

Overlooked Oxalotrophs: Oxalotrophy in Dryland and Marine Ecosystems



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Abstract

The diversity and ecological roles of oxalotrophic microorganisms remain poorly understood outside host-associated and terrestrial systems. In soils, oxalotrophs mitigate oxalate mineral accumulation and can contribute to carbon sequestration via the oxalate–carbonate pathway, but their presence and activity in other environments have received limited attention. Analysis of publicly available metagenomic and metatranscriptomic datasets suggests that oxalotrophs may be active across a broad range of environments, including marine ecosystems. To investigate oxalotroph diversity and physiology across contrasting ecosystems, we isolated novel oxalotrophic microorganisms from dryland soils of the urban Sonoran Desert and from Pacific Northwest hydrothermal vent plume waters using calcium oxalate as the sole carbon source in solid and liquid media. While oxalotrophy has been demonstrated without the presence of canonical genes for oxalate metabolism (*oxc*, *frc*), genome sequencing of our isolates confirmed the presence of these genes. Dryland isolates exhibited high temperature tolerance, with growth observed as high as 45°C. Our work also provides a novel demonstration of marine oxalotrophy in cultured isolates. Together, these findings suggest that oxalotrophic metabolism is more environmentally widespread than previously recognized, resilient to elevated temperatures, and may represent an underappreciated component of carbon cycling in marine ecosystems.

Motivation

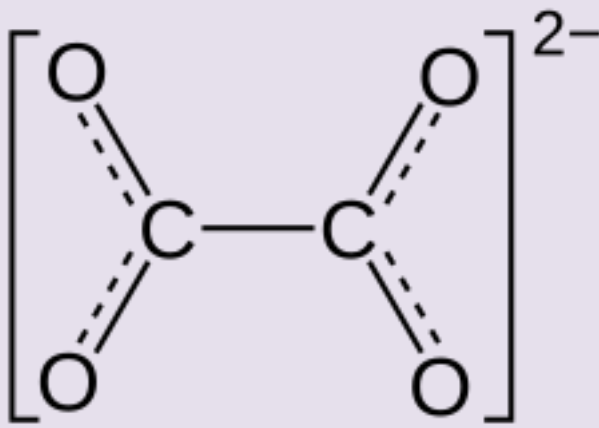
Oxalate is one of the **most abundant organic acids** in the biosphere; however, our understanding of its sources, sinks, and cycling in diverse ecosystems is limited or absent.

- Isolation and characterization of oxalotrophs has focused on:
1. Ecosystems associated with plants or animals (e.g., human gut)
 2. And rarely includes the more environmentally relevant form of calcium oxalate (CaOx).

What makes oxalate unique?

Oxalate is uniquely positioned at the boundary of organic and inorganic carbon.

- An organic carbon anion that forms an inorganic mineral (e.g., CaC_2O_4).



- The only low molecular weight (LMW) organic compound that has a stable and common mineral form under conditions relevant to Earth's biosphere.
- Easily complexed with metals.

Therefore, oxalate is more challenging to categorize as a DIC, DOC, POC, or mineral than other carbonate or carboxylate (e.g., acetate, citrate, succinate, or formate) species.

Metabolic pathways

- Genes encoding oxalate metabolism occur across all domains of life.

Gene	Name	KO(s)	Bac; Arc
oxo	Oxalate oxidase	K28780	0 (plants)
oxdC	Oxalate decarboxylase	K01569	2,649; 19
oxc	Oxalyl-CoA decarboxylase	K01577	2,374; 0
frc	formyl-CoA transferase	K07749	10,553; 144
oxlT	Oxalate / formate antiporter	K08177	19,734; 811
oor	Oxalate oxidoreductase	K19070	46; 0
		K19071	106; 0
		K19072	22; 0

Table 1: Oxalate genes and associated KEGG Orthology and Annotree (Mendler et al. 2019) hits out of 80,789 bacterial and 4,416 archaeal genomes.

- The most common bacterial pathways involve four key genes in either a two-step (1, 2, 3) or one-step (4) pathway.

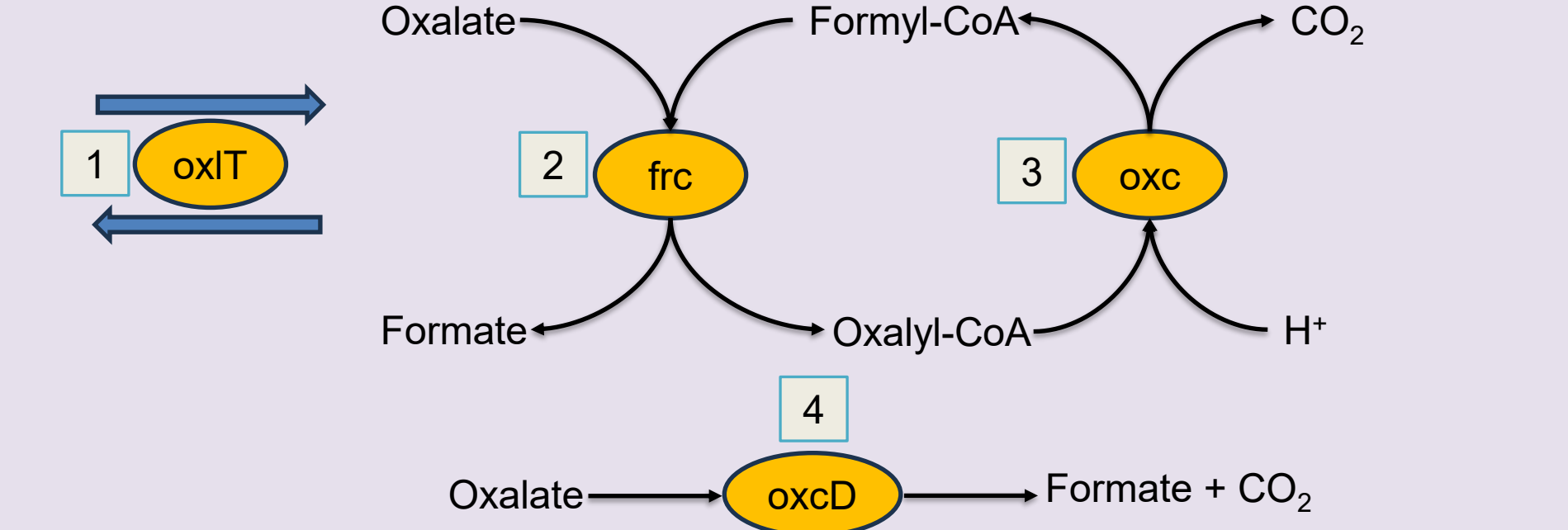


Figure 1. Bacteria can encode (1) *oxlT* to bring oxalate into the cell and (2) *frc* for converting it back to formate to export while generating oxalyl-CoA. Oxalyl-CoA can then be converted back to formyl-CoA and generate a molecule of CO_2 using (3) *oxc*. There is also a single step process that uses (4) *oxdC* to directly generate formate and carbon dioxide from oxalate. Figure adapted from Stewart et al. 2004 and Dutton et al. 1996.

Why drylands?

- The Oxalate-Carbonate Pathway is well established in soils (Cowan et al. 2024)
- Dryland plants produce CaOx (hundreds of Kg of CaOx in a single saguaro cactus; Garvie 2006).
- Since CaOx is very stable over geologic time, it requires biological breakdown to maintain the low concentrations observed in soils.
- If oxalotrophic microorganisms can metabolize CaOx, the resulting inorganic carbon can be sequestered as calcium carbonate.

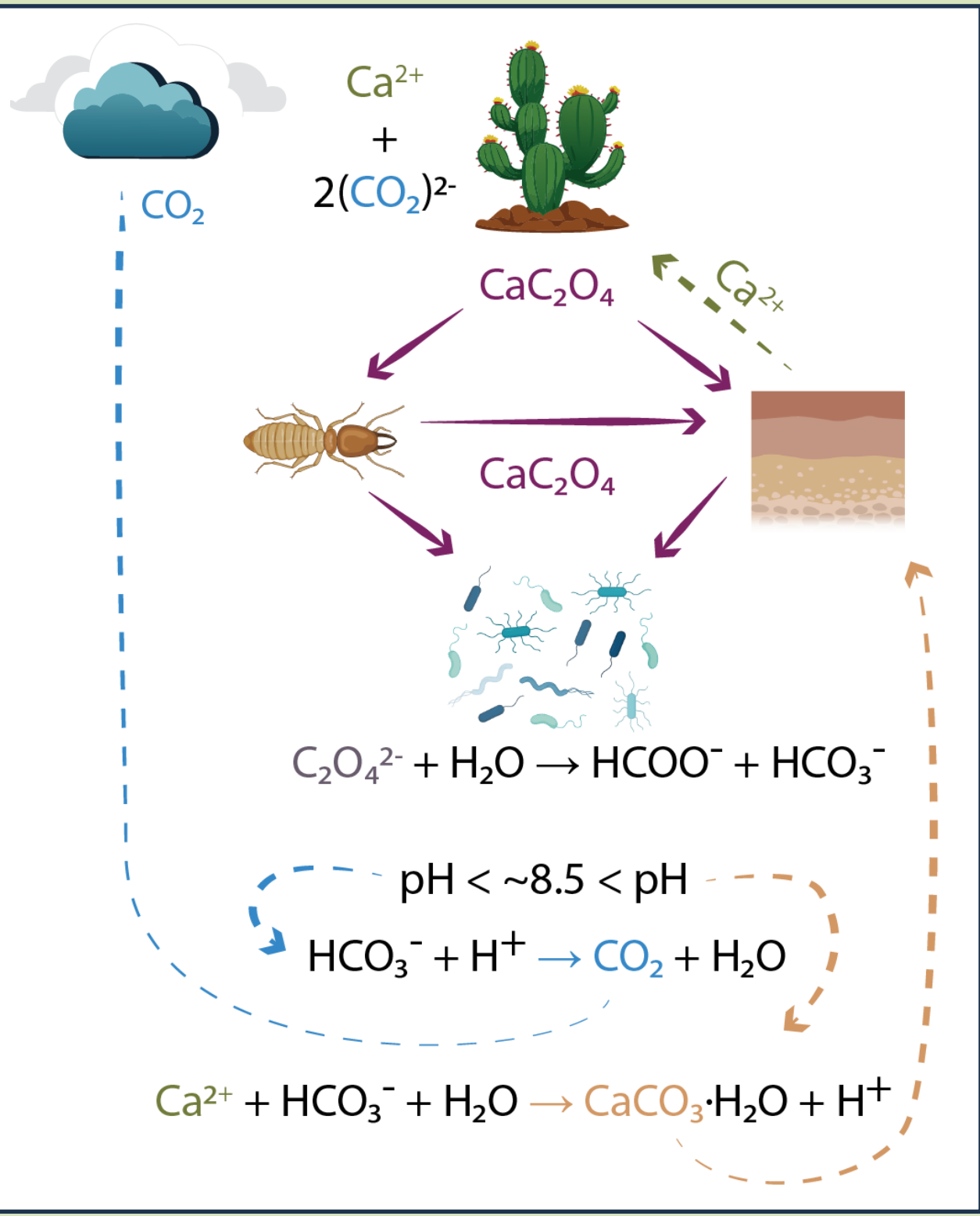
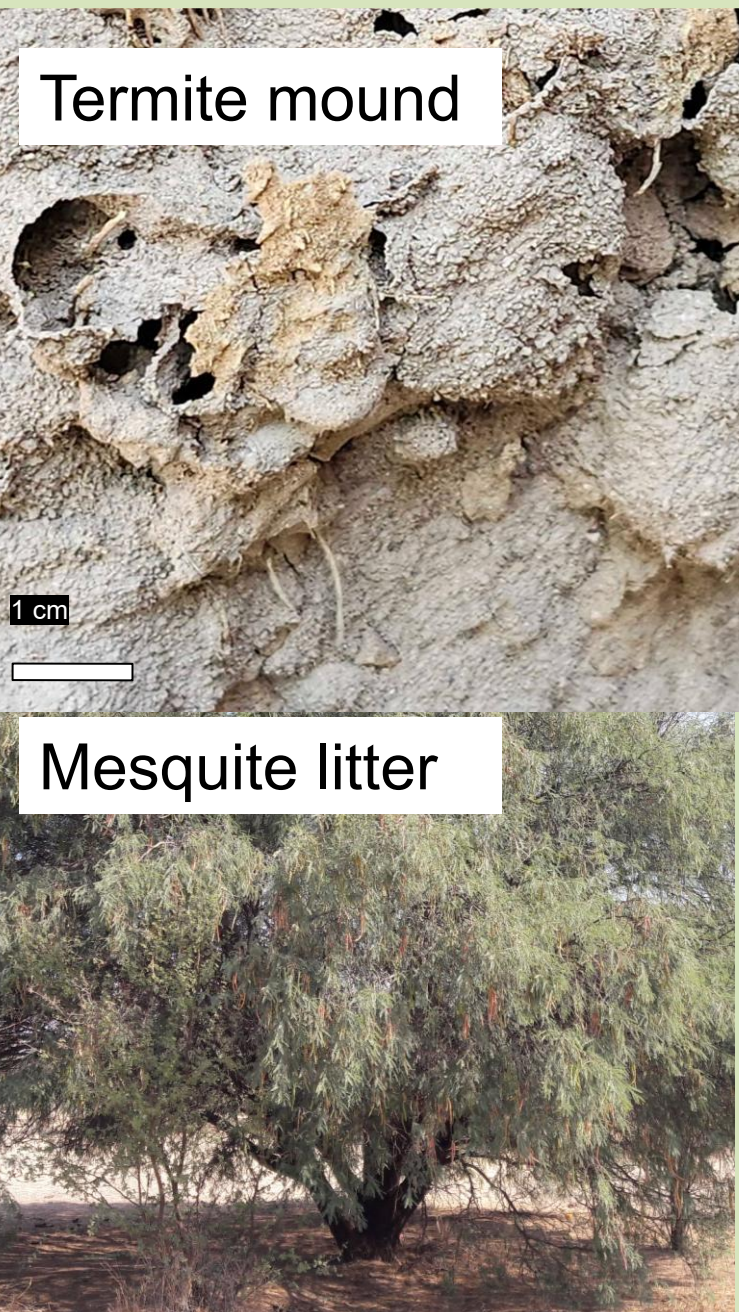
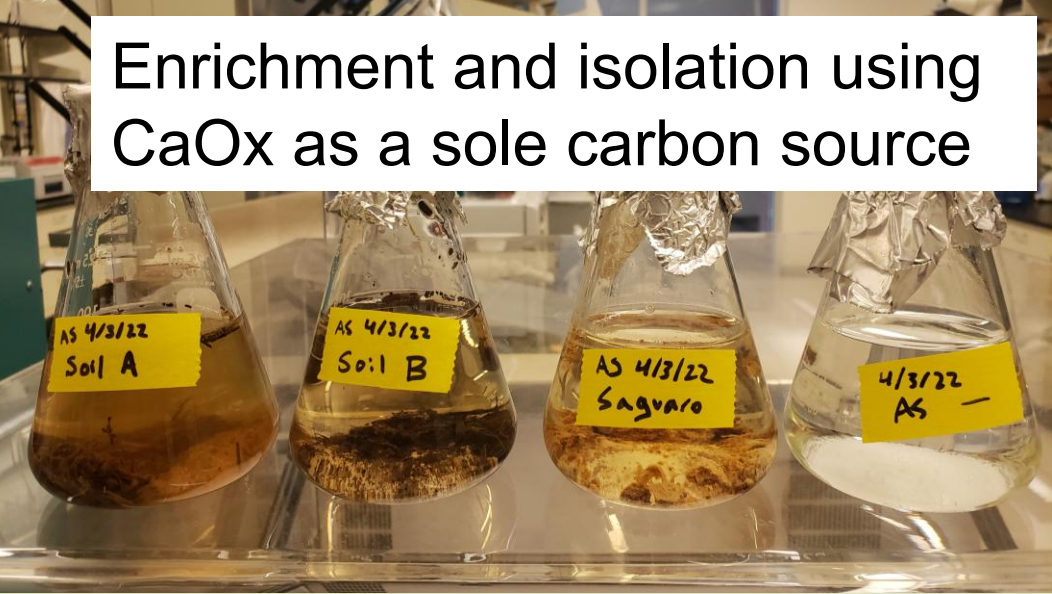


Figure 2. Diagram of Oxalate-Carbonate Pathway in drylands.

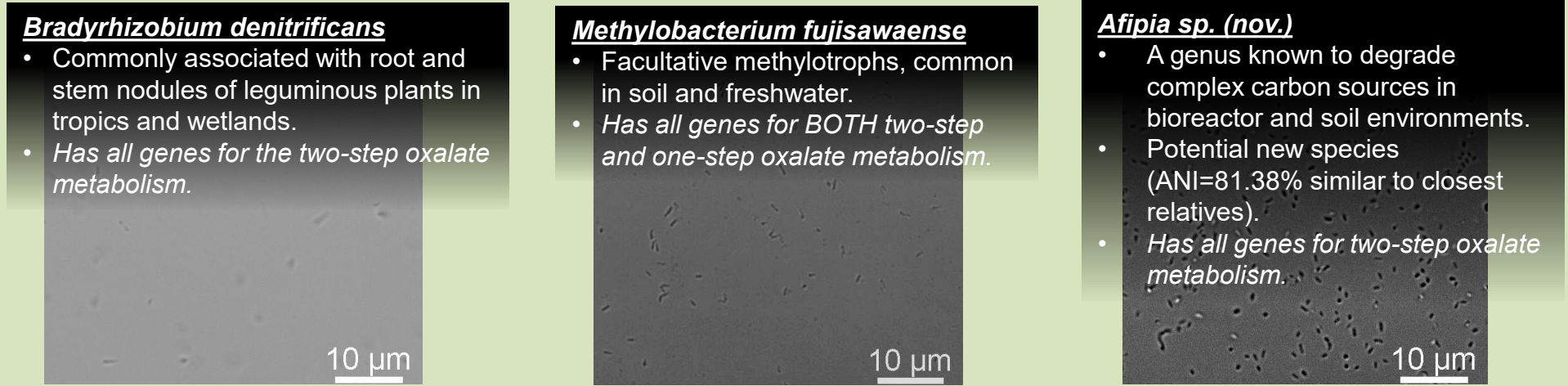
Approach

- Enrich oxalotrophic organisms from dryland soils using Schlegel Mineral Medium with CaOx as a sole carbon source.
- Characterize their growth under different temperatures and CaOx concentrations.
- Characterize enrichment genomes for oxalate metabolic pathways.



Results

- Three genera were enriched from SMM agar plates, and their complete genomes were sequenced, with either one or two complete oxalate pathways.



- A novel temperature range and optimum were observed across all dryland enrichment genera.

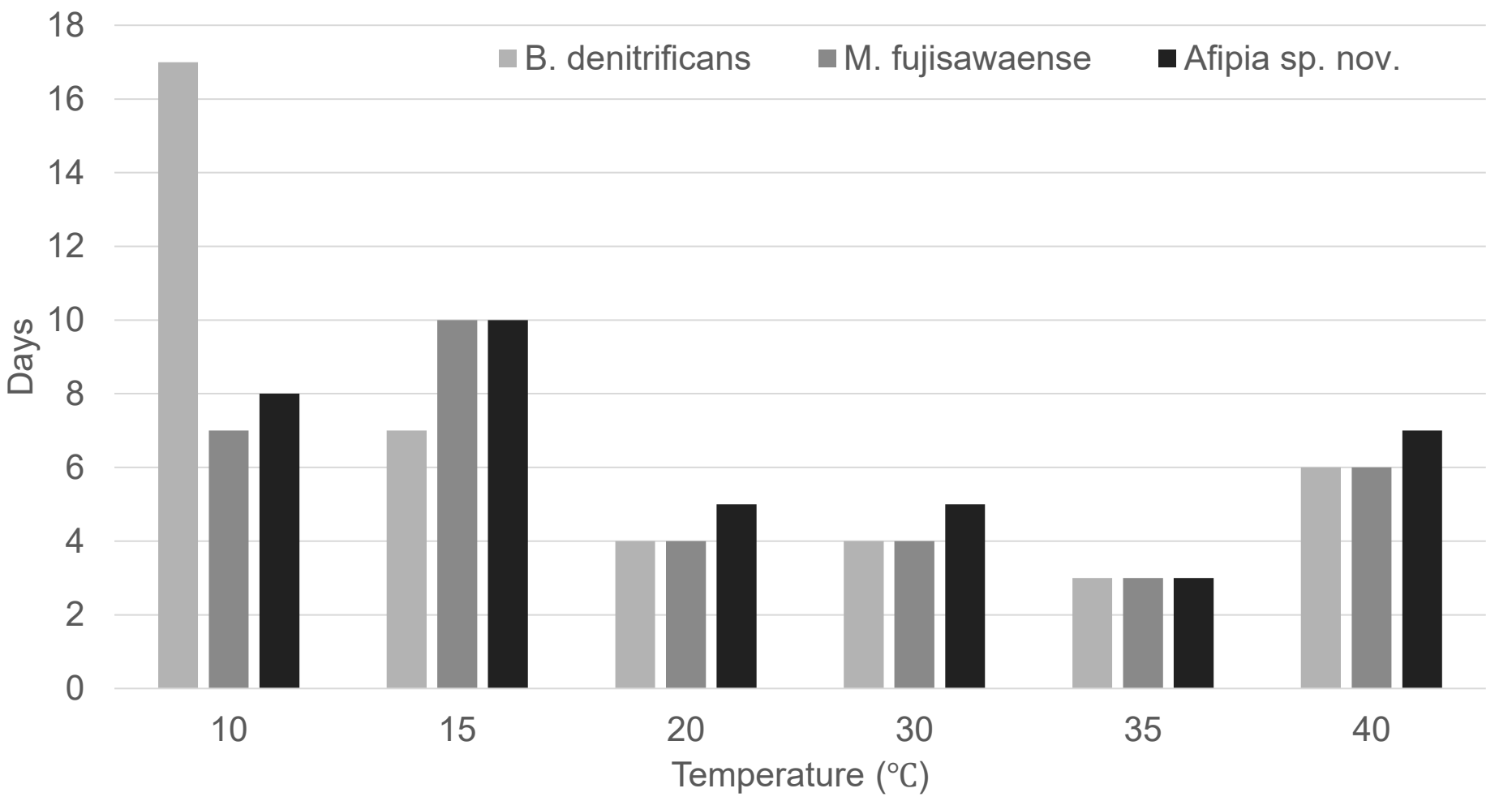


Figure 3. Days until first colony observed across different growth temperatures on SMM agar plates.

- Oxalate concentration correlated with growth, but growth was still observed with no oxalate added. Growth was not observed on agar alone (data not shown).

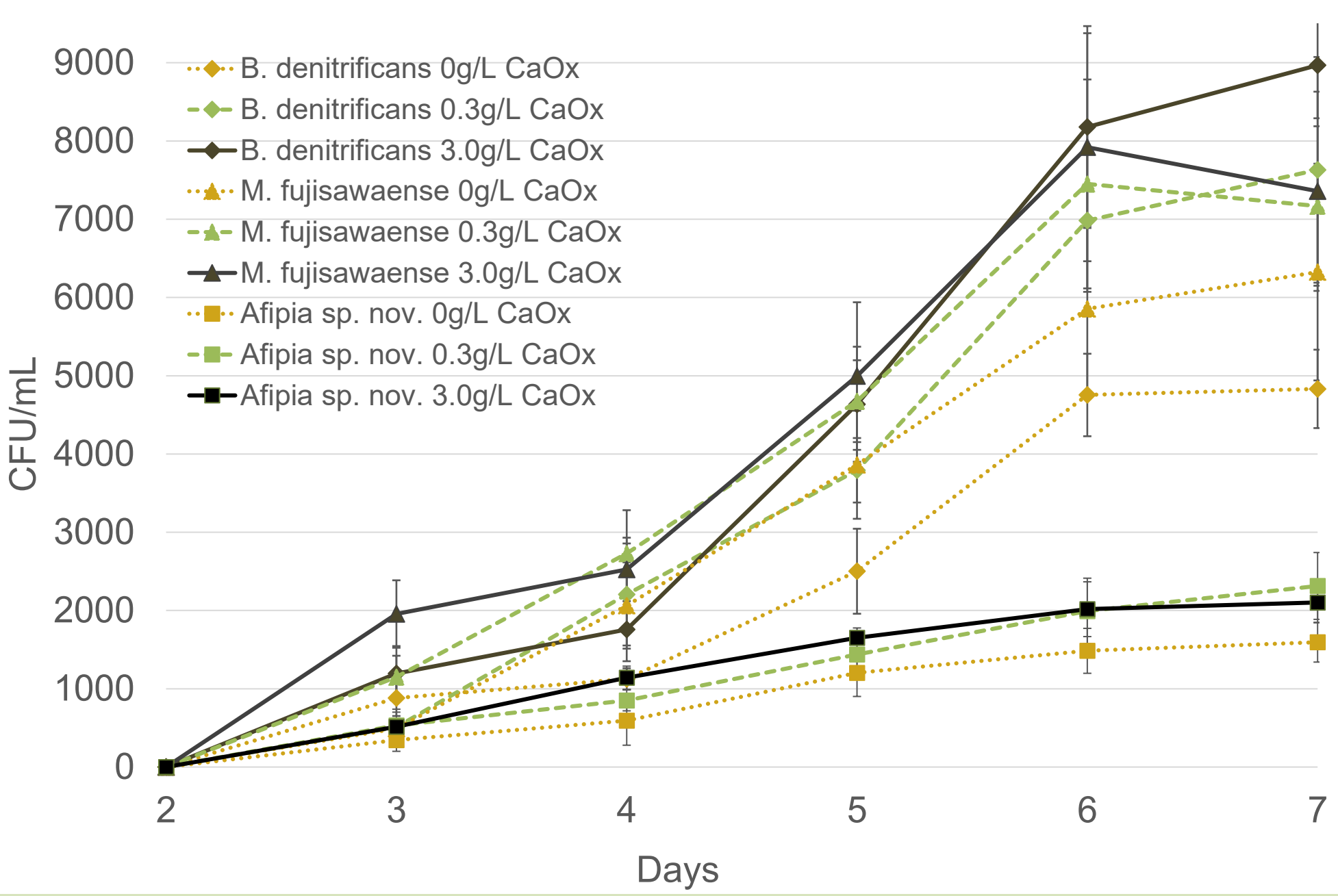
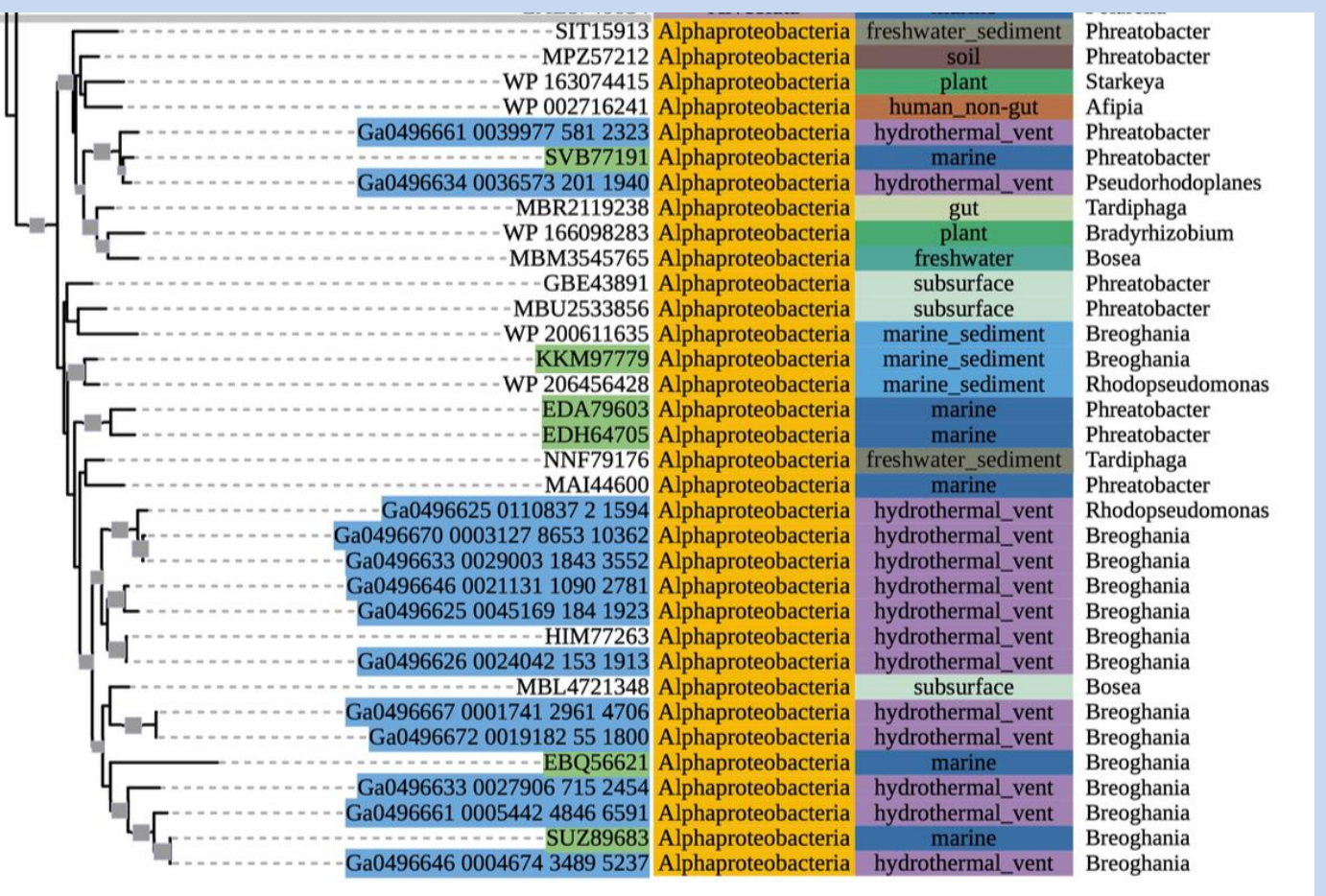


Figure 4. Growth of three dryland enrichments with three oxalate concentrations on SMM agar at preferred growth temperature of 35°C.

Why the ocean?

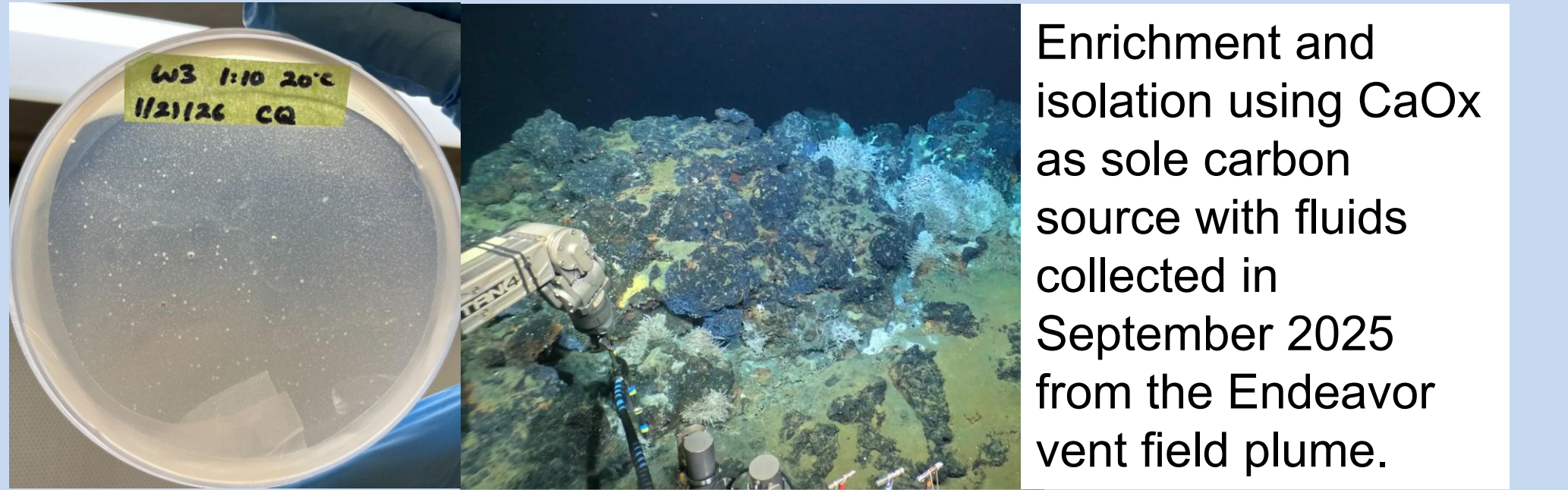
- RNA transcripts were recovered from hydrothermal vent plume waters of the East Pacific Rise.
- This suggested oxalotrophy may also be a marine metabolism.



Phylogenetic tree of *oxc* genes from BLAST hits (Sonke & Trembath-Reichert 2023).

Approach

- Same methods as dryland enrichments.



Results

- Marine organisms can metabolize oxalate.
- Marine enrichments exhibit a wider temperature range, but a lower temperature optimum compared to dryland enrichments.

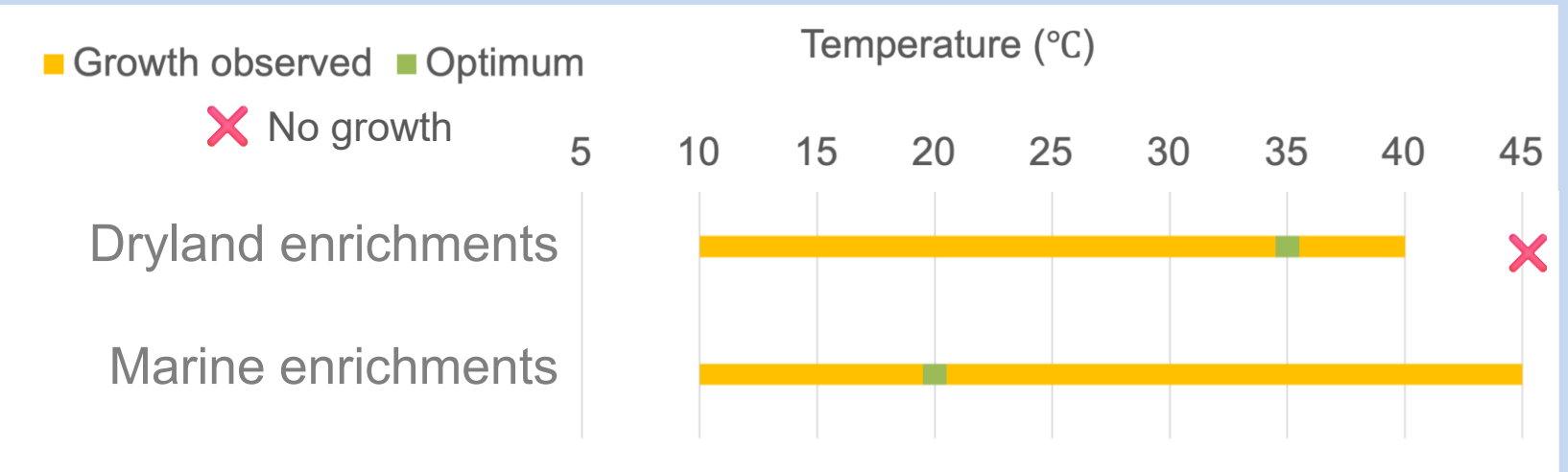


Figure 5. Temperature comparison between dryland and marine enrichments.

- Oxalate concentration correlated with growth.
- No growth on SMM without oxalate.

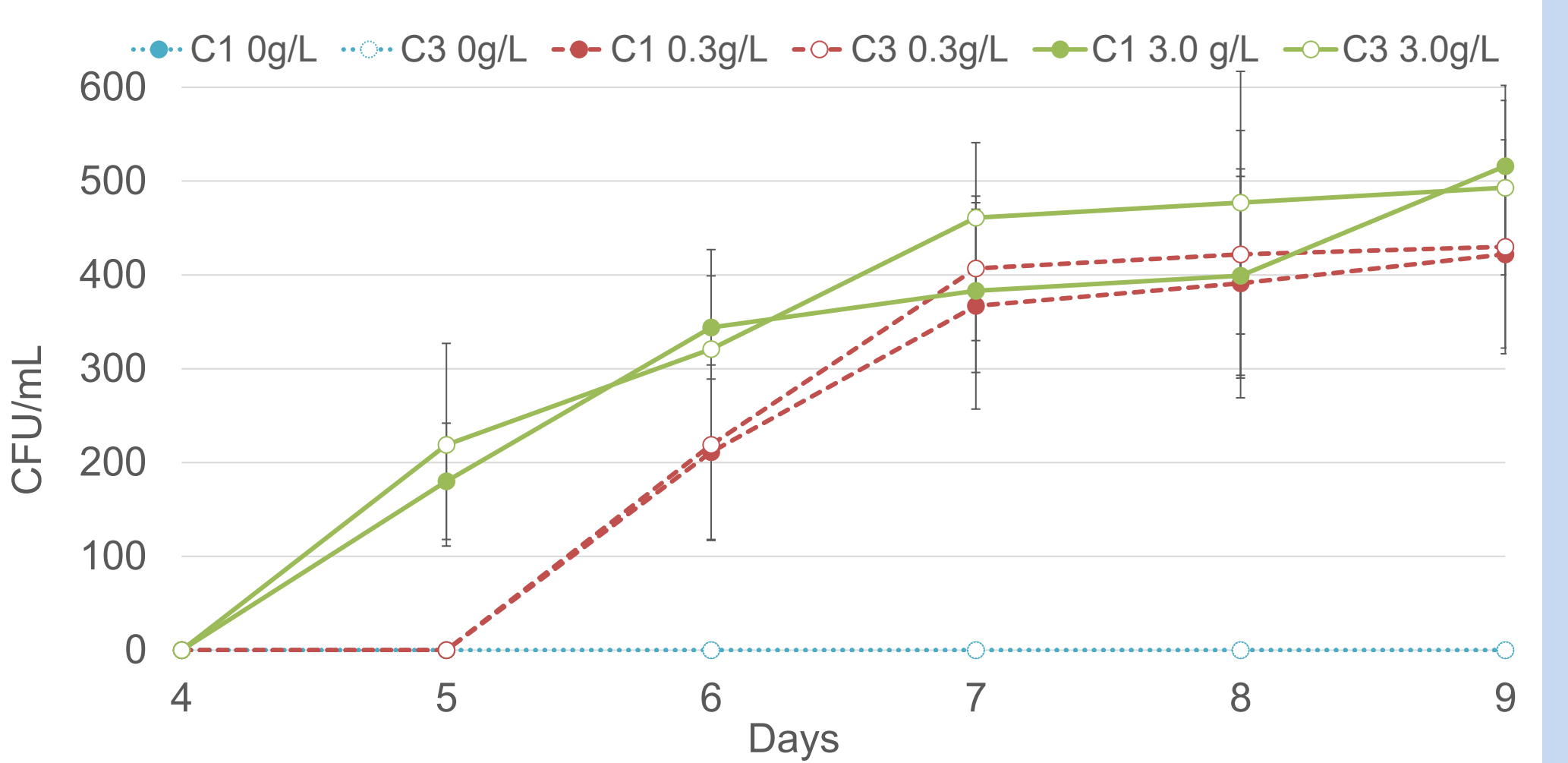


Figure 6. Growth of marine enrichments with three oxalate concentrations on SMM agar at preferred growth temperature of 20°C.

Future Work

- Further isolation and genome sequencing of marine enrichments.
- Develop oxalotroph marine media and evaluate further enrichment potential.
- Measure rates of oxalate consumption using FTIR.

References

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