Project title:
DiSeMiNation – Digging into Sediments and Microbes for Nature conservation

Project number: K218/2016

Executive Summary

Mangroves sequester more carbon in stable anoxic sediments than most other marine or terrestrial ecosystems, contributing to the mitigation of CO_2-driven climate change. This service, based on above- and belowground biomass production and decomposition processes, strongly depends on the diversity and species composition of the sediment microbiota and fauna. Based on a cross-continental comparison, DiSeMiNation provides detailed information on microbe-driven sediment processes related to organic matter turnover and C-sequestration in mangrove areas, and how they are affected by the flora and fauna and their use by local populations. Understanding these relationships will help to develop management plans for future sustainable exploitation of mangroves for natural resources, and will serve to ensure efficient C-sequestration in tropical coastal sediments through providing a sound scientific basis for spatially planning protected areas and networks thereof according to where relevant services are provided most efficiently.

Thus, DiSeMiNation aimed at unravelling how the floral and faunal communities and environmental conditions govern service-relevant microbial processes in mangrove ecosystems, and how related services spatially contribute to the benefit of users. At the same time, human resource-use and anthropogenic pressure affect fauna and flora, as well as the microbiota, both directly and indirectly. While both directions of this mutual dependence have rarely been studied simultaneously, such knowledge is pivotal for the development of sustainable management plans. The innovative combination of cutting-edge technologies and methods in biology, chemistry and socio-ecological sciences into a "conservation-omics" framework has the potential to provide a sound basis for knowledge-driven spatial conservation planning not solely in mangroves, but will also be easily transferrable to other coastal ecosystems.

Preliminary analyses of these multi-disciplinary data indicate an effect of both the dominant mangrove species and the site (as well as, on a larger spatial scale the region) on sediment characteristics and the composition (labile vs. recalcitrant compounds) of the organic matter in the upper sediment layers, as well as on the abundance and species richness of the bacterial sediment community. Analyses about which of these mutually dependent parameters are directly vs. indirectly affected by human resource-use or pressure are ongoing.

Among these different ways and goals of resource-use exists a clear prioritization for some of them by local or regional stakeholders. While details in the perception of mangrove services and relevance differed across regions and among stakeholder groups, an across-region pattern of most valued ecosystem services provided by mangroves became obvious: provisioning of food along with providing nursery habitats for fish, provisioning of firewood, climate change-mitigation through CO_2-storage, and coastal stabilization and protection.

1. Achievement of objectives and milestones

DiSeMiNation successfully sampled and analyzed (partly still ongoing) sediment samples from a total of 38 mangrove stands in 6 countries (Annex 1). This resulted in detailed knowledge of

-environmental parameters of sediments from mangrove forests in different regions and environmental conditions, with different mangrove species, under different conditions of human use and pressure

-the perception of mangrove forests and their ecosystem services, as well as anthropogenic pressures and threats, in different regions and biogeographic realms (=cultural backgrounds)

-taxonomic and functional community composition of the sediment microbiota from mangrove forests in different regions and environmental conditions, with different mangrove species, under different conditions of human use and pressure (c.f. Annex 1)

From the same mangrove stands, leaves of different mangrove and associate species were sampled and are being analyzed as reference material for the determination of the origin of organic matter in the above mangrove sediments. The latter analysis could not yet be finalized, resulting also in a delay of jointly analyzing all findings from organic chemical, microbiological and socio-ecological sub-projects (see 2.).

2. Activities and obstacles

Field sampling campaigns were performed as planned in Singapore, Brazil, Colombia and South Africa (Annex 1); the project plan changed from Oman to Senegal because of permit issues in Oman, and from Australia to Fiji because of permit issues (and competing interests of potential local collaboration partners).

In parallel to field sampling campaigns, IÖR & ZMT organized onsite socio-ecological stakeholder analysis, workshops, and IÖR analyzed questionnaires about ecosystem services regionally provided by mangrove forests (and used by human actors).

For data exchange within the project, cloud storage at ZMT and IPB were used. A metadata capture template and corresponding spreadsheet template were designed at IPB to include all information relevant for data processing and eventual data publication. Sample naming conventions were developed to allow the linking between samples, metabolomics and corresponding sequencing data at DSMZ. The procedure for the conversion of the mass spectrometry raw data from vendor file formats into open file formats was performed at IPB. Open formats are required for reproducible data processing with Open Source software. Together, the metadata templates and open file formats allow for FAIR data publication.

For the Pyrolysis GC/MS metabolomics platform, a data processing and quality control pipeline was developed by IPB and ZMT. For algorithm development and testing, preliminary and test data sets on the same analytical platform were used: In-depth analysis of requirements for reproducible analysis of GC/MS data from different samples (plant tissue and sediment) from different regions led to the development of a data-handling pipeline for comparative analyses of metabolomic environmental fingerprints from sediments, including, e.g., the identification of plant species-specific biomarkers for the determination of different origins of organic matter in the sediment (e.g., Bakkar et al. 2017; Bakkar et al. in prep.). This pipeline has been tested on several datasets and will be applied to the dataset of DiSeMiNation as soon as the final GC/MS data will be available (see below).

While pyrolysis-GC/MS has been used to analyse the chemical composition of organic matter found in solid samples, the output data are a mix of intact compounds that transform into the gas phase without breaking apart, and of pyrolysates, i.e. parts of molecules that have been broken upon heating prior to entering the gas phase. For the latter, it is difficult to reconstruct the compounds of origin found in the sediment.

Therefore, an evaluation was devised to evaluate two distinct methodological approaches:
1) direct pyrolysis-GC/MS of sediment samples, the technique used in routine at the ZMT;
and 2) extraction of sediment samples, followed by GC/MS analysis of the extract, followed by py-GC/MS of the remnant of the extracted sediment (i.e. sediment sample with the OM that was not extractable, such as lignins, cellulose). The two protocols differ in terms of time and effort for preparing the samples in the lab, the ability to measure different compound classes (based on their solubility) and their usability in an upstream analysis (e.g. compound identification). With the devised experimental design, we can use the approach from Trutschel et al. (2015) to separately calculate technical and sample variance. The procedure with the lowest variances can be considered the most suitable one.

In the past years, new DevOps strategies emerged in the software development industry. While those strategies were adopted rapidly in the private sector, the uptake in the academic world was much slower. We have used modern continuous integration and cloud strategies for our bioinformatics software development, which allow to scale them on-demand.

A Mangrove-omics mini-workshop was held at the annual conference of the German Center for Biodiversity research (iDiv) on 11./12.12.2018, presenting how chemical and molecular mechanisms link with biodiversity aspects of important ecosystem-engineering organisms, such as mangroves.

<u>Obstacles:</u>
Lab analyses for organic matter (ZMT) was hampered by initial problems in developing an appropriate analysis pipeline (see above for final success), as well as the decision to analyze all samples in one GC/MS session, both postponing the start of the analyses to after the last sampling campaign in 2019 – at this point in time, the chemistry lab of ZMT encountered technical problems with the GC/MS that took several months to be solved. Upon starting the GC/MS analysis, the unexpected lockdown of the ZMT laboratory due to the pandemic situation rendered the analysis impossible. Upon partial opening of the laboratory in summer 2021, a long queue of samples to-be-analyzed postponed the analysis further. The difficulties in finally getting ABS compliance for samples from Senegal halted the analysis of these (and thus all other samples, including those microbiological analyses) for another couple of months – compliance was finally achieved in summer 2021.

The initially approved sampling permit for Fiji got somehow lost between our hosting/partnering institute of the University of the South Pacific in Fiji and the state authorities in charge – we are still in the process of negotiating a retrospective permission for taking the samples and exporting them to Germany: in fact the samples are being stored at ZMT and DSMZ and even have even been analyzed through GC/MS, but the data cannot be analyzed, before the permits will have been re-issued. Thus, the data integration of environmental parameters, human use and pressures, organic matter content and structure and bacterial (taxonomic and functional) community composition, having been planned as one of the key outcomes of the project, could not yet have been performed.

3. Results and successes

<u>Environmental/Sediment characteristics at different sampling locations/regions and sites:</u>
-clear differences across locations (countries), both within and between the biogeographic realms of the Atlantic-East Pacific (AEP) and Indo-West Pacific (IWP)
-site- versus specific-specific differences, e.g.:

Fiji: higher clay and silt content, lower sand content, in Bruguiera gymnorhiza sediments than in Rhizophora stylosa sediments; lower TDS (and individual mineral contents) in Bruguiera gymnorhiza than in Rhizophora stylosa sediments in Western and eastern estuarine channel, but not in the central channel; higher C:N ratio in Bruguiera gymnorhiza stylosa sediments than in Rhizophora sediments; higher total OC content along the central estuarine channel than along the eastern and western.

Brazil: clear trend in decreasing clay content from the mainland towards the tip of the peninsula (and increasing, with higher sand and lower silt/clay contents in Avicennia germinans stands; higher sediment salinity in Rhizophora mangle stands; higher content of moderately labile OM (and total OM) in Rhizophora mangle and Laguncularia racemosa than Avicennia germinans sediments, with lower OM (and particularly moderately labile OM) content in dwarf stands of the latter; higher nutrient contents in Rhizophora mangle than in dwarf Avicennia germinans sediments;

Higher clay content in the mangrove sediments of Brazil than of Senegal or Colombia (essentially none in the latter two regions), with little effect of the species on this trend; markedly higher OM and nutrient contents in sediments of Colombian than of Brazilian or Senegalese mangrove stands.

These comparisons within and among regions and biogeographic realms are detailed in the BSc theses of Jessica Dohr, Soyla Kraus, Anja Ruhl and Janika Schuster, and a MSc thesis by Mondane Fouqueray. The results will be published in a series of papers (see 8.) that is still under elaboration.

<u>Bacterial community composition:</u>
Both RNA and DNA were extracted from sediment and root and rhizosphere samples for all locations in order to analyse active and total communities, respectively. Targeted amplicon sequencing of the V3 region of the 16 rRNA was performed and a total of 648,525 sequence variants (SVs) were detected in 174,919,874 sequences post quality filtering. Sequence coverage estimates were between 97 – 100%, which shows that the inventory covered the majority of taxa in the samples. Sediment samples were generally more diverse than root and rhizosphere samples, and RNA samples were slightly more diverse than DNA samples. Some influence of location was also observed, with South African samples being less diverse than all other locations. Plant species also showed an effect; particularly, the samples obtained from the dwarf Avicennia germinans *were less rich than all others, with exception of* Rhizophora mucronata*. For three locations in Senegal (plots 1, 23 and 35), stimulation experiments were performed by adding different substrates (and appropriate controls) labelled with bromodeoxyuridine (BrdU) to sediment samples. Coupled with subsequent immunocapture and high-throughput sequencing, this approach allowed to discriminate between organisms which utilized the different substrates. Bacterial communities after immunocapture were very distinct from the original ones and also very distinct from the controls, which validated the results. Organisms growing on the substrates supplemented were affiliated mainly to the proteobacterial class Gammaproteobacteria and the phyla Firmicutes and Fusobacteriota. Interestingly, though, the plot location had a bigger influence on the growing bacteria than the individual substrates. The results will be published in a series of papers (see 8.) that is still under elaboration.*

<u>Stakeholders and service-use at different locations/regions:</u>
Stakeholder analyses were carried out in five of the six study areas (Singapore, Brazil, South Africa, Colombia, and Fiji), partly using workshops, and everywhere through surveys or interviews of key stakeholders that had been selected with the help of local partners. During the workshops, huge differences in political framework conditions, governance, and valuing concepts between the countries appeared. However, it is possible to transfer knowledge about how to optimize protection and governance concepts in mangrove areas to ensure the services and benefits they provide to the people.

Ecosystem services (ES) are of increasing importance for environmental governance and science. To safeguard the essential ES in endangered ecosystems, we identified the key stakeholders benefitting and threatening them, determined the most important ES for them, and examined how these ES can be used sustainably and governed wisely through social-empirical studies of stakeholders' opinions. The responses of a total of 73 stakeholders were examined quantitatively and qualitatively. In contrast to the prevalent consideration of mangrove ecosystems as a single type of socio-ecological system, our study areas differed considerably in the appreciation of, and threats to, their ES. The analyses allow distinguishing stakeholder groups (regarding threatening, and benefiting from, the mangrove ecosystem). We found pros and cons among the different governance systems and delivered hints for better protection of the mangrove ecosystems there.

Of course, stakeholder behavior is site-specific. Nevertheless, the meaning of sustainable use and ideas of better management are more similar than expected. The results will be published in a paper that is still under elaboration.

Mangrove forests around the island of San Andrés, Colombia, were examined in-depth through surveys among stakeholders such as scientists, conservationists, government officials, community leaders, educators, resource-users, police, and coastal managers. The most appreciated services of mangrove ecosystems are storm protection, sediment protection, and water decontamination, as well as local and global climate regulation (carbon sequestration). Nursery was appreciated only to a medium 16th rank in San Andrés among a total of 31 services studied. Representatives of local communities also highly appreciated the possible extraction of food and other life-supporting goods. Stakeholder opinions about governance differed considerably even on this relatively small island. The often only small-scale uses of food and timber are seen as the most threatening ones, but also the hotels and other facilities causing pollution and waste, which is a huge problem for mangrove conservation on San Andrés. Surprisingly, the coast guard was marked as a potentially threatening user, causing littering with oil bottles and others. Touristic entrepreneurs, scientists, and the employees of conservation and management organizations, such as CORALINA, were considered the most benefitting users. Although these pose little threat on mangrove forests, their activity sometimes lacks the desired positive effects. Therefore, better coordination between the leading actors and solutions for several conflicts are strongly demanded for the future of mangrove ecosystems.

The legal situation and governmental action were scrutinized, with a range of critical outcomes, which in turn have the potential to drive beneficial changes. The respondents stated that many stakeholders disobey the regulations and that the governance of mangrove areas takes place in a top-down manner. Even though most people know who to contact if they want to influence decision-making processes, these governance instruments do not work properly, mainly because of the missing confidence in the governance mechanisms. A report about the survey results is under elaboration and will be published together with the local partners of Universidad Nacional de Colombia, Sede Caribe.

Based on a previous survey on the most relevant ES for inhabitants, we consider five selected ES: provisioning of animal food (esp. by mangrove crab Ucides cordatus*), provisioning of firewood from mangrove trees for bioenergy, regulation of global climate by CO_2-storage, coastal stabilization, and nursery for fish populations. For these ES, the project team developed GIS-based transferable ES assessment methods that can be used everywhere in the world by combining open-source data with local knowledge and (if any) findings of surveys on site. The economic valuation used income factor and land opportunity costs as a comparison. The developed methodical framework was implemented for the study area RESEXM Caeté-Taperaçu in Pará, North Brazil as a case study (Gutting et al. 2021): besides worldwide databases related to global mangrove distribution, aboveground biomass, and canopy height, regional literature and statistics provided applicable parameters, allometric equations, and further information needed for the quantification of the above-mentioned ES. The distribution of ES within the study area was refined by local data, of which the map of species distribution is the most important one. In total, the area delivers the basic nutrition of about 1,400 households, which equals 2.7 million US$. The biomass contains 2.1 million Mg C, amounting to 50.9 million US$ if it were paid as certificates.*

4. Equal opportunities, career development and internationalisation

The major measure for ensuring equal opportunities applied was the employment of the most suitable candidate for each position to be filled, irrespective of gender or origin. Thus, while the PIs are all male, three female postdoctoral researchers were employed, and thesis projects related to DiSeMiNatuon were all performed by female candidates.

5. Structures and collaboration

In all six sampling countries, the Leibniz-consortium was supported –during all phases of planning, organizing and performing field work and sampling trips– by local collaboration partners. Sampling campaigns were organized and coordinated by ZMT, and sampling was performed by ZMT and DSMZ and (for Fiji) IPB, always in close collaboration with, and marked contribution by, local collaboration partners. During field campaigns, stakeholder communication was performed by IÖR, usually following workshops with local stakeholders organized by ZMT and IÖR.

Difficulties in obtaining research permits for sampling in Oman resulted in shifting the sampling region and partner to Senegal (also leading to a balanced study design across biogeographic realms); difficulties in realizing initially planned and agreed-upon collaboration with Australian partners resulted in changing the sampling region and partner to Fiji (also better balancing the ratio of island-to-mainland locations). Both decisions proved to be for the better of the project, because of (in addition to the above balanced design)

smoother performance during the field campaigns and afterwards. However, the administrative hurdles in Fiji proved to be more difficult to handle than anticipated.

6. Quality assurance

An extensive QC scheme was developed by IPB and ZMT to ensure that data can be normalised across measurements. The use of QC mixes, pooled over all study samples and replicated across all analytical runs, allow for assessing the instrumental and environmental variability across samples and study sites. Further, these QC mixes became an essential component of the analysis pipeline, as they allow for taking temporal shifts in analytical performance into account.

Common Open Data Format in metabolomics for GC/MS data are netCDF and mzML. In addition, experimental metadata need to be captured in a machine-readable way, such as the Investigation-Study-Assay (ISA-Tab) format. Such Open Formats are particularly important for the deposition of research data in repositories. DiSeMiNation will submit published research data sets to the domain-specific repository MetaboLights at the European EMBL-EBI. These submissions ensure that the published research data meets the FAIR10 standards.

Beyond metabolomics data, all chemical and sequence data, as well as corresponding metadata, will be submitted to open data repositories and made available upon publication.

DiSeMiNation did not encompass any handling of, or experiments with, animals.

7. Additional resources

Beyond the direct funding from the Leibniz Competition Program, in-kind contributions of the different partners consisted of time investment of PIs (5-20%) and laboratory personnel (5-10%). The value of consumables and services covered by the participating institutes was around 5-10% of the corresponding budgets.

8. Outlook

As described above, not all analytical and synthesis steps could be completed during the runtime of the project. Hence, several analyses remain to be performed, as soon as the metabolomics analyses have been finished, and the corresponding data have been quality-checked and interpreted:

Correlate site and sediment parameters
-with bacterial community composition and activity
-with vegetation type
-with sediment community (fauna, microbiota beyond bacteria, based on eDNA)
These analyses will contribute to the development of models for spatially predicting
-bacterial communities and activities
-sediment infauna
-sediment processes (focusing on OM-turnover)
in mangrove sediments across the world.

The global study design across the two major biogeographical mangrove realms (IWP, AEP: see Annex 1) will allow for several future analyses, based on sediment characteristics and flora.

*-**AEP**: two/three species in common at three locations (Brazil, Colombia, Senegal), along*
-different temperatures (among locations: Colombia, Brazil, Senegal)
-salinities (among locations; among sampling sites for Colombia, Senegal)
→transregional map of bacterial communities (DSMZ), sediment communities (ZMT) and sediment processes (OM-turnover) (IPB), based on flora and environment;
→predict what people mostly value in mangroves (IÖR)
→validate these predictions with (country-wise) existing information from the literature (e.g., FAO), expecting that many other factors (e.g., cultural, history, …) will also affect mangrove-use

*-**IWP**: (South Africa, Singapore, Fiji) more demanding and speculative, as we find the same genera in Singapore and South Africa (only partly in Fiji) but not the same species…*

Thus, no species-specific analysis of floral effects will be possible. The consortium is still discussing, and working on, what can be concluded from the corresponding data.

The above findings and conclusions will be presented to, and discussed with, all local partners in a virtual closing workshop upon having finished all analyses.

Anlagen:

Annex 1: geographical study design

Realm	Region	Year	Sampling Sites	Mangrove Species	Human Use	Stressors
Atlantic-East Pacific, AEP	**Colombia (San Andrés Island)**	**2018**	Old Point (3 plots)	*Avicennia germinans* *Laguncularia racemosa* *Rhizophora mangle* in mono-specific stands	moderate (tourism)	moderate (national park next to settlement and industrial port)
			Sound Bay (3 plots)	*Avicennia germinans* *Laguncularia racemosa* *Rhizophora mangle* in mono-specific stands	none	high (untreated sewage)
			Smith Channel (3 plots)	*Avicennia germinans* *Laguncularia racemosa* *Rhizophora mangle* in mono-specific stands	none	low
	Brazil (Ajuruteua Peninsula, Pará)	**2017**	5 sites (with three species plus "dwarf" growth of one of them/plots, each) along the main axis of the peninsula	*Avicennia germinans* *A. germinans* (dwarf form) *Laguncularia racemosa* *Rhizophora mangle* in mono-specific stands	low (RESEX: crab fisheries, small-scale wood extraction)	low (RESEX; locally disturbed hydrodynamics upon road construction in 1970s)
	Senegal (Sine Saloum)	**2019**	10 sites (with two species/plots, each) along an (inverse) estuarine salinity gradient	*Avicennia germinans* *Rhizophora mangle* in mono-specific stands	low small-scale crab fisheries, fisheries, dead wood-extraction for firewood)	low
Indo-West Pacific, IWP	**South Africa (East Coast, Kwazulu Natal)**	**2018**	iSipingo (2 plots)	*Avicennia marina* *Bruguiera gymnorhiza* in mono-specific stands	low (recreation)	moderate (garbage)
			Durban Harbor (2 plots)	*Avicennia marina* *Bruguiera gymnorhiza* in mono-specific stands	none	high (industrial port)
			uMgeni River (1 plot)	*Avicennia marina* in mono-specific stand	low (homeless, drug traffic, prostitution)	high (garbage and untreated sewage)
			Beachwood (2 plots)	*Avicennia marina* *Bruguiera gymnorhiza* in mono-specific stands	low (recreation, drug traffic)	low
			uMlalazi (2 plots)	*Avicennia marina* *Bruguiera gymnorhiza* in mono-specific stands	high (tourism)	low (nature park)
			uMhlathuze (2 plots)	*Avicennia marina* *Bruguiera gymnorhiza* in mono-specific stands	low ((illegal) fisheries)	low
			Richards Bay (3 plots)	*Avicennia marina* *Bruguiera gymnorhiza* *Rhizophora mucronata* in mono-specific stands	none	moderate (industrial port)
	Singapore	**2017**	Sungei Buloh (3 plots)	*Avicennia alba* *Bruguiera gymnorhiza* *Rhizophora mucronata* in mixed stands	high (tourism)	low (protected area)
			Mandai (3 plots)	*Avicennia alba* *Bruguiera gymnorhiza* *Rhizophora mucronata* in mixed stands	none	high (industrial park)
			Pasir Ris (3 plots)	*Avicennia alba* *Bruguiera gymnorhiza* *Rhizophora mucronata* *in mixed stands*	moderate (recreation)	high (dense settlement)
			Pulau Ubin (3 plots)	*Avicennia alba* *Bruguiera gymnorhiza* *Rhizophora mucronata* *in mixed stands*	moderate (recreation)	low (recreational area)
	Fiji (Viti Levu, Rewa River Delta)	**2019**	9 sites (with two species/plots, each) along estuarine salinity gradients of three estuarine channels	*Bruguiera gymnorhiza* *Rhizophora stylosa* in mono-specific stands	low (crab fisheries, fisheries)	low

Annex 2: References

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