7. Planetary Biology

Background

Planetary biology at EMBL is a new and ambitious initiative to understand life in its natural context. Organisms live in complex and dynamic ecosystems where biological communities – whether of microbes, animals, or plants – interact with each other, and with physical and chemical factors. All living systems are exposed to constantly changing, naturally occurring or anthropogenic environments. To decipher how life adapts, prospers, or declines in different contexts, the biotic and abiotic environmental factors that influence organisms must be identified. The responses that these factors trigger, as well as the underlying molecular mechanisms, must be elucidated to gain a true understanding of the basis of life. Untangling the complex relationships between organisms and their environments remains a longstanding scientific challenge. Exploring these relationships in molecular terms at the ecosystem scale represents an even greater challenge, yet this is the context in which life happens. Indeed, just as the cell is the basic functional unit of life, ecosystems are the basic unit of nature.

To better understand ecosystem function, new information at the molecular, cellular, and organismal levels will be key. For example, recent studies to unravel the mechanisms of coexistence between microbial communities and their hosts (holobionts) have required a real-time understanding of the gene regulatory networks and metabolic pathways that respond to nutritional variables. The emergence of new viruses and the spread of antibiotic resistance can only be understood by following microbial dynamics in situ in their naturally occurring habitats. The processes of rapid adaptation, pathogenicity, or mutualism between organisms require an understanding of community composition, genetic and epigenetic processes (such as horizontal gene transfer, transposable element activation, and environmentally sensitive gene expression), and defence pathways (such as RNA interference and immune responses).

In this highly collaborative endeavour, EMBL will leverage its expertise in molecular biology to study ecosystems, an area until now mainly studied by ecologists, epidemiologists, geobiologists, and environmental biologists. EMBL will employ a cross-disciplinary approach involving experimental and theoretical collaborators from various fields. Planetary biology research will be carried out in: (1) natural contexts (Figure PB1) using longitudinal, multidimensional sampling of microbes, animals, and plants, particularly at land–water interfaces; (2) laboratories at EMBL and at collaborating institutes around Europe for analysis and experimentation; and (3) environmentally controlled ecosystems (ecotrons, mesocosms). Carefully selected natural and experimental ecosystems will be studied to generate repertoires of biodiversity coupled with environmental variables to understand function and perform experiments to elucidate mechanisms.
Figure PB1 | Example ecosystems, their biotic and abiotic components, and processes that are within the scope of EMBL’s planetary biology research projects.

One of the main challenges of characterising ecosystem function is identifying how the biotic and abiotic components interact to influence processes such as nutrient cycles and energy flows. Adapted from Cavicchioli R, Ripple WJ, Timmis KN, et al. Scientists’ warning to humanity: microorganisms and climate change. *Nat Rev Microbiol*. 2019;17(9):569-586. doi:10.1038/s41579-019-0222-5.

Planetary biology research should provide new molecular tools and expertise to track and understand biodiversity and ecosystem functions, including primary productivity and nutrient cycling, which are linked to environmental sustainability. The identification of new model organisms that are either keystone species (those that play a crucial role in ecosystem function) or dominant organisms (the most abundant species within an ecosystem by biomass) will also have far-reaching implications for scientific discovery and applications in human health and other areas, such as in fisheries or agriculture, where knowledge of ocean, freshwater, and soil health is critical. Aside from the fundamental importance of understanding life in its natural context, ecosystem function is crucial for human survival (for example, for food production, medicine, consumer goods, and materials). However, as biodiversity declines at alarming rates due to habitat destruction, deforestation, urbanisation, pollution, climate change, and other anthropogenic effects, natural ecosystems are changing on a massive scale. Some consequences include the emergence of new infectious diseases that affect humans and animals; the spread of antimicrobial resistance (AMR), which is predicted to cause 10 million deaths each year by 2050 due to drug-resistant infections; climate change and the erosion of soils, which are causing acidification and increased agriculture risks around the world; and the loss of forest and ocean life, which is impacting natural resources. To stop and reverse these effects, an understanding of the factors that destabilise ecosystems, and the impacts of these factors, is essential.

Just as scientists have developed molecular diagnostics and therapies for human health, molecular approaches can provide potential diagnostics and potential therapies for planetary health, which is intrinsically linked to human health. For example, obtaining fundamental molecular mechanistic insights into zoonotic diseases, horizontal transfer of AMR genes, or eutrophication would be major steps in the search
for methods to overcome or prevent these phenomena. EMBL will seek to build bridges not only between scientific disciplines but also with policymakers to ensure that evidence-based actions and decisions are taken (Chapter 15: Public Engagement, Communications, and Outreach).

Through its initiatives in planetary biology, EMBL will seek to drive a new era for the life sciences in Europe, combining data-driven and hypothesis-driven research in partnership with ecologists and other experts. As Europe’s only intergovernmental life sciences research institution, EMBL regards this as a unique and compelling opportunity to form new interdisciplinary collaborations with scientists across its member states, fulfilling its pan-European mandate. The knowledge gained from EMBL’s planetary biology research will enable scientists to answer fascinating fundamental questions about the impact of the environment on biological processes, and will pave the way for solutions to pressing environmental and societal challenges, including global warming, environmental pollution, harmful zoonotic diseases, and AMR.

The Opportunity

The remarkable technological advances of the past half century, as well as the recent unprecedented capacity to collect, store, and analyse data, mean that molecular biologists can now effectively study life in its natural context. These advances coincide with urgent environmental and societal challenges relating to the rapid deterioration of ecosystems, which require solutions based on scientific discovery and evidence. With its planetary biology theme, EMBL will build upon its strengths in fundamental molecular biology research to capture and explore environmental information and the responses of organisms to environmental variations. Although the goal is ambitious, EMBL has a long track record of successful technology development, multidisciplinary collaborations, and advanced data integration and provision.

Planetary biology research will build on EMBL’s ongoing research and past experience. EMBL has a successful track record in global ecosystem exploration, thanks to highly collaborative projects with the Tara Ocean Foundation and recent soil microbiome studies (Figure PB2). The Tara Oceans expedition was devised by EMBL scientist Eric Karsenti and took place from 2009–2013. This pioneering, cross-continent scientific survey of planktonic and coral ecosystems in the context of climate change produced a tremendous amount of data, which has enabled major discoveries and secondary research around the world. The publicly available resources represent the largest sequencing effort in marine science and the most expansive description of the world’s largest cohesive ecosystem – the ocean. The success of Tara Oceans was in part due to standardised sampling and analysis protocols, deep environmental surveillance, and open data policies. The Tara Oceans data led to a multitude of discoveries about the diversity of viral, prokaryotic, and eukaryotic species, their genetics, and their interactions, including novel symbiotic relationships. Major ecological insights included the discovery of latitudinal gradients of biodiversity in the ocean, how diatom evolution relates to changing climates, and how plankton are dispersed by ocean currents. Recent groundbreaking contributions from the Tara Oceans data include estimates of the distribution of the world’s biomass, new discoveries about the origins of mitochondria, identification of heterotrophic nitrogen-fixing bacteria in the ocean, the discovery of a new class of microbial rhodopsins with potential applications in optogenetics, and the discovery of ‘giant’ marine phages with new CRISPR–Cas systems.

EMBL has also participated in major projects that involved sampling global soil microbiomes. Soil microbiomes are among the most diverse on the planet and carry out critical processes including support of plant growth, nutrient cycling, and carbon storage. Sampling studies at EMBL have provided remarkable insights into fungal and bacterial communities with diverse responses to precipitation or pH, and evidence for bacterial–fungal antagonism (inferred from antibiotic production), indicating the importance of biotic interactions in shaping microbial communities. These examples illustrate the remarkable contributions and opportunities that systematic large-scale ecosystem exploration can provide.
EMBL has a strong tradition of research in studying model organisms, both unicellular and multicellular. The study of how these organisms adapt to environmental changes will be an important pillar of EMBL’s planetary biology research. Current research includes investigating developmental dynamics and phenotypic plasticity, as well as responses to environmental signals in diverse organisms such as the marine annelid *Platynereis*, the sea anemone *Nematostella*, and the ascidian *Phallusia* (Chapter 3: Cellular and Multicellular Dynamics). These studies have been made available to the scientific community through multimodal atlases that combine whole-organism, cellular, and subcellular imaging with single-cell transcriptomics, epigenomics, and metabolomics. EMBL also has a distinguished history of carrying out studies on genetics and developmental biology in fruit flies (*Drosophila*) and mice (*Mus*). These foundations are complemented by more recent metabolic analyses and experimental setups to study phenotypic diversity due to genetic variation and environmental factors, including microbiota. EMBL’s expertise in using such models, combined with the array of technologies now available for establishing and studying new model organisms, will be crucial to EMBL’s planetary biology research.

EMBL’s aim is not to explore all ecosystems, but to focus on dedicated collaborative projects that will serve as paradigms for future endeavours to study ecosystems at the molecular level. The intention is not to become an ecological research organisation, but rather to collaborate and build bridges with communities of scientists to build a new era of European life sciences together.

Research Aims

**A new era of molecular and cellular approaches to ecosystems**

Most natural variation studied to date has been limited to observable characteristics and genomic information. Today, technologies exist to explore molecular and subcellular information that varies in response to the environment, including at the levels of stress and metabolic responses. Spatial phenomics, which combines single-cell metabolomics, gene expression, and epigenomics with advanced imaging at the molecular, subcellular, whole-organism, and community levels, has opened up a new era of investigation.
The main objectives of the planetary biology theme will be to **recognise and understand phenotypic changes that are induced in the natural environment**, using the array of tools available for molecular, structural, genomic, cellular, and developmental biology, and the powerful technologies that enable visualisation and perturbation of processes. Genotype–phenotype relationships and the contributions of genetic variation (both between and within species), and environmental factors in influencing phenotypes will be explored in their natural context on the molecular and cellular scales and at the ecosystems level. By measuring the tempo of genetic, phenotypic, and metabolic variation of organisms in their communities; their interactions with each other (e.g. infection, symbiosis, mutualism, competition); and responses to natural variables and environmental stressors, the complex relationships between organisms and their environment can be untangled.

To gain molecular mechanistic insights into organisms within ecosystems, EMBL will first determine ‘who is there?’ (Figure PB3) via sampling, measurement, and analysis. This will be followed by exploration of mechanisms – ‘what do they do?’ and ‘how do they do it?’ – through isolation, cultivation, and perturbation studies in the lab and in controlled settings. Advanced data analyses (including AI) linked to EMBL’s Data Sciences programme (Chapter 8: Data Sciences), as well as theoretical approaches (Chapter 9: Theory at EMBL), will be crucial to fully identify and understand significant correlations and to create testable predictions. The discovery of indicator species which reflect environmental states, and new biomarkers and biosensors to measure the states of ecosystems in nature, can then inform further experiments, enabling new measurements and further perturbation methods.

![Figure PB3 | Planetary biology processes and questions.](image)

EMBL’s planetary biology processes will enable questions (left) to be asked using the experimental, computational, and theoretical approaches applied in natural and laboratory contexts (right). The processes by which scientists measure (blue), perturb (purple), and predict (orange) can encompass various combinations of relevant tools, techniques, or parameters.

Today, molecular ecology – the application of molecular techniques to ecosystems research – focuses mainly on the use of metagenomic information (sequencing of DNA in environmental samples to obtain a (meta) genomic blueprint), metatranscriptomics (sequencing of RNA in environmental samples to observe gene expression states), and barcoding (16S rRNA for prokaryotes, 18S rRNA for eukaryotes, and the internal
transcribed spacer (ITS) region for fungi) to identify species. Although metagenomics provides repertoires of genes and species within ecosystems, it cannot provide multidimensional spatio-temporal information about how organisms coexist (in communities, as symbionts, etc.) or how they change their molecular and cellular phenotypes in different environmental conditions, be they nutritional variations, natural seasonal changes, or environmental insults such as abnormal temperature or the presence of pathogens or chemical toxins.

To meet this need for multidimensional measurements, planetary biology research at EMBL will combine advanced omics technologies (including single-cell transcriptomics and metabolomics), with multiscale imaging (e.g. high-resolution fluorescence microscopy and correlative light and electron microscopy). Quantitative and dynamic measurements of these multimodal parameters need to be generated in living systems (Chapter 3: Cellular and Multicellular Dynamics). Importantly, these multiscale phenomics technologies at EMBL will have to incorporate and monitor chemical and physical variables at the same time. Parameters such as temperature, weather conditions, salinity, pH, oxygen levels, water, abundance of macro- and micronutrients, and biomass of organisms will be measured in conjunction with levels of sulfides, nitrous oxide, nitrate, nitrite, hydrogen, and pollutants from human activities such as micro- and nanoplastics, antibiotics, herbicides, pesticides (e.g. endocrine disruptors), and fertilisers.

High-throughput molecular data, structural biology studies, and high-resolution imaging on a range of scales will lead to many hypotheses, which will need to be tested. Measuring and predicting the response to stressors requires the controlled introduction of perturbations. The tools already available at EMBL to manipulate genomes, RNA, or proteins under different conditions and to visualise and measure the impact of perturbations both in vitro and in vivo will be used (Chapter 2: Molecular Building Blocks in Context). New tools that enable combinations of approaches (e.g. optogenetics, dCas9, and single-cell omics) to perturb and measure molecules will also be developed.

To test predictions and gain mechanistic insights into how organisms exist in communities and respond to environmental factors, a range of approaches will be applied. Customised, untargeted metabolomics and chemical screens with a large library of known biotic and xenobiotic substances will be performed using EMBL’s Chemical Biology Core Facility, in collaboration with other institutes and networks in Europe. Combinations of substances will be tested, together with physical parameters. Novel assays will be developed using organisms (e.g. insect and plant models), mammalian cells and plant cells in culture, marine organisms in aquaria, and bacterial communities in bioreactors (see Chapter 4: Microbial Ecosystems).

**Specific research aims**

Some general questions include how responsive or resistant organisms are to environmental changes in natural contexts; for example, how and why biodiversity changes within an ecosystem, how stable ecosystems are, or how reversible the impact of environmental changes can be. Such questions are being asked by ecologists, evolutionary biologists, and epidemiologists. EMBL aims to bring new perspectives and methods to help answer these questions in three broad areas of study. **EMBL aims to:**

I. **Explore how organisms in communities are affected by natural and anthropogenic environmental variables.** EMBL aims to undertake longitudinal biotic and abiotic sampling with advanced technologies in mobile labs. This will form the basis of multiple projects focusing on microbial ecosystems, as well as specific animals and plants in soil, sediment, aquatic, or arid land contexts. A major area of interest will be capturing biodiversity and accompanying natural and anthropogenic environmental variables at interfaces such as coastal regions, lakes, and rivers, where environmental gradients exist.
Specific studies will include surveying plankton communities at various temporal and spatial scales, across various environmental gradients, to unveil their diversity and phenotypic plasticity; identifying key taxa in microbial communities using molecular methods, to understand how nutrients moving from land to water lead to eutrophication in seas, lakes, and rivers; studying soil microbes to understand the productivity of soils and the impact of synthetic products such as pesticides; understanding the spread of AMR by following microbes in changing soil and water ecosystems to see how antibiotic resistance genes are disseminated; and exploring microbial biodiversity shifts in regions undergoing rapid aridification. To understand gradual versus acute changes induced by the external environment, the variables that affect potential adaptations must be monitored over time, followed by investigations of changes in communities in controlled laboratory settings (see Aim III below) to derive mechanistic insights from the molecular to the systems levels.

II. Understand responsiveness to natural and anthropogenic environmental changes using model organisms. Model organisms studied at EMBL can be used to investigate the impact of specific environmental variations. The aim is to identify the cellular mechanisms that underpin responsiveness and the environmental factors that drive organism adaptations and ultimately their evolution. For example, the marine annelid *Platynereis*, with its highly stereotypic development, can be used to investigate the impact of multiple environmental variables, including light, temperature, acidity, nutrition or the relationship between associated microbiomes and cellular morphology, physiology, metabolism, and behaviour. The sea anemone *Nematostella*, with its remarkable phenotypic plasticity, can be used to understand the impact of food availability on tentacle development and other features (Chapter 3: Cellular and Multicellular Dynamics). Medaka fish (Chapter 6: Human Ecosystems) can be used to examine the impact of environmental toxins on multiple phenotypes, including phenotypes relating to reproduction and behaviour. To understand how animals can adapt to environmental effects, such as temperature, nutrition, or toxins, the classic *Drosophila* model can be used for controlled evolution experiments. Other important models for the study of environmental factors that are not currently used at EMBL – such as plants – will be explored through collaborations and by potential new faculty recruits. More advanced goals will be to identify key organisms or communities that can be used as new models to answer particular questions related to the environment. These organisms can be made genetically tractable and amenable to exploration through modern isolation and culturing techniques, and by the development of chemical and genetic perturbation approaches, to enable new mechanistic insights and the development of tools for further exploration. These new model organisms will be studied in labs, *in natura*, and in the context of environmentally controlled settings. Within ecosystems of interest, the identification of keystone species and dominant organisms that can be rendered genetically tractable, would provide a crucial starting point for studying interactions within and between species, and for modeling ecosystems in the lab, to understand how they work.

III. Study specific communities under controlled environmental conditions. By culturing communities and organisms under controlled variations of environmental conditions, the aim is to explore ecosystems in natural contexts, or to create simple synthetic ecosystems in the lab, to analyse metabolic repertoires, interaction networks, and response or resistance to changing environments. This is already a reality at EMBL in studies of selected small ecosystems, such as the microbial subcommunities in the human gut, which are being carried out in defined, closed settings (anaerobic chambers). In these studies, chemical perturbations are employed to dissect microbial interactions and to measure the impact of environmental factors, including the effects of therapeutic drugs with direct clinical relevance (Chapter 4: Microbial Ecosystems, and
Chapter 5: Infection Biology). EMBL will seek to advance this framework and apply it to other ecosystems.

Select ecosystems will be analysed in bioreactors, mesocosms, or ecotrons to understand specific environmental effects of nutrients or hydrological, morphological, thermal, or xenobiotic factors. EMBL’s experience in building bioreactors will be leveraged to usher in a new era of ecosystem investigation. One example study is an investigation of the biotic and abiotic variables in arid soil ecosystems that are in danger of losing their capability to support productive ecosystems. A specific goal would be to identify the variables that could be used to detect vegetation decline and the onset of the soil disruption phase, to better predict systemic collapse.

By defining biotic and abiotic parameters and how they vary over time in natural ecosystems, ecotron research infrastructures can be used to study multiple questions about when, where, how, and why mutualistic, pathogenic, or symbiotic relationships occur, particularly in the context of environmental changes such as temperature, nutritional variation, toxin exposure, or stress. Example communities include bacteria and fungi in soil, plants and their microbial communities, or oceanic microalgal symbionts in acantharian hosts. These studies can lead to significant progress in explaining what underlies certain types of phenotypic plasticity in nature, how zoonotic diseases and AMR spread under various conditions, and the impact of the environment on early development, which can lead to phenotypic changes in later life and intergenerational effects in some organisms.

**Planetary biology research principles:**

- **Collaborative research.** To maximise scientific discovery and societal impact, EMBL will work with scientists from many disciplines through a scientific advisory board and networks of collaborators, including molecular biologists, population geneticists, ecologists, geobiologists, engineers, systems biologists, and data scientists. New expertise will be brought in via collaborations and new recruitments (Chapter 1: Introduction, Figure IN2). By deploying mobile lab services, EMBL will join forces with scientists from its member states in new collaborations, and will provide training via postdoctoral schemes and specialised sabbaticals (Chapter 11: Training), equipping a new generation of scientists to carry out planetary biology research. To maximise cooperation, EMBL will publish calls for joint ecosystem research projects and will organise stakeholder meetings to select and develop projects that would be timely, would foster excellent research, and would ensure the highest scientific impact.

- **International scope and pan-European mandate.** Research on ecosystems is often limited by national borders. Planetary biology at EMBL provides an opportunity to link national environmental science projects that might otherwise be geographically restricted. EMBL also aims to link to international networks and infrastructures such as European marine biology stations, via the European Marine Biological Resource Centre (EMBRC-ERIC); ecotrons, via the Analysis and Experimentation on Ecosystems (AnaEE) infrastructure; and the UK’s Darwin Tree of Life project and the international Earth BioGenome Project. As an intergovernmental organisation, EMBL is ideally situated to collaborate with national initiatives to pursue and further such societally relevant research in an integrative manner.

- **Technology development.** Profiling ecosystems at the molecular and organismal levels will require technical innovations such as automated environmental sampling, advanced omics technologies (nanopore sequencing or metabolomics), single-cell phenomics, improved genetic and chemical screening, and customised modern microscopy techniques (from cryo-EM
to super-resolution microscopy and live-cell imaging with chemical probes). Equipment for sample preparation and some in situ analyses will also be needed when field studies are performed. This will enable data to be simultaneously captured at multiple levels – genetic, transcriptomic, epigenetic, metabolic, and morphological – and to be analysed in an integrated way.

- **Advanced data integration and provision.** EMBL is a world-renowned hub for the integration and provision of various types of molecular data, with expertise and critical mass in data storage, processing, handling, harmonising, and analysis. This will be essential for capturing and leveraging the enormous and ever-growing quantities of data obtained from global high-resolution surveys of ecosystems. EMBL’s commitment to providing open access data and analysis tools will guide these activities.

**EMBL’s Approach**

**I. Explore how natural communities are affected by natural and anthropogenic environmental variables**

EMBL will undertake longitudinal sampling projects, applying advanced molecular and cellular approaches to describe ecosystems exposed to natural and anthropogenic variables. The focus will initially be on specific soil and aquatic ecosystems at land–water interfaces. These interfaces are important for biodiversity and ecosystem functioning, and several recent studies have shown that coastline areas have suffered rapid declines in biodiversity and higher rates of antimicrobial resistance spread, with gradient effects going from land to sea. Synchronised land and aquatic sampling will allow evaluation of the impact of natural and anthropogenic factors on soil, rivers, and possibly air, as well as the impact of wind and floods on land. The focus will be on understanding the effects of these factors on terrestrial and aquatic microbial biodiversity and ecosystem functions.

To realise this ambitious endeavour of studying ecosystems at scale, EMBL proposes to establish mobile labs, with state-of-the-art technologies, to conduct carefully planned sampling expeditions in collaboration with scientists from EMBL’s member states, developing standardised protocols and applying unified data science concepts. **Mobile labs** will combine research and services, together with training and outreach activities. The container-based mobile services (Chapter 10: Scientific Services) will bring advanced equipment to the participating and collaborating labs throughout each expedition. Science and service will be combined, with numerous training and outreach events in the member states (Chapter 11: Training, and Chapter 15: Public Engagement, Communications, and Outreach).

**Standardised protocols** will be developed to measure environmental variables, capturing a maximum of information in situ. The concentrations and fine-scale chemical gradients of oxygen, sulfide, nitrous oxide, nitrate, nitrite, and hydrogen in the natural environment can all be monitored using microsensors, enabling high-quality microscale measurement of analytes. Measurements will be used to resolve this environmental information in heterogeneous settings, such as the root surfaces of plants, animal burrows in marine sediments, microbial communities, porous soils, and wastewater locations. Alongside this, the consumption rate of oxygen over time, or the oxygen exchange rate across the water–sediment interface, can be investigated in different geographical locations. Other environmental variables of relevance include temperature, weather conditions, salinity, and levels of macro- and micronutrients. As the relationships between organisms and the environment continue to be studied, technological developments to create new biosensors will be enabled by collaborations between molecular biologists, engineers, and chemists at EMBL and within its member states. These biosensors could potentially be used as novel diagnostic tools to help identify ecosystems under threat (see also Tools for Planetary Health below).
An ocean–land sampling pilot project was carried out by EMBL in collaboration with the Stazione Zoologica Anton Dohrn in Naples in October 2019, to test some of these approaches, including the appropriate tempo of sampling. A longer-term planetary biology project is being developed. This example flagship project will develop planetary biology approaches and protocols, and will pioneer the use of mobile labs. Selected communities and model systems will be further analysed in the lab under controlled conditions (see sections II and III).

**TREC Flagship Project:** Based on EMBL's experience in global ecosystem surveys of oceans and soils (Figure PB2), EMBL aims to initiate a European coastline expedition called TREC (Traversing European Coastlines), to study microbial communities and populations of selected macroscopic model organisms. Simultaneously serving as a proof of principle and as a flagship project, TREC will aim to characterise marine and terrestrial ecosystems along the European coastline, extending also to freshwater–land interfaces such as rivers and lakes. In cooperation with multiple partners (Figure PB4), the project aims to address the impact of various environmental factors – including pollutants – on microbial communities, as well as on key organisms about which EMBL has expertise. The strategy couples cross-sectional molecular profiling and imaging with in-depth analyses of selected organisms, communities, and contextual environmental data. Numerous locations are being considered, where scientific interest exists and where a wide variety of organisms, including bacteria, fungi, viruses, micro-eukaryotes, plants, and animals, could be collected from land–water interfaces (Figure PB5).

The TREC expedition will rely on core resources, infrastructure, and expertise provided by the core partners, and there are initial plans for three projects (described below). This core support will also be used to facilitate numerous plug-in projects with member state scientists who wish to undertake cross-sectional or longitudinal studies. These plug-in projects will be selected based on existing consortia or open calls. EMBL will partner with scientific institutions and link up to national projects or complementary consortia.

**Figure PB4 | Organisational structure of the TREC expedition.**

The central ring (green) represents the core expedition, comprising core infrastructure and core activities. Current core partners are the Tara Oceans Consortium, the Tara Ocean Foundation, and the European Marine Biological Research Centre (EMBRC-ERIC). Additional partners (yellow) will support and leverage TREC infrastructure, technologies, and expertise. Plug-in collaborative projects (blue) from member state scientists and others can be integrated to maximise the scientific and societal impact of the expedition. Core partners will support the expedition with resources, infrastructure, and expertise. EMBL will provide much of the land-based infrastructure (mobile labs, vehicles, and equipment). The Tara Ocean Foundation and the Tara Oceans Consortium will provide the Tara research vessel and associated equipment and sampling expertise. EMBL will also collaborate with marine stations with EMBRC-ERIC infrastructure and with local scientific communities.
Three examples of **TREC core scientific projects** that could be carried out in parallel are described below (two in this section and the third in Section II of EMBL’s Approach). In each case, additional partners, institutions, or consortia could join to expand and leverage the proposals described below.

**TREC Core Project 1: Sampling Coastal Plankton Communities**

Plankton communities, comprising for example diatoms, dinoflagellates, or other microalgae (phytoplankton), play a key role in Earth’s biosphere as photosynthetic producers in marine waters, yet are still poorly understood. Microalgae have fascinating endosymbiotic states that vary with climatic and nutritional contexts. Given their high protein and oil content, they can also be cultured easily for use as biofuels or animal feeds. The TREC project will sample coastal plankton communities at various scales, across various environmental gradients, to unveil the diversity and phenotypic plasticity of microalgae and zooplankton, including their architecture and metabolism at subcellular levels. By integrating organismal abundance data and data on functional traits, it should be possible to deduce environment-dependent interactions between species. These interactions range from tight symbiotic or parasitic relationships to indirect associations, reflecting nutrient dependencies or contributions to interspecies molecular pathways.

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**Figure PB5 | TREC expedition potential sampling sites.**

This map highlights the coastline of the EMBL member states. EMBL scientists and collaborators will explore marine and terrestrial ecosystems along the European coastline, extending also to freshwater–land interfaces such as rivers (indicated in blue) and lakes. EMBL will continue to work with member state scientists, collaborators, and representatives across multiple disciplines to select sampling sites along the coast and inland.
Sampling sites will be selected to provide a balance between relatively undisturbed habitats and those affected by human activity. The former will require extensive systematic screening for pollutants such as microplastics. Sampling and processing protocols will be developed and standardised as was previously done for the Tara Oceans expedition. Protocols will also include measurements of temperature, pH, oxygen saturation, and salinity, as well as quantities of trace elements. Methodologies combining measurements of environmental parameters with high-resolution imaging and single-cell transcriptomic and metabolomic analyses will enable a direct correlation of imaged phenotypes with molecular biotic and abiotic information.

TREC Core Project 2: Sampling Coastal Soil and Sediment Microbiomes

Microbial soil communities along coastlines and in sediments represent dynamic ecosystems that can provide insights into the natural biodiversity at land–water interfaces, the productivity of soils, and the impact of synthetic products such as microplastics, antibiotics, herbicides (e.g. glyphosate), pesticides, and endocrine disruptors. Soil ecosystems contain viruses, bacteria, archaea, fungi, protozoa, and nematodes. Topsoil microbiomes are critical for the support of plant growth and the cycling of carbon, nitrogen, and other nutrients. To understand soil functioning, it is necessary to model the distribution patterns and functional gene repertoires of soil microorganisms, as well as the biotic and environmental associations between the diversity and structure of soil communities.

Recent studies have revealed that both competition and environmental filtering affect the abundance, composition, and encoded gene functions of bacterial and fungal communities, indicating that the relative contributions of these microorganisms to nutrient cycling varies spatially. These studies have also revealed the existence of bacterial–fungal antagonism in topsoil and ocean habitats, inferred from antibiotic resistance genes (ARGs). To outcompete bacteria, many fungal taxa secrete substantial amounts of antimicrobial compounds, which may select for antibiotic-resistant bacteria and effectively increase the relative abundance of ARGs. These genes can provide indications about correlations between antibiotic producers and associated species, but their abundance profiles can also reveal patterns of antibiotic biogeography and dispersal. In particular, it has been proposed that chemical pollution in urban and industrial areas, as well as the extensive use of antibiotics in farming, have largely contributed to the spread of AMR and the emergence of multidrug-resistant bacteria (Chapter 5: Infection Biology).

The goal of this sampling programme will be to gain a deeper understanding of diverse soil microbial communities, their adaptations to different environmental factors – including pollution – and their exchanges with adjacent marine microbiomes. Studies will also explore microbial communities for antibiotic production and associated resistance. EMBL will collaborate with local partners, including marine stations and agricultural institutes, to join forces with local centres of ecological and geobiological expertise. The study of sediment is also important, since it can be key to understanding the reproductive habitat of many organisms and the formation of beaches, sandbars, and estuaries. Importantly, sediment provides unique insights not only into living ecosystems at land–water interfaces, but also into historical biodiversity, since ancient DNA can now be collected and analysed to obtain insights into human impacts during industrialisation, environmental impacts during the Medieval Warm Period or Little Ice Age, and deeper in time. The resulting knowledge could be used to develop molecular biomarkers for pollution states and to explore bioremediation strategies.
II. Understand responsiveness to natural and anthropogenic environmental changes using model organisms

To conduct in-depth analyses and establish a baseline understanding, EMBL will investigate classic model systems and establish new model systems. These will range from individual species with close symbiotic relationships to selected microbial communities, which will be characterised in their environmental context in natura and under controlled lab conditions. Model species, model systems, and selected ecosystems will be characterised using systemic approaches that include perturbations and comprehensive profiling of molecular, cellular, and phenotypic features. A molecular and mechanistic understanding underlying interactions within an ecosystem is a prerequisite to predict biotic and abiotic interactions in a context-dependent manner and abstract those interactions into more comprehensive models which, in turn, must be validated. Studies of experimental evolution can also be performed to observe evolutionary processes in real time and under various conditions, to study adaptation and estimate evolutionary parameters.

EMBL also aims to identify key organisms or communities that can be used as new models to answer questions relating to the environment, and which can be made genetically tractable. Collaborations with other large national or international initiatives to characterise biodiversity on Earth will also support the identification of ecologically important keystone species and dominant organisms. Initial collaborative efforts include the Earth BioGenome Project (a global effort to sequence all 1.5 million known species of animals, plants, protozoa, and fungi) and the Darwin Tree of Life project (which aims to sequence the genomes of all 60,000 species of eukaryotic organisms in the UK and Ireland). One aim of EMBL’s planetary biology theme will be to dissect ecosystem function, either by using single organisms or simple communities exposed to different biotic and abiotic variables, including those defined in longitudinal sample initiatives such as TREC (for example, photosymbiosis between eukaryotic microalgae and unicellular hosts along environmental gradients; interactions between fungi and soil bacterial communities in the context of antibiotic exposure and antibiotic resistance). Some illustrative examples are described below.

TREC Core Project 3: Sampling Selected Animal and Plant Species Along Environmental Gradients

EMBL will use its knowledge and expertise to select model species and sample and analyse them in more depth along European coasts. For example, the marine annelid Platynereis dumerilii has been extensively studied in evolutionary developmental biology and neurobiology, and is responsive to environmental signals such as light and photoperiod, and chemical cues including alcohols, esters, amino acids, and sugars. P. dumerilii usually inhabits bright, shallow marine environments but it can be found in less favourable environments, such as thermal vents or polluted or acidic areas. Subpopulations of P. dumerilii larvae have been found in environments with various pH values, indicating that Platynereis seems to adapt to a wide range of environmental conditions. Widespread along European coasts, P. dumerilii shows multiple haplotypes adapted to varying conditions. It is now possible to map allelic variants, differential gene expression, metabolites, and morphological variation over the entire body of a Platynereis larva with cellular resolution (Figure PB6). This also includes measurements of distinct microbiomes to analyse the variable composition and spatial distribution of microbiota contributing to the Platynereis holobiont. Platynereis can be grown and manipulated in the lab, and is one of several powerful models at EMBL for exploring the impact of environmental variables on development, cellular morphology, physiology, and metabolism, and for studying the links between genetic variants and environmentally induced phenotypic changes. Correlating multiple modalities to sample sites (and thus to environmental conditions) will make it possible to establish links between differing ecological parameters and cellular assimilation and adaptation in natura, and will generate hypotheses that can then be functionally tested in the laboratory. In addition to Platynereis, several other marine invertebrate model species could be sampled and investigated.
Figure PB6 | The Platynereis cell atlas.
This is a unique and collaboratively driven resource that builds on the synchronous and stereotypical development of Platynereis, and combines gene expression and ultrastructural information. Cells and nuclei have been segmented and mapped to a cell-type atlas so that segmented cells and nuclei can be correlated with full gene expression information for each cell type in the body. This resolution will be applied to several selected environments to study phenotypic plasticity and adaptation to the environment at cellular resolution. Cutting-edge imaging techniques, including automated electron and light microscopy and X-ray tomography, will be integrated to link morphometry of phenotypes to genetic variation (see Chapter 10: Scientific Services, Tech Dev Box TD3_SS).

Terrestrial organisms that will be investigated include plants, nematodes, and insects. Plants in particular are of major interest, as they are key biosphere producers by fixing CO₂ and producing oxygen for the planet. They represent the majority of biomass on Earth, and due to their sessile nature they are exquisite biosensors of environmental change. They have also evolved with a plethora of microorganisms that play important roles in their growth – both above and below ground (phyllosphere and rhizosphere, respectively). A considerable amount of information is now available on the structure and dynamics of plant microbiota, and on the functional capacities of isolated community members. However, much innovation is needed to connect the functional potential of plant microbiomes with plant physiology in natural contexts. The longitudinal sampling of plants and their microbiota along coastlines as part of TREC represents a unique opportunity. Although no plant scientists currently work at EMBL, new group leaders in this area may be recruited in the future, and plug-in projects will lead to collaborations with numerous experts around Europe.

Free-living nematodes including the classic lab model Caenorhabditis elegans are important components of terrestrial ecosystems. They live in complex habitats (e.g. soil, rotting fruit) in which they routinely experience large fluctuations in temperature and nutrient availability, and are exposed to diverse pathogens including viruses, bacteria, and fungi. C. elegans has been an invaluable model organism in research fields including evolutionary and developmental biology and more recently in ecology. Due to its high sensitivity to different pathogens and contaminants, its ease of use for experimentation, and its important functional roles in ecosystems, it is an ideal model for exploring organisal adaptations to changing environments. In a biomedical context, the implication of pesticides in the aetiology of several neurodegenerative diseases has been extensively investigated using C. elegans as a primary model organism, with C. elegans likely to be extensively integrated into environmental risk assessment procedures. New nematode models are now being...
established in many laboratories in Europe due to their interesting biology, including variations in pathogen responses and defence strategies. They can also be used to tackle environmental challenges: for example, the root-knot nematode *Meloidogyne incognita* causes considerable damage to global agriculture and could serve as a model of plant parasitism and pathogenicity.

Insects are the largest and most diverse group of organisms on Earth. A classic model used over the past century by scientists, including those at EMBL is the fruit fly *Drosophila melanogaster*. Fruit flies can now be used to measure the impact of environmental fluctuations and invasive species *in natura*, in part due to the immense power of genetic, developmental, and phenotypic approaches that exist to analyse *Drosophila* species and their microbiota. *Drosophila* can also serve as an easy and rapid indicator for monitoring local species diversity across ecosystems. These indicators are based on standardised sampling procedures focusing on the identification of key morphologies. During TREC, *Drosophila* species will be collected at each site and analysed for genetic, microbial, and metabolic diversity, each at high resolution. Phenotypic changes due to environmental variables, including insecticide exposure and nutritional changes, can be taken back to the lab and used in a predictive context (see Chapter 3: Cellular and Multicellular Dynamics). The environmental context will be studied by molecular dissection of gene regulatory networks, metabolism, and proteomics at the population and ecosystem levels. In a pilot project, EMBL scientists are using MALDI imaging mass spectrometry for high-throughput metabolic screening of thousands of *Drosophila* embryos to characterise how their metabolism is reprogrammed upon genetic and environmental perturbations. The aim is to understand metabolic profiles and changes, and link these directly to the uptake of exogenous environmentally critical molecules such as pollutants, herbicides, and unwanted drugs. Experimental evolution in the presence of these toxins will enable the systematic analysis of metabolic evolution in a complex animal.

### III. Explore specific communities under controlled environmental conditions

Quantifying the effects of changing environmental conditions at scale and over time by molecular profiling will generate new knowledge about the contributions of genetic variation (both between and within species) and environmental factors in influencing phenotypes. This will, in turn, generate hypotheses that need to be tested and validated under complex but carefully controlled conditions. To fully model natural and anthropogenic environmental changes, ecosystems of interest from planetary biology research will need to be studied in large-scale specialised research infrastructures. Such infrastructures are needed to replicate natural environments and enable scientists to effectively control, measure, and perturb the effects on organism function of specific environmental variables such as levels of nutrients or hydrological, thermal, or xenobiotic factors. The results can be used to create models of complex interactions, ideally moving from perturbation to prediction. This will enable scientists to test for conditions that can change ecosystem properties, and allow desired properties to be introduced or resilience to detrimental factors to be increased. Experiments can start in simple communities, such as carbon fixation in certain algae that form symbiotic relationships with coral, but can gradually build up to more complex ones, such as modelling ecological tipping points in soil and plant microbiomes in arid regions. Depending on the type and complexity of the ecosystem under study, multifaceted microcosms, mesocosms, or ecotrons will be used for ecosystem exploration.

### Ecosystem Research Infrastructures

**Microcosms** are small, artificial, simplified ecosystems that are used to simulate and predict the behaviour of natural ecosystems under carefully controlled laboratory conditions. Associations with environmental factors observed by longitudinal sampling can be followed up in on-site microcosms, with the goal of
performing tests for functional traits or organismal interactions. EMBL has expertise in building and maintaining microcosms and has developed bioreactors to optimise yields for particular microbial products or to stably and reproducibly grow small, defined bacterial communities (Chapter 4: Microbial Ecosystems). The development of bioreactors was a prerequisite for comparing defined perturbations of individual species with synthetic and ex vivo gut microbial communities, with the plan to also incorporate host interactions (Chapter 4: Microbial Ecosystems). While robotic systems to automate perturbations are already in place, technology is being developed to increase throughput and to incorporate integrated molecular profiling and imaging technologies of different resolutions to span spatial scales, from viruses to bacterial communities.

**Mesocosms** are medium- to large-sized, artificially constructed model ecosystems, which provide a link between field surveys and highly controlled laboratory experiments. Mesocosm studies are normally conducted outdoors to incorporate natural variation. Mesocosms provide control over multiple parameters that often confound experiments in natural settings. They also allow controlled testing of toxins that cannot be released into nature, and nutrients that can profoundly disturb ecosystems. Mesocosms are, for example, suited for studying soils, as could be done with samples obtained from the TREC expedition.

**Ecotrons** are large infrastructures designed to reproduce naturally occurring ecosystems and elements in a simplified way. Advanced equipment and technology make it possible to combine different environmental variables according to a defined time cycle, while avoiding undesired variability. On a technical and operational level, an ecotron consists of large chambers, offering the possibility of studying agro-ecosystems. The variables that can be regulated include light (spectrum, intensity, and photoperiod), air (temperature and humidity), rainfall, wind, CO$_2$ and ozone concentrations, and boundary conditions. Ecotrons can also be designed to incorporate genetically modified organisms.

EMBL will create such structures when needed, but will preferentially use existing facilities through partnerships. To gain expertise in these infrastructures and explore potential projects and collaborations, EMBL will engage with existing infrastructures in Europe and beyond (for example, member state ecotrons including AnaEE in France, or AQUACOSM, which brings together 19 aquatic inland and marine ecology experimental platforms from 12 European countries). Small model laboratories are sufficient for chemotrophic and anaerobic microbial communities, but other, more complex communities require experimental ecosystems that are closer to natural settings. In this context, plans are under way to establish microcosms and even a small mesocosm in a new building, the Molecular Biology Centre for Human and Planetary Health, at EMBL’s Heidelberg site.

An example of a controlled environment study in collaboration with researchers in Spain relates to **arid ecosystems** (defined by an average annual precipitation of <250 mm), which make up a large proportion of the Earth’s surface and are in danger of being pushed over an ecological tipping point, beyond which they collapse into desert and are no longer capable of supporting productive ecosystems. As with many ecosystems, there is currently a limited understanding of the molecular processes involved, or the repercussions of this ecosystem collapse. To obtain mechanistic insights and develop molecular biomarkers, *in situ* systems-level analyses will be complemented with studies in controlled experimental settings. EMBL wants to partner with ecologists and geobiologists to take the first steps in exploring **ecological engineering**, or terraformation, of arid soil ecosystems. The plan is to use mesocosms to bring these intact soil ecosystems into partially controlled laboratory conditions. Here, temperature and CO$_2$ levels can be increased, and humidity decreased, to model changes in the soil system in response to induced global warming conditions. Longitudinal metagenomic sampling will allow microbe biodiversity to be surveyed, while metabolomics will be used to explore whether there are metabolic signatures that could be used as biomarkers to warn of ecological collapse. The long-term goal is to increase the resilience of ecosystems to climate change by modelling and predicting the functional traits required by microbes in these ecosystems, which can then be introduced by the microbes carrying them.
Data Collection, Processing, Integration, Analysis, and Dissemination

Longitudinal sampling projects and the characterisation of model species and selected ecosystems under controlled lab conditions will generate data that must be analysed and translated into knowledge. Genomic data must be linked with data from other increasingly utilised molecular profiling techniques such as metatranscriptomics, metabolomics, and metaproteomics (Tech Dev Box TD1_PB), or targeted and untargeted chemical profiling approaches. EMBL also intends to develop technologies to integrate molecular profiling data with molecular, cellular, and environmental imaging (such as X-ray tomography of soil), which requires novel bioinformatics approaches. EMBL is developing data management systems to unify and harmonise workflows, and to integrate and disseminate organismal and environmental data (Chapter 8: Data Sciences). Open science will allow data access and reuse, and will stimulate further research in various fields. Data will be publicly released via specialised integrative data portals (Chapter 10: Scientific Services) to facilitate data analyses by scientific communities.

Technology Development Box TD1_PB | Towards integration of multiple molecular profiling technologies by bioinformatics.

Several omics technologies will be employed to profile model systems in vitro and in natura. Metagenomics, transcriptomics, proteomics, and metabolomics data from marine and soil samples must be integrated using bioinformatics methods, accounting for the strengths and weaknesses of different molecular profiling technologies. For example, metagenomics provides an unprecedented genetic catalogue of biodiversity and functional potential, but metatranscriptomics is better suited for discriminating between ancient DNA and that in living cells, and for quantifying functionality. In turn, both of these techniques give only indirect clues about chemical pollution, which can be more directly measured by targeted and untargeted metabolomics. As each profiling method has its own biases, significant efforts must be made to integrate data from these different approaches. Some measures are more reproducible than others (as shown by the ‘Within technical replicates’ key in the figure), as illustrated in a mock experiment with a synthetic microbial community of gut bacteria. The analysis of this experiment shows the agreement between different methods in terms of the similarity across 80 samples after different perturbations of this community (as shown by the ‘Between datasets’ key in the figure). Considerable efforts are ongoing to normalise these data to add value to their interpretation. Proper quantification is a stepping stone towards modelling interactions between species, and their interactions with the environment.

Benchmarking as prerequisite for integration of molecular profiling: (meta) -genomics, -transcriptomics, -proteomics and metabolomics as well as 16S RNA profiling.

Based on 57 samples of drug-perturbed communities, where consistency in clustering samples by each method independently is measured.

Between datasets

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Based on 50 technical replicates.

Within technical replicates

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Tools for Planetary Health

Detailed knowledge of ecosystem functioning not only serves as a basis for unravelling the crucial molecular mechanisms underlying macroscopic changes, but can also support the development of novel biomarkers for the diagnosis of ecosystem states. As well as the development of novel biosensors (see Section I in EMBL’s Approach), planetary biology research has the potential to contribute greatly to biomonitoring methods. These methods can be used to determine the presence of chemicals, and – more significantly – the way organisms respond to exposure to such environmental chemicals, for example from plastic degradation or pesticides. Molecular biomarkers that can be used to measure the effects of environmental pollutants on organisms and communities at the molecular, cellular, organ, and organism level, and can be integrated with chemical monitoring or population studies to determine the toxic effects of pollutants even when they are present at low, sublethal concentrations. Molecular biomarkers may therefore provide a sensitive early warning of adverse effects that could occur later within populations.

Examples of existing biomarkers of exposure include parameters that reflect exposure to a specific class of pollutants in bivalves or fish as sentinel organisms. Such parameters could be tissue levels of metallothioneins, inhibition of cholinesterase activity, peroxisomal proliferation, or mixed function oxidation. However, these biomarkers may not identify the toxic agent responsible, or may not make a distinction between the exposure to or detrimental effect of a pollutant. With the application of molecular biology technologies and planetary biology approaches, it may now be possible to develop more sophisticated molecular pollutant biomarkers. EMBL’s expertise in genomics, transcriptomics, proteomics, metabolomics, metagenomics, and high-throughput imaging could contribute to the discovery and further development of improved, sensitive molecular pollution biomarkers. In parallel, complex yet controlled laboratory environments such as microcosms and mesocosms could be used to validate and test results from the field. New molecular biomarkers could be specifically associated with a toxicant’s mechanism of action, and could be sufficiently well characterised to relate the degree of biomarker modification to the degree of the adverse effect. In this way, EMBL’s expertise in fundamental research and technology development could contribute to solving societally relevant challenges that are currently being tackled by EU environmental risk assessments.

Impact

A fundamental molecular and mechanistic understanding of biological processes in complex ecosystems will have a far-reaching impact, from both a scientific and societal perspective. New scientific knowledge gained through EMBL’s collaborative networks and research strategies will usher in a new era of life science research. This knowledge will help to provide solutions for some of the major global challenges of our time, such as climate change, pollution, and increased AMR.

Biotic and abiotic environmental perturbations in model organisms, and modelling and modulation in microcosms, mesocosms, and ecotrons, will be key to moving from repertoires and descriptions to molecular mechanisms. Data on global biodiversity and functional traits from planetary biology research projects will be made openly available, and will advance the collective understanding of biology and evolution. EMBL will sample and analyse communities to study the effects of natural and anthropogenic environmental variables, gaining an experimentally derived mechanistic understanding at the molecular level. This will have a wide impact on a number of scientific fields including ecology, the environmental sciences, zoology, and geobiology. The resulting data will help to foster secondary research to bolster conservation efforts, protect and restore biodiversity, and consequently create new benefits for society and human welfare.

Molecular surveys of ecosystems, together with measurements of relevant environmental factors, are also likely to reveal organisms and functional traits of biotechnological relevance, such as those that can increase
carbon fixation. Some highly efficient microalgae have been extensively studied in this respect, but even more efficient groups may exist. These could be revealed using such approaches, as might entirely new functionalities that make the core process of carbon fixation more effective. EMBL's aim to understand the mechanisms by which antibiotic resistance spreads in the environment may facilitate the development of new strategies to combat the emergence of multidrug-resistant pathogens.

Molecular biomarkers may also be identified using field experiments, and validated and further investigated in microcosm and mesocosm settings. These biomarkers could provide an improved understanding of biotic and abiotic interactions within ecosystems, and could enable research to quantify the states of ecosystems. Such discoveries could also have practical applications and be used for assessments of planetary health (Chapter 12: Innovation and Translation). These practical applications would help scientists to understand the effects of pollution levels on an ecosystem and provide an early warning system for potentially irreversible ecological changes in specific at-risk ecosystems. In turn, this could inform policy initiatives across Europe and around the world. The development of diagnostic capabilities and the identification of solutions for specific problems can then be powerfully leveraged by EMBL's member states.

Through this novel scientific endeavour, EMBL in close collaboration with its member states will help to address fundamental and pressing scientific questions about the impact of the environment on biological processes, while also addressing societal questions about planetary health. Planetary biology research represents an extraordinary fusion between intrinsically fascinating biological questions and modern environmental challenges, which EMBL can and must help to overcome.