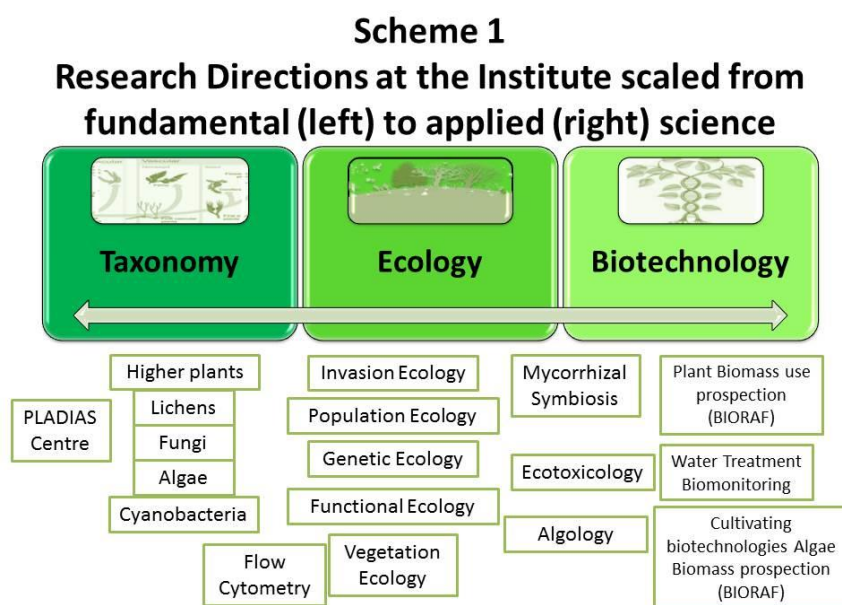


## Characteristics of main research directions investigated at the institute and the achievements 2010–2014

Institute	Institute of Botany of the CAS, v. v. i.
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The major research topics at the IB focus on species, populations and communities of plants. The following diverse fields of study are represented: taxonomy, biosystematics and phytogeography of higher and lower plants (e.g., algology, ecotoxicology, mycology and lichenology), plant ecology (population biology, synecology, comparative and production ecology, ecophysiology), mycorrhizal symbioses and geobotany of large complexes of vegetation, including vegetation mapping and paleoecology. Research is especially concerned with biodiversity and evolutionary trends among plants, ecology of invasive species, responses of plants and vegetation to environmental changes and the mechanisms that enable species to coexist in ecosystems. An important aspect of our research is also searching for applicable topics and their practical exploitation in environmental policy and decision-making for bioconservation and nature protection, and in biotechnology using algae and fungi.



As is shown in **Scheme 1**, research at the IB covers a very broad range. The majority of disciplines are interconnected, and due to that networking, truly fundamental scientific disciplines have potential to be applied and brought to commercial exploitation. There are numerous explicit examples of this interlinking and details are given in reports of particular research groups. For example, fundamental taxonomic work on dandelion (*Taraxacum*) can be exploited in the selection of species of the genera that have potential for production of natural rubber as an alternative to that from *Hevea*. The isolation and characterization of new strains of algae and cyanobacteria also represent a fundamental advance, not only for ecological and physiological sciences, but for bioprospecting and future practical uses of these organisms in nutraceuticals. The discovery of the unique omega acid-producing algae, *Trachydiscus*, in cooling ponds of a nuclear power-station is an example. Numerous

other examples can be highlighted, where thorough taxonomical knowledge and expertise has served as an essential tool for further ecophysiological studies and other research groups have bridged the gap for exploitation of that knowledge (research on mycorrhizal symbiotic fungi, paleobotany, dendrochronology, ecotoxicological research or research on GIS). Between fundamental research and biotechnological research there are several overlapping ecological disciplines. Studies of plant invasiveness, genetic ecology, functional ecology and population ecology are topics where further fundamental knowledge on behaviour and functions of target organisms is acquired and applied in nature conservation, cultural landscape management, policy making etc. These ecological topics lead, for example, to recommendations on efficient land use of abandoned fields, optimal protection of biodiversity and plant community structures, or bioindicative values of organisms (algae, lichens etc). The ecology of invasive plants can reveal crucial data on the dangers of alien species spreading through a landscape. Full details of achievements are described in reports of each group, whereas in this summary, only the most significant topics and achievements are highlighted.

Regarding particular achievements, the most outstanding research has been carried out by the **plant invasion ecology and GIS group**, focused on projections of future trends for plant invasions in Europe under contrasting socioeconomic scenarios, interactions between alien plants and organisms of other trophic levels on an historical time scale, forest history and its implications for management and biodiversity, population ecology of invasive species, and evolution of invasive traits and local adaptations. Their work included a global assessment of the impacts of plant invasions on resident species, communities and ecosystems, although their focus was on locally important issues such as alien plants in the Czech Republic, taxonomic challenges related to biological invasions, and implications for management and nature conservation. A classification of alien plant and animal species based on impacts of their invasions - "towards an International Union for Conservation of Nature Black List" - resulted in a proposal for a standardized method to evaluate the magnitude of environmental impact, as driven by mechanisms recognized by the IUCN. The most prolific research team has published their studies in the highest impact factor journals such as Science, Nature, PNAS, Ecology Letters and others. The international recognition of research leader prof. Pyšek speaks for itself, and he was ranked as one of the best researchers in the Czech Republic (ISI Highly Cited Researcher in ecology) in 2014, and is 15th amongst the most cited European researchers in plant sciences.

Another flagship of research at the IB is the research group in **taxonomy** and the associated department of **flow cytometry**. This part of the IB is a major national research centre dealing with biosystematics, phylogeography, evolution and population biology of different groups of autotrophic and heterotrophic organisms. A combination of classical taxonomic approaches and cutting edge molecular biology has promoted advanced and internationally recognised research. The group is responsible for completion of major monographs, including 9 volumes of "Flora of the Czech Republic" – a truly intergenerational work completed within the last research period. This is the first Czech multi-authored flora elaborated as a set of monographs of each genus or family, based on up-to-date biosystematic knowledge and expert taxonomic re-evaluation of field observations, experiments, herbarium and literature data. It is an essential work of the Czech botanical literature, of long-term value, containing a vast wealth of information about native and introduced plants. Another major monograph, in which numerous research groups took part for a long period, is "Phytocartographical synthesis of the Czech Republic".

Among major scientific achievements in **flow cytometry** were studies on evolutionary, ecological and taxonomic consequences of variations in nuclear genome size, in a number of plant groups originating from four continents. The data serve as a taxon-specific marker for prediction, as genome size is associated with several phenotypic, physiological and/or ecological characteristics, and for understanding the dynamics of genomic evolution. Their study of alien species provided the first quantitative support for an association of small genome size with invasiveness. A robust test of how genome size and trait variation can influence the performance of Australian acacias (introduced around the world) was presented. In addition, major limitations of flow cytometry in field research were overcome due to the development of a protocol (based on glycerol-preserved nuclei) that is now the most reliable method of sample preservation for genome-size research. Another important topic for this group was a study of reproductive systems, their evolutionary importance, and the role of hybridization in evolution and plant diversity. The application potential of these taxonomic studies in interspecific hybridization lies in conservation. Hybridization may pose a threat to endangered species as it can cause genetic erosion or even amalgamation of endangered species into hybrid swarms. Results have very important implications for protection of endemic species. The plant flow cytometry group are indeed global leaders in this field.

One of the major achievements in **taxonomic work** was related to evolution and phylogeny of complex plant groups. Phylogenetic reconstructions based on molecular sequence data are, at present, the dominant method for studying evolutionary relationships among taxa. A combination of molecular and karyological data has allowed us to assess the evolution of karyotypes and the role of genomic duplication and chromosomal change in several groups of plants (e.g. Potamogetonaceae, Asteraceae). Taxonomic monographs of critical groups are the basis of taxonomic work at IB. The importance of alpha-taxonomy cannot be underestimated because biological material is frequently misidentified and the large databases for species and DNA sequences contain an alarming proportion of incorrect information. Taxonomic monographs are comprehensive accounts of all taxonomic data relating to particular plant groups. They are based on a thorough analysis and exhaustive synthesis of information from a wide range of approaches. At the global level, they are an irreplaceable source of information. For example, the genus *Taraxacum* is one group that is difficult to treat taxonomically, and thus was investigated in several different regions including Central China, the Himalayas and Central Asia. Results were published in the prestigious compendium of the "Flora of China". Involvement of the team in prestigious international monographs was, and still is, very significant (Euro+Med PlantBase, Rothmaler's Exkursions flora von Deutschland, The Jepson Manual of Vascular Plants of California, Flora vascolare della Calabria, and Catalogue of the Vascular Plants of Madagascar). Team members conducted numerous studies addressing currently unexplored topics in lichen taxonomy, ecology and evolution. Recent field surveys yielded dozens of new country records and rediscoveries of species previously regarded as vanished. An extensive synthesis of available data resulted in a checklist and the Red List of lichens of the Czech Republic (over 1500 species of which nearly 40% are threatened and 9% extinct). Team members contributed to exploration of lichen diversity in other countries (participation in German lichen flora studies and exploration of the high Andean lichen flora). A significant contribution was also made in the field of Ascomycetes. Multidisciplinary research, including multigene phylogenetic analyses, bioinformatics, architecture of secondary structural elements of ribosomal RNA and novel approaches

to structural phylogenetics revealed 12 new life histories in the Sordariomycetes and identified 14 new holomorph genera.

The department of **population ecology** is a major national group dealing with plant population biology, ecology and genetics, community ecology and evolutionary biology. Their results have also found applications in conservation as their work is conducted in close collaboration with national park administrations, and the National Agency for Nature and Landscape Protection. Their major scientific finding, through spatially explicit modelling, is that spatiotemporal dynamics are not an outcome of niche differentiation. Species replacement patterns resemble a neutral process with sets of species differing in the speed at which they fill empty spaces. The results of ecological and dynamics research also served as a guide for management of dry grasslands - the species-richest and most valuable habitats in Europe. Past and present landscape structures were explored and current plant species dynamics were used for predicting future species dynamics. Research on ecology, population biology and evolution of polyploidy systems showed that to understand species dynamics in the landscape requires an understanding of the evolutionary potential of dispersal traits. The oceanic islands, as an analogy to a fragmented landscape on the mainland, was used in the study. Other research showed that polyploidization is a major factor driving species diversification. While many previous studies explored morphological differences between different cytotypes, researchers have only relatively recently started exploring the distribution of cytotypes in landscapes, attempting to understand the distribution and migration of different polyploidy taxa. Collaboration with a team from Stockholm University, Sweden, showed the crucial importance of cytotype for plant-insect interactions. One of the cutting edge topics of the team is epigenetics - transgenerational plasticity and epigenetic variation in the ecology and evolution of plant populations. Research revealed that heritable epigenetic variation can influence not only individuals but also their interactions with other organisms and the evolution of natural populations. Strong maternal effects demonstrated that offspring always performed better if they were exposed to the original maternal environment. A complementary study on *A. thaliana* clearly demonstrated that epigenetic diversity of populations can improve the stability of plant populations and consequently the functioning of whole ecosystems. In studies on biotic interactions and determinants of invasion success of exotic plants, the group found that exotic plants are influenced by soil pathogens and leaf herbivores similarly to native species. They do not differ in competitive ability or in allelopathic effects when compared with native, closely related plants. They demonstrated interesting mechanisms of biotic interactions, working through plant - soil feedbacks, which are responsible for the successful invasion of species. Invasive plants suppressed native species by accumulation of soil pathogens in younger invasive populations (*Heracleum mantegazzianum*), or by means of changes in behaviour of invasive populations where they lost susceptibility to their own pathogens. A strong effect of invader on soil quality was also demonstrated in the study of *Parthenium hysterophorus*, a major invader in Nepal with a substantial impact on plant diversity and the economy. Understanding the determinants of biological invasiveness is a key prerequisite for successful eradication of invasive species. Two botanical studies were notable; one from Nepal brought new insights into traditional herbal medicine and the other, on an isolated tropical inselberg in South America, revealed the importance of cockroach pollination for plant reproductive success.

Research on **genetic ecology** focused on various aspects of plant biology using tools of molecular biology as well as population ecology, particularly on (i) genomic studies, (ii) biogeography and phylogeography, (iii) evolution and phylogeny and (iv)

biosystematics. Genomic studies in allopolyploid wheatgrasses represent fascinating examples of reticulate evolution. The ability of many species of this group to hybridize with other species is remarkably high and most likely accounts for the reticulate structure of the tribe. The tribe includes economically important grasses such as cereal and forage crops, as well as troublesome weeds of worldwide distribution. Significant innovation in traditional and low resolution fossil-based studies, using modern population genetics and phylogeography, has produced molecular data of high resolution that has enhanced our knowledge in this area. The development of novel molecular markers for further phylogenetic and population genetic studies has been carried out for three groups of plants, Hieracium, Alnus and Cardamine. Research on the Hieracium, traditionally subdivided into three subgenera, resulted in the discovery of unknown taxa from the territory of the Czech Republic and detection of several rare morphotypes. Biodiversity hot-spots are regions harboring evolutionary heritage from ancient or recent geological epochs, i.e. evolutionary museums or cradles. Results showed that species-pools of most habitat types were characterized by over- or under-representation of lineages from at least one epoch, dating back to the origin of angiosperms. These patterns are not captured by mean phylogenetic distances. Habitat types vary in the way they represent diversity of lineages from different epochs. Abiotic environments explained, on average, 53% and up to 75% of the variance in the number of lineages per epoch. Specifically, warm and dry habitats tended to harbour lineages dating back to warm and dry epochs. Human impact during the 20th century correlates with major declines in habitats harboring lineages from the Early-Cretaceous to Paleogene. The taxonomy and ecology of Bolboschoenus species were extensively studied and the unifying effect of a water environment, softening climatic extremes, frequently contributed to the formation of large circumpolar areas of distribution of aquatic and wetland plants.

Research into **mycorrhizal symbioses** produced a wealth of original data on the diversity of mycorrhizal fungi and the role of mycorrhizal symbioses in many ecosystems. In addition to fundamental science, there were very significant collaborations with industrial partners. Though the publishing achievements have not been as high as other teams, the department has been, by far, the most successful in receiving financial support for applied projects (in total over 160 M Czech koruna in the last 5 years). The major scientific achievements were in the role of arbuscular mycorrhiza (AM) in plant stress tolerance (interaction with heavy metals, adaptation to serpentine soils, ecology of AM in post mining sites). Important applied research, carried out in collaboration with Chinese partners, was on the diversity of fungi in vegetable farming in China. Fundamental research was also carried out on the occurrence of fungi in aquatic environments, where surprisingly frequent colonisations by AM and endophytic fungi were recorded. Research on communities of AM fungi, as affected by invasions of non-native plants with potentially serious consequences, revealed that comparable numbers of ectomycorrhizal fungi interacted with invasive as with native trees. This is in contrast to the situation in the Southern hemisphere. A very significant achievement was that AM fungi were identified in the field using mitochondrial DNA analysis of the large ribosomal subunit as a suitable tool to distinguish between genotypes of *Rhizophagus irregularis*. The field-inoculated strain was specifically traced, for the first time in the history of this research, and its survival was verified after two years in the field. As there are only very few groups, worldwide, investigating ericoid mycorrhizal fungi, all research carried out in this field is unique. A new type of mycorrhizal symbiosis was also discovered.

Research in **functional ecology** focused on the role of plant ecophysiological traits, reproductive strategies, morphological and anatomical features for plant functions (reproduction, growth and survival) in a changing environment, including socio-economic changes in traditional land-use practices in Europe, and global climate changes in warming-prone alpine and arctic areas. Functional traits, any characteristic of an organism linked to their fitness, provide a powerful means of addressing crucial theoretical and applied ecological questions, through their dual roles as indicators of mechanisms driving differences in species assemblies into communities and as predictors of ecosystem-services. The functional trait composition therefore represents the interface between the formation, and the effect, of biodiversity on ecosystems. The department has a long-standing tradition in contributing to developments in this key aspect of plant community research, particularly developing and testing new mathematical techniques. In several studies on the importance of functional traits in the assembly of communities and biodiversity, reviews of existing data provided insights into key methodological issues. This resulted in the production of a special issue on the importance of functional traits and functional trait diversity between, and within, species. Their research showed that biodiversity can affect ecosystem services in different ways, but mostly this occurs via species traits, both in terms of dominant trait values, or trait diversity, in a community. These effects are expected both within one trophic level and across trophic levels, as further demonstrated by following publications showing the importance of trait connections between different types of organisms in controlling ecosystem services. Significant research occurs in the Arctic archipelago Svalbard and on the ecology of Paramo in the high Andes. Moreover, this department was responsible for linking international long-term interdisciplinary research on plant functional biodiversity with its dynamics in Ladakh, W Himalayas. An extensive floristic database (>4200 georeferenced plots covering the elevational range from 2640 to 6150 m) throughout Ladakh was assembled during 1997-2014, together with information on individual species life history traits (eco-physiological trait database for 500 species and species habitat preferences and their evolutionary relatedness). The Department played a key role in ecological restoration in central Europe, cooperating in this research with the Department. of Botany, University of South Bohemia, and with the Institute for Nature Environment, Charles University in Prague. The database now contains nearly 3000 phytosociological records, on about 1200 species, and it also includes main environmental data and species functional traits. Such a data set is unique in the world and its expansion promotes research on vegetation succession to a new qualitative level. Results have contributed to a novel successional theory, as well as to practical ecological restoration. Several studies on oligotrophic meadows, focused on biodiversity, as well as functional aspects of meadow species in response to short term variations in management, were conducted. Their research showed that response to management depends on a productivity gradient, and once a meadow is not limited by nutrient availability, changes in species composition and diversity after cessation of management are rapid, and may be irreversible. Although the research showed some general patterns in responses of functional traits to abandonment, they also disproved some earlier generalizations and failed to show the same pattern of trait responses over regions and time scales. Specifically, storage economies of carbohydrate compounds in roots and rhizomes of meadow plants, although responding to management changes, were mainly species specific. The department has a 25-year tradition in ecophysiological research on aquatic carnivorous plants, in particular *Utricularia* and *Aldrovanda*. Research is oriented mainly towards growth, traits of turions (photosynthesis, mineral content, frost-

resistance), photosynthesis and respiration of shoots, N<sub>2</sub> fixation by shoots and traps, K<sup>+</sup> reutilization in senescent shoots, and on biophysical and biochemical aspects of *Utricularia* trap functions. Significant research was also carried out on the ecophysiology of photosynthesis in peatland bryophytes, particularly in *Sphagnum* mosses, and the nature of calcium tolerance in peatland mosses. The main results were (i) characterization of the relationship between mineral-nutrient availability and the stoichiometry of moss biomass with respect to environmental alkalinity; (ii) clarifying the physiological nature of calcium tolerance in mosses; (iii) clarifying the global genetic diversity of the key calcium-tolerant *Sphagnum* species; and (iv) distinguishing between environmental and competitive effects in the ecology of fen mosses. Interesting international research conducted in montane tropical forests of Western Africa has identified these areas as biodiversity 'hotspots' with extraordinary species diversity and exceptional concentrations of endemic species. This research focused on 1) plant community composition, structure and diversity using a system of permanent research plots; 2) diurnal and seasonal courses of radial tree growth using a Dendrometer Increment sensor and X-ray wood densitometry; 3) relationships between forest tree and bird diversity; 4) plant-pollinator interactions. This study showed that pollination systems occurring in the Old World followed similar evolutionary trends as systems in the New World, including hovering hummingbirds. Significant research was also conducted in the field of dendro- and herbo-chronology. The dendrochronological laboratory of the Department of Functional Ecology is now the best equipped in the country, utilizing the most up-to date facilities such as an automatic dendrometer increment sensor, X-ray wood densitometry, wood and herb microtome sectioning, and wood cell analyses using WinCELL and PAST software. This group deals with different aspects of tree-ring research and forest ecology in different parts of the world (Czech Republic, Korea, Indian Himalayas, Cameroon). The main scientific topics over the past five years were: 1) the impact of climate change on forests; 2) reconstruction of forest disturbance regimes; 3) analysis of spatiotemporal recruitment and mortality processes in forests; and 4) comparative analysis of anatomical variation in Himalayan plants. The department is focused on clonal and bud bank traits, with a long term perspective, and it hosts the only global database storing these traits; this is widely used by many scientists.

**The Phycological research group** is one of the major centres in its field and it is a global leader in the systematics of cyanobacteria. Taxonomic work covers the whole globe. Members of the team authored handbooks on cyanobacteria and algae that are widely used by other scientists worldwide. The highest impact is recent work of prof. Komárek - a completion of the third (final) part of the monograph on cyanobacteria, which was published within a compendium "Süsswasserflora von Mitteleuropa". This book was long awaited by researchers and students working with cyanobacteria worldwide. Research on diversity of freshwater coccoid types in Antarctica and islands of the NW Weddell Sea was also a great achievement as this topic had not previously been studied. Taxonomic revisions of many cyanobacterial and algal groups are of great importance e.g. clarification of *Gloeobacter violaceus*, a primitive thylakoid-less cyanobacterium is commonly used as a model organism for cyanobacterial phylogenies. Economically important cyanobacterial types forming water-blooms were studied. From the phytogeographical and ecological point of view, attention was paid to plankton dominant in Central Europe, including invasive species from western tropical parts of the Americas, or South America, as well as dominant species from both Polar Regions. From non-planktonic heterocytous types, economically important members of the family Scytonemataceae and

Stigonemataceae were investigated. With their ability to fix atmospheric nitrogen, they play an important role in autotrophic soil microflora worldwide, but their growth and impact is especially pronounced in tropical regions. In other studies, numerous cyanobacteria have been found as new species. A complete checklist (505 taxa) of cyanobacteria reported from the Czech Republic was compiled for the first time. It includes recent records, as well as critically evaluated historical data, and reflects up to date taxonomy of the group. The microflora of cooling towers and other man-made environments colonized by cyanobacteria were also studied and it was revealed that such habitats may be highly specific in terms of species composition, i.e. they harbour organisms not present in the surroundings, or contain completely unique taxa. Research dealing with taxonomy and ecology of particular species is urgently needed in order to avoid force-fitting items into incorrect taxonomic groups, which may lead to incorrect biogeographical conclusions. Diatoms, green algae and cyanobacteria all around the world have been studied from different perspectives. An almost complete compendium of diatom flora from several Maritime Antarctic Islands (James Ross Island Group and the South Shetland Islands) was constructed, including seven new species. A set of 18 freshwater and morphologically similar marine samples of *Ulva* were collected from inland and coastal waters throughout Europe to assess their taxonomic identity and invasive potential. An unknown green alga (order Ulvales) was also identified as a dominant macroalga in the rocky littoral zone of Lake Garda and morphological and phylogenetic analyses indicated that the alga was from the genus *Jaoa*, considered endemic to China. Taxonomic revisions were performed and several novel algal and cyanobacterial genera from the Polar Regions were described, e.g. *Wilmottia*. Research in Antarctica was focused on the ice-free parts of the Ulu Peninsula, and the James Ross Islands, where the J.G. Mendel Czech Antarctic Station is located. The first comprehensive limnological survey of this region was performed, describing the origin, bedrock geology, geomorphology, hydrological stability and physical and chemical characteristics of a representative set of freshwater lakes. This study represents a background for further ecological research in this area. A unique microflora, dominated by filamentous cyanobacteria, and including a high proportion of endemic species, was reported from the lakes. In several old shallow lakes, three new species of cyanobacteria and one green alga were described. The lakes represent a biodiversity hotspot in the extreme conditions of Antarctica. Experiments were performed in Svalbard, Río Tinto in Spain and in Iceland. Research was conducted on unique biological soil crusts that are crucial for local ecosystems and improve resistance to wind and water erosion, supplying nutrients to the environment. High-elevation ecosystems, especially their subnival zones, are among the most extreme environments. Knowledge on the functioning of these outlying ecosystems is still very sparse. High-elevation cold deserts of the Himalayas are the least explored environments on Earth. Following up on projects from a previous period, research on the diversity of snow algae was performed in the Czech Republic, Bulgaria and Spain. Except for snow algae, these high-mountain cyanobacterial flora were studied in cooperation with Slovak partners in the High Tatra Mountains, or the previously unexplored Chirripó National Park, Costa Rica where 52 morphotypes of cyanobacteria were found; 48 of these taxa are new species records for Costa Rica. An important subject in algal research has been the study of polyunsaturated fatty acids in eustigmatophycean algae, particularly in *Trachydicus minutus*. Its physiology, morphology, life cycle and biogenesis of lipids were described and its phylogenetic position was revised. A monograph dealing with physiological aspects of this species was published. Due to the relatively high growth rate and its eicosapentaenoic acid



(EPA) content, this species proved to be of potential for commercial exploitation and the original strain was subsequently patented. Furthermore, the effects of nutrient deficiency on the production of EPA were studied in this strain using a specific combination of cultivation conditions.

Research in **vegetation ecology** was mainly focused on Holocene vegetation development in the Carpathians and mountain ecosystems in the Sudetes, and the reconstruction of Holocene ecosystems on the basis of modern analogues in Siberia. Various methods and a multi-proxy approach were used for the reconstruction of past vegetation and environments. Research in the southern Ural Mountains searched for recent parallels with Holocene vegetation in central Europe. The hypothesis was that open pine and larch forests in Central Europe during the late glacial were rich in light-demanding species. We showed that the spread of birch, aspen and oak in the early Holocene did not affect the diversity of vascular plants. Another part of the palaeoecological research dealt with lowland regions where the record of the Komořanské Lake was evaluated. Three main research fields were the historical development of forests, changes in biodiversity in the 20<sup>th</sup> century and related conservation issues. The historical research involved application of specific approaches (studies of old manuscripts and maps) and GIS analysis. Recently, this research was developed through the work of an interdisciplinary team, consisting of palaeoecologists, ecologists, historians and archaeologists, and supported recently (2012–2016) by an ERC frontier research grant, LONGWOOD, lead by P. Szabó. The project team, consisting of about 15 people, carried out unique research on the long-term history (Neolithic to present) of Czech forest ecosystems. Joint dendroecological and historical ecological research proved the dominant role of coppice management in growth and recruitment of oaks in the same region. A significant part of the research was devoted to wetland vegetation. Peatbogs are important natural habitats providing ideal model ecosystems for the study of biogeographical patterns. The long-term development of mires was studied in the central European mountain regions. The Department participated substantially in a project on a modern national vegetation survey, resulting in four volumes of “Vegetation of the Czech Republic”. The project was coordinated by the Institute of Botany and Zoology, Masaryk University. The first volume of “Vegetation.” covered grassland and heathland vegetation, the second surveyed vegetation synanthropic vegetation units. In 2010–2014, the last two volumes of this monograph were published. “Vegetation of the Czech Republic” vol. 3 focused on wetland vegetation. Considerable research effort of the Department was devoted to variations in soil chemistry and vegetation. Soil chemistry was experimentally manipulated in order to study processes in whole ecosystems and reactions of selected species. The main focus was on anthropogenic impacts on sensitive environments, including processes of atmospheric acidification, eutrophication and disturbances by skiing.

The department of **experimental phycology and ecotoxicology** was active in the ecology of cyanobacterial blooms, ecology and fate of cyanobacterial toxins and their role in the stability and diversity of aquatic ecosystems. Significant achievements were related to research on the effect of cyanotoxins on human health and *in vitro* toxicology. Whereas tumor promoting and carcinogenic activities of major cyanobacterial toxins have been recognized, little was known about cancer-relevant effects of other metabolites, and detailed mechanisms of cyanotoxin action at the cellular and tissue levels. These original findings significantly contributed to our mechanistic understanding of tumor promotion. As new topics, the department initiated studies on mechanisms of P-recycling, P-pollution prevention in the aquatic ecosystem

and with extremely important topics in the ecotoxicology of newly-emerging pollutants, including metal nanoparticles and estrogens. Major achievements were the development of environmentally friendly methods for management of cyanobacterial blooms. Phthalocyanines were identified as natural inhibitors of cyanobacterial growth. The work also focused on mechanisms of action of phthalocyanines against living organisms. This strategy of searching for new chemical methods against cyanobacteria will help to deal with cyanobacterial blooms all over the world. The model for cyanobacterial bloom prevention was developed and is already used in the Brno reservoir, based on destratification and oxygenation of reservoir water to prevent cyanobacterial blooming. In 2011, this so called “Aeration Tower” system was awarded the prestigious prize, ‘Czech Head’, the highest scientific award in the Czech Republic, and was protected by a Czech utility model in 2012. Research activities have also focused on the occurrence of cyanotoxins in the environment. Novel fluorescence-based techniques, suitable for *in situ* quantitation of phytobenthic assemblages, were developed. These techniques can be effectively applied to monitor producers of potent cyanobacterial toxins. A novel method, based on passive sampling, was also developed, optimized and evaluated for sampling and detection of the major cyanobacterial toxins, microcystins. This method, in combination with LC-MS/MS detection, allowed cost-effective, sensitive and integrated monitoring of microcystin concentrations in the field, and was successfully applied for monitoring microcystins in drinking water reservoirs and water treatment plants. Immunoassays allowing rapid and extremely sensitive detection of cyanotoxins were employed in a monitoring study focusing on the occurrence of the major cyanotoxin types in Czech water reservoirs. The effects of cyanotoxins on vertebrates were investigated in fish embryos. Experiments with Japanese medaka eggs showed that environmentally relevant levels of cyanobacterial bloom extracts affected hatching rate and development, and may have negative effects on freshwater fish communities. In collaboration with Masaryk University (RECETOX) and the Veterinary and Pharmaceutical University, effects of toxic cyanobacterial biomass were investigated using an animal bird model - Japanese quails. The study revealed that exposure to cyanobacterial toxins significantly enhanced the sensitivity of bird populations to other chemical and biological stressors, possibly contributing to bird mortalities.

In summary the achievements in applied research, and collaborations with industry, resulted not only in a number of papers but in several commercial products (three pharmaceutical products containing stilbenes), manuals for farmers on recultivation of degraded land and alternative use of contaminated land for fast growing coppist, technologies for algal cultivation, removal of cyanobacterial blooms etc. Novel data on P recycling and cleaning of waste waters by means of membrane bioreactors, in both pilot and real operations, were obtained and many other achievements with an outcome of utility patterns and patents.

Noteable achievements are also numerous awards obtained by employees of the IB within the evaluation period.

***CAS award for young scientists for outstanding results***

2014 – doc. Mgr. Jan Vondrák, Ph.D., 2013 – RNDr. Vít Latzel, Ph.D., 2012 – Ing. Václav Mahelka, Ph.D., 2011 – RNDr. Linda Nedbalová, Ph.D.,

***G.J. Mendels honor medal for achievements in biological sciences***

2014 – RNDr. Vlasta Jankovská, CSc., 2013 – RNDr. Jan Květ, CSc.

***Otto Wichterle Premium*** for the most prospective young scientists

2012 – Mgr. Ing. Pavel Trávníček, Ph.D., 2010 – Mgr. Jiří Doležal, PhD.

***Purkyne Fellowship for the outstanding scientist from abroad*** Dr. Igor Bartish  
***PRAEMIUM ACADEMIAE – financial support for the most excellent scientists***

2010 – prof. RNDr. Petr Pyšek, CSc.

***Czech head, project for support of scientific and technical excellence***

2011 – prof. Ing. Blahoslav Maršálek, CSc. – Award of Ministry of Environment

***Hlavka Foundation – for extraordinary young scientists***

2013 – Mgr. Magdalena Lučanová, 2010 – Mgr. Petr Vít

***Awards of the Minister of Education***

2012 – prof. RNDr. Jiří Komárek, DrSc.; Award of F. Běhounek for outstanding research and promotion of Czech science abroad (the first award in history)

2013 – prof. RNDr. Petr Pyšek, CSc.; Award for extraordinary scientific achievements

***Award of the Technological Agency of the Czech Republic*** - The best project of the year in category usefulness of the solution: 2014 – doc. ing. Jan Wild, Ph.D.

***ISI THOMSON REUTERS***

2014 – prof. RNDr. Petr Pyšek, CSc. - ISI Highly Cited Researcher

## Research Report of the team in the period 2010–2014

Institute	Institute of Botany of the CAS, v. v. i.
Scientific team	Department of Invasion Ecology & Department of GIS and Remote Sensing

### **PREAMBLE: RESEARCH METRICS, INTERNATIONAL COOPERATION, ACHIEVEMENTS AND RECOGNITION**

During the assessment period, members of the Department of Invasion Ecology & Department of GIS and Remote Sensing (herewith referred to as INV-GIS) published 128 papers (17, 26, 31, 28, and 28 in the respective years of 2010–2014) indexed in ISI WoS Core Collection. A substantial proportion of those papers have been published in high-profile journals such as PNAS (8 papers), Nature Communications (1), Annual Reviews series (2), Trends in Ecology and Evolution (4), Ecology Letters (3), Global Ecology and Biogeography (4), Diversity and Distribution (5), New Phytologist (3), and included commentaries in Science (3) and Nature (2). The most prolific members of INV-GIS over that period were P. Pyšek (91 papers), the late V. Jarošík (36), J. Pergl (33), M. Hejda (16), J. Wild (13), P. Petřík (13), L. Moravcová, J. Sádlo and C. Alba (8 each).

These papers, over the short period of four years, received 2476 citations (as of 6 March 2015), on average 20 per item. The three most cited reached 257 (Vilà et al. 2011), 205 (Vilà et al. 2010) and 169 (Blackburn et al. 2011), and six had over 100 citations (including also Simberloff et al. 2013, Pyšek & Richardson 2010, and Pyšek et al. 2010). The annual citation rate of the 2010–2014 INV-GIS papers in 2014 was 922 citations. An important metric of recognition of the INV-GIS research over this period is that two papers were highlighted by Nature (Aronson et al. 2013, Driscoll et al. 2014) and three selected for recommendation by the prestigious Faculty 1000 (Dullinger et al. 2013, Parker et al. 2013, Colautti et al. 2014). In the 2014 evaluation of the top results of Czech science, four INV-GIS publications were assigned A-class, representing ~10% of all A-labelled results in the respective research panel EP-10, comprising biological sciences. We also delivered five keynote/plenary talks at conferences abroad and numerous invited talks.

As to the international recognition of team members, Petr Pyšek was ranked as an ISI Highly Cited Researcher in ecology/environment (<http://highlycited.com>) in 2014, and is 15th amongst most cited European researchers in plant sciences by Lab Times (<http://www.labtimes.org>) in 2013. Other individual awards reflecting achievements over the assessment period include Praemium Academiae (Academy of Sciences of the Czech Republic, 2010) to P. Pyšek, Creative Award (now Bedřich Hrozný Award, Charles University Prague) 2010 to P. Pyšek and 2013 to V. Jarošík, and Minister of Environment of the Czech Republic Award for Outstanding Results in Research, Development and Innovations (2013) to P. Pyšek. Over the period, Jan Pergl completed his Wichterle Prize (Academy of Sciences of the Czech Republic, 2009–2014) and P. Pyšek became an Elected Fellow of the Learned Society of the Czech Republic in 2011.

Besides primary studies conducted at INV-GIS, which represent our core effort, an important part of our research occurs in broad international cooperation with groups from all over the world, some of which rank among the world leaders in current invasion

biology. Ongoing cooperations established during the work on EU projects ALARM, DAISIE and PRATIQUE in the previous period, and partly supported by currently running COST projects with our participation, resulted in the publication of numerous papers in prestigious high-profile journals. The most important international cooperations over the assessment period include: Centre for Invasion Biology, Stellenbosch University, South Africa (D. M. Richardson; with formally established cooperation in 2012, and P. Pyšek being CIB Research Associate); Bio-Protection Research Centre, Lincoln University, New Zealand (P. E. Hulme; with P. Pyšek and V. Jarošík long-term visit in 2011); Synthesis Centre for Biodiversity Sciences (sDiv) within the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig (M. Winter); Estación Biológica de Doñana, Seville, Spain (M. Vilà); Department of Conservation Biology, University of Vienna, Austria (F. Essl); Institute of Ecology and Evolution, University of Bern, Switzerland (W. Nentwig; J. Pergl's long-term stay in 2010–2011); Institute of Low Temperature Sciences, Hokkaido University, Japan (T. Hara; J. Wild's long-term visit as invited Associate Professor in 2014); Institute of Photogrammetry and Remote Sensing, University of Dresden, Germany (E. Csaplovics). Nationally, we had the closest cooperation with the group of Milan Chytrý, Institute of Botany and Zoology, Masaryk University, Brno.

The international standing of our research was also reflected in long-term international visitors interested in working at INV-GIS over the study period (Laura Meyerson, University of Rhode Island, USA; Llewellyn Foxcroft, South African National Parks, South Africa; Grant Hamilton, Queensland University, Australia) and foreign postdocs and students (Christina Alba, USA; Margherita Gioria, Italy; Ana Novoa, Spain; Thaísa Michelin, Brazil; Kirsty McGregor, UK).

During the assessment period, INV-GIS suffered a major loss to its scientific capacity. In 2013, a distinguished member of our team, Vojtěch Jarošík sadly passed away, and we had to cope, in particular, with loss of his statistical expertise.

## **THE MOST IMPORTANT RESEARCH TOPICS IN 2010–2014**

In the following overview, topics studied by INV-GIS are arranged according to the approach taken and reflect the level of biological organization, from large-scale macroecological analyses (Topics 1–2), to community-ecology oriented research (Topics 3–4), and then those addressing population- and evolutionary ecological questions (Topic 6). Some themes are cross-organizational, and are hence embedded within the biological organization to which they relate most closely, such as the impact of biological invasions (Topic 5) to community ecology and species invasiveness, and the role of biological traits in invasion to the population level (Topic 7). Much attention has been paid to conceptual issues, including formulation of general frameworks of species invasiveness and habitat invasibility (Topic 8). Primary botanical research has always been the focus of our team, represented especially by the updated catalogue of alien plants in the Czech flora (Topic 9). Finally, practically oriented research with management implications, including studies on plant invasions in protected areas globally, is captured within Topic 10, and transfer to policy recommendation in Topics 11–12.

### **Macroecological determinants of biological invasions and trends in biological diversity: interplay of socio-economic and historical factors**

An important part of INV-GIS research focused on continental scale macroecological patterns of plant and animal diversity for the whole of Europe, in particular, identifying

factors that determine the regional richness of alien floras as well as that of endangered species, the relative importance of those factors and how their effects changed on a time scale of decades to centuries.

The importance of biogeographic, climatic, economic, and demographic factors as drivers of biological invasions that can disrupt ecosystems and cause severe ecological or agricultural damage is increasingly recognized, but our two studies, based on the occurrence of invasive species from a wide range of taxonomic groups (fungi, plants, invertebrates, vertebrates) in terrestrial and aquatic habitats of 55 European regions, were the first to show that economic and demographic factors are key drivers across a range of invasive taxa and that these factors are more important than regional differences in geography and climate. Next to human population density, the most important factor determining how many species of invasive plants and animals a country will harbour is the long-standing national wealth. This is because mechanisms of species invasion are often associated with human-induced disturbances that create landscapes suitable for invasions, and with international trade. Other possible factors, such as climate, geography or land cover, were less significant than population density and wealth, and those secondary causes may have been overestimated in previous studies (Pyšek et al. 2010). However, the majority of extant invasive species were introduced to Europe long ago and there is often a delay between the first introduction of a species to a new territory and its establishment and spread. By taking an historical approach, we provided an explicit test of this phenomenon, by demonstrating that current alien species richness is better explained by socio-economic data (human population density, per capita GDP, and trade intensity) from 1900 than from 2000. This suggests that invasions triggered by current economic behavior will take a long time to become fully realized, a phenomenon for which we introduce the term “invasion debt” (Essl et al. 2011).

In a study on extinction risk, based on extensive data from 22 European countries where we also took an historical approach, we provided circumstantial evidence that there are substantial time-lags between human pressure and population decline of threatened plant and animal species. We showed that the proportions of plants, vertebrates and invertebrates facing medium to high extinction risks were more closely matched to country-specific indicators of socio-economic pressures from the early or mid, rather than the late 20th century, indicating a considerable historical legacy of species’ population losses. A related analysis revealed that current spending on environmental conservation only has a weak mitigating effect, which implies that current conservation actions are effective, but inadequate in scale, to halt species losses. The broad taxonomic and geographic coverage indicates that the so-called ‘extinction debt’ is a widespread phenomenon and the full impact of current high levels of anthropogenic pressures on biodiversity will only be realized decades into the future (Dulling et al. 2013).

The contributions of INV-GIS to the three papers above, all published in PNAS, were key. We played a major role in collecting data on alien species richness, conceiving the ideas, conducting data analyses for the first one, and consulting on the others, interpretation of results and writing the papers; this is reflected in the first and last positions of a team member on the two studies, respectively.

### **Projection of future trends of plant invasions in Europe under contrasting socioeconomic scenarios**

In our previous studies, we were the first to provide rigorous systematic evidence that local levels of alien plant invasions are, to a great extent, determined by land-use and

climate (Pyšek et al. 2010, Pyšek & Chytrý 2014). Therefore, future policy orientations towards either deregulation or sustainability will affect plant invasions through their effects on land-use and climate change.

In this study (Chytrý et al. 2012), based on our previously published map of plant invasions in Europe, we integrated the European land-use projections for years 2020, 2050 and 2080 under three socio-economic scenarios with data on plant invasions in different land-use categories, to produce spatially explicit projections of the future levels of plant invasions across Europe. We showed that for all scenarios, the level of invasion is expected to increase, especially in North-Western and Northern Europe, and decrease in some agricultural areas of Eastern Europe. However, a net increase in the level of invasion throughout Europe is predicted under all scenarios. Sustainability-oriented policies that support agriculture in areas where it is less profitable, would further increase the level of invasion by alien plants, since many of them are associated with agricultural landscapes. As the overall level of invasion on the continental scale is correlated with the occurrence of highly invasive species that cause both environmental and economic damage, plant invasions will remain a serious problem in Europe even if sustainability policies are implemented. Hence, proactive strategies to combat invasive alien plants are urgently needed (Chytrý et al. 2012).

INV-GIS had a substantial intellectual input into previous publications within the ALARM project that yielded a basic map upon which the above predictions were based. Here we played crucial roles in conceiving the idea, we conducted the technical treatment of European habitat data using GIS expertise, made important contributions to the interpretation of results, and writing of the paper.

### **Interactions between alien plants and organisms at other trophic levels on a historical time scale**

When studying determinants of invasiveness of plant species, it needs to be taken into account that the relationships between plants and organisms at other trophic levels result from centuries of mutual interactions, of which pollination is one of the most important.

In the first study on historical changes in these relationships, we have shown that alien flora introduced to central Europe contained a higher proportion of insect-pollinated species than did the central European native flora, and hosted a higher diversity of pollinators per species. However, the frequency of pollination modes in the introduced alien flora gradually changed during the process of naturalization, becoming more similar to that of native species, and eventually, the naturalized species that became invasive did not differ in their frequency of pollination modes from native species. The results further suggest a remarkable role of pollination mode in successful invasions; self pollination tends to support the spread of invasive species more than any other modes of pollination. Moreover, groups of plants that have been provided with a longer time to sample a wider range of habitats than recently arriving alien species have formed more associations with native pollinator species occurring in those habitats (Pyšek et al. 2011). To obtain insights into an opposite type of historical relationship, we analyzed fungal and viral pathogen species richness in 124 plant species in both their native European and introduced North American ranges. Plants introduced 400 years ago supported six times more pathogens than those introduced 40 years ago. Introduced species have accumulated pathogens at rates that are slow relative to most ecological processes, and contingent on geographic and historic circumstance (Mitchell et al. 2010).

For the first study, published in *Ecological Monographs*, foreign contributors from the ALARM consortium provided data on insect pollinators, consulted on results and commented on the manuscript draft. Otherwise, the paper was produced by INV-GIS. For the latter paper, P. Pyšek and V. Jarošík provided unique plant data for central Europe, with characteristics of invasion history spanning several centuries, V. Jarošík advised on statistical analyses and both were involved in writing and commenting on the paper.

### **Forest history and its implications for management and biodiversity**

European forests have been shaped by humans for millennia and knowledge of forest history is key to understanding the recent forest state and its future development. In this research, we focused on various historical managements in lowland broadleaved-to-mountain coniferous forests with the aim to (i) reconstruct long-term spatio-temporal dynamics in forest management and (ii) reveal relationships between management practice and forest growth dynamics.

Coppicing was one of the most important management systems in broadleaved forests in Europe, documented from prehistory as well as in the Middle Ages; its gradual abandonment by the mid-20th century altered the ecosystem structure, diversity and function of coppice woods. Based on an analysis of forest archival sources spanning the past seven centuries (Müllerová et al. 2014), and detection of historical coppicing events from oak tree-rings, we were able to link coppicing with the recruitment of mature oaks (Altman et al. 2013). Both studies demonstrate the importance of humans in maintaining broadleaved forest ecosystem biodiversity.

Replacement of natural tree species by trees more suitable for commercial wood production, both native and alien, was a common management practice in the last two centuries. We used archival written documents and historical forest management plans to document such forest changes from the beginning of 19th century in a large transboundary area of Bohemian-Saxon Switzerland (Seiler et al. 2013). The resulting geodatabase of forest age and tree species composition became an indispensable tool for nature conservation planning in this area, as well as background for further scientific research, and was used to identify forest stands of high nature value, to understand the historical distribution of invasive white pine (*Pinus strobus*) and to predict the areas most vulnerable to its future spread.

For a full picture of forest dynamics, management history should be combined with an understanding of natural forest dynamics. However, it is hard to infer natural processes operating at the landscape scale from scarce remnants of natural forests in Europe. Therefore, we again used different archival materials (maps, but also paleoecological data) to reveal long term natural dynamics of coniferous forests (less affected by humans). We proved that large scale disturbances (windstorms, bark beetle outbreaks) are within the historical range of variability of mountain spruce forest dynamics in central Europe (Brůna et al. 2013). Further, we showed that fire affects long-term forest development in sandstone landscapes (Bobek 2013) and should thus be considered a natural driver of forest vegetation patterns, even in temperate regions. We also significantly contributed to knowledge on a local scale of natural spruce forest dynamics, were the first to demonstrate that forests mirror the tree spatial pattern after the stand-replacing disturbance, and proposed possible mechanisms for this process (Wild et al. 2014).

The research on coppicing was carried out in close within-institutional co-operation with the Department of Vegetation Ecology and the Department of Functional Ecology. INV-GIS member J. Müllerová led one of the papers and participated in



conceiving the studies, field work, historical data collection, data analysis and article writing. The building of a forest history geodatabase was a part of European project Ziel 3, within which J. Wild led the Czech team and co-edited the project book, and other team members participated on conceiving the studies, data collection and writing of papers. Other studies were conceived by team members and they also took a leading role in paper writing.

### **Global assessment of the impacts of plant invasions on resident species, communities and ecosystems**

Invasive species pose risks to native biodiversity worldwide, and the impact of biological invasions starts to be increasingly obvious, even in scarcely populated pristine areas such as Antarctica where scientific personnel rather than tourists pose the highest risk of introducing alien species on their clothes and equipment (Hulme et al. 2012). To cope with the consequences of plant invasions, we need to know which species are likely to cause profound changes in the ecosystems of invaded areas, but Europe is currently the only continent with solid information on the impacts of its invasive species (Pyšek & Hulme, 2011). The study of impacts of invasions is a rather new topic in invasion ecology and analysis of comprehensive global data has been missing until now (Pyšek & Richardson 2010).

To reduce this gap, we conducted the first global overview of how often, and under what circumstances, plant invasions cause significant impacts on resident species, communities and ecosystems. The study was based on 287 publications that addressed the impact of 167 invasive plant species and showed that there is no universal measure of impact and that the outcome depends on what we measure. Invasive plants exert consistent significant impacts on some measured outcomes, whereas for others, such as species richness, diversity and soil resources, the significance of impacts is determined by interactions between species traits and the biome invaded. Invasive plants are far more likely to cause significant impacts on species richness on islands rather than mainlands. Species with certain biological traits, however, cause significant impacts regardless of the type of habitat or geographical region invaded. These results provide a means to predict the likely impact of invasive species based on their traits, so that managers can focus on those species that are likely, following introduction, to reduce local biodiversity and to change functions of resident ecosystems (Pyšek et al. 2012). In another study, we conducted a global meta-analysis of the same data set and showed that alien plants had a significant effect in 11 of 24 different types of impacts assessed. Abundance, diversity and fitness of resident species generally decreased in invaded sites, whereas primary production and several ecosystem processes were enhanced; however, by the time changes in nutrient cycling are detected, major impacts on plant species and communities are likely to have already occurred. Overall, impacts of alien species are heterogeneous and not unidirectional, even within particular impact types (Vilà et al. 2011).

The elaboration of the global database of impacts of alien plants was organised and led by INV-GIS, in close cooperation with the team of Montse Vila (Estacion Biologica Doñana). The first analysis, published in *Global Change Biology*, was led by our team, the second in *Ecology Letters* by hers, with corresponding emphasis on our respective roles. INV-GIS substantially contributed to all parts of this research.

## **Population ecology of invasive species, evolution of invasive traits and local adaptations**

To explore trait divergence between native and introduced populations, and identify which biotic and abiotic factors are associated with such divergence, we studied the evolutionary ecology of *Verbascum thapsus*, a plant species that was introduced to North America from Europe more than 400 years ago. This introduction event served as a model system, with particular focus on herbivore effects on plant chemistry and performance (Alba et al. 2013, 2014, Wilbur et al. 2013), and on how traits associated with defense and performance can change following introduction to a new range with a different suite of herbivores (Alba et al. 2012, Kumschick et al. 2013). The research also includes a large biogeographic component, based on a field census of 50 populations distributed across the species' native range in Europe and introduced range in North America (Alba & Hufbauer 2012). Some major findings reveal that native and introduced populations have diverged in a number of traits associated with competitive ability (Kumschick et al. 2013) and defense against generalist and specialist herbivores (Alba et al. 2012), and that such divergence is associated with differences in biotic (herbivory and competition) and abiotic factors between the two ranges (Alba & Hufbauer 2012).

In other research, we studied local adaptations of populations of non-native and native congeners, using a model system of the genus *Impatiens*. We found some evidence that local adaptations, based on differences in germination dynamics and seedling frost resistance between the native and two invasive congeners, were explained by climatic characteristics of the source localities from which seed was taken (Skálová et al. 2011, 2013). In other studies, we explored ecological differences and habitat requirements within our model system (Skálová et al. 2012, Čuda et al. 2014).

C. Alba was the team leader on the first topic, playing a crucial role in data collection, analysis, and paper writing, and this is reflected by her position as first author on four of the six published papers. The local adaptation research was carried out exclusively by INV-GIS, and led by H. Skálová.

## **Biological traits supporting invasiveness and demography of invasive plants and animals in their native and invaded ranges**

A fundamental assumption in invasion biology is that most invasive species exhibit enhanced performance in their introduced range relative to their home ranges. The success of invasive species has been thus explained by two contrasting but non-exclusive views, that (i) intrinsic factors make some species inherently good invaders, and (ii) species become invasive as a result of extrinsic ecological and genetic influences following introductions. To improve tests of these hypotheses of invasion success, we introduced a simple mathematical framework to quantify invasiveness and applied it to a sample dataset of 1,416 plant species across Europe, Argentina, and South Africa. Invasiveness, as measured by occurrence data, was better explained by inter-specific variation in invasion potential than post-introduction biogeographical changes in performance (Colautti et al. 2014). However, there are surprisingly few data testing the underlying assumption that the performance of introduced populations, including organism size, reproductive output, and abundance, is enhanced in their introduced compared to their native range. In another paper we combined data from published studies to test this hypothesis for 26 globally invasive plant and 27 animal species. On average, individuals were indeed larger, more fecund, and more abundant in their introduced ranges. The overall mean, however, belied significant variability among species, as roughly half of the investigated species performed similarly when

compared to conspecific populations in their native range. Thus, although some invasive species perform better in their new ranges, the pattern is not universal, and just as many perform largely the same across ranges (Parker et al. 2013).

In related research, we addressed the role of species' biological traits that are not usually considered in comparative studies using large datasets of many species, typically because of lack of data such as genome size (Kubešová et al. 2010), the wide range of reproductive traits (Moravcová et al. 2010), ploidy level (te Beest et al. 2012) and the ability to build a persistent seed bank (Gioria et al. 2012).

Research on species traits has been done exclusively by INV-GIS, with the exception of the genome size study done in cooperation with Laboratory of Flow Cytometry; the other studies were part of a broad international cooperation. The papers originated at joint meetings and workshops, where members of INV-GIS contributed to the development of ideas, and later, contributed to collating literature data and writing of papers.

### **Conceptual issues and general frameworks**

Over the assessment period, we were involved in broad global activities aimed at formulating conceptual frameworks addressing various aspects of biological invasions. In these papers, the INV-GIS was represented by P. Pyšek, who contributed, in various degree, to all aspects of paper production, from debates where ideas were conceived, to paper writing and editing.

P. Pyšek was part of an international team that proposed an integrated unified framework for the invasion process, in which we combined previous stage-based and barrier models and provided a terminology and categorization for populations at different points in the invasion process (Blackburn et al. 2011). In another paper, we pointed out that studies should focus on the phase of naturalization, which is key in the process of invasion (Richardson & Pyšek 2012).

Habitat invasibility was addressed in another study, where we recommended two invasion indices: relative alien species richness and relative alien species abundance, and suggested that these should be adopted as a standard metric of habitat invasion (Catford et al. 2012). We were also involved in a recent debate, arguing that when assessing the risks associated with ongoing global change, one cannot ignore the differences between native and alien species (Hulme et al. 2011, Simberloff et al. 2011). Finally, in a conceptual paper focusing on model systems in invasion biology, we argued that a complex strategy of integrated research, with a systematic focus on selected species and ecosystems, could contribute to a better understanding of complex interactions between factors affecting outcomes of particular invasions (Kueffer et al. 2013).

### **Alien plants in the Czech Republic, taxonomic challenges and biological invasions**

Over the assessment period, we continued in the tradition of botanical research on alien plants in the Czech Republic that we started at the beginning of 2000s. We updated the national catalogue of alien plants that was first published in 2002; this work yielded a complete survey of 1454 alien taxa, among which, 350 (24%) are archaeophytes introduced before the end of the Medieval period, and 1104 (76%) neophytes, introduced after that date. We also reported on 44 new alien taxa for the first time in the Czech Republic. Of the total number reported, 408 taxa were considered naturalized, i.e. permanently established in the flora, and 61 invasive. All recorded alien taxa were classified with respect to their historical dynamics in the

landscape, relation to propagule sources from cultivation, and habitats in which they occur (Pyšek et al. 2012a, b).

In another study addressing the situation in botanical research of alien plants, we showed that a lack of taxonomic expertise, and by implication, a dearth of taxonomic products such as identification tools, has become a trend and has hindered progress in understanding and managing biological invasions. This is because biosecurity strategies, legislation dealing with invasive species, quarantine, weed surveillance and monitoring all depend on accurate and rapid identification of non-native taxa. However, such identification can be challenging because the taxonomic skill base in most countries is diffuse and lacks critical mass; identifications can therefore impede ecological studies. A better integration of classical alpha taxonomy and modern genetic taxonomic approaches will improve the accuracy of species identification, and further refine taxonomic classification at the level of populations and genotypes in the field and laboratory. Taxonomy not only plays a critical role in the study of plant invasions, but in turn benefits from the insights gained from these studies; biological invasions have provided important tests of basic theories about species concepts (Pyšek et al. 2013).

The national inventory of alien flora was organised, led, elaborated and written by the INV-GIS team, with important contributions of taxonomic expertise from other departments of the institute. P. Pyšek was a leading author of the latter paper, where J. Suda of the Flow Cytometry Lab contributed with data on genome sizes, and all other coauthors contributed to writing the manuscript and commenting. The role of INV-GIS in both papers was fundamental.

### **Implications of research for management and nature conservation**

Some topics of our research over the evaluation period included a strong practical aspect, being aimed at management and control of alien species, with special emphasis on protected areas. This also refers to testing of new technological tools that have potential for management.

The first published quantitative analysis of factors that determine whether eradication of an invasive species will be successful (based on the data from 173 campaigns against 94 species of invasive organisms, of which about half eradications were successful) showed that it is much more difficult to succeed in natural habitats, and that the probability of success is enhanced by international cooperation and implementation of measures before the invasion exceeds a critical threshold (Pluess et al. 2012a, b).

An important part of our research was on plant invasions in protected areas. In a paper based on data from Kruger National Park, South Africa, we showed that natural vegetation along the park boundaries acts as a barrier against colonization by invasive plants, and we identified the relative importance of factors that drive this process (Foxcroft et al. 2011). This makes it possible to predict, in a quantitative way, the threat of plant invasions in the park and suggest measures to mitigate the invasion load (Jarošík et al. 2011).

In 2013, we were involved in publication of a synthesis of the current state of knowledge of problems with invasive plants in protected areas (Foxcroft et al. 2013); part of this effort was the suggestion of measures to reduce the risks of new invasions (Meyerson & Pyšek 2013) and mapping the situation in protected areas in Europe, for which we illustrate that continent-wide rigorous data on the distribution and abundance of invasive alien species in protected areas are lacking. Managers there are well aware of the seriousness of the problem and of threats imposed by invasive plant species,

but are constrained in their efforts by the lack of resources, both staff and financial, and of rigorous scientific information that would be translated into practical guidelines (Pyšek et al. 2013). In another study we analysed a global database of quantitative studies to evaluate existing knowledge on alien plant impacts within and outside protected areas. We found that only a minority of studies provided any subsequent management recommendations. There is therefore considerable scope to improve the evidence base on alien plant management in protected areas (Hulme et al. 2014).

A potential powerful tool for management of invasive plants is remote sensing based on both aerial and satellite data, which we previously successfully applied to the monitoring and assessment of the spread of a problematic invasive species, giant hogweed (*Heracleum mantegazzianum*). In another paper we compared data with different spatial and spectral resolutions, such as historical panchromatic, color and multispectral aerial data, and satellite imagery of coarser spatial and higher spectral resolution, and tested the different classification approaches and imageries acquired in different phenological stages of population development. Based on this we have suggested an efficient method for monitoring invasive species, applicable in large areas and to other problematic species (Müllerová et al. 2013). The potential for remote-sensing based information acquired from a longitudinal series of aerial photographs, over 50 years, was again proven by studies that we conducted with members of another department of the Institute of Botany on changes of impact of giant hogweed over time (Dostál et al. 2013, Jandová et al. 2014); these studies could not have been carried out without our remote sensing data.

## **TRANSFER OF OUR RESEARCH ON INVASIONS INTO POLICY RECOMMENDATIONS**

### **Black List of invasive species in the Czech Republic**

To make our research relevant for society at large, INV-GIS maintains a wide cooperation with state and government authorities in charge of invasive alien species (IAS). Our cooperation with the Ministry of Environment of the Czech Republic, established during the Czech presidency of the EU when J. Pergl served as an advisor on IAS and participated in the process of preparation of EU Council conclusions, continued through the assessment period. J. Pergl provided expert assistance for preparing and applying Regulation no 1143/2014 of the European parliament and of the EU Council, of 22 October 2014, on the prevention and management of introduction and spread of invasive alien species. We also coordinated work on preparing the Black List of alien species of the Czech Republic, and delivered an internal report to the Ministry in 2013, which is already being used as a tool for allocation of funds in landscape management. The Black List has, since then, been updated and was recently submitted as a regular research paper.

INV-GIS provided the Czech Phytosanitary Agency with results of our mapping of selected alien species, and, with the Nature Conservation Agency of the Czech Republic, we are developing guidelines on the management of selected alien plant species that will serve as an official basis for management in the Czech Republic. For local nature protection authorities in cities, we provided a science-based information service for assessing newly introduced species, especially for biofuel.

### **Classification of alien plant and animal species based on impacts of their invasions: towards an IUCN Black List**

In terms of international policy, our research in this field was motivated by the fact that alien species impacts vary greatly across species and ecosystems (Hulme et al. 2013), cannot be predicted based on species' invasiveness (Horáčková et al. 2014) and studies are often biased by using unclear methodology (Jeschke et al. 2014).

We propose a standardized method to evaluate the magnitudes of environmental impacts (Blackburn et al. 2014), driven by mechanisms recognized by the International Union for Conservation of Nature (IUCN). The classification applies to different levels of ecological complexity, and spatial and temporal scales. Currently, an implementation process for the scheme is under negotiation with IUCN.

## Research Report of the team in the period 2010–2014

Institute	Institute of Botany of the CAS, v. v. i.
Scientific team	Department of Taxonomy & Department of Flow Cytometry

The Department of Taxonomy and Flow Cytometry is a major national research centre dealing with biosystematics, phylogeography, evolution and population biology of different groups of autotrophic and heterotrophic organisms, including vascular plants, fungi and lichens. It combines traditional alpha taxonomy with modern molecular, cytogenetic and experimental approaches in order to gain a holistic insight into the diversity and evolution of investigated groups. The team is responsible for major national monographs, including the multi-volume Flora of the Czech Republic and Phytocartographical syntheses of the Czech Republic. The group of plant flow cytometry belongs among the leaders in its field on a global scale. Major scientific achievements for the period of 2010-2014 may be grouped under the following categories:

### Revision and synthesis of national plant diversity

The Department of Taxonomy and Flow Cytometry is responsible for research, synthesis and assembling information about plant diversity in the country. A comprehensive taxonomic inventory is currently being undertaken within the project Květena České Republiky (Flora of the Czech Republic). This is the first Czech multi-authored floral compendium, elaborated as a set of monographs of each genus or family, based on up-to-date biosystematic knowledge and expert taxonomic re-evaluation of field observations, experiments, herbarium and literature data. It is an essential work of the Czech botanical literature, of a long-term value, containing a vast wealth of knowledge about native and introduced plants. It underpins further biological research, education, conservation priorities and all environmental and land-use work in the country, but is also of high value internationally. The eighth volume was published in 2010 (Štěpánková et al. 2010), and preparation of the ninth volume is in progress.

Distribution maps, as a visual representation of phytogeographical records, are fundamental sources of information in botany. Grid-based atlases of plant distribution in particular, hold tremendous potential for large-scale biogeographical and macroecological analyses, and provide an important source of information for conservation of endangered species. The team produces a multi-volume grid atlas based on a template of 10 × 6 arc minutes. The fourth volume, which contains 252 distribution maps of taxa of 137 genera and 23 families, was published in 2012 (Štěpánková 2012). Comprehensive data sets and final distribution maps enable the Czech Republic, and the IBOT in particular, to serve as a national centre in large international projects such as Euro+Med PlantBase or Atlas Florae Europaeae.

A critical synthesis of current knowledge resulted in the first comprehensive survey of general features of Czech flora, including a phytogeographic outline and assessment of its historical development. Special attention was paid to endemic species and their evolution (Kaplan 2012). A complete checklist of vascular plants of

the flora of the Czech Republic was compiled for the first time in modern history. It includes all new findings and taxonomic novelties, and lists 3557 taxa in total (Danihelka et al. 2012).

Karyological characteristics of plants serve as one of the basic sources of information required for assessing species relationships and interpretation of molecular data. A synthesis of European karyological diversity in the taxonomically very challenging genus, *Rubus*, was produced (Krahulcová et al. 2013). Additional chromosomal counts and detailed analyses of karyotypes in various angiosperms of both European and extra-European origins contributed to resolving species relationships, major speciation events and delimitation of taxa within taxonomically complex groups or groups that include endangered species and/or endemics (e.g. Hoták et al. 2013, Kaplan et al. 2013, Prančl et al. 2014, Rooks et al. 2011, Štěpánek et al. 2011, Záveská Drábková 2013).

All three editors of the Flora of the Czech Republic (Jitka Štěpánková, Jindřich Chrtek & Zdeněk Kaplan) are members of the team. They are responsible for management of the project, collaboration with authors and illustrators, editorial works and publication of the Flora. Together with 10 other staff members from IBOT (mainly from the team), they wrote most of the text. The team members were the sole authors or they substantially contributed to all above-cited publications. They were particularly responsible for study design, identification of plant material, karyological analyses, taxonomic conclusions, evolutionary interpretations and article writing.

### **Evolutionary, ecological and taxonomic consequences of variation in nuclear genome size**

With the aid of DNA flow cytometry, we explored variations in nuclear genome size in a number of plant groups originating from four continents (Europe, Africa, South America and Australia), and used the data in three different ways (reviewed in Loureiro et al. 2010): (i) for taxonomy as a taxon-specific marker; (ii) for prediction, as genome size is associated with several phenotypic, physiological and/or ecological characteristics, and (iii) for understanding the dynamics of genomic evolution.

Our study of a large set of alien species provided the first quantitative support for association of small genome sizes with invasiveness (Lučanová et al. 2010). A comprehensive investigation of *Fallopia* sect. *Reynoutria* (one of the most serious alien plants in the European flora) revealed that the amount of nuclear DNA can be used as a reliable marker for the identification of all species and hybrids, even at early developmental stages (Suda et al. 2010). We also presented a robust test of how genome size and trait variation influence the performance of Australian acacias, which have been introduced around the world (Gallagher et al. 2011). After investigating the Australian genus *Anigozanthos*, we found that species with compatible genomes show similarities in DNA C-values, providing the first evidence that inter-specific differences in genome sizes are correlated with hybrid fitness (Le Roux et al. 2010).

As a part of a broader study re-examining the classical example of allopolyploid speciation using molecular and cytogenetic tools, we provided support for the origin of the allotetraploid species *Galeopsis tetrahit* (Bendiksby et al. 2011). In the Andean genus *Lasiocephalus*, we found that ecological conditions have played a role in driving shifts in genome size between closely related species inhabiting different environments (Dušková et al. 2010). Quite surprisingly, regional abundance of central-European species, particularly of annuals, was found to be weakly but significantly correlated with genome size (Herben et al. 2012). This indicates that predictors of regional abundance should also be sought among variables that are linked to cell size and cell division rate.



Knowledge of genome size is essential for estimating the cost and time for whole genome sequencing projects and for selecting protocols for DNA fingerprinting studies. We thoroughly investigated variations in DNA C-values in several groups, including *Myrtus* (by comparing cultivated and wild representatives; Serçe et al. 2010), *Oldenlandia* (the first step of genomic analysis using expressed sequence tags; Qin et al. 2010) and the subfamily Apostasioideae of Orchidaceae (i.e., the rare and difficult to obtain basal group, which is essential for evolutionary interpretations of genome size change in this largest angiosperm family; Jersáková et al. 2013). We also contributed significantly to the debate concerning intraspecific variation in genome size by revealing a substantial variation in *Taraxacum stenocephalum* at both intraspecific and intrapopulation levels (Trávníček et al. 2013).

Accurate C-value estimates are the key prerequisite for any genome size study. We reviewed the process of standardization, which is one of the most important issues currently facing the field of genome size research (Suda & Leitch 2010). In addition, we overcame one of the major limitations of flow cytometry in field research (i.e., the need for fresh tissue) and developed a protocol based on glycerol-preserved nuclei (Kolář et al. 2012). Our methodology currently represents the most reliable way of sample preservation for genome size research.

In total, 14 WoS-indexed articles on this topic were published between 2010–2014. Eight of these were led by members of the team (i.e., they conceived the study, collected the samples, performed analyses, interpreted data and wrote the manuscript). In the remaining six articles, we estimated genome size values using high-resolution flow cytometry, wrote corresponding parts of the manuscripts and commented on earlier versions of the text.

### **Patterns and processes in mixed-ploidy populations**

We investigated several diploid-polyploid plant groups in order to elucidate the evolutionary history of zones of sympatry and assess the role of different pre- and post-zygotic mechanisms in generating ploidy diversity and maintaining ploidy mixtures.

The study of the challenging species complex *Galium pusillum*, in northern and central Europe, revealed independent evolution in deglaciated and unglaciated areas, and a high level of eco-geographic segregation, which supports the genetic integrity of particular species (Kolář et al. 2013). In *G. valdepilosum*, a narrow zone of ploidy contact was detected in the Czech Republic. The cytotype distribution was driven by different ecological preferences of di- and tetraploids (Kolář et al. 2014). Both primary and secondary contact zones were revealed in *Knautia arvensis* in central Europe (the first polyploid system for which the incidence of both cytotype contacts was supported by molecular evidence). The evolutionary history of the group has been shaped by a variety of processes, including isolation in Holocene refugia, repeated colonization, hybridization and recurrent polyploidization (Kolář et al. 2012). In subsequent research we assessed the role of ecologically adaptive versus non-adaptive processes in the establishment of *Knautia* neopolyploids (Hanzl et al. 2014). The most marked example of ploidy coexistence (sympatric growth of up to five different cytotypes) was recorded in the *Gymnadenia conopsea* complex (Trávníček et al. 2011a). The gene flow in mixed-ploidy populations is limited by a distinct spatial structure. Rare minority cytotypes substantially contribute to ploidy diversity in fragrant orchids (Trávníček et al. 2012). Both the ongoing production of unreduced gametes and heteroploid hybridization are involved in the origin of minority ploidies. Cytotype distribution that was shaped by Pleistocene glaciations was revealed in a high-alpine plant *Senecio carniolicus* (Sonnleitner et al. 2010). Using different molecular markers, we provided

evidence for survival of diploids in both peripheral and inferior refugia, a scenario that has only rarely been seen previously (Escobar-García et al. 2012). The frequency and long-term stability of contact zones is largely determined by cross-compatibility patterns among 2x, 4x and 6x cytotypes (Sonnleitner et al. 2013).

In addition to the four above-mentioned model systems studied in detail, we also assessed intra- and inter-population ploidy variation and distributional patterns in several other vascular and non-vascular plant groups, including *Vicia cracca* (Trávníček et al. 2010), invasive *Centaurea stoebe* (Otisková et al. 2014) and a moss *Tortula muralis* (Košnar et al. 2012). The study of *Pilosella echioides* (Trávníček et al. 2011b) deserves particular attention. This is the first sexually-reproducing plant system with prevailing triploid cytotypes. Based on published data on population cytotype structure, we developed a general gametic model that predicts equilibrium ploidy frequencies in natural populations of diploid-polyploid systems (Herben & Suda 2012). Our results imply that conditions for cytotype coexistence in natural populations are likely to be less restrictive than previously assumed.

We have also embarked on a detailed cytological investigation of the unique Cape flora (South Africa). In contrast to previous assumptions, our investigation of the key geophytic genus *Oxalis* revealed that karyological diversity in the Cape region may be much greater than currently believed. We also found ecological sorting of different cytotypes at both landscape (Krejčíková et al. 2013a) and local (Krejčíková et al. 2013b) levels. Representative sampling revealed striking differences in ploidy variation between primary and secondary ranges of the invasive *O. pes-caprae* (Krejčíková et al. 2013c). As a side result of our extensive field work, two new species were discovered and described (Suda et al. 2014).

We published 18 articles on this topic and are one of the three most productive groups on an international scale (in addition to researchers in Guelph, Canada and Innsbruck, Austria). Members of the team led 12 studies, conducted flow cytometric analyses (ploidy estimates using both fresh and silica-dry material) and took part in article preparation in five other studies. The theoretical paper was accomplished with equal contributions from members of our Department and the Department of Population Ecology.

### **Reproductive systems and their evolutionary importance**

Variation in reproductive systems has important consequences for patterns and pathways of evolution. Detailed knowledge of acting reproduction modes is a key to understanding intra- and inter-population variation and in resolving phylogeny and taxonomy. Allogamy, autogamy and agamospermy have evolved in the genus *Taraxacum*, often showing distinct patterns of geographical parthenogenesis, such as in mountain dandelions of central Europe (Štěpánek et al. 2011). Ancient hybridity, another specific feature of dandelions, manifests itself even among sexual diploid species to such an extent that phylogenetic relationships in *Taraxacum* are blurred by repeated reticulations (Kirschner et al. 2014).

Apomixis may be combined with residual sexuality that involves occasional sexual mating and, less frequently, haploid parthenogenesis, increasing variation among progeny (Krahulcová & Rotreklová 2010). Expression of apomixis was found to be unstable even in non-segregating ( $2n$ ) genomes of the apomictic maternal parent, depending on the genetic background of  $2n + n$  hybrids in *Pilosella* (Krahulcová et al. 2011). Residual sexuality in *Pilosella* hybrids, arising from reciprocal artificial crosses between an apomictic and a sexual biotype, contributed to gene flow towards the sexual parent (Rosenbaumová et al. 2012). This direction of introgression was also

confirmed in mixed apomictic-sexual populations in the field (Krahulcová et al. 2014, Krahulec et al. 2014, Urfus et al. 2014). Surprisingly, when the range of ploidy levels is considered, the capacity to produce variable progeny is even higher in facultatively apomictic mothers than in their sexual counterparts (Krahulcová et al. 2014). Polyhaploids may also represent a source of variation in facultatively apomictic plants. In *Pilosella* we discovered a novel two-step process (polyploid – polyhaploid – polyploid) that autonomously generates new genotypes. This process involves meiosis but excludes any paternal contribution (Krahulec et al. 2011). Our finding is of general importance, admitting autonomous reproduction as a source of considerable variation.

Pollination biology is strikingly diverse in the aquatic genus *Callitriche*, with modes ranging from hypohydrogamy to geitonogamy, and also including a unique mode of self-pollination referred to as internal geitonogamy. A thorough investigation of this group revealed the incidence of several previously undetected hybrids (incl. the first ever record of hybridization between hydrogamous and non-hydrogamous species in angiosperms), some of which are evolutionarily very successful (Prančl et al. 2014).

Members of the team were either the sole authors or substantially contributed to the above-cited publications. They were particularly responsible for crossing experiments, karyological, cytometric, molecular and phylogenetic analyses, detection of reproduction modes, data interpretation and writing of the texts.

### **Role of hybridization in evolution and constituting of plant diversity**

We successfully applied molecular and cytogenetic techniques to elucidate causes and evolutionary consequences of interspecific hybridization. DNA analyses were employed to investigate genetic variation in Potamogetonaceae, one of the most diverse and taxonomically difficult families of aquatic plants. We revealed considerable cryptic diversity, including previously unknown interspecific hybrids (Kaplan & Uotila 2011, Kaplan et al. 2013) and the first hybridization event detected in the Southern Hemisphere (Kaplan et al. 2011). Some hybrids may persist vegetatively *in situ* even when their putative parents disappeared. Parental ITS copies preserved in genomes of these relic hybrid clones provides unique insights into past but now extinct local plant diversity (Kaplan & Fehrer 2011). Three octoploid species were shown to be evolutionary successful allopolyploids, which at present have distribution ranges reaching continental levels (Kaplan et al. 2013). However, detailed comparative screening in Central Europe revealed that much of this diversity vanished during the 20th century (Kaplan 2010, Kaplan & Fehrer 2013). Recurrently formed hybrids may differ in their subsequent evolution, with some clones remaining intermediate but others exhibiting maternally driven expression of morphological characters (Zalewska-Gałosz et al. 2010).

Using flow cytometry, we detected frequent occurrence of hybrids in another challenging group of aquatic plants, the genus *Callitriche*. Although hybrids have only rarely been recorded in this group previously, our results suggest that both polyploidization and hybridization have interacted in its evolution. Unlike other plant groups, triploid hybrids are often vital and form abundant populations over large spatial scales (Prančl et al. 2014).

An analysis of Central European pines revealed that most taxa originally distinguished in the group of *Pinus uncinata* are referable as neglected hybrids. Because correct discrimination of hybrids and non-hybrid pines is highly important for efficient conservation of the protected endemic *P. uncinata* subsp. *uliginosa*, all previously described forms were revised and newly interpreted (Businský & Kirschner 2010).

The lack of inter-ploidy crosses, challenging previous views, was found in the genus *Chenopodium* (Mandák et al. 2012). Nuclear DNA amounts suggested an allopolyploid origin of the widespread 6x *C. album*, which most likely arose by hybridization between still unknown di- and tetraploid species.

Although past reticulation has played an important role in the evolutionary history of the genus *Hieracium*, with dominant polyploids, convincingly documented cases of recent interspecific hybridization are very rare. Recent natural hybridization was detected between two diploid species, resulting in dominant diploids but also one tetraploid. This is the first record of spontaneous polyploidization following interspecific crossing in this genus, suggesting an important role of hybridization in the evolutionary history of polyploid *Hieracium* species (Mráz et al. 2011).

Team members designed most of the cited studies, and either were responsible for, or contributed substantially to plant sampling and identification, crossing experiments, karyological and cytometric analyses, taxonomic conclusions, evolutionary interpretations and writing the texts.

### **Conservation consequences of interspecific hybridization**

Hybridization may pose a threat to endangered species as it can cause genetic erosion or even amalgamation of endangered species in hybrid swarms. We conducted several studies that involved rare, endangered or endemic plants. The results have important implications for species protection.

We resolved a long-held controversy about the rate and direction of hybridization in a flag-ship species of the Czech endemic flora, *Cerastium alsinifolium* (Vít et al. 2014). Our results showed weak reproductive barriers between this critically endangered species and a widespread sympatric congener *C. arvense*, and reshaped traditional views on habitat preferences of the Czech endemic. The same conceptual basis, i.e. the combination of flow cytometry and multivariate morphometrics, was adopted in other studies, incl. *Dryopteris* (both homoploid and heteroploid hybridization was detected; Ekrt et al. 2010) and *Spergularia* (Kúr et al. 2012). After confirming species-specific genome sizes in water lily samples collected across Eurasia (Volkova et al. 2010), we carried out a detailed biosystematic study of the genus *Nymphaea* in Central Europe (Kabátová et al. 2014). Interspecific hybridization under natural conditions was found to be quite rare, and involved both reduced and unreduced gametes.

Team members led three of the cited studies. In the remaining two, we conducted flow-cytometric analyses that served as a foundation for taxonomic interpretations (recognition of parental species and both heteroploid and homoploid hybrids). Our strength lies in flow cytometric analyses with very high resolution (low coefficients of variation of resulting fluorescence peaks), which makes it possible to discriminate between samples differing only slightly in genome size. There are just a few research groups worldwide that can achieve analyses with comparable accuracy.

### **Ploidy-specific interactions of host plants with symbiotic mycorrhizal fungi**

Although polyploidy has been shown to modulate interactions between plants and other organisms such as pollinators and herbivores, no information was available on whether it can also shape the functioning of mycorrhizal symbiosis. We pioneered this field and, using three different obligate mycotrophic angiosperm species, demonstrated that plant responses to arbuscular mycorrhizal fungi can indeed be ploidy-specific (Sudová et al. 2010). Interactions between plants and mycorrhizal fungi are complex, and functioning of the symbiosis may be modulated not only by ploidy

level but also by edaphic conditions (Doubková et al. 2011, Sudová et al. 2014). We also provided the first evidence for divergence of mycorrhizal fungi according to ploidy level of the host plant (in *Gymnadenia* orchids; Těšitelová et al. 2013). This divergence may contribute to niche partitioning and facilitate establishment and co-existence of different cytotypes.

Our studies were the first to address the topic of plant ploidy-mycorrhizal fungi interactions. The research was carried out in close co-operation with the Department of Mycorrhizal Symbioses. Members of our team conceived most studies, participated in the field work, estimated ploidy levels and contributed to article writing. Most experimental work was done by our collaborators.

### **Evolution and phylogeny of complex plant groups**

Phylogeny reconstructions based on molecular sequence data are, at present, the predominant method for studying evolutionary relationships among taxa at various taxonomic levels. A combination of molecular and karyological data allowed us to assess the evolution of karyotypes and the role of genome duplication and chromosomal change in the highly diverse family Potamogetonaceae. The results disproved previous hypotheses about frequent transitions between karyotypes and indicated only rare polyploidization events, which mainly shaped the early evolutionary history of the family (Kaplan et al. 2013).

Phylogeny reconstructions are particularly difficult in closely related species complexes with high degrees of hybridization and polyploidy. To assess strengths and limitations of individual molecular markers, we investigated the predominantly apomictic genus *Hieracium* s. str. Multi-copy nuclear markers provided the best approximation of the species tree. Basal polytomies and merging or splitting of species groups of non-hybrid taxa in other datasets were explained by a rapid diversification of the genus combined with ancestral polymorphism and incomplete lineage sorting (Krak et al. 2013). Three newly developed low-copy nuclear markers have great potential for phylogeny reconstruction in Asteraceae (Krak et al. 2012).

Robust phylogenetic analysis, based on the screening of multiple DNA regions, and an exceedingly rich selection of species, was conducted in order to assess relationships in the species-rich and widely distributed family Juncaceae. *Luzula* was revealed as monophyletic, while *Juncus* appeared to be a paraphyletic genus (Záveská Drábková 2010). A very basal lineage of the family was detected, which was traditionally included in the genus *Juncus*. As the lineage proved to be sufficiently distinct, it was described as a new genus, *Oreojuncus* (Záveská Drábková & Kirschner 2013). Detailed investigation of *Luzula* revealed that the traditionally accepted subgenera and sections appear to be non-monophyletic, sharing ancient haplotypes and ribotypes with their descendants. Both recent hybridizations and incomplete lineage sorting of ancestral polymorphisms explained the incongruence between chloroplast and nuclear data (Záveská Drábková & Vlček 2010).

All studies on Potamogetonaceae and Juncaceae were conceived by members of the team, who also were responsible for plant sampling, identification of plant material, karyological analyses, most molecular analyses, taxonomic conclusions and evolutionary interpretations, and wrote most of the text. In *Hieracium* publications, Jindřich Chrtek was responsible for sampling and determination of plant material, participated in study design, data interpretation and manuscript preparation.

### **Taxonomic monographs of critical groups**

The importance of alpha-taxonomy cannot be underestimated because the biological material is frequently misidentified, and the large species and DNA sequence databases are weed-infested by an alarming proportion of incorrect information. Taxonomic monographs are comprehensive accounts of all taxonomic data relating to particular plant groups. They are based on a thorough analysis and exhaustive synthesis of information from a wide range of approaches. At a global level they are an irreplaceable source of information as they combine otherwise incompatible concepts adopted in different countries into a coherent result based on profound taxonomic expertise.

The genus *Taraxacum* is among the groups most difficult to treat taxonomically. The lack of information is particularly obvious in underexplored regions of the world, such as Central China, the Himalayas and Middle Asia. In a series of taxonomic works, backed by population, reproductive and phylogenetic studies, an enormous unexplored diversity of dandelions in the vast region spanning from China and Mongolia to the Himalayas and Middle Asia was evaluated (Kirschner & Štěpánek 2011, Kirschner et al. 2014). Five new sections and more than fifty new species were recognized and described. A part of the results were published in the prestigious compendium of the multivolume Flora of China (Ge et al. 2011). Further revision included taxonomic treatments of plants from southern Europe, the Mediterranean area and the South Caucasus (Štěpánek & Kirschner 2012, 2013a, b, 2014, Štěpánek et al. 2010, 2013).

Several other papers were published, evaluating variation and diversity within taxonomically difficult groups of Potamogetonaceae (e.g. Kaplan 2010, Kaplan & Marhold 2012, Kaplan & Reveal 2013, Lastrucci et al. 2010) and Juncaceae (Rooks et al. 2011).

The monographers Zdeněk Kaplan, Jan Kirschner and Jan Štěpánek acted as taxonomic experts in all studies. Either they were sole author(s) or played important roles. Vlasta Jarolímová contributed with karyological analyses.

Besides the outputs listed above, members of the team have contributed to several foreign and international projects focused on expert assessment of plant diversity, including Flora of China, Euro+Med PlantBase, Rothmaler's Exkursionsflora von Deutschland, The Jepson Manual Vascular Plants of California, Flora vascolare della Calabria, and Catalogue of the Vascular Plants of Madagascar.

### **Plant diversity research for economic utilization**

A potential crisis of *Hevea rubber* on the world commodity markets led to a revival of interest in alternative sources of rubber, particularly from the Kazakh Dandelion, *Taraxacum koksaghyz*. Because it is a sexual species, its cultivation and utilization requires controlled reproduction. Contamination of the original samples in seed banks, botanical gardens and germplasm collections worldwide by achenes of the triploid agamospermous *T. brevicorniculatum* resulted in total replacement of *koksaghyz* by the asexual species, exhibiting very limited rubber production. Achenes of true *T. koksaghyz* were distributed to selected germplasm collections to replace the material erroneously preserved under the latter name. This result is of enormous importance for the future exploitation of this species. It also shows that basic taxonomic work may have a direct economic impact on business-based projects (van Dijk et al. 2010, Kirschner et al. 2013).

Jan Kirschner worked together with Jan Štěpánek on an equal basis; they were responsible for most data collation and writing of texts, and entirely for the biosystematic investigations and taxonomic interpretations.

## **Diversity, ecology and evolution of lichenized fungi**

Team members conducted numerous studies addressing several hot topics of lichen taxonomy, ecology and evolution. Modern methods combining molecular analyses and sophisticated phenotypic approaches (incl. chemotaxonomy) were applied.

The first world-wide taxonomic reappraisal with phytogeographical outcomes was done for the crustose genus *Myrionora* (Palice et al. 2013). Large-scale molecular phylogeny works of selected species groups within collective genera *Caloplaca* and *Lecanora* contributed significantly to understanding their evolution and relationships (Vondrák et al. 2010c, 2011, 2013a, Šoun et al. 2011, Pérez-Ortega et al. 2010).

Despite long-term research, central-European lichen flora is still insufficiently understood. Recent field surveys yielded dozens of new country records and rediscoveries of species regarded as vanished (Malíček et al. 2014). A detailed inventory in old-growth forests indicates that the number of lichen taxa in contemporary biodiversity studies is underestimated and more detailed sampling is required to fully understand their diversity (Malíček & Palice 2013). Important changes in the distribution of many species (namely epiphytes) due to air pollution were found in the 1970s and 1980s. However, even very sensitive species could survive in relict habitats such as in deep river valleys, and may shift their ecological preferences (Vondrák & Liška 2010). Several published studies dealt with taxonomically difficult and ecologically unique groups of microlichens (Jabłońska et al. 2011, Vondrák et al. 2013b).

An extensive synthesis of available data resulted in the checklist and the Red List of lichens of the Czech Republic (Liška & Palice 2010). Of the 1526 species listed, 37.3% are threatened and 9% are extinct. The Red List represents an important tool for nature conservation authorities in conservation assessments of particular areas. Nomenclature contributions helped to clarify previously unresolved issues in lichen taxonomy (e.g. Liška 2011b, 2013b, Palice et al. 2011). Another review summarized the history of lichen research in the Czech Republic, and compared lichen diversity with data from neighbouring countries (Liška 2012b).

Team members contributed to explorations of lichen diversity in other European and extra-European countries (e.g. Fačkovcová et al. 2014, Joshi et al. 2011, Vondrák & Liška 2013, Vondrák et al. 2010). They also participated in the preparation of the recent German lichen flora (Palice in Wirth et al. 2013, Vondrák & Wirth 2013). Particular attention was paid to underexplored remote areas with specific ecological conditions such as the high Andean páramos ecosystems (e.g. Jørgensen & Palice 2010, 2012, Sipman & Palice in Lumbsch et al. 2011, Sklenář et al. 2010).

The organization of fungal and algal partners into specific vertical stacks constitutes interesting eco-physiological adaptations in rock-dwelling lichens from continental arid areas. It helps more efficient use of light irradiance by the photobiont and enhances the overall fitness of the lichen in extreme conditions (Vondrák & Kubásek 2013).

Almost no information was available on viruses in lichens. Several plant viruses were isolated from lichens and it was shown that more viruses can infect the lichen simultaneously (Petrzik et al. 2013).

The team members published 64 papers and book chapters in 2010–2014. Either they were the sole author(s) or contributed significantly by their expertise. In particular, they collected and identified lichen samples and contributed to, or were responsible for, interpretation of data and taxonomic evaluation.

## **Systematics and phylogenetic trends in pleomorphic microscopic fungi (Ascomycota)**

Mycological investigations were aimed at molecular-systematic studies of fungi from different poorly known groups. Special attention was paid to pleomorphic fungi that reproduce both sexually and asexually, i.e. from independent spore-stages in their life cycle. They represent the long-standing problem of integrating asexual morphs into the main framework of sexual morph-based fungal systematics. The multidisciplinary research includes multigene phylogenetic analyses, bioinformatics, architecture of secondary structure (2D) elements of ribosomal RNA and novel approaches in structural phylogenetics (Réblová & Réblová 2013). A robust phylogeny-based taxonomy was constructed and interpreted with emphasis on *in vitro* experiments and original morphology-based hypotheses.

Novel evolutionary fungal lineages, comprising saprobic and important plant, animal and human pathogens, were revealed in two classes. The impossibility of reliably identifying them has complicated the study of their biology and ecology worldwide. Therefore, the discovery of their life histories, and correlation of sexual and asexual morphologies with phylogenetic data, significantly broadened our knowledge. These lineages include the new order Glomerellales and four new families in the Sordariomycetes (Réblová et al. 2011). A novel lineage, the Cyphellophoraceae, comprising medically important species involved in long-term skin infections of humans and animals, was discovered (Réblová et al. 2013). A previously unknown evolutionary lineage, only distantly related to all known fungal organisms and consisting of a single taxon, *Cirrosporium novae-zelandiae*, endemic to New Zealand, was revealed (Réblová & Seifert 2012). The lineage reproduces only asexually and is characterized by a rare type of meristem-arthritic conidiogenesis.

We significantly contributed to the topic of amalgamating asexual morphs into the overall ascomycete system by discovering 12 new life histories in the Sordariomycetes and identifying 14 new holomorph genera (Jaklitsch et al. 2013, Réblová 2011, 2013, 2014a, b, Réblová & Seifert 2011, Réblová et al. 2012, 2014).

All cited studies were designed by Martina Réblová, who performed phylogenetic analyses, collected material and wrote most of the text. Kamila Réblová was responsible for 2D homology modelling of the ribosomal RNA and wrote corresponding parts of the text. Keith Seifert and Walter Jaklitsch wrote the text, collected partial material, helped to interpret phylogenies and the mode of respective conidiogenesis, and supervised the nomenclatorial parts. Jacque Fournier collected fresh fungal material and Václav Štěpánek carried out PCR experiments.



## Research Report of the team in the period 2010–2014

Institute	Institute of Botany of the CAS, v. v. i.
Scientific team	Department of Population Ecology

The Department of Population Ecology is a major national research center dealing with plant population biology, ecology and genetics, community ecology and evolutionary biology. Researchers in this group present their results in high quality international journals and collaborate with top researchers from all over the world (mainly from Universities in Sweden, Switzerland and USA). In addition to this, we are also attempting to use the results of our research for direct conservation actions and thus we work in close collaboration with administrations of national parks, the national agency for nature and landscape protection, and NGO's performing practical management at localities of various protected species. Our major scientific achievements for the period of 2010-2014 may be grouped under the following categories:

### Grassland ecology

The main focus of the grassland ecology group is diversity in grasslands, with particular reference to species-rich mountain grasslands. We have been building on an existing long-term spatiotemporal data series from two mountain grasslands, which contains a wealth of data on species spatiotemporal dynamics on a fine scale, species replacements etc. We used data extracted from this time series to identify a guild structure of the grassland community, and have tested this guild structure using a series of removal experiments. We demonstrated that spatiotemporal dynamics is not an outcome of niche differentiation; species replacement patterns resemble a neutral process with sets of species differing in the speed at which they fill empty spaces (Herben et al. 2013). Results of this research has also served as a guide for richness-centered management of these grasslands (Pecháčková et al. 2010).

Further, we are studying root systems of grassland communities. In particular, we are examining responses of root systems of individual species in the field to removal of the main dominant. As roots are notoriously difficult to identify, we are using quantitative PCR-based techniques to assess the amount of root biomass (after proper calibration). This research is currently underway and we expect the first results to be submitted for publication by the end of 2015.

One of the main tools for understanding the driving forces of spatiotemporal dynamics of grassland systems is spatially explicit modelling. We built on earlier research on the topic, and parameterized a process-based, fully spatially explicit model of one of the study grasslands. The model was constructed in such a way to be able to take account of clonal growth, and the existence of clonal connections belowground (this made it possible to link model parameterization and predictions to already existing data on rhizome structure). Using the parameterized model, we were able to perform virtual experiments ("in silico") to ask questions that cannot be examined otherwise (e.g. such as those requiring manipulation of trait values of component species (Herben & Wildová 2012, Yu et al. 2012). Here we were able to show how functional

diversity of individual plant traits affects species diversity of the system, and to examine different effects depending on the type of trait in question. In particular we showed that effects of traits that are associated with positioning in a competitive hierarchy are very different from those of traits associated with niche differences (Wildová et al. 2012, Herben & Goldberg 2014). Our results strongly suggest that inferences of community assembly processes taken from patterns of trait dispersion can be misleading. In addition, our department participated in a critical review of spatially explicit simulations of clonal plants (Oborny et al. 2012).

### **Comparative biology of clonal plants**

The main focus of the group for the study of clonal plants is the comparative biology and ecology of clonal plants. This is done in collaboration with Jitka Klimešová. Here we managed to link the existing database on clonal traits (CLOPLA) with extensive data on patterns of vegetative and seed reproduction in culture, and also with data on genome sizes and ploidies (Herben et al. 2012 Ann Bot). The research has hitherto shown that functional differences between clonal and nonclonal plants are deeper than previously thought (Zobel et al. 2010, Herben et al. 2014a, Klimešová et al. 2015). These groups strongly differ in their reproductive biology, in key constraints on their demographic processes, in traits that predict their overall abundance, and frequency in individual habitat types (Herben et al. 2014b, Klimešová et al. 2015). Different clonal growth habits are distributed in a non-random fashion across communities in Central Europe (Herben et al. 2015 JVS).

### **Ecology and dynamics of dry grasslands**

Dry grasslands belong among the most species-rich habitats throughout Europe. At the same time, they are endangered by changes in land use, abandonment of traditional management and habitat fragmentation. Detailed knowledge about grassland ecology and about the effects of environmental and landscape changes on dynamics of a grassland community are necessary for successful protection of these valuable habitats. Over the period 2010-2014, we were involved in two large projects concerning grassland dynamics.

In the first large project, we determined the relative importance of stochastic and deterministic factors for community composition in dry grasslands, with a special focus on dominant plant species. We found evidence for both deterministic and stochastic processes that influence species richness and form species compositions in plant communities. While species richness can, to large extent, be explained by habitat conditions and/or landscape structure (Knappová et al. 2012, Čepelová & Münzbergová 2012), species composition could be more sensitive to stochastic factors, especially at the early stages of community assembly. In particular, experimentally induced differences in seed density, mimicking different intensities of natural seed rain, may explain differences in the composition of natural communities that cannot be attributed to habitat conditions (Münzbergová 2012). Seed availability also seems to be much more important than species traits for successful colonization of a new habitat (Knappová & Münzbergová 2015). Particularly grassland specialists with narrow habitat requirements and poor competitive abilities are unlikely to find suitable microsites within a dense canopy unless they arrive in sufficient density. Their successful establishment thus depends on microsite and seed availability rather than on dispersal ability (Knappová & Münzbergová 2015). According to our combined field and garden experiment, the four involved dominant species likely produce strong negative plant-soil feedback, i.e. modification of the biotic and abiotic soil environment

by plant roots or plant litter leading to suppression of individuals of the same species (Hemrová et al., in prep.). This feedback might complicate the assessment of habitat suitability.

In the second project, we explored the relationship (past and present) between landscape structure and current plant species dynamics, and we use this information for predicting future species dynamics. We demonstrated that past landscape structure is important for determining species distributions, as well as within species genetic diversity, and its effects are in fact stronger than the effects of present landscape structure (Münzbergová et al. 2013b, Husáková & Münzbergová 2014, Hemrová and Münzbergová in press). In addition, we demonstrated that species responses to past landscape structures can be predicted by species traits (Hemrová & Münzbergová in press). An important prerequisite for modelling future species dynamics is proper identification of suitable habitats for a species and we thus tested different approaches to fulfill this aim (Hemrová & Münzbergová 2012, Tajek et al. 2011). We demonstrated that best predictions can be obtained by combining information on species composition and environmental conditions of the studied localities.

In addition, we explored the ability of dry grassland species to colonize newly created habitats in the landscape and demonstrated that abandoned fields are suitable habitats for some, but not all, dry grassland species (Knappová et al. 2012, Knappová et al. 2013).

To explore landscape dynamics of a species, we built a model and used it to identify to what extent individual species are affected by present and past landscape structures (area and isolation). We also demonstrated the importance of large herbivores on species dynamics in the landscape (Hemrová et al. 2012). All the studies enabled us to fully understand the system and to estimate the importance of individual parameters necessary to model future dynamics of a species.

### **Importance of soil communities for diversity of grassland communities**

One important finding of our research on dynamics of dry grassland species is that not all grassland species are able to colonize newly abandoned fields in the area. In cooperation with the Department of mycorrhizal symbiosis we focused on the importance of arbuscular mycorrhizal fungi (AMF) for growth of these dry grassland species.

First, we looked at the adaptation of plants to their native single AMF species and the whole community. The results demonstrated that local genotypes of our model species, *A. amellus*, were able to adapt to local conditions and that this adaptation was mediated by AMF (Pánková et al., 2011). This result was also confirmed in a subsequent experiment, which additionally demonstrated that plants of a given origin supported a specific abundance of particular morphological structures of AMF in their roots, independent of where they were actually grown (Pankova et al. 2014a). Genetic determination of the degree and type of root colonization by AMF was also confirmed in a 5 year field transplant experiment (Pankova et al. 2014b).

Furthermore, we extended this research to the level of plant community, and tested the impact of AMF community on the establishment and structure of a plant community on dry grassland as well as on an abandoned field. To be able to test the effect of an AMF community on the establishment and structure of a plant community, we firstly evaluated the AMF species composition using molecular techniques and functional parameters (such as mycorrhizal inoculation potential and the number of infective propagules in the soil) of the AMF community in the grassland and abandoned field. The results showed that the AMF community composition was influenced by

biotope and the AMF species varied in their distribution across the dry grassland and abandoned field (Voříšková et al. – in prep.). The methodology for such a molecular evaluation of soil communities was also published (Kohout et al., 2014). A field fungicide experiment subsequently demonstrated a strong effect of suppression of grassland species after fungicide application and an increasing coverage of species with low or no mycorrhizal dependence (Dostalek et al., 2013). A specific test of the importance of the AMF community on establishment of dry grassland species and on the abandoned field is currently under way.

### **Evolution of dispersal traits**

Understanding species dynamics in the landscape also requires an understanding of the evolutionary potential of dispersal traits. To study this, we used oceanic islands as an analogy to a fragmented landscape on the mainland. Evolution was studied at two different levels. Firstly, traits between closely related endemic and non-endemic species were compared to test for the existence of changes in their dispersal ability. Changes were revealed, mostly in the opposite direction than we expected – many pairs showed a better dispersal ability of endemics than for non-endemics (Vazačová & Münzbergová 2014a). Secondly, we focused on the importance of dispersal traits for further species dispersal among islands and the colonization of new habitats. The results showed that no dispersal trait alone can explain the distribution of species among islands. Species presence on the islands was the result of a combination of both good dispersal ability and traits related to species persistence at the locality, as well as the abundance of the species in the archipelago (Vazačová & Münzbergová 2014b). The methodology of inferring dispersal potential of island species was complemented in Vazačová & Münzbergová (2013).

### **Ecology, population biology and evolution of polyploidy systems**

Polyploidization is a major factor driving species diversification and, as such, it has received a lot of attention in the past. While many previous studies explored morphological differences between different cytotypes, researchers have only relatively recently started exploring the distribution of cytotypes in the landscape and attempted to understand the distribution and migration of different polyploidy taxa. We studied a unique diploid-hexaploid system, *Aster amellus*, and demonstrated a diffuse contact zone between cytotypes with only a single mixed ploidy population detected out of several hundred studied (Castro et al. 2012). In a subsequent genetic study, we demonstrated only limited gene flow between the cytotypes, with consistent patterns across a wide spatial range (Münzbergová et al. 2013a). This fits well with the observed existence of strong breeding barriers between cytotypes (Castro et al. 2011). All knowledge on this peculiar system was summarized in a large review paper (Münzbergová et al. 2011).

In addition to working on the *Aster amellus* system, we were generally interested in consequences of polyploidy for species performance. We (Černá & Münzbergová 2013) demonstrated that habitat conditions are more important than cytotype for performance of the *Anthericum* species. In contrast, we found strong effects of cytotype on performance of *Vicia* species (Eliášová & Münzbergová 2014). This fits with strong differences in genetic diversity and degree of inbreeding of these two cytotypes (Eliášová et al. 2014).

The dynamics of plant populations are strongly affected by a wide range of organisms. As this topic received only limited attention up to now, we thus attempted to understand the importance of various biotic interactions on performance of different

polyploid species. In collaboration with a team from Stockholm University, Sweden, we demonstrated the crucial importance of cytotype on plant-insect interactions in the tetraploid-octoploid system of *Cardamine amara* (Arvanitis et al. 2010). This was also confirmed in a study in a diploid-tetraploid system of *Centaurea phrygia* (Münzbergová et al. in press). We also studied the importance of cytotype for species responses to AM fungi (Sudova et al. 2010, Sudova et al. 2014). These studies, carried out together with the Department of Mycorrhizal symbiosis, demonstrated that the importance of cytotype for plant-AMF interaction is largely species specific and that it may be significant, but not the main player driving performance of species in polyploid systems.

Overall, these results demonstrate that changes in ploidy level may have important consequences for species dynamics, and that these differences may be confounded by differences in habitats in which the cytotypes originated or currently occur. To deal with this, we initiated work towards creating synthetic polyploids of our model species, which will be used for our future experiments. First progress in this work was presented as the master thesis of Z. Pavlikova (2014).

Finally, we attempted to understand the genetic basis of polyploidy. During her stay at Harvard University, USA M. Šurinová compared the evolution of 70 meiotic genes of polyploid and diploid *Arabidopsis arenosa* lineages (Wright et al. 2014). The results showed strong selection for meiotic genes in tetraploid lineages, leading to amino acid changes. Our results indicate selection on structural proteins involved in sister chromatid cohesion, the formation of meiotic chromosome axes and synapsis.

### **Transgenerational plasticity and epigenetic variation in ecology and evolution of plant populations**

Plants often react to environmental variation by phenotypic plasticity. This plasticity may just represent a passive response to resource limitation, for instance limited growth under water deficiency. More typically, plasticity is a functionally appropriate adjustment of the phenotype that acts to enhance fitness under current environmental conditions. Moreover, the expressed phenotype of parents, and sometimes also grandparents, could be extended to their offspring. This aspect is often called transgenerational plasticity or transgenerational effects. These transgenerational effects can be facilitated by heritable epigenetic variation, such as methylation of cytosine. Because epigenetic variation influences phenotypic expression of traits that usually contribute to individual fitness, these traits can be subject to natural selection. Therefore, heritable epigenetic variation can influence not only individuals themselves but also their interactions with other organisms, and also evolution of natural populations. We therefore investigated the role of transgenerational plasticity and tested the role of epigenetic variation in functioning of plant populations. Indeed we revealed strong maternal effects in our system (Latzel et al. 2010, Latzel & Klimešová 2010a), (Latzel & Klimešová 2010b) and demonstrated that an offspring always performed better if exposed to its maternal environment (Latzel et al. 2014a). In particular, our study provided strong evidence for transgenerational effects of different nutrient conditions on the phenology, biomass production and carbon storage of *P. lanceolata*. Our results thus strongly support the idea that transgenerational plasticity can be adaptive, which can alter evolutionary trajectories of plant species. As a byproduct of the study, we also tested how the levels of soil nutrients affect carbon storage policy in plants (Latzel et al. 2014b).

In another study (Latzel et al. 2013), we tested how the epigenetic diversity of genetically uniform *A. thaliana* populations can alter their functioning and how diversity affects other species of experimental communities. This is an absolutely novel

approach that has never been studied because epigenetic diversity is considered as another component of biodiversity. Indeed, our study (Latzel et al. 2013) clearly demonstrated that epigenetically diverse populations produced more biomass than epigenetically uniform populations and moreover, that epigenetic diversity improved resistance of plant populations to pathogens and invasions. Thus, our study clearly demonstrated that epigenetic diversity of populations, similarly to genetic and species diversity, can improve the stability of plant populations and consequently the functioning of whole ecosystems.

### **Biotic interactions and determinants of exotic plant invasion success**

Invasion success of non-native plants is explained by their advantage when interacting with native environments and species. However, in our tests of multiple exotic plants, as well as interacting native species from several trophic levels, we found that, on average, exotic plants are similarly influenced by soil pathogens and leaf herbivores, and do not differ in competitive ability or in allelopathic effects when compared with native, closely related plants (Dostál 2010, Dostál 2011, Dostál & Palečková 2011, Dostál et al. 2013a, Jandová et al. 2015). We demonstrated that interesting mechanisms of biotic interactions, working through plant - soil feedback, were responsible for species invasion success. The invasive plants suppressed the native species by accumulation of soil pathogens, either temporarily in younger invasive populations (*Heracleum mantegazzianum* study, Dostál et al. 2013b) or by means of changes in behaviour of invasive populations that had lost susceptibility to their own pathogens and increased their growth (*Rorippa austriaca* study, Dostálek et al., under review in Plos One). Such a loss of negative intraspecific soil feedback and increased growth of the invasive populations contributed to further successful invader range expansion. A strong effect of the invader on soil quality was also demonstrated in the study of *Parthenium hysterophorus*, a major invader in Nepal, with a substantial impact on species diversity and economy (Timsina et al., 2011).

Understanding the determinants of biological invasions is a key prerequisite for successful eradication of invaders. One possible way to reduce invaders or noxious weeds is to make use of their natural enemies. To explore the importance of natural enemies on species invasion ability, we studied the importance of natural enemies on performance of *Cirsium arvense*, a major invader in North America. While we demonstrated that the insect communities in the native range can be extremely diverse (Abela-Hofbauerova et al. 2011), we have also shown that the impact of natural enemies on plant performance are quite limited. Still, there are strong differences in performance of plants from the two ranges (Abela-Hofbauerova and Münzbergová 2011) and these may at least partly be explained by differences in production of secondary metabolites between plants from the two ranges (as shown in the Master's thesis of I. Karska 2014 supervised by Z. Münzbergová).

Tree species invasions are not so frequent but when they happen, they influence large areas and have strong economic effects. Such a type of invader is the conifer tree species, *Pinus strobus*, the major invader in sandstone areas in the Czech Republic. To assess all aspects of the invasion dynamics we studied species genetic diversity in the native and invasive ranges, population dynamics, ability of long-distance dispersal and effects on soil communities. The research team comprised scientists from four departments of IB ASCR (Departments of genetic ecology, population ecology, mycorrhizal symbioses and GIS and remote sensing). This enabled us to cover the broad scale research area resulting in a range of publications (Mandák et al 2013, Wild et al. 2013, Kohout et al. 2011, Münzbergová et al. 2010, Münzbergová et al. 2013c).

Our data show, that the species possesses great genetic diversity in the adventive area comparable to that in the native range. This probably supports the great invasive success of the species. We found that it is able to spread several hundreds of metres over one generation, to regenerate and grow along a wide range of environments with a population growth rate always above one (although different stages were important in different environments), suggesting that the species would further spread without management measures. The results were also used in decision making on management of this species in the most invaded National park, as V. Hadincová and J. Wild are members of the NP forest committee.

### **Ethnobotanical studies from Nepal**

To understand the distribution patterns and uses of medicinal plants in Nepal, we initiated a large field and literature survey of this topic and demonstrated that the distributional patterns of medicinal plants deviated from distributional patterns of all plant species from Nepal by being more abundant in lower elevations, where they are also most endangered (Rokaya et al., 2012b). As information on medicinal plants is still scarce in many regions of Nepal, we performed a detailed study of medicinal plants used in the Humla district, west Nepal (Rokaya et al., 2010), in central Nepal (Luitel et al., 2014) and used for specific diseases such as gastrointestinal problems in the whole of Nepal (Rokaya et al., 2014). We found that different regions were exceptionally rich in plant diversity and people used herbal remedies for various kinds of ailments.

After prioritizing important plant species, a detail study on *Rheum* was carried out (Rokaya et al., 2012c). To understand the possibilities for cultivation of these species, we conducted germination experiments with these species (Rokaya & Münzbergová, 2012) and concluded that seeds of both species will germinate better if shifted to lower altitudes, where temperatures are higher than in their actual habitats. In a detailed study on secondary metabolites in these plants, five different anthraquinones (chrysophanol, emodin, aloe-emodin, physcion and rhein) and two stilbene compounds (piceatannol and resveratrol) were determined and compared in cultivated and naturally grown plants (Rokaya et al., 2012a). The results suggest that the two species could be used interchangeably; the cultivated plants could be used when high levels of piceatannol and resveratrol are desired, whereas plants from natural habitats could be used to obtain higher levels of the other compounds. Finally, we studied population dynamics of the two *Rheum* species and designed a strategy for their sustainable harvest from natural populations (Rokaya et al., under review). In addition to medicinal plants, we explored the diversity of orchid species found in Nepal (Rokaya et al., 2013).

### **Population biology and genetics of rare endangered species within central Europe**

Descriptions of local population dynamics and the distribution of genetic diversity within a species are major steps in designing efficient conservation plans for endangered and protected species. Using a range of critically endangered and endangered species as models, we attempted to understand these processes in populations of these species and to detect generalities in their performance that would allow generalization of our results across species.

In studies of species population dynamics, we attempted to understand differences in population dynamics between different populations of the same species occurring in geographically isolated area (Dostálek and Münzbergová, 2013) or in ecologically distinct habitats (Heinken-Smidova & Münzbergová, 2012), and between

closely related species occurring in the same habitat (Bucharová et al. 2010, Černá & Münzbergová 2013, Münzbergová 2013). These studies generally demonstrated local habitat conditions are more important for species performance than species identity and suggest that knowledge of critical life cycle stages can be transferred between closely related species within the same habitat. By using information on population dynamics of species from years with different climatic conditions, we were also able to assess the effects of future climate change on performance of one of our model species (Bucharová et al. 2012).

As the population studies imply major effects of habitat conditions on species dynamics, we attempted to study ecological requirements for closely related species, aiming to reveal possible causes of species rarity. We did this by studying habitat requirements and host specificity of three *Thesium* hemiparasitic species (Dostálek & Münzbergová 2010). The study did not detect any major differences between rare and common species and suggested that *Thesium* species do not specifically select hosts, but rather they occur in microhabitats with specific conditions.

In addition to the studies of plant population dynamics, we also attempted to understand the drivers of the observed species dynamics. To do this, we explored the ecological factors determining female reproductive success of *D. austriacum* and the impact of pollen limitation on population growth (Castro et al. in press). The results revealed that fruit and seed production was significantly pollen-limited in all populations studied. However, despite the positive effect of pollen supplementation on seed production, the resulting increase in seed number did not significantly increase population growth rates in any of the populations.

An important aspect in rare species conservation is not only maintaining sufficient number of individuals at a locality but also retaining high genetic diversity. Although this concept is generally accepted, the information about genetic diversity of rare species is still rather limited in many areas. Within Europe, these data are missing, even for many high priority species that are considered to be the most endangered plant species throughout Europe. We thus estimated the genetic diversity and assessed its importance for plant fitness in species belonging to the most endangered species in Europe, *Dracocephalum austriacum* L. (Dostálek et al. 2010), *Thesium ebracteatum* (Dostálek et al. 2014) and *Ligularia sibirica* (Smidova et al. 2011). The results indicated high between-population genetic variations in all three systems and allowed us to select the most valuable populations for conservation of genetic diversity in the studied regions. In addition they demonstrated that genetic diversity of populations is closely correlated to plant fitness and support the notion that conservation of sufficient genetic diversity should be a top priority in all systems.

Most of the above work was carried out within a large project dealing with priorities of species conservation that was running in cooperation with the Agency for Nature and Landscape Protection. In addition to studies on single species cited above, we also demonstrated key differences in life history traits between critically endangered species and common species in the Czech flora (Gabrielova et al. 2013). In addition to publication of papers in scientific journals, we also published a summary publication, summarizing all the research on critically endangered species of the Czech Republic in a local journal published by the Agency of Nature and Landscape Protection (Münzbergová et al. 2001 eds. - 372p.). Our research was also directly implemented into methodology for the protection of various critically endangered species (Pánková 2011, Pánková & KlauDIS 2010) and for preparation of rescue programmes for *Gentianella bohemica*.



### **The consequences of cockroach pollination for plant reproductive success**

Four orders of species are commonly recognized as typical plant pollinators: Coleoptera, Diptera, Hymenoptera and Lepidoptera. Information on pollination by members of other insect groups is fragmentary although it might not be, in fact, that infrequent. We studied specialization in a cockroach pollination system on an isolated tropical inselberg (a rocky outcrop) in South America pollinating a species that we described as a new species *Clusia blattophila* (Vlasakova & Gustafsson 2011). The results demonstrated that cockroaches have no specialized structures for pollen transport, their foraging ranges are limited to several tens of meters, and visitation rates are very low (Vlasáková 2015). As a result, seed production of *C. blattophila* is pollen limited (Vlasakova & Jarau 2011). Consequently, the rate of regeneration from seeds is low. Hence, the dominance of *C. blattophila* on the inselberg must be attained by other means than sexual reproduction, in particular, extensive vegetative growth. This is typical for the persistence strategy of survival, which is characteristic for harsh environments with limited recruitment sites and low productivity such as inselbergs (Vlasáková 2013). Three diploma students participated in the project, and one diploma thesis has been defended.

## Research Report of the team in the period 2010–2014

Institute	Institute of Botany of the CAS, v. v. i.
Scientific team	Department of Genetic Ecology

The Department of Genetic Ecology focuses on various aspects of plant biology using tools of molecular biology and population ecology, namely on (i) genomic studies, (ii) biogeography and phylogeography, (iii) evolution and phylogeny and (iv) biosystematics.

### Genomic studies in allopolyploid wheatgrasses (Poaceae – Triticeae)

Grasses from the tribe Triticeae (Poaceae) represent fascinating examples of reticulate evolution. The ability of many species of this group to hybridize with other species is remarkably high, and likely accounts for the reticulate structure of the tribe. The tribe includes economically important grasses, such as cereal and forage crops, as well as troublesome weeds of worldwide distribution. On the one hand, the ease with which some species hybridize, is exploitable in crop breeding programmes. On the other hand, it may have a serious impact on natural plant communities if, for example, transgene flow from a crop to a wild relative takes place. Intermediate wheatgrass (*Thinopyrum intermedium*) possesses many desirable agronomic traits, that, together with the ability to cross with wheat, makes it an invaluable source of genetic material useful in wheat improvement. In Central Europe, intermediate wheatgrass readily crosses with *Elymus repens*, a serious weed of the agrosystem. Therefore, gene flow from wheat through *Th. intermedium* and *E. repens* is possible. Both *Th. intermedium* and *E. repens* are predominantly allohexaploids ( $2n = 6x = 42$ ), whose origins and genome constitutions have not been satisfactorily resolved so far. Understanding the genomic constitutions and evolutionary histories of both polyploid wheatgrasses are basic prerequisites for understanding gene flow within the whole species complex. Both species are also excellent model species to study the evolution of allopolyploid plant genomes at the DNA level.

To achieve this goal, accessions of *E. repens* and *Th. Intermedium*, from their native distribution areas in Central Europe, were analyzed using sequencing of single-copy (granule-bound starch synthase I gene) and multi-copy (5S and 45S families of nuclear ribosomal DNA) DNA in concert with molecular cytogenetics (rDNA-FISH, GISH). In both species, new and interesting discoveries clarified their evolutionary histories. Our results showed that at least six distinct lineages were involved in the formation of *E. repens* (Mahelka, Kopecký 2010). *Pseudoroegneria* and *Hordeum* represent the major genome constituents, where *Pseudoroegneria* contributed two subgenomes out of three. Furthermore, two additional gene variants from within the Triticeae were discovered: one was *Taeniatherum*-like, but the identity of the other was unknown. Their presence was interpreted as introgression. Apart from these, the presence of further unexpected alien genetic material, originating from species outside the Triticeae and close to *Panicum* (Paniceae) and *Bromus* (Bromeae), were revealed. In particular, *E. repens* contained ITS rDNA and a small chromosomal segment corresponding to *Panicum*. It resided in the *Hordeum* subgenome and probably represents an old

acquisition of a *Hordeum* progenitor. Obviously, *E. repens* has experienced a very complex evolutionary history, likely involving multiple hybridizations, introgressions and allopolyploidization.

Unlike in *E. repens*, the genome of *Th. intermedium* consists of three distinct subgenomes (Mahelka et al. 2011, Mahelka et al. 2013). We showed that these three subgenomes were most likely donated by *Pseudoroegneria*, *Dasypyrum* and *Aegilops*. In particular, the contribution from *Aegilops* is a new and notable finding with implications for wheat improvement. The study of Mahelka et al. (2013) showed that both rDNA families in *Th. intermedium* have undergone different patterns of evolution. While the 45S rDNA family has evolved in a concerted manner towards almost rDNA sequence homogenization, no interlocus homogenization has likely occurred in the 5S rDNA family. Therefore, the 5S rDNA in *Th. intermedium* has rather evolved according to a mixed model of concerted and birth-and-death evolution.

### **Biogeography and phylogeography**

Attempts to understand the impact of climate and climatic changes on ecological, genetic, and evolutionary processes have long been an active research area in biology. These processes have traditionally been studied through analyses of fossil records. Although an important tool in macro-evolutionary studies, fossil data lack resolution on shallow evolutionary scales. Modern population genetics and phylogeography employ molecular data of high resolution and can fill this gap. There are several projects concerning phylogeography of various species using tools of both palaeoecology and molecular biology, i.e. (1) phylogeography of *Alnus glutinosa* and *A. incana*, (2) phylogeography of North American invasive tree species *Pinus strobus* and (3) phylogeography of *Hippophaë rhamnoides*, (4) biogeography of *Pringlea antiscorbutica* and (5) pantropical biogeography of Chrysophylloideae.

(1) *Alnus* species are keystones of alluvial and wetland habitats distributed through the European forest zones from the northern treeline to the Mediterranean. Understanding their last glacial occurrence and postglacial migration patterns may shed light upon the resistance and resilience of wetland forest habitats in the course of global climate change. Douša et al. (2014) reviewed migration pattern of subgenus *Alnus* (i.e. *A. glutinosa* and *A. incana*) in Europe on the basis radiocarbon dated pollen and macrofossil sites and showed that expansion of *Alnus* during the Late Glacial and early Holocene periods supports the presence of alders during the LGM (Last Glacial Maximum) in southern peninsulas and northerly areas in western Europe, the foothills of the Alps, the Carpathians and northeastern Europe. After glaciers withdrew, the ice-free area of Europe was likely colonized from several regional refugia; the deglaciated area of Scandinavia was likely colonized from a single refugium in northeastern Europe. In the more northerly parts of Europe, we found a scale-dependent pattern of *Alnus* expansion characterised by a synchronous increase of *Alnus* within individual regions, though with regional differences in the times of the expansion. In southern peninsulas, the Alps and the Carpathians, by contrast, it seems that *Alnus* expanded differently at individual sites rather than synchronously in whole regions. We broadly confirmed LGM refugial areas and the general pattern of the postglacial expansion of *Alnus* as presented in the Huntley and Birks (1983) "Pollen Maps". In addition to Huntley and Birks (1983) we emphasized the importance of northern LGM refugial areas for *Alnus* expansion.

(2) North American *Pinus strobus* is a highly invasive tree species in Central Europe. Using ten polymorphic microsatellite loci we compared various aspects of the large-scale genetic diversity of individuals from 30 sites in the native distribution range with

those from 30 sites in the European adventive distribution range. To investigate the pattern of genetic diversity of this intercontinental comparison further, we surveyed fine-scale genetic diversity patterns and changes over time within four highly invasive populations in the adventive range. Our data show that on a large scale, the genetic diversity found within the relatively small adventive range in Central Europe, surprisingly, equals the diversity found within the sampled area in the native range, which is about thirty times larger. Bayesian assignment grouped individuals into two genetic clusters separating North American native populations from the European, non-native populations, without any strong genetic structure shown over either range. In the case of the fine scale, our comparison of genetic diversity parameters among the localities and age classes yielded no evidence that genetic diversity increased over time. We found that spatial genetic structure (SGS) differed across age classes within the populations under study. Old trees in general completely lacked any SGS, which increased over time and reached its maximum in the sapling stage. Based on (i) the absence of differences in genetic diversity between the native and adventive ranges, together with the lack of structures in the native range, and (ii) the lack of any evidence of any temporal increase in genetic diversity at four highly invasive populations in the adventive range, we conclude that population amalgamation probably first happened in the native range, prior to introduction. In such a case, there would have been no need for multiple introductions from previously isolated populations, but only several introductions from genetically diverse populations (Mandák et al. 2013).

(3) The first comprehensive phylogeny of *Hippophaë* with high resolution and strong support for particular nodes has been reconstructed. This phylogeny was calibrated using paleobotanical records together with an equally well-resolved phylogeny of Rosales (order of Angiosperms). The ages of stem and crown nodes for particular taxa in both trees were estimated. The dated tree of *Hippophaë* was used for reconstruction of evolutionary history and biogeographical events in the genus. Our findings highlight the impact of different stages in uplift of the Qinghai-Tibetan Plateau (QTP) and Eurasian mountains and climatic changes in the Neogene on diversification and range shifts in highland flora on the continent. The results provide support to the idea of an immigration route for some European highland plants from their ancestral areas on the QTP across central and western mountain ranges of Eurasia in the late Miocene (Jia et al. 2012).

(4) The origins and evolution of sub-Antarctic island floras are not well understood. In particular there is uncertainty about the ages of the contemporary floras and the ultimate origins of the lineages they contain. *Pringlea* (Brassicaceae) is a monotypic genus endemic to four sub-Antarctic island groups in the southern Indian Ocean. We used a sample of populations across the whole range of the genus to study its biogeography. Our results lend support to the hypothesis that angiosperms persisted on the sub-Antarctic islands throughout the Pliocene and Pleistocene. Taken together with evidence from other sub-Antarctic island plant groups, they suggest the extant flora of sub-Antarctic is likely to have been assembled over a broad time period and from lineages with distinctive biogeographic histories (Bartish et al. 2012).

(5) Continental disjunctions in pantropical taxa have been explained by vicariance or long-distance dispersal. The relative importance of these explanations in shaping current distributions may vary, depending on historical backgrounds or biological characteristics of particular taxa. We aimed to determine the geographical origin of the pantropical subfamily Chrysophylloideae (Sapotaceae) and the roles vicariance and dispersal have played in shaping its modern distribution. Our results indicate that long-distance dispersal has been the dominant mechanism for range expansion in the

subfamily Chrysophylloideae. Vicariance could explain the South American–Australian disjunction via Antarctica, but not exchanges between Africa and South America and between New Caledonia and Australia, or the presence of the subfamily in Madagascar. We found little support for the hypothesis that the North Atlantic land bridge facilitated range expansions at the Palaeocene/Eocene boundary (Bartish et al. 2011).

### **Development of novel molecular markers**

Development of novel molecular markers for further phylogenetic and population genetic studies has been carried out for three groups (1) *Hieracium*, (2) *Alnus* and (3) *Cardamine*. (1) Krak et al. (2012) identified potentially single-copy genes in a cDNA library of *Lactuca* and explored their copy number and level of variability in the subtribe Hieraciinae (comprising the genera *Hieracium* s. l., *Andryala* and *Hispidella*). Three of these candidates were proven to be single-copy and showed higher variability (2.1–4.5 times) than nrDNA ITS in this subtribe. Moreover these markers were successfully cross-amplified to representatives of seven other Asteraceae tribes. Problems with paralogy or pseudogenization were observed only in three cases, indicating wider applicability of these markers in the Asteraceae. (2) In order to obtain a useful set of microsatellite markers for analysis of population genetic structure of *Alnus glutinosa* and *A. incana* populations, we investigated 39 previously developed *Betula*, *Alnus*, and *Corylus* simple sequence repeat (SSR) markers (Drašnarová et al. 2014). Of these markers, ten loci had successful amplification within the *Alnus* species. Finally, we designed two multiplexes composed of eight and nine loci for *A. glutinosa* and *A. incana*, respectively. By comparing sequences of source species and both *Alnus* species, we concluded that repeat motifs of five of these ten loci differed from those described for the source species. These differences represent mainly modifications of the original motifs and affected compound or interrupted repeats as well as pure ones. The repeat motifs of three loci of the two alder species also differed. These mutations could lead to erroneous estimates of allele homology, because alleles with identical lengths will not have the same number of repeat units. Hence, before using microsatellite markers in studies comparing two or more species, they should be carefully examined and sequenced to ensure that allele homology is really stable and not affected by various inserts that change the sequence. (3) Specific microsatellite markers discriminating between *Cardamine amara* and *C. rivularis* were developed using low coverage 454 sequencing of both species. These markers were used to trace *C. amara* and *C. rivularis* in the genomes of triploid *C. × insueta* and pentaploid and hexaploid *C. schultzei*. Using these markers, the genomic composition, frequency of origin and evolutionary success of the allopolyploid *Cardamine* species was inferred (Zozomová-Lihová et al. 2014).

### **Microevolutionary processes and phylogeny of closely related plant taxa**

Research on the genus *Hieracium*, traditionally subdivided into three subgenera: (i) *Hieracium* s. str.; (ii) *Pilosella* and (iii) *Chionoracium* was in several parallel directions: (i) analysis of populations of subgenus *Pilosella* in particular areas with special emphasis on their taxonomic identity, chromosomal numbers, reproductive mode and genotype composition. This resulted in discovery of unknown taxa from the territory of the Czech Republic and detection of several rare morphotypes (Křišťálová et al. 2010; Krahulec & Krahulcová 2011; Krahulcová et al. 2013). (ii) Analysis of mixed populations of apomictic and sexual *Pilosella* species to detect the role of residual sexuality of facultative apomicts. This was studied both in the field and also in experimental

gardens by hybridization experiments (Krahulcová et al. 2012, 2014; Krahulec et al. 2014; Urfus et al. 2014; Rosenbaumová & Krahulec 2015 – published online early 2014). This resulted in an explanation for the role of facultative apomicts regularly producing hybrids and increasing population genetic diversity. Surprisingly, apomicts act as drivers of polyploidization and in fact produce more diverse progeny than sexual plants with respect to ploidy levels. (iii) On the basis of hybridization experiments we also analysed reproductive modes of several categories of progeny (Krahulcová et al. 2011, Rosenbaumová et al. 2012). These results showed that expression of apomixis is unexplained by the existence of one or a few genes: hybrids containing the same and whole genome of a good apomict varied in penetration of apomixis from 0 to 100%. Reciprocal hybridization produced  $n+n$  hybrids, which differed in penetration of apomixis. By studying polyhaploid progeny of facultative apomicts we detected an autonomous cycle, polyhaploid – polyhaploid – new polyploid, producing new genotypes involving only meiosis with no gamete fusion (Krahulec et al. 2011). (iv) Phylogenetic relationships within the predominantly European subgenus *Hieracium* s. str. were studied using three cpDNA intergenic spacers (*trnV-ndhC* and *trnT-trnL*), low-copy nuclear marker (*sqs*) and a multi-copy nuclear marker (nrDNA ETS). This study focused on the predominantly European subgenus *Hieracium* s. str. comprised mostly of obligatory apomictic triploid and tetraploid taxa. Diploid species represent only a small part of the taxa and they are confined to certain geographic areas that often represent never-glaciated refuges. The hybrid origins of several taxa (diploid as well as polyploid) were successfully reconstructed, albeit not confirmed in all cases by all markers. Despite the same terminal clades being identified based on different markers, basal relationships were largely incongruent between the three phylogenies. The cpDNA and *sqs* phylogenies showed basal polytomies, whereas, according to ETS, two major lineages correlating with genome size and geographic origin of the species were recognized. Moreover the cpDNA and *sqs* trees showed merging or splitting of species groups, even of non-hybrid taxa, indicating a rapid diversification of the genus with ancestral polymorphism and incomplete lineage sorting. This study is a valuable example of how incorporation of seemingly contradictory data can help to understand species origins and evolutionary patterns in groups with abundant hybridization and polyploidy (Krahulec et al. 2013).

As a part of a broader study examining the phylogeny and gene expression of polyploid taxa within *Chenopodium album* agg., a first paper concerning genome size variation focused on (a) the origin of polyploid species and (b) the frequency of hybridization between species with different ploidy levels. We did not find any direct evidence for the existence of hybrids between two species with different ploidy levels within the *C. album* group. The sample/standard ratio of tetraploid and hexaploid species does not equal multiples of diploid species, which suggests that (i) tetraploids are not diploid autopolyploids and that (ii) hexaploids have not evolved from diploid species alone. Moreover, we have not found any hybrid plant, either in the field or in offspring resulting from our experimental crosses. In view of these results, we adhere to the opinion that *Chenopodium* species do not hybridize freely across ploidy levels. Our analysis of DNA levels, however, suggests that hexaploid *C. album* is an allopolyploid that has arisen by hybridization between a diploid and a tetraploid species, the identity of which are unknown (Mandák et al. 2012).

Vít et al. (2014) focused on morphology and variations in karyology of serpentine endemic *Cerastium alsinifolium*, with special emphasis on interspecific hybridization with *C. arvense*. The extent of interspecific hybridization significantly differed among habitat types and occurred predominantly in grassland habitats. On the other hand,

hybrids were almost absent in serpentine forest populations. This fact led us to build a novel hypothesis – serpentine forests as a primary habitat of endemic species. Based on genome size and morphometrics, only primary hybridization events were confirmed and former speculations about backcrossing were rejected. Interspecific hybridization has probably no direct influence on species integrity of rare endemic species.

Substantially different patterns were observed within Central European *Diphasiastrum* populations (Hanušová et al. 2014). We detected a continuous pattern in both morphological variation and genome size (strongly correlated together), suggesting extensive levels of interspecific gene flow within this region, including several large hybrid swarm populations. The secondary character of habitats of Central European hybrid swarm populations suggests that man-made landscape changes might have enhanced unnatural contact of species, resulting in extensive hybridization within this area. On the contrary, a distinct pattern of genomic size variation among individuals from northern parts of Europe indicates that pure populations prevail outside Central Europe. Introgressive hybridization among *Diphasiastrum* species in Central Europe represents a unique case of extensive interspecific gene flow among cryptogamic vascular plants that cause serious complications of taxa delimitation.

Studies of *Allium* were in two different directions: (i) participation in large projects of floras (Krahulec & Duchoslav 2010; Duchoslav & Krahulec 2012; Krahulec 2014), within the framework of which determination keys, plant descriptions and distributions, and ecological demands were prepared. These contributions help other botanists in correct plant determinations. (ii) Parallel line of studies concentrated on the distribution of cytotypes of *Allium oleraceum* (3x, 4x, 5x, 6x) in the Czech Republic and Slovakia. The pattern of coexistence of polyploids in *Allium oleraceum*, at the landscape and locality scale, and their ecology, were studied. Plants of different ploidy levels were found to be ecologically differentiated and the ruderal character of cytotypes increased in the direction 4x-5x-6x, with the largest realized niche differences being between tetra- and hexaploids. The results provide evidence for adaptive differences between ploidy levels, which may contribute to their complex pattern of distribution (Duchoslav et al. 2010; Šafářová et al. 2011).

### **Macroecological analyses of species pools**

Biodiversity hot-spots are regions harboring evolutionary heritage from ancient or recent geological epochs, i.e. evolutionary museums or cradles, respectively. We hypothesize (i) there are also museums and cradles within regions: species-pools of particular habitat-types harboring angiosperm (flowering plants) lineages from ancient, others from recent, geological epochs; (ii) habitat-specific abiotic factors control numbers of angiosperm lineages maintained from a given epoch in a given habitat species-pool. We found that species-pools of most habitat types were characterized by over- or under-representation of lineages from at least one epoch, dating back until the origin of angiosperms. These patterns are not captured by mean phylogenetic distances. Habitat types very differently represent diversity of lineages from different epochs. Abiotic environments explained on average 53% and up to 75% of the variance in numbers of lineages per epoch, but with opposing effects of the same factor for different epochs. Specifically, warm and dry habitats tend to harbour lineages dating back to warm and dry epochs. Human impact during the 20<sup>th</sup> century correlates to major declines in habitats harboring lineages from the Early-Cretaceous to Paleogene (Bartish et al. 2010; Gerhold et al. 2011; Hermant et al. 2012; Ozinga et al. 2013).

### Genetics of seed banks

We attempted to confirm that seed banks can be viewed as an important genetic reservoir by testing the hypothesis that standing (aboveground) plants represent a nonrandom sample of the seed bank. We sampled multilocus allozyme genotypes from three species with different life history strategies: *Amaranthus retroflexus*, *Carduus acanthoides*, *Pastinaca sativa*. In four populations of each species we analysed the extent to which allele and genotype frequencies vary in consecutive life history stages, including the summer seed bank, which has been overlooked up to now. We compared the winter seed bank (i.e., seeds collected before the spring germination peak), seedlings, rosettes, the summer seed bank (i.e., seeds collected after the spring germination peak) and fruiting plants. We found that: (1) All three species partitioned most of their genetic diversity within life history stages and less among stages within populations and among populations. (2) All genetic diversity parameters, except for allele frequencies, were similar among all life history stages across all populations in different species. (3) There were differences in allele frequencies among life history stages at all localities in *Amaranthus retroflexus* and at three localities in both *Carduus acanthoides* and *Pastinaca sativa*. (4) Allele frequencies did not differ between the winter and summer seed bank in most *Carduus acanthoides* and *Pastinaca sativa* populations, but there was a marked difference in *Amaranthus retroflexus*. In conclusion, we have shown that the summer seed bank is not genetically depleted by spring germination and that the majority of genetic diversity remains in the soil through summer. We suggest that seed banks in the species investigated play an important role by maintaining genetic diversity sufficient for recovery rather than by accumulating new genetic diversity at each locality.

### Taxonomy and ecology of *Bolboschoenus* species

The unifying effect of a water environment, softening climatic extremes, frequently contributed to the formation of large circumpolar areas of distribution of aquatic and wetland plants. Nevertheless, local habitat conditions, ways of spread and hybridization during evolutionary processes may influence the occurrence of wetland plants to a considerable extent. In such processes, ecological and physiological adaptations may arise as the first step, and morphological adaptations may be delayed. The groups of morphologically similar taxa (species) need not include taxa closely related in ecology and biological properties. The genus *Bolboschoenus* may serve as an example of such groupings: similar morphotypes occur in distant continents – North America, Eurasia, Australia and New Zealand, and the five European species were formerly included into one broadly conceived species, *Bolboschoenus maritimus*. Nevertheless, important differences appeared among species within the European *Bolboschoenus maritimus*-group in ecology, and, consequently, in their role in plant communities; a new phytocoenological classification of *Bolboschoenus* communities was included into a vegetation survey of the Czech Republic (Hroudová et al. 2011a, b, Šumberová & Hroudová 2011a, Hroudová 2011a, b, c).

Corresponding to differences in ecology, different biological traits were found in European *Bolboschoenus* species (Hroudová et al. 2014): in the facultative halophyte *Bolboschoenus maritimus*, genetically fixed tolerance to increasing salinity but lowered ability to utilize increased nutrient input were found. By contrast, the hybridogenous freshwater species *B. laticarpus* was found to be best able to use nutrient input for growth and biomass production, corresponding to its wide ecological amplitude and weedy character.



During a given period, studies on the variation and distribution of *Bolboschoenus* species also continued on a global scale: Areas of distribution of *Bolboschoenus* species were mapped and determination keys published for Iran (Amini Rad et al. 2010) and Slovakia (Hroudová et al. 2014). Complementary distinguishing anatomical characters were found in Iranian *Bolboschoenus* species (Amini Rad & Hroudová 2013) and in the micromorphology of Indian species (Mishra et al. 2014). Anatomical characters in the nutlet pericarp enabled us to determine *Bolboschoenus glaucus* in charred archaeological findings from the ancient Near East (recent Turkey, Syria, Iraq, Iran); this freshwater species was probably used by ancient people as a food or was used in other ways (Wollstonecroft et al. 2011). Maps of distribution in the southern part of Bohemia for *Bolboschoenus yagara* and *B. laticarpus* were made, their threat evaluated and a suitable management strategy was proposed for maintaining their populations (Ducháček & Hroudová 2013a, b). Basic information on collection of cultivated *Bolboschoenus* species from their worldwide distribution was presented (Hroudová et al. 2014), and the importance of weedy *Bolboschoenus* species on arable land was demonstrated (Hroudová & Zákřavský 2014a).

Besides *Bolboschoenus* species, the results of studies of other wetland plants were also published, especially concerning taxonomy and distribution of plants adapted to fluctuating water levels or nature conservation. This includes taxonomy and distribution of the genus *Butomus* (*Butomaceae*) in “Flora of the Czech Republic” (Slavík & Hroudová 2010a, b), and other floristic contributions to the flora of the Czech Republic (Pířová et al. 2013, Hroudová 2014, Hroudová & Zákřavský 2014b). Characteristics of plant communities occurring in habitats with fluctuating water levels were included in the vegetation survey of the Czech Republic (Hroudová & Šumberová 2011a, b, Šumberová & Hroudová 2011b, c, d, e, Hroudová, Ekrť, Ekrťová & Šumberová 2011). The effect of controlled fishpond management on reed-bed restoration in protected areas was evaluated – a positive effect of decreasing water levels during the first year of a two year management cycle, and alternative or reduced fish stock, was found (Hroudová et al. 2010).

## Research Report of the team in the period 2010–2014

Institute	Institute of Botany of the CAS, v. v. i.
Scientific team	Department of Mycorrhizal Symbioses

The Department of Mycorrhizal Symbioses is a major national research centre dealing with diversity of mycorrhizal fungi and the role of mycorrhizal symbioses in a range of ecological contexts. Because mycorrhizal symbioses themselves are very complex and diverse in nature, research pursued in the Department is also broad, comprising a wide range of topics. Although we are predominantly involved in basic research, we have also participated in research & development projects.

We strongly emphasize the integration of students into all stages of scientific work and have a good track record in successful completion of BSc., MSc. or PhD. theses. We productively collaborate with other research groups in the Institute of Botany and with universities and research institutions in the Czech Republic and world-wide. The main research topics are described in the following six chapters.

### **Role of arbuscular mycorrhiza in plant stress tolerance**

The occurrence of arbuscular mycorrhiza (AM) in stressful environments and its contribution to plant stress tolerance has been a traditional topic of the Department of Mycorrhizal Symbioses. Finalizing research projects from previous periods, we addressed mechanisms of altered heavy metal (HM) tolerance and uptake patterns of AM plants and showed that the presence and exudation of extraradical mycelia of AM fungi (AMF) substantially decrease cadmium availability in the substrate (Janoušková et al. 2010). Consistently, ectomycorrhiza was shown to alleviate HM stress and/or decrease HM uptake in willows and poplars (Mrnka et al. 2012, Trakal et al. 2013) confirming that mycorrhiza may be a supporting agent in revegetation and phytoremediation projects. On the other hand, mycorrhizal inoculation did not favorably affect the process of EDDS-enhanced phytoextraction of copper from a contaminated soil (Komárek et al. 2010), demonstrating the limits of mycorrhizal technology in some plant-environment systems. Moreover, stress tolerance of AMF, as a pre-requisite for alleviation of stress to plants, may quickly get lost without the selective pressure of the stress (Oliveira et al. 2010). Members of our team designed and led two of these studies and wrote the corresponding texts. In another two studies, they contributed to the design of experiments, were responsible for mycorrhizal parts of the experiments and contributed to manuscript writing. In one study (Oliveira et al. 2010), our team member was involved in designing the experiment and commented on the manuscript.

In the last 5 years, the main focus of our research has, however, shifted from model systems and anthropogenically contaminated sites to natural habitats with multiple abiotic stresses. Specifically, we addressed the role of AMF in the adaptation of their hosts to the unique environment of serpentine soils, which are characterised by multiple abiotic stresses (primarily low calcium/magnesium ratio, increased HM-concentrations, and low water-holding capacity). In a series of experiments involving a model plant species (*Knautia arvensis*; Doubková et al. 2011, 2012, 2013a, 2013b; Doubková & Sudová 2014), we clearly showed that AM symbiosis belongs to the plant

adaptation mechanisms to serpentine soils. Improved phosphorus acquisition and drought stress alleviation were likely the crucial mechanisms behind this beneficial effect of AMF, while no substantial modifications in calcium, magnesium or nickel uptake were recorded. Edaphic differentiation was proven, not only for the selected serpentine and non-serpentine plant populations and AMF isolates, but also for the whole plant-mycobiont complex. This topic was studied in close cooperation with the Department of Flow Cytometry. Members of our department were responsible for most of the experimental work, data analyses and manuscript writing. All co-authors contributed to the design of the studies, participated in the field work and data interpretation and commented on the manuscripts.

### **Co-evolution of plant populations and their arbuscular mycorrhizal symbionts**

In collaboration with the Department of Population Biology, we examined reciprocal adaptations of plants and AMF on a model of *Aster amellus*, an endangered species of dry grasslands. We found that local genotypes of the model plant had the highest benefits from mycorrhiza under their local conditions, i.e. in their native soil and in association with a native AMF isolate (Pánková et al. 2011). Moreover, plants from different geographic regions established a specific level of mycorrhizal colonization, and AMF from each region formed specific abundances of particular morphological structures in roots (Pánková et al. 2014). In the same habitats, nutrient poor dry grasslands, we tested how AMF contribute to plant community structure using fungicide application: While mycorrhiza-dependent forbs, including some rare and endangered species, decreased their cover or even became extinct after fungicide application, the cover of graminoids with low mycorrhizal dependence increased (Dostálek et al. 2013). Members of our department participated in designing the studies, helping to interpret the results and commenting on manuscript texts.

In close cooperation with the Department of Flow Cytometry and Department of Population Biology, we addressed the interaction of AMF with ploidy level of their host plants. On a model of three angiosperms with intraspecific ploidy variation, we demonstrated a ploidy-specific mycorrhizal growth response in *Aster amellus* (Sudová et al. 2010), although the effects of the ploidy-AM interaction were weaker than the main effects of these factors *per se* (Sudová et al. 2014). Members of our department were responsible for most experimental work, data analyses and manuscript writing. All co-authors contributed to the design of the studies, participated in the field work and data interpretation and made comments on the manuscripts.

Development of relationships between AMF and plants during ecosystem development in vegetation succession has been a traditional research topic of the Department of Mycorrhizal Symbioses. Results of long-term research on the impact of AM on the ecology of individual plant species, as well as on structuring plant communities during vegetation succession in post mining sites were summarized in a book chapter (Rydlová et al. 2013). Another published book chapter reviewed research on mycorrhiza and gentians published to date (Sýkorová 2014). Co-evolution of plants from the family Gentianaceae and their mycorrhizal fungi resulted in a specific morphology of AM structures in roots of gentians and AMF may also play an important role in their life cycle. These two publications were prepared solely by the members of the Department of Mycorrhizal Symbioses.

### **Community ecology of mycorrhizal fungi**

The composition of communities of mycorrhizal fungi may significantly affect plant host physiology and fitness as well as ecosystem processes. Therefore, understanding their

assembly rules plays a pivotal role in recent mycorrhizal research. Several projects, focused on communities of AMF, ectomycorrhizal fungi (EcMF) and orchid mycorrhizal fungi, as well as on root associated fungal endophytes, have been addressed by our team members over the period 2010-2014.

In one of the projects, we focused on fresh water lakes because aquatic habitats have received little attention in mycorrhizal research until now. Concentrating on isoetids, a specific group of submerged aquatic macrophytes, frequent colonisation by AMF and endophytic fungi was recorded, with the level of colonisation being dependent on host plant identity (Sudová et al. 2011; Kohout et al. 2012). A detailed analysis of root-associated fungi, based both on culture-dependent and culture-independent approaches, then revealed surprisingly high fungal diversity (Kohout et al. 2012). Nevertheless, the diversity of AMF communities seems to be lower in aquatic habitats as compared to terrestrial ones (Kohout et al. 2014).

Communities of mycorrhizal fungi might be significantly affected by invasions of non-native plants, with potentially serious consequences for the functioning of mycorrhizal symbioses. We focused on the effects of pine invasions on communities of EcMF in the Czech Republic and northern Iran (Kohout et al. 2011; Bahram et al. 2013) and found, interestingly, comparable numbers of EcMF interacting with invasive as with native trees, which is in contrast to the situation in the Southern hemisphere. These contradictory observations could be explained by the Holarctic distribution of pines and their long co-evolution with local mycorrhizal fungi.

Besides biotic interactions, mycorrhizal communities are also affected by abiotic environmental characteristics, most of which change during the vegetation season. We described for the first time, a substantial shift in orchid mycorrhizal fungal community composition during the growing season: Fungal species, which are known to form orchid mycorrhizal symbiosis, dominated during summer time, but they were replaced by non-mycorrhizal fungal endophytes during the autumn (Kohout et al. 2013).

In addition to analyzing the species composition of fungal communities in different habitats, we also addressed the introduction of AMF inocula into soils and how the inoculated strains interact with the resident AMF. We were involved in a pilot study that showed the large subunit of mitochondrial DNA to be a suitable tool to distinguish between genotypes of the AM fungus *Rhizophagus irregularis*, which may be highly differentiated between biotopes (Börestler et al. 2010). Subsequently, we showed that competition between two closely related isolates of this AMF species alters the dynamics of fungal development in roots (Krak et al. 2012), which is potentially important when assessing the development of mycorrhiza after inoculum application to a soil, where other genotypes of the same species are present. We specifically traced, for the first time in the history of mycorrhizal research, a field-inoculated isolate and verified its survival and propagation at the site for at least two years (Sýkorová et al. 2012). Quantitative molecular tools, however, also revealed that inoculation has only short-term effects on the abundance ratios of AMF species in a resident community, indicating that the composition of AMF communities is robust against the introduction of AMF propagules (Janoušková et al. 2013).

In eight of the ten cited articles, our team members were mainly responsible for study design, data analysis and text writing; our collaborators contributed by providing plant material, developing molecular tools, were involved into the design of the study, contributed to data analysis and/or commented on the manuscript. In two studies (Börestler et al. 2010, Bahram et al. 2013), our team members were involved in the collection and analysis of data and contributed to the writing of the text.

## **Microbial interactions in the rhizosphere of ericoid mycorrhizal and ectomycorrhizal plants**

Despite ericoid mycorrhizal plants co-dominating large areas in temperate, boreal and subarctic ecosystems, ericoid mycorrhiza (ErM) have been investigated by only a few research groups worldwide. Since the 2000s, this represents a well-established topic of our department with several interesting discoveries.

Traditionally, ErM were thought to be formed by Ascomycetes. We revealed that the spectrum of ericoid mycorrhizal fungi (ErMF) is much broader, including Sebaciniales, an order of basal Hymenomycetes (Basidiomycota) and non-sebacinaceous basidiomycetous fungi related to common wood-decaying saprobes. We described a new type of mycorrhizal symbiosis, a sheathed ErM, formed by these fungi, with a rich enzymatic apparatus that may confer adaptations distinct from those provided to their hosts by the so far investigated ascomycetous ErMF (Vohník et al. 2012). Besides ErMF, Ericaceae roots are systematically colonized by dark septate endophytes and their colonization frequencies seem to be negatively correlated, suggesting competition for the living space. Some mycobionts of Ericaceae are capable of simultaneous formation of both ErM and dark septate endophytic associations (Vohník and Albrechtová 2011). ErMF from the *Rhizoscyphus ericae* aggregate, especially *Meliniomyces variabilis*, also form a substantial part of fungal endophytes colonizing basidiomycetous ectomycorrhizae of conifers. At sites where Ericaceae are absent, conifer ectomycorrhizae may serve as alternative niches for ErMF (Vohník et al. 2013). The team members designed and conducted the studies, conducted all isolations of mycobionts and molecular analyses and statistically analyzed data. They also wrote the major part of the manuscripts. The collaborators performed phylogenetic analyses and wrote respective parts of the manuscripts. Blueberries (*Vaccinium* spp., Ericaceae) represent a valuable commercial commodity. ErMF may support blueberry growth, especially under conditions resembling natural ecosystems (Nestby et al. 2014), which substantially differ from plantations with intensive application of artificial fertilizers. We showed that, to a certain degree, fertilizers may in the long term be substituted by organic matter (pine bark and wood chips) coupled with inoculation by beneficial soil microorganisms. Because ericaceous root symbionts do not completely degrade lignin, we also attempted inoculation with lignin-degrading saprobic basidiomycetes, which significantly improved both growth and berry production (Vohník et al. 2012). In the first study, team members prepared inocula, conducted inoculations and wrote respective parts of the manuscript. They also led and worked out the major part of the second study.

As revealed in our previous research, fungi associating with ericaceous roots can affect community composition of testate amoebae, an ecologically significant group of soil protists that can play a significant role in the biodegradation of organic matter and nutrient cycling. We further extended our research to saprotrophic fungi decomposing conifer needle litter and showed that they may affect the composition of testate amoebae communities. We also questioned mycophagy of one of the most frequent soil testate amoeba, *Phryganella acropodia*. It was significantly associated with fungal mycelium but seemed to feed rather on bacteria colonizing the hyphosphere than on the mycelium itself (Vohník et al. 2011). We further confirmed a significant effect of vegetation and root symbiotic fungi on the composition of soil testate amoeboid communities, since those occurring in the plant mycorrhizosphere significantly differed from those occurring in the surrounding soil (Vohník et al. 2012). Team members conceived and led the studies and wrote major parts of the manuscripts.

### **Methods used for determination of mycorrhizal fungi**

Studies focused on mycorrhizal community ecology have to deal with several methodological challenges. Isolation of DNA from host plant roots or soil, followed by amplification of the so called barcoding region by PCR with fungal-specific primers, and then sequencing of the resulting products, is the most common method now used. Sequences are subsequently compared with a known sequence database to obtain fungal species names. However, each of the steps in this protocol might be biased and could potentially affect our observations. In a series of articles, we focused on understanding and overcoming some of these biases. In one study, we showed that a selected method of material preservation may substantially bias quantification of fungal DNA in colonised plant material (Janoušková et al. 2014). In another study, we performed the first comprehensive comparison of Glomeromycota-specific primers, together with a newly designed one, and found a trade-off between the tested primer systems with no universal solution (Kohout et al. 2014). Our study highlighted potential disadvantages of the most commonly used primer pairs. Exploring DNA-fingerprinting methods, which are even more prone to potential errors, members of our team participated in a study aimed at identifying the effects of GC content on the detected length of labelled DNA fragments (Bukovská et al. 2010). In addition to the previously described problems, a comparison of obtained sequences with known fungal sequence databases represents another critical step, because public databases contain around 10 % of wrongly annotated sequences (Tedersoo et al. 2011). To overcome this problem, we joined an international scientific effort and contributed to the expert annotation of all available fungal ITS rDNA sequences in a publicly available database called UNITE (Tedersoo et al. 2011). A similar international effort established a new system of sorting and naming of molecularly detected fungal taxa. This system sorts all high quality ITS rDNA fungal sequences into the so called Species Hypothesis, which might be used by scientist for easier communication and, together with all available metadata, represents a strong tool for meta-analysis (Koljalg et al. 2013). This system was recently implemented into Quime, the most widely used pipeline for analysing microbial next-generation sequencing data. In two of the cited studies (Janoušková et al. 2014, Kohout et al. 2014), members of our department were responsible for most experimental work, data analyses and manuscript writing; our collaborators contributed by providing material, commenting on the study design and the manuscript. For other published papers, team members contributed to data analyses and text writing.

### **Projects of research & development**

The Department of Mycorrhizal Symbioses has long-term experience with applied research and collaboration with industrial partners and a number of R&D projects have recently been initiated in cooperation with research institutions or commercial enterprises, both national and international, covering a wide range of research themes.

During the last five years, the Department has been involved in numerous projects focused on collaboration with industrial partners, and nearly the whole department was involved in the Applied Centre for Bioindication and Revitalization (2005-2011, over 100 M CZK funding plus co-funding by industrial partners) where collaboration with over 20 private companies was focused on biotechnologies of revegetation of brownfields (mining spoil banks and fly ash deposits, biotization of horticultural substrates and commercial applications of mycorrhizal inoculants). A series of experiments tested whether mycorrhizal inoculation can compensate for the

reduced inputs of organic matter, making the reclamation more cost-effective. We found that AMF stimulated growth and quality of several technical crops employed in agricultural reclamation of spoil banks (Rydlová et al. 2011, Püschel et al. 2011). Experiments with pot-cultivation of ornamental plants in peat-based substrates revealed a significant positive effect of AMF on growth and visual attractiveness of certain ornamental plants, as well as increasing their drought resistance (Püschel et al. 2014). Further, our wide experience in the application of mycorrhizal inoculations, e.g. in nursery practices (Oliveira et al. 2010), in production of vegetables (Albrechtová et al. 2012) or herbs (Hernádi et al. 2012) enabled a critical review of mycorrhizal inoculation technology for a wide range of applications (Vosátka et al. 2013). The majority of published work was performed by our team (including experimental design, data processing and preparation of the manuscripts). In joint experiments, members of our team significantly contributed to the experimental setup, interpretation of results and writing respective manuscripts.

We studied the production of bioactive compounds, stilbenes and emodin, in the knotweed hybrid *Reynoutria × bohemica* under a range of conditions and found nitrogen and phosphorus levels inversely influenced stilbene levels. The best knotweed clones, in terms of production of these bioactive compounds, were identified and the optimal time of harvest was determined. Our research yielded three commercial nutraceutical products containing stilbene and resveratrol compounds (developed in collaboration with Czech pharmaceutical company Favea) and three publications (Kovářová et al. 2010 & 2011, Frantík et al. 2013). Our team designed and conducted the experiments, participated in data acquisition and interpretation, and publication writing.

We evaluated the phytoremediation potential of fast growing tree plantations on contaminated land. This project, funded by Norway Financial mechanisms, was focused on investigating alternative land use in the Příbram region (about 60 km SW of Prague, a large area contaminated with heavy metals due to long-term smelter activities). Apart from the listed publications (Mrnka et al. 2012, Trakal et al. 2013, Zárubová et al. 2012) we protected the developed phytoremediation biotechnology (utility model no. 22842 at Industrial Property Office) and published a farmers cultivation manual for fast growing trees on heavy metal-contaminated land (Mrnka et al. 2011). Our team led the project, substantially contributed to the experimental design, setup, data acquisition and processing and to publication/patent writing.

Another two projects funded by the Ministry of Industry and Trade were focused on the development of stimulants and plant extracts, based on the newly discovered phytohormones strigolactones, to be used for stimulating the yield of important agricultural crops (wheat, rape seed and others). Part of the research was focused on applications in Africa, to eliminate the dangerous parasitic plant *Striga hermontica*, which is devastating agricultural crops in numerous African countries. The research resulted in two patents (no. CZ201200796-A3 and CZ201200746-A3) and one certified methodology.

We participated in long-term monitoring of endangered dry grassland vegetation, addressing questions of the impact of changing weather and grazing management. Weather conditions affected dominance and species richness of graminoids and forbs differently, depending on the local soil conditions (Dostálek & Frantík 2011). Spring, but not summer grazing of the dry grasslands, was shown to suppress expansive grass and woody species (Dostálek & Frantík 2012). Another long-term (40 y) study monitored the spread of alien plants along roads in the Orlické hory Mts. and revealed that the spread is significantly influenced by both adjacent land

use and the intensity of anthropogenic disturbances. The spread of individual species was shown to be strongly habitat dependent (Dostálek et al. 2014a). Our department also contributed to research dealing with optimization of woody stock for landscape restoration. Optimal size of native tree transplants was determined for each woody species used (Dostálek et al. 2014b). A member of our team participated (in equal proportion) to experimental design, implementation and data processing, as well as in publication writing.

Overall, our applied research resulted in four certified methodologies, one utility model and two patents.



## Research Report of the team in the period 2010–2014

Institute	Institute of Botany of the CAS, v. v. i.
Scientific team	Centre for Phycology

The Centre for Phycology is one of the major national research centres dealing with diversity, systematics, evolution, ecology and the biotechnological use of microalgae and Cyanobacteria. Beside the Czech Republic, the team has wide coverage across the world – the members carry out or participate in research projects in e.g. both Polar Regions, North, Central, as well as South America, India, Thailand, Bulgaria, and other countries. The team in cyanobacterial taxonomy is among the leaders in the field on a global scale. Members of the team authored handbooks on cyanobacteria or certain groups of algae widely used by either scientists, or field specialists' worldwide. Major scientific achievements for the period of 2010-2014 may be grouped under the following categories, although they often overlap:

### **Diversity, ecology, and taxonomy of Cyanobacteria**

Between the years 2010 and 2014 the group mainly focused on taxonomic evaluation and reclassification of cyanobacteria following modern methods (polyphasic approach). The widest impact and importance from all publications finished during this time period is likely to be a completion of the third (final) part of the monograph on cyanobacteria, which was published within a compendium "Süßwasserflora von Mitteleuropa" in 2013 (Komárek 2013a). This comprehensive work is dedicated to heterocytous cyanobacterial taxa. In addition to keys and a summary of knowledge on this group of cyanobacteria in Europe, it includes an overview (with appropriate descriptions) of all heterocytous cyanobacteria around the world. This book was long awaited by researchers and students working with cyanobacteria worldwide.

Of overall importance are also publications presenting recent results of a polyphasic reassessment of cyanobacterial classification worldwide. Of particular importance is a comprehensive overview of taxonomic classification of cyanobacterial genera based on a polyphasic approach (Komárek et al. 2014, Komárek 2014a), which updates previous studies on taxonomic and nomenclatoric changes in cyanobacteria with the advances of molecular methods (Komárek 2010, Komárek 2012).

Attention was given to selected coccoid types of cyanobacteria, especially those previously not well characterized. It was found that *Gloeobacter violaceus*, a primitive thylakoid-less cyanobacterium that forms a basal group in a cyanobacterial tree of life is a common inhabitant on rock surfaces (Mareš et al. 2013b) and its name was conserved against other commonly used names of the identical organism (*Aphanothece caldariorum*, *Gleothoece coerulea*, and *Gleothoece linearis*) (Mareš et al. 2013c, 2013d). This formal step is quite consequential, as *Gloeobacter* is commonly used as a model organism for various other studies as well as being an outgroup in cyanobacterial phylogenies. The name of another coccoid cyanobacterium – *Gleothoece* – was also conserved to clarify previously unclear, and for years confused, nomenclature of these types (Mareš et al. 2013a). A new genus, *Anathece*, was separated from planktonic *Aphanothece* types (Komárek et al. 2011) and another new

genus *Chalicogloea*, with a type species *C. cavernicola* from low-light aerophytic environments, was described (Roldán et al. 2013). The diversity of freshwater coccoid types was also intensively studied in Antarctica and islands of the NW Weddell Sea (Komárek 2013b, Komárek 2014c).

Revision of the main genera of simple filamentous cyanobacteria from the families Phormidiaceae / Microcoleaceae was conducted, including revision of the genera *Phormidium* (Strunecký et al. 2010, Strunecký et al. 2012a, Strunecký et al. 2012b) and *Microcoleus* (Strunecký et al. 2013). In the former genus *Phormidium*, several distinct subgroups were identified, of which some were already described as separate genera, e.g. *Wilmottia* (Strunecký et al. 2011), *Oxynema* (Chatchawan et al. 2014), or *Kamptonema* (Strunecký et al. 2014).

In oscillarioid types (family Oscillatoriaceae), another group of simple filamentous cyanobacteria, characterized by wider filaments and a higher width to length cell ratio, a new genus *Limnoraphis*, with a type species *L. robusta* from Lake Atitlán, Guatemala, was described and characterized (Komárek et al. 2013b). A new genus *Moorea*, was separated from a marine environment based on genetic evidence from *Lyngbya majuscula* (Engene et al. 2012b).

New information on the taxonomy of the family Pseudanabaenaceae, also belonging to a group of simple filamentous cyanobacteria, was published (Kling et al. 2012). A new genus *Oculatella* was described (derived from *Leptolyngbya*), which possess a unique feature of red tips (Osorio-Santos et al. 2014), and a new genus *Plectolyngbya* with a type species *P. hodgsonii* from Antarctic lakes (Taton et al. 2011) was also reported; occurrence of the not very well known genus *Glaucospira* was also studied (Simić et al. 2014).

Intensive research was conducted especially, on the group of heterocytous cyanobacteria. These economically important cyanobacterial types form water-blooms especially the families Aphanizomenonaceae and Nostocaceae. From the phytogeographical and ecological point of view, attention was paid to plankton dominant in Central Europe, including invasive species (Komárek 2014b, Kaštovský et al. 2010b) in western tropical parts of the Americas (Forastier et al. 2014, Komárek & Komárková 2010, Rejmánková et al. 2011), or South America (Montoya et al. 2014), as well as dominant species in both Polar Regions (Komárek et al. 2012b, Komárek 2013b). The taxonomy of freshwater planktonic heterocytous cyanobacteria was updated (Komárek & Mareš 2012).

Concerning work on nostocacean types in general, the revised genus *Nodularia* (Řeháková et al. 2014), was found to be phylogenetically uniform with variable morphotypes, and in *Cylindrospermum* (Johansen et al. 2014), three new species were recognized. New genera described were *Desmonostoc*, (Hrouzek et al. 2013), *Cronbergia* (Komárek et al. 2010), and *Limnoraphis* from Lake Atitlán, Guatemala (Komárek et al. 2013b), which was later observed forming a water-bloom in Lake Titicaca, Perú (Komárková et al. in press). A new species of *Anabaena*, *A. fuscovaginata*, was discovered in the periphyton of the freshwater alkaline marsh in the Everglades, USA (Mareš 2010). A benthic, biofilm forming *Hydrocoryne* from Antarctica was also characterized (Genuario et al. 2013).

From non-planktonic heterocytous types, we investigated especially economically important members of the family Scytonemataceae and Stigonemataceae (Mareš et al. in press, Sant'Anna et al. 2013). With their ability to fix atmospheric nitrogen, they play an important role in autotrophic soil microflora worldwide, but their growth and impact is especially pronounced in tropical regions. An investigation and the gathering of more detailed information on the family

Scytonemataceae were also supported during the years 2010 to 2014, with funding from the GA CR in a project entitled: Phylogenetic and phenotypic evaluation of heterocytous cyanobacteria of the family Scytonemataceae (P506/12/1818). Within this project, one new genus from alkaline marshes in Belize – *Chakia* – with a type species *C. ciliosa* (Komárková et al. 2013), and several species of the genus *Scytonema*, including *Scytonema santannae* (Hentschke & Komárek 2014), *S. papillicapitatum* and *S. chorae* (Komárek et al. 2013) were described. A study summarizing knowledge of the *Scytonema* species from the “Mata Atlântica” ecosystem, SE Brazil was also published. The populations of 14 studied *Scytonema* morphotypes were found to be ecologically significant, as they hold the dominant position in the microvegetation communities of lateritic and forest soils and of stony substrates (Komárek et al. 2013). Advancement in knowledge was also achieved for a relatively recent tropical genus, *Brasilonema* (Sant’Anna et al. 2011a). During the project, a large amount of data on the taxonomy, distribution and ecology of the genera *Scytonema*, *Brasilonema* and related genera such as *Scytonematopsis* or *Petalonema* were collected and are now being processed to produce further publications (see below).

A large effort was also put into an investigation of complicated relationships between members of the traditional morphological group, Microchaetaceae. It was found that the family is based on a type that cannot be verified and that many genera previously classified into this family belong to relatively distantly related groups of Nostocaceae, or close to Nostocaceae, when molecular methods are applied. Moreover, it has been demonstrated that the concept of the genus *Tolypothrix* differs substantially between the International Code of Botanical Nomenclature, and Bergey’s Manual of Systematic Bacteriology used by a large group of researchers. In a partial review of the group, two new families, Tolypotrichaceae and Godleyaceae were established (Hauer et al. 2014). In the same study, three new species of the genus *Fortiea*, *F. contorta*, *F. laiensis*, and *F. Coimbrae*, were described. Other studies also thoroughly describe new genera, which were identified within the *Microchaete*-like isolates. Genus *Calochaete*, with a type species *C. cimrmanii* was isolated from a massif of the Chirripó Mountain, Costa Rica (Hauer et al. 2013).

Other studies contributed to overall knowledge on the ecology, diversity and distribution of cyanobacteria worldwide (Cantonati et al. 2012, Engene et al. 2012a), in tropical regions (Kaštovský et al. 2011), in Polar Regions (results are included below in the appropriate section), and in the Czech Republic (Kaštovský et al. 2010a). A complete checklist of cyanobacteria reported from the Czech Republic was compiled for the first time. It includes recent records as well as critically evaluated historical data and reflects up to date taxonomy of the group. It lists 505 taxa in total (Kaštovský et al. 2010b).

Microflora of cooling towers and other man-made environments colonized by cyanobacteria were also studied (Hauer 2010, Chatchawan et al. 2011). It was revealed that such habitats may be highly specific in terms of species composition, i.e. due to specific environmental variables they harbor organisms not present in very wide surroundings, or completely unique taxa.

### **Diversity, ecology and taxonomy of eukaryotic algae**

Over the last decades, there has been a significant amount of interest in understanding the ecology and biogeography of microorganisms. Previously, the prevailing theory stated that “everything is everywhere the environment selects”, but there is an important scientific challenge to find evidence contradicting this statement. The only

way to succeed in this challenge is by applying good fine-tuned taxonomy. Therefore primary research dealing with taxonomy and ecology of particular species is necessary and urgently needed to avoid force-fitting items into incorrect taxonomical groups, leading to incorrect biogeographical conclusions.

Between the years 2010 and 2014, diatoms, green algae and cyanobacteria all around the world have been studied from different perspectives. Diatoms (Bacillariophyta) are one of the most diverse algal groups and play an important role in almost all freshwater and terrestrial ecosystems. Despite this overall occurrence, little is known about the diversity, ecology and biogeography of this group in the Maritime Antarctic Region. The main objective of diatom research in the Antarctic Region has been therefore to refine the taxonomical, ecological and biogeographical characterization of aquatic, semi-aquatic and moss-inhabiting diatom communities (e.g. Kopalová et al. 2013). During the last 5 years, an almost complete diatom flora from several Maritime Antarctic Islands (such as the James Ross Island Group and the South Shetlands Islands) was obtained. As a result, many taxonomical papers describing seven new species (e.g. Van de Vijver et al. 2013, 2014) were published and a complete Iconographia Diatomologica, presenting revised and well-illustrated diatom flora from this region, will be published in 2015.

Besides research on polar (mainly pennate) diatoms, important work on the voluminous series of the "Atlas of Freshwater Centric Diatoms with a brief Key and Descriptions", meant for specialists as well as for field workers dealing with identification and evaluation of freshwater centric diatoms, was continued (Houk et al. 2010, 2014) and also a new species *Discostella tatrica* was described (Procházková et al. 2012).

Except for diatoms, other algal groups were also studied. A joint project of the Inst. of Botany CAS and the Inst. of Plant Physiology and Genetics BAS, Sofia, Bulgaria, "Study of biotechnological potential of extremophilic and extremotolerant algae and cyanobacteria" was focused on cryoseston and thermophilic cyanobacteria of Bulgaria and several scientific papers were published on this topic e.g. Lukavský et al. (2011). Small collections of extremophilic algae from both types of localities were isolated and are available in our cultures. Except for Pirin and Rila Mountains, cryoseston were determined for the first time, including a few new species from Bulgaria (Lukavský & Cepák 2010, 2014).

Other studies concerned macroscopic green algae. A set of 18 freshwater and morphologically similar marine samples of *Ulva* were collected from inland and coastal waters throughout Europe, to assess their taxonomic identity and invasive potential. Also an unknown green alga (order Ulvales) was a dominant macroalga in the rocky littoral zone of Lake Garda (southeastern Alps), and morphological and phylogenetic analyses indicated that the alga was in the genus *Jaoa*, considered endemic to China. This alga belongs to a little-known genus, but is easily observed and was discovered in Italy's largest lake, a tourist resource of outstanding importance. It is a striking example of the degree to which lake benthic algae are understudied in many areas (Mareš et al. 2011, Mareš et al. 2014). During biodiversity exploration of James Ross Island, Antarctica a conspicuous green alga was found growing on stones in the littoral of two lakes. Based on results of thorough analyses, a new species, *Hazenia broadyi*, was described (Škaloud et al. 2013).

### **Biodiversity and ecology of the Polar Regions and other extreme habitats**

Research in the Polar Regions and other extreme habitats could be divided into three overlapping areas, focusing on biodiversity and biogeography of algae and

cyanobacteria in the Polar Regions, *in situ* manipulation experiments and a detailed ecophysiological investigation of extremophilic phototrophic microorganisms. The biodiversity and biogeography of cyanobacteria and algae were studied intensely in both Polar Regions. In 2010-2014, the biodiversity of freshwater and terrestrial algae and cyanobacteria was evaluated. Samples collected during polar expeditions originated from the Antarctic Peninsula (maritime Antarctica) (e.g. Komárek 2013, Strunecký et al. 2012) and Svalbard (e.g. Komárek & Kováčik 2013, Pushkareva & Elster 2013). The polar cyanobacteria were specific and partial differences were observed between individual polar biotopes and between the Arctic and the Antarctic (Strunecký et al. 2010). Taxonomic revisions were performed and several novel algal and cyanobacterial genera from the Polar Regions were described, e.g. *Wilmottia* (Strunecký et al. 2011). The habitats studied included wet walls, cryoseston, plankton, littoral and benthos of lakes, stream benthos and wetlands (Komárek et al. 2012).

Research in Antarctica was focused on the ice-free parts of the Ulu Peninsula, James Ross Island (NE Antarctic Peninsula), where the J.G. Mendel Czech Antarctic Station is located. The first comprehensive limnological survey of this region was performed, describing the origin, bedrock geology, geomorphology, hydrological stability and physical and chemical characteristics of a representative set of freshwater lakes. This study represents a background for further ecological research in this area (Nedbalová et al. 2013). A unique microflora with the predominance of filamentous cyanobacteria and a high proportion of endemic species was reported from the lakes. In several old shallow lakes, three new species of cyanobacteria (Komárek et al. 2012) and one green alga (Škaloud et al. 2013) were described. The lakes represent a biodiversity hotspot in the extreme conditions of Antarctica.

On-going climatic change in the Arctic and Antarctic will lead to an increased temperature and changes in moisture regimes in polar ecosystems. In order to simulate effects of mild warming on primary producers and decomposition, three passive open-top chambers (OTCs) were installed in a wet tundra meadow in Svalbard in 2009 -2013 (Elster et al. 2012). Despite minor differences in mean temperatures between OTCs and control, the vegetation season in OTCs was prolonged for approximately 2 weeks. However, no warming effects were observed on photosynthesis or nitrogenase activity in *Nostoc* sp. colonies, or on decomposition rates. Potential changes in plant community structures were probably masked by herbivory.

The ecophysiological studies were performed in the field as well as in the laboratory. The field experiments were performed in Svalbard, Río Tinto in Spain and in Iceland. In Svalbard, a desiccation experiment with *Nostoc* sp. colonies revealed that the colonies are able to fix nitrogen, and photosynthetic activity was detectable, even after loss of ca 80 % of their fully-hydrated weight (Kvídiová et al. 2011). The desiccation experiment focused on the green filamentous alga *Zygnema* spp., and revealed the formation of pre-akinetes during the late Arctic summer. Pre-akinetes formation was not triggered by desiccation, but hardening during slow dehydration was required for the pre-akinetes to become stress-resistant (Pichrtová et al. 2014). In the extremely acidic locality of Río Tinto, variable fluorescence imaging was used to detect the presence of photosynthetic microorganisms in samples and proved suitable for acclimatization/adaptation of biofilms to the environment (Gómez et al. 2011).

In the laboratory experiments, growth requirements of *Chlorella*-like algae and thermophilic cyanobacteria were evaluated. Several experimental series were focused on evaluating individual stress factors in selected strains. Resistance to freezing was evaluated in snow and lichen algae using ice nucleation measurements. The lichen algae were more resistant to freezing than the snow ones. In polar *Zygnema* sp.,

exposure to increased UV light led to production of UV-screening phenolic substances, most likely stored in vacuoles and other vesicles at the cell periphery, providing protection for other organelles (Pichrtová et al. 2013). Formation of pre-akinetes, observed in late summer in the field, was found to be induced by nitrogen starvation. In general, viability and recovery rates after experimental desiccation depended on pre-cultivation conditions and rates of drying. Moreover, the pre-akinetes survived even rapid drying (at 10% relative air humidity) when hardened by mild dehydration stress (Pichrtová et al. 2014). An extremophilic red alga, *Cyanidium* sp., was exposed to a pH gradient and its response to stress was measured by chlorophyll fluorescence. The measurements demonstrated an optimum pH between 1.5 and 2.5, thus reflecting adaptation/acclimatization to the original environments. To assess the viability of cells during various experiments, a novel method of triple fluorescence staining (DAPI/SYTOX Green/CTC) was developed (Tashyreva et al. 2013).

### **Research on biological soil crusts and other phototrophic assemblages in high mountains**

Biological soil crusts (BSCs) are important components of arid and semiarid ecosystems worldwide. The BSCs are well adapted for primary colonization of dry environments due to their extraordinary ability to survive desiccation and extreme temperatures. Their occurrence can be crucial for local ecosystems – they improve resistance to wind and water erosion and supply nutrients to the environment. High-elevation ecosystems, especially their subnival zones, are among the most extreme environments. Our knowledge of the functioning of these outlying ecosystems is still very sparse. High-elevation cold deserts of the Himalayas are still the least explored environments on the Earth.

Research on BSCs has proceeded since 2008. Our study is the first to try to discover the microbial composition in the high-mountain range in Ladakh (W Himalaya). The investigation of BSCs have been carried out at different altitudes varying in aridity. Over the five years, we have investigated the microbial diversity of BSCs, physiological features of particular components of the microbial community and BSC ecophysiology.

The heterotrophic bacterial assemblage was defined from the BSCs as well as from bare soil and the rhizosphere of higher plants. We have determined the occurrence of methanogens in newly deglaciated soils. In cooperation with the laboratory of Max-Planck Institute of Terrestrial Microbiology, Germany, we performed analyses of archaeal communities and potential activity of methanogens (Aschenbach et al, 2013). Cyanobacteria predominate in microbial communities of BSCs in Ladakh. We carried out several detailed studies of cyanobacterial community structures (Řeháková et al. 2011, Janatková et al. 2013). In all these studies, the influence of altitude and soil physico-chemical parameters on the composition and biovolume of microbial communities were tested. The biovolume of Cyanobacteria increased with elevation. On the other hand, species richness decreased significantly.

Physiological features such as nitrogen fixation, respiration and pigment content of microbial communities were also explored. Up to the present, we have determined the ability of high-altitude phototrophs to fix molecular nitrogen and surveyed their contribution to nitrogen input in the subnival ecosystem (Janatková et al. 2013).

Research on the diversity of snow algae, following up projects from the previous period, was performed in the Czech Republic, Bulgaria (three regions), and Spain. According to actual results, the most diverse were communities in Bulgaria, with six to ten taxa recorded (Lukavský & Cepák 2013, Cepák & Lukavský 2014). Except for snow

algae, the high-mountains cyanobacterial flora were studied in cooperation with Slovak partners in the High Tatra Mountains, Slovakia (results of long-term research are being prepared for publication), or the previously unexplored Chirripó National Park, Costa Rica (Mühlsteinová & Hauer 2013) where 52 morphotypes of Cyanobacteria were found; 48 of these taxa are new species for Costa Rica.

### **Biotechnological utilization of algae, life cycles**

This important subject of research has involved the study of polyunsaturated fatty acids (PUFA) in eustigmatophycean microalgae, particularly in the hitherto poorly studied species *Trachydiscus minutus*. We described its physiology, morphology, life cycle and biogenesis of lipids, and revised its phylogenetic position (Přibyl et al. 2012a). A monograph dealing with some physiological aspects of this species was published as well (Lukavský 2012). Due to the relatively high growth rate and eicosapentaenoic acid (EPA) content, this species proved to be a prospective producer of this valuable compound (Řezanka et al. 2010, Iliev et al. 2010) and an original strain was subsequently patented (Lukavský et al., 2010). Furthermore, we studied the effects of nutrient deficiency on fatty acid composition (Řezanka et al. 2011), and optimized production of EPA in this strain by a specific combination of cultivation conditions (Cepák et al. 2014).

Lipid production by microalgae has been another topic of our research. We focused on rapidly growing *Chlorella*-like species. Ten species of *Chlorella* and *Parachlorella* were tested with respect to their lipid content and productivity. One lipid hyper-accumulating strain of *Chlorella vulgaris* was studied in detail. The pattern of lipid globule biogenesis, fatty acid composition and conditions for maximizing its lipid productivity were described. Cultivation and lipid production of this strain was subsequently successfully up-scaled in a thin-layer photobioreactor (150 L); lipid synthesis was induced by natural depletion of diluted medium (Přibyl et al. 2012b). Moreover, we described the biogenesis of lipid bodies and their mobilization with respect to nitrogen availability during culture growth (Přibyl et al. 2013). Production of lipids in another strain of *Parachlorella kessleri* was studied under individual nutrient limitation. This strain was also successfully grown in a thin-layer photobioreactor; for lipid induction a two-phase cultivation regime (nutrient sufficient/deficient) was applied (Li et al. 2013). Moreover, photosynthetic carbon partitioning into starch (growing cultures) and neutral lipids (stationary nutrient depleted cultures) was studied in this strain. This effect was fully reversible after nutrient replenishment of depleted cultures (Fernandes et al., 2013). Extensive knowledge on microalgal lipids was compiled as a review in a book chapter (Přibyl et al. 2014).

Extremophilic microalgae, cyanobacteria and specific microalgal metabolites have been another field of study of our group. Several species were isolated from cryoseston and thermal mineral springs in Bulgaria and selected strains were tested for their growth characteristics or anti-microbial activities (Najdenski et al. 2013, Hindák et al. 2013). Prospective healing effects of polysaccharides from the red microalga *Rhodella grisea* were also reported (Nosál'ová et al. 2011). A chapter for a monograph series focused on cryoseston algae of Bulgaria, and their practical applications in biotechnology, has been prepared. Other aspects of our research covered the use of flow cytometry in microalgal biotechnological processes (Hyka et al. 2013) and heterotrophic and mixotrophic cultivation of microalgae (Kovář et al. 2014).

Ecophysiology of microalgae covered studies of life cycles of some green zoosporic microalgae; sexual reproduction was reported for the first time in some cases. The roles of red and blue light in zoosporogenesis and gametogenesis in *Botryosphaerella*

*sudetica* were investigated in detail (Příbyl 2013). Additionally, we monitored microorganisms in cooling pools in the Temelín nuclear power-plant and tested various algicides for their suppressive effects. A new method for evaluating algicide effects based on variable chlorophyll fluorescence was developed (Kvíděrová 2010). We also successfully tested a biological way of controlling phytoplankton by supporting natural zooplankton (top-down trophic cascade effect).



## Research Report of the team in the period 2010–2014

Institute	Institute of Botany of the CAS, v. v. i.
Scientific team	Department of Functional Ecology

The Department of Functional Ecology deals with the ecology and evolution of plant functional traits and adaptive strategies as key drivers of plant responses to climate and land-use changes. Understanding how different organisms, including vascular plants, cope with these changes and their synergistic effects are now major topics in ecology and conservation biology. Our department focuses on the role of plant ecophysiological traits, reproduction strategies, morphological and anatomical features for plant functioning (reproduction, growth and survival) in a changing environment, including socio-economic changes in traditional land-use practices in Europe, and global climate changes in warming prone alpine and arctic areas.

Knowledge of ecological and ecophysiological strategies of vascular plants allows us to take part in multidisciplinary research projects on community assembly, plant-pollinator interactions, dendroclimatology, plant-soil feedbacks and management of protected areas. Our main collaborators are profs. Jan Lepš and Klára Řehoučková from University of South Bohemia, České Budějovice, prof. Tomáš Herben from Charles University, Prague, prof. Fritz Schweingruber from WLS Zurich, and prof. Jong-Suk Song from Andong University, Korea. We are active in student training at the University of South Bohemia in České Budejovice. A number of ongoing student projects clearly benefit from the scientific interactions with staff members, field data collection and lab training. This is reflected in the numbers of defended PhD (12), MSc (16) and BSc (16) theses over the past five years.

### **Linking community assembly and biodiversity effects on ecosystem functioning**

Functional traits, any characteristics of an organism linked to their fitness, provide a powerful means of addressing crucial theoretical and applied ecological questions through their dual role as an indicator of mechanisms driving differences in species assembly into communities, and as a predictor of ecosystem-services. Functional traits provide a means of testing mechanisms behind species assembly within communities because environmental filtering, competition and disturbance influence species fitness via their traits. Functional traits also provide a link between species and multiple ecosystem-level processes, such as primary productivity, nutrient fluxes and resilience, since species influence these processes via their traits. Functional trait composition therefore represents the interface between the formation of biodiversity and its effect on ecosystems.

The Department of Functional Ecology has a long standing tradition in contributing to developments in this key aspect of research on plant communities, particularly developing and testing new mathematical techniques and proposing new tools to transfer these dimensions freely. For the latter see existing webpages ([http://www.butbn.cas.cz/francesco/Webpage/R\\_Functions.html](http://www.butbn.cas.cz/francesco/Webpage/R_Functions.html)) for available functions in the free software R. This tradition has been enhanced by the recruitment of Dr. Francesco de Bello in 2009 into the Department.

In several studies, Dr. de Bello and his collaborators tested the importance of functional traits on the assembly of communities and their biodiversity, reviewing both existing data (Gotzenberger et al. 2012), analysing new data (de Bello et al. 2013, two papers) and providing insight into key methodological issues (de Bello 2012; de Bello et al. 2012). This work resulted in organizing a special issue in the *Journal of Vegetation Science* on the importance of functional traits and functional trait diversity between and within species (Mason & de Bello 2013). Several studies also show the importance of intraspecific trait variability in community structures (de Bello et al. 2011; Leps et al. 2011; Albert et al. 2012; Le Bagousse Pinguet et al. 2014), a component often neglected in the majority of published works.

At the same time, another set of works has been developed to understand the mechanisms by which biodiversity affect several ecosystem processes and services. Firstly a review was conducted to show links between functional traits and a variety of ecosystem processes (de Bello et al. 2010). This paper shows that biodiversity can affect ecosystem services in different ways, but mostly this is done via species traits, both in terms of the dominant trait values in a community and trait diversity in that community. These effects are expected both within one trophic level and across trophic levels, as further demonstrated by following publications showing the importance of trait connections between different organism types in controlling ecosystem services (Lavorel et al. 2013; Moretti et al. 2013). Other works showed a way to tease apart the role of dominant trait values in a community from trait diversity in that community (Dias et al. 2013; Bila et al. 2014).

A long term collaboration has been established with Meelis Partel (University of Tartu, Estonia), with Sandra Lavorel and Wilfried Thuiller (CNRS, France), Marco Moretti (WLS, Switserland), Matty Berg (University of Amsterdam, Netherland) and various colleagues in Brazil where an official collaboration is ongoing. In the Czech Republic, collaboration with the University of South Bohemia, particularly with Jan Lepš will be continued.

### **Functional ecology of alpine and arctic plants**

Arctic and alpine ecosystems are threatened by global climate warming, namely shortage of snowfall and higher summer temperatures. This has accelerated deglaciation and colonization of new substrates by microbial communities and vascular plants, whose establishment is, to a large extent, driven by plant traits, often those below ground. The Department of Functional Ecology is a major national research center dealing with ecology, evolution and adaptation of plants in cold arctic and alpine areas and their responses to global warming. Our members, in the last five years, took part in projects of the University of South Bohemia, in the Arctic archipelago Svalbard (K. Prach & J. Klimešová) and a project led by P. Sklenář from Charles University in Prague on ecology of Paramo in the high Andes (A. Kučerová and P. Macek). Moreover, our department was responsible for a Himalayan project (J. Doležal – project leader, K. Řeháková, J. Klimešová, F. de Bello, and postdoc and PhD students P. Macek, M. Dvorský, J. Altman, K. Janátková) aimed at continuing, and internationally linking the long-term interdisciplinary research on plant functional biodiversity and its dynamics in Ladakh, W Himalayas, started by the late Dr. Leoš Klimeš in 1997.

Ladakh is the region on Earth with the highest recorded and predicted temperature increases, while presently sustaining a substantial alpine and subnival flora, including some vascular plant species that ascend to near the highest elevation (6150 m) on record. An extensive floristic database (>4200 georeferenced plots

covering an elevational range from 2640 to 6150 m) throughout Ladakh was assembled during 1997-2014 (Klimeš 2004; Dvorský et al. 2011, 2013) together with information on individual species life history traits (eco-physiological trait database for 500 species, Chlumská 2010; Dvorský et al. 2015 online), species habitat preferences (Klimeš & Doležal 2010) and their evolutionary relatedness (Klimeš 2008). Our activities focused on water and nutrient status of mountain plants (Macek et al. 2012), the importance and diversity of belowground organs (Klimešová et al. 2011, de Bello et al. 2011, Štastná et al. 2012, Klimešová et al. 2013), the role of competition and positive interactions between plants (de Bello et al. 2011; Dvorský et al. 2013) and the diversity of soil bacterial, cyanobacterial and microalgal communities as first colonizers of high-mountain soils affecting nutrient availability for vascular plants (Řeháková et al. 2011; Janatková et al. 2013; Aschenbach et al. 2014). Ecophysiological adaptations to low temperatures were also studied in the high Andes (Sklenář et al. 2010, 2012).

The dry mountains of Ladakh, located in the rain-shadow north of the Great Himalayan Range, NW India, provide unique opportunities for studying plant responses to climate change at one of the highest (ca. 6000 m) elevations in the world inhabited by vascular plants. We conducted two transplant experiments at 5800-6150 m to test if there was a gap between the current upper distributional limit of subnival plants and their climatically determined potential elevational distribution, by transferring plants above their current limits and tracing their survival. We found that the highest elevation at which transplanted flowering plants survived corresponded well to the observed elevational limit, suggesting there is no gap between the current and climatically determined upper distributional limit (Klimeš & Doležal 2010). Also repeated mapping of vegetation in the High Arctic (Svalbard) over 70 years revealed that contemporary climatic changes still have not resulted in changes in plant cover, probably due to dispersal limitation (Prach et al. 2010).

Cushion plants represent one of the most suitable survival strategies under cold, windy and nutrient-poor conditions in alpine and arctic zones. Cushion plants often act as nurse plants, facilitating the establishment of other species within their canopy by offering microhabitats that are more favorable for seed germination and seedling recruitment than their surroundings. We carried out two studies (de Bello et al. 2011, Dvorský et al. 2013) at high elevations (4800-5900 m) that showed that at the very extreme end of the stress gradient, facilitative effects need not manifest and that the role of cushion plants as nurse species might not be as general as expected.

Germination of seeds, growth and colonization processes of vascular plants in the subnival and alpine zones are affected by microbiotic soil crusts that includes cyanobacteria, algae, microfungi, lichens and bryophytes. These microbial assemblages play a key role in the fixing and retaining of carbon, nitrogen and liquid water and protect against erosion, which is important for subsequent plant and animal succession. We found that not only cyanobacteria (Řeháková et al. 2011, Janatková et al. 2013) but also methanogens (Aschenbach et al. 2014) play important roles in the development of desert biocrusts in newly deglaciated soils in the Western Himalayas. In total, 15 Web of Science (WoS)-indexed articles on this topic were published in 2010–2014. In another 3 articles, members of our department substantially contributed to data collection and writing.

### **Practical ecological restoration and spontaneous succession**

The Department of Functional Ecology plays a key role in ecological restoration in central Europe, cooperating in this research with the Department of Botany, University of South Bohemia (K. Řehounková) and with the Institute for Nature Environment,

Charles University in Prague (J. Frouz). It takes advantage of expert knowledge and the building of an extensive database of vegetation records from various post-mining, ex-arable and abandoned sites and their vegetation successional series in the Czech Republic. The database now contains nearly 3000 phytosociological records, and about 1200 species, and it also includes the main environmental data and species functional traits. Such a data set is unique in the world and its elaboration elevates research on vegetation succession to a new qualitative level (Prach et al. 2013, 2014). Results contribute to successional theory, as well as to practical ecological restoration. When looking at the problem from the point of view of community assembly during succession, we performed trait analyses that revealed that traits of seed dispersal are more important at the beginning of succession whereas clonal and bud bank traits became important in later stages (Latzel et al. 2011).

It has repeatedly appeared that spontaneous succession is usually the best and cheapest way of restoring disturbed ecosystems (Prach & Walker 2011), as was shown on different mining sites over central Europe (e.g. Řehouňková & Prach 2010, Konvalinková & Prach 2010) and on former arable land. Conversion of arable fields to meadows and pastures can be accomplished by spontaneous natural colonization. Reaching the target meadow community in this way, however, may be hindered by noxious arable weeds or the early spread of woody species (Jírová et al. 2012). Directed conversion involves various techniques, ranging from sowing species-rich mixtures (Prach et al. 2012, 2014) to transplanting so-called stepping-stones (small meadow blocks taken from target communities, Klimeš et al. 2010); all methods can substantially speed up the restoration process but it appeared repeatedly that the surrounding landscape, its structure and local species pools, substantially influenced the course of restoration. Therefore maintenance of already existing species-rich meadows as sources of diaspores is crucial for restoration and biodiversity protection generally.

An important role in restoration of mining sites is played by soil development and a member of our department, O. Mudrák, studied this problem under the supervision of J. Frouz (Mudrák et al. 2010, 2012, Mudrák and Frouz 2012, Roubíčková et al. 2012, 2013). Soil development was proven to play an important role in spontaneous succession of post-mining sites, which cannot be revealed by only studying aboveground processes. Leaving an ecosystem to its own development does not restore its productivity, but is able to restore biodiversity and should be taken as an alternative to technical recultivation.

In total, 30 WoS-indexed articles on this topic were published in 2010–2014, led by members of the team (Karel Prach, Ondřej Mudrák, Alena Jírová), with the highest IF reached 15.7 (TREE).

### **Species rich meadows as a model system for practical and theoretical ecology: maintenance and understanding biodiversity**

Temperate meadows are the most species-rich small scale communities on Earth and are often used as a model ecosystem for community ecology. To understand the basic mechanisms of species coexistence, or finding appropriate ways to maintain or restore meadow diversity, we performed several studies on oligotrophic meadows, led by members of our department (J. Klimešová, F. de Bello), or in cooperation with researchers from other institutions, namely J. Lepš. We focused on biodiversity as well as functional aspects of meadow species in response to short term variations in management.

Our research shows that responses to management depends on the productivity gradient, and once a meadow is not limited by nutrient availability, changes in species composition and species diversity after cessation of management are fast and may be irreversible (e.g. Mason et al. 2011, Horník et al. 2012, Klimeš et al. 2013, Janeček et al. 2014, Mudrák et al. 2013, Carboni et al. 2014). When we have employed functional traits in our studies, we were able to show some basic mechanisms that are behind species responses to relaxed management (Lanta et al. 2011, Doležal et al. 2011, de Bello et al. 2012, Klimešová et al. 2010, 2011, 2013). For example, we showed that plant species of large stature lost a greater proportion of their aboveground biomass by mowing than did small plants but they were additionally penalized by losing flowering and fruiting stalks that cannot resprout after mowing, due to morphological and phonological constraints. Although we were able to identify some general patterns in the responses of functional traits to abandonment (e.g. benefit for large erosulate species) we also disproved some earlier generalizations and failed to show the same pattern of trait responses over regions and scales (e.g. Janeček et al. 2010, Bartoš et al. 2011, Lanta et al. 2011, Janeček & Klimešová 2014). Specifically, storage economies of carbohydrate compounds in roots and rhizomes of meadow plants, although responding to management changes, were mainly species specific.

In cooperation with J. Lepš, we performed some experiments examining a role of hemiparasitic plants in the coexistence of meadow plants. Annual hemiparasites reduce the growth of competitive strong grass species and, therefore, support small and rare species (Mudrák and Lepš 2010, Mudrák et al. 2014). This ability can be used in the restoration of meadows invaded by grasses, fertilized meadows; additional seeding of hemiparasitic species of *Rhinanthus* thus may be viewed as a cheap tool for meadow restoration.

In total, 30 WoS-indexed articles on this topic were published in 2010–2014, led by members of the team (J. Klimešová, J. Doležal, Š. Janeček, O. Mudrák, F. de Bello).

### **Ecophysiological research on aquatic carnivorous plants**

The Department of Functional Ecology has a 25-year tradition in ecophysiological research on aquatic carnivorous plants, in particular *Utricularia* and *Aldrovanda*, led by Dr. Lubomír Adamec. The research is oriented mainly on their growth, traits (photosynthesis, mineral content, frost resistance) of turions, photosynthesis and respiration of shoots, N<sub>2</sub> fixation by shoots and traps, K<sup>+</sup> reutilization in senescent shoots, and also on biophysical and biochemical aspects of *Utricularia* trap functioning.

A photosynthetic comparative study on 3 aquatic *Utricularia* species with linear shoots and 3 rosette or rhizomatous shoot species proved that the net photosynthetic rate (O<sub>2</sub>) was higher in the former group, including rapid growers, than in the latter group with slow growers (Adamec 2013). The photosynthetic rate in some linear-shoot species was at a record high (as a requirement for very rapid growth) as compared to literature data on aquatic non-carnivorous plants, and their sprouting turions were similarly high. A complex study on N<sub>2</sub> fixation (using <sup>15</sup>N<sub>2</sub>) by shoots of 4 aquatic *Utricularia* species and *Aldrovanda* revealed a relatively low molecular-N<sub>2</sub> fixation rate, which increased with shoot age and indicated the participation of periphytic organisms in N<sub>2</sub> fixation (Sirová et al. 2014). Gene expression associated with N<sub>2</sub> fixation in the model, *Utricularia vulgaris*, was very low and only confirmed the low fixation rate. As calculated, this low N<sub>2</sub> fixation rate was not physiologically important for the plants but it could be ecologically important in oligotrophic habitats as an additional source of N<sub>2</sub>.

In a molecular-genetic study, the genome size and genomic GC-pair content were compared in 119 species of the Lentibulariaceae family of the genera *Pinguicula*,

*Genlisea* and *Utricularia*. We found that several species of the genera *Genlisea* and *Utricularia* had genomes (1C) even smaller than a model plant, *Arabidopsis thaliana*, with a miniature genome (Veleba et al. 2014). They represent the absolute world record in genome miniaturization in plants. However, no relationship was found between genome size and phylogeny of a single genera or species, or between the ecology of single taxonomic groups.

In two studies, the compositions of organic substances (sugars, organic acids, amino acids, alcoholic sugars) were described in the trap fluid of some aquatic *Utricularia* species and was dependent on photosynthetic conditions (Sirová et al. 2011; Borovec et al. 2012). Another study in two *Utricularia* species proved that a great proportion of organic substances from photosynthesis was secreted into the trap fluid, probably to support microbial commensals (Sirová et al. 2010). Using a linear position sensor, some studies on traps of several aquatic *Utricularia* species proved that traps can also fire spontaneously, without any mechanical stimulation, after a period of several h (Adamec 2011a,b). The characteristics of spontaneous firings were identical with stimulated ones. Moreover, it was proven that trap firing is not regulated electrophysiologically, but only mechanically (Adamec 2012). A comparative study on 13 *Utricularia* species confirmed that globally spread species also possess the most efficient traps in terms of firing and resetting rates. Traps also pump out water continuously, without any lag-period, just after firing. On the basis of new biophysical findings on water flows in *Utricularia* traps, a new ecological model on the ingress of mineral nutrients N and P into the traps was designed. It assumes N and P trap secretion into the trap fluid to support the commensals: according to this model, commensals in prey-free traps surprisingly have the character of parasites.

During the past 5 years, a total of 34 impacted papers or chapters in world monographs were published. The main national collaborators on the topic were Dr. Dagmara Sirová and Prof. Jaroslav Vrba from the University of South Bohemia, České Budějovice and Dr. Helena Štorchová from the Institute of Experimental Botany of CAS in Prague. The main foreign collaborator was Dr. Bartosz J. Piłchno from the Jagiellonian University, Cracow, Poland.

### **Ecophysiological research of peatland bryophytes**

The Department of Functional Ecology is also focusing on research of bryophyte ecophysiology, with eight papers published on this topic over the last five years by Dr. Tomáš Hájek. The main focus is on ecophysiology of photosynthesis in peatland bryophytes, particularly in *Sphagnum* mosses, and the nature of calcium tolerance in peatland mosses. The main results are (i) characterization of the relationship between mineral-nutrient availability and the stoichiometry of moss biomass with respect to environmental alkalinity; (ii) clarifying the physiological nature of the (in)tolerance of mosses to calcium; (iii) clarifying the global genetic diversity of the key calcium-tolerant *Sphagnum* species; and (iv) distinguishing between the environmental and competition effects in the ecology of fen mosses (Hájek et al 2014, Hájek & Vicherová 2014, Kangas et al. 2014, Pichrtová et al. 2014). Field observations and laboratory experiments were combined to study desiccation tolerance of peatland bryophytes and revealed inducible mechanism of tolerance, elucidating contradictions published in the literature. We used a similar experimental approach and found similar results in a hydro-terrestrial filamentous alga in the Arctic (Pichrtová et al. 2014). In a different study, we measured the rate of photosynthetic induction of mosses exposed to sunflecks, i.e. under rapidly changing light intensities; the results stressed the effective utilization of sunflecks in the forest floor, which contrasted with that in vascular plants

whose photosynthetic induction is reduced by slow stomatal opening (Kubásek et al. 2014). Another field of interest is the chemical composition of moss cell walls, with the respect to the litter quality that affects decomposition rate and thus peat accumulation. We proved that *Sphagnum* mosses (but not other mosses that are capable of litter accumulation) have decay-resistant cell-wall polysaccharides (but not decay-resistant/inhibiting phenolics as was traditionally assumed). Moreover, decomposing *Sphagnum* releases pectic polysaccharides that inhibit extracellular enzymes of decomposers. A close collaboration exists with Finnish universities in Helsinki and Joensuu, with participation in several ongoing projects that evaluate moss photosynthesis, including student supervision; this cooperation resulted in three publications (e.g. Kangas et al. 2014).

### **Species and functional diversity of plants in tropical montane forests: links to avifaunal diversity and plant-pollinator interactions**

Montane tropical forests of Western Africa have been identified as biodiversity 'hotspots' with extraordinary species diversity and exceptional concentrations of endemic species. However, most of the primary vegetation has been destroyed. It is estimated that 96% of the original montane forests in western Cameroon may have been lost. The remaining forests provide a unique natural laboratory for studying plant diversity, functional adaptations and coevolution processes. The Department of Functional Ecology is conducting a long-term systematic research in Cameroon, recently in two sites: Mt. Cameroon National Park and Bamenda Highlands, led by Š. Janeček and J. Doležal. Š. Janeček collaborates with Dr. Mark Brown from University of KwaZulu (South Africa), Kevin Yana Njabo from UCLA Institute of the Environment and Sustainability (Los Angeles, USA) and Dr. Olivier Lachenaud, Meise Botanic Garden (Belgium).

The research focuses on 1) plant community composition, structure and diversity using a system of permanent research plots; 2) diurnal and seasonal course of radial tree growth using a Dendrometer Increment sensor and X-ray wood densitometry; 3) relationships between forest tree and bird diversity, 4) plant-pollinator interactions. In Janeček et al. (2011) we show that the long-peduncle Cameroonian *Impatiens sakeriana* is not capable of autonomous selfing and can be pollinated only by two, often hovering, sunbirds, the Cameroon sunbird *Cyanomitra oritis* and the northern double-collared sunbird *Cinnyris reichenowi*. Our study revealed that this plant is highly specialised for pollination by *C. oritis*. *Cinnyris reichenowi* hovers less frequently and often thieves nectar by piercing the flower spur when perching. This study shows that pollination systems occurring in the Old World follow similar evolutionary trends as systems, including hovering hummingbirds, in the New World.

We also studied the nectar properties of *Impatiens sakeriana* and we compared the nectar traits of *I. sakeriana* with six other co-flowering insect-visited plant species (Bartoš et al. 2012). We showed that many nectar properties are specific to *I. sakeriana* compared to the insect-visited plants. These are in accordance with the most recent theory that nectar properties of the sunbird-pollinated plants are similar to those pollinated by hummingbirds.

We studied the behaviour of three sunbird species feeding on the nectar of five plant species in afro-montane vegetation, with and without consideration of plant abundance (i.e. diet selectivity and diet composition, respectively). The results showed (Janeček et al. 2012) that although sunbirds frequently feed on both morphologically adapted and nonadapted plants, food selectivity data are consistent with the hypothesis of phenotypic complementarity. Moreover, we found that the type

of plant abundance measurement can change conclusions in some cases, as individual plants differ in their growth habits and nectar production. In Bartoš & Janeček (2014) we presented a simple but ingenious adaptation of *Impatiens frithii*, a bird-pollinated plant that shares its main pollinator with four other *Impatiens* species on Mt. Cameroon. Our observations demonstrate that a minute change in floral morphology can effectively overcome constraints resulting from the basal floral architecture early in the group's evolution. We assume that such adaptations can not only help the plants to avoid interspecific competition, but, as the adaptations create strong reproductive barriers, they may also contribute to plant speciation.

In Bamenda Highlands region we described the reproductive and pollination system of *H. aristata* (Padyšáková et al. 2013) to show that the apparently generalized pollination system is highly specialized and that the effective pollinators are in agreement with the pollination syndrome of this plant. Due to our experimental approach, we were able to determine not only the pollinator effectiveness but also the negative impact of visitors on the studied plant's reproduction. The large bees were effective pollinators whereas the relatively smaller bee *A. mellifera* had a negative effect on *H. aristata* reproduction. Among the three sunbird species visiting *H. aristata*, *C. reichenowi* was the most frequent visitor, but it did not effectively pollinate the flowers.

During the past 5 years, a total of 7 impacted papers were published.

### **Tree physiology, radial growth and wood anatomy as tools to understand plant growth responses to a changing environment**

Annual growth (tree-ring widths) increments and other wood (xylem and phloem) anatomical features such as early and late wood widths, proportion of conductive (vessels), supportive (fibers) and storing (parenchyma) tissues serve as a chronicle of the past environment and provides long-term records about plant responses to external drivers such as climate change, volcanic eruptions, glacial activity, wind disturbance and many others processes. The Department of Functional Ecology has become one of the major national research centers for basic and applied dendro- and herbo-chronological research, participating recently in four multidisciplinary research projects.

The dendrochronological laboratory of the Department of Functional Ecology is now the best equipped in the country, utilizing the most-up-to date facilities such as Automatic Dendrometer Increment sensor, X-ray wood densitometry, wood and herb microtome sectioning, and wood cell analyses with WinCELL and PAST software. We deal with different aspects of tree-ring research and forest ecology in different parts of the world (Czech Republic, Korea, Indian Himalayas, Cameroon). The main scientific topics in the past five years were: 1) the impact of climate changes on forests; 2) reconstruction of forest disturbance regimes; 3) analysis of spatiotemporal recruitment and mortality processes in forests; and 4) comparative analysis of anatomical variation in Himalayan plants. A close collaboration with prof. Fritz Schweingruber from WSL Birmensdorf, Zurich proved crucial in our recent research.

We studied forest disturbance history on the Korean peninsula using oak tree rings (Altman et al. 2013a). In general, high Korean forest diversity is being linked to the high frequency of typhoons that open up the forest canopy and enable survival of light-demanding forbs. To elucidate a role of the spatiotemporal pattern of forest disturbance was the aim of a study based on analyses of growth releases of dominant oaks, indicating tree-stand disturbance and improved light conditions for surviving trees. Our results show that the releases were associated with typhoons and also



indicate the differential impact of typhoons on the forests during the past two centuries and along a latitudinal gradient of decreasing typhoon activity (Altman et al 2013a). We also studied glacial retreat history in Kamchatka using birch tree rings (Dolezal et al. 2014). A fast retreat of glaciers in Kamchatka reflects global warming. We used the 230-year record of birch growth in Kamchatka to analyze temporal variations of climate as well as glacial advances and retreats. In Europe, we used tree-rings of oak standards to reconstruct coppicing events and to link them with the recruitment of oaks (Altman et al. 2013b). Coppicing was one of the most important forest management systems in Europe, documented in prehistory as well as in the middle Ages. However, coppicing was gradually abandoned by the mid-20th century, which has altered the ecosystem structure, diversity and function of coppice woods. We found large peaks in radial growth for the periods 1895–1899 and 1935–1939, which matched with historical records of coppice harvests. After coppicing, the number of newly recruited oak standards markedly grew in comparison with the preceding or following periods. The last significant recruitment of oak standards was after the 1930s following the last regular coppicing event. Our historical perspective carries important implications for oak management in Central Europe and elsewhere. Relatively intense cutting, creating open canopy woodlands, either as in the coppicing system or in the form of selective cutting, is needed to achieve significant radial growth in mature oaks. It is also critical for the successful regeneration and long-term maintenance of oak populations.

Studies using tree-rings to reconstruct forest disturbance dynamics has increased in recent years. Despite the evident need for a common set of tools for verification, replication and comparison across studies, only a few DOS programs for disturbance detection exist. We developed the TRADER (Tree Ring Analysis of Disturbance Events in R), an open-source software package for R that improves research efficiency and facilitates replication of previous studies (Altman et al. 2014). In total, 10 WoS-indexed articles using tree-ring data were published in 2010–2014, led by members of the team (J. Doležal, J. Altman).

### **Clonal and Bud bank traits in CLO-PLA database**

Our department is focused on clonal and bud bank traits with a long term perspective and host the only database storing these traits worldwide. Since launching a new version of the database in 2006, new data are collected by J. Klimešová and her team. The data in the database are free to access and are used by scholars from different groups of the Institute of Botany as well as from other institutions over the world (55 citations on Google scholar in the past 5 years). From November 2014, CLO-PLA is available also through TRY database, the largest world trait database (see <https://www.try-db.org/TryWeb/Home.php>). CLO-PLA database enables us to test basic hypotheses on clonal plant distribution, habitat preferences; on this particular task we cooperated with T. Herben from Charles University in Prague who conducted the analyses and with R. van Diggelen from University in Antwerp who provided data on species composition of wetlands in the Netherlands.

Clonal and bud bank traits clearly explain plant abundance in Czech flora and vegetation (Herben et al. 2014). As a proof of functionality we tested whether morphologically defined clonal traits reflected clonal spread in a large set of plants (nearly 1000) growing in botanical garden of Charles University and showed that lateral spread positively affected clonal spread of herbaceous plants but was also negatively correlated with their seed reproduction (Herben et al. 2012). We also tested allometry and habitat preferences of plants with different lateral spreads and confirmed previous observations that in wet conditions, rhizome increments tend to be longer and short

lived (Klimešová et al. 2011 in cooperation with M. Sammul from University of Tartu, Estonia who contributed data).

In stressful environments, clonality provides plants with reproductive insurance and conservation of limiting nutrients. Hence, clonal growth is expected to be more frequent in floras of cold environments, such as the alpine and arctic regions, than in temperate regions. Evidence for this is largely based on comparisons of the ratio of clonal to non-clonal vascular plant species in local floras, as it is difficult to obtain reliable data that assess the extent of clonality in plant communities. We reviewed this problem by pointing out methodological pitfalls of simple comparisons (Klimešová & Doležal 2011). We also applied methods of clonal and bud bank traits assessment used in CLO-PLA for central European flora to other regions – high altitudinal ecosystems in Scandinavia, Ladakh, and high latitudinal ecosystems on Svalbard (Rush et al. 2011, Klimešová et al. 2011, 2013). On both sites we showed that undeveloped soils of extreme altitudes and latitudes, rather than hosting a high proportion of clonal plants, are dominated by non-clonals. Especially long rhizomes seem to be vulnerable to soil movements and do not occur on slopes and habitats with stony substrates but thrive at lower elevations and in wet places.

Apart from large scale comparative studies mentioned above we focused our research on the often overlooked ability of plants to form potential bud banks on roots by mean of adventitious sprouting from this organ, typically devoid of buds. As the adventitious buds on roots or the hypocotyle are usually formed after injury we tested, in a series of experiments, what other triggering factors exist beyond plant injury, and how season, nutrients etc. affect resprouting capacity in short lived species that often behave as weeds (Bartušková & Klimešová 2010, Latzel et al. 2011, Martínková et al. 2011, Malíková et al. 2012, Sosnová & Klimešová 2013). We used the knowledge attained for a modelling study where we discovered that only spring injury, but not sooner or later in the season, causes root-sprouting ability in short lived weeds of wet arable land and is important for population maintenance and spread. When the population is disturbed at other times, recovery of plants depends on the soil seed bank (Sosnová et al. 2014, T. Herben from Charles University, Prague did the modelling).

Examining numerous populations of 21 species of short lived adventitious sprouters, under field conditions, we found that contrary to expectations, their resprouting ability was not affected by environmental conditions but rather by plant size (Malíková et al. 2010, Klimešová et al. 2014). We also confirmed that adventitious buds provide short lived plants capable of forming them, additional insurance against bud depletion if they lose all stem parts together with axillary buds. We published 17 WOS-indexed papers in 2010-2014 (J. Klimešová, M. Sosnová, J. Martínková, A. Bartušková, L. Malíková.)

## Research Report of the team in the period 2010–2014

Institute	Institute of Botany of the CAS, v. v. i.
Scientific team	Department of Vegetation Ecology

The Department of Vegetation Ecology (<http://ekolbrno.ibot.cas.cz/?lang=en>) is one of the leading national research centers in vegetation ecology. The focus is on a wide range of topics in vegetation diversity, ecology and dynamics. The Department participates in international networking in Europe, Asia and North America, and intensively cooperates with national academic and government institutions. The Department of Vegetation Ecology continues the rich heritage of vegetation research at the Institute of Botany. It was established as a part of the Geobotanical Laboratory of the Czechoslovak Academy of Sciences in its current location in Brno in 1955, only one year later than the main part of the Laboratory in Průhonice. Since its establishment, the Department has developed mainly in two research directions, vegetation ecology and palaeoecology ([http://ekolbrno.ibot.cas.cz/?page\\_id=70&lang=en](http://ekolbrno.ibot.cas.cz/?page_id=70&lang=en)). The six-decade long tradition of vegetation ecology research was a foundation for the current activities of the Department of Vegetation Ecology.

In the past decade (since ca. 2005), the research strategy has emphasized interdisciplinary cooperation between the sciences (botany, ecology, palaeoecology, soil science) and the humanities (history, archaeology). The department's researchers represent graduates in all these subjects. The accent is on human shaping of environments, and applications of basic research to nature conservation. The research structure encompasses three core areas: palaeoecology, historical ecology and vegetation ecology proper. Vegetation properties are studied on a wide range of spatio-temporal scales, from the Holocene development across the historical human impact on vegetation to recent dynamics including the effects of global change on biodiversity, from the level of microsites to whole continents.

The scientific outputs for the years 2010–2014 can be classified in three areas: palaeoecological reconstruction of past vegetation, diversity and dynamics of forest vegetation, and ecology, biogeography and diversity of wetland vegetation. In addition, relationships between soil chemistry and vegetation were studied using field experiments. A major vegetation ecology research output, the monograph of national vegetation survey, benefited substantially from contributions of the team members.

### **Palaeoecological reconstruction of past vegetation and environments**

The palaeoecological research resulted in several major achievements in 2010–2014. Connecting to previous research areas, it focused on three general topics: Holocene vegetation development in the Carpathians, Holocene development of mountain ecosystems in the Sudetes, and the reconstruction of Holocene ecosystems on the basis of modern analogues in Siberia. Various methods and a multi-proxy approach were used for the reconstruction of the past vegetation and environments. The research was conducted in various habitats including mires and lakes (due to fossil data resources), alpine vegetation and forests. We consider the palaeoecological

research so characteristic for the Department that we decided to present the respective outputs as a separate group of results. It has to be emphasized however that palaeoecology should not be perceived as a stand-alone subject. On the contrary, it is firmly embedded in general vegetation ecology research, and in more recent periods (since about 2008), benefits from close cooperation with historical ecological research (use of archival resources).

A large part of palaeoecological research in 2010–2014 focused on the Carpathian Mountains. The results showed rich patterns of prehistoric to historic development of this region. Our results revealed that the environments and vegetation diversity were strongly shaped by a combination of natural factors and long-term human influence. On the geographical scale, we documented an essential dependence of current species assemblages on Holocene development, in particular on the age of sites revealed by palaeoecological analysis. On the other hand, strong and sometimes a decisive influence of prehistoric human presence was found in calcareous fen vegetation. We discovered and described a unique reverse succession process (Hájková et al. 2011a). The same study demonstrated the existence of ombrotrophic bogs already in the Preboreal, pushing the history of such ecosystems further back in time than was previously thought. Through a combination of archaeological, palaeobotanical and recent botanical data, a paper demonstrated that the unique species composition and richness of meadows in the White Carpathians is the result of a combination of local conditions and specific Holocene history (Hájková et al. 2011b). We also managed to demonstrate the early expansion of mesophilous trees (*Ulmus*, *Fraxinus*, *Tilia* and *Quercus*) in northern parts of the Carpathian-Pannonian boundary and the existence of open steppes and/or dry grasslands and open wetlands in this region throughout the Holocene (Hájková et al. 2013). Since the Bronze Age, there are coincidences in the history of human settlement, local development of fens and regional changes in the representation of particular habitats, including managed wet, mesic and semi-dry grasslands. Using plant macrofossil and mollusc analyses, a wider scale study of 49 undisturbed calcareous fens showed that in the Western Carpathians many of the extant fen sites originated fairly recently, with two peaks in the High Middle and Roman Period, the former corresponding to human-induced changes of the landscape. A conspicuous number of fens also appeared in the Late Glacial and later in the Bronze Age, due to suitable environmental conditions (Hájková et al. 2012). The team analyzed more recent periods as well. We explored interactions among human activities, landscape development and changes in biotic proxies in two small calcareous spring fens in the Slovak Eastern Carpathians, which date back to AD 930. Results of pollen, plant macrofossil and mollusc analyses were compared with settlement history (Jamrichová et al. 2014).

Another geographical focus of the palaeoecological research was on the mountain ecosystems in the Sudetes. It included two main mountain ranges, the Hrubý Jeseník Mts. and the Krkonoše Mts. Our results revealed that vegetation history was dominated by shifting of forest vegetation types and the relatively late occurrence of significant human impact. A unique 15 m long profile from the Labský důl glacial cirque in the Krkonoše Mts. reflects the environmental history for the last 30,000 years (Engel et al. 2010). Studies of fossil pollen, macrofossils, magnetic susceptibility and organic matter provided the first continuous record of Late Glacial and the entire Holocene vegetation history in the Sudetes. Another long peat profile from the Rejvíz pine bog in the Hrubý Jeseník Mountains showed three cycles of advance and retreat in forest cover. At this site, the first human impact was detected only half a millennium ago (Dudová et al. 2010). A multiproxy study examined the vegetation history of a raised

bog in the same mountain range over the past 6000 years with the help of macrofossils, testate amoebae and peat characteristics (Dudová et al. 2013). In this case, mixed spruce-elm-hazel woodland was recorded close to the treeline in the middle Holocene. Later on, a gradual transition to mixed spruce-fir woodlands with an admixture of beech took place, and from approximately 800 BC, spruce and fir became dominant. Major human impact started in approximately AD 1230 and was connected with settlement and mining in the foothills. Another multiproxy study analyzed the first peat section covering the entire Holocene in the Hrubý Jeseník Mountains using pollen, plant macrofossils, testate amoebae, peat stratigraphy and chemistry. From a methodological viewpoint, the study demonstrated how using multiple biotic proxies and extensive training sets in transfer functions may overcome taphonomic problems (Dudová et al. 2014).

Research in the southern Ural Mountains searched for recent parallels with the Holocene vegetation in central Europe. The concept of historical analogues with the vegetation in Siberia was first proposed by the Department's senior scientist Dr. V. Jankovská and further developed. The hypothesis was that open pine and larch forests in Central Europe during the late glacial were rich in light-demanding species. The spread of birch, aspen and oak in the early Holocene did not affect the diversity of vascular plants. This happened only later, after the spread of elm, lime, maple and ash. These trees also enriched the soil with calcium, and enhanced nutrient circulation. This resulted in species-rich assemblages of terrestrial snails. A comparison of current snails with Central European palaeo-assemblages showed that Central Europe was not covered exclusively by dry loess steppes in the glacial maximum (Chytrý et al. 2010, Horsák et al. 2010a, 2010b). Palaeoreconstructions were also aided by the study of surface pollen-vegetation relationships in the same region (Pelánková & Chytrý 2010).

Another part of the palaeoecological research dealt with lowland regions. In the resulting paper, we reevaluated the palaeoenvironmental record of the Komořanské Lake (Jankovská et al. 2013). Finally, two WoS papers, with the participation of team members, addressed more theoretical issues. One dealt with the way present vegetation can help in improving pollen models of past vegetation, while the other discussed how better cooperation between plant ecology and Quaternary palaeoecology could be achieved (Abraham et al. 2014, Reitalu et al. 2014).

The palaeoecological research resulted in publication of 18 papers in leading journals of the subject (indexed in the WoS). In 10 of the papers, team members participated as lead authors, otherwise as co-authors responsible for pollen analysis, macrofossil analysis, historical data and their interpretation, as well as for interpretation of vegetation data. The research was developed in close cooperation with the Department of Botany and Zoology, Masaryk University, Brno. Other cooperations yielding in the results presented here were with the Charles University in Prague, Czech Republic, the Universities of Nijmegen and Groningen in the Netherlands, and the Adam Mickiewicz University in Poland.

### **Diversity and dynamics of forest vegetation**

Research in forest vegetation integrates all three main disciplines developed and currently promoted in the Department of Vegetation Ecology. It resulted in a major publication output from 2010–2014. Three main research fields were the historical development of forests, the biodiversity changes in the 20<sup>th</sup> century and the related conservation issues. The historical research involved an application of specific approaches (study of old manuscripts and maps) and GIS analysis. Biodiversity

change research benefited from the rich heritage of old vegetation records and became a subject fully integrated in international networking (institutions in Europe and North America; <http://www.forestreplot.ugent.be>). Cooperation between the disciplines regarding the historical and present forest dynamics resulted in novel findings, significantly improving our knowledge in forest ecology and beyond. It can be said that the team based in the Department of Vegetation Ecology has become a leading national and internationally recognized body in historical forest ecology research. This achievement is relatively new, established in 2008 in a grant project supported at the national level (Lowland woodland in the perspective of the historical development, 2008–2012, led by R. Hédli). This research was further developed through the work of an interdisciplinary team of the Department, consisting of palaeoecologists, ecologists, historians and archaeologists, supported most recently (2012–2016) in the framework of the ERC frontier research grant LONGWOOD, led by P. Szabó ([www.longwood.cz](http://www.longwood.cz)). The project team, consisting of about 18 people, carries out unique research on the long-term history (Neolithic to present) of Czech forest ecosystems.

The central concept in this research is that the vegetation of Central Europe has been directly influenced by humans for at least eight millennia, during which time the original forests have been gradually transformed into today's landscape. Forests have been brought under various management regimes, which profoundly altered their structure and species composition. The abandonment of traditional management in the 20th century has also had a massive impact on composition and biodiversity of woodland vegetation. These processes and their consequences are poorly understood because research requires cooperation between at least four scientific disciplines on appropriate temporal and spatial scales.

As a basis for analyses and modelling, three databases, fully integrated in GIS, were built in 2010–2014 for the territory of Moravia (ca. 27,000 km<sup>2</sup>). The historic vegetation database ([http://longwood.cz/?page\\_id=165](http://longwood.cz/?page_id=165)), which is unique in Europe regarding the spectrum of data types, the volume of archival sources and its spatial scale and resolution, contains ca. 15,000 records with ca. 36,000 pieces of information on individual forests in 3,500 parishes. The archaeological database records prehistoric human activities in 935 parishes, and altogether we identified 4,643 sites with 9,347 archaeological components defined by their function and date. The vegetation database ([http://longwood.cz/?page\\_id=219](http://longwood.cz/?page_id=219)) currently contains about 1,500 resampled semi-permanent plots, allowing for reconstruction of changes in biodiversity and environments during the 20<sup>th</sup> century. Furthermore, we established eight sets of 600 strictly standardized permanent plots in biodiversity-valued forest vegetation. They became a part of the Czech national LTER monitoring ([www.lter.cz](http://www.lter.cz)). We also established experimental plots to study the possibility of restoration of historical management forms and their implications for forest biodiversity.

We carried out detailed studies on the history of key forest biodiversity hotspots in the Czech Republic, especially focusing on management as a possible driver of vegetation changes (Szabó 2010a, 2010b, 2012a, 2012b, 2013a, 2013b; Szabó & Hédli 2013b). Through interdisciplinary studies, we were able to demonstrate that management was an integral part of the long-term evolution of forested landscapes. According to our results, management changes in the 14<sup>th</sup> century were directly responsible for the formation of current thermophilous oakwood communities in the southeastern Czech Republic (Jamrichová et al. 2013). Joint dendroecological and historical ecological research proved the dominant role of coppice management

in the growth and recruitment of oaks in the same region (Altman et al. 2013). Connections between human settlement history and silver fir dynamics at the landscape level were also demonstrated, bringing new insights to the ecology of this important tree species (Volařík & Hédli 2013). Application of the knowledge of historical landscape structures was through two studies showing the role of past land use on the composition of forest vegetation (Hofmeister et al. 2013, 2014). Furthermore, our results underlined the importance of the reintroduction of traditional management techniques for the purposes of nature conservation. This topic was studied in a separate field experiment in the Czech Republic (Vild et al. 2013) and an observational study in northern England (Vild & Rotherham 2011).

Furthermore, we also contributed to the theoretical debate on the role of historical legacies in vegetation ecology. Historical ecology is currently in the making and position papers will likely influence research directions for several decades. We compiled a review and synthesis asking why history matters in ecology, from the perspectives of both general disciplines (Szabó 2010c), and also published two papers that concerned particular means of cooperation between ecology and history, with the aim of fostering conservation efforts (Szabó & Hédli 2011, 2013a).

Research on modern threats to vegetation in forest ecosystems is another significant component of our work. It largely focused on the abandonment of traditional management (in lowland regions mostly coppicing) and demonstrated the dramatic effects of this on woodland vegetation. To better understand this, we reconstructed the process of abandonment, which turned out to be largely spontaneous (Müllerová et al. 2014). Based on repeated surveys of semi-permanent plots, we demonstrated that this process led to non-random extinctions of many sensitive plant species, resulting in greatly impoverished, homogenized communities (Hédli et al. 2010, Kopecký et al. 2013). However, the assumed negative effects of biotic invasions were comparatively minor to the process of the abandonment of traditional management.

Through this research, the team substantially participated in a pan-European analysis of resurveyed forest plots, focusing on the effects of nitrogen deposition on temperate forest herbaceous biodiversity (Verheyen et al. 2012). This initiative later turned into the ForestREplot network ([www.forestreplot.ugent.be](http://www.forestreplot.ugent.be)), coordinated by an international committee including the Head of Department, R. Hédli. This cooperation has resulted in three further joint papers that brought international recognition. The effects of climate change on the herbaceous vegetation of temperate forests in Europe and N America were studied by De Frenne et al. (2013) revealing an important temperature buffering capacity of the closing forest canopy. Related research on the combined effects of soil and climate warming on a forest grass *Milium effusum*, was published by De Frenne et al. (2014). An innovative methodological paper dealing with the process of biotic homogenization (Baeten et al. 2014) was co-authored by a team member. A study by Hofmeister et al. (2012) also showed that foliar concentrations of nitrogen can be used as an indicator of the tendencies of forest ecosystems to eutrophication and expansion of nitrophytes.

Finally, two WoS papers dealing with the effects of topographical gradients in forest vegetation were published. Gilliam et al. (2014) demonstrated linkages between topography, tree overstory, soil chemistry and herbaceous diversity in the Appalachian Mts., North America. An elevation gradient in beech forests in Slovakia (Hrivnák et al. 2014) indicated the role of mass-effect and the influence of a dominant tree species on herb layer diversity. A more general perspective on natural gradients underlying diversity in forest vegetation was the subject of a special issue of *Folia Geobotanica*, co-edited by R. Hédli (Ewald & Hédli 2014).

Members of the team were lead authors in most of these publications. In total, no less than 21 papers in journals were indexed in the WoS, and an additional 7 peer-reviewed papers resulted through research on forest vegetation in 2010–2014. The team members were generally responsible for archaeological, historical, palynological and vegetation ecological data, analysis and interpretation. In data analysis, we closely cooperate with members of the Department of GIS and Remote Sensing, several of whom are partly financed through our projects.

### **Ecology, biogeography and dynamics of wetland vegetation**

A significant part of the research in 2010–2014 by the Department of Vegetation Ecology was devoted to wetland vegetation. Wetlands form relatively small patches of specific habitats in a landscape matrix, which makes them a good model ecosystem to study various aspects of dynamics, community and biogeographical patterns, water and soil chemistry patterns, and human impact on the environment. A large part of our wetland vegetation research was devoted to mires – fens and bogs, along with research on springs, wet meadows and fishponds. The long-term development of mires was studied in the central European mountain regions (see description of palaeoecological research above). In this section, outputs from research oriented to other aspects of wetlands will be described.

Peatbogs belong to important natural habitats, providing ideal model ecosystems for the study of biogeographical patterns. These were studied in Europe, focusing on vascular plants and mosses. By analyzing large datasets effects of species dispersal modes in several taxonomic groups on the constitution of mire communities, was examined (Hájek et al. 2011). We have shown that propagule size, rather than body size, is a predictor of the response of a given taxonomic group to geographical gradients. Macroscopic mosses had a weak response similar to microscopic diatoms. In contrast, vascular plants and molluscs strongly followed geographic gradients. The historical effects of dispersal were studied in fen sites in the Western Carpathian Mountains with known radiocarbon-dated ages spanning Late Glacial to the present (Horsák et al. 2012). Species richness data from the same plots were analyzed for vascular plants, bryophytes, and molluscs. Our results showed that only habitat specialist richness was influenced by habitat age and/or site area, with habitat age becoming more important as species dispersal ability decreased. Effects of habitat age, in the geographical context, were studied in Bulgarian peatbogs. They harboured a large numbers of rare species during dry glacial periods, providing refugia for these species. Our research showed that the phenomenon of isolated plant species populations is more widespread in Bulgaria than it was thought previously (Hájek et al. 2009). A conspicuous concentration of these species in just a handful of sites was observed in sites unknown to science, therefore not protected by law and vulnerable to destruction (Hájek et al. 2010). Geographical distribution and ecology of a rare plant *Trichophorum pumilum*, were studied by Dítě et al. (2013).

Several field studies documented linkages between mire biota and the environment. Our knowledge on the functioning of mires was improved through a joint study of vascular plants, mosses, desmids, diatoms, and water chemistry. Regarding environmental conditions, a decisive role of water acidity and mineral content was confirmed for the bog ecosystems. Vegetation of mires is generally considered a sensitive indicator of environmental changes. Central European mountain bogs were exposed to intense human impact during the 20<sup>th</sup> century. By analysis of permanent plots in the mountain bogs in the Sudetes Mts., effects of atmospheric deposition of



sulphur and nitrogen were assessed (Hájková et al. 2011c). We demonstrated that these bogs are relatively stable environments, although experiencing relatively large changes in sensitive species. Evidence was provided regarding pasturing impact in 1918–1947 and chemistry changes due to aerial liming in the early 1990s. When the water regime is not affected, the bog vegetation is rather resistant to high atmospheric deposition and climate fluctuation. A significant change in species composition occurs only in the long-term perspective. Particular species could, however, decrease or increase their frequencies more rapidly. For some of these species, a positive or negative response to nitrogen availability was also found in other studies, whereas for other species further research is needed in order to separate the effects of atmospheric deposition from internal ecosystem dynamics (Jiroušek et al. 2013). To better elucidate vegetation-chemistry relations, we analysed element concentrations in apical parts of dominant peat and brown mosses, along the complete pH/calcium gradient, in fens of three Central European regions (Hájek et al. 2014). Water chemistry substantially determined the concentration of elements in plant tissues. Ca and K concentrations were best predicted by water chemistry, while concentrations of N, P, and Fe were least predictable. The indication capacity of element concentration in plant tissues was also studied by Rozbrojová & Hájek (2010).

Studying the variation of European mires, three main groups were identified (Jiménez-Álfaro et al. 2014). They are diversified along gradients of temperature, precipitation and latitude. Geographically, more focused studies of mountain mires in the Western Carpathians and in the Alps showed that the vegetation patterns at regional levels were due to variations in composition of bryophyte and vascular plant communities. Significant differences were identified for acidic and base-rich mires. Contrary to previously explored alpine springs of the same regions, local species richness and composition were determined rather by pH than mineral richness, and all patterns were more consistent between regions and taxonomic groups (Sekulová et al. 2011, 2013).

Formalized classifications were applied for cataloguing of other types of wetland vegetation in the Czech Republic. The results were put in a broader geographical context. Some wetland vegetation types have a cosmopolitan distribution, such as reed vegetation. They are plastic to environmental conditions. There are, however, types of wetland vegetation concentrated in Central Europe. This specific distribution can be linked to the fishpond industry. These are mainly communities of bare fishpond bottoms, a survey of which was published by Šumberová & Hrivnák (2013). This vegetation emerges regularly from soil seed banks during summer fishpond revitalization. Part of their variability can be contributed to dispersal by fishery equipment (Šumberová et al. 2011). Ecology and colonization abilities of two related species of wetland annuals from the genus *Lindernia* were also studied by Šumberová et al. (2012). They are characteristic for bare fishpond bottoms. Finally, a study of wetlands across the southernmost Chilean Patagonia identified variations in vegetation types, including a description of new associations. Differentiation of vegetation types along gradients of soil chemistry was described (Filipová et al. 2013). It was shown that not only natural factors, but also human impact, mainly pasturing, over the past 150 years significantly affected the Patagonian wetlands. In addition, a survey of soil types in the wetlands of the region was published (Filipová et al. 2010).

Methodological aspects were studied in two papers. The importance of measurements of soil moisture were demonstrated by Hájek et al. (2013) and the potential for diatom sampling from herbaria collections for reconstruction of environmental change was described by Pouličková et al. (2013).

Research on wetland vegetation yielded 19 papers in journals indexed in the WoS, and one paper in another peer-reviewed journal. Members of the Department of Vegetation Ecology were either leading authors or significantly contributed to these papers. Many of them resulted from a joint effort with the Institute of Botany and Zoology, Masaryk University in Brno. Significant contributions were from the Bulgarian Academy of Sciences, Palacký University in Olomouc, Czech Republic, and the Chilean Institute of Agricultural Research (INIA). In addition to these research activities, M. Hájek co-edited a special issue of *Preslia*, which focused on oligotrophic wetlands (Hájek & Pyšek 2013).

### **The national vegetation survey and plant checklists**

The Department of Vegetation Ecology substantially participated in a modern national vegetation survey, resulting in four volumes of the *Vegetation of the Czech Republic*. The project was conceived and coordinated by prof. M. Chytrý from the Institute of Botany and Zoology, Masaryk University in Brno, the main cooperating institution of the Department of Vegetation Ecology. The approach used in the *Vegetation* is essentially following the Braun-Blanquet tradition of phytosociology, utilizing recent achievements in data analysis and a large vegetation database covering the whole Czech Republic (the Czech National Phytosociological Database). It benefited from coordinated efforts of a large team of vegetation scientists from several institutions. The first volume of *Vegetation* covered grassland and heathland vegetation, the second surveyed synanthropic vegetation units. Both volumes were published before 2010. In 2010–2014, last two volumes of this monograph were published.

The *Vegetation of the Czech Republic* vol. 3 (Chytrý et al. 2011) focused on wetland vegetation. A comprehensive survey of vegetation units was compiled, with the crucial contribution of four of the Department's members; K. Šumberová was a single author, sometimes a co-author, of most of the chapters; as a leading expert on vegetation of aquatic herbs, annual wetlands and marshes, she contributed substantially to this 828 page monograph. Furthermore, M. Hájek and P. Hájková authored or co-authored comprehensive chapters on the vegetation of marshes, springs and mires. The *Vegetation of the Czech Republic* vol. 4 (Chytrý et al. 2013) focused on forest vegetation. Three members of the Department worked on this volume, authoring or co-authoring significant parts of the 552-page book. J. Roleček was responsible for the vegetation of oakwoods, R. Hédl for the vegetation of beech forest and ravine forests.

Next to the *Vegetation*, members of the Department were also responsible for compiling two plant checklists. One comprises the vascular plant species of the Czech Republic (Danihelka et al. 2012). The other provides an extensively commented updated list of alien plants of the Czech Republic (Pyšek et al. 2012).

### **Soil chemistry and processes in vegetation through field experiments**

Considerable research effort of the Department of Vegetation Ecology was devoted to variations in soil chemistry and vegetation. Soil chemistry was experimentally manipulated in order to study the processes in whole ecosystems, and the reactions of selected species. The main focus was on the anthropogenic impact on sensitive environments, including the processes of atmospheric acidification, eutrophication, and disturbances by skiing. Several thematically similar studies examined the anthropic impact in grassland and forest ecosystems, regarding nutrient balance. Using field fertilization experiments, competition relationships between structurally dominant grass species, including the expansive *Calamagrostis epigejos*, were the

subject of three papers (Fiala et al. 2011, Holub et al. 2012 a 2012b). Root production in wet grassland was also experimentally studied (Holub et al. 2013b). An effect of atmospheric acidification on soil and vegetation was the topic of two papers. Soil chemistry patterns resulting from long-term acid pollution was documented at the landscape level, along a combined elevation-forest type gradient in the Eastern Sudetes Mts. (Hédl et al. 2011). The ability of the locally widespread fern *Athyrium distentifolium* to mitigate the effects of soil acidification was experimentally demonstrated in the Beskydy Mts. (Tůma et al. 2012). Another paper resulted from a 3-year precipitation manipulation field experiment (Holub et al. 2013a). Lowered precipitation significantly affected dry grasslands, in contrast to precipitation-change in more resilient mountain grassland communities. The last of eight papers from this research area studied the effects of skiing on soil properties along a slope gradient of vegetation types, including the dominance of *Athyrium distentifolium* in the Hrubý Jeseník Mts. (Hédl et al. 2012). Significant impacts of skiing-related activities were documented.

The team members contributed to 8 papers in journals indexed in the WoS. They were responsible for the design and running of field experiments, collection of all data, and authorship or co-authorship of resulting papers. Experimental studies on the relationships between vegetation and environment, in particular soil chemistry, have constituted a major research area of the Department of Vegetation Ecology over the past decades. The last classical field experiments manipulating nutrients, precipitation etc. were completed in 2010. Currently there are several active long-term field experiments focusing on the restoration of traditional forest management, such as coppicing or litter raking.

### **Other topics and studies**

Members of the Department of Vegetation Ecology widely participate in research led by other teams. This cooperation resulted in no less than 19 papers over the period 2010–2014, in journals indexed in the WoS. The majority of these papers were led by the Institute of Botany and Zoology, Masaryk University, Brno, Czech Republic. The research also included international cooperation with academic institutions from the Russian Federation, United Kingdom, Brunei Darussalam, Ukraine, Germany and other countries. A short commentary should be made to several research topics. The most frequent topic was plant species diversity at the community level and its explanatory mechanisms (Cachovanová et al. 2013, Chytrý et al. 2010, Chytrý et al. 2012a, Fajmonová et al. 2013, Michalcová et al. 2014, Roleček et al. 2014). Productivity-diversity relationships were studied in both central Europe and Siberia (Axmanová et al. 2012, 2013). Various aspects of urban habitats were addressed in several papers (Chytrý et al. 2012b, Lososová 2011, 2012a, 2012b). Perhaps the most “exotic” paper resulted from the research expedition to rainforests in Brunei Darussalam, where a new species of mycoheterotrophic plant was described (Dančák et al. 2013). Taxonomic issues and individual species biology were addressed by another 5 papers (Danihelka et al. 2010, Marcussen et al. 2012, Wagner et al. 2012, Slancarova et al. 2013, van den Hof et al. 2010). Our team also contributed to a worldwide analysis of arctic greening (Walker et al. 2012).

## Research Report of the team in the period 2010–2014

Institute	Institute of Botany of the CAS, v. v. i.
Scientific team	Department of Experimental Phycology and Ecotoxicology

Over the last 5 years, the Department of Experimental Phycology and Ecotoxicology was the leading Centre for cyanobacteria and their toxins, having an historic close cooperation with the RECETOX research centre at the Masaryk University. The whole Department was active in the ecology of cyanobacterial blooms, the ecology and fate of cyanobacterial toxins, and their role in the stability and diversity of aquatic ecosystems. ... XXX ... As a new topic, we have focussed on the ecosystem role of phosphorus, research into mechanisms of P-recycling, P reuse, and P-pollution prevention in aquatic ecosystems. The ecotoxicology of new-emerging pollutants, including e.g. metal nanoparticles or estrogens, was another important part of our research activities.

Major scientific achievements for the period of 2010-2014 may be grouped under the following categories:

### **Environmentally friendly methods for management of cyanobacterial blooms**

Cyanobacterial water blooms are the ecosystem's response to nutrient pollution, especially by phosphorus in the aquatic system. Our research dealing with management of cyanobacterial blooms is focused mainly on understanding the ecological consequences of eutrophication and on the development of new, environmentally friendly methods for bloom management, or detection systems for quantification of biomass and toxins.

Our team focused on prospective natural compounds that may inhibit cyanobacterial growth (cyanostatic compounds). As an example, we have examined extracts from both terrestrial and aquatic macrophytes, alkaloids and other chemicals synthesized or extracted from plants, or nature-inspired dyes called phthalocyanines. For the evaluation of management methods, we usually use traditional ecotoxicological biotests with endpoints of growth inhibition of cyanobacteria and algae, immobilization assays with invertebrate organisms (for example *Daphnia magna*, *Heterocypris inconguens*, *Artemia salina* etc.), growth assays with higher plants (*Lemna minor*, *Myriophyllum* sp. and others) and Gram-positive and Gram-negative bacteria. With respect to differences between laboratory conditions and the *in situ* environment, our research was moved from the laboratory to real aquatic mesocosms and was focused on field studies. Extrapolating data to real ecosystem effects is crucial from an ecosystem stability point of view (Jančula and Maršálek, 2011). We have developed equipment for such testing, applying our knowledge from mesocosm experiments, and we have developed a utility model for this outdoor testing device (Maršálek and Jančula 2012). This device helps us to better understand responses of various aquatic organisms to stress provoked by addition of different chemical substances, and has great potential for application in a broad spectrum of experiments in the aquatic ecosystem.

As mentioned above, we see phthalocyanines (PCs) as prospective molecules with amazing properties for management of algal/cyanobacterial blooms in technological facilities (we have tested phthalocyanines against filamentous algae *Cladophora glomerata* in cooling towers of nuclear power plants, for example), swimming pools, ponds etc. In fact, our department was the first, in the world to focus on the complex ecotoxicological evaluation of PCs in order to characterize their potential for reducing the consequences of eutrophication. Searching for the mode of action (Mikula et al., 2014) of PCs provides key information for the development of new PCs that will be able to act at very low concentrations (tens of micrograms per liter) and will be capable of inhibiting the growth of cyanobacteria. At the same time PCs should be (and usually are) safe for non-target species in the environment as for example aquatic plants (Jančula et al., 2010), fish or human skin (Jančula et al., 2013). In our studies on phthalocyanines, we usually collaborate with the Research Institute of Organic Synthesis (<http://vuos.com/en/>) (VUOS), Rybitvi. Whereas VUOS is responsible for synthesis, purification and molecular characterization of phthalocyanines, our team evaluates the ecotoxicological properties of PCs. A key element of our work is also focused on mechanisms of action of phthalocyanines against living organisms, which is an interesting alternative to metal nanoparticles in several technological applications such as prevention of biofouling.

Because phthalocyanines act in various ways, including production of reactive oxygen species (ROS), our research also deals with other generators of ROS. A typical representative is hydrogen peroxide, which was found to be effective against the bloom-forming cyanobacterium *Microcystis aeruginosa*. In our research we have discovered crucial elements of hydrogen peroxide action against blue-green algae in a study dealing with metabolic activity and membrane integrity changes in *Microcystis aeruginosa* (Mikula et al., 2012). Although, oxidative stress is not apparently the main mode of action of another promising tool - nanoparticles of zero-valent iron (NZVI), we have identified various activities of this treatment with potential for cyanobacterial management. NZVI was found to have multiple modes of action, including the removal of bioavailable phosphorus, the destruction of cyanobacterial cells, and the immobilization of microcystins, preventing their release into the water column (Maršálek et al., 2012). This research was performed in cooperation with the Regional Centre of Advanced Technologies and Materials (RCPTM), Palacky University, Olomouc. RCPTM characterized nanoparticles of ZVI and our department assessed their ecotoxicological properties, their effects on cyanobacteria and phosphorus removal. A partnership between our institution and RCPTM was very productive in understanding the effects of newly synthesized material for various applications. Our strategy in searching for new chemicals active against cyanobacteria will help to deal with cyanobacterial blooms all over the world.

As conventional methods like polyaluminum chloride (PAC) are used globally for management of cyanobacterial blooms, we established several monitoring systems to evaluate the ecotoxicological effects of PAC on the aquatic ecosystem. Although PAC is used world wide, information about potential risks for aquatic ecosystems is rare. Thus, we have monitored five reservoir restorations in the Czech Republic, including the first ever large-scale application in our country (Jančula and Maršálek 2012). Although applications of PAC did not cause any fundamental long-term changes in the composition of phytoplanktonic species or phosphorus load, it caused fast and acute removal of the cyanobacterial community, and may act as a rapidly acting, high efficiency algicidal compound. In contrast, PAC application may cause short-term removal of non-target organisms, as shown in another study under controlled

conditions (Jančula et al., 2011a). Processes of coagulation and flocculation using non-metal materials have also been studied in our work focused on organic molecules (Jančula et al., 2011b). These non-toxic molecules, working on the principle of flocculation, are used in a practical way without a basic understanding of ecotoxicological and hydrobiological consequences. We therefore provide critical evaluation of these compounds, not only for scientific research, but also for practical technological management of eutrophicated ecosystems.

Although chemical compounds are often used because of their simple manipulation and low price, risks connected with toxic effects on non-target species are relatively high. Over a long period, we have been developing new methods and strategies for preventing cyanobacterial blooms using physical and physico-chemical methods in order to protect non-target aquatic species. One of the models is based on hydraulic jet cavitation (Jančula et al., 2014). This method has several advantages. The method a) acts via disruption of cyanobacterial gas vesicles, resulting in removal of cyanobacteria from the water column to the bottom of a reservoir; b) cyanobacterial cell membranes are not harmed and thus toxins are not released into the surrounding water; c) it does not affect other species such as green algae, which are important ecological competitors of blue-greens. The other model for cyanobacterial blooms, prevention based on destratification and oxygenation of reservoir water, was developed and is already used in the Brno reservoir to prevent cyanobacterial bloom development. The so called “Aeration Tower” system was awarded the prestigious Česká Hlava (Czech Head) prize, the highest scientific award in the Czech Republic in 2011. The method was protected by a Czech utility model in 2012 (Šoukal et al., 2012).

### **Toxic cyanobacteria and cyanotoxins - environmental occurrence and toxicology**

Considerable research at the Department of Experimental Phycology and Ecotoxicology has been focused on the investigation of cyanobacterial toxins, whose environmental occurrence represents a serious environmental and human health hazard and may be associated with ecological and human health risks. The research activities within the Department have focused on the evaluation of cyanobacterial and cyanotoxin occurrences in the environment, their ecotoxicology and the toxicology of cyanobacteria.

Information on cyanobacteria and cyanotoxin occurrence in the environment are critical for understanding cyanobacterial ecology, to identify causes of mass proliferation of cyanobacteria, factors affecting production, the environmental fate of cyanotoxins, and, for assessment of ecological and human health, risks of toxic cyanobacteria and cyanotoxins.

The Department has contributed to the development of novel fluorescence-based techniques suitable for *in situ* quantitation of phytobenthic assemblages. These techniques can be effectively applied for monitoring benthic cyanobacteria, which represent producers of potent cyanobacterial toxins (Carpentier et. al, 2013). A review of advanced optical techniques suitable for rapid, real-time and *in situ* detection and monitoring of harmful cyanobacterial and algal blooms was prepared and published in Encyclopaedia of Aquatic Ecotoxicology (Marsalek and Babica, 2014). Regarding ecology of planktonic cyanobacteria, a book chapter providing a comprehensive summary of the ecology and life cycle of the most important bloom-forming toxic cyanobacterium, *Microcystis*, was also published (Sejnovhova and Marsalek, 2012).

Research into the occurrence of cyanotoxins and monitoring was primarily carried out in collaboration with RECETOX, Masaryk University, which provided the analytical infrastructure and relevant expertise in environmental analytical chemistry. A novel method based on passive sampling was developed, optimized and evaluated for the sampling and detection of major cyanobacterial toxins, microcystins (Kohoutek et al., 2010). This method, in combination with LC-MS/MS detection, allows cost-effective, sensitive and integrated monitoring of microcystin concentrations in the field, and was successfully applied for monitoring microcystins in drinking water reservoirs and drinking water treatment plants (Jasa et al., in preparation). Instrumental analytical techniques were further applied for identification of novel cyanotoxin structural variants in various strains of toxic cyanobacteria (Addico et al. 2010, 2011), and for studying temporal and spatial dynamics of microcystin concentrations in three major water reservoirs in the Czech Republic (Blaha et al., 2010). Immunoassays allowing rapid and extremely sensitive detection of cyanotoxins were employed in a monitoring study focusing on the occurrence of the major cyanotoxin types in Czech water reservoirs, confirming that microcystins represent the most frequently occurring and most abundant cyanotoxins in the Czech Republic. However, other hazardous cyanotoxins can also be found in the Czech water reservoirs, including cylindrospermopsin and saxitoxins, the latter being detected for the first time in this country (Jancula et al. 2014).

### **Toxic cyanobacterial effects related to human health**

Tumor promoting and carcinogenic effects probably represent the most significant human health hazards associated with chronic exposure to toxic cyanobacteria and cyanotoxins. Whereas tumor promoting and carcinogenic activities of the major cyanobacterial toxins, microcystins and cylindrospermopsin, have been recognized, little is known about cancer-related effects of other cyanobacterial metabolites, as well as about detailed mechanisms of tumor promoting activity of cyanotoxins at the cellular and tissue levels. Research in the Department focused on the effects of cyanobacterial extracts on *in vitro* biomarkers of tumor promotion, namely inhibition of gap-junctional intercellular communication (GJIC), and activation of mitogen-activated protein kinases (MAPKs) ERK1/2 in a rat liver epithelial cell line. Our experiments demonstrated, for the first time, that cyanobacterial extracts, (but not green algal extracts), specifically induced rapid effects on GJIC and MAPKs (Blaha et al., 2010). However, these effects did not correlate with the content of the well-recognized cyanotoxins, microcystin and cylindrospermopsin, and were thus most likely induced by not-yet-identified cyanobacterial metabolites, contributing to cyanobacterial tumor promoting activities. Follow-up studies revealed that such effects were elicited not only by natural cyanobacterial bloom extracts, but also by extracts and exudates of laboratory cultures of the most common bloom-forming cyanobacteria, such as *Microcystis*, *Anabaena*, *Aphanizomenon*, *Planktothrix* or *Cylindrospermopsis* (Novakova et al. 2011). Tumor promoting effects of cyanobacterial extracts were also synergistically potentiated by co-exposure to other environmental contaminants such as polychlorinated biphenyls or polycyclic aromatic hydrocarbons (Novakova et al. 2012).

These studies represent original findings, significantly contributing to our understanding of mechanisms of tumor promotion by toxic cyanobacteria. The experiments were performed in collaboration with Masaryk University (RECETOX), where the Department provided important information on the relevant and representative selection of cyanobacterial bloom samples, cyanobacterial strains and

cyanobacterial toxins, their concentrations as well as experimental design and data interpretation. ... XXX ...

### **Ecotoxicology of cyanobacterial blooms**

Massive proliferation of cyanobacteria has a significant impact on the structure and function of aquatic ecosystems. Aquatic organisms may be negatively affected not only by changes in physico-chemical conditions, energy fluxes and nutrient cycles induced by cyanobacterial blooms or scums, but also directly by production of toxic cyanobacterial metabolites.

Effects of cyanobacterial secondary metabolites on photosynthesizing organisms competing with toxic cyanobacteria were investigated by the Department. Our experiments revealed, for the first time, that the well-recognized cyanobacterial toxins microcystins, affect cell differentiation of the filamentous cyanobacterium *Trichormus variabilis*, suggesting possible signaling or allelopathic functions of microcystins (Bartova et al. 2011a). However, it was also documented in a study with a green alga *Pseudokirchneriella subcapitata* that effects of microcystins on biochemical parameters of oxidative stress and detoxication were qualitatively and quantitatively different from the effects of complex cyanobacterial extracts and exudates, which indicates that biological activity of other cyanobacterial metabolites may significantly modulate microcystin effects (Bartova et al. 2011b). This research was carried out in collaboration with RECETOX, Masaryk University, which contributed with expertise in cyanobacterial toxin purification, cyanobacterial extract fractionation and characterization, and evaluation of biochemical markers, whereas the Department of Experimental Phycology provided knowledge regarding cyanobacterial and algal cultures and bioassays.

Effects of toxic cyanobacteria and cyanotoxins on vertebrates were investigated in fish embryos, which represent a life-stage that is extremely vulnerable to toxin exposure. Experiments with Japanese medaka (*Oryzias latipes*) eggs showed that environmentally relevant concentrations of cyanobacterial bloom extracts affected hatching rate, hatching onset and embryo-larval development of Japanese medaka, and that cyanobacterial blooms may have negative impacts on freshwater fish communities (Adamek et al., 2011). Another study carried out in collaboration with the Veterinary and Pharmaceutical University, Masaryk University (RECETOX) and Mendel University focused on the accumulation and effects of microcystins in tissues of Nile tilapia (*Oreochromis niloticus*). Although microcystins significantly accumulated in the hepatopancreas and were only slowly eliminated, their concentrations in muscles were mostly undetectable, and exposure to cyanobacterial toxins did not have any negative effects on the dietetic quality of muscles (Palikova et al. 2011). In addition, exposure to toxic cyanobacteria had no significant effects on detoxification and physiological performance of exposed Nile tilapias (Zikova et al. 2010).

Although mass deaths of birds associated with cyanobacterial blooms are often reported, there are few experimental data on cyanotoxin effects in birds. In collaboration with Masaryk University (RECETOX) and the Veterinary and Pharmaceutical University, effects of toxic cyanobacterial biomass were investigated using an animal bird model, Japanese quails. The study revealed that exposure to cyanobacterial toxins significantly enhanced the sensitivity of bird populations to other chemical and biological stressors, possibly contributing to bird mortalities (Pikula et al. 2010). In these studies, members of the Department provided critical expertise



regarding selection of cyanobacterial material and toxins, their preparation, study designs and evaluated endpoints, as well as data discussion and interpretations.

### **Cyanotoxin removal**

The presence of the potent cyanotoxin, microcystin-LR, in drinking water sources poses a serious risk to both aquatic ecosystems and public health. In our research, we focused on degradation of this toxin using ferrates. The kinetics of the reactivity of ferrate with microcystin, using waters of different compositions, were reported. The analysis of protein phosphatase (PP1) activity, an important bio-marker in human toxicity of microcystins, showed that degradation by-products of microcystin should not possess significant biological activity and thus should be safe. This research is the result of international cooperation, where the ideas of our team, who prepared the experimental design and analytical background, was extended by analytical end-points used at the Department of Chemistry and Biochemistry, Florida the International University, and the Department of Environmental and Occupational Health, School of Public Health, Texas A&M University. This joint research was published in *Environmental Sciences and Technology*, an ACS Journal.

Similar research focussed on cyanotoxin degradation using a photosensitiser group of phthalocyanine compounds. Our experiments revealed new information that phthalocyanines, under conditions of light, can break the circle of microcystins. Moreover we proved that the molecular mode of action is based on production of the singlet oxygen radical (Jancula et al 2010). This research can bring new technologies and alternatives for cyanotoxin removal from drinking water treatment plants with potential for scientific cooperation with partners.

### **Phosphorus pollution prevention and phosphorus recycling**

A reduction in the level of nutrients available in aquatic ecosystems is highly important to prevent problems concerning cyanobacterial blooms. Development and application of new technologies that would significantly reduce nutrient levels during waste water treatment can contribute considerably to a decrease in the quantity of nutrients entering the environment and thus to minimize the effect of one of the basic factors influencing cyanobacterial blooms. Studies performed by our Department, in collaboration with other institutes, brought valuable information on cleaning of waste waters by means of membrane bioreactors in both pilot and full scale operation.

Data about long-term monitoring at one small municipal wastewater treatment plant (WWTP) and one of the biggest membrane-using WWTPs in the Czech Republic (cca 400 EI), including operating and qualitative indicators (COD, BOD, N-NH<sub>4</sub><sup>+</sup>, total P) of both WWTPs, and concentrations of organic extracellular polymers causing membrane clogging were published. The results demonstrated highly efficient removal of all basic qualitative indicators and proved a correlation between the composition of the biotic community in activated sewage sludge and concentrations of extracellular polymers. Another interesting discovery was an increase in the concentration of hydrocarbons and DNA in the absence of organic substrate (Holba et al. 2012, Gómez et al. 2012). The topic of membrane utilization in wastewater treatments has been discussed in another two papers (Gómez et al. 2013; Škorvan et al. 2012).

Phosphorus recycling principles, new mechanisms and potential technologies for phosphorus recovery is an area in which we are also active at the international level (EU initiative European Sustainable Phosphorus Platform, and in the European Innovation Partnership as the action group ARREAU (Accelerated Resource Recovery from Water Cycle) where P-reuse and recycling is the dominant and highly promising

topic not only for high quality scientific papers, but also for new IP protection by patents and know-how protection.