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## 9. Environmental Biogeosciences

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# 9. Environmental Biogeosciences

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## 9.1

### *In situ* microbial oxidation of H<sub>2</sub> in the deep subsurface

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Autotrophic growth is metabolic strategy that is well described but for which there are few studies, particularly in the deep subsurface. The ability of microorganisms to carry out this type of metabolism is of importance due to their independence from sunlight-driven biogeochemical cycling. We performed an *in situ* experiment in which we amended a 25m long borehole, 300 meters deep in a Clay Rock formation in Western Switzerland with hydrogen. The goal of the experiment was to characterize the response of the microbial community to this amendment and to document the succession or parallel metabolisms that ensue.

Within a month of the start of the amendment, Fe(II) and sulfide appeared in the porewater, suggesting microbial Fe(III) reduction and sulfate reduction. Repeated H<sub>2</sub> amendments supported these findings. Isotopic fractionation of S, H, C and O was also evaluated and generally supported the dominance of microbial processes. We evaluated the rate of the microbial sulfate reduction using S isotope fractionation. Additionally, we monitored geochemical changes such as the depletion of sulfate in this low conductivity rock, the rapid consumption of H<sub>2</sub> and resumption of sulfate reduction after a few days of amendment interruption.

Community changes were monitored using 16S rRNA pyrosequencing as well as metagenomic and metatranscriptomic approaches. Pyrosequencing results to date confirm the rapid establishment of sulfate-reducing conditions and the predominance of one or several representative(s) of the family Desulfobulbaceae, Gram-negative sulfate reducing bacteria (SRB) with known genera able to grow on Fe(III), sulfate as well as autotrophic representatives. In fact, analysis of the porewater as a function of time (3 days, 48 days and 101 days after the start of H<sub>2</sub> amendment) shows a steady progression towards an increased contribution of this subset of microorganisms with time. This result is striking in light of the dominance of Gram-positive SRB in anoxic boreholes sampled at the same site. This works suggests that H<sub>2</sub> supports an active microbial community and strongly selects for a subset of SRB. The absence of H<sub>2</sub> at other boreholes precludes the growth of these autotrophic Gram-negative SRB. Current investigations focus on metagenomic analysis of the community to further describe the metabolic diversity within this family as well as to understand the biochemical basis for autotrophic growth. H<sub>2</sub> amendment has been ongoing for approximately 200 days and will continue with the aim to reach methanogenic conditions in the borehole and to further characterize the Archaeal community able to thrive *in situ*. This study is unique due to the unfettered access to the subsurface provided by the Mont Terri facility in Switzerland and the ability to monitor the processes as they unfold and to correlate concurrent geochemical and microbial changes.

## 9.2

# Evaluating the importance of microbial mats for dolomite formation in the Dohat Faishakh sabkha, Qatar

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Research conducted in recent years has demonstrated that microbes can induce dolomite formation at low temperature, providing a possible solution for the long-standing enigma surrounding the origin of sedimentary dolomite. The goal of this study was to evaluate the relationship between microbial activity and dolomite precipitation in the Dohat Faishakh sabkha, Qatar. This hypersaline coastal area was one of the first settings recognized as a rare modern geological environment where dolomite formation occurs (Illing et al., 1965). In previous studies, dolomite formation in this area was considered to be the result of a penecontemporaneous replacement of aragonite. However, no conclusive evidence confirms this hypothesis. To evaluate whether a “microbial factor” is important in this evaporitic environment, we collected core samples and microbial mats along a transect from the lower intertidal to the supratidal zone of the sabkha. X-ray diffraction analysis and measurements of total organic carbon (TOC) revealed the existence of a direct correlation between portions of the cores rich in buried organic material (likely the biomass of partially degraded mats) and dolomite. Oxygen and carbon stable isotope analysis of bulk carbonate samples indicates a strong positive co-variation, suggesting precipitation in isotopic equilibrium within a closed evaporative hydrologic system. Moreover, scanning electron microscopy (SEM) investigations showed that dolomite is not exclusively forming in the supratidal zone, but also in the lower intertidal zone, nucleating within the extracellular polymeric substances (EPS) of living microbial mats. EPS are recognized as an important component for the formation of Mg-rich carbonates. We, therefore, hypothesize that the main factor controlling the occurrence of dolomite within the sediments of the Dohat Faishakh sakha is the presence of EPS (constituting living and buried microbial mats) and not a replacement process transforming primary aragonite into dolomite, as proposed in earlier studies.

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## 9.3

### Impact of microbial surfaces on biogenic birnessite reactivity

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Birnessite minerals (layer-type MnO<sub>2</sub>) are known for their ability to adsorb heavy metals in the environment. They are found in different soil compartments and can be precipitated by a wide range of microorganisms. When produced by bacteria, biogenic birnessite particles are typically deposited in a biofilm matrix comprised by bacteria, extracellular polymeric substances and mineral particles. However, the impact of the biofilm on the sorption behavior of heavy metals by biogenic birnessite is poorly understood. For example, Zhu et al., (2010) showed that heavy metals adsorbed preferentially to biomass when the mineral fraction was small relative to the biomass fraction (<4%). Other researchers using a biomass-mineral assemblage containing 10-20% birnessite by mass found that birnessite significantly enhanced Zn and Ni sorption relative to biomass alone, with trace metals adsorbing preferentially to birnessite cation vacancy sites (Peña et al., 2011; Peña et al., 2010; Toner et al., 2005). Based on these studies we hypothesize that changing the mineral-biomass ratio may cause birnessite-organic interactions that modify the mineral structure and/or the total number of surface sites that can bind metals.

The goal of this study is to evaluate the impact of the biofilm on the reactivity of biogenic birnessite at varying MnO<sub>2</sub>:biomass ratios. The experiments were performed with cultures of *Pseudomonas putida* strain GB-1, known for its ability to oxidize Mn. The birnessite-biomass sorbents were produced using bacterial cultures containing different concentrations of Mn to obtain sorbents with 0 to 14.5 % (on a mass basis) birnessite. We combined adsorption experiments for Ni at pH 6 with electron microscopy and X-ray absorption spectroscopy to better understand the molecular-scale reactivity of biogenic birnessite.

Our results indicate a difference in the adsorption reactivity of the composite biomass-birnessite assemblage depending on the mineral to organic ratio. Larger Ni uptake (molNi.mol<sup>-1</sup>Mn) was observed at low birnessite:biomass ratios than when birnessite is present in greater quantity. Our results suggest that the biomass fraction (bacteria and EPS) of the sorbent and the bacterial growth conditions play an important role in governing Ni removal. The impact of bacterial growth conditions on the composition of the biomass, mineral structure (e.g., vacancy content) and the interaction between mineral and organic matter should be accounted for in predicting the sorption behavior of complex microbe-mineral assemblages.

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## 9.4

# Utilizing natural abundance variations in radiocarbon to probe microbial metabolism in the environment

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Recent studies have revealed a remarkable diversity of microorganisms in the environment, as well as surprisingly diverse environmental conditions that appear conducive to sustain microbial life. A key challenge related to such findings concerns the elucidation of microbial metabolisms associated with specific environmental conditions and biogeochemical processes. The natural abundance of radiocarbon, with its half-life of approximately 5730 years, provides a powerful tool to probe metabolism of carbon-containing compounds in systems where distinct age contrasts exist in potential carbon sources available to microbial communities. With emerging methods that enable radiocarbon measurements on microgram quantities of carbon, it is now feasible to target an increasingly broad range of environmental systems, and to examine radiocarbon signals associated with individual organic compounds that may be characteristic of specific members of a microbial community. This presentation will provide an overview that seeks to highlight the manner in which natural-abundance radiocarbon measurements can help to shed light on carbon sources and processes associated with microbial communities in terrestrial and marine environments. Examples will be provided both for natural environment systems as well as those impacted by anthropogenic activity.

## 9.5

## Use of a trait-based framework for predicting where microbial adaptation to climate change will affect peatland functioning

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As the earth system changes in response to human activities, a critical objective is to predict how biogeochemical process rates (e.g. carbon cycling) and ecosystem function (e.g. microbial decomposition) will change under future conditions. An important question within this context is the effect of warming on the microbial communities which control carbon cycling (Wallenstein & Hall 2012). Understanding linkages between microbial diversity and function is then essential if we are to increase our knowledge of the control mechanisms involved in biogeochemical cycling in ecosystems. Although microbial communities can adapt to changes in temperature and precipitation regimes, they are typically not optimally adapted to their local environment. Here we addressed the above by examining the effect of climate warming on the microbial food web and belowground functioning in temperate peatlands using a trait-based framework.

We conducted two different climate experiments in peatlands situated in the Jura and Alps mountains (France and Switzerland): a warming manipulation experiment by Open Top Chambers (OTCs) and an altitudinal experiment. The complex interplay between temperature increase, microbial community structure, microbial traits, microbial food web structure and ecosystem processes such as carbon cycling was studied in both studies. We focus our work on main microbial consumers within the microbial food web in peatlands: the testate amoebae, a diverse group of common soil protozoa. Samples of water were collected to determine the concentrations of dissolved organic carbon (DOC) - a sensitive indicator of changes in C cycling (Neff and Asner 2001).

We show that the community could be separated into two main categories, high vs. low trophic position taxa based on their shell-aperture size/body size ratio. This approach combined with the Community Size Spectrum (CSS) approach show that warming by OTCs reduced the size structure of testate amoebae. We found warming by OTCs excludes specialist microbial taxa such as top-predators (-70%) over two warming years. This, in turn, increased the functional redundancy of the main microbial consumer community. Similar tendencies were found along the altitudinal gradient, where the abundance of high trophic level testate amoeba species as well as the CSS increased from lowest to highest elevation (respectively by 3.7x and 6x). We further found in both experiments that changes in CSS of testate amoebae were concomitant to increases in DOC (Fig. 1), suggesting that decreased testate amoeba size structure enhance microbial decomposition and thus increase C turnover within the food web. These findings suggest that climate-induced changes in microbial community structure represent a mechanism controlling the carbon balance of peatlands.

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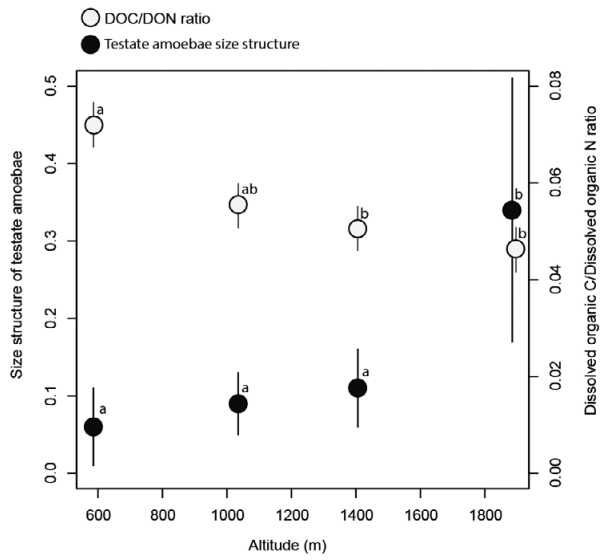


FIGURE 1: Size structure of testate amoebae and ratio of dissolved organic carbon over dissolved organic nitrogen in pore water at different altitude. Different superscripts indicate significant differences between sites for each variable ( $P < 0.05$ ,  $n = 77$ ).

## 9.6

# Natural organic matter as a fully regenerable terminal electron acceptor for anaerobic microbial respiration in temporarily anoxic systems

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### Introduction

Natural organic matter (NOM) plays a key role in electron transfer reactions in many element cycles, including the carbon cycle. NOM is particularly important in temporarily anoxic environments with a high NOM content, such as wetlands and peats. In these system, NOM may serve as terminal electron acceptor (TEA) in anaerobic microbial respiration under anoxic conditions. Reduction of NOM may competitively suppress electron transfer to other TEAs, including CO<sub>2</sub>, and hence suppress hydrogenotrophic methanogenesis. Upon re-aeration, reduced NOM moieties may be re-oxidized by the transfer of electrons to O<sub>2</sub>. Despite the overall importance of NOM redox cycling to the biogeochemistry of temporarily anoxic environments, the changes in NOM redox state over successive microbial reduction and O<sub>2</sub> re-oxidation cycles have not been explicitly investigated. In previous studies, such changes in NOM redox states were difficult to quantify due to the lack of appropriate methods.

In this study we applied a novel electrochemical method, to (i) assess the reversibility of electron transfer to/from NOM over microbial reduction and O<sub>2</sub>-re-oxidation cycles (ii) to determine the sustainability of electron transfer over repeated redox cycles and (iii) to elucidate the thermodynamics of microbial NOM reduction.

### Method

We recently introduced mediated electrochemical analysis as a novel approach to quantify the redox states of dissolved NOM (Aeschbacher et al. 2010). This method employs electron transfer mediators to facilitate electron transfer between NOM and the working electrode of an electrochemical cell. The redox states of NOM are characterized by mediated reduction and oxidation towards defined reductive and oxidative potentials. The number of transferred electrons is measured directly as reductive and oxidative current responses which, upon integration, yield the electron accepting (EAC) and electron donating capacities (EDC). The EAC and EDC measurements are complemented by mediated potentiometric redox potential ( $E_h$ ) measurements in which small amounts of the mediators are used to facilitate  $E_h$  equilibration.

For this study; we conducted laboratory incubation experiments with four humic acids (HAs), which were both from aquatic and terrestrial sources and were chosen to represent a broad range of NOM. The HAs were inoculated with the facultative anaerobe *Shewanella oneidensis* MR-1 and lactate as electron donor. Using the electrochemical approach described above, we quantified the changes in redox states of the HAs over successive cycle of microbial reduction under anoxic conditions, followed by O<sub>2</sub> re-oxidation (Klöpffel et al, 2013, submitted).

### Results & Discussion

Anoxic incubations of HAs with *S. oneidensis* resulted in extensive microbial HA reduction, as evidenced by decreasing EAC and increasing EDC values. No changes in the redox states of the HAs were observed in sterile controls incubated under the same conditions. Introduction of O<sub>2</sub> resulted in re-oxidation of the microbially reduced NOM, as shown by increasing EAC and decreasing EDC values. The sum of EAC and EDC (=electron exchange capacity, EEC) in the inoculated samples stayed constant over the time course of the experiment (3 months) and was statistically indistinguishable from the EEC of the HAs in the sterile control samples. A constant EEC and hence number of redox active moieties in HAs implies that no redox active groups in the HA were being destroyed or created during the redox cycling. In addition, the constant EEC demonstrate reversible electron transfer to and from HA in single redox cycles and sustainable redox buffering by the HAs over consecutive redox cycles.

The use of mediated  $E_h$ -measurements revealed that the extents of microbial reduction of the different HAs was limited by system thermodynamics: Even though the four HAs used in the experiment exhibited very differing redox properties (e.g., EACs), they were reduced by *S. oneidensis* to comparable redox potentials,  $E_h$ , between -0.18 V and -0.2 V (pH 7; reported against the Standard Hydrogen Electrode). These potentials were much lower than the potentials of the unreduced HAs (0 V <  $E_h$  < +0.1 V). This implies that the tested HAs accepted electrons over wide  $E_h$  ranges, including iron reducing, sulfur reducing and methanogenic conditions.

### Implications

To assess the importance of NOM redox cycling for carbon cycling in temporarily anoxic environments, we compared methane fluxes reported for northern peatlands with the number of electrons that can be transferred to NOM under anoxic conditions and subsequently to O<sub>2</sub> under oxic conditions. For this calculation, we assumed 20 cm of annual water table fluctuation, which was reported in literature for peatlands, and EAC values for dissolved organic matter (from this work) and particulate organic matter (Roden et al. 2010). This estimation showed that NOM redox cycling may lead to a suppression of methane fluxes of 190'000 mol CH<sub>4</sub> per km<sup>2</sup> a<sup>-1</sup>, which corresponds to between 10 and 166% of reported average CH<sub>4</sub> fluxes from northern peatlands.

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## 9.7

# Utilization of Silicate Minerals for pH Control during *In Situ* Bioremediation of Chlorinated Solvents

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Chloroethenes such as tetrachloroethene (PCE) and trichloroethene (TCE) are among the most prevalent contaminants in groundwater due to their extensive use in industrial processes. *In situ* bioremediation (ISB) is an attractive technology for the removal of these compounds. This technology has been widely used for treatment of chloroethene plumes and recent studies have shown promising results for removal source zones containing dense non-aqueous phase liquids (DNAPLs). However, application of source zone ISB is still a significant technical challenge. One of the main issues is groundwater acidification due to organohalide respiration and fermentation processes, which can inhibit the activity of dehalogenating micro-organisms. The main objective of this work was to develop an efficient pH control strategy for chloroethene ISB by using the acid-neutralizing potential of silicate minerals. These minerals are of particular interest as their dissolution rate and their solubility is pH-dependent with faster kinetics and higher solubility in the acidic range. In addition, they persist in the subsurface and are long term alkalinity sources. Their usage therefore potentially reduces the frequency of injection of buffering solution.

To assess the potential of this technology, modeling and experimental approaches were combined. A geochemical model, implemented within PHREEQC, was developed to select appropriate buffer candidates and to help determine main parameters influencing mineral buffering capacity. The model included microbial degradation kinetics, mineral dissolution kinetics and chemical speciation. Anaerobic microcosm experiments were conducted to compare the buffering capacity of five silicate minerals in presence of organohalide-respiring bacteria (OHRB). Finally the long-term buffering capacities of three minerals (forsterite, fayalite and diopside) in porous media were investigated in flow-through column experiments. The columns were bioaugmented with an organohalide-respiring consortium containing *Dehalococcoides* and were operated at close to saturation concentrations to reproduce the conditions found in the contamination source zone. The distribution of the microbial communities at the end of the experiment was evaluated by molecular biology analyses.

The results of model simulations confirmed that silicate minerals are promising candidates for pH control of groundwater undergoing ISB. The efficiency of the system is dependent on mineral dissolution kinetics, equilibrium constants, temperature, and reactive surface area. The developed model allows the estimation of the amount of mineral needed to maintain the pH in the neutral range for specified site characteristics. Results of microcosm experiments with silicate minerals demonstrated that, under the selected conditions, diopside, fayalite, forsterite and andradite were able to maintain the pH in the appropriate range for PCE degradation, i.e., between 5.5 and 7.5. However, minerals containing iron III such as andradite had inhibitory effect on organohalide respiration due to their influence on the redox potential.

The results the column experiment showed that olivine minerals (such as fayalite and forsterite) are suitable agents for long-term pH control. They successfully maintained the pH in the neutral range (7.5 for forsterite and 6.5 for fayalite) and sustained the activity of OHRB. In contrast, the buffering potential of diopside rapidly decreased due to the formation of a less-reactive cation-depleted leached layer at the mineral surface. This study demonstrated the potential of silicate minerals to act as a long-term source of alkalinity release for groundwater pH control. This technology was applied here to the particular case of chlorinated solvent ISB but can be extended to any groundwater remediation technology requiring near neutral pH conditions.



## 9.8

# Toxicity of mercury towards *Chlamydomonas reinhardtii* in presence of perfluorooctane sulphonate

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In natural waters, aquatic organisms are exposed to cocktails of numerous inorganic and organic micropollutants. However, if algal response to one toxicant at a time is now better known, their sensitivity to mixture of pollutants is still poorly understood. The present study aims to fill this gap and examines the effect of mercury (Hg) and perfluorooctane sulphonate (PFOS) mixtures towards the unicellular green algae, *Chlamydomonas reinhardtii*. PFOS is a synthetic surface-active compound, which, such as Hg, is environmentally persistent and accumulates in wildlife around the globe (Giesy & Kannan 2002; Houde *et al* 2006). PFOS is also known to increase algal membrane permeability (Liu *et al* 2008). Our working hypothesis is thus that the presence of PFOS will modify algal Hg accumulation and as such increases its toxicity.

To that end, *C. reinhardtii* is exposed for 48 h to increasing concentrations of Hg (0.1  $\mu\text{M}$  to 1  $\mu\text{M}$  – 92% under  $\text{HgCl}_2^0$  form) and three concentrations of PFOS (0.01  $\mu\text{M}$ , 1  $\mu\text{M}$  and 100  $\mu\text{M}$ ), alone and in combination. Algal growth as well as pollutant impact on its physiology is determined using flow cytometry and fluorescence microscopy. Cell dying with propidium iodide is used to assess modifications of algal membrane permeability. Additionally, intracellular Hg concentrations are measured with an Advanced Mercury Analyser after washing algal cells with cysteine.

Algal growth remains optimal up to 0.2  $\mu\text{M}$  Hg, whereas higher Hg concentrations (0.4  $\mu\text{M}$ ) lead to 27 % of growth inhibition. No additional physiological effects such as modifications of cell size, granularity or chlorophyll *a* content are measured at the studied Hg concentrations. PFOS alone does not affect algal growth or the studied physiological parameters up to 100  $\mu\text{M}$  whereas no effect on algal membrane permeability is observed. Mixtures of 0.2  $\mu\text{M}$  Hg or 0.4  $\mu\text{M}$  Hg with 0.01  $\mu\text{M}$  PFOS and 1  $\mu\text{M}$  PFOS induce similar growth inhibition as exposure to Hg alone. However, exposure to 0.4  $\mu\text{M}$  Hg or 0.6  $\mu\text{M}$  Hg with 100  $\mu\text{M}$  PFOS leads to about 2.5 times higher toxicity towards algae than exposure to Hg alone. This result could be partly explained by a 1.5-time increase of intracellular Hg concentration in these conditions. The present study demonstrates that Hg can be more toxic to algae when exposed in combination with other pollutants (here PFOS) than alone and that aquatic organisms may not be fully protected by established legislation due to mixture effects.

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## 9.9

# Hot topics in environmental biogeosciences; the microbiology of the nuclear fuel cycle

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Microorganisms are able to colonise some of the most extreme environments on Earth, including highly radioactive environments associated with the nuclear fuel cycle. In nuclear facilities, their proliferation as biofilms or planktonic “blooms” can cause operational challenges and concerns regarding the long-term stability of stored nuclear waste. However, in contaminated land they can have a controlling influence on the solubility of actinides and fission products, and here microbial activity can be harnessed for non-invasive bioremediation applications. In the “far field” deep geosphere surrounding underground nuclear repositories, microorganisms can also immobilise redox active radionuclides via respiratory processes that either change directly the oxidation state of the element, or produce new biogenic phases for enhanced sorption. In the “near field” of the repository, where higher level wastes are located, the direct and indirect impacts of microbial metabolism are less well characterised but have the potential to have a significant impact on waste-form evolution and radionuclide mobility, and must be incorporated into the safety case of the repository.

Recent work on the molecular ecology of a range of nuclear facilities will be discussed, and the impact of microbial metabolism on various steps of the nuclear fuel cycle discussed. Focusing on contaminated land and geodisposal scenarios, this talk will also discuss the biogeochemistry and redox cycling of priority radionuclides including U, Np, Pu and Tc in the subsurface, including both reduction and oxidation reactions and their impact on soluble and insoluble radionuclide inventories. The roles of proteins, secreted electron shuttles and other microbial products will be discussed alongside additional controls coupled to bulk element cycles e.g. the production of new mineral phases or significant changes in the geochemical environment such as pH. Studies from a range of contrasting natural and engineered systems will highlight how microbial communities can respond to the radioactive inventory and the extreme (radio)chemistry of some disposed wastefoms, and ultimately control the biogeochemical fate of key radioactive elements, including new studies on microbial gas metabolism, metal chelate (ISA) biodegradation and radionuclide biotransformations mediated via direct and indirect interactions with microbial systems.

## 9.10

### Hints towards a biogenic origin of Asperge Cave speleothems

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Asperge Cave (Hérault, FR) is 7 km long, 126 m deep, and opens at the contact between schist and marble (Alabouvette et al., 1982). Limited zones of the cave present acicular-, coralloid-, and bulb-shaped aragonitic speleothems, as well as beaded aragonite which formation is still unexplained (Davis, 2012), (Fig. 1). The presence of blue aragonite is related to the important copper and uranium concentrations in the ceiling schists, as revealed by XRF analyses. Atypical morphologies such as U-loops, as well as field observations of mycelium-like filaments and microbial biofilms (Fig. 2) suggest that the speleothems could be biogenic. In addition, SEM analyses (Fig. 2C) showed that the coralloid concretions present extracellular polymeric substances (EPS) on the inner and outer parts, suggesting that calcite precipitation could be mediated by these EPS.

In order to investigate the microbial diversity, environmental DNA was sampled in portions of the cave presenting the speleothems. The bacterial diversity was proven different from other European caves diversity and consisted in 30 to 60% of putative new species, genera or higher clades. Cultivable bacteria isolated from the biofilms were checked for their ability to induce carbonate precipitation. Strains belonging to the phylum Actinobacteria were able to induce carbonate precipitation, in agreement with similar cave studies (Diaz-Herraiz et al., 2013).

Due to the high concentrations of copper in the ceiling schists, copper tolerance was assessed for the cultivable bacteria isolated from the biofilms. Most of the strains were tolerant to high concentrations of cupric ion, with some strains resisting up to 1g Cu<sup>2+</sup>.L<sup>-1</sup>. Uranium tolerance has not yet been assessed, but genetic analyses showed that a third of the strains from the concretions room were closely related with strains sequenced in highly radioactive environments.

We hypothesize that the heavy metal tolerances could be linked to detoxification via co-precipitation of calcite and heavy metals. This precipitation could be mediated by biofilms' EPS as it has been shown in other studies (Decho, 2010).

Further investigations would allow to (1) inventory new diversity; (2) understand these speleothems' genesis and mineralization mechanisms; (3) search for bioremediation processes for heavy-metal and radioactive polluted environments.

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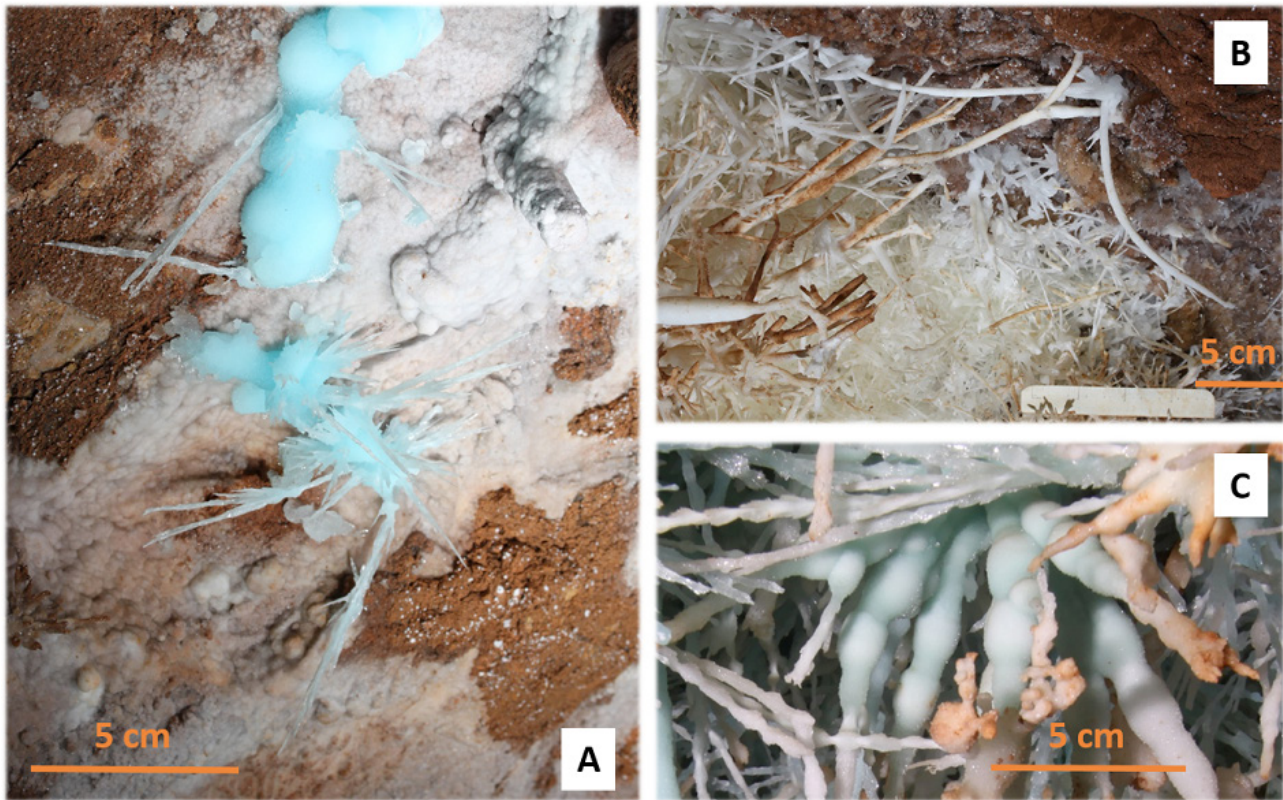


Figure 1. Various aragonite morphologies. A) Acicular and bulb-shaped aragonite; B) Coralloid aragonite; C) Beaded and acicular aragonite. Credits: Nicola Tisato

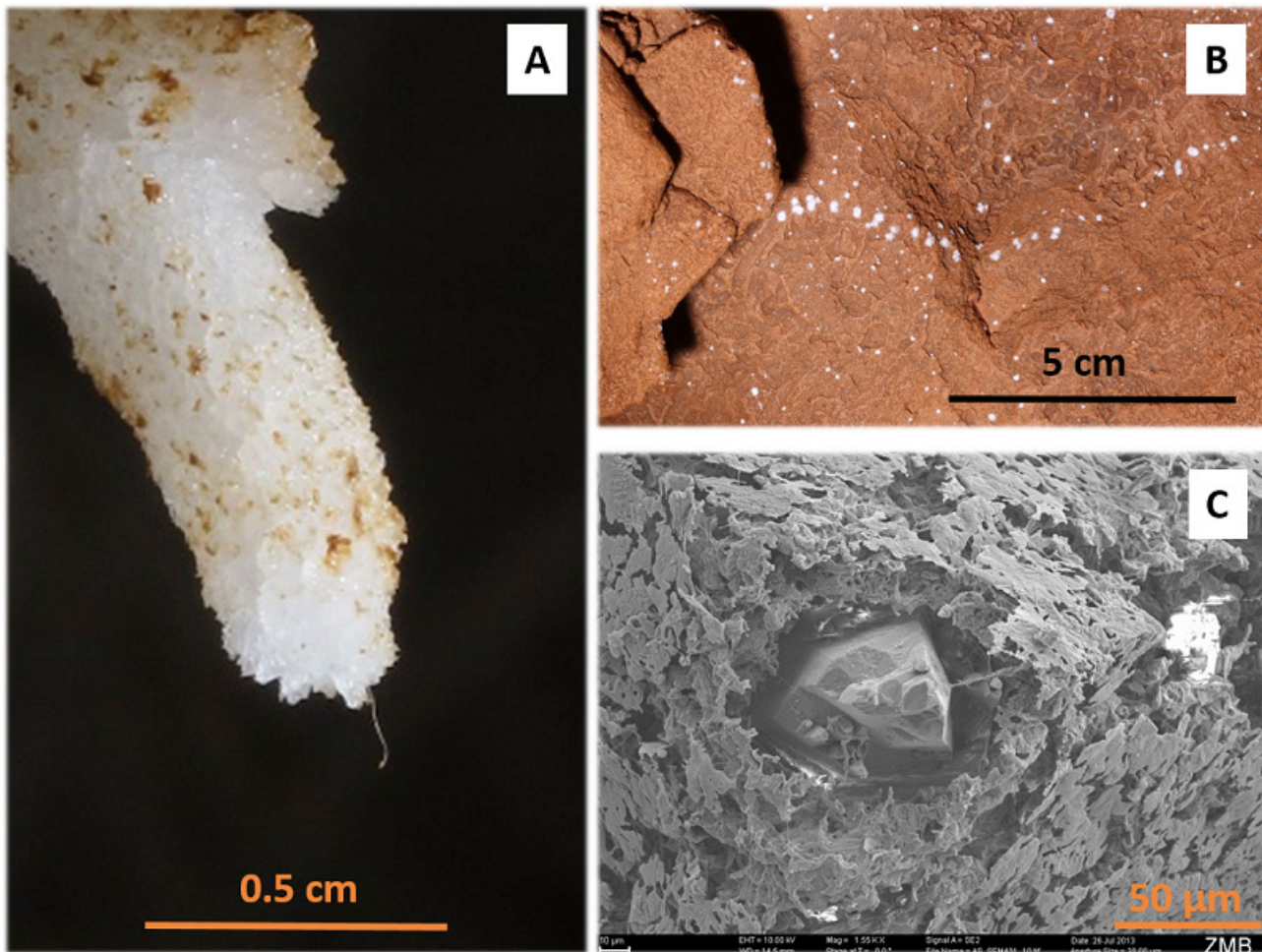


Figure 2. Hints towards a biogenic origin. A) Mycelium-like filament at the tip of a coralloid concretion; B) Microbial biofilms in the vicinity of the concretions; C) Calcite crystal of a coralloid concretion surrounded by extracellular polymeric substances. Credits: Nicola Tisato



## 9.11

### Composition and structure of fresh and aged Fe oxidation products

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The oxidation of dissolved Fe(II) by O<sub>2</sub> leads to the formation of amorphous to poorly crystalline Fe(III)-precipitates. These precipitates control the fate of major and trace elements at redox-interfaces and play an important role in many natural and technical systems. Dissolved phosphate (P), silicate and Ca are major factors controlling composition and structure of fresh Fe(III)-precipitates [1, 2, 3]. In this study, we therefore investigated (i) the interdependent effects of P/Fe ratio, Si and Ca on the composition and structure of fresh Fe(III)-precipitates formed by oxidation of 0.5 mM Fe(II) in bicarbonate-buffered solution at pH 7.0 and (ii) changes in precipitate composition and structure during aging for 30 days at 40 °C.

During Fe(II) oxidation, mostly lepidocrocite (Lp) formed at initial P/Fe ratios below ~0.1. Phosphate was nearly completely co-precipitated with Fe(III) up to an initial molar P/Fe ratio of ~0.55 in the absence of Ca and ~0.75 in the presence of dissolved Ca. Above these P/Fe ratios, only amorphous Fe(III)-phosphate formed and phosphate removal was incomplete. Enhanced co-precipitation of phosphate and Ca with Fe(III) was attributed to electrostatic effects and to the formation of mixed Ca-Fe(III)-phosphates, with a fraction of Fe(III)-octahedra exhibiting mitridatite-like corner- and edge-sharing linkage. Silicate did not interfere with the initial phosphate uptake but inhibited Lp formation at low P/Fe ratios, instead promoting the formation of hydrous ferric oxide (HFO; ferrihydrite-like polymers with limited corner-sharing linkage of Fe(III)-octahedra). Continuing Fe(III) polymerization during aging led to the remobilization of phosphate, especially in the absence of Ca and silicate. Phosphate remobilization was limited in the presence of Ca, which stabilizes mixed Ca-Fe(III)-phosphate, and especially silicate, which inhibits Fe(III) polymerization into crystalline Fe(III)-precipitates.

The results from this study form the basis for an improved mechanistic and quantitative understanding of Fe(III)-precipitate formation and trace element co-sequestration at aquatic redox-interfaces and in technical systems, for example drinking water treatment for As removal.

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## 9.12

## Time-resolved *in situ* investigation of metal sorption mechanisms in layer-type manganese oxides

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Manganese oxides are minerals commonly found in aquifers, soils, and sediments and are thought to originate mostly from microbially catalyzed Mn(II) oxidation. They are characterized by a layer-type crystal structure with hexagonal sheet symmetry and abundant vacant Mn(IV) sites. Due to their high sorption capacity for metals, these minerals strongly influence the distribution, mobility and bioavailability of the metals in the environment.

Traditionally, the mechanisms of trace metal sorption on mineral surfaces are studied on pre-equilibrated samples by bulk X-ray absorption spectroscopy (XAS). In general, metals have been shown to sorb via the following mechanisms: 1) sorption at the vacancy sites via triple-corner sharing (TCS) coordination; 2) incorporation into the vacancy site (Inc); 3) sorption at the particle edges via double-corner or double-edge sharing coordination (DCS or DES). The coordination geometry of adsorbed metals varies with pH, mineral structure, and nature of a metal. For example, at the surface of both biotic and abiotic birnessite Co(II) is sorbed as a TCS complex, which can be oxidized to Co(III) and incorporated into the MnO<sub>2</sub> sheet resulting in a mixture of TCS and Inc (Manceau et al, 1997). In contrast, the bonding environment of Ni highly depends on the type of birnessite and pH. At pH 6 at the surface of biogenic birnessite Ni(II) can sorb as TCS complex, while at pH 8 it occurs as a mixture of Inc and TCS species (Peña et al, 2010). In the case of abiotic birnessite ( $\delta$ -MnO<sub>2</sub>), the surface speciation of Ni significantly differs at pH 6 where Ni is found as a mixture of TCS, DCS and DES, while at pH 8 the bonding mechanism closely resembles that observed at the biogenic birnessite (Simanova et al 2013).

Detailed knowledge of trace metal sorption mechanisms is crucial for predicting the mobility, bioavailability and thus environmental fate of heavy metals. However, for more complete understanding we also need to acquire knowledge about the time scale over which different surface species are formed. Previously, the sorption speciation has been probed using the bulk XAS measurements of samples pre-equilibrated for hours to days, while evolution of different surface species may lie in second-to-minute time scale. In this study, we apply quick X-ray absorption spectroscopy (QXAS) to gather time-resolved *in situ* XAS spectra of Co or Ni sorbed at the birnessite particles at different surface loadings and pH values. We collected Co and Ni K-edge QXAS spectra to follow the changes in the bonding environment and Mn K-edge QXAS spectra to monitor any changes in Mn oxidation states and birnessite structure. Information on time-resolved evolution of different surface complexes will help us better understand the reactivity of different surface sites (e.g., vacancy sites, particle edges) towards metal sorption.

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## 9.13

## Plant oils used to maintain bone and antler tools in the Neolithic lakeshore settlement, Zurich Opera Parking

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A not rare and still unclear observation in Neolithic Alpine lakeshore settlements is that a large number of archaeological bone and antler tools with clearly different functions like massive chisels made of bones from cattle or red deer (used for processing wood or antler), small points made of sheep or goat bones and bigger points made of bones from cattle or red deer (for processing leather or textiles), combs made of ribs from cattle or deer (for processing plant fibres), scrapers made of red deer antler and even sockets or sleeves (used to connect axes with the wooden handles) made of red deer antler show clear shiny and apparently polished surfaces (e.g. Schibler 2013). The so different materials that were worked with a variety of tools could not have produced these brightness areas, and other explanations must be sought.

We explore the origin of such shiny surfaces by molecular and isotopic analyses of the lipids staining freshly unearthed and not conserved bone and antler artifacts from the recent excavation at the underground parking garage for the Zurich opera house dating to about 3230 to 2729 BC. A set of 10 artifacts, covering distinct osteological and typological features was selected for this study. Most of the artifacts are made of bones or antler from red deer (*Cervus elaphus*) or bones from large ruminants, such as cattle (*Bos taurus*) or red deer. One artifact is made of a metatarsus from roe deer (*Capreolus capreolus*). For each tool the lipids were extracted separately from the tapered thinner working part and the wider handling part. The saponifiable and unsaponifiable lipids were analysed by GC-MS and GC-C-IRMS. The overall extractable lipid distribution is characterized by an important amount of animal sterols (cholesterol, cholestanol, coprostanol, epicholestanol), waxy *n*-alkanes, *n*-alkan-1-ol and phytosterols ( $\beta$ -sitosterol, sitostanol), and abundant saturated, mono- and polyunsaturated fatty acids in the C<sub>14</sub>-C<sub>24</sub> range. The high concentrations of C<sub>18</sub> polyunsaturated acids, linoleic (18:2) and linolenic (18:3) acids, clearly indicate that oil plants are overprinting the indigenous animal lipid signature. The  $\delta^{13}\text{C}$  values of the main fatty acids confirm that the archaeological samples were stained with C<sub>3</sub> plant oils. C<sub>3</sub> vegetable oils rich in 18:2 and/or 18:3 may be obtained from seeds of poppy (*Papaver somniferum*; 50-75% 18:2), flax (*Linum usitatissimum*; 15-30% 18:2, 45-50% 18:3), rape (Brassicaceae; 8-30% 18:2, 0.1-3% 18:3), sunflower (*Helianthus annuus*; 48-74% 18:2), safflower (*Carthamus tinctorius*; 83% 18:2), *Sesamum indicum* (sesame; 48% 18:2), and hazelnut (*Corylus avellana*; 55% 18:2) (Gunstone and Harwood, 2007). Flax and poppy seeds are extremely frequent in important amounts in the Neolithic lakeshore settlements in central Europe (Jacomet 2007). Both, flax and poppy were domestic oil and/or fiber plants. Seeds from the wild oil plant turnip (*Brassica rapa*) and also hazelnut shells are common in the Neolithic lakeshore settlements, whereas the other above-mentioned 18:2 rich oil plants were not present in Neolithic Europe (*Helianthus annuus*, *Sesamum indicum*) or extremely rare (*Carthamus tinctorius*) (e.g. Jacomet 2007). The fatty acid concentrations, fatty acid and sterols concentration ratios, and  $\delta^{13}\text{C}$  values of individual fatty acids show significant correlations. Principal component analysis was used to define the chemical associations characterizing the indigenous animal lipid signature, the exogenous staining lipids, and (bacterial) alteration of the primary lipids at the archaeological site.

The results indicate that most of the shiny and polished surfaces of the bone and antler artifacts is not the result of the use but is due to a combination of preserved indigenous animal lipids and plant oils staining the tools.

Now we have archaeological and chemical evidence that the Neolithic craftsmen of the late 4<sup>th</sup> and early 3<sup>rd</sup> millennium BC, used specific tools deliberately fashioned, pre-treated and maintained with a preservative material based on plant oils most likely obtained from seeds of *Linum usitatissimum* and *Papaver somniferum*, with probably some minor contribution of *Brassica rapa*.

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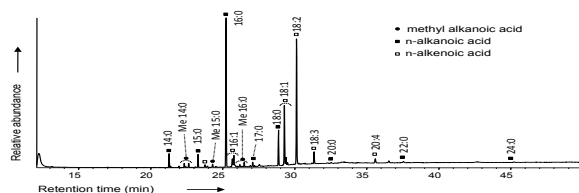


Figure 1. Representative GC-MS total ion chromatogram of the fatty acid methyl esters in the lipids extracted from the bone or antler artifacts recovered at Zurich opera parking.

## 9.14

### Uranium(IV) mobility in a mining-impacted wetland

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Wetland soils are known to accumulate uranium and other heavy elements. The sorption of U(VI) onto solid phases in soil and precipitation of sparingly soluble U(IV) minerals under reducing conditions are thought to be the main processes of U immobilization within wetlands. We studied a wetland, located near a former U mine in central France (Limousin), which was impacted by mining activity and exhibited a few U hotspots (~4,000 ppm). We observed U release into a stream flowing through the wetland, as evidenced by a steady increase in the concentration of U in the stream as a function of distance from the entry point into the wetland. To elucidate U speciation and the U release mechanism, soil and porewater samples were collected from two selected U hotspots in the wetland as a function of depth and season and in such a way as to preserve the redox state. High porewater Fe(II) concentrations and decreasing  $\text{SO}_4^{2-}$  concentrations with depth were indicative of the onset of Fe(III) and  $\text{SO}_4^{2-}$  reduction, respectively. Microbial analysis showed sulfate-reducing bacteria (i.e., Desulfobacteraceae and Thermodesulfobionaceae) and microorganisms potentially capable of iron and uranium reduction (e.g., Clostridiales), supporting the microbial origin of  $\text{SO}_4^{2-}$  and Fe reduction. Surprisingly given the prevailing reducing conditions, high U concentrations (~1  $\mu\text{M}$ ) were observed throughout the porewater profiles while high soil U content was restricted to the top 30 cm. Using laser fluorescence spectroscopy (LFS) and X-ray absorption spectroscopy (XAS) at low temperature, we showed that tetravalent U is predominant in both porewater and soil, and U in the soil occurs primarily as a non-crystalline U(IV) species: U(IV) adsorbed onto amorphous Fe-Al-P-Si aggregates. Transmission electron microscopy (TEM) and ultrafiltration analysis demonstrated U association with colloidal  $\text{Fe}(\text{OH})_2$ -organic matter assemblages in porewater, which could lead to the observed U(IV) mobility. Hence, U(IV) species in the porewater are distinct from those present in the soil, suggesting the release of a labile form U(IV) from soil. Moreover, U(IV) association with mobile colloids ultimately results in its release into the stream, despite the reducing conditions and the low solubility of U(IV) phases. The unexpected mobility of U(IV) in this system brings into question the often assumed immobilization of U through (bio)reduction, particularly in high organic matter environments such as wetlands.



## 9.15

### Metabolic interdependencies between phylogenetically novel fermenters and respiratory organisms in an unconfined aquifer

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Fermentation-based metabolism is an important ecosystem function often associated with environments rich in organic carbon, such as wetlands, sewage sludge, and the mammalian gut. The diversity of microorganisms and pathways involved in carbon and hydrogen cycling in sediments and aquifers and the impacts of these processes on other biogeochemical cycles remain poorly understood. Here we used metagenomics and proteomics to characterize microbial communities sampled from an aquifer adjacent to the Colorado River at Rifle, Colorado, USA, and document interlinked microbial roles in geochemical cycling. The organic carbon content in the aquifer was elevated *via* two acetate-based biostimulation treatments. Samples were collected at three time points, with the objective of extensive genome recovery to enable metabolic reconstruction of the community. Fermentative community members include genomes from a new phylum (ACD20), phylogenetically novel members of the Chloroflexi and Bacteroidetes, as well as candidate phyla genomes (OD1, BD1-5, SR1, WWE3, ACD58, TM6, PER, and OP11). These organisms have the capacity to produce hydrogen, acetate, formate, ethanol, butyrate, and lactate, activities supported by proteomic data. The diversity and expression of hydrogenases suggests the importance of hydrogen currency in the subsurface. Our proteogenomic data further indicate the consumption of fermentation intermediates by Proteobacteria can be coupled to nitrate, sulfate, and iron reduction. Thus, fermentation carried out by previously unstudied members of sediment microbial communities may be an important driver of diverse subsurface biogeochemical cycles.

## P 9.1

# When a century is enough to invert a million years' pedogenesis: the oxalate carbonate pathway in tropical agro-ecosystems

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Ferralsols are intertropical soils that spread over ca. 750 millions of ha on three continents (WRB, 2006) and develop over long time span, up to million years. A relative geomorphological stability combined with warm-humid conditions and a very weak relief allow ferralitization to take place. Ferralitization involves intense weathering processes that concentrate Al, Si, and Fe in the soil in comparison to other cations such as Na, Mg, K, and Ca, which are eliminated from the solum by leaching. These soils are consequently highly acidic with a low nutrient availability. However, a yet overlooked pedogenic process is able, in less than a century, to mitigate the effect of ferralitization. This process, called the oxalate-carbonate pathway, can be described as the transformation of the sunlight energy into pedogenic carbonate. Briefly, the oxalate produced by primary producers is oxidized by soil oxalotrophic bacteria, leading to a local increase of soil pH, which can further induce carbonate precipitation (Braissant et al., 2004; Verrecchia et al., 2006; Cailleau et al., 2011).

In a coffee plantation from the Western Province of Cameroon, the influence of a ca. 150 yrs old Iroko tree (*Milicia excelsa*) has been studied along a 30 m long transect. Five soil pits, and for each, three surrounding coffee plants (*Coffea arabica* L.), were collected in order to compare their elemental content.

Oxalotrophy leads to an increase of up to 2.2 pH units close to the Iroko tree (pH: 7.4) compared to a control soil at 45 m (pH: 5.2). The total amount of Ca, Mg, K, and P in the soil close to the Iroko tree is more important than at distance. A similar trend is observed for exchangeable cations like Ca<sup>2+</sup>, Mg<sup>2+</sup>, and K<sup>+</sup>. Furthermore, other trends have been noted in coffee plants tissues. At a distance of 0.5 to 2.5 m from the Iroko tree, Ca, and K contents are higher than at a distance of 8 to 30 m, while at 45 m Ca content increases up to the level close to the iroko whereas K continues to decrease. This suggests a slight Ca impoverishment between both ends of the transect (in the 8 to 30 m interval). Finally, while P does not show any trend in the coffee plant transect, N/P ratio increases, indicating P deficiency for plants all along the transect from the Iroko tree. These results suggest that the Iroko tree ecosystem tends to be less dependent from the organic matter turnover, by constituting a more resilient nutrient's storage in the soil.

This study emphasizes drastic changes in biogeochemical soil characteristics in the relationship with the oxalate carbonate pathway induced by an iroko tree. Beyond the importance of understanding nutrient cycling in such ecosystems, a question arises if these iroko ecosystems could constitute a soil improving system, which could be used in a context of agroforestry.

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## P 9.2

# Long-term Organic Management Induced Changes in Soil Organic Matter Stability and Enzyme Activities

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Launched in 1976 by the Swiss Research Station Agroscope ACW - Changins, Nyon (VD), a long-term experiment investigates the capacities of different organic fertilizers to maintain soil fertilities and improve crop yield, in particular in relation to soil organic matter “SOM” (Maltas et al., 2012a, 2012b). The experiment consists of 6 treatments: “control” with only PK fertilizers, mineral NPK fertilizers “Min”, cereal straw “Str” + NPK fertilizers, green manure “GM” (mustard applied every 2 years) + NPK fertilizers, solid farmyard manure “Ma” (35t ha<sup>-1</sup> applied every 3 years)+ NPK fertilizers, and liquid farmyard manure “Slu” (60m<sup>3</sup> ha<sup>-1</sup> applied every 3 years) + NPK fertilizers. The main objective of this study is to evaluate the effect of the replacement of farmyard manures by mineral fertilizers and/or fresh organic matter (straw and green manure) on SOM quality and soil biological activity.

Organic fertilizers and SOM quality were analyzed by infrared spectroscopy (FTIR). For soils, humic acids (HA) were extracted by sodium pyrophosphate. FTIR signatures (Fig. 1) indicate that green manure is rich in carboxylic acids, which may increase its solubility in the soil solution. Cereal straw seems to be enriched in aliphatic compounds (fats, wax, or lipids), contributing to its water-repellent property. Solid (Ma) and liquid (Slu) farmyard manures, in contrast, contain more aromatic compounds, indicating an advanced stage of humification. Soil treated with green manure, “GM” soil, had the most degraded HA due to its lowest aromatic and carboxylic contents, while HA of “Min” soil seems to be the most stabilized with its highest carboxylic content. HA of “Str”, “Ma” and “Slu” soils demonstrated an intermediate degree of stabilization between “GM” soil and “Min” soil.

To better understand the mechanisms by which the organic fertilizations contribute to the SOM stability, C-, N- and P-degrading enzyme activities ( $\beta$ -1,4-glucosidase (BG), phenol-oxidase (PO);  $\beta$ -1,4-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP); phosphatase (AP)) were also assayed. Relatively high BG, NAG (Fig. 2) and AP (data not shown) activities were observed in “GM” soil as well as “Str” soil. Green manure being easily decomposable, its application probably stimulated these enzymes, resulting in the most unstable SOM. Water-repellent straw, in contrast, may have helped the SOM stabilization despite of the enzyme activity. In addition, enzymatic activity seems to increase due to a lower availability of nitrogen and phosphorus compared to carbon in straw. Therefore, our study demonstrates the effect of organic inputs on SOM quality, closely mediated by hydrolytic enzymes. It is to note that the enzyme activity seems to be strongly influenced by the decomposability and the nutrient balance of organic inputs.

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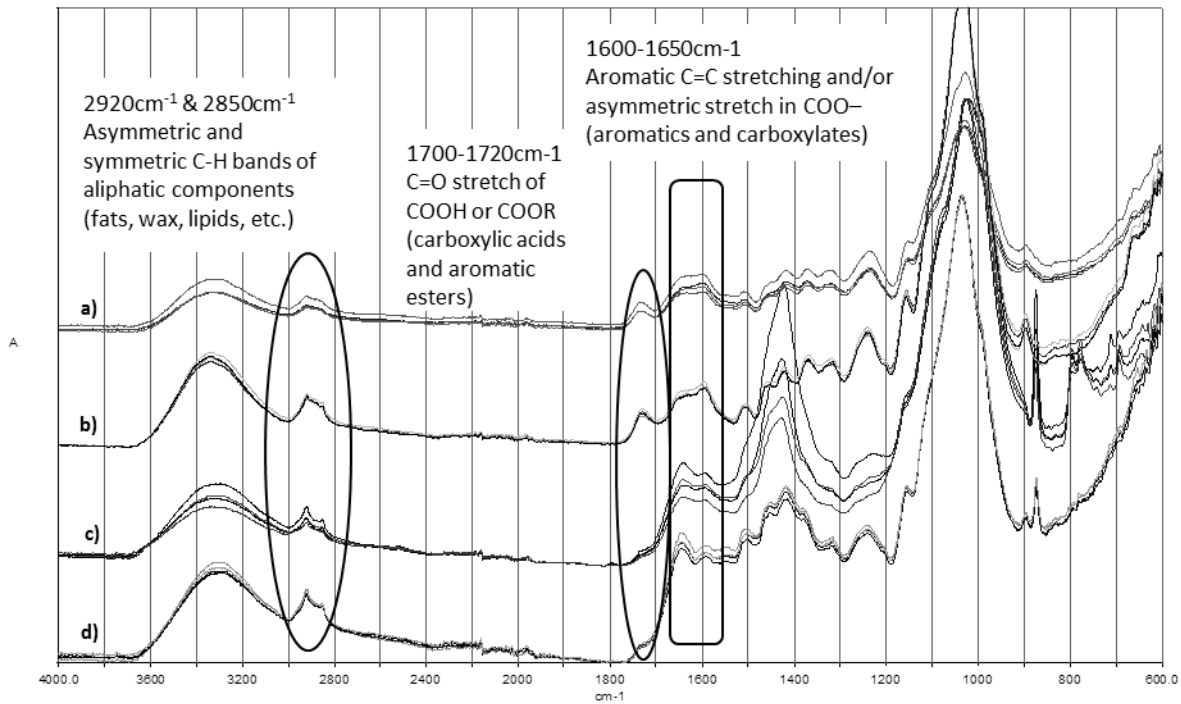


Figure 1. FTIR major signatures of organic fertilizers: a) green manure, b) cereal straw, c) solid farmyard manure and d) liquid farmyard manure.

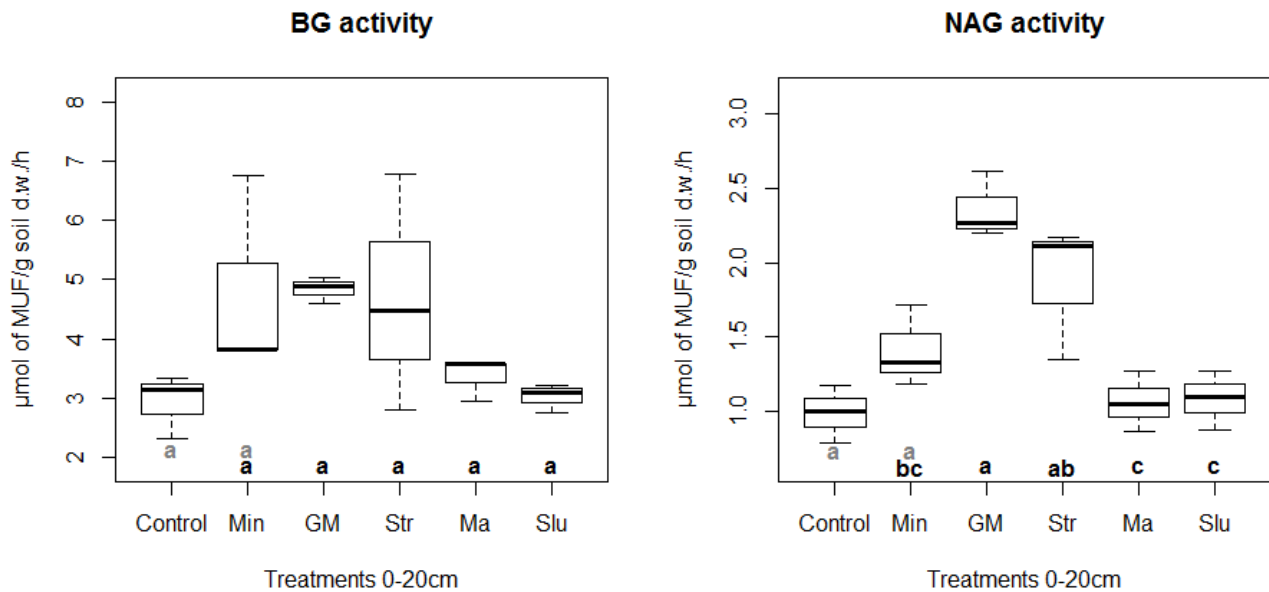


Figure 2. Enzyme study in soils: C-degrading BG and N-degrading NAG activity in Control: no N fertilization, Min: mineral fertilizer, GM: green manure, Str: cereal straw, Ma: solid farmyard manure and Slu: liquid farmyard manure, n = 4.

## P 9.3

# Mineralogical and chemical controls on the photoreductive dissolution of birnessite minerals

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Of the different known manganese oxides, layered-type Mn(IV) oxide phases (birnessites) are amongst the most environmentally significant. Birnessite minerals play a key role in elemental cycles due to their high sorption reactivity, oxidizing potential and important role in microbial metabolisms. Furthermore, birnessite minerals are commonly produced by the enzymatic oxidation of Mn(II) to form Mn(IV) oxides. Manganese oxides are also known to have semiconducting properties. Spectroscopic and computational studies (Sherman, 2005; Kwon et al., 2009) have shown that the band gap – the energetic separation between valence band and conduction band of birnessites – is in an energy range that allows electronic excitation by visible light. Upon excitation by light, electrons may be injected into the mineral structure and cause re-equilibration through valence change (i.e., Mn reduction) if the excited state has a long enough lifetime (Gilbert, 2005). Furthermore, metal impurities, structural defects and organic matter – all of which are characteristic of birnessite minerals in natural environments – can enhance the photoreactivity of semiconductors.

The goal of this research is to evaluate the fate of birnessite minerals and metal contaminants in sunlit aquatic environments. We are studying the effect of structural defects (Mn(IV) vacancy sites), particle size and adsorbed trace metals on the photoreductive dissolution of birnessites by comparing the dissolution rates obtained for three chemically-synthesized birnessites: microcrystalline K-birnessite, characterized by Mn(IV) layers with approximately 12% vacancies and Mn(III) in the interlayer; nanocrystalline triclinic birnessite characterized by layers of alternating Mn(IV) and Mn(III) and low to no vacancy content; and nanocrystalline  $\delta$ -MnO<sub>2</sub>, with Mn(IV) rich layers and approximately 6% structural vacancies. By doping the minerals with various metals (Ni, Co, Zn) we plan to probe the effect of chemical impurities on the dissolution rates.

Preliminary experiments carried out under a custom-built fluorescent light setup suggest possible competition between reductive dissolution and sorption of produced Mn(III) or Mn(II), as inferred from wet chemical measurements (ICP-OES, potentiometric titrations). The role of metal impurities suggest an increase of mineral reactivity towards metal sorption under the effect of light. This research furthers our knowledge of the Mn cycle, with important implications for the mobility and availability of metal contaminants associated with mineral surfaces.

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## P 9.4

# Typology of alkaline-earth metal precipitates in meso-oligotrophic lake (Léman, Switzerland)

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In the course of a routine water-quality survey in meso-oligotrophic Lake Léman (Switzerland), suspended matter was collected by filtration on 0.2 µm membranes between July 2012 and August 2013, mostly at the depth of maximal Chla concentration. In spring and summer samples, scanning electron microscopy revealed the presence of numerous dark and gelatinous patches occluding the pores of the membranes, containing high numbers of picoplanktonic cells and, in places, clusters of smooth microspheres 0.5-2 µm in diameter (fig. 1).

Their chemical composition, determined by semi-quantitative, energy-dispersive X ray spectroscopy (EDS) shows Mg, Ca, Sr and Ba (alkaline earth metals) to be the dominant cations. Carbon (as carbonate) and phosphorus (presumably as phospho-carbonate) are present as anions. The carbonate microspheres have been subdivided into four types represented in a Ca-Sr-Ba ternary space. All types are confined within a domain bound by Ca > 40, Sr < 10 and Ba < 50 (in mole %). Type I, the most frequent, displays a broad variability in Ba/Ca, even within a given cluster. Types II and III are devoid of Ba, but may incorporate P and S. Type IV contains only Ca. In contrast to the other types, phosphorus-rich microspheres are smaller and occur as isolated individuals or loose aggregates. The Type I composition resembles that of benstonite, a Group IIA carbonate that was recently found as intracellular granules in a cyanobacterium from alkaline Lake Alchichica (Mexico).

Lake Léman microspheres are solid, featureless and presumably amorphous. They are embedded in a mucilage-looking substance in the vicinity of picoplanktonic cells (Fig. 1), morphologically similar to small eukaryotes (*Chlorella* sp) or cyanobacteria (*Synechococcus* sp). In summer 2012, the macroscopic physico-chemical conditions in Lake Léman epilimnion were such as to allow precipitation of calcite, but not strontium and barium carbonates. For these, favorable conditions did exist, though, in the micro-environment provided by the combination of active picoplankton and a mucilaginous envelope.

A multidisciplinary research program is ongoing to gain a proper understanding of this intriguing process of alkaline-earth metals sequestration. By means of techniques such as SEM/EDX/TEM, epifluorescence microscopy, flow cytometry and genomics, the following questions will be addressed: the spatio-temporal distribution of the precipitates, their exact mineralogical composition, and the taxonomy and role of picoplankton in the precipitation processes.

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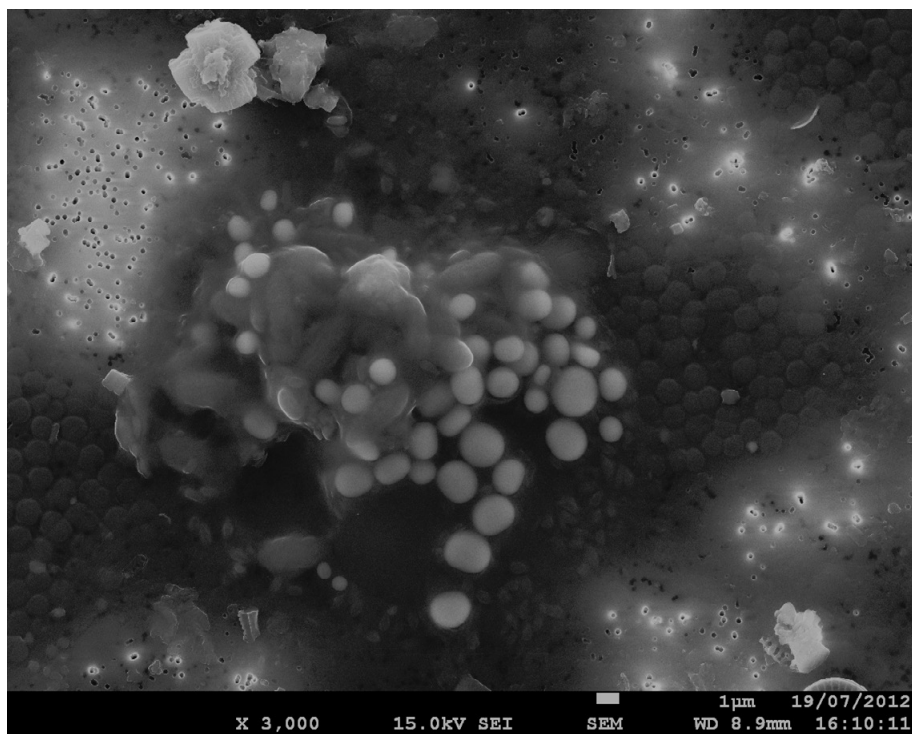


Figure 1. Cluster of smooth microspheres [Ca (approx. 50 mole %) / Ba (>45%) / Sr (>4%)], surrounded by picoplanktonic cells. Sample taken at 6 m under the water surface of lake Léman in July 2012. Photograph: Department of Earth Sciences, University of Geneva.

## P 9.5

## Effects of salinity on TiO<sub>2</sub> nanoparticles behavior and toxicity towards natural plankton. A case study: the Venice Lagoon, Italy

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Manufactured TiO<sub>2</sub> nanoparticles are widely used in many commercially available products (food, cosmetics, clothes...). This massive consumption could lead to their large input into the environment and to their potential impact on aquatic life. The physico-chemical composition of ambient waters determines the behavior of nanoparticles in aquatic systems as well as their interactions with organisms (Battin et al., 2009; Scown et al., 2010). The presence of salts can for example modifies the zeta potential of nanoparticles in making them more neutral, and as such promoting their aggregation (Brunelli et al., 2013). The aim of the present study was thus to examine the toxicity of manufactured TiO<sub>2</sub> toward natural plankton. Our working hypothesis was that the negative effects of TiO<sub>2</sub> towards plankton would decrease when salinity will increase due to the increase of nanoparticles aggregation.

To that end, the aggregation and zeta potential of TiO<sub>2</sub> nanoparticles were analyzed in artificial and natural seawaters with a Zetasizer Nano ZS (Malvern). The effects of TiO<sub>2</sub> on a model organism, the protozoa *Tetrahymena thermophila* exposed to three concentrations (1, 10, 100 mg / L) were examined using flow cytometry and the fluorescent dye propidium iodide as a marker of cell viability. Finally two fractions of natural plankton (one lower than 45 μm and one higher than 45 μm,) were collected in the lagoon of Venice at five different salinities and exposed to three concentrations of TiO<sub>2</sub> (1, 10 and 100 mg/L). The two planktonic fractions were also analyzed for their viability using propidium iodide but with fluorescence microscopy.

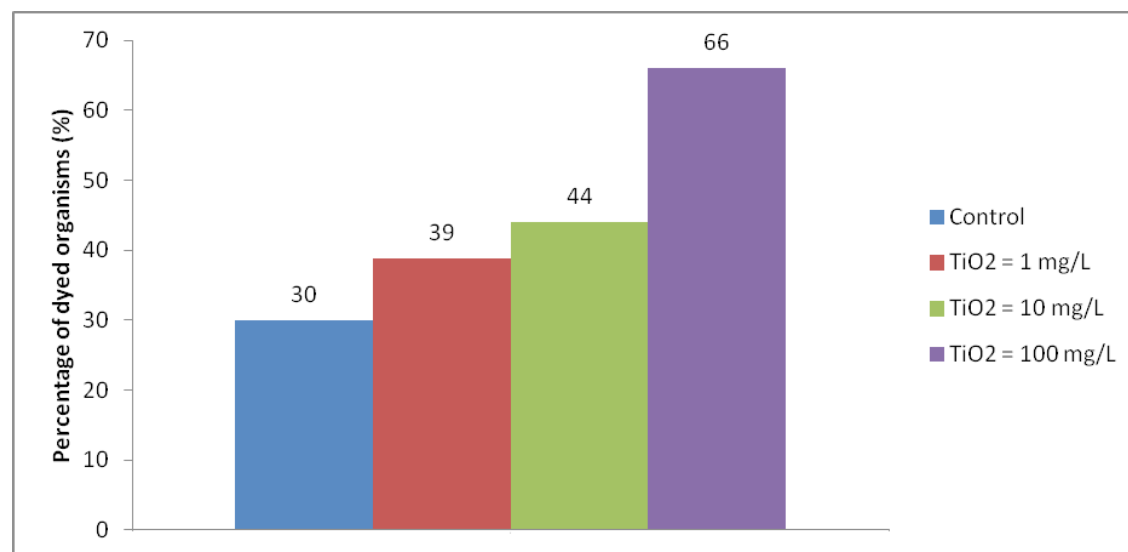


Figure 1 : Percentage of dyed organisms with propidium iodide after 24 hours of exposure to TiO<sub>2</sub> nanoparticles. Test performed with natural plankton of Venice lagoon (fraction higher than 45 μm, salinity = 12 ‰).

The fluorescent dye propidium iodide was successful to assess the toxicity of TiO<sub>2</sub> nanoparticles in natural plankton (Figure 1). In the control, about 30 % of the population was dyed, possibly due to a stress experienced during the sampling. Increase of TiO<sub>2</sub> nanoparticles concentration up to 100 mg/L in the ambient water of planktonic organisms doubled the number of stressed organisms. This method was further used to evaluate the effects of nanoparticles along a salinity gradient.

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## P 9.6

### The impacts of acidic in-situ recovery of uranium in southern Kazakhstan on geochemistry and microbial community structure

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Acidic *in situ* recovery (ISR) of uranium is one of the major mining processes utilized worldwide (Kazakhstan, USA, Australia, South Africa...) for low ore grade deposits. ISR is suited for deposits typically located in porous rock, and confined in impermeable rock layers. Among the main advantages of this technology, compared to traditional physical mining techniques, are: the reduction of hazards for the employees from accidents, dust, and radiation, the absence of mill tailings and its low cost. ISR circulates acid below the surface of the Earth within a closed loop recovery system within a mineralized aquifer. Once mining operations cease, some mines practice natural attenuation. Hence, in order to recover the initial physico-chemical conditions of the aquifer, clay minerals are expected to sorb metals and buffer the acidic pH while the native Fe and SO<sub>4</sub><sup>2-</sup> reducing bacteria will also raise the pH and generate reduced conditions favorable for uranium precipitation. The main risks of ISR coupled with natural attenuation are groundwater contamination and a long timeframe of pH recovery. In order to evaluate the rate of such 'natural processes', first the effect of the acid on both geochemistry and the native microbial communities must be understood. The initial sampling mission from this collaboration revealed that the acid not only liberates metals and salts, as predicted, but also has an affect on the indigenous microbes. These results will be presented along with future plans to compare natural attenuation vs. biostimulation *in situ*.

## P 9.7

### Distribution and dynamics of SOM on carbonate rocks (Jura Mountains): a geochemical approach

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Soil organic matter (SOM) is considered as a key factor in ecosystem dynamics. Its degradation state depends on several interactive factors, including bedrock lithology, vegetation, litter, temperature, and humidity. The geochemistry of SOM on carbonate substrata is little studied (e.g. Grünewald et al 2006). An on-going multi-proxy geochemical study of soil profiles on two carbonate lithologies in the Swiss Jura Mountains (Ballens) aims to fill this gap. The bedrocks include Kimeridgian limestone and calcareous moraine and a C<sub>3</sub> vegetation as a mixed forest (Abieti-Fagetum association) with beeches and conifers as the dominant trees. In order to evaluate the potential combined influence of litter and carbonate bedrock lithology on SOM, four bedrock-vegetation combinations ("soil groups") were considered: limestone-beech (L/B), limestone-conifer (L/C), moraine-beech (M/B) and moraine-conifer (M/C). Twelve soil profiles (triplicates for each combination) were described and systematically sampled. Soil geochemical analyses include mineralogical composition (XRD), major and trace element contents (XRFA), SOM characterization by Rock-Eval pyrolysis, bulk C and N isotope analyses, distribution of the soil lipids and compound specific C isotope analysis of the main fatty acids.

Soils are referenced as mollic Umbrisols, calcaric Cambisols, and hypereutric Cambisols, and have similar texture (silty) and pH (~6). Major element concentrations reflect soil mineralogy defined mainly by the quartz and calcite contents. The TOC for L/B and L/C soil decreases with depth from 37 wt.% (holorganic horizons) to 25 wt.% (mineral horizons), and for M/B and M/C from 16 to 0.07 wt.%. Rock-Eval HI and OI indexes (214 to 371 mg HC/g TOC and 92 to 151 mg CO<sub>2</sub>/g TOC, respectively) show small but still significant differences between the different soil groups. The δ<sup>13</sup>C<sub>org</sub> and δ<sup>15</sup>N<sub>org</sub> values increase from holorganic horizons to mineral horizons, with a clear separation between both horizon types (Figure 1). The holorganic horizons have the more negative values (δ<sup>13</sup>C<sub>org</sub>: -32 to -27‰, δ<sup>15</sup>N<sub>org</sub>: -7.6 to -3.5‰). The discriminant role of

either litter or lithology remains unclear. The mineral horizons have a slightly different  $\delta^{13}\text{C}_{\text{org}}$  values according to bedrock lithology (-27 to -25.2‰ for SOM on calcareous moraine, -25.4‰ to -24.5‰ for SOM on Kimmeridgian limestones). Similar  $^{15}\text{N}$  enrichment was also measured for the SOM on Kimmeridgian limestone. There were two controls of SOM isotopic compositions: new litter inputs and overall isotopic fractionation during decomposition on different lithologies. Decomposition increased the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values: surface SOM (high litter contribution) with low  $\delta^{13}\text{C}_{\text{org}}$  and  $\delta^{15}\text{N}_{\text{org}}$  values and mineral soils with high  $\delta^{13}\text{C}_{\text{org}}$  and  $\delta^{15}\text{N}_{\text{org}}$  values. The distribution of fatty acids (FA, carboxylic acids abbreviated as x:y, where "x" is the number of carbon atoms and "y" the number of double C-C bonds in the chain) are characterized by straight chains in the  $\text{C}_{12}$  to  $\text{C}_{32}$  range, with clear even-over-odd C number preference, maximizing at 16:0 for the beech forest soils and at 22:0 for the conifer forest soils (Figure 2). The relative concentration ratios of the FA decrease severely with depth. All samples contain small to trace amount of odd chain acids in the  $\text{C}_{13}$  to  $\text{C}_{33}$  range (maxima at 23:0) and terminally branched iso and anteiso methyl alkanolic chains in the  $\text{C}_{12}$  to  $\text{C}_{18}$  range. These acids are abundant in lipids from soil microbial communities. The molecular concentration ratios and the  $\delta^{13}\text{C}_{\text{org}}$  and  $\delta^{15}\text{N}_{\text{org}}$  values show differences between the SOM on moraine and on limestone, suggesting different edaphic conditions, development and decomposition pathways of SOM on the Kimmeridgian limestone and calcareous moraine. On-going compound specific C isotope analyses combined with the information from neutral lipids (*n*-alkanes, *n*-alcohols, sterols) will provide further information on SOM transformations with depth in both lithological contexts.

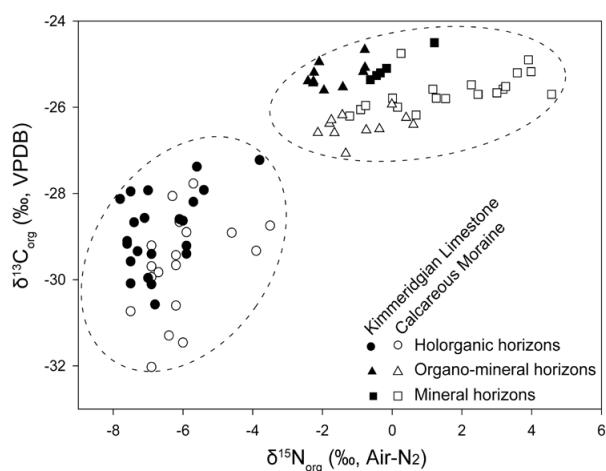


Figure 1.  $\delta^{13}\text{C}_{\text{org}}$  vs.  $\delta^{15}\text{N}_{\text{org}}$  of SOM on carbonate rocks of the Jura Mountains.

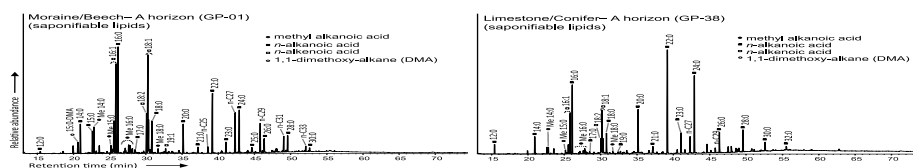


Figure 2. Distribution of saponifiable lipids extracted from surficial M/B and L/C soils.

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## P 9.8

# Building a global database on organic geochemical characteristics of surface marine sediments: design & call for input

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A growing and more diverse community is focused on studying the organic geochemical characteristics of marine sediments. The results of such studies are increasingly rich both in terms of data density and also in the variety of the parameters measured. At present, however, most of the resulting data remains disseminated in the literature and is not readily accessible for broader scale assessments. In particular, the fact that the data are not available in a single public source renders global-scale evaluations of composition and distribution of the sedimentary organic matter impractical. For this reason we have initiated a project to build a global database as a free instrument to be utilized by the whole international community. The database, called MOSAIC (Modern Ocean Sediment Archive and Inventory of Carbon), will be a resource for detailed organic geochemical and related information on marine sediments. MOSAIC will incorporate geospatial analysis tools in order to provide regional to global scale views on organic matter content and composition.

Key characteristics of MOSAIC include:

- Greatest emphasis is given on continental margin sediments since they represent the major loci of carbon burial, represent the interface between the terrestrial and oceanic realm, and are spatially highly heterogeneous;
- Organic geochemical parameters include concentrations and isotopic compositions at the bulk and molecular level and associated properties;
- The database will incorporate extensive contextual data regarding the depositional setting, and in particular, incorporating sedimentological properties;
- MOSAIC will be online and freely consultable.

The database is structured in a way that users will be able to provide and recover data and contextual parameters. The procedure will involve data incorporation into pre-configured spreadsheets (tables) available on the MOSAIC website. Completed tables will be imported via the Structural Query Language (SQL) into MOSAIC. The database is written in PostgreSQL, an open-source database management system. In order to visualize the data geographically, each element/datum must be associated to a latitude, longitude and altitude, enabling PostGIS (the spatial extension for PostgreSQL) to create a spatial database in a manner that can be interfaced to a Geographic Information System (GIS). In order to make the database broadly accessible, a HTML-PHP language-based website will ultimately be created and connected directly to the database. From the website it will be possible to both visualize and save data (in txt format) for utilization using common software (e.g. Excel, Word, PPT, Illustrator, ODV).

This contribution will outline the structure of the database, solicit feedback on desirable features, request data for inclusion in MOSAIC, and provide examples of data output.

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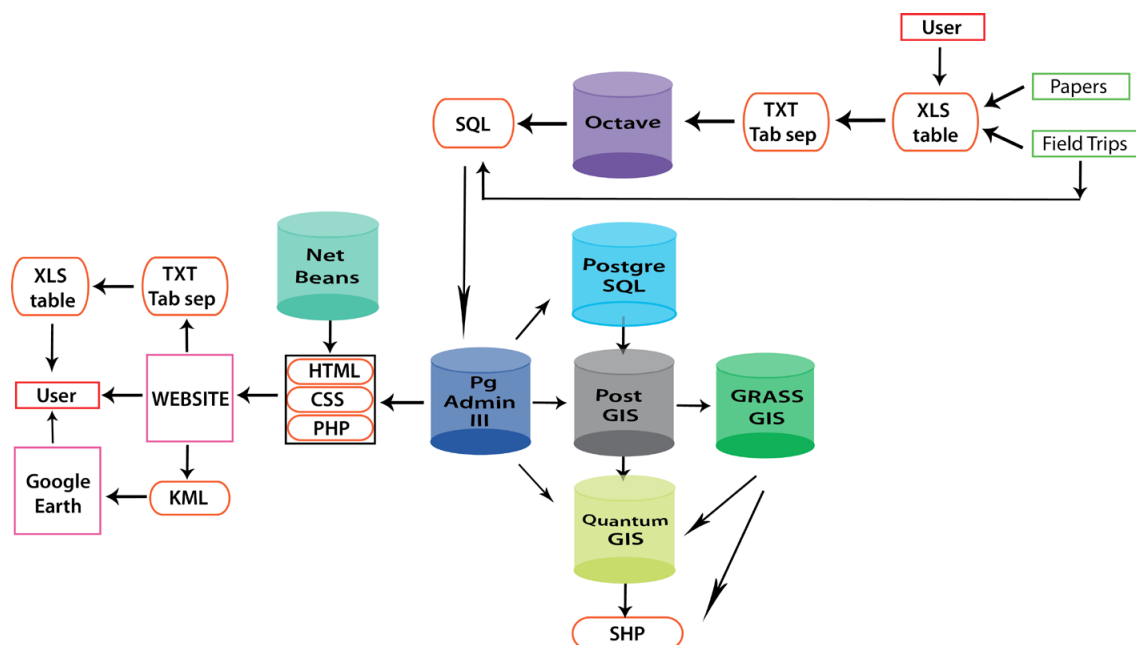


Figure 1. Architecture of MOSAIC database

## P 9.9

### Archaeal 16S rRNA gene sequences in the deep extreme Dead Sea sediments: determining who is there and why?

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The ICDP Dead Sea Deep Drilling Project (DSDDP) is an internationally funded initiative aiming to reconstruct the paleo-environmental and paleoseismicity histories of the Dead Sea Basin, in the Levantine region. Here we present preliminary results of the first geomicrobiological investigation in this extreme environment based on 16S rRNA gene sequences. We aim to obtain a comprehensive view of the Dead Sea sediment subsurface biosphere to identify and better understand the interaction between sediment and microbes and its imprint in the sedimentary record.

A 454m long sedimentary core retrieved from the center of the present Dead Sea displays distinctive facies that can be correlated to different lake regimes under contrasting climatic conditions that induced the preferential precipitation of evaporitic minerals. During rather dry periods as today in the Levant, the lake is holomictic and halite deposits as the main evaporitic phase. The lacustrine basin displays maximum salinities under such conditions. During more humid intervals, like those experienced in the late glacial period, or punctually during the Holocene, the lake becomes stratified, and aragonite precipitates forming varve-like laminae alternating with detritus laminae (aad facies) brought by incoming freshwater. Transitional periods of increasing aridity are further characterized by massive gypsum precipitation. We investigated the 16S rRNA gene sequences in several samples from key lithologies. Knowing the prevalent environmental conditions and the state of the lake when it happens we can propose some hypothesis to explain the presence of the identified archaeal sequences down to 200m below today's lake floor.

We have attempted to characterize as largely and completely as possible the microbial subsurface biosphere using DNA extractions and amplifications. We present here libraries of archaeal 16S rRNA sequences from two halite samples of 0.2 and 204m depth below lake floor (blf), one from a gypsum sample of 96 m blf, and one from an aad facies at 2.35 m blf. In order to give a good insight into the specific archaeal assemblages of the Dead Sea, we also compare the identified microbial assemblages with those of a microbial mat found in the saline shores of the present Dead Sea.

Our results show a microbial population largely dominated by obligate halophiles of the *Euryarcheota* family. Sequences retrieved from the halite and gypsum samples show very strong similarities with each other, with maximum richness found in the shallowest sediments. They also share their main phylotypes with those identified in the modern microbial mat, and previously described in the modern Dead Sea water column (Bodaker et al., 2010). While those populations show relatively little variation between each other, the aad sample, deposited in a completely different setting, under stratified lake conditions display a completely different population. It is almost exclusively composed of members of the MSBL1 candidate division first described in the Mediterranean deep brine basins (Van der Wielen et al., 2005). They are potential candidates for the production of methane found in high quantities in those environments. The Dead Sea water during aragonite precipitation shares with these sites extremely high salinity and high divalent cation concentrations, forming sharp gradients against less saline water, together with anoxic conditions.

The identified assemblages do not show major variations in the archaeal communities of the Dead Sea and lake ancestors sediments. The similarity between sequences found in the oldest and present day halite and gypsum point towards very low activity within the sediments. Thus, it is actually more likely that most of the microbial diversity and metabolic changes occur in the water column and the most recent sediments. Once buried, *Archaea* may have very low metabolic rates, and probably do not influence much their environments when deposited in halite and gypsum sediments of a holomictic lake.

On the other hand, the aad facies shows a unique archaeal assemblage with very specific phylotypes. The MSBL1 Candidate Division is here identified for the first time in a continental setting. Its unique recovery in this specific lithology advocates for the importance of specific sedimentary conditions and pore water chemistry for their development. It appears that the prevalence of sharp salinity gradients and anoxic conditions in the lake water at the moment of sedimentation are the prerequisites for their presence. Their activity and potential metabolism is nevertheless still to be expressed.

Finally, although primarily controlled by the salinity, it seems that physico-chemical conditions found in the (paleo)lake water, which are originally driven by climatic variations, are responsible for the development and preservation of specific archaeal assemblages recovered in the Dead Sea Basin sediments.

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## P 9.10

# Structure of biofilm communities and their sensitivity to metals. A case study of the Lagoon of Venice

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Biofilms are communities of microorganisms, which colonize the bottom of aquatic systems such as rivers, lakes and lagoons. They are mainly composed of algae, bacteria, fungi and viruses, which are wrapped in a matrix of exopolymeric substances (Paul S. Giller, 1998). As primary producers, they form the basis of aquatic food chains and represent a major source of carbon for grazers and fish. Moreover, biofilms are known to be very sensitive to environmental changes and as such could be used as biomarkers of metal contamination (Barranguet, et al., 2000). The present study aims to further evaluate the use of biofilms as a biomarker of water quality.

The biofilms growing in the Lagoon of Venice have been chosen for this study because the site provides a set of different environmental conditions. First, the lagoon is subject to a salinity gradient due to the mixing of freshwaters coming from twelve main rivers with seawater (Collavini, et al., 2005, Zonta, et al., 2005). In addition, a large variety of pollutants (trace metals, organic pollutants, fertilizers) occur in the Lagoon as a result of the proximity of the city (transport, wastewater), industrial activities situated in Porto Marghera (petrochemical, metallurgy, refinery) and agricultural activities which take place on 70 % of the watershed of the Lagoon (Collavini, et al., 2005). Our working hypothesis is that (i) the structure of biofilm communities will change according to metal concentrations and environmental conditions in the Venice lagoon (ii) their sensitivity to metals will depend on the composition of the biofilm i.e. EPS quantity and (iii) their metal content is directly proportional to the concentration of metals in ambient waters.

Colonisation boxes containing 32 microscope slides were immersed in water between 60 to 110 cm deep in five different sites representing various sources of pollution, i.e. a less-impacted site, which was considered as a control station (Santa Maria del Mare), an industrial site (Porto Marghera), an urban site (in a channel of Venice city) and two agricultural sites (in the Dese River). Two weeks later, slides colonized with biofilms were collected and kept undisturbed until treatment with fluorescent dyes for microscopy analysis. A combination of DAPI for biotic quantification and Concaviline A Texas Red for EPS quantification (Battin, et al., 2003) was used to determine biofilm structures whereas the algal autofluorescence was used to assess algal fraction. At least ten images per parameter were processed with Image J and the color thresholding method. Chlorophyll *a* was also measured in the samples after extraction with acetone. Total and intracellular metal concentrations (after washing with EDTA) were measured by ICP-MS after acidic digestion. Finally, biofilms were exposed to 100 µM of cadmium for 24 hours using microcosms and analysed for their concentration of bioaccumulated cadmium. Their sensitivity to cadmium was assessed using a fluorescent marker of lipid peroxidation.

Structural differences between communities were successfully assessed using fluorescent microscopy (Figure 1). Biofilms grown in Santa Maria del Mare (control site) were observed to be essentially composed of a biotic fraction (91 %) whereas a lower percentage (7 %) was found in biofilms collected in the Dese river in which abiotic fractions were predominant. The percentage of EPS was 7 times higher in the biofilms collected in the Dese River than in the one collected on the control site. Further analysis will be performed to relate water quality with the structure of biofilm communities.

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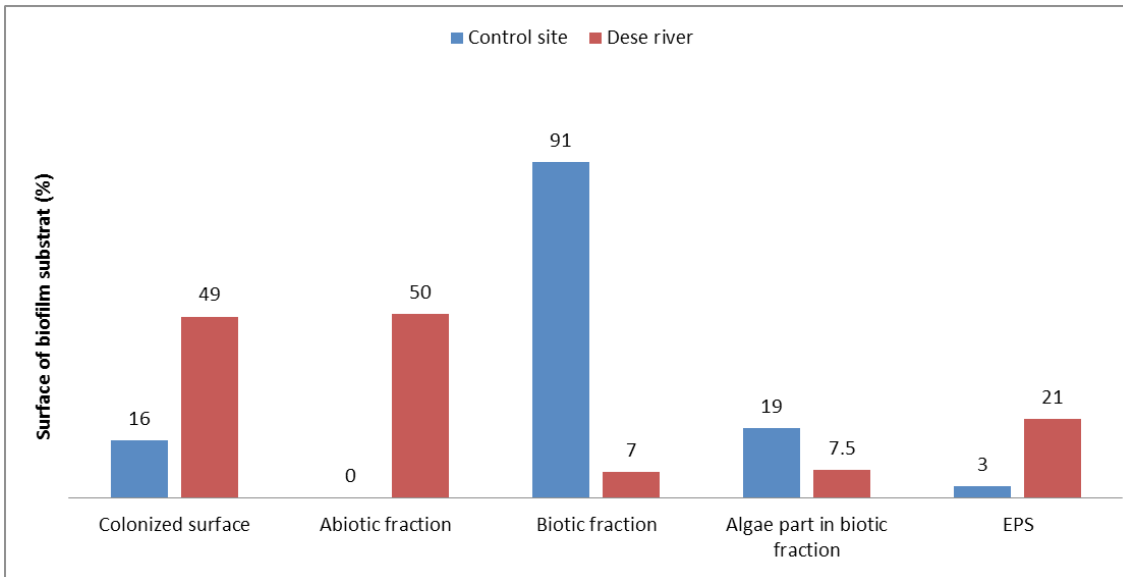


Figure 1: Structure of biofilm communities collected at the control site and at the Dese river site in the Venice Lagoon.



## P 9.11

# The influence of the biological nitrogen cycle on stromatolite formation

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Comparative studies of different shaped stromatolites that were built through geologic times, from the early Precambrian to the present in varying environments, and studies of stromatolite forming organisms and satellite images of recent stromatolite locations, indicate that the biologic nitrogen cycle plays a key role for an explanation and the understanding of stromatolite formation.

Nitrogen is a key element in biological cycles, particularly for the creation of proteins. However, nitrogen has to be present in a biologically available form such as nitrogen oxide or ammonia. Abiotic nitrogen oxide occurs in volcanoes, in black smokers and other blowouts. Biological nitrogen fixation occurs when atmospheric nitrogen is converted to ammonia.

The characteristic layers of stromatolites arise when procaryotes form microbial mats with biofilms in which minerals precipitate. Biofilms are exclusively a product of photosynthesis. Since many procaryotes use protons for their metabolism it was an evolutionary pressure to develop a strategy to ensure a proton source. The result was the invention of photosynthesis by which protons became separated either from hydrogen sulfide ( $H_2S$ ) or water ( $H_2O$ ). Early shallow marine basins near blowouts containing nitrogen oxide may have been composed of solutions of  $H_2S$ . Under such circumstances the early sulfur photosynthesis evolved. Extracellular polymer substances (EPS) formed the first biofilms in which minerals precipitated, building the first dense stromatolitic layers. Progressively, condensing water filled the ocean basins and began to cover the landmasses of the early cratons. Around the blowouts the necessary nitrogen oxide was still abundantly available, the sunlight, however, reached only the water covered areas of the landmasses. For an autotrophic life it became necessary to invent a new form of photosynthesis, the oxygen photosynthesis, by which not only protons were separated from oxygen but also a new global energy currency was formed, ATP – adenosinetriphosphate, produced by cyanobacteria.

The increasing distance of the shallow water areas from the energy rich blowouts became problematic for the early prokaryotes. A new nitrogen source was necessary, and found in the atmosphere – elemental nitrogen. However, with a huge amount of ATP production cyanobacteria changed the elemental nitrogen into the biologically available form of ammonia. Consequently, life explosively developed at the water-atmosphere boundary in intertidal areas. Cyanobacteria formed biofilms and microbial mats that subsequently led to the formation of stromatolitic layers.

For the next two billion years cyanobacteria changed the world not only with their production of oxygen but also with the conversion of elemental nitrogen into ammonia.

Because nitrogen fixation is very energy-consuming it is strongly regulated by organisms and only processed if there is no other possibility for a biologically available nitrogen supply. Thus, cyanobacteria, too, use ammonia if existent. As a consequence, with increasing concentration of ammonia in the open ocean stromatolites developed in deeper waters. The occurrence of conophyton stromatolites towards the end of the Precambrian is an indicator of such circumstances. They formed at slopes in a deeper marine environment and their shapes point towards the sunlight.

As a requirement for the evolution of eucaryotes ammonia was distributed in the seas by oceanic currents subsequently, leading to an explosion of life, and announcing the descent of the stromatolites.

In modern reefs cyanobacteria are as abundant as in microbial mats of Precambrian stromatolites, but they are accompanied by eucaryotes. This new assemblage led to new reef structures beginning with thrombolites and ending in fascinating coral reefs. Undisturbed biofilms that form stromatolitic microbial mats only occur if there is a need for nitrogen fixation under conditions of depleted biologically available nitrogen. A co-existence of stromatolites and evolved reef types under similar conditions is impossible. Thus, there are only two marine environments known today in which stromatolites grow, Shark Bay in Western Australia and the Exuma Cays in the Bahamas.

