



Genus-specific and microanatomical controls on element incorporation in coralline calcification revealed by Ocean Alkalinity Enhancement experiments

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Ocean liming is a promising marine carbon dioxide removal (mCDR) method with substantial potential for carbon sequestration. This technique involves dispersing CO₂-reactive alkaline minerals onto the ocean surface, increasing the flux of atmospheric CO₂ into the ocean and counteracting ocean acidification by elevating pH levels. Coralline algae of the subfamily Corallinophycidae are vital habitat engineers found in ecosystems ranging from tropical to polar regions. These globally distributed algae inhabit environments extending from intertidal zones to the lower limits of the photic zone. Despite their complex role in the global carbon cycle, the mechanisms governing their cell wall calcification remain poorly understood. Additionally, the potential impacts of ocean liming on these organisms require further investigation.

Mesocosm experiments were conducted in Vigo, Spain, and Crete, Greece, using Ca(OH)₂ treatment at two different concentrations (Low and High) at each site, with three replicates per treatment. The study examined genus-specific factors influencing magnesium (Mg) incorporation into the calcified cell walls of coralline algae as a proxy for growth and active calcification. The tested specimens included three genetically identified species: *Phymatolithon calcareum* and *P. lusitanicum* from Vigo, and *Lithothamnion corallioides* from Crete. SEM-EDS, Raman spectroscopy and XRD techniques were integrated to investigate in detail our coralline thalli mineralogy. The results revealed significant variations in Mg and other ion distributions between primary (PCW) and secondary (SCW) cell walls, emphasizing the role of microanatomical features over the broader temperature-driven trends in Mg concentrations within coralline thalli. Specifically, *Phymatolithon* species exhibited higher Mg content in SCWs compared to PCWs, whereas *L. corallioides* showed equal or lower Mg concentrations in SCWs.

High Ca(OH)₂ treatments caused a decrease in Mg content in shallow-water *Phymatolithon* specimens from Vigo, suggesting inhibited growth due to reduced water circulation and

smothering by aragonite precipitation within the mesocosms. In contrast, deep-water *L. corallioides* in Crete displayed no significant changes in Mg levels under either Low or High treatments. These findings suggest a non-significant impact of ocean liming on the tested coralline species, and underscore the intricate nature and variate response of calcification in coralline algae. The results highlight the importance of microanatomical features, environmental conditions, and species-specific traits in determining the impact of such mCDR interventions on marine calcifiers.