Further investigations of sorghum for biomass on marginal lands - hybrid genotypes, growth-promoting microbes, and plant usable nitrogen (Biomass Cluster Activity 7)

Lining Tian, Kevin Vessey, Julia Lu

May 19, 2022

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Project Overview

- Canada has large marginal land
- Not suitable for many crops
- Sorghum can adapt to different types of soils and growth conditions
- Could grow and produce high biomass on marginal lands

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Part I: Sorghum Hybrids

London Research and Development Centre
Agriculture and Agri-Food Canada

Lining Tian

Acknowledgement

- Government of Canada under the Canadian Agricultural Partnership (CAP)
- Biomass Cluster holder: BioFuelNet Canada
- Partners: AERC Inc
- Research: Mimmie Lu, Josee Kelly, Rawan Al-Nasery, Sufyan Tahir, Albert Asztalos and the farm crew

Goals

- Evaluate different sorghum hybrids
- Identify hybrid(s) for high biomass production on marginal lands

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Approaches

- Grow sorghum and evaluate sorghum biomass yield
- Three sorghum hybrids, namely, 10AX118, 10AX131, CSSH45
- Fields: Simcoe, Ottawa, and London (control)
- Nitrogen was applied at 120kg/ha

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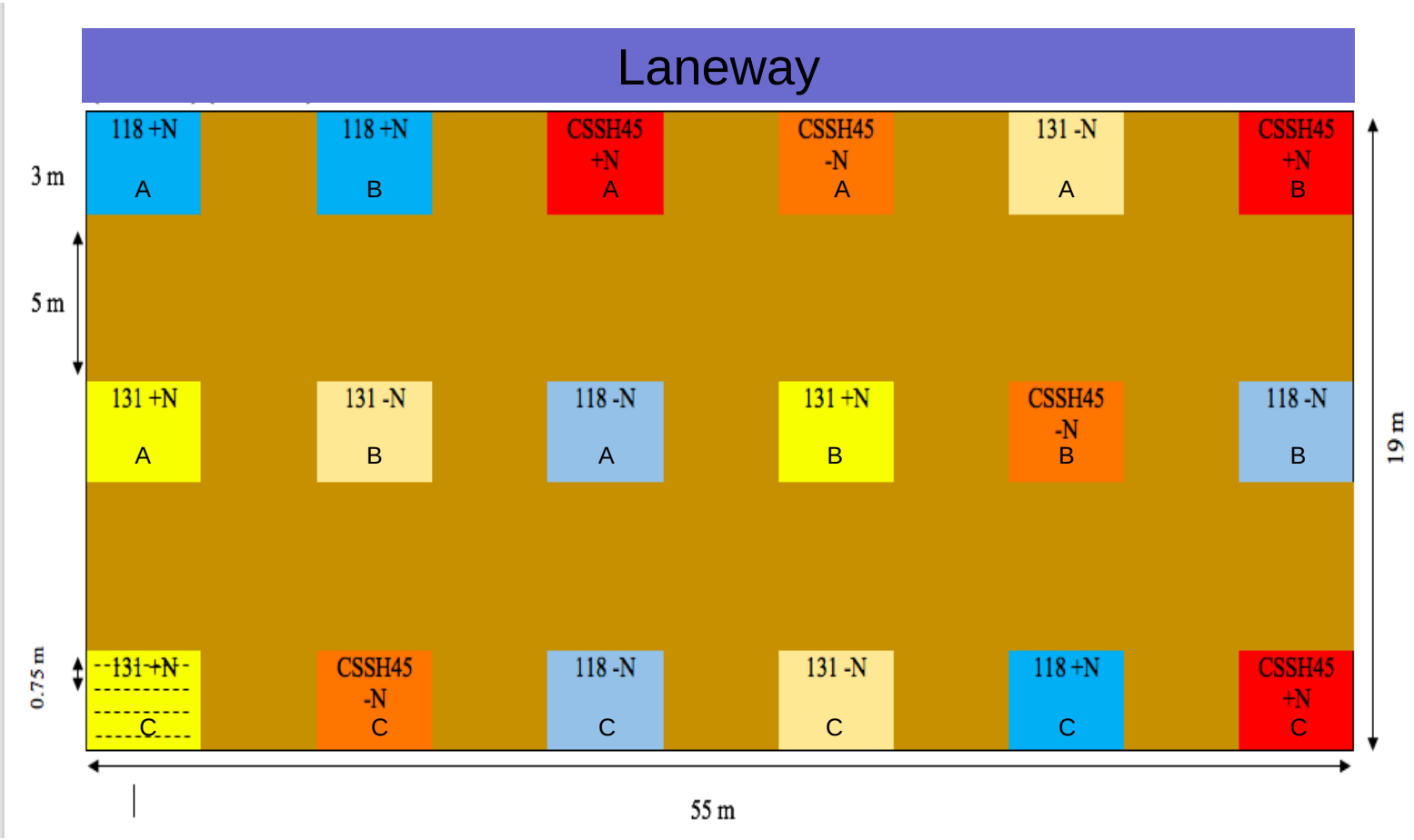
Plot Design



- Three replicates for each treatment (with or without nitrogen) per hybrid, in randomized plots

Plot Preparation, Simcoe

Plant development, Simcoe

Nitrogen fertilization

First application

- When plants were about 2-4 inches high

Second application

- When plants were about 2 feet high

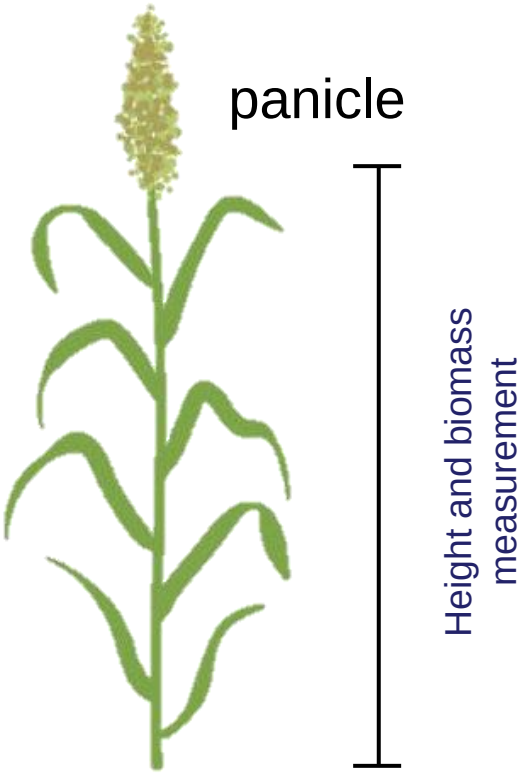
Total: 120 kg/ha during the plant growth season

Plant growth, Simcoe field

Progress and Results

Measure plant growth

- Height
- Fresh weight
- Dry weight



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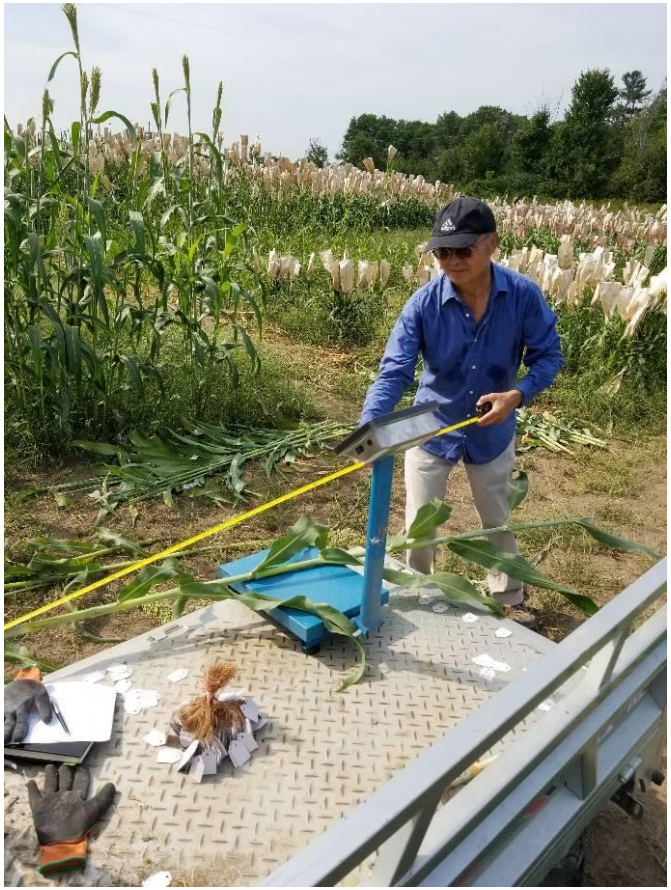
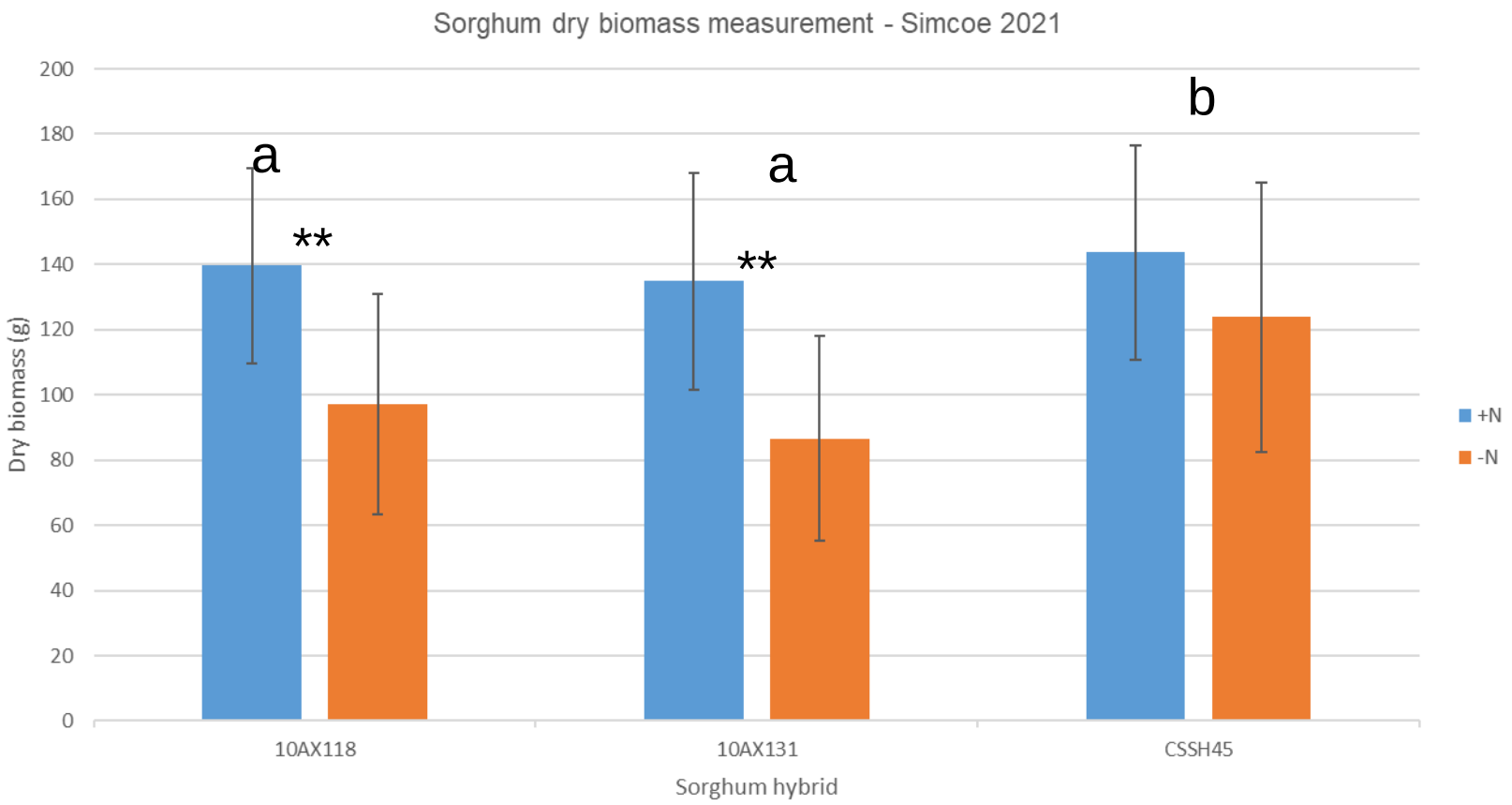
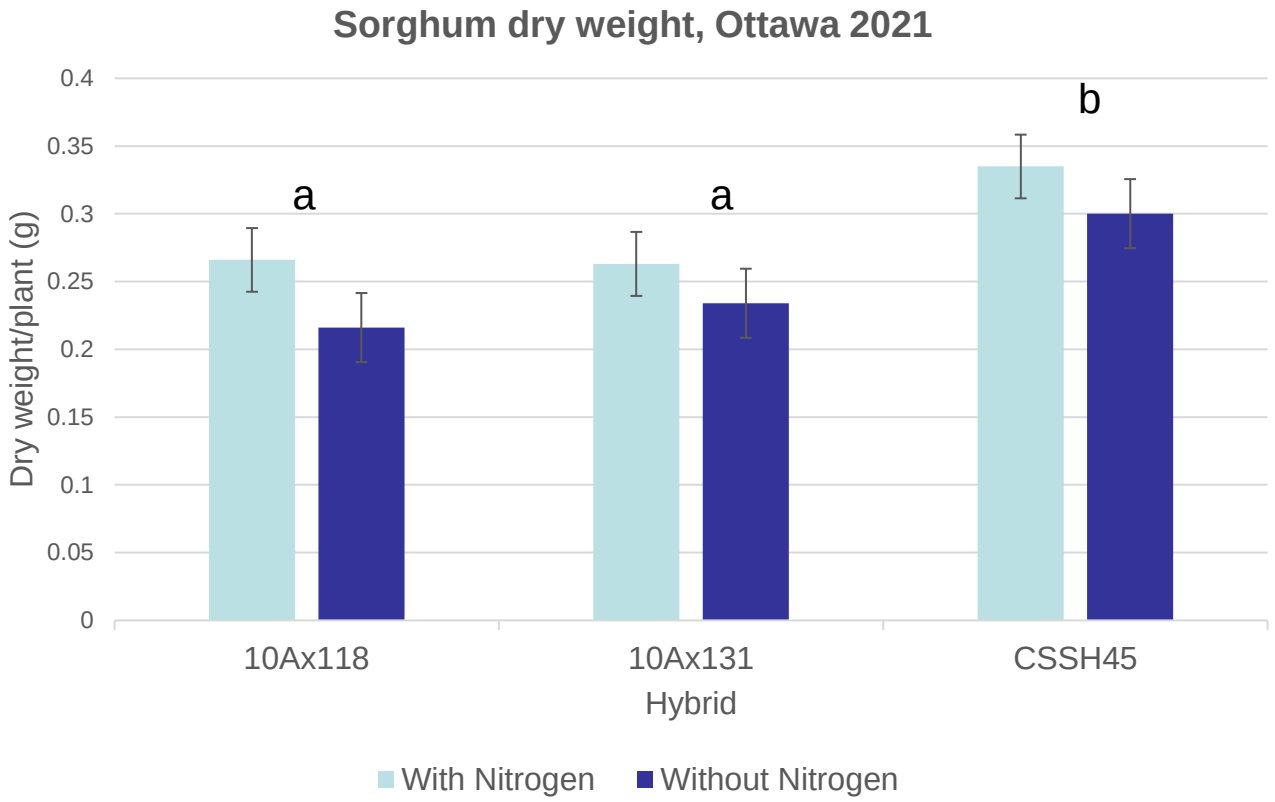


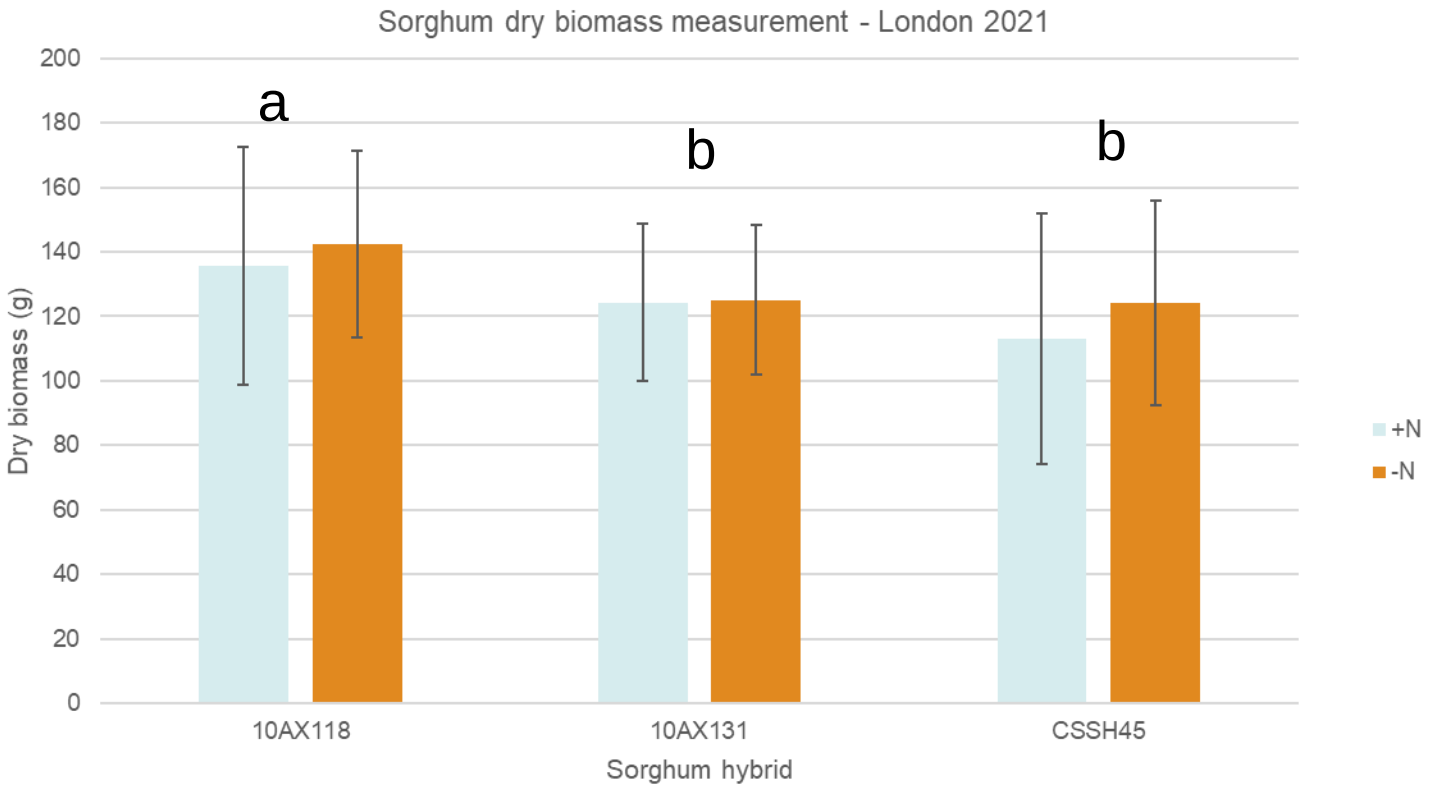
Plant measurement, Ottawa Aug., 2021

Dry weight, Simcoe field





There is significant difference among hybrids (indicated by different letters). There is no significant difference for nitrogen application (p=0.05). Two-Way ANOVA with Tukey test.


Sorghum dry biomass measurement - London 2021
Dry biomass (g)
200
180
160
140
120
100
80
60
40
20
0
a
b
b
10AX118
10AX131
CSSH45
Sorghum hybrid
+N
-N

Summary

- Some sorghum hybrids grow better and produce higher biomass than others on marginal lands
- The hybrid(s) that grow well on prime agriculture lands do not necessarily grow well on marginal land(s)
- Sorghum has deep root system and may absorb nitrogen in deep soil.
  - Sorghum could use nitrogen more efficiently
  - Less nitrogen loss to environment
  - Nitrogen fertilization can be optimized

Future Work

- Evaluate more and new sorghum hybrids for biomass production
- Identify other hybrid(s) that produces high biomass on marginal lands.

Part II: Plant Growth-Promoting Microbes

J. Kevin Vessey[1] and Yousef Papadopoulos[2]
[1]Saint Mary's University, Halifax, NS
[2]Agriculture and Agri-Food Canada, Truro, NS

BioMass Canada Webinar Series
19 May 2022

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Research Goal

- The goal of this component of Project 7 is to determine if plant-growth-promoting rhizobacteria (PGPR) can stimulate the growth of hybrid sorghum genotypes grown on marginal lands.

Overview

Previous research has shown that the PGPR, *Gluconacetobacter diazotrophicus,* can infect some sorghum genotypes

Plant Soil
DOI 10.1007/s11104-015-2653-8

REGULAR ARTICLE

Colonization efficiency of different sorghum genotypes by *Gluconacetobacter diazotrophicus*

Vanessa Yoon · Gang Tian · J. Kevin Vessey · Sheila M. Macfie · Om P. Dangi · Anand K. Kumer · Lining Tian

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This bacterium as well as numerous other PGPR have been shown to stimulate the growth of various biomass crops

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: www.elsevier.com/locate/biombioe

Research paper

Enhancing the productivity of hybrid poplar (*Populus* × *hybrid*) and switchgrass (*Panicum virgatum* L.) by the application of beneficial soil microbes and a seaweed extract

Houman Fei[a], Matthew Crouse[b], Yousef Papadopoulos[b], J. Kevin Vessey[a,*]

Biocatalysis and Agricultural Biotechnology 10 (2017) 247–255

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Improving a "Generation 1.5" biofuel feedstock crop: Colonization and growth enhancement of energy beet (*Beta vulgare* L. Beta 5833R) by inoculation with *Gluconacetobacter* spp.

Houman Fei, Abuduxikuer Abudureheman, J. Kevin Vessey*

Can. J. Plant Sci. Vol. 100, 2020

NRC Research Press

ARTICLE

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Improving biomass yield of giant Miscanthus by application of beneficial soil microbes and a plant biostimulant

Houman Fei, Matthew Crouse, Yousef A. Papadopoulos, and J. Kevin Vessey

Approach

- Using greenhouse and field studies, combinations of sorghum genotypes (cultivars and breeding lines) and PGPR are being tested for potential enhancement of plant growth compared to uninoculated control plants/plots at two levels of N fertility. Evidence of N_2 fixation in the symbioses is being tested using the ^{15}N-isotope dilution technique in some trials.

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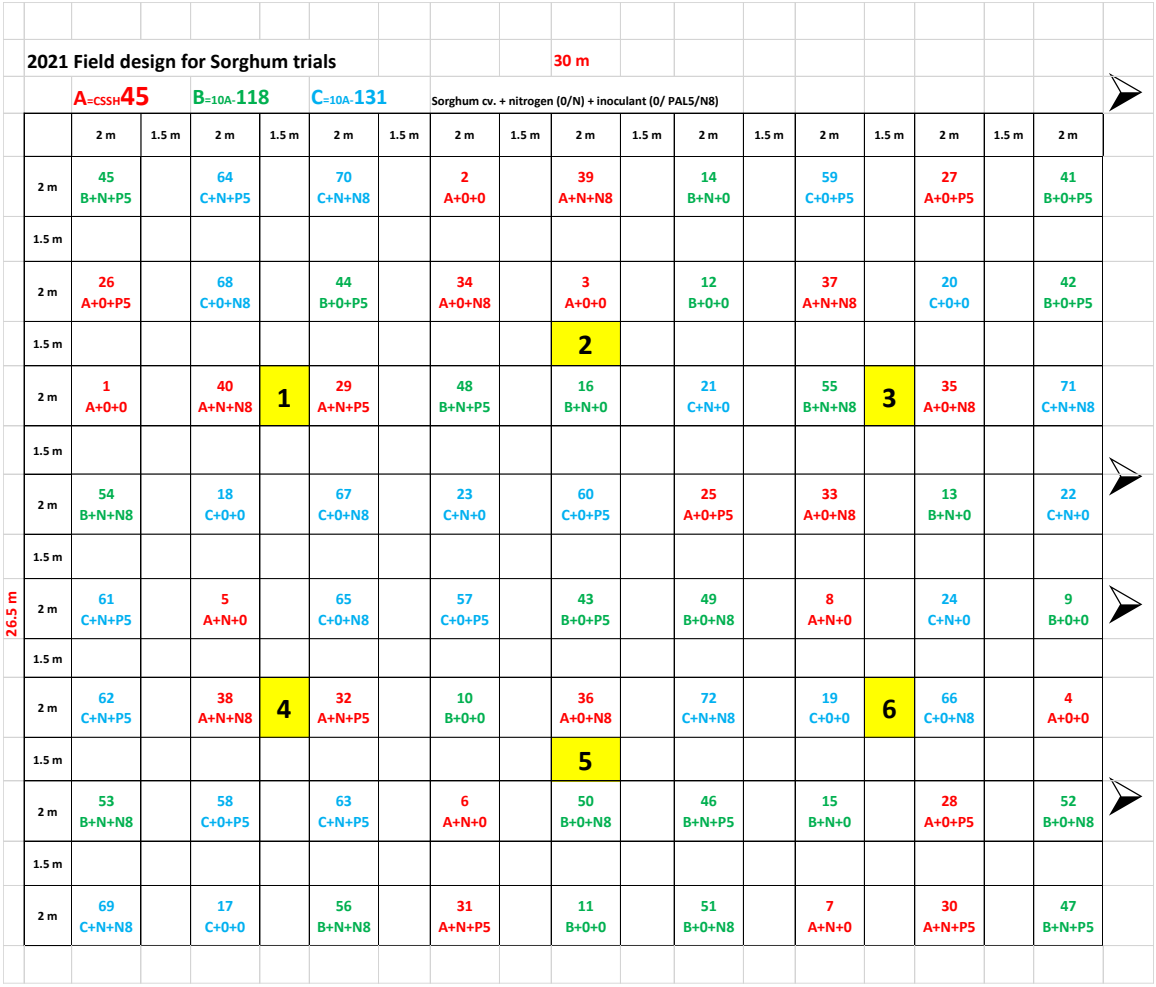
Approach

- Plant-microbe combinations test under greenhouse conditions (2019-2020):
  - Four genotypes of sorghum (CSSH 45; CFSH 30; 10Ax131; 10Ax118)
  - Five potential plant-growth-promoting microbes: *G. diazotrophicus* PAL5T; *G. azotocaptans* DS1; *Azospirillum lipoferum* N7; *A. brasilense* N8; and *Penicillium bilaii.*
- Plant-microbe combinations test under field conditions (2020-2021):
  - Three genotypes of sorghum (CSSH 45; 10Ax131; 10Ax118)
  - Four potential plant-growth-promoting microbes: *G. diazotrophicus* PAL5T; *G. azotocaptans* DS1; *Azospirillum lipoferum* N7; and *A. brasilense* N8.

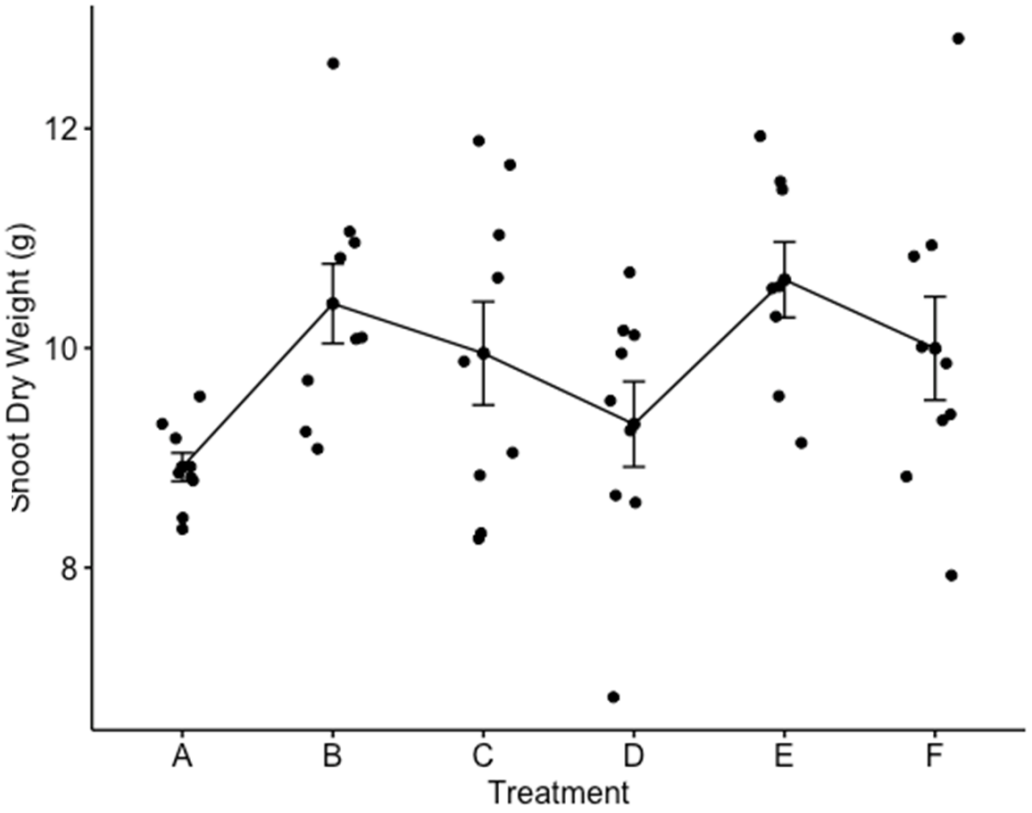
Field Experimental Design 2021



	2 m	1.5 m	2 m	1.5 m	2 m	1.5 m	2 m	1.5 m	2 m	1.5 m	2 m	1.5 m	2 m	1.5 m	2 m	1.5 m	2 m
2 m	45 B+N+P5		64 C+N+P5		70 C+N+N8		2 A+0+0		39 A+N+N8		14 B+N+0		59 C+0+P5		27 A+0+P5		41 B+0+P5
1.5 m																	
2 m	26 A+0+P5		68 C+0+N8		44 B+0+P5		34 A+0+N8		3 A+0+0		12 B+0+0		37 A+N+N8		20 C+0+0		42 B+0+P5
1.5 m									2								
2 m	1 A+0+0		40 A+N+N8	1	29 A+N+P5		48 B+N+P5		16 B+N+0		21 C+N+0		55 B+N+N8	3	35 A+0+N8		71 C+N+N8
1.5 m																	
2 m	54 B+N+N8		18 C+0+0		67 C+0+N8		23 C+N+0		60 C+0+P5		25 A+0+P5		33 A+0+N8		13 B+N+0		22 C+N+0
1.5 m																	
2 m	61 C+N+P5		5 A+N+0		65 C+0+N8		57 C+0+P5		43 B+0+P5		49 B+0+N8		8 A+N+0		24 C+N+0		9 B+0+0
1.5 m																	
2 m	62 C+N+P5		38 A+N+N8	4	32 A+N+P5		10 B+0+0		36 A+0+N8		72 C+N+N8		19 C+0+0	6	66 C+0+N8		4 A+0+0
1.5 m									5								
2 m	53 B+N+N8		58 C+0+P5		63 C+N+P5		6 A+N+0		50 B+0+N8		46 B+N+P5		15 B+N+0		28 A+0+P5		52 B+0+N8
1.5 m																	
2 m	69 C+N+N8		17 C+0+0		56 B+N+N8		31 A+N+P5		11 B+0+0		51 B+0+N8		7 A+N+0		30 A+N+P5		47 B+N+P5

- 72 plots in a completely randomized design: 3 genotypes × 3 microbial treatment × 2 fertilizer levels × 4 replicates.
- Each subplot was 2m × 2m ($4m^2$).
- Each subplot contained three rows of sorghum plants.
- Target density was 42 plant/plot = 105,000 plants/ha.

Greenhouse Experiment #1: CSSH 45 Shoot Dry Weight

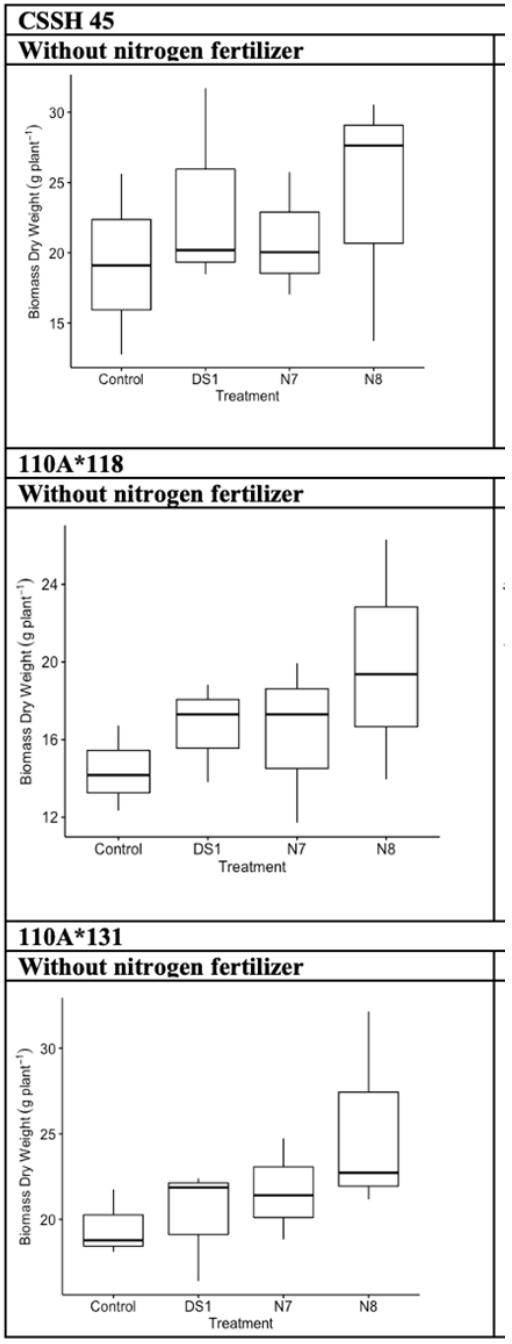


A - Control
B - DS1
C – N7
D – N8
E - PAL5T
F – *P. bilaii*

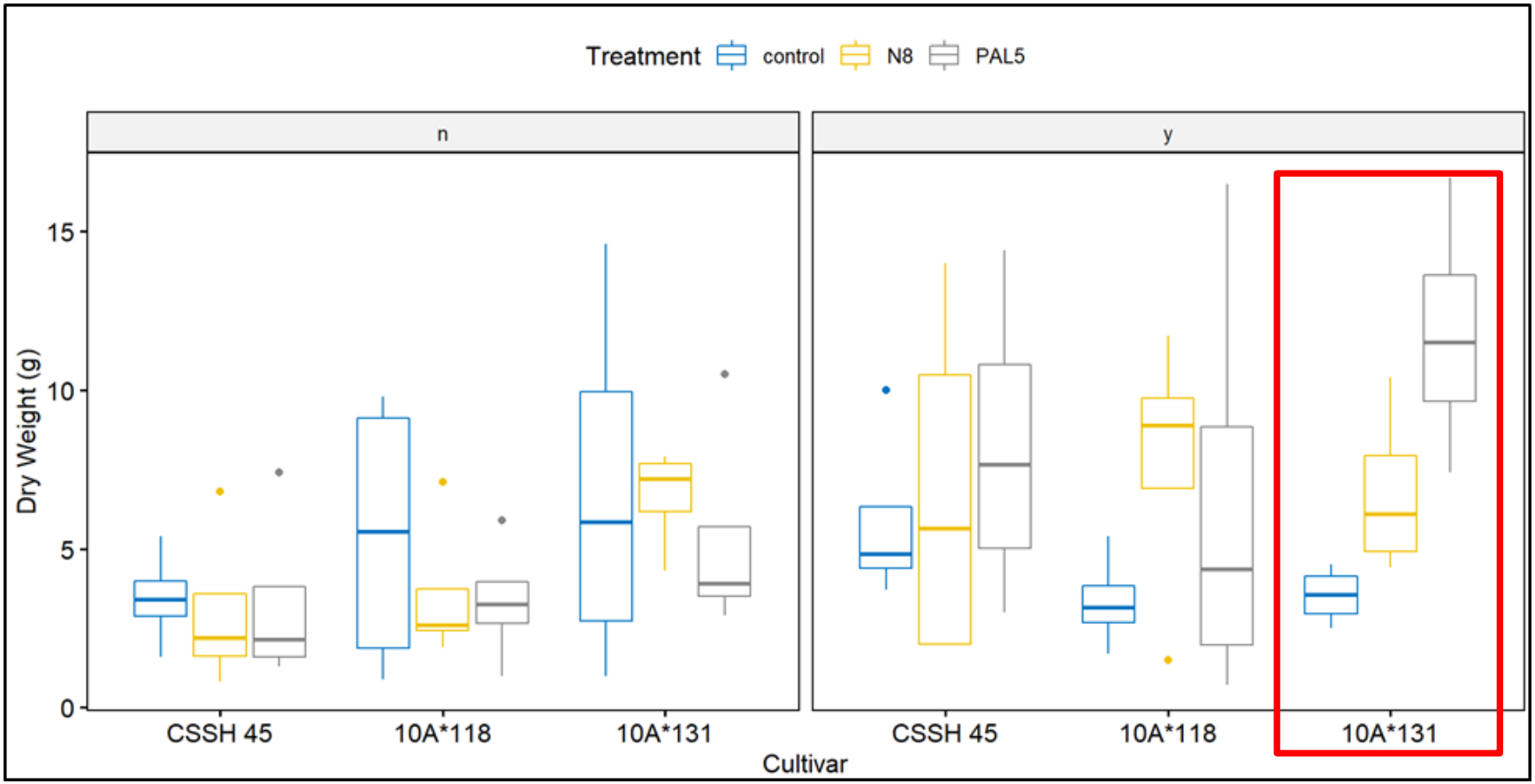
- ANOVA (α = 0.05) indicated a significant difference in mean shoot DW among treatments.
- **According to Tukey's Post Hoc test, the Control & PAL5T were significantly different at p= 0.0357 and Control & DS1 significantly different at p= 0.0766**
- No evidence of N_2 fixation in any treatments.

Field Experiment 2020: Effect of inoculation and N fertilizer treatments on shoot DW of three *S. bicolor* genotypes.

- Although there were no significant effects of inoculation on shoot DW, in treatments with no N fertilizer additions, microbial treatments increased shoot DW in a consistent pattern within each sorghum genotype (i.e. N8 > N7 > DS1 > Control).



Field Experiment 2021: Effect of inoculation and N fertilizer treatments on shoot DW of three *S. bicolor* genotypes



Relevance

- This research will determine the potential of producing an annual biomass crop on marginal lands with little or no N fertilizer. This may greatly improve the life-cycle greenhouse gas emissions associated with producing this crop.
- A sustainably-produced annual biomass crop such as sorghum on marginal lands could greatly increase the flexibility of agricultural producers to fit a biomass crop into their crop rotations.
- If one or more of the tested PGPR are found to effectively increase the growth of the sorghum, there may be opportunities to develop a commercial inoculant(s) for the marketplace.

Critical Success Factors

- While *G. diazotrophicus* PAL5 has indicated some enhancement of sorghum genotypes, our testing to date has not shown a clear and consistent effect of any of the PGPRs on sorghum yield.
- A clear and consistent effect of *G. diazotrophicus* PAL5 on sorghum yield would be necessary to pursue the development of a commercial inoculant for the crop.

Future Work

- During the growing season of 2022, we will again test the potential effects of *G. diazotrophicus* PAL5 and *A. brasilense* N8 on the growth of three genotypes of sorghum under field conditions at the AAFC Research Farm in Nappan, NS.

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Summary

- Greenhouse and field studies have indicated a potential enhancement of hybrid sorghum growth on marginal lands in Nova Scotia by inoculation with PGPR, but more research is necessary to determine the conditions under which there is a clear and consistent effect of these inoculants on sorghum growth.

With special thanks to . . .

- SMU: Dr. H. Fei, Research Associate; S. Thewage, MSc candidate; C. Dalzell, Research Associate; L. Laskey, Undergraduate student
- AAFC Nappan: M. Crouse, Plant Breeding Technician; S. Fromm, Agronomy Technician

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Part III: Plant Usable Nitrogen Species in Soil

Julia Lu

Department of Chemistry and Biology

Toronto Metropolitan University

May 19, 2022

BioMass Canada Webinar Series

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Acknowledgement

- Researchers: Zhongwei Shi, Tim Ho, David Lewis
- Dr. Vessey's group at SMU for collecting soil samples from the Nappan site

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Goal

The goals of this component of Project 7 are

to study the effect of *Gluconacetobacter* bacteria, plant, and fertilizer application on water soluble N-species in soil during the growing season

Project Overview

- Nitrogen is important for plant (sorghum) growth
- Plants get nitrogen from soil
- Plant-usable nitrogen species are water soluble

↓

This study analyzes water soluble nitrogen species in soil during the plant growth season

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Activity summary

Sample collection:

- London, ON
- Simcoe, ON
- Ottawa, ON
- Nappan, NS

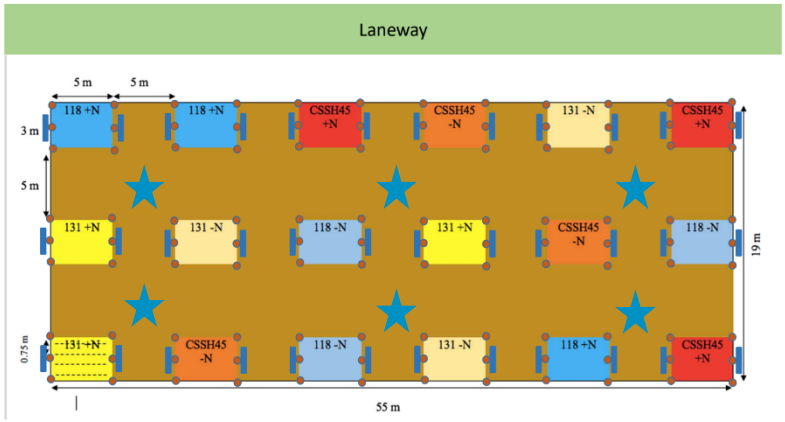
Background
After Planting (after N-application)
At Harvest

Experimental design:

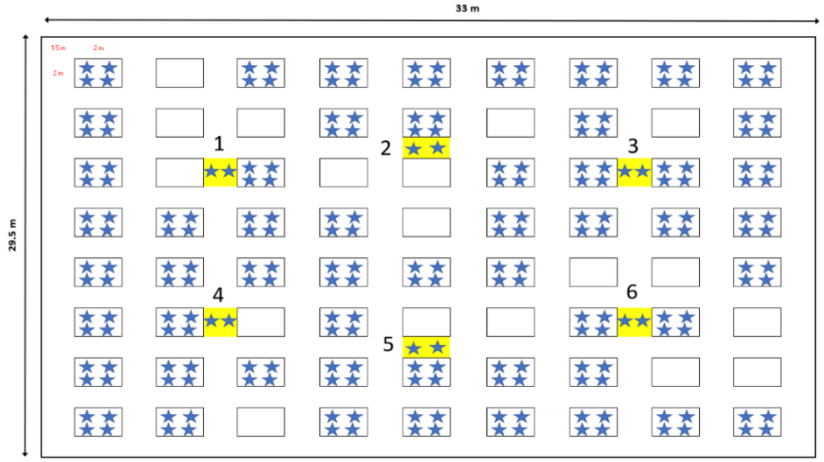
- Sorghum hybrids:CSSH45, 10AX118, 10AX131
- N-fertilizer application: 120 kg/ha
- *growth-promoting microbes*: N8, PAL5T

Sample collection

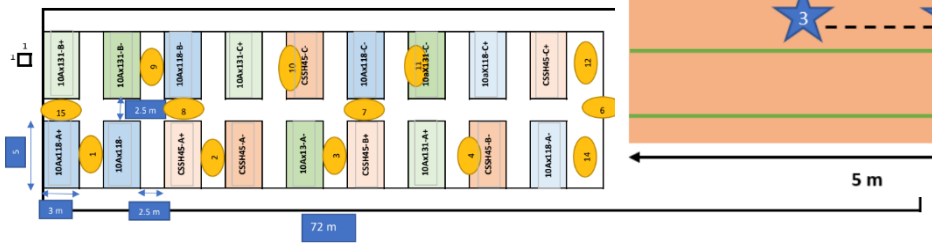
Simcoe & London plots - 2021

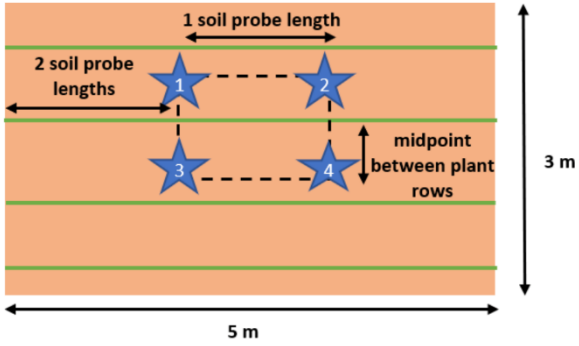


Nappan plots - 2021



Ottawa plots - 2021





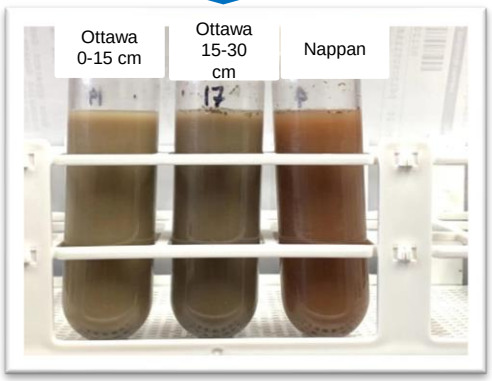
Sample Preparation

Homogenization:
- Pulverize & mix
- Sieving (2mm)

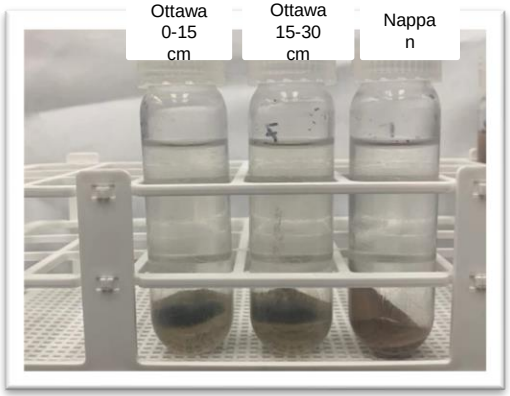
Heating & weighing

- Moisture
- Total Organic Matter

Extraction:
- EPA Method 1312
- water of pH 5.00 ± 0.05
- End-over-end mixing, 18 hrs



Separation by Centrifugation



Clear solution

Analysis of N-species

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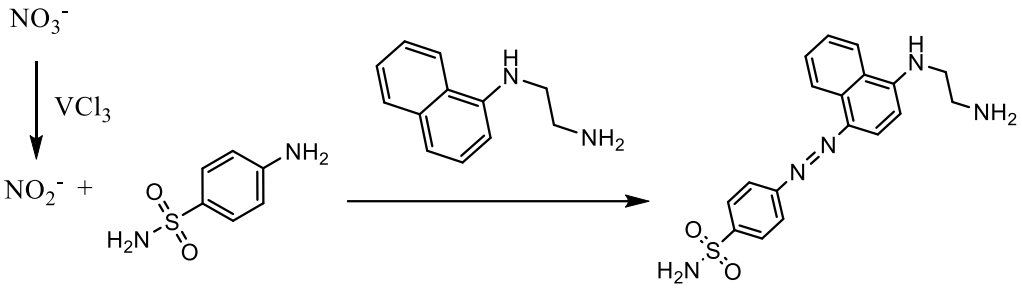
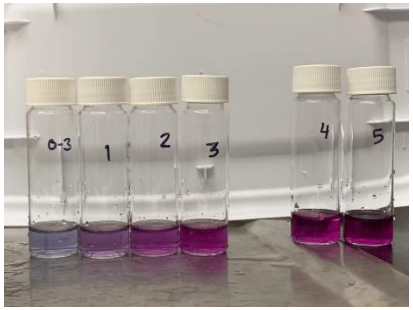
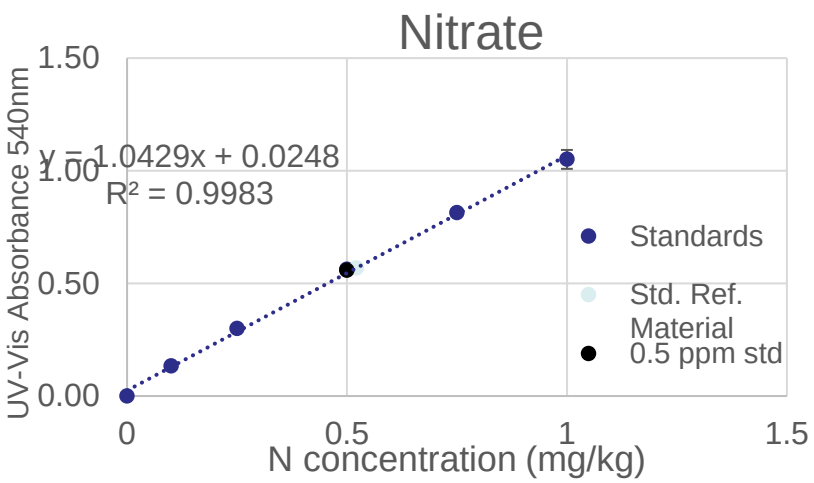
Colorimetry Analysis - NO_3^-/NO_2^-

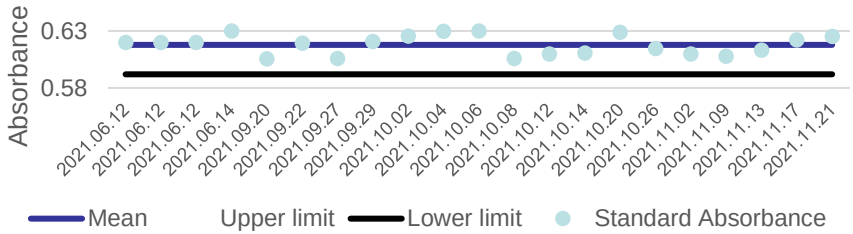


Spectrophotometric analysis at 540 nm

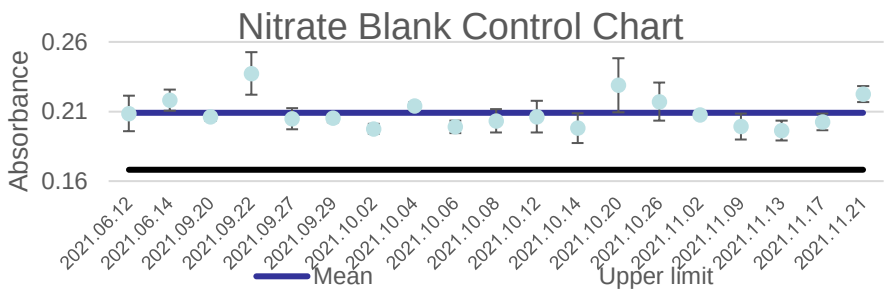




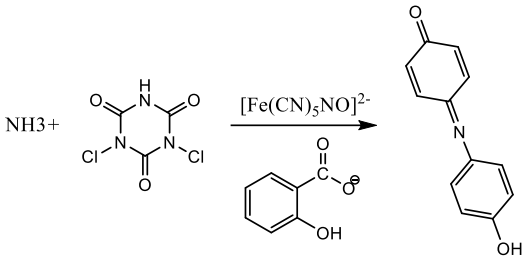
Nitrate Standard Control Chart



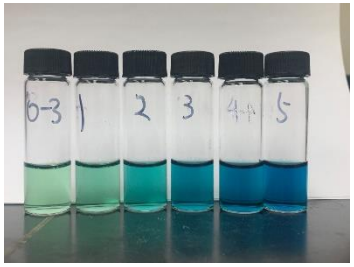
Range defined as Mean ± 3*Standard Deviation

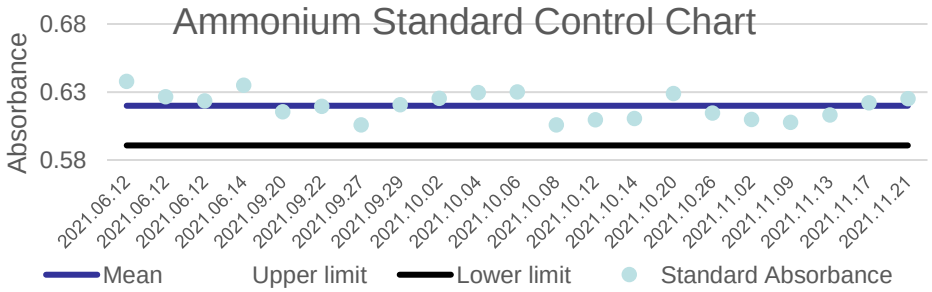


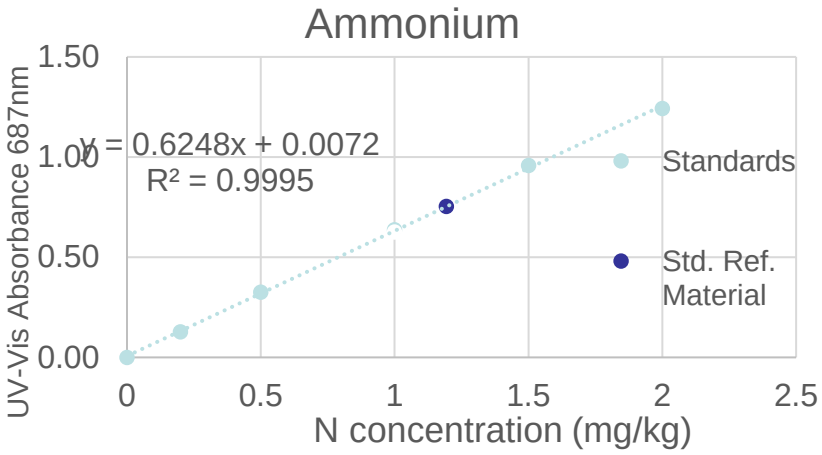
Colorimetry Analysis - NH_4^+

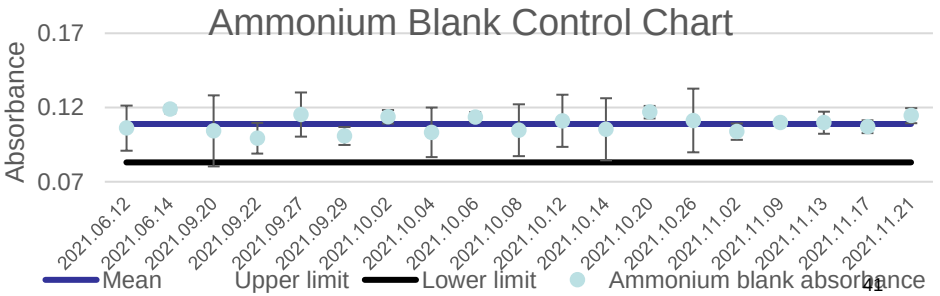


Spectrophotometric analysis at 687 nm





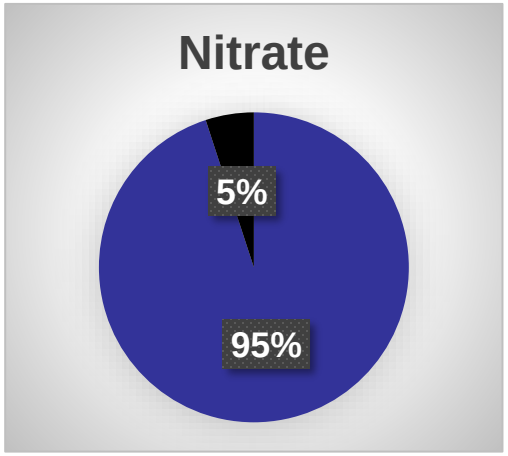


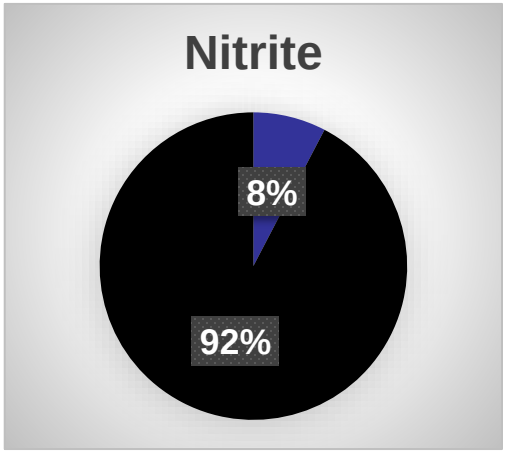


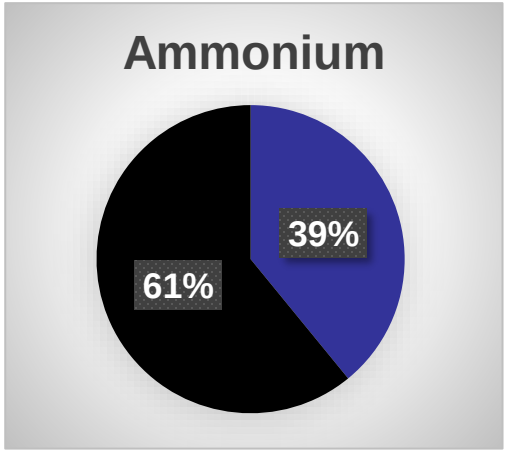
Results Preview

	Nitrate	Nitrite	Ammonium
LOD (mg/kg)	1.09	0.11	1.47
Sample Analyzed (n)	576	196	504

$$LOD = \frac{3 * Blank\ Std.Dev.}{Calibration\ Sensitivity}$$

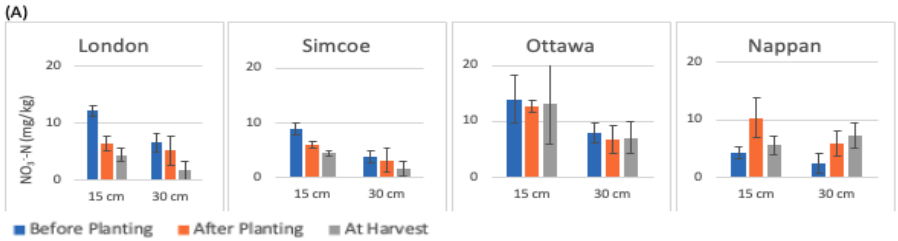






- **> LOD**
- **< LOD**

NO_3^--N] in the soils collected from the 4 sites in 2021



Background

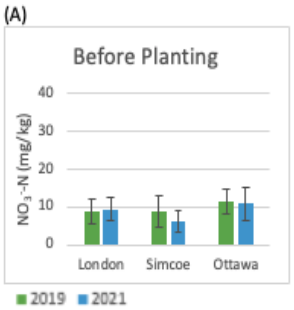
Statistical Results from ANOVA using R

	Soil depth	Hybrids	Fertilizer Application	Temporal Effect	Land Type	Bacteria Treatment
London	*	ns	*	*		N/A
Simcoe	*	*	ns	ns		N/A
Ottawa	*	*	*	*		N/A
Nappan	*	*	*	*	*	*

* denotes significance at p-value < 0.05.
ns denotes non-significance as p-value ≥ 0.05.
N/A denotes Not Applicable.

Comparisons of [NO_3^--N] in the soil samples between 2019 and 2021

N-fertilizer application rate: 2019: 45 kg/ha 2021: 120 kg/ha



Background

Results from t-test by Excel for the effect of fertilizer application rate on $[NO_3^-\text{-}N]_{soil}$ in 2019 (45 kg $ha^{-1)}$ and 2021 (120 kg ha^{-1})

	Before Planting	After Planting	At Harvest
London	ns	*	ns
Simcoe	ns	ns	ns
Ottawa	ns	*	ns

* denotes significance at p-value < 0.05.
ns denotes non-significance as p-value ≥ 0.05.

Summary

2021:

- Higher $[NO_3^-\text{-}N]_{soil}$ in top layer soil (0-15 cm)
- Significantly increased $[NO_3^-\text{-}N]_{soil}$ after N-Fertilizer application
- No significant effect on $[NO_3^-]_{soil}$ and $[NH_4^+]_{soil}$ by sorghum hybrid
- $[NO_3^-]_{soil+N8} > [NO_3^-]_{soil+PAL5T}$ with 10AX118 after N-fertilizer application

2019 vs. 2021

- Rate of N-fertilizer application has no significant effect on $[NO_3^-]_{soil}$

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5. Future Work

- Repeating the experiments and confirm 2021 research results
- Test soil nitrogen species and biomass production of new sorghum hybrid(s)
- Information exchange in forms of presentation and publication

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