读书报告

汇报人：张玲玉

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Introduction

微生物促进海草吸收溶解有机氮

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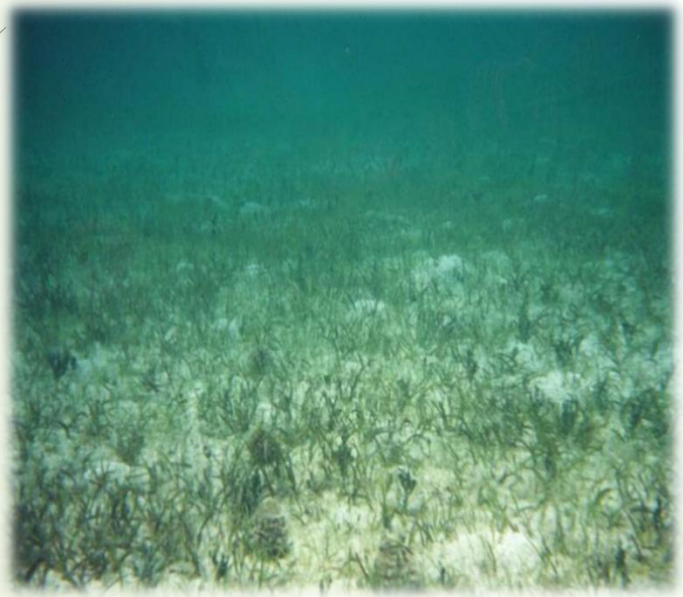
Seagrass

Introduction

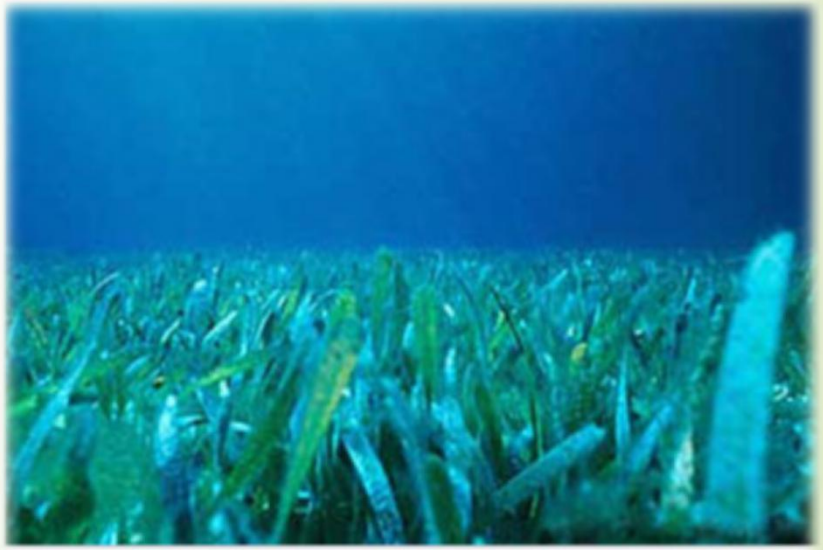
Seagrasses are flowering plants (angiosperms) belonging to four families (Posidoniaceae, Zosteraceae, Hydrocharitaceae and Cymodoceaceae), all in the order Alismatales (in the class of monocotyledons), which grow in marine, fully saline environments. Twelve genera comprising some 60 species are known.



Seagrass

Introduction

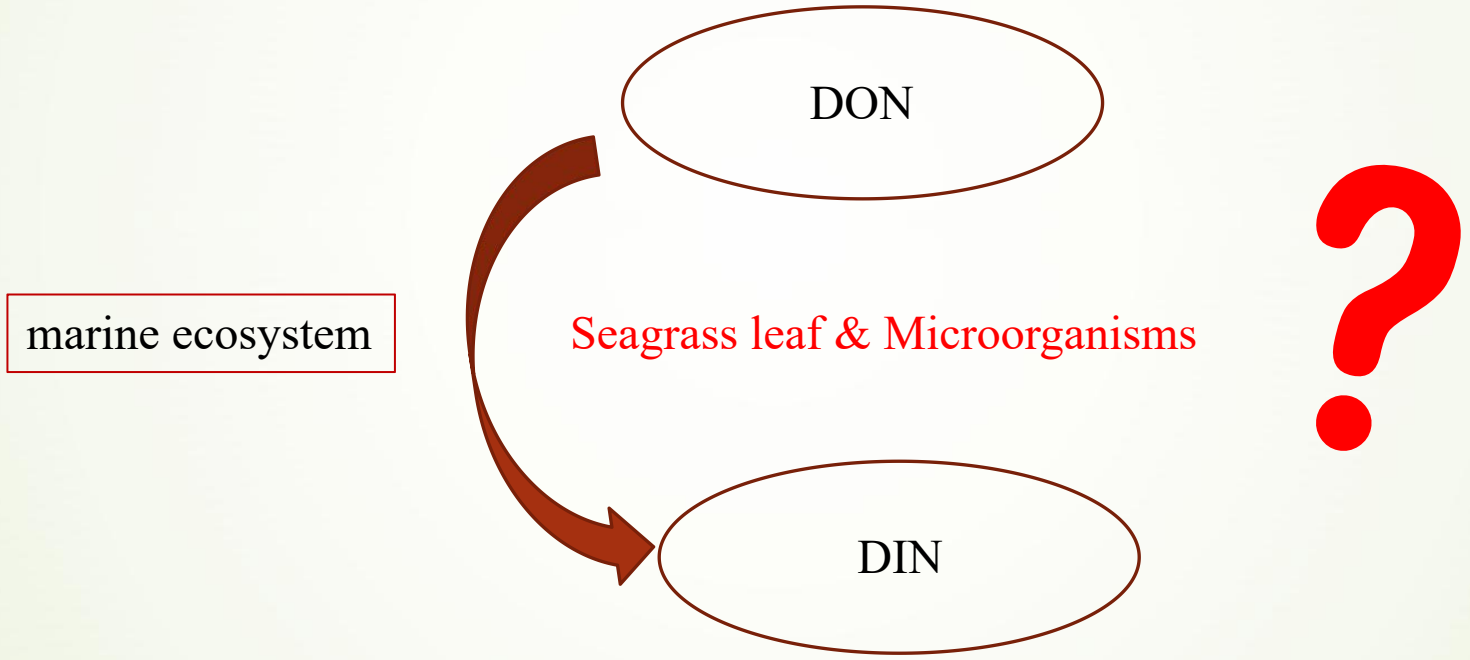
- Seagrasses beds/meadows can be either monospecific (made up of a single species) or in mixed beds.
- Seagrass meadows are extremely productive aquatic habitats that provide important ecological functions, such as refuge and nutrient sources for a great variety of marine organisms.
- Nitrogen (N) is an essential element for maintaining seagrass growth and productivity , with foliar uptake of N often contributing more of the plant total N requirement compared with roots (up to 74%).
- Within the marine environment, microorganisms on the surface of plants and animals can be highly abundant.



Floridian seagrass bed

Introduction

Microorganisms play a critical role in nitrogen cycling by mineralising dissolved organic nitrogen (DON) compounds into bioavailable inorganic forms (DIN).



Material & Methods

Field collection and ^{15}N Experiment

Sample site: Marmion Marine Park (31° 48.240'S 115° 43.123'E)

Sample: *Posidonia sinuosa* shoots (2.1 to 2.7g): 48, transported in aerated water at ambient temperature (22ºC) in the shade before being placed in 30L aquaria under natural illumination for 24h to acclimatize.

experiment treatment

24 seagrass shoots: remove obvious epiphytic organisms, washed in 0.2µm prefiltered artificial seawater (AS) and placed into individual incubation cylinders containing 1L AS (with 5mL L-1 of antibiotic mixture (comprising 10,000 units penicillin, 10mg mL-1 streptomycin and 25µg mL-1 amphotericin B),

Another 24 seagrass shoots: with associated microorganisms

Twelve seagrass leaves with and 12 without microorganisms were randomly selected to be incubated in 50µM ^{15}N (98%^{15}N) spike of algal derived aa mixture (NLM-2161, Cambridge Isotope Laboratories).

Another 12 with microorganisms and 12 without incubated with 50µM ^{14}N-aa (98%^{14}N; ULM-2314, Cambridge Isotope Laboratories).

Material & Methods

Sample collection for IRMS and NanoSIMS

Sample collection: 3 seagrass leaves, 1cm^2 kept at 4° C in PBS

For NanoSIMS: Cutting and fixation of samples for NanoSIMS analyses, was collected and immediately stained and fixed with the LIVE/DEAD® BacLightTM kit in order to verify the antibiotic effect for the treatment.

All remaining leaf material (for treatments and controls) was stored at -20° C and processed for IRMS analyses.

Material & Methods

Isotope ratio mass spectrometry analysis

Sample treatment: Frozen leaves were oven-dried at 58ºC for 48h before being ground to fine powder, and weighed (~1.5mg) into 6 x 4mm tin capsules

Nitrogen elemental composition (%) and isotope ratios ($\delta^{15}N$) : Automated Nitrogen Carbon Analyser-Mass Spectrometer system

LIVE/DEAD® BacLight™

Sample treatment: wash twice with 0.1M PBS, incubated for 15min in the dark at room temperature with 200µL of LIVE/DEAD® BacLightTM reagents, mixed as per manufacturer's instructions.

Then wash twice with 0.1M PBS, 200µL 10% natural buffered formalin for 45 minutes, then rinsed twice with 0.1M PBS

confocal scanning laser microscopic imaging

Material & Methods

NanoSIMS sample preparation and analysis.

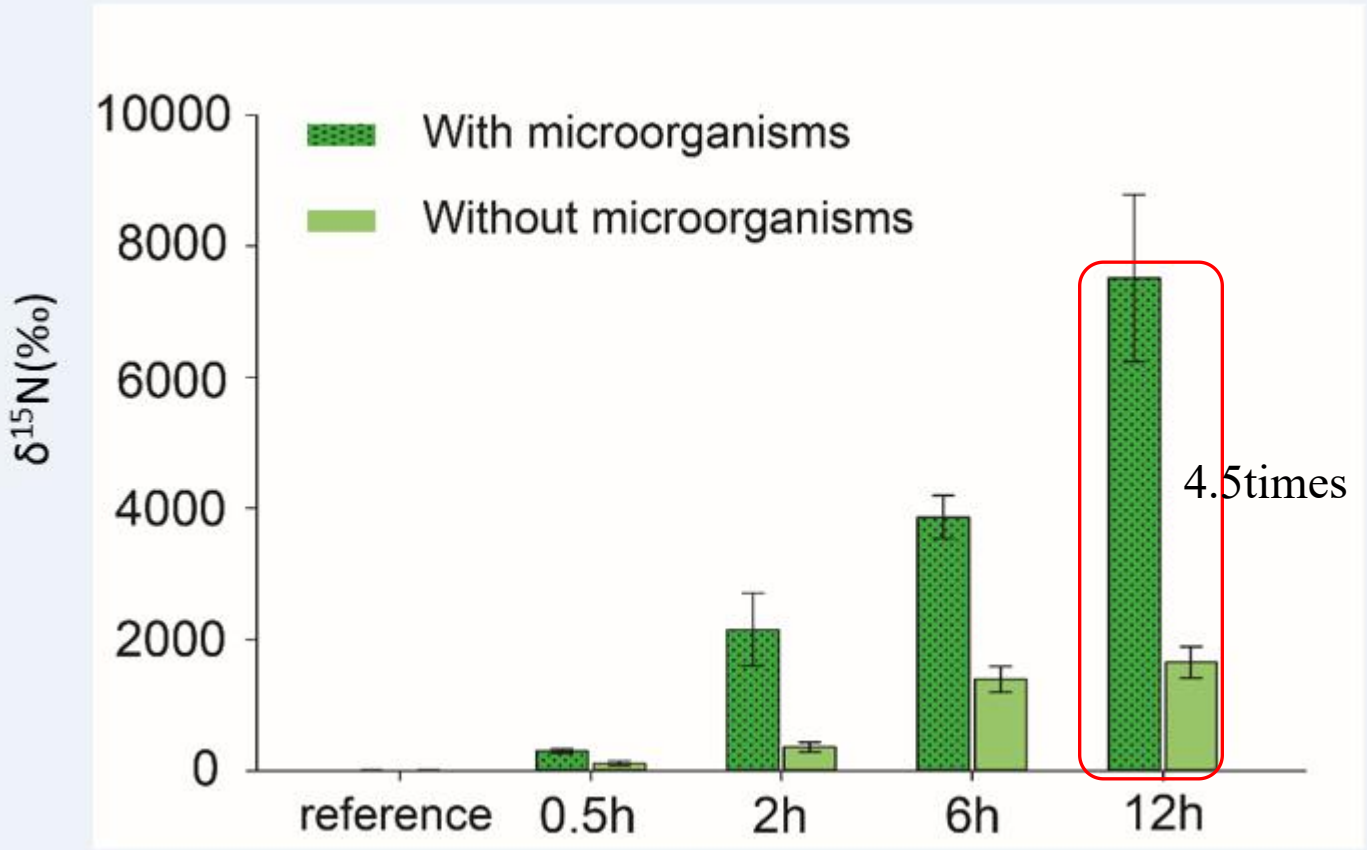
Sample treatment:Samples were dehydrated in a graded series of ethanol (50%, 70%, 90%, 100%, anhydrous 100%) and anhydrous acetone (100%).

Fixing: araldite resin mixtures at 60° C for 24h, Leica EM UC6 Ultramicrotome

Analysis: Wet cut sections were mounted either on a Silicon wafer for NanoSIMS analysis (150nm), a glass slide for optical imaging to map sample ultrastructure (150nm) or formvar TEM grids (100nm). Silicon wafers with adhered (air dried) samples were coated with 5nm gold for subsequent analysis in a CAMECA NanoSIMS-50 ion microprobe.

Image Sample analysis and Statistical analyses

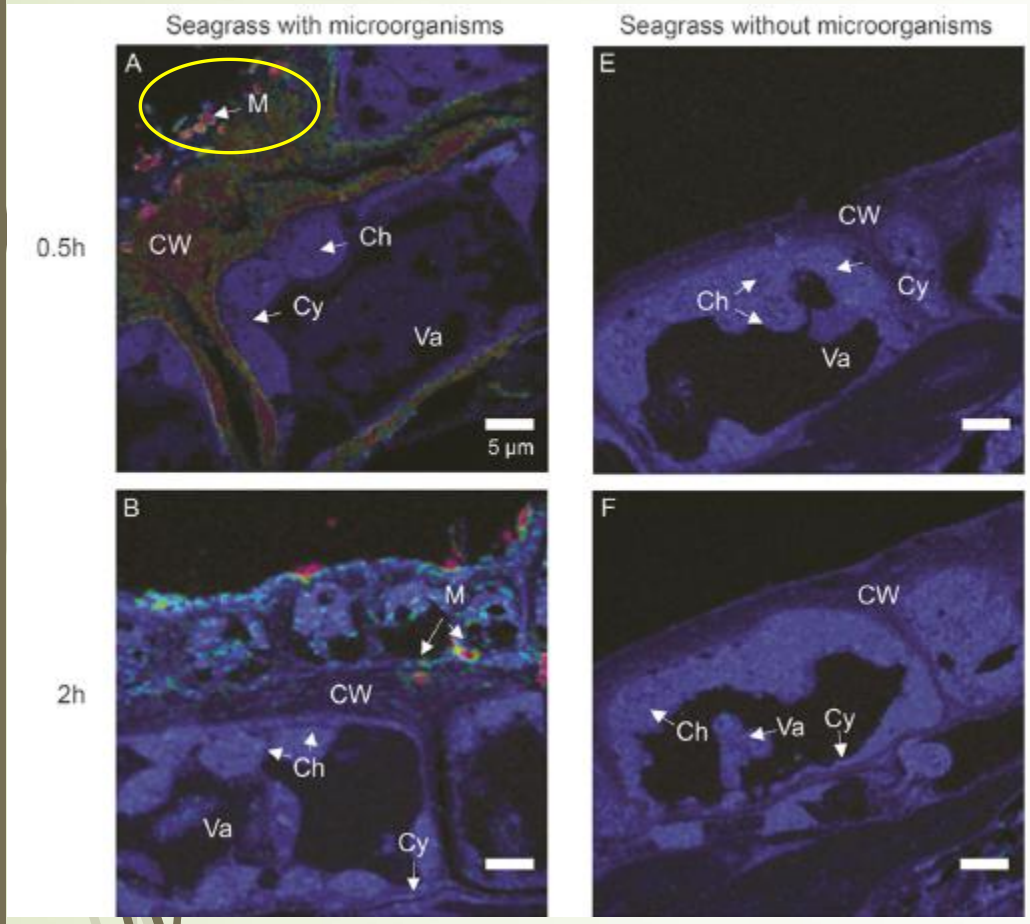
Results & Discussion



Evidence that microorganisms associated with *P. sinuosa* leaves facilitate seagrass uptake of ^{15}N derived from aa was provided by our results of bulk tissue analysis (IRMS) of seagrass leaves with and without intact microorganisms.

Fig.S3 Isotope ratio mass spectrometry (IRMS) $\delta^{15}N$ (‰) values of seagrass leaves with and without associated microorganisms after incubation in ^{15}N-aa (98% ^{15}N).

Results & Discussion

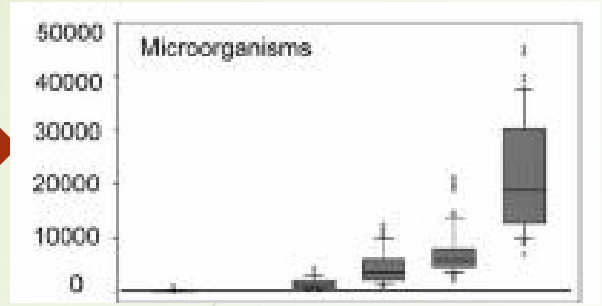


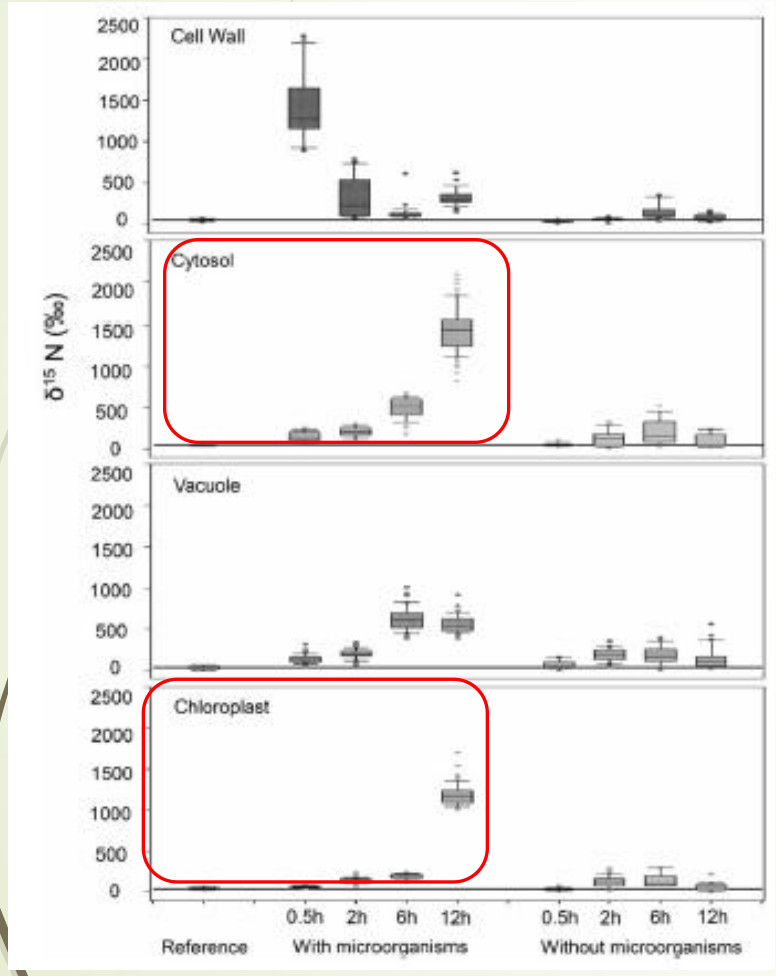
CW: cell wall, Ch: chloroplast, Va: vacuole, Cy: cytosol

After 0.5 h incubation in 15N-aa, analysis showed microorganisms on the surface of the seagrass leaf were highly enriched compared to the adjacent seagrass cells

Fig. 1 ^{15}N enrichment images of seagrass (P. sinuosa) leaf cells after incubation in enriched ^{15}N aa mixture with (a–d) or without (e–h) microorganisms on their leaves. Seagrass leaves were incubated for 0.5 h (a,e), 2 h (b,f), 6 h (c,g) and 12 h(d,h).

Results & Discussion





CW: cell wall, Ch: chloroplast, Va: vacuole, Cy: cytosol

The microorganisms remained more ^{15}N enriched than adjacent seagrass cells at each time, and their enrichment was characterised by an exponential accumulation of ^{15}N over time (Fig. 2). By 12 h, microorganisms were ~200 times more ^{15}N-enriched than unlabelled reference samples, indicating the efficiency of microorganisms to utilise aa through the activity of intracellular and extracellular enzymes that break down aa to produce DIN.

Reference values (reference), representing $\delta^{15}N$ natural abundance for microorganisms and seagrass sub-cellular compartments obtained from incubation with non-enriched ^{14}N aa, are displayed for each graph.

Results & Discussion

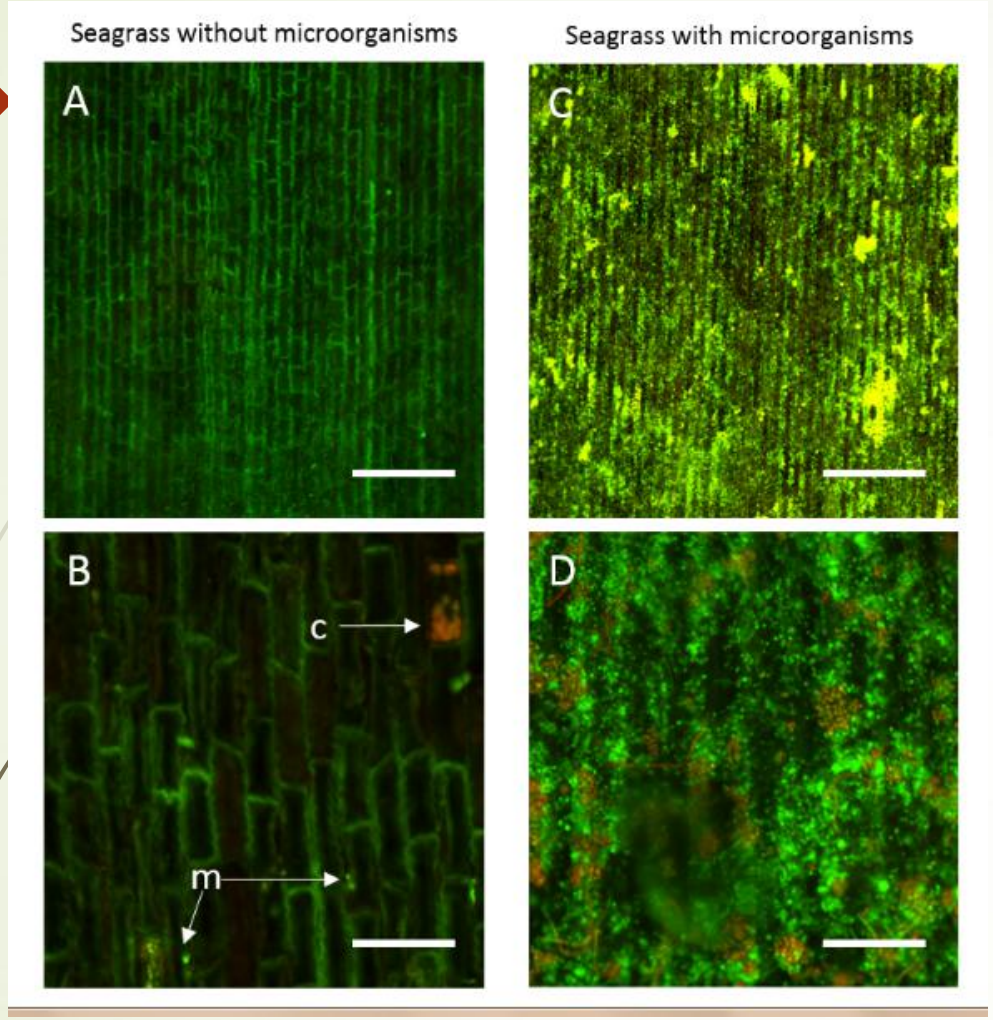


Fig.S1

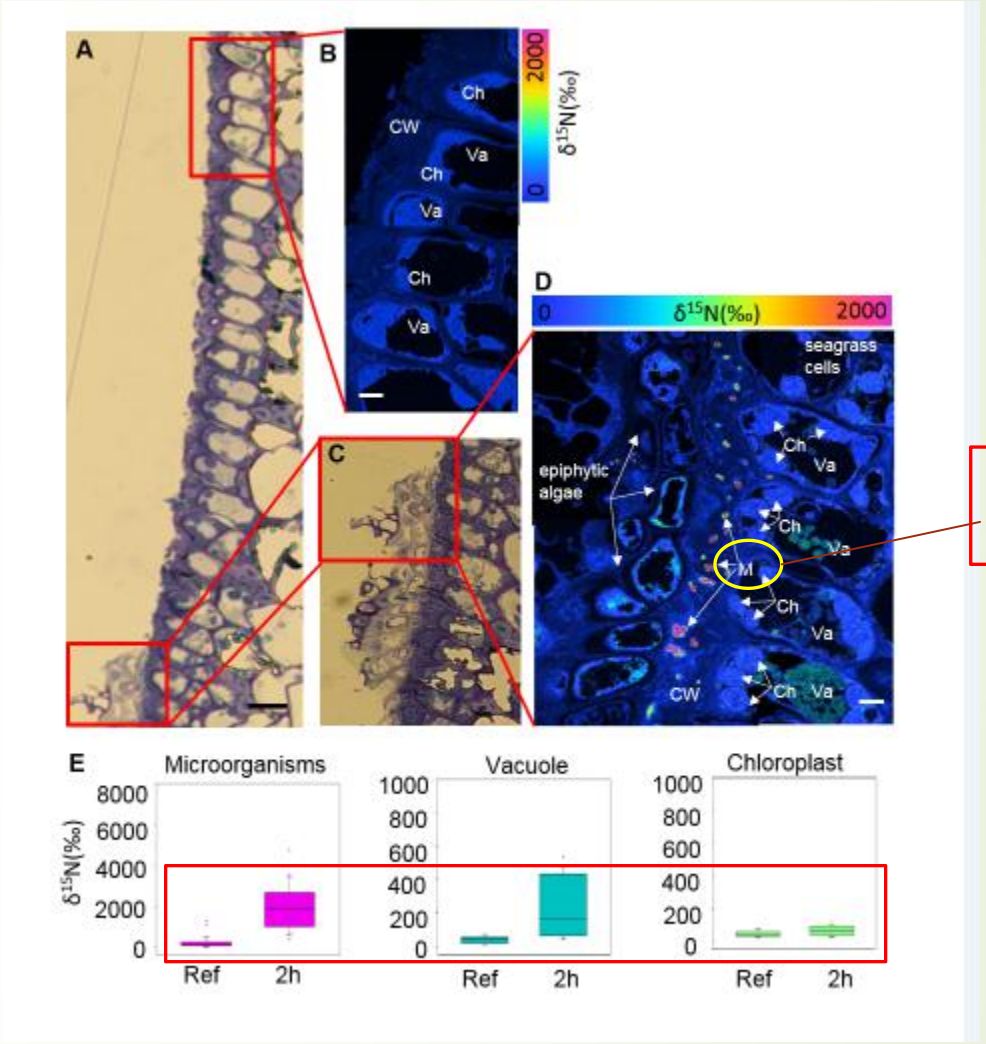


Fig.S6

m: bright green spots, microbes

c: red dots, chloroplasts of the seagrass epidermal cells

NanoSIMS analysis, and corresponding live/dead cell counts, revealed a few examples where microorganisms were not entirely removed.

Conclusion

- In this study, we show that the association between microorganisms and the leaves of *P. sinuosa* can provide an alternative source of N for uptake by seagrass from the abundant organic nitrogen pool.
- By mineralising amino acids, epiphytic microorganisms on *P. sinuosa* leaves link the nitrogen elemental cycle in seagrass meadows via heterotrophic metabolism and are likely to contribute significantly to the high productivity of seagrass meadows.

THANKS