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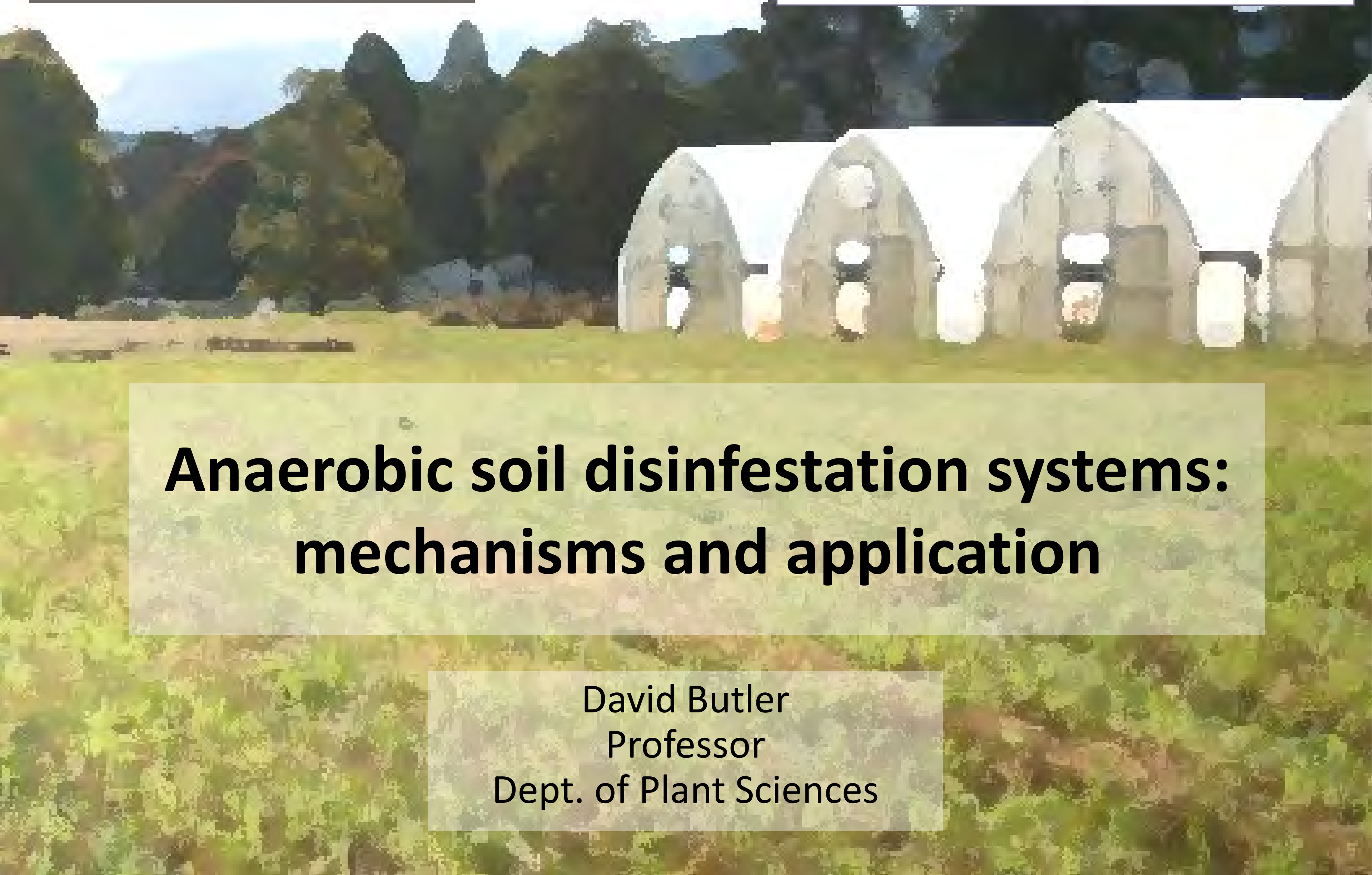

Anaerobic soil disinfestation systems: mechanisms and application

David Butler
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Outline

- Soil disinfestation background
- Amendment effects
- VFA/reduced metal effects
- Biocontrol effects
- Crop rotation effects
- Implementation

(photo: U. Shrestha)

Non-fumigant alternatives

- Generally rely on system changes/practices to alter soil chemical, physical & biological properties directly and indirectly
  - Crop rotation and/or cover crop use
  - Soil heating (solarization, steam)
  - Organic amendments (anaerobic/biological soil disinfestation, brassica biofumigation, biosolarization)
  - Biological controls

Solarization only

Solarization, ASD 6wks

(photos: Shennan et al., 2011; U. Shrestha)

Non-fumigant systems

- Many advantages, but require more research & development
- Anaerobic soil disinfestation (ASD) or anaerobically-mediated biological soil disinfestation
  1. Incorporate easily-decomposable organic material
  2. Tarp with polyethylene mulch
  3. Irrigate to saturation of topsoil

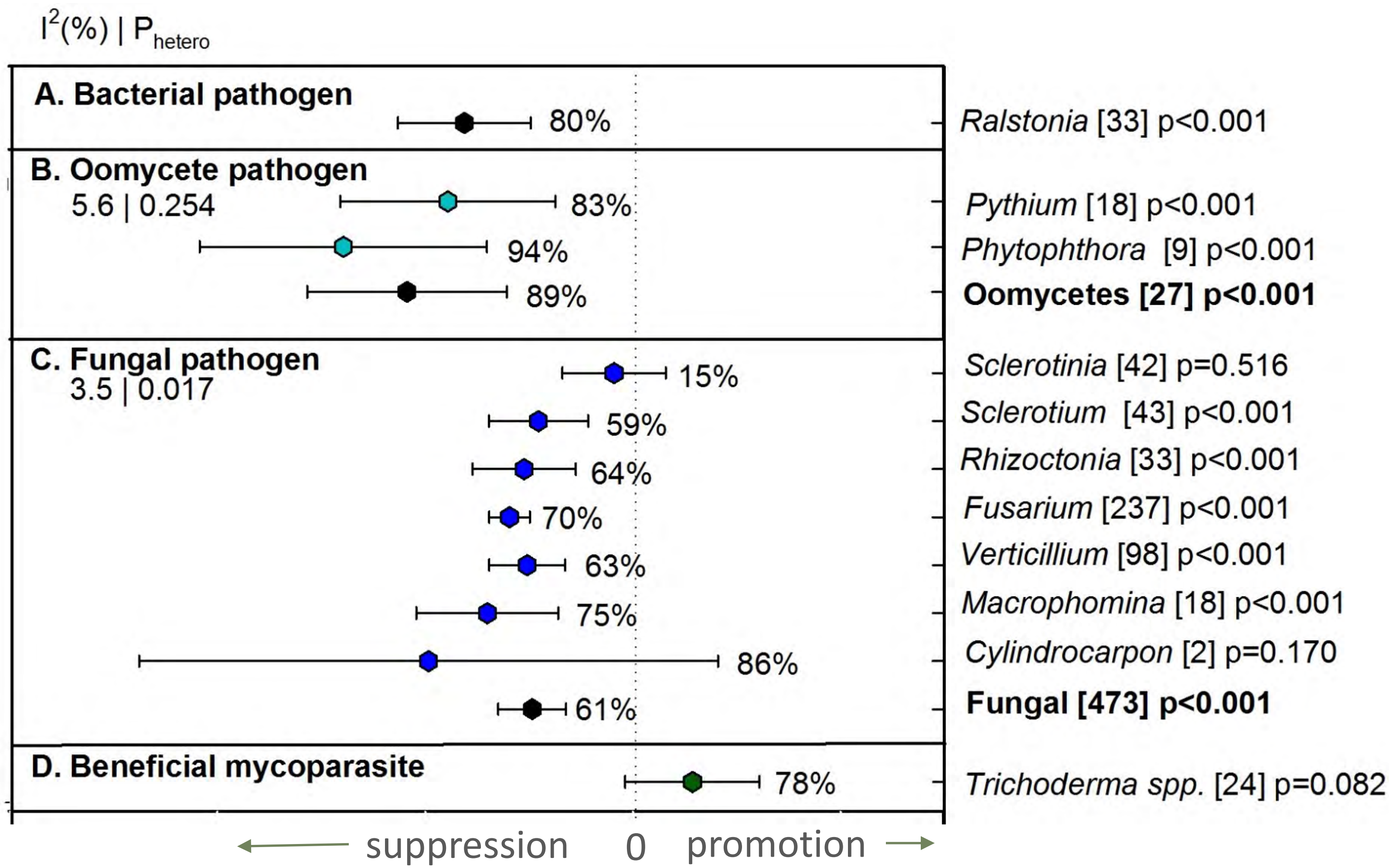
(Butler et al., *Plant & Soil*, 2012)

ASD treatment mechanisms

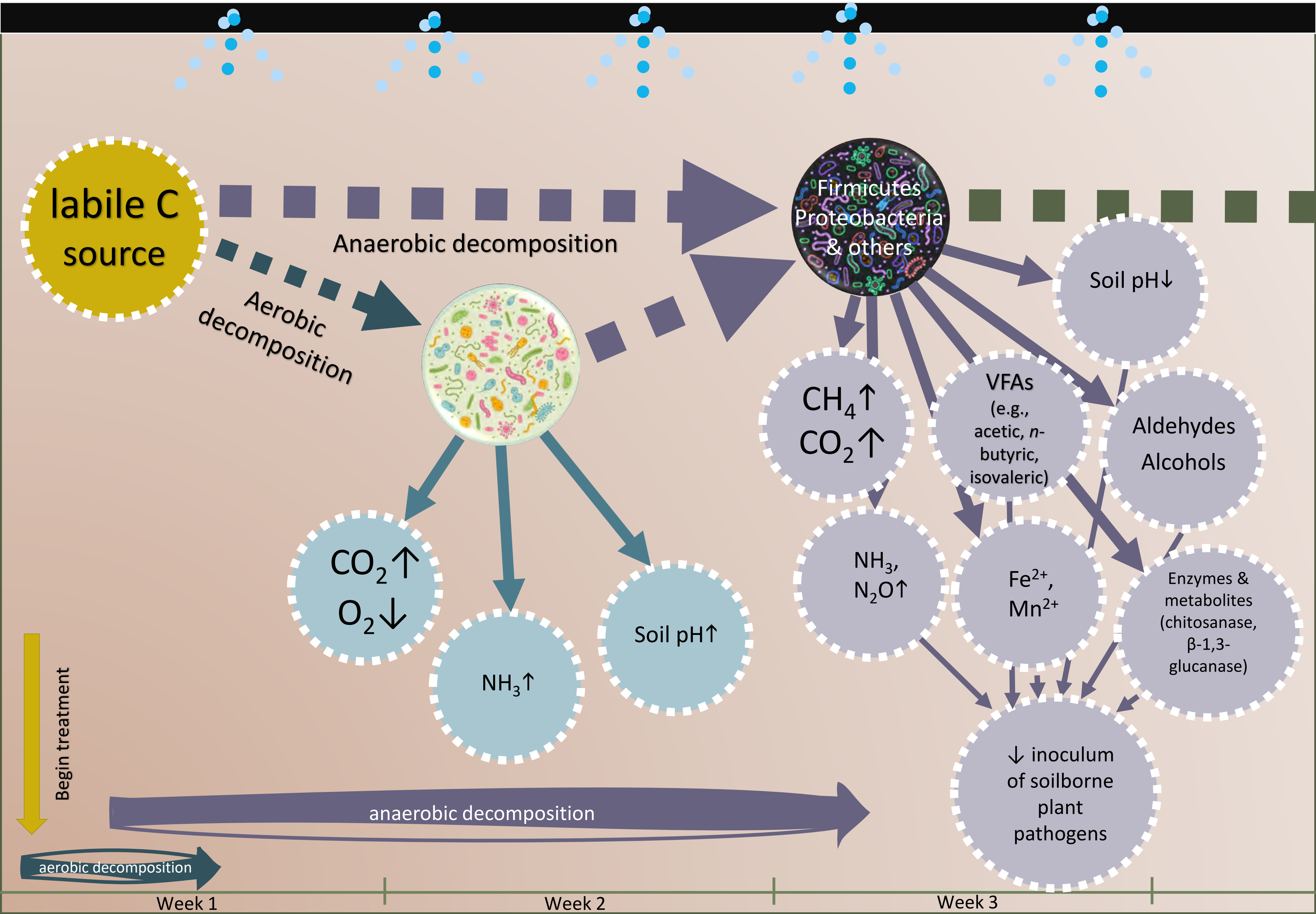
- Microbial growth and respiration creates anaerobic conditions
- By-products of anaerobic decomposition accumulate and have activity against soilborne pathogens
- Creates environment favorable for many soil biocontrol microbes during and post treatment (e.g., *Trichoderma* spp.)
- Multiple impacts on soil chemical, physical and biological properties

(Butler et al., *Plant & Soil*, 2012; Shrestha et al., *Phytopathology*, 2018; Momma et al., 2006, *J. Gen. Pathol*.)

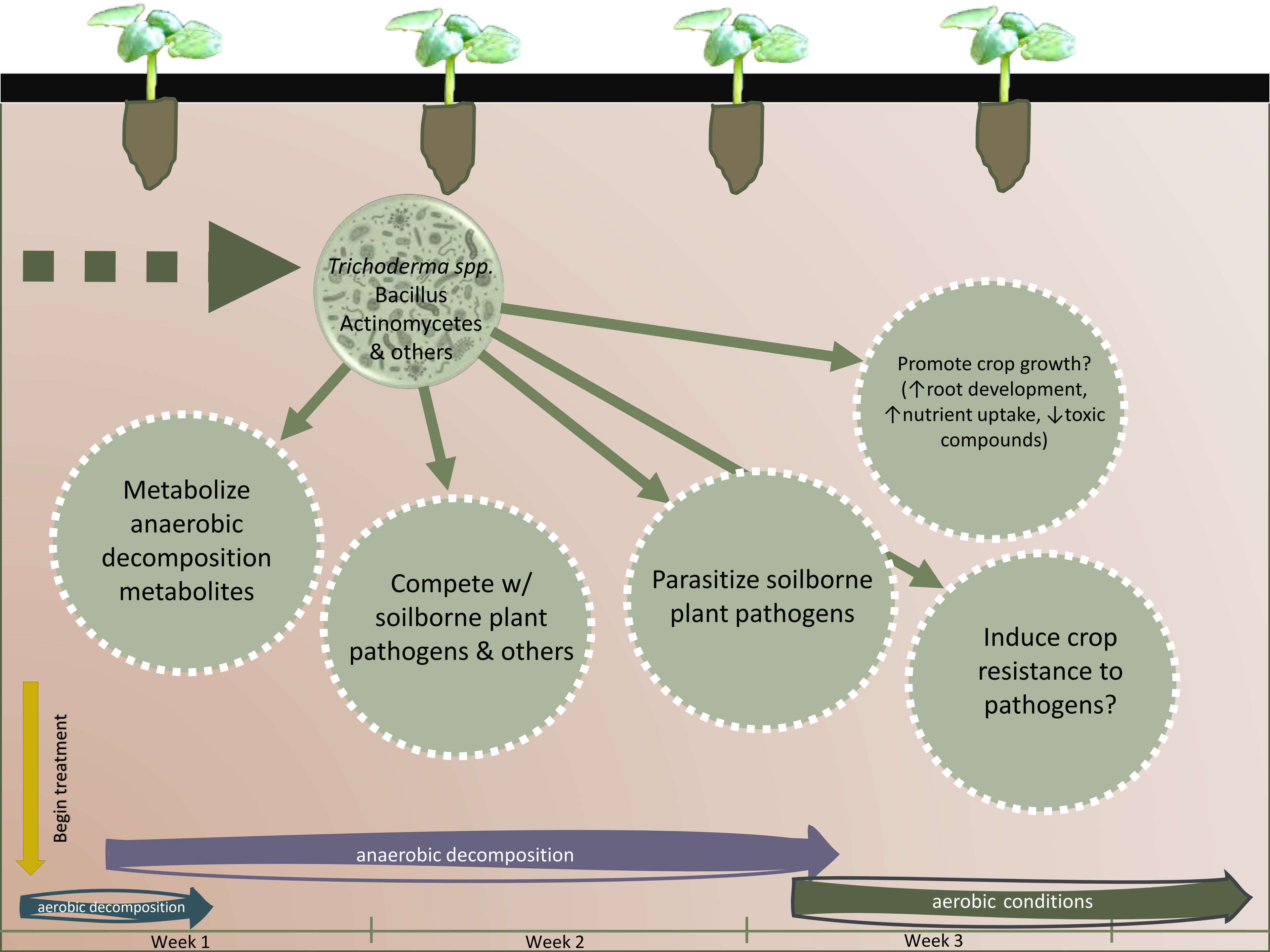
Meta-analysis: pathogen effect sizes



(Shrestha et al., *Front. Plant Sci.*, 2016)



labile C source
Anaerobic decomposition
Aerobic decomposition
Firmicutes Proteobacteria & others
Soil pH↓
CH_4↑ CO_2↑
VFAs (e.g., acetic, *n*-butyric, isovaleric)
Aldehydes Alcohols
CO_2↑ O_2↓
NH_3↑
Soil pH↑
NH_3, N_2O↑
Fe^{2+}, Mn^{2+}
Enzymes & metabolites (chitosanase, β-1,3-glucanase)
↓ inoculum of soilborne plant pathogens
Begin treatment
anaerobic decomposition
aerobic decomposition
Week 1
Week 2
Week 3



Trichoderma spp.
Bacillus
Actinomycetes
& others
Metabolize anaerobic decomposition metabolites
Compete w/ soilborne plant pathogens & others
Parasitize soilborne plant pathogens
Promote crop growth? (↑root development, ↑nutrient uptake, ↓toxic compounds)
Induce crop resistance to pathogens?
Begin treatment
anaerobic decomposition
aerobic decomposition
aerobic conditions
Week 1
Week 2
Week 3

Background

- Fermentation biochemistry has been well-described in industrial fermentation research
- Fermentation in soils inherently more complex
  - Environmental conditions (e.g., pH, temperature, moisture, nutrients) are variable in space and time
  - Soil colloids affect chemical properties of soil solution (e.g., pH, nutrients, VFA concentration, etc.)
  - Diverse indigenous microbial community, that changes with C input and resulting metabolite production/environmental conditions in space & time
  - Available soil amendments have variable chemical and physical properties
  - Post-treatment soil effects not well-described for crop production

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Background

- ASD treatment efficacy is affected by type and rate of amendment
  - Direct impacts on soil microbial activity or activity of potential mycoparasites such as *Trichoderma* spp.
  - Direct and indirect effects on formation of anaerobic decomposition by-products (e.g., rice straw → acetic acid and phenols, green manure → isovaleric acid and alcohols, and ethanol → acetic acid)

(Hewavitharana et al., 2014; Huang et al., 2016; Fernando et al., 2005; Kai et al., 2009; Tsutsuki & Ponnamperuma, 1987; Shrestha et al., 2016)

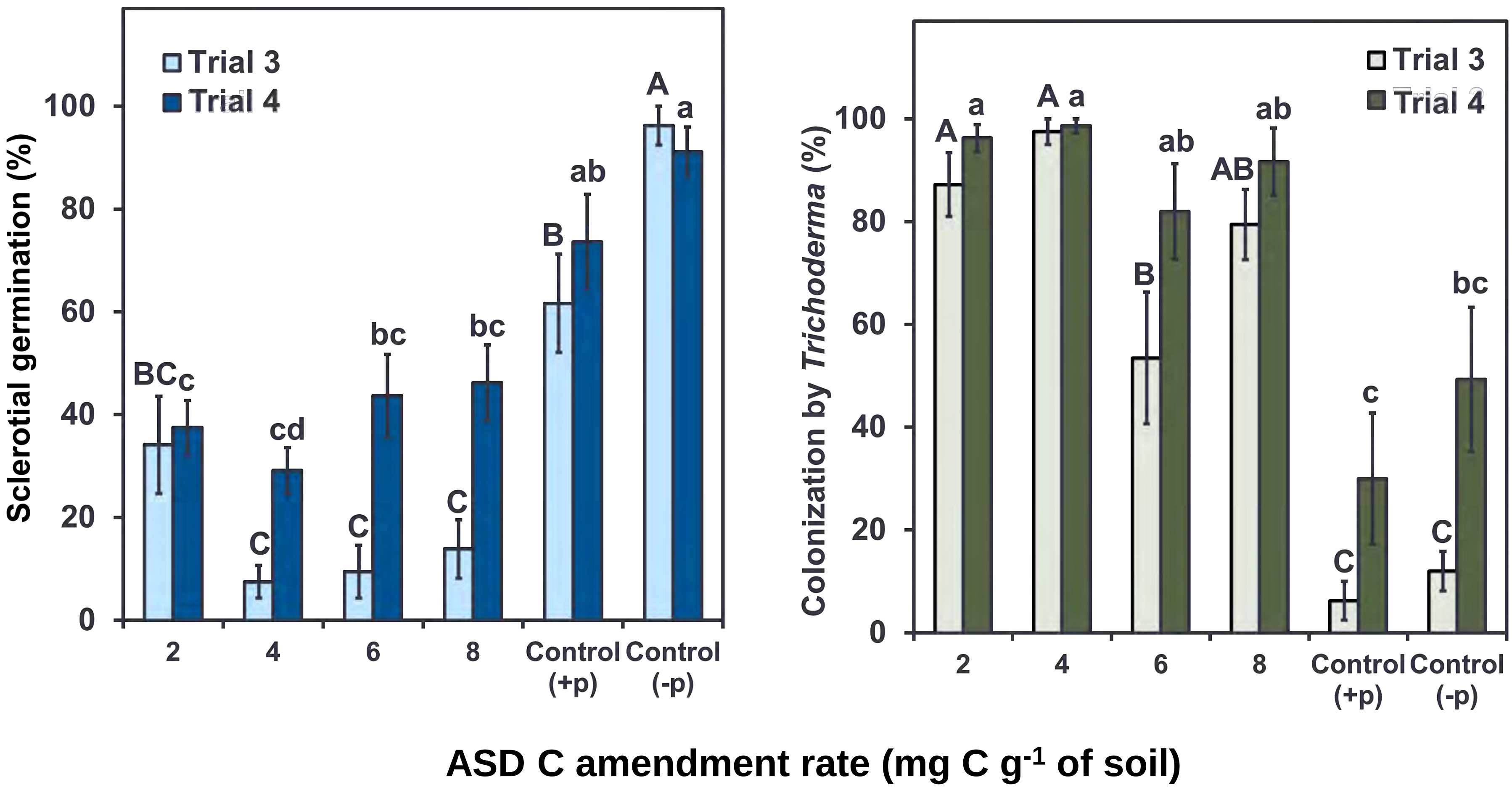
Methods: C rates and C:N ratios

- C rates, pot studies (C source: dry molasses)
  - ASD C rates: 2, 4, 6 & 8 mg C g^{-1} soil
  - Controls (0 mg C g^{-1} soil)
    - (A) Irrigated, polyethylene-mulched (anaerobic control)
    - (B) Non-irrigated, bare (aerobic control)
- C:N ratio, pot and field studies
  - ASD (C-source mixtures)
    - 10:1 C:N ratio, 4 mg C g^{-1} soil
    - 20:1 C:N ratio, 4 mg C g^{-1} soil
    - 30:1 C:N ratio, 4 mg C g^{-1} soil
    - 40:1 C:N ratio, 4 mg C g^{-1} soil
    - 30:1 C:N ratio, 2 mg C g^{-1} soil (field only)
  - Control (irrigated, polyethylene-mulched)
  - Fumigant (MeBr/chloropicrin, field only)
- Pests: *S. rolfsii*, *F. oxysporum*, yellow nutsedge

(Shrestha et al., *Phytopathology,* 2018)

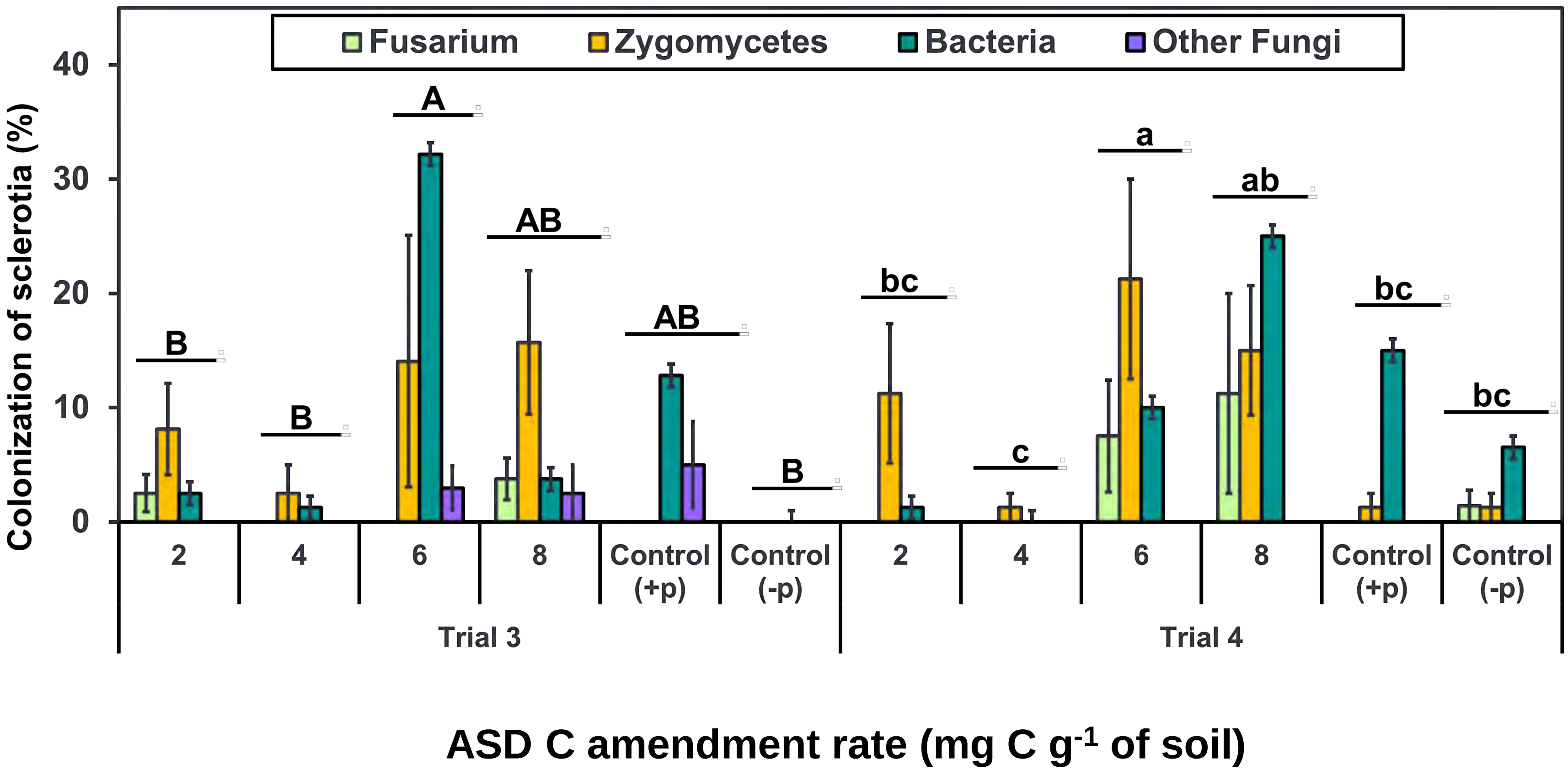
(Photos: U. Shrestha, J. Leduc)

C rate effects



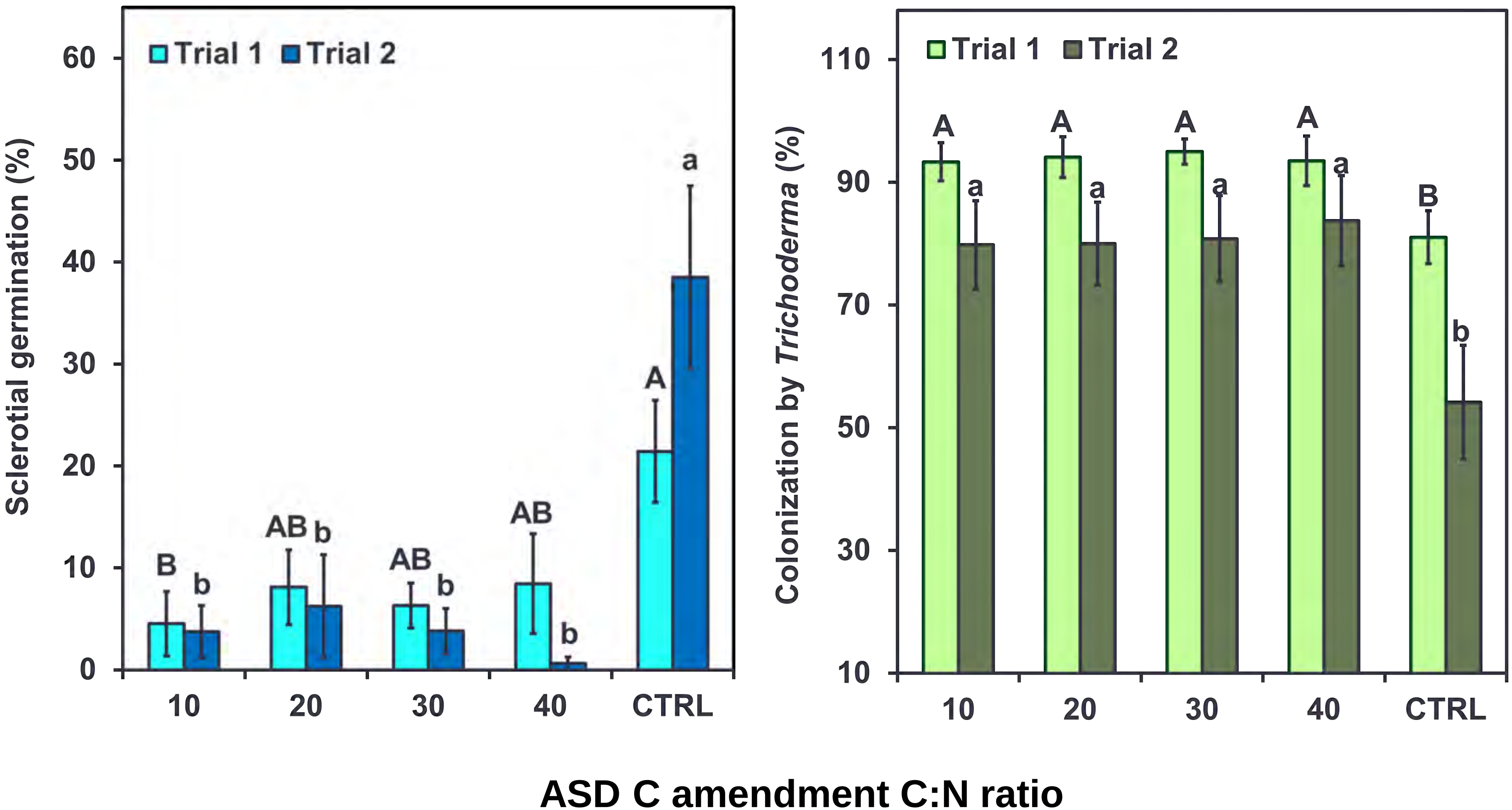
(Shrestha et al., *Phytopathology,* 2018)

C rate effects



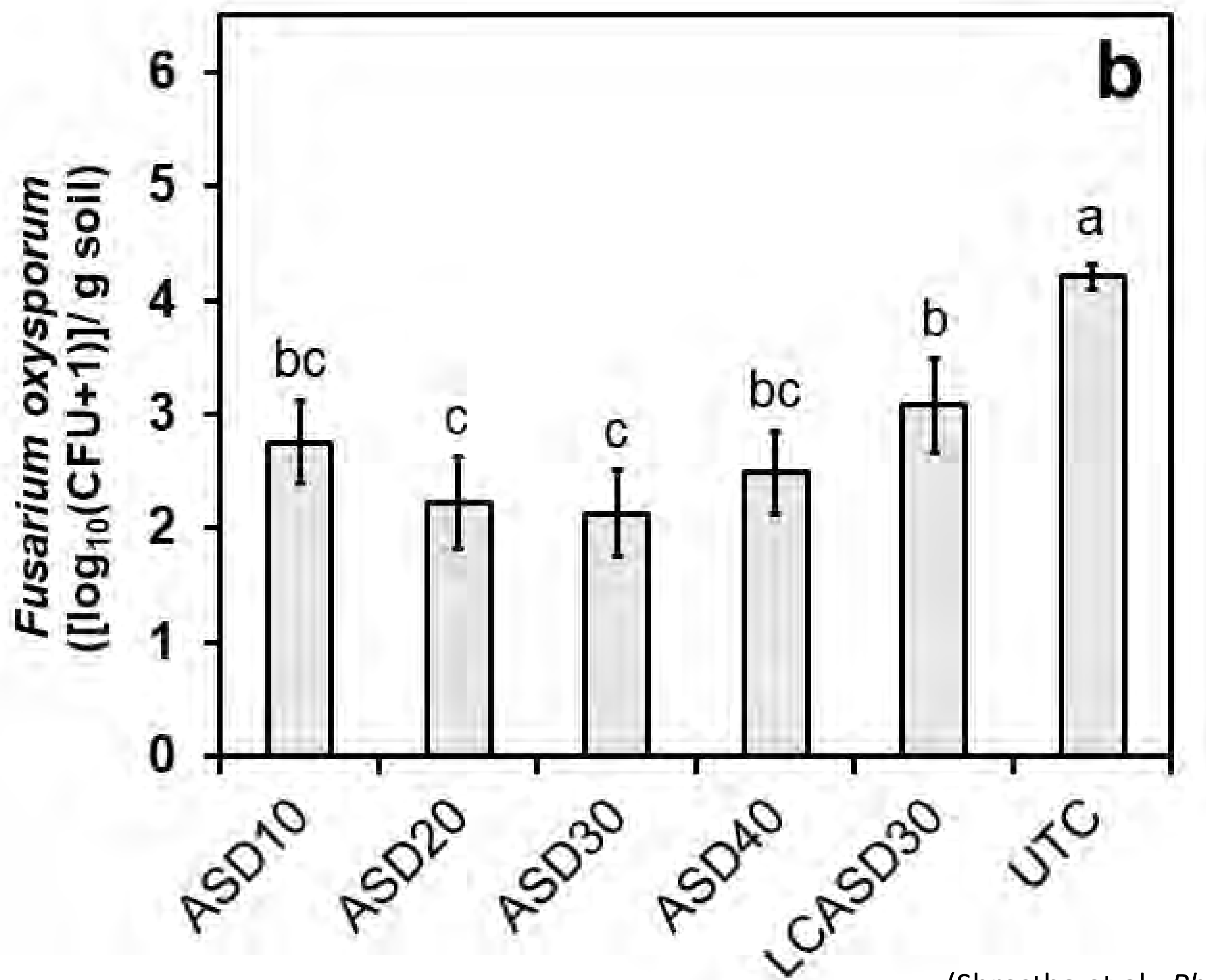
(Shrestha et al., *Phytopathology,* 2018)

C:N ratio effects



(Shrestha et al., *Phytopathology,* 2018)

Amendment C:N ratio, field studies



(Shrestha et al., *Phytopathology,* 2021)

Temperature and C source effects

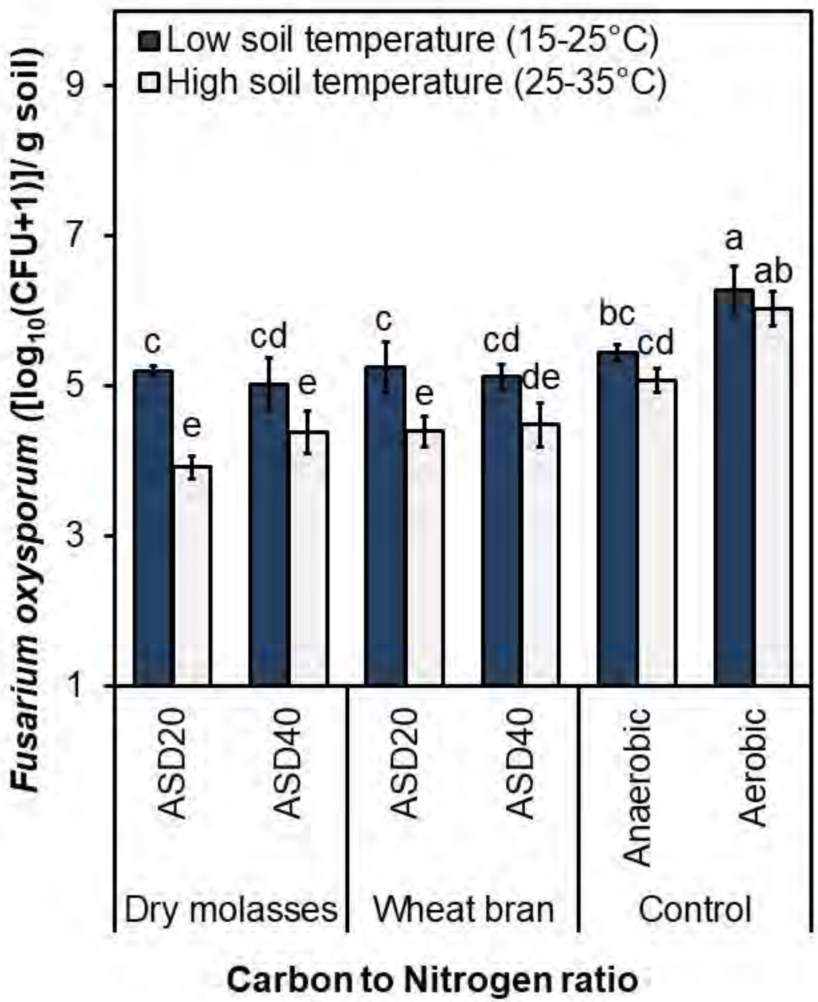
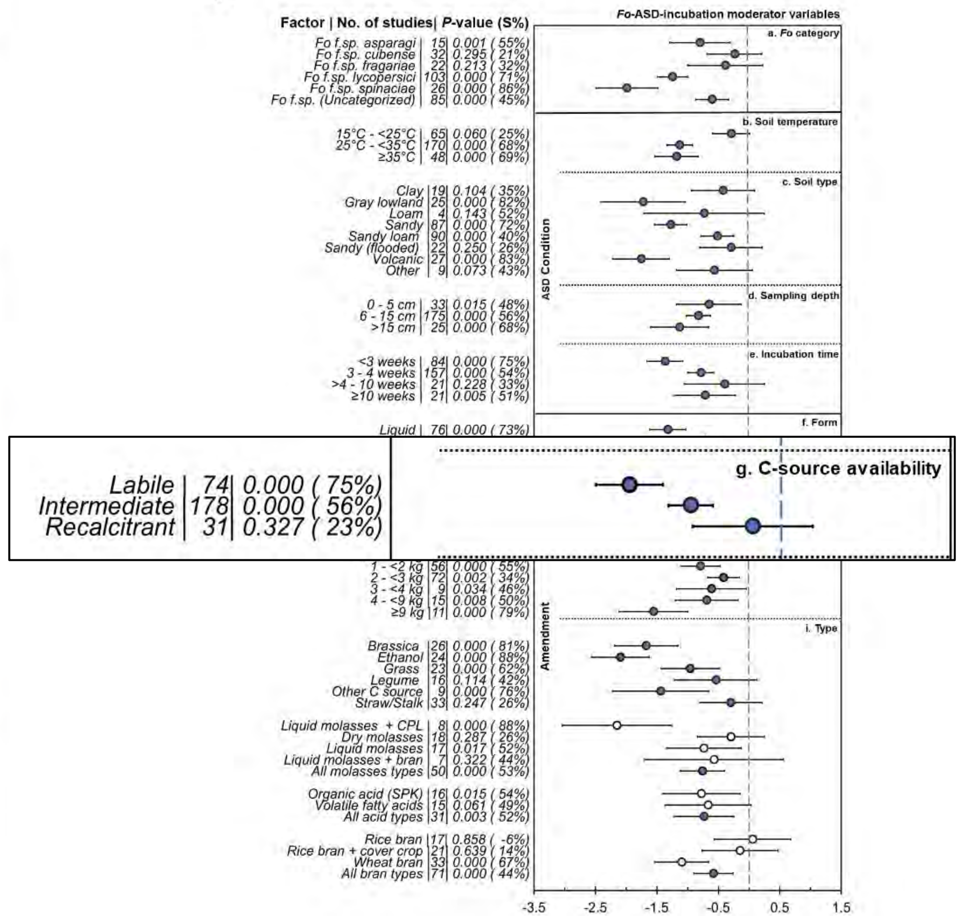
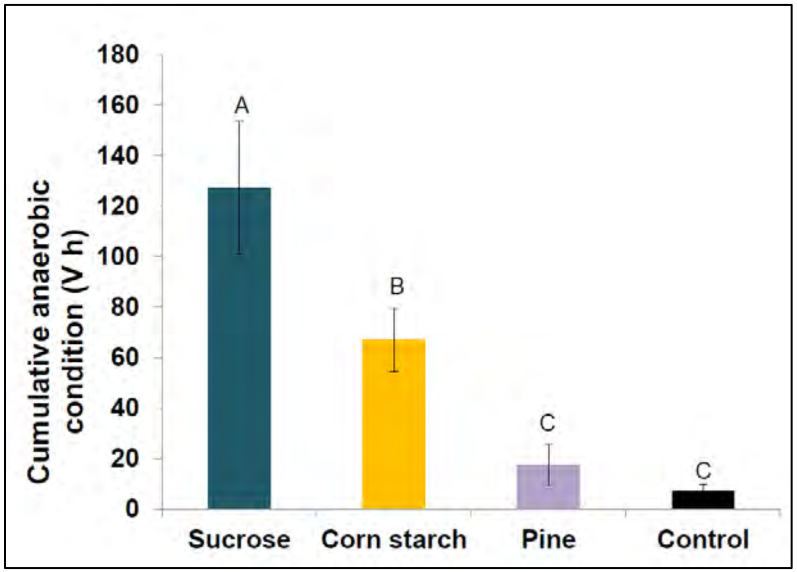


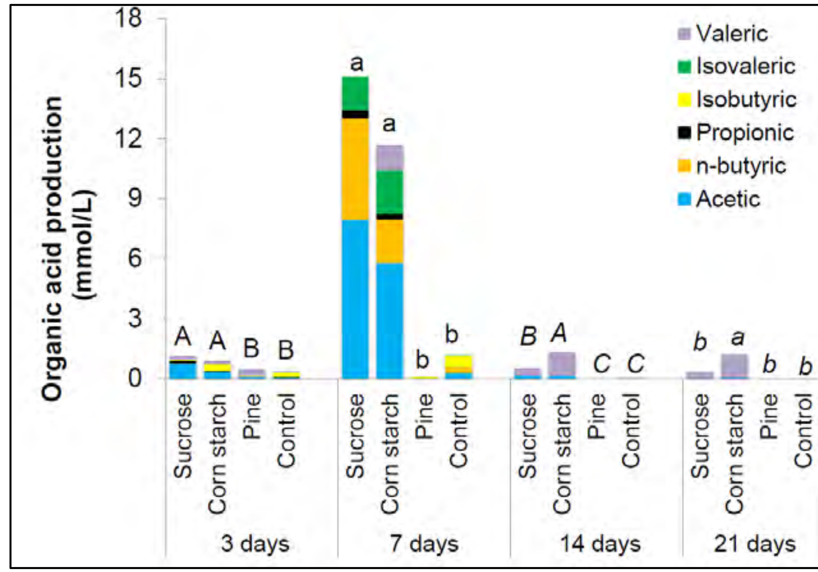
Fig. 2

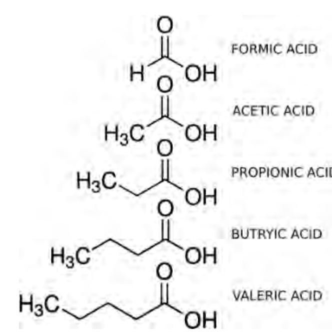


(Shrestha et al., *Phytopathology,* 2021)

C source effects







(Shrestha et al., *Acta Horticulturae,* 2020; VFA structure image from Krzyzowski et al., 2019)

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(Photo: U. Shrestha)

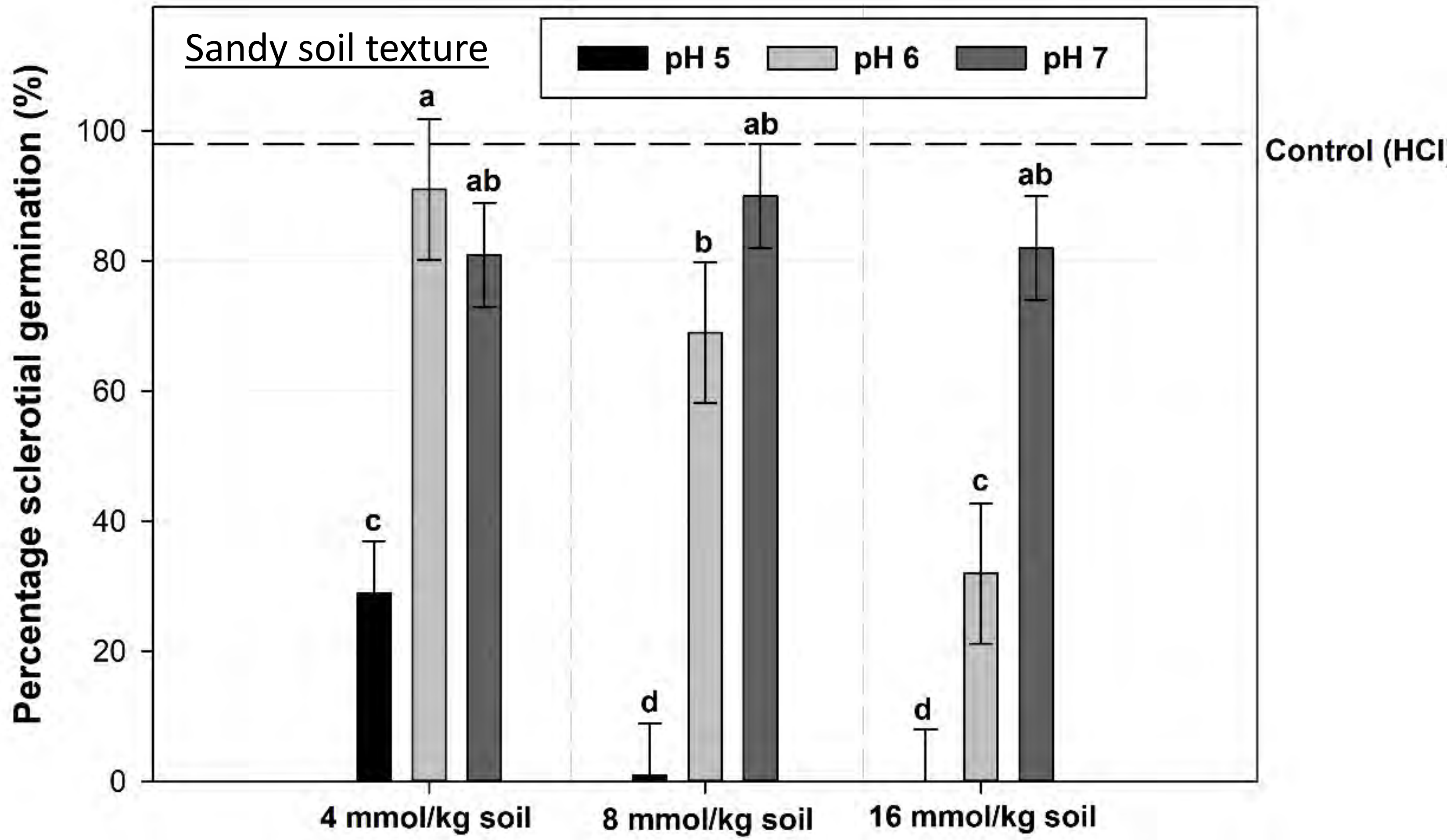
VFAs and *S. rolfsii* germination

- Soil solutions
  - VFAs (acetic, n-butyric, etc.)
  - Inorganic acid control (HCl)
  - Sterile water control
- Concentrations
  - 4, 8, and 16 mmol VFA/kg dry soil
- Soil pH
  - 4, 5, 6
- Soil texture
  - Sandy, sandy loam, silt loam

(Swilling et al., 2021, *Eur. J. Plant Pathology;* photos: K. Swilling)

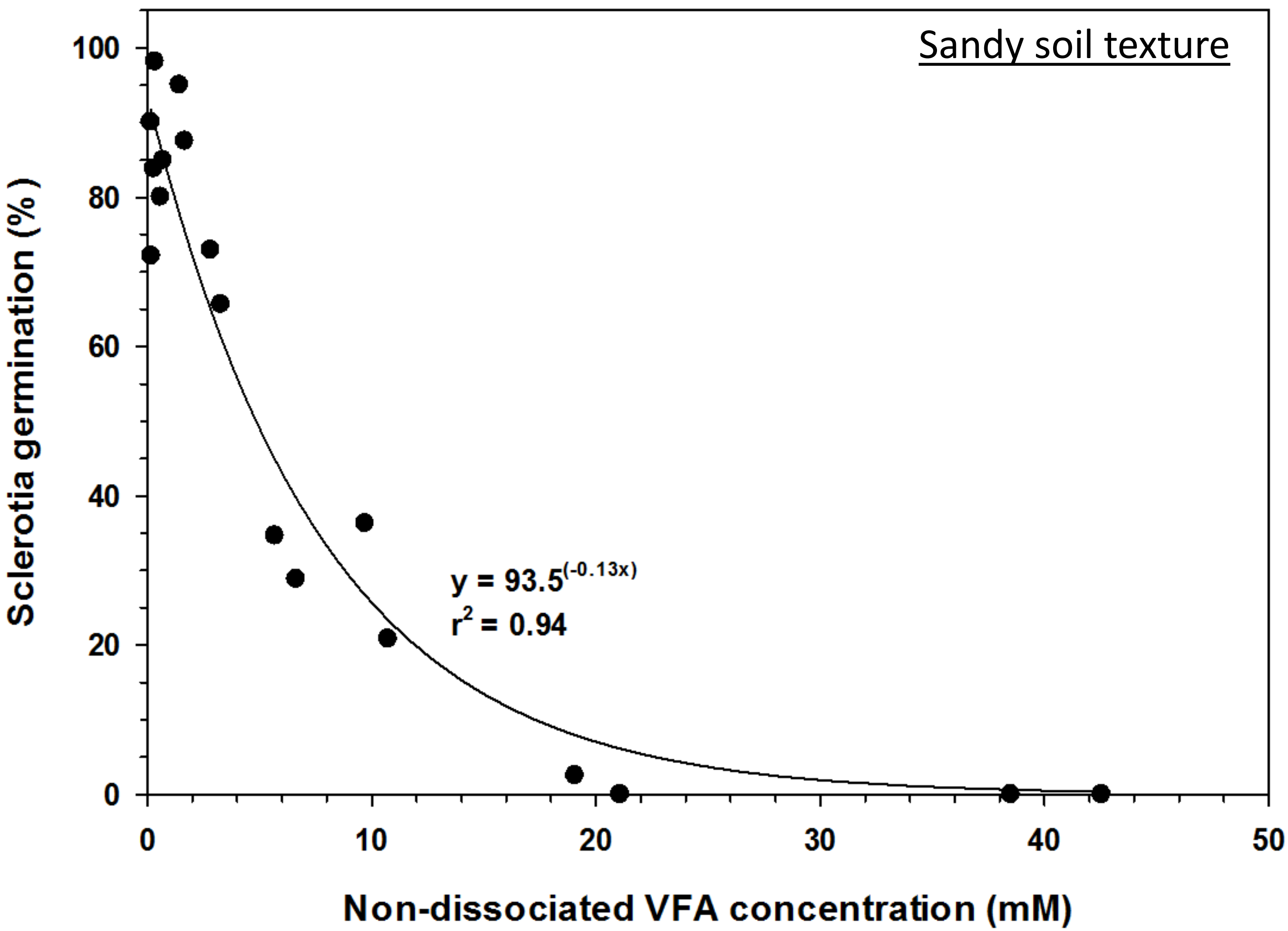
VFAs and *S. rolfsii* germination

Acetic & *n*-butyric



(Swilling et al., 2021, *Eur. J. Plant Pathology*)

VFAs and *S. rolfsii* germination



(Swilling et al., 2021, *Eur. J. Plant Pathology*)

VFAs and *S. rolfsii* germination

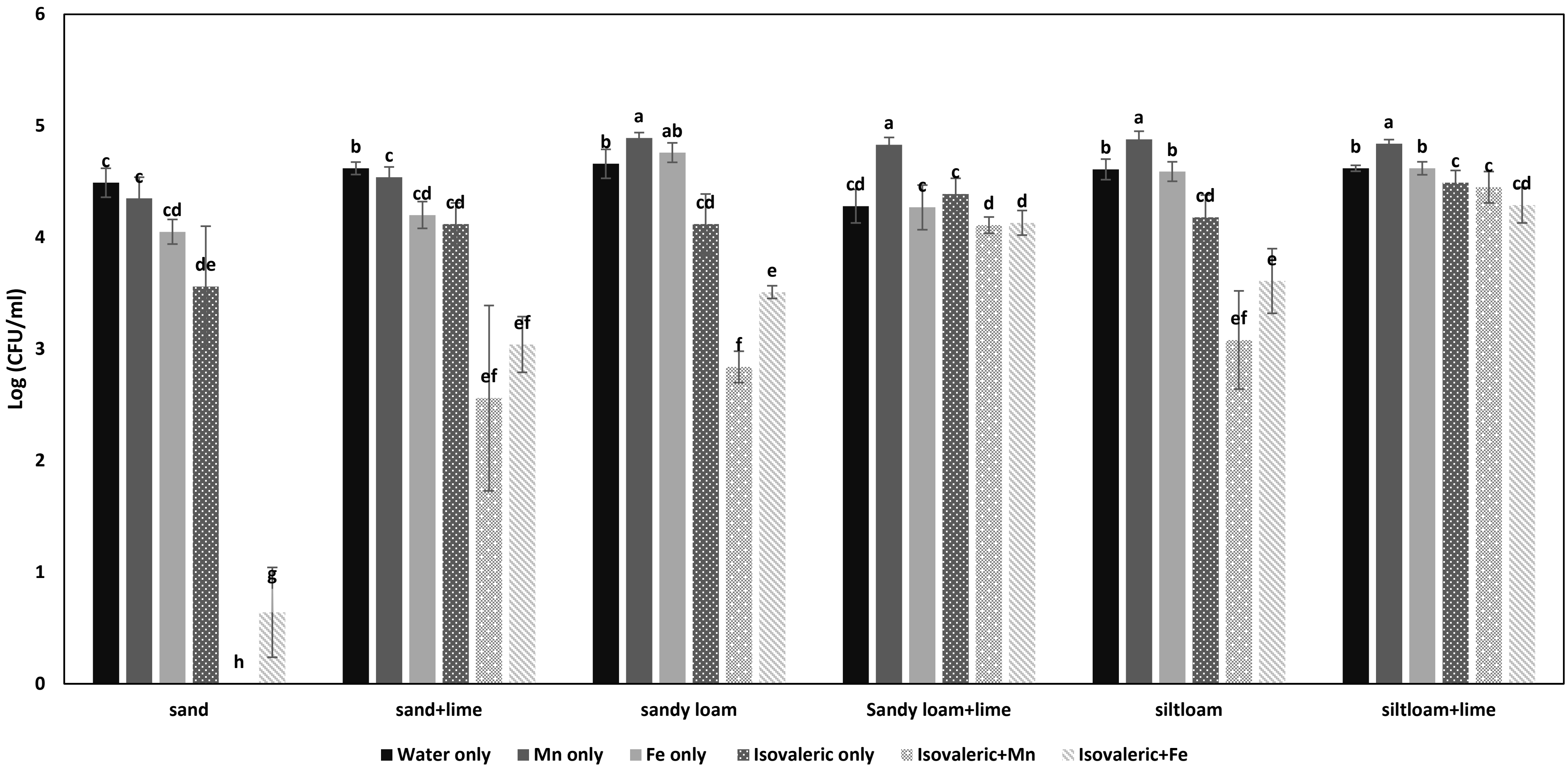
	Sandy, pH 5.0	Sandy loam, pH 5.0	Sandy, pH 6.0	Sandy loam, pH 6.0
	--------------*S. rolfsii* germination (%)--------------			
Acetic acid				
16 mmol/kg soil	0 h*	0 h	0 h	28 f
4 mmol/kg soil	1.3 gh	55 e	19 fg	80 bc
n-butyric acid				
16 mmol/kg soil	0 h	0 h	0 h	2.5 gh
4 mmol/kg soil	0 h	63 cde	0 h	61 de
Control**				
HCl (16 mmol/kg soil)	100 a	100 a	N/A	N/A

*Differences between means according to Tukey's HSD, $P < 0.05$

**Unbuffered water controls, 81% (ab; sand) and 89% (a; soil)

(Swilling et al., 2021, *Eur. J. Plant Pathology*)

VFAs, Fe, Mn and *Fusarium* suppression



(Littrell et al., 2022, *in preparation*)

Outline

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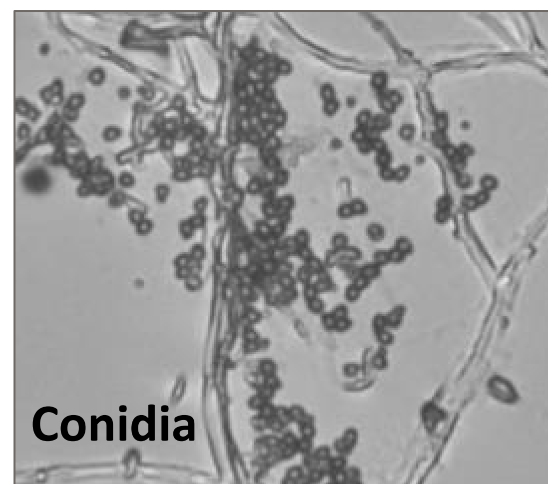
(Photo: U. Shrestha)

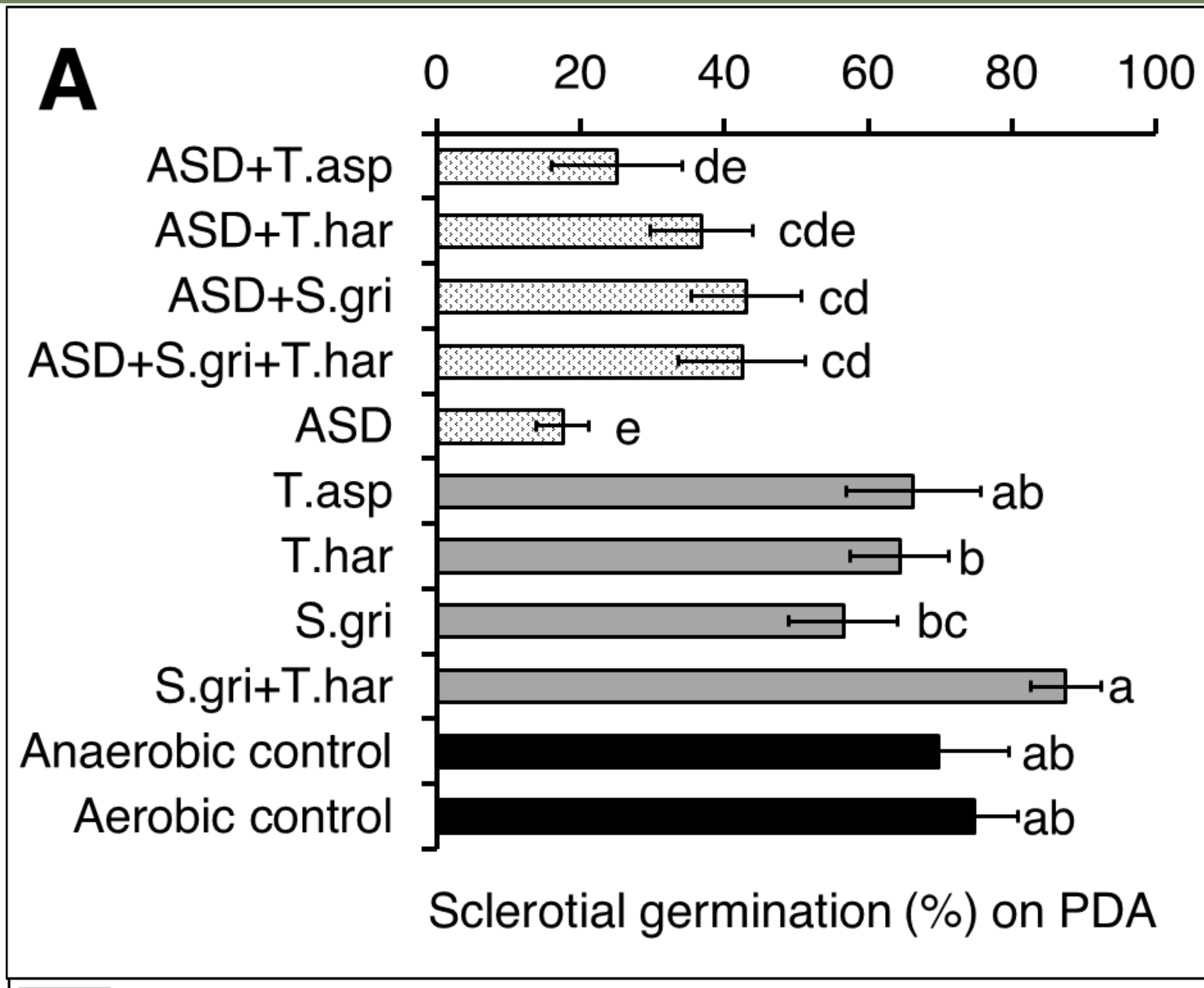
Methods

- Treatments
  - ASD with/without antagonists <u>at time of ASD treatment</u>
    - *T. asperellum* (field isolate)
    - *T. harzianum* (RootShield®)
    - *Streptomyces griseoviridis* (Mycostop®)
    - *T. harzianum + S.griseoviridis*
  - Antagonists only (aerobic)
  - Anaerobic control
  - Aerobic control



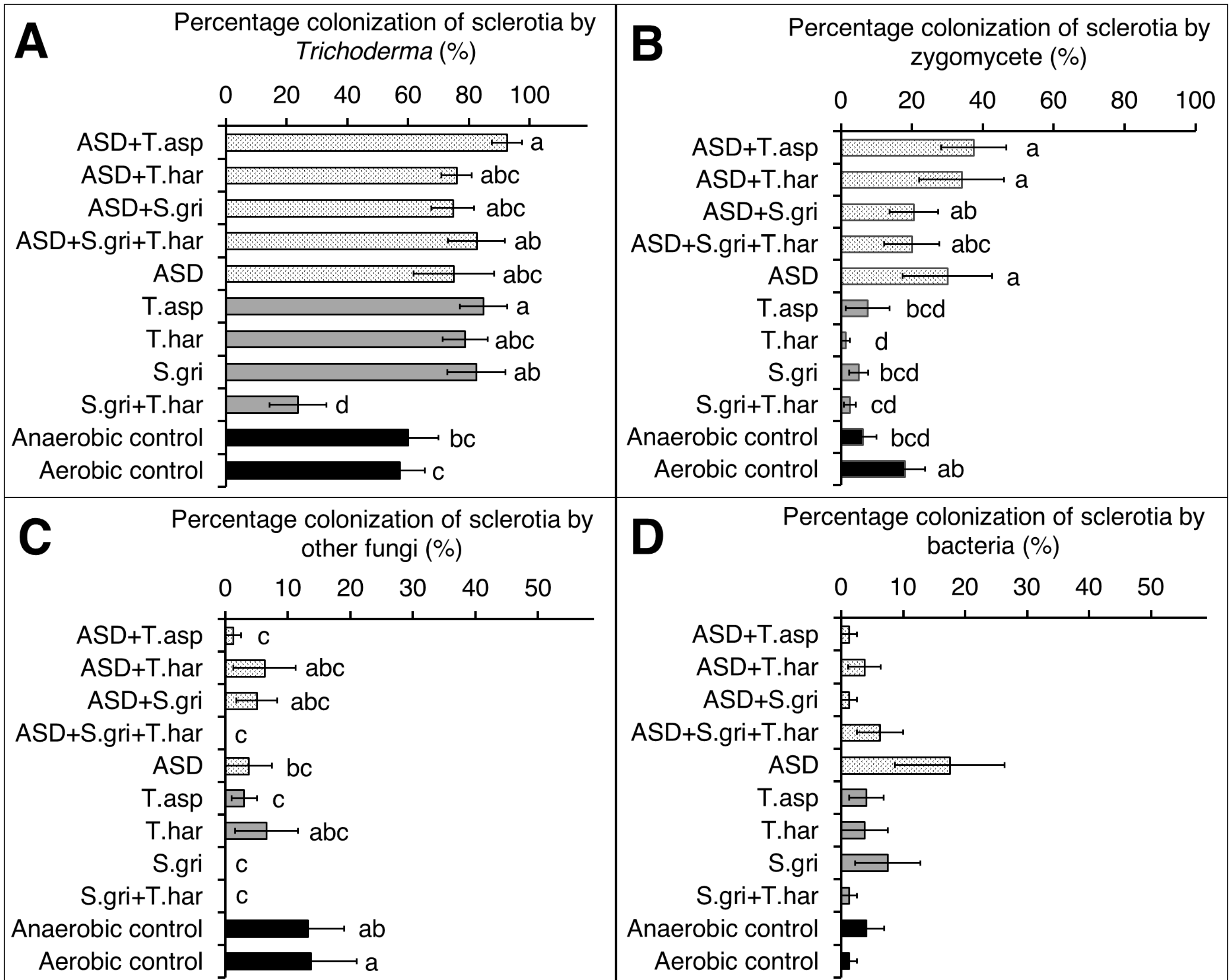
(Photos: U. Shrestha)

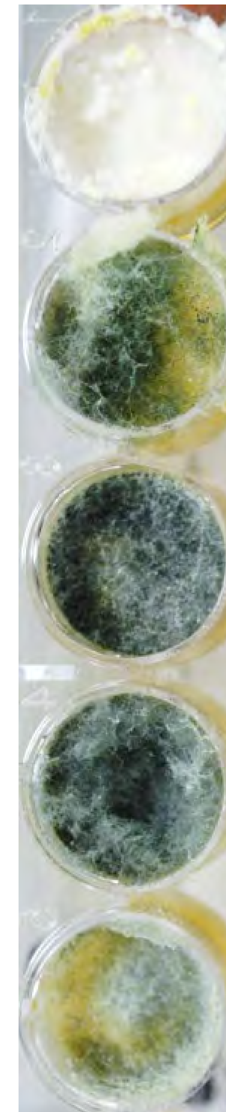
S. rolfsii germination/colonization



(Shrestha et al., *Applied Soil Ecology,* 2020)

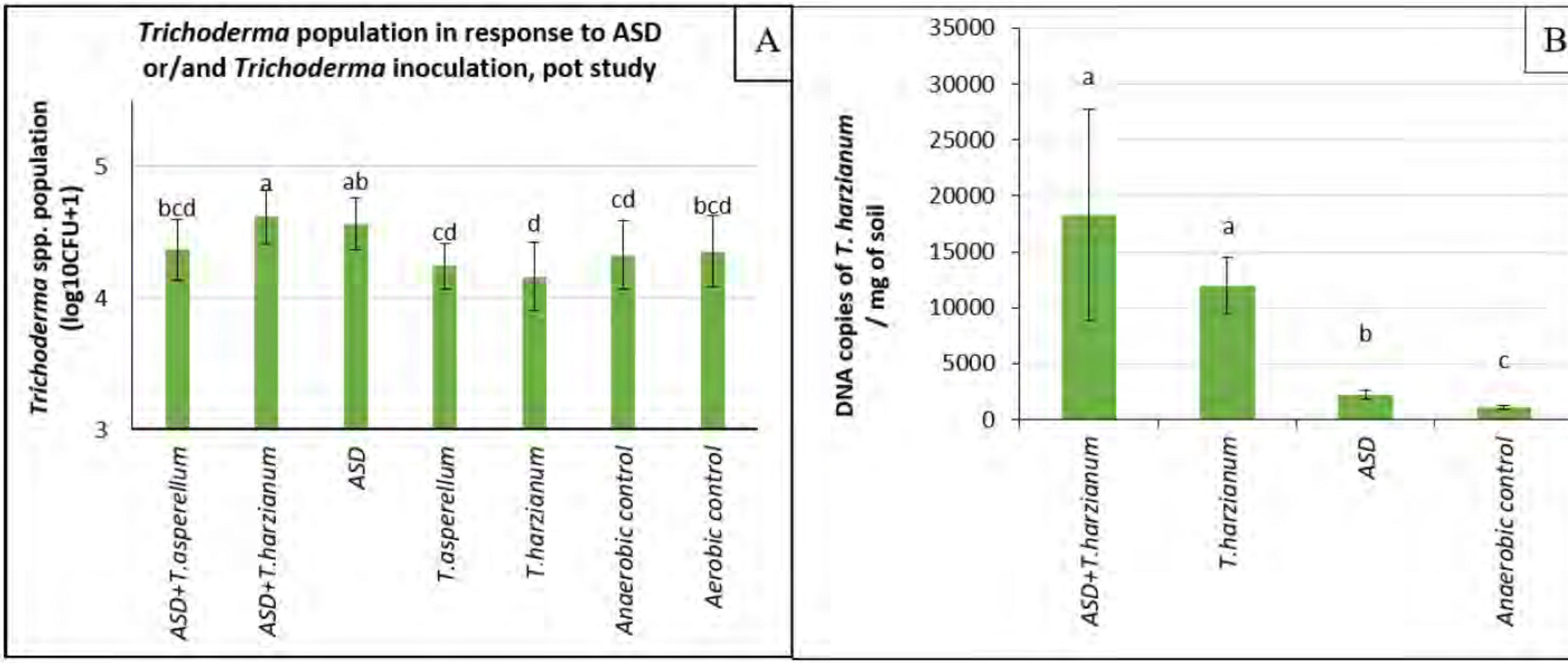
S. rolfsii germination/colonization





(Shrestha et al., *Applied Soil Ecology,* 2020)

Trichoderma soil abundance



(adapted from Shrestha et al., *Applied Soil Ecology,* 2020)

Outline

- Soil disinfestation background
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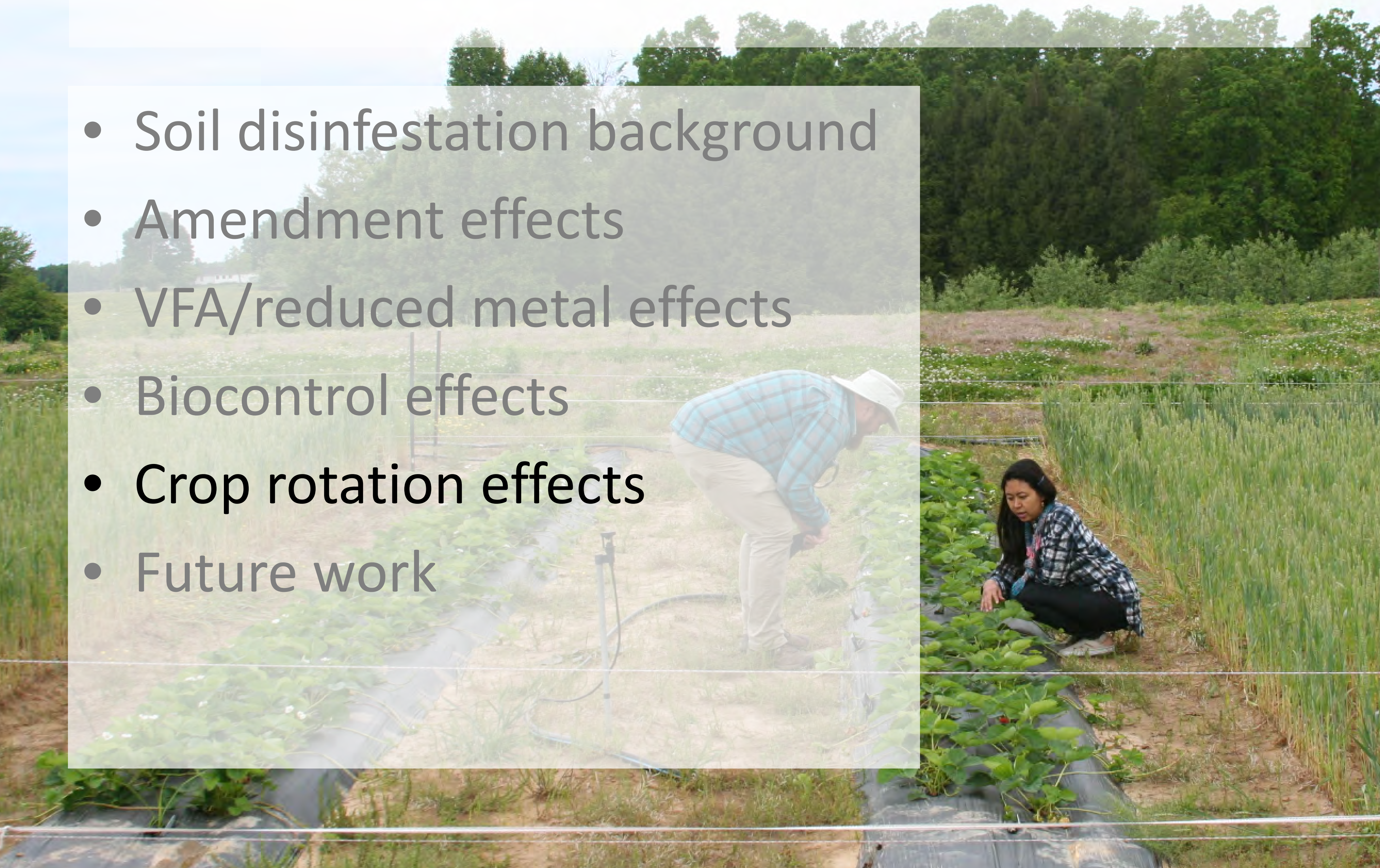
- Biocontrol effects
- **Crop rotation effects**
- Future work



Objectives

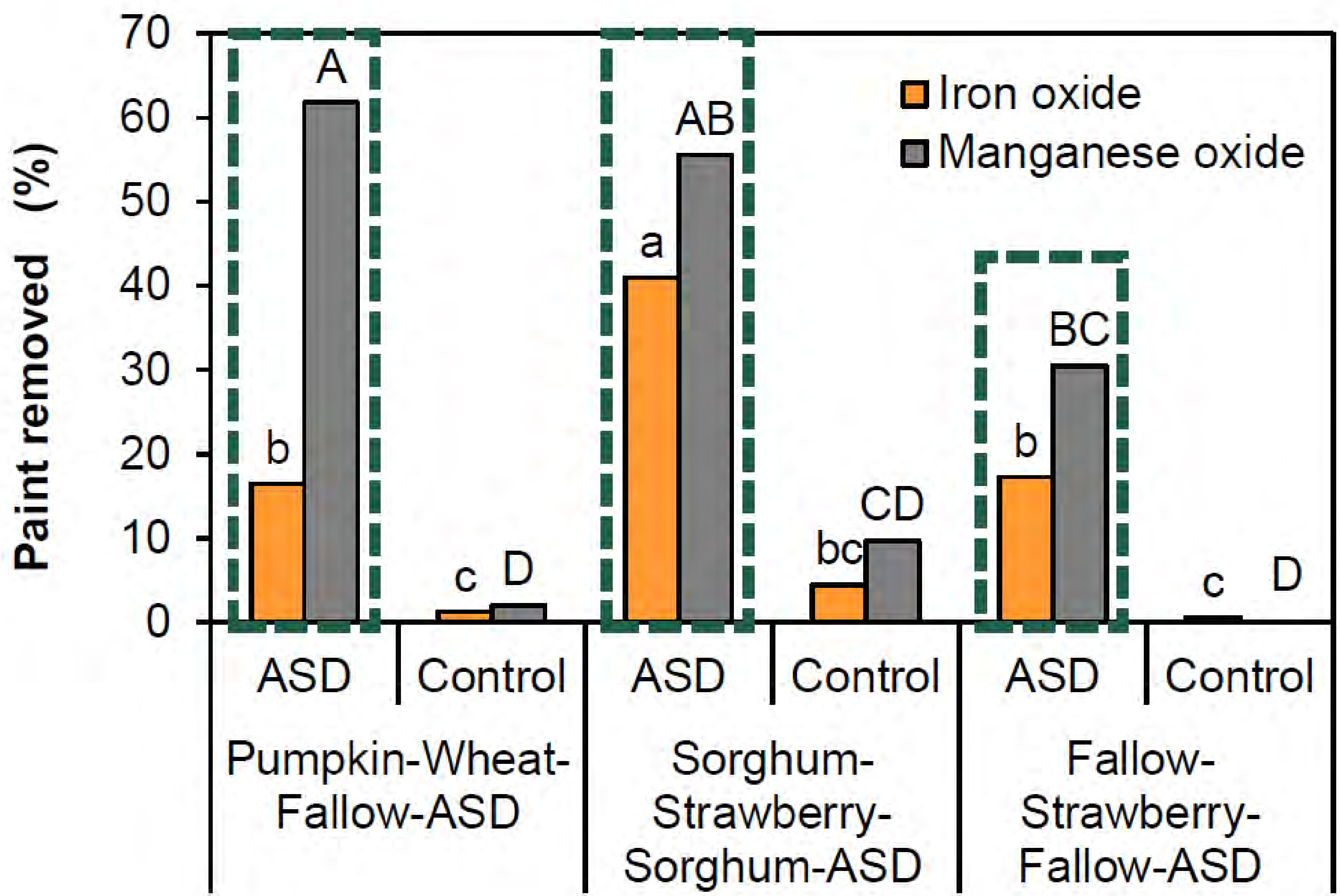
- Evaluate optimized ASD under varying rotation systems [systems: a) strawberry-cucurbit/wheat cover crop, b) strawberry-summer cover crop, and c) continuous strawberry]

a. Cucurbits/winter cover crop

b. Summer cover crop

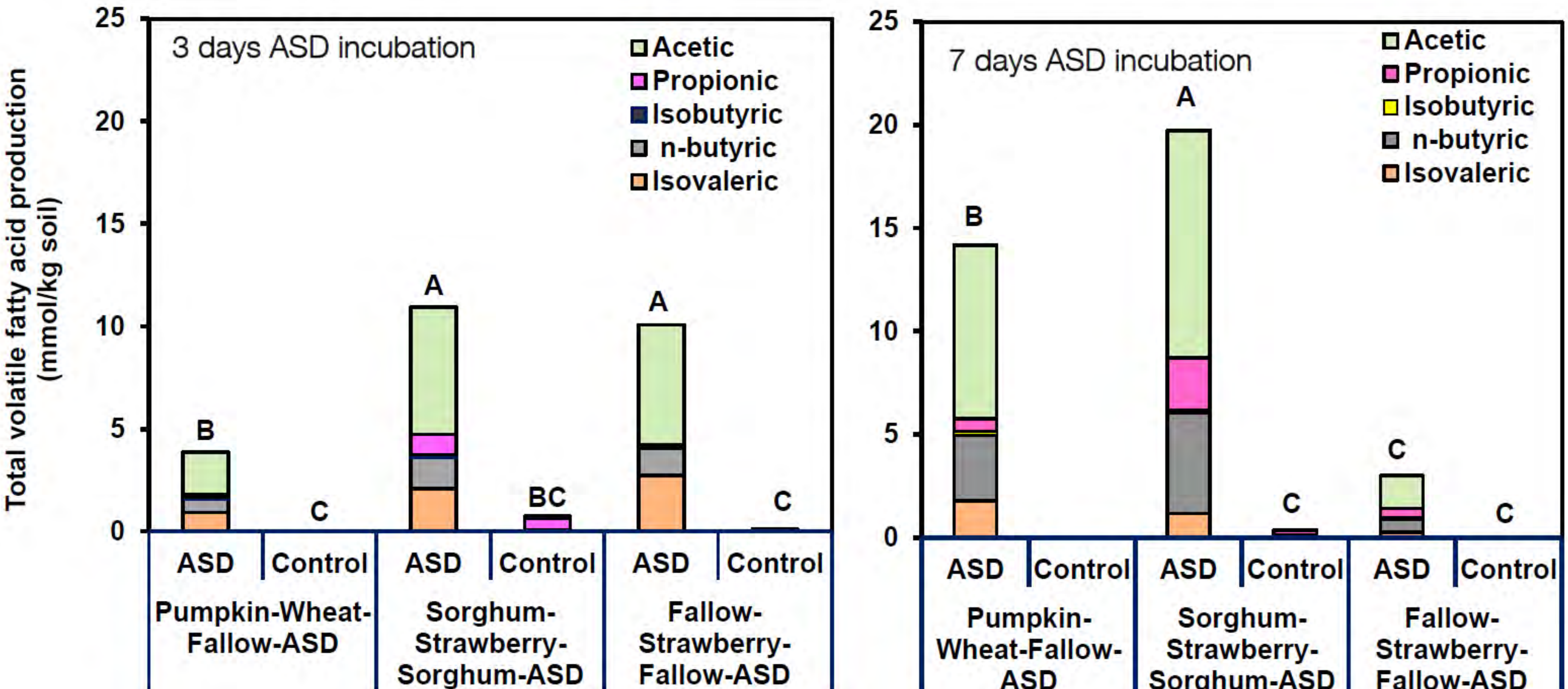
c. Fallow-Continuous strawberry

Anaerobic conditions



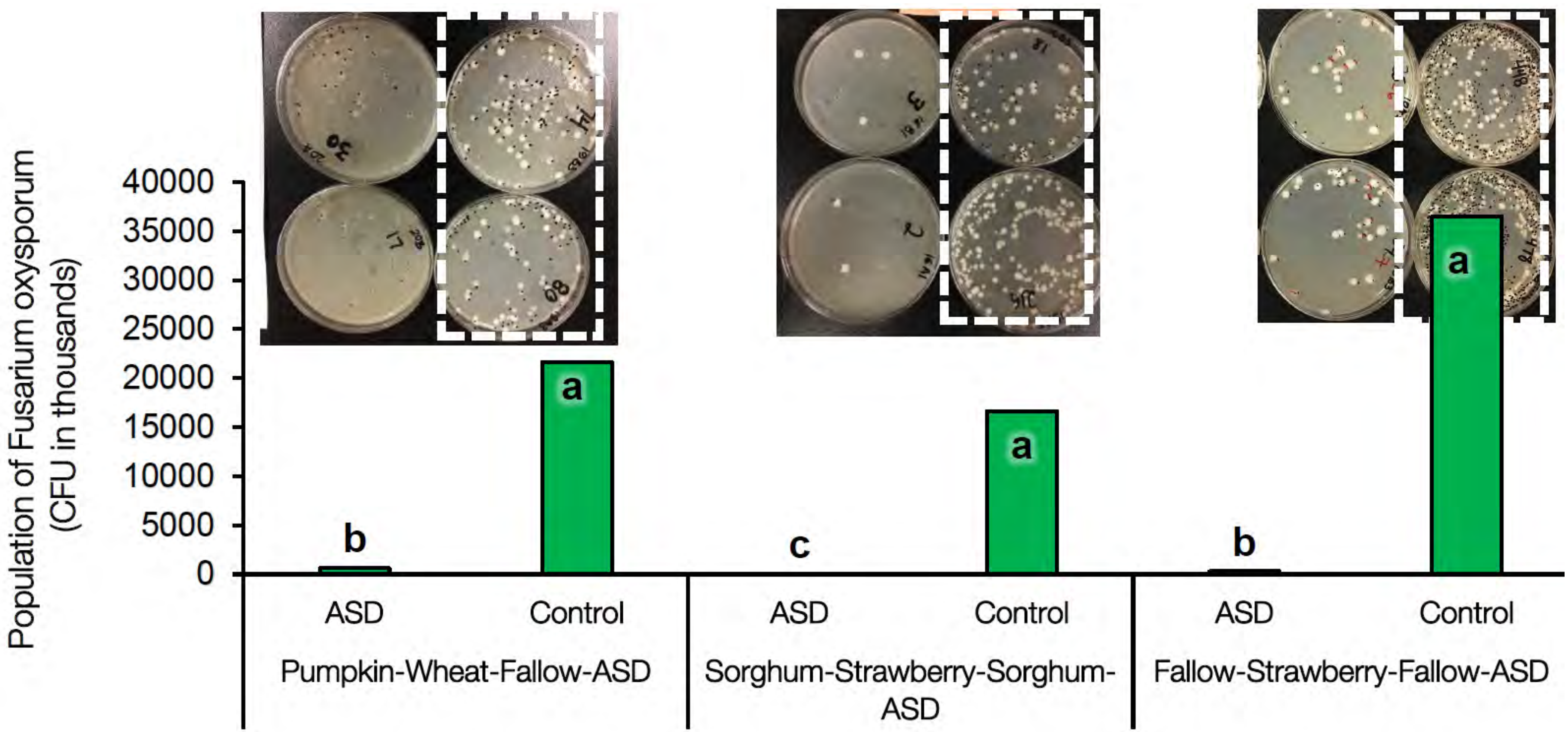
(Shrestha et al., 2022, *in preparation*)

VFA production



(Shrestha et al., 2022, *in preparation*)

F. oxysporum mortality



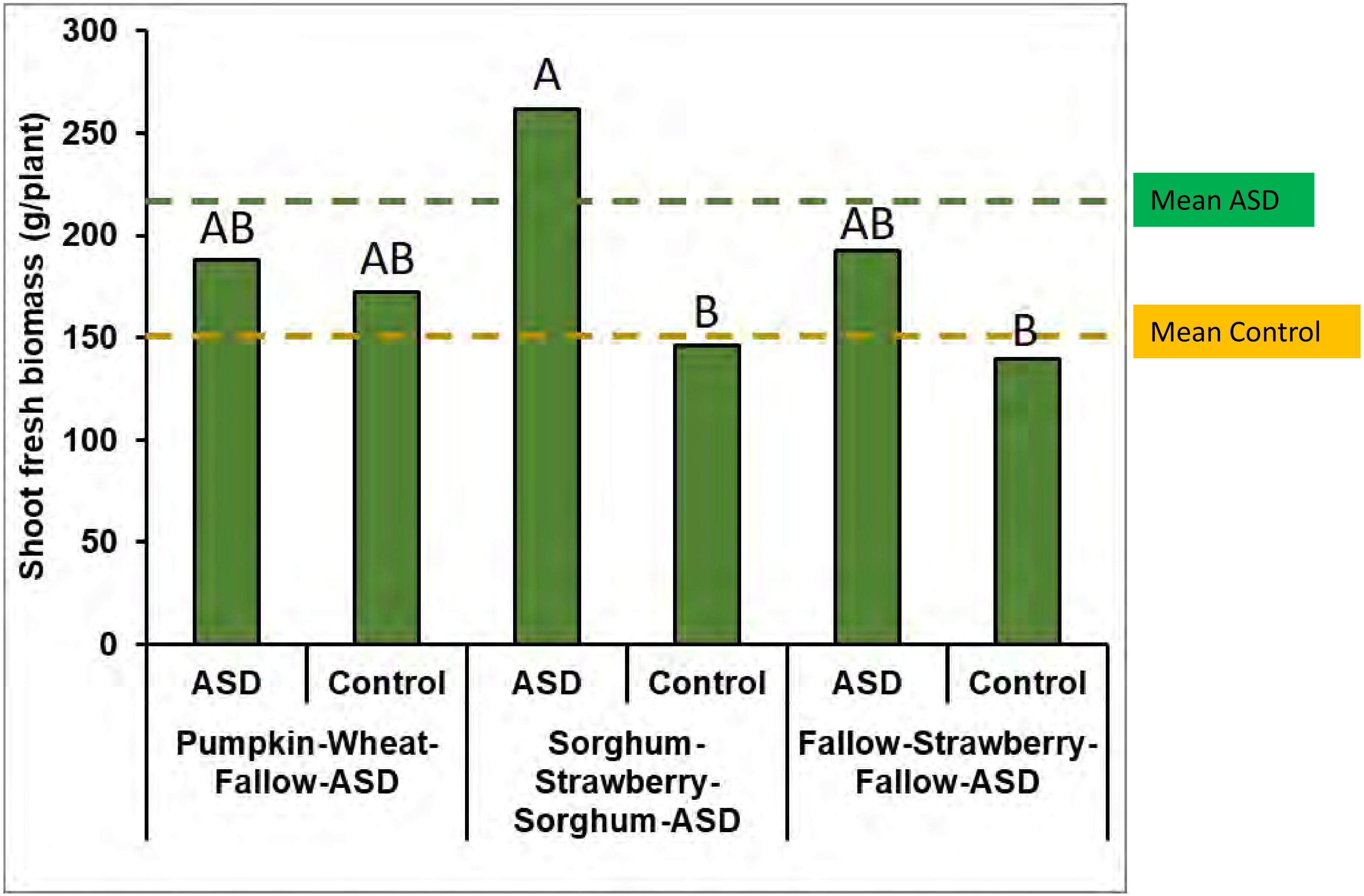
(Shrestha et al., 2022, *in preparation*)

ASD effect on strawberry biomass

ASD treatment

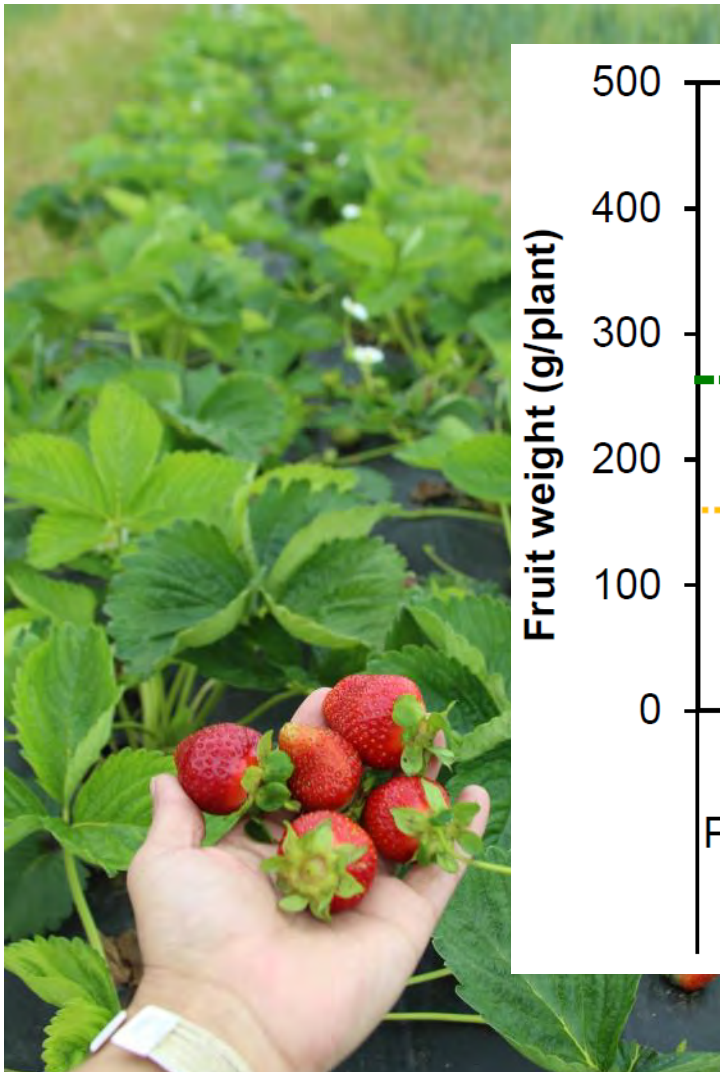
Control treatment

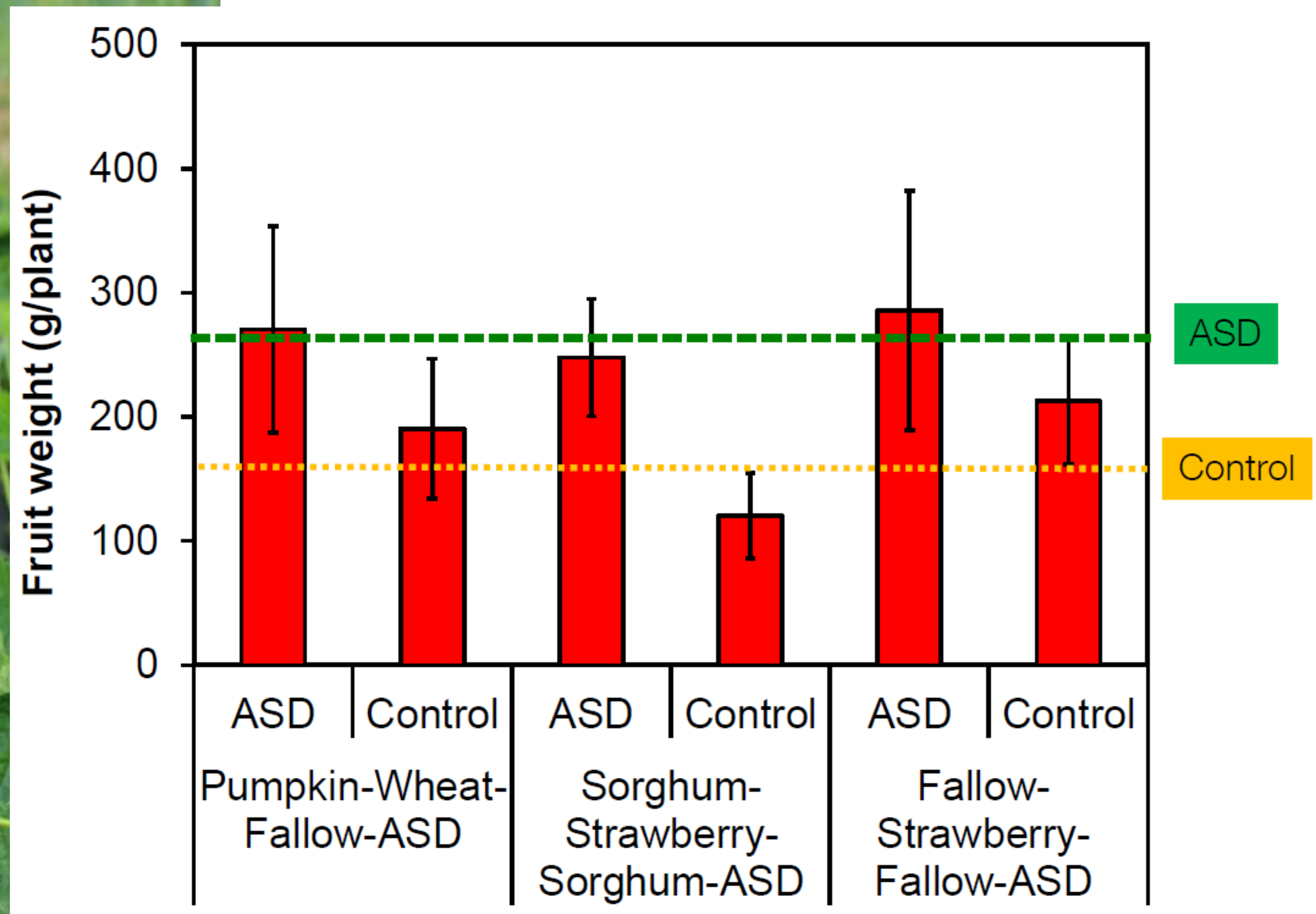
ASD effect on strawberry biomass



(Shrestha et al., 2022, *in preparation*)

ASD effect on strawberry yield





(Shrestha et al., 2022, *in preparation*)

How to implement ASD treatment

- ASD methods generally need optimized to specific production systems and amendment availability
  - Weed control likely not adequate alone
  - Be mindful of soil fertility
  - How can it be integrated with other pest management tactics?
- Recommend producers trial on a <u>small area </u>(a few beds) in the first year in comparison to standard practices to modify ASD to fit production system

Resources

UT Extension
SP 765-A

Introduction to Anaerobic Soil Disinfestation as a Fumigant Alternative

Utsala Shrestha, Graduate Research Assistant
Annette L. Wszelaki, Associate Professor and Commercial Vegetable Extension Specialist
David M. Butler, Assistant Professor
Department of Plant Sciences

What is soil disinfestation?

Preplant soil disinfestation refers to several methods often utilized in high-value specialty crop production systems (such as vegetables, fruits, nursery crops, ornamentals and herbs) to eliminate or reduce soilborne pests and weeds prior to planting a crop. These methods can include fumigation with gaseous pesticides (such as methyl bromide), application of steam, soil solarization (mulching of moist soils with clear plastic for extended periods of time in hot, sunny conditions), biofumigation (incorporation of residues of various mustard family plants), and flooding. The focus of this article is a more recently developed method, referred to as anaerobic soil disinfestation (ASD). The term disinfestation is used to refer to all of these methods of soil treatment rather than sterilization because it best describes the biological state in treated soils — a reduction or elimination of pests of concern but not a complete elimination of all soil microorganisms. While many soil organisms will be killed when soils are treated with synthetic fumigants, the soil is rapidly recolonized by various soil fungi and bacteria. Other disinfestation methods, such as ASD, rely on populations of soil microorganisms to create conditions favorable for control of certain soilborne pests.

When is soil disinfestation appropriate?

Soil disinfestation treatment costs can be substantial due to materials and supplies, application equipment, and labor needed to implement soil treatments, which suggests that its use should be limited to situations where other less economically intensive pest control methods are not effective or reliable. Crop rotation can help to control many soilborne pests where producer land, infrastructure and cropping system options make it economically viable. At the same time, the wide host range of many fungal, bacterial and nematode pests of several specialty crops makes reliance on crop rotation alone impractical in some situations, such as production on high value land, limited land availability, limited economically viable crop options, or greenhouses

Figure 1. Greenhouse and high tunnel production systems may be especially suitable for soil disinfestation treatments due to limited rotation and repeated production of high-value crops.
Photo credit: David Butler.

and high tunnels (Figure 1). The diversity of crops produced in many specialty crop production systems also limits the availability of registered non-fumigant pest control compounds in these crops due to the potential for adverse impacts to nontarget crops in terms of drift or residue persistence in rotation. Preplant soil disinfestation with non-persistent soil fumigants has been very valuable to producers who have come to rely on this single tactic that controls a broad-spectrum of pests and can be utilized on nearly all specialty crops produced.

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UT Extension
SP 765-B

Implementing Anaerobic Soil Disinfestation in Tennessee

Utsala Shrestha, Graduate Research Assistant
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David M. Butler, Assistant Professor
Department of Plant Sciences

What is anaerobic soil disinfestation?

Anaerobic soil disinfestation (ASD), as the name implies, is a process of disinfesting soil by creating anaerobic soil conditions with the incorporation of easily decomposable soil amendments, covering with plastic (polyethylene) mulch, and irrigating to saturation to begin a two- to six-week treatment period prior to planting certain high value crops, such as fruits or vegetables. ASD was developed independently in Japan and the Netherlands in the 1990s and 2000s, and more recently has been researched as a potential fumigation alternative in the United States. ASD has also been referred to as biological soil disinfestation, soil reductive sterilization, reductive soil disinfestation and anaerobically-mediated biological soil disinfestation.

During ASD treatment, the easily available carbon from the organic soil amendments used in ASD provides a substrate (food source) for rapid growth and respiration of soil microbes. As a consequence, available soil oxygen is reduced as soil is irrigated to fill soil pore space and plastic mulch is used to limit gas exchange between the soil and the ambient atmosphere above the mulch. This creates anaerobic conditions that persist until the carbon source is utilized or soil moisture content drops (typically one to two weeks). Anaerobic decomposition of the added soil amendment allows many toxic byproducts to accumulate such as organic acids (e.g., acetic and butyric acids) and other volatile compounds that serve to decrease soilborne pests. Preliminary research has also indicated that ASD treatment enhances populations of beneficial biocontrol microbes in soils, which also likely play a role in the effectiveness of treatment.

For more background information on ASD, please read "SP 765-A Introduction to Anaerobic Soil Disinfestation as a Fumigant Alternative," by U. Shrestha, A.L. Wszelaki and D.M. Butler, 2014, a UT Extension Publication.

ASD treatment steps

Step 1: Incorporate easily decomposable organic material.

Amendment types and properties. Various organic materials and agricultural byproducts have been shown to be effective soil amendments for ASD, including molasses (liquid or dried), wheat or rice bran, cover crop residues, fresh crop residues, or crop processing residues and byproducts. When choosing an amendment, consider the cost and availability, rate of decomposition, and nitrogen content or carbon to nitrogen ratio. In general, amendments that decompose rapidly in soil can be effectively utilized in ASD. Amendments that decompose very slowly (such as sawdust or wheat straw) will not create adequate anaerobic conditions due to the slow growth of soil microbes on these amendments. Likewise, amendments such as wheat straw with a relatively high carbon to nitrogen ratio (low relative nitrogen content) can slow decomposition and create nitrogen limitation issues with growing the crop and increased nitrogen fertilization will be required. While research is still ongoing on this topic, amendments with a carbon to nitrogen ratio in the approximate range of 10:1 to 35:1 are considered acceptable for use in ASD (Table 1). However, post treatment management of soil fertility will differ depending on C:N ratio of amendment(s) chosen (see section on post treatment management).

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  - Tennessee Ag. Experiment Station
  - Southern Region Small Fruit Consortium

USDA NIFA

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