Pink methylotrophic bacteria aid in coastal sage scrub and grassand restoration

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INTRODUCTION

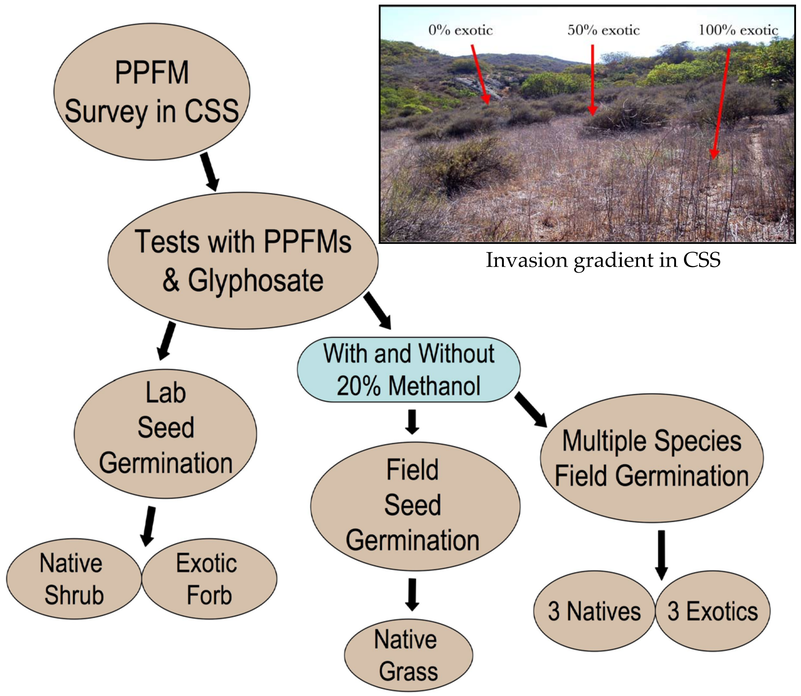
Pink-Pigmented Facultative Methylotrophic bacteria (PPFM, *Methylobacterium*) **are well studied in agriculture due to their importance in seed germination, growth, crop yield, pathogen resistance and drought stress tolerance** (Trotsenko et al. 2001) **but are not as well studied in natural systems.** PPFMs are phytosymbionts that could be as important in natural systems as they are in agriculture. PPFMs utilize C_1 compounds generated by growing plants, such as methanol. Studies have shown that crop species have different abundances of PPFMs in their phyllosphere, suggesting that there may be an optimal number of PPFMs for plants. Plants that harbor PPFMs are thought to have competitive advantages that could be important factors in restoration success.

Further, since restorations often include the use of herbicides we need to understand how glyphosate, a commonly used herbicide, affects plants and their microbial mutualists (Harris 2009). No previous work has tested the PPFM response to glyphosate. To begin to understand how PPFMs interact with plants in natural communities we surveyed PPFM abundance in the rhizosphere of native and exotic plant species in coastal sage scrub (CSS) habitat. We found that PPFM abundance varies between species and that there tend to be fewer PPFMs in the 50/50 mixed species zone in an invasion. With that information we asked:

- **Question 1:** Are there fitness consequences to native plant germination with PPFMs?
- **Question 2:** Since glyphosate is often used in restorations, are PPFMs damaged by glyphosate application with resulting reductions in seed germination and seedling fitness? If so, is methanol application a feasible remediation after glyphosate use?

- **Hypothesis 1:** PPFMs benefit native species relatively more than exotics.
- **Hypothesis 2:** Glyphosate inhibits PPFMs and native species suffer negative effects as a result.

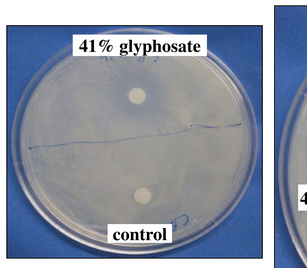
METHODS



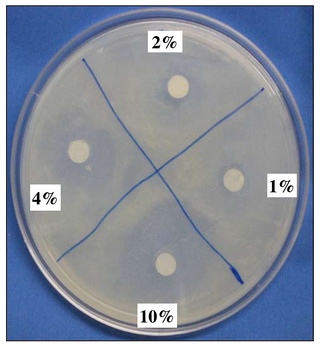
Invasion gradient in CSS

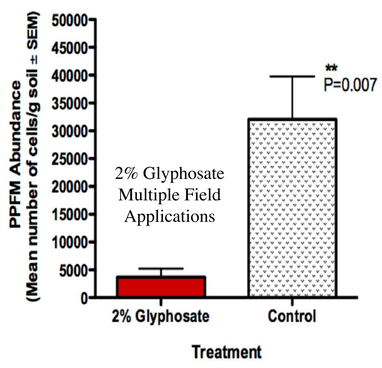
RESULTS

Glyphosate effects on PPFMs

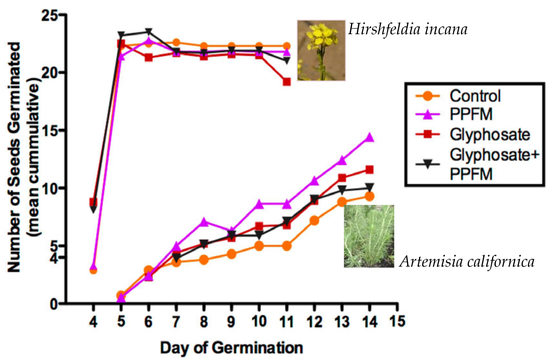


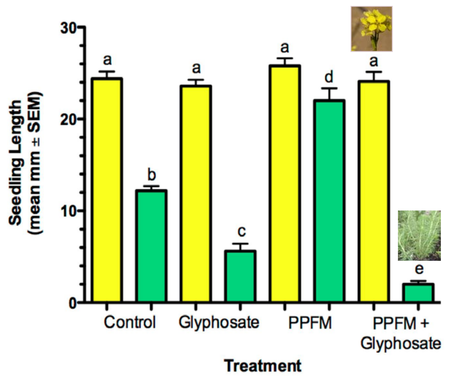
Culture & Sensitivity assay
Larger zone of clearing = more sensitive to concentration



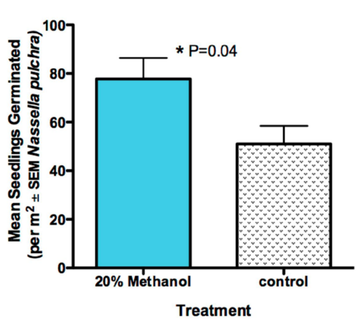


Glyphosate & PPFMs on *in vitro* germination rates & seedling size

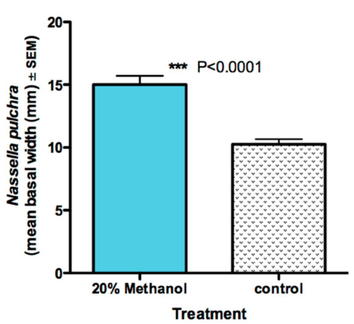




20% Methanol application: effects on *Nassella pulchra* field germination & seedling size (Cheeseboro Canyon, Santa Monica Mountains National Recreation Area)



May 2009



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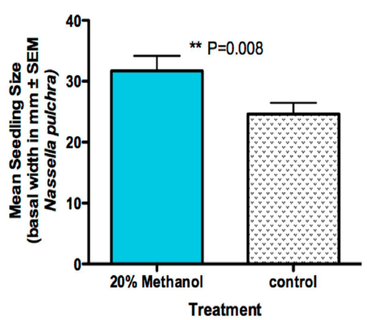

July 2009

DISCUSSION

- PPFM abundance can be manipulated to improve the outcome of restorations in CSS and grasslands.
- A 20% methanol application is an inexpensive, effective remediation tool after glyphosate has been used.
- We found that glyphosate application in the field and the laboratory significantly lowered PPFM abundances with resulting negative effects on native seed germination and seedling growth.
- We found that PPFMs had a positive impact on native seedling emergence and growth and no impact on non-native seedling emergence and growth.
- By inoculating native seeds or seedlings bound for restoration sites with PPFMs, we may further improve the natives' ability to compete with exotics, especially early on.
- The effects of other herbicides on PPFMs should also be tested.
- Our finding that mixed zones in an invasion gradient harbor fewer PPFMs is intriguing because PPFMs may be responding to increasing organics, perhaps allelopathic, exuded by roots during competition (Reinhart & Callaway 2006).
- Further work into the mechanisms allowing plants and PPFMs to interact may offer new insights into invasion dynamics and belowground plant competition.

RESTORATION IMPLICATIONS

Exploration of plant-microbe feedbacks is revealing intricate linkages between plants and important microbial mutualists. Restoring ecosystem functioning should include not only the plant community but also their microbial partners to achieve the best outcomes.

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Harris, J. (2009) Soil microbial communities and restoration ecology: facilitators or followers? *Science* 325:573-574
Reinhart, K.O. & R.M. Callaway (2006) Soil biota and invasive plants. *New Phytologist* 170:445–457
Trotsenko, Y.A.; Ivanova, E.G. and N.V. Doronina (2001) Aerobic methylotrophic bacteria as phytosymbionts. *Microbiology* 70:623–632.

ACKNOWLEDGMENTS

We wish to thank the following people for their generous help and advice:
- The Martiny Lab
- The Suding Lab
- John Tiszler, SMMNRA
- Randy Philips, SMMNRA
- Stephanie Raymond, SMMNRA

and for financial support: