in branch of ecology and silviculture in Mendel University in Brno.

For terrestrial ecosystems research we mostly focused on development of methods for post-processing and exploitation of airborne hyperspectral data from reflectance and thermal domains (VNIR, SWIR, and TIR), waveform LiDAR airborne scanner data, and field spectroscopy measurements and development of methodologies for applying new categories of airborne information in the assessment of biochemical properties of forests, and in agricultural applications.
Despite the fact that the large part of the infrastructure was only finished at late phase of the project, several important results have been achieved during this period:
1. A new method for retrieval of spruce leaf chlorophyll content from airborne image data using continuum removal and radiative transfer was developed in cooperation with foreign partners. Radiate transfer modelling is one of key orientations of the team in image spectroscopy.
2. There are two methodologies resulting from cooperative projects in applied agricultural research. The first one contributes to identification of sensitive zones for water vegetation stress on the base of remote sensing methods. The second one, which will become a part of nonlegislative directives for pasturing systems in the Czech Rep., uses airborne hyperspectral and LiDAR information to assess grassland biomass and sensitivity of vegetation cover to the degradation and soil erosion.
3. A monograph “Airborne remote sensing: theory and practice in assessment of terrestrial ecosystems” was published in 2014. The monograph was an output of the HYDAP project (http://hydap.czechglobe.cz/en) in which six Czech and seven foreign partners participated and the team acts as coordinator. This was a particularly important project for the team because it brought together scientists across many disciplines dealing with assessment of different ecosystems, as well as remote sensing specialists. The monograph, which can be considered a contribution to hyperspectral and Lidar education materials, is also a high gain for the RS team from the viewpoint of establishing collaborations on both the national (Mendel Univ. in Brno, Technical Univ. in Brno) and international levels (Idaho State Univ., University of Colorado at Boulder, Forschnungszentrum Jülich).

To involve molecular biology techniques in our research, that are necessary for deeper understanding of complex biosystem network structures and functions, we established during second half of 2014 molecular laboratory equipped with two instruments for polymerase chain reactions (PCR). Classical PCR instrument for standard qualitative targeted DNA sequence amplification. The other, state of the art PCR instrument for quantitative gene expression analysis and for high resolution melting analysis that allows to use a powerful technique for detection of mutations, polymorphisms and epigenetic differences in double-stranded DNA. Vertical and horizontal electrophoresis

for separation and analysis of DNA, RNA and proteins based on their size and charge were also installed in the lab. In collaboration with experts from network of our international collaborators we were able to establish on short time scale optimized protocols for DNA and RNA extraction from various cyanobacterial and algal strains.

Industrial collaboration and applied research

The team established strong collaboration with Czech SME company Photon Systems Instruments, spol. s r.o. This collaboration led to development of novel instrumentation for biotechnology, joint project proposals, scientific advisory and technology transfer. Additionally, these instruments are actively used in education. The web-based tools www.e-photosynthesis.org and www.e-cyanobacterium.org offer repository of experimental data generated using the company instruments and repository of models related to parameters measured by these instruments.

There is also ongoing … XXX … development of green factory biotechnologies for in-house assimilation of waste water phosphorous contamination and flue gas carbon dioxide assimilation. The team members supervise undergrad students on joint topics with the company.