



Original Research Paper

Soil and root carbon storage is key to climate benefits of bioenergy crops

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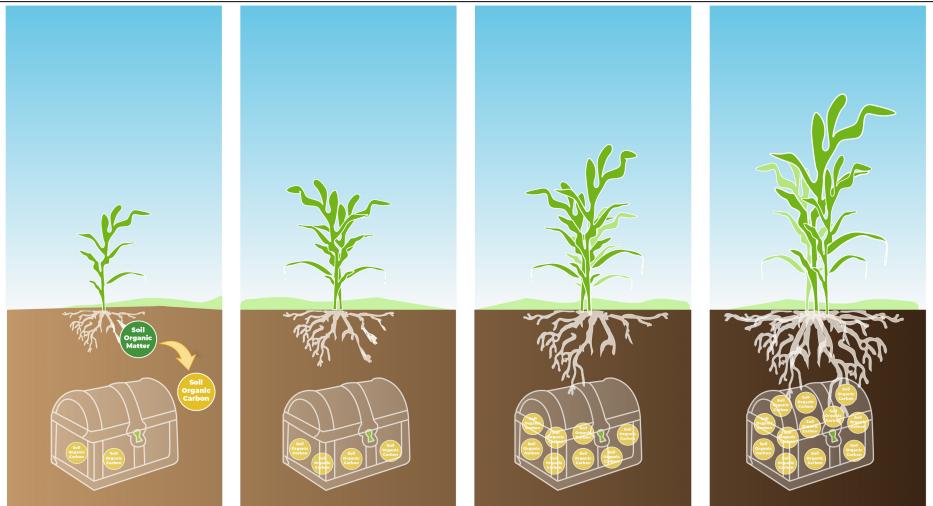
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HIGHLIGHTS

- Perennial grasses grown on degraded and abandoned cropland were analyzed.
- Soil and root C storage was found to be key to GHG reductions of biofuels.
- Soil and root C storage rates tend to be higher in high-diversity species mixtures.
- Climate benefit of aboveground biomass can be reduced by fuel market rebound effect.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 20 February 2020

Received in revised form 7 April 2020

Accepted 9 April 2020

Available online 1 June 2020

Keywords:

Perennial crops
Plant diversity
Climate change mitigation
Life-cycle GHG
Consequential-LCA

ABSTRACT

Most bioenergy feedstock studies focus on maximizing aboveground biomass production. Cropland with fertile soils can produce high aboveground biomass yields but its diversion to bioenergy causes greenhouse gas emissions from direct and indirect land use changes. Here, we analyze three grassland experiments that minimize land use changes by using abandoned and degraded agricultural land. We find that soil and root carbon storage is a greater determinant of the climate change mitigation potential of biofuels than aboveground biomass, and tends to be higher for treatments with high plant diversity. Aboveground biomass yield ranged from 450-650 g ha⁻² yr⁻¹ for the productive treatments with moderate intensification, but its climate benefit *via* converting into biofuels and displacing fossil fuels can be substantially reduced by the rebound effect of fuel market. Because of high soil and root C storage rates (152-483 g CO₂ ha⁻² yr⁻¹), many treatments are carbon negative even without the fossil fuel displacement benefit. To effectively mitigate greenhouse gas emissions, we should focus on increasing belowground carbon storage and explore the potential benefits of high-diversity plant species mixtures.

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1. Introduction

Diverting food producing croplands to bioenergy crop production can lead to direct and indirect land clearing and large releases of CO₂ associated with it (Fargione et al., 2008; Searchinger et al., 2008). Legally mandated use of transportation ethanol in the US, for example, has diverted ~40% of domestic corn to ethanol and contributed to the conversion of ~1.5 million hectares of US grasslands into croplands between 2008 and 2012, with a release of ~700 Tg of CO₂ (Spawn et al., 2019). As agricultural commodities are traded internationally, diverting crops in one country to biofuel use can drive up global food prices, triggering farmers around the world to bring new land into production (or indirect land use change) (Searchinger et al., 2008). For the complexity of the global agroecosystem, indirect land use changes and associated CO₂ releases are highly uncertain but potentially large, and may more than offset the carbon benefit of food-based biofuels (Plevin et al., 2010).

Alternatively, growing perennial grasses on marginal lands unsuited for crops has been proposed as a viable approach to sustainable biofuels (Gelfand et al., 2013). In particular, the use of already degraded and abandoned agricultural lands would minimize the greenhouse gas (GHG) emissions from land use change effects associated with food-based biofuels (Tilman et al., 2006a; Field et al., 2008; Campbell et al., 2008). Biofuels from perennial grasses mitigate climate change *via* sequestering CO₂ from the atmosphere in soil and roots belowground and *via* displacing fossil fuels and their GHG emissions when aboveground biomass is converted into biofuels (Tilman et al., 2006a). However, degraded lands are infertile, which limits the productivity of aboveground biomass and the amount of fossil fuels that can be displaced (Yang et al., 2018). In addition, the effectiveness of reducing GHG emissions *via* displacing fossil fuels may be further reduced by the rebound effect of fuel market if increases in biofuels use reduces total oil demand, thus lowering its price and potentially increasing oil consumption globally (Rajagopal, 2013; Yang, 2016). In other words, 1 MJ biofuels may ultimately displace less than 1 MJ of petroleum fuels. A recent review suggests a displacement ratio of ~0.5 because of the rebound effect (Hill et al., 2016).

Experiments of perennial bioenergy grasses have generally focused on (maximizing) aboveground biomass productivity (or yield), with relatively few measurements on belowground carbon (C) storage (Agostini et al., 2015). Here, we show that soil and root C storage is, however, central to the capacity of biofuels from perennial grasses to mitigate climate change. We analyze data from three grassland experiments conducted in an abandoned agricultural site. The experiments i) include a wide range of species diversities from highly productive switchgrass monocultures to high-diversity mixtures of native prairies, ii) receive several intensification levels from no annual inputs to moderate irrigation and N fertilization, and iii) span different durations from short term (6 yr) to long term (>20 yr). There are in total 25 diversity-intensification treatments. Our goal is to examine the relationships between yield and belowground C storage, on the one side, and the life-cycle GHG emissions and savings of each treatment when converted to biofuels, on the other. The novelties of our study include 1) analyzing experiments with a variety of diversity and agronomic properties and conducted on abandoned cropland and 2) exploring the key determinant of the GHG mitigation potential of biofuels.

2. Material and Methods

2.1. Study site and experiment design

Our study is based on three experiments carried out at the Cedar Creek Ecosystem Science Reserve located in east-central Minnesota on a glacial outwash sand plain. Farming at Cedar Creek started in late 1800s and abandonment occurred after 1920. Abandoned farmlands undergo ecological succession and are dominated by exotic annuals and perennials in the early stage of succession and then gradually by native prairie perennial grasses, legumes, and forbs. The climate at Cedar Creek is continental, with a mean annual temperature of 6°C and mean annual precipitation of 660 mm. Soils at are 92 to 97% sand, with low soil fertility (C<1% and nitrogen (N) <0.1%). Below we describe the three experiments used in our analysis. Detailed, plot-level data are available at <http://www.cedarcreek.umn.edu/research/data>.

The first is a switchgrass experiment designed to test effects of increasing plant species diversity on switchgrass biomass production. Switchgrass has been proposed as a major cellulosic feedstock in the US for its high productivity

and adaptability. The experiment begun in 2012 and has 64 plots, 9 m by 9 m each, randomly assigned to a combination of diversity and intensification treatments. There are four diversity levels: switchgrass monocultures (each plot planted with four varieties of switchgrass, SG), switchgrass plus three other C4 species (*Andropogon gerardii* (with two varieties), *Sorghastrum nutans*, and *Schizachyrium scoparium*; SG+C4), switchgrass plus legumes (i.e., red clover and alfalfa; SG+L), and all combined (SG+C4+L). Each diversity treatment received 4 levels of intensification: no annual input (or ambient), irrigation only (2 cm per week over the growth season from June to August), N fertilization only (at a moderate 7 g m⁻²), and both irrigation and N. Thus, there are in total 16 diversity-intensification treatments, each with 4 replicates.

The second is a biodiversity experiment designed to examine the various effects of plant species biodiversity, without fertilization nor irrigation. The experiment begun in 1994 and has ~160 plots (9 m by 9 m), assigned to a combination of 1, 2, 4, 8, and 16 species randomly selected from a pool of 18 native grassland species consisting mostly of C4 grasses, C3 grasses, legumes and forbs. Because it is mainly C4 grasses, such as switchgrass and big blue stem, that have been proposed as bioenergy feedstocks (Yang et al., 2019a), only plots planted with at least one C4 grass species (~100 plots) are analyzed. All monoculture plots are thus C4 perennial grasses. Additional details on the experiment have been published before (Tilman et al., 2001 and 2006a).

The third is a high-diversity intensification experiment designed to evaluate the importance of both water and N addition in high-diversity restored prairie grassland planted with 32 perennial grassland species. The experiment begun in 2006 and has 36 plots (9 m by 9 m), randomly assigned to one of the six combinations of two irrigation treatments (un-irrigation and irrigated) and three N fertilization treatments (at 0, 7, and 14 g m⁻² yr⁻¹). To be comparable with the other two experiments, only plots with no or moderate N input (7 g m⁻²) are included in our analysis. Additional details on the experiment have been published before (Yang et al., 2018).

2.2. Sampling of aboveground biomass, root biomass, and soil C

For the switchgrass experiment, sampling of aboveground biomass was done annually from 2012 to 2013 and from 2015 to 2017, of root biomass in 2017, and of soil C in 2012 (before planting) and in 2017. For the biodiversity experiment, sampling of aboveground biomass was done annually, of root biomass periodically, and of soil C in 1994 before planting, 2000, 2004, 2006, and 2015 (Yang et al., 2019b). And for the high-diversity experiment, sampling of aboveground biomass was done annually from 2007 to 2011 and from 2015 to 2017, of root biomass in 2017, and of soil C in 2010, 2015, and 2017 (Yang et al., 2018).

Aboveground biomass was sampled in early to mid-August by clipping four 10 cm × 600 cm strips of vegetation that were parallel and evenly spaced in each plot. Strips were 600 cm long, instead of 900 cm, to avoid edge effects. Vegetation was dried at 40°C and then weighed to determine total aboveground biomass per plot. To sample root biomass, eight soil cores, 5 cm in diameter and at depths of 0–30 and 30–60 cm, were collected per plot, from eight sites evenly spaced within the strips clipped for vegetation. Each soil core was rinsed gently on a fine mesh screen to remove soil. Roots were dried at 40°C, placed in a sieve, gently shaken to remove any remaining soil, and then weighed to determine total root biomass. To sample soil C, nine soil cores, 2.5 cm in diameter and at the depth of 0–20 cm, were collected per plot. They were sieved to remove roots, combined, and thoroughly mixed per plot; then were dried, mixed again, and subsampled for grinding and archiving; and finally, were dried again at 104°C and analyzed for total carbon by combustion and gas chromatography (ECS 4010; Costech Analytical).

2.3. Life cycle analysis

We estimate the life-cycle GHG emissions and savings of each treatment, from feedstock production, transport, to biofuel conversion (Tables S1 and S2). We focus on ethanol as it is the main liquid biofuel on the market. Results are expressed in two measures (Tables S3 and S4). One is life-cycle GHG emissions per MJ of energy consumed, a measure that is commonly used to compare biofuels with fossil fuels to determine the mitigation potential of the former (Farrell et al., 2006; Wang et al., 2007).

The other is net GHG savings per m² of land harvested, which include displacement of fossil fuels and indicates the total mitigation potential of land (Tilman et al., 2006a; Gelfand et al., 2013).

The main life-cycle GHG savings of ethanol from perennial bioenergy grasses are i) direct C storage belowground in soil and roots and ii) displacement of petroleum and its life-cycle GHG emissions (Robertson et al., 2017). The amount of petroleum which can be displaced depends partly on aboveground biomass productivity. Soil C, root C, and aboveground biomass are directly measured in the experiments above (Tables 1, S5, S6, and S7 in the Supporting Information). Soil C sequestration rates for the switchgrass, the biodiversity, and the high-diversity experiments are based on, respectively, 6, 22, and 7 years of biomass growth (section 2.2.).

GHG emissions occur at various points across the life cycle of ethanol, including fertilization, irrigation, planting and harvesting at feedstock production, feedstock transportation, and ethanol conversion. These are all estimated based on previous works (Tilman et al., 2006a; Murphy and Kendall, 2015; Ruan et al., 2016; Vora et al., 2017; Yang et al., 2018). Details are available in Tables S1 and S2.

Table 1.
Mean yield, soil C storage rates (0-20 cm of soil), and total root biomass (0-60 cm).

Diversity treatment	Intensification treatment	Yield (g m ⁻² yr ⁻¹)	Soil C storage (g m ⁻² yr ⁻¹)	Root biomass (g m ⁻²)
SG	0	377.0	-16.7	1470.3
SG+L	0	403.5	7.6	1249.8
SG+C4	0	360.4	50.8	1013.6
SG+C4+L	0	377.5	41.8	1262
SG	N	451.1	39.3	1417.9
SG+L	N	433.9	15.5	1429.4
SG+C4	N	441.4	24.8	1123.6
SG+C4+L	N	465.1	115.1	1238.4
SG	I	508.0	12.7	1561.9
SG+L	I	442.2	1.4	1513.5
SG+C4	I	481.3	5.4	1351.2
SG+C4+L	I	433.4	-10.3	1146.1
SG	N+I	615.3	53.4	1818.1
SG+L	N+I	648.2	26.4	1674.7
SG+C4	N+I	644.5	-53.8	1203
SG+C4+L	N+I	555.7	40.8	1245.8
32-species	0	290.5	28.3	1357.6
32-species	N	345.2	48.7	1449.5
32-species	I	452.6	56.5	1968.2
32-species	N+I	586.9	83.2	2190.7
1-species	0	73.6	15.9	761.9
2-species	0	167.8	22	1041.6
4-species	0	194.8	21.8	1060.6
8-species	0	256.6	25.4	1332.8
16-species	0	322.2	31.7	1464.1

L: denotes legume grasses; N: nitrogen application at 7 g per m² yr⁻¹; I: irrigation at 2 cm per week over the growth season.

Detailed information on sample size and standard error can be found in Tables S5, S6, and S7.

3. Results and Discussion

3.1. Importance of soil and root C storage

The rate of net C storage in soil and roots is a major determinant of the climate mitigation potential of cellulosic ethanol, whereas aboveground biomass (or yield) is poorer indicator (Fig. 1). In terms of life-cycle GHG emissions per MJ of ethanol produced, estimates range from -94 to 123 g CO₂e across treatments. In regressions, soil and root C storage rates alone explain ~80% of the variation in life-cycle GHG emissions (P<0.001), and observed variation in biomass yields alone have no statistically significant effect on these emissions (P=0.7080). Life-cycle GHG emissions for 18 of 25 treatments are lower than 39 g CO₂e per MJ of fuel produced, the level required by the US

Renewable Fuel Standard for cellulosic biofuels, and for 8 treatments are <0 g CO₂e MJ⁻¹ or carbon negative. In terms of total GHG savings per m² of land converted to biomass production, including petroleum displaced by ethanol (assuming 1 MJ of ethanol displaces 1 MJ of petroleum), results range from -90 to 505 g CO₂e yr⁻¹. Soil and root C storage rate alone explains ~93% of the variation (P<0.0001) and there is a weak positive effect of yield alone on GHG savings (P=0.1260).

A multiple regression analysis shows that, controlling for soil and root C storage, GHG savings per m² of land increase with yield (P=0.001), because more yield means more ethanol and can potentially displace more petroleum and its GHG emissions. But this benefit can be considerably diminished by the rebound effect of fuel market (Fig. 1), although estimating rebound effect is highly uncertain as it depends on a suite of factors from the cost of biofuels, policy interventions, and elasticities of supply and demand (Rajagopal and Plevin, 2013).

Uncertainties about fossil fuel displacement affect the total climate mitigation potential of biofuels, but even without such displacement, some treatments still yield positive net GHG savings because of high soil and root C storage rates (Fig. 2; red triangles indicate net GHG savings). Under a 1:1 displacement ratio (1 MJ of biofuel displacing 1 MJ of fossil fuel), for example, the three best-performing treatments have net GHG saving of ~350 to 500 g CO₂e m⁻² yr⁻¹, with ~250 to 330 g CO₂e m⁻² yr⁻¹ from fossil fuel displacement and ~300-480 g CO₂e m⁻² yr⁻¹ from soil and root C storage. Under a 1:0.5 displacement ratio, as suggested by a recent review (Hill et al., 2016, p. 201), GHG savings from fossil fuel displacement would halve, but the net GHG savings would still range from ~220-380 g CO₂e m⁻² yr⁻¹. Even assuming an unlikely scenario of zero displacement (or no biofuel production), the three treatments would reduce ~100 to 250 g CO₂e m⁻² yr⁻¹ because of soil and root C storage.

3.2. Importance of plant diversity

We also find that high-diversity mixtures tend to store soil and root C at greater rates and thus have lower life-cycle GHGs per MJ and greater GHG savings per m² (Fig. 2). In the switchgrass experiment, the most diverse treatment (with switchgrass + other C4 species + legume species), with or without intensification, has the highest storage rate of 232 g CO₂e m⁻² yr⁻¹. In the biodiversity experiment, which receives no fertilization or irrigation, mean annual soil and root C storage rates range are 77, 106, 106, 126, and 152 g CO₂e m⁻² yr⁻¹, respectively, for 1-, 2-, 4-, 8-, and 16-species combinations. In the high-diversity experiment with 32 planted species, the treatment that also receives no annual inputs has a storage rate of 170 g CO₂e m⁻² yr⁻¹. The high-diversity treatments as a whole, with or without intensification, have an average storage of 284 g CO₂e m⁻² yr⁻¹, the highest of all diversity treatments. As a result, 7 of the 8 treatments that are carbon negative are either high-diversity mixtures of native prairies (16- or 32-species) or the mixture of switchgrass with C4 and legume species. Further, we find that C stored in roots can be significant. In the high-diversity experiment, for example, root C amounts to 66-107 g CO₂e m⁻² yr⁻¹ in the upper 60 cm of soil.

3.3. Implications and future research needs

While life-cycle studies of bioenergy crops generally show that both aboveground biomass and belowground C storage are important for climate change mitigation (Robertson et al., 2017), our results suggest that belowground C storage is more critical. This is partly because C stored in soil and roots means a direct reduction of CO₂ in the atmosphere. And it can remain belowground in the long run if soils are properly maintained. On the other, the climate benefit through converting biomass to bioenergy and then displacing fossil fuels and their life-cycle GHG emissions involves a long chain of various assumptions and uncertainties (Yang and Heijungs, 2018). Especially, this could cause the fuel market to rebound, substantially reducing the amount of fossil fuels that can be displaced (Rajagopal and Plevin, 2013). Furthermore, in our analysis we only included soil C in the top 20 cm soil profile and additional C is likely stored in deeper soils (Follett et al., 2012). Overall, our study suggests, as also argued recently by DeCicco and Schlesinger (2018), that more attention be paid to carbon storage belowground.

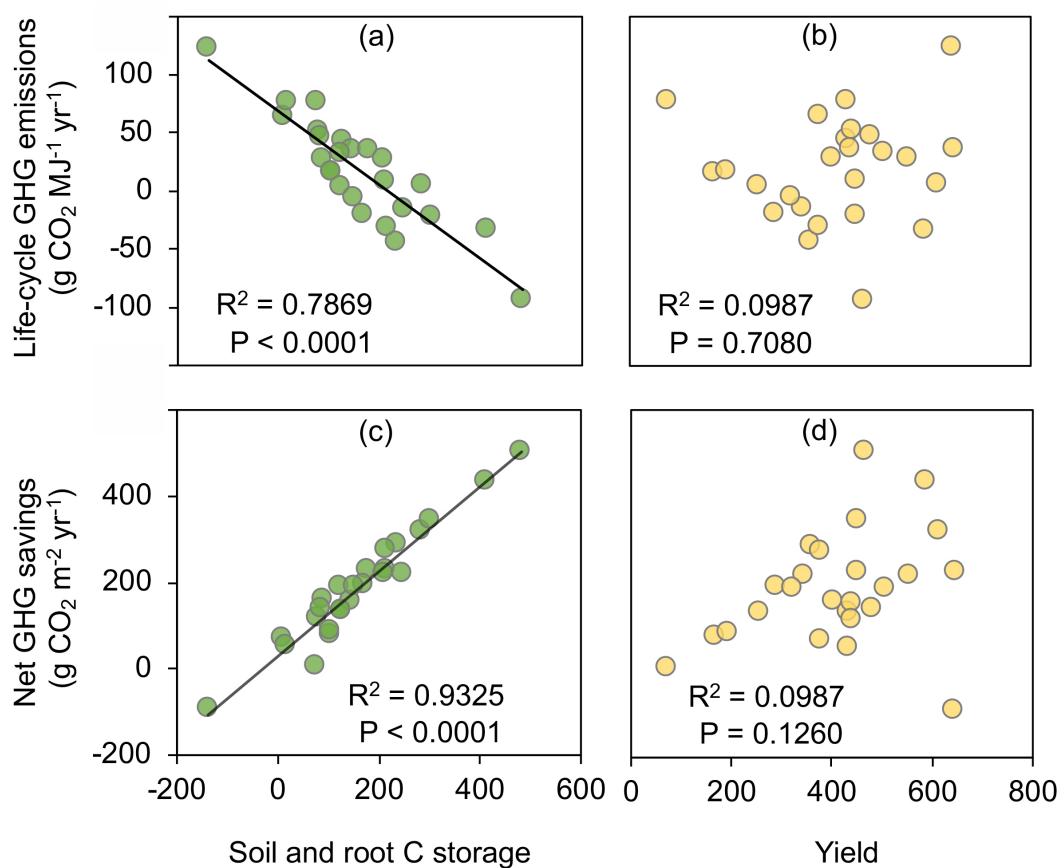


Fig. 1. Life-cycle GHG emissions per MJ of ethanol consumed (a and b) and net GHG emissions per m² of land harvested (c and d), and their relationships with rates of soil and root C storage or aboveground biomass (yield). Each dot indicates a diversity-intensification treatment. Net GHG savings per m² reflects the magnitude of GHGs that can be saved per m² of abandoned agricultural land used to make cellulosic biomass and ethanol, which displaces petroleum and its life-cycle GHG emissions (1:1 displacement ratio assumed here).

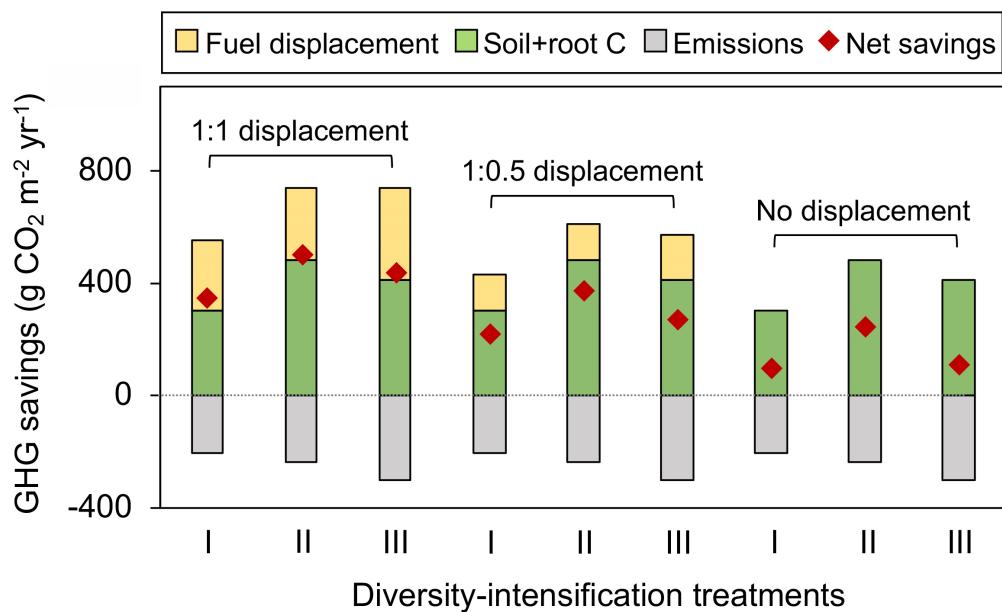


Fig. 2. Main contributors to GHG savings of the three best-performing treatments under different displacement ratios (I – 32 species with irrigation; II – a mixture of switchgrass, C4, and legume with N fertilization; III – 32 species with both irrigation and N fertilization). Red triangles indicate net GHG savings, calculated as the sum of the positive savings from fossil fuel displacement and from net carbon storage in roots and soil carbon minus the GHG emissions associated with biomass production, transportation, and ethanol conversion.

Biofuel policies have traditionally focused towards the production and use end of the biofuel life cycle, such as mandating biofuel production volumes, issuing blender's tax credit, and financially supporting biomass production, collection, harvesting, storage, and transportation to biorefineries (Carriquiry et al., 2011). We suggest future policies shift focus to incentivizing the adoption of conservation practices on biomass farmland that increase soil C storage. In particular, high rates of soil C storage can make biofuels carbon-negative even without considering the potential benefit of fossil fuel displacement (Fig. 3). For annual crops, many practices can potentially add to soil C, including crop diversification and rotation and planting cover crops (Paustian et al., 2016). For perennial crops, high plant diversity can help accelerate soil C sequestration in the long run (Lange et al., 2015; Sprunger and Robertson, 2018; Yang et al., 2019b), and so does the application of biochar (Han Weng et al., 2017).

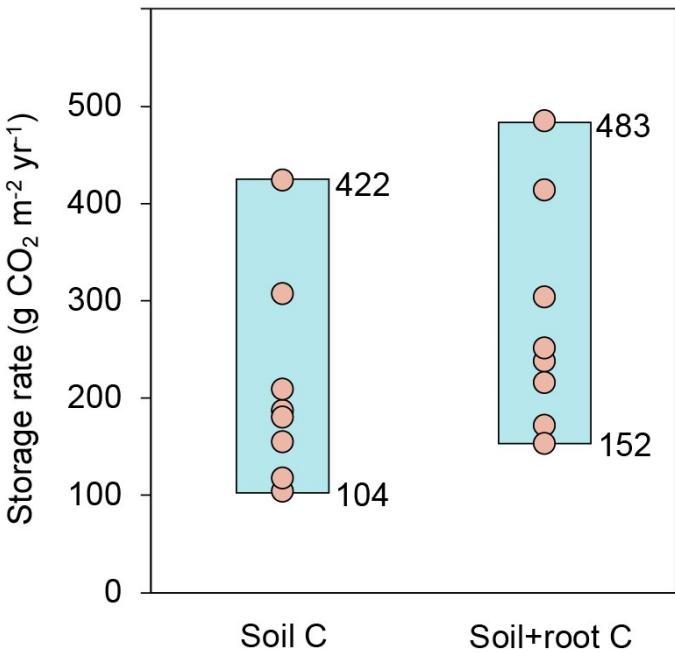


Fig. 3. Soil and root C storage rates by treatments that are carbon negative without considering GHG savings from displacing fossil fuels.

Given the dispersed and variable nature of soil, it is challenging to accurately measure and monitor changes in soil properties at large scales (Paustian et al., 2016). More funding can be directed to basic and applied research aimed at improving the accuracy, reliability, and cost-effectiveness of measuring soil C. In addition, there are many other environmental and ecological benefits associated with increasing plant diversity in perennial bioenergy crops, including weed suppression, lower soil N₂O emissions, ecosystem stability, and resilience against climate variability (Tilman et al., 2006b; Isbell et al., 2017; Yang et al., 2019a). Few studies have quantified these other aspects under the life cycle assessment framework to determine the totality of biodiversity benefits. This can also be a focus of future research, as the negative impacts of biodiversity loss on the sustainability of the Earth are being increasingly recognized (Isbell et al., 2011; Cardinale et al., 2012).

4. Conclusions

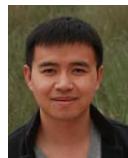
Our study shows that belowground C storage in soil and roots is the major cause of the observed climate benefit of cellulosic biofuels produced from perennial biomass grown on degraded lands. Our study also adds to the literature and highlights the importance of plant diversity in increasing soil C stocks. The policy implication of our study is that biofuel policies should focus more on promoting soil conservation and crop diversification practices that increase soil and root C. Rather than the current focus on percentage differences between fuel life cycle emissions, such policies should target achieving high

rates of soil and root C sequestration as a primary means for assuring GHG reductions for bioenergy and biofuels. Overall, degraded lands managed for high-diversity mixtures of perennial plant species, be they herbaceous or woody species, present a unique synergic opportunity of land restoration, climate change mitigation, and ecosystem services.

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Supplementary Data

Table S1.Annual GHG sequestration (positive values) or release (negative values) at biomass production stage (per m² of land harvested).

Experiment	Diversity	Irrigation	N addition	Biomass production GHGs (g CO ₂ e m ⁻² yr ⁻¹) ^a			
				Soil+root CO ₂	N fertilizer	Irrigation	Others
e276	SG	0	0	10	0	0	-109
e276	SG+L	0	0	89	0	0	-110
e276	SG+C4	0	0	236	0	0	-108
e276	SG+C4+L	0	0	215	0	0	-109
e276	SG	0	1	213	-77	0	-111
e276	SG+L	0	1	127	-77	0	-110
e276	SG+C4	0	1	146	-77	0	-110
e276	SG+C4+L	0	1	483	-77	0	-111
e276	SG	1	0	123	0	-50	-112
e276	SG+L	1	0	79	0	-50	-111
e276	SG+C4	1	0	86	0	-50	-111
e276	SG+C4+L	1	0	18	0	-50	-110
e276	SG	1	1	285	-77	-50	-115
e276	SG+L	1	1	179	-77	-50	-116
e276	SG+C4	1	1	-138	-77	-50	-116
e276	SG+C4+L	1	1	210	-77	-50	-113
e248	32-species	0	0	170	0	0	-107
e248	32-species	0	1	249	-77	0	-108
e248	32-species	1	0	303	0	-50	-111
e248	32-species	1	1	412	-77	-50	-114
e120	1-species	0	0	77	0	0	-101
e120	2-species	0	0	106	0	0	-104
e120	4-species	0	0	106	0	0	-104
e120	8-species	0	0	126	0	0	-106
e120	16-species	0	0	152	0	0	-108

^a GHG emissions associated with N fertilizer include emissions from N fertilizer production (-39 g CO₂e m⁻² yr⁻¹), transport (-0.8 g CO₂e m⁻² yr⁻¹), application (-0.8 g CO₂e m⁻² yr⁻¹), as well as soil N₂O emissions (-37 g CO₂e m⁻² yr⁻¹). GHG emissions associated with irrigation are primarily from energy use. Others include GHG emissions from 1) producing prairie seed, planting, harvesting, and transporting bales; 2) pesticide production; and 3) farm capital and machinery production. Others also include grassland soil background N₂O emissions and CH₄ uptake (-16 and 15 g CO₂e m⁻² yr⁻¹, respectively), as well as foregone C (75 g CO₂e m⁻² yr⁻¹), i.e., C that would have been stored in soil and roots had the abandoned farmland continued to undergo natural succession. Details on these estimates were published before (Yang et al., 2018).

Table S2.Annual GHG savings (positive values) or release (negative values) at biorefining stage (per m² of land harvested).

Experiment	Diversity	Irrigation	N addition	Biorefining GHGs (g CO ₂ e m ⁻² yr ⁻¹) ^a			
				Transport	Pre-treatment	Refining	Coproduct credit
e276	SG	0	0	-16	-44	-10	31
e276	SG+L	0	0	-17	-47	-11	33
e276	SG+C4	0	0	-15	-42	-10	30
e276	SG+C4+L	0	0	-16	-44	-10	31
e276	SG	0	1	-19	-52	-13	37
e276	SG+L	0	1	-18	-50	-12	36
e276	SG+C4	0	1	-18	-51	-12	36
e276	SG+C4+L	0	1	-19	-54	-13	38
e276	SG	1	0	-21	-59	-14	42
e276	SG+L	1	0	-19	-51	-12	37
e276	SG+C4	1	0	-20	-56	-13	40
e276	SG+C4+L	1	0	-18	-50	-12	36
e276	SG	1	1	-26	-71	-17	51
e276	SG+L	1	1	-27	-75	-18	54
e276	SG+C4	1	1	-27	-74	-18	53
e276	SG+C4+L	1	1	-23	-64	-15	46
e248	32-species	0	0	-12	-34	-8	24
e248	32-species	0	1	-14	-40	-10	29
e248	32-species	1	0	-19	-52	-13	37
e248	32-species	1	1	-25	-68	-16	48
e120	1-species	0	0	-3	-9	-2	6
e120	2-species	0	0	-7	-19	-5	14
e120	4-species	0	0	-8	-23	-5	16
e120	8-species	0	0	-11	-30	-7	21
e120	16-species	0	0	-13	-37	-9	27

^a Biorefining GHG data reflect an average ethanol conversion technology estimated by Murphy and Kendall (2015), with ethanol yield being 265 L/ton dry mass, GHG emissions for biomass transport, pretreatment, and ethanol refining being 0.16, 0.44, 0.1 kg CO₂e/L, and GHG savings from coproduct credit being 0.31 kg CO₂e/L.

Table S3.Net GHG savings, including avoided GHG emissions from displacing petroleum (g CO₂e m⁻² yr⁻¹).

Experiment	Diversity	Irrigation	N addition	Avoided GHGs ^a (g CO ₂ e m ⁻² yr ⁻¹)	Net GHG savings ^b (g CO ₂ e m ⁻² yr ⁻¹)
e276	SG	0	0	209	72
e276	SG+L	0	0	224	162
e276	SG+C4	0	0	200	290
e276	SG+C4+L	0	0	209	277
e276	SG	0	1	250	229
e276	SG+L	0	1	241	135
e276	SG+C4	0	1	245	158
e276	SG+C4+L	0	1	258	505
e276	SG	1	0	282	190
e276	SG+L	1	0	245	118
e276	SG+C4	1	0	267	142
e276	SG+C4+L	1	0	240	54
e276	SG	1	1	341	321
e276	SG+L	1	1	359	229
e276	SG+C4	1	1	357	-90
e276	SG+C4+L	1	1	308	221
e248	32-species	0	0	161	195
e248	32-species	0	1	191	220
e248	32-species	1	0	251	347
e248	32-species	1	1	325	436
e120	1-species	0	0	41	9
e120	2-species	0	0	93	78
e120	4-species	0	0	108	90
e120	8-species	0	0	142	136
e120	16-species	0	0	179	190

^a Avoided GHG emissions from displacing petroleum are calculated by multiplying the life-cycle GHG emissions of petroleum (98.2 g CO₂e MJ⁻¹) by the energy content of ethanol produced per m² of land. This assumes an ideal 1:1 displacement, meaning 1 additional MJ of ethanol displaces 1 MJ of petroleum. Due to the rebound effect of global fuel market, however, 1 MJ of ethanol is likely to displace <1M of petroleum (Rajagopal, 2013; Yang and Heijungs, 2018). See Figure 2 in the main text for more discussion.

^b Net GHG savings are the sum of GHGs at biomass production and biorefining stages and GHG savings from petroleum displacement.

Table S4.Net GHG savings per MJ of ethanol consumed (g CO₂e MJ⁻¹).

Experiment	Diversity	Irrigation	N addition	Life-cycle GHG emissions ^a (g CO ₂ e MJ ⁻¹)	Percentage reduction ^b
e276	SG	0	0	45	54%
e276	SG+L	0	0	19	81%
e276	SG+C4	0	0	-31	132%
e276	SG+C4+L	0	0	-22	122%
e276	SG	0	1	6	94%
e276	SG+L	0	1	30	69%
e276	SG+C4	0	1	25	75%
e276	SG+C4+L	0	1	-66	167%
e276	SG	1	0	22	78%
e276	SG+L	1	0	36	63%
e276	SG+C4	1	0	32	67%
e276	SG+C4+L	1	0	54	45%
e276	SG	1	1	4	96%
e276	SG+L	1	1	25	75%
e276	SG+C4	1	1	86	12%
e276	SG+C4+L	1	1	19	81%
e248	32-species	0	0	-14	114%
e248	32-species	0	1	-10	110%
e248	32-species	1	0	-26	126%
e248	32-species	1	1	-24	124%
e120	1-species	0	0	54	45%
e120	2-species	0	0	11	89%
e120	4-species	0	0	12	88%
e120	8-species	0	0	3	97%
e120	16-species	0	0	-4	104%

^a These estimates reflect the total life-cycle GHG emissions per MJ of ethanol consumed, a measure used by the federal policy Renewable Fuel Standard in the US to determine whether a biofuel meets the requirement of GHG reductions to qualify as a particular type of renewable fuel. Negative values indicate carbon negative.

^b Percentage reduction relative to petroleum life-cycle GHG emissions at 98.2 g CO₂e MJ⁻¹, assuming again an idealized 1:1 displacement ratio (Yang, 2016).

Table S5.

Aboveground biomass or yield (SG - switchgrass, L - legume).

Experiment	Diversity	Irrigation	N addition	Sample size	Yield (g m ⁻²)	Standard error
e276	SG	0	0	4	377.0	26.5
e276	SG+L	0	0	4	403.5	13.6
e276	SG+C4	0	0	4	360.4	69.5
e276	SG+C4+L	0	0	4	377.5	11.3
e276	SG	0	1	4	451.1	23.4
e276	SG+L	0	1	4	433.9	28.3
e276	SG+C4	0	1	4	441.4	63.2
e276	SG+C4+L	0	1	4	465.1	64.7
e276	SG	1	0	4	508.0	41.9
e276	SG+L	1	0	4	442.2	54.9
e276	SG+C4	1	0	4	481.3	36.4
e276	SG+C4+L	1	0	4	433.4	22.8
e276	SG	1	1	4	615.3	62.8
e276	SG+L	1	1	4	648.2	67.3
e276	SG+C4	1	1	4	644.5	68.3
e276	SG+C4+L	1	1	4	555.7	55.6
e248	32-species	0	0	18	290.5	21.3
e248	32-species	0	1	18	345.2	25.5
e248	32-species	1	0	18	452.6	31.1
e248	32-species	1	1	18	586.9	26.2
e120	1-species	0	0	30	73.6	4.2
e120	2-species	0	0	51	167.8	10.9
e120	4-species	0	0	60	194.8	9.4
e120	8-species	0	0	69	256.6	8.2
e120	16-species	0	0	105	322.2	9.0

Table S6.

Root biomass (top 60 cm of soil).

Experiment	Diversity	Irrigation	N addition	Sample size	Root biomass (g m ⁻²)	Standard error
e276	SG	0	0	4	1470.3	73.4
e276	SG+L	0	0	4	1249.8	32.7
e276	SG+C4	0	0	4	1013.6	132.7
e276	SG+C4+L	0	0	4	1262.0	94.0
e276	SG	0	1	4	1417.9	110.8
e276	SG+L	0	1	4	1429.4	133.2
e276	SG+C4	0	1	4	1123.6	78.2
e276	SG+C4+L	0	1	4	1238.4	84.9
e276	SG	1	0	4	1561.9	108.2
e276	SG+L	1	0	4	1513.5	132.6
e276	SG+C4	1	0	4	1351.2	71.8
e276	SG+C4+L	1	0	4	1146.1	174.0
e276	SG	1	1	4	1818.1	84.5
e276	SG+L	1	1	4	1674.7	82.0
e276	SG+C4	1	1	4	1203.0	62.1
e276	SG+C4+L	1	1	4	1245.8	210.9
e248	32-species	0	0	6	1357.6	142.4
e248	32-species	0	1	6	1449.5	223.1
e248	32-species	1	0	6	1968.2	104.2
e248	32-species	1	1	6	2190.7	157.1
e120	1-species	0	0	20	761.9	53.9
e120	2-species	0	0	34	1041.6	57.1
e120	4-species	0	0	40	1060.6	61.5
e120	8-species	0	0	46	1332.8	48.9
e120	16-species	0	0	70	1464.1	40.4

Table S7.

Soil carbon storage rates (top 20 cm of soil).

Experiment	Diversity	Irrigation	N addition	Sample size	Soil C storage ($\text{g m}^{-2} \text{yr}^{-1}$)	Standard error
e276	SG	0	0	4	-16.7	30.4
e276	SG+L	0	0	4	7.6	16.6
e276	SG+C4	0	0	4	50.8	24.7
e276	SG+C4+L	0	0	4	41.8	27.4
e276	SG	0	1	4	39.3	37.9
e276	SG+L	0	1	4	15.5	27.2
e276	SG+C4	0	1	4	24.8	28.3
e276	SG+C4+L	0	1	3	115.1	46.8
e276	SG	1	0	4	12.7	31.3
e276	SG+L	1	0	4	1.4	54.3
e276	SG+C4	1	0	4	5.4	46.8
e276	SG+C4+L	1	0	4	-10.3	18.7
e276	SG	1	1	4	53.4	46.9
e276	SG+L	1	1	4	26.4	20.4
e276	SG+C4	1	1	4	-53.8	36.1
e276	SG+C4+L	1	1	4	40.8	17.5
e248	32-species	0	0	6	28.3	14.7
e248	32-species	0	1	6	48.7	29.0
e248	32-species	1	0	5	56.5	17.1
e248	32-species	1	1	6	83.2	56.2
e120	1-species	0	0	10	15.9	4.8
e120	2-species	0	0	17	22.0	7.6
e120	4-species	0	0	20	21.8	4.3
e120	8-species	0	0	23	25.4	3.4
e120	16-species	0	0	35	31.7	3.1

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