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Effects of Soil Amendments on the Germination and Health of Panicum virgatum L. Grown on Soils Polluted by Copper Mining Activities

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Abstract

Copper mining was an essential extractive economy that drove the colonization of the Upper Peninsula of Michigan. These mining operations were responsible for vast volumes of pollution in the form of coastal dumping of a mining talus called stamp sand. Stamp sands are both biologically toxic and highly transient. This study presents the emerging technology of agricultural phytomanagement as a method for establishing a vegetative cap over coastal deposits of stamp sand. The biofuel feedstock *Panicum Virgatum L.* (Switchgrass), was chosen for its capabilities as an agricultural crop. A 25 day growth trial was conducted to evaluate the germination and health of *P. virgatum* seedlings grown on increasing gradients of organic matter (OM) amendments. Stamp sand soil amended with High levels of OM and an additive VAM fungi *Rhizophagus irregularis* yielded seedlings with significantly greater root development, shoot length and biomass. However, all three concentrations of OM additions Low, High, and High+VAM achieved much lower germination than expected, with 43, 57, and 54 %, while a control of pure stamp sand achieved 93% of the expected germination.

Introduction

The Upper Peninsula of Michigan in the mid-19th and 20th century was the site of several lucrative copper mining operations. The vast majority of these mining operations existed within the copper belt of Keweenaw Peninsula, where an estimated 12 billion metric tons of copper have been extracted within the last two and a half decades (Bornhorst et. al 1983). The predominant technique used for the extraction of copper metal from its respective ore, was a technology known as "stamp milling". The stamp milling process utilizes large steam powered hammers to crush ore bearing rock in preparation for an acid extraction process that dissolves away the metal of interest. An essential byproduct of stamp milling is the fine sandy material known as “stamp sand” that remains after the process of acid leaching. Initially, these stamp sands were believed to be an inert byproduct of the mining process. This misconception proved to be unfortunate, as mining companies dumped billions of metric tons of the waste into Lake Superior and its surrounding watersheds (Ramussen et. al 2002). Recent studies by Sidhu et. al 2016 have shown that the inefficient metal extraction processes used during 19th and 20th century copper ore processing left stamp sands with residual levels of Copper (Cu), Iron (Fe), and Arsenic (As). Furthermore, the presence of heavy metals in combination with the physical size (1-2 mm) of stamp sand particles makes for a dynamic pollutant that is both biologically toxic and transient with coastal currents (Ramussen et. al 2002, Hakk 2011).

The emerging technology of agricultural phytomanagement may be a cost-effective method for addressing both the translocation and toxicity of coastal stamp sand deposits (Brown et. al 2015; Asensio et. al 2013). Agricultural phytomanagement utilizes the principles of phytoremediation, wherein plants are used to degrade, sequester, or volatilize pollutants. This technology is paired with agricultural crop economics in an aim to provide no cost or low cost solutions for addressing pollution (Pandey and Bauddh 2019). In order for this technology to be viable, potential crop species need to overcome the challenges of decreased yield and translocation of pollutants into above ground vegetation (Bauddh et. al 2015). The biofuel crop, *Panicum Virgatum L.* (Switchgrass) may be an economically viable crop for use in the agricultural phytomanagement of stamp sands (Pogrzeba et. al 2017). This study looked to evaluate the effects of three common soil amendment, vermicompost, Miracle Grow potting soil, and mycorrhizal fungi, on the health and germination of switchgrass grown on soils polluted with stamp sands.

Materials and Methods

Soil collection and amendments

Stamp sand was collected from the Sand Point site located near L'anse, Upper Peninsula Michigan. A 1:1 mix of Miracle Grow potting soil (San Antonio, Texas, United States of America) and Wiggle Worm Soil Builder Vermi-compst (Unco Industries inc, Union Grove, WI United States of America) was used as an organic matter (OM) amendment. Dry inoculant culture of *Rhizophagus irregularis* was purchased from the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (VAM). Three amended soil mediums were mixed at increasing rates of amendment additions, based on recommendations found in (Sidhu et. al 2016). A Low amendment treatment (25% OM, 75% stamp sand), High amendment treatment (50% OM, 50% stamp sand), and a High amendment + VAM treatment (48% OM; 45% stamp sand; 7% VAM) was made (Table 2). A control of 100% stamp sand was used to represent an unamended analog of a potential site for remediation.

Planting, growth and observational data collection

Live seed of the *P. virgatum* variety "Cave In Rock" was purchased from (Roundstone Native Seed LLC, Upton, KY). Treatments and control were sown in 25.5 x 52.5 cm trays, at a rate of 128 seeds/m^2, totaling 17 individuals per tray. Each seed was sown 1 cm below the surface using a homemade seed drill prepared from a 5mm diameter plastic tube. Seeding rate and depth were both in accordance with values recommended by industry standards and the seed manufacture (Bughrara et. al 2007). After sowing, trays were placed into a greenhouse and kept at 25 °C. Trays were covered with a transparent plastic lid and watered as needed. After 25 days of growth, seedlings were delicately removed from their respective soils with roots intact. Immediately after removal, each seedlings' roots were washed with deionized water to get rid of root-bound soil particles. After washing, wet weight was recorded (g). After the recording of wet weight, max shoot length (mm) and max root length (mm) were measured. Seedlings were then left to air dry at room temperature for 12 days. At the end of the 12 day drying period weight was again recorded (g).

Statistical Analysis

Data were analyzed using Microsoft Excel 2016 and SPSS 26.0 (Microsoft, USA) (IBM, USA). A one-way ANOVA, followed by a post-hoc comparison using Tukey's test of honestly significant differences, was used to analyze the effects of soil amendments on seedling shoot length, root length, wet weight, and dry weight.

Results

Low, High, and High+VAM amendment soils only yielded half of the expected total germination, with 43, 57, and 54 %, according to the manufactures PLS (Table 2). In contrast, control soil achieved 93% of the expected germination, nearly double that of amended soils. Switchgrass seedlings in High and High+VAM amendment soils had significantly greater root length than seedlings grown on control soil (P-value=0.043; P-value=0.014), with High+VAM amended soil exhibiting a stronger level of significance (Figure. 1a). High+VAM amended soil also exhibited significantly longer mean shoot length when compared to control (P-value=0.002), whereas Low and High amended soils did not when compared to control soil (P-value=0.179; P-value=0.203) (Figure. 1b). Mean wet and dry weight of seedlings grown in High+VAM soil possessed a strong significant difference when compared to control (P-value=0.001; P-value=0.003) (Figure. 1cd). Data collected from seedlings grown in Low amendment soils did not exhibit any significant difference when compared to control, however there was marginally greater wet weight when compared to control (P-value=0.08) (Figure. 1a).

Discussion

The seed manufacturer provided a Pure Live Seed rate (PLS) of 83%, meaning 83% of planted seeds are expected to germinate under a standardized lab conditions. Thus, with n =17 individuals, we should have seen n=14 individuals germinate in both treatments and control. That was not the case, roughly half of the expected germination was achieved in all three treatments, whereas nearly 93% of the expected germination was achieved in control. Research has shown that the seeds of *P. virgatum* varieties that inhabit coastal dunes of the Great Lakes will enter a dormant state when buried at depths greater than 15 cm (Zhang and Maun 1990). Zhang and Maun hypothesized that this response was directly correlated with the lack of temperature fluctuations experienced at greater soil depths and exists as a mechanism for establishment of a seed bank. Furthermore, the high porosity of organic matter has been shown to decrease the thermal conductivity of soil and in turn minimize daily fluctuation of soil temperature (Abul-Hamdeh and Reeder 2000). Thus, it is plausible that the inhibition of seed germination seen in treatments was due to the minimized soil temperature fluctuation experienced as a result of organic matter rich amendments.

Seedlings grown in Treatment 3 exhibited significantly higher biomass, shoot length and root length when compared to control, indicating that some advantage was conferred by the soil amendments (Figure 2). It seems as though the addition of VAM fungi in treatment 3 was responsible for the significant increase in biomass, root length, and shoot length observed. This can be inferred from the lack of significant increases in biomass, root length and shoot length observed in treatment 2. This seems plausible as the VAM fungus used, *Rhizophagus irregularis,* has been shown to enhance essential nutrient uptake and resistance to heavy metal toxicity in higher plants (Li et. al 2014, Mishra et. al 2017).

For *P. virgatum* to be a viable crop in the agricultural phytomanagement of sites polluted with copper mining waste, further research needs to be conducted into amendment recipes that will improve biomass without affecting germination. Furthermore, studies similar to Geiger et. al 2019 and Pogrzeba et. al 2017 must be conducted to address the ecological and economic impacts associated with the translocation of pollutants into above ground vegetation of crops used in agricultural phytomanagement.

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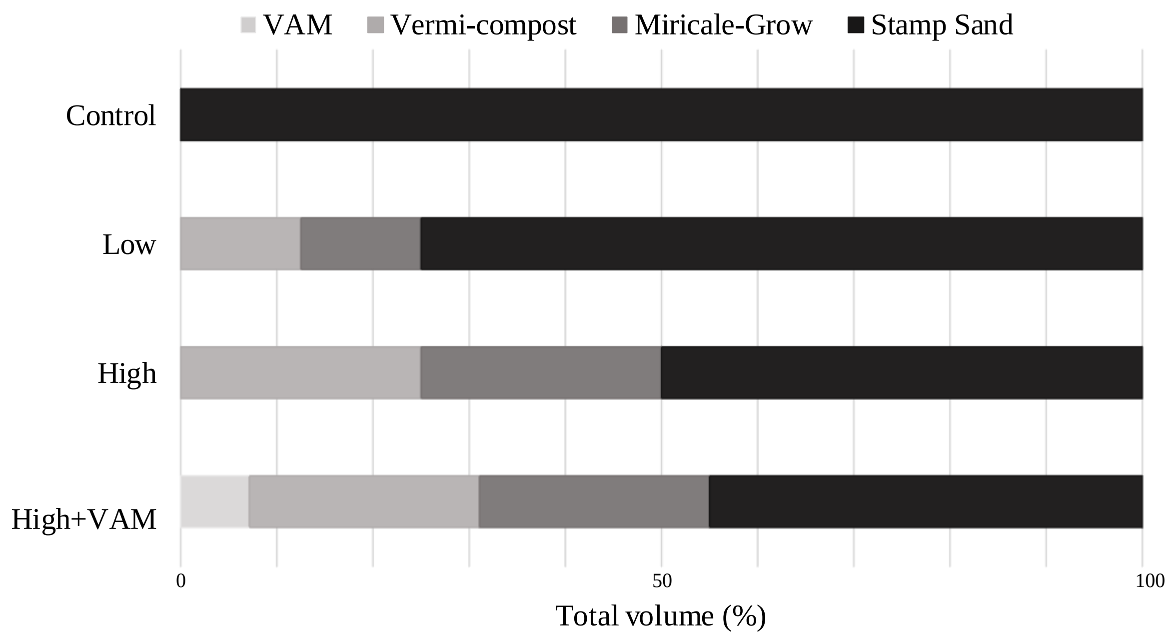

Table 1: Soil amendment recipes for treatments and control. Volumes are expressed as a percentage of the total volume of soil used; 2100 ml total for each Treatments and Control.

Treatment	Experimental Germinations	Expected Germinations	Expected Germination Achieved (%)
Control	13	14	93
Low	6	14	43
High	8	14	57
High+VAM	7	14	50

Table 2: Germinations for varying concentrations of amendment additions were compared to the manufactures PLS of 83%; n=17 individuals per tray, expected germination was n=14.

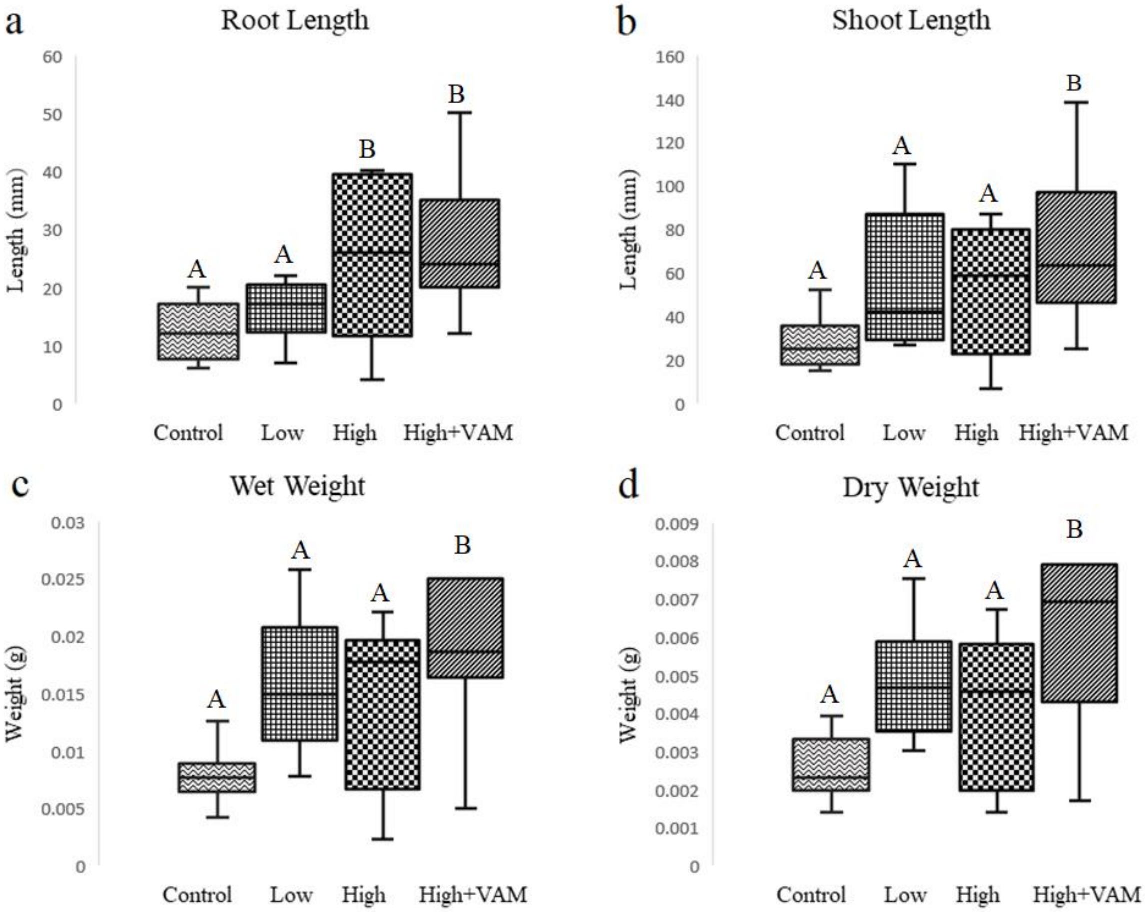

Figure 1: Top (left) root length was significantly greater in seedlings grown on both High and High+ VAM soils when compared to the control (P<0.05). Top (right) shoot length was significantly greater for seedlings grown on High+VAM soil (P<0.05). Bottom (left) wet weight of switchgrass seedlings grown on High+VAM soil were vary significantly different from the control (P<0.001). Bottom (right) dry biomass was significantly different for seedlings grown in High+VAM soil when compared to the control.

Figure 2: (Left) An example of a seedling pulled from control soil (Right) an example of a seedling pulled from Treatment 3 soil.