A Novel Method to Facilitate Biodethatching Using Fungal Laccases

Final Report

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INTRODUCTION

The proposed research was designed to explore the feasibility of utilizing extracellular laccases produced by white rot fungi to enhance the biodegradability of thatch. Through the study, we intend to develop the enzymatic pretreatment method that will significantly enhance the effectiveness of the bio dethatching processes.

Thatch is a layer of organic matter consisting of tightly intermingled dead and living leaves stem and roots that develop between the soil surface and the green vegetation. Thatch layer intermixed with sand or soil is known as Mat layer (Beard 1973). High organic matter accumulation in form of thatch or mat causes depletion of oxygen and decreased saturated hydraulic conductivity and increased water content (Hartwiger 2004). This further leads to problems like welt wilt, soft surface, black layer, limited rooting etc. (Carrow 2004; O'Brien and Hartwiger 2003).

Lignin, a 3- dimensional amorphous polymer consisting of methoxylated phenyl propane structure limits the degradation of organic matter (Beard 1973). It resists most microbial degradation mechanisms and serves as a barrier in the cell walls to limit the accessibility to the more biodegradable plant materials, such as cellulose and hemicelluloses, by microbial degraders. Oxidative enzymes such as laccases, lignin peroxidases and manganese peroxidases produced by white rot fungi attack the aromatic components of lignin and leads to its effective degradation.

White rot fungi are recognized as the most active lignin degrading microorganisms among few in the nature (Boyle, et al 1992; Gold and Alic 1993). Oxidative enzymes produced by fungi are able to attack the aromatic contents in lignin and produce free radicals, leading to effective degradation of lignin (Nakayamaa and Kamachi 1999). We hypothesize that thatch that has been directly treated with lignin-degrading enzymes will be more amenable for microbial

degradation because the lignin barrier that restricts the microbial accessibility have been effectively removed.

Laccases, lignin peroxidases, and manganese peroxidases are enzymes that have been known to be involved in lignin degradation (Nakayamaa and Kamachi 1999). They have been widely studied and used in pulp and paper industry to remove lignin, which serves as strong basis supporting the hypothesis mentioned above. Laccases, the multi copper oxidases are known to act on a wide variety of aromatic compounds by reducing oxygen to water (Baldrian 2006). The capability of degrading lignin utilizing oxygen as well as their strong extracellular activity makes laccases potentially suitable material for bio dethatching.

The proposed research is aimed at verifying the following hypothesis: 1) degradation of organic matter can be enhanced by applying laccase to the thatch layer; and 2) laccase has no appreciable adverse effects on turf quality.

OBJECTIVES

To test the hypothesis listed above represents the overarching goal of this study, and this has been achieved in a three phase studies. Phase 1 was a laboratory study aimed to verify the ability of laccase to facilitate the degradation of the organic matter in thatch layer; Phase 2 was a green house study with bentgrass pots to determine the effects of laccase application on thatch layer and on turf quality; Phase 3 was a field study to evaluate the overall dethatching effect under field conditions.

RESULTS

Phase 1 (2008) and 2 (2008-09) of the study has been completed and the results have been discussed in the previous reports submitted during November 2009 – November 2010. Another greenhouse experiment as part of the phase two was conducted on a dead bentgrass from December 2009 to June 2010 and the results were discussed in report submitted during November 2010.

For phase 3, the field study, started in June 2010. The main objectives of the field study are 1) To determine the effectiveness of laccase enzyme in facilitating organic matter degradation under field conditions. 2) To optimize the laccase application rate as well as application frequency. 3) To determine the effectiveness of laccase on bentgrass, bermudagrass

and Zoysiagrass. 4) To compare laccase application in comparison to and ain combination with the cultural management practice (Core aeration followed by topdressing).

Based on the results of the greenhouse experiments, following treatments were finalized and laccase was applied for seven months from June to December 2010.

Bentgrass:

1. Cultural Management Control (CMC) – Only Cultural Management Practice
2. Control (Untreated i.e. no enzyme or CMC)
3. Rate 0.1, frequency 2 weeks
4. Rate 0.2, frequency 2 weeks
5. Rate 0.4, frequency 2 weeks
6. Rate 0.8, frequency 2 weeks
7. Rate 0.4, frequency 4 weeks
8. Rate 0.4, frequency 8 weeks
9. Rate 0.4, frequency 12 weeks
10. Rate 0.4, frequency 2 weeks Chinese enzyme (Sample 3)
11. Rate 0.4, frequency 2 weeks Chinese enzyme (Sample 2)
12. Rate 0.4, frequency 4 weeks with Cultural Management Practices
13. Untreated

Bermudagrass:

14. Control
15. Rate 0.4, frequency 2 weeks

Zoysiagrass:

16. Control
17. Rate 0.4, frequency 2 weeks

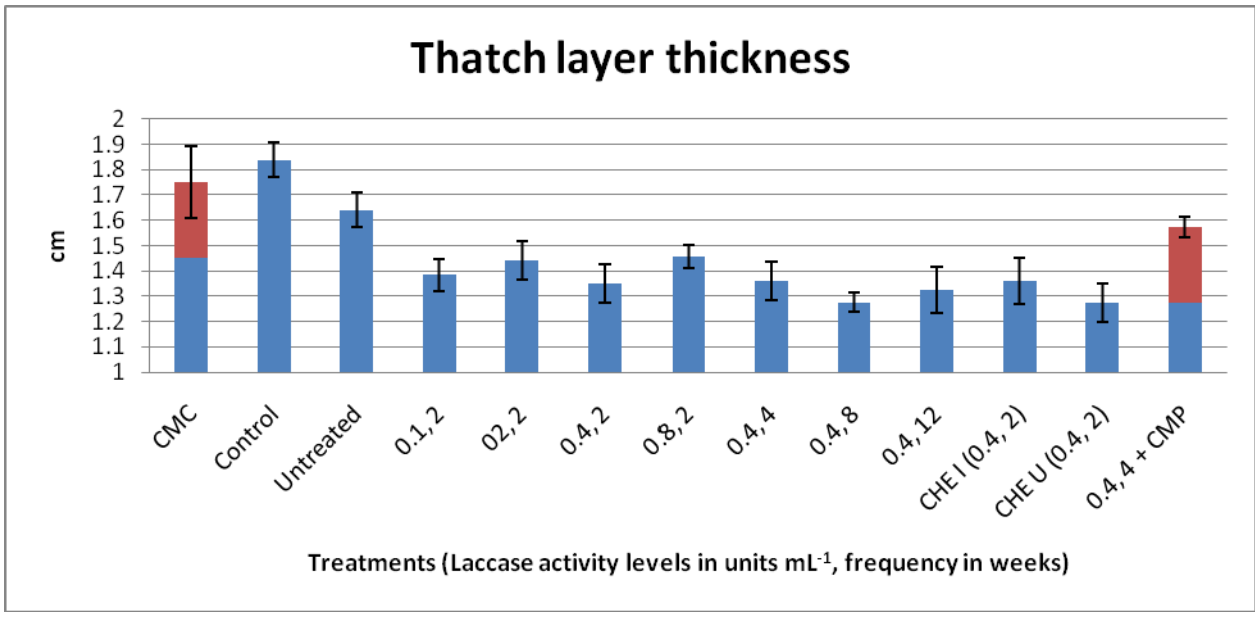
Cultural Management Practices:

1. Core aeration 2 times per year (04-05-2010 and 09-27-2010)
   Sand top-dress along with core aeration

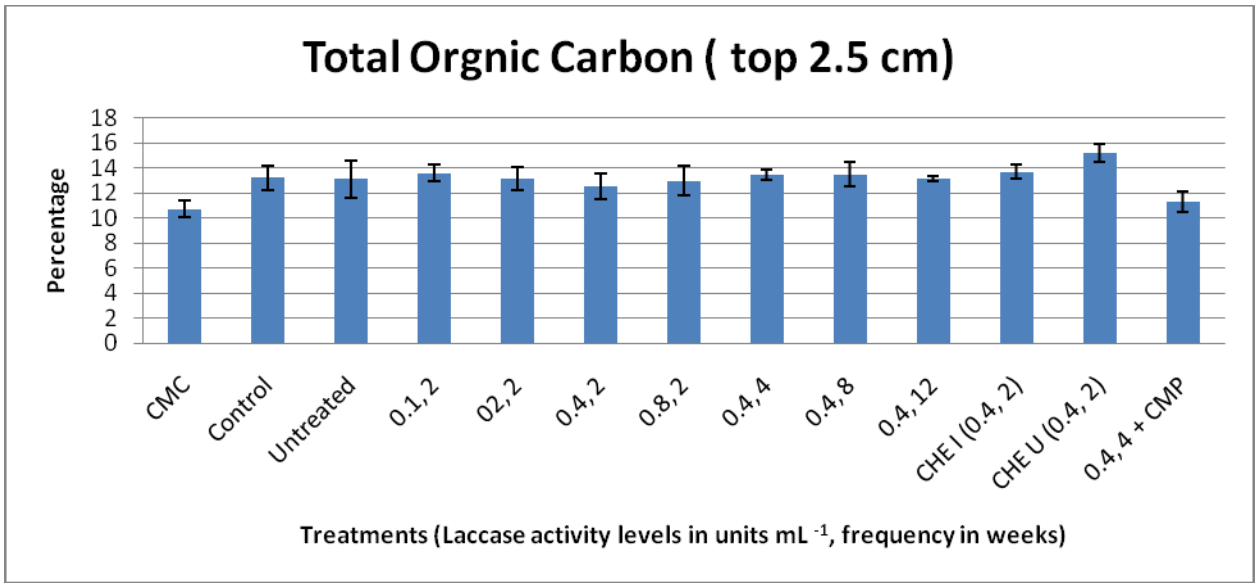
In bentgrass field study, a split plot experiment was started with four replications. The area of each plot was two square feet and laccase was applied as 410-mL solution. The activity level of laccase is represented by units mL^{-1}. As mentioned earlier in the treatments that laccase was applied at different frequencies i.e. at 2, 4, 8, and 12 weeks. Two different sources of laccase enzyme from China were also used to compare the effectiveness of these sources to the laccase enzyme used in this study from Sigma Aldrich. The Chinese laccase sources are from industry and university and are so mentioned as CH I and CH U, respectively.

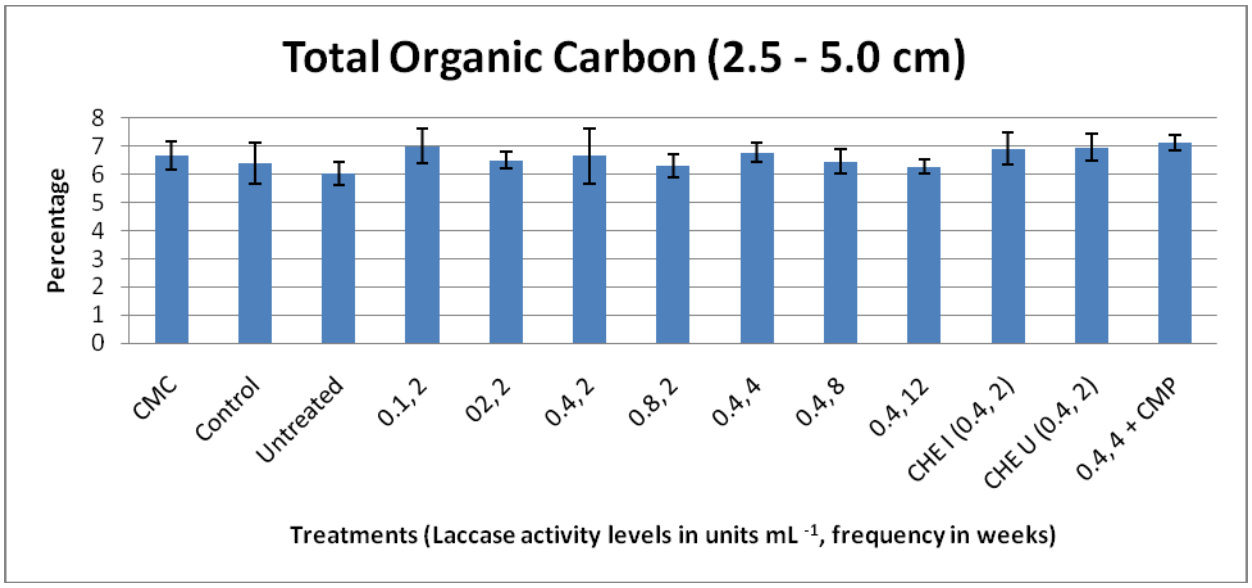
However in Bermudagrass and Zoysiagrass, only one laccase activity level at two weeks of application frequency was used to observe the effectiveness of laccase on these two grass types. Sampling was done in January and February 2011 and the samples were analyzed for thatch layer thickness, total organic carbon at two depths; 0 - 2.5, and 2.5 – 5 cm, and saturated hydraulic conductivity. Two sub samples were collected from each plot for analysis. The results from field experiments are summarized in below graphs.

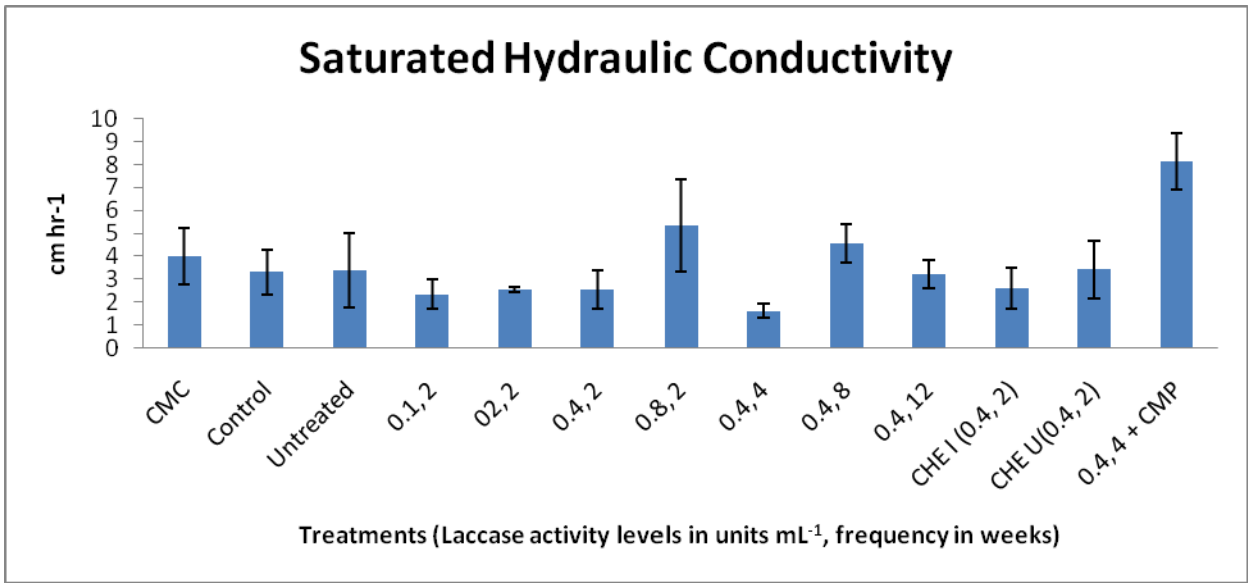
RESULTS: Bentgrass



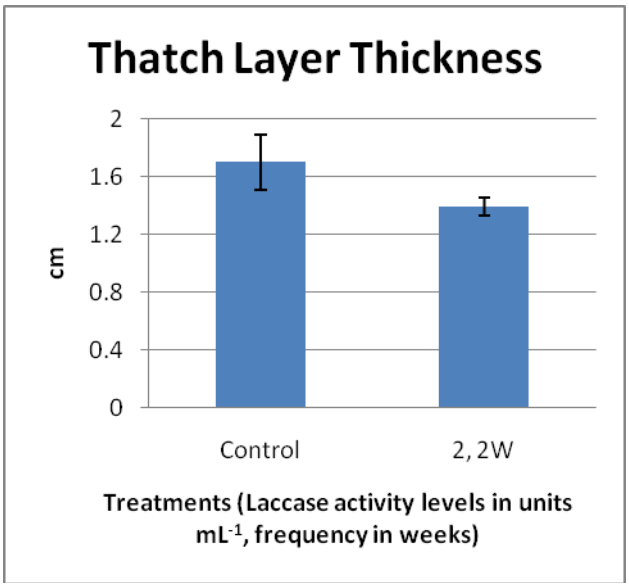
Red color represents the sand layer approximately 3 mm in depth over the thatch layer due to sand top dressing.

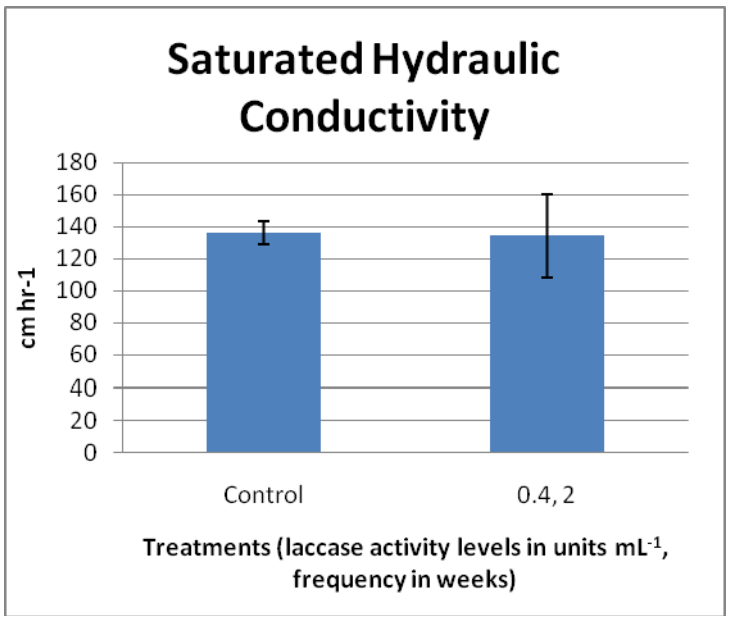

Total Orgnic Carbon (top 2.5 cm)
Percentage
18
16
14
12
10
8
6
4
2
0
CMC
Control
Untreated
0.1,2
02,2
0.4,2
0.8,2
0.4,4
0.4,8
0.4,12
CHE I (0.4, 2)
CHE U (0.4, 2)
0.4, 4 + CMP
Treatments (Laccase activity levels in units mL -1, frequency in weeks)

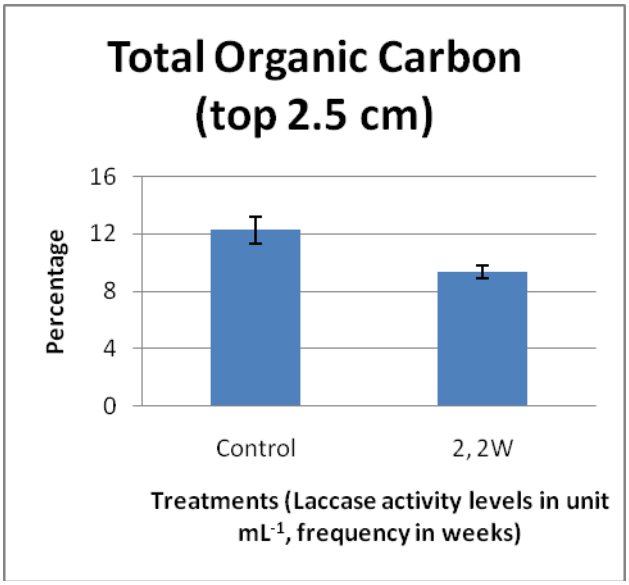

Total Organic Carbon (2.5 - 5.0 cm)
Percentage
0
1
2
3
4
5
6
7
8
CMC
Control
Untreated
0.1,2
02,2
0.4,2
0.8,2
0.4,4
0.4,8
0.4,12
CHE I (0.4, 2)
CHE U (0.4, 2)
0.4,4 + CMP
Treatments (Laccase activity levels in units mL -1, frequency in weeks)

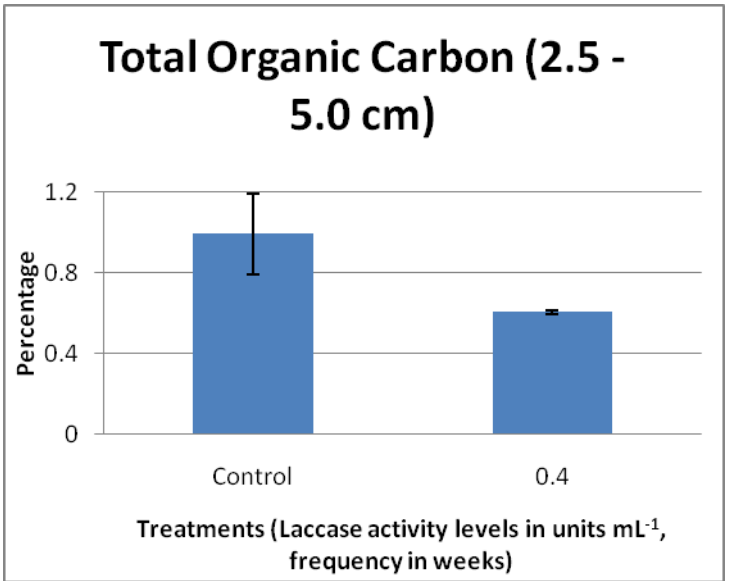

Saturated Hydraulic Conductivity
cm hr-1
0
1
2
3
4
5
6
7
8
9
10
CMC
Control
Untreated
0.1,2
02,2
0.4,2
0.8,2
0.4,4
0.4,8
0.4,12
CHE I (0.4, 2)
CHE U(0.4, 2)
0.4,4 + CMP
Treatments (Laccase activity levels in units mL-1, frequency in weeks)

RESULTS: Bermudagrass

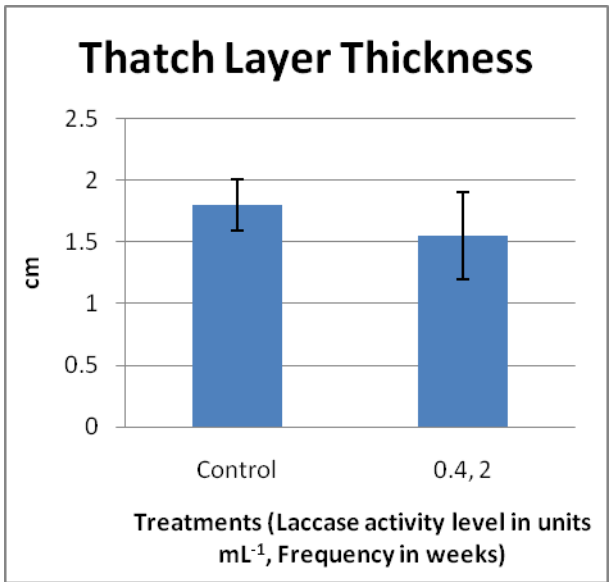


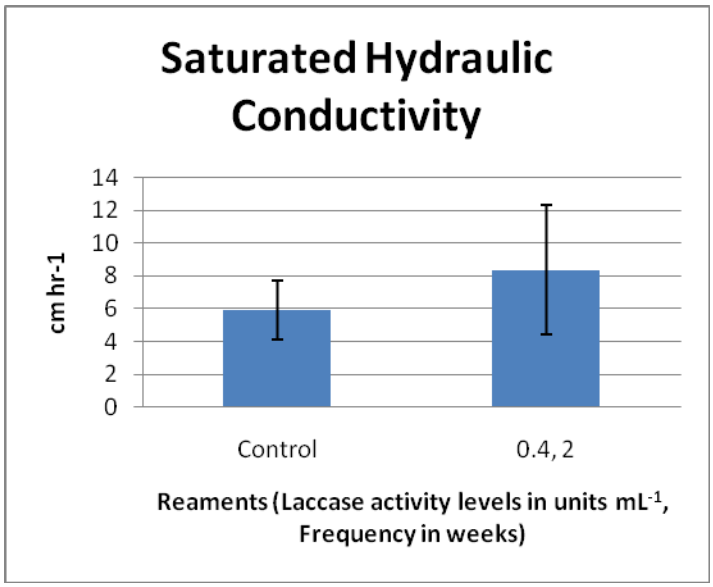


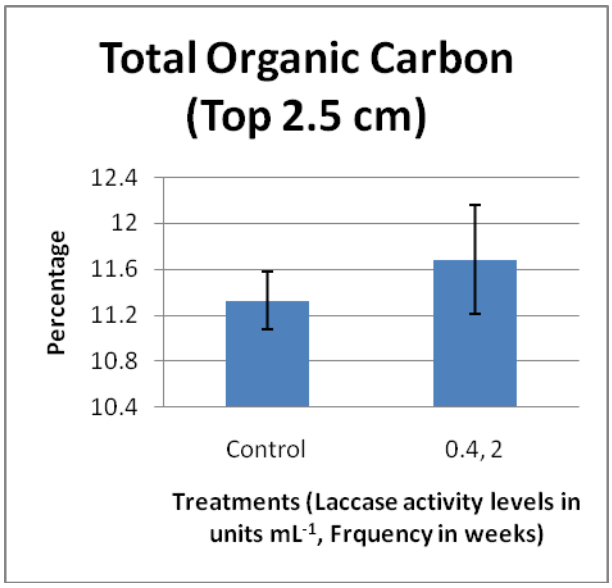


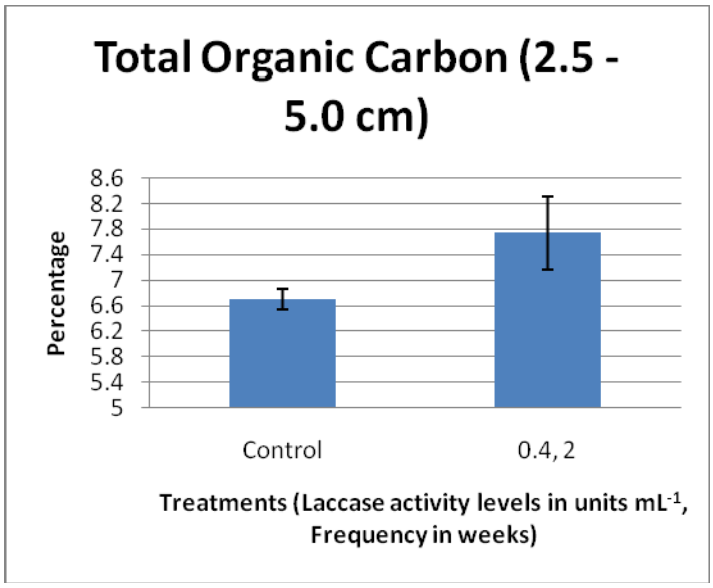


RESULTS: Zoysiagrass






Total Organic Carbon (Top 2.5 cm)
Percentage
12.4
12
11.6
11.2
10.8
10.4
Control
0.4, 2
Treatments (Laccase activity levels in units mL-1, Frquency in weeks)


Total Organic Carbon (2.5 - 5.0 cm)
Percentage
8.6
8.2
7.8
7.4
7
6.6
6.2
5.8
5.4
5
Control
0.4, 2
Treatments (Laccase activity levels in units mL-1, Frequency in weeks)