N dynamics Following Anaerobic Soil Disinfestation (ASD)

J. Muramoto[1], C. Shennan[1], M. Zavatta[1], G. Baird[1], S.T. Koike[2], M.P. Bolda[2], K. Klonsky[3] and M. Mazzola[4]

[1] Department of Environmental Studies, University of California, Santa Cruz
[2] University of California, Cooperative Extension
[3] Department of Agricultural and Resource Economics, University of California, Davis
[4] USDA-ARS, Tree Fruit Lab, Wenatchee, WA

Outline

1. ASD Basics and Update
2. Exp. 1: Rotation/mustard seed meal/ASD trials

   ….organic site and conventional site

3. Exp. 2: ASD carbon-source trial

   …..conventional site

ASD Basics

- ✓ Biological alternative to fumigants developed in the Netherlands (Block et al., 2000) and Japan (Shinmura et al., 2000)
  - ✓ **Control a range of soilborne diseases and enhance yield in many crops**
- ✓ Acid fermentation in anaerobic soil
- ✓ In CA, being optimized for strawberry and cane berries
  - ✓ being tested for strawberry nurseries, Brussels sprout, almond, walnuts

ASD Process

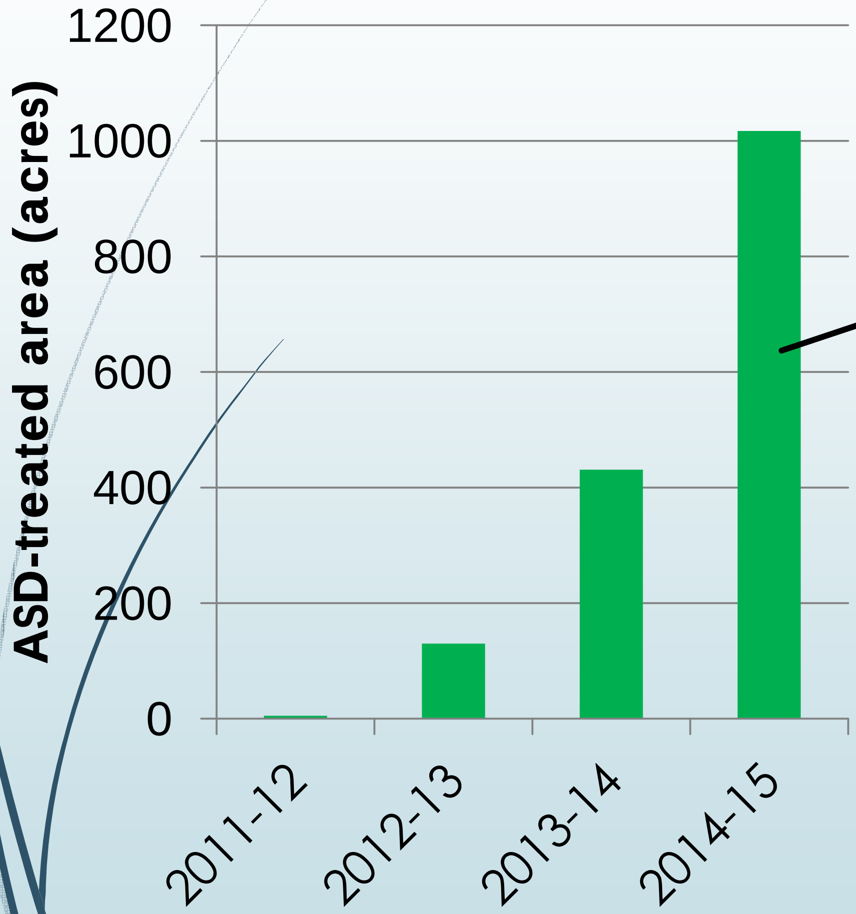
1. Incorporate readily available organic matter
   - **Provide C source for soil microbes**
2. Cover with oxygen impermeable tarp
3. Irrigate to saturate soil then to maintain field capacity
   - **Water-filled pore space**
   - **Create anaerobic conditions and stimulate anaerobic decomposition of incorporated organic material**

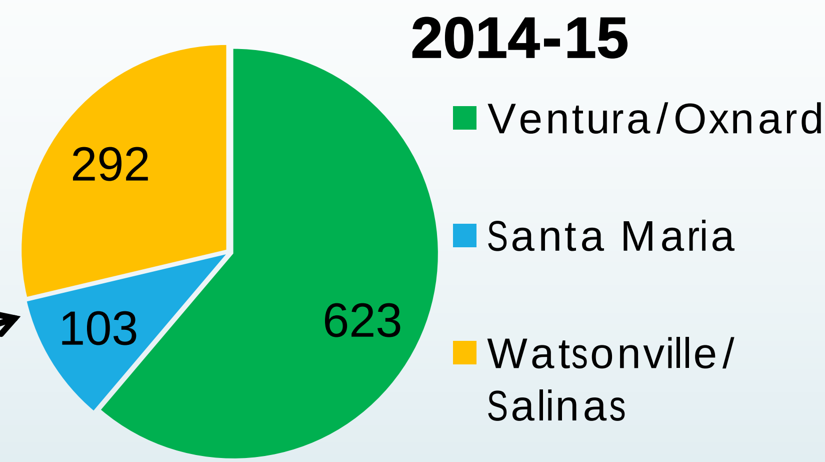
Anaerobic Soil Disinfestation (ASD)

(Shennan et al., 2007)

1. Broadcast rice bran at 9 tons/ac
2. Incorporate bran
3. List beds
4. Cover w/ plastic mulch
5. Drip irrigate total 3 -5 ac-inches over 3 wks
6. Leave 3 wks and monitor soil Eh and temp

ASD-Treated Fields in California





- 80% organic sites
- 20% conventional sites

- ~20% of CA organic strawberry acreages
- ~2.5% of CA total strawberry acreages

(Farm Fuel Inc. Personal communication)

Potential Mechanisms

- Production of organic acids toxic to some pathogens
- Production of volatiles toxic to some pathogens
- Reduction of iron and manganese – Fe^{2+} and Mn^{2+} toxic to some pathogens
- Shifts in microbial communities to create competition or antagonism that suppress pathogens
- Lack of oxygen, low pH,
- Combination of the above – all interrelated!

How are each of these processes related to suppression of specific pathogens?

How are processes affected by C source used, soil moisture and temperature, and initial microbial community?

Summary of Findings to 2014 ~field trials~

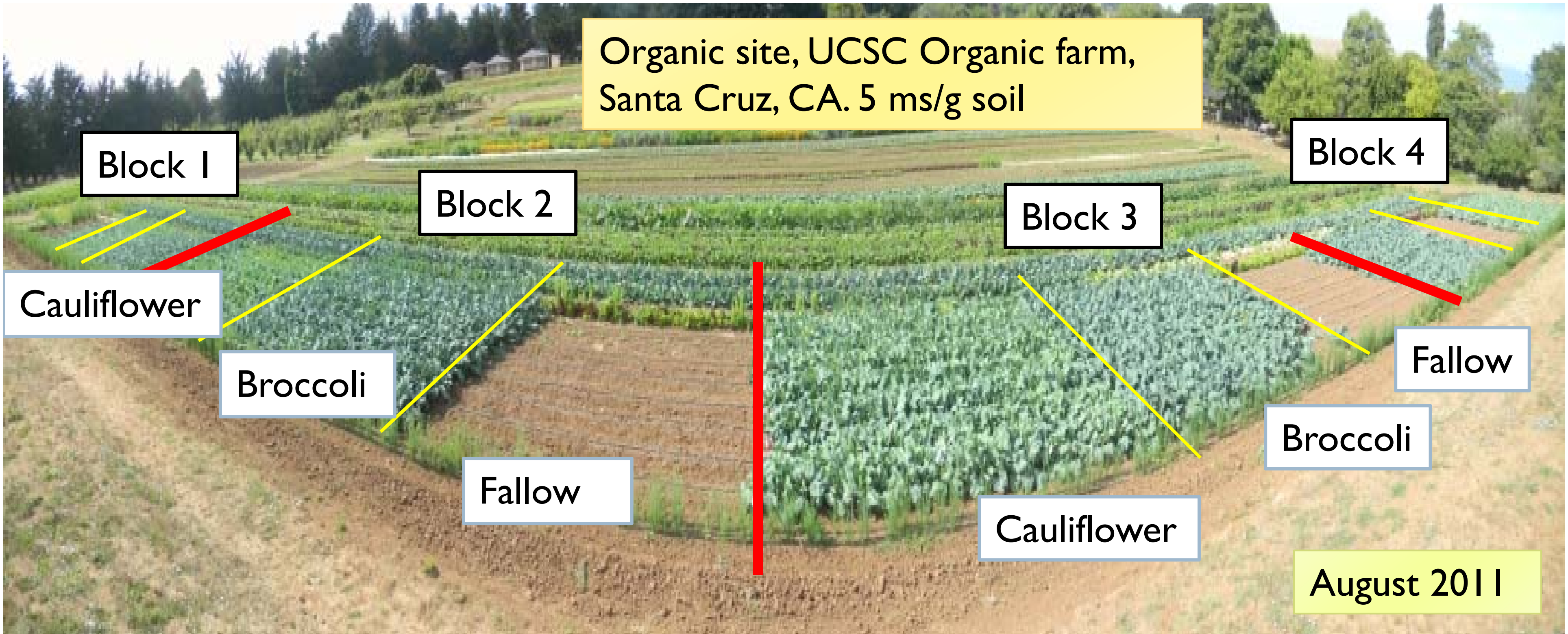
- Good yields obtained with 9 t/ac rice bran in field trials averaged 99% (82 – 114%) of fumigant yields in 10 replicated field trials in Watsonville, Castroville, Salinas, Santa Maria, and Ventura
- Got consistently good *V. dahliae* suppression; 80 to 100% decrease in # microslerotia in soil, using 9 t/ac rice bran
- Weed suppression limited in the central coast of CA
- May not need pre-plant fertilizer with 6-9 t/ac rice bran as C-source, but probably will with lower N sources
- May provide excess N to strawberries with 9 t/ac rice bran
    - Need to monitor N dynamics!

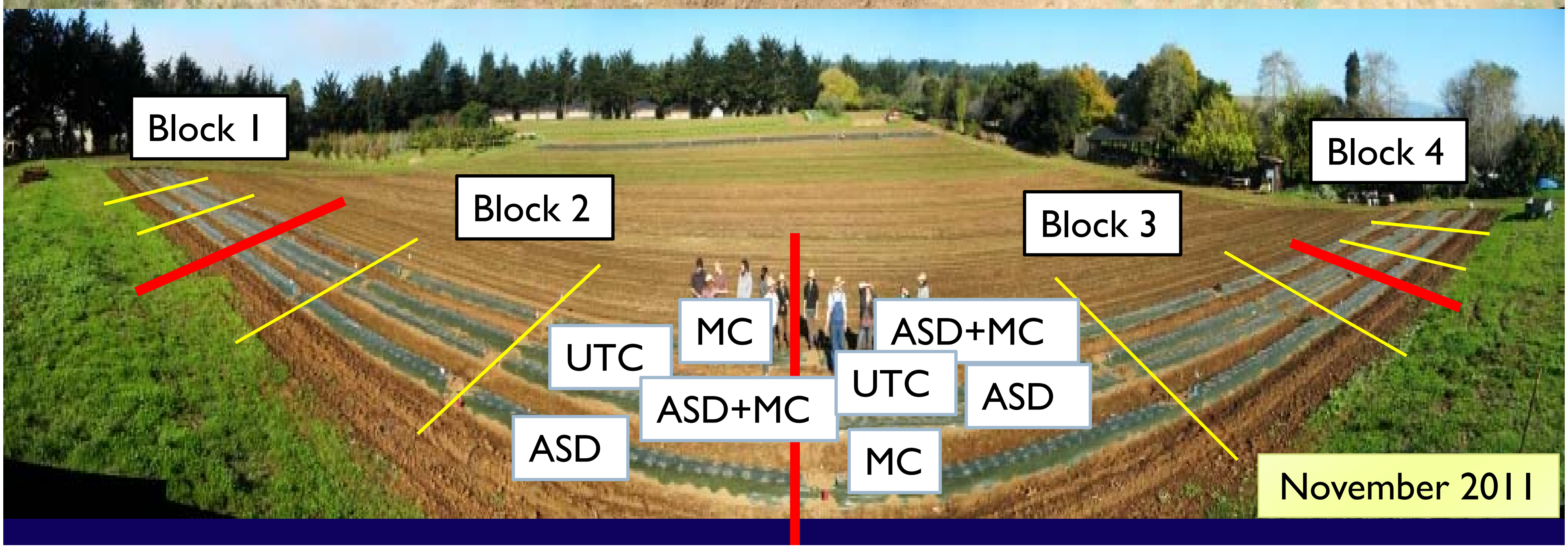
Exp. 1: Rotation/Mustard seed meal/ASD trials

- **Randomized block split plot** designed field trials with **4 replicates** at **2 sites** (organic and conventional)
  - **Main plot**: pre-crop of strawberry
    - **broccoli**-strawberry-winter cover crop*-lettuce*
    - **cauliflower**-strawberry-winter cover crop*-lettuce*
    - **fallow**-strawberry-winter cover crop*-lettuce*

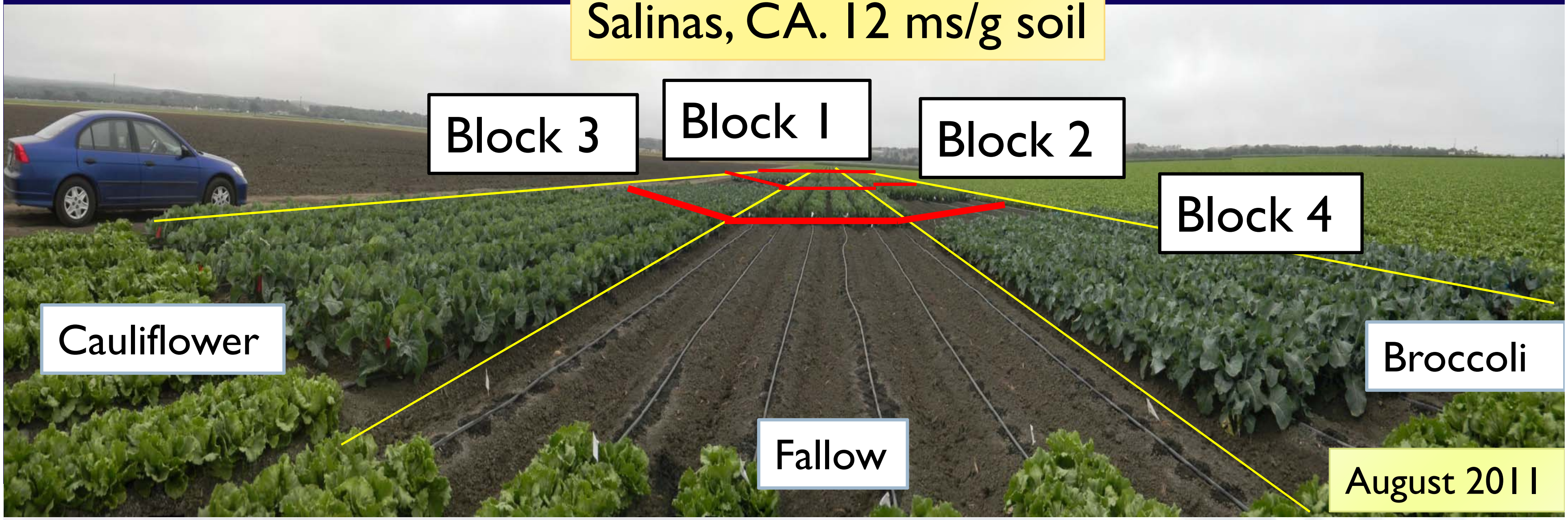
    * Organic only
  - **Split plot**: soil treatment for strawberries
    - **MC** (*B. juncea* : *S. alba* = 1:1 weight) 1.5 t/ac
    - **ASD** w/ rice bran 9 t/ac
    - **MC** 1.5t/ac **+ ASD** w/ rice bran7.5t/ac
    - untreated check (**UTC**)
    - **fumigation** w/ Pic-clor60**

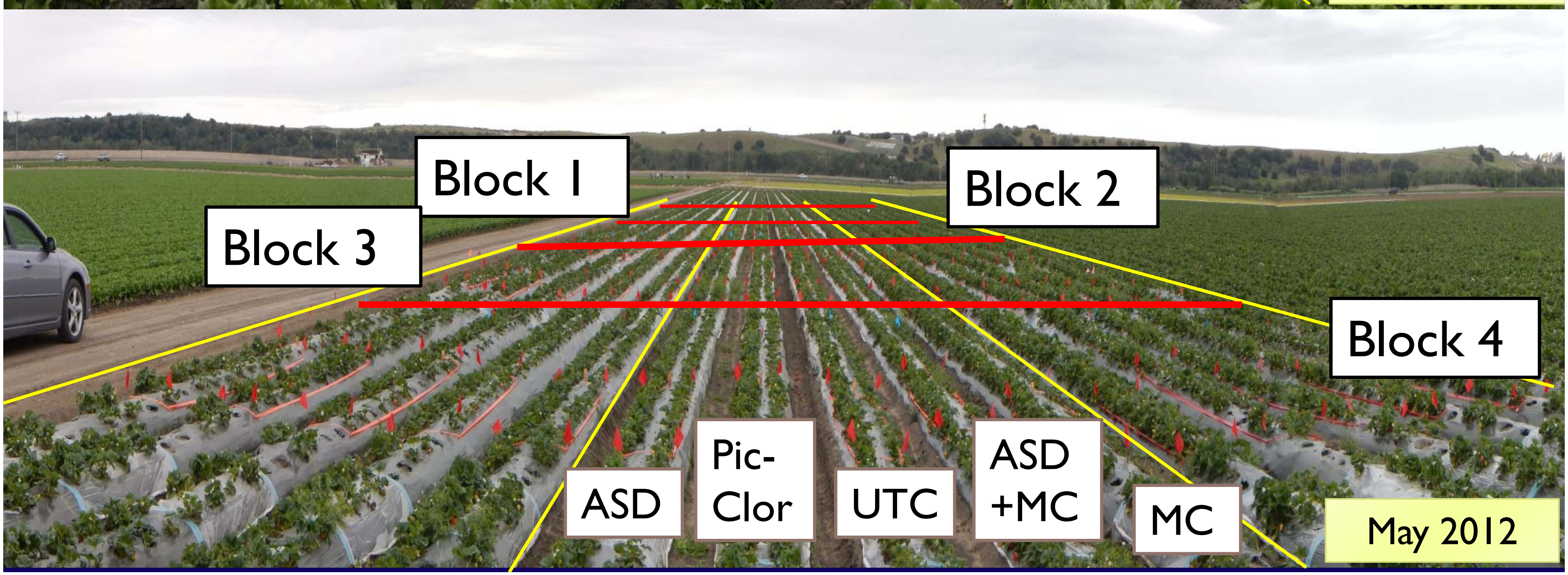
    ** Conventional only


Organic site, UCSC Organic farm,
Santa Cruz, CA. 5 ms/g soil
Block 1
Block 2
Block 3
Block 4
Cauliflower
Broccoli
Fallow
Fallow
Broccoli
Cauliflower
August 2011


Block 1
Block 2
Block 3
Block 4
MC
UTC
ASD+MC
ASD
ASD+MC
UTC
ASD
MC
November 2011

Conventional site, Salinas, CA. 12 ms/g soil

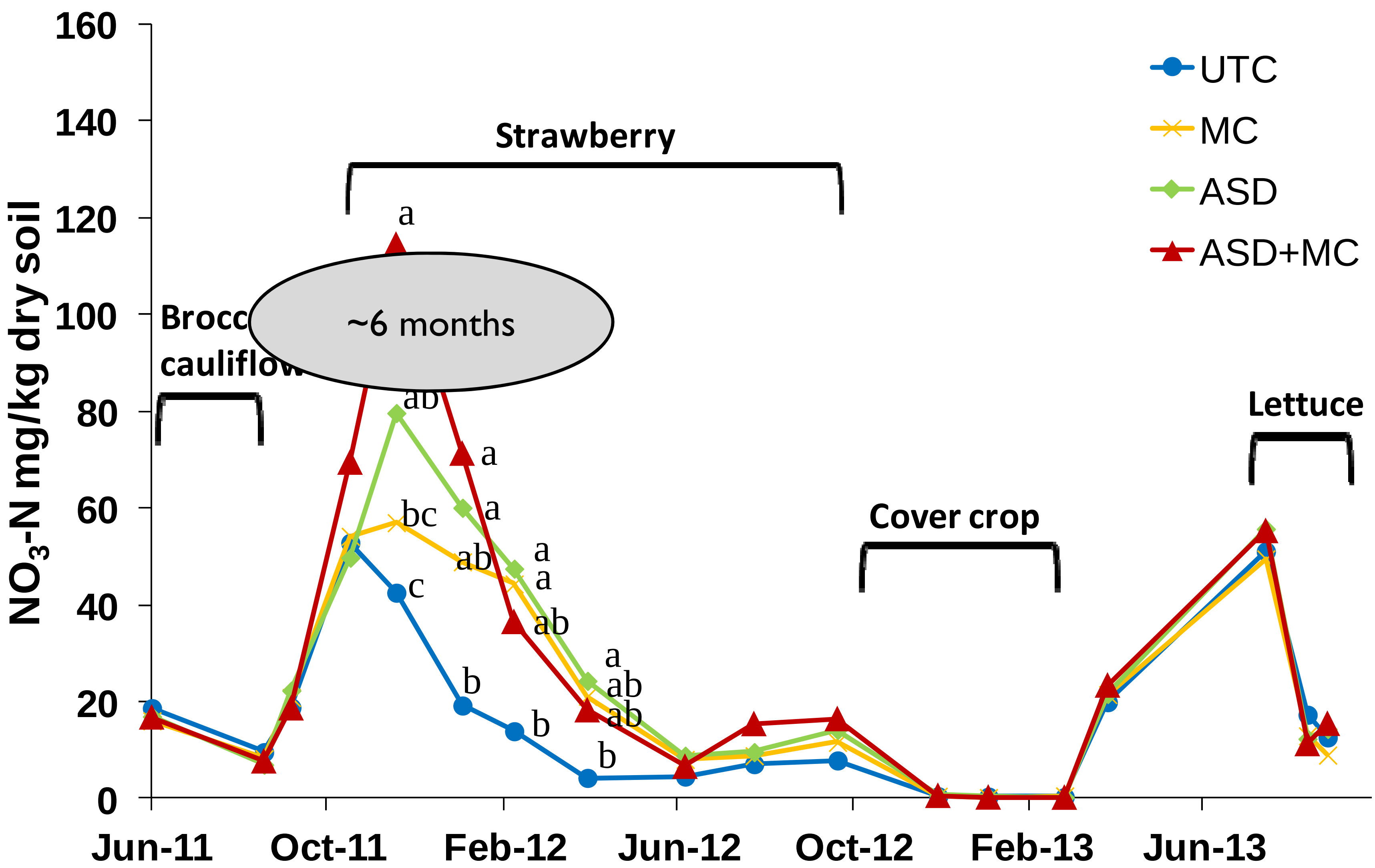


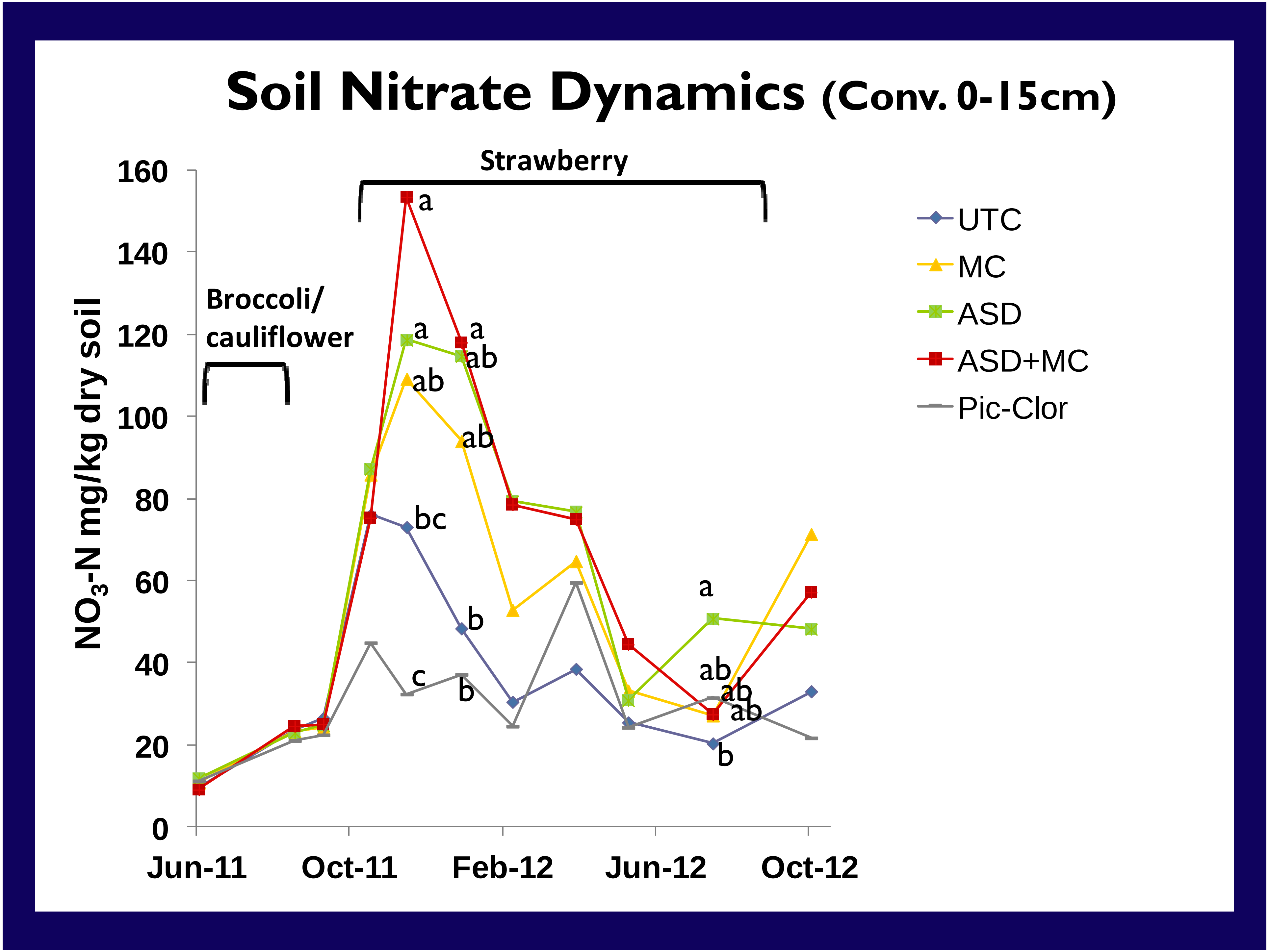


Variables Monitored

- N dynamics
  - Soil nitrate (0-6” depth)
- *Verticillium dahliae*
  - Viable microsclerotia in soil (ms/g soil)
  - Wilt score: 1 (healthy) – 8 (dead)
  - Infection rate of strawberry plant (%)
- Crop yield and biomass

Soil Nitrate Dynamics (Org. 0-15cm)



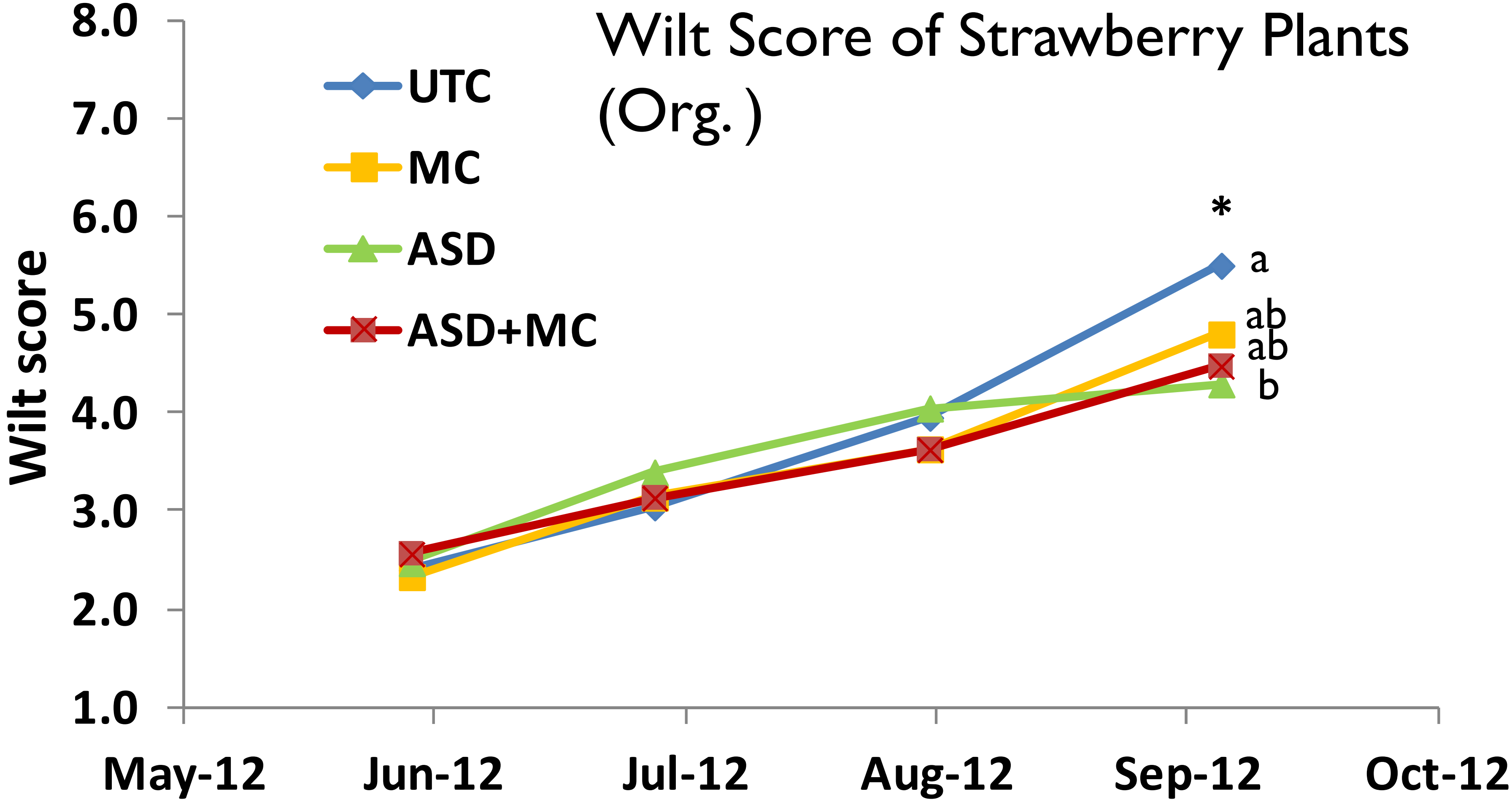


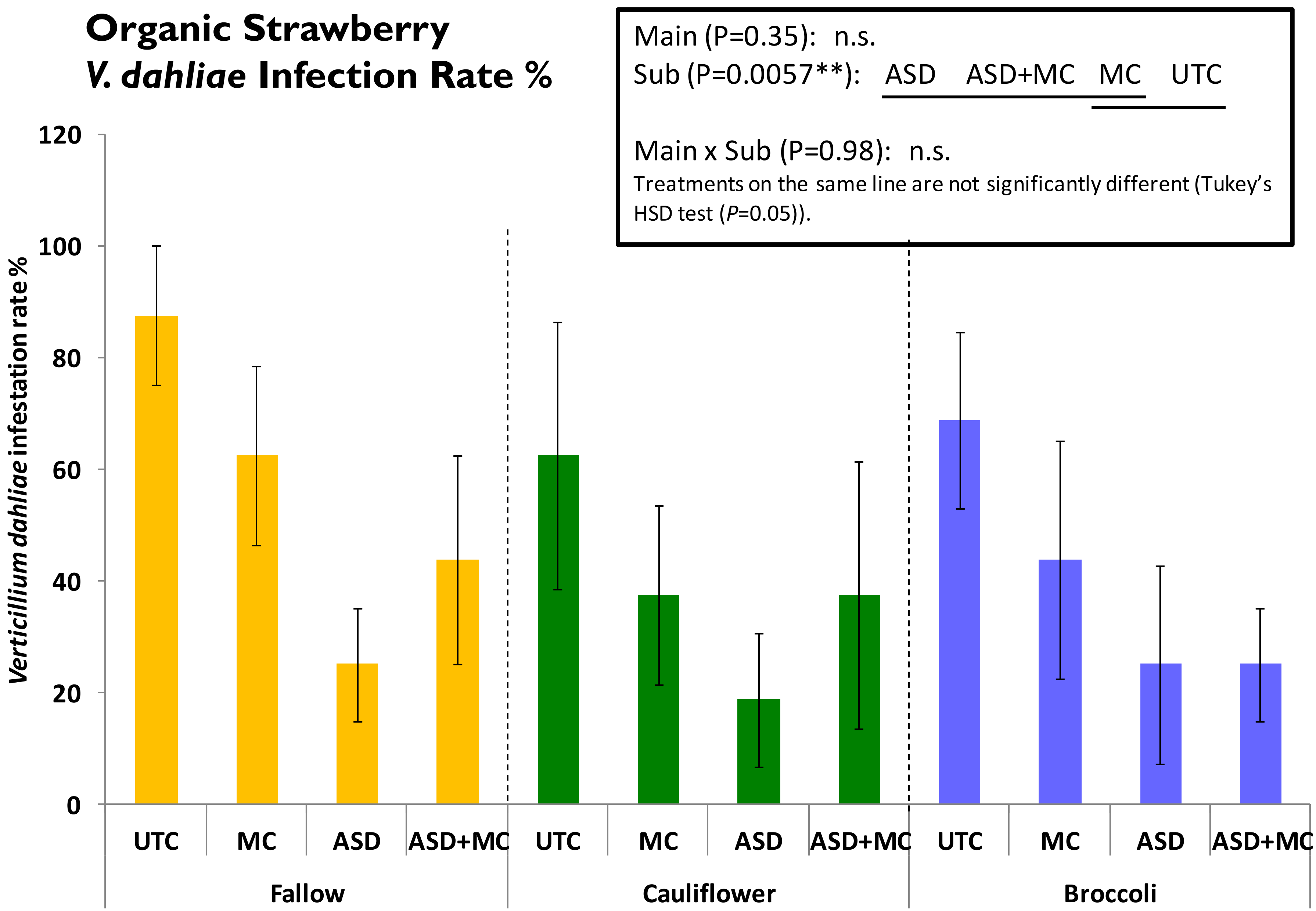
Soil Nitrate Dynamics (Conv. 0-15cm)
Strawberry
Broccoli/
cauliflower
NO3-N mg/kg dry soil
160
140
120
100
80
60
40
20
0
Jun-11
Oct-11
Feb-12
Jun-12
Oct-12
UTC
MC
ASD
ASD+MC
Pic-Clor
a
a
a
ab
ab
ab
bc
b
c
b
a
ab
ab
ab
b

Salt Burn Damage at ASD plots (Conv. Jan. 2012)

ASD w/ 9t/ac rice bran (N 2%) -> total 360 lbs-N/ac
MC 1.5t/ac (N 6%) -> total180 lbs-N/ac
ASD w/ 7.5t/ac rice bran + MC 1.5t/ac -> total 480 lbs-N/ac
…….plus inorganic N from pre-crop residues!

Wilt Score of Strawberry Plants (Org.)

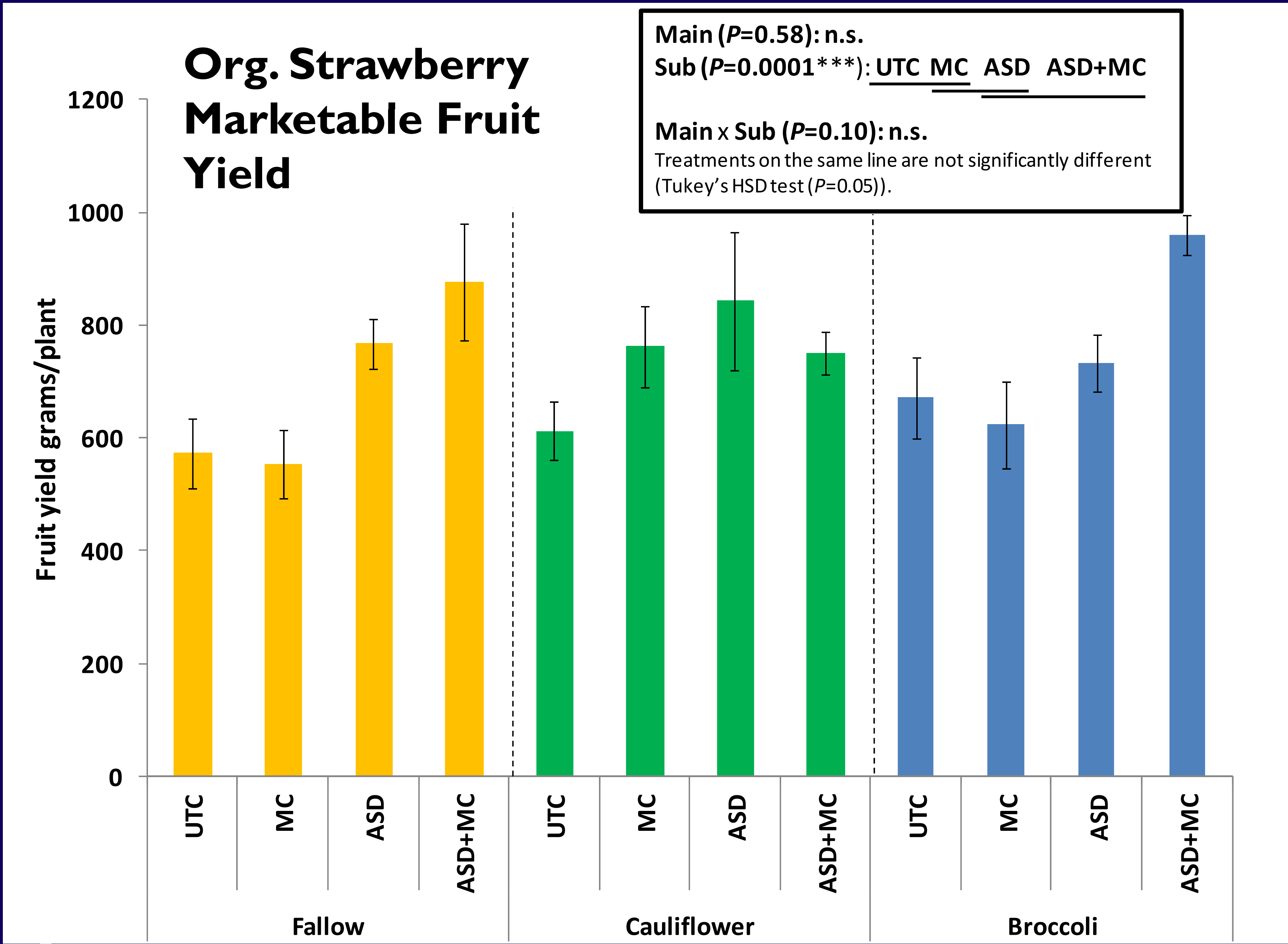



Organic Strawberry
V. dahliae Infection Rate %
Main (P=0.35): n.s.
Sub (P=0.0057**): ASD ASD+MC MC UTC
Main x Sub (P=0.98): n.s.
Treatments on the same line are not significantly different (Tukey's HSD test (P=0.05)).
Verticillium dahliae infestation rate %
120
100
80
60
40
20
0
UTC
MC
ASD
ASD+MC
Fallow
Cauliflower
Broccoli

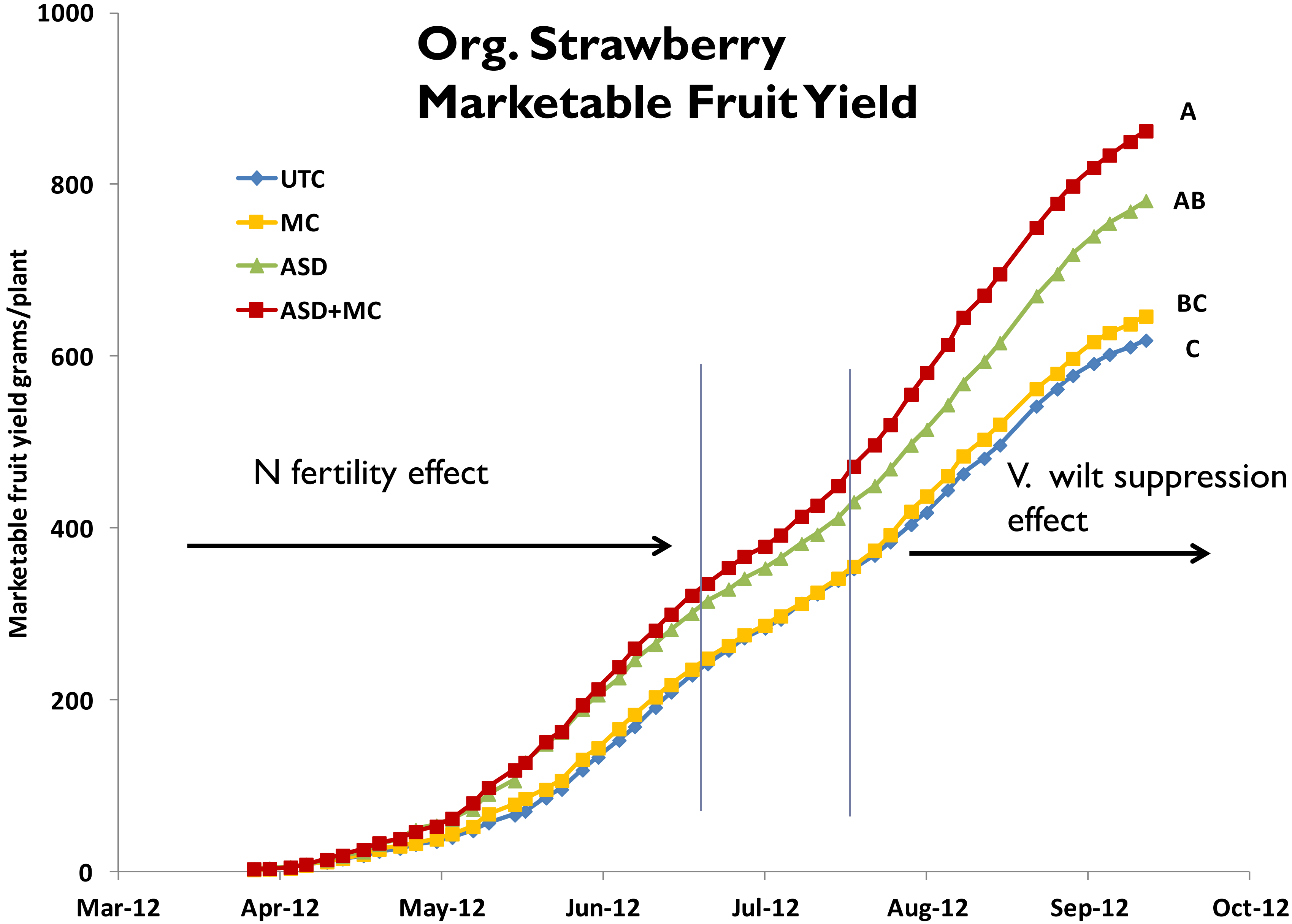
Changes in *Verticillium dahliae* Microsclerotia Number in Topsoil of Organic site.

Treatment	Sampling Date				
	Baseline June 2011	Post-Broccoli/Cauliflower Sep. 2011	Pre-ASD/MC Sep. 2011	Post-ASD/MC Oct. 2011	Post-Strawberries Sep. 2012
Main plot					
Fallow	0.6	0.0	1.5	0.5	0.0
Cauliflower	0.4	0.4	2.1	0.0	0.0
Broccoli	0.5	0.3	1.0	0.1	0.0
ANOVA (*P*)	*0.81*	*0.24*	*0.51*	*0.35*	-
Sub plot					
UTC	1.2	0.7	1.5	0.5	0.0
MC	0.0	0.2	1.7	0.3	0.0
ASD	0.5	0.0	1.3	0.0	0.0
ASD+MC	0.3	0.0	1.7	0.0	0.0
ANOVA (*P*)	*0.06*	*0.08*	*0.99*	*0.15*	-

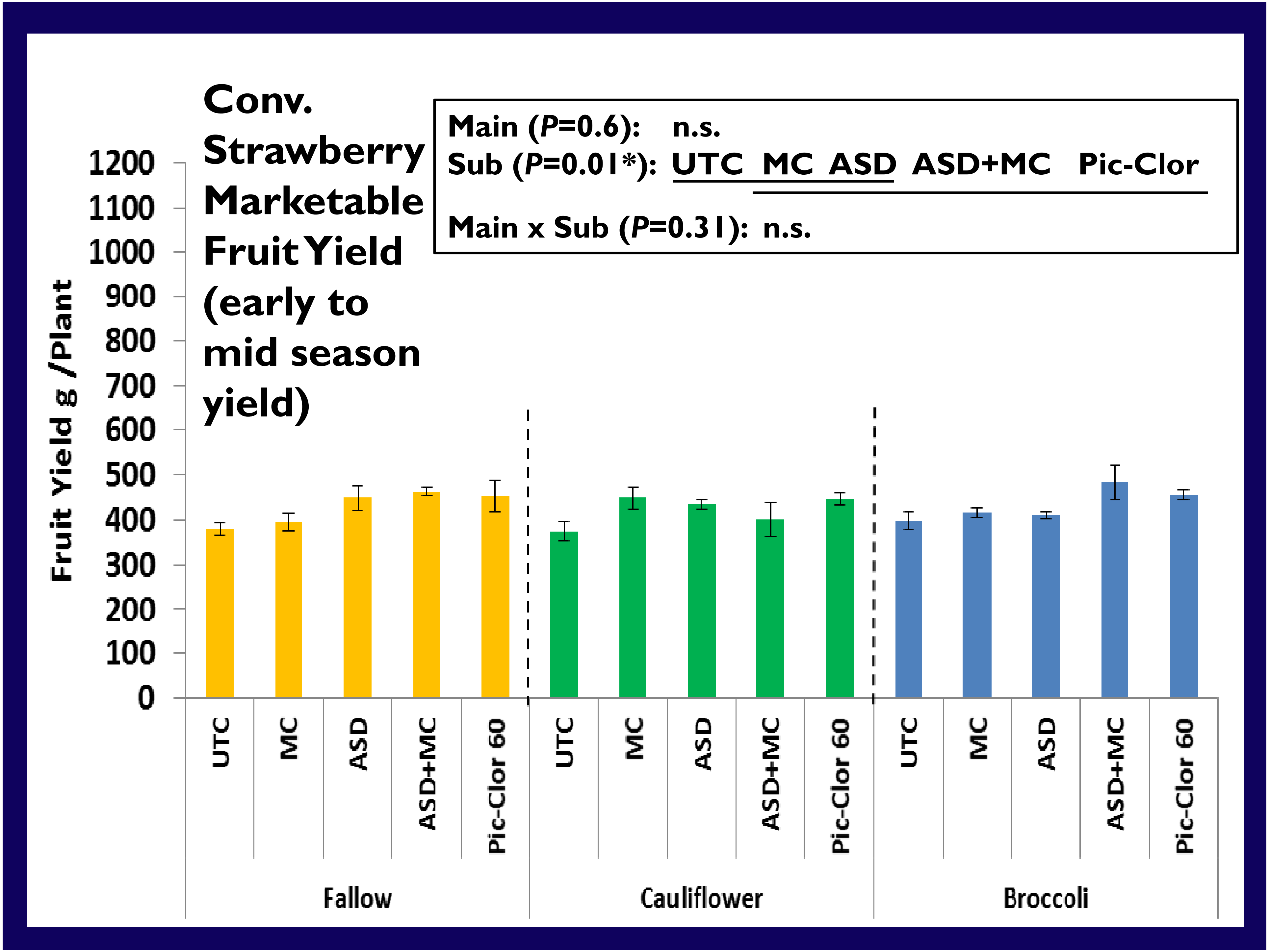
Statistical analysis was performed for log-transformed data. Numbers in the table show the back-transformed populations of viable *V. dahliae* microsclerotia/gram soil.

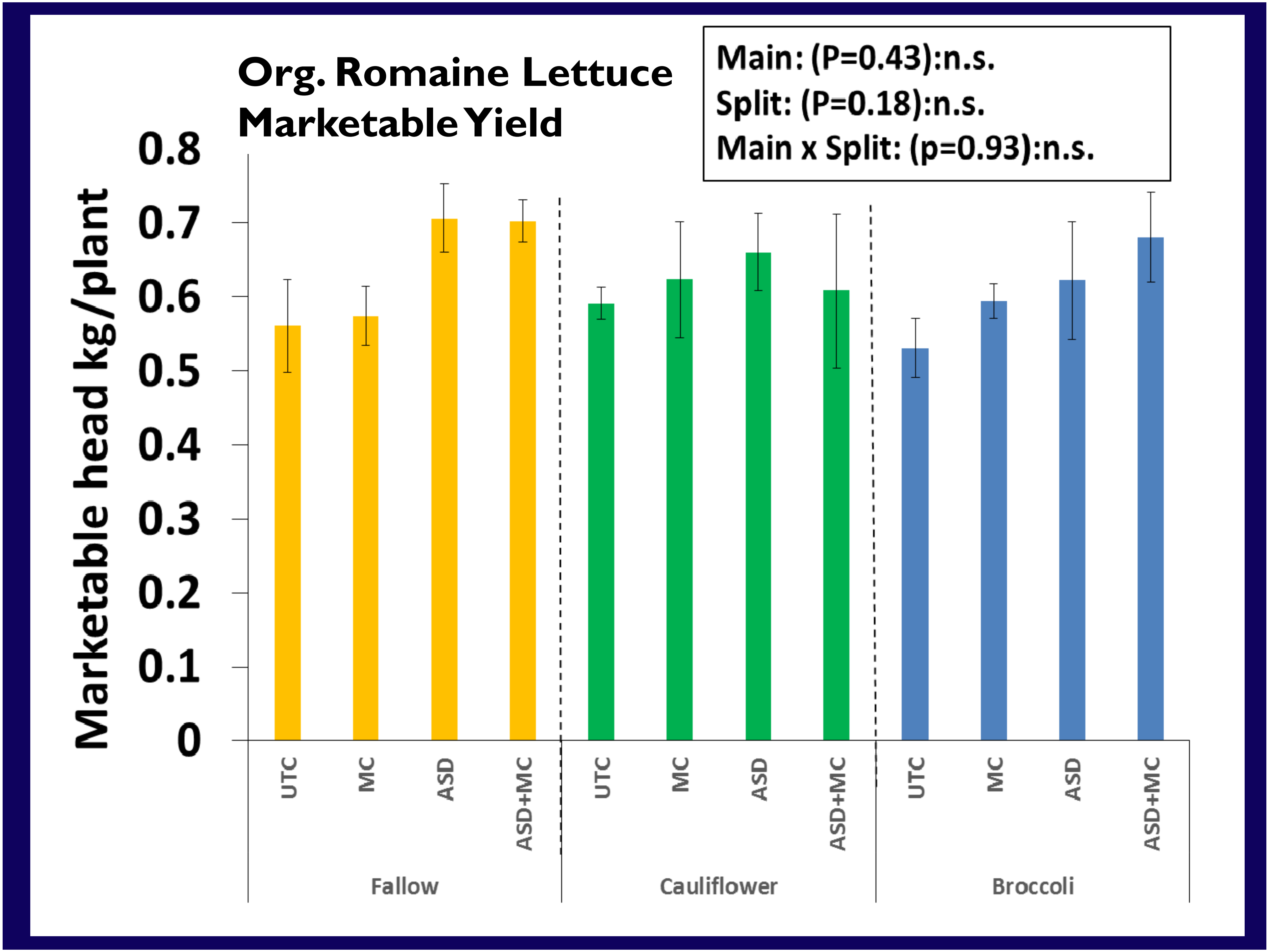


Org. Strawberry Marketable Fruit Yield
Main (P=0.58): n.s.
Sub (P=0.0001***): UTC MC ASD ASD+MC
Main x Sub (P=0.10): n.s.
Treatments on the same line are not significantly different (Tukey's HSD test (P=0.05)).
Fruit yield grams/plant
1200
1000
800
600
400
200
0
UTC
MC
ASD
ASD+MC
Fallow
Cauliflower
Broccoli

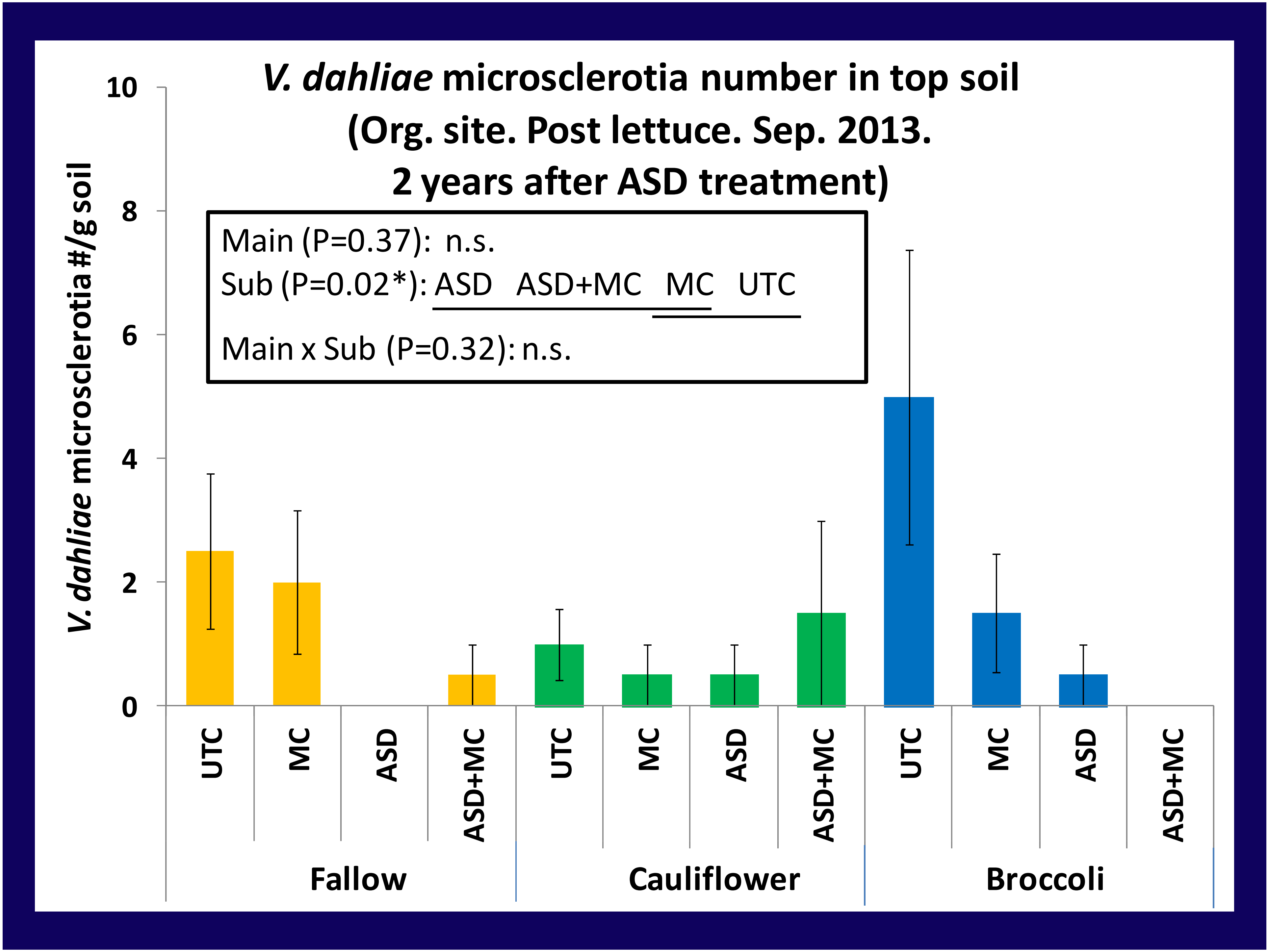


Org. Strawberry
Marketable Fruit Yield
Marketable fruit yield grams/plant
1000
800
600
400
200
0
UTC
MC
ASD
ASD+MC
A
AB
BC
C
N fertility effect
V. wilt suppression
effect
Mar-12
Apr-12
May-12
Jun-12
Jul-12
Aug-12
Sep-12
Oct-12


Conv. Strawberry Marketable Fruit Yield (early to mid season yield)
Main (P=0.6): n.s.
Sub (P=0.01*): UTC MC ASD ASD+MC Pic-Clor
Main x Sub (P=0.31): n.s.
Fruit Yield g /Plant
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
UTC
MC
ASD
ASD+MC
Pic-Clor 60
Fallow
Cauliflower
Broccoli



Org. Romaine Lettuce
Marketable Yield
Main: (P=0.43):n.s.
Split: (P=0.18):n.s.
Main x Split: (p=0.93):n.s.
Marketable head kg/plant
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
UTC
MC
ASD
ASD+MC
UTC
MC
ASD
ASD+MC
UTC
MC
ASD
ASD+MC
Fallow
Cauliflower
Broccoli


V. dahliae microsclerotia number in top soil
(Org. site. Post lettuce. Sep. 2013.
2 years after ASD treatment)
Main (P=0.37): n.s.
Sub (P=0.02*): ASD ASD+MC MC UTC
Main x Sub (P=0.32): n.s.
V. dahliae microsclerotia #/g soil
10
8
6
4
2
0
UTC
MC
ASD
ASD+MC
UTC
MC
ASD
ASD+MC
UTC
MC
ASD
ASD+MC
Fallow
Cauliflower
Broccoli

Summary

- N dynamics:
  - Rice bran provided significant amount of nitrate for ~6 months from ASD treatment
  - Benefitted early growth and yield
  - Excess N caused salt damage to strawberry plants (esp. Conv. site)
  - Need to reduce N inputs in ASD by using lower-N carbon source or lowering rate of rice bran

Summary (Cont.)

- *V. dahliae* suppression:
  - ASD+MC and ASD showed reduction of *V. dahliae* infection in strawberry plant (Org. site)
  - *V. dahliae* microsclerotia number in soil was lower at ASD and ASD+MC even after 2 years from treatment

Summary (Cont.)

- Crop yield:
  - Strawberry's marketable fruit yield at ASD and ASD+MC was highest in Org. and ASD+MC was comparable to fumigation in Conv. (early-mid season yield)
- Overall:
  - ASD reduced V. wilt and increased yield but no additive or synergistic effect of MC and/or broccoli rotation was observed
  - Yield increase in ASD appears to be caused by a combination of N provision (early season) and disease suppression (late season)

Exp. 2: Carbon source trial (PSI, Watsonville)

- Rhizoctonia-infested field
- RB split plot. 4 reps

  Main plots:
  - ASD RB 9 t/ac
  - ASD RB 6 t/ac
  - ASD ground dry grape pomace (GP) 9 t/ac
  - Methyl bromide/chloropicrin
  - UTC

  Split plots:
  - With and with pre-plant fertilizer (PPF. 650 lb/ac of 6-month slow-release 18-6-12)
- In-season fertilizer (all plots)
  March-Aug. 45-19-51 lbs/ac

Bed top application!

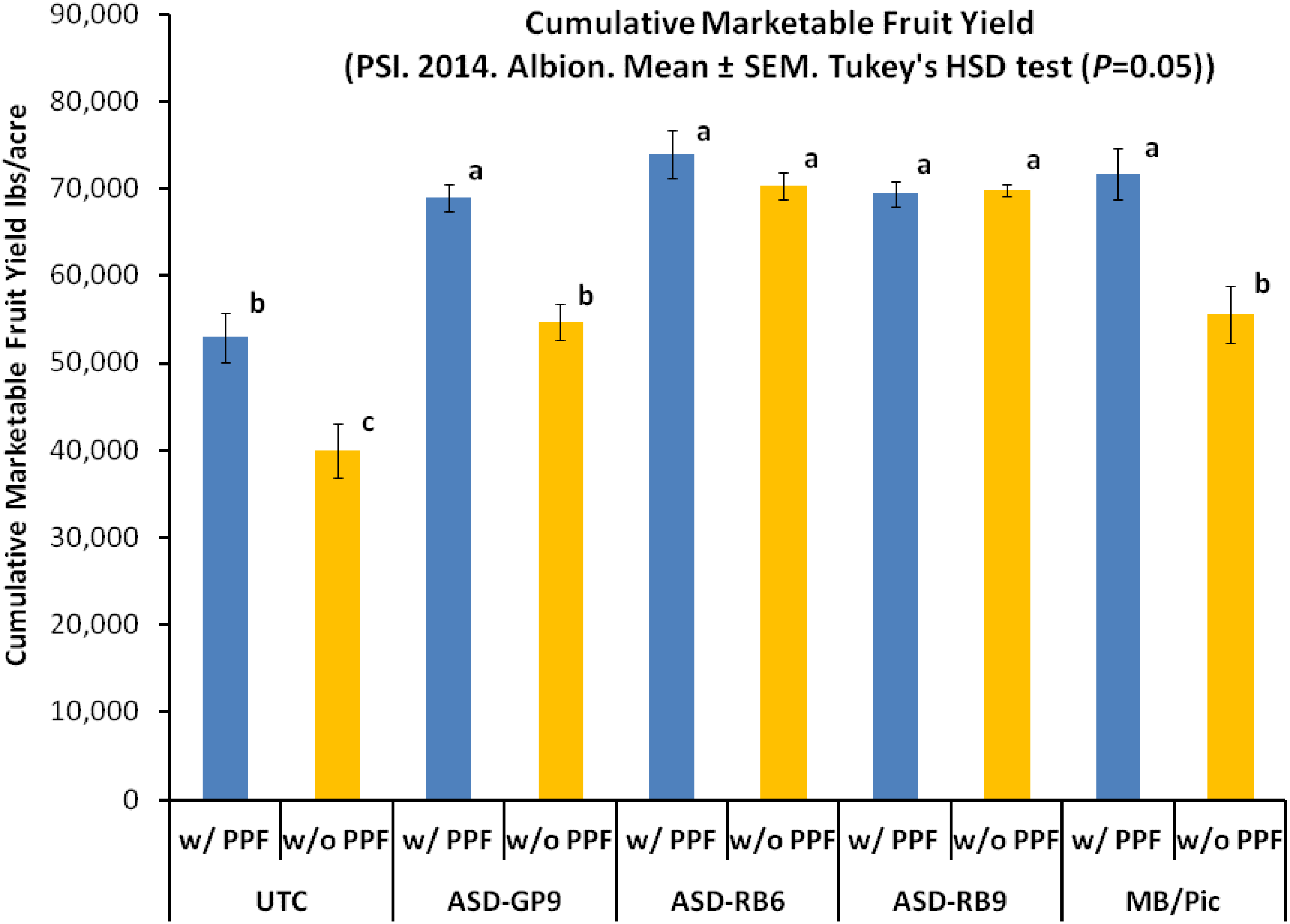
Albion plants

Ground Dry Grape Pomace
(grape skin + seeds)

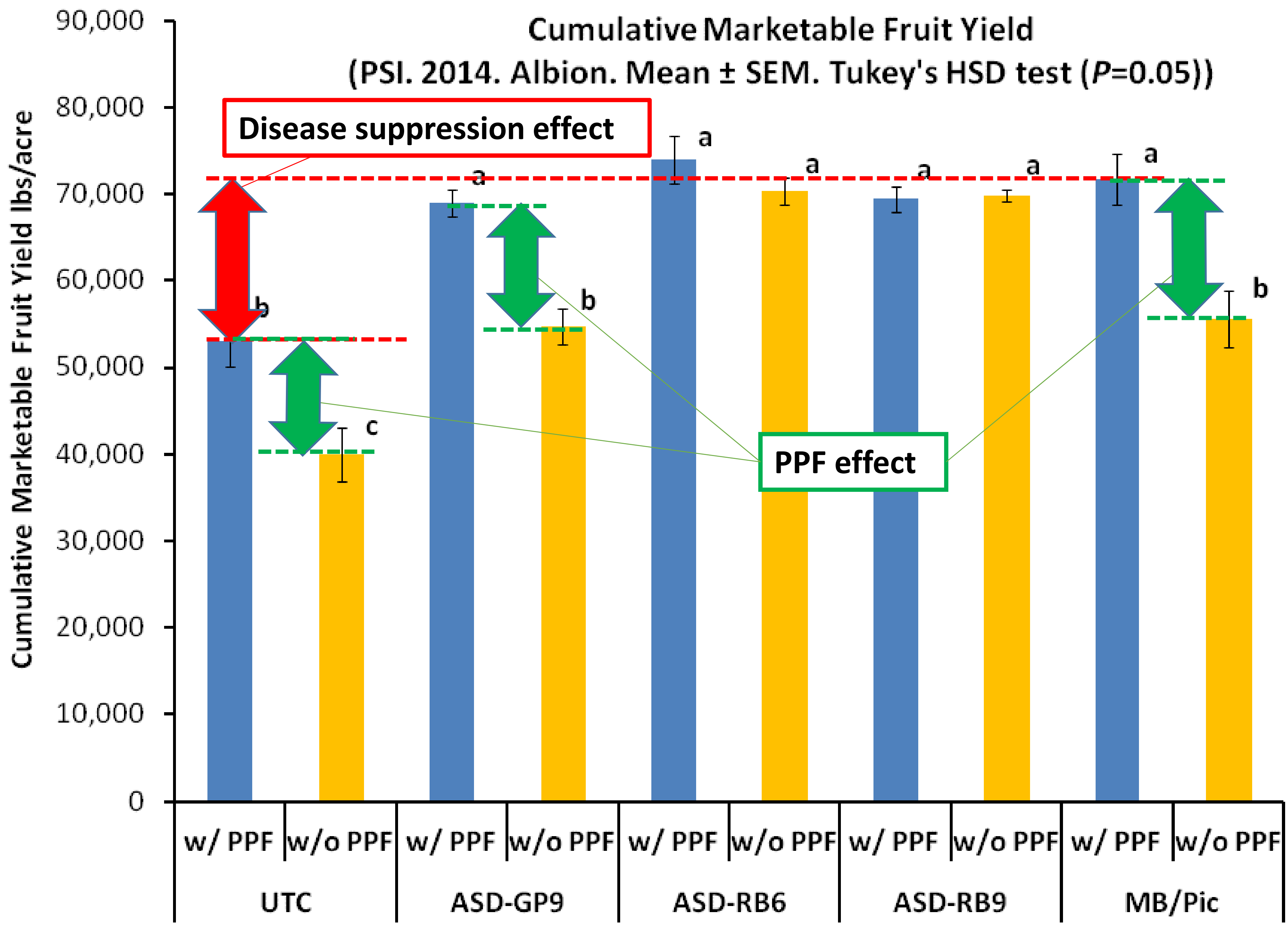
Rice Bran

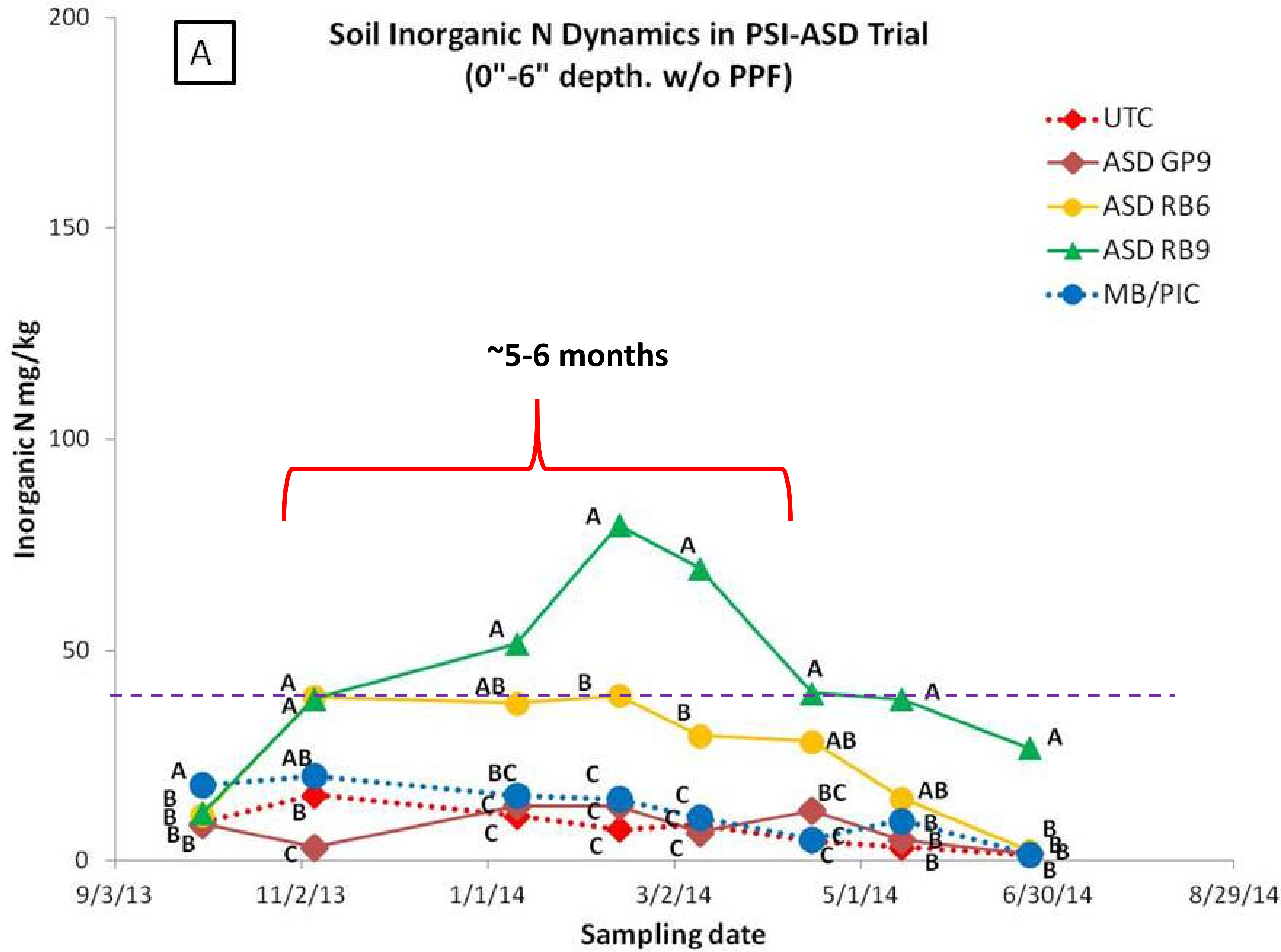
N:2.1%, C:49%, C/N:23

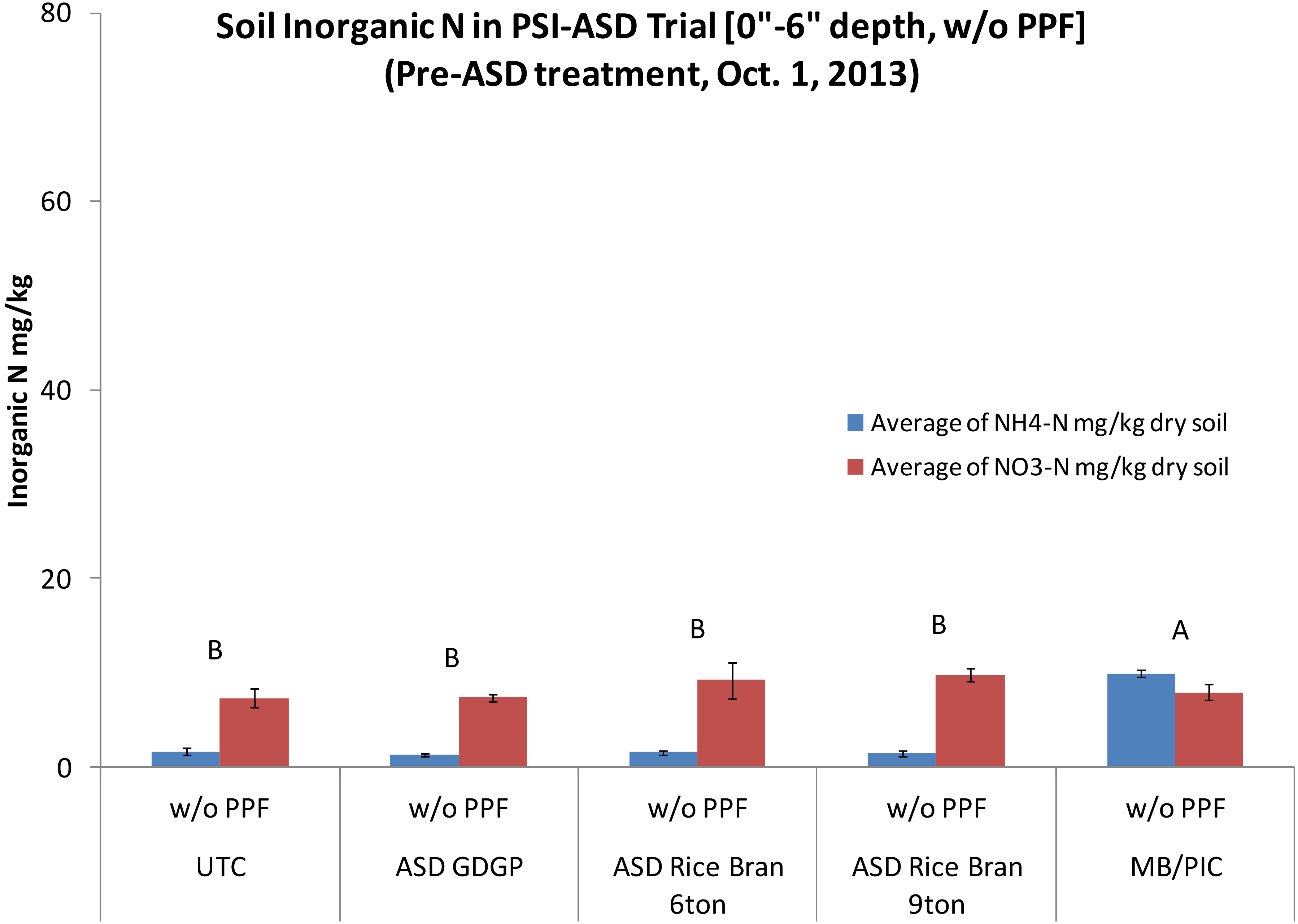
N:2.3%, C:41%, C/N:18



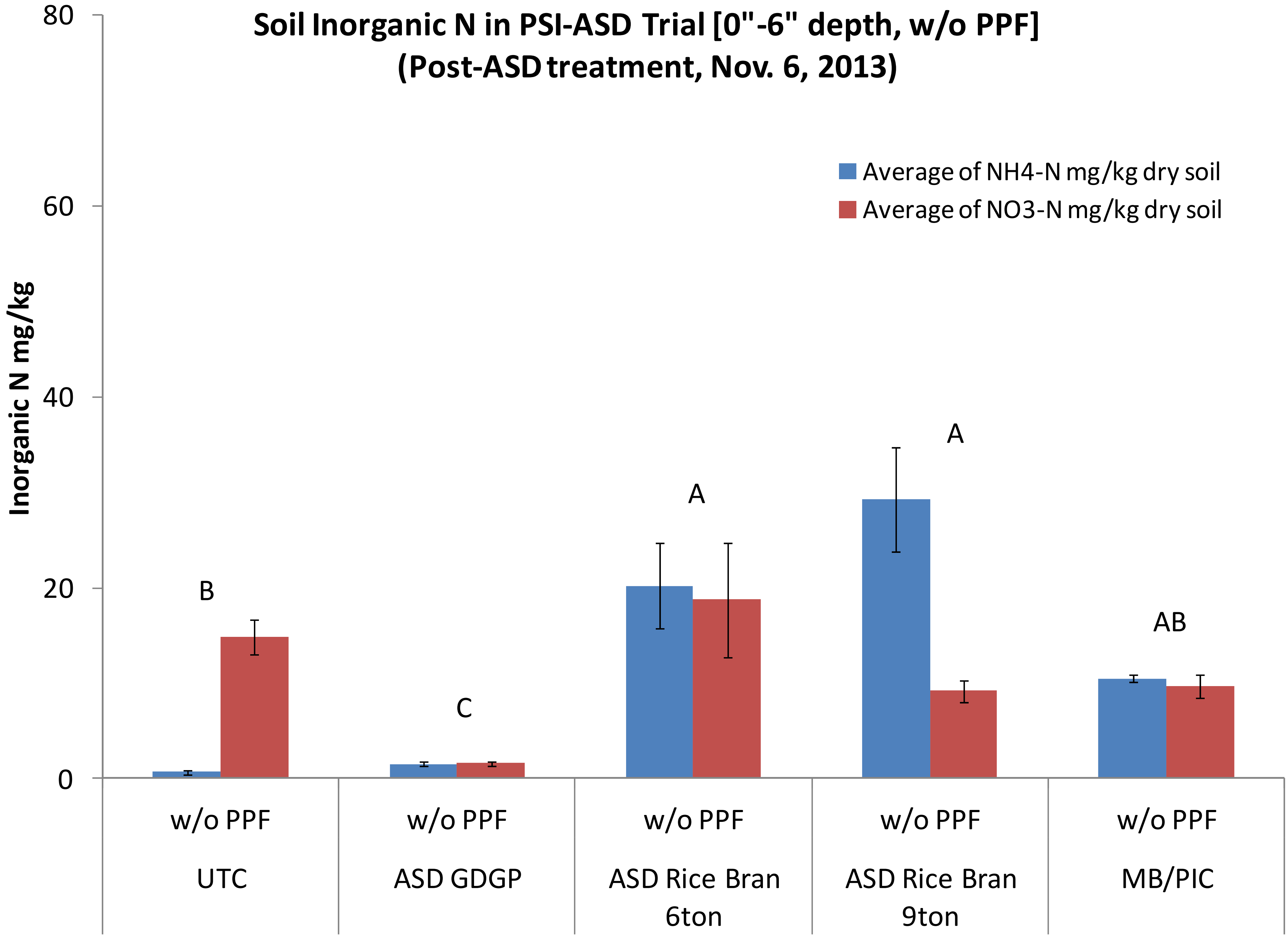
Cumulative Marketable Fruit Yield
(PSI. 2014. Albion. Mean ± SEM. Tukey's HSD test (P=0.05))
Cumulative Marketable Fruit Yield lbs/acre
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
b
c
a
b
a
a
a
a
a
b
w/ PPF
w/o PPF
UTC
ASD-GP9
ASD-RB6
ASD-RB9
MB/Pic

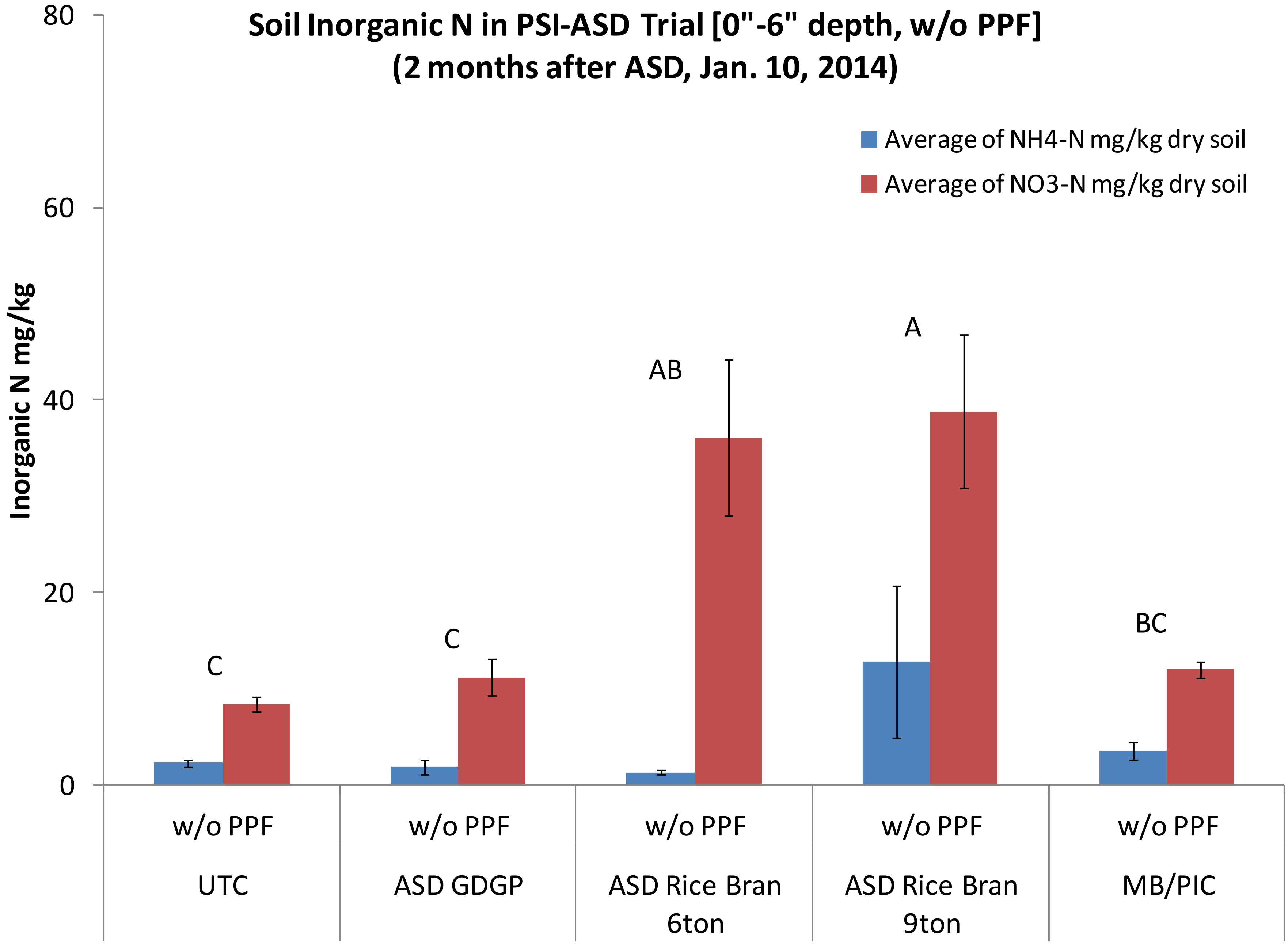

Cumulative Marketable Fruit Yield
(PSI. 2014. Albion. Mean ± SEM. Tukey's HSD test (P=0.05))
Cumulative Marketable Fruit Yield lbs/acre
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
Disease suppression effect
PPF effect
a
b
c
w/ PPF
w/o PPF
UTC
ASD-GP9
ASD-RB6
ASD-RB9
MB/Pic

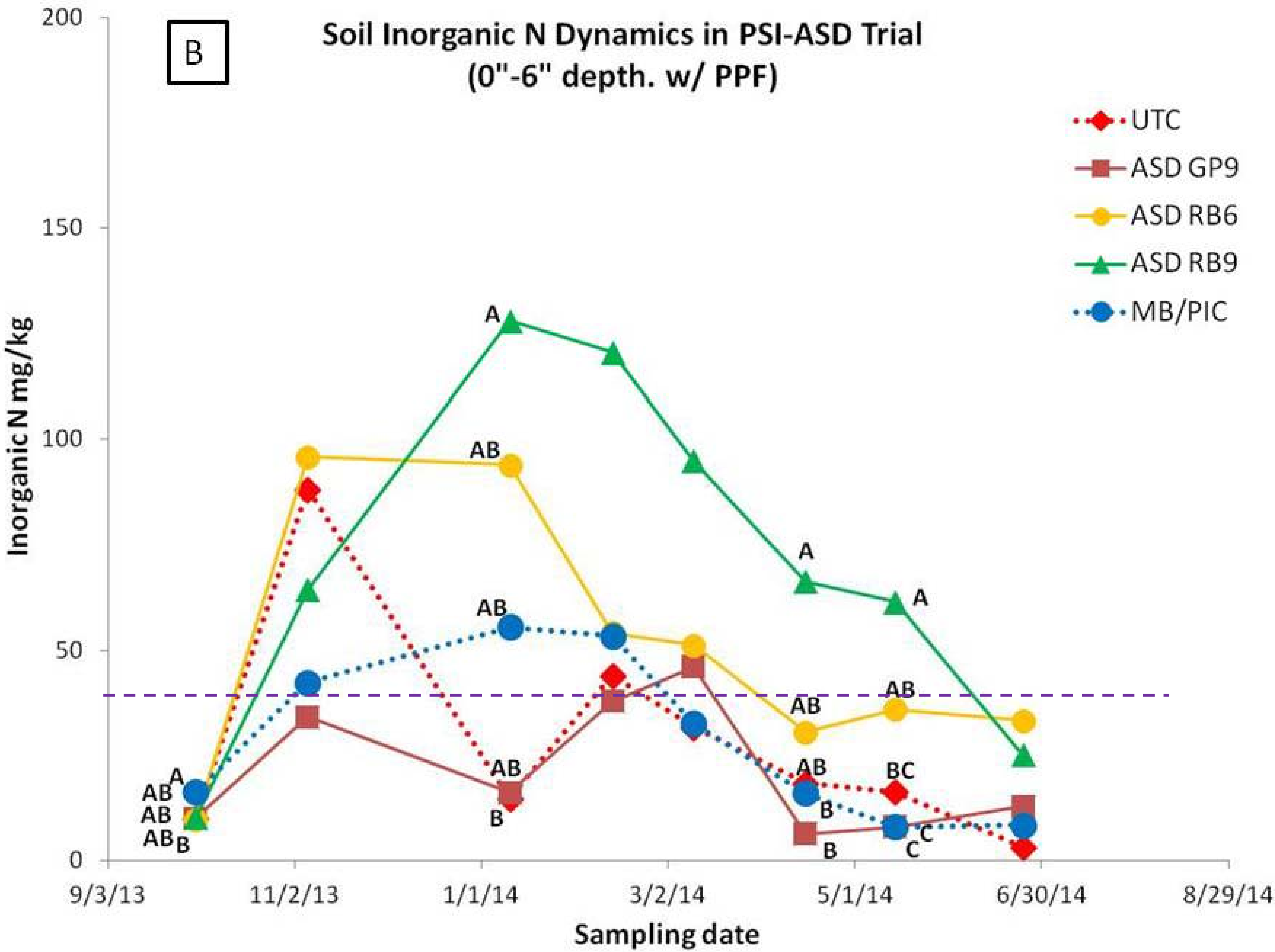

A
Soil Inorganic N Dynamics in PSI-ASD Trial
(0"-6" depth. w/o PPF)
UTC
ASD GP9
ASD RB6
ASD RB9
MB/PIC
~5-6 months
Inorganic N mg/kg
Sampling date
200
150
100
50
0
9/3/13
11/2/13
1/1/14
3/2/14
5/1/14
6/30/14
8/29/14



Soil Inorganic N in PSI-ASD Trial [0"-6" depth, w/o PPF]
(Pre-ASD treatment, Oct. 1, 2013)
Inorganic N mg/kg
80
60
40
20
0
Average of NH4-N mg/kg dry soil
Average of NO3-N mg/kg dry soil
B
B
B
B
A
w/o PPF
UTC
w/o PPF
ASD GDGP
w/o PPF
ASD Rice Bran 6ton
w/o PPF
ASD Rice Bran 9ton
w/o PPF
MB/PIC


Soil Inorganic N in PSI-ASD Trial [0"-6" depth, w/o PPF]
(Post-ASD treatment, Nov. 6, 2013)
Average of NH4-N mg/kg dry soil
Average of NO3-N mg/kg dry soil
Inorganic N mg/kg
80
60
40
20
0
B
C
A
A
AB
w/o PPF
UTC
w/o PPF
ASD GDGP
w/o PPF
ASD Rice Bran 6ton
w/o PPF
ASD Rice Bran 9ton
w/o PPF
MB/PIC


Soil Inorganic N in PSI-ASD Trial [0"-6" depth, w/o PPF]
(2 months after ASD, Jan. 10, 2014)
Average of NH4-N mg/kg dry soil
Average of NO3-N mg/kg dry soil
Inorganic N mg/kg
80
60
40
20
0
C
C
AB
A
BC
w/o PPF
UTC
w/o PPF
ASD GDGP
w/o PPF
ASD Rice Bran 6ton
w/o PPF
ASD Rice Bran 9ton
w/o PPF
MB/PIC

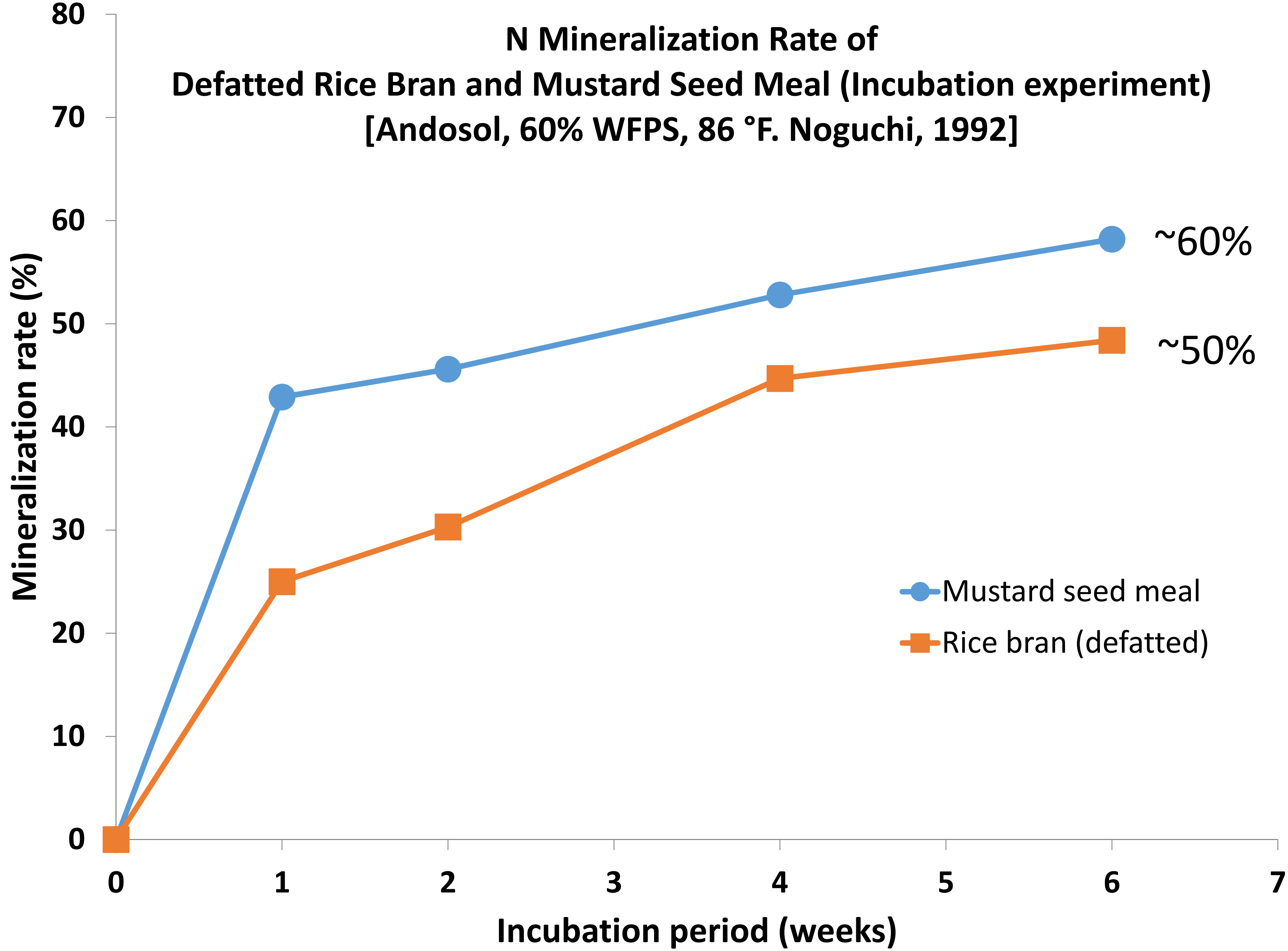


B
Soil Inorganic N Dynamics in PSI-ASD Trial
(0"-6" depth. w/ PPF)
UTC
ASD GP9
ASD RB6
ASD RB9
MB/PIC
Inorganic N mg/kg
Sampling date
200
150
100
50
0
9/3/13
11/2/13
1/1/14
3/2/14
5/1/14
6/30/14
8/29/14
A
AB
AB
AB
B
AB
AB
AB
B
A
A
A
AB
AB
AB
BC
B
B
C
C

Estimated Mineral N Input

Treatment	PPN total N lbs/ac	PPN minrl. rate %	PPN minrl. N est. lbs/ac	In-season N lbs/ac	Total minrl. est. N lbs/ac	Mrkt. Fruit yield lbs/ac (%)
ASD GP9 w/o PPF*	378	0	0	45	45	54,698 (76)
ASD GP9 w/ PPF*	378 + 117	0 + 100	0 + 117	45	162	69,024 (96)
ASD RB6 w/o PPF*	276	50	138	45	183	70,435 (98)
ASD RB9 w/o PPF*	414	50	207	45	252	69,871 (97)
MB/Pic w/PPF*	117	100	117	45	162	71,741(100)

* PPF: Pre-plant fertilizer. 650 lb/ac of 6-month slow-release 18-6-12


N Mineralization Rate of
Defatted Rice Bran and Mustard Seed Meal (Incubation experiment)
[Andosol, 60% WFPS, 86 °F. Noguchi, 1992]
Mineralization rate (%)
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Incubation period (weeks)
~60%
~50%
Mustard seed meal
Rice bran (defatted)

Estimated Mineral N Input

Treatment	PPN total N lbs/ac	PPN minrl. rate %	PPN minrl. N est. lbs/ac	In-season N lbs/ac	Total minrl. est. N lbs/ac	Mrkt. Fruit yield lbs/ac (%)
ASD GP9 w/o PPF*	378	0	0	45	45	54,698 (76)
ASD GP9 w/ PPF*	378 + 117	0 + 100	0 + 117	45	162	69,024 (96)
ASD RB6 w/o PPF*	276	50	138	45	183	70,435 (98)
ASD RB9 w/o PPF*	414	50	207	45	252	69,871 (97)
MB/Pic w/PPF*	117	100	117	45	162	71,741(100)

* PPF: Pre-plant fertilizer. 650 lb/ac of 6-month slow-release 18-6-12

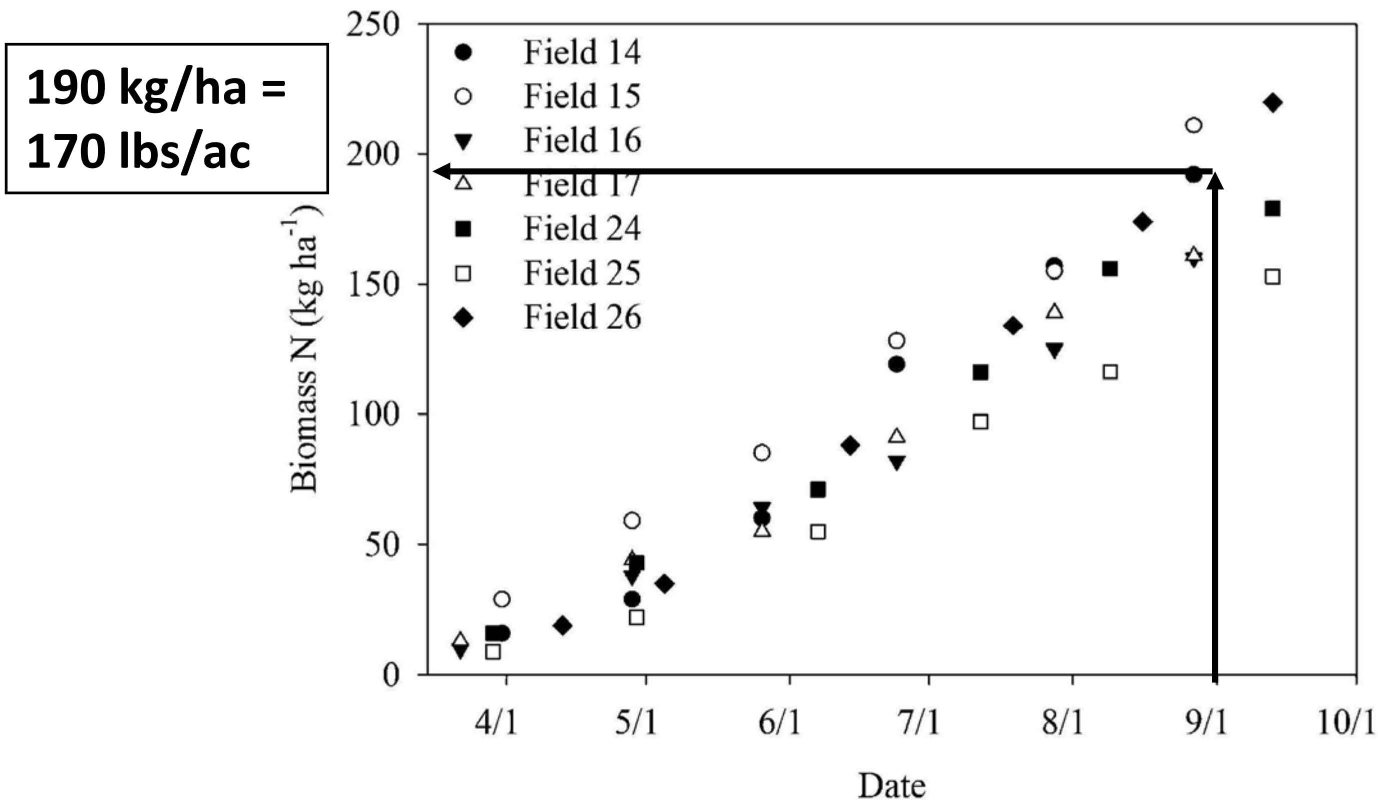


Fig. 2. Aboveground crop biomass nitrogen (N) accumulation (vegetative tissue and marketable fruit) over the growing season in seven strawberry fields; measurements made in 2010 and 2011.

(Bottoms et al., 2013)

Summary

1. ASD with rice bran 6 t/ac worked well without sacrificing fruit yield and having excess soil inorganic N
2. Ground dry grape pomace 9 t/ac worked but only with pre-plant fertilizer
3. Pre-plant fertilizer was not necessary when rice bran 6 to 9 tons/ac was used
4. All above have to be examined in flat rice bran application/incorporation systems
5. NH_4-N dominates immediately after ASD, then gradually nitrified
6. ~40 mg/kg of soil inorganic N (0”-6” depth) until April to May was sufficient to achieve the highest yield
7. For N balance in fields using organic amendments, mineralization rates of materials have to be considered

ASD: On-going Studies/Challenges

- Controlling emerging diseases caused by *Fusarium oxysporum* and *Macrophomina phaseolina*
- Reducing N input from C-sources
  - Cover crop + Low rate of rice bran
  - Revisit molasses mixes
- Evaluating environmental impacts
  - Greenhouse gas emission, nitrate leaching, phosphorus accumulation
- Ineffective in heavy soils?
  - Large clods in beds prevent development of anaerobic condition
- Understanding biological mechanisms
  - Changes in functional diversity of soil microorganisms?

Acknowledgements

We gratefully acknowledge funding for this work from the following:

- USDA NIFA MBTP Award # 2012-51102-20294
- USDA WSARE Award # SW11-116
- Organic Farming Research Foundation

And the many growers, extension and industry people who have made this work possible

- Gary Tanimura, Glenn Noma, Tanimura and Antle Fresh Foods Inc.
- Liz Mirazzo, Andy Webster of CASFS, UCSC
- Luis Rodriguez, Patti Wallace, Mike nelson, Plant Science inc.
- K. Kammeijer, L. Murphy, P. Ayala, UCCE
- Lab assistants, interns, and volunteers of the Shennan lab, UCSC

Questions?

joji@ucsc.edu