Title: Can winter cover crops influence weed density and diversity in a reduced tillage vegetable system?

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Abstract

Weeds are a major constraint for organic crop production. Previous research has found that cover crops in reduced tillage systems can provide weed interference, subsequently reducing inputs and improving crop yield. However, questions remain about effects of cover crop species identity and cover crop biomass on weed suppression and crop yield. This four-year study investigated how winter cover crops grown alone or in mixture influenced weed presence and crop yield in a reduced tillage organic vegetable system. Treatments were barley (*Hordeum vulgare* L.), crimson clover (*Trifolium incarnatum* L.), mixed barley + crimson clover, and a no-cover crop control. Plots were flail-mowed and strip-tilled prior to planting main crops (2011 and 2012: broccoli *Brassica oleracea* L.; 2013 and 2014: crookneck squash *Cucurbita pepo* L.). We measured density, diversity, and community composition of weeds and viable weed seeds, changes in weed percent cover within growing seasons, and crop yield. We found that the presence of barley, crimson clover, or barley + crimson clover reduced weed density by 50% relative to the control. Cover crop biomass negatively influenced weed density and weed seed diversity, and positively influenced squash yield. Weed percent cover within growing seasons did not respond differentially to cover crop treatment. Cover crop treatment and cover crop biomass had no influence on weed or weed seed community composition. These results suggest that reduced tillage winter cover crops in mixture or monoculture can similarly suppress weeds and improve yield, primarily due to biomass effects.

Nomenclature: barley, *Hordeum vulgare* L.; crimson clover, *Trifolium incarnatum* L.; broccoli, *Brassica oleracea* L.; crookneck squash, *Cucurbita pepo* L.

Key words: polyculture; monoculture; strip tillage; Cucurbitaceae; Brassicaceae; mulch

1. Introduction

Weeds are one of the most problematic issues facing organic crop production (Oerke 2006). Weeds **interfere with crops chemically** (allelopathy) and via competition for resources including soil nutrients, water, and light (Knezevic et al. 1994, Einhellig 1996, Evans et al. 2003, Belz 2007, Lindquist et al. 2010). In organic production, weed management tactics include hand weeding, organic herbicides, tillage, and crop rotation (Swanton and Weise 1991, Bond and Grundy 2001). However, hand weeding **incurs high labor costs** and organic herbicides **have** limited product availability (Peruzzi et al. 2007). **Tillage** comes with a high initial cost and can lead to soil compaction **and the loss of soil organic matter, stability, resilience, and quality** (Havlin et al. 1990, Lal 1993, Hamza and Anderson 2005).

An **additional weed management** tactic is the use of winter cover crops (Moore et al. 1994). **When** winter cover crops **are** mowed, **rolled or burned down with herbicide and combined with reduced tillage practices such as strip tilling or no-tilling, the surface residue suppresses weeds by preempting** space and resources **and** reducing light transmittance and soil temperatures (Teasdale and Mohler 1993, Brandsæter et al. 1998, den Hollander et al. 2007, Lawley et al. 2012). **Practices that prevent** weed emergence and growth can reduce **subsequent** weed seed rain **resulting in** long-term weed **suppression** (Liebman and Gallandt 1997, Norris 1999, Mirsky et al. 2010). Winter cover crop residue is used for weed control in organic field crop production (Teasdale 1996, Mirsky et al. 2013), but has received less attention in organic vegetable production. Where use of cover crop residue in organic vegetables has been investigated, residue provided greater weed suppression than **bare ground** (Leavitt et al. 2011, Wayman et al. 2015). **Although negative effects of cover crops on yield have been documented** (Creamer et al. 1996, Dhima et al. 2006), **cover crops can enhance crop yield by preventing soil erosion, adding soil**

organic matter, and improving soil structure subsequently enhancing soil quality (Hartwig and Ammon 2002).

The ability of cover crop residue to suppress weeds and improve yield can be influenced by cover crop species. Although performance of cover crop species varies by region, in general cereals such as rye (*Secale cereale* L.) and barley (*Hordeum vulgare* L.) have a relatively high carbon to nitrogen ratio (C:N ~33:1), slowing residue decomposition and prolonging physical interference with weeds (Creamer et al. 1997). Leguminous winter annuals such as hairy vetch (*Vicia villosa* Roth) and crimson clover (*Trifolium incarnatum* L.) fix nitrogen and have a relatively low carbon-to-nitrogen ratio (C:N ~15:1), allowing more rapid decomposition and nitrogen return to the soil (Dabney et al. 2001, Clark 2007, Manzonei et al. 2008, Nascente et al. 2015). Comparatively, a cereal-legume cover crop mixture should provide an intermediate C:N and period of weed suppression. Furthermore, the allelopathic potential of cover crops against weeds differ according to cover crop and weed species. For example, crimson clover suppresses *Solanum ptychanthum* Dunal, *Ipomoea lacunose* L., and *Sinapis arvensis* L., allelopathically and barley suppresses *Setaria glauca* (L.) Beauv allelopathically (White et al. 1989, Creamer et al. 1996). Physical interference by cover crop residue can be species-specific, as physical structure of cover crop residue may influence weed growth (Teasdale and Mohler 1993). Complementarity of these species-specific chemical and physical traits may allow cover crop species mixtures to suppress weeds better than single-species counterparts.

Cover crop biomass, which may differ among species, plays an important role in weed suppression (Bàrberi and Mazzoncini 2001, Tonitto et al. 2006). For example, rye and barley establish quickly and accumulate biomass rapidly relative to hairy vetch and crimson clover (Clark 2007). Because functional group complementarity promotes productivity in natural

systems (Tilman et al. 1996, Loreau and Hector 2001, Loreau et al. 2001), cover crop mixtures of cereals and legumes are expected to accumulate more biomass than single-species counterparts at similar seeding rates. This expectation is complicated by evidence that increased productivity in mixtures may be due to increased likelihood of encountering a single highly productive species within species mixtures (Smith et al. 2014, Finney et al. 2016), that very high species diversity can diminish productivity (Wortman et al. 2012), and that variation in biomass production across year and location is ubiquitous (Akemo et al. 2000, Hayden et al. 2012). Despite this, mixed-species cover crops have been shown to provide more effective weed suppression relative to single-species cover crops (Teasdale and Abdul-Baki 1998, Akemo et al. 2000, Hayden et al. 2012, Webster et al. 2013). However, instances where mixed-species winter cover crops show no advantage over single-species in weed suppression (Creamer et al. 1996, Leavitt et al. 2011, Wayman et al. 2015) suggest that additional research is needed understand the mechanisms by which cover crop species mixtures interact with weeds.

While organic vegetable growers prioritize reducing weed density, cover crops may also promote changes in the composition of weed communities, which can be challenging if communities shift toward weed species that are difficult to control (Davis et al. 2005) such as a shift to perennial weeds. Changes in weed species diversity across cover crop species has been demonstrated for oat (*Avena sativa* L.), hairy vetch, and mixed oat + hairy vetch residues (Campiglia et al. 2012), however species-specific effects make predicting the direction of weed community responses to cover crops difficult. Suppression of certain weed species may decrease weed species diversity, but suppression of a competitively dominant weed might release weaker competitors from competition and increase weed species diversity. A more diverse weed

community may not be detrimental to vegetable production (Clements et al. 1994), and indeed may support important ecosystem functions (Clements et al. 1994, Radicetti et al. 2013).

Optimizing weed suppression and crop yield requires teasing apart the respective roles of cover crop species and cover crop biomass. The objective of this study was to investigate the effects of cover crop species alone in reduced-tillage vegetable production. Using methodologies similar to those used by small organic vegetable farms, we tested effects of four cover crop treatments: crimson clover, barley, barley + crimson clover mixture, and a no-cover crop control on weeds and crop yield in broccoli (*Brassica oleracea* L.) and crookneck squash (*Cucurbita pepo* L.). We tested the following hypotheses: (1) weed and weed seed density and diversity will be reduced in cover crop treatments relative to the control; (2) weed and weed seed density and diversity will be reduced in barley + crimson clover relative to single-species cover crops; (3) barley + crimson clover will reduce weed percent cover more consistently within seasons relative to single-species cover crops; (4) cover crops will increase crop yield relative to the control; and (5) species-specific interference by cover crops will result in different weed and weed seed communities across treatments. Although biomass manipulations were not part of the study design, cover crop biomass measurements allowed us to further explore whether (6) cover crop biomass irrespective of cover crop species influenced weed presence or crop yield.

2. Materials and Methods

2.1 Experimental Design

Four trials were conducted at the Central Maryland Research and Education Center in Upper Marlboro, MD from fall 2010-summer 2014. In a ~ 66 x 71 m field previously growing field corn, 16 ~ 17 x 17 m plots were plowed and seeded with one of four treatments in a Latin square design; once assigned a treatment, plots received that treatment each year. Treatments

were barley (145-171 kg/ ha), crimson clover (30-35 kg/ha), barley + crimson clover mixture (73-86 kg/ha + 15-22 kg/ha), and a no-cover crop control which was cultivated in fall and either flail mowed (2013) or cultivated (2014) in spring. Alleyways between plots (~ 6.7 - 9.1 m wide) were seeded with fescue in 2010 and regularly mowed thereafter.

2.2 Field and crop management

Dates of field events and data collection are given in Table 1. In spring, all plots were flail mowed (John Deere® 360, Moline, IL) and strip-tilled in one or two passes of a two-row tiller (12 30.5 cm wide rows, 1 m spacing, Ferguson Manufacturing®, Suffolk, VA). In 2014 only, control plots received light cultivation after flail mowing. Prior to planting, newly germinated weeds in the tilled rows were removed by hand-hoeing or killed with a hand-sprayed organic herbicide (2013 only, Avenger® Weed Killer, Cutting Edge Formulations, Inc., Buford GA).

Broccoli and crookneck squash were chosen to represent two commonly grown vegetable families. In 2011 and 2012, 6-8 week-old broccoli ('Green Magic F1' (2011) and 'Blue Wind' (2012), Johnny's Selected Seeds®, Winslow ME) seedlings were transplanted at 0.6 m spacing (16 plants/row, 14,769 plants/hectare). In 2013 and 2014, crookneck squash ('Yellow Organic', Johnny's Selected Seeds®, Winslow ME) were direct seeded at 1.2 m spacing (10 plants/row, 9,230 plants/hectare). All plots were fertilized with pelletized organic chicken manure (MicroSTART60, Perdue AgriRecycle®, Sussex Co. DE), 7-2-2 N-P-K formulation in 2011 and 2012 and 3-2-3 formulation in 2013 and 2014. Broccoli received 65 kg/ha N in 2011 and 112 kg/ha N in 2012. Squash received 78 kg/ha N in 2013 and 90 kg/ha in 2014. In 2012, one block inadvertently received an additional 64 kg/ha N, which is accounted for by blocking effects in the analysis. Fertilizer amounts were adjusted yearly based on previous years' crop

performance, and were applied at the low end of the recommended ranges for these crops to emphasize potential nutrient effects of the treatments. Soil nutrient testing at the end of the experiment in 2014 showed no differences in soil C:N (mean $\pm$ SEM 9.99 ± 0.57) or cation exchange capacity (mean $\pm$ SEM 11.23 ± 2.38 meq/100g) across treatments. Broccoli harvest was followed by soil cultivation in preparation for a green bean double crop, which is not discussed in this paper.

In order to simulate some methodologies commonly used by organic vegetable growers, weeds were managed by hand pulling or hoeing throughout the plots after weed surveys, and with full-plot light cultivation following final harvest. Hand weeding in 2014 was limited to 0.5 m around each squash plant, and the remainder of the plot area was mowed.

2.3 Data collection

Before cover crops were mowed in the spring, plant material was collected in four 0.3 x 0.3 m quadrats per plot. All quadrat sampling used a stratified haphazard placement to avoid clustering. Aboveground plant material within each quadrat was clipped at the soil surface and in all years except 2011, weeds and cover crops were separated. Plant material was dried at 60 °C (>2 weeks) and weighed. All samples from each plot were combined, ground to powder, and analyzed for C:N (A&L Laboratories®, Memphis, TN).

Before main crops were planted, soil cores were collected to assess the weed seedbank. Fifteen 7.3 cm diameter x 10 cm deep soil cores (2011-2013) or 30 2.2 cm diameter x 10 cm deep soil cores (2014) were collected from each plot. Soil was homogenized and coarsely sifted to remove roots and transported to the University of Maryland greenhouse facility, College Park, MD. Soil samples were spread across 2-3 perforated flat trays per plot, on top of 1 cm of potting soil (Sunshine mix LC1, Sun Gro®, Agawam, MA). Trays were watered daily and seedlings

were counted, identified, and removed every two to three weeks. After one month, the soil was allowed to dry for two weeks, crumbled, and re-watered to encourage another germination flush. Weed counts continued until germination ceased, approximately three months in the greenhouse. A negligible number of seedlings could not be identified (19 out of 104,987 total individuals) and were excluded from analyses.

After main crops were planted, weeds in 16 0.3 x 0.3 m quadrats **per plot**, evenly divided into between-row and within-row locations, were counted (2011, 2013 and 2014) and identified to species (2013 and 2014). Weed percent cover in eight 0.3 x 0.3 m quadrat samples, evenly divided into between-row and within-row locations, was measured twice per season in 2013 and 2014.

A subset of broccoli plants was harvested on two and four dates in 2011 and 2012, respectively. Sample sizes of harvested subsets were unbalanced, and are given in Table A1. Broccoli heads were assessed for marketability due to insect damage and weighed. Crookneck squash were harvested three times per week. All squash plants in the inner 10 plot rows were sampled. Fruits were assessed for marketability due to size, insect damage, or rot, and weighed. Yield for broccoli and squash were converted to kg/ha based on planting density.

2.4 Data analysis

Analyses were performed in R versions 3.0.2-3.2.2 (R Core Team 2015). Row-wise and column-wise Latin square spatial blocks were included in all models (Quinn and Keough 2002). Tukey's HSD was performed among treatments when treatment effects were $P < 0.05$.

Weed seed density, **weed** seed Shannon-Wiener diversity, early-season weed density (excluding 2012), early-season weed Shannon-Wiener diversity (2013 and 2014), broccoli yield, and squash yield were analyzed with linear mixed effects models (**R package nlme, function lme;**

(Pinheiro et al. 2011). Predictor variables were treatment (fixed effect), and year, spatial blocks, and plot (random effects). Early-season weed density and Shannon-Weiner diversity analyses also included location between or within rows as a fixed effect predictor. All response variables were log-transformed to conform to model assumptions, except broccoli yield which was BoxCox-transformed. All figures show untransformed means $\pm$ SEM.

Seedbank and early-season weed species composition (2013 and 2014) were analyzed with non-metric multidimensional scaling (R package `vegan`, function `adonis`; (Oksanen et al. 2015). Non-metric multidimensional scaling (NMDS) displays relationships among communities in ordination space, where distance among points represents dissimilarity. Bray-Curtis distances were used, and goodness-of-fit was estimated with stress (S) and fit (adonis R) values. A vector describing the relationship of biomass and community composition was created with the `envfit` function; vector direction indicates association with cover crop biomass. Seedbank species composition was grouped by treatment constrained by year, and by year. Early-season weed composition was grouped by treatment and plot location constrained by year, and by year.

As proportion data, percent cover estimates were analyzed with generalized linear mixed models (GLMM) with a binomial distribution (R package `MASS`, function `glmmPQL`; (Venables and Ripley 2002). Early-season and late-season weed percent cover were analyzed in response to treatment, date, location, and treatment-by-date interaction (fixed effects), and to year, spatial blocks and plot (random effects). For this analysis, the treatment-by-date interaction is the focal term to determine whether treatments influenced how percent weed cover changed over a season, and is the only term which will be interpreted.

To account for effects of cover crop biomass on weed presence independent of cover crop treatment, weed seed density, weed seed Shannon-Weiner diversity, in-field weed density

(averaged across plot location and excluding 2012), weed percent cover, broccoli yield, and squash yield were analyzed in response to cover crop biomass with linear models (R function lm), excluding the no-cover crop control treatment. Cover crop biomass was not separated from weed biomass in 2011, so that year was excluded from all analyses.

3. Results

3.1 Weed density and diversity

Weed density was reduced by all cover crops ($\chi^2 = 24.7$, $df = 3$, $P < 0.001$; Fig 1), with 50% more weeds in the no-cover crop control plots (mean $\pm$ SEM 458.3 ± 46.1 weeds/m²) relative to barley, crimson clover, or barley + crimson clover plots (mean $\pm$ SEM 223.7 ± 28.8 weeds/m²; Tukey's HSD $P < 0.001$). Cover crop treatment did not influence weed Shannon-Weiner diversity ($\chi^2 = 3.2$, $df = 3$, $P = 0.4$), weed seed density ($\chi^2 = 0.5$, $df = 3$, $P = 0.9$), or weed seed Shannon-Weiner diversity ($\chi^2 = 0.7$, $df = 3$, $P = 0.9$). Cover crop treatment did not influence weed percent cover across season (interaction term $\chi^2 = 0.8$, $df = 3$, $P = 0.8$). However, plot location did influence weed percent cover ($\chi^2 = 57.1$, $df = 1$, $P < 0.001$), where weeds comprised $52 \pm 4\%$ (mean $\pm$ SEM) of between-row ground cover and $32 \pm 2\%$ (mean $\pm$ SEM) within-row ground cover.

Figure 1. Weed density (mean $\pm$ SEM) averaged across years (2011-2014) and plot locations (within and between rows) was greater in the no-cover crop control relative to the three treatments with cover crops (Tukey's HSD $P < 0.001$). Weed density was measured with 16 0.3 x 0.3 meter quadrats per plot.

Cover crop biomass negatively influenced weed density ($F = 4.3$, $df = 1, 22$, adj. $R^2 = 0.13$, $P < 0.05$; Fig 2A) and weed percent cover ($F = 79.8$, $df = 1, 94$, adj. $R^2 = 0.45$, $P < 0.001$; Fig 2B), but did not influence weed diversity ($F = 0.6$, $df = 1, 22$, adj. $R^2 = -0.02$, $P = 0.4$). Cover crop biomass did not influence weed seed density ($F = 1.0$, $df = 1, 34$, adj. $R^2 = -0.001$, $P = 0.3$) but positively influenced weed seed Shannon-Weiner diversity ($F = 4.4$, $df = 1, 34$, adj. $R^2 = 0.09$, $P < 0.05$; Fig 2C). Cover crop and weed biomass and C:N provided by treatments in each year are given in Table A2. Cover crop biomass was highest in barley + crimson clover, which along with the no-cover crop provided an intermediate C:N relative to barley and crimson clover. No-cover crop control plots had the greatest weed biomass, nearly three times that of crimson clover and nearly ten times that of the barley + crimson clover cover crop.

Figure 2. Cover crop biomass (g/m^2) correlated negatively with (A) weed density ($F = 4.3$, $df = 1, 22$, $P < 0.05$) and (B) weed percent cover ($F = 79.8$, $df = 1, 94$, $P < 0.001$), and positively with (C) seedbank weed diversity ($F = 4.4$, $df = 1, 34$, $P < 0.05$), and (D) squash yield ($F = 40.1$, $df = 1, 22$, $P < 0.001$). Weed density was measured with 16 0.3×0.3 meter quadrats per plot; weed seeds were sampled from 10cm-deep soil cores each spring; squash were harvested from all plants in the inner 10 rows, every three days from maturity.

3.2 Crop yield

Cover crop treatments did not influence broccoli yield ($\chi^2 = 1.2$, $df = 3$, $P = 0.8$; average yield 1131 kg/ha) or squash yield ($\chi^2 = 5.7$, $df = 3$, $P = 0.1$, average yield 3305 kg/ha). Cover crop biomass did not influence broccoli yield ($F = 0.9$, $df = 1, 10$, $R^2 = -0.01$, $P = 0.4$), but positively influenced squash yield ($F = 40.1$, $df = 1, 22$, $R^2 = 0.6$, $P < 0.001$; Fig 2D).

3.3 Weed community composition

Observed weed species in the field and seedbank are listed in Table 2. Cover crop treatments influenced weed community composition in the field ($S = 0.26$, $R^2 = 0.1$, $df = 3$, $P < 0.001$), where 95% confidence intervals (CI) for the no-cover crop control and barley + crimson clover do not overlap (Fig 3). Weed communities in all treatments were dominated by the summer annuals *Mollugo verticillata* L., *Digitaria ischaemum* (Schreb.) Schreb. ex Muhl., *Digitaria sanguinalis* (L.) Scop., and *Eleusine indica* (L.) Gaertn., the perennial *Cyperus esculentus* L., and *Oxalis* spp.. These species made up 90% of all individuals in each treatment. Communities in the no-cover control treatment notably differed from other treatments in being dominated by a single species, *M. verticillata*, which made up over 50% of the individuals observed. Cover crop biomass as a fitted vector was not significantly associated with community composition ($r^2 = 0.1$, $P = 0.09$).

Cover crop treatments did not influence community composition of the seedbank ($S = 0.17$, $R^2 = 0.03$, $df = 3$, $P = 0.8$). The seedbank community changed from the first sampling date in 2011 to a different composition that remained similar across the next three years of the experiment, 2012-2014 (Fig 4). However, within each year weed seed communities were similar across cover crop treatments. Seven species composed 90% of weed seeds, all summer annuals or perennials: *Oxalis* spp. (39%), *M. verticillata* (26%), *E. indica* (9%), *Cerastium vulgatum*

278 Thuill. (7%), *C. esculentus* (6%), *Panicum dichotomiflorum* Michx. (3%), and *D. sanguinalis*
 279 (2%). Cover crop biomass as a fitted vector was significantly associated with weed seed
 280 community composition ($r^2 = 0.3$, $P < 0.01$), and was more associated with communities
 281 characterized by summer annuals *Chenopodium album* L., *P. dichotomiflorum*, and *Amaranthus*
 282 *retroflexus* L., and perennial *C. esculentus*.

283 Figure 3. Multidimensional scaling ordination plot (distance = Bray-Curtis, $d = 2$, $S = 0.26$) of in-field
 weed communities in 2013 and 2014, where there was a treatment effect on species composition (P
 < 0.05). + symbols indicate each location within each plot in each year. Ellipses indicate 95%
 confidence intervals for treatment (black = control, blue = barley, red = crimson clover, grey = mixed
 barley + crimson clover). Text labels species occurring in at least 75% of observations, accounting
 for 88% of total weed abundance. DIGSA = *Digitaria sanguinalis* L. Scop., DIGIS = *Digitaria*
ischaemum L. (Schreb.) Schreb. Ex Muhl., ELEIN = *Eleusine indica* L., OXASP = *Oxalis* spp., MOVE
 = *Mollugo verticillata* L., CYPES = *Cyperus esculentus* L.

Figure 4. Multidimensional scaling ordination plot (distance = Bray-Curtis, dimensions = 2, $S = 0.25$) of weed seedbank species in cover crop treatments (barley, crimson clover, and barley + crimson clover) in 2012-2014. There was significant directionality of cover crop biomass (red arrow) across weed communities ($P < 0.05$), but no treatment effect. + indicates a single plot in a single year. Text labels indicate species occurring in at least 50% of observations, accounting for 98% of total viable weed seed abundance. LAAM = *Lamium amplexicaule* L., STEME = *Stellaria media* L. Vill., CERVU = *Cerastium vulgatum* Thuill., PANDI = *Panicum dichotomiflorum* Michx., SOLNI = *Solanum nigrum* L., DIGSA = *Digitaria sanguinalis* L. Scop., DIGIS = *Digitaria ischaemum* L. (Schreb.) Schreb. Ex Muhl., AMARE = *Amaranthus retroflexus* L., CHEAL = *Chenopodium album* L., ELEIN = *Eleusine indica* L. Gaertn., OXASP = *Oxalis* spp., MOVE = *Mollugo verticillata* L., CYPES = *Cyperus esculentus* L.

4. Discussion

We found partial support for our hypotheses that cover crops would suppress weeds and increase yield. Winter cover crop residue reduced weed density by 50%, but did not influence weed seed density. Although greater biomass accumulation and intermediate C:N of the species mixture suggest that the two species demonstrated complementarity (Finney et al. 2016), the

mixed-species cover crop did not suppress weeds better than single-species cover crops, nor did the mixed-species cover crop provide better weed suppression across the season. Cover crop biomass negatively influenced weed density and weed percent cover, but did not influence weed seed density. Cover crops did not influence weed or weed seed diversity or community composition, but cover crop biomass positively influenced weed seed diversity. Despite cover crop treatment effects on weed density, there was no subsequent influence on broccoli or squash yield. Biomass had a positive effect on yield in squash but not broccoli.

In this study, cover crop species appeared to be less important than biomass in influencing weeds and yield, suggesting that selection of cover crop species should be aimed at optimal biomass production in addition to potential species-specific interference effects or complementarity of function. Cover crop species specificity has been shown to be important in previous studies, which often focused on allelopathic effects. For example, crimson clover and barley have demonstrated allelopathic effects specific to *S.ptychanthum* and *S. glauca* (Creamer et al. 1996), however these weed species were not common in our study. Other species-specific characteristics include differences in competitive ability, growth habit, and tolerance for environmental extremes (Teasdale and Mohler 1993, Akemo et al. 2000, Snapp et al. 2005, Flower et al. 2012, Hayden et al. 2012). Cereal cover crops such as barley are often stronger competitors and more winter-hardy than legumes such as crimson clover (Akemo et al. 2000, Snapp et al. 2005, Flower et al. 2012, Hayden et al. 2012). Evidence of these effects were not found in our study, despite differences in plant architecture, growth habit, and C:N across treatments. It is possible that species-specific effects were present, but were rendered undetectable by biomass effects or inter-annual variation in biomass. Previous studies finding stronger weed suppression by mixtures relative to single-species cover crops were rarely

consistent among years (Teasdale and Abdul-Baki 1998, Akemo et al. 2000, Leavitt et al. 2011, Hayden et al. 2012, Wayman et al. 2015).

Although extremely high biomass, particularly when coupled with high C:N, can be detrimental to crop yield (Finney et al. 2016), in general cover crops achieving an optimal intermediate biomass are most likely to suppress weeds and increase crop yield (Bàrberi and Mazzoncini 2001, Tonitto et al. 2006). Biomass levels for effective weed suppression have been reported between 4500-8000 kg/ha (450-800 g/m²) (Teasdale and Mohler 1993, Clark 2007), a level that was reached in this study only in 2013. Effects of cover crop biomass on weeds and squash yield in our study, despite generally low cover crop biomass accumulation, indicate that results are a conservative estimate of the influence of cover crops and cover crop biomass, and that stronger effects on weed suppression and yield should be expected with more optimal cover crop biomass accumulation.

While the presence of cover crops reduced weed density, they appeared to suppress all weed species equally, as diversity and community-level metrics were similar in plots with and without cover crops. A comprehensive functional trait-based study of cover crop species and mixtures (Smith et al. 2015) found little evidence that cover crop species identity played a strong role in weed community composition, although we did expect that the presence of cover crops themselves might influence community composition based on previous findings of cover crop effects on weed diversity (Campiglia et al. 2012). Only cover crop biomass influenced weed communities, increasing diversity of the weed seedbank, perhaps due to cover crop biomass accumulation protecting seeds of certain species that would have been lost to germination, predation, or other factors prevalent with lower cover crop biomass. Cover crops in combination

with reduced tillage have been found to increase weed diversity relative to conventionally tilled land (Bàrberi and Mazzoncini 2001).

Reduced tillage may have played a role in the lack of cover crop treatment or biomass effects on weed seed density in our study. Because cover crops reduced in-field weed density, we would expect to find fewer viable weed seeds in plots with cover crops. However, this was not the case. Reduced tillage systems may be less likely than systems with more intense disturbance, such as chisel ploughing or disking, to reduce seedbank density and diversity (Feldman et al. 1997, Bàrberi and Lo Cascio 2001, Gallandt 2006, but see Murphy et al. 2006), especially when imposed for several years (Moonen and Bàrberi 2004, Shrestha et al. 2015). An exception to this could be where strips are tilled for crop planting. Disturbance and removal of surface residue by strip tilling was expected to promote weed growth (Brainard et al. 2013). In the current study, however, weed percent cover was lower within tilled strips than between, perhaps due to shading by crop foliage.

Future research aimed at resolving the respective effects of biomass and cover crop species identity or functional group should seek to describe optimum levels of biomass production for a variety of cover crop species and mixtures. This information, in addition to addressing how temporal and spatial variability in cover crop productivity can be predicted or managed, will help growers prepare appropriate or alternative weed management tactics for given environmental conditions. Results from such studies would be of particular value for organic vegetable production, where many growers have small diversified farms and limited equipment, and depend on reliable management tactics.

5. Acknowledgements

Thanks to the facilities crew at the University of Maryland Central Maryland Research and Education Center; G. Chen, L.G. Hunt, and many field technicians. Thanks to P.L. Coffey, Z. Szendrei, the MSU Vegetable Entomology Lab, A.W. Leslie, and anonymous reviewers for comments. This project was funded by USDA NIFA OREI grant number 2010-51300-21412.

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516

517 Table 1. Timeline of field and sampling events. DAP = days after planting

Date	Event	DAP
10/11/2010	Cover crops seeded	-217
5/6/2011	Plant cover biomass collection	-10
5/7/2011	Flail mow & strip till	-9
5/16/2011	Transplant broccoli	0
5/25/2011	Seedbank soil collection	9
6/2/2011	Weed density survey	17
6/8/2011	Fertilize planted rows	23
7/18/2011	Begin broccoli harvest	63
9/16/2011	Field cultivated	123
10/10/2011	Cover crops seeded	-182
4/3/2012	Plant cover biomass collection	-6
4/3/2012	Flail mow	-6
4/5/2012	Seedbank soil collection	-4
4/6/2012	Strip till	-3
4/9/2012	Transplant broccoli and fertilize planted rows	0
5/23/2012	Fertilize planted rows	44
5/30/2012	Begin broccoli harvest	51
7/23/2012	Field cultivated	105
9/30/2012	Cover crops seeded	-264
5/1/2013	Seedbank soil collection	-51
5/6/2013	Plant cover biomass collection	-46

5/7/2013	Flail mow	-45
5/14/2013	Strip till	-38
6/21/2013	Seed squash	0
6/24/2013	Fertilize planted rows	3
6/29/2013	Weed density, diversity, and percent cover survey	9
7/26/2013	Begin squash harvest	35
8/23/2013	Weed percent cover survey	63
8/27/2013	Field cultivated	67
<hr/>		
9/20/2013	Cover crops seeded	-269
5/7/2014	Seedbank soil collection	-40
5/8/2014	Plant cover biomass collection	-39
5/12/2014	Flail mow	-35
5/12/2014	Strip till	-35
6/16/2014	Seed squash	0
6/17/2014	Fertilize planted rows	1
6/18/2014	Weed density and diversity survey	2
7/1/2014	Weed percent cover survey	13
7/22/2014	Weed percent cover survey	34
7/28/2014	Begin squash harvest	40
9/2/2014	Field cultivated	78

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519

520 Table 2. Weed species observed in field and weed seedbank across all treatments and years. A =
 521 annual, B = biennial, P = perennial

Weed species name	Taxon	Life history
<i>Abutilon theophrasti</i> Medik.	Malvaceae	A
<i>Acer rubrum</i> L.	Aceraceae	P
<i>Allium canadense</i> L.	Liliaceae	P
<i>Amaranthus retroflexus</i> L.	Amaranthaceae	A
<i>Ambrosia artemisiifolia</i> L.	Asteraceae	A
<i>Bromus tectorum</i> L.	Poaceae	A
<i>Capsella bursa-pastoris</i> (L.) Medik.	Brassicaceae	A
<i>Cenchrus longispinus</i> (Hack.) Fern.	Poaceae	A
<i>Cerastium fontanum</i> ssp. <i>vulgare</i> Hartman Greuter & Burdet	Caryophyllaceae	B/P
<i>Chamaesyce maculata</i> (L.) Small	Euphorbiaceae	A
<i>Chenopodium album</i> L.	Chenopodioideae	A
<i>Chenopodium glaucum</i> L.	Chenopodioideae	A
<i>Convolvulus arvensis</i> L.	Convolvulaceae	P
<i>Conyza canadensis</i> (L.) Cronq.	Asteraceae	A/B
<i>Croton capitatus</i> Michx.	Euphorbiaceae	A
<i>Cyperus esculentus</i> L.	Cyperaceae	P
<i>Dactylis glomerata</i> L.	Poaceae	P
<i>Digitaria ischaemum</i> (Schreb.) Schreb. ex Muhl.	Poaceae	A
<i>Digitaria sanguinalis</i> (L.) Scop.	Poaceae	A

<i>Echinochloa crus-galli</i> (L.) Beauv.	Poaceae	A
<i>Eleusine indica</i> (L.) Gaertn.	Poaceae	A
<i>Elymus repens</i> (L.) Gould	Poaceae	P
<i>Erigeron annuus</i> (L.) Pers.	Asteraceae	A
<i>Galeopsis tetrahit</i> L.	Lamiaceae	A
<i>Galinsoga quadriradiata</i> Cav.	Asteraceae	A
<i>Geranium carolinianum</i> L.	Geraniaceae	A/B
<i>Hieracium caespitosum</i> Dumort.	Asteraceae	P
<i>Ipomoea hederacea</i> Jacq.	Convolvulaceae	A
<i>Ipomoea lacunosa</i> L.	Convolvulaceae	A
<i>Juncus tenuis</i> Willd.	Juncaceae	P
<i>Lamium amplexicaule</i> L.	Lamiaceae	A/B
<i>Lamium purpureum</i> L.	Lamiaceae	A
<i>Lepidium virginicum</i> L.	Brassicaceae	A/B/ P
<i>Lolium arundinaceum</i> (Schreb.) S.J. Darbyshire	Poaceae	P
<i>Medicago lupulina</i> L.	Fabaceae	A/P
<i>Mollugo verticillata</i> L.	Aizoaceae	A
<i>Oenothera laciniata</i> Hill	Onagraceae	A/P
<i>Oxalis</i> spp.	Oxalidaceae	A/P
<i>Panicum capillare</i> L.	Poaceae	A
<i>Panicum dichotomiflorum</i> Michx.	Poaceae	A
<i>Phytolacca americana</i> L.	Phytolaccaceae	P
<i>Plantago lanceolata</i> L.	Plantaginaceae	A/B/ P

<i>Poa annua</i> L.	Poaceae	A
<i>Polygonum convolvulus</i> L.	Polygonaceae	A
<i>Polygonum pensylvanicum</i> L.	Polygonaceae	A
<i>Polygonum persicaria</i> L.	Polygonaceae	A/P
<i>Portulaca oleracea</i> L.	Portulacaceae	A
<i>Potentilla arguta</i> Pursh	Rosaceae	P
<i>Scleranthus annuus</i> L.	Caryophyllaceae	A
<i>Setaria faberi</i> Herrm.	Poaceae	A
<i>Setaria pumila</i> (Poir.) Roemer & J.A. Schultes	Poaceae	A
<i>Setaria viridis</i> (L.) Beauv.	Poaceae	A
<i>Sida spinosa</i> L.	Malvaceae	A/P
<i>Silene latifolia</i> Poir.	Caryophyllaceae	B/P
<i>Solanum carolinense</i> L.	Solanaceae	P
<i>Solanum nigrum</i> L.	Solanaceae	A/B
<i>Sorghum halepense</i> (L.) Pers.	Poaceae	P
<i>Stellaria media</i> (L.) Vill.	Caryophyllaceae	A/B
<i>Taraxacum officinale</i> G.H. Weber ex Wiggers	Asteraceae	P
<i>Trifolium pratense</i> L.	Fabaceae	B/P
<i>Trifolium repens</i> L.	Fabaceae	P
<i>Triodanis perfoliata</i> (L.) Nieuwl. var. <i>perfoliata</i>	Campanulaceae	A
<i>Verbascum thapsus</i> L.	Scrophulariaceae	B
<i>Veronica arvensis</i> L.	Scrophulariaceae	A
<i>Vicia sativa</i> L.	Fabaceae	A

522 **Appendix. Supplemental tables and figures**

523

524 Table A1. Number of harvested plants used in broccoli yield analysis in each year

Year	Treatment	n
2011	Control	40
	Barley	38
	Crimson clover	58
	Barley + crimson clover	205
2012	Control	403
	Barley	444
	Crimson clover	408
	Barley + crimson clover	420

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529 Table A2. Dry biomass and C:N (mean $\pm$ SEM) of cover crops and weeds prior to flail mowing,
530 averaged across treatments. In 2011, weeds and cover crops were not separated before weighing;
531 values for treatments containing cover crops are biomass of weeds + cover crops.

Year	Treatment	Weed biomass (g/m ²)	Cover crop biomass (g/m ²)	Weed + cover crop C:N
2011	Control	13.2 $\pm$ 1.0	NA	29.3 $\pm$ 1.1
	Barley	Total biomass = 61.8 $\pm$ 3.4		42.3 $\pm$ 2.4
	Crimson clover	Total biomass = 113.7 $\pm$ 11.9		17.9 $\pm$ 0.7
	Barley + crimson clover	Total biomass = 96.9 $\pm$ 12.8		24.6 $\pm$ 2.0
2012	Control	54.8 $\pm$ 1.6	NA	22.5 $\pm$ 2.9
	Barley	6.1 $\pm$ 1.9	138.2 $\pm$ 7.1	30.9 $\pm$ 2.1
	Crimson clover	8.0 $\pm$ 1.6	245.9 $\pm$ 14.5	22.1 $\pm$ 7.4
	Barley + crimson clover	3.5 $\pm$ 0.6	316.0 $\pm$ 7.9	29.1 $\pm$ 5.1
2013	Control	194.8 $\pm$ 13.3	NA	28.3 $\pm$ 1.7
	Barley	17.3 $\pm$ 6.7	528.9 $\pm$ 76.8	49.0 $\pm$ 2.1
	Crimson clover	66.2 $\pm$ 36.2	537.5 $\pm$ 25.0	18.5 $\pm$ 0.6
	Barley + crimson clover	10.7 $\pm$ 3.9	789.7 $\pm$ 21.4	26.9 $\pm$ 2.8
2014	Control	111.3 $\pm$ 5.5	NA	25.9 $\pm$ 3.3
	Barley	27.7 $\pm$ 9.0	100.5 $\pm$ 23.4	27.9 $\pm$ 3.6
	Crimson clover	55.6 $\pm$ 3.6	167.5 $\pm$ 6.3	17.3 $\pm$ 0.5
	Barley + crimson clover	23.2 $\pm$ 4.2	221.3 $\pm$ 13.75	21.0 $\pm$ 1.0

532