Utilization of Spent Microbial Biomass as an Alternative Crop Nitrogen Source

Casey T. Sullivan, Rebecca M. Harman, Neal S. Eash,* James A. Zahn, John J. Goddard, Forbes R. Walker, Arnold M. Saxton, Dayton M. Lambert, David W. McIntosh, William E. Hart, Robert S. Freeland, and John E. Morrison, Jr.

ABSTRACT

Spent microbial biomass (SMB), a nutrient-rich co-product of industrial white biotechnology processes, is produced in substantial quantities alongside high-value products and most often disposed of in landfills or incinerated. Alternatively, SMB could be reused as a land-applied N source in agricultural crop production, reducing the environmental and economic footprint of synthetic fertilizers. This research compares SMB applied at different rates to current farmer practice (FP) fertilizer use in tall fescue (*Festuca arundinacea* Schreb.) and corn (*Zea mays* L.) production on a Dewey silty clay in Lenoir City, TN. The effect of SMB on tall fescue was measured over three harvests through plant biomass production, crop N status using the normalized difference vegetation index (NDVI), and forage quality by near-infrared reflectance spectroscopy (NIRS). Corn productivity was measured by crop height, leaf chlorophyll content using a handheld meter, and grain yield. Tall fescue data showed the mineralization and release of SMB N over time compared with the rapidly available fertilizer N, and the highest SMB application rate in tall fescue was not statistically different from the FP in plant biomass, leaf NDVI, or any measured forage quality parameters. Despite differences in corn leaf chlorophyll contents between SMB and fertilizer treatments during the growing season, no differences in final grain yields were found. This research substantiates the potential of SMB as a soil N amendment from a nutrient source and yield perspective. Additional studies are needed to understand SMB mineralization rates and to confirm the material's nutrient contribution to crop production.

Core Ideas

- Nutrient-rich spent microbial biomass has potential for reuse in agriculture.
- Tall fescue data from three harvests showed release of spent microbial biomass nutrients over time.
- Highest spent microbial biomass rate yielded greater tall fescue biomass than fertilizer in July.
- All five spent microbial biomass rates produced corn yields consistent with the fertilizer control.
- The renewable disposal of spent microbial biomass in agriculture may be expanded to other industries.

Published in Agron. J. 109:1870–1879 (2017)
doi:10.2134/agronj2016.12.0742

5585 Guilford Road, Madison, WI 53711 USA

Nitrogen fertilizers are an essential input for commercial crop production. However, inefficient fertilizer applications in terms of rate, timing, type, and method have resulted in significant environmental degradation and costs to farmers (Cameron et al., 2013; Ribaudo et al., 2012; Robertson and Vitousek, 2009; Vitousek et al., 1997). An alternative crop N source is available in the form of an organic waste product from white biotechnology processes. White biotechnology is a rapidly growing sector of industrial chemical production which uses live bacterial, fungal, or plant cells as biocatalysts to synthesize products. This technology has developed as a more environmentally sustainable alternative to petroleum-driven methods in the production of bio-based chemicals, textiles, pharmaceuticals, and biofuels (Frazzetto, 2003; Tang and Zhao, 2009).

Spent microbial biomass, a nutrient-rich organic co-product, is generated in substantial quantities alongside these high-value products. After microbial conversion is completed, SMB is removed, inactivated with heat or chemical treatment, and then reused, disposed of in a landfill, or incinerated (Halter and Zahn, 2016; Mathias et al., 2014). While biomass from processes including biofuel and brewing is often recycled as animal feed

---

C.T. Sullivan, R.M. Harman, N.S. Eash, F.R. Walker, W.E. Hart, R.S. Freeland, and J.E. Morrison, Jr. (deceased), Dep. of Biosystems Engineering and Soil Science, Univ. of Tennessee, 2506 E.J. Chapman Dr., Knoxville, TN 37996; J.A. Zahn, DuPont Tate and Lyle Bio Products, 198 Blair Bend Rd., Loudon, TN 37774; J.J. Goddard, Loudon County Extension, Univ. of Tennessee, 100 River Rd., Suite 112, Loudon, TN 37774; A.M. Saxton, Dep. of Animal Science, Univ. of Tennessee, 2506 River Dr., Knoxville, TN 37996; D.M. Lambert, Dep. of Agricultural and Resource Economics, Univ. of Tennessee, 2621 Morgan Circle, Knoxville, TN, 37996; D.W. McIntosh, Dep. of Plant Sciences, Univ. of Tennessee, 2431 Joe Johnson Dr., Knoxville, TN 37996. Received 30 Dec. 2016. Accepted 2 May 2017. *Corresponding author (eash@utk.edu).

Abbreviations: ADF, acid detergent fiber; aNDF, neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash; CP, crude protein; DDM, dry matter digestibility; DM, dry matter; DMI, dry matter intake; dNDF48h, digestible neutral detergent fiber 48 hours; ENE, estimated net energy; FP, farmer practice; GM, genetically modified; IVTDMD48h, in-vitro true dry matter digestibility 48 h; ME, metabolizable energy; NDFD, neutral detergent fiber digestibility; NDVI, normalized difference vegetation index; NEg, net energy for gain; NEl, net energy for lactation; NEm, net energy for maintenance; NFC, non-fibrous carbohydrates; NIRS, near-infrared reflectance spectroscopy; PDO, 1,3-propanediol; RBD, randomized block design; RFQ, relative forage quality; RUP, rumen undegradable protein; SMB, spent microbial biomass; T, tassel; TDN, total digestible nutrients; V, vegetative.

or soil nutrient amendments (Mathias et al., 2014; Shurson et al., 2004), many of the biocatalysts used in industrial chemical production have been genetically modified (GM), limiting reuse in these fields due to concerns over horizontal gene transfer (Andersen et al., 2001; Halter and Zahn, 2016; Yu et al., 2013).

Although research into GM SMB land application is limited, a few examples of successful implementation have demonstrated its potential as a crop nutrient source. The biotechnology company Novozymes began distributing their chemically inactivated SMB to farmers in Brazil over 20 yr ago in the form of the organic fertilizer NovoGro, and has since expanded this system to sites in other countries (Andersen et al., 2001; Novozymes, 2006, 2011). Last year, Novozymes reported that they were able to recover and convert 97% of their total waste SMB to NovoGro (Novozymes, 2016). In the United States, Wortmann et al. (2015) studied the land application of Novozymes' lime-stabilized SMB on corn and soybean [*Glycine max* (L.) Merr.], and found potential for its use as a crop N source. The abundant quantities of SMB resulting from white biotechnology processes in the United States indicate there is still great opportunity for reuse as a crop nutrient source if farmers can maintain profitable crop yields, and if GM transfer concerns are addressed.

An industrial fermentation process in Loudon, TN, employs a GM biocatalyst to convert corn starch into the organic compound 1,3-propanediol (PDO) (Kurian, 2005; Nakamura and Whited, 2003). Approximately 12,000 t of SMB from this process are produced on an annual basis, and the material is disposed of in sanitary landfills. Analyses of this SMB indicate the potential for its reuse as a crop N source. The SMB contains ~50% C on a dry matter (DM) basis, ~11% N (DM), and trace amounts of other essential plant nutrients including Ca, Cl, Cu, K, Mo, P, and Zn (Halter and Zahn, 2016). Further validating the opportunity for land application reuse, a 2015 study tracked the DNA degradation of SMB from PDO production in laboratory and field environments (Halter and Zahn, 2016). The researchers found that DNA presence was not detectable in the field 14 d after application, and saw no evidence of gene transfer within local microbial communities.

The reuse of SMB in crop production as a supplemental or alternative nutrient source to synthetic fertilizers could have several positive impacts including the recycling of a waste product, reduced nutrient costs for farmers, and decreased reliance on synthetic fertilizers and the environmental degradation associated with their use. Further, beneficial utilization of SMB would improve the life cycle assessment for PDO production by reducing the environmental footprint of the manufacturing process and decreasing the cost of landfill disposal (EPA, 2016; Halter and Zahn, 2016).

In this study, we evaluated the use of SMB from PDO production as a nutrient source in tall fescue and corn production to determine whether the material could serve as an alternative N source to fertilizer and be recycled as a value-added product. The results indicate the potential for SMB use from a nutrient and yield standpoint.

MATERIALS AND METHODS

Site Description and Preliminary Sampling

This study was conducted on a private farm in Lenoir City, TN (35°44′6.48″ N, 84°11′2.23″ W) during the 2015 growing season. The soil type was a Dewey silty clay (fine, kaolinitic, thermic Typic Paleudult) with 8% sand and 50% silt (top 15 cm) (Soil Survey Staff, 2015). The average annual precipitation at the site is 1300 mm, and the mean annual temperature is 15°C (1981–2010) (NCDC, 2015).

Prior to planting, composite soil samples from the top 15 cm were taken from the tall fescue and corn field sites for analysis of soil water pH and routine soil test macro- and micronutrients (P, K, Ca, Mg, Zn, Fe, Mn, B, and Na) used statewide to develop lime and fertilizer recommendations (Mehlich, 1953) (Soil, Plant, and Pest Center, Nashville, TN). Field trials utilized heat-inactivated SMB produced from a GM derivative of *Escherichia coli* K12 (Genbank accession no. U00096.3) that lacked λ-DNA sequence and *F* plasmid (*F*-). A composite sample of SMB was chemically analyzed by Galbraith Laboratories Inc. (Knoxville, TN) using American Society for Testing and Materials (ASTM), Environmental Protection Agency (EPA), and U.S. Pharmacopoeia (USP) standard methods before it was transported to the field (Table 1) (ASTM, 1992; ASTM, 1996; EPA, 1994; EPA, 1996; Pfaff, 1993; USP, 2015). The SMB composition was similar to that found by Halter and Zahn (2016) with C and N contents of 51.9 and 11.8% (DM), respectively. Cell viability measurements indicated that no live cells were present in the SMB after the inactivation process (Halter and Zahn, 2016).

Experimental Design

The tall fescue study was a randomized block design (RBD) with five treatments and four blocks, with each block running parallel to the hillslope. Each plot was 1.83 by 9.14 m and received one of five nutrient rate treatments: three SMB application rates—1.34 (SMB 1), 2.69 (SMB 2), and 4.03 t DM SMB ha^{-1} (SMB 3); a farmer practice treatment receiving 337 kg 19–19–19 fertilizer ha^{-1} (64 kg N ha^{-1}) (FP); and a control receiving 0 kg N ha^{-1} (Control). The SMB rates were selected based on N content and to represent simplified rates which farmers in the United States would likely apply in the field (1, 2, and 3 U.S. tons wet mass SMB acre^{-1} at the measured moisture content of 40%, for the SMB 1, 2, and 3 rates, respectively). Based on SMB composition (Table 1), the total N rates applied in tall fescue SMB treatments were 158, 316, and 475 kg N ha^{-1}, for the SMB 1, 2, and 3 rates, respectively. In addition to the treatment plots, an 84-m^2 N-rich reference strip was established parallel to the study for handheld crop sensor calibration, which received 112 kg N ha^{-1} as granular urea (46–0–0) (Trimble, 2012).

Despite the high total N application rates and the low C/N ratio of the SMB, much of the N is found in organic forms which must be mineralized before becoming available for crop uptake. Additionally, SMB was surface applied and was not incorporated. For these reasons, we expected SMB N to mineralize slowly, similar to manure rates, with only a fraction of total SMB N expected to become available for plant uptake in the first year following application (Castellanos and Pratt, 1981).

The corn study was also a RBD, with six treatments and four topographical blocks (blocks parallel to slope). Each plot was 4.56 by 9.14 m, included six rows of corn, and received one of six nutrient rate treatments: five SMB application rates—1.34 (SMB 1), 2.69 (SMB 2), 4.03 (SMB 3), 5.38 (SMB 4), and 6.72 t DM SMB ha^{-1} (SMB 5); and a farmer practice treatment

Table 1. Chemical analysis of spent microbial biomass (SMB) land-applied to tall fescue and corn in Lenoir City, TN.

Analysis	Unit	Method†	SMB composition, at application‡	SMB composition, DM basis
Solids§	%	–	59.87	100.00
Water	%	USP < 921 > Method 1c (USP, 2015)	40.13	0.00
C	%	ASTM D5291-16 (ASTM, 1996)	31.09	51.93
H	%	ASTM D5291-16	8.10	13.53
S	%	ASTM D4239-83 (ASTM, 1992)	7.46	12.46
N-total	%	ASTM D5291-16	7.04	11.76
NH_3	%	GLI Procedure E7-7 (H2SO4 titration)	1.18	1.97
P	%	EPA SW-846 Method 6010B (EPA, 1996)	0.52	0.86
K	%	EPA SW-846 Method 6010B	0.13	0.21
Mg	%	EPA SW-846 Method 6010B	0.12	0.20
Co	%	EPA SW-846 Method 6010B	nd (MDL < 7.2)	nd
Cu	%	EPA SW-846 Method 6010B	nd (MDL < 7.2)	nd
Mo	%	EPA SW-846 Method 6010B	nd (MDL < 7.2)	nd
Ca	mg L^{-1}	EPA SW-846 Method 6010B	282	471
Na	mg L^{-1}	EPA SW-846 Method 6010B	150	251
Cl	mg L^{-1}	EPA Method 300.0 (Pfaff, 1993)	83	139
Zn	mg L^{-1}	EPA SW-846 Method 6010B	37	62
NO_3–N	mg L^{-1}	EPA Method 300.0	nd (MDL < 10.0)	nd
Ni	mg L^{-1}	EPA SW-846 Method 6010B	nd (MDL < 7.2)	nd
Ag	mg L^{-1}	EPA SW-846 Method 6010B	nd (MDL < 5.0)	nd
As	mg L^{-1}	EPA SW-846 Method 6010B	nd (MDL < 1.0)	nd
Ba	mg L^{-1}	EPA SW-846 Method 6010B	nd (MDL < 1.0)	nd
Cd	mg L^{-1}	EPA SW-846 Method 6010B	nd (MDL < 1.0)	nd
Cr	mg L^{-1}	EPA SW-846 Method 6010B	nd (MDL < 1.0)	nd
Pb	mg L^{-1}	EPA SW-846 Method 6010B	nd (MDL < 1.0)	nd
Se	mg L^{-1}	EPA SW-846 Method 6010B	nd (MDL < 1.0)	nd
Hg	mg L^{-1}	EPA SW-846 Method 7471A (EPA, 1994)	nd (MDL < 0.0094)	nd

† ASTM; American Society for Testing and Materials; EPA, Environmental Protection Agency; GLI, Galbraith Laboratories, Inc.; USP, United States Pharmacopoeia.

‡ nd, not detected; MDL, minimum detection limit; DM, dry matter.

§ DNA and lipid composition of this sample were not determined at the time of this analysis, but prior analyses of spent microbial biomass (SMB) from the same PDO production process have found a mean lipid composition of 4.70±0.10% and DNA composition of 2.25±0.26% dry matter.

receiving 213 kg N ha^{-1} as urea (FP). Again, these values were selected to represent rates which U.S. farmers would realistically apply in the field and convert to 1, 2, 3, 4, and 5 U.S. tons wet mass SMB ha^{-1} at 40% moisture, respectively. Total N rates applied in the corn SMB treatments were 158, 316, 475, 632, and 760 kg N ha^{-1} for SMB 1, 2, 3, 4, and 5, respectively. A 250-m N-rich reference strip receiving 280 kg N ha^{-1} as urea was also established parallel to the treatment plots for chlorophyll meter calibration (Peterson et al., 1993; Shapiro, 1999).

The KY-31 tall fescue used in this study was planted in the 1960s and has since been under continuous production with maintenance N, P, K, and lime applied according to soil test recommendations. The cornfield has been managed using conservation agriculture practices, including 15 yr of no-till, maintained near-continuous residue cover over the soil surface, and a corn–soybean rotation. Corn (Becks 6347 hybrid corn seed) was planted on 12 Apr. 2015 using a John Deere 1790 16-row planter (John Deere Co., Moline, IL) with 76-cm row spacing at a density of 84,000 plants ha^{-1}. Nitrogen fertilizers and SMB were hand applied to tall fescue and corn plots on 21 Apr. 2015. In addition, P and K were hand-applied based on soil test recommendation rates to all tall fescue and corn plots as triple superphosphate (0–45–0) and potash (0–0–60) (Savoy and Joines, 2009a, 2009b).

Tall Fescue

The tall fescue was harvested at the full-head stage on three dates in the 2015 growing season: 12 May, 27 July, and 9 September. Prior to the first and last harvests, the NDVI was measured in three locations per plot using a GreenSeeker handheld crop sensor (Trimble Navigation Ltd., Sunnyvale, CA) to provide an indicator of crop N status (Trimble, 2012). At each harvest, a 1.06 by 9.14 m strip in the middle of each plot was cut to 5-cm stubble using a Troy-Bilt Trailblazer sickle bar mower (Garden Way Incorporated, Troy, NY). The total mass harvested per plot was measured in the field using a portable balance, and a sample was dried at 49°C for 72 h, from which forage DM and water content were determined (Barnhart, 2009). These samples were then further dried for lab analysis at 55°C for 24 h, and ground using a Thomas Model 4 Wiley Mill with a 1 mm mesh size screen (Novotny et al., 2013) (Thomas Scientific, Swedesboro, NJ).

A subsample of ground tall fescue was analyzed for forage quality using NIRS with a NIRS 5000 Feed and Forage Analyzer (FOSS, Hillerød, Denmark) and WinISI II software (Infrasoft International, LLC, Port Matilda, PA). The following forage quality constituents were analyzed: acid detergent fiber (ADF), ash, Ca, crude protein (CP), digestible neutral detergent fiber 48 h (dNDF48h), DM, dry matter digestibility (DDM), dry matter intake (DMI), estimated net energy (ENE), fat, in-vitro true dry matter digestibility 48 h (IVTDMD48h), K, lignin, Mg, metabolizable energy (ME), moisture, neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash (aNDF), neutral detergent fiber digestibility (NDFD), net energy for gain (NEg), net energy for lactation (NEl), net energy for maintenance (NEm), nonfibrous carbohydrates (NFC), P, relative forage quality (RFQ), rumen undegradable protein (RUP), and total digestible nutrients (TDN) (Bates et al., 2015).

Equations for this analysis were standardized and checked for accuracy using the 2012 grass hay equation developed by the NIRS Forage and Feed Consortium. The Global *H* statistical test compared each sample against the model and other samples within the database for accurate results, where all forage samples fit the equation with $H < 3.0$ and were reported accordingly (Murray and Cowe, 2004).

Corn

Plant population stand counts from three 5.32-m lengths of row (Gibson, 1998) and residue cover using the line-transect method (Morrison et al., 1993) from each plot were collected at the vegetative (V) 4 crop stage. Crop height from the soil surface to the end of the tallest fully extended leaf was measured on eight crops per plot from the interior four rows at the V5 and V12 crop stages.

Leaf chlorophyll content was measured at the V12 and VT (tassel) crop stages using a Minolta SPAD-502Plus chlorophyll meter (Konica Minolta, Tokyo, Japan). The SPAD meter was used according to methods recommended by Salmerón et al. (2011), Víg et al. (2012), and Yang et al. (2012). At the V12 crop stage, the SPAD meter was used on five randomly selected plants per plot. Four points per plant moving down one side of the youngest fully expanded leaf were measured and averaged. At the VT crop stage, the SPAD meter was used on the ear leaf instead (as is recommended after tasseling) and five measurements per plant were obtained and averaged. The maximum SPAD value from the N-rich reference strip was used to calculate a sufficiency index from each treatment plot, with a 95% limit for sufficiency (Peterson et al., 1993).

Corn was hand harvested on 17 Sept. 2015 and plot yields were estimated similar to the ear weight method described by Lauer (2002). All stalks from a 5.3-m length in the middle of each of the two interior rows (10.6 m in total) per plot were counted, and all ears from these same plants were counted and harvested. Ears were transported to a lab at the University of Tennessee, Knoxville, where the total dehusked ear weight for each plot harvest sample was measured, and ears were shelled by hand using a Maximizer corn sheller (Pleasant Hill Grain, Hampton, NE). The empty cobs were weighed again, and grain weight was calculated. Grain moisture was measured on three samples of shelled grain for each plot using a Dickey-John mini GAC plus moisture tester (Dickey-John Corp., Auburn, IL). To calculate yield, the total grain weight from each plot was multiplied by 1233 (harvest area was 1/1233 of a hectare [1/500th of an acre]) and corrected to 15.5% moisture using the measured values (Lauer, 2002).

Statistical Analysis

Mixed models ANOVA was used to test for differences between treatment N uptake as indicated by the measured variables in the tall fescue and corn studies, with each crop analyzed separately (SAS 9.4, Cary, NC). Repeated measures ANOVA was used for all tall fescue variable analyses and the corn variables repeated over time (crop height and SPAD values) to determine the impact of SMB degradation and nutrient mineralization on crop production throughout the growing season. Treatments were analyzed in the whole plot, and harvest/crop stage were analyzed in the subplot. Least squares means were separated using Dunnett's test at the 0.05 significance level to compare SMB and control treatments to the FP in each study (Dunnett, 1980) (SAS 9.4). Orthogonal polynomial contrasts were also used to examine regression trends across SMB application rates for both crops, with the control as the 0 SMB rate in the tall fescue study (SAS 9.4).

RESULTS AND DISCUSSION

Tall Fescue

Plant Biomass

Treatment means supported the use of SMB as an alternative N source to fertilizer from a plant biomass perspective. Differences in tall fescue DM plant biomass were found by treatment, harvest date, and the treatment × harvest date interaction ($P < 0.05$) (Table 2). The plant biomass from the SMB 1 and 2 treatments, as well as the control, did not differ significantly from FP plant biomass at any of the three harvests (Table 3). SMB 3 produced significantly greater tall fescue plant biomass than the FP in July, but was not different in May and September.

In Tennessee, cool-season perennial grass plant biomass is typically greatest at the first harvest (Bates, 1999), which was not the case for our tall fescue. The May plant biomass was low across all treatments, ranging from 0.65 to 0.94 Mg ha^{-1}. This harvest occurred only 3 wk after SMB and fertilizers were applied, so it is unlikely that a significant portion of SMB N had mineralized at this point for crop uptake. In addition, <6 mm of precipitation fell between treatment application and the first harvest, which likely also affected fertilizer N availability (Table 4, U.S. Climate Data, 2016). By the second harvest in July, N uptake across all treatments improved and overall plant biomass increased. SMB 3 produced 74% more plant biomass than the FP at this time ($P < 0.01$) (Table 3). At the third and final harvest in September, all treatment plant biomass production had again declined and no significant differences were found (Table 3).

Orthogonal contrasts of SMB treatments indicated that the amount of SMB applied affected tall fescue plant biomass production, with a predominately linear trend representing 90% of treatment differences, caused by high plant biomass at the second harvest ($P < 0.05$) (Table 2). When means were combined across all harvest dates, SMB treatment plant biomass increased rapidly from 0.81 to 1.41 Mg ha^{-1} with the increase in SMB application rate from 0 in the control to 2.69 t DM SMB ha^{-1} in SMB 2. From SMB 2 to SMB 3 the increase in plant biomass production moderated to 1.45 Mg ha^{-1} with an SMB application rate of 4.03 t DM SMB ha^{-1}.

Table 2. Analysis of variance and orthogonal polynomial contrasts of treatment tall fescue data collected in Lenoir City, TN, over three harvests in 2015.

	Source															Random source		
				Treatment‡§			May			July			Sept.					
Variable†	Treatment (T)	Harvest (H)	T × H	L	Qd	C	L	Qd	C	L	Qd	C	L	Qd	C	Block (B)	Error a	Error b
	P value¶															Variance estimate#		
Biomass, Mg ha^{-1}††,‡‡	0.034	<0.001	0.033	<0.001	0.180	0.603	0.673	0.708	0.327	<0.001	0.794	0.461	0.104	0.086	0.396	0.00	0.06*	0.11***
NDVI, unitless	<0.001	<0.001	<0.001	<0.001	0.045	0.874	0.004	0.547	0.983	–	–	–	0.002	0.042	0.862	0.00	0.00	0.00
ADF, %	0.004	<0.001	0.217	0.008	0.588	0.393	0.219	0.971	0.369	0.016	0.600	0.665	0.015	0.112	0.703	0.37	0.11	1.65
aNDF, %	0.004	<0.001	0.010	0.002	0.463	0.994	0.017	0.522	0.793	0.075	0.624	0.930	0.009	0.141	0.872	0.00	0.03	2.24
Ash, %	0.084	<0.001	0.925	0.037	0.096	0.700	0.353	0.168	0.207	0.148	0.333	0.862	0.064	0.361	0.693	0.00	0.00	1.11***
Ca, %	0.319	<0.001	0.002	0.441	0.904	0.334	0.233	0.124	0.051	0.035	0.358	0.818	0.012	0.700	0.881	0.01	0.01	0.01***
CP, %	<0.001	<0.001	0.001	<0.001	0.077	0.625	0.018	0.216	0.273	<0.001	0.065	0.971	<0.001	0.377	0.923	0.08	0.18	0.83
DDM, %	0.004	<0.001	0.216	0.008	0.587	0.394	0.221	0.971	0.369	0.016	0.604	0.662	0.015	0.112	0.704	0.22	0.07	0.99
DM, %	0.552	<0.001	0.095	0.347	0.834	0.219	0.715	0.356	0.775	0.012	0.626	0.789	0.623	0.306	0.026	0.00	0.00	0.13***
DMI, %	0.002	<0.001	0.025	0.001	0.275	0.940	0.032	0.611	0.791	0.028	0.473	0.894	0.020	0.145	0.805	0.00	0.01	0.01
dNDF48h, %	0.207	<0.001	0.009	0.935	0.263	0.075	0.174	0.157	0.033	0.040	0.012	0.212	0.549	0.377	0.948	0.94	0.23	2.80*
ENE, %	0.004	<0.001	0.220	0.008	0.585	0.392	0.221	0.971	0.369	0.016	0.606	0.665	0.015	0.112	0.704	0.41	0.11	1.82
Fat, %	0.039	<0.001	0.458	0.491	0.071	0.171	0.182	0.103	0.500	0.662	0.722	0.399	0.786	0.224	0.031	0.01	0.00	0.03***
IVTDMD48h, %	0.016	<0.001	0.043	0.009	0.438	0.033	0.353	0.927	0.156	0.001	0.028	0.075	0.346	0.409	0.300	0.74	0.29	1.08**
K, %	0.015	<0.001	0.193	0.162	0.139	0.030	0.737	0.764	0.031	0.002	0.179	0.265	0.836	0.063	0.205	0.01	0.01	0.04**
Lignin, %	0.032	<0.001	0.101	0.020	0.131	0.122	0.434	0.246	0.198	0.004	0.008	0.660	0.867	0.529	0.559	0.08	0.00	0.23***
ME, %	0.003	<0.001	0.240	0.004	0.547	0.331	0.235	0.926	0.343	0.013	0.710	0.739	0.008	0.124	0.589	0.01	0.01	0.01
Mg, %	0.018	<0.001	0.003	0.009	0.339	0.117	0.180	0.215	0.205	0.337	0.718	0.102	<0.001	0.159	0.300	0.00	0.01*	0.01**
Moisture, %	0.552	<0.001	0.095	0.347	0.834	0.219	0.715	0.356	0.775	0.012	0.626	0.789	0.623	0.306	0.026	0.00	0.00	0.13***
NDFD, %	0.012	<0.001	0.028	0.026	0.187	0.024	0.786	0.196	0.025	0.001	0.003	0.098	0.537	0.768	0.892	3.61	0.26	6.92**
NEg, Mcal kg^{-1}	0.013	<0.001	0.043	0.003	0.414	0.329	0.270	0.913	0.312	0.015	0.664	0.697	0.010	0.093	0.770	0.01	0.00	0.01***
NEl, Mcal kg^{-1}	0.004	<0.001	0.313	0.008	0.495	0.429	0.218	1.000	0.428	0.019	0.799	0.649	0.025	0.137	0.820	0.01	0.01	0.01
NEm, Mcal kg^{-1}	0.003	<0.001	0.240	0.006	0.597	0.375	0.211	0.768	0.318	0.023	0.624	0.826	0.014	0.088	0.661	0.01	0.01	0.01
NFC, %	0.032	<0.001	0.015	0.024	0.040	0.677	0.696	0.365	0.280	0.001	0.063	0.985	0.037	0.030	0.887	0.00	0.00	3.21***
P, %	<0.001	<0.001	0.090	<0.001	0.117	0.210	0.196	0.744	0.249	<0.001	0.081	0.942	0.001	0.111	0.249	0.01	0.01	0.01**
RFQ, unitless	0.022	<0.001	0.302	0.407	0.385	0.097	0.425	0.252	0.040	0.072	0.400	0.249	0.570	0.171	0.876	14.89	5.38	43.65**
RUP, %	0.097	<0.001	0.013	0.068	0.277	0.158	0.673	0.086	0.032	0.020	0.042	0.808	0.242	0.152	0.929	0.75	0.00	7.71***
TDN, %	0.004	<0.001	0.220	0.008	0.584	0.392	0.220	0.971	0.369	0.016	0.605	0.664	0.015	0.111	0.705	0.48	0.13	2.15
df	4	2	8	1	1	1	1	1	1	1	1	1	1	1	1	3	12	24

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

† NDVI, normalized difference vegetation index; ADF, acid detergent fiber; aNDF, neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash; CP, crude protein; DDM, dry matter digestibility; DM, dry matter; DMI, dry matter intake; dNDF48h, digestible neutral detergent fiber 48 h; ENE, estimated net energy; IVTDMD48h, in-vitro true dry matter digestibility 48 h; ME, metabolizable energy; NDFD, neutral detergent fiber digestibility; NEg, net energy for gain; NEl, net energy for lactation, NEm, net energy for maintenance; NFC, nonfibrous carbohydrates; RFQ, relative forage quality; RUP, rumen undegradable protein; TDN, total digestible nutrients.

‡ Orthogonal polynomial contrasts only include SMB treatments and the control, farmer practice (FP) is excluded.

§ L, linear; Qd, quadratic; C, cubic.

¶ *P* values for all nutrient treatment and harvest levels.

Variance associated with block, block × treatment interaction, and block × treatment × harvest interaction.

†† Variable units provided for variance estimates.

‡‡ Plant biomass and all near-infrared reflectance spectroscopy (NIRS) analysis values are reported on a 100% DM basis.

Table 3. Tall fescue data treatment means collected in Lenoir City, TN, over three harvests in 2015.

	Harvest														
	May‡					July					September				
Variable†	FP	Control	SMB 1	SMB 2	SMB 3	FP	Control	SMB 1	SMB 2	SMB 3	FP	Control	SMB 1	SMB 2	SMB 3
Biomass, Mg ha^{-1}§¶	0.85	0.87	0.76	0.94	0.65	1.48	0.90	1.41	2.15	2.58**	0.86	0.66	1.19	1.13	1.09
NDVI, unitless	0.68	0.56***	0.58**	0.60**	0.63	–	–	–	–	–	0.73	0.73	0.77	0.79*	0.78
ADF, %	36.33	39.30*	38.29	38.76	37.81	35.29	37.28	35.80	35.38	34.65	37.10	40.37**	40.46**	39.98*	37.73
aNDF, %	57.50	62.18***	60.95*	59.77	59.56	52.36	51.86	51.53	50.97	49.87	57.74	61.00*	61.09*	60.27	57.96
Ash, %	6.36	5.63	6.02	7.24	6.02	9.74	9.23	10.26	10.49	10.40	7.74	6.85	8.06	8.25	8.41
Ca, %	0.45	0.35	0.36	0.52	0.38	1.16	1.26	1.16	1.12	1.11	0.86	0.62**	0.66*	0.71	0.79
CP, %	12.74	9.68**	10.30**	11.47	10.96	15.55	13.31*	16.08	18.03**	19.09***	14.36	11.43**	13.29	14.66	15.72
DDM, %	60.60	58.29*	59.07	58.70	59.45	61.41	59.86	61.01	61.34	61.91	60.00	57.45**	57.38**	57.75*	59.51
DM, %	95.22	95.08	95.17	95.21	94.97	89.97	89.54	89.88	90.03	90.20	93.17	93.77	93.17	93.69	93.46
DMI, %	2.09	1.93**	1.97*	2.01	2.02	2.29	2.32	2.33	2.36	2.41*	2.08	1.97*	1.96*	1.99	2.07
dNDF48h, %	31.56	33.44	33.37	29.86	32.60	26.69	22.13**	26.76	26.03	25.47	28.65	29.92	30.59	30.24	29.16
ENE, %	51.73	48.61*	49.67	49.17	50.17	52.81	50.73	52.28	52.72	53.49	50.91	47.48**	47.39**	47.89*	50.26
Fat, %	2.36	2.15	2.29	2.42	2.28	3.50	3.34	3.42	3.31	3.33	2.99	2.60*	2.88	2.64*	2.71
IVTDMD48h, %	73.03	70.32*	71.30	70.38*	71.47	77.47	75.26*	78.65	78.20	78.84	71.85	71.16	71.48	70.89	72.21
K, %	1.84	1.65	1.84	1.55	1.80	2.38	2.35	2.44	2.14	1.96*	2.42	2.26	2.57	2.41	2.34
Lignin, %	4.04	4.04	3.95	4.31	3.62	4.41	5.60**	4.40	4.20	4.47	6.24	6.01	5.73	5.95	6.00
ME, %	1.00	0.95*	0.97	0.96	0.98	1.02	0.99	1.01	1.02	1.04	0.99	0.93**	0.93**	0.94*	0.98
Mg, %	0.19	0.15	0.15	0.18	0.16	0.30	0.31	0.29	0.33	0.32	0.30	0.24*	0.28	0.33	0.34
Moisture, %	4.78	4.92	4.83	4.79	5.03	10.03	10.46	10.12	9.97	9.80	6.83	6.23	6.83	6.31	6.54
NDFD, %	54.94	53.77	54.74	49.97	54.77	50.94	42.61**	51.92	51.06	51.09	49.63	49.03	50.04	50.18	50.33
NEg, Mcal kg^{-1}	0.77	0.67*	0.70	0.69*	0.72	0.81	0.74	0.79	0.80	0.83	0.34	0.29**	0.28**	0.29*	0.33
NEl, Mcal kg^{-1}	1.38	1.30*	1.33	1.31	1.34	1.41	1.35	1.40	1.41	1.43	0.62	0.57**	0.57**	0.58*	0.61
NEm, Mcal kg^{-1}	1.35	1.24*	1.28	1.26	1.29	1.39	1.31	1.37	1.39	1.41	0.60	0.54**	0.54**	0.55*	0.59
NFC, %	22.89	22.01	22.23	21.02	22.96	21.84	25.10	21.63	20.00	20.14	19.67	20.22	17.05	16.32*	17.41
P, %	0.26	0.23*	0.23	0.22*	0.24	0.30	0.25***	0.29	0.31	0.32	0.28	0.23***	0.26	0.26	0.27
RFQ, unitless	129.02	117.60	121.66	112.50**	125.02	114.08	108.54	112.42	103.68	101.38	110.73	106.72	101.22	99.53	104.18
RUP, %	38.30	38.40	38.34	42.96	37.78	34.65	37.90	32.95	31.85	33.02	43.41	40.51	37.75*	36.81**	38.27*
TDN, %	61.13	57.74*	58.89	58.35	59.44	62.31	60.05	61.73	62.21	63.05	60.25	56.52**	56.42**	56.96*	59.54

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

† ADF, acid detergent fiber; aNDF, neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash; CP, crude protein; DDM, dry matter digestibility; DM, dry matter; DMI, dry matter intake; dNDF48h, digestible neutral detergent fiber 48 h; ENE, estimated net energy; IVTDMD48h, in-vitro true dry matter digestibility 48 h; ME, metabolizable energy; NDFD, neutral detergent fiber digestibility; NDVI, normalized difference vegetation index; NEg, net energy for gain; NEl, net energy for lactation, NEm, net energy for maintenance; NFC, nonfibrous carbohydrates; RFQ, relative forage quality; RUP, rumen undegradable protein; TDN, total digestible nutrients.

‡ FP, farmer practice (337 kg 19–19–19 fertilizer ha^{-1}); Control, 0 kg N ha^{-1}, SMB, spent microbial biomass; SMB 1, 1.34 t DM SMB ha^{-1}; SMB 2, 2.69 t DM SMB ha^{-1}; SMB 3, 4.03 t DM SMB ha^{-1}.

§ Means separated across each row and within each harvest using Dunnett's test to compare SMB and control treatment means to the FP.

¶ Plant biomass and all near-infrared reflectance spectroscopy (NIRS) analysis values are reported on a 100% DM basis.

Table 4. Monthly weather data for Lenoir City, TN, in 2015 compared to historical climate averages.

Month	High temperature† 2015	High temperature† Average	Low temperature 2015	Low temperature Average	Total precipitation 2015	Total precipitation Average
	°C				mm	
Apr.	22.26	21.67	8.62	7.78	112.01	111.00
May	27.62	26.11	14.13	12.78	76.20	120.90
June	31.08	30.00	19.24	18.33	142.24	98.04
July	30.75	31.67	20.24	20.56	196.60	123.95
Aug.	30.10	31.67	18.44	19.44	120.90	90.93
Sept.	27.97	28.33	16.22	15.56	86.36	91.95

† 2015 and historical climate means (1981–2010) from U.S. Climate Data (2016).

Normalized Difference Vegetation Index

GreenSeeker NDVI readings from the first and third harvest dates suggested increasing tall fescue N uptake at the higher SMB rates as the season progressed (Table 2). At the first harvest in May, SMB 1, SMB 2, and the control had significantly lower NDVI than the FP (Table 3), indicating that the readily available N supplied by the fertilizer facilitated greater nutrient uptake and chlorophyll production at this time. However, the SMB 3 treatment mean NDVI reading was not different from the FP on this date, suggesting that this application rate provided a similar amount of plant available N to the crops.

By the third harvest in September, treatments SMB 2 and 3 had significantly greater NDVI readings than the FP with mean values of 0.79, 0.78, and 0.73, respectively (Table 3). The control treatment mean NDVI value was equal to the FP, indicating that significant fertilizer nutrients were no longer present for crop uptake. The improvement in chlorophyll production across all SMB treatments relative to the fertilizer-treated plots later in the season further supports the idea that the SMB acts as a slow release nutrient source.

The SMB treatment NDVI differences were significant with linear and quadratic trends, 91 and 9% of treatment differences, respectively. There were strong positive linear trends on both dates as leaf chlorophyll production and NDVI readings increased at the higher SMB rates ($P < 0.05$) (Table 2).

Forage Quality

The NIRS analysis indicated a trend similar to plant biomass and NDVI in the overall improvement of forage quality in SMB treatments over time (Table 2). Forage CP contents provided a good indicator of SMB decomposition and N mineralization compared to the fertilizer, as the control CP was significantly lower than the FP at all three harvests (Table 3). In May, the only SMB rate which differed in CP from the FP was SMB 1, which was significantly lower. By July, more SMB N had mineralized, and tall fescue from the SMB 2 and SMB 3 treatments contained greater CP than the FP with means of 18.03, 19.09, and 15.55%, respectively. At the last harvest, all SMB CP contents were again similar to the FP.

Other differences in SMB forage nutrient content occurred throughout the growing season, but were not confined to a specific rate (Table 3). In May, SMB 2 contained lower P than the FP, and SMB 3 was lower in K in July, despite all treatments receiving the recommended P and K rates from the soil test, plus additional nutrients mineralized from the SMB. The SMB 1 treatment contained lower Ca than the FP in September. No differences between SMB treatment Mg contents compared to the FP occurred at any harvest.

The digestibility parameters measured by the NIRS analysis also showed improvements in tall fescue quality later in the season (Table 3). Measures of fescue indigestible or slowly digestible components were ADF, aNDF, lignin, and RUP, with lower numbers indicating better animal digestibility and forage uptake. The highest SMB rate, SMB 3, did not generate significant differences in ADF, aNDF, or lignin when compared to the FP on any of the harvest dates, though differences in the lower SMB rates did occur. In September, all SMB treatments contained significantly lower RUP than the FP and control, indicating that more protein from the SMB was degradable compared to the fertilizer-treated plots. Measures of tall fescue digestible components (digestible DM, protein, fiber, and sugar) included DDM, dNDF48h, IVTDMD48h, NDFD, NFC, and TDN. The SMB 3 treatment forage was not different from the FP in any of these parameters at any of the three harvests (Table 3). Consistent with patterns seen in forage indigestible components, digestibility declined in the SMB 1 and 2 rates and more differences were seen at the last harvest.

The fescue energy indicators, ENE, ME, NEg, NEl, and NEm, were all significantly lower in the control in May and SMB 1, SMB 2, and the control in September (Table 3). In addition, SMB 2 was lower in NEg in May compared to the FP.

Lastly, the RFQ provided an overall indicator of forage quality (Table 3). The significantly lower RFQ in the SMB 2 treatment in May was the only incidence of SMB treatment RFQ difference from the FP ($P < 0.01$). All treatments in May fell within the southeastern forage quality categorization system range for "good quality" (RFQ 110–139) and were either of "good" or "fair quality" (RFQ 90–109) in July and September (Hancock, 2011).

When NIRS variable means were analyzed by harvest date, many variables followed plant biomass trends (Table 2). Most of the NIRS analysis traits were found to be greatest across all treatments at the second cutting (nutrients, CP, fat, energy, and digestibility), and the indigestible components were found to be lowest. Dry matter, dNDF48h, NDFD, and RFQ were all significantly greatest during the May harvest, while K, lignin, and ADF were greatest in the September harvest.

Orthogonal polynomial contrasts within SMB treatments identified predominantly strong positive linear trends within the forage quality parameters as SMB rate increased, mostly as linear trends in the July and September harvests (Table 2). Crude protein and DMI had strong positive linear trends by treatment. Acid detergent fiber, aNDF, ash, DM, DDM, lignin, moisture, Mg, P, TDN and all energy components (ENE, ME, NEg, NEl, NEm) also had linear trends by treatment.

Some quadratic and cubic trends were also observed, with quadratic trends apparent in the July and Sept ember harvests, and cubic trends evident in the May and September harvests (Table 2). Forage K content had cubic trends by treatment, resulting from differences at the first harvest. IVTDMD48h and NDFD exhibited linear and cubic trends by treatment. Calcium, DM, dNDF48h, fat, moisture, RFQ, and RUP had no overall linear, quadratic, or cubic trends by treatment but some trends did occur within individual harvest dates.

Treatment Summaries

When treatment means over the three harvests were combined, the SMB 3 means for plant biomass, NDVI, and the 26 forage quality variables were never different from the FP ($p > 0.05$). Differences between SMB rates and the FP increased as SMB rate declined, with 8 and 10 NIRS variables different from the FP in the SMB 2 and SMB 1 treatments, respectively. These treatments exhibited lower overall RFQ, energy, and digestible components, and higher indigestible components.

Compared to the FP, the control was significantly different in 16 forage quality parameters. The control had lower NDVI, CP, P, fat, energy, and digestibility; and contained greater indigestible components. No differences in plant biomass, ash, Ca, DM, dNDF48h, K, lignin, Mg, moisture, NFC, or RUP treatment means between any SMB treatments or the control and the FP were found.

Corn

Growing Season Data

The mean population density across all treatments was 77,500 plants ha^{-1}. No significant differences between treatment residue covers were found ($P > 0.05$), with an overall mean of 67% cover. Crop height was significant by crop stage ($P < 0.001$), but not by treatment (Table 5).

SPAD meter values differed among treatments and by crop stage ($P < 0.01$) (Table 5). When treatment means were combined across the two crop stages, SMB 4 had a greater overall SPAD value than the FP, with values of 60.24 and 57.20, respectively ($P < 0.01$). This was due to differences at the V12 crop stage, where again the SMB 4 mean SPAD value was significantly greater than the FP. All treatment SPAD values increased from the V12 to VT crop stage as the leaf measured was changed following crop tasseling, but no significant differences between the SMB rates and the FP were found.

The maximum SPAD values measured by the N-rich reference strip were 56.80 and 63.90 at the V12 and VT crop stages, respectively. When sufficiency indices of the treatments were calculated, the FP was the only treatment below 95% at the V12 crop stage; and at VT, SMB 1 and 2 were below 95% sufficiency.

Table 5. Indicators of corn N uptake (2015) as affected by nutrient source and crop stage in Lenoir City, TN.

	Means					
	Crop height			SPAD-502†		
Treatment	Overall	V5‡	V12	Overall	V12	VT
	cm			unitless		
FP§¶	92.73	17.13	168.33	57.20	53.41	61.00
SMB 1	92.20	15.73	168.67	57.71	55.28	60.15
SMB 2	94.09	16.34	171.84	56.59	54.01	59.17
SMB 3	95.43	16.15	174.70	59.12	56.90	61.33
SMB 4	94.86	16.11	173.60	60.24*	58.94**	61.54
SMB 5	91.28	16.11	166.45	59.31	56.40	62.22

		ANOVA					
		P value#					
Source of variation							
	Treat (T)	0.908	1.00	0.687	0.009	0.003	0.273
	Crop stage (S)	<0.001	–	–	<0.001	–	–
	T × S	0.886	–	–	0.249	–	–
	L††,‡‡	0.896	0.963	0.816	0.003	0.014	0.024
	Qd	0.197	0.938	0.079	0.649	0.301	0.676
	C	0.763	0.942	0.618	0.009	0.003	0.335
	Qt	0.989	0.973	0.958	0.385	0.861	0.304
CV, %§§	83.80	6.68	6.33	5.85	5.01	2.62	

* P < 0.05.

** *P* < 0.01.

† SPAD-502, Minolta SPAD-502 Chlorophyll Plus Meter indexed chlorophyll content.

‡ V5, vegetative crop stage 5; V12, vegetative crop stage 12; VT, tasseling.

§ FP, farmer practice (213 kg N ha^{-1}); SMB, spent microbial biomass; SMB 1, 1.34 t DM SMB ha^{-1}; SMB 2, 2.69 t DM SMB ha^{-1}; SMB 3, 4.03 t DM SMB ha^{-1}; SMB 4, 5.38 t DM SMB ha^{-1}; SMB 5, 6.72 t DM SMB ha^{-1}.

¶ Means separated down each column using Dunnett's test to compare SMB treatment means to the FP.

\# *P* values for all nutrient treatment and harvest date levels.

†† Orthogonal polynomial contrasts only include SMB treatments, FP is excluded.

‡‡ L, linear; Qd, quadratic; C, cubic; Qt, quartic.

§§ CV, coefficient of variation.

Orthogonal polynomial contrasts within SMB treatments suggested that the amount of SMB applied did affect SPAD values, represented by moderate positive linear and cubic trends. This was caused by the decline in overall mean SPAD values from SMB 1 to SMB 2, followed by a rapid increase in values at the SMB 3 and 4 rates. Between SMB 4 and 5, overall SPAD values declined, as SMB 5 chlorophyll content at V12 was not as prolific as the SMB 4 rate, but no differences between these two treatments were found at VT. The relationship between SMB application rate and SPAD values could perhaps have been better represented with more data collected over the growing season than just these two dates.

Corn Yield

Despite some differences in SPAD values across treatments, no differences in dry grain yields were detected between SMB and FP treatments ($P > 0.05$), with mean yields of 14.18, 14.60, 15.06, 14.37, 14.07, and 15.08 Mg ha^{-1} for the SMB 1, 2, 3, 4, and 5, and FP treatments, respectively. These findings are consistent with studies by Blackmer and Schepers (1995) and Shapiro (1999) who found that N deficiencies detected by chlorophyll meters were not always great enough to cause a reduction in yield. This outcome indicates that SMB used in the place of fertilizer did not limit grain production, and there was no difference between the lowest SMB application rate of 1.34 t DM ha^{-1} and the highest rate of 6.72 t DM ha^{-1}. This is somewhat surprising, as SMB 1 was estimated to supply <150 kg N ha^{-1}, which is below the TN recommended rate of 235 kg N ha^{-1} for equivalent yields (Savoy and Joines, 2009a), and not all the total applied N was expected to become available for crop uptake in the first year. It is possible that higher than average temperatures during the growing season (Table 4) increased SMB mineralization, allowing more N to become plant available than was previously estimated. It should also be noted that grain quality was not considered in this study, but any differences were minor enough to escape visual detection while hand threshing.

Ear/stalk ratios between SMB treatments and the FP were also similar ($P > 0.05$), with means of 0.96, 0.99, 0.96, 0.95, 0.97, and 0.96, for SMB 1, 2, 3, 4, 5, and the FP, respectively. No significant trends were observed for either grain yield or ear/stalk ratios when SMB rates were contrasted.

CONCLUSIONS

Spent microbial biomass applied at a rate of 4.03 t DM ha^{-1} in tall fescue and 1.34 t DM ha^{-1} in corn provided sufficient N to maintain tall fescue forage quality and plant biomass production, and corn yields compared to a fertilizer treatment. These results may be different in other agricultural landscapes, including soil types, crop cultivars, and climatic conditions. The reuse of SMB in land application offers a sustainable alternative to the current method of landfill disposal, which could expand beyond the PDO production process to other areas of white biotechnology. The successful implementation of SMB use as a crop nutrient source will depend on finding farmers who are willing to participate and adapt their management practices. Future research on the use of SMB will need to further consider the timing and rate of application to optimize nutrient mineralization and crop uptake, and will need to consider the logistics of large-scale application including transportation to farmers, machinery to spread the material, and methods to preserve and store the material until it can be used.

ACKNOWLEDGMENTS

The authors would like to thank Don Bailey for his cooperation, patience, and the use of his fields for this research. David Bilderbeck, Mat Halter, and Geoff Card provided invaluable field work assistance. The authors gratefully thank DuPont Tate and Lyle Bio Products for providing financial support for this research. The SMB was tested as an agricultural soil amendment under approval by the Tennessee Department of Agriculture.

REFERENCES

American Society for Testing and Materials. 1992. ASTM D4239 14e2. Standard test method for sulfur in the analysis sample of coal and coke using high-temperature tube furnace combustion. ASTM Int., West Conshohocken, PA. 05.05:484-495.

American Society for Testing and Materials. 1996. Method 5291. Standard test methods for instrumental determination of carbon, hydrogen, and nitrogen in petroleum products and lubricants. ASTM Int., West Conshohocken, PA. Book of Standards Volume: 05.02.

Andersen, J.T., T. Schäfer, P.L. Jørgensen, and S. Møller. 2001. Using inactivated microbial biomass as fertilizer: The fate of antibiotic resistance genes in the environment. Res. Microbiol. 152:823–833. doi:10.1016/S0923-2508(01)01266-9

Barnhart, S.K. 2009. PM 1758: Estimating available pasture forage. Iowa State Univ. Ext. USDA Natural Resources Conserv. Serv., Washington, DC.

Bates, G. 1999. Tall fescue, orchardgrass, and timothy, cool season perennial grasses. Cool-season perennial grasses. University of Tennessee Agricultural Extension Service. https://extension.tennessee.edu/publications/Documents/SP434-E.pdf (accessed 28 Feb 2017).

Bates, G., A. Riues, J. Rhinehart, R. Nave, T. Mulliniks, and D. McIntosh. 2015. Forage analysis definitions. Univ. of Tennessee Inst. of Agric., Ext. http://utbfc.utk.edu/Content%20Folders/Forages/Hay%20and%20Silage/Publications/SP%20774.pdf (accessed 27 July 2016).

Blackmer, T.M., and J.S. Schepers. 1995. Use of a chlorophyll meter to monitor nitrogen status and schedule fertigation for corn. J. Prod. Agric. 8:56–60. doi:10.2134/jpa1995.0056

Cameron, K.C., H.J. Di, and J.L. Moir. 2013. Nitrogen losses from the soil/plant system: A review. Ann. Appl. Biol. 162(2):145–173. doi:10.1111/aab.12014

Castellanos, J.Z., and P.F. Pratt. 1981. Available nitrogen from animal manures. Calif. Agric. 35:24.

Dunnett, C.W. 1980. Pairwise multiple comparisons in the homogeneous variance, unequal sample size case. J. Am. Stat. Assoc. 75:789–795. doi:10.1080/01621459.1980.10477551

Environmental Protection Agency. 1994. Method 7471A (SW-846): Mercury in liquid waste (manual cold-vapor technique). Revision 1. EPA. https://www.epa.gov/homeland-security-research/epa-method-7470a-sw-846-mercury-liquid-wastes-manual-cold-vapor-technique (accessed Apr. 2017).

Environmental Protection Agency. 1996. Method 6010B. Inductively coupled plasma-atomic emission spectrometry. EPA. https://www.epa.gov/sites/production/files/documents/6010b.pdf (accessed Apr. 2017).

Environmental Protection Agency. 2016. Design for the environment life-cycle assessments. EPA. https://www.epa.gov/saferchoice/design-environment-life-cycle-assessments (accessed 18 Feb. 2017).

Frazzetto, G. 2003. White biotechnology. EMBO Rep. 4(9):835–837. doi:10.1038/sj.embor.embor928

Gibson, L.R. 1998. Calculate stand density from stand counts. Iowa State University Agronomy 212-Grain and forage crops. Iowa State Univ. http://agron-www.agron.iastate.edu/Courses/agron212/calculations/Stand_counts.htm (accessed 1 May 2015).

Halter, M.C., and J.A. Zahn. 2016. Degradation and half-life of DNA present in biomass from a genetically-modified organism during land application. J. Ind. Microbiol. Biotechnol. 44(2):213–220. doi:10.1007/s10295-016-1876-x

Hancock, D.W. 2011. Using relative forage quality to categorize hay. Univ. of Georgia Coop. Ext., Colleges of Agric. and Environ. Sciences and Family and Consumer Sci. http://georgiaforages.caes.uga.edu/pubs/RFQcategorization.pdf (accessed 21 Dec. 2016).

Kurian, J.V. 2005. A new polymer platform for the future—Sorona® from corn derived 1, 3-propanediol. J. Polym. Environ. 13:159–167. doi:10.1007/s10924-005-2947-7

Lauer, J. 2002. Methods for calculating corn yield. Agronomy advice. Univ. of Wisconsin-Madison. http://corn.agronomy.wisc.edu/AA/pdfs/A033.pdf (accessed Aug. 2015).

Mehlich, A. 1953. Determination of P, Ca, Mg, K, Na, and NH4. North Carolina Soil Test Division (Mimeo 1953). North Carolina Dep. of Agric., Raleigh.

Morrison, J.E., C.H. Huang, D.T. Lightle, and C.S. Daughtry. 1993. Residue measurement techniques. J. Soil Water Conserv. 48:478–483.

Murray, I., and I. Cowe. 2004. Sample preparation. In: C. Roberts et al., editor, Near-infrared spectroscopy in agriculture. Agron. Monogr. 44. ASA, CSSA, and SSSA, Madison, WI. p. 75–112.

Nakamura, C.E., and G.M. Whited. 2003. Metabolic engineering for the microbial production of 1, 3-propanediol. Curr. Opin. Biotechnol. 14:454–459. doi:10.1016/j.copbio.2003.08.005

NCDC. 2015. Data tools: 1981-2010 Normals. NOAA. http://www.ncdc.noaa.gov/cdo-web/datatools/normals (accessed Aug. 2015).

Novotny, L., T. Griggs, J. Karlen, P. Laskowski-Morren, and L. Bauman. 2013. Guidelines for optimizing accuracy and consistency in the NIRSC laboratory. NIRS Forage and Feed Testing Consortium. https://www.dropbox.com/s/t60fs3s86evsjip/Guidlines_Document.pdf?dl=0 (accessed Apr. 2015).

Novozymes. 2006. Neighbourliness: An investment in the local environment. Novozymes. http://www.novozymes.com/en/news/news-archive/2006/06/42184 (accessed 21 Feb. 2017).

Novozymes. 2011. A sustainable partnership: Biomass from Novozymes used as free fertilizer. Novozymes TV. http://www.novozymes.tv/video/1769913/a-sustainable-partnership (accessed 21 Feb. 2017).

Novozymes. 2016. 7.4 Waste. Environmental data. Novozymes. https://report2016.novozymes.com/-/media/9F43399909284A61B505E870789B3FAA.ashx (accessed 1 May 2017).

Peterson, T.A., T.M. Blackmer, D.D. Francis, and J.S. Schepers. 1993. Using a chlorophyll meter to improve N management. NebGuide G93-1171-A. Univ. of Nebraska, Lincoln.

Pfaff, J.D. 1993. Method 300.0. Determination of inorganic anions in water by ion chromatography. U.S. Environmental Protection Agency. https://www.epa.gov/sites/production/files/2015-08/documents/method_300-0_rev_2-1_1993.pdf (accessed Apr. 2017).

Ribaudo, M., M. Livingston, and J. Williamson. 2012. Nitrogen management on U.S. corn acres, 2001-10. USDA Economic Brief no. EB-20. USDA. https://www.ers.usda.gov/publications/pub-details/?pubid=42867 (accessed 12 Dec. 2016).

Robertson, G.P., and P.M. Vitousek. 2009. Nitrogen in agriculture: Balancing the cost of an essential resource. Annu. Rev. Environ. Resour. 34:97–125. doi:10.1146/annurev.environ.032108.105046

Mathias, T.R.S., P.P.M. Mello, and E.F.C. Sérvulo. 2014. Solid wastes in brewing process: A review. J. Brew. Distill. 5(1):1–9. doi:10.5897/JBD2014.0043

Salmerón, M., R. Isla, and J. Cavero. 2011. Effect of winter cover crop species and planting methods on maize yield and N availability under irrigated Mediterranean conditions. Field Crops Res. 123:89–99. doi:10.1016/j.fcr.2011.05.006

Savoy, H.J., and D. Joines. 2009a. Lime and fertilizer recommendations for the various crops of Tennessee. ch. II. Agronomic crops. Univ. of Tennessee Ext. https://ag.tennessee.edu/spp/SPP%20Publications/chap2-agronomic_mar2009.pdf (accessed 21 Sept. 2016). p. 1.

Savoy, H.J., and D. Joines. 2009b. Lime and fertilizer recommendations for the various crops of Tennessee. ch. III. Pasture/hay/silage crops. Univ. of Tennessee Ext. https://ag.tennessee.edu/spp/SPP%20Publications/chap3-pasturehay_mar2009.pdf (accessed 21 Sept. 2016). p. 7.

Shapiro, C.A. 1999. Using a chlorophyll meter to manage nitrogen applications to corn with high nitrate irrigation water. Commun. Soil Sci. Plant Anal. 30:1037–1049. doi:10.1080/00103629909370266

Shurson, G., M. Spiehs, and M. Whitney. 2004. The use of maize distiller's dried grains with solubles in pig diets. Pig News and Information 25(2):75–83.

Soil Survey Staff. 2015. Web soil survey. USDA-NRCS. http://websoilsurvey.nrcs.usda.gov/ (accessed Apr. 2015).

Tang, W.L., and H. Zhao. 2009. Industrial biotechnology: Tools and applications. Biotechnol. J. 4:1725–1739. doi:10.1002/biot.200900127

Trimble. 2012. GreenSeeker® handheld crop sensor quick reference card. Trimble Navigation Ltd., Sunnyvale, CA. http://www.farmworks.com/files/pdf/GreenSeeker%20HCS/GreenSeekerQRC_91500-00-ENG_Screen.pdf (accessed June 2015).

U.S. Climate Data. 2016. Climate Lenoir City-Tennessee. Your Climate Service. http://www.usclimatedata.com/climate/lenoir-city/tennessee/united-states/ustn0284. (accessed 3 Aug. 2016).

U.S. Pharmacopeia. 2015. < 921 > Water determination. Method 1c. Physical tests. USP 37. https://hmc.usp.org/sites/default/files/documents/HMC/GCs-Pdfs/GC_pdf_USP38/c921.pdf (accessed Apr. 2017).

Víg, R., L. Huzsvai, A. Dobos, and J. Nagy. 2012. Systematic measurement methods for the determination of the SPAD values of maize (*Zea mays* L.) canopy and potato (*Solanum tuberosum* L.). Commun. Soil Sci. Plant Anal. 43:1684–1693. doi:10.1080/00103624.2012.681740

Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler et al. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecol. Appl. 7:737–750.

Wortmann, C.S., J. Sawyer, and S. Tonn. 2015. Research identifies attributes of industrial byproduct for soils. Univ. of Nebraska-Lincoln. CropWatch. http://cropwatch.unl.edu/research-identifies-attributes-industrial-byproduct-soils (accessed Apr. 2015).

Yang, Y., D.J. Timlin, D.H. Fleisher, S.B. Lokhande, J.A. Chun, S.H. Kim et al. 2012. Nitrogen concentration and dry-matter accumulation in maize crop: Assessing maize nitrogen status with an allometric function and a chlorophyll meter. Commun. Soil Sci. Plant Anal. 43:1563–1575. doi:10.1080/00103624.2012.675393

Yu, J., M. Porter, and M. Jaremko. 2013. Generation and utilization of microbial biomass hydrolysates in recover and production of poly (3-hydroxybutyrate). Biomass now-cultivation and utilization. InTech, New York. p. 33–48.