Enhancing soil quality, plant health, and disease management in organic production with Brassica cover crops used as biofumigants

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Abstract/Summary: Brassica cover crops are cool season species that fit nicely into many crop rotation systems, especially where there is a short growing season (as in the North Central region of the U.S.). These cover crops produce natural antimicrobial compounds (glucosinolates) that suppress soilborne pathogens and weeds, and they have been shown to change soil biology through selective activity on specific soil microorganisms. Unfortunately, their performance has been inconsistent, and can be negative, with several extreme cases of 100% crop-stand reduction. To better understand the cover crops' impact in organic production systems, studies were conducted at an MSU research field and Michigan organic farmer's field to (1) Determine the role of native soil biology of the Brassica cover crops and subsequent cash crop performances, (2) Determine the role of allelopathy (plant growth inhibition due to natural compounds produced by the cover crops) on the performance of Brassica cover crops in cropping systems and identify safe plant back periods for susceptible crops, (3) Determine the change in populations of major soilborne pathogens and soil microbial activity following Brassica biofumigation, (4) Quantify the nutrient cycling potential of Brassica cover crops for use in crop nutrient management programs, and (5) Deliver the results and observations to growers through extension activities.

Data collected from 2007 to 2013 show that the cover crops are phytotoxic and could affect cash crops if the cash crop is planted soon after cover crop incorporation. Environmental conditions that prevent adequate biomass production (cool and wet weather, insect damage) reduce the biofumigation effects of the cover crops. Adequate soil moisture during cover crop incorporation is critical to avoid loss of isothiocyanates. The choice of brassica variety that is used as a cover crop affects biomass production and efficacy of biofumigation. Finally, brassica cover crops should fit into the whole farm crop rotation planning and should be avoided when there are brassica cash crops included in the rotation system.

Introduction

Cover crops in the Brassicaceae (brassica) family such as oilseed radish, oriental mustard, and yellow mustard have been shown to decrease plant pathogen populations in the soil (Sarwar et al., 1998). Cover crops in general provide a variety of additional benefits in a production system: they reduce erosion, aid nutrient cycling, preserve soil quality, and suppress weeds (Mutch and Snapp, 2003). Use of brassica species in crop rotations could be one of the practices that may help gain these benefits.

Brassica cover crops that possess compounds inhibitory to microorganisms and weeds may also be phytotoxic to cash crop seeds. Haramoto and Gallandt (2005), determined that brassica cover crops decreased cash crop emergence by 23%-34% and delayed germination for two days, though their impact on germination was similar to that of other short-season cover crops such as red clover. Laboratory experiments have shown that compounds produced by brassica cover crops can inhibit seed germination (Norsworthy and Meehan, 2005a, 2005b; Norsworthy et al., 2006). Studies with black mustard (*Brassica nigra)* aqueous extracts have shown that extracts inhibit germination and growth of lentils (*Lens culinaris*) and wild oats (*Avena fatua*) (Turk and Tawaha, 2002, 2003). Phytotoxicity would typically be a problem in production systems where the cover crop is tilled under and another crop is immediately planted, as in a muskmelon/eggplant (*Solanum melongena*), wherein the cover crop is planted in April and tilled under in late May. It is for this reason that cover crop management recommendations include allowing adequate time between cover crop incorporation and sowing a crop.

Brassica cover crops produce multiple classes of compounds that have allelopathic properties. The primary class of interest is glucosinolates, which when degraded by hydrolysis produce biologically-active compounds called isothiocyanates (ITCs) (Kirkegaard and Sarwar, 1998). Some of these glucosinolate by-products are water-soluble, while some are highly volatile (Brown and Morra, 1996). Other compounds from glucosinolate hydrolysis that may be allelopathic are organic cyanides and oxazolidinethione (Brown and Morra, 1996).

Allelopathy is not the only mechanism by which brassica cover crops could impact germination. Cohen and Mazzola (2006) and Hoagland et al. (2008) have demonstrated that some low-glucosinolate canola (*Brassica napus*) seed meals can induce a shift in populations of soil microbe communities, leading to elevated *Pythium* spp. populations.

Because of their allelopathic effects, brassica cover crops have the potential to be a valuable tool in vegetable cropping systems. However, more information is needed on their impact on cash crops (especially those that are direct-seeded) to ensure they are safe tools to use in vegetable production. The main objectives of this study are to:

1. *Determine the role of native soil biology on the performance of Brassica cover crops and subsequent cash crop performance.*
2. *Determine the role of allelopathy (plant growth inhibition due to natural compounds produced by the cover crops) on the performance of Brassica cover crops in cropping systems and identify safe plant back periods for susceptible crops.*
3. *Determine the change in populations of major soilborne pathogens and soil microbial activity.*
4. *Quantify the nutrient cycling potential of Brassica cover crops for use in crop nutrient management programs.*
5. *Deliver the information to growers through extension activities.*

Methods and Materials

Laboratory experiments

Bioassay with cover crop residue

Bioassay tests using oilseed radish tissue were conducted in the laboratory. Oilseed radish plants collected from a grower field were rinsed with tap water, then with deionized water to remove soil, air-dried for 1 d at room temperature (23˚ C), and weighed. Roots were separated from shoots and processed separately. The tissue was processed in a commercial grade blender with deionized water (1 L to 1 kg biomass) for 90 to 120 s. The resulting extracts were strained through cheesecloth. Both extracts were then filtered through Whatman #4 filter paper. The extract was diluted with deionized water to make solutions of 0%, 5%, 12.5%, 25%, 50%, and 100% strength. Deionized water was the control. Crops tested were 'Journey' cucumber, 'Athena' muskmelon, and 'Earlibrew' honeydew. Ten seeds of each crop were placed in 10-cm petri dishes on Whatman #4 filter paper, and then 3.0 mL of each extract dilution was placed into each petri dish. Dishes were sealed with Parafilm. Seeds were incubated in a Conviron growth chamber at 21°C in the dark for 6 d.

DNA extraction

Soil samples were collected during the growing season from each of the replicated treatments of the field experiments described below. Soil samples were placed into separate plastic bags, transported to the laboratory on ice, and stored at 4°C pending further analysis.

Subsequently, total genomic DNA was extracted from half a gram of soil from each composite soil sample using the FastDNA SPIN Kit for Soil (MP Biomedicals LLC, Solon, OH) according to the manufacturer's instructions. DNA samples were stored at -20°C until analysis.

Polymerase chain reaction and restriction fragment length polymorphism

Polymerase chain reaction (PCR) was conducted, with primer 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1387R (5'-GGG CGG WGT GTA CAA GGC-3'), universal primers for amplification of the 16S rDNA gene region in bacteria. Each PCR reaction had total volume of 25 µl, containing 5 U Taq DNA polymerase, 5 × Taq polymerase PCR buffer (Promega, Madison, WI, USA), 200 mM dNTP mixture, 0.2 mM of each primer, and 1 µl (2 to 25 ng/µl) of template DNA. Amplifications were performed on a BioRad i-cycler thermocycler (Hercules, CA) with an initial denaturation step of 5 min at 94°C, followed by 36 cycles of 40 s at 94°C, 40 s at 58°C, and 1.5 min at 72°C, and a final extension of 7 min at 72°C. PCR products were resolved by electrophoresis on 1.5% (w/v) agarose gel. Restriction fragment length polymorphism (RFLP) of PCR products for DNA fingerprinting of microbial communities was performed with BstUI restriction enyzme (New England Biolabs Inc, Ipswich, MA) following the manufacturers instructions. Subsequently restriction digest products were resolved by electrophoresis on 2.5% (w/v) agarose gel.

Field experiments

Field studies experiments were conducted from 2011 to 2013 at two locations in Michigan.

Field Experiment No1. Experiment station
This experiment was conducted at the Horticulture Teaching and Research Center (HTRC) of Michigan State University. The experiment tested the impact of biofumigation with brassica cover crops and anaerobic soil disinfestation. The experiment was designed as a factorial with two factors: cover crop (Oilseed radish, Oriental mustard, yellow mustard and bare ground control), and anaerobic soil disinfestation (standard polyethylene mulch and virtually impermeable film-VIF). In spring 2011 and 2012, the cover crops were seeded in the respective plots. At flowering stage, the cover crop were flailed and incorporated immediately. Raised beds covered with black plastic mulch were formed after cover crop incorporation. Soil samples were collected in each treatment before cover crop incorporation and at two-week intervals following cover crop incorporation, and analyzed for microbial biomass. Cucumber and tomatoes were used as cash crops.

Field Experiment No 2. Experiment station

This experiment was conducted at the Southwest Research and Extension Center (SWMREC) in Benton Harbor, MI following the procedure outlined in 2011. A new site was used in 2012 to address the issue of proper crop rotation. Several Brassicaceae cover crops were compared to the control to evaluate their biofumigation potentials. These include, yellow mustard 'Tilney' and IDA Gold'; oriental mustard 'Forage' and 'Pacific Gold'; and oilseed radish 'Defender'. A cover crop of oat was also included in 2012 to help separate the allelopathic effects from glucosinolates degradation in brassica species from the simple biomass effect.

Cover crops were drilled at each species respective rates, yellow mustards at 8 lbs/A, oriental mustards at 6 lbs/A, and oilseed radish at 10 lb/A. Cover crops were flail mowed, then rototilled to incorporate; after cover crop incorporation plastic mulch beds were placed on 5.5 foot centers, the beds ran perpendicular to cover crop plots, in effect catching a 30 foot cover crop cross-section. Muskmelon, 'Athena', was direct seeded at 0, 5, 10, 15, 20, 25 days after incorporation (DAI), respectively, to measure feedback inhibition of ITC production. To address the issue of dry soil conditions that limited the effects of the cover crops in 2012, the site received overhead irrigation the day prior to cover crop incorporation.

Field data collection consisted of cover crop stand counts and biomass weight; muskmelon stand germination counts, and yield..

Soil populations of major soilborne pathogens and beneficial microorganisms following Brassica crops were analyzed. Groups of microorganisms were analyzed on semi-selective media, including general fungi on rose Bengal agar, general bacteria on tryptic soy agar (TSA), Trichoderma spp. THS medium, florescent pseudomonads on S1, *Bacillus* spp. on TSA, *Phytophthora* spp. and *Pythium* spp. on PARP. The colonies of microorganisms were enumerated.

Fig. 1. Studies on biofumigant cover crops in summer 2012 at Michigan State University. Left: Spring planted brassica cover crops at flowering stage and ready for incorporation. Right: Simultaneous flail mowing and incorporation of the cover crops to maximize the biofumigation effects

Fig. 2. Effect of Brassica cover crop residue on melon stand at the SWMREC site in 2012. Left- Emergence of 'Athena' muskmelon seeded 0 days after cover crop incorporation (DAI) of the cover crop *B. juncea cv*. 'Pacific Gold'. Right- Emergence of 'Athena' melon seeded 25 DAI (photo by Aaron Yoder).

On-farm Study

This experiment was conducted in Bangor, MI on a certified organic farm. Treatments were yellow mustard 'Tilney' and IDA Gold'; oriental mustard 'Pacific Gold'; oilseed radish 'Defender' and the control of a bare fallow. All cover crops were managed as indicated above. However, no plastic mulch was used to cover the beds after cover crop incorporation. Cover crop biomass was evaluated prior to incorporation and summer squash was direct seeded about 3 weeks after cover crop termination. This was mainly a demonstration plot with no yield data collected since the grower cooperator managed the entire experiment. This strategy was used on purpose to confirm grower interest in the brassica cover crops.

Fig. 3. On-farm trial. Cover crop seeding by the grower cooperator, in Bangor, MI. 2011.

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Fig. 4. Flea beetle damage on Oilseed radish 'Defender' or Oriental mustard 'Pacific Gold' (right) are more prominent compared to damage on yellow mustard 'Ida Gold' (left).

Fig. 5. Due to flea beetle damage yellow mustard 'Ida Gold' (left) was the only cover crop with good stand at the on-farm study in Bangor, 2011.

At the end of the season a meeting was organized with the grower cooperator to access project outcomes and major findings. The discussion centered around time commitment with the practice of cover cropping, impacts on soil quality, impacts on diseases, insects, and weeds, and future research interests.

Results

Laboratory experiments

Bioassay studies with cover crop residue

Both root and shoot aqueous extracts significantly reduced germination of honeydew, muskmelon, and cucumber (Fig. 6). As concentration of the extract increased from 0% to 100%, inhibition became more pronounced. Whereas 100% of seeds germinated at 0% extract concentration, at 100% extract concentration germination rates ranged from 0% to 3.4%. Muskmelon germination was not affected by root and shoot extracts of oilseed radish at low concentrations.

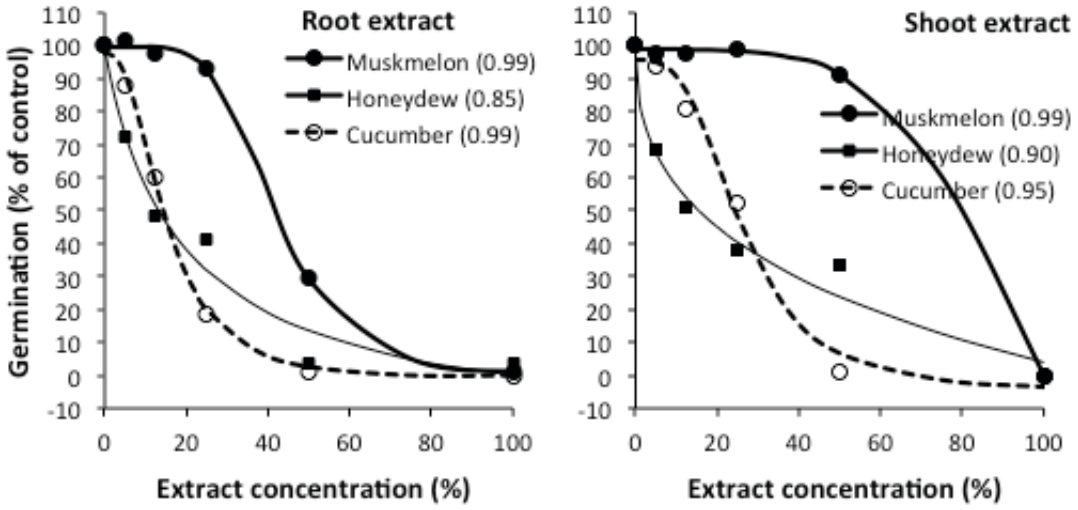

Fig. 6. Germination percentages of three cucurbit crops exposed to six extract concentrations oilseed radish root and shoot aqueous extracts. Germination was expressed as a percentage of the value obtained for the control to allow for comparison among crops.

Total genomic DNAs were all extracted, purified. These DNAs are currently being analyzed using PCR technique. Soil populations of some groups of microorganism were enumerated on agar media and the result is expected to come in two months.

DNA extraction

Approximately 150 soil samples from the HTRC and SWMREC were successfully processed for DNA extraction. The quality and quantity of the soil DNA extractions were determined using a NanoDrop (Cole Parmer Inc. Vernon Hills, IL). The DNA samples concentrations were standardized and various dilutions were tested using PCR analysis to optimize the subsequent reactions (data not shown). It was established that a 1:10 dilution was the optimum for PCR analyses of DNA from soil samples (Fig. 7).

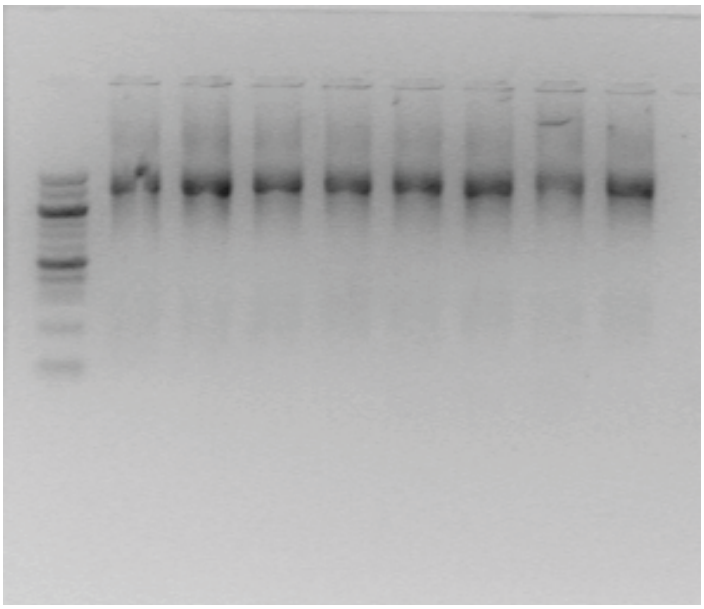

Fig. 7. Gel electrophoresis [1.5% (w/v) agarose gel] of selected DNA samples at a 1:10 dilution after PCR analysis using with primer 63F and 1387R, universal primers for amplification of the 16S rDNA gene region in bacteria.

Polymerase chain reaction and restriction fragment length polymorphism

A total of 66 soil samples from the SWMREC field trial were successfully analyzed using PCR amplification of the 16S rDNA gene region (Fig. 8). The resulting samples showed differing DNA RFLP fingerprint profiles, which indicates polymorphic differences among soil communities from the different treatments (Fig. 9). The results are promising; the remaining samples DNA samples from the HTRC field treatments will be analyzed using PCR-RFLP. Subsequently all DNA samples from both field trials will be analyzed using next-generation sequencing technologies to achieve finer scale characterization of soil microbial communities among field treatments.

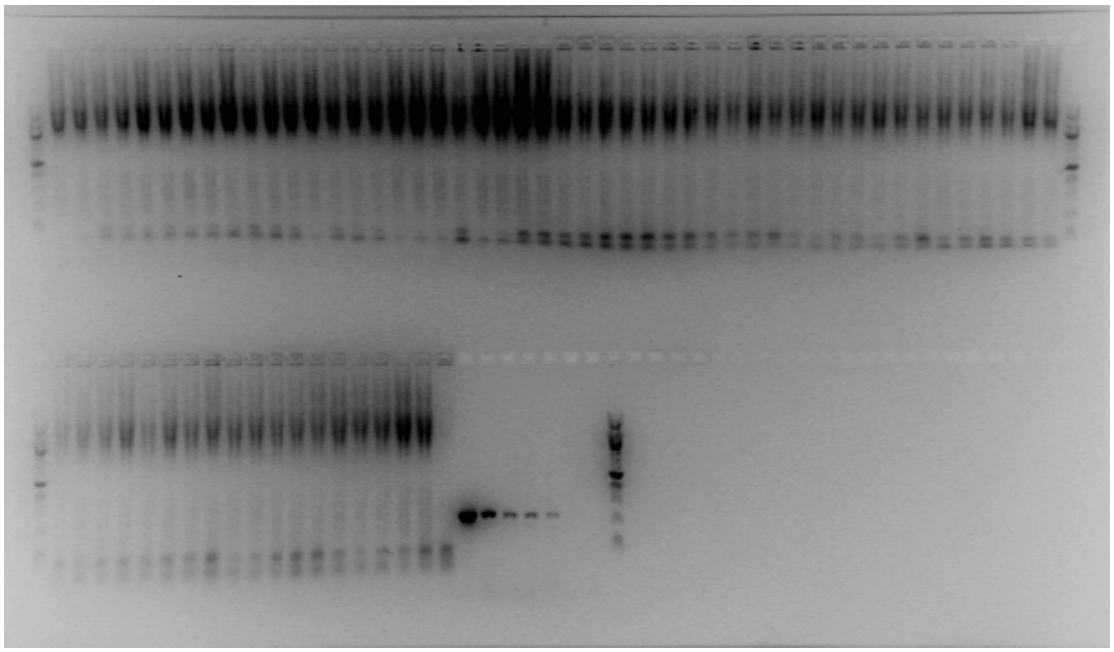

Fig. 8. Gel electrophoresis [1.5% (w/v) agarose gel] of SWMREC DNA samples at a 1:10 dilution after PCR analysis using with primer 63F and 1387R, universal primers for amplification of the 16S rDNA gene region in bacteria.

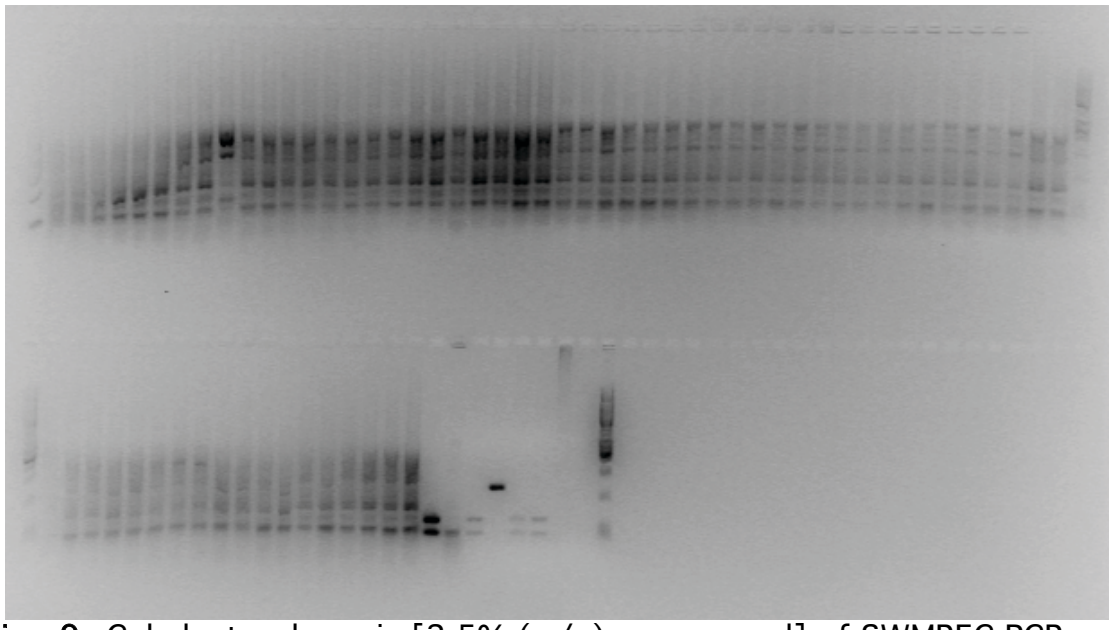

Fig. 9. Gel electrophoresis [2.5% (w/v) agarose gel] of SWMREC PCR reactions dilution after RFLP analysis using BstUI restriction enzyme.

Field experiment#1

Crop germination: Unlike 2011, brassica cover crop residue affected muskmelon seed germination and emergence in 2012 (Fig. 10). The lack of effect in 2011 was attributed to environmental conditions that reduced cover crop biomass production and dry soil conditions at the time of cover crop incorporation. In 2012, the plot was irrigated prior to cover crop incorporation. The greatest inhibitory effects were observed when melon was seeded immediately after cover crop incorporation. Cover crop residue toxicity declined over time and about two weeks after incorporation have released a majority of their toxin, making it feasible to plant the crops after this time.

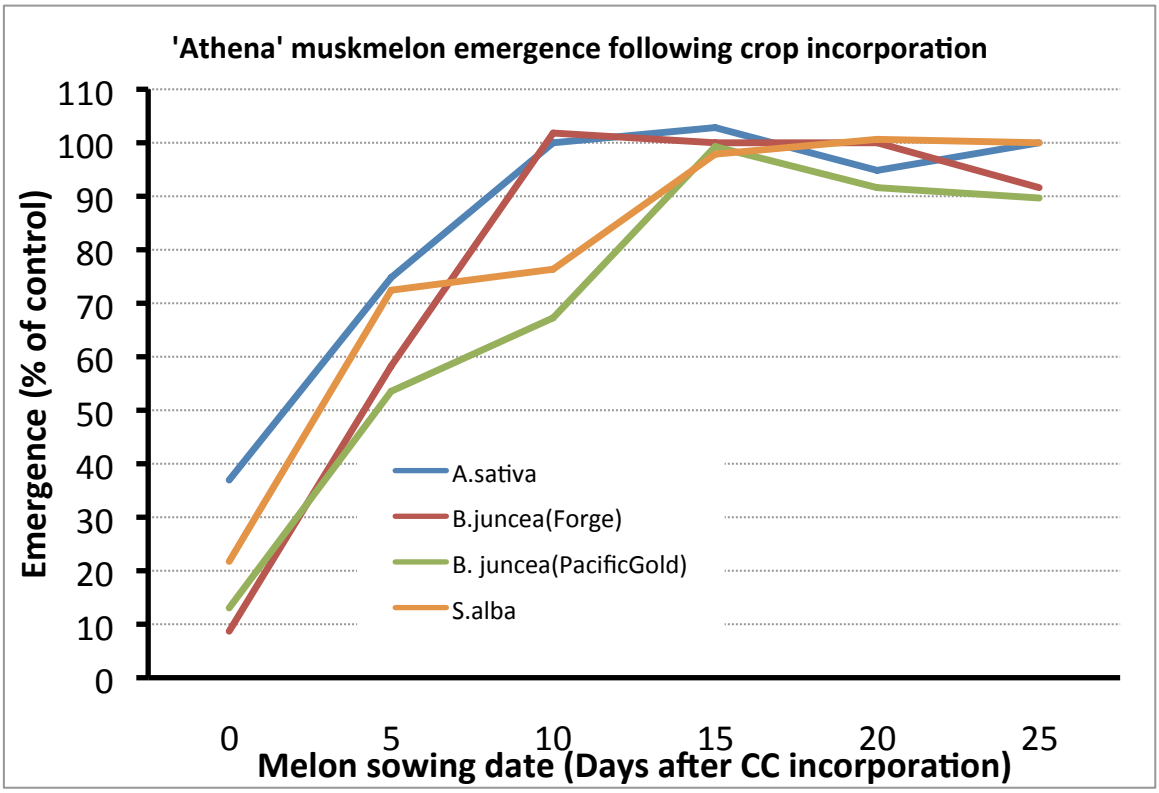

Fig. 10. Effect of Brassica cover crop residue on melon emergence (%) at the SWMREC site in 2012.

Field experiment#2

Anaerobic disinfestation: The cover crops were planted on April 2nd and 4th 2012, in Benton Harbor and East Lansing, respectively. Cover crops were flail-mowed and incorporated on May 31st and June 4th 2012 at flowering. The study was repeated in 2013. Subplot treatments included bare ground, conventional black plastic and virtually impermeable film (VIF). Soil CO_2 measurement five days after cover crop incorporation showed about five and ten-fold increase in soil CO_2 concentration for the conventional and VIF mulches, respectively compared to bare ground. Soil temperature also increased rapidly under VIF film. These preliminary data suggest that combining spring-planted brassica cover crops with anaerobic soil disinfestation could minimize losses in vegetables in our region.

Cover crop biomass: Although seeding methods seemed to improve cover crop stand establishment in 2013, in both years dry weight biomass of cover crops was substantially lower than anticipated (Table 1). Previous studies using spring seeded brassica cover crops in southern Michigan have demonstrated biomass yields of 6068, 3641 and 3487 kg/ha for oilseed radish, Oriental, and yellow mustard respectively (Ackroyd et al., 2011), a notable difference from the 2291, 1235 and 1133 kg/ha generated in this study (Table 1). Cultivars like 'Pacific Gold' are noted to be quite sensitive to day-length and can begin to flower before substantial biomass has accumulated (Snapp et al., 2006). Oilseed radish accumulated the greatest quantity of biomass for the brassicas (Table 1) while yellow and oriental mustards had the lowest in both years. Among all of the cover crops seeded, oats accumulated the most biomass in 2012, and had substantially lower mean biomass in 2013, although biomass was quite variable from plot to plot (Table 1).

Redox potential and soil gas monitoring during ASD: Establishing anaerobic conditions proved to be more challenging than anticipated. Of the 32 sensors installed in the field, only three reported Eh values below the CEh (182-198mV): two were in VIF+M plots (35-10,513 mVh beneath CEh), and another was under VIF (268 mVh beneath CEh). Also, it is worth noting that these sensors were also all placed within the cover crop treatments which may have contributed to the lower Eh from the added biomass. Although biomass estimates from the yellow mustard plot were lower than anticipated (1063 kg/ha), in a greenhouse study, Butler et al. (2011) showed that similar rates of cover crop biomass produced high cumulative anaerobicity, although the authors noted that anaerobic conditions are often more challenging to establish under field conditions than in greenhouse pot studies (Butler et al., 2011). Other studies have demonstrated that anaerobic conditions can be maintained under field conditions, where mVh beneath the CEh can exceed 50,000 within two weeks depending on soil type, irrigation and plastic characteristics

(Shennan et al., 2010). To attain cumulative anaerobicity closer to those values needed for successful ASD (as determined by previous research) more work could be focused on manipulating irrigation techniques, timing, and evaluating different carbon sources suitable for use in the Michigan climate.

CO_2 concentrations were observed to be much higher under VIF mulch treatments than under standard black plastic during the entirety of ASD confirming that VIF mulch is less permeable than the standard black plastic mulch (Figure 7). The addition of molasses also created substantially higher concentrations of CO_2 under the mulch. N_2O concentrations followed similar patterns where by VIF mulch with molasses generated the highest concentration of N_2O throughout the ASD period. Methods used to determine gas concentrations do not permit quantification of the actual generation of gases over time among plastic mulch treatments (fluxes), but the data suggest that the molasses amendment generated greater quantities of N_2O under VIF mulch (Figure 8). Interestingly, cover crop treatments all diverged from no cover treatments on the sampling date prior to ASD termination (June 17).

Nitrogen dynamics and microbial biomass: Differences in NO_3^- and NH_4^+ concentrations were observed at various times throughout the 2012 and 2013 growing seasons among mulch treatments, while cover crop treatment differences were not significant within each year (α=0.05). Two mulch treatments were monitored in 2012 (NM and VIF), while all 4 were monitored in 2013 (NM, BP, VIF, VIF+M). NO_3^- and NH_4^+ were significantly higher during ASD and the first four (for NO_3^-) and two (for NH_4^+) weeks after transplanting (Fig. 14). For the last four weeks, this trend was reversed where NM treatments sustained significantly higher levels of NO_3^- and NH_4^+, although the magnitude of these differences was less substantial than at earlier sampling dates. Differences in NH_4^+ concentrations were not significant after the first four weeks in 2012 (Fig. 13). In 2013, NO_3^- was significantly higher in VIF and BP treatments than NM and VIF+M during ASD and the first two weeks after transplanting (Fig. 14). This trend was reversed from 7/18-7/31 where NM and VIF+M soils had significantly higher NO_3^- than under BP, and again from 8/14-8/28 NM plots had higher NO_3^- than all other plots. NH_4^+ concentrations were highest in VIF+M treatments for the first two weeks following ASD and the last two weeks of the season. NH_4^+ data proved to be quite variable, particularly under mulched treatments although these differences were significant (α=0.05) on the second and last sampling date in 2013 (Fig. 14), where VIF+M treatments were the highest. Mean microbial biomass carbon and soil respiration were the highest in the VIF+molasses treatments, although differences were not significant (α=0.05) (Fig. 16).

Tomato yields and quality: In 2012 and 2013, cover crop treatments did not have significant effects on tomato yields, while mulching treatments did (Table 2). In 2012, early marketable yields (from first four harvests) were highest in plastic mulch treatments compared with no mulch, while late marketable yields (last four harvests) were significantly higher in no mulch plots than in both black plastic and VIF treatments. While early marketable yields were substantially greater under plastic, the fraction of the total marketable yield accounted for by early yields was substantially less than that of later yields in no mulch (7% vs. 75%), black plastic (15% vs. 55%) and VIF (10% vs. 67%). This demonstrates that the majority of tomato yields are harvested later in the season, a potential trade-off faced by producers wanting to maximize early yields while also attaining high cumulative yields. 2013 marketable yields followed similar trends based on these three mulching treatment, although total marketable yield differences were not significant (α=0.05).

VIF+M treatments yielded significantly less total marketable yields than all other mulch treatments where moderate early and late yields did not compensate overall for the differences in high early (BP, VIF) and high late (NM) yields of the other mulching treatments. Interestingly, total unmarketable yields were greater in VIF treatments than in black plastic in 2012 while in 2013 no significant differences in total unmarketable yields were observed among plastic mulch treatments (although they were significantly greater in NM plots). Several studies have noted that extreme heat under black plastic mulch in causes reductions in total yields of tomato when compared with other plastic (Ngouajio et al., 2005) and organic-residue mulches (Tindall et al., 1991; Teasdale et al., 1995). While black plastic is currently the standard mulch for tomato production in Michigan, increases in early yields may be offset by lower late yields, particularly during warm years as observed in this study. Using plastic or organic mulches that transfer less heat to the root-zone than black plastic mulches might be a management strategy worth adopting by growers in this region.

Cucumber yields : Overall cucumber yields were substantially higher in 2012 than in 2013 across all treatments. In 2012, there was no significant difference among cover crop or mulch treatments on marketable or unmarketable yields (Table 3). However, in 2013, plants mulched with VIF had significantly higher marketable and unmarketable yields. Unlike tomato yields, cucumbers yields were not signifcantly affected by VIF mulch in the unusually warm year of 2012 although mean yields under no mulch treatments were higher than under VIF. Although optimal root-zone temperatures have been cited as being relatively similar to tomatoes at 24-30°C (75-86°F) (Gosselin et al., 1985), it is possible that cucumber leaves shade soil more effectively than tomatoes to

reduce soil heat accumulation, reducing the impact of excessive temperatures on crop yields grown on black plastic mulch. Many of the harvested cucumber fruits in 2012 from the VIF plots had symptoms of heat exposure (white, bleached areas) that were not common in NM plots which contributed to the higher unmarketable yields under VIF. While nitrogen was not monitored under cucumber plots, it seems likely that NO_3^- availability would be higher under cucumber plots as increases in soil temperature under black plastic would increase mineralization and subsequently plant available nitrogen early in the season as was seen in tomato plots; this could help to explain greater yields under VIF in the cooler 2013 season.

On-Farm Study

Volunteer cover crops are usually a major concern among organic growers. In this study, the grower acknowledged that there was no volunteer cover crop in the field. While the use of mustard cover crops required more time and field passes, in general the soil was more ready for planting following the cover crops. He direct seeded summer squash three weeks after the cover crops without any major problem. Weed suppression was observed but as usual not enough to reduce routine weed management practices. The grower indicated a strong interest to see how observations may be impacted by crop rotations, especially crop rotations that do not include brassicas. Also grower concluded that more precise crop rotations should be designed to target specific use of cover crops. Selecting a cover crop with a purpose then designing the crop rotation around this cover species.

The major negative effect of the cover crops was flea beetle infestation. The farm had many brassica cash crops in the rotation. Adding a brassica cover crop especially in a year that favored flea beetle infestation was not good and indeed an excellent lesson for all organic growers. One finding from this situation was the low flea beetles infestation in the yellow mustard 'Ida Gold' compared to Oriental mustard 'Pacific Gold' and oilseed radish 'Defender'. This difference was probably due to the presence of hair-like structures on the leaves and stem of 'Ida Gold'.

For future studies, the grower was interested in using cover crops in perennial crops like asparagus, and testing alternative cover crops and seeding methods (hand vs. earthway vs. slurry seeding).

Table 1. 2012 and 2013 Cover crop seeding rates, mean dry weight cover crop and weed biomass at incorporation, accumulated total N, and residual soil N prior to ASD[1]

Site#2. East Lansing

Cover Crop/ variety	Seeding rate (lbs/ac)	Cover crop biomass (kg/ha)	Weed biomass (kg/ha)	[2]Accumulated N (kg/ha) from cover crop biomass	[3]Accumulated N (kg/ha) from weed biomass	Soil NO_3^-(mg/kg) at incorporation	Soil NH_4^+(mg/kg) at incorporation
2012							
Control (no cover)	-	-	819 ± 99	0	-	-	-
Oilseed radish/ 'Defender'	10	2291 ± 301	148 ± 27	44.3 ± 7.2	-	-	-
Oat /'Excel'	120	2418 ± 431	136 ± 24	36.9 ± 4.2	-	-	-
Yellow mustard / 'Ida gold'	7	1133 ± 165	388 ± 103	28.9 ± 4.5	-	-	-
Oriental mustard / 'Pacific gold'	7	1235 ± 216	311 ± 19	26.8 ± 4.6	-	-	-
2013							
Control (no cover)	-	-	732 ± 114	0	13.8 ± 4.5	3.43 ± 0.44	1.53 ± 0.22
Oilseed radish/ 'Defender'	10	2097 ± 408	133 ± 31	51.3 ± 8.2	3.9 ± 0.8	2.91 ± 0.23	1.36 ± 0.13
Oat /'Excel'	120	1459 ± 968	122 ± 28	27.3 ± 5.8	3.2 ± 0.5	2.99 ± 0.19	2.19 ± 0.62
Yellow mustard / 'Ida gold'	10	1002 ± 183	347 ± 119	24.6 ± 2.8	11.3 ± 3.2	3.26 ± 0.34	2.05 ± 0.34
Oriental mustard / 'Pacific gold'	7	1246 ± 348	278 ± 22	28.3 ± 7.4	8.3 ± 0.9	3.32 ± 0.33	1.35 ± 0.10

[1] Cell values (except for seeding rates) represent mean values followed by standard errors.

[2]Accumulated N was calculated by multiplying the dryweight biomass estimates by plant %N data derived from subsamples of dried biomass.

[3] N accumulated from weed biomass was estimated in 2013 only.

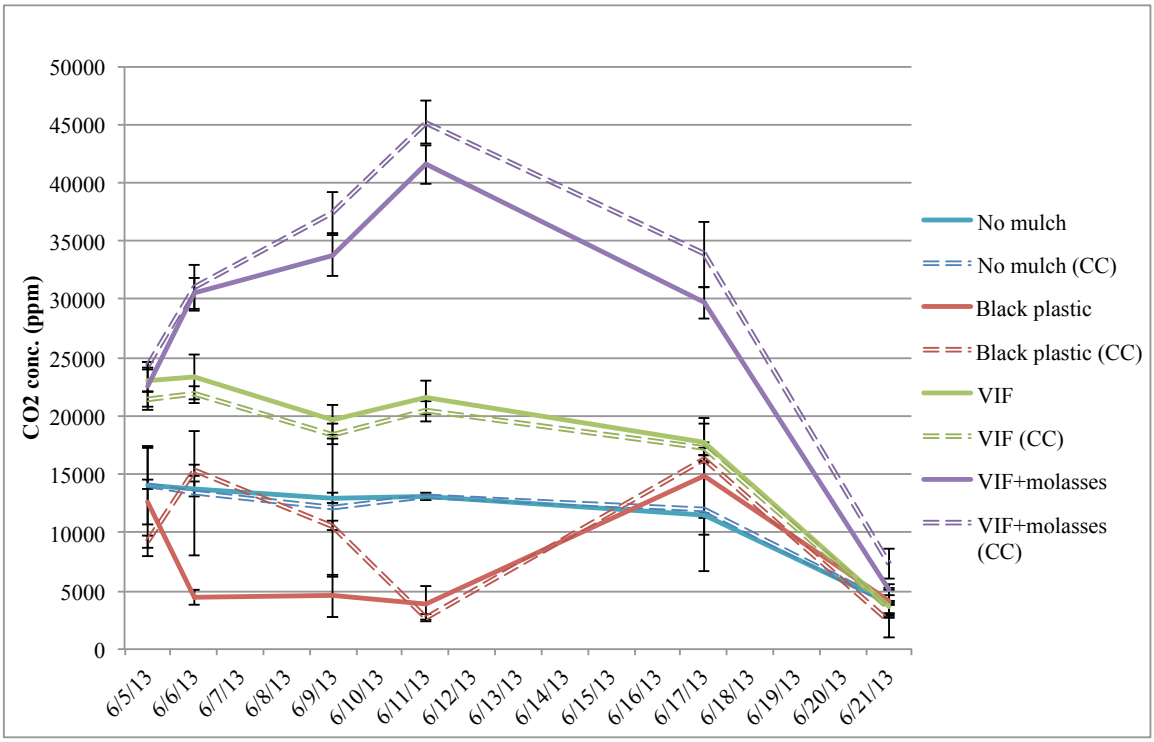

Fig. 11. Concentrations of CO_2 collected from beds with various mulch and cover crop treatments. Samples were collected immediately following the initiation of ASD (6/5) and sampled intermittently until transplants were set (6/19; indicated by the dashed line. Error bars indicate standard errors from 4 replications of each treatment combination sampled.

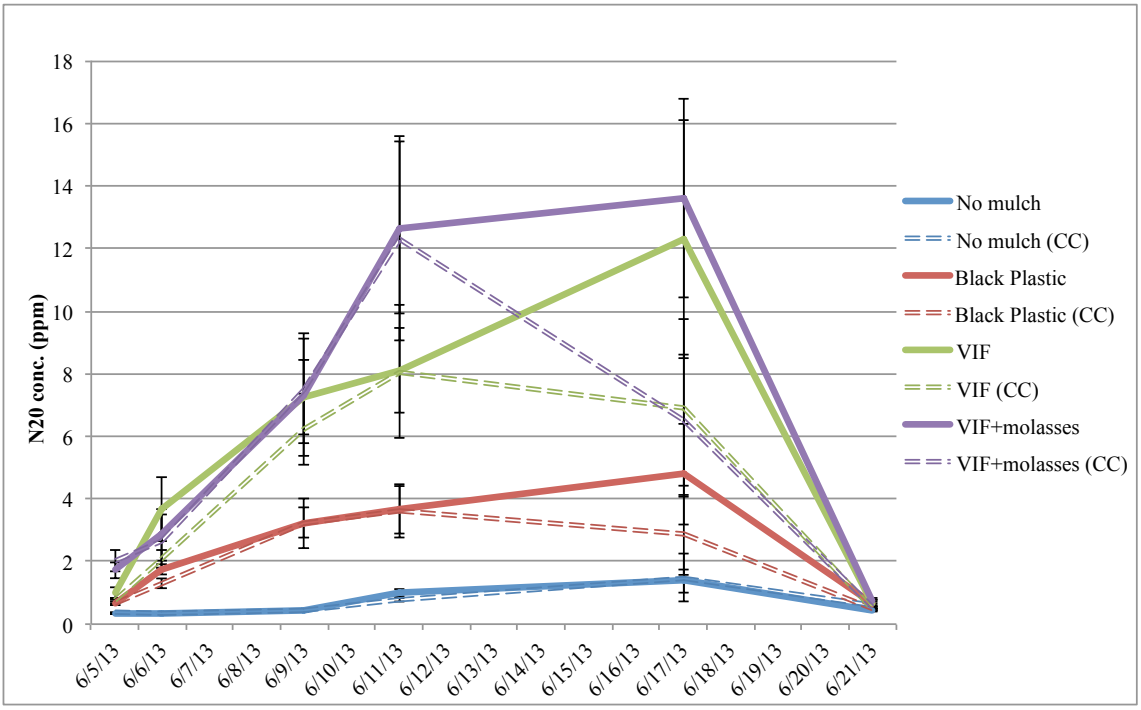

Fig. 12. Concentrations of N_2O collected from beds with various mulch and cover crop treatments. Samples were collected immediately following the initiation of ASD (6/5) and sampled intermittently until transplants were set (6/19; indicated by the dashed line. Error bars indicate standard errors from 4 replications of each treatment combination sampled.

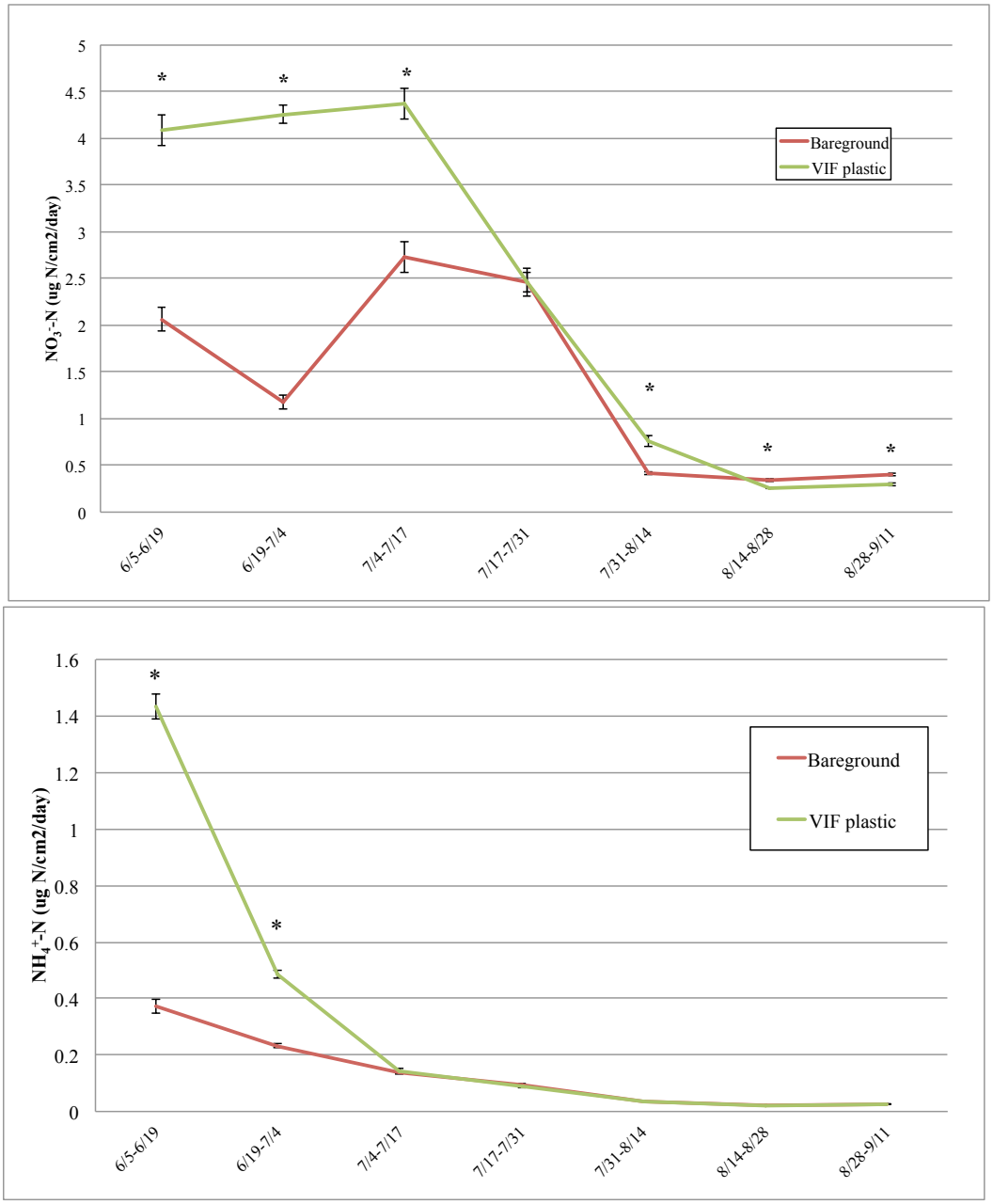

Fig. 13. NO_3^- (above) and NH_4^+ (below) extracted from ion exchange resin strips in 2012. Main effects of mulch treatment were analyzed for each sampling date after determining lack of significance among cover crop treatments analyzed. Note different scales between NO_3^- and NH_4^+ graphs.

*Indicates significant difference detected (α=0.05)

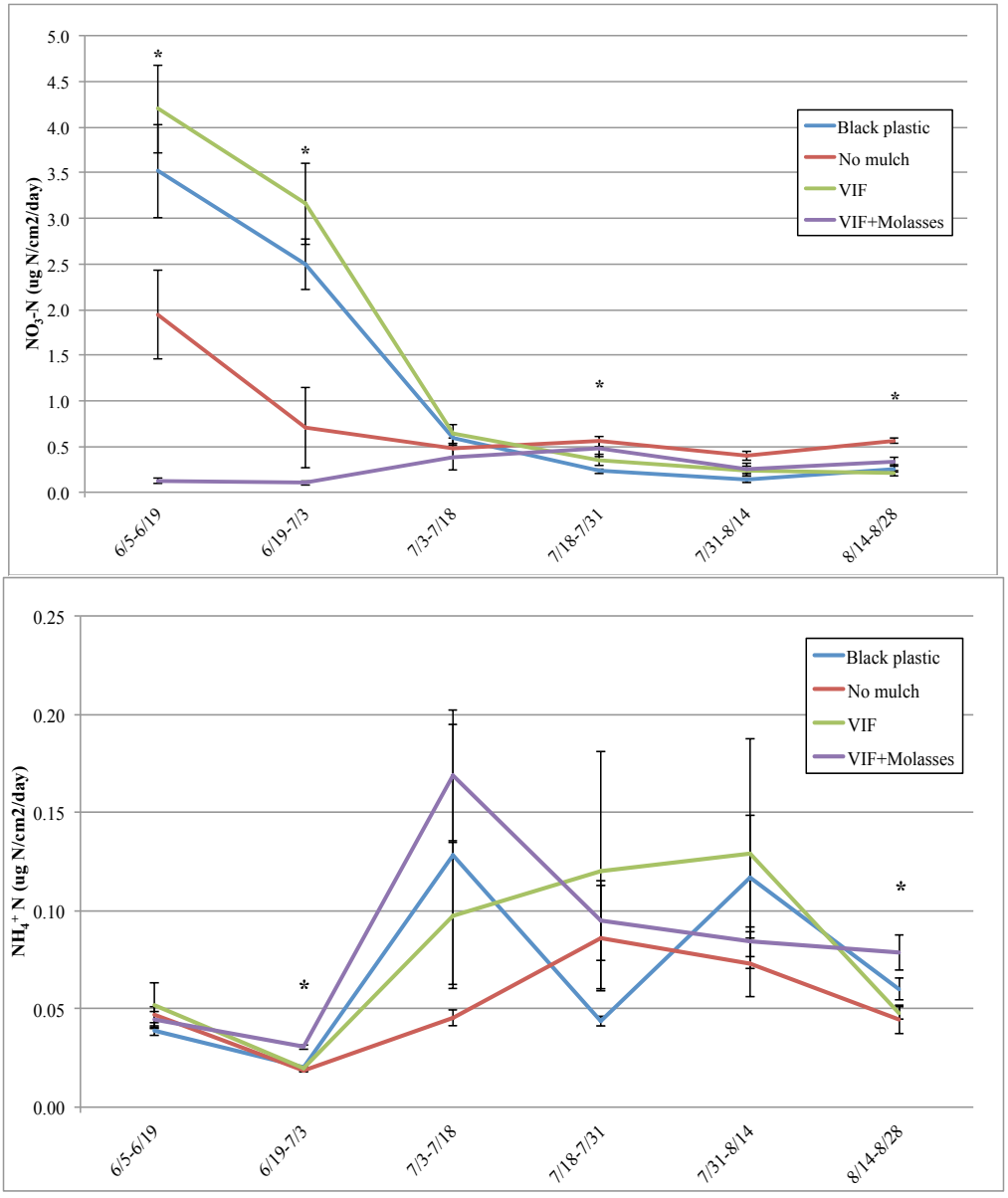

Fig. 14. NO_3^- (above) and NH_4^+ (below) from ion exchange resin strips in 2013. Main effects of mulch treatment were analyzed for each sampling date after determining lack of significance among cover crop treatments analyzed. Note different scales between NO_3^- and NH_4^+ graphs.

*Indicates significant difference detected (α=0.05)

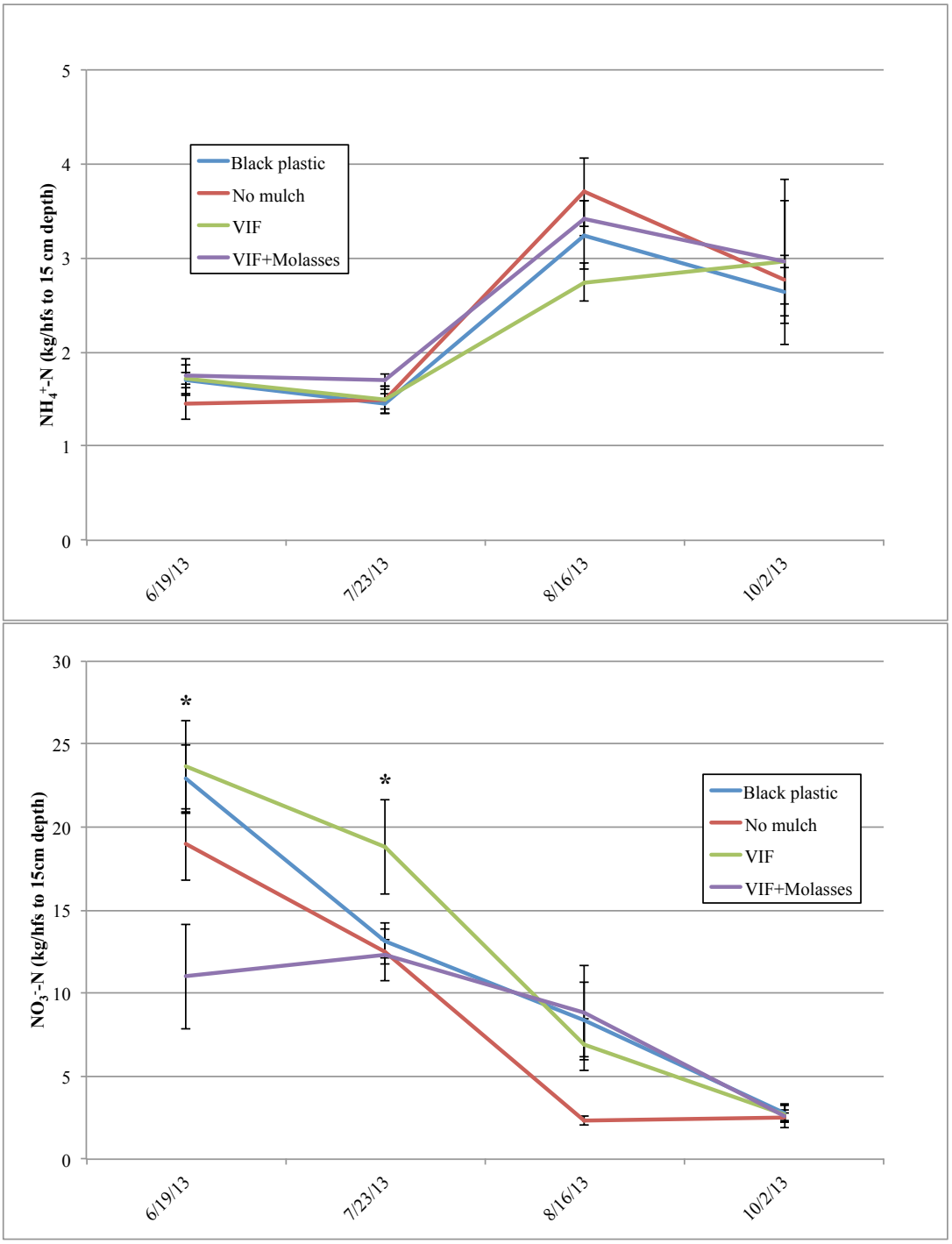

Fig. 15. Soil NO_3^- (above) and NH_4^+ (below) collected from soil cores during the 2013 growing season. Main effects of mulch treatment were analyzed for each sampling date after determining lack of significance among cover crop treatments analyzed. Note difference in scale between

NO_3^- and NH_4^+ graphs. *Indicates significant difference detected (α=0.05)

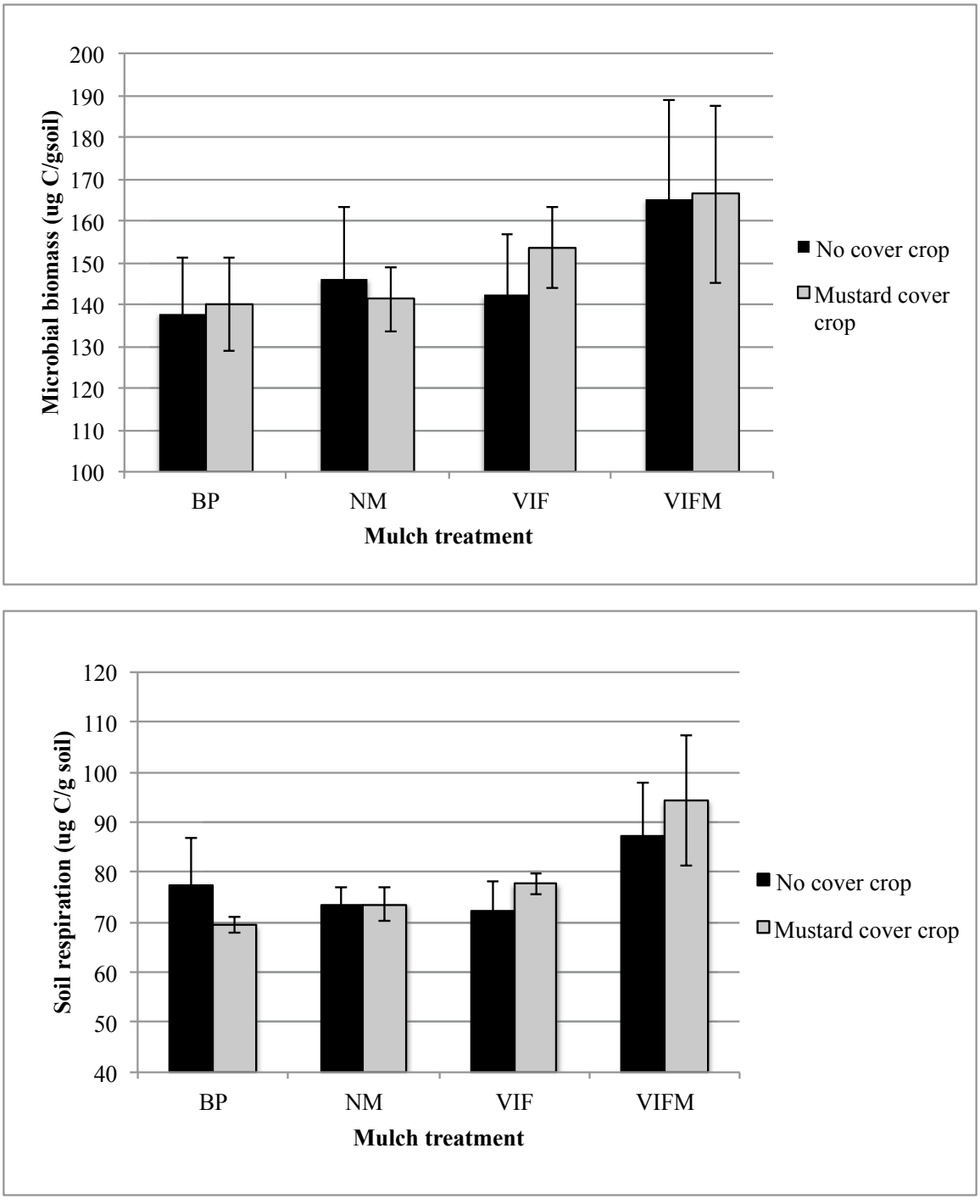

Fig. 16. Microbial biomass carbon (above) and soil respiration (below) collected from soil samples immediately after ASD treatment. No significant differences were detected among mulching x cover crop treatment combinations (α=0.05) likely due to the high variability among field replicates.

Table 2. Fresh market tomato yields under various mulch treatments in 2012 and 2013

Mulch Treatment	[1]Early yields (Mg/ha)		[2]Late yields (Mg/ha)		[3]Cumulative yields (Mg/ha)	
	Marketable	Cull	Marketable	Cull	Marketable	Cull
2012						
No Mulch	[4]3.7 **C**	1.6 **B**	41.9 **A**	29.5 **A**	55.5 **A**	31.9 **A**
Black Plastic	6.9 **A**	4.4 **A**	31.5 **B**	18.0 **B**	47.5 **B**	23.7 **C**
VIF	4.3 **B**	3.7 **A**	30.9 **B**	22.7 **C**	45.9 **B**	28.0 **B**
Pr > F	*<0.0001*	*<0.0001*	*<0.0001*	*0.0003*	*0.0014*	*0.0041*
2013						
No Mulch	9.4 **B**	1.6 **B**	51.1 **A**	27.8 **A**	60.5 **A**	29.4 **A**
Black Plastic	21.9 **A**	3.5 **A**	33.1 **B**	15.2 **B**	55.0 **A**	18.8 **B**
VIF	18.8 **A**	2.8 **A**	39.6 **B**	17.6 **B**	58.5 **A**	20.4 **B**
VIF+molasses	10.6 **B**	1.6 **B**	33.5 **B**	18.7 **B**	44.2 **B**	20.3 **B**
Pr > F	*<0.0001*	*0.0002*	*<0.0001*	*<0.0001*	*0.0002*	*<0.0001*

[1]Early yields included the first four harvests in 2012 and the first three harvests in 2013

[2]Late yields included the last four harvests from 2012 and the last three harvests from 2013

[3]Yields were collected weekly for a total of 9 harvests in 2012 (Aug. 13-Oct. 3) and 6 harvests in 2013 (Sept. 4

[4]Means followed by different letters within columns are significantly different (α=0.05)

Table 3. Slicing cucumber yields under mulch treatments in 2012 and 2013

Mulch Treatment	Cumulative yields (Mg/ha)	
	Marketable	Cull
2012		
No Mulch	22.3	12.4
VIF	20.5	13.8
Pr > F	*0.1781*	*0.1522*
2013		
No Mulch	[1]5.2 **B**	1.5 **B**
VIF	10.1 **A**	3.3 **A**
Pr > F	*<0.0001*	*<0.0001*

[1]Means followed by different letters within columns are significantly different (α=0.05)

Conclusions

Organic agriculture continues to be the fastest growing segment of agriculture. However, improving soil quality remains one of the top challenges for all growers and that could be achieved by appropriate cover crop practices. Because of their biofumigation effect Brassica cover crops have great potential to improve soil quality in organic systems. However, their performance has been inconsistent due to environmental conditions. Therefore, understanding the impact of environmental conditions on the performance of these cover crops in agro-ecosystems is a critical step for efforts designed to improve their service. Results of this study confirmed the phytotoxicity of these cover crops further, confirming their biofumigation potential, as well as the risk of crop damage that may occur from a poorly managed cover crop. Furthermore, the need to produce adequate biomass for effective biofumigation was demonstrated. Environmental conditions in the spring affected biomass production and the overall efficacy of the biofumigation process. While not yet confirmed by a scientific experiment, we also found in 2011 that adequate soil moisture at the time of cover crop incorporation is critical for effective biofumigation. In 2012 and 2013, plots at MSU's field research station were irrigated prior to cover crop incorporation and results were much better compared to 2011. Finally, the on-farm experiment in 2011 provided a unique opportunity to test the cover crops under commercial production. As a result of the on-farm study, it became more evident that the cover crop used by growers should fit within the whole production system. Therefore, growers using brassica cash crops in the same field should avoid brassica cover crops as they may attract flea beetles as was the case in our on-farm study. The VIF film was used effectively to create anaerobic soil conditions in 2012 and 2013 as shown by a rapid increase in soil CO_2 concentration. Table 4 provides an outline of major project outputs to date.

Project Outputs

Table 4. Project outputs-scholarly and educational products disseminated to date.

Topic	Output
Thesis	Yoder A.Y. 2014. Assessment of the Impact of Biofumigation and Anaerobic Soil Disinfestation on Soil Biology, Nitrogen Cycling, Crop Establishment and Yield in Vegetable Cropping Systems. Master of Science Thesis, Department of Horticulture Michigan State University.
Presentations at Growers meeting (Mainly Organic producers)	The Midwest Organic and Sustainable Education Service annual conference (poster); LaCrosse, WI, 2013 and 2014 Great Lakes Fruits, Vegetable and Farm Market Expo, Grand Rapids MI., 2012 and 2013. Empire State Fruit and Vegetable EXPO. Syracuse, New York, Jan 23-26, 2012. Fruit and Vegetable Grower Association of Delaware (FVGAD) to present at the Delaware Ag Week: Jan 18-20, 2011 Oceana County Summer Research Tour Sept 6, 2011. West Central Spring Horticulture Meeting, Hart, Michigan. March 15, 2011.
Conference presentations	Ngouajio M. 2012. Practical ways to use biofumigant cover crops for soil health improvement. Great Lakes Fruits, Vegetable and Farm Market Expo, Grand Rapids MI. Dec 5, 2012. Yoder A and M Ngouajio. 2012. Determining optimal planting dates following spring cover crop incorporation. Poster Presentation, Great Lakes Fruits, Vegetable and Farm Market Expo, Grand Rapids MI. Dec 4, 2012. Ngouajio M. 2012. Practical ways to use cover crops as biofumigants. Midwest Cover Crop Council (MCCC) Annual Meeting, West Lafayette, Indiana, Feb., 29. 2012. Ngouajio M. 2012. Feature Presentation: Improving productivity of onions grown on muck with mustard cover crops in Michigan. Empire State Fruit and Vegetable EXPO. Syracuse, New York, Jan 23-26, 2012. Ngouajio M. 2012. Improving tomato, pepper, and eggplant rotation

	with cover crops: Experience from Michigan. Empire State Fruit and Vegetable EXPO. Syracuse, New York, Jan 23-26, 2012 Ngouajio M. 2011. Cover Crops as Biofumigants. Invited by Midwest Cover Crop Council (MCCC) to present at the Conservation Tillage and Technology Conference, Ada, Ohio, Feb 24-25, 2011. Ngouajio M. 2011. Research on cropping systems, cover crops, soil amendments and vegetable production. Invited by the Fruit and Vegetable Grower Association of Delaware (FVGAD) to present at the Delaware Ag Week: Jan 18-20, 2011 Nair A. and M. Ngouajio. 2011. Integrating Brassica Cover Crops Into Onion Cropping Systems: Implications for Plant Population, Stand Establishment, and Yield. American Society for Horticultural Science Annual Conference (Oral presentation). Ngouajio M. 2011. Effect of compost and brassica cover crops on soil quality and asparagus performance. Oral Presentation, International Symposium: Organic Matter and Compost Use in Horticulture. 4-7 April, 2011, Adelaide, Australia Ngouajio M and D Clark. 2011. With Current Cover Crop Seeding Recommendations Achieving Appropriate Plant Populations is a Challenge With Small Seeded Species. Presentation at the Oceana County Summer Research Tour Sept 6, 2011. Ngouajio M. 2011. Brassica Cover Crops: Tools for Biofumigation and Soil Quality Improvement. West Central Spring Horticulture Meeting, Hart, Michigan. March 15, 2011. Ngouajio M. 2010. 'Veggie 201' Workshop March 19, 2010 Jackson MI. March 19, Jackson MI
Publications	Nair A., M. Ngouajio and J Biernbaum. 2012. Quality Ingredients: Choosing the right growing medium and providing proper nutrition are key to producing healthy organic transplants. American Vegetable Grower Magazine. June issue P24-25. Ngouajio M. 2012. Stress Defenders: Acting as biofumigants, Brassica cover crops help reduce insects, weeds, and disease. American Vegetable Grower Magazine. May issue p. 16 Ngouajio M., J.W. Counts, and D. Clark. 2011. Effect of Compost and Brassica Cover Crops on Soil Microbial Biomass and Asparagus Performance. Acta Hort. 950:65-71.

	Ackroyd J.V. and M. Ngouajio. 2011. Brassicaceae cover crops affect seed germination and seedling establishment in cucurbit crops. HortTechnology 21:525-532 Nair A. and M. Ngouajio. 2011. Integrating Brassica Cover Crops Into Onion Cropping Systems: Implications for Plant Population, Stand Establishment, and Yield. HortScience 46(9):S203. (Abstr.) (Abstr. Oral presentation). Ngouajio M., J.W. Counts, and D. Clark. 2011. Effect of compost and brassica cover crops on soil quality and asparagus performance. Abstr. Book of Abstracts Intl. Symp. Organic Matter and Compost Use in Horticulture. 4-7 April, 2011, Adelaide, Australia. P55. Ngouajio M. 2011. Why should growers observe a plant-back period following cover crop kill?. Michigan State University Extension News for Agriculture; May 18, 2011. Available at URL: http://news.msue.msu.edu/news/article/why_should_growers_observe_a_plant_back_period_following_cover_crop_kill Ngouajio M. 2011. Cover crops need to be managed properly. The Vegetable Growers News (Print Version only). September 2011, P9. Ngouajio M. 2011: Cover Crops. Spudman Magazine. July/August Issue 2p. Available at http://spudman.com/index.php/magazine/article/cover-crops.

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