Mesocosm experiments in ocean alkalinity enhancement research

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Abstract. An essential prerequisite for the implementation of ocean alkalinity enhancement (OAE) applications is their environmental safety. Only if it can be ensured that ecosystem health and ecosystem services are not at risk will the implementation of OAE move forward. Public opinion on OAEs will depend first and foremost on reliable evidence that no harm will be done to marine ecosystems and licensing authorities will demand measurable criteria against which environmental sustainability can be determined. In this context mesocosm experiments represent a highly valuable tool in determining the safe operating space of OAE applications. By combining biological complexity with controllability and replication they provide an ideal OAE test bed and a critical stepping stone towards field applications. Mesocosm approaches can also be helpful in testing the efficacy, efficiency and permanence of OAE applications. This chapter outlines strengths and weaknesses of mesocosm approaches, illustrates mesocosm facilities and suitable experimental designs presently employed in OAE research, describes critical steps in mesocosm operation, and discusses possible approaches for alkalinity manipulation and monitoring. Building on a general treatise on each of these aspects, the chapter describes pelagic and benthic mesocosm approaches separately, given their inherent differences. The chapter concludes with recommendations for best practices in OAE-related mesocosm research.

Preface

The authors would like to emphasize that this chapter does not intend to cover all aspects of mesocosm experimentation in its full breadth, but rather tries to address aspects specific to research on ocean alkalinity enhancement (OAE) or aspects we consider important to reiterate here. For a more comprehensive presentation of recommendations and guidelines on mesocosm experiments the reader is referred to Chapter 6 of the *Guide for Best Practices on Ocean Acidification Research and Data Reporting* (Riebesell et al. 2010) and references therein as well as Stewart et al. (2013).

Although the general approach to mesocosm experiments is straightforward and basically involves enclosing a body of water with or without sediment in order to monitor responses of the enclosed communities and related

processes to the manipulated perturbation over an extended period of time, the specifics of conducting such experiments can vary considerably. These include factors such as the materials, design and location of the enclosures, e.g. fixed structures on land or flexible wall enclosures in situ, as well as the procedures for mesocosm filling, operation, mixing and sampling. While the dimensions of the experimental enclosures can range from less than 1 m^3 to >1000 m^3 depending on the requirements of the experiment, we here adopt the classification set out by the SCOR Working Group 85 in 1991: Microcosms (less than 1 m^3), mesocosms (between 1 and 1000 m^3) and macrocosms (more than 1000 m^3). We note that benthic experimental enclosures can have different size categories.

1 Placing mesocosms in the context of OAE research

Mesocosm experiments provide an essential bridge between the tightly controlled but poorly realistic laboratory culture experiments and the complexity of natural systems. This is particularly important for possible OAE implementations, in order to achieve a sound understanding of the entire process of the proposed OAE strategies, from the dissolution kinetics and effectiveness of the alkalinisation technique, to the potential environmental impacts, risks and co-benefits. This knowledge is crucial prior to any form of OAE application to safeguard the protection of marine ecosystems functioning, biodiversity and related ecosystem services. Moreover, should OAE prove to be a viable approach for marine carbon dioxide removal (mCDR), it will also be crucial to achieve social acceptance for potential OAE implementations. Also in this context mesocosm experiments can serve as a useful tool for proof of concept, the results of which can play an important role in the public discourse about the risks and benefits of mCDR implementation.

Functional redundancy and species richness in ecosystems allow for some degree of resistance to withstand disturbances and resilience to recover once a disturbance has ended or dissipated. To determine the actual ecological impacts of OAE it is essential, therefore, to test suggested applications at the community/ecosystem level. Doing this in field trials, however, poses serious difficulties, given the hydrographic complexity of most marine systems, with lateral advection (currents, tides), vertical flow (convection, up- and downwelling) and wave-driven mixing. Determining dose-response relationships for environmental impacts is extremely challenging under such conditions. Mesocosm experiments, on the other hand, enable the combination of biological complexity needed for testing resistance and resilience of communities/ecosystems in their natural setting and seasonal succession (in a single experiment where succession occurs on short time scales, e.g. a phytoplankton bloom, or multiple experiments in different seasons using the exact same experimental set-up) with a reasonable degree of control and replication and hence the statistical power to reach reliable conclusions. At the same time, they allow testing the chemical kinetics of mineral dissolution and secondary carbonate precipitation, thereby providing vital information on the efficacy of the suggested OAE applications in a natural setting under a range of environmental conditions (salinity, temperature, carbonate chemistry, inorganic nutrient concentrations, dissolved and particulate organic carbon concentrations etc). Testing them in mesocosm enclosures has the additional benefit of minimizing public concern and regulatory requirements when compared to field trials.

Environmental impacts of OAE will be scale- and context-dependent in terms of the physical (e.g. timescales of mixing and CO_2 equilibration, point source vs. diluted release), chemical (e.g. amount/type of alkaline substance, impurities), and biological characteristics (e.g. seasonal succession and related ecosystem vulnerability).

74 Biological impacts are determined by exposure time and dose, ranging from acute shock responses on transient
75 and local scales at point sources to chronic effects associated with possible transitions of ecosystem structure and
76 performance at the regional and long-term scale. Key research questions which can be addressed adequately
77 through mesocosm experiments are:

78 - What is the safe operating space for OAE applications with respect to possible impacts on marine
79   ecosystems functioning, biodiversity, and ecosystem services?
80 - How could OAE be implemented to reduce the risk of inadvertent negative environmental effects, and
81   maximize co-benefits?
82 - Which biological indicators can serve as early warning signals or proxies for OAE environmental
83   impacts?
84 - How do different OAE approaches perform in terms of efficiency (e.g. mineral dissolution, CO_2 uptake)
85   and permanency (e.g. secondary precipitation)?
86 - Which application sites are most appropriate for which OAE approach?

87 **2 Strengths and weaknesses of mesocosm experimentation**

88 Mesocosm experiments offer a salient advantage over laboratory-based investigations, as they allow a realistic
89 replication of natural communities. Multiple trophic levels can be confined under natural environmental
90 conditions over a long period of time in a self-sustaining manner. Thereby, the same community can be sampled
91 repeatedly over time. Furthermore, these experiments permit straightforward validation in the context of field
92 research. Mesocosms, in essence, are closer to representing natural ecosystems characterized by carefully defined
93 dimensions and monitored conditions and processes. To ensure realistic ecological boundary conditions,
94 mesocosm experiments should be exposed to meteorological conditions resembling those of the target
95 environment. Notably, the logistical flexibility of mesocosms affords researchers the opportunity to conduct
96 investigations beyond the geographical confines of the environment under investigation. Consequently,
97 mesocosms provide an invaluable avenue for the controlled study of specific environments and the impact of
98 controlled manipulations therein. Given the diverse range of natural processes encountered in mesocosm
99 experiments, external influences may be challenging to control, necessitating a robust monitoring strategy to
100 achieve statistical power by either treatment replication or treatment gradients. Moreover, mesocosm experiments
101 provide extensive multidisciplinary datasets that allow for a high degree of scientific integration and
102 interdisciplinary collaboration. These datasets are valuable for parameterisation and assessment of marine
103 ecosystems and biogeochemical models.

104 While mesocosm experiments can be considered the preferred tool for the assessment of environmental impacts
105 of OAE applications, they have several weaknesses that need to be considered when interpreting the data and
106 extrapolating the results to the real world. These weaknesses include unnatural mixing and turbulence (in pelagic
107 mesocosm), unnatural flow of bottom water across the sediment (in benthic mesocosms), wall effects and the
108 growth of periphyton and other organisms on the mesocosm walls, spatial heterogeneity in the enclosed sediments
109 and the related difficulties in obtaining representative samples. The larger and more expensive the enclosures
110 become, the more difficult it becomes to have a sufficient number of replicates in a replicated design or treatments
111 in a gradient design. The fact that even the largest mesocosms enclose truncated communities, i.e. exclude higher

112   trophic levels and highly migratory organisms make it difficult to adequately represent the responses of organisms
113   with longer life cycles and the associated impacts on the food web. Another drawback of mesocosm experiments
114   is their limited duration, due to the gradual diversion from their natural counterparts, e.g. due to community shifts,
115   nutrient depletion, and the consequent progressive loss of biological realism. The increasing variability between
116   mesocosms in this process makes it increasingly difficult to identify treatment effects with statistical significance.

117   **3 Experimental design**

118   The primary purpose of a mesocosm experiment is to obtain "near-natural" conditions, that is to say, keeping the
119   abiotic and biotic factors as close to the environment as possible in order to maximize the realism of the tested
120   conditions. In general, time scale is related to mesocosm volume: the shorter the time needed for a controlled
121   experiment, the smaller the enclosure size. Careful consideration should be given to the experimental design to
122   adequately address the specific research questions, account for ecosystem- and site-specific characteristics as well
123   as seasonal variability. The choice of the experimental configuration includes the three key dimensions of time,
124   space and biological complexity, along with the required level of replication. Preference should be given to mimic
125   the natural seasonal succession rather than provoking out-of-season events, e.g. triggering phytoplankton blooms
126   through nutrient addition.

127   Considering the often limited number of experimental units, a critical consideration concerns the level of
128   replication (Kreyling et al. 2018). The choice is between two basic approaches: (1) replicated (n≥3) treatments,
129   with limited treatment levels (e.g. Riebesell et al., 2006); (2) a gradient approach with a larger number of non-
130   replicated treatment levels (e.g. Taucher et al., 2017). The statistical power of the two options, using ANOVA
131   statistics for the replicated design and regression statistics for the gradient design, is similar for the small number
132   of experimental units typically available in mesocosm studies (Havenhand et al., 2010). If large within-treatment
133   variation is expected, e.g. due to strong environmental variability or spatial heterogeneity, the replicated approach
134   is recommended. In fact, strong within-treatment variability can easily mask subtle treatment effects. An important
135   advantage of the gradient approach, on the other hand, is that it enables the identification of non-linearities,
136   thresholds and tipping points in biological responses to OAE applications, relevant information for model
137   parameterizations in terms of community functional responses. Knowledge about thresholds and possible tipping
138   points is crucial also in the context of regulatory considerations for OAE implementation.
139
140   Pelagic mesocosms
141   When aiming to investigate OAE applications in the free water column, pelagic mesocosms are the research tool
142   of choice. Among the various proposed strategies, ocean liming in the wake of ships would consist of sparging
143   high-alkalinity fluids or mineral particles within the surface layer in offshore settings. In this scenario, any
144   chemical perturbation is expected to affect in the first instance the pelagic domain and the planktic component of
145   the marine ecosystem. Also OAE applications at fixed locations with a discharge of alkalinity-enriched water into
146   coastal waters, e.g. desalination plants or sewage treatment plants, are best simulated in pelagic mesocosms. A
147   suitable simulation of OAE approaches in which the alkalising mineral is released in particulate form should
148   ideally have the dissolution rate of the particles known in advance. If the rate is fast enough to ensure complete
149   dissolution in the water column, pelagic mesocosms are well suited. In cases where the dissolution rate is slow

150 compared to the particle sinking rate and particles sink to the seabed before dissolving, the experimental design
151 may require a benthic component.

152 A missing component in all closed-system mesocosm experiments is the dilution through mixing with non-
153 perturbed waters. Switching to an open system, where the enclosed water is partially replaced by non-alkalised
154 water, places much greater demands on monitoring and complicates the interpretation of the observed responses,
155 to the extent that it may be impossible to establish a reliable dose-response relationship. This experimental artifact
156 is exacerbated when repeated additions of alkalinity are applied. Incorporating naturally occurring dilution in the
157 experimental design can be done by applying the OAE treatment to only part of the enclosed water column and
158 allowing for gradual mixing with the untreated water. The time until mixing can be controlled by stratifying the
159 water column through a salinity gradient (adding fresh water into the upper layer or brine into the bottom layer,
160 whereby the salinity change should be at a low enough level not to cause a biological response, e.g. a few tens of
161 a salinity unit) or via a temperature stratification. Break-off of the stratification can be gradual or abrupt through
162 active mixing. Parallel sampling of the OAE treated and untreated water bodies can provide insights about the
163 compensating effect of dilution.

164 There is a wide range of enclosure volumes and structures used in pelagic mesocosm experimentation. Among
165 the various available solutions, the most obvious difference is the placement of the mesocosm: 1) stable,
166 permanent structures on land, or 2) floating bags in the water. All materials that come into contact with the
167 enclosed water/sediment must be chemically inert, i.e. they must not leach or actively absorb any substances.
168 Some technical details of the mesocosm design can markedly affect some abiotic factors, such as thermal
169 characteristics, light conditions or mixing intensity of the enclosed water column. Most pelagic mesocosm
170 enclosures are made of transparent material supported by a mini-mal rigid framework, with the intent to keep light
171 conditions as in nature. Most materials, however, change the spectrum of the transmitted light, for example are
172 not transparent for UV-light. As enclosure depth is often lower than the mixed layer depth of the natural
173 environment, natural light conditions are not well represented in mesocosms, with light intensities averaged over
174 the mesocosm depth often higher than those averaged over the mixed layer depth.

175 Benthic mesocosms
176 Benthic mesocosm experiments offer the unique chance to study OAE-mineral addition to the seafloor in a
177 controlled set-up. In comparison to experiments in laboratory settings, often small in scale with respect to mineral
178 weathering, benthic mesocosms are more likely to mimic natural seafloor conditions and allow the coupling of
179 biogeochemical processes at larger spatial and temporal scales. Key research questions on seabed alkalinisation
180 to be addressed in benthic mesocosm experiments include: 1) What are alkaline mineral dissolution rates under
181 mesocosm conditions? 2) Do secondary minerals form that may compromise the net CO_2 sequestration efficiency
182 of this method? 3) How are microbial communities and macrofauna affected by mineral dissolution? 4) Is there a
183 release and accumulation of heavy metals related to addition of silicate-based minerals and how does their toxicity
184 affect the community/ecosystem?
185
186 Continuous water flow system: In this set-up, a continuous flow of ambient seawater, preferably bottom water,
187 over the sediment (Fig. 2), likely best resembles natural seafloor conditions. It is recommended to remove larger

188 debris that could obstruct the water supply using a sediment trap (Fig. 2), whilst allowing small particulate matter

189 to enter the mesocosms. The supply of particulate matter is essential to sustain natural microbial metabolism in

190 the sediments and to provide food for filter-feeding macrofauna that colonize the sediment surface within a short

191 period of weeks to months (Fig. 2). A relatively high flow rate is required (between 5000 to 10000 L d^{-1}) to keep

192 the seawater well oxygenated and guarantee the survival of fauna and for maintaining the natural microbial

193 communities as closely as possible to in situ conditions. With this set-up, the bottom water should be monitored

194 to trace seasonal changes in physical and chemical properties of the incoming seawater.

195 Water circulation approach: The benthic mesocosm set-up with a seawater circulation approach consists of two

196 tanks stacked on top of each other, with the upper tank housing the benthic ecosystem with sediments and

197 organisms and the lower tank is functioning as a seawater reservoir from which water is pumped into the upper

198 tank (Fig. 3). Thus, a constant flow of water is generated through the water in- and outflow and the height of the

199 water column in the upper tank can be controlled by the vertical positioning of the outflow. The tanks for the

200 benthic mesocosms have a volume of approximately 1 m^2 and are situated outdoors and exposed to natural

201 temperature fluctuations.

202 Based on the water circulation approach, the closed system allows for the detection and accumulation of

203 weathering products and to focus on a specific process or reaction, such as the dissolution kinetics of silicate

204 minerals in the case of the University of Antwerp study (Fig. 3). After a defined timespan (flux session) the total

205 amount of water is replaced and accumulation of weathering products starts again from initial values. In terms of

206 this experiment design, ≥3 replicates of benthic mesocosms are crucial to ensure that results are statistically

207 significant and can be generalized to the broader ecosystem being studied (e.g. Wadden Sea).

208 The total experiment duration as well as the sampling strategy is defined by the research questions and longer

209 experiments may be necessary to capture seasonal or long-term trends in the system. The use of natural sediment

210 and the inclusion of a dominant bioturbating organism (e.g. *Arenicola marina*) in benthic mesocosm experiments

211 is a crucial step toward making the experimental setup more representative of real-world conditions. However,

212 it's important to emphasize that the choice of sediment type and benthic organisms should be aligned with the

213 specific research objectives and questions being addressed.

214

215 In OAE studies involving benthic mesocosms, various types of sediments can be considered, ranging from fine-

216 grained sediments to rocky substrates. The selection of sediment type should be guided by factors such as the

217 local environmental conditions, the availability of sediment types that reflect the targeted ecosystem, and the

218 specific geochemical interactions being investigated. For studies related to carbonate dissolution and alkalinity

219 enhancement as given above, fine-grained or sandy sediments are most suitable, given their potential to facilitate

220 mineral dissolution and subsequent alkalinity release.

221

222 Similarly, the choice of benthic organisms should be tailored to the research objectives. While many benthic

223 organisms can be tested in mesocosms, it's important to consider the life history, behavior, and ecological role of

224 the selected species (Bach et al. 2019; Flipkens et al. 2023). For instance, if the experiment spans a year and aims

225 to study the recruitment and life cycle of benthic organisms that have a pelagic phase, careful planning is required.

Monitoring larval settlement, growth, and interactions with the sediment during their benthic phase becomes integral to such investigations.

As an illustrative example, consider an OAE study targeting the enhancement of carbonate precipitation through the addition of alkalinity. In a coastal setting, sandy sediments rich in carbonate minerals might be chosen, given their potential for mineral dissolution and subsequent bicarbonate formation. Benthic organisms like filter-feeding mollusks and burrowing polychaetes could be tested to assess their responses to altered alkalinity levels.

Finally, the water circulation approach should be carefully designed to ensure consistency in water flow rates and initial seawater chemistry. Sedimentation in the water reservoir tank has to be prevented to avoid secondary sediment surfaces and a continuous monitoring system (salinity, temperature) is recommended to estimate evaporation rates. In addition, regular sampling of environmental conditions (humidity, pCO_2) as well as carbonate system parameters and nutrients, can ensure that the experiment proceeds as planned and that the results are reliable.

4 Mesocosm operation: filling, sampling, wall cleaning

Filling of the mesocosms is a delicate process that, if not done with care, can jeopardize the entire experiment. A key aspect is to ensure identical starting conditions, both for the abiotic and biotic conditions in all mesocosms. Between mesocosm differences in baseline conditions can cause divergence of the enclosed communities and severely hamper the detection of treatment effects. As the filling often represents a major perturbation itself, some time of equilibration may be needed before applying the treatment manipulation and starting the actual experiment. The time for equilibration may differ for pelagic and benthic habitats as well between different ecosystems and seasons. Adequate monitoring during this pre-manipulation phase can determine when a new steady state is reached and confirm whether all mesocosms have similar starting conditions. Key parameters for which equal starting conditions among mesocosms need to be ensured include temperature, salinity, inorganic nutrient concentrations, the carbonate chemistry (pH, pCO2, DIC TA), dissolved and particulate organic matter concentrations, community composition and diversity, and standing stocks of the dominant taxonomic groups across trophic levels.

Another critical aspect of mesocosm operation is taking representative samples. The enclosed water bodies and sediments typically show spatial heterogeneity (vertical gradients in the water column and sediments, patchiness in the distribution of larger organisms). The spatial variability of the target variables of the enclosed system should be determined prior to deciding on the best sampling strategy. Sampling bias related to vertical gradients, e.g. water column nutrient concentration and phytoplankton biomass, can be overcome by taking depth-integrated water samples (Fig. 4). Some species may even perform diurnal vertical migration, which also should be accounted for in the sampling strategy.

Mesocosm enclosures are always associated with additional surfaces, the mesocosm walls, that are not present in the natural environment. The smaller the mesocosms, the larger the additional surface area relative to the enclosed volume. Free surfaces are generally subject to rapid biofilm formation, followed by colonization of larger

organisms. The associated microbial community can significantly influence water column processes, which is of particular concern in pelagic mesocosms. To minimize such wall effects, cleaning of the mesocosm walls can be useful. Specific to OAE mesocosm experimentation is that under conditions where the water column is highly oversaturated with respect to calcium carbonate, mesocosm walls can provide free surfaces for secondary precipitation of carbonates. Under these circumstances, wall cleaning can scrape off these carbonates, creating additional precipitation nuclei in the water column. If wall cleaning is continued under these circumstances, possible effects caused by this, e.g. enhancement of secondary precipitation in the water column and increased ballasting of particulate matter, should be seen as artifacts and interpreted as such. If wall cleaning is discontinued and the biofilm on the walls grows to a significant biomass compared to the suspended biomass, this may limit the duration of the experiment. The decision for or against wall cleaning must be made on a case-by-case basis and depends, among other things, on the severity of wall growth, the duration of the experiment and the specific research questions to be investigated.

Pelagic mesocosms

Different techniques have been employed for filling pelagic mesocosms, including (1) direct pumping from the sea in cases where mesocosms are placed *in situ* or close to natural waters, (2) collection in tanks when source waters need to be transported over some distance and subsequent pumping from the tanks into the mesocosm, (3) lowering a flexible bag like a curtain over an undisturbed water column. In all cases care should be taken to fill the mesocosms with identical source waters. Considering that water masses may change over the filling procedure, this can best be achieved by filling the mesocosms in parallel through a distributor system (Fig. 4). Likewise, if several tanks are needed to obtain the required source water volume, the water of each tank should be distributed evenly into all mesocosm units. The source water should be representative for the targeted ecosystem. This concerns the depth at which the source water is collected and, when diurnally vertically migrating organisms are present, the time of day. When pumping is applied some damage to fragile organisms, e.g. gelatinous zooplankton, is unavoidable. It is therefore recommended to use pumps that ensure a smooth flow of pumped water, e.g. peristaltic pumps (Fig. 4). To prevent large and rare organisms from entering and being unevenly distributed in the mesocosms, some screening can be applied at the intake of the pumping hose.

As mentioned above a typical artifact of mesocosm enclosures is the reduced level or absence of turbulence. In mesocosms with solid wall structures it may be useful to apply some form of mixing of the water column, considering that turbulence (including its absence) is known to strongly affect the plankton community composition and succession. In floating enclosures with flexible walls some turbulence is induced by surface wave action, below surface water movement and variability in water currents, but the vorticity of the enclosed water is still always much reduced compared to that of the natural environment. Somewhat related to the mixing regime is another potential artifact in mesocosms where settling particulate matter is continuously resuspended from the bottom. Resuspension of degrading organic matter, which under natural conditions would sink out of the upper mixed layer, exaggerates the heterotrophic processes in the system. Collecting and removing the sedimented matter in cone-shaped sediment traps which form the bottom of the mesocosms can avoid this problem (Fig. 4).

Benthic mesocosms

A particular challenge in benthic mesocosm experiments concerns the filling with sediment from the seafloor. Depending on the size of the tanks and the sediment height, it may be necessary to transfer several hundreds of kilograms of sediment from the seafloor to the tanks. Near intact sediments (undisturbed vertical stratification) may be collected relatively easily in sub-tidal areas. At sea, undisturbed sediments may be retrieved using a box corer or similar device, although this may be a tedious exercise involving multiple deployments of the coring equipment. Large amounts of sediment can be gathered relatively easily and quickly using a sediment grab, but disturbance of the sediment matrix is inevitable, and longer equilibration times for the sediment geochemistry to stabilize will be required before experiments can be started. In any case, benthic communities within mesocosms may be altered from those in natural ecosystems and a sound understanding of the equilibration period is crucial to allow for changes in benthic communities and the establishment of a new steady state within the benthic mesocosm. This equilibration period should be determined based on the specific conditions of the mesocosm experiment, including the number of replicates, environmental parameters, and the selected organisms. Adequate monitoring and sampling during the equilibration period are essential to ensure that the experimental conditions have stabilized and the ecosystem has reached a new steady state which in turn increases material and labour requirements. Robust control units are crucial in benthic mesocosm experiments and should ideally consist of the same number of replicates as the treatment group to ensure that any observed changes are due to the experimental treatments rather than natural variability. Sampling and monitoring should be in the same manner as the treatment group.

5 Alkalinity manipulation and monitoring

Different minerals, waste materials and electrochemical products have been suggested as feedstock for ocean alkalinity enhancement (for a comprehensive introduction to potential source materials see Eisaman et al. 2023). Most source materials do not come as pure alkalinity, but contain other substances, such as silicate, calcium, magnesium and various trace metals (e.g. iron, nickel, cobalt, chromium). OAE can be achieved by addition in dissolved form, which requires dissolution of the feedstock before its release into the sea, or in particulate form, after grinding of the feedstock, with the grain size being one important factor determining the dissolution rate. OAE can further be conducted in a CO_2-equilibrated mode, which involves some form of active injection of CO_2 into the alkalinity-enriched source water prior to its release, or in a non-equilibrated mode, which relies on air-sea gas exchange to provide the additional CO_2 that the alkalinized seawater can absorb. In case of the latter it is important to keep in mind that the time scales for CO_2 equilibration are on the order of months and can only occur as long as the alkalinized seawater is in contact with the atmosphere. (see Schulz et al., 2023 for further details)

Taken together, this results in a wide range of possible application scenarios, not all of which can be tested with the same scrutiny in mesocosm experiments due to the high financial and personnel costs involved. Hence, it is important to focus on those OAE application scenarios which are most likely to be implemented. As the field of OAE R&D is developing rapidly and dynamically, there will likely be changes in what is considered the most suitable OAE application approaches, in terms of cost, efficiency, environmental safety, friendliness in terms of monitoring, verification and reporting (MRV), technological readiness, as well as the regulatory requirements for their implementation. Mesocosm research in this field should maintain sufficient flexibility to respond to those changes and aim for testing 'real-world' scenarios of OAE applications. On the other hand, because the results

340 obtained from mesocosm studies will likely be context-specific (depending on e.g. ecosystem type, time of year,
341 latitudinal location, hydrographic setting and depend on the mesocosm set-up and operation itself, it takes multiple
342 such studies for a given OAE approach to reach robust conclusions about its environmental safety. To facilitate
343 inter-comparison between results it would be favorable to use standardized mesocosms and follow common
344 protocols for mesocosm experimentation.

345

346 From an experimental perspective, there is a trade-off between testing pure alkalinity enhancement and feedstocks
347 which involve the release of other biologically active components. While the latter is more in line with real-world
348 applications, it complicates the interpretation of the observed responses due to confounding factors and limits the
349 extrapolation of the findings, considering that the stoichiometric composition differs between feedstocks. As the
350 field is currently still at an early stage and considering that the number of mesocosm studies will likely be small
351 due to their high costs, it seems beneficial to first establish a basic understanding of alkalinity effects in isolation,
352 before turning to more feedstock-specific testing. This being said, we note that the above-mentioned confounding
353 effects may actually be the intended research question or that the focus may be on a specific feedstock likely to
354 be utilized widely. In general, we recommend designing mesocosm experiments with a more generic approach
355 first and address feedstock-specific in smaller scale laboratory-based experiments.

356

357 Pelagic mesocosms

358 Alkalinity manipulations in pelagic mesocosms are fairly straightforward when done in dissolved form.
359 Dissolving the alkaline feedstock in freshwater or deionized water prevents secondary carbonate precipitation
360 during preparation of the concentrated solution (we note that the use of freshwater for feedstock dissolution may
361 not be practical for large-scale implementation of OAE). To avoid confounding effects of the freshwater addition
362 on the mesocosm community, the volume should be kept to a minimum. Using source materials with a high
363 solubility in water, such as $NaHCO_3$, Na_2CO_3, $Ca(OH)_2$ or NaOH enables highly concentrated alkaline source
364 water (Hartmann et al., 2023). To simulate CO_2-equilibrated alkalinisation $NaHCO_3$ and Na_2CO_3 can be combined
365 in appropriate proportions (Subhas et al., 2022), for non-equilibrated alkalinisation carbonate-free source
366 materials such as NaOH and $Ca(OH)_2$ can be used (Moras et al., 2021). To avoid prolonged pH peaks and
367 secondary precipitation during the injection procedure it needs to be assured that the concentrated solution is
368 mixed in rapidly. One way to achieve a uniform alkalinity enhancement across the water column is to move a
369 distribution device with multiple outlets up and down the mesocosms at a constant speed (Fig. 5). Flocculent
370 precipitates that form directly at the injection site are usually not stable and disappear quickly when further diluted
371 through mixing. Care should be taken to ensure that the added alkalinity is evenly distributed throughout the
372 enclosed water column.

373

374 Alkalinity enhancement in particulate form is far less practical. If the particles sink faster than they dissolve, they
375 accumulate on the mesocosm floor or sink directly into the trap in mesocosms with a sediment trap at the bottom.
376 Accumulation and subsequent dissolution at the bottom might lead to highly concentrated alkalinity enrichment,
377 enhancing the risk of secondary precipitation and of strong negative impacts in bottom waters. Alkaline particles
378 sinking into the sediment trap would be lost from the mesocosm enclosure during the next trap sampling. In both
379 cases it would be considered an experimental artifact. It is therefore recommended to use minerals with high

380   dissolution rates (e.g. NaOH, CaO, Ca(OH)$_2$, Mg(OH)$_2$) and small grain sizes to ensure dissolution before the mineral

381   particles reach the bottom of the mesocosms (see Eisaman et al. 2023 for a detailed description of technical aspects

382   of OAE).

384   Monitoring of seawater carbonate chemistry in the water column should adhere to the guidelines provided in

385   Schulz et al., 2023. High levels of non-equilibrated alkalinisation can lead to secondary precipitation, triggering

386   a process termed "runaway precipitation" (Moras et al., 2022; Hartmann et al., 2023), whereby carbonate

387   formation can consume more alkalinity than initially added. It seems that the initiation of this process can occur

388   both in the water column and on the mesocosm walls. As the carbonate crystals grow in size, their sinking velocity

389   increases. When incorporated in organic matter aggregates they serve as ballast, thereby increasing the vertical

390   flux of organic matter. In addition, carbonate crystals could affect mobility and feeding of plankton organisms,

391   with possible adverse effects on food web interactions and trophic transfer. Secondary precipitation also increases

392   seawater turbidity, affecting light attenuation and possibly primary production. Collecting this sinking particulate

393   matter in sediment traps at the bottom of the mesocosms enables the quantification and identification of the

394   precipitates and provides information about the chemical reactions leading to their formation. In mesocosms

395   without integrated sediment traps, simple traps can easily be set up on the bottom and sampled through a tube that

396   reaches the surface.

398   Benthic mesocosms

399   Alkalinity enhancement in the benthic mesocosm approach is achieved by mineral addition, which dissolves in

400   the surface sediment over time. In general, the addition of sedimentary OAE source materials (e.g. siliciclastic

401   minerals, carbonates; Eisaman et al., 2023) modifies the grain size distribution of the sediment and thus affects

402   the porosity, permeability, and water flow through the sediment. The changing sediment structure can impact

403   living conditions for organisms, as well as the distribution and abundance of organisms living in the sediment and

404   their behavior and ecology. With respect to mineral addition, the grain size selection is important, as a trade-off

405   between grain size and production costs is required (e.g. Hartmann et al., 2013). Previous studies have investigated

406   the relationship between CO_2-sequestration efficiency and grain sizes and there is a general assumption that small

407   grain sizes reveal higher dissolution rates and CO_2 sequestration rates due to larger reactive surface areas, whereas

408   more grinding energy is required generating a higher CO_2 footprint and lower CO_2-sequestration efficiencies

409   (Köhler et al., 2010; Renforth and Henderson, 2017; Foteinis et al., 2023). Clearly, the CO_2 emissions during

410   production and transport must be significantly lower than the potential CO_2 sequestration of benthic mineral

411   dissolution (see Eisaman et al., 2023). The selection of appropriate grain sizes for the addition of alkaline minerals

412   is a critical consideration for experimental studies, particularly in the context of the target environment's

413   geological setting. From an environmental perspective, it is recommended to choose comparable grain sizes that

414   are stable under in-situ hydrodynamic conditions. For highly dynamic ecosystems such as the Wadden Sea,

415   estuaries and wave-dominated coastal areas, a range of grain sizes from fine to coarse sand (0.075 to 2 mm) may

416   be appropriate for experimental approaches. However, in low-dynamic systems such as lagoons, enclosed bays,

417   or shelf regions, grain sizes from silt to very fine sand (<0.075 mm) can be considered for investigation. This

418   approach would also help to ensure that the sedimentary structure and settings for organisms in the mesocosms

419   are representative of the natural conditions of the target environment.

It may be practical to interrupt the water circulation system during mineral deployment in order to allow sedimentation of the suspended matter. To achieve a uniform alkalinity enhancement in the benthic mesocosms, minerals should be evenly distributed. To induce a measurable effect on alkalinity changes in the envisioned experimental time, grain sizes smaller than 1 mm are desirable (Strefler et al., 2018). The addition to the marine environment could best be achieved through a mixture of natural seawater, marine sediments, and OAE source materials. This may ensure a more uniform distribution and reduce the purity of industrially produced OAE source materials, which are poor in nutrients and microbial organisms. Thus, this approach is also recommended for the addition of silicates to benthic mesocosms. By using a mixture, the potential effects of silicate addition can be more accurately evaluated because the experimental conditions are more similar to those in the natural environment.

For calcium carbonate, it may be reasonable to use the annual flux of POC to the seafloor as an upper estimate of the required mineral to be added. The underlying assumption here is that the added mineral can completely neutralize the natural CO_2 produced from organic matter degradation. However, this assumes that mineral dissolution efficiency is close to 100 %, which may not be the case if it is mixed below the undersaturated layers. Adding minerals in large excess risks clogging the surface layer and creating a physical barrier against effective benthic-pelagic coupling of solute fluxes. Finding the optimal mineral dosage to achieve a balance between dissolution efficiency and dissolution rate would likely be specific to the local environmental characteristics and require testing at each potential mineral addition site. For silicate minerals (e.g. olivine), the upper limit of mineral addition per square meter will also depend on the trace metal concentrations (Flipkens et al., 2021). Based on the variation in Ni content of marine sediments (prior to the addition of olivine), this implies that the allowable range for the addition of olivine is between 0.059 and 1.4 kg per square meter of seafloor without posing a risk to benthic biota. This threshold is based on Environmental Quality Standards (EQS), which are derived from metal toxicity data using methods such as species sensitivity distributions (SSDs). They provide threshold metal concentrations in seawater or sediment that are considered protective for the aquatic environment and are used by industries, governments, and environmental agencies to guide regulations. So far, these guidelines are only appropriate to specific regions and environments and may need to be re-evaluated for a broader use in OAE applications.

Monitoring of mineral dissolution will be determined by the experimental design. A major drawback of a high through-flow is that rapid dilution and flushing of geochemical tracers emitted from the sediment compromises the analytical detection of dissolving alkaline minerals in the overlying water and the reliable assessment of the effectiveness of the method (see also section 4.4.3). In this case, alternative ways of mineral dissolution detection may be required. For instance, alkalinity enhancement may be detectable in pore fluids, which can be extracted using filters (e.g., rhizones) inserted horizontally through holes pre-drilled vertically in the tank (Fig. 6). However, the vertical sampling resolution may be too coarse to detect mineral dissolution close to the sediment surface. Microelectrodes for O_2, pH and H_2S are arguably a better alternative to detect changes in surface geochemistry in the uppermost centimeters after mineral addition. An advantage of the high dilution factors is the potential suppression of secondary mineral formation such as phyllosilicates and/or carbonates, that could reduce the net CO_2-sequestration efficiency of OAE (Fuhr et al., 2022, Moras et al., 2022, Hartmann et al., 2023). Secondary mineral formation is a common process in marine seafloor sediments, potentially impacting global carbon and

458 element cycles on a global scale, the controlling factors are not unambiguously identified to date (e.g. Rahman et
459 al., 2017; Torres et al., 2020; Geilert et al., 2023).

460 The deployment of benthic incubation chambers within the mesocosms themselves is a non-invasive method for
461 detecting alkalinity release following mineral addition (Fig. 6). These benthic chambers enclose a certain area of
462 the surface sediment and allow the accumulation of alkalinity and other components of interest over time, from
463 which benthic fluxes can be determined. Mineral dissolution rates can be estimated by comparison with control
464 mesocosms where no minerals were artificially added. Fluid sampling can be achieved by hand via suction using
465 connected tubing and syringes. Care is needed to prevent hypoxia or anoxia inside the chambers due to respiration
466 by benthic biota, which may be observable by a blackening of the sediment surface due to precipitation of iron
467 sulfide minerals. Low oxygen levels will result in an interruption to the normal respiration rates of animals causing
468 them to resurface. This may alter natural sediment mixing rates as well as mineral saturation states via changes in
469 biogeochemical turnover rates and pathways in the sediment. Together, these undesired artifacts may be reflected
470 in unrealistic fluxes of alkalinity and other solutes from the sediment. Completely interrupting the water flow to
471 the whole benthic mesocosm in order to detect changes in bottom water alkalinity will only serve to magnify these
472 side effects.

473 **Recommendations**
474 General
475   - Use inert materials for mesocosm hardware (e.g. plastics, stainless steel)
476   - Select the mesocosm size and experimental duration according to the enclosed community and processes
477   studied
478   - Choose the experimental design to maximize the statistical power and report it
479   - Maximize similarity in starting conditions between mesocosms during enclosure filling
480   - Monitor starting conditions before applying experimental treatment
481   - Allow for the natural (e.g. seasonal) succession and avoid out-of-season events
482   - Avoid confounding factors and perturbations other than the intended treatments
483   - Adapt the sampling frequency to the dynamics of the processes studied
484   - Determine spatial heterogeneity and take account of it in the sampling strategy
485   - Apply depth-integrated sampling in case of vertical gradients (pelagic mesocosms)
486   - Minimize wall growth, e.g. by regularly cleaning the walls
487
488 OAE-specific
489   - Test real-world OAE scenarios, focusing on those most likely to be implemented
490   - Keep some flexibility to respond to changes in the OAE R&D field
491   - Monitor carbonate chemistry with at least two carbonate system parameters and watch out for
492   secondary precipitation
493   - Maximize transferability of results by testing generic OAE approaches
494   - Take note of the context-specificity of the observed ecosystem responses
495   - Provide detailed information of the feedstock composition utilized for experimental manipulations

496   -   Closely monitor signs of potential barriers to OAE implementation, e.g. long-term restructuring of
497   community composition and functioning, decline in ecosystem productivity, proliferation of harmful
498   species, disruption of trophic transfer, changes in elemental cycling

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Competing interests

None of the authors has any competing interests.

Author Contributions

UR scoped and edited the contents of the manuscript. UR drafted the general text, with contributions from all co-authors. UR drafted to the sections specific to pelagic mesocosms, with contributions from DB. SG, AD, and MK drafted the sections specific to benthic mesocosms. All authors contributed to revising the manuscript.

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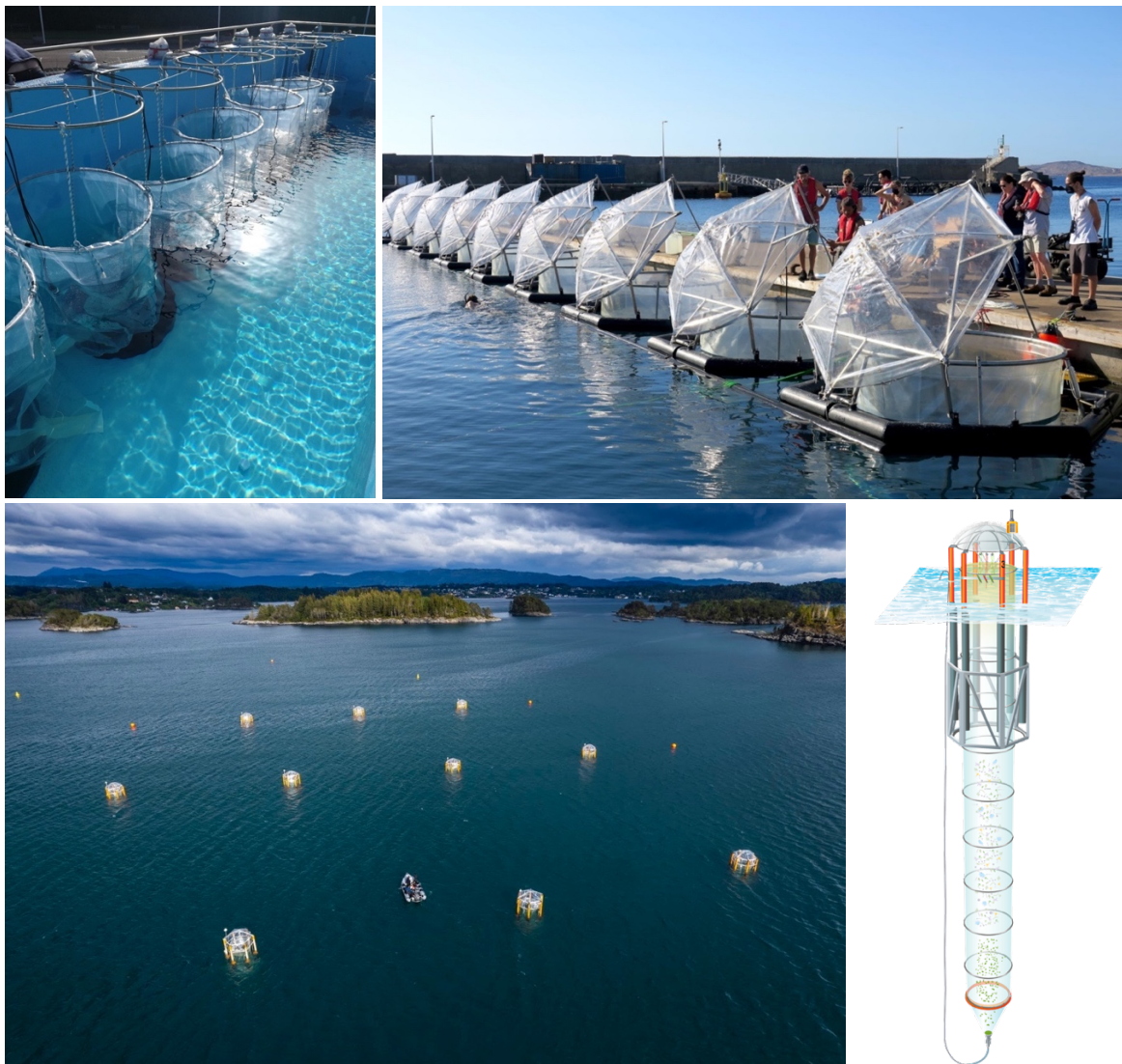
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600 Figure 1. Pelagic mesocosm facilities currently used in OAE research. *top left:* Land-based mesocosms (1 m3)

601 at the University of Vigo, Spain. *top right:* In situ on-shore mesocosms (10 m3) operated by GEOMAR, here

602 employed on Gran Canaria, Spain. *bottom left:* Kiel Off-Shore Mesocosms for Ocean Simulations (KOSMOS),

603 here employed in the Raunefjord, Norway. *bottom right:* Sketch of a KOSMOS mesocosm unit (55 m3).

604 Photo/graphic sources: *ul:* Daniela Basso, University of Milano-Bicocca, *ur:* Ulf Riebesell, GEOMAR, *bl:* Uli

605 Kunz, *br:* Rita Erven, GEOMAR.

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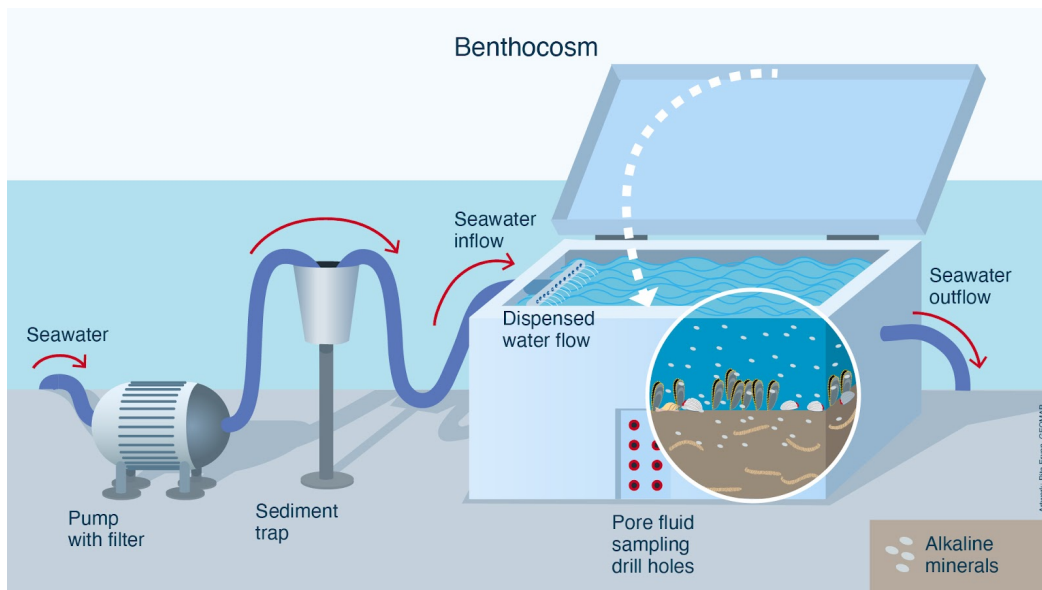
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608   Figure 2. *top:* Benthic mesocosm units currently (2022-2023) installed at the Kiel Fjord, Germany. *bottom:*

609   Sketch of the experimental set-up for the benthic mesocosms shown in top picture. Photo/graphic source: top:

610   Sonja Geilert; bottom: Rita Erven, GEOMAR.

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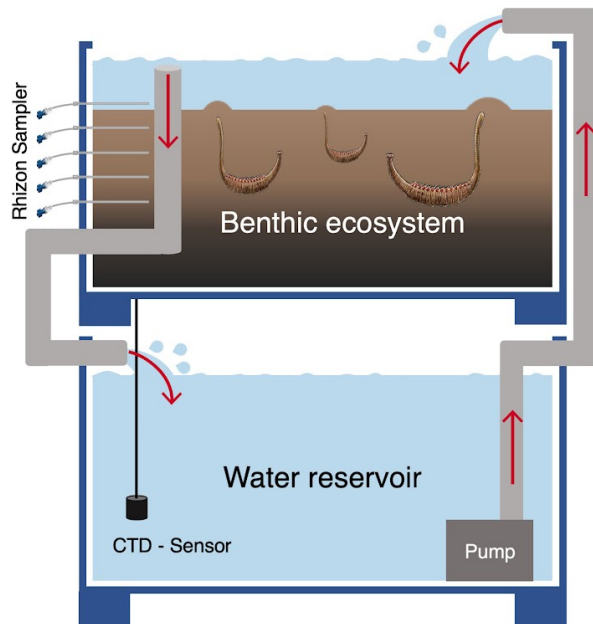
Figure 3: In the benthic mesocosms at the University of Antwerp the dissolution kinetics of silicate minerals and the impacts on the benthic fauna in coastal environments are monitored since 2019. The system comprises 20 units with two stacked tanks, the upper tank is housing the benthic ecosystem, and the lower tank is functioning as a water reservoir. Natural sediment of 40 sediment height with a mean grain size of 123 μm (3.0 phi) was collected from an intertidal sand flat in the Oosterschelde (Netherlands) and mixed with olivine sand of similar grain size. Water from the Easter Scheldt Estuary (salinity 32-35) is used to conduct flux-sessions of 5 weeks (weekly sampling). At the end of each session, the total volume of water in each unit (~500 L) is renewed (Drawing: A. Hylén, Photo: M. Kreuzburg https://www.coastal-carbon.eu/, Geobiology, University of Antwerp).

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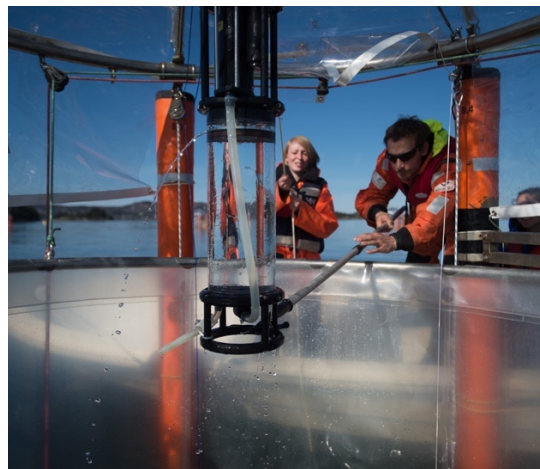
Figure 4: *Upper left:* Distributor control system enabling parallel filling of all mesocosms. *Upper right:* Peristaltic pump ensuring smooth flow of source water during filling of the mesocosms, keeping damage to fragile organisms at a minimum. *Lower left:* Sediment traps forming the bottom of in situ mesocosm enclosures. *Lower right:* Programmable water sampler, enabling dept-integrated water samples over the entire mesocosm depth (or parts thereof). (Photo sources: *ul, ur:* Ulf Riebesell, *ll:* Michael Sswat, *lr:* Solvin Zankl)

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642 Figure 5: *Left:* Distribution device used for alkalinity addition; by moving it up and down in the water column
643 during alkalinity injection at constant speed a uniform alkalinity enhancement can be achieved. *Right:* Milky water
644 at the outlet of the injection tubes indicates temporary precipitation which, however, quickly disappears as the
645 highly concentrated alkalinity solution dilutes. Photo sources: Ulf Riebesell

654 Figure 6. *left:* Pore fluid sampling using rhizons. *right:* benthic incubation chamber to assess alkalinity
655 enhancement with respect to mineral dissolution in benthic mesocosm experiments. Photo sources: left Sonja
656 Geilert, right Michael Fuhr, GEOMAR.