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Living Mulch for Sustainable Maize Stover Biomass Harvest

Cynthia A. Bartel

Iowa State University, cabartel@iastate.edu

Chumki Banik

Iowa State University, cbanik@iastate.edu

Andrew W. Lenssen

Iowa State University, alenssen@iastate.edu

Kenneth J. Moore

Iowa State University, kjmoore@iastate.edu

David A. Laird

Iowa State University, dalaird@iastate.edu

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Abstract

The Renewable Fuels Standard mandate provides enhanced opportunity for maize (*Zea mays* L.) stover use as a bioenergy feedstock. Living mulch (LM) offers a possible solution for the natural resources constraints associated with maize stover biomass harvest. A two site-year study was conducted near Boone and Kanawha, IA, in both maize following maize (MM) and maize following soybean [*Glycine max* (L.) Merr.] (SM) sequences to evaluate the impact of established and chemically suppressed Kentucky bluegrass (*Poa pratensis* L.) (KB) 'Ridgeline', 'Wild Horse', 'Oasis', and 'Mallard' blend and creeping red fescue (*Festuca rubra* L.) (CF) 'Boreal' as LM on three maize hybrids (population sensitive, population insensitive, and yield stable). Maize grain yield for the no LM treatments in the MM and SM sequences was 12.0 and 13.2 Mg ha⁻¹, respectively, at Boone and 12.8 and 14.8 Mg ha⁻¹, respectively, at Kanawha, 23-73% greater than the LM treatment. Ethanol yield (L ha⁻¹) was 12-119% greater, protein concentration was ≤9% greater, and starch concentration was ≤1% lower in the no LM treatment maize than LM treatment maize. Maize hybrid by cover interaction was significant for parameters including total aboveground biomass and protein concentration at Boone, with inconsistent maize hybrid responses to the LM system. Stover yield, stover quality, stover C and N, leaf area index (LAI), maize plant density, maize maturity, and sequence year in the MM sequence were also evaluated. Results emphasize the need for maize hybrid and LM system compatibility and effective LM suppression techniques.

Disciplines

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Authors

Cynthia A. Bartel, Chumki Banik, Andrew W. Lenssen, Kenneth J. Moore, David A. Laird, Sotirios V. Archontoulis, and Kendall R. Lamkey

Living mulch for sustainable maize stover biomass harvest

Cynthia A. Bartel*, Chumki Banik, Andrew W. Lenssen, Kenneth J. Moore, David A. Laird, Sotirios V.

Archontoulis, Kendall R. Lamkey

C.A. Bartel*, C. Banik, A.W. Lenssen, K.J. Moore, D.A. Laird, S.A. Archontoulis, K.R. Lamkey, Dep. of Agronomy, Iowa State University, Agronomy Hall, 716 Farmhouse LN, Ames, IA 50011-1051.

*Corresponding author (cabartel@iastate.edu).

Abbreviations: ADF, acid detergent fiber; CF, creeping red fescue; GDD, growing degree days; KB, Kentucky bluegrass; LAI, leaf area index; LM, living mulch; MM, maize following maize; NDF, neutral detergent fiber; SFA, sequential fiber analysis; SM, maize following soybean; SOC, soil organic carbon; SOM, soil organic matter; TAB, total aboveground biomass.

ABSTRACT

The Renewable Fuels Standard mandate provides enhanced opportunity for maize (*Zea mays* L.) stover use as a bioenergy feedstock. Living mulch (LM) offers a possible solution for the natural resources constraints associated with maize stover biomass harvest. A two site-year study was conducted near Boone and Kanawha, IA, in both maize following maize (MM) and maize following soybean [*Glycine max* (L.) Merr.] (SM) sequences to evaluate the impact of established and chemically suppressed Kentucky bluegrass (*Poa pratensis* L.) (KB) 'Ridgeline', 'Wild Horse', 'Oasis', and 'Mallard' blend and creeping red fescue (*Festuca rubra* L.) (CF) 'Boreal' as LM on three maize hybrids (population sensitive, population insensitive, and yield stable). Maize grain yield for the no LM treatments in the MM and SM sequences was 12.0 and 13.2 Mg ha⁻¹, respectively, at Boone and 12.8 and 14.8 Mg ha⁻¹, respectively, at Kanawha, 23-73% greater than the LM treatment. Ethanol yield (L ha⁻¹) was 12-119% greater, protein concentration was $\leq 9\%$ greater, and starch concentration was $\leq 1\%$ lower in the no LM treatment maize than LM treatment maize. Maize hybrid by cover interaction was significant for parameters including total aboveground biomass and protein concentration at Boone, with inconsistent maize hybrid responses to the LM system. Stover yield, stover quality, stover C and N, leaf area index (LAI), maize plant density, maize maturity, and sequence year in the MM sequence were also evaluated. Results emphasize the need for maize hybrid and LM system compatibility and effective LM suppression techniques.

Maize stover is harvested for a variety of uses, including as a feedstock for cellulosic ethanol production or for use in livestock operations as bedding or feed. The use of 136 billion liters per year of renewable fuels by 2022 is mandated by the Renewable Fuels Standard as established in the Energy Independence and Security Act of 2007, of which cellulosic biofuels would comprise 61 billion liters (Perlack and Stokes, 2011). The promulgated rules identify maize stover as a cellulosic biofuels feedstock (Schnepf, 2013), thus expanding long-term opportunities for stover as an additional revenue stream in the renewable fuels arena. Other uses also enhance the economic incentive to harvest maize stover. For example, chemical processing of residues can increase digestibility by 35 to 62% by decomposition of lignocellulosic bonds (Shreck et al., 2011), making maize stover more attractive for use as a livestock feed, especially when grain prices escalate (Meteer, 2014). The increased harvest of maize stover, however, compounds the already hefty challenges of natural resources conservation in conventional systems.

Maize stover provides myriad ecosystems services, and returning stover to soil recycles plant nutrients. Standard fertilization practices can be insufficient to compensate for nutrient loss following residue removal or when soil erosion approaches the soil loss tolerance rate (Lindstrom, 1986). Maize stover retention in maize-based cropping systems adds C to soils (Wilhelm et al., 2007) and helps to maintain soil organic matter (SOM) levels (Johnson et al., 2006; Wilhelm et al., 2004). Soil organic C (SOC) also impacts soil aggregation and stability (Tisdale and Oades, 1982), which in turn affect water infiltration, movement, and storage (Franzluebbers, 2001). Stover needed to maintain SOC, estimated at 5.25 to 12.50 Mg ha⁻¹ at 155 g kg⁻¹ moisture content, functions as an even greater restriction than the levels of stover needed to mitigate wind or water erosion (Wilhelm et al., 2007).

Residue removal can increase both water runoff and soil erosion (Pimentel et al., 1995). Crop residue protects the soil surface against raindrop impact (Wilhelm et al., 2004), as raindrop impact exacerbates erosion through the dislodging of exposed soil particles (Pimentel et al., 1995). In 1982, 68

million hectares, or 40% of all cropland, exceeded the soil loss tolerance rate (USDA, 2009). While the amount of cropland surpassing the soil loss tolerance rate has declined, in 2007 there remained 40 million hectares, or 28% of all cropland, which exceeded this rate (USDA, 2009). While quantification of soil erosion is difficult (Trimble and Crosson, 2000), cropland soil erosion is estimated at 10.3 Mg ha⁻¹ yr⁻¹ (USDA, 2012) while soil regeneration rates are only 0.02 to 1.9 Mg ha⁻¹ yr⁻¹ (Alexander, 1988).

Residue removal negatively impacts soil health and stability, and also affects the productivity of the primary crop of economic interest (Wilhelm et al., 2004). The relationship between residue management and maize grain yield is dependent on climate, weather, and tillage practices (Wilhelm et al., 2004). In the upper Midwest, for example, while stover removal may enhance maize grain yield in the short term, residue removal may damage soil quality long term through enhanced erosion and SOM loss (Rogovska et al., 2016). Although crop residue is credited as a feedstock capable of fueling growth in the ethanol sector in sufficiently available quantities, the environmental ramifications of its removal must be reconciled (DiPardo, 2000). Realization of continued crop yield increases requires improved management of nutrients and water (Mueller et al., 2012). Alternative cropping systems must therefore be designed to both facilitate sufficient supply of biofuels feedstocks and foster natural resources management (Karlen et al., 2011).

Living mulch offers a potential solution for reconciling stover removal with natural resources preservation, and grass species have been evaluated for their use as LM. While grain yield reductions were still documented in the LM treatment maize, *Poa* and *Festuca* species were identified as compatible groundcovers for a maize row crop system (Flynn et al., 2013). Substantial benefits of cover crops in a natural resources management capacity occur through diminished soil erosion, enhanced soil fertility, and water infiltration (Teasdale, 1996). Hall et al. (1984) reported that perennial covers with birdsfoot trefoil (*Lotus corniculatus* L.) and crownvetch (*Coronilla varia* L.) curtailed soil erosion 96.7 to 100% and also effected an 86.3 to 98% reduction in surface runoff. Effective weed control by perennial

grass covers has also been documented with rye (*Secale cereale* L.) (Ateh and Doll, 1996) and both Chewings fescue (*Festuca rubra* L. subsp. *commutata* Markgr.-Dann. 'Shadow') and Ladino clover (*Trifolium repens* L.) (Echtenkamp and Moomaw, 1989). Living mulch which effectively minimizes weed competition will often compete with the grain crop, requiring suppression and management of the LM itself (Teasdale, 1996). Similar maize grain yield has been documented between maize in strip tilled KB LM with effective suppression and the no LM treatment maize (Wiggans et al. 2012), and maize grain yield was highest among LM treatments with not more than 60% perennial cover in a chemically-suppressed grass sod of tall fescue (*Festuca arundinacea* Schreb.), orchardgrass (*Dactylis glomerata* L.), or smooth brome grass (*Bromus inermis* Leyss.) (Elkins et al., 1983). Another study reported that effective chemical suppression for approximately eight weeks was integral to reduce competition between the LM and maize (Elkins et al., 1979). The well-documented critical period for weed control (Hall et al., 1992; Knezevic et al., 2002) emphasizes the importance of suppression and LM management during early season maize growth.

Bartel et al. (2017) assessed i) the impact of establishing LM on developmental morphology and yield of soybean and maize and ii) the success of LM establishment specifically during the LM establishment year in both soybean and maize row crop systems. We built upon this previous work by conducting field studies in the early post-establishment production years with the objective of: i) assessing the impact of established LM on developmental morphology and yield of the maize crop, ii) evaluating persistence of established LM, and iii) assessing impact of established LM on weed community.

MATERIALS AND METHODS

The experimental design and many of the materials and methodologies used in this study were similar or identical to those included in a related study by Bartel et al. (2017). A two-site year study was conducted in successive years, in 2015 at the Agronomy and Agricultural Engineering Sorenson Research

Farm, 11.9 km southeast of Boone, IA, (42°0'N; 93°44'W) and in 2016 at the Northern Research Farm, 0.5 km south of Kanawha, IA, (42°56'N; 93°47'W), respectively. Climate data were obtained from the Iowa Environmental Mesonet stations closest to the research sites, approximately 3 km northwest of Boone and 17 km north of Kanawha (Iowa Environmental Mesonet Network, 2017). Growing degree days (GDD) were calculated from planting as follows:

$$\text{GDD} = \sum [(\text{daily maximum air temperature} \leq 30^{\circ}\text{C} + \text{daily minimum air temperature} \geq 10^{\circ}\text{C})/2] - 10^{\circ}\text{C} [1]$$

where 30°C comprises the maximum and 10°C the base temperature for maize development.

The Boone experiments in 2015 were located on soils dominated by Webster clay loam (0-2% slope, L107, fine-loamy, mixed, superactive, mesic Typic Endoaquolls), Clarion loam (2-6% slope, L138B, fine-loamy, mixed, superactive, mesic Typic Hapludolls), and Canisteo clay loam (0-2% slope, fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls). Average soil pH at the Boone site in 2015 was 6.3 and 6.6 in the 0- to 5- and 5- to 15-cm depths, respectively. Soil test (Mehlich-3) levels of P were 27 and 10 mg kg⁻¹ in the 0- to 5- and 5- to 15-cm depths, respectively. Soil test (Mehlich-3) levels of K were 160 and 80 mg kg⁻¹ in the 0- to 5- and 5- to 15-cm depths, respectively. The Kanawha experiments in 2016 were located on Clarion loam (2-6% slope, 138B, fine-loamy, mixed, superactive, mesic Typic Hapludolls) and Nicollet clay loam (1-3% slope, 55, fine-loamy, mixed, superactive, mesic Aquic Hapludolls). Average soil pH at the Kanawha site in 2016 was 5.1 and 4.9 in the 0- to 5- and 5- to 15-cm depths, respectively. Soil test (Mehlich-3) levels of P were 34 and 13 mg kg⁻¹ in the 0- to 5- and 5- to 15-cm depths, respectively. Soil test (Mehlich-3) levels of K were 243 and 111 mg kg⁻¹ in the 0- to 5- and 5- to 15-cm depths, respectively.

Two experiments were conducted, each with a different crop sequence. Experimental design consisted of a randomized complete block with three replications in each experiment. The first experiment included an MM crop sequence and the second experiment included an SM crop sequence.

Results are presented for both experiments for the second sequence year of maize. In the MM sequence, first and second year maize data were available to analyze impact of sequence year from both sites from a related study by Bartel et al. (2017). The 9.14- by 12.19-m research plots for both experiments were designed to illustrate application of the proposed system on a commercial scale, each plot accommodating 12 maize rows with 0.76-m interrow spacings. Cropping systems included maize-soybean rotations prior to the establishment of the LM plots in 2014. The plots used in the experiments were the identical plots from Bartel et al. (2017), where LM was established in either soybean or maize. Permanent rows for both maize and soybean were established with 0.76-m interrow spacings. The LM was neither controlled nor suppressed during the establishment year. The MM experiment was an incomplete factorial with 12 unique treatments in each block (Table 1). The no LM treatments with conventional tillage served as the control with each of three maize varieties used and maize stover residue retention. The impact of maize stover residue removal from no LM treatments in the MM sequence was assessed in three additional plots per block, with conventional tillage and each of three maize varieties used. The groundcover treatments each included one species of LM, either KB (Pennington Smart Seed Kentucky bluegrass blend, Madison, GA) or CF (La Crosse Forage & Turf Seed LLC, La Crosse, WI) with chemical suppression immediately after maize planting, paired with each of the three maize varieties, zone tillage, and residue removal in the MM sequence. In the SM sequence, treatments were similar to those in the MM sequence except that no maize stover existed from the previous year for removal. The SM experiment was an incomplete factorial with nine unique treatments in each block. To assess variation in maize germplasm response to the LM system, three glyphosate [*N*-(phosphonomethyl)glycine]-resistant maize varieties were planted, including yield stable DKC60-67RIB Blend, 110-d relative maturity; population sensitive DKC61-16RIB Blend, 111-d relative maturity; and population insensitive DKC57-75RIB Blend, 107-d relative maturity (Monsanto, St. Louis, MO). The DKC60-67RIB Blend is well suited for MM production as a yield stable hybrid with consistent

performance across growing regions, with a recommended planting rate of medium-high to high (Monsanto, 2013). Population sensitive DKC61-16RIB Blend is also well suited for MM production and requires higher plant density to economically optimize yield, generating best performance at medium-high to high densities (Monsanto, 2012). Population insensitive DKC57-75RIB Blend has a recommended planting rate of medium to high (Monsanto, 2014).

First Experimental Year

Cool season grasses at Boone were overseeded on 6 May 2014. The KB seed and CF seed were planted with a Tye 104-4204 Pasture Pleaser no-till seeder (AGCO Corporation, Duluth, GA). Seed was planted no deeper than 0.6 cm. The overseeding for CF was completed at 17.9 kg ha⁻¹ while KB was seeded at 20.7 kg ha⁻¹ to ensure adequate establishment after previous failed plantings. Initial grass planting at Boone in spring 2013 failed due to summer drought and winterkilled after replanting in September 2013.

On 1 Dec. 2014 approximately 90% of maize stover was removed from appropriate plots for the MM experiment at Boone with a John Deere 972 flail chopper (Deere & Company, Moline, IL). Soil preparation included chisel plowing to a 25-cm depth on 12 May 2015 in the no LM treatment plots with a custom chisel plow. Subsequent tillage was completed on 18 May 2015. Strip tillage was performed to a 20-cm depth in LM plots with an Unverferth Ripper Stripper 330 (Unverferth Manufacturing Co, Inc., Kalida, Ohio). The no LM treatment plots were tilled to a 20-cm depth with a John Deere 210 tandem disk (Deere & Company, Moline, IL). Field cultivation was completed in conventional plots to a 9-cm depth with a Stan-Hoist cultivator (Stan-Hoist Manufacturing Co., Standard Engineering, Fort Dodge, Iowa).

Fertilizer was broadcast at 90 kg P ha⁻¹ as P₂O₅ and 112 kg K ha⁻¹ as K₂O (both pre-planting) with a Befco 209 HOP Fertilizer Spreader (Befco Inc., Rocky Mount, NC) on both conventional and LM plots on 28 Apr. 2015. Nitrogen as S-coated urea (43-0-0-4) at 191 kg ha⁻¹ was banded on LM plots and on

conventional plots was broadcast on 19 May 2015 with a Gandy Orbit-Air Test plot applicator (Gandy Company, Owatonna, MN) with a 4.6 m boom.

Planting was completed with a John Deere 7100 planter equipped with Maxemerge units at 80,300 seeds ha⁻¹ on 19 May 2015. Broadcast applications of 0.56 kg a.i. ha⁻¹ paraquat [N,N'-dimethyl-4,4'-bipyridinium dichloride] (Gramoxone SL 2.0, Syngenta Canada Inc., Guelph, ON) for LM suppression were completed in LM plots immediately after maize planting on 19 May 2015 with a tractor-mounted Fimco sprayer (Fimco Industries, North Sioux City, SD) with 4.6 m boom and TeeJet XR 8002 flat fan tips (Spraying Systems Co., Wheaton, IL). Plots were sprayed on 22 May 2015 with 2.17 kg a.i. ha⁻¹ acetochlor [2-chloro- N-ethoxymethyl-N-(2-ethyl6-methylphenyl)acetamide] and 1.75 kg a.i. ha⁻¹ atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] and related triazines (Keystone, Dow AgroSciences, Indianapolis, IN) for control of grasses and broadleaf weeds and 0.05 kg a.i. ha⁻¹ flumetsulam [N-(2,6-difluorophenyl)-5-methyl-1,2,4-triazolo-(1,5a)-pyrimidine-2-sulfonamide] and 0.13 kg a.e. ha⁻¹ clopyralid [3,6-dichloro-2-pyridinecarboxylic acid] (Hornet, Dow AgroSciences, Indianapolis, IN) herbicide for broadleaf weed suppression. Glyphosate [N-(phosphonomethyl)glycine] (Drexel Imitator Plus, Drexel Chemical Company, Memphis, TN) was applied on 9 June 2015 to all plots at 0.83 kg a.e. ha⁻¹.

Unless otherwise noted, broadcast applications of herbicides for in-season weed control were completed in conventional plots with a John Deere 790 tractor and Fimco sprayer with 4.6 m boom and TeeJet XR 8002 flat fan tips. Herbicides were applied in LM plots with a John Deere 790 tractor and custom 4-row Red Ball hooded band sprayer. The 4-row hooded band sprayer ensured shielded application in a 25.4-cm width over each maize row, not applying chemical in 50.8 cm of each inter-row spacing so as to protect the living mulch. Both standard, full-boom width and banded applications were completed at 207 kpa and 159 L ha⁻¹ water for the treated areas.

Second Experimental Year

Cool season grasses at Kanawha were planted on 29 Apr. 2015 with the same no-till seeder and at the same depth as at Boone. Kentucky bluegrass was seeded at 5.6 kg ha⁻¹ and CF was seeded at 13.5 kg ha⁻¹. Approximately 90% of maize stover was removed from appropriate plots in the MM experiment at Kanawha on 3 Nov. 2015 after chopping with a John Deere H15 mower and raking with a New Holland 56 (New Holland Agriculture, New Holland, Pennsylvania). All plots were strip tilled with a John Deere 6125R tractor and custom strip tillage toolbar to a 20-cm depth with Yetter 2984 strip tillage row units (Yetter Manufacturing Inc., Colchester, IL) in fall of 2015. Spring tillage was completed on 25 Apr. 2016 prior to planting. Conventional plots were field cultivated to a 10-cm depth with a John Deere 6125R tractor and Wil-Rich 2500 Field Cultivator (Wil-Rich LLC, Wahpeton, ND). Living mulch plots were strip tilled to a 20-cm depth with the identical tractor and cultivator. Maize following maize plots were strip tilled again to a 20-cm depth with the same strip tillage equipment to manage residue for the planter.

Maize plots were planted at Kanawha on 26 Apr. 2016 at 82,800 seeds ha⁻¹ with a John Deere 6105R tractor and John Deere 1705 planter. An application of 0.56 kg a.i. ha⁻¹ paraquat [N,N'-dimethyl-4,4'-bipyridinium dichloride] (Gramoxone SL 2.0, Syngenta Canada Inc., Guelph, ON) for LM suppression was done in LM plots immediately after maize planting. All chemical applications in both LM and no LM treatment plots were broadcast with a John Deere 6125R tractor and Century 300 three point sprayer (Hiniker Agricultural Equipment, Mankato, MN) with custom hydraulic pump and GPS capability at 276 kpa and 187 kg ha⁻¹ water.

Fertilizer was applied on 6 May 2016 to all plots at 65 kg P ha⁻¹ as P₂O₅ and 45 kg K ha⁻¹ as K₂O was broadcast with a Gandy Orbit-Air Test plot applicator with a 4.6 m boom. An application of 168 kg N ha⁻¹ as S-coated urea (43-0-0-4) was banded on LM plots and was broadcast on conventional plots with a Gandy Orbit-Air Test plot applicator with a 4.6 m boom.

An application of 1.77 kg a.e. ha⁻¹ 2,4-D [2,4-dichlorophenoxyacetic acid] (Corn Belt 6 lb. Lovol Ester, Van Diest Supply Company, Webster City, IA) and 1.06 kg a.e. ha⁻¹ pendimethalin [N-(1-

ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine] (Prowl H₂O, BASF Corporation, Research Triangle Park, NC) were completed for LM plots on 16 May 2016. Conventional plots were sprayed on 16 May 2016 with 0.05 kg a.i. ha⁻¹ dimethenamid-P [(S)-2-chloro-N-[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethyl-thien-3-yl)-acetamide] (Outlook, BASF Corporation, Research Triangle Park, NC) and 0.99 kg a.e. ha⁻¹ glyphosate [N-(phosphonomethyl)glycine] (Buccaneer Plus, Tenkoz, Inc., Alpharetta, GA) with 2.23 kg ha⁻¹ spray grade ammonium sulfate spray adjuvant (Cornbelt Premium AMS, Van Diest Supply Company, Webster City, Iowa).

Conventional plots were sprayed at 1.04 kg ha⁻¹ glyphosate [N-(phosphonomethyl) glycine] (Abundit Extra, Nufarm, Inc., Burr Ridge, IL) with 2.23 kg ha⁻¹ spray grade ammonium sulfate spray adjuvant (Cornbelt Premium AMS, Van Diest Supply Company, Webster City, Iowa), 0.02 kg ha⁻¹ rimsulfuron [N-((4,6-dimethoxypyrimidin-2-yl) aminocarbonyl)-3-(ethylsulfonyl)-2-pyridinesulfonamide] and 0.09 kg ha⁻¹ mesotrione [2-(4-Mesy-2-nitrobenzoyl)-1,3-cyclohexanedione] (Dupont Realm Q, E. I. du Pont de Nemours and Company, Wilmington, DE) on 10 June 2016.

Measurement Procedures

Maize plant density was measured at both the V2 and R6 stage in 2015 and 2016. Maize maturity was assessed on a biweekly basis beginning at V6 in 2015 and V4 in 2016. Maize maturity was determined by the leaf collar method (Abendroth et al., 2011). Eight plants per plot were tagged between the V5 and V6 leaves from rows six and seven from which the mean plot growth stage was obtained. The husks were peeled back during the reproductive stages to assess kernel development and maturity.

Leaf area index and light interception were measured biweekly using a Decagon AccuPAR (Decagon Devices Inc., Pullman, WA) beginning at V9 in 2015 at Boone and V8 in 2016 at Kanawha. In each plot, four measurements were taken diagonally between 1000 and 1400 h on clear days with a

minimum of $900 \mu\text{mol m}^{-1} \text{s}^{-1}$ across the two center rows and from under the maize canopy, from which an average LAI per plot was determined.

The four center maize rows were harvested for grain yield data in both experiments with a four-row head attached to a John Deere 9450 combine at Boone and a John Deere 9410 combine at Kanawha. Maize grain was machine harvested on 16 Oct. 2015 at Boone and on 21 Oct. 2016 at Kanawha. Combines were outfitted with HarvestMaster systems (Juniper Systems, Inc., Logan, Utah) for grain moisture, weight, and yield. Maize grain yield data were standardized to 150 g kg^{-1} moisture content. A 1.32-m row of maize (equivalent to 1.0 area m^2) was hand harvested from the first row out from the four center rows at maize physiological maturity (R6) on 22 and 25 Sept. 2015 at Boone, on 20 Sept. 2016 at Kanawha, and again at machine harvest at both locations. The whole plant to soil surface was harvested. Plant number and ear number from the 1.32-m row, as well as fresh weight of stover (husks, stalks, and leaves), and fresh weight of ears were recorded for a random six-plant subsample from each plot. Stover and harvested ears from the six-plant subsample were separated, retained, and dried at 70°C until a constant weight was achieved as described by Dobermann (2005). In both years, grain was separated from cobs at all harvest dates and cobs were added back to dried stover for total stover weight (husks, stalks, leaves, and cobs) as defined by Wilhelm et al., 2004. Grain moisture was analyzed using a grain moisture analyzer (Model GAC 2000, DICKEY-john, Auburn, IL). The dried stover samples were representatively subsampled and ground to pass a 1-mm sieve on a Wiley Mill (Model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ). Stover yield and harvest index (HI) were calculated from the dried weights obtained for each treatment. Harvest index is defined as the grain dry weight divided by the total aboveground biomass dry weight.

Maize grain quality was evaluated with a Foss Infratec-1229 Whole Grain Analyzer (Foss North America, Eden Prairie, MN) with transmittance near infrared spectroscopy. Parameters evaluated included crude protein, oil, and starch on a dry matter basis, and both ethanol yield and density (specific

gravity) on a 150 g kg^{-1} moisture basis. Iowa State University Grain Quality Laboratory calibration model CN201301 for maize was used in both years for whole grain samples. As depicted by Dr. Charles Hurburgh and Glen Rippke of the Iowa State University Grain Quality Laboratory in the following language, the Iowa State University calibration process as described by Rippke et al. (1995) was subsequently the basis for the standard method of the American Association of Cereal Chemistry (AACC, 1999). The present calibrations are based the Artificial Neural Network (ANN) algorithm as adapted for Infratec analyzers by Foss (Büchmann et al., 2001).

Final harvest maize stover was analyzed using sequential fiber analysis (SFA) for neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, lignin, and ash (Van Soest et al., 1991). Both R6 (data not presented) and final harvest maize stover in both years were analyzed for C and N content using a vario Micro Cube Analyzer (Elementar Americas, Mt. Laurel, NJ).

A 5 by 5 frequency grid (15 cm by 15 cm per square) was used at the completion of the maize growing season, post-harvest in 2015, and both pre-planting and post-harvest in 2016 to assess groundcover persistence under maize with 100 cells counted per plot (Vogel and Masters, 2001). The grid was placed over the living mulch within the interrow spacing. The number of squares with grasses was counted out of total squares possible to generate percent cover, yielding frequency of persistence. Weeds were assessed by counting the number of weeds per species in five randomly-distributed 0.1-m^2 hoops per plot. Weed counts were taken in the spring on 7 June 2016 at Kanawha prior to the first in-crop herbicide application and fall post-harvest at both sites on 25 Oct. 2015 at Boone and 21 Oct. 2016 at Kanawha. The number of weeds per species counted in the five hoops were added and multiplied by a factor of two to estimate the weed density (weeds per m^2) and number of weeds per species per m^2 .

Statistical Analysis

Data were analyzed with the PROC GLM and MIXED procedures in SAS version 9.4 (SAS Institute, 2011). For the continuous maize sequence (MM), analysis of variance was used to assess significant

affects in the linear additive model. Location, treatment, and sequence year were considered fixed effects in the model, with block nested in location. Since the treatment structure of the experiments were incomplete factorials, estimates and differences of least square means were used for comparisons of treatments at $\alpha = 0.05$. First year maize data for the MM sequence were obtained from a related study (Bartel et al., 2017). The SM sequence was analyzed with a similar linear additive model, but without sequence year or resulting interactions because of the single year of maize data available from the SM sequence. Because of significant location by treatment interactions (Table 2; Table 3), data were subsequently analyzed within each site year with treatment as a fixed effect and block as a random effect, and presented as such.

RESULTS AND DISCUSSION

Weather Conditions

The Boone research site logged 2848 GDD during the growing season from planting to harvest (19 May to 16 Oct.) in 2015; the Kanawha research site logged 2893 GDD during the growing season from planting to harvest (26 Apr. to 21 Oct.) in 2016 (Fig. 1). Boone logged 714 mm precipitation during the 2015 growing season. Kanawha accumulated 825 mm precipitation during the 2016 growing season (Fig. 1). Annual totals included 3642 GDD and 1132 mm precipitation at Boone in 2015; and 3293 GDD and 1032 mm precipitation at Kanawha in 2016. Rainfall from Apr. to Oct. exceeded the trailing 30-yr average by 171 mm in 2015 at Boone and by 221 mm in 2016 at Kanawha. Average monthly high and low air temperatures did not depart substantially in either year from the trailing 30-yr averages at either site during the growing season (Fig. 1).

Living Mulch Persistence in Maize

A significant treatment by location interaction was observed for LM frequency in both sequences (Table 2). The CF maintained greater LM frequency (stand frequency) than KB in both crop sequences at Boone at the fall collection date in 2015, averaging 95 and 100% in the MM and SM

sequences, respectively, compared to 57 and 94% in the MM and SM sequences, respectively, for KB. Kentucky bluegrass frequency exceeded CF frequency in both sequences at the spring and fall collection dates in 2016 at Kanawha. Kentucky bluegrass averaged 48% in the spring and 94% in the fall, compared to 28% in the spring and 78% in the fall in the MM sequence for CF. In the SM sequence, KB averaged 66% in spring and 93% in the fall, compared to 44% in the spring and 82% in the fall for CF.

While rhizomatous propagation is more robust for KB than CF, red fescues are more shade tolerant, require less N and other nutrients, and establish more rapidly than KB (Beard, 1972), supporting second year results at Boone in 2015. In 2016, where KB persistence was superior to CF persistence, Kanawha received 272 and 93% more rain than the trailing 30-yr averages for Sept. and Oct., respectively. Because red fescues are less tolerant of wet soils than KB (Beard 1972), the greater-than-average fall precipitation was likely disadvantageous for CF persistence at the time of post-harvest data collection.

Grass was overseeded at Boone on 6 May 2014 because of sparse observed LM stands. The forage-type 'Park' KB and tall fescue initially planted as LM were older cultivars (Hall, 1996), which failed to establish after planting in the spring of 2013 because of summer drought and winterkilled after replanting in September 2013. The cultivars were selected for the experiments because similar maize grain yield has been documented between chemically suppressed forage-type 'Park' KB and the no LM treatment maize (Wiggans et al., 2012). We hypothesize that the existing LM stands at the time of overseeding contributed to the reductions in maize grain yield in the LM treatment maize at the Boone research site during the establishment year in 2014 (Bartel et al., 2017). At Kanawha where LM was planted concurrently with the maize crop, maize grain yield was similar between the no LM treatment maize and the LM treatment maize during the establishment year in 2015 (Bartel et al., 2017). Weather conditions impacted LM establishment success, which imply that additional research is needed to

identify LM species which establish consistently, ensure compatibility between the LM and the maize crop, and enhance system resiliency for system adoption by growers.

Maize Maturity and Plant Density

Differences were observed at Boone between the no LM treatment maize and CF maize in both sequences in the vegetative stages prior to R1 ($P \leq 0.01$), with CF maize delayed by as much as one vegetative stage at biweekly collection dates (data not presented). No differences were detected between treatments for the onset of R1 or R6. At Kanawha, in both sequences the KB maize was slower to mature through vegetative stages than either the no LM treatment maize or CF maize ($P \leq 0.01$), with KB maize delayed by as much as two vegetative stages compared to the no LM treatment maize at biweekly collection dates (data not presented). The LM treatment maize was largely at late vegetative stages (V13, V14, or VT) when the no LM treatment maize reached R1 at Kanawha. Maturity for the no LM treatment maize with residue removal was superior to no LM treatment maize with residue retention in the MM sequence until V14 at Kanawha and V13 at Boone ($P \leq 0.05$), as was maize plant density at Kanawha at V2, averaging 79,800 and 76,600 plants ha⁻¹, respectively ($P \leq 0.05$).

Maize plant density was similar at Boone for the MM sequence at V2, averaging 74,500 plants ha⁻¹. Maize plant density at R6 was greater for both the no LM treatment maize and KB maize than the CF maize, averaging 75,700, 75,100, and 68,900 plants ha⁻¹ for the no LM treatment maize, KB maize, and CF maize, respectively. For the SM sequence at Boone, maize plant density was greater for the no LM treatment maize than the CF maize at both the early vegetative and R6 stage, while maize plant density was greater for the no LM treatment maize than KB maize at the V2 collection date. Maize plant density at V2 averaged 77,800, 72,900, and 70,300 plants ha⁻¹ for the no LM treatment maize, KB maize, and CF maize, respectively. Density at R6 averaged 74,400, 70,300, and 67,300 plants ha⁻¹ for the no LM treatment maize, KB maize, and CF maize, respectively.

At Kanawha, differences in plant density were more pronounced throughout the season and in both sequences. For the MM sequence, the no LM treatment maize plant density was greater at V2 and R6 than the CF maize, but only greater at the R6 collection date compared to the KB maize. Densities at V2 and R6 averaged 78,200 and 77,900 plants ha⁻¹, respectively, for the no LM treatment maize, 75,900 and 74,000 plants ha⁻¹, respectively, for KB maize, and 74,000 and 72,100 plants ha⁻¹, respectively, for CF maize. For the SM sequence at Kanawha, the no LM treatment maize plant density was greater than both the KB maize and CF maize at both collection dates. Density for V2 and R6 averaged 81,100 and 81,200 plants ha⁻¹, respectively, for the no LM treatment maize, 73,600 and 72,900 plants ha⁻¹, respectively, for CF maize, and 73,300 and 72,100 plants ha⁻¹, respectively, for KB maize.

These findings are consistent with earlier reports, which found that consistent and uniform maize stands in maize-sod systems were challenging to achieve (Stanley and Gallaher, 1980), observed delayed maturity in intercropped maize (Klocke et al., 1989), and reported that greater LM cover generally resulted in lower maize plant density than the control (Flynn et al., 2013). Observed reductions in plant density in the no LM treatment maize with residue retention compared to no LM treatment maize with residue removal is consistent with other reports regarding lower emergence rates for maize treatments with residue retention across various tillage methods (Dam et al., 2003), and reduced maize plant growth when the maize plant was in close proximity to residue (Yakle and Cruse, 1983).

Maize Leaf Area Index

Leaf area index was greater for the no LM treatment maize than LM treatment maize throughout the season in both sequences and at both sites in the second sequence year (data not presented). Except for the final two collection dates, the LAI values for the MM sequence at Boone were greater for the no LM treatment maize than the LM treatment maize ($P \leq 0.01$). While the no LM treatment maize and KB maize LAI values were similar after the R1 collection date, the no LM treatment

maize and CF maize LAI values differed for every collection date ($P \leq 0.05$). Leaf area index of the population sensitive hybrid was greater than the population insensitive hybrid in LM from the R1 to the R5 collection date ($P \leq 0.05$). At Boone, no LM treatment maize LAI values were greater than CF maize LAI values in the SM sequence for every collection date ($P \leq 0.01$). Leaf area index values for no LM treatment maize were similar to the KB maize at the majority of the collection dates, differing only at the V13 and R6 dates ($P < 0.05$).

At Kanawha, LAI values were greater for the no LM treatment maize than the KB maize in the MM sequence at every collection date ($P \leq 0.05$). The CF maize and no LM treatment maize were similar at several collection dates, including at V9, V14, and R5. At Kanawha, no LM treatment maize LAI values were greater at every collection date than either KB maize or CF maize in the SM sequence ($P < 0.001$) and also greater for CF maize than KB maize at every collection date ($P \leq 0.01$).

The reductions in LAI values for LM treatment maize may have resulted from several factors, including the lower maize plant density for the LM treatments, competition from the LM for the maize within the LM treatments, and/or N insufficiency. Previous reports have documented reductions in maize LAI where competition with weeds was observed compared to weed free controls (Bonilla, 1984; Hall et al., 1992). In our experiment, rapid post-suppression recovery of the LM during early season maize growth was observed. Early exposure to weeds and the maize shade avoidance response, resulting from a low red to far-red light ratio shift, impedes maize biomass accumulation and leaf development (Page et al., 2009). Previous reports have also documented a relationship between available N and leaf area, in that larger leaf area values were observed with greater N levels (Radin, 1983). Smaller leaf area values had at least some deleterious resulting effect on radiation use efficiency (Sinclair and Horie, 1989). Reductions in maize leaf area were observed in conjunction with lower N flux ($\text{mmol N ear}^{-1} \text{ day}^{-1}$), C flux ($\text{mmol C ear}^{-1} \text{ day}^{-1}$), and resulting grain weight in low-N compared to high-N treatments (Lemcoff and Loomis, 1986). Differences in LAI observed from the late vegetative stages

coincides with timing of enhanced N demand by maize (Bender et al., 2013). This conclusion regarding the effect of N insufficiency in LM treatment maize is supported by related research (D.A. Laird, personal communication, 2017), which depicts reduced end of season stalk nitrate levels for the LM treatment maize in our study compared to the no LM treatment maize.

Maize Total Aboveground Biomass, Stover Yield, Grain Yield, Yield Components, and Harvest Index

The location by treatment interaction was significant in the MM sequence for maize total aboveground biomass, stover yield, and grain yield in the MM sequence, and for maize grain yield in the SM sequence (Table 2). Total aboveground biomass, stover yield, and grain yield were greater for the no LM treatment maize than LM treatment maize in both sequences and at both sites, excepting the no LM treatment maize and KB maize were similar for total aboveground biomass and stover yield in the MM sequence at Boone, averaging 17.47 and 6.94 Mg ha⁻¹, respectively (Table 4; Table 6). At Boone, the KB maize grain yield (10.7 Mg ha⁻¹) was greater than the CF maize grain yield (8.9 Mg ha⁻¹) in the MM sequence (Table 4). At Kanawha, the CF maize grain yield was greater than the KB maize grain yield in both the MM and SM sequences, averaging 6.7 and 9.7 Mg ha⁻¹ for the KB maize and CF maize, respectively, in the MM sequence and 6.7 and 10.4 Mg ha⁻¹ for the KB maize and CF maize, respectively, in the SM sequence (Table 4; Table 6). Total aboveground biomass and stover yield at Kanawha were greater in the CF than KB maize in the SM sequence by 29 and 37%, respectively, and in the MM sequence by 22 and 21%, respectively (Table 4; Table 6).

The hybrid by cover treatment interaction was significant for total aboveground biomass in both sequences at Boone (Table 4; Table 6). In the MM sequence at Boone, the population insensitive hybrid had greater total aboveground biomass in the no LM treatments than LM treatments, at 18.79 and 13.95 Mg ha⁻¹, respectively. In the SM sequence at Boone, the yield stable hybrid produced greater total aboveground biomass in no LM treatments than LM treatments, at 20.20 and 14.33 Mg ha⁻¹, respectively.

The maize grain yield reduction for LM treatment maize is similar to other reports, in that excessive competition from LM resulted in lower grain yield than conventional maize (Adams et al., 1970; Carreker et al., 1972; Flynn et al., 2013; Robertson et al., 1976). Box et al. (1980) observed that maize grain yield was lower in strip-killed sod than completely killed sod, and while water availability was not a limitation, observed differences may have been due to phytotoxicity. Previous reports attribute observed yield suppression of maize in LM to early season stresses (Bartel et al., 2017; Flynn et al., 2013). Grasses in our study had rapid post-suppression recovery and expanded into the strip tilled maize rows, exceeding the 60% cover or effective chemical suppression which other reports have found integral to support maize yield in LM systems (Elkins et al., 1979; Elkins et al., 1983). Yellow nutsedge (*Cyperus esculentus* L.) observed in the KB plots within the strip tilled maize rows during the summer growing season (Bartel, personal observation) likely provided enhanced competition within KB plots for maize growth at Kanawha in 2016.

The N insufficiencies for the LM treatment maize documented by Banik et al. (2016) likely contributed to LM treatment maize yield reduction. Bennett et al. (1976) observed positive relationships between N application and maize grain yield in a sod-maize system and a generally positive relationship between the sod-applied atrazine rate and maize grain yield, although the relationship was to some extent sod species-specific. Carreker et al. (1972) reported a proportionality between grain yield and both N applied in a sod-maize system and fescuegrass [*Schedonorus arundinaceus* (Schreb.) Dumort.] cover when irrigated; however, N rates applied elicited no maize grain yield response in live sod without irrigation.

The results from Wiggans et al. (2012), in which grain yield was similar between LM treatment maize and the no LM control, may have resulted from the enhanced compatibility of the LM species or cultivars used in that study. Similar maize grain yield observed under a variety of tillage methods was attributed to LM species compatibility (Beale and Langsdale, 1964). Effective chemical suppression in

conjunction with cool season species dormancy facilitates an advantage for maize in LM in the competition for resources (Elkins et al, 1979). The inadequately suppressed LM in our experiment likely functioned as an early season weed, which can initiate the shade avoidance responses in maize during the critical period for weed control regardless of resources abundance (Page et al., 2009). Cool season grass species have been identified as superior for shade tolerance to warm season grasses, and cool season grasses are more shade tolerant in the summer and fall (Lin et al., 1999). This enhanced shade tolerance roughly coincides with row crop canopy closure timeframes and depletion of photosynthetically active radiation for the LM grass species. Thus, compatibility and summer dormancy within a cool-season, C3 grass species may be two key aspects of successful LM integration into row-cropped maize.

In the MM sequence, no LM treatment maize with residue removal had statistically significantly greater total aboveground biomass and stover yield than no LM treatment maize with residue retention at Boone by 21 and 23%, respectively (Table 4). In the MM sequence, year one and year two differed for total aboveground biomass (20.27 Mg ha⁻¹, 16.44 Mg ha⁻¹), stover yield (9.28, 6.86 Mg ha⁻¹), and grain yield (12.2 Mg ha⁻¹, 10.7 Mg ha⁻¹) (Table 2). The reduction in productivity in the second year maize with residue retention is consistent with the well-established yield penalty in continuous maize production (Dam et al., 2003), in part attributed to lower N availability from reduced net soil N mineralization and/or enhanced immobilization from maize biomass residue (Gentry et al., 2013).

The HI was similar in both sequences at both sites, at 0.58. Sequence year was significant in the MM sequence, with a lower HI for year one maize (0.54) (Table 2). The greater HI in year two also reflects the lower total aboveground biomass produced in the MM sequence in that same year, and maize HI is affected by both genetics and environmental stresses (Prihar and Stewart, 1990).

Maize Grain Quality, Stover C/N, and Sequential Fiber Analysis

The location by treatment interaction was significant for ethanol yield (L kg^{-1}) in the SM and MM sequences (Table 2). Ethanol yield (L ha^{-1}) was 12% and 34% greater for the no LM treatment maize than KB maize and CF maize, respectively, and greater for KB maize ($4,538 \text{ L ha}^{-1}$) than CF maize ($3,790 \text{ L ha}^{-1}$) in the MM sequence at Boone (Table 4). No LM treatment maize was 28% greater for ethanol yield (L ha^{-1}) than the LM treatment maize in the SM sequence at Boone, but CF maize and KB maize ethanol yield (L ha^{-1}) were similar, averaging $4,318 \text{ L ha}^{-1}$ (Table 6). Ethanol yield (L ha^{-1}) was 98% and 33% greater for the no LM treatment maize than KB maize and CF maize, respectively, and greater for CF maize ($4,119 \text{ L ha}^{-1}$) than KB maize ($2,767 \text{ L ha}^{-1}$) in the MM sequence at Kanawha (Table 4). Ethanol yield (L ha^{-1}) was 41 and 119% greater for the no LM treatment maize than CF maize or KB maize, respectively, and greater for CF maize ($4,466 \text{ L ha}^{-1}$) than KB maize ($2,880 \text{ L ha}^{-1}$) in the SM sequence at Kanawha (Table 6).

In the SM sequence in both site-years, oil concentration was similar, averaging 34 g kg^{-1} at Boone and 32 g kg^{-1} at Kanawha (Table 6). The no LM treatment maize was greater than the LM treatment maize for protein concentration and maize grain density by 9 and 1%, respectively, in the SM sequence at both sites (Table 6). Ethanol yield (L kg^{-1}) was greater for the LM treatment maize, averaging 0.43 and 0.42 for the LM treatment maize and the no LM treatment maize, respectively, in the SM sequence at both sites (Table 6). The population insensitive hybrid in LM was greater than the population sensitive hybrid in LM for starch concentration, grain density, and ethanol yield (L kg^{-1}) in the SM sequence at Boone, but only greater in grain density in the SM sequence at Kanawha (Table 6).

More maize grain quality parameters were significantly different between the no LM treatment maize and the CF maize than the KB maize in the MM sequence at Boone (Table 4), resulting from greater LM cover within CF plots than KB plots. The no LM treatment maize grain was greater for protein concentration than the LM treatment maize, averaging 64 and 60 g kg^{-1} , respectively. The no LM treatment maize grain also greater for density and lower for both starch concentration and ethanol yield

(L kg⁻¹) than the CF maize (Table 4). The no LM treatment maize and CF maize averaged 1.21 and 1.20 g cc⁻¹, 637 and 641 g kg⁻¹, and 0.42 and 0.43 L kg⁻¹, respectively, for grain density, starch concentration, and ethanol yield (L kg⁻¹) (Table 4). The population sensitive hybrid in LM had greater maize grain protein but lower oil concentration, starch concentration, and ethanol yield (L kg⁻¹) than the population insensitive hybrid in LM (Table 4).

Few differences were observed for measured grain quality parameters in the MM sequence at Kanawha. The location by treatment interaction for grain density was significant in the MM sequence (Table 2). Grain density was greater for the no LM treatment maize than the KB maize, averaging 1.22 and 1.20 g cc⁻¹, respectively. (Table 4). No differences were observed for protein concentration, starch concentration, and ethanol yield (L kg⁻¹), averaging 61 g kg⁻¹, 634 g kg⁻¹, and 0.43 L kg⁻¹, respectively (Table 4). Sequence year was significant for every grain quality parameter in the MM sequence, lower in year two for all parameters (data not presented).

The hybrid by cover treatment interaction was significant for protein concentration in both sequences at Boone and for starch concentration in the MM sequence at Boone (Table 4; Table 6). The population sensitive and yield stable hybrids had greater protein concentration in the no LM treatments than the LM treatments by 10 and 11%, respectively, and greater by 8% for both hybrids in the MM sequence (Table 4; Table 6). Starch concentration was lower in the no LM treatment than LM treatment for the population sensitive hybrid, at 638 and 641 g kg⁻¹, respectively, and yield stable hybrid, at 638 and 640 g kg⁻¹, respectively, in the MM sequence.

Differences in grain quality between treatments may be attributed to both a reduction in assimilate supply in the LM system and genetic differences in N utilization in the maize hybrids. While maize grain oil content is influenced by factors other than assimilate supply, restrictions in assimilate supply of N and sucrose have been found to concurrently reduce maize grain protein content and enhance maize grain starch content (Borrás et al., 2002). Disparities in response to assimilate supply for

maize grain protein have also been attributed to genetic differences in maize hybrid N remobilization and utilization efficiency (Wyss et al., 1991).

Stover N (g kg^{-1}) was greater and the C/N ratio was lower for the no LM treatment maize than the LM treatment maize in the SM sequence at both sites (Table 6). Stover C (g kg^{-1}) was greater for the no LM treatment maize than the KB maize in the SM sequence at Kanawha, averaging 459 and 453 g kg^{-1} , respectively (Table 6). Stover N (g kg^{-1}), stover C (g kg^{-1}), and the C/N ratio were similar between the no LM treatment maize and LM treatment maize in the MM sequence at Kanawha, averaging 463 g kg^{-1} , 5.5 g kg^{-1} , and 86.3, respectively. The CF maize had lower stover N (g kg^{-1}) and a greater C/N ratio than the no LM treatment maize at Boone, likely attributable to the frequency of CF cover (Table 4). Stover N (g kg^{-1}) was greater and the C/N ratio was lower for the no LM treatment maize with residue removal than both the no LM treatment maize with residue retention and LM treatment maize in the MM sequence at Boone (Table 4). In both sequences, stover N and C accumulation (kg ha^{-1}) were greater for the no LM treatment maize than LM treatment maize ($P \leq 0.05$) (data not presented). In the MM sequence, sequence year was significant for stover N concentration, lower for second year maize (5.4 g kg^{-1}) than first year maize (7.3 g kg^{-1}) (Table 2). Sequence year was also significant for stover C and N accumulation, both greater in first year maize ($P < 0.001$) (data not presented). The hybrid by cover treatment interaction was significant for stover C (g kg^{-1}) in the MM sequence at Boone (Table 4). The population insensitive hybrid had greater stover C in the no LM treatment than LM treatment, at 475 and 465 g kg^{-1} . The yield stable hybrid had greater stover C in the LM treatment than no LM treatment, at 475 and 485 g kg^{-1} .

These findings suggest that more N was available to the maize in the SM sequence than the MM sequence, which would be consistent with previous reports concerning the enhancement of N availability by legumes in crop rotations for the subsequent crop (Peoples et al., 2009). Although the contribution has been challenging to quantify (Peoples and Craswell, 1992; Varvel and Wilhelm, 2003),

the soil N generated through Bradyrhizobium N-fixing bacteria in leguminous crops is well documented (Keyser and Li, 1992; Salvagiotti et al., 2008). Legume residues also have a lower C/N ratio (Gomes et al., 2009; Turner et al., 2016) which enhances the net N mineralization rate (Gentry et al., 2001), in contrast with the immobilization resulting from the greater C/N ratio observed in maize residue (Kaboneka et al., 1997).

The location by treatment interaction was significant for the SFA measurements (g kg^{-1} , 100% DM) of NDF and ADF for both sequences, and also cellulose in the MM sequence (Table 3). At Boone, ADL and lignin were greater and hemicellulose was lower for the no LM treatment maize than LM treatment maize in both sequences, with ADF also greater for no LM treatment maize in the MM sequence (Table 5; Table 7). At Kanawha in both sequences, ADF and cellulose were greater for the no LM treatment maize than the LM treatment maize, with lower total ash and hemicellulose for the no LM treatment maize (Table 5; Table 7). Sequence year was significant for all parameters in the MM sequence, greater in year two, except total ash (data not presented). Differences were also observed between the KB and CF maize in both site-years. In both sequences at Kanawha, ADF was lower and hemicellulose was greater for KB maize than CF maize. In the MM sequence at Boone, ADF was greater and hemicellulose was lower for KB maize than CF maize (Table 5; Table 7).

The hybrid by cover treatment interaction was significant for ADL and lignin in the MM sequence at Boone and for hemicellulose in the MM sequence at Kanawha (Table 5). In the MM sequence at Boone, ADL was 22, 17, and 8% greater for the population sensitive, population insensitive, and yield stable hybrid, respectively, in the no LM treatment than LM treatment (Table 5). In the MM sequence at Boone, lignin was 24, 17, and 6% lower for the population sensitive, population insensitive, and yield stable hybrid, respectively, in the LM treatment than no LM treatment (Table 5). In the MM sequence at Kanawha, hemicellulose was 9, 4, and 6% greater for the population sensitive, population insensitive, and yield stable hybrid, respectively, in the LM treatment than no LM treatment (Table 5).

Results observed are consistent with previous reports which documented an inverse relationship between maize ear fill and stover quality. Coors et al. (1997) found that NDF, ADF, and ADL concentration increased, while hemicellulose concentration decreased, with enhanced ear fill. Results are meaningful as biomass feedstock quality influences biofuels production. Slagging tendency is influenced by ash composition of biomass feedstock (Xiong et al., 2010), and lignin and hemicellulose removal through biomass pretreatment liberates cellulose for biofuels production (Sun and Cheng, 2002). Research has focused on modifying lignin and cellulosic content to achieve desirable composition for the purposes of biofuels production (Ragauskas et al., 2010).

Weed Community

At the spring collection date at Kanawha, total weed density (weeds m⁻²) was greater in the KB plots than either the no LM plots ($P < 0.001$) or CF plots ($P < 0.001$) in the MM sequence. Mean weed density (minimum-maximum) in the MM sequence for the no LM plots, KB plots, and CF plots was 1.2 (0-12), 12.7 (4-34), and 2.2 (0-10) weeds m⁻², respectively. In the SM sequence, total weed density was greater in the CF plots than no LM plots ($P < 0.05$). Mean weed density for the SM sequence for the no LM plots, KB plots, and CF plots was 0.9 (0-4), 26.0 (0-142), and 44.4 (2-258) weeds m⁻², respectively. The weed community at Kanawha consisted of 59% green foxtail [*Setaria italica* (L.) P. Beauv.], 14% shattercane [*Sorghum bicolor* (L.) Moench], 13% toothed spurge (*Euphorbia dentata* Michx.), and 14% from 10 other species. The weed community at Kanawha was comprised of 81 and 19% grass and broadleaf species, respectively.

At the post-harvest collection date in the MM sequence at Boone and Kanawha, total weed density was similar, averaging 10.2 (0-38) and 4.3 (0-24) weeds m⁻², respectively. In the SM sequences, total weed density was greater in the KB plots than no LM plots at Boone ($P < 0.05$), but was greater in both KB plots and CF plots than no LM plots at Kanawha ($P < 0.05$). Mean weed density in the SM sequence at Boone for the no LM plots, KB plots, and CF plots was 7.7 (0-42), 15.8 (8-36), and 9.6 (2-26)

weeds m⁻², respectively. Mean weed density in the SM sequence at Kanawha for the no LM plots, KB plots, and CF plots was 5.2 (0-32), 20.2 (0-70), and 22.4 (4-50) weeds m⁻², respectively. The weed community at Kanawha consisted of 52% grass species (neither KB nor CF), 16% corn speedwell (*Veronica arvensis* L.), 12% giant foxtail (*Setaria faberi* Herrm.), 5% wild buckwheat [*Fallopia convolvulus* (L.) Á. Löve], and 14% from six other species. The weed community at Kanawha was comprised of 65 and 35% grass and broadleaf species, respectively. The weed community at Boone included 54% dandelion (*Taraxacum* sp. L.), 21% other grass species (neither KB nor CF), 6% West Indian nightshade (*Solanum ptychanthum* Dunal), 6% clover (*Trifolium* spp. L.), and 13% from seven other species. The weed community at Boone included 79 and 21% broadleaf and grass species, respectively.

Our findings are consistent with previous research, in that weed suppression efficacy in LM systems is generally attributable to level of LM cover. Weeds compete with the grain crop for available resources, restricting crop yield, and may also produce allelopathic chemicals which in turn limit crop growth. Ateh and Doll (1996) observed that rye as LM reduced the shoot biomass of weed species, including common lambsquarters (*Chenopodium album* L.), velvetleaf (*Abutilon theophrasti* Medik.), and giant foxtail, by 60 to 90% in each of three years as compared to a no rye control without weed suppression. Previous reports indicate significant weed control by increasing the rye cover crop seeding rate (Ateh and Doll, 1996; Nagabhushana et al., 1995). Ernache and Ilnicki (1990) documented that LM systems with subterranean clover (*Trifolium subterraneum* L. 'Nangeela') controlled weeds better than either dead mulch or mulch-free systems. Decreased weed pressure may reduce input expenses through reduced herbicide applications. Living mulches which minimize weed pressure usually themselves require suppression so as not to impact the companion grain crop (Teasdale, 1996). Overall herbicide use would therefore be contingent on levels also used for LM suppression.

CONCLUSIONS

Greater grain yield and ethanol yield were observed consistently for the no LM treatment maize than LM treatment maize in both the MM and SM sequences at both sites, in addition to reductions in LAI, plant density, total aboveground biomass, and stover yield within the LM treatment maize. The significant maize hybrid and living mulch interaction observed in measured parameters particularly at Boone in the MM sequence underscore the importance of further research to identify crops for living mulch systems which are stable and compatible. In both experiments, rapid recovery of the LM species following suppression encouraged early season competition with the maize crop. The LM planting failures at the Boone site presented challenges with LM establishment, and LM species persistence was inconsistent and location dependent. These conclusions emphasize the importance of both enhancing system resiliency and identifying effective suppression techniques to minimize LM competition especially during the early critical period of growth for the maize row crop. We propose that ideal traits for the LM cover would include summer dormancy, receptivity to chemical or mechanical suppression, faster green up in the spring for nitrate-N (NO_3^-) recycling and reduction of leaching, tolerance for crop canopy shade, a compatible root structure with both strong rhizome development and shallow root systems, and low-growing so as to interfere to a lesser extent with crop emergence and growth.

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Fig. Captions:

Fig. 1. (A) Growing degree days (GDD) and departure from the 30-yr trailing average by month in 2015 for Boone and in 2016 for Kanawha; and (B) total precipitation and departures from the 30-yr trailing average by month in 2015 for Boone and in 2016 for Kanawha.

1 **Table 1. Treatments for the maize following maize (MM) and maize following soybean (SM) sequences at Boone and Kanawha in 2015 and 2016,**
 2 **respectively, and residue removal protocol exclusively for the MM sequence.**

Treatment†	Living mulch	Tillage method	Hybrid characteristic	Residue removal	N fertilizer application
1	None	Conventional	Population sensitive	Removed	Broadcast
2	None	Conventional	Population sensitive	Not removed	Broadcast
3	None	Conventional	Population insensitive	Removed	Broadcast
4	None	Conventional	Population insensitive	Not removed	Broadcast
5	None	Conventional	Yield stable	Removed	Broadcast
6	None	Conventional	Yield stable	Not removed	Broadcast
7	Kentucky bluegrass	Zone tillage	Population sensitive	Removed	Banded
8	Kentucky bluegrass	Zone tillage	Population insensitive	Removed	Banded
9	Kentucky bluegrass	Zone tillage	Yield stable	Removed	Banded
10	Creeping red fescue	Zone tillage	Population sensitive	Removed	Banded
11	Creeping red fescue	Zone tillage	Population insensitive	Removed	Banded
12	Creeping red fescue	Zone tillage	Yield stable	Removed	Banded

3 † Treatments 1 and 2, 3 and 4, and 5 and 6 were duplicates within the SM sequence.

4 **Table 2. Type III tests of significance for fixed sources of variation for MM† and SM measurements including GY, TAB, stover yield, HI, stover C, stover N,**
5 **C/N, grain protein, grain oil, grain starch, and grain density, and EY.**

Source of variation	GY	TAB	Stover	HI	Stover C	Stover N	C/N	Protein	Oil	Starch	Density	EY (L kg ⁻¹)	EY (L ha ⁻¹)	LM Frequency
							<u>MM sequence</u>							
Location (L)	NS‡	NS	NS	NS	NS	*	*	NS	*	**	NS	NS	**	**
Treatment (T)	***	NS	NS	NS	NS	***	***	***	***	***	**	NS	***	***
L × T	**	**	**	NS	NS	NS	NS	NS	NS	NS	*	NS	***	***
Sequence Year (Y)	***	***	***	***	NS	***	***	***	***	***	**	**	***	***
L × Y	***	***	NS	***	***	***	***	NS	***	***	NS	NS	***	***
T × Y	***	**	*	NS	NS	NS	NS	**	NS	NS	*	NS	***	***
L × T × Y	***	**	*	NS	NS	NS	NS	NS	NS	NS	***	NS	***	***
							<u>SM sequence</u>							
L	NS	NS	*	NS	***	NS	NS	NS	*	**	NS	NS	**	**
T	***	***	***	NS	NS	*	*	***	*	***	***	NS	***	***
L × T	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	***	**

6 * Significant at the 0.05 probability level.

7 ** Significant at the 0.01 probability level.

8 *** Significant at the 0.001 probability level.

9 † EY, ethanol yield; GY, grain yield; HI, harvest index; LM, living mulch; MM, maize following maize; SM, maize following soybean; TAB, total aboveground biomass.

10 ‡ NS, nonsignificant.

12

13 **Table 3. Type III tests of significance for fixed sources of variation for MM† and SM measurements including total ash, NDF, ADF, ADL, hemicellulose,**
 14 **cellulose, and lignin.**

Source of variation	Total ash	NDF	ADF	ADL	Hemicellulose	Cellulose	Lignin
<u>MM sequence</u>							
Location (L)	NS‡	*	NS	*	NS	NS	*
Treatment (T)	NS	NS	***	NS	***	***	NS
L × T	NS	*	*	NS	NS	*	NS
Sequence Year (Y)	NS	***	***	**	***	***	**
L × Y	NS	***	***	NS	**	***	NS
T × Y	NS	NS	NS	NS	*	NS	NS
L × T × Y	NS	NS	*	NS	NS	**	NS
<u>SM sequence</u>							
L	**	*	*	NS	NS	**	NS
T	NS	NS	**	NS	***	*	NS
L × T	NS	*	*	NS	NS	NS	NS

15 * Significant at the 0.05 probability level.

16 ** Significant at the 0.01 probability level.

17 *** Significant at the 0.001 probability level.

18 † ADF, acid detergent fiber; ADL, acid detergent lignin; MM, maize following maize; NDF, neutral detergent fiber; SM, maize following soybean.

19 ‡ NS, nonsignificant.

20 **Table 4. Treatment means and significance for MM† measurements including GY, TAB, stover yield, HI, stover C, stover N, C/N, grain protein, grain**
 21 **oil, grain starch, and grain density, and EY at Boone and Kanawha in 2015 and 2016, respectively. Grain yield was obtained from combine harvest and**
 22 **is expressed with grain density and EY at 150 g kg⁻¹ moisture content. Total aboveground biomass, stover yield, HI, stover C, stover N, grain protein,**
 23 **grain oil, and grain starch are expressed on an oven-dry basis.**

Treatment‡	GY	TAB	Stover	HI	Stover C	Stover N	C/N	Protein	Oil	Starch	Density	EY	EY
	Mg ha ⁻¹				g kg ⁻¹			g kg ⁻¹			g cc ⁻¹	L kg ⁻¹	L ha ⁻¹
						<u>2015</u>							
1	11.3	17.48	6.98	0.60	480	5.3	91.6	65	32	638	1.21	0.42	4786
2	10.3	15.96	6.25	0.62	469	4.9	95.7	64	34	636	1.20	0.42	4359
3	12.7	21.02	8.90	0.58	474	7.0	68.6	65	37	636	1.22	0.42	5350
4	11.5	16.56	7.62	0.54	475	6.0	79.6	59	36	641	1.22	0.43	4895
5	13.4	20.77	8.37	0.60	471	5.5	85.9	65	33	637	1.22	0.42	5660
6	12.8	16.36	5.81	0.64	479	4.5	108.7	63	33	638	1.21	0.42	5418
7	10.6	17.60	6.65	0.62	479	5.8	82.2	64	34	636	1.21	0.42	4486
8	10.3	12.61	4.76	0.66	463	5.9	81.0	58	38	640	1.21	0.43	4383
9	11.1	18.83	7.08	0.62	489	4.6	107.6	60	33	640	1.21	0.43	4745
10	9.6	13.56	5.23	0.61	477	5.0	96.7	60	33	639	1.19	0.43	4085
11	8.3	15.28	6.71	0.56	467	5.0	93.8	57	36	643	1.20	0.43	3537
12	8.8	14.23	5.51	0.61	481	4.7	104.7	58	34	641	1.20	0.43	3747
SE	0.72	1.51	0.81	0.04	5.08	0.37	6.83	1.26	0.69	1.20	0.006	0.001	300.73
	<i>P > F</i>												
No LM vs. LM Hybrid	***	**	**	NS	NS	NS	NS	***	NS	**	**	***	***
Cover × hybrid	NS§	NS	NS	NS	NS	**	*	*	***	NS	NS	NS	NS
No LM vs. KB	*	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	*
No LM vs. CF	***	**	*	NS	NS	*	*	***	NS	***	***	***	***
CF vs. KB	**	NS	NS	NS	NS	NS	NS	*	NS	*	NS	NS	**
LM insensitive vs. LM sensitive	NS	NS	NS	NS	*	NS	NS	***	***	**	NS	*	NS
R1 vs. R2	NS	*	*	NS	NS	*	*	*	NS	NS	NS	NS	NS
R1 vs. LM	***	***	**	NS	NS	**	*	***	NS	**	***	***	***
						<u>2016</u>							
1	13.2	18.90	7.77	0.59	461	5.9	79.1	62	31	634	1.21	0.43	5631
2	12.7	20.81	8.89	0.57	462	5.7	82.8	62	32	632	1.20	0.42	5375
3	12.3	18.03	7.60	0.58	461	5.9	79.6	59	34	635	1.24	0.43	5282
4	11.8	14.61	6.77	0.54	458	6.1	76.6	58	33	636	1.23	0.43	5073
5	13.1	17.32	7.47	0.57	513	5.5	95.9	63	30	632	1.23	0.43	5607
6	13.7	18.85	9.17	0.51	463	4.8	96.3	62	31	632	1.22	0.43	5830
7	6.7	13.04	5.12	0.61	456	5.1	89.9	60	33	633	1.18	0.43	2836

8	6.9	13.46	6.04	0.55	454	6.6	73.1	58	34	635	1.22	0.39	2727
9	6.4	12.10	5.60	0.53	455	4.4	102.9	60	31	634	1.19	0.43	2739
10	11.0	17.86	7.35	0.59	458	5.2	88.8	63	31	633	1.22	0.43	4682
11	8.8	15.18	6.68	0.55	457	6.7	69.2	60	33	633	1.21	0.43	3738
12	9.2	14.12	6.29	0.55	455	4.5	101.3	61	31	634	1.20	0.43	3936
SE	0.76	1.23	0.61	0.021	14.58	0.63	6.84	1.53	0.66	1.90	0.009	0.01	348.64

P > F

No LM vs. LM Hybrid	***	***	***	NS	NS	NS	NS	NS	NS	NS	**	NS	***
Cover × hybrid	NS	NS	NS	*	NS	NS	**	NS	***	NS	**	NS	NS
No LM vs. KB	***	***	***	NS	NS	NS	NS	NS	NS	NS	***	NS	***
No LM vs. CF	***	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	***
CF vs. KB	***	**	*	NS	NS	NS	NS	NS	NS	NS	*	NS	***
LM insensitive vs. LM sensitive	NS	NS	NS	*	NS	*	*	NS	*	NS	NS	NS	NS
R1 vs. R2	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
R1 vs. LM	***	***	**	NS	*	NS	NS	NS	NS	NS	**	NS	***

24 * Significant at the 0.05 probability level.

25 ** Significant at the 0.01 probability level.

26 *** Significant at the 0.001 probability level.

27 † CF, creeping red fescue; EY, ethanol yield; KB, Kentucky bluegrass; HI, harvest index; LM, living mulch; MM, maize following maize; R1, no LM treatment with residue removal; R2, no LM treatment with residue retention; TAB, total aboveground biomass.

29 ‡ Treatments 1 to 6 are maize with no LM, with residue removal for treatments 1, 3, and 5. Treatments 1 and 2 are the population sensitive hybrid, treatments 3 and 4 are the population insensitive hybrid, and treatments 5 and 6 are the yield stable hybrid. Treatment 7 to 9 are maize with KB. Treatment 7 is the population sensitive hybrid, treatment 8 is the population insensitive hybrid, and treatment 9 is the yield stable hybrid. Treatments 10 to 12 are maize with CF.

32 Treatment 10 is the population sensitive hybrid, treatment 11 is the population insensitive hybrid, and treatment 12 is the yield stable hybrid.

33 § NS, nonsignificant.

34 **Table 5. Treatment means and significance for MM† measurements including sequential fiber parameters, including total ash, NDF, ADF, ADL,**
 35 **hemicellulose, cellulose, and lignin, all expressed on a g kg⁻¹ basis, at Boone and Kanawha in 2015 and 2016, respectively.**

Treatment‡	Total ash	NDF	ADF	ADL	Hemicellulose	Cellulose	Lignin
	g kg ⁻¹						
	<u>2015</u>						
1	72	811	489	63	322	427	60
2	79	803	475	51	328	424	47
3	77	801	469	53	332	415	50
4	74	809	477	52	333	425	48
5	86	799	479	53	320	426	49
6	85	802	471	51	331	419	47
7	84	799	469	46	329	424	42
8	74	814	469	47	345	422	44
9	82	798	469	53	329	416	49
10	80	802	460	48	341	413	44
11	77	803	453	43	350	410	39
12	77	805	456	44	349	412	42
SE	5.63	6.14	6.74	3.09	5.74	5.84	3.26
	<i>P > F</i>						
No LM vs. LM Hybrid	NS§	NS	**	***	***	NS	**
Cover × hybrid	NS	NS	NS	NS	*	NS	NS
No LM vs. KB	NS	NS	NS	*	NS	NS	*
No LM vs. CF	NS	NS	***	***	***	*	**
CF vs. KB	NS	NS	*	NS	**	NS	NS
LM insensitive vs. LM sensitive	NS	NS	NS	NS	*	NS	NS
R1 vs. R2	NS	NS	NS	*	NS	NS	NS
R1 vs. LM	NS	NS	**	***	***	NS	***
	<u>2016</u>						
1	81	806	483	55	323	428	50
2	83	796	472	53	324	419	46
3	91	788	455	54	333	401	46
4	91	774	446	45	328	401	37
5	87	802	478	54	324	424	47
6	88	805	480	52	325	428	45
7	89	797	440	59	358	381	53
8	97	780	432	39	348	393	32
9	102	782	435	50	347	385	44
10	83	804	459	47	345	412	42

11	96	778	436	77	342	360	70
12	94	796	453	49	343	404	42
SE	4.19	5.61	5.90	6.81	3.80	9.10	6.79
	<i>P > F</i>						
No LM vs. LM Hybrid	**	NS	***	NS	***	***	NS
Cover × hybrid	*	**	**	NS	NS	NS	NS
No LM vs. KB	NS	NS	NS	NS	*	NS	NS
No LM vs. CF	**	*	***	NS	***	***	NS
CF vs. KB	NS	NS	***	NS	***	***	NS
LM insensitive vs. LM sensitive	NS	NS	**	NS	*	NS	NS
R1 vs. R2	*	***	*	NS	NS	*	NS
R1 vs. LM	NS	NS	NS	NS	NS	NS	NS
	*	*	***	NS	***	***	NS

36 * Significant at the 0.05 probability level.

37 ** Significant at the 0.01 probability level.

38 *** Significant at the 0.001 probability level.

39 † ADF, acid detergent fiber; ADL, acid detergent lignin; CF, creeping red fescue; KB, Kentucky bluegrass; LM, living mulch; MM, maize following maize;
40 NDF, neutral detergent fiber; R1, no LM treatment with residue removal; R2, no LM treatment with residue retention.

41 ‡ Treatments 1 to 6 are maize with no LM, with residue removal for treatments 1, 3, and 5. Treatments 1 and 2 are the population sensitive hybrid, treatments 3
42 and 4 are the population insensitive hybrid, and treatments 5 and 6 are the yield stable hybrid. Treatment 7 to 9 are maize with KB. Treatment 7 is the
43 population sensitive hybrid, treatment 8 is the population insensitive hybrid, and treatment 9 is the yield stable hybrid. Treatments 10 to 12 are maize with CF.
44 Treatment 10 is the population sensitive hybrid, treatment 11 is the population insensitive hybrid, and treatment 12 is the yield stable hybrid.

45 § NS, nonsignificant.

46 **Table 6. Treatment means and significance for SM† measurements including GY, TAB, stover yield, HI, stover C, stover N, C/N, grain protein, grain**
 47 **oil, grain starch, and grain density, and EY at Boone and Kanawha in 2015 and 2016, respectively. Grain yield was obtained from combine harvest and**
 48 **is expressed with grain density and EY at 150 g kg⁻¹ moisture content. Total aboveground biomass, stover yield, HI, stover C, stover N, grain protein,**
 49 **grain oil, and grain starch are expressed on an oven-dry basis.**

Treatment‡	GY	TAB	Stover	HI	Stover C	Stover N	C/N	Protein	Oil	Starch	Density	EY	EY
	Mg ha ⁻¹				g kg ⁻¹			g kg ⁻¹			g cc ⁻¹	L kg ⁻¹	L ha ⁻¹
<u>2015</u>													
1	13.2	18.33	7.58	0.59	477	6.6	73.6	67	32	635	1.21	0.42	5539
2	11.7	18.84	8.63	0.55	476	6.9	70.0	61	35	639	1.22	0.42	4957
3	14.6	20.20	8.39	0.58	474	6.2	78.1	68	33	635	1.24	0.42	6135
4	9.6	15.70	6.99	0.56	476	5.2	95.7	59	34	639	1.20	0.43	4093
5	10.7	18.23	8.32	0.54	475	6.0	81.1	59	35	642	1.21	0.43	4579
6	11.8	13.36	5.51	0.59	469	5.4	86.7	60	33	640	1.21	0.43	5015
7	9.4	16.60	6.54	0.60	481	5.5	88.8	63	33	638	1.20	0.42	3984
8	8.5	18.56	8.29	0.55	470	5.4	87.2	57	35	642	1.21	0.43	3664
9	10.8	15.30	5.88	0.62	476	5.6	86.0	62	34	638	1.21	0.42	4572
SE	0.79	1.50	0.84	0.02	4.10	0.54	6.95	1.70	0.85	1.07	0.006	0.001	327.79
<i>P > F</i>													
No LM vs. LM Hybrid	***	**	*	NS	NS	**	**	***	NS	***	***	***	***
Cover × hybrid	*	NS	NS	NS	NS	NS	NS	*	NS	**	NS	NS	*
No LM vs. KB	NS	*	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
No LM vs. CF	***	**	*	NS	NS	*	**	***	NS	***	**	***	***
CF vs. KB	***	*	*	NS	NS	**	*	***	NS	***	*	**	***
LM insensitive vs. LM sensitive	NS§	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	*	*	NS
<u>2016</u>													
1	14.6	20.99	8.47	0.60	461	6.3	76.6	65	32	629	1.22	0.42	6177
2	14.9	20.95	8.82	0.58	457	7.3	63.1	62	34	632	1.24	0.43	6373
3	15.0	20.74	9.38	0.55	458	6.5	72.2	65	31	631	1.25	0.43	6356
4	5.9	11.54	4.31	0.62	456	5.4	85.0	58	32	635	1.20	0.43	2534
5	8.2	14.13	5.57	0.60	453	5.9	78.3	58	32	636	1.23	0.43	3533
6	6.0	10.21	4.34	0.56	449	5.9	78.5	59	33	634	1.22	0.43	2572
7	10.1	14.13	5.37	0.62	460	5.3	89.2	58	33	636	1.21	0.43	4323
8	10.2	15.78	6.87	0.56	452	6.4	71.1	57	34	636	1.24	0.43	4403
9	11.0	16.21	7.21	0.57	456	5.4	85.0	63	31	633	1.23	0.43	4672
SE	0.99	1.75	0.96	0.03	2.72	0.64	8.56	2.31	1.00	2.05	0.01	0.002	424.62
<i>P > F</i>													
No LM vs. LM Hybrid	***	***	***	NS	**	**	*	***	NS	**	*	***	***
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS

Cover × hybrid	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
No LM vs. KB	***	***	***	NS	**	*	NS	**	NS	**	**	**	***
No LM vs. CF	***	***	**	NS	NS	*	NS	**	NS	**	NS	**	***
CF vs. KB	***	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	***
LM insensitive vs. LM sensitive	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	**	NS	NS

50 * Significant at the 0.05 probability level.

51 ** Significant at the 0.01 probability level.

52 *** Significant at the 0.001 probability level.

53 † CF, creeping red fescue; EY, ethanol yield; KB, Kentucky bluegrass; HI, harvest index; LM, living mulch; SM, maize following soybean; TAB, total
54 aboveground biomass.

55 ‡ Treatments 1 to 3 are maize with no LM. Treatment 1 is the population sensitive hybrid, treatment 2 is the population insensitive hybrid, and treatment 3 is the
56 yield stable hybrid. Treatment 4 to 6 are maize with KB. Treatment 4 is the population sensitive hybrid, treatment 5 is the population insensitive hybrid, and
57 treatment 6 is the yield stable hybrid. Treatments 7 to 9 are maize with CF. Treatment 7 is the population sensitive hybrid, treatment 8 is the population
58 insensitive hybrid, and treatment 9 is the yield stable hybrid. The first 3 treatments were duplicated within each block from which the averages were derived.

59 § NS, nonsignificant.

60 **Table 7. Treatment means and significance for SM† measurements including sequential fiber parameters, including total ash, NDF, ADF, ADL,**
 61 **hemicellulose, cellulose, and lignin, all expressed on a g kg⁻¹ basis, at Boone and Kanawha in 2015 and 2016, respectively.**

Treatment‡	Total ash	NDF	ADF	ADL	Hemicellulose	Cellulose	Lignin
	g kg ⁻¹						
	<u>2015</u>						
1	75	803	482	53	321	430	49
2	81	801	469	51	331	418	48
3	78	796	474	52	322	422	48
4	75	801	466	43	335	423	40
5	73	820	475	45	345	430	43
6	76	805	471	46	334	424	44
7	74	800	463	45	337	418	43
8	70	814	478	49	336	429	47
9	76	787	453	44	333	409	41
SE	5.62	9.74	8.87	2.86	6.62	7.95	2.83
	<i>P > F</i>						
No LM vs. LM Hybrid	NS§	NS	NS	**	**	NS	**
Cover × hybrid	NS	NS	NS	NS	NS	NS	NS
No LM vs. KB	NS	NS	NS	**	**	NS	**
No LM vs. CF	NS	NS	NS	*	*	NS	*
CF vs. KB	NS	NS	NS	NS	NS	NS	NS
LM insensitive vs. LM sensitive	NS	NS	NS	NS	NS	NS	NS
	<u>2016</u>						
1	85	799	482	55	317	427	48
2	97	773	445	49	328	395	41
3	93	800	478	53	322	425	45
4	94	795	439	55	356	384	50
5	102	778	429	34	348	395	28
6	111	776	429	40	348	388	34
7	85	804	465	44	339	421	39
8	102	785	446	58	340	387	50
9	97	792	455	53	337	401	45
SE	5.98	8.00	9.28	6.36	4.44	10.20	6.05
	<i>P > F</i>						
No LM vs. LM Hybrid	*	NS	***	NS	***	**	NS
Cover × hybrid	NS	NS	NS	NS	NS	NS	NS
No LM vs. KB	*	NS	***	NS	***	**	NS

No LM vs. CF	NS	NS	NS	NS	***	NS	NS
CF vs. KB	NS	NS	**	NS	**	NS	NS
LM insensitive vs. LM sensitive	*	*	NS	NS	NS	NS	NS

62 * Significant at the 0.05 probability level.

63 ** Significant at the 0.01 probability level.

64 *** Significant at the 0.001 probability level.

65 † ADF, acid detergent fiber; ADL, acid detergent lignin; CF, creeping red fescue; KB, Kentucky bluegrass; LM, living mulch; NDF, neutral detergent fiber; SM,
66 maize following soybean.

67 ‡ Treatments 1 to 3 are maize with no LM. Treatment 1 is the population sensitive hybrid, treatment 2 is the population insensitive hybrid, and treatment 3 is the
68 yield stable hybrid. Treatment 4 to 6 are maize with KB. Treatment 4 is the population sensitive hybrid, treatment 5 is the population insensitive hybrid, and
69 treatment 6 is the yield stable hybrid. Treatments 7 to 9 are maize with CF. Treatment 7 is the population sensitive hybrid, treatment 8 is the population
70 insensitive hybrid, and treatment 9 is the yield stable hybrid. The first 3 treatments were duplicated within each block from which the averages were derived.

71 § NS, nonsignificant.