Environmental Science & Technology

ARTICLE

pubs.acs.org/est

Biochemical Suitability of Crop Residues for Cellulosic Ethanol: Disincentives to Nitrogen Fertilization in Corn Agriculture

Morgan E. Gallagher,*[,†] William C. Hockaday,[‡] Caroline A. Masiello,[†] Sieglinde Snapp,[§,⊥] Claire P. McSwiney,[⊥] and Jeffrey A. Baldock[∥]

[†]Department of Earth Science, Rice University, 6100 Main Street, MS 126, Houston, Texas 77005, United States

[‡]Department of Geology, Baylor University, One Bear Place #97354, Waco, Texas 76798, United States

[§]Department of Crop and Soil Sciences, Michigan State University, 576A Plant and Soil Sciences, East Lansing, Michigan 48824, United States

[⊥]W.K. Kellogg Biological Station, Michigan State University, 3700 East Gull Lake Drive, Hickory Corners, Michigan 49060, United States

[∥]CSIRO Land and Water/Sustainable Agriculture Flagship, Urrbrae Laboratory, PMB 2, Glen Osmond SA 5064, Australia

Supporting Information

ABSTRACT: Concerns about energy security and climate change have increased biofuel demand, particularly ethanol produced from cellulosic feedstocks (e.g., food crop residues). A central challenge to cropping for cellulosic ethanol is the potential environmental damage from increased fertilizer use. Previous analyses have assumed that cropping for carbohydrate in residue will require the same amount of fertilizer as cropping for grain. Using ^{13}C nuclear magnetic resonance, we show that increases in biomass in response to fertilization are not uniform across biochemical classes (carbohydrate, protein, lipid, lignin) or tissues (leaf and stem, grain, reproductive support). Although corn grain responds vigorously and nonlinearly, corn residue shows only modest increases in carbohydrate yields in response to high levels of fertilization (25% increase with 202 kg N ha^{-1}). Lignin yields in the residue increased almost twice as much as carbohydrate yields in response to nitrogen, implying that residue feedstock quality declines as more fertilizer is applied. Fertilization also increases the decomposability of corn residue, implying that soil carbon sequestration becomes less efficient with increased fertilizer. Our results suggest that even when corn is grown for grain, benefits of fertilization decline rapidly after the ecosystem's N demands are met. Heavy application of fertilizer yields minimal grain benefits and almost no benefits in residue carbohydrates, while degrading the cellulosic ethanol feedstock quality and soil carbon sequestration capacity.

INTRODUCTION

Rising oil prices and growing concerns over greenhouse gas emissions and climate change have increased demand for biofuels, particularly for corn grain ethanol.[1−3] Increasing demand for corn grain ethanol is increasing corn acreage (8% increase in acreage in the U.S. from 2004 to 2009[4]) leading to decreased crop diversity,[5] increased soil erosion, and increased use of nitrogen (N) fertilizers, pesticides, and herbicides.[6−8] However, ∼1.02 billion people are undernourished,[9] making the use of corn grain for ethanol controversial.[6] A second-generation biofuel, cellulosic ethanol, has the potential to circumvent some of these problems by using plant residue (e.g., corn stover, wheat straw, or nonfood biomass) instead of corn grain.[3,10] Still, further improvements to cellulosic ethanol production are necessary before it can become a viable substitute for grain ethanol. Finding biochemically labile feedstocks for cellulosic ethanol is a necessary step in this process. Agricultural ecosystems are also under pressure to increase their capacity to sequester soil organic matter (SOM), and this capacity is, in part, a function of the amount and biochemical composition of the crop residue that remains after harvest. Here we consider the biochemical profiles of corn tissues from the perspectives of grain production, cellulosic ethanol production, and soil carbon sequestration.

Feedstock biochemical profiles influence the efficiency of cellulosic ethanol production, with high carbohydrate and low lignin levels being preferable (carbohydrate is the cellulosic ethanol feedstock[11]). The lignin−hemicellulose copolymer is extremely difficult for enzymes and microbes to depolymerize and slows the conversion of carbohydrates into simple sugars.[12] To achieve the highest conversion efficiency, lignin must be physically or chemically removed from biomass.[11] Current lignin management techniques include improving biomass chemical processing,[13,14] genetically altering enzymes to break down lignin,[15] and genetically engineering low-lignin plants.[16]

It has been assumed that cropping for both grain and residue (straw, stover, etc.) will require the same nutrient management practices as cropping exclusively for grain (e.g., ref 17). However, high levels of fertilizer application may not produce residues suitable for cellulosic ethanol production because N fertilization is unlikely to increase carbon (C) allocation equally to all plant tissues (i.e., grain, leaves, stems, and roots) and biochemicals (i.e., carbohydrate, protein, lignin, lipid). Previous studies have

Received: September 24, 2010
Accepted: January 17, 2011
Revised: January 4, 2011
Published: February 25, 2011

observed plant biochemical shifts in response to fertilization using biological assays and near-infrared spectroscopy. A typical response to N fertilization is increased protein and decreased carbohydrate concentrations.[18] Shifts in biomass allocation (e.g., from stems to roots) can also cause shifts in plant biochemical stocks since plant tissues each have a specific biochemical profile.[19,20]

In addition to its effects on crop biochemistry, excess fertilization has negative environmental consequences.[21−23] N fertilizers are the main cause of hypoxic "dead zones",[24] and nitrate runoff into drinking water supplies has been linked to a number of health problems.[25] Furthermore, the use of synthetic fertilizers contributes to greenhouse gas emissions through (1) carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) emissions during production,[26] and (2) nonlinear increases in N_2O emissions from soils in response to the presence and cycling of inorganic N.[27] Emissions of fossil fuel derived CO_2 and N_2O (GWP = 310[28]) from biofuel crop systems can significantly reduce the climate benefits associated with the use of biofuels.[29,30] In this rapidly changing food and energy landscape, we must understand how to maximize crop yield while minimizing fertilizer application and energy consumption.

Our goal in this paper is to quantify the impact of N fertilization on biochemical yields in the whole-plant harvest of a conventional corn agricultural ecosystem. We hypothesize that corn grain and residue respond differently to N fertilization, with yields diminishing in response to fertilization. Because the metabolic pathway for plant lignin production requires phenolic amino acids, we also hypothesize that limiting N fertilization may be an effective way to reduce fuel feedstock lignin content. Finally, we recognize that plant nutrient status affects the decomposability of biomass, influencing the ability of soil to sequester carbon. Previous studies have considered only the effects of N fertilization on corn grain yield; here we additionally consider the effects of N fertilization on the quantity and quality of a potential cellulosic ethanol feedstock.

EXPERIMENTAL SECTION

We used corn grown at the Kellogg Biological Station Long-term Ecological Research (KBS-LTER) Site (42°24′ N, 85°24′ W) in southwest Michigan during the 2006 growing season. Plants were fertilized with ammonium nitrate at seven rates (0, 34, 67, 101, 134, 168, and 202 kg N ha^{-1}) in four replicate blocks (R1−R4; Figure S1 in the Supporting Information). The appropriate N fertilization rate for any particular agricultural ecosystem will depend on soil type, cropping history, and the crop being planted. For this field site, 0−67 kg N ha^{-1}, 101 kg N ha^{-1}, and 134−202 kg N ha^{-1} are characterized as deficient, sufficient, and excessive, respectively.[31] Corn plants were split into three tissue types: (1) grain, (2) reproductive support (husk, shank, tassel, and cob), and (3) leaf and stem (corn roots were not collected since roots are not typically harvested). Corn residue is the combination of the reproductive support and leaf and stem tissues. Further details on the field site design, sampling, and processing can be found in the Supporting Information (SI).

We measured C and N concentrations using Costech 4010 and Perkin-Elmer 2400 series CHNS/O Elemental Analysis Systems (Table S2). We assessed carbohydrate, protein, lignin, and lipid concentrations using a rapid and accurate ^{13}C nuclear magnetic resonance (NMR) technique[32,33] developed for the organic geochemistry community,[34−36] but equally applicable to plant biochemical analyses. NMR has been recognized as a more accurate and effective technique for litter biochemical characterization[37] since studies in the late 1990s showed that wet extractive "proximate" analyses often extracted materials chemically unrelated to the target compounds. For example, "lignin" extractions have been shown to be enriched in cutins and tannins.[38] Comparisons of wet chemical assays for lignin show differences of as much as 150% in yield,[39] pointing to the need to use modern analytical chemistry tools to assess plant biochemical composition. Solid-state ^{13}C NMR nondestructively quantifies organic chemical functional groups present in plant biochemicals (e.g., the phenolic functional groups present in lignin monomers), without chemical extraction or pretreatment. ^{13}C NMR is a robust method compared with other techniques, which are usually compound-specific and when combined, can detect as little as 20% of C present in a sample.[32,37]

Solid-state ^{13}C NMR spectra were collected on each corn tissue sample using a Bruker 200 MHz NMR. We performed cross-polarization (CP) experiments using a 4-mm magic angle spinning (MAS) probe spun at 5 kHz frequency, with a 1 ms contact time and a recycle delay >2 s on all four replicates at fertilization rates 0, 67, 134, and 202 kg N ha^{-1} (see SI Figures S3−S5). A single replicate (R1) was additionally analyzed at fertilization rates 34, 101, and 168 kg N ha^{-1}. The four replicates at 0, 67, 134, and 202 kg N ha^{-1} allow full error analysis, including instrumental variability as well as the natural variability of this agricultural site. Spinning sideband (SSB) intensity was integrated and quantified. NMR signals were then mathematically corrected for spinning sideband intensity by assuming that the upfield and downfield sidebands were of equal intensity. We also spin-counted[40] each spectrum to quantitate the NMR data. An average of 77.7 ± 6.8% of the C was detected in the samples, and no spectrum detected less than 64.6% of the sample C. A low-temperature (220 K) CP experiment was performed to determine if there was a systematic underdetection of mobile C structures (e.g., lipids). From this experiment we concluded all underdetection in observability is accounted for in mobile aliphatic structures (Figure S6).

To determine which C functionalities were systematically overdetected in the CP experiments, we collected direct polarization (DP) spectra on 14 samples (7 grain and 7 leaf and stem samples, e.g., Figure S7 and Figure S8). All DP spectra were corrected for SSB and spin-counted, with an average of 87.8 ± 19.2% C detected. DP pulse sequences are more accurate than CP; however, they are much noisier and often generate poorer quality spectra. To improve DP spectral quality, we collected DP spectra for 6 times as long as CP spectra (12 versus 2 h per sample, respectively). This generated DP spectra of sufficient quality to determine that CP spectra overestimated carbohydrate yields by ∼7%; less than natural variability noted between replicates for all but one of our samples. DP spectra did not yield recognizable peaks (above 3× noise) for the spectral regions used to calculate lipid, lignin, or protein concentrations, so we could not use DP spectra to generate these data. Because a linear offset of 7% for any of our data points has no effect on our results, we have chosen not to apply a correction factor to any results reported here.

^{13}C Nuclear Magnetic Resonance Spectrometry and the Molecular Mixing Model. A molecular mixing model (MMM) was used to determine the biochemical composition of each corn biomass sample.[32,33] The MMM uses a sample's C:N ratio (Table S2) and the signal distribution across seven predefined ^{13}C NMR spectral regions to calculate the relative abundance of

Table 1. Absolute Increases in Total Biochemical Yields (Mg ha^{-1}) and Error (Natural Variability) of Carbohydrate, Protein, Lignin, and Lipid for the Corn Tissues (Grain, Reproductive Support, and Leaf and Stem), Crop Residue, and the Total Corn Plant under Seven Different Fertilization Rates[a]

	carbohydrate			protein			lignin			lipid		
fertilization rate (kg N ha^{-1})	rep 1 yield (Mg ha^{-1})	average yield (Mg ha^{-1})	error (Mg ha^{-1})	rep 1 yield (Mg ha^{-1})	average yield (Mg ha^{-1})	error (Mg ha^{-1})	rep 1 yield (Mg ha^{-1})	average yield (Mg ha^{-1})	error (Mg ha^{-1})	rep 1 yield (Mg ha^{-1})	average yield (Mg ha^{-1})	error (Mg ha^{-1})
					corn grain							
0	3.12	3.53	0.41	0.17	0.21	0.04	0.19	0.22	0.04	0.08	0.07	0.03
34	5.25			0.35			0.31			0.10		
67	6.68	6.53	0.15	0.45	0.45	0.01	0.28	0.34	0.06	0.14	0.14	0.03
101	6.47			0.49			0.44			0.09		
134	6.95	7.17	0.38	0.49	0.54	0.05	0.38	0.36	0.02	0.12	0.15	0.06
168	6.58			0.56			0.26			0.08		
202	7.20	7.53	0.31	0.54	0.56	0.03	0.41	0.41	0.04	0.15	0.17	0.04
					reproductive support							
0	0.72	0.82	0.08	0.02	0.03	0.01	0.16	0.17	0.03	0.02	0.02	0.01
34	0.87			0.03			0.20			0.01		
67	0.98	1.04	0.04	0.04	0.03	0.00	0.24	0.24	0.01	0.02	0.02	0.01
101	1.11			0.03			0.31			0.01		
134	1.38	1.17	0.18	0.04	0.04	0.01	0.29	0.24	0.04	0.02	0.02	0.01
168	1.14			0.04			0.26			0.04		
202	1.10	1.26	0.13	0.03	0.04	0.01	0.23	0.29	0.04	0.03	0.02	0.01
					leaf and stem							
0	2.85	3.27	0.44	0.08	0.10	0.03	0.56	0.77	0.14	0.12	0.12	0.03
34	3.22			0.13			0.83			0.12		
67	3.63	3.49	0.12	0.19	0.17	0.02	0.93	0.87	0.06	0.12	0.13	0.02
101	3.10			0.20			0.71			0.19		
134	3.58	3.57	0.27	0.18	0.23	0.04	0.95	0.92	0.08	0.11	0.15	0.05
168	3.29			0.22			0.82			0.10		
202	3.98	3.85	0.14	0.27	0.28	0.02	0.98	1.03	0.05	0.17	0.17	0.04
					crop residue							
0	3.57	4.09	0.51	0.10	0.13	0.03	0.72	0.94	0.15	0.14	0.14	0.03
34	4.09			0.16			1.03			0.13		
67	4.61	4.53	0.09	0.23	0.20	0.02	1.17	1.11	0.07	0.14	0.15	0.01
101	4.22			0.23			1.02			0.20		
134	4.96	4.74	0.40	0.22	0.27	0.05	1.24	1.16	0.10	0.12	0.18	0.05
168	4.43			0.26			1.08			0.13		
202	5.08	5.11	0.18	0.30	0.32	0.03	1.21	1.32	0.09	0.20	0.19	0.05
					total corn plant							
0	6.69	7.62	0.90	0.26	0.34	0.07	0.91	1.16	0.17	0.21	0.21	0.05
34	9.34			0.51			1.34			0.22		
67	11.3	11.1	0.17	0.68	0.65	0.03	1.46	1.45	0.04	0.29	0.28	0.03
101	10.7			0.72			1.46			0.29		
134	11.9	11.9	0.67	0.71	0.80	0.10	1.62	1.52	0.12	0.25	0.33	0.10
168	11.0			0.82			1.34			0.22		
202	12.3	12.6	0.42	0.84	0.88	0.05	1.61	1.73	0.10	0.35	0.36	0.08

[a] Crop residue is the combination of the reproductive support and leaf and stem tissue. Rep 1 yield (Mg ha^{-1}) represents biochemical yields in replicate 1. Average yield (Mg ha^{-1}) represents the average biochemical yields for replicates 1, 2, 3, and 4 at the fertilization rates 0, 67, 134, and 202 kg N ha^{-1}. The error (Mg ha^{-1}) is the standard deviation for this average and represents the natural variability of this corn ecosystem. See Calculations and Error section for details.

four biochemical classes (carbohydrate, protein, lignin, and lipid; Table S9[32,33]). The model simultaneously solves a suite of linear equations to divide each of the seven spectral regions of a sample into its biochemical components based upon the NMR spectra of standards that are representative of each biochemical class (e.g., cellulose for carbohydrate). Carbon-13 NMR experiments

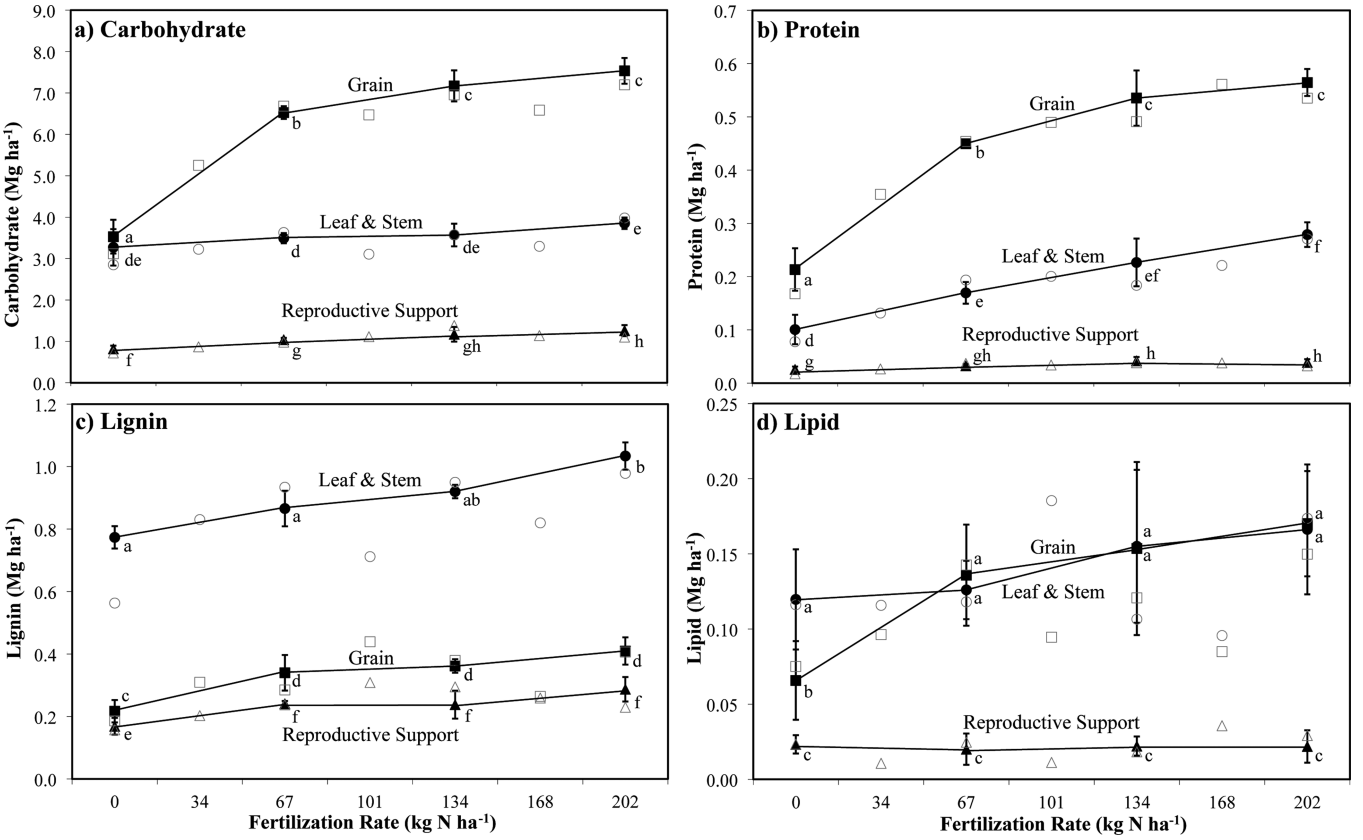

Figure 1. Total biochemical stocks for each tissue type. The change in total biochemical stock yields ((a) carbohydrate, (b) protein, (c) lignin, and (d) lipid) for grain, leaf and stem, and reproductive support in Mg ha^{-1} over seven nitrogen fertilization rates. The black symbols (■ grain, ▲ reproductive support, and ● leaf and stem) represent the average of replicates 1−4 for the biochemical yield at four fertilization rates (0, 67, 134, 202 kg N ha^{-1}). Natural variability is the standard deviation of the biochemical stocks for replicates 1−4 at these four fertilization rates and is represented by the error bars on these yields. Open squares, triangles, and circles represent grain, reproductive support, and leaf and stem replicate 1 data, respectively. Different letters, or combination of letters, represent stocks that are significantly different from each other (two-tailed *t* test; *p*-value <0.05). Lipid concentrations are difficult to constrain using ^{13}C NMR and the MMM due to their high molecular mobility.

conducted in our lab using known mixtures of purified biopolymers indicate that the MMM results are accurate to ±1% (Table S10). Further validation of the MMM can be found in Nelson and Baldock 2005.[33]

Calculations and Error. We used the MMM to determine the biomolecular content of three different corn tissue types over seven nitrogen fertilization rates (Table S9). The percent composition of biochemicals in each sample, measured using ^{13}C NMR and the MMM, have errors of ±1%. Weighting the biomolecule mass percent with net primary production (NPP) data (i.e., yield data Mg ha^{-1} [31]) gives the total biochemical yield (carbohydrate, protein, lignin, or lipid; Table 1; Figure 1) of the aboveground corn ecosystem (eq 1).

$$\text{Biochemical Yield (Mg ha}^{-1}\text{)} = \text{Corn Tissue Yield (Mg ha}^{-1}\text{)} \times \left[\frac{\text{Biochemical in Corn Tissue (\%)}}{100\%}\right] \quad (1)$$

Natural variability is calculated as the standard deviation of replicates 1 through 4 for four fertilization rates. Biochemical yields for corn residue and the total plant for each replicate were calculated by summing the biochemical yields of their component tissues (e.g., eq 2).

$$\text{Residue Lignin Yield (Mg ha}^{-1}\text{)} = \text{Leaf and Stem} + \text{Reproductive Support Yield (Mg ha}^{-1}\text{)} \quad (2)$$

Percent lignin, C, and N for corn residue were calculated as the weighted averages of the component tissue (leaf and stem and reproductive support) values with the yield data (NPP) for each replicate (e.g., eq 3), and these values were used to calculate environmental parameters (C:N and lignin:N ratios) for the corn residue (eq 4-5).

$$\%\text{Lignin}_{\text{residue}} = \frac{\%\text{Lignin}_{\text{LeafStem}} \times \text{NPP}_{\text{LeafStem}} + \%\text{Lignin}_{\text{Rep.Support}} \times \text{NPP}_{\text{Rep.Support}}}{\text{NPP}_{\text{LeafStem}} + \text{NPP}_{\text{Rep.Support}}} \quad (3)$$

$$\text{C : N Ratio} = \frac{\text{Carbon (\%by mass)}}{\text{Nitrogen (\%by mass)}} \quad (4)$$

$$\text{Lignin : N Ratio} = \frac{\text{Lignin (\%by mass)}}{\text{Nitrogen (\%by mass)}} \quad (5)$$

Table 2. Percent Change in Total Yields of Each Biochemical with the Addition of 67, 134, or 202 kg N ha^{-1} of Nitrogen Fertilizer Compared to the Biochemical Yields of the Unfertilized Plots[a]

	increase with nitrogen fertilization				
	grain	reproductive support	leaf and stem	crop residue	total plant
67 kg N ha^{-1}					
carbohydrate	85%***	27%**	7%	11%	45%**
protein	111%**	27%^	68%**	60%**	92%**
lignin	57%*	43%**	12%	17%	25%*
lipid	106%*	−14.0%	5%	2%	35%^
134 kg N ha^{-1}					
carbohydrate	103%****	43%*	9%	16%^	56%***
protein	151%****	64%*	125%**	113%**	137%***
lignin	67%**	41%*	19%	23%^	31%*
lipid	133%*	−5%	30%	24%	58%^
202 kg N ha^{-1}					
carbohydrate	114%****	54%**	18%^	25%*	66%***
protein	165%****	52%*	177%****	152%***	160%****
lignin	89%***	70%**	34%*	40%**	49%**
lipid	158%**	−6%	39%	32%	72%*

[a] Calculated using eq 6. Asterisks represent values that are significant changes from the unfertilized plot based on different p-values for a two-tailed t test (*p-value <0.05; **p-value <0.01; ***p-value <0.001; ****p-value <0.0001; ^p-value <0.1).

Percent change in biochemical yield is the percent increase in biochemical yield at a particular fertilization rate when compared to the unfertilized plot and was calculated using eq 6 (Table 2).

$$\text{Change}\ (\%) = \frac{[\text{Fertilized}\ (\text{Mg ha}^{-1}) - \text{Unfertilized Biochemical Yield}\ (\text{Mg ha}^{-1})]}{\text{Unfertilized Biochemical Yield}\ (\text{Mg ha}^{-1})} \times 100\% \quad (6)$$

Statistical analyses were performed using the data analysis tools in Microsoft Excel software. Statistical significance was determined using a two-tailed, t test (two-sampled, unequal variance) to compare between fertilization rates for grain and residue biochemical yields, and environmental parameters. A p-value of 0.05 was used as the significance threshold, above which results were not treated as statistically significant. Our complete data set, including statistical tables (Tables S11 and S12), is presented in the SI.

RESULTS AND DISCUSSION

To determine the level of fertilizer application where yields of grain and corn residue ceased to increase significantly, we estimated biochemical yields (Mg ha^{-1}) for each plant tissue type (grain, leaf and stem, and reproductive support; Figure 1) for all four field replicates at fertilization rates 0, 67, 134, and 202 kg N ha^{-1} (Table 1; Figure 1). Replicate 1 samples were analyzed at fertilization rates 34, 101, and 134 kg N ha^{-1} to clarify the trend between these rates. In agricultural production, the commodities of interest are the corn grain and corn crop residue. The leaf and stem and reproductive support tissues (Figure 1) make up the corn crop residue (Figure 2).

Although we detected significant increases in total plant mass yields of carbohydrate with increased fertilization, carbohydrate yield from the corn residue changed only slightly in response to increased N fertilization (Table 1; Figure 2), with maximum total carbohydrate yield (25% increase; Table 2) occurring at the highest fertilization rate (202 kg N ha^{-1}). The majority of the carbohydrate increase in the total plant occurs within the grain, which saw 73% of the increase in yield with 202 kg N ha^{-1}. Without fertilization, plant carbohydrate was dominated by residue carbohydrate (54%). When fertilized the dominant source of carbohydrate shifted to the grain (60%).

For grain production, both carbohydrate and protein respond nonlinearly to N fertilization, plateauing between 67 and 134 kg N ha^{-1} (Figure 2a and b). Increasing fertilization rates from 134 to 202 kg N ha^{-1} does not increase grain yield for either carbohydrate or protein (p-values = 0.189 and 0.405, respectively). Increasing fertilization from 67 to 134 kg N ha^{-1} only increases grain carbohydrate yields by 0.64 Mg ha^{-1} (a 10% increase; p-value = 0.034); small compared to the 85% increase in yield between 0 and 67 kg N ha^{-1} (p-value = 0.0002).

For corn residue, carbohydrate yield (the majority of cellulosic ethanol feedstock) responded slowly to N fertilization. Carbohydrate yields in the corn residue at fertilization rates 0, 67, and 134 kg N ha^{-1} were all statistically the same (Table 1; p-values >0.09). Carbohydrate yields at low fertilization rates (0 and 67 kg N ha^{-1}) were lower than yields at the highest fertilization rate of 202 kg N ha^{-1} (p-values = 0.022 and 0.004, respectively). Corn residue carbohydrate yields at the high fertilization rates, 134 and 202 kg N ha^{-1}, were statistically indistinguishable (p-value = 0.160). Even at the highest fertilization rate, the crop residue only responded with a 25% increase in carbohydrate yield (Table 2).

In combination, the grain and residue data imply a fertilization threshold between 67 and 134 kg N ha^{-1}, beyond which yield returns are not likely to justify the cost of additional fertilizer, regardless of the biochemical desired (carbohydrate for fuel or protein for food). When the trends in Figure 2a and b (based upon the analysis of a single replicate (R1)) are considered we can speculate that the threshold is closer to 67 kg N ha^{-1} than 134 kg N ha^{-1}.

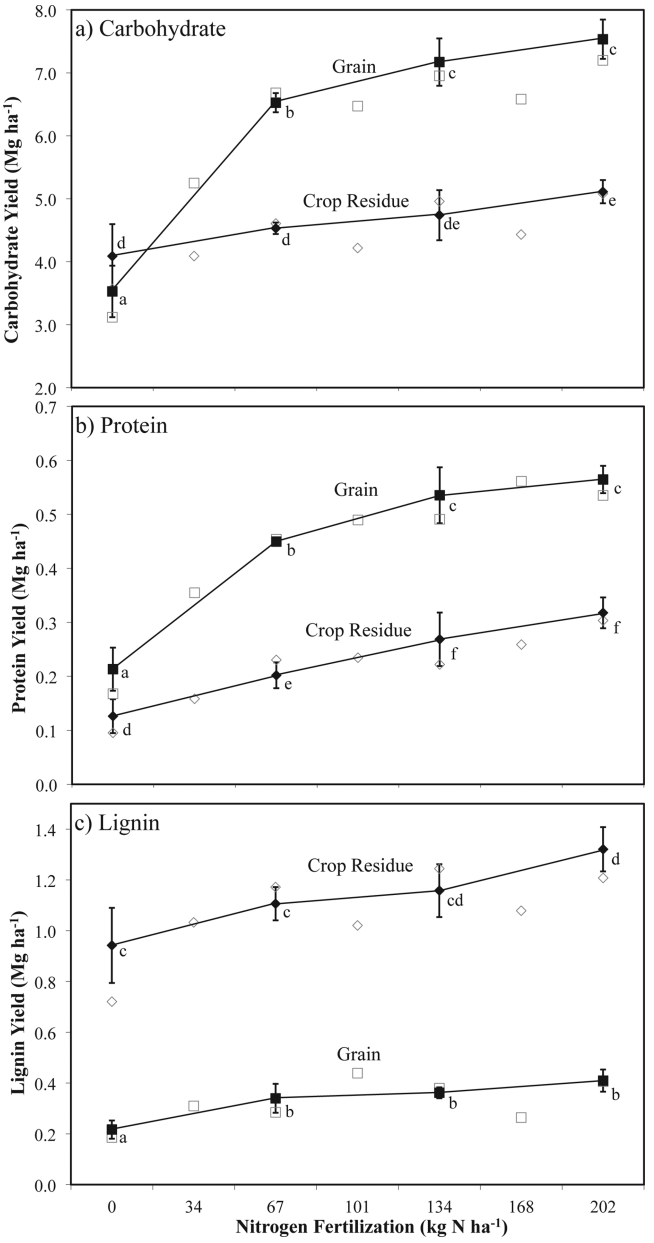

Figure 2. Corn grain and crop residue biochemical stocks. Variations in biochemical yields in the corn grain and crop residue with increasing N fertilization rates: (a) carbohydrate, (b) protein, and (c) lignin stocks. Crop residue is made up of the corn leaf and stem and reproductive support tissue types (see Figure 1). Squares and diamonds represent biochemical stocks for the grain and the corn residue, respectively. The black symbols (■ grain and ♦ corn residue), connected with black lines, represent the average of replicates 1−4 for the biochemical yield at four fertilization rates (0, 67, 134, 202 kg N ha^{-1}). Natural variability is the standard deviation of the biochemical stocks for replicates 1, 2, 3, and 4 at these four fertilization rates and is represented by the error bars on these yields. Different letters, or combination of letters, represent biochemical stocks that are significantly different from each other (two-tailed *t* test; *p*-value <0.05; Table S11). The open gray symbols represent replicate 1 data. See SI for more details.

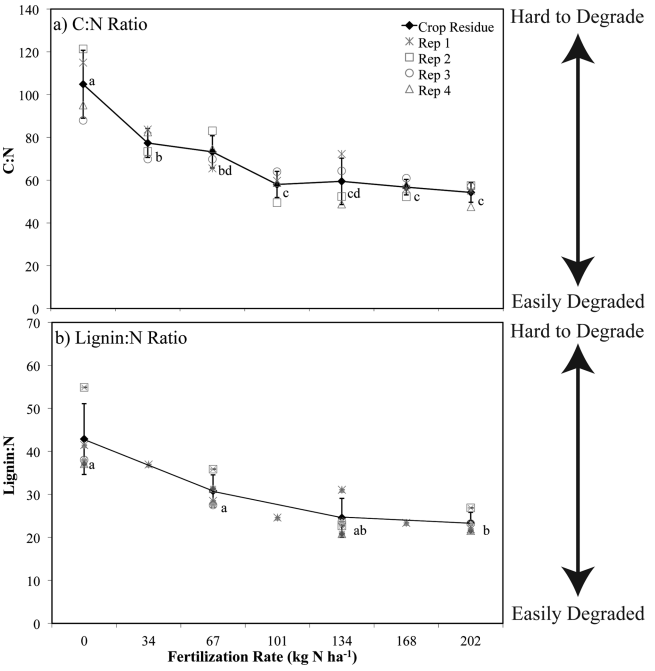

Figure 3. Indicators of environmental recalcitrance. The parameters C:N ratios (a) and lignin:N ratios (b) are indicators of biomass decomposability in the soil. Different letters, or combination of letters, represent ratios that are significantly different from each other (two-tailed *t* test; *p*-value < 0.05; Table S12). For cropping systems that harvest part of the crop residues for cellulosic ethanol production and leave some residue in the soil, lower fertilization rates produce residues that convert more efficiently to ethanol and also generate more stable organic matter if left in the soil.

If crops are grown for cellulosic ethanol, plant lignin content must also be considered. In cellulosic ethanol production, feedstock carbohydrate (primarily cellulose) is depolymerized into a mixture of simple sugars, which microbial fermentation then converts to ethanol.[11] Plant lignin content influences cellulosic ethanol production efficiency. Lignin is extremely resistant to hydrolytic enzymes and slows the conversion of cellulose and hemicellulose to simple sugars.[12] To achieve the highest efficiency of conversion, lignin must be physically or chemically removed from plant biomass before fermentation.[11] Current approaches to reducing lignin recalcitrance include improving biomass chemical processing,[13,14] genetically altering enzymes to break down lignin,[15] and genetically engineering low-lignin plants.[16] Below, our results suggest that the precise management of N fertilization (i.e., precision agriculture) may also be an effective way to reduce fuel feedstock lignin content (Figure 2c).

When fertilized, corn residue lignin yield increased by 17−40%, while carbohydrate yield only increased by 11−25% (Table 2; Figure 2c; Figure 2a). Fertilizing at a low rate, 67 kg N ha^{-1}, did not significantly increase carbohydrate yields (11%; 0.44 Mg ha^{-1}; *p*-value = 0.179), or lignin yields (17%; 0.17 Mg ha^{-1}; *p*-value = 0.097). Applying a high fertilization rate (202 kg N ha^{-1}) increased carbohydrates by 25% (1.02 Mg ha^{-1}; *p*-value = 0.022), while lignin increased 40% (0.38 Mg ha^{-1}; *p*-value = 0.006). The increase in lignin yield with fertilization (17−40% increase; Table 2) is likely due to the dependence of lignin biosynthesis upon the availability of aromatic amino acid precursors (phenylalanine and tyrosine[41]). Phenylalanine availability is rate-limiting in lignin biosynthesis at the cellular level.[41] With the addition of fertilizer at even the lowest rate (34 kg N ha^{-1}), lignin production is stimulated as plant N requirements are met (Figure 2c). Hence, overfertilization could produce a less biochemically favorable cellulosic ethanol feedstock, since crop

residue lignin increases more rapidly than carbohydrate in response to nitrogen. There may be potential for fertilizer management to improve efficiency of cellulosic ethanol production through controlling plant lignin production.

To assess sustainability of the entire biofuel cropping system, soil C must also be considered. If all corn residues are not harvested as feedstock for cellulosic ethanol, some can be left in the field to help build SOM. Maintaining SOM is critical to sustainability as organic matter maintains soil moisture, retains nutrients, and supports beneficial microbiota and invertebrates.[42] Slower decomposition rates of residue allow SOM to accumulate and these rates are influenced by litter biochemistry.[43−45] Two parameters used to predict decomposition rates are C:N and lignin:N ratios.[43,46] Higher C:N and lignin:N ratios indicate that the residue will have slower decomposition rates in the soil.[43−46] Residue N content is a control of decomposition rates since it is utilized by decomposers for their cell growth.[45] At our field site, the highest plant C:N and lignin:N ratios (best for soil C storage) occur at the lowest fertilization levels (Figure 3a; Figure 3b; Figure S13). As N fertilization increases, the corn residue becomes easier for microbes to decompose, allowing more of the residue C to be oxidized to CO_2 and released to the atmosphere rather than stored in soil. In a carbon-based economy, this would create further disincentives to overfertilization.

We show that crop biochemical profiles can be managed, in part, by altering N fertilization rates, and that optimizing agricultural practices for carbohydrate yields may reduce the environmental impact of cellulosic ethanol production. However, before large-scale introduction of this process, other issues need attention. Plant response to N fertilization varies within and between species, likely leading to significant diversity in plant biochemical yield. Our approach to optimizing biochemical yields will need to be tested in other viable cellulosic biofuel crops (e.g., switchgrass) before our results can be extrapolated to other agrisystems. In addition, it will be very important to determine the effects of residue harvesting practices on soil erosion, sustainability of soil C pools, and net agricultural greenhouse gas balances.

■ ASSOCIATED CONTENT

Supporting Information. Additional information regarding the field site, ^{13}C NMR spectra, and biochemical stocks data. This information is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 713-348-5087; fax: 713-348-5214; e-mail: morgan.gallagher@rice.edu.

■ ACKNOWLEDGMENT

Funding for this project was provided by the National Science Foundation (DEB-0445282; DEB-0614524). Support for this research was also provided by the NSF Long-Term Ecological Research Program at the Kellogg Biological Station and by the Michigan Agricultural Experiment Station. We thank Dr. Phil Robertson, the KBS-LTER site's staff, and Dr. Larry Alemany for assistance with the Rice Bruker 200 MHz NMR Spectrometer.

■ REFERENCES

(1) Solomon, B. D.; Barnes, J. R.; Halvorsen, K. E. Grain and cellulosic ethanol: History, economics, and energy policy. *Biomass Bioenergy* **2007**, *31* (6), 416–425.

(2) Hill, J.; Nelson, E.; Tilman, D.; Polasky, S.; Tiffany, D. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proc. Natl. Acad. Sci.* **2006**, *103* (30), 11206–11210.

(3) Service, R. F. Cellulosic ethanol: Biofuel researchers prepare to reap a new harvest. *Science* **2007**, *315* (5818), 1488–1491.

(4) National Agricultural Statistics Service, U.S. Department of Agriculture. *Field corn national statistics*. 2009. Available at http://www.nass.usda.gov/QuickStats/.

(5) Landis, D. A.; Gardiner, M. M.; van der Werf, W.; Swinton, S. M. Increasing corn for biofuel production reduces biocontrol services in agricultural landscapes. *Proc. Natl. Acad. Sci.* **2007**, *105* (51), 20552–20557.

(6) Pimentel, D.; Marklein, A.; Toth, M. A.; Karpoff, M. N.; Paul, G. S.; McCormack, R.; Kyriazis, J.; Krueger, T. Food versus biofuels: Environmental and economic costs. *Hum. Ecol.* **2009**, *37* (1), 1–12.

(7) McLaughlin, S.; Walsh, M. Evaluating environmental consequences of producing herbaceous crops for bioenergy. *Biomass Bioenergy* **1998**, *14* (4), 317–324.

(8) Patzek, T. Thermodynamics of the corn-ethanol biofuel cycle. *Crit. Rev. Plant Sci.* **2004**, *23* (6), 519–567.

(9) Food and Agriculture Organization of the United Nations. *More people than ever are victims of hunger*. 2009. Available at http://www.fao.org/news/story/0/item/20568/icode/en/.

(10) Ugarte, D. G. D. L. T.; English, B. C.; Menard, R. J.; Walsh, M. Conditions that influence the economic viability of ethanol from corn stover in the Midwest of the USA. *Int. Sugar J.* **2006**, *108* (1287), 152–156.

(11) Sanchez, O. J.; Cardona, C. A. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour. Technol.* **2008**, *99* (13), 5270–5295.

(12) Hamelinck, C. N.; van Hooijdonk, G.; Faaij, A. P. Ethanol from lignocellulosic biomass: Techno-economic performance in short-, middle- and long-term. *Biomass Bioenergy* **2005**, *28* (4), 384–410.

(13) Sun, Y.; Cheng, J. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresour. Technol.* **2002**, *83* (1), 1–11.

(14) Wyman, C. E. What is (and is not) vital to advancing cellulosic ethanol?. *Trends Biotechnol.* **2007**, *25* (4), 153–157.

(15) Zhang, M.; Eddy, C.; Deanda, K.; Finkelstein, M.; Picataggio, S. Metabolic engineering of a pentose metabolism pathway in ethanologenic *Zymomonas mobilis*. *Science* **1995**, *267* (5195), 240–243.

(16) Sticklen, M. B. Plant genetic engineering for biofuel production: Towards affordable cellulosic ethanol. *Nat. Rev. Genet.* **2008**, *9* (6), 433–443.

(17) Simpson, T. W.; Martinelli, L. A.; Sharpley, A. N.; Howarth, R. W. Impact of ethanol production on nutrient cycles and water quality: The United States and Brazil as case studies. In *Biofuels: Environmental Consequences & Implications of Changing Land. Proceedings of the Scientific Committee on Problems of the Environment (SCOPE) International Biofuels Project Rapid Assessment*; Howarth, R. W., Bringezu, S., Eds.; Cornell University: Ithaca, NY, 2009; pp 153−167.

(18) Zhang, F.; Mackenzie, A. F.; Smith, D. L. Corn yield and shifts among corn quality constituents following application of different nitrogen fertilizer sources at several times during corn development. *J. Plant Nutr.* **1993**, *16* (7), 1317–1337.

(19) Poorter, H.; Bergkotte, M. Chemical composition of 24 wild species differing in relative growth rate. *Plant, Cell Environ.* **1992**, *15* (2), 221–229.

(20) Poorter, H.; Nagel, O. The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: A quantitative review. *Aust. J. Plant Physiol.* **2000**, *27* (6), 595–607.

(21) Carpenter, S. R.; Caraco, N. F.; Correll, D. L.; Howarth, R. W.; Sharpley, A. N.; Smith, V. H. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* **1998**, *8* (3), 559–568.

(22) Bergstrom, L.; Brink, N. Effects of differentiated applications of fertilizer N on leaching losses and distribution of inorganic N in the soil. *Plant Soil* **1986**, *93* (3), 333–345.

(23) Power, J. F.; Wiese, R.; Flowerday, D. Managing nitrogen for water quality - lessons from Management Systems Evaluation Area. *J. Environ. Qual.* **2000**, *29* (2), 355–366.

(24) Turner, R. E.; Rabalais, N. N.; Justic, D. Gulf of Mexico hypoxia: Alternate states and a legacy. *Environ. Sci. Technol.* **2008**, *42* (7), 2323–2327.

(25) Rao, E. V. S. P.; Puttanna, K. Strategies for combating nitrate pollution. *Curr. Sci.* **2006**, *91* (10), 1335–1339.

(26) Wood, S.; Cowie, A. *International Energy Agency bioenergy task 38: A review of greenhouse gas emissions for fertilizer production*; Cooperative Research Centre for Greenhouse Accounting, International Energy Agency, 2004.

(27) McSwiney, C. P.; Robertson, G. P. Nonlinear response of N_2O flux to incremental fertilizer addition in a continuous maize (*Zea mays* L.) cropping system. *Global Change Biol.* **2005**, *11* (10), 1712–1719.

(28) Forster, P.; Ramaswamy, V.; Artaxo, P.; Berntsen, T.; Betts, R.; Fahey, D. W.; Haywood, J.; Lean, J.; Lowe, D.; Myhre, G.; et al. Chapter 2. Changes in atmospheric constituents and in radiative forcing. In *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*; Solomon, S., Qin, M., et al. , Eds.; Cambridge University Press: Cambridge, U.K. and New York, 2007; p 212.

(29) Hill, J.; Polasky, S.; Nelson, E.; Tilman, D.; Huo, H.; Ludwig, L.; Neumann, J.; Zheng, H.; Bonta, D. Climate change and health costs of air emissions from biofuels and gasoline. *Proc. Natl. Acad. Sci.* **2009**, *106* (6), 2077–2082.

(30) Robertson, G. P.; Dale, V. H.; Doering, O. C.; Hamburg, S. P.; Melillo, J. M.; Wander, M. M.; Parton, W. J.; Adler, P. R.; Barney, J. N.; Cruse, R. M.; et al. Sustainable biofuels redux. *Science* **2008**, *322* (5898), 49–50.

(31) McSwiney, C. P.; Snapp, S. S.; Gentry, L. E. Use of N immobilization to tighten the N cycle in conventional agroecosystems. *Ecol. Appl.* **2010**, *20* (3), 648–662.

(32) Baldock, J. A.; Masiello, C. A.; Gélinas, Y.; Hedges, J. I. Cycling and composition of organic matter in terrestrial and marine ecosystems. *Mar. Chem.* **2004**, *92* (1−4), 39–64.

(33) Nelson, P. N.; Baldock, J. A. Estimating the molecular composition of a diverse range of natural organic materials from solid-state ^{13}C NMR and elemental analyses. *Biogeochemistry* **2005**, *72*, 1–34.

(34) Hedges, J. I.; Baldock, J. A.; Gelinas, Y.; Lee, C.; Peterson, M.; Wakeham, S. G. Evidence for non-selective preservation of organic matter in sinking marine particles. *Nature* **2001**, *409* (6822), 801–804.

(35) Gelinas, Y.; Baldock, J. A.; Hedges, J. I. Demineralization of marine and freshwater sediments for CP/MAS ^{13}C NMR analysis. *Org. Geochem.* **2001**, *32* (5), 677–693.

(36) Sanderman, J.; Baldock, J. A.; Amundson, R. Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. *Biogeochemistry* **2008**, *89* (2), 181–198.

(37) Harmon, M. E.; Lajtha, K. Analysis of detritus and organic horizons for mineral and organic constituents. In *Standard Soil Methods for Long-Term Ecological Research*; Robertson, G. P., Coleman, D. C., et al. , Eds.; Oxford University Press: New York, 1999; p 147.

(38) Preston, C.; Trofymow, J.; Sayer, B.; Niu, J. C-13 nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning investigation of the proximate-analysis fractions used to assess litter quality in decomposition studies. *Can. J. Bot.* **1997**, *75* (9), 1601–1613.

(39) Hatfield, R.; Fukushima, R. Can lignin be accurately measured?. *Crop Sci.* **2005**, *45* (3), 832–839.

(40) Smernik, R. J.; Oades, J. M. Solid-state ^{13}C-NMR dipolar dephasing experiments for quantifying protonated and non-protonated carbon in soil organic matter and model systems. *Eur. J. Soil Sci.* **2001**, *52* (1), 103–120.

(41) van Rensburg, H.; Anterola, A. M.; Levine, L. H.; Davin, L. B.; Lewis, N. G. Monolignol compositional determinants in loblolly pine: Aromatic amino acid metabolism and associated rate-limiting steps. In *Lignin: Historical, Biological, and Materials Perspectives*; Glasser, W. G., Northey, R. A., et al. , Eds.; American Chemical Society: Washington, DC, 2000; Vol. 742, pp 118−144.

(42) Tiessen, H.; Cuevas, E.; Chacon, P. The role of soil organic matter in sustaining soil fertility. *Nature* **1994**, *371* (6500), 783–785.

(43) Melillo, J. M.; Aber, J.; Linkins, A. E.; Ricca, A.; Fry, B.; Nadelhoffer, K. J. Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. *Plant Soil* **1989**, *115* (2), 189–198.

(44) Melillo, J. M.; Aber, J.; Muratore, J. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* **1982**, *63* (3), 621–626.

(45) Singh, J. S.; Gupta, S. R. Plant decomposition and soil respiration in terrestrial ecosystems. *Bot. Rev.* **1977**, *43* (4), 499–528.

(46) Johnson, J. M.-F.; Barbour, N. W.; Lachnicht Weyers, S. Chemical composition of crop biomass impacts its decomposition. *Soil Sci. Soc. Am. J.* **2007**, *71* (1), 155–162.