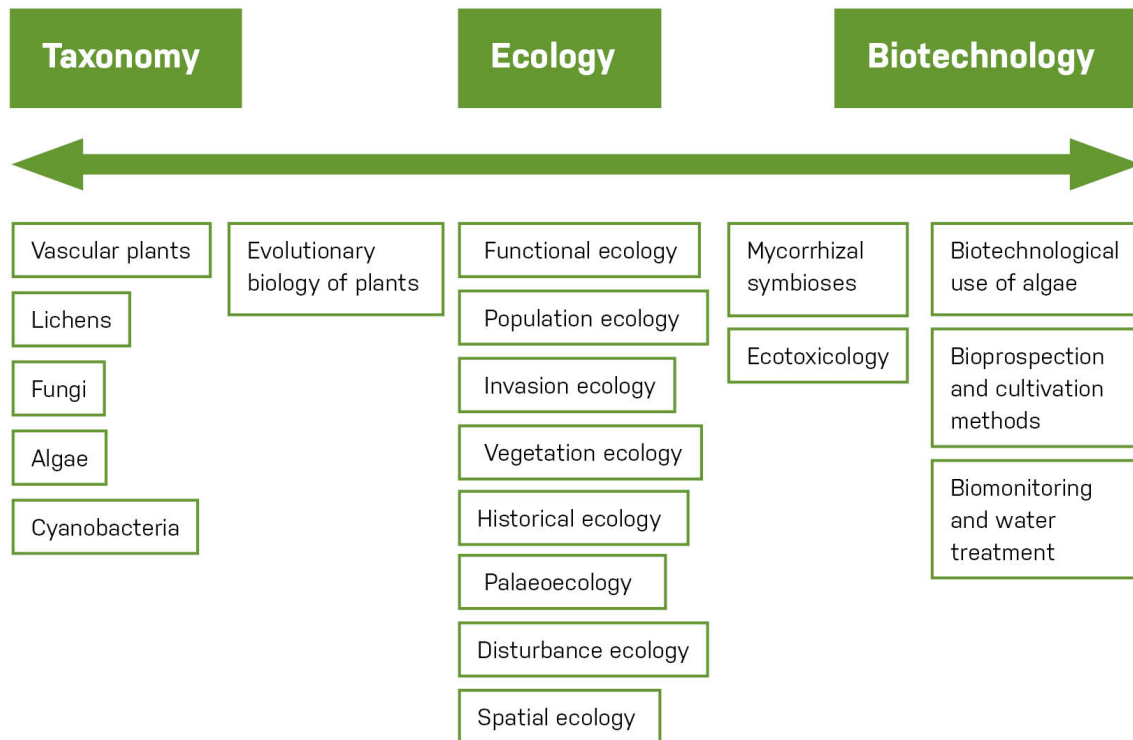


Description of the main research directions investigated by the institute



The main research directions of the IB are stabilized and thus did not change significantly from the last evaluation period. We focus on research in a wide range of botanical disciplines conducted at various levels of the biological organization, from individuals and populations to ecosystems. The main backbone is formed by **taxonomy**, biosystematics and the evolution of **vascular plants, fungi, lichens, algae, and cyanobacteria**. It combines traditional alpha taxonomy with state-of-the-art molecular, cytogenetic and experimental approaches to gain a holistic insight into the diversity and evolution of the investigated groups. The study of complex evolutionary processes, particularly genome duplication, gene transfer or micro-evolutionary processes forms a key specialization in this broad research field. The deep knowledge of taxonomy also allows us to set national standards in botanical nomenclature and systematically work on canonical national monographs such as Flora of the Czech Republic and plant identification guides.

A broad range of ecological research stems from the taxonomical background. Starting at the individual plant level, the field of **functional ecology** is essential. It focuses on the morphological and physiological characteristics of plants (functional traits) that are linked to their fitness. We mainly seek to discover how the plants cope with abiotic stress, disturbances such as extremely low temperature, water deficit, mechanical injuries and biotic interactions such as herbivory and insect outbreaks, competition or facilitation. Both irreversible evolutionary adaptations and reversible acclimation are in our focus. Linking data collected at the individual level (e.g. functional traits, (epi)genetic variability) with the functioning of whole communities falls within the scope of **population ecology**. We use this connection to seek for drivers of community dynamics and determinants of species abundance and coexistence.

In the center of our interest are also biotic interactions both with other plant species and with organisms at other trophic levels, such as herbivores, pathogens or symbionts. In all these researches we prefer the comparative approach focused on populations of many species which allows us to address both very general processes, and processes that are specific to individual species and environments.

Further ecological research scales up from individual to ecosystem level. Our work in the field of **invasion ecology** gained us worldwide recognition. It encompasses a wide range of topics from the population, community and evolutionary aspects of plant invasion to macroecology. Special focus is devoted to mechanisms driving species invasiveness, pathways of plant invasion and habitat invasibility as well as the impact of biological invasions. The field of **vegetation ecology** is focused on spatiotemporal patterns in plant communities studied largely in the context of long-term interactions with human societies. The connection of the study of plant communities and other research fields such as **paleoecology**, environmental archeology and **historical ecology** plays a key role. **Disturbance ecology** is a topic investigated across several teams, which focuses particularly on forests and their main disturbance agents: fire, wind and insects. Several teams deal with **spatial ecology** benefiting mainly from their experience in remote sensing and geoinformatics. Through interdisciplinary research combining earth and geo sciences with ecology, we seek to understand how plant communities react to environment and global change at different spatial and temporal scales.

Other directions investigated traditionally at our Institute deviate slightly from the narrow definition of field botany, but nicely complement other topics and naturally put more emphasis on applied research. The research on **mycorrhiza** pursues two main directions: the identity and community ecology of root-associated fungal symbionts and the context-dependence of the symbiotic functioning of plant-fungal associations. Within the latter direction, we mainly focus on the contribution of mycorrhizas to the tolerance of plants to abiotic stresses, but also on important phenomena of plant-soil interactions such as plant-soil feedback or successional dynamics. The in-depth study of diversity, systematics, evolution and ecology of **microalgae** and **cyanobacteria**, often in extreme conditions, lays the foundations for a range of applied research directions. One direction is the **biotechnological utilization** of algae, specifically for the production of extracellular polysaccharides, polyunsaturated fatty acids and carotenoids under various cultivation regimes. The optimization of the cultivation processes themselves is also in our focus. Within the frame of **ecotoxicological research**, we mainly address the prevention of the proliferation of cyanobacterial biomass in water bodies, the development of technologies for the detection and destruction of cyanotoxins and the ecotoxicology of nanomaterials and nanoparticles. The majority of disciplines are connected and thanks to that networking even truly fundamental research has the potential for transferring knowledge to practice, whether in nature protection or in bioprospection and biotechnologies. There are numerous examples of this connection and details are given in the reports of particular research groups.

Research activity and characterisation of the main scientific results

During the evaluated period the team published 253 research papers, including 155 papers indexed in the Web of Science. Many of these papers were published in leading international journals in the particular field. We also authored or co-authored four books and five additional book chapters. Major achievements are detailed below.

Evolutionary consequences of whole genome duplication

Whole genome duplication (WGD, polyploidization) is a massive genome-wide mutation whose ubiquity across eukaryotes suggests an adaptive benefit, though no mechanism has been identified. Consequently, a large controversy dominates whether WGD represents net benefit or detriment to evolutionary success. Our research used wide range of field, experimental and (cyto)genetic and genomic methods in order to decipher costs and benefits of polyploidy in nature. We started by thorough screens of phenotypic, genetic and ecological diversity in naturally ploidy-variable systems (Kolář et al. 2015a, b, 2016a, b, Zahradníček & Chrtek 2015, Morales-Briones et al. 2018, Knotek & Kolář 2018, Němečková et al. 2019). In *Knautia* we brought the first empirical evidence that WGD aids local establishment of the novel polyploid mutant (Čertner et al. 2019). We also performed relevant taxonomic re-assessments of the studied groups in order to facilitate diversity surveys and efficient conservation (Kolář et al. 2015b, Nunvářová Kabátová 2019, Šrámková et al. 2019). Finally, we reviewed relevant topics on ploidy diversity in natural populations (Kolář et al. 2017, Baduel et al. 2018).

The principal development in the past 2–3 years focused on establishing of population genomic approaches in the study of polyploid populations. Despite overall rapid development in genomics, polyploidy remained understudied because mainstream research tends to avoid complex polyploid genomes. We pioneered studies of genomic consequences of WGD in naturally ploidy variable *Arabidopsis*. First, we phenotyped natural diploid and tetraploid populations and found negligible effects of polyploidy on phenotypic variation, perhaps due to massive gene flow across ploidy border (Wos et al. 2019). Then we conducted the very first empirical investigation of genomic consequences of whole genome duplication in natural populations. Based on ~300 genomes across native range of wild *Arabidopsis* we demonstrated multifarious effects of ploidy on selection processes. Contrary to the theoretical expectations, the effects of ploidy were rather subtle, challenging both traditional competing views of polyploids as either genetically variable evolutionary winners or maladaptive dead-ends (Monnahan et al. 2019). We also detected a significant role of introgression on adaptation towards challenges posed by WGD in two *Arabidopsis* species (Marburger et al. 2019). Finally, we collaborated on further development of methods in polyploid data analysis (Stift et al. 2019).

In most of these studies we designed and led the research, analysed data and wrote the manuscripts. We initiated one of the reviews and led meta-analysis, while we contributed with ecological sections to the other.

Role of hybridization in evolution and constituting of plant diversity

Hybridization is an important evolutionary phenomenon that can generate new diversity and even lead to speciation. We studied hybridization in several plant groups, using a suite of several approaches (flow cytometric estimation of genome size, chromosome counting, DNA sequencing, RFLP). In *Batrachium* we discovered numerous previously unknown hybrids and identified their exact parentages. Our

detailed screening revealed that 15% of the studied individuals were of hybrid origin. However, involvement in hybridization is species-specific, indicating that breeding barriers are more effective among diploids and ecologically specialized species. Some of the detected hybrids represent F1 generations while others contribute to the evolution of allopolyploid species, which also differ in their ecological behaviour. This complex pattern is further obscured by occasional backcrossing (Prančl et al. 2018).

Several hybrids were detected also in *Potamogeton* (Aykurt et al. 2017, Kaplan et al. 2018, 2019). The disentangling of the identity of an Australian hybrid was particularly surprising because one of the parental species currently does not occur in the continent. This indicates a complex history of this hybrid that may involve its relictual occurrence from a time when both the parental species co-occurred there or long-distance dispersal of a hybrid seed from Eurasia (Kaplan et al. 2019). The North American *P. floridanus* was considered a rare endemic species and ranked as endangered in the IUCN lists. We identified it as a clonal primary hybrid, which also explains its sterility. This study also exemplifies that thorough taxonomic evaluation is indispensable for understanding patterns of biological diversity and identification of conservation priorities and adequate conservation measures (Kaplan et al. 2018).

We conducted the first comparison of hybridization pathways in *Pilosella*, a polyploid complex of diploid sexual and polyploid facultatively apomictic biotypes, at a geographical scale. Individual regions markedly differed in diversity in species-mixed populations due to different proportions of stabilized hybridogenous species and polyploid sexual biotypes that would potentially become drivers of ongoing hybridization. Our results enable prediction of future dynamics of species-mixed populations (Krahulcová et al. 2016, 2018). Molecular markers linked to the two loci controlling apomixis were screened across *Pilosella* individuals from natural and experimental cross populations, to examine their conservation, inheritance and association with reproductive modes. The markers were essentially absent within sexual plants, but they were not conserved in all apomictic individuals, and both loci appeared to be inherited independently. Our results represent a valuable contribution to understanding the origin and inheritance of apomixis (Hand et al. 2015).

Most of the work was done by the team, with the contribution from the DNA lab staff. Foreign co-authors of the *Potamogeton* studies contributed mainly with plant samples and commented on the manuscripts. In the last *Pilosella* study the Australian co-authors conducted lab analyses of the experimental hybrids produced and characterised by our team.

Cryptic diversity in aquatic plants

Cryptic diversity constitutes a considerable part of the total biodiversity but it still remains neglected in vascular plants, for which few cryptic species were detected so far. We investigated genetic diversity in three model groups of aquatic plants that are characterised by reduced morphology, but high phenotypic plasticity. Using a multi-disciplinary approach involving flow cytometry, phylogeny based on multiple DNA markers and alpha taxonomy we revealed and documented the occurrence of several cryptic species and previously unknown cytotypes and hybrids. In addition, we untangled the evolutionary histories of these groups, which included both hybridization and polyploidization events (Kaplan et al. 2018, Prančl et al. 2018).

The team members conceived and designed the studies and played major roles in conducting analyses, data interpretation the data and writing the papers.

Taxonomic revisions of critical groups of vascular plants

Taxonomy provides basic understanding of the components of biodiversity, which is indispensable for every field of biology, for effective decision-making in conservation and sustainable use. Now the taxonomic studies are even more critical when biodiversity is being lost at an unprecedented rate. At the global scale they are irreplaceable sources of information as they combine incompatible concepts adopted in different countries into a coherent result based on profound taxonomic expertise.

The genus *Taraxacum* is one of the most challenging angiosperms to be treated taxonomically. The lack of information is particularly obvious in underexplored regions of the world. 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 the Mediterranean to China was evaluated and 18 species new to science described (Kirschner & Štěpánek 2017, Kirschner et al. 2015, 2016, 2017a, b, Štěpánek & Kirschner 2015, 2017, 2018, Zeisek et al. 2015).

Due to Quaternary climatic oscillations, the vascular flora of the Czech Republic is poor in endemic taxa, and these are usually microspecies with an apomictic mode of reproduction. However, using a combination of molecular, cytogenetic, morphological and ecological approaches on *Knautia* we discovered two new sexual species endemic to central Europe. Our study highlights the importance of ecologically stable Holocene refugia for preserving unique plant diversity. In addition, it demonstrates the value of multi-disciplinary taxonomic research even in botanically well-researched areas (Kolář et al. 2015).

Aquatic plants are phenotypically plastic and often remain in a vegetative state throughout the season, which impedes their morphological identification. We discovered that the genome size in *Batrachium* is a reliable marker for distinguishing its species. We set the standard for their determination by flow cytometry, which mostly allows identification of even small vegetative fragments (Prančl et al. 2018).

Several other papers were published, evaluating variation and diversity within taxonomically difficult genera such as *Potamogeton* (Lastrucci et al. 2015, Aykurt et al. 2016, Zalewska-Gałosz et al. 2018), *Stellaria* (Lepší et al. 2019), *Spiraea* (Záveská Drábková et al. 2017) and *Valeriana* (Kirschner & Zeisek 2017).

The team members had substantial input in all publications, mostly conceived the idea, collected and interpreted data and wrote most of the manuscripts, or acted as taxonomic experts for studies of foreign co-authors.

Revision and synthesis of national plant diversity

Countries hold the primary responsibility for the exploration and protection of their national biodiversity. Plant diversity is changing due to direct interventions and climate change. Simultaneously, our knowledge on plants is improving, mainly due to the applications of molecular methods. All this progress needs to be periodically reflected in national inventories of plant diversity. As the principal national centre for taxonomic research in the Czech Republic we were responsible for re-evaluation of the national plant diversity within the PLADIAS project (see below). The most important output of this task is a book that includes the expert revision of the total diversity of vascular plants (>3700 species) in the Czech Republic (Kaplan et al. 2019). Authors from the team wrote most of the text, the main editor of the book is a team member, and the majority of the chapter editors are also the team members.

Another national monograph is Flora and Vegetation of the Czech Republic (Chytrý et al. 2017). This is the first comprehensive botanical summary about this country. It includes accounts of the history of botanical research, diversity of vascular

plants, bryophytes and lichens, vegetation development from the late Pleistocene to the present, impact of alien species and conservation of threatened plants. This book is an essential reference for botanists and plant ecologists who are working in Central Europe or searching for information on flora and vegetation in this part of the world. Jiří Danihelka and Zdeněk Kaplan were co-editors of the monograph, Zdeněk Kaplan wrote the chapter Flora and phytogeography, Zdeněk Palice wrote the chapter on lichens, Jiří Danihelka and Zdeněk Palice were the co-authors of the chapter History of botanical research, 7 of 12 authors are from IBOT.

A comprehensive taxonomic revision is undertaken within the long-term project Flora of the Czech Republic. This is the first Czech multi-authored 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 specimens and literature. 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. Manuscripts for the 9th volume of the Flora of the Czech Republic were prepared by taxonomic experts, and edited by the team members (Jitka Štěpánková, Jindřich Chrtek, Zdeněk Kaplan).

We collaborated on the integration of so far isolated plant distribution records into a single national database, in which we were responsible for the taxonomic backbone. Based on examined herbarium specimens and critically evaluated database records we produced grid-based distribution maps of 780 vascular plants (Kaplan et al. 2015–2019). These datasets open novel ways of addressing fundamental questions of phytogeography, plant ecology and macroecology.

The flora of the Czech Republic is continuously enriched by introduced plants. These need to be detected and identified. The team includes prominent Czech taxonomists that play the principal role in the taxonomic assessment of alien plants. During the evaluated period they discovered several new introduced species (e.g. Prančl 2015, Danihelka & Sedláček 2017, Danihelka et al. 2017, Danihelka 2019).

Biotechnology exploitation of vascular plants

Because of the vulnerability of the current production of natural rubber (NR), one of the most important world natural commodities, we continued in studies of alternative NR sources. We discovered a group of *Taraxacum* species that is a plausible alternative for rubber production (Zeisek et al. 2019). On the basis of a unique set of KASP markers, mapping the allelic make-up of the genes relevant for rubber biosynthesis, these species proved to be a promising NR production alternative.

Diversity and ecology of forest lichens

During the last few years, general knowledge about diversity of epiphytic and epixylic lichens in European forests has considerably enlarged, with the principal contribution from the team (Vondrák et al. 2015, 2018a, 2019a, Malíček et al. 2018a, Ismailov et al. 2017, Berger et al. 2018). In order to make ecological-diversity studies more effective, we designed, optimized and standardized a new simple and cost-effective method for thorough surveying of forest lichens (Vondrák et al. 2016a, Vondrák et al. 2018a). Intensive studies of the most valuable old-growth and primeval forests across Central and Eastern Europe yielded unexpectedly high numbers of lichens (Vondrák et al. 2018a, Ismailov et al. 2017, Malíček et al. 2018a, 2019), with new diversity records in the Caucasus Mts (Vondrák et al. 2019a). Our studies demonstrated that these old-growth forests harbour much higher phylogenetic, taxonomic and functional

diversity of lichens than managed forests, and serve as a refuge for rare niche specialists (Hofmeister et al. 2015, 2016, 2019, Malíček et al. 2019).

Taxonomy and phylogeny of lichens

Revisions and phylogenetic reconstructions of complex groups are fundamental stones of taxonomic work. The team members were leaders in or substantially contributed to numerous studies (e.g., Resl et al. 2015, Frolov et al. 2016, Malíček et al. 2017a, Muggia et al. 2017, Vondrák et al. 2018b, 2019b, Brodo et al. 2019, Launis et al. 2019). Other studies included associated changes in taxonomy and nomenclature, some dealt with on a global scale (e.g., Arcadia & Vondrák 2017, Jørgensen & Palice 2015, 2016; Knudsen et al. 2019, Malíček et al. 2018c, Vondrák et al. 2016b, 2016c, 2019b). During the evaluated period we participated in discovery, mostly supported by molecular or chemical data, of 20 new lichen species (Arup et al. 2015, Frolov et al. 2016, Holien et al. 2016, Printzen et al. 2016, Malíček et al. 2017, 2018c, Vondrák et al. 2017a, 2017b, 2018b, 2019b, Launis et al. 2019).

Systematics and phylogeny of microscopic fungi

Mycological research focused on the understanding of fungal systematics and biodiversity incorporating multi-gene phylogenetic analyses, bioinformatics and structural phylogenetics. Extensive sampling and synthesis of other available data led to the discovery of several novel evolutionary lineages in the *Ascomycota*, three new orders characterised by unique sexual and asexual morphological traits, and four new genera (Réblová et al. 2015a, 2016a, 2018). Implementation of the one-fungus-one-name concept (1F1N) led to a comprehensive re-evaluation of generic names in *Sordariomycetes* including recommendations, which of the competing generic names, typified by sexual and asexual morphs, should be used or require formal protection (Réblová et al. 2016b). Following the 1F1N system, we introduced new life histories in genera *Barbatosphaeria* (Réblová et al. 2015b), *Ascotaiwania* and *Melanotrigonum* (Réblová 2016a), and closely related *Lentomitella* and *Spadicoides* (Réblová et al. 2018). Because species accommodated in some of these genera exhibit a low degree of morphological variation, we performed in-depth analyses of RNA secondary structures of ITS1 and ITS2 regions to assess their relationships. We disentangled hidden lineages in the *Rhizoscyphus ericae* aggregate, an ecologically important complex whose members live in various symbiotic relationships with plant roots (Fehrer et al. 2019). Using in vitro mycorrhizal experiments together with multi-locus phylogenies and cultivation studies, we discovered additional members and ecological lineages of sexually and asexually reproducing fungi.

The team members designed most of these studies and took the lead in sampling, cultivation and identification of fungi, performing phylogenies, RNA structural analyses, drawing taxonomic conclusions and writing manuscripts.

Research activity and characterisation of the main scientific results

1. Hybridization and horizontal gene flow

We successfully applied a suite of biosystematic techniques to elucidate causes and evolutionary consequences of hybridization. The task was targeted on (i) interspecific hybridization and its potential threat for the maintenance of the genetic diversity of endemic (*Knautia carinthiaca* – Čertner et al. 2015, *Dianthus arenarius* subsp. *bohemicus* – Vítová et al. 2015) or rare (*Prunus fruticosa* – Macková et al. 2018) species; (ii) unravel gene flow among ploidy levels (*Senecio carniolicus* – Hülber et al. 2015); (iii) sum-up the role of adaptive introgression and horizontal gene flow (Schmickl et al. 2017).

Special emphasis was also placed on horizontal gene transfer (HGT), i.e. a movement of nuclear DNA among organisms without fertilization. The phenomenon was thoroughly investigated on the genus *Hordeum*, where the presence of Panicoideae ITS sequences was corroborated (Mahelka et al. 2017). The study unequivocally showed (i) the presence of HGT among lineages that split before ca 60 Mya, (ii) multiple occurrences of a distinct copy of the target sequence, up to five within one diploid barley, therefore a quite common and repeated phenomenon, and (iii) an undistinguishable nature of some HGTs, because none of the non-native sequences was transcribed so it did not affect the phenotype.

The members of the team contributed substantially to most of the cited studies or were responsible for specific tasks of their expertise (flow cytometry, molecular analyses). They namely participated in the evolutionary interpretations and in writing texts. A total of 10 IF publications on all aspects and consequences of hybridization were produced with the participation of our team in the target period.

2. Genome size – its role, variability and consequences

Our long-lasting expertise in the field of flow cytometry resulted in several papers on the sources and evolutionary consequences of genome size variation. The review on the role of genome size in plant invasions (Suda et al. 2015) is amongst the most cited achievements in the field. The study presented a comprehensive overview of knowledge based on extensive analyses of published data and outlined the possibilities for future research on plant invasiveness.

The evolutionary consequences of genome size variation were revealed in several plant groups of our special interest – the genus *Chenopodium* (Mandák et al. 2016; Vít et al. 2016) and the genus *Anthoxanthum* (Chumová et al. 2015, Chumová et al. 2016). Those studies evaluated either the usability of genome size as species specific marker for taxa delimitation, or the role of B-chromosomes in genome size variability.

We also paid attention to orchids, the only known plant group with partial endoreplication, that challenges proper genome size estimation (Trávníček et al. 2015). However, a more methodological, yet important piece of work showed the pitfalls in the correct genome size interpretation in plants with endoreplication.

The members of the team designed and supervised all the cited studies, analysed the data and were mainly involved in writing of the papers. A total of 8 IF publications aimed to assess the role of genome size variation in plant evolution was produced by team members.

3. Phylogeography

The extent of knowledge at the ploidy level and the assessment of genetic diversity at population level enabled us to trace the Pleistocene history of numerous ploidy complexes and to evaluate their post-glacial re-colonization of target areas. Based on it, we were able to elucidate a surprising pattern in *Galium pusillum* polyploid complex, where three independent lineages colonizing deglaciated areas have a common history involving stepwise differentiation in distinct periglacial refugia (Kolář et al. 2015).

Unexpected results were also observed in *Alnus glutinosa*, where the comprehensive assessment of European populations revealed two hotspots of tetraploids in potential southern refugia (Havrdová et al. 2015; Mandák et al. 2016). The study thus discovered two completely new tree species for Europe, which were subsequently taxonomically described as *Alnus lusitanica* and *A. rohlenae* (Vít et al. 2017). The research of closely related *Alnus incana* pointed to the existence of Central European refugia that served as the source populations for postglacial re-colonization of northern parts of Europe (Mandák et al. 2016).

The members of our team further participated in the research of the post-glacial history of two members of the genus *Arabidopsis* – *A. arenosa* (Kolář et al. 2016) with the utilization of genome-wide SNPs analysis and *A. halleri* (Šrámková-Fuxová et al. 2017) with the usage of a wide spectrum of molecular techniques combined with morphometrics. A long-term study of *Chenopodium album* aggregate was crowned by the research on the importance of human-mediated dispersal of ruderal and weed plants in Europe (Krak et al. 2019).

The members of the team were mostly responsible for designing studies, analysing data and writing articles. A total of 9 IF publications aimed at the elucidation of post-glacial recolonization was (co)authored by team members. Studies on *Arabidopsis* were conducted in co-operation with the Department of Botany, Faculty of Sciences, Charles University, and the team members were responsible for the parts of their expertise.

4. Cape Floristic Region

One of the research directions that has been outlined almost 15 years ago and has been implemented to our development strategy is the research of the Cape Floristic Region. Here we would like to highlight an overview of the role of whole-genome duplication in the formation of exceptional species richness of Cape Flora (Oberlander et al. 2016). The study showed a significant disproportion between the expected number of polyploids and species richness compared to similar biodiversity hotspots on Earth.

Another keystone study concerns the genus *Oxalis*, a prominent eudicot geophyte that serves as a model plant group for phylogeny reconstruction via the application of the new NGS based method Hyb-Seq (Schmickl et al. 2016). The study shows that phylogeny based on hundreds of loci can be conducted with a relatively low effort and budget even for non-model taxa. In this respect, the study is a milestone because it substantially affected ongoing research for our team and others in the Institute.

Besides those two prominent papers, the members of the team were involved for example in collaboration on the research of the phylogenetic significance of leaf anatomical traits of southern African *Oxalis* (Jooste et al. 2016) or evolutionary consequences of polyploidy in Cape Restionaceae (Linder et al. 2017).

In sum, our effort resulted in the publication of 8 IF studies (co)authored by team members. The two highlighted papers were designed, processed and written with leading involvement of team members. The rest of them was done with partial but substantial input of the members of the team.

5. Polyploid complexes

The flow cytometry laboratory is an integral part of our Department. Attention was mainly paid to studies of polyploid complexes. Data on cyto geography, cytotype differentiation, mating interactions, etc. were mostly supplemented by molecular data to get a better insight into the evolution of such complexes. In the *Chenopodium album* aggregate, the allopolyploid origin of hexaploid *C. album* s. str. was detected (Krak et al. 2016) and the role of hybridization and polyploidization on the formation of taxa within the aggregate was assessed (Habibi et al. 2018; Mandák et al. 2018).

Complex research of *Arabidopsis arenosa* revealed the lack of ecological segregation in the formation of parapatric distribution patterns of cytotypes (Kolář et al. 2016). A detailed study on 39 populations of *A. arenosa* showed subtle but pervasive impact of polyploidy onto population genomic processes (Monnahan et al. 2019).

The research of the genus *Anthoxanthum* proved that Mediterranean area is the place of origin of the European taxa and that climatic changes in the Miocene triggered the taxa differentiation (Chumová et al. 2017). The ongoing polyploid evolution started in Pleistocene and newly arisen polyploids were able to colonize the mountains of tropical East Africa (Tusiime et al. 2017).

The members of the team participated also in the research aimed at various agamic complexes like *Potentilla crantzii* (Paule et al. 2015), *Sorbus* subgenus *aria* (Lepší et al. 2015), and *Pilosella* (Rosenbaumová & Krahulec 2015). Long-term experience with apomictic taxa also allowed us to participate in the review of their position in the current taxonomy (Majeský et al. 2017).

14 IF publications (co)authored by members of the team can be assigned to this task. Some studies were conducted almost exclusively by team members, some of them resulted from broad international cooperation, but with our substantial participation.

6. Mixed-ploidy populations

Mixed-ploidy populations offer a unique opportunity to trace the mutual interactions between cytotypes and provide a natural laboratory for the elucidation of polyploid origin, maintenance, and further evolution. Similar studies were so far confined to the perennial plant models. Our team used an annual herb *Tripleurospermum inodorum* for the first time to get a better insight into the dynamics of cytotype co-occurrence (Čertner et al. 2017). The model plant and its features enabled the easy distinction of neotetraploid mutants and long-established tetraploids, thus the population dynamics in contact zones of diploids and tetraploids could be separately viewed from the point of neopolyploid formation or maintenance of starting situation. The data analysis showed that besides fertile triploids, capable of contributing to gene flow and bringing evolutionary novelty, neopolyploids were incapable of the local establishment. The follow-up research on the drivers of diploid-tetraploid coexistence has shown climatic conditions acting at large (continental) scale but the co-occurrence of cytotypes at fine-scale is much more affected by founder effect and human activities (Čertner et al. 2019).

The members of our team also researched ecological niche displacement, which may play a crucial role in cytotype co-existence. We showed that microhabitat

differentiation of the cytotypes of *Senecio carniolicus* is promoted in contact zones that lead to divergence of the cytotypes niches in comparison to homopoloid populations (Sonnleitner et al. 2016). The second study showed that polyploidization in *Knautia serpentinicola* can promote a better response for higher competition stress and provide an adaptive advantage in the colonization of new habitats (Čertner et al. 2019).

Notwithstanding, the main achievement in the research on the cytotype-coexistence is the review by Kolář et al. (2017) which, in cooperation with colleagues from Innsbruck (Austria) and Guelph (Canada), summarized up-to-date studies on mixed-ploidy species and outlined the possible avenues of future research of polyploids. This work concluded the long-term research in the field, where the Laboratory of Flow Cytometry of the Institute of Botany played a leading role after its foundation in 2000.

The members of our team designed and supervised all but one cited study, analysed the data and were mainly involved in the writing of the papers. Although only 5 IF publications can be attributed to this task, they represent a significant contribution to the knowledge of plants with ploidy heterogeneity.

7. Orchids

Our focus on orchids was motivated by their unique feature: they exhibit the so-called partial endoreplication which means that only a species-specific fragment of nuclear DNA is replicated in the course of the cell endocycle. This phenomenon was traced in four out of five subfamilies except for the basalmost Apostasioideae (Trávníček et al. 2015) and means that orchids are exceptional among other plants.

The follow-up research on the phenomenon showed the nature of partial endoreplication. A complete elimination of particular elements of DNA, like retrotransposons, was not observed. The hypothesis of incomplete DNA replication instead of the *ad hoc* elimination of replicated DNA was thus corroborated (Hřibová et al. 2016).

In the next study, we tried to clarify the evolutionary consequences of whole-genome associated traits like genome size, GC-content and partial endoreplication in the most diverse plant family on Earth. The study showed unparalleled variability namely in GC-content, and new limits for plants and even eukaryotic organisms were set. Variability in both GC-content and genome size exhibited a tight link to the type of endoreplication and some life-history traits and climatic conditions. We therefore concluded that diversity in whole-genome traits in orchids has adaptive consequences (Trávníček et al. 2019).

The members of our team also co-authored new descriptions of two orchid species – one from Vietnam (Ponert et al. 2016) and one from Brazil (Ponert et al. 2019).

The members of the team crucially contributed to the design and supervision of all cited studies. One of them was conducted in close co-operation with the Institute of Experimental Botany, Olomouc. In all cases, we were involved in final data analyses and paper writing. A total of 5 IF publications dealing with orchids were (co)authored by the team members.

Research activity and characterisation of the main scientific results

Unless specified below, all the work presented below was done primarily by members of our Department, with both the first and last authors as well as the majority of the team being from the Department.

Species adaptation to climate change

Within our own work using a unique climatic grid allowing to study independent temperature and moisture gradients and their interactions, established by our colleagues from the University of Bergen, we demonstrated that shifts in temperature and precipitation are likely to dramatically affect plant performance. We obtained novel insights into the interplay between genetic differentiation and plasticity in response to temperature and precipitation and elucidated their effects on responses of species to climate change. Such complex responses will affect how climate-change impacts scale up to community and ecosystem levels (Münzbergová et al. 2017). We also demonstrated that species performance is not only affected by shifts between species original and current climate but also by conditions experienced by maternal plants in the previous season (Münzbergová & Hadincová 2017) and provided novel evidence that species responses to climate may be due to changes in DNA methylation (Münzbergová et al. 2019). By combining these data with data on genetic differentiation, we provided novel evidence that populations of the model long-lived species have a potential for evolutionary response to the changing conditions (Stojanova et al. 2019). In collaboration with colleagues from Switzerland, we also obtained novel evidence that plants from different climates are differentiated in the intensity of tritrophic interactions (Kergunteuil et al. 2019). This together with changes in constitutive and inducible defences (Knappová et al. 2018) makes these plants more vulnerable to increased herbivore pressures, as one possible consequence of climate change.

Our work on plant-herbivore interactions along altitudinal gradients in the Himalayas was one of the first studies to simultaneously measure intraspecific variation in plant growth, direct and indirect defences and plant tolerance as a consequence of herbivory providing novel insights into the complex interplay among the different mechanisms (Dostálek et al. 2016). Despite higher production of defences by plants from lower altitudes, Rokaya et al. (2016) found that plants from higher altitudes are less palatable. This may be due to the production of metabolites which primarily serve as protection against extreme climatic conditions but simultaneously have an anti-herbivore function. This clearly illustrates the need for complex studies combining field and laboratory approaches to obtain insight into the complex mechanisms driving species responses to novel climates.

In addition to studying recent climate changes, we also studied effects of climate change during past 20 million years using migrations from Qinghai-Tibetan Plateau (QTP) to other temperate regions as a model (Jia and Bartish 2018). We demonstrated the impact of different stages in the uplift of QTP and Eurasian mountains and climatic changes in Neogene on diversification and range shifts in highland flora.

Transgenerational plasticity and epigenetic variation in ecology and evolution

Phenotypic plasticity belongs among the most effective mechanisms of plants in coping with changing environment. The plastic response can occur not only within a life-span of an individual but can also be "passed" to offspring generations. Such

transgenerational plasticity (TP) can play an important role in plant evolution because it can allow for rapid adaptation to novel conditions without a change in the DNA sequence. TP is enabled by various mechanisms including hormonal transmission from mother to offspring or via epigenetic mechanisms. We provide novel insights into the role of TP in plant behaviour and adaptation to changing environment by focusing on clonal plants, which are underrepresented in studies on TP, are dominant in many ecosystems and environmentally induced epigenetic variation can be easily passed from mother to clonal offspring due to lack of meiotic barrier during clonal reproduction. Epigenetic memory can also enable plants to engage an intelligent behaviour (Latzel et al. 2016) as demonstrated in our unique study for wild strawberry (Latzel & Münzbergová 2018). TP functions as a form of memory on the past environment and is likely mediated by epigenetic variation (Rendina González et al. 2016, 2018). Growth and behaviour of clonal plants might thus be affected not only by the ambient environment but also by environments experienced by maternal plants as demonstrated in our climate adaptation studies (Münzbergová & Hadincová 2017, Münzbergová et al. 2019). Such behaviour and memory can be adaptive and thus evolutionarily very important for clonal plants (Rendina González et al. 2017). Our experience with experimental alteration of epigenetic status of adult clonal plants via their chemical demethylation led to the development of a new method that is highly effective without unwanted side effects compared to original method (Puy et al. 2018). Our experience on ecological epigenetics resulted also in a large review published together with a range of foreign colleagues (Richards et al. 2017).

Plant-soil interactions as drivers of species performance and community dynamics

Plant-soil feedback (PSF) is becoming an important concept used for explaining plant competition and coexistence or plant invasions. However, many aspects of PSF remain unclear. We performed a number of studies to increase our understanding of the relationship between PSF and plant invasiveness, of the drivers of PSF and factors affecting the results of PSF experiments. Using controlled comparative studies, we provided novel evidence that, contrary to expectations, invasive plants did not create more positive intraspecific PSF compared to native plants, and plants from the invasive range did not increase but diminished their negative effects on other species via soil modification (Dostálek et al. 2016, Florianová & Münzbergová 2018, Aldorfová & Münzbergová 2019).

To generalize the results of PFS studies, we used a meta-analytical study (Kuťáková et al. 2018) and demonstrated that heterospecific PSF can be explained by plant traits and is reflected in species co-occurrence patterns. Also, phylogeny is a significant predictor of feedbacks over short phylogenetic distance in the meta-analysis as well as in an extensive experiment (Münzbergová & Šurinová 2015). In addition, we provided novel evidence that plant traits and the effect of the conditioning plant on soil chemistry may be useful factors allowing us to predict the intensity of PSF in natural systems.

We also performed methodological experiments. Hemrová et al. (2016) obtained unique evidence that intraspecific negative PSF may be a key factor determining the performance of plants in field translocation experiments and this feedback could be replicated in controlled conditions using local soil. Florianová & Münzbergová (2018), Aldorfová & Münzbergová (2019) and Lepinay et al. (2018) questioned the common methodologies of PSF studies and provided novel insights

into how cultivating conditions and temporal duration affect the PSF results providing guidelines from optimal methodologies. In collaboration with colleagues from IDIV, Germany, Kuřáková et al. (2018) also showed that presence of soil microarthropods in a PSF experiment can substantially alter the composition of soil microbial and nematode communities and alter results of PSF studies.

Importance of soil biota for the diversity of grassland communities

In the last 25 years, we have seen a strong increase in the number of abandoned agricultural fields. While some grassland plant species are able to colonize these fields, others are not. We suspected that these effects may be due to changes in AMF communities. In line with this, our garden and fields studies provided important evidence that AMF are crucial for species diversity and composition in grasslands (Knappová et al. 2016, Pánková et al. 2018) and are also important for reducing the intensity of competition in soil from the abandoned field (Knappová et al. 2016). In our eight-year experiment, we also found that recovery of altered AMF community may be very slow (Pánková et al. 2018). All this indicates that AMF are crucial for the diversity of grasslands and their loss may persist over many years. Indeed, Voříšková et al. (2016) showed functional differences between the fungal communities in both habitats by evaluating the composition and infectivity of AMF communities. Based on this, we assumed that inoculation with grassland soil could improve seed germination or seedling performance of species which are not able to colonize abandoned fields. However, neither a greenhouse experiment (Pánková et al. 2018) nor a field experiment supported this hypothesis (Lepinay et al. 2019).

Functional traits as determinants of plant abundance at different spatial scales

One hypothesis to explain the large variation in the local and regional commonness of species predicts that commonness is positively related to phenotypic plasticity because it may contribute to species' capacity to occupy broader ranges of conditions and optimally exploit resource-rich environments. We tested this using over 100 perennial species. In contrast to expectations, plasticity was negatively related to niche breadth and abundance (Dostál et al. 2016, Dostál et al. 2017). We also found that high values of generative and vegetative reproduction pre-adapt species for their global success. The success is driven by the independent effect of the two reproductive modes (Klinerová et al. 2018).

Drivers of the composition of plant communities

One long-term aim of our research is to understand mechanisms structuring grassland communities with special focus on belowground interactions. We developed a unique technique to quantify root biomass into species using qPCR for 14 co-occurring species and analysed the vertical distribution of their roots in a meadow. In combination with an existing removal experiment, it permitted us to examine whether root spatial arrangement changes in response to the presence of a dominant species (Herben et al. 2018). While our findings showed strong vertical differentiation of individual species, this vertical differentiation was not due to plastic response to the dominant species. A likely explanation lies in the low predictability of nutrient availability in the soil (Březina et al. 2019), which precludes individual species to forage for nutrients (even if they are capable to do this in culture).

We further sought for mechanisms of species interactions and coexistence using long-term data series of fine-scale aboveground data. We used these data to

extract main parameters of population dynamics using a Bayesian approach, and demonstrate that coexisting species differ along two main directions (Herben et al. 2019). Further, we showed the existence of linked cycles of legume abundance, grass abundance and nitrogen content in biomass in a 30-year time series and demonstrated how it is driven by dynamics of the nitrogen content of dominants. While it is a well-known fact that nitrogen fixers show recurrent population cycles, these cycles have almost never been demonstrated using hard long-term data, nor linked to long-term nutrient dynamics (Herben et al. 2017).

At the scale of vegetation classes, plant communities differ also in the evolutionary history of their species pools. Some can be characterized as museums while others - as cradles of phylogenetic diversity (Bartish et al. 2016). Maintenance of particular clades of closely related species in habitat types with similar environments may be facilitated by eco-evolutionary feedbacks (Prinzing et al. 2017). The feedbacks can be important determinants of functional synergy in communities (Barbe et al. 2018). This suggests that functionality of ecosystems depends on both environments and evolutionary history of incumbent pools of interacting species and that current ecological interactions may be stable at a macroevolutionary scale.

Drivers of landscape-level dynamics of species

At the landscape scale, we obtained important support for the fact that knowledge of past landscape structure is crucial for explaining species distribution patterns (Husáková et al. 2016) and among species differences to landscape past are strongly driven by species traits (Hemrová & Münzbergová 2015). In contrast to previous assumptions on importance of dispersal traits in a fragmented landscape, we provided multiple pieces of unique evidence that other factors such as local habitat conditions (Knappová et al. 2017), seed production (Knappová & Münzbergová 2015), local population dynamics (Hemrová et al. 2017) and dispersal of orchid associated fungi (Hemrová et al. 2019) are more limiting for species colonization and spread than their dispersal abilities.

Ecology, population biology and evolution of polyploid systems

Most studies on consequences of polyploidization are not able to separate real effects of polyploidization from effects of the subsequent evolution of the polyploid lineage. We did this using synthetic polyploids and also explored the effects of the polyploidization agent per se and assessed generality of results using multiple mixed ploidy populations as the starting material. We obtained important novel results that there is high variation between populations in effects of polyploidization on growth-related traits (Münzbergová 2017, Eliášová and Münzbergová 2017) and production of photosynthetic and antioxidative pigments (Münzbergová and Haisel 2019). All this indicates that the consequences of polyploidization are very complex and simple controlled experiments cannot appreciate the real patterns observed in nature.

To generalize the findings on consequences of polyploidization, we used a larger set of species and demonstrated that polyploidization is closely linked to the ability of vegetative reproduction, namely spatial extent of lateral spreading, confirming thus an old hypothesis, which has not been tested previously (Herben et al. 2017). We also demonstrated that polyploidization has many important consequences for plant physiology (Münzbergová and Haisel 2019, Pavlíková et al. 2017), but these changes are not always translated into changes in plant fitness (Pavlíková et al. 2017). Polyploidization may also have significant consequences for plant-herbivore interactions (Münzbergová et al. 2015, Münzbergová and Skuhrovec 2017).

We also attempted to understand the origin and demographic history of polyploid systems using a unique autopolyploid aggregate consisting of diploid and hexaploid types. By applying macroevolutionary, microevolutionary and niche modelling tools, we illustrated that polyploidization led to shift in species ecological niche of the hexaploid and created a new entity, which is currently undergoing demographic expansion (Mairal et al. 2018). In another model aggregate, we used a combination of field data, experiments and modelling to identify mechanisms that maintain coexistence of several ploidy levels at one site, namely clonal reproduction and free mixing of parental gametes of both sexes (Herben et al. 2016, Chrtek et al. 2017).

Comparative analyses of clonality-related plant traits

In collaboration with the group of J. Klimešová of Department of Functional Ecology, we performed comparative analyses of traits of plant clonality. While clonality is a key behavioural capacity of many plants, it has been largely overlooked in comparative analyses. Using flora-wide data sets on several thousands of species, we showed relative roles of LHS and clonal traits in disturbance response (Herben et al. 2018). We also showed that clonality-related traits are largely independent of seed-dispersal traits, although both constitute well-defined syndromes and they critically differentiate growth strategies of woody plants and herbs (Herben et al. 2016). Using comparative data on plant clonality and data on plant life spans permitted us to show how architectural reiteration and clonality contribute to the absence of senescence (Klimešová et al. 2016). We also showed that organization of the plant shoots and correlations of key shoot traits are linked through shape and size of the shoot apical meristem, a fact that has been long suspected but never demonstrated using a comparative analysis (Schnáblová et al. 2017). We also contributed several conceptual papers on plant clonality (Janovský et al. 2017, Klimešová et al. 2018, Ottaviani et al. 2017).

Plant structural-functional modelling for scaling up ecophysiological mechanisms

We investigated how traits and physiological mechanisms affecting water flow in transpiring vascular plants scale up from individuals to determine population and ecosystem dynamics. Our first study in this area employed water flow simulations on xylem tissue reconstructed from micro-computed tomography scans. We were able to explain unexpected *in vivo* flow observations but only after discarding classical theoretical assumptions in the simulations. These results challenge elements of the classical understanding of water transport in xylem and present novel hypotheses concerning constraints on the evolution of xylem (Bouda et al. 2019).

Population biology and genetics of rare species

One of our long-term ambitions is to perform studies on various rare species applicable in practical species conservation. Within this, the most important project is LIFE for *Minuartia* (2016-2020). Up to now, our work on this project was presented in popular journals and various presentations for the general public. Scientific publications will be presented in the next evaluation period. In a genetic study of another critically endangered species (Münzbergová et al. 2018), we provided important evidence that restoration of small populations of short-lived species with permanent seed bank is possible as these populations may still be genetically diverse. In addition to working with single species, we aimed to uncover general determinants of species rarity by

comparing germination and seed mass in a larger group of rare and common species and demonstrated that rare species are better adapted to extreme mountain conditions than their common congeners suggesting that germination may not be the key to explaining species rarity (Paulů et al. 2017). We also performed work with conservation focus in the Himalayas and suggested optimal harvesting for the survival of populations of highly used medicinal plants (Rokaya et al. 2017).

Research activity and characterization of the main scientific results

The research topics we addressed in 2015–2019 are arranged in several main themes (A–F), each characterized by the most important results. The themes reflect key aspects of the invasion process, from large-scale macroecological analyses, regional to global (A), to introduction pathways with a focus on horticulture (B), community-ecology oriented research (C), and the role of functional traits in invasions (D). Two topics are practically oriented – impacts of biological invasions (E), and research related to management and policy (F). Additional topics that form coherent distinct items in our research (theoretical frameworks and conceptual papers; case studies; animal invasions) are embedded within respective themes to which they belong, and so are discussions within the scientific community on controversial topics (Hulme et al., PNAS 2015; Wilson et al., NeoBiota 2016; Gordon et al., Inv PI Sci Manage 2016; Ricciardi et al., Trends Ecol Evol 2017; Pauchard et al., PLoS Biol 2018; Peterson et al., Divers Distrib 2019; Kehoe et al., Science 2019).

Note on INV team's share on papers resulting from cooperation: In general terms, our contribution to particular themes was in the majority of cases important and can be inferred from the authorship. The majority of our outputs originate from cooperation with other teams, mostly international but also with other Czech institutions (see 'Cooperation within international research area' section). On many multiauthored papers, we were part of the core team and if not being the first or last author, we are part of the front group of authors. In particular, we played a key role in studies related to the GloNAF database that P. Pyšek and J. Pergl have built with colleagues from abroad.

A. BIG PICTURE: MACROECOLOGICAL DRIVERS OF BIOLOGICAL INVASIONS

A1. Global exchange and continental accumulation of non-native plants. In 2015, we published the first comprehensive analysis of the global accumulation and historical exchange of alien plant species based on a unique database of naturalized alien plant species in over 1,000 world regions, including ~400 islands (Global Naturalized Alien Flora – GloNAF). Currently, ~14,000 plant species, corresponding to ~4% of the extant vascular flora, are naturalized somewhere on the globe as a result of human activity. Continents in the northern hemisphere are the major donors of naturalized alien species to southern continents (van Kleunen et al., Nature 2015, Seebens et al. Glob Change Biol 2015). The GloNAF database was being built by eight core team members since 2011 and used in ~25 papers published in 2015–2019. Among them is the first comprehensive analysis of the naturalized alien flora of the world (Pyšek et al., Preslia 2017). Since 2019, GloNAF is freely available after being released in a data paper (van Kleunen et al., Ecology 2019). Associated with collecting data for GloNAF were collaborations in understudied regions that so far yielded comprehensive, updated catalogues of naturalized and/or invasive species for India (Inderjit et al. 2018), Ghana (Ansong et al. 2018), Russia (Vinogradova et al. 2018) and Turkey (Uludag et al., NeoBiota 2017).

A2. Accumulation of alien plant and animal species creates global invasion hotspots across biota. One trend in invasion biology since the last decade is the search for generalities across taxa. GloNAF database provided plant data for research comparing invasion patterns of nine taxonomic groups, including also invertebrates and vertebrates, and showed that across such an unprecedented range of taxa, coastal areas and islands are at the highest risk of biological invasions (Dawson et al., Nature Ecol Evol 2017). This is especially warning since the dynamics

of introduction over the last centuries of plant and animal species that have subsequently become naturalized show no signs of saturation, or slowing down (Seebens et al., *Nature Comm* 2017). A European-wide analysis of the rates of alien insects spread indicated that the political changes following the collapse of the Iron Curtain in 1989, and the further dismantling of customs checkpoints within an enlarged European Union speeded up the spread of alien insects (Roques et al., *Biol Inv* 2016).

A3. Changes in species richness in the Anthropocene: from theoretical concepts to global inventories. Biological invasions are a defining feature of the Anthropocene, but the factors that determine the spatially uneven distribution of alien plant species vary among taxa. The first global analysis of the effects of biogeographic factors, the physical environment, and human influences on the richness of naturalized and invasive alien plants showed that socioeconomic factors were more important in explaining the invasion than naturalization. Islands have more naturalized and invasive species than mainland regions, and 26% of all islands harbour more naturalized aliens than native plants (Essl et al., *AoB Plants* 2019). A separate analysis revealed that North and South America might face contrasting challenges in terms of potential threats to biodiversity posed by alien plant species, because of the different past and present dynamics of invasions and predictions of future development. Since considerable increases in naturalized plant numbers are expected in the next 20 years for emerging South American economies, the present state of North America being more invaded can be reversed (Pyšek et al., *Glob Ecol Biogeogr* 2019). Introducing a novel theoretical concept, we argued that it is important to distinguish species for which human-induced environmental changes are important indirect drivers of range expansion into new regions and that such species will become an essential feature for biodiversity management and science in the Anthropocene (Essl et al., *BioScience* 2019). The fact that invasions by different taxa are often driven by specific mechanisms opens discussion about current colonization processes and where is the dividing line between natural and human-caused changes in distribution. This can be illustrated by the beginning of the golden jackal establishment in central Europe; the attitude towards this species in EU countries varies (Pyšková et al., *ZooKeys* 2016).

A4. Naturalization of central-European plants in North America results from the interplay of habitat legacy, propagule pressure, and species traits. Naturalization of central European plants in North America most strongly depends on the time since the species' introduction and on the habitat niche it occupies in its native range (Pyšek et al., *Ecology* 2015, see also Kalusová et al., *PNAS* 2017). Several biological traits contribute to the invasion success indirectly, via their effect on the number of native range habitats occupied and on the probability it is cultivated in the native range, but the importance of the biological traits is an order of magnitude less than that of the above main factors (Pyšek et al., *Ecology* 2015). The worst plant invaders recruit from habitats in their native range that occupy large areas, such as forest, grassland, and riparian (Hejda et al., *Divers Distrib* 2015). In another paper, we highlight that by including information on species traits that are usually not available for multispecies analyses, such as quantitative data on reproductive output, we can increase the explanatory power of our models (Moravcová et al., *PLoS One* 2015).

A5. Remoteness promotes the worldwide invasion of islands. One of the best-known general patterns in island biogeography is a decrease in the number of native species with increasing island isolation due to lower rates of natural dispersal to remote oceanic islands. However, the anthropogenic introduction of alien species altered the composition and richness of island species pools. Using a large dataset for alien and native plants, ants, reptiles, mammals, and birds on 257 (sub)tropical islands,

we revealed that the number of naturalized alien species increases with isolation for all taxa but birds, a pattern that is opposite to native species. The reversal of the species–isolation relationship for alien species is due to reduced diversity and increased ecological naiveté of native biota on more remote islands (Moser et al., PNAS 2018).

B. PATHWAYS OF PLANT INVASIONS IN EUROPE

B1. Horticulture as a driver of plant invasions. Horticulture is the most important pathway for the introduction of alien plants (van Kleunen et al., Biol Rev 2018). We estimated the direct effects of geographical origin and biological traits on naturalization, and their indirect effects mediated by year and/or frequency of introduction. The number of botanical gardens where a species is planted increased naturalization success directly, while most species traits had indirect effects on naturalization – failure to recognize such introduction biases could thus impair our ability to explain the success of alien plants (Maurel et al., Glob Ecol Biogeogr 2016). In another paper, using quantitative data about the pool of species planted in private gardens in the Czech Republic, we quantified so-called propagule pressure; almost 80% of the 1842 recorded taxa are aliens from which future invaders can recruit (Pergl et al., Preslia 2016).

B2. Will climate change boost the naturalization of garden plants? We identified 165 ornamental plant species that are not yet naturalized but under the current climate would find suitable conditions in more than 5% of Europe. Although climate change substantially increases the potential range of many species, some will lose climatically suitable areas, particularly those native to boreal and Mediterranean biomes (Dullinger et al., Glob Ecol Biogeogr 2017). While suitable ranges of garden plants will on the average increase, those of their native congeners will remain constant or shrink; hybrids between potential future invaders and resident species will thus not emerge more frequently in Europe as the climate warms. This makes the risk of genetic erosion and generation of new invaders unlikely (Klonner et al., Divers Distrib 2017).

C. INVASIONS INTO PLANT COMMUNITIES

C1. Alien plants invade communities that are phylogenetically clustered. We asked whether the phylogenetic structure of extant plant species pools is related to the availability of corresponding habitats in the geological history, and found that the effect of habitat was strong (Lososová et al., J Veg Sci 2015). In another study, we show that phylogenetic relatedness of invaders to native species promotes invasion because of their shared adaptations to the same environments. Alien species more strongly invade community types that are phylogenetically clustered, and because they tend to be related to native species, invaded communities become even more clustered (Lososová et al., Glob Ecol Biogeogr 2015).

C2. Integration of alien species into plant communities. Alien plants generally tend to occupy phylogenetic and functional space within the range formed by the native species in a community, either by filling empty niches or by excluding natives from their existing space (Loiola et al., J Ecol 2018). However, these processes depend on the stage of the invasion process. Across six different habitat types in central Europe, naturalized non-invasive species are functionally similar to native species occurring in the same habitat type, but invasive species are different as they occupy the edge of the plant functional trait space. This pattern is driven mainly by the invasive species being on average taller than natives (Divišek et al., Nature Comm 2018). To determine the appropriate complex metrics for the integration of naturalized species into plant communities, Grime's concept of adaptive strategies (competitors, stress-tolerators,

and ruderals) proved insightful. Using a global dataset of 3,004 vascular plant species, we showed that competitiveness was positively and stress-tolerance negatively associated with both the probability of naturalization and the number of regions where the species has naturalized globally, while R-scores had positive effects on the probability of naturalization only (Guo et al., Ecol Lett 2018).

D. SPECIES FUNCTIONAL TRAITS ASSOCIATED WITH INVASION

D1. Testing the role of species traits in plant invasions: towards a new level of robustness. The Global Naturalized Alien Flora (GloNAF database) opened new ways for robust testing of the role of traits in plant invasions. We illustrated the potential of this data set by testing one of the first general predictions in plant invasion biology that plants with selfing ability are more likely to establish outside their historical range because they can reproduce from a single individual when mates or pollinators are not available. Using our global breeding-system database of 1,752 angiosperm species we indeed found that species with a high selfing ability are likely to be naturalized in more regions of the world (Razanajatovo et al., Nature Comm 2016). In another paper using the GloNAF database, niche shifts were shown to be frequent in plant invasions but evolutionary potential may not be of major importance for such shifts. Niche dynamics rather appear to be driven by changes in the realized niche without adaptive change of the fundamental climatic niche (Dellinger et al., New Phytol 2016).

D2. Small genome contributes to the invasiveness of plants. By intercontinental comparison of native and invasive populations of *Phragmites australis*, we revealed a distinct relationship between genome size and invasiveness at the intraspecific level. Relative to native populations, the European populations that successfully invaded North America have a smaller monoploid genome that is associated with plant traits favouring invasiveness (Pyšek et al., Ecology 2018). The ecology of this particular invasion has been further explored in several papers focusing on latitudinal patterns in the invaded range (Meyerson et al., Biol Invas 2016), physiology (Pyšek et al., Preslia 2019), and evolution of invasiveness (Guo et al., Ecol Evol 2018).

D3. Seed-related functional traits as drivers of plant invasion. The ability to form persistent seed banks contributes to the invasion potential of alien plants. Using the largest seed bank dataset to date that we collated (14,293 records for 2,566 species), we found that invasive species are more likely to form longer-persisting and denser seed banks than non-invasive congeners in their native range, but not in their alien range. This is the first quantitative evidence of preadaptation resulting in the formation of a persistent seed bank in invasive species (Gioria et al., Biol Inv 2019). Heteromorphic species were more likely to naturalize outside their native range than monomorphic, but both groups did not differ in the number of world regions where they became naturalized. When life history and height were included in the models, the effect of fruit heteromorphism became non-significant (Fennesi et al., Ann Bot 2019).

D4. Mycorrhizal fungi and other symbionts as drivers of plant geography, and implications for invasions. Island biogeography has traditionally focused more on abiotic drivers of colonization, extinction, and speciation, than on biotic drivers, such as the absence of symbionts. We provided evidence for a mycorrhizal filter, with mycorrhizal associations being less common among native island plants than native mainland plants (Delavaux et al., Nature Ecol Evol 2019). Mycorrhizal plants are more likely to be naturalized and naturalize to a greater extent than non-mycorrhizal plants, and those with facultative mycorrhizal associations are more successful than those with obligate mycorrhizal associations (Pyšek et al., Ecosphere 2019). As to other

symbionts, we found that the cointroduction of Australian acacias with their rhizobia is more prevalent than previously thought. A single rhizobium cointroduction event may be sufficient to facilitate the establishment of effective mutualisms in many *Acacia* species, leading to an invasion meltdown (Warrington et al., J Biogeogr 2019).

E. IMPACTS OF BIOLOGICAL INVASIONS

E1. Impact scoring as a basis for the management of invasive plants. We conducted the first quantitative scoring of environmental and socioeconomic impacts of alien plants in Europe, using 128 species. Environmental impacts are most often manifested via competition with native species, while socioeconomic impacts are mostly on human health (Rumlerová et al., Biol Inv 2016). This approach made it possible to elaborate the first national checklist of noxious plant and animal invaders (Black List) as well as of those that may become dangerous in the future (Watch List) (Pergl et al., NeoBiota 2016). At the European scale, this issue was addressed in a horizon-scanning procedure to derive a ranked list of potential future invaders to Europe; 66 species not yet established in EU that are considered as very high (8 species), high (40 species), or medium (18 species) risk appeared on the list (Roy et al., Glob Change Biol 2019). Yet, data that cannot properly inform about impacts are still being used in the literature which leads to inappropriate analyses and misleading inferences regarding the impacts of alien species (Hulme et al., PNAS 2015).

E2. Impact of biological invasions: the origin matters. To contribute to a debate about whether alien species cause greater negative impacts than strong native dominants we compared impacts of the same species in their native and invaded ranges, and show that the dominant species suppress diversity more if they are introduced invaders (Hejda et al., J Biogeogr 2017). Another important topic is whether alien species introduced by certain pathways are more likely to have impacts. In a pioneering study, we show that plants introduced as contaminants of commodities are less likely to have an impact than those introduced by other pathways, and that arrival by multiple pathways increases the probability of impact. This supports management strategies aimed at targeting the riskiest pathways (Pergl et al., NeoBiota 2017).

F. TRANSFER OF KNOWLEDGE TO PRACTICE: MANAGEMENT AND POLICY

F1. Conflict of interests: a differentiated approach to invasive trees. European woodlands are prone to plant invasions (Wagner et al., Diversity Distrib 2017) and one invasive tree with wide-ranging impacts across trophic levels is *Robinia pseudoacacia* (Hejda et al., Diversity Distrib 2017). Besides negative environmental impacts, it also has positive economic impacts, which creates conflicts of interest between nature conservation, forestry, urban landscaping, and beekeepers (Vítková et al., For Ecol Manage 2017). This is a rather frequent situation in biological invasions where many impacts differ widely in how they are perceived by various interest groups (Essl et al., BioScience 2017). For *Robinia*, we suggested a complex management strategy that takes into account habitat, its local ability to spread, as well as economic, cultural, and biodiversity aspects. We categorized black locust stands in Europe into eight groups and proposed a stratified approach to the management based on decisions that reflect local context. Our complex management strategy provides a comprehensive guideline for the management of alien trees in Europe (Sádlo et al., NeoBiota 2017).

F2. Towards the European list of invasive aliens: how many there are out there? So-called “100 worst” lists of alien species are based on expert opinion and primarily aimed at representativeness of the taxonomic and habitat diversity rather than at quantifying the harm caused by alien species. We used the Generic Impact Scoring

System (GISS) to identify those with the highest environmental and socioeconomic impacts in Europe. The resulting list contains 149 alien species, comprising 54 plants, 49 invertebrates, 40 vertebrates, and six fungi (Nentwig et al., *Biol Inv* 2018).

During the assessed period members of INV published 174 papers (29, 23, 47, 36, and 39 in the respective years of 2015–2019) indexed in ISI WoS Core Collection, taking a leading role in 44% of them (on 54 and 23 papers as first and last authors, respectively). Compared to the previous 5-year period this represents an increase by 46 papers. A substantial proportion of those papers have been published in high-profile interdisciplinary journals such as *Proceedings of the National Academy of the USA* (5 papers), *Nature* (2), *Nature Communications* (3), *Nature Ecology and Evolution* (2), and in top ecological/plant sciences journals, e.g., *Trends in Ecology and Evolution* (4), *Ecology Letters* (2), *Global Ecology and Biogeography* (10).

The papers published by INV, over the short period of four years, received a total of 3,726 citations (as of 11 March 2020), on average 21 per item. The three most cited papers reached 296 (van Kleunen et al., *Nature* 2015), 285 (Seebens et al., *Nature Communications* 2017), and 149 citations (Kumschick et al., *BioScience* 2015). The annual citation rate of the 2015–2019 papers in 2019 was 1,590 citations. In total, 13 papers reached the status of Highly Cited on Web of Science. Another important metric of recognition of the INV research over this period is that two papers were highlighted by *Nature* (Seebens et al., *Nature Communications* 2017; Gallardo et al., *Global Change Biology* 2017), *Nature Plants Research Highlights*, and *Science News* (Seebens et al., *Global Change Biology* 2015) and two papers were selected as Editor's Choice of their respective journals (Wagner et al., *Diversity and Distributions* 2017; Pipek et al., *Ecography* 2018).

Research activity and characterisation of the main scientific results

Microclimate

After 10 years of development and testing, we published the peer-reviewed description of the new TMS microclimatic logger developed by the Department of GIS and RS in cooperation with the Czech private company TOMST (Wild et al. 2019). The TMS logger represents a substantial innovation and overcomes several major drawbacks of other available microclimatic loggers. Immediately after publication, the paper received considerable international attention and there are now thousands of TMS loggers in use all around the world.

We used several years of continuous microclimate monitoring to find that the maximum temperature is the most important temperature variable affecting plant species composition in temperate forest understories (Macek et al. 2019). Using geostatistics and modelling, we showed that topography is the main environmental driver of these temperatures and that digital terrain modelling can be used to create microclimatic grids with better predictive performance than widely used climatic grids.

We also cooperated on microclimatic research with other research groups in Europe. Using our measurements from the Czech Republic, we showed that local variation in canopy cover coupled with distance to the sea coast modifies temperature offset between forest understoreys and free-air temperature measured at standard meteorological stations (Zellweger et al. 2019). These microclimatic measurements together with our database of repeatedly observed vegetation plots allow us together with other European scientists to analyse the effects of the microclimate on the forest plant community reaction to ongoing climate change. The resulting paper was published in *Science* and three members of the Department (M. Kopecký, M. Macek, P. Petřík) are among the authors.

Finally, we joined the global research network focused on soil temperature modelling called SoilTemp (www.soiltemp.weebly.com). We have been actively participating in the SoilTemp initiative since its beginning in 2019. The Head of the Department is a member of the SoilTemp Core Group, consisting of 9 people coordinating the whole initiative. Four members of the Department were actively participating in the first SoilTemp workshop in Antwerp, Belgium, in 2019. The first publication resulting from the SoilTemp was already published in *Global Change Biology* and several others are in preparation.

Forest vegetation dynamics

We explored forest vegetation dynamics through recent decades, mostly using resurveys of historical vegetation plots.

First, to explore the robustness of the vegetation resurvey to the inherent uncertainty in the historical plot location, we performed a methodological comparison between vegetation changes on exactly and approximately relocated historical vegetation plots (Kopecký & Macek 2015). We found that the resurvey of historical vegetation plots is robust to plot-location uncertainty. This novel finding provides support for an increasingly used vegetation resurvey, which provides a unique opportunity to explore decadal changes in plant species diversity and community composition in response to global change. Since its publication, the paper has received considerable attention and it is now used as a methodological reference within the field. M. Kopecký also contributed to the important review of the vegetation resurvey studies (Kapfer et al. 2017), which has quickly become a highly cited source within the field.

Working together with the Department of Vegetation Ecology, we described patterns and explored processes driving changes in forest plant communities in the Czech Republic (e.g. Müllerová et al. 2015, Vild et al. 2018). We focused mostly on lowland broadleaved forests, but we also explored managed coniferous forests at higher elevations (Prach & Kopecký 2018).

Through collaboration within the ForestREplot network, we contributed to a number of studies exploring drivers of forest plant community changes across the temperate zone of Europe (e.g. Bernhardt-Römermann et al. 2015, Perring et al. 2018, Maes et al. 2019).

Forest succession after disturbances

To provide much-needed data about forest regeneration in response to large-scale bark beetle outbreaks, we established a network of permanent plots across the Bohemian Forest National Park. Since 2010, we have been annually monitoring the survival and growth of almost 10 000 seedlings and saplings of the Norway spruce in these plots.

In addition to these permanent plots, we performed detailed monitoring of the forest regeneration after stand-replacing disturbances caused by bark beetle outbreaks also in the Bavarian Forest NP, Germany. Using the resulting dataset based on the data about life and death of 2552 Norway spruce seedlings collected repeatedly over 12 years after the bark-beetle caused the dieback of canopy trees, we found that spruce trees were able to regenerate naturally even after the most severe bark-beetle outbreak owing to advance and particularly disturbance-related regeneration (Macek et al. 2017). Our study thus discovered the so far unrecognized importance of disturbance-related regeneration for stand recovery after bark-beetle outbreaks.

We also combined several approaches to provide novel insights into the so-far overlooked role of fire in the forest ecology of Central Europe. For example, we combined contemporary forest ecology with paleoecology to show that fire is an important driver of forest vegetation in Central Europe (Adámek et al. 2015). This novel finding challenges the traditional view that forest fires are rare and unimportant in European temperate forests, and provides a novel perspective for forest conservation and management. We further collected and analysed a unique dataset of vegetation plots covering 200 years of post-fire succession in temperate pine forests which allow us to describe the general pattern in post-fire succession and vegetation development (Adámek et al. 2016). Finally, we used geostatistics to explore drivers of forest fires across the whole Czech Republic, providing new insights not only for forest science but also for fire management and nature conservation (Adámek et al. 2018). Our paper highlighted the important role of terrain topography and tree species composition as natural drivers of forest fire ignition in temperate Europe. Together, the results published in these papers redefined the role of fire in the ecology of Central European forests. Contrary to previous beliefs, we showed that fire always has been and remains an integral part of forest dynamics in Central Europe.

Finally, we established a successful collaboration with global leaders in the research of forest disturbances and ecological modelling. Among the results of this collaboration is e.g. an important finding that climate change could markedly modify future forest disturbance regimes at the global scale and that increasing disturbance risks for forests have to be expected in the future. In particular, the indirect climate impacts, such as the alteration of tree species and the interactions between different disruptive factors were collated in a comprehensive manner (Seidl et al. 2017). Since its publication in 2017, the paper has been highly cited as it represents a knowledge

base for the whole field. Another methodologically important paper compares outputs from a wide range of currently used forest simulation models and suggests future avenues for their advancement (Bugmann et al. 2019).

Species distribution patterns

We put considerable effort into unifying information so far scattered about plant distribution in the Czech Republic. During the last five years, we have built the PLADIAS database with more than 13 million records of almost 5000 taxa of vascular plants occurring in the Czech Republic (<https://pladias.cz>) and the DALIBOR database with distributional information about bryophytes and lichens in the Czech Republic (<https://dalibor.ibot.cas.cz>).

In the PLADIAS database, we integrated vascular plant occurrence data from the Czech Republic and built up one of the largest databases of this kind globally (Wild et al. 2019). The PLADIAS database forms a basic pillar of further analyses of biodiversity based on linking the occurrence data to data on species traits and environmental conditions. It also showed the grey areas in the knowledge of plant distribution in the Czech Republic. The Department of GIS and RS was responsible for building up the database structure, programming the environment and integrating fragmented databases sources on occurrence data.

Besides major work on the PLADIAS and DALIBOR databases, we also contributed our database and GIS skills to other projects focused on large-scale patterns in species distribution. For example, we helped to assemble a global spatially-explicit mycobiome dataset and used it to describe the global distribution of fungal taxa and explore climate, soil and vegetation productivity as potential drivers of fungal distribution (Větrovský et al. 2019). We found that climate is an important driver of different aspects of fungal biogeography, including the global distribution of common fungi and the composition and diversity of fungal communities. The global fungal distribution will thus be likely affected by ongoing climate change. Besides contributing to the writing and revising of the paper, authors from the Department of GIS and RS conceived geospatial methods used in the paper, processed all geospatial data, analysed drivers of fungal distribution and performed all the multivariate analyses.

We also intensively cooperated with the Department of Functional Ecology on topics related to plant distribution in Ladakh, a Himalayan region with one of the highest known occurrences of vascular plants in the world. Besides fieldwork during demanding field expeditions, we contributed to numerous resulting papers, for example, we used 95812 species occurrence records from Ladakh to test the Asymmetric Abiotic Stress Limitation hypothesis (Dvorský et al. 2017). Our results supported predictions of the hypothesis for species upper distributional limits, but not for lower limits. This allowed us to refine the hypothesis, to describe the general increase in plant niche asymmetry with elevation and to disentangle its main drivers. We also explored drivers of the upper elevational limit of trees growing in this so far unexplored region (Doležal et al. 2019).

Remote sensing of plant invasions

We explored the so-far little-used remote sensing approaches for the detection and monitoring of invasive species. To explore the pros and cons of various remote sensing methods to detect invasive species, we performed their detailed comparison (Müllerová et al. 2017a). As such, the paper sets the basis for a new and dynamic field on the border between invasive ecology and earth sciences. In several other papers, we also showed the large potential of Unmanned Aerial Vehicles (UAV) to detect both

invasive species through remote sensing (e.g. Müllerová et al. 2017b) as the well as the potential of UAVs for environmental studies in general (e.g. Manfreda et al. 2018).

Research activity and characterisation of the main scientific results

The main research activities during the past five years involved:

- 1) exploring mechanisms of organization and functioning of plant communities in response to climate and land use change, improving thus our ability to predict the impact of environmental drivers on biodiversity and ecosystem functioning,
- 2) implementing state-of-the-art morphological, physiological and anatomical methods to identify top-down and bottom-up forces in a changing environment,
- 3) quantification of ecosystem functioning and stability and their linkage with bottom-up processes related to site-specific diversity changes and species-specific growth responses and plant adaptive mechanisms,
- 4) explaining the key growth processes (primary, secondary growth, clonal multiplication) and their inter-annual changes using climate, soil and biotic interactions for a large number of species from contrasting habitats,
- 5) considering ongoing environmental changes as selection factors that exclude certain species from communities and bring novel species to the system and promotes the evolutionary adaptation of persistent species,
- 6) combining quantitative trait ecology with an experimental approach to plant-plant interactions, addressing poorly understood issue of how biotic interactions shape plant responses to environmental change,
- 7) searching for generalities by research syntheses involving contrasting habitats from different ecosystems.

From here on, we present the key research topics of the team members and major publications, where in most cases team members are the first or last authors.

Plant clonality, bud banks, plant resprouting

We conducted a set of analyses of plant clonality distribution along environmental gradients showing that clonal and bud bank traits determine plant ecology. We supported the idea that clonal plants produce fewer seeds than other growth forms (Herben et al. 2015) and that spread by clonal growth is independent of the spread realized by the dispersal ability of seeds (Herben et al. 2016). We also analyzed how clonal growth is considered in demographic studies and recognized that it is in majority of cases either ignored or treated erroneously so that we lack reliable data about the role of clonality in the demography of plants (Janovský et al. 2017).

We analyzed the distribution of clonal and bud bank traits in vegetation types of central Europe and found out that clonal plants prevail in wetlands and alpine zone but are rare on arable land (Klimešová and Herben 2015). Moreover, clonal and bud bank traits explain the distribution of plants in central Europe better than usually used traits of leaf economic spectrum, plant height and seed mass (Klimešová et al. 2016; Vojtkó et al. 2017; Ladoceur et al. 2019). This is probably because herbaceous plants are prevailing in central European flora and for them, clonality is more important than for trees.

As clonality may serve as insurance for failed sexual reproduction we tested its role in the evolution of polyploids and supported the idea that clonality enabled the successful establishment of polyploids (Herben et al. 2017). We also confirmed the hypothesized positive role of disturbance in the evolution of root sprouting plants (Klimešová et al. 2017). We assessed the distribution of plant traits including clonal and bud bank traits along disturbance gradients that we newly defined for the

vegetation of central Europe. We confirmed that disturbance frequency and severity play a large role in structuring flora and vegetation (Herben et al. 2018a,b). Although clonal plants are decreasing in their occurrence in severely and frequently disturbed habitats, those few who are adapted for such conditions are characterized by long lateral spread and high multiplication rate. To highlight the relevance and importance of coarse belowground organs for different ecological questions, we published several forum pieces (Ottaviani et al. 2017; Klimešová et al. 2018, 2018) and we also have been building an international network of experts working with clonal, bud bank and storage organs and traits. The first publication from this network are standardized methods for selected overlooked traits like bud bank size and location, clonal traits and clonal organs, carbohydrate storage and anatomical structure (Klimešová et al. 2019). We also published a book synthesizing plant architecture for the temperate herbs and providing examples of belowground organs for central European flora (Klimešová 2018).

We performed a set of experiments focused on the role of root sprouting in plant life history. We show that severe disturbance causes slight postponement of senescence in potentially monocarpic herbs (Martínková et al. 2016). We also tested the effect of ploidy level, soil origin and nutrient availability on resprouting of *Knautia arvensis* after disturbance and revealed that ruderal taxa spreading over Europe are not more adapted to disturbance than serpentine specialists (Martínková et al. 2015). For weedy non-clonal species capable to survive root fragmentation and resprout we introduced the term „enforced clonality“. We evaluated an overall fitness of such plants including sexual and clonal reproduction and confirmed fitness advantage of this combined strategy (Martínková and Klimešová 2016). We confirmed the idea that there is transgenerational plasticity in the response of root sprouting herb to disturbance and that descendants of plants that experienced disturbance are better at tolerating damage and it depends on the habitat of origin (Malíková et al. 2016). In a comparative study with numerous root sprouting clonal plants, we did not support the hypothesis that root sprouting clonal plants are able to forage by ramets for nutrients in the heterogeneous soil environment (Martínková et al. 2018).

Tree growth responses to climate change: dendrochronology

The Dendrochronological laboratory is mainly dealing with the impact of 1) increasing number of extreme events on tree growth, 2) changes in forest management or 3) climate changes. We focused on the implementation of modern approaches of tree-ring analyses as isotopic analysis, densitometry, blue intensity analysis or quantitative wood anatomy. We used the tree-ring-based approach for determination of past tropical cyclone (TC) variability in northeast Asia (Altman et al. 2017). Our results indicate a significant increase in TC activity in the northern latitudes over the past century. In addition, we focused on the impact of TCs on long-term forest dynamics in northern Japan to link spatial and temporal disturbance history with tree regeneration and diversity. We identified large-scale disturbances reducing tree recruitment and diversity by promoting the spread of dwarf bamboos in the forest understories. We also performed two dendroclimatological studies in the Himalayas. We found that the combination of winter frost, summer floods, and strong summer diurnal temperature fluctuations control annual and intra-annual growth dynamics (Dolezal et al. 2016). The relative importance of these drivers is, however, changing with ongoing climate change. We investigated the climatic growth factors of three conifers along an elevational gradient (Sohar et al. 2017). We found that all three species were sensitive to pre-monsoon temperature, and therefore the continuous increase in temperature

and evapotranspiration, which in combination with the decrease in precipitation possibly lead to enhanced drought stress and reduced growth in the region. We also performed four studies to determine the impact of climate changes and acid depositions on the growth of *Picea abies* in the whole Czech Republic (Altman et al. 2017; Cienciala et al. 2016, 2018; Tumajer et al. 2017). We found that at lower elevations, trees are limited by water deficit, whereas direct temperature limitations concern the higher elevations. Similarly, Dolezal et al. (2016) identified the decline of oak induced by mistletoe, competition and climate change in the Czech Republic.

Functional ecology of alpine plants

We conducted several studies in Western Himalayas to find out how alpine plants have adapted to harsh conditions and how they responded to ongoing climate change. We found that alpine plant responses to recent climate warming are modulated by biotic interactions (Dolezal et al. 2019a), abiotic constraints (Dolezal et al. 2016) and anatomical, physiological and morphological trait adaptations (Dolezal et al. 2018). We found that rapid warming in the Himalayas increases plants upper distributional limits, vegetation cover and abundance of species adapted to a warmer climate (Dolezal et al. 2016). However, individual species responses to ongoing climate change are not unidirectional upward range shifts but rather multi-dimensional, species-specific and spatially variable (Liancourt et al. 2017). We revealed that the alpine tree line in the arid Himalayas is determined by sink (growth) and not source (photosynthesis) limitation (Dolezal et al. 2019a) and that climate warming may push tree limit upward via enhanced growth. We also discovered that alpine cushion plants, dominant pioneers of subnival zones, are threatened by warming and expansion of competitively strong graminoids and herbaceous perennials from alpine grasslands (Dolezal et al. 2019b). We also explored interactions between soil phototrophs and plants in High Himalayas to find out that expanding vascular plants directly reduce cover and diversity of soil cyanobacterial communities and their biological soil crusts and indirectly reduce them through modification of soil chemistry (Rehakova et al. 2017). We also explored how plants ability to survive drought and cold is related to anatomical adaptations. We found that Himalayan grasses and sedges have evolved small water transporting conduits and large storage parenchyma tissues at colder or drier places to cope with freezing- and drought-induced damages (Dolezal et al. 2019c). We also found that Himalayan alpine plants form symbiotic relationships with arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) up to 5800 and 6150 m asl, respectively, with the highest AMF in mid-elevation steppe and alpine vegetation (Kotilinek et al. 2017).

Assembly of plant communities

The field of trait and phylogeny-based community assembly is quickly progressing. We have contributed to critically reassessing the concepts and pointing out caveats and gaps (de Bello et al. 2015, Hortal et al. 2015, Shipley et al. 2016). Another important contribution has been made by developing and testing statistical methods based on trait probability densities, which allow for the inclusion of intra-specific trait variability and the decomposition of diversity across different scales (Carmona et al. 2016). In studies of community assembly, both traits and phylogeny are used to quantify ecological and evolutionary differentiation between species. However, how to meaningfully combine these two types of information is still an open question to which we have proposed a new answer by suggesting a promising method that enables to estimate the ‘decoupled’ and ‘joined’ contributions of functional and phylogenetic

dissimilarities (de Bello et al. 2017). Also crucial in community assembly is the use of null models, which provide a reference point to establish if community parameters like functional or phylogenetic diversity are deviating from random patterns. We conducted an evaluation of commonly used null models, providing guidelines for the use in trait-based community assembly studies (Götzenberger et al. 2016).

Ecophysiological research of peatland bryophytes and aquatic carnivorous plants

Alkaline fens are rare but valuable peatland habitats, which are directly endangered by expansion of acidophilic *Sphagnum* mosses triggered by landscape nitrogen and potassium eutrophication (Hájek et al. 2015). This phenomenon was observed more frequently in fens in the agricultural landscape of Bohemian Massif than in fens of Slovak Western Carpathians (Plesková et al. 2016). Laboratory moss cultivation revealed that potassium alleviates the toxic effect of calcium in acidophilic *Sphagnum* species, while the basophilic (calcium-tolerant) mosses avoid the toxicity thanks to the physiological mechanism at plasmalemma (Vicharová et al. 2015). We focused on *Sphagnum warnstorffii*, which is one of the few sphagnum species with partial calcium tolerance (Vicharová et al. 2015). Although it is widespread across Northern hemisphere it has small genetic variability referring to its high dispersal ability (Mikulášková et al. 2015). Results of a 4-year field moss transplant experiment revealed that this species can establish on elevated, less alkaline but drier fen microhabitats (hummocks) and later expand, particularly when supported by humid weather conditions (Vicharová et al. 2017). The expansion results in microhabitat acidification thanks to water retention, which may facilitate the establishment of further, more acidophilic and competitively stronger species.

We have a 30-year tradition in ecophysiological research of aquatic carnivorous plants, resulting in a monograph by Ellison and Adamec (2018) dealing with all aspects of their biology, evolution, taxonomy, physiology, biochemistry, genomics, ecology, their prey and trap commensals, and conservation issues.

Ecological restoration and spontaneous succession

Traditional approaches of ecosystem restoration are based on expensive reclamation measures including the return of the topsoil on disturbed site or planting the dense tree stands. We found that this practice is not needed, and less expensive spontaneous natural processes can bring similar results in terms of the site productivity (common target of reclamation measures) and can be fairly more efficient in restoring biodiversity. We have demonstrated that spontaneous succession directs towards the natural potential vegetation (Prach et al. 2016) resulting in more diverse plant communities than technical reclamation (Šebelíková et al. 2019). This indicates the high efficiency of spontaneous processes in the restoration of natural communities. Comparison of worldwide patterns in the succession revealed that the rate of successfully ongoing succession increases with latitude (Prach et al. 2019). The potential of spontaneous succession to restore the ecosystems is, therefore, higher in temperate regions than in e.g. tropical regions, where large human intervention in ecosystem restoration will be needed.

On the experimental level, we documented the high importance of less obvious belowground interactions in the restoration of the postmining site. For instance, an experiment manipulating the aboveground and belowground competition between the tree *Salix caprea* and the understory of its stand on postmining site indicated that while

aboveground competition for light shifts species composition of the understory, belowground composition is essential for understory productivity (Mudrák et al. 2016). We have also highlighted the importance of earthworms in plant primary succession. While in the early successional stages earthworms create persistent soil structures and affect plants through a number of other more direct effects, e.g. nutrient mobilisation, in late successional stages soil structures are already created and earthworms affect plants predominantly only via these direct effects. The experiment also indicated that competitively strong species profit from earthworm presence, which fastens species replacement during the succession (Mudrák et al. 2018). Both these experiments indicated that complex processes start spontaneously in the soil of postmining sites without the need for expensive human intervention.

Another important research activity concerns the restoration of species-rich grasslands. We evaluated the results of the most extensive (>500 ha) grassland restoration in the region of Bílé Karpaty Mts., where grasslands were re-established with the use of species-rich regional seed mixture. After initial sowing, restored plant communities progress towards the target communities (Prach et al. 2015; Mudrák et al. 2017). Mainly the species with higher capacity for vegetative reproduction showed successful performance, while non-clonal species establish in a short window of opportunity after the sowing and do not spread afterwards. This result is important for the preparation of seed mixtures for grassland restoration (Albert et al. 2019; Mudrák et al. 2018).

Research activity and characterisation of the main scientific results

Origins of the diversity of Central European landscapes

The diversity of Central European landscapes has its roots in the Holocene history. To understand this complex topic, large databases of various types of information were compiled and analysed, combining palaeoecological, archaeological and historical methods aided by up-to-date modelling approaches. We focused on several topics including the Late Glacial and Holocene vegetation history of the Western Carpathians, the Pannonian-Carpathian interface and the Danube Lowland, Holocene history of montane vegetation of the Bohemian Massif, and influence of fire on vegetation during the Holocene.

One of the most significant outputs supported the hypothesis that glacial and postglacial refugia are the key to understanding the current biogeographical patterns in Central Europe. We concluded that humid and relatively warm mountains might have acted as glacial refugia of temperate forest species, while lowlands and leeward basins might have acted as postglacial refugia of steppe grasslands. Synthesis study by Jamrichová et al. (2017) compiled data from 112 pollen profiles in the Western Carpathians and adjacent areas.

Widening the perspective of palaeoecological research and focusing on the Carpathian Mountains, we brought evidence that the palaeoenvironmental history may have influenced general biogeographical trends (Hájek et al. 2016, Gálová et al. 2016). We were able to explain the recent vegetation diversity patterns and distribution of relict species (Hájková et al. 2015a, b), reconstruct the past climatic conditions (Hájková et al. 2016), and reveal the importance of anthropogenic disturbances for the long-term survival of ancient meadow communities (Hájková et al. 2018).

Using the combination of palaeoecological and archaeological approaches, we revealed significant effects of prehistoric human activities on the Holocene vegetation. Results were published in renowned archaeological and interdisciplinary journals (e.g. Kuneš et al. 2015; Kolář et al. 2016; Kolář et al. 2018). These results largely modify the previous views on the human impact in the past, emphasizing active roles in shaping the European environments for many thousands of years. This research was funded by the ERC project LONGWOOD, enabling us to set up an archaeological database of sites covering the whole Holocene in the area of Moravia (Czech Republic). At the end of the project (12/2016) we identified in the published and grey literature more than 9.000 archaeological sites with ca. 18.000 temporal and functional components.

Projects on fire history revealed the role of fire disturbances in forest dynamics in Central Europe. We reconstructed the Holocene fire history using micro-charcoal influx in sedimentary records and related them to the long-term vegetation changes. Our results provoked an increased scientific interest in fire history in the temperate zone of Europe (Adámek et al. 2015). The research on the consequences of past fire disturbances was further developed by exploring the influence of fire in the ecosystems of Bohemian sandstone areas. They have undergone a profound transformation of the soil environment and our results demonstrated a significant impact of fire on temperate forest ecosystems (Bobek et al. 2019).

Our team substantially contributed to the presented results. We conceived ideas, collected most of the data by using our expert knowledge and the Institute infrastructure and co-wrote the papers. This research was achieved through close cooperation with the Department of Botany and Zoology, Masaryk University in Brno

(Czech Republic). Fire history was studied in collaboration with the Department of GIS and Remote Sensing (IBOT).

Historical ecology of coniferous forests: two sides of the same coin

We reviewed interactions between the research fields of ecology and history (Szabó 2015 and Szabó et al. 2018), demonstrating the benefits and challenges of interdisciplinary research. Following this theoretical background, we examined one of the classic paradigms of vegetation ecology by focusing on the past distribution of conifers in Central European landscapes.

The proportion of coniferous trees was significantly higher than suggested in the map of the potential natural vegetation in the higher-elevated regions before the introduction of modern forest management (Szabó et al. 2017), as well as in the north-western edge of the Pannonian Basin, indicating the substrate-conditioned dominance of pine throughout the entire Holocene (Jamrichová et al. 2019). Two other studies from the Western Carpathians concluded that spruce and fir occurred naturally in mountain forests and fens, while its proportion can be overestimated in other habitats (Hájková et al. 2019, Wiezik et al. 2019).

The other side of the same coin is the unforeseen consequences of conifer planting in European forests. Collaborative studies with historical climatologists (Brázdil et al. 2017a, b; Brázdil 2018a, b) showed that the intensity of forest damage by windstorms has intensified since the mid-20th century. This is probably related to the intensification of forest management since the 19th century, which favoured single-species plantations of spruce and pine, sensitive to the effects of extreme winds.

The team members played significant roles in conceptual preparation and realization of the outputs. We wrote or co-wrote the papers, partly in cooperation with two departments of the Masaryk University in Brno in the framework of joint grant projects.

Changing biodiversity of temperate forest ecosystems

Global biodiversity crisis calls for a better empirical understanding of long-term changes in biodiversity and species distribution across spatial and temporal scales. Large databases of resurveyed vegetation plots are intensively used to analyse how anthropogenic factors changed biodiversity over the past decades. Our research focused on temperate forest ecosystems and covered scales from local and regional (forests in the Czech Republic) to the continental scale, the latter by collaboration exploring the international database forestREplot.

We found that the biodiversity and compositional shifts in forest plant communities resulted from the combination of three main factors: atmospheric nitrogen deposition, land use history and forest management (Bernhardt-Römermann et al. 2015, Chudomelová et al. 2017, Vild et al. 2017, Perring et al. 2018). The observed long-term biodiversity changes in temperate forests across scales therefore largely resulted from interactive effects of only a few prevailing anthropogenic factors. This finding has not only theoretical but also practical implications for the future management of European forests.

The impact of forest management changes on biodiversity was extensively studied in the context of coppicing. This traditional forest management contributed to the development of the current forest ecosystems. For the first time, evidence for the Late Middle Ages prevalence of coppicing in Central European landscapes was brought by using modelling with historical data (Szabó et al. 2015). Traditional coppicing abandonment in the 19th–20th centuries negatively affected forest vegetation

and threatened the existence of many species (Müllerová et al. 2015). Several cases of the traditional coppicing restoration in the Czech Republic and Germany were examined in this context, demonstrating a positive impact on the diversity of plant communities, but also invertebrates (Hédl et al. 2017, Roleček et al. 2017, Šipoš et al. 2017, Ewald et al. 2018). Similar positive effects on biodiversity were revealed for the modern restoration of litter raking, another once widespread traditional forest use (Vild et al. 2015, Vild et al. 2018).

In connection with this topic, we have developed conceptual and methodological standards of the long-term biodiversity dynamics research. The resulting papers were partly published in journals' special issues we organized and co-edited (Hédl et al. 2017, Kapfer et al. 2017, Verheyen et al. 2017, 2018).

Our team members have substantially contributed to this research direction. Most presented results were achieved through collection and analyses of the original field and archival data. We wrote or participated in the writing of the papers.

Ecology of aquatic and wetland ecosystems

Research on the water and wetland plant communities resulted in publication of several high profile papers involving cooperation at national and international levels (e.g. Marcé et al. 2016, Fránková et al. 2017, 2019, Šumberová & Ducháček 2017, Rutegwa et al. 2019, Seelen et al. 2019). Citizen science was an important part of this research. As national experts on the wetland flora, members of the Department participated in a large project of vascular plant species distribution mapping PLADIAS; it included also ecology and threat assessment for the vascular plant species of the Czech flora (Kaplan et al. 2015–2018). The published results will serve as a baseline for the planned distribution atlas of the Czech flora. In relation to that, two vegetation classification papers brought important methodological insights (Landucci et al. 2015a, b).

Our team members contributed significantly to this research, which was partly related to the national COST project run in 2014–2016. This project deepened the international cooperation (see NETLAKE COST Action), enabled collecting of unique data on the environment (water parameters including of several ones monitored continually with sensor systems) and biota (vascular plants, charophyta, diatoms, zooplankton and phytoplankton) of a large fishpond serving as a model locality.

Research activity and characterisation of the main scientific results

Context-dependence of mycorrhizal functioning

In a series of experiments conducted in controlled greenhouse conditions and employing isotope labelling, we examined how the availability of soil nutrients affects the mutualistic functioning of AM symbiosis. Limited availability of nitrogen (N) in the soil can turn AM symbiosis, generally beneficial for plant growth, highly disadvantageous (Püschel et al. 2016). Under low P availability, AM fungi increased phosphorus (P) nutrition and simultaneously also plant N acquisition by stimulated rhizobial N fixation. Under ample P availability, however, AM fungi were less important and competed with Rhizobacteria for the plant-assimilated carbon (Püschel et al. 2017). Continuous monitoring of both the below- and aboveground CO₂ respiration using a novel CO₂ collection system revealed that 2.3-2.9% of the plant gross photosynthetic production was allocated to the AM fungus (Slavíková et al. 2017). Even short periods of shade can have important consequences for the transfer of P from the fungus to the plant due to the "P for C" basis of mycorrhiza functioning (Konvalinková et al. 2015). All studies were performed in long-term, equal-share collaboration with members of the Laboratory of Fungal Biology (Institute of Microbiology of the Czech Academy of Sciences). Our team had a major contribution, including full responsibility for the experiments and manuscript preparation in the former two articles, and minor contribution in participating at the experiment design and commenting on manuscript drafts in the two latter articles.

To assess the natural variability in mycorrhiza functioning, isotopic signatures of arbuscular mycorrhizae were analyzed also in collaboration with the University of Basel (Switzerland). Natural ¹³C/¹²C and ¹⁵N/¹⁴N ratios in the tissues of C3 and C4 plants, as well as in the intraradical and extraradical structures of a range of phylogenetically dispersed AM fungal strains revealed specific isotopic abundances in each AM fungus/plant interaction (Courty et al. 2015). We were fully responsible for the experiment and contributed to manuscript writing, our colleagues developed the study concept and conducted the isotopic analyses.

We also explored some practical aspects of mycorrhiza functioning. We described significant stimulation of the synthesis of a wide range of essential oils (EO) by arbuscular mycorrhiza in two commercially important aromatic plants, coriander and dill. The effect was species-specific and dependent on the pool of AM fungi present in the soil (Rydlová et al. 2016). This study was conducted in close cooperation with colleagues from the Department of Gene Bank (Crop Research Institute, Olomouc), who were responsible for the garden experiment and conducted the EO analyses.

We had a major role in testing the application potential of AM fungi in the protection of a rare plant species in its natural habitat. For the first time, the occurrence of AM fungi and their effect on the growth of wild cyclamens (endemic *Cyclamen purpurascens* subsp. *immaculatum*) were examined (Rydlová et al. 2015). AM significantly increased plant fitness and should be considered in *Cyclamen* conservation programs.

Role of AM fungi in plant stress alleviation

The role of mycorrhiza in alleviating a wide range of abiotic stresses has been our traditional research topic. We conducted a few studies – often on the ground of invitation to collaboration from researchers abroad – directly comparing stress tolerance of mycorrhizal and non-mycorrhizal plants in specific contexts. Testing the

effect of AM on cold stress resistance of cucumber revealed that AM fungi continue supplying the host plant with P under cold stress and mycorrhiza alleviates the impact of cold stress on the plant photosynthetic apparatus (Ma et al. 2015, Ma et al. 2019a). This study was conducted in collaboration with the Institute of Vegetables and Flowers of the Chinese Academy of Agricultural Sciences, our team member participated in designing the experiments, data analysis and manuscript writing.

Another study explored the effect of AM symbiosis on the heavy metal (HM) uptake and tolerance by plants potentially usable for phytoremediation (Mnasri et al. 2017). It was conducted during an internship of a PhD student from Centre de Biotechnologies de la Technopole de Borj Cedria in Tunisia. Our team members significantly contributed to designing the experiment, data analysis and manuscript preparation.

The potential use of mycorrhizal inoculation in the revegetation of HM-contaminated sites was also behind a study conducted solely by the members of our department, which addressed the interaction of HM tolerance of the fungal and plant partner. Despite certain positive effects of a HM tolerant AM strain on the physiological state of its hosts, our data provide little support for inoculation as an economically feasible strategy for *A. capillaris*-based revegetation of HM contaminated sites (Doubková and Sudová 2016).

Assembly rules of soil fungal communities

Composition of fungal communities is structured by various processes, and their understanding can significantly advance our knowledge on ecosystem processes. We found that in homogenous environments, the composition of free-living fungal communities (represented by saprotrophs) is driven by stochastic processes, while vegetation drives the composition of EcM fungal communities (Bahram et al. 2016). The composition of vegetation, particularly the identity of the interacting host plant species also structures the community composition of ErM fungi (Kohout et al. 2017), AM fungi (Polme et al. 2017) and of orchid mycorrhizal fungi (Oja et al. 2015; Polme et al. 2018). Nevertheless, no differentiation of AM fungal communities was observed in coexisting cytotypes of the same plant species (Sudová et al. 2019). Besides the biotic interactions, abiotic factors associated with soil chemistry (Kohout et al. 2015) and/or soil moisture (Kohout and Tedersoo, 2017) can also significantly affect the composition of mycorrhizal fungal communities. The composition of root-associated fungal communities was shown to affect the fate of decaying plant roots, which can lead to shifts in carbon cycling in ecosystems (Kohout et al. 2018). In five of the nine cited articles, our team members were mainly responsible for the study design, data analysis and text writing; our collaborators contributed to the design of the study and data analysis, and/or commented on the manuscript. In four other studies, our team members were involved in the collection and analysis of the data and contributed to manuscript writing.

Factors affecting the composition of AM fungal communities were also studied in the model conditions of greenhouse experiments. We utilized and further developed our methodical know-how for exact quantification of AM fungal species within communities by quantitative PCR (Janoušková et al. 2015, Voříšková et al. 2017), which enabled us to monitor the composition of synthetic or real AM fungal communities. In one study, we described the suppressive effect of inoculation on root colonization by native soil fungi and documented, for the first time, horizontal spread of an inoculant between root systems (Janoušková et al. 2017). In a series of experiments, we related the composition of AM fungal communities to the functioning of the mycorrhiza at the continuum between mutualism and parasitism, thus

interconnecting our research lines of community ecology and mycorrhizal physiology. The first published results show that spontaneously established AM fungal communities are highly stable against changes in mycorrhiza functioning, being their compositions mainly determined by competitive relationships of the fungal community members (Voříšková et al. 2019). This research line is being developed in collaboration with Laboratory of Fungal Biology, Institute of Microbiology, thus utilizing the synergy of the excellent cultivation facilities and molecular methods know-how of the IBOT team, and facilities for physiological measurements of our partners.

The development of fungal communities in plant succession

Our research comprised both primary and secondary succession. The development of mycorrhizal and other root-associated fungi over the course of primary succession was studied on a unique model of coal-mine spoil bank composed of a diverse mosaic of differently aged and managed sites. Successional patterns within communities of AM, EcM and plant pathogenic fungi were more related to plant community succession than to shifts in soil chemistry. In contrast, variation in the communities of root endophytes remained largely unexplained by the measured environmental variables (Kolaříková et al. 2017; Krüger et al. 2017). A clear asymmetric competitive relationship between AM and EcM was evidenced during the successional shift of vegetation cover, as an EcM host decreased the abundance of AM fungi, while the effect was negligible in the opposite direction (Knoblochová et al. 2017). In a field experiment, we found a similar impact of belowground competition between EcM and AM plants on the biomass of AM understory and AM fungal infectivity (Mudrák et al. 2016). Interestingly, such asymmetric competition between AM and EcM hosts can be considerably moderated by the activity of earthworms (Frouz et al. 2019). In controlled greenhouse experiments, we found, e.g., that mycorrhizal growth response of *C. epigejos* was closely related to soil N-P ratios that undergo distinct changes along the succession (Rydlová et al. 2016), or that certain fungal taxa are stimulated by soil mesofauna, leading to shifts in the fungal community composition (Janoušková et al. 2018).

Our team members were fully responsible for the whole research in five of the seven cited articles. Collaborators from the Institute for the Environmental Studies (Charles University, Prague) provided us with access to and detailed information on the experimental sites. In one study (Frouz et al. 2019), our team members conducted the experiment and contributed to writing the manuscript. Our team member partly collaborated on one article (Mudrák et al. 2016). Further, we also contributed to similarly focused studies conducted in other laboratories, namely: Harantová et al. (2017) from the Laboratory of Environmental Microbiology (Institute of Microbiology) and Krüger et al. (2015), focusing on a retrogressive >2-million-year old dune chronosequence in Australia (University of Western Australia, Perth).

The role of mycorrhizal fungi in secondary succession on abandoned fields was addressed in close collaboration with the Department of Population Ecology. We found differences in the mycorrhizal responsiveness between grassland specialist and generalist plant species, as well as differences in infectivity between the AM fungal communities of undisturbed dry grasslands and abandoned fields. However, persistent changes in AM fungal community composition do not seem a reason for the inability of some specialist plants to colonize abandoned fields (Voříšková et al. 2016, Pánková et al. 2018, Lepinay et al. 2019). Our contribution consisted in analyzing the mycorrhizal parameters and AM fungal community composition, drafting one publication and commenting on the drafts of the other two.

Fungal partners of ericoid mycorrhizal plants

Despite the co-dominance of ErM plants in boreal and subarctic ecosystems, ErM is investigated by only a few research groups worldwide. In our department, it represents a well-established line of research.

We examined the colonization potential of dark septate endophytic (DSE) fungi in Ericaceae roots and found that although some of them form structures resembling ErM, they decrease the growth of the ErM host in comparison with true ERM fungi (Lukešová et al. 2015). We described the spectra of the cultivable root mycobionts of Ericaceae in NW Patagonia, Argentina (Bruzzone et al. 2015, 2017). For the first time, we reported the occurrence of the typical ErM fungus *Hyaloscypha hepaticicola* (syn. *Rhizoscyphus ericae*) in the roots of Southern Hemisphere's Ericaceae (Bruzzone et al. 2017). We provided the first experimental evidence that some members of the Serendipitaceae family (Sebacinales, Basidiomycota) form ericoid mycorrhizae (Vohník et al. 2016). A basidiomycetous mycobiont forming sheathed ericoid mycorrhiza was identified as *Kurtia argillacea* (= *Hyphoderma argillaceum*). It so far represents the only confirmed non-sebacinoid ErM fungus in the Basidiomycota and its intriguing placement among mostly saprobic and parasitic Hymenochaetales begs further investigation of its eco-physiology (Kolařík and Vohník 2018). We were involved in a breakthrough discovery that the prominent ErM fungus belongs to *Hyaloscypha* (Fehrer et al. 2019).

Members of our department were responsible for designing the studies, field and experimental work, data analyses and writing the manuscripts. In the last two studies, they were involved in designing the studies, data processing and manuscript writing.

Root-associated fungi in aquatic ecosystems

Fungi colonize not only roots of terrestrial plants, but also of numerous aquatic plants in both freshwater and marine habitats – a phenomenon that remains largely unexplored. We described an anatomically and morphologically unique association with DSE fungi in the dominant Mediterranean seagrass *Posidonia oceanica* (Vohník et al. 2015). We detected very narrow fungal communities lacking typical terrestrial root endophytes, which indicates a close symbiotic relationship between the dominating pleosporalean mycobiont and *P. oceanica* (Vohník et al. 2016, 2017). This dominant DSE root mycobiont, so far ignored from other hosts or ecosystems, was described as *Posidoniomyces atricolor* gen. et sp. nov. in Aigialaceae, Pleosporales (Vohník et al. 2019). For the first time in the seagrasses, we documented an ontogenetic substitution of dense root hair cover in seedlings by abundant fungal root colonization in adults (Borovec and Vohník 2018). We also provided new insight into the ecology, phylogeny and distribution of an overlooked phytomyxid endosymbiont of the alien seagrass *Halophila stipulacea* (Vohník et al. 2017, Kolátková et al. 2019). Members of our department had a major contribution to all these achievements: they designed the studies, conducted field work, discovered the symbiosis, analysed data and wrote the manuscripts.

Concerning the diversity of root-associated fungi in freshwater habitats, our study of fungi inhabiting isoetid roots in Norwegian freshwater lakes indicated surprisingly diverse fungal communities with a high proportion of yet undescribed taxa. A new species of AM fungus was isolated and described as *Rhizoglosum melanus* (Sudová et al. 2015). Morphological and molecular characterization of another AM fungal species isolated from isoetid habitats helped to delimit a new *Acaulospora* species (Pereira et al. 2016). Both studies were performed in collaboration with foreign specialists in AM fungal taxonomy, who were responsible for the species descriptions.

Members of our department performed field work and isolation of the AM fungi and were involved in manuscript writing and finalization.

Applied research

Members of our department were engaged also in applied research with the active participation of commercial subjects. In this type of research, patents or certified methodologies were usually the envisaged outputs, rather than scientific publications. We present our endeavour in applied research in condensed form, listing each topic, participating partner(s) and the reached outputs. Our team members either had the sole responsibility or major contribution to these studies:

- Potential of inoculation with AM and/or EcM fungi to improve the growth and survival of 13 planted tree species during forest reclamation of fly ash deposits. ČEZ, a.s. (Sýkorová et al. 2016).
- Infectivity changes of AM fungal inocula in peat-based horticulture substrates during storage under different temperature regimes. Rašelina, a.s. (Püschel et al. 2019).
- Enhancement of plant productivity in marginal and HM-contaminated soils using symbiotic microbes. University of Chemistry and Technology Prague, Ecofuel Laboratories, Rabbit Trhový Štěpánov a.s. (Schmidt et al. 2017a, 2018).
- Identification and development of innovative high-value products such as nutritional supplements, animal feed additives, novel biofertilizers, biopesticides, bioplastics and new generation biofuels from microbial, plant and animal biomass. Institute of Chemical Process Fundamentals; University of Chemistry and Technology; Ecofuel Laboratories; Rabbit Trhový Štěpánov a.s.; Agra Group a.s.; Briklis Ltd. (Bhave et al. 2017, Schmidt et al. 2017b; two patents; two certified methodologies).
- Antifungal properties of plant methanolic extract against oomycete pathogens, causal agents of late blight disease in tomato and pepper. University of Tuscia (Viterbo, Italy) and ENEA C.R. (Casaccia, Rome, Italy), (Švecová et al. 2017).

Furthermore, our team members were consultants of the following research:

- Effects of composite membrane carpets for the remediation of heavy metal polluted soils, including microbial components and zerovalent nanoiron. Nordmanns Fir Ltd. (Wu et al. 2018).
- Development of a seed-coating technology for inoculation with AMF fungi. University of Coimbra, Portugal (Oliveira et al. 2016b and 2017b, Ma et al. 2019c, Rocha et al. 2019a-e).

Research activity and characterization of the main scientific results

The scientific work for the period 2015-19 may be grouped under the following categories, although they often overlap.

1. Diversity, ecology, and taxonomy of Cyanobacteria

We continued in the description of new taxa on different taxonomical levels using the polyphasic approach, which is the combination of phylogenetical analyses together with morphological features of studied taxa and their ecological demands (Komarek et al. 2016). This combination brings unique knowledge about investigated organisms and helps us to describe Cyanobacteria in a more complex way.

1.1. Taxonomy of Chroococcales

The systematics of coccal cyanobacteria represents a challenge due to morphological convergence and the application of various taxonomic concepts. The genus *Cyanothece* is one of the most problematic. We analyzed a set of complementary genetic and phenotypic evidence to disentangle the relationships among *Cyanothece*, *Gloeothece*, and *Aphanothece*. We described new genera, *Rippkaea* and *Zehria*, to characterize two distinct phylogenetic lineages outside the known genera (Mares et al. 2019).

Detailed studies of new taxa from the order Chroococcales have been conducted and a new species *Nephrococcus serbicus* was described. Genus *Nephrococcus* has only three records in the whole world (Popovic et al. 2016). A review of genus *Eucapsis*, family Merismopediaceae, and the most related genera *Synechocystis* and *Limnococcus* was published (Komarek et al. 2016).

1.2. Taxonomy of Nostocales

Taxonomic evaluation and reclassification were conducted also in the order Nostocales at different continents.

Dominant morphotypes from the deglaciated area of the Antarctic were investigated. Eleven species of heterocytous cyanobacteria were recognized and studied in detail. These species were dominant in the studied habitats, and four novel species were described. All have an endemic Antarctic distribution (Komarek et al. 2015). Morphological, phylogenetical and eco-physiological analyses of four benthic *Anabaena* from the Czech Republic were carried out. The results confirmed deep heterogeneity within the genus *Anabaena* and pointed out a necessity to study *Anabaena* in more detail (Kust et al. 2015).

We also studied the morphological diversity and population dynamics of *Anabaenopsis* from artificial shallow ponds in Argentina. The species richness recorded in this study suggests that there is likely much more diversity remaining in this genus to be reported in Argentina (Aguilera et al. 2016).

New genus *Roholtiella* belonging to the Nostocales was described in collaboration with colleagues from John Carroll University in the USA (Bohunicka et al. 2015). Another new genus *Macrochaete* was established as a freshwater taxon sister to the freshwater *Calothrix* lineage (Berrendero Gomez et al. 2016).

1.3. Validation of scientific names

Our taxonomic work also included the validation of scientific names, when they have been introduced twice for different organisms covered by the ICN. This is the case of *Cyanospira*, which was once described as euglenoid alga and the second time as heterocytous cyanobacterium. We validated the generic name and the names of its

currently included species and proposed the conservation of *Cyanospira* G. Florenz. & al. against *Cyanospira* Chodat (Juran et al. 2015).

Other taxonomical work is neotypification and epitypification, which helped to resolve the taxonomy in polyphyletic genus *Pleurocapsa*. Strains putatively named *Pleurocapsa* were scattered throughout the orders Pleurocapsales and Chroococcales, indicating a need for a clear definition of the genus so that revisionary work and alpha-level taxonomy can move forward. To satisfy this need, *P. fuliginosa* HA4302-MV1 and *P. minor* HA4230-MV1 were chosen as neotype and epitype, respectively, establishing the genus based on molecular sequence data (Shaligyn et al. 2019).

Despite the progress in the description of new cyanobacterial taxa, problems in their taxonomy occur. One of the most pressing is that their taxonomy is still heavily lagging behind the progress in genome sequencing. Besides nomenclatural issues and difficulties in species identification, this problem is largely a consequence of missing reference strains and sequences for many species. The study by J. Mares (Mares 2018) presented a robust multilocus phylogenetic tree based on 23 conserved proteins from the current set of available genomes and compared to a respective 16S rRNA gene tree. The comparison supports the careful use of this gene in distinguishing cyanobacterial genera, whereas its application at other taxonomic levels is limited.

1.4. Methodical improvement of the sequencing of uncultivable taxa

We developed an optimized protocol for single cell/filament isolation and 16S rRNA gene sequencing of uncultivable taxa which highly extends the number of taxa which can be characterized by a polyphasic approach because a higher proportion of cyanobacteria is uncultivable. We tested our method on terrestrial cyanobacteria and applied it to determine the phylogenetic position of the genera *Petalonema* and *Stigonema*. We elucidated the phylogenetic relation of genus *Petalonema*, which was in the past classified as heteropolar nostocalean cyanobacteria, but newly belongs to the family Scytonemataceae (Mares et al. 2015).

1.5. Ecological studies of cyanobacteria

Ecological studies about cyanobacteria from various habitats were conducted. The results highlight the ecological demands of single taxa and their importance in the ecosystems. For example, the cyanobacteria from soil crust stabilize soil, decrease soil erosion and improve water retention in the soil. These are important features, which can be used in combating the drought associated with global climate changes. Also, these studies can show us the potential candidate with specific ecological demands and usually with the production of specific substances such as fatty acids interesting for bioprospection.

A review about terrestrial cyanobacteria brings complex information about biodiversity of aerial and subaerial cyanobacterial assemblages, mainly in Europe, and identifies some of the main knowledge gaps in this field of study. The summary could be used by wider scientific communities, which try to protect buildings and important cultural heritage from deterioration caused by microalgae (Hauer et al. 2015). The ecological studies about cyanobacteria from ambient spring were conducted in the collaboration with a researcher from Italy (Cantonati et al. 2015). Attention was paid to extreme habitats such as the subnival zone of Himalaya Mts. (Capkova et al. 2016) or biological soil crusts from the Arctic (Pushkareva et al. 2015) and Antarctica (Pushkareva et al. 2018) or thermal springs in Bulgaria (Kopejtka et al. 2019).

The water-bloom species have not been overlooked and special attention was paid to *Limnorphis* which forms water-bloom in oligotrophic lakes. The factors, which could cause its proliferation were discussed (Komarkova et al. 2016). Taxa from eutrophic ecosystems were investigated together with a limnologist from Poland (Plinski M. & Komárek J. 2017) and Macedonia (Krstic et al. 2017). The obtained knowledge is important for the prediction of toxicity of water blooms and could serve to water authorities in deciding how to manage the water bodies.

1.6. Taxonomical identification keys – chapters and books

We took a significant part in preparing publications (listed below) for students and professional public, and also the non-scientific audiences who deal with the determination of cyanobacteria in North America; with cyanobacteria and algae in the Czech Republic. The determination key for the Czech Republic cyanobacteria and algae can be used as an identification key for the taxa from central Europe. These books can also be useful for water quality and water management authorities.

- Updated chapters on coccoid Cyanobacteria and filamentous Cyanobacteria for the second edition of the “Freshwater Algae of North America” reflecting new findings and taxonomic changes (Komarek and Johansen 2015).
- Chapters Cyanobacteria and Biofilm in Encyclopaedia of Astrobiology (Kviderova 2015).
- Catalog of microscopic organisms of the Everglades, Part 1—The cyanobacteria (in collaboration with Caribbean Florida Water Science Center, Rosen and Mares 2016).
- Determination keys of Czech cyanobacteria and algae (in collaboration with University of South Bohemia, Kastovsky et al. 2018a, b).

2. Diversity, ecology and taxonomy of eukaryotic algae

2.1. Taxonomy and ecology of Diatoms

Diatom research was mainly done in the Polar Regions, where the knowledge about their taxonomy is still insufficient and where global climate change is most pronounced and causes the loss of original ecosystems. The diatom community of cryoconite holes was investigated at Svalbard. The results revealed similar or greater average genus-richness than adjacent freshwater habitats. Overall, cryoconite hole diatom communities differed significantly from those observed in lakes, suggesting that other sources actively contribute to these communities (Vinsova et al. 2015).

Taxonomic revision of the freshwater and limno-terrestrial diatom flora of the Maritime Antarctic Region which has been started in the previous five years continues. The new genus *Humidophila* was established during taxonomic revision (Kopalova et al. 2015). Unknown taxa belonging to the genus *Nitzschia* were also found. Microscopical observations allowed their separation from the known taxa and resulted in the description of eight new species (Hamsher et al. 2016). Furthermore, four taxa belonging to *Achnanthes*, *Psammothidium* and *Planothidium* were found and new species were described (Kopalova et al. 2016). New aerophilic diatoms from the genus *Hantzschia* were described in collaboration with colleagues from Belgium (Bulinova et al. 2018). *Humidophila*, *Nitzschia*, *Hantzschia* are genera present worldwide, but a large number of *described* taxa have a restricted distribution within the Antarctic, showing clear bioregionalism. Iconographia Diatomologica, Volume 24: Diatoms from the

Antarctic Region, Maritime Antarctica was finished with the contribution of our scientist K. Kopalova (Zidarova et al. 2016).

A number of centric diatoms were described under the supervision by V. Houk in cooperation with colleagues from different parts of the world. A new centric diatom, *Aulacoseira kruegeriana*, was discovered from high-elevation Andean streams (Morales et al. 2015) and a new centric diatom *Cyclotella longirimoportulata* was described from Bolivia (Morales et al. 2018). *Cyclotella hinziae* was described from a subalpine lake Schliersee, Germany (Houk et al. 2015). Large, subfossil populations of an unknown centric, planktonic diatom were observed in a lake sediment core from Lake Kinneret (Israel), which was described as *Cyclotella paleo-ocellata* sp. nov. (Vossel et al. 2015). Another new taxon for science is *Angusticopula rowlingiana*, a melosiroid diatom from Ascension Island in the South Atlantic Ocean (de Vijver et al. 2019).

The ecophysiological study of tolerance to the freezing of vegetative and resting cells of diatoms from the polar and temperate zone was conducted. The results were surprising because no differences in growth recovery had been observed between polar and temperate taxa (Hejdukova et al. 2019).

2.2. Taxonomy and ecology of Chlorophyta

Exploration of biodiversity of Antarctica continued in class Chlorophyta at James Ross. The identity and ecology of green planktic algae from ice-covered lakes were studied. The dominant taxon *Monoraphidium* was identified and waits for the description as a new taxon. It is a psychrotolerant taxon with a high content of polyunsaturated fatty acids. This feature makes these isolates interesting candidates for biotechnological applications (Nedbalova et al. 2016, Rezanka et al. 2017). Attention was paid to other parts of Antarctica as well, and an inventory of bacterial and eukaryotic diversity was done in various habitats. A relatively large amount of sequences new to science was detected, which underscores the need for additional biodiversity assessments in Antarctic inland locations (Obbeis et al. 2016). A taxonomical study was conducted in another cold place too, at Svalbard. New psychrotolerant algae, *Chloromonas svalbardensis* and *Ch. arctica* were established. Our results demonstrated the paraphyletic origin of *Chloromonas* within Chloromonadinia with genetically, morphologically and ecologically well-defined clades. We suggested a scenario of a possible *Chloromonas* split and revision (Barcyte et al. 2018a, 2018b).

Another publication was devoted to the distribution of *Chlorella*- and *Stichococcus*-like species, which has so far been inferred only from morphology. We contributed a phylogenetic analysis of an expanded SSU and ITS2 DNA sequence representing *Chlorella*- and *Stichococcus*-like species from terrestrial habitats of polar, temperate and tropical regions. Our data suggest that terrestrial *Chlorella* and *Stichococcus* might be capable of intercontinental dispersal; however, their actual distributions exhibit biogeographical patterns (Hodac et al. 2016).

2.3. Cryoseston algae

Algal flora of cryoseston was studied on snow fields in various countries. Cryoseston algae and microfungi were recorded, for the first time, on Mt. Olympus, Greece. In total 16 species were recorded, some of them rare in cryoseston and *Xenococcus kernerii* was new record for cryoseston (Cepak et al. 2016). The investigation of cryoseston continued in collaboration with colleagues from Switzerland and Austria.

Spherical cysts of Chlamydomonadales sampled from mountains in Europe, N- and S- America and both Polar Regions were investigated. Molecular analyses

revealed the presence of an independent lineage within the Chlamydomonadales. The genus *Sanguina* was described, with *Sanguina nivaloides* as its type. The cosmopolitan distribution with an absence of geographical structuring was recorded, indicating an effective dispersal with the cysts being transported all around the globe, including trans-equatorially (Prochazkova et al. 2019).

2.4. Taxonomy and ecology of Euglenophyta

A newly studied class of eukaryotic algae at our Institute is Euglenophyta under the supervision of J. Juran. The rare euglenoid taxa *Trachelomonas bituricensis* var. *lotharingia* and *Trachelomonas saccasii* were discovered in a small mesotrophic pond in the Czech Republic. The work provides new information about the worldwide distribution of these taxa and their ecology (Juran 2016, Juran and Coute 2018).

An extremely high diversity of Euglenophyta was recorded in Poland (Poniewozik and Juran 2018). J. Juran proposed compiling the Red list of microscopic algae of the Czech Republic as a starting point for the evaluation of microalgal diversity. It should highlight their importance for ecosystems and contribute to the protection of their habitats (Juran and Kastovsky 2019).

2.5. Taxonomy and ecology of Eustigmatophyceae

The study on a higher taxonomical level was conducted in collaboration with colleagues from the University of Ostrava, using Eustigmatophyceae members as model organisms. The phylogenomic analyses of a concatenated protein revealed that eustigmatophytes form a clade with Chrysophytas, exhibiting an unexpectedly elevated rate of plastid gene evolution. Our analyses also indicate that the root of the ochrophyte phylogeny falls between the recently redefined Khakista and Phaeista assemblages (Sevcikova et al. 2015).

Eustigmatophyceae prepared other surprising for scientists and show up the interaction on the molecular level (gene transfer event) between Eustigmatophyte and a novel lineage of endosymbiotic bacteria (Yurchenko et al. 2018).

The Handbook of protists was issued in 2017. One chapter is about the overlooked class Eustigmatophyceae are a distinct lineage of ochrophyte (stramenopile) algae with a relatively small number (~30) of described species, but with evidence for a substantial taxonomic diversity yet to be explored, which was written with the contribution of our worker P. Pribyl (Elias et al. 2017).

3. Biotechnological utilization of algae

The group led by J. Lukavsky studied the extracellular polysaccharides, polyunsaturated fatty acids (PUFAs) and carotenoid production under various cultivation regimes and exploited a wide range of strains from various extreme habitats obtained by other team members within their taxonomical work (e.g. Nedbalova et al. 2016, Rehakova et al. 2019). The group mainly contributed to bioprospection of new potential candidates for the production of valuable substances, which could grow in less demanding growth conditions (e.g. lower temperature or light intensity) than nowadays commercially used strains.

3.1. Exopolysaccharide production

The nanostructure of exopolysaccharide (EPSs) of *Dictyosphaerium chlorelloides* was studied and it was found that the nanostructure is dependent on the concentration of EPSs (Cybulska et al. 2016). This strain was used also for the biotechnological production of exopolysaccharides and the optimal conditions for the maximal

production of EPS were determined (Kumar et al. 2017). For the production of EPSs 17 different microalgal species, not commonly used in biotechnologies, were screened with the aim to find new suitable candidates for EPS production. Dominant producers were selected for cultivation on a larger scale to obtain EPSs for detailed chemical studies and potential biological activities (Halaj et al. 2018). The other candidate from Trebuxiophyceae was screened for EPS (Cepak and Pribyl 2018).

3.2. New strain for carotenoid production

A brand-new isolate of *Scenedesmus* sp. was introduced to the international community of algal biotechnologists. This strain has a high specific growth rate and carotenoid productivity, is highly tolerant to temperatures up to 40°C, which makes it an excellent candidate for large-scale production of carotenoid-rich biomass without the need for cooling the up-stream process (Pribyl et al. 2015, 2016).

Long-chain PUFAs were recorded in other classes of microalgae in Dinophyceae and Trebuxiophyceae, Chlorophyta, and their lipidomic profile was identified (Rezanka et al. 2017a, 2017b, 2019).

3.3. Polyunsaturated fatty acids in Eustigmatophyceae

Fatty acid and PUFAs optimal concentration and ratio in the eustigmatophycean alga *Trachydiscus minutus* were investigated under the different temperatures, light regimes, sequestration of nitrates and phosphates. The optimal growth conditions were characterized to obtain the required concentration of fatty acids (Rezanka et al. 2015, Cepak et al. 2016). The experimental work focused on the effect of rare earth elements on the growth and production of valuable pigments and fatty acids in microalgal cultures. The positive effect on the production was noted only for fatty acids, pigment production did not change (Goecke et al. 2017).

3.4. Algae and nanotechnologies

The application of green synthesis in nano-science and technology is of great importance in the area of the preparation of various materials. In this work, three selected algal species were successfully used for the preparation of silver nanoparticles in collaboration with Pavol Jozef Šafárik University in Košice, Slovakia (Sedlakova-Kadukova et al. 2017). Microalgae occupy all territories and their products represent a rich source of phytochemicals for human beings. The pharmacodynamics properties of extracellular biopolymers from Cyanobacteria and Chlorophyta were tested. Biopolymers from both tested organisms revealed antitussive and bronchodilatory effects (Sutovska et al. 2017, Halaj et al. 2018, Uhliarikova et al. 2019).

3.5. Mixotrophic and heterotrophic growth of algae

We tested the ability of microalgal strains to grow under heterotrophic and mixotrophic conditions to select suitable candidates for well-controlled cultivation processes and possibly significantly higher production of the target compound (Pribyl and Cepak 2019). The ability of cultivated strains to grow heterotrophically can decrease the costs of the production of valuable compounds.

CCALA – culture collection of autotrophic organisms

CCALA collection as an integrated part of the Institute maintains nearly 950 strains from different habitats from more than 50 countries. The strains are regularly checked, documented and their molecular characterization has started. The collection is regularly extended with new isolates.

Research activity and characterisation of the main scientific results

The major scientific outcomes of our Department for 2015-2019 can be grouped into the following categories and scientific areas: i) cyanobacterial blooms and cyanotoxins, ii) ecotoxicology of nanomaterials and nanoparticles, iii) patents and research for practice.

Cyanobacterial blooms and cyanotoxins

Between 2015 and 2019, our long-term topic – the ecology and ecotoxicology of cyanobacterial water blooms was influenced by the CYANOCOST project, which saw the cooperation of 32 countries. We were active in the group of scientists focusing on preventive measures and technologies for the prevention of blooms formation and cyanotoxins removal. The international cooperation resulted for instance in the papers “Existing and emerging cyanocidal compounds: new perspectives for cyanobacterial bloom mitigation” (Matthijs et al. 2016) and “Controlling internal phosphorus loading in lakes by physical methods to reduce cyanobacterial blooms: a review” (Bormans et al. 2016), both published in the journal *Aquatic Ecology*. The issue of the journal *Aquatic Ecology* was a special issue on Cyanobacterial blooms. The invited review was based on our international cooperation with a distinguished expert from France. Together we prepared a critical review of measures which are available such as in-lake technology for internal phosphorus management. The papers clearly document our position in the international context and represent our topics as well as the fact that we are considered as partners within the international scientific community.

Most of the paper (80% to be exact) was prepared in Brno, Prof. Bormans edited and processed the paper.

Another paper delivering the scientific outputs of our team in the field of cyanobacterial toxins was published in the journal *Water Research* and is entitled “Application of passive sampling for sensitive time-integrative monitoring of cyanobacterial toxins microcystins in drinking water treatment plants” (Jaša et al. 2019). This is a groundbreaking paper in the technology of microcystin detection. We developed an original composition of sorbent, membrane and device for the passive sampling of cyanobacterial toxins. The technology is capable of documenting the dynamic of microcystin production and integrate the microcystin concentration in drinking water process, or (in) aquatic ecosystem(s). We decided to publish in the journal *Water Research* because we consider it as the best journal in this field. All of the authors of the paper are members of our Department.

Ecotoxicology of nanomaterials and nanoparticles

Several papers were published in the fields of ecotoxicology of nanomaterials and nanoparticles. We would like to mention just two in more detail.

The first is “Toxicity of graphene oxide against algae and cyanobacteria: Nanoblade-morphology-induced mechanical injury and self-protection mechanism” (Malina et al. 2019). In this paper, we were the first to document the mechanism of graphene oxide interaction with algal and cyanobacterial cells and to describe the molecular mode of action of cell defence. Papers published so far describe the strong cell toxicity of GOs. However, algae have been compromised by natural carbon nanoparticles for millions of years and were able to establish the defence mechanism. The international scientific community presumed what the defence mechanism was but we were the first to present experimental evidence. All experiments were conducted in our Department,

the first and second authors as well as corresponding authors are all members of our Department.

The other paper "Iron and iron oxide nanoparticles synthesized with green tea extract: differences in ecotoxicological profile and ability to degrade malachite green" proved that nanoparticles can be produced and stabilised in an environment-friendly manner. It also proved that a small change in the procedure can seriously change the toxicological properties and effectivity to degrade pollutants. This simple but effective idea has already been discussed at several conferences and the beneficial effect for technology and the scientific community is reflected in citations.

All experiments were conducted in our Department, the first and corresponding authors are members of our Department.

Patents and research for practice

We focus not only on papers with a high impact factor but also on the so-called social impact factor and activity. This is reflected in our cooperation with industry, in the development of methods for practical use and also in the production of novel technologies protected by patents.

We patented a new *Coccomyxa mucigena* strain of algae, useful in water purification technologies, for the production of energy-rich algae products, and for the production of significant quantities of oil and glycerol including diglycerides and triglycerides (Patent Number CZ306896, 2017). The *Coccomyxa mucigena* strain is characterized by high production of extracellular polysaccharides and especially by the type of arrangement of extracellular polysaccharides (having 3.5-7 times greater surface area than conventional extracellular polysaccharides algae coated with a continuous, spherical arrangement). It is thanks to this feature that this strain of algae can be used in the treatment of industrial wastewater to remove metals, or in the treatment of municipal wastewater to remove estrogens, pharmaceuticals and pesticides. Its function is based on the principle of enhanced sorption and partial biodegradation.

The mobile device for non-destructive fluorescence resolution, imaging and quantification of microorganisms on the surface of materials (Patent Number CZ308044, 2019) should attract special attention because it has a promising future. This patent has currently been submitted as an international patent. The system is to be used in early warning detection of organisms causing the bio-destruction of materials and can detect and quantify the bacteria, fungi, algae, cyanobacteria, lichens and mosses.

A part of our scientific activities and experience is also dedicated to the services used by water authorities and municipalities (consultancy of the measures for the prevention of cyanobacterial water blooms in Tábor and Cheb in CZ, or Košice and Prešov in Slovakia, the Quarapiranga reservoir in Sao Paulo, Brasil, the ancient reservoir in Axum, Ethiopia, or Grahamstown in South Africa). Special attention was dedicated to the cooperation with the largest Czech energy company ČEZ. We provided consultancy on the bio-destruction of concrete and plastic materials by cyanobacteria and other microorganisms in the cooling towers of all coal power plants, both nuclear power plants and all big hydroelectric power plants. The survey provided serious information which is currently used in the driven ageing of technologies and in increasing the safety of alarm systems (in the case of the bio-destruction of cable insulation materials). Our services were highly appreciated by the company's general

managers and we will continue such activities because we have excellent equipment and experts. Also, it gives us pure scientific-human satisfaction, that our expertise and advice are directly used in practice.