Carbon-Negative Biofuels from Low-Input High-Diversity Grassland Biomass

David Tilman,1,* Jason Hill,1,2 Clarence Lehman1

Biofuels derived from low-input high-diversity (LIHD) mixtures of native grassland perennials can provide more usable energy, greater greenhouse gas reductions, and less agrochemical pollution per hectare than can corn grain ethanol or soybean biodiesel. High-diversity grasslands had increasingly higher bioenergy yields that were 238% greater than monoculture yields after a decade. LIHD biofuels are carbon negative because net ecosystem carbon dioxide sequestration (4.4 megagram hectare−1 year−1 of carbon dioxide in soil and roots) exceeds fossil carbon dioxide release during biofuel production (0.32 megagram hectare−1 year−1). Moreover, LIHD biofuels can be produced on agriculturally degraded lands and thus need to neither displace food production nor cause loss of biodiversity via habitat destruction.

Globally escalating demands for both food (1) and energy (2) have raised concerns about the potential for food-based biofuels to be sustainable, abundant, and environmentally beneficial energy sources. Current biofuel production competes for fertile land with food production, increases pollution from fertilizers and pesticides, and threatens biodiversity when natural lands are converted to biofuel production. The two major classes of biomass for biofuel production recognized to date are monoculture crops grown on fertile soils (such as corn, soybeans, oilseed rape, switchgrass, sugarcane, willow, and hybrid poplar) (3–6) and waste biomass (such as straw, corn stover, and waste wood) (7–9). Here, we show the potential for a third major source of biofuel biomass, high-diversity mixtures of plants grown with low inputs on agriculturally degraded land, to address such concerns.

We performed an experiment on agriculturally degraded and abandoned nitrogen-poor sandy soil. We determined bioenergy production and ecosystem carbon sequestration in 152 plots, planted in 1994, containing various combinations of 1, 2, 4, 8, or 16 perennial herbaceous grassland species (table S1) (10). Species composition of each plot was determined by random draw from a pool of species. Plots were unfertilized, irrigated only during establishment, and otherwise grown with low inputs (10). The 16-species plots are the highest-diversity, or the LIHD (low-input, high-diversity), treatment. All plots were burned in early spring to remove aboveground biomass before growth began. Soil samples, collected before planting in 1994 and again in 2004, determined carbon sequestration in soil. Plots were sampled annually from 1996 to 2005 for aboveground biomass production.

Annual production of aboveground bioenergy (i.e., biomass yield multiplied by energy released upon combustion) (10) was an approximate log function of planted species number (Fig. 1A). On average for the last 3 years of the experiment (2003–2005), 2-, 4-, 8-, and 16-species plots produced 84%, 100%, 157%, and 238% more bioenergy, respectively, than did plots planted with single species. In a repeated measures multivariate analysis of variance, annual bioenergy production was positively dependent on the number of planted species (F15, 155 = 68.4, P < 0.0001), on time (F0.9, 147 = 8.81, P < 0.0001), and on a positive time-by-species number interaction (F9, 147 = 11.3, P < 0.0001). The interaction occurred because bioenergy production increased more through time in LIHD treatments than in monocultures and low-diversity treatments, as shown by the ratio of bioenergy in LIHD (16 species) plots to those in 8-, 4-, 2-, and 1-species plots (Fig. 1B).

The gross bioenergy yield from LIHD plots was 68.1 GJ ha−1 year−1. Fossil energy needed for biomass production, harvest, and transport to a biofuel production facility was estimated at 4.0 GJ ha−1 year−1 (table S2). Different biofuel production methods capture different proportions of bioenergy in deliverable, usable forms (Fig. 2) (10). Cocombustion of degraded land LIHD biomass with coal in existing coal-fired electric generation facilities would provide a net gain of about 18.1 GJ ha−1 as electricity (11). Converting LIHD biomass into cellulosic ethanol and electricity is estimated to net 17.8 GJ ha−1 (12). Conversion into gasoline and diesel synguels and electricity via integrated gasification and combined cycle technology with Fischer-Tropsch hydrocarbon synthesis (IGCC-FT) is estimated to net 28.4 GJ ha−1 (10, 13). In contrast, net energy gains from corn and soybeans from fertile agricultural soils are 18.8 GJ ha−1 for corn grain ethanol and 14.4 GJ ha−1 for soybean biodiesel (14). Thus, LIHD biomass converted via IGCC-FT yields 51% more usable energy per hectare from degraded infertile land than does corn grain ethanol from fertile soils. This higher net energy gain results from (i) low-energy inputs in LIHD biomass production because the crop is perennial and is neither cultivated, treated with herbicides, nor irrigated once established and likely requires only phosphorus replacement fertilization because nitrogen is provided by legumes; (ii) the more than 200% higher bioenergy yield associated with high crop biodiversity; and (iii) the use of all aboveground biomass, rather than just seed, for energy. LIHD biofuels also provide much greater net energy outputs per unit of fossil fuel input than do current biofuels [net energy balance (NEB) ratios of Fig. 2]. Fertile lands yield about 50% more LIHD biomass (and bioenergy) than our degraded soils (15, 16).

Annual carbon storage in soil was a log function of plant species number (Fig. 1C). For 1994–2004, there was no significant net sequestration of atmospheric CO2 in monoculture plots [mean net release of CO2 of 0.48 ± 0.44 Mg ha−1 year−1 (mean ± SE)], but, in LIHD plots, there was significant soil sequestration of CO2 (2.7 ± 0.29 Mg ha−1 year−1). Soil carbon storage occurred even though all aboveground biomass-based organic matter was removed annually via burning. Periodic resampling of soils in a series of prairie-like agriculturally degraded fields found C storage rates similar to those of the LIHD treatment and suggested that this rate could be maintained for a century (17). The observed annual rate of change in soil C at a particular soil depth declined with depth (P = 0.035), suggesting that an additional 5% more

Reference

1Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN 55108, USA. 2Department of Applied Economics, University of Minnesota, St. Paul, MN 55108, USA.

*To whom correspondence should be addressed. E-mail: tilman@umn.edu

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C may be stored in soils deeper than we measured (below 60 cm depth).

In 2004, after 10 years of growth, atmospheric CO2 sequestration in roots was a log function of plant species numbers (Fig. 1D). On an annual basis, 0.62 Mg ha\(^{-1}\) year\(^{-1}\) of atmospheric CO2 was sequestered in roots of species grown in monocultures, and 160% more CO2 (1.7 Mg ha\(^{-1}\) year\(^{-1}\)) was captured in roots of 16-species plots. Multiple regression showed that root CO2 sequestration (Mg ha\(^{-1}\) of CO2) increased as a log function of plant species number (\(S\)), as a log function of time (Year), and their interaction:

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C_{\text{root}} = -1.47 + 6.16\log_{10}(S) + 9.64\log_{10}(\text{Year}) + 9.60[\log_{10}(S) - 0.613][\log_{10}(\text{Year}) - 0.782]
\]

where Year = 3 for 1997, the first time roots were sampled; overall \(F_{3, 1260} = 191, P < 0.0001\); for log10(S), \(F_{1, 1260} = 398, P < 0.0001\); for Year, \(F_{1, 1260} = 148, P = 0.0001\); for \(S \times \text{Year}\), \(F_{1, 1260} = 27.3, P = 0.0001\). This regression suggests that most root carbon storage occurred in the first decade of growth; during the second decade, roots of 16-species plots are projected to store just 22% of C stored during the first decade. Measurements at greater depths in 10 LIHD plots suggest that 43% more C may be stored in roots between 30 and 100 cm.

LIHD plots had a total CO2 sequestration rate of 4.4 Mg ha\(^{-1}\) year\(^{-1}\) in soil and roots during the decade of observation. Trends suggest that this rate might decline to about 3.3 Mg ha\(^{-1}\) year\(^{-1}\) during the second decade because of slower root mass accumulation. In contrast, the annual rate of CO2 sequestration for monocultures was 0.14 Mg ha\(^{-1}\) year\(^{-1}\) for the first decade and projected to be indistinguishable from zero for subsequent decades.

Across their full life cycles, biofuels can be carbon neutral [no net effect on atmospheric CO2 and other greenhouse gases (GHG)], carbon negative (net reduction in GHG), or carbon sources (net increase in GHG), depending on both how much CO2 and other greenhouse gases, expressed as CO2 equivalents, are removed from or released into the atmosphere.

**Fig. 1.** Effects of plant diversity on biomass energy yield and CO2 sequestration for low-input perennial grasslands. (A) Gross energy content of harvested aboveground biomass (2003–2005 plot averages) increases with plant species number. (B) Ratio of mean biomass energy production of 16-species (LIHD) treatment to means of each lower diversity treatment. (C) Annual net increase in soil organic carbon (expressed as mass of CO2 sequestered in upper 60 cm of soil) increases with plant diversity as does (D) annual net sequestration of atmospheric carbon (as mass of CO2) in roots of perennial plant species. Solid curved lines are log fits; dashed curved lines give 95% confidence intervals for these fits.

**Fig. 2.** NEB for two food-based biofuels (current biofuels) grown on fertile soils and for LIHD biofuels from agriculturally degraded soil. NEB is the sum of all energy outputs (including coproducts) minus the sum of fossil energy inputs. NEB ratio is the sum of energy outputs divided by the sum of fossil energy inputs. Estimates for corn grain ethanol and soybean biodiesel are from (14).
during crop growth and how much fossil CO$_2$ is released in biofuel production. Both corn ethanol and soybean biodiesel are net carbon sources but do have 12% and 41% lower net GHG emissions, respectively, than combustion of the gasoline and diesel they displace (14). In contrast, LIHD biofuels are carbon negative, leading to net sequestration of atmospheric CO$_2$ across the full life cycle of biofuel production and combustion (table S3). LIHD biomass removed and sequestered more atmospheric CO$_2$ than was released from fossil fuel combustion during agriculture, transportation, and processing (0.32 Mg ha$^{-1}$ year$^{-1}$ of CO$_2$), with net life cycle sequestration of 4.1 Mg ha$^{-1}$ year$^{-1}$ of CO$_2$ for the first decade and an estimated 2.7 to 3 Mg ha$^{-1}$ year$^{-1}$ for subsequent decades. GHG reductions from use of LIHD biofuels in lieu of gasoline and diesel fuel are from 6 to 16 times greater than those from use of corn grain ethanol and soybean biodiesel in lieu of fossil fuels (Fig. 3A).

LIHD biofuel production should be sustainable with low inputs of agrichemicals, as in our study. Legumes in LIHD plots can supply nitro-

Fig. 3. Environmental effects of bioenergy sources. (A) GHG reduction for complete life cycles from biofuel production through combustion, representing reduction relative to emissions from combustion of fossil fuels for which a biofuel substitutes. (B) Fertilizer and (C) pesticide application rates are U.S. averages for corn and soybeans (29). For LIHD biomass, application rates are based on analyses of table S2 (10).