methodologies in detecting stress gradients (including heavy metal values), from a multiple stressor perspective. The possibility of synergic effects due to the cumulative effect of pressures will also be tested.

05-P Dissolved greenhouse gas concentrations as proxies for emissions: first results from a survey of 43 alpine lakes.

Sylvie Pighini [1] - Georg Wohlfahrt [2] - Franco Miglietta [3]

Fondation Edmund Mach, San Michele all'Adige, Italy [1] - Institute of Ecology, Innsbruck, Austria [2] - CNR IBIMET, Florence, Italy [3]

Up to very recently, freshwater ecosystems were neglected in assessments of the global carbon cycle and considered merely as passive 'pipes' which transport carbon from the land to the oceans. This view has been challenged by an increasing number of studies showing that freshwater ecosystems may negate a substantial fraction of the carbon sink through carbon dioxide (CO_2) and in particular methane (CH_4) emissions and thus rather should be viewed as 'reactors' which process a large fraction of the terrigenous carbon. Most of our knowledge on freshwater CO_2 and CH_4 emissions to date derives from studies in tropical and boreal regions, while temperate freshwater ecosystems are understudied.
This study is focused on lakes from the Alpine area and their content in dissolved greenhouse gases, CH_4 and CO_2. We mostly aim to assess the content of dissolved methane and carbon dioxide from the Alpine lakes in order to understand whether Alpine lakes could be potential CH_4 and CO_2 emitters. We also would like to relate concentrations to lake characteristics and potential biotic and abiotic driving forces.
A diverse set of 43 lakes, from Trentino, South Tirol (Italy) and North Tirol (Austria), was selected resulting in a gradient with respect to elevation (from 240 to 1700 m a.s.l.) and latitude (from 45.52° to 47.38°). Complementary to dissolved CH_4 and CO_2 surface water samples, dissolved oxygen and temperature were measured. Only water surface samples were considered. Analyses were done with a gas chromatographer equipped with a flame ionization detector (FID) for CH_4 and a thermal conductivity detector (TCD) for CO_2 determination.
The first results show that all the sampled lakes were super-saturated in dissolved methane and carbon dioxide concentrations, at least partly to a degree that in the literature has been shown to result in substantial emissions to the atmosphere. To estimate emissions, CO_2 and CH_4 fluxes will be quantified using the floating chamber technique on a subset of the investigated lakes in a next step. Results will indicate which parameters lead to greenhouse gases emissions in the Alpine area.

05-P Phytoplankton response to winter warming modified by large-bodied zooplankton: an experimental microcosm study.

Hu He [1] - Zhengwen Liu [1] - Erik Jeppesen [2]

Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing, China [1] - Department of Bioscience, Aarhus University, Aarhus, Denmark [2]

While several field investigations have demonstrated significant effects of cool season (winter or spring) warming on phytoplankton development, the role played by large-bodied zooplankton grazers for the responses of phytoplankton to winter warming is ambiguous. We conducted an outdoor experiment to compare the effect of winter warming (heating by 3°C) in combination with presence and absence of *Daphnia* grazing (*D. similis*) on phytoplankton standing crops and community structure under eutrophic conditions. When *Daphnia* were absent, warming was associated with significant increases in phytoplankton biomass and cyanobacterial dominance. In contrast, when *Daphnia* were present, warming effects on phytoplankton dynamics were offset by warming-enhanced grazing, resulting in no significant change in biomass or taxonomic dominance. These results emphasize that large-bodied zooplankton like *Daphnia* spp. may play an important role in modulating the interactions between climate warming and phytoplankton dynamics in nutrient rich lake ecosystems.

05-P Proteomic analysis of aquatic microbial responses to nanoparticulate and ionic silver.

Diana Barros, Cláudia Pascoal, Fernanda Cássio

Centre of Molecular and Environmental Biology, Department of Biology, Univ. of Minho, Braga,

Environmental biomarkers are the most promising next generation risk assessment tools, augmenting measurements of direct and highly sensitive responses at the cellular and sub-cellular levels. Silver nanoparticles (AgNPs) are among the mostly used nanoparticles and likely to be released in significant amounts to aquatic environments. Due to their antimicrobial properties, it is relevant to examine whether AgNPs can pose a risk to aquatic microbes in natural ecosystems. We used a bacterial strain, *Pseudomonas* sp. M1 (PsM1), isolated from sediments in a metal-polluted stream, to gain insights into the molecular mechanisms underlying its ability to deal with the toxic effects of AgNPs using a proteomic approach. We identified changes in the protein expression at AgNP concentrations inhibiting biomass production in 20% (EC_{20}). After SDS-PAGE, the LC-MS/MS identified almost 200 proteins, about 50% of which increased its abundance under stress induced by AgNPs and Ag^{+}. Silver is known to react with proteins by combining with the thiol groups of enzymes, leading to protein inactivation. After AgNPs exposure, some of the upregulated proteins were associated with the degradation of transiently denatured and unfolded proteins, accumulated in the periplasm under stress conditions (e.g. periplasmic serine endoprotease). Exposure to AgNPs also induced proteins related to stress response, in particular, antioxidant enzymes, such as catalase-peroxidase and superoxide dismutase. The antioxidant response was consistent with our previous work suggesting that the ability to initiate an efficient antioxidant response is essential for the bacterium to cope with AgNP toxicity. We also found an increase in the proteins involved in amino acid (e.g. ornithine carbamoyltransferase) and energy metabolism (e.g. fructose-bisphosphate aldolase), which may reveal an AgNP-induced reorganization of the metabolic fluxes, that is compatible with an increased need of the bacterial cells to generate energy to support the defense mechanisms against AgNPs toxicity. An increased amount of chaperones (e.g. chaperone protein ClpB) was also found. These proteins play an essential role in the cell by assisting the correct folding of nascent and stress accumulated misfolded proteins and preventing their aggregation. AgNPs can likewise interact with elements of bacterial membranes, causing structural changes, dissipation of the proton motive force, which is consistent with the increase in a specific porin with serine protease activity. Overall, PsM1's response to the stress induced by AgNPs involved, among others, stress response proteins, proteins of the energy metabolism and transport proteins. Since the risk of the appearance of bacterial strains with augmented silver resistance is growing, it is highly recommended that the knowledge obtained from PsM1's response to AgNPs be considered in future studies.

05-P Quantitative weight of evidence approach for hazard evaluation of engineered nanoparticles in freshwaters.

Arunava Pradhan, Cláudia Pascoal, Fernanda Cássio

Centre of Molecular And Environmental Biology (CBMA), Department of Biology / Institute For Science and Innovation for Bio-sustainability (IB-s), University of Minho, Braga, Portugal

Release of engineered nanoparticles (ENPs) into natural waters raises concern about risks to aquatic biota and ecosystem functions. Hazard screening of ENPs is a key step for environmental risk assessment (ERA). But the lack of knowledge and complex nature of ENPs in aquatic environment limit the regulatory frameworks. Moreover, unreliable measures induce impaired and inadequate risk assessment of ENPs in freshwaters.
Weight of evidence (WoE) is a useful framework for constructing individual lines of evidence (LoE) to build conclusions on the degree of impairment. WoE frameworks were suggested for ERA of ENPs since conventional approaches had huge limitations. However, most of the WoE frameworks are qualitative lacking transparency.
We applied quantitative WoE approach based on multi-criteria decision analysis (MCDA) as a transparent and scientifically robust decision analytical framework for hazard evaluation of ENPs in freshwaters to integrate heterogeneous information. We used silver nanoparticles (Ag-NPs) as a proxy of ENPs, because Ag-NPs are among the most abundant ENPs seeking ERA. Peer-reviewed articles providing 140 measurement endpoints (LoE) and information on data quality, exposure, properties and toxicity of Ag-NPs (at different biological organization and trophic levels) in freshwaters were used. The WoE established the LoE-specific "hazard index" of Ag-NPs in freshwaters by calculating "hazard score" based on 3 criteria indices: "physicochemical properties", "toxicity" and "data quality" and their aggregation using "weighted sum". The "physicochemical properties" index was composed of 2 sub-criteria indices based on the influence to exposure or effects of Ag-NPs. Each LoE-specific sub-criterion index was further divided into sets of sub-sub-criteria and so on creating a hierarchy. For instance, "influencing effects" was divided to sub-sub-criteria: "purity", "reactivity", "shape", "biosorption/bioaccumulation", "toxic substance in coating" and "ionic fraction" of ENPs. Each index of the last division in this multi-criteria hierarchy consists of classes (alternatives) assigned with discrete value in [0,100] range. For "toxicity" index, 5 hazard classes were assigned. The "Data quality" index was composed of "adequacy", "reliability" and "relevance" which are also divided into sub-sub-criteria and so on. The "data quality" scores were normalized in the scale [0,1] to construct relative weight in the information pool. The "weights" of decision criteria, sub-criteria or alternatives were assigned by a widely used tool, analytical hierarchy process (AHP), based on