Effect of acidic biochar extracts and biofertilizers on seed germination

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INTRODUCTION

- At least 33% of all croplands are moderately or highly degraded due to synthetic fertilizers, pesticides, intensive tillage, monocropping, and yield-based management systems (FAO, 2015).
- Soil degradation has led to mineral and nutrient decline in foods, as well as reduced soil-water holding capacity and microbiological diversity (Montgomery & Biklé, 2022).
- Most agricultural soils globally have lost 30-75% of their original organic carbon, resulting in atmospheric CO_2 (Global Carbon Project 2019).

In response to these global challenges, biochar is being investigated as a soil amendment to restore degraded soils. Biochar is produced from organic waste material (e.g. woody materials, crop residues, manures) that is partially combusted with limited oxygen. Biochars have been shown to improve soil health, plant growth and soil microbial dynamics; sequester carbon; and reduce greenhouse gas emissions (Lehman and Joseph, 2015). While biochar has been used for millennia to improve soil health and plant productivity, gaps in applying soil to alkaline soils, such as those in Texas, remain.

Figure 1. Left: Biochar added to a field; Right: Improved wheat growth due to biochar (credits: Michigan State University, Kristin Trippe, USDA ARS).

Modifications to biochar, such as nutrient addition (*charging*) or microbial inoculation (*activation*), have promoted seedling germination (Banu et al., 2022; Zhang et al., 2022) as well as plant and beneficial microbial growth (Sales et al., 2022). Specifically, biochar inoculations of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) have reported beneficial effects (Ipek and Eşitken, 2022; Lu et al., 2023). However, no studies have investigated inoculated biochar on alkaline soils, like those in Texas. Thus, the objective of this study was to determine if an acidic biochar inoculated with biofertilizers, namely a) PGPR, b) PGPR and arbuscular mycorrhizal fungi (AMF), or c) effective microbes (EM) including yeast, actinobacteria, photosynthetic bacteria, and *Lactobacillus casei* (Figure 2), could enhance seedling germination. This study is the first step before exploring biochar applications to crops in soils and soilless mixes.

EM (e.g., *Lactobacillus casei*) PGPR (e.g., *Bacillus thuringiensis*) AMF (e.g., *Glomus spp*)

Figure 2. Microbial consortia present in biofertilizers (credit: Horst Neve).

METHODOLOGY

This experiment involves:
1) treatment of seeds with acidic biochar extracts at different rates, 1% (1B), 2% (2B), 5% (5B), 10%(10 B),
2) treatment of seeds with beneficial microorganisms (biofertilizers), including PGPR, PGPR + AMF, and EM, and
3) evaluating the sensitivity of different crop seeds to the water-soluble compounds from biochar.

The results from this study will inform a forthcoming greenhouse experiment with microbially inoculated biochars to assess their influence on soil nutrient availability and plant uptake.

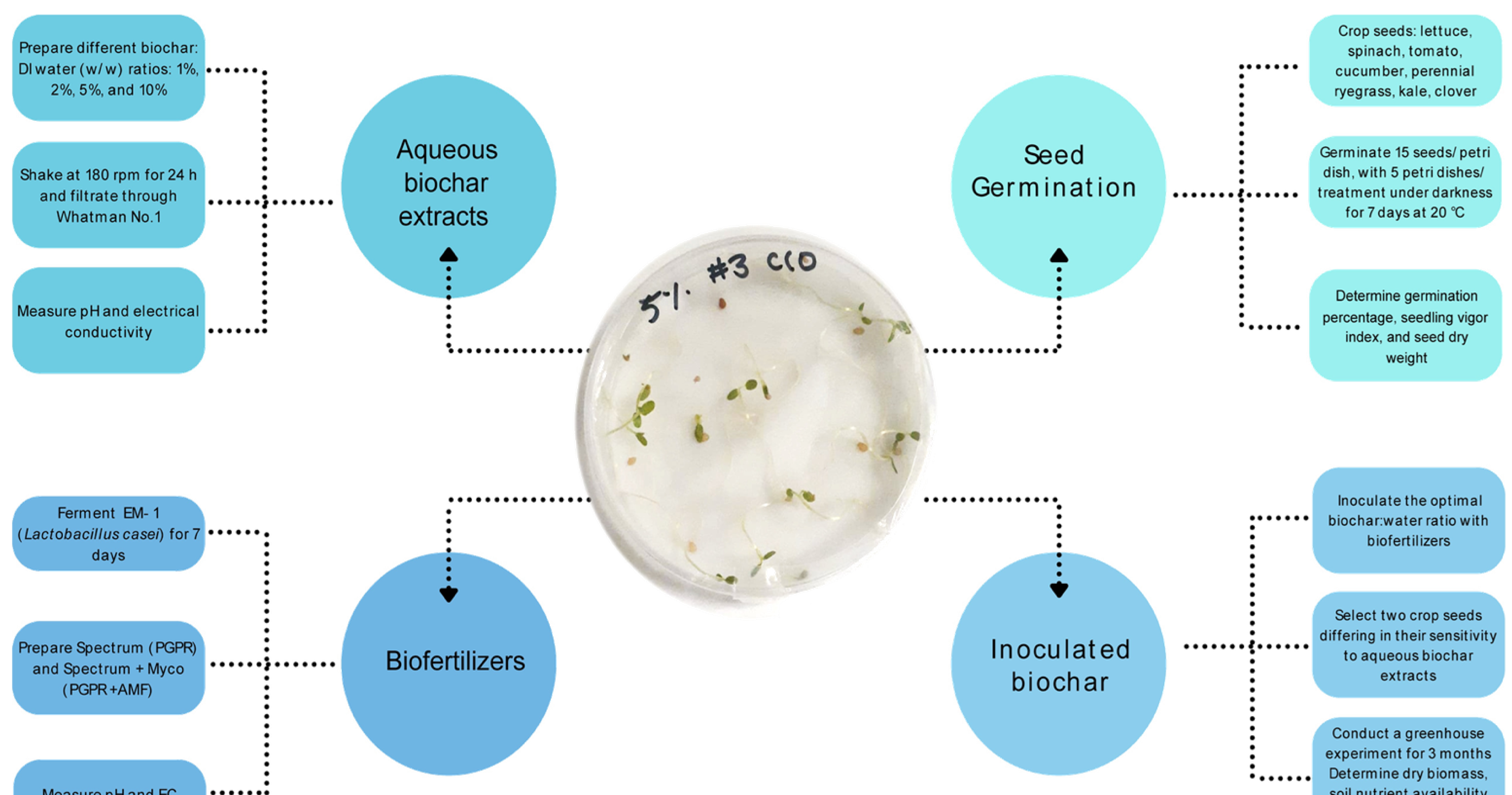

Figure 3. Experimental design diagram.

RESULTS

pH and electrical conductivity (EC) values play a crucial role in a plant's ability to absorb nutrients. High values can hinder root growth due to the presence of potentially toxic salts or osmotic stress, thereby impacting crop germination (Milon et al., 2022). Despite studies showing pH increases with biochar application, our results didn't exceed pH 7.5 for the biochar rates tested (Table 1), suggesting acidic biochar can be used as a soil amendment without causing negative alkaline conditions. However, EC, a measure of chemical salt concentrations, increased with higher biochar rates.

Table 1. pH and electrical conductivity (EC) of different biochar rates (1%, 2%, 5%, and 10%), microbial inoculants and deionized water only (control, C), where E = EM, M = PGPR + AMF, and S = PGPR. The data represent the mean of three replicates.

Parameters	1B	2B	5B	10B	E	M	S	C
pH	7.14	7.11	7.25	7.18	5.05	7.30	7.59	6.14
EC (µS/cm)	15	21	122	209	8.4	6.3	5.8	5.5

Phytotoxicity germination serves as a sensitive and reliable plant assay for fertilizer evaluation and dosage estimation (Milon et al., 2022). Differing biochar rates did not significantly affect germination percentage compared to the control (Figure 4). The seed vigor index, based on phytotoxicity quantification, indicated that almost all treatments did not promote phytotoxicity, as they presented values >80%. The control with clover exhibited a moderate level of vigor index, which may be attributed more to the absence of nutrients in DI water than phytotoxicity. According to Gasco et al. (2016), fertilizers yielding seedling vigor values >100% can be considered phytostimulants or phytonutrients. For lettuce, three ratios could be classified as phytostimulants (Figure 5a). While lettuce treated with 10% biochar showed a lower seedling dry weight than the control (Figure 6a), clover showed no significant differences among treatments, possibly due to species-specific responses.

RESULTS *(continued)*

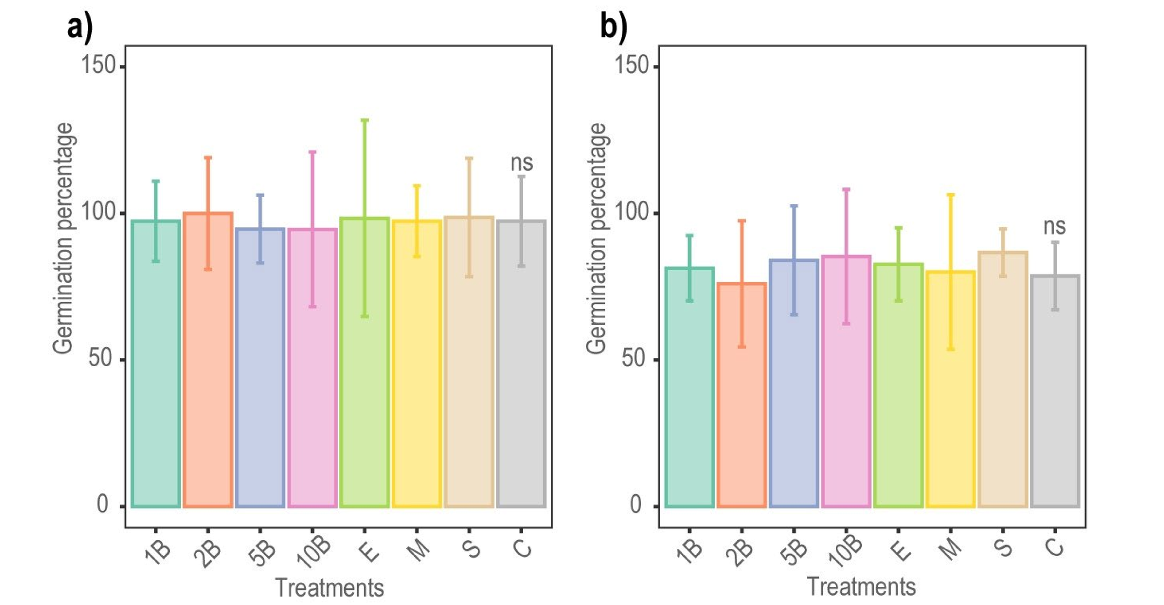

Figure 4. Effect of different biochar ratios (1B, 2B, 5B, and 10B) and microbial inoculations (E, M, S) compared to the control (C) on the germination of a) lettuce and b) clover seeds after seven days. Error bars indicate the standard error (N=5). Different letters represent significant differences among treatments according to Nemenyi's test ($p \leq 0.05$ level). ns, not significant.

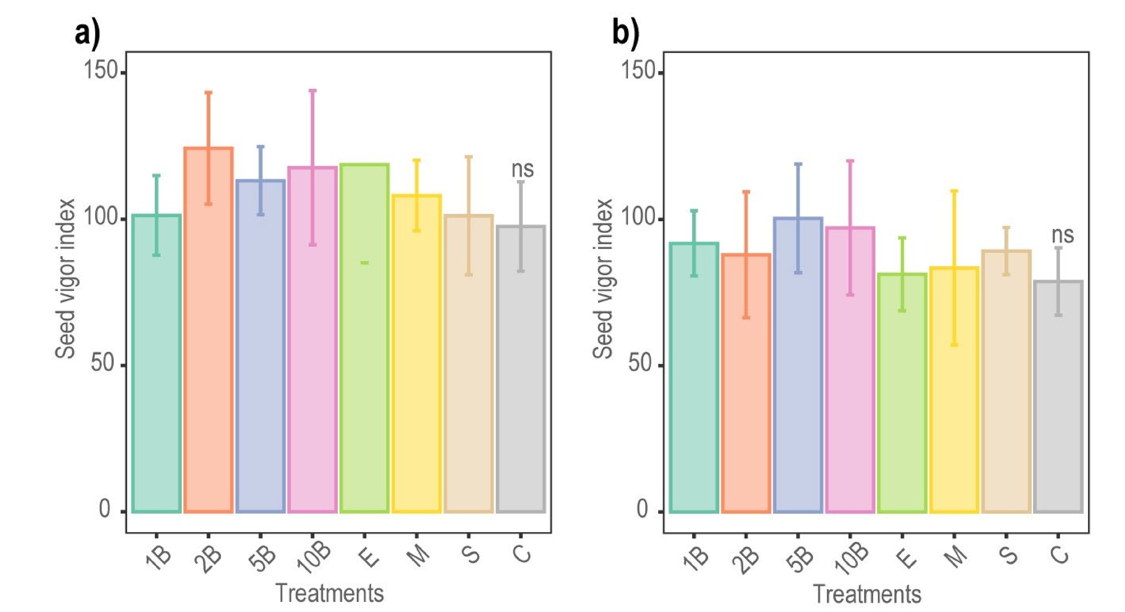

Figure 5. Effect of different biochar ratios (1B, 2B, 5B, and 10B) and microbial inoculations (E, M, S) compared to the control (C) on seed vigor index of a) lettuce and b) clover seeds after seven days. Error bars indicate the standard error (N=5). Different letters represent significant differences among treatments according to Nemenyi's test ($p \leq 0.05$ level). ns, not significant.

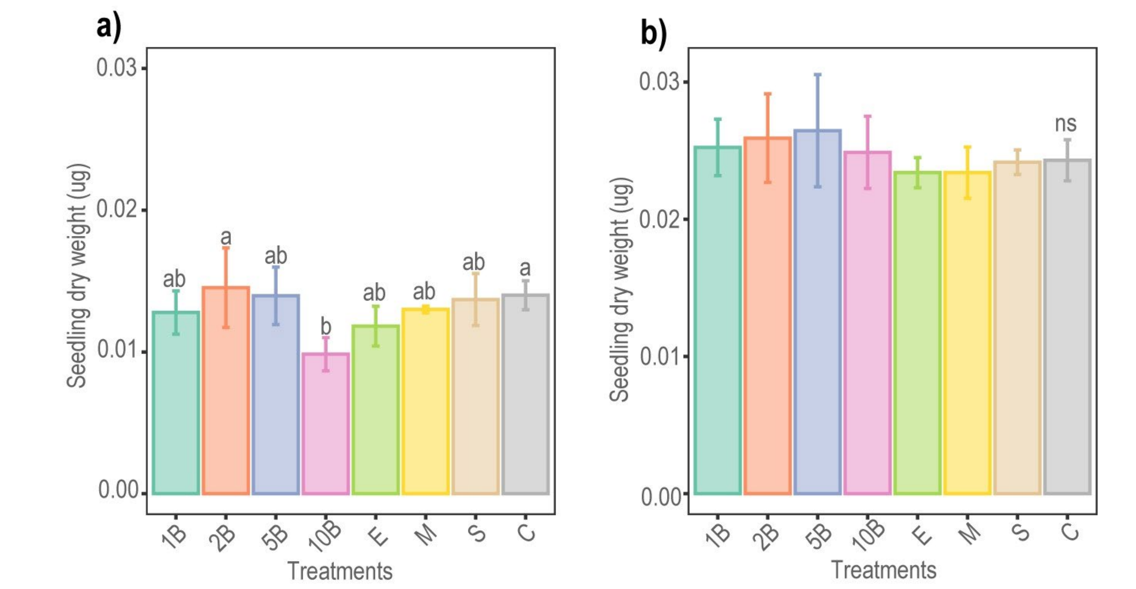

Figure 6. Effect of different biochar (1B, 2B, 5B, and 10B) and microbial inoculations (E, M, S) compared to the control (C) on seedling dry weight of a) lettuce and b) clover seeds after seven days. Error bars indicate the standard error (N=5). Different letters represent significant differences among treatments according to Nemenyi's test ($p \leq 0.05$ level). ns, not significant.

CONCLUSIONS

The variation in germination percentage among seeds may result from their varying sensitivity to treatments. While this study demonstrates the phytostimulant effect of some biochar ratios on lettuce seeds, further research is needed as no significant differences among treatments were observed. Our future research will explore methods to enhance the effectiveness of biochar, such as charging it with organic fertilizers or inoculating it, given the absence of significant differences compared to the control treatments in this study.