PREFACE

In this book, the authors discuss the ecology, biological implications and environmental impact of microcosms. Topics include using microcosms to investigate aspects of plant-bacterial interactions and bacterial evolution; multifactorial microcosm experiments to predict how plant species and assemblages respond to changes in the availability and spatio-temporal heterogeneity of resources like water, light and nutrients; development and optimization of an aquatic laboratory microcosm for ecotoxicological risk assessment; assessment on transformation of organic pollutants in microcosms; bacterial evolution in simple microcosms; ecology and environmental side-effects of pesticides in tropical microcosms; floating dish microcosms to study the developments of biofilm communities; the role of semiosis and cohesion and sustainability inside microcosms; and soil microcosms and biogeographical research.

Chapter 1 – The fast pace of scientific research often means that contemporary investigators are unaware of critical developments resulting from the work of previous generations of scientists. The authors were superficially aware of Sergei Winogradsky (1856 – 1953) through his columns, currently in teaching to illustrate aspects of microbial succession and community function, but were unaware of his importance as the founder of microbial ecology and the first to use microcosms to study bacterial physiology. Here the authors take the opportunity to remind fellow microbiologists of his work, presenting in homage columns constructed using water and sediments from the Dnipro (Dnieper) collected close to his place of birth. They then provide a review of their own research using microcosms to investigate aspects of plant-bacterial interactions, bacterial evolution, biofilm-formation, and soil colonization, as well as recent advances in developing plastic and transparent soils to investigate root development and fungal hyphae invasion in soil pore networks, to show that more than one hundred years after the invention of the microcosm, they are still being used profitably in microbial research.

Chapter 2 – An important part of ecological research has been devoted to understand and predict how plant species and assemblages respond to changes in the availability and spatio-temporal heterogeneity of resources like water, light and nutrients. In the natural world, spatial heterogeneity in the availability of soil-based resources at various scales (soil heterogeneity) is the norm, rather than the exception, in most ecosystems. Albeit soil heterogeneity is a key feature of most terrestrial ecosystems, and plays a crucial role in shaping their structure, productivity, composition and diversity, it has often been ignored when evaluating the effects of global change drivers on plant performance and associated

ecosystem processes. Thus, it is virtually unknown whether observed plant responses to global change drivers are modified by soil heterogeneity. This chapter synthesizes a series of multifactorial microcosm experiments aiming to evaluate the joint effects of soil heterogeneity and global change drivers (biotic diversity, nutrient availability, $[CO_2]$ and changes in rainfall patterns) on the response of individuals and assemblages (in terms of biomass production and allocation and nutrient uptake patterns) formed by different combinations of *Lolium perenne* L., *Plantago lanceolata* L., *Trifolium repens* L., *Anthoxanthum odoratum* L. and *Holcus lanatus* L. The authors tested the hypothesis that soil heterogeneity modulates the magnitude of the response of both individuals and assemblages to multiple global change drivers (i.e., that individual and assemblage responses to soil heterogeneity and global change drivers will not be predictable from the observed responses to any one of the evaluated factors in isolation). In all the experiments conducted the authors did find a series of two- and three-term interactions determining key responses at both the individual and assemblage level. Thus, the authors' working hypothesis was supported by their results. Their synthesis strongly suggest that single-factor studies should not be used to extrapolate how grassland ecosystems may respond to global change, which is composed of multiple—and potentially interacting—drivers.

Chapter 3 – Ecotoxicological assessment of chemical substances is mainly based on standard bioassays, most often single-species tests covering the range of acute and chronic toxicity. In the authors' laboratory the authors have been developping since 1997 a protocol of ecotoxicological bioassay in 2-L laboratory microcosms and applied it to the study of various pollutants and scenarios of ecotoxicological risk assessment in the field of urban facilities and transport infrastructures. Effects are assessed on five different organisms (micro-algae, duckweeds, daphnids, amphipods, chironomids) using endpoints such as growth, emergence (chironomids), reproduction (daphnids), survival, with a duration exposure of 3-4 weeks. For specific studies, some variants of the 2-L protocol have been developed in higher volumes (up to 100 L) with increasing biological diversity and different hydraulic conditions. More recently, a flow-through microcosm assay (dynamic assay) was developped for the 2-L protocol to improve conditions inside the microcosms, resulting in stabilisation of physico-chemical parameters, increase of organisms fitness and reduction of variability.

This chapter will present the various protocols, their application to different ecotoxicological risk assessment studies, their limitations (variability and sensitivity, ecological relevance, organism fitness, ...), and the improvement of the 2-L protocol through continuous water renewal. Finally, recent works and perspectives in modelling will be presented.

Chapter 4 – Investigation on transformation of organic pollutants in microcosm is an important way of understanding their fate and behavior in the environment. The common methods used in microcosm experiment include (1) determination of concentration change of organic pollutants during the whole process by gas chromatography (GC) or high performance liquid chromatography (HPLC); (2) identification of degradation products by gas chromatography-mass spectrometry (GC-MS) or high performance liquid chromatography-mass spectrometry (HPLC-MS).

Over the past decades, a new and innovative method of compound-specific isotope analysis (CSIA) using gas chromatography coupled isotope ratio mass spectrometry via a combustion interface (GC-C-IRMS) has been established as a powerful tool for assessing

transformation of organic pollutants based on the fractionation of stable isotopes during degradation process.

Two stable isotopes, ^{13}C and ^{2}H, have been extensively exploited to evaluate the transformation processes of contaminants. The enrichment of $\delta^{13}C$ and $\delta^{2}H$ offer a direct evidence of transformation, which does not prominently influenced by physical processes such as volatilization and sorption. Moreover, isotopic fractionation effect is closely associated with the reaction mechanism and the type of bond being broken. In this chapter, the authors review studies on degradation pathway of organic pollutants in microcosm by mean of CSIA. Especially with diethyl phthalate (DEP) as an example, the authors discuss how the CSIA technique combined with GC-MS analysis was used for studying the degradation pathway of organic pollutants in microcosm.

Chapter 5 – Microcosms have been used extensively to investigate ecological factors, evolutionary dynamics and the underlying molecular biology of adapting populations of bacteria in experimental evolution studies. One such experimental system has combined simple glass vials containing King's B liquid growth medium with the model soil and plant-associated pseudomonad, *Pseudomonas fluorescens* SBW25. In these, evolution reproducibly generates the Wrinkly Spreader genotype, a novel class of adaptive mutations that enable *Pf.* SBW25 to produce a robust, physical cohesive–class biofilm at the air-liquid interface. The analysis of Wrinkly Spreader mini-Tn*5* mutants led to the identification of cellulose as the main matrix component of the biofilm, and ultimately to the discovery of the mutation in a chemosensory-like system that activated the Wrinkly Spreader phenotype through the over-production of cyclic-*di*-GMP. Parallel ecological investigations have shown the importance of O_2 availability for the evolution and success of the Wrinkly Spreader. The initial colonisers establish an O_2 gradient, dividing the microcosm into a 100 – 200 μm high-O_2 zone at the air-liquid interface, and second, deeper low-O_2 zone in the rest of the liquid column. Growth is O_2–limited in King's B medium, and it is the domination of the air-liquid interface that guarantees access to O_2 and underlies the fitness advantage the Wrinkly Spreader has over non-biofilm–forming competitors including the ancestral wild-type *Pf.* SBW25. However, recent experiments demonstrate that Wrinkly Spreader fitness is reduced in static microcosms containing lower levels of nutrients, or in those in which King's B liquid viscosity has been increased. These findings demonstrate that the Wrinkly Spreader biofilm is a finely-balanced solution to the colonisation of the air-liquid interface in static microcosms, and changing environmental conditions will impact directly on Wrinkly Spreader fitness. This work highlights the importance of environmental factors, which along with competition, help guide the evolution of *Pf.* SBW25 in simple microcosms, invariably leading to the rise of the Wrinkly Spreaders.

Chapter 6 – It has often been discussed that research into the environmental fate and effects of pesticides has been almost exclusively conducted under non-tropical conditions. Furthermore, those studies conducted in tropical regions have focused almost exclusively on single species laboratory tests and very few microcosm studies under tropical settings are currently available. Given the differences in ecology and climatic conditions between tropical and non-tropical conditions, differences in pesticide fate, direct and indirect effects and recovery potential of impaired ecosystems may be anticipated. In the present chapter, findings from studies conducted in tropical Thailand are included to illustrate these possible differences. Indications for the methodology and research needs of future tropical microcosm studies are discussed.

Chapter 7 – Bacteria in the environment are organized in communities and show often high degrees of worksharing and cooperation. They are resilient against noxious agents and their functions are redundant to compensate changing environmental conditions. The authors' understanding of these functional communities, however, is limited, especially because only a minority of strains present in the environment can currently be isolated. Microcosms are powerful tools for the study of microbial communities without the need for prior isolation of strains. The authors became especially interested in microbial communities growing on interfaces forming so called biofilms. To study functional communities organized in biofilms they developed floating dish microcosms. Here soil or sediment samples from the environment are placed in a pot, covered with sterile water and a dish carrying different materials underneath is floated on the surface. Bacteria now migrate through the water column to these substrata and form complex biofilms. The authors used these biofilms to i) isolate degraders of pollutants used as substrata (polychlorinated biphenyls, hexachlorocyclohexanes, tributyltinoxide), ii) monitor biofilm development over time observing biofilm structure and composition and the authors identified the succession of degraders of different congeners, iii) compare biofilm communities from different sites and found similar activities but different species composition from different sites and iv) manipulate biofilm composition by using different substrata or adding compounds known to control biofilm formation. The floating dish microcosms can easily be adapted to specific research requirements making the whole approach very flexible. The authors optimized the protocol so that the microcosms can be used to grow biofilm communities which can be characterized by confocal laser microscopy, phylogenetic fingerprinting of the community composition and chemical analysis of pollutant degradation. The microcosms can also be adapted for the screening of natural compounds or novel materials for their ability to shape biofilm composition, structure and activity. In this chapter they present different floating dish microcosms and their scientific results. They also discuss the potential but also the limitations these microcosms have.

Chapter 8 – The dialectic relationship that characterises the semiotic process inherent to any form of cognition is responsible for the cohesion and sustainability of the microcosms defined by living entities in their interaction with the surrounding world.

The coupling that arises as a result of the plasticity of both living organism and its environmental bubble produces an autonomous and strictly bounded unit that has nevertheless been shaped extensively in the course of its evolutionary and developmental history.

Semiosis is consequently an embodied, embedded and always situated phenomenon. This means it involves a living entity endowed with a particular physical architecture interacting with the specific world it is immersed in, producing a dynamics that defines events taking place in a particular microcosm and perceived by a human observer as anchored in space/time.

Uexkull (1909) introduced the fundamental concepts of Umwelt and Innenwelt stressing their strict interdependent nature. The present paper stems from these fundamental concepts but it goes further by equating them with the semiosis inherent to any form of cognition. Plus, by proposing the mathematical modelling of a prototypical instance of individual cognition and by introducing the concept of Umwelt Overlap the paper attempts to identify the essential parameters of ecological sustainability.