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ECOSYSTEM ECOLOGY – ORIGINAL RESEARCH

Carbon storage potential increases with increasing ratio of C_4 to C_3 grass cover and soil productivity in restored tallgrass prairies

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Abstract

Long-term soil carbon (C) storage is essential for reducing CO_2 in the atmosphere. Converting unproductive and environmentally sensitive agricultural lands to grasslands for bioenergy production may enhance C storage. However, a better understanding of the interacting effects of grass functional composition (i.e., relative abundance of C_4 and C_3 grass cover) and soil productivity on C storage will help guide sustainable grassland management. Our objective was to examine the relationship between grass functional composition and potential C storage and how it varies with potential soil productivity. We estimated C inputs from above- and belowground net primary productivity (ANPP and BNPP), and heterotrophic respiration (R_H) to calculate net ecosystem production (NEP), a measure of potential soil C storage, in grassland plots of relatively high- and low-productivity soils spanning a gradient in the ratio of C_4 to C_3 grass cover (C_4:C_3). NEP increased with increasing C_4:C_3, but only in potentially productive soils. The positive relationship likely stemmed from increased ANPP, rather than BNPP, which was possibly related to efficient resource-use and physiological/anatomical advantages of C_4 plants. R_H was negatively correlated with C_4:C_3, possibly because of changes in microclimate or plant–microbe interactions. It is possible that in potentially productive soils, C storage can be enhanced by favoring C_4 over C_3 grasses through increased ANPP and BNPP and reduced R_H. Results also suggest that potential C storage gains from C_4 productivity would not be undermined by a corresponding increase in R_H.

Keywords Aboveground/belowground net primary production · Carbon sequestration · Grassland · Heterotrophic respiration · Net ecosystem production

Introduction

Long-term carbon (C) storage in soils is a key part of climate stabilization as greenhouse gasses continue to accumulate in the atmosphere (Scurlock and Hall 1998; Smith 2004; Lal 2004; Powlson et al. 2011). As such, the Paris Climate Agreement requires that C sources equal C sinks by the second half of this century (UNFCCC 2015). Restoring temperate grasslands, which are increasingly being replaced by annual crops (net C sources) in the Midwestern US, has potential for increasing C storage (Jobbágy and Jackson 2000; Lal 2003; Lark et al. 2015). For example, switching from annual crops to perennial grasslands for bioenergy production on less productive or marginal land may promote C sequestration, while contributing to US energy needs (Lal 2004; Tilman et al. 2006; Gelfand et al. 2013). But sustainable management of perennial bioenergy cropping systems or prairie habitat restorations requires balancing production, biodiversity preservation, and ecosystem services such as C storage. A better understanding of how factors such as the functional composition of grasslands (e.g., the ratio of C_4 to C_3 grasses) and soil productivity affect C storage will help guide management for these goals.

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Grasslands can be C sinks if C inputs from net primary production (NPP) exceed C losses from heterotrophic respiration (R_H). This difference between NPP and R_H is net ecosystem production (NEP), which is often used as a measure of the rate of C storage (Randerson et al. 2002). In a given climate, NPP is dependent on soil productivity. But because plants with C_4 or C_3 photosynthetic pathways can perform optimally or allocate resources for growth depending on environmental context (Teeri and Stowe 1976; Sage and Pearcy 1987; Sheehy et al. 2007; Kocacinar et al. 2008; Von Fischer et al. 2008; Atkinson et al. 2016), both functional composition and potential soil productivity should be important for determining grassland NPP. Although R_H can increase with greater NPP, thus mitigating C storage gains (Raich and Tufekciogul 2000; Carney et al. 2007; Nie et al. 2013), R_H may also depend on grassland functional composition (Cahill et al. 2009). Plant functional composition and potential soil productivity are, therefore, crucial for determining the rate of C storage in grassland ecosystems by affecting the response of C inputs (Jarchow et al. 2012), which can affect C outputs (Carney et al. 2007).

Carbon inputs to soil from NPP derive from both above- and belowground production (i.e., ANPP and BNPP). Although ANPP is sometimes used to estimate overall NPP, a separate measure of BNPP should not be overlooked because allocation of resources to ANPP or BNPP can vary between C_4 and C_3 plants and depend on biotic and abiotic conditions (Angelo and Pau 2015). For example, the greater water-use efficiency of C_4 plants allows for more growth in shoots when water availability is high and more growth in roots when availability is low (Long 1999; Taylor et al. 2010). The lower leaf tissue density and vascular structure of C_4 compared to C_3 plants allows for greater ANPP in nutrient-rich environments because more leaves can be produced at the same C cost, which can be structurally supported (Kocacinar and Sage 2003; Atkinson et al. 2016). However, the greater N- and water-use efficiency of C_4 plants (Sage and Zhu 2011) suggests that they may be more productive than C_3 plants when these resources are limiting. Therefore, the functional composition of grasses and soil properties that affect factors such as water and nutrient availability in grasslands, are key to understanding patterns of NPP and NEP. Examining the component (i.e., ANPP, BNPP, and R_H) responses of NEP to functional composition and soil productivity may provide a more informative picture of how to include C storage with other management goals, such as biomass production.

We investigated how the functional composition of grasses was related to C storage (NEP) in plots with either relatively high or low potential soil productivity. We asked three questions: (1) What is the relationship between grass functional composition and C storage? To address this question, we conducted a study in temperate mixed grasslands across a gradient of C_4 to C_3 grass cover (C_4:C_3), where we expected a positive relationship between NEP and C_4:C_3. (2) How does potential soil productivity affect the relationship between functional composition and C storage? Our study was conducted in two soil types: potentially more productive silt and clay loam soils and less productive sandy soils, in which we expected the strength of the relationship between NEP and C_4:C_3 to be greater in more productive soils. (3) How does the response of each NEP component (ANPP, BNPP, and R_H) to functional composition and soil productivity relate to the overall response of NEP? We expected that a positive relationship between NEP and C_4:C_3 would reflect similar positive relationships of ANPP and BNPP with C_4:C_3, and a negative relationship between R_H and C_4:C_3.

Materials and methods

Study sites

This study was conducted in grasslands in central Wisconsin, USA, over 2 years in 2007 and 2008. Because of land cover change and agricultural intensification, this area of the Midwestern US has high potential for increasing soil C storage in grasslands (Tilman et al. 2006; Robertson et al. 2008; Gelfand et al. 2013). Moreover, mean high temperatures during the growing season in this region are near the crossover temperature (22 °C), above which C_4 grasses are more productive and below which C_3 grasses are more productive (Collatz et al. 1998) making this an ideal location to assess the effects of grass functional composition on NEP.

We established research plots on two tallgrass prairie restoration sites: Bison Ridge Ranch (BRR) in Marquette County (89°27′W, 43°44′N) and the Wisconsin Integrated Cropping Systems Trial (WICST) at the University of Wisconsin-Madison's Arlington Agricultural Research Station in Columbia County (89°19′W, 43°18′N). Study plots at BRR were established within a 4-ha restored grassland, which was seeded with a diverse prairie mix in 1990. The soils of BRR are Gotham loamy fine sand and Metea fine sandy loam on 2–6% slopes (Soil Survey Staff, NRCS, USDA 2017), which are well-drained with rapid permeability. BRR grasslands were subject to annual hay harvest on 31 August 2007 and 1 September 2008 after each year's sampling was complete. Annual total precipitation at BRR during 2007 and 2008 was 727 and 738 mm, respectively (University of Wisconsin Agricultural Extension 2017). Historical mean annual precipitation was 803 mm and mean temperatures in July and January were 21 and − 11 °C, respectively (NOAA 2017).

Study plots at WICST were established across six 0.33-ha fields. In 1999 these fields were seeded with either a low- or high-diversity seed mix, which contained 6 and 25 species of forbs and grasses, respectively (Simonsen 2004). WICST soils are Plano silt loam on 0–2% slopes (Soil Survey Staff, NRCS, USDA 2017), which retain more water and nutrients for plant growth and have a greater cation exchange capacity than the sandier soils at BRR. WICST plots were burned prior to this study in the spring of 2003 and again in spring 2007, just before the study. Annual total precipitation at WICST in 2007 and 2008 was 892 and 938 mm, respectively (University of Wisconsin Agricultural Extension 2017). Historically, mean annual precipitation was 833 mm and mean temperatures in July and January were 21 and − 9 °C, respectively (NOAA 2017).

Sampling design

We set up 30 research plots at each site, 15 plots at each site in each of the years 2007 and 2008, for a total of 60 plots. Plot locations within each site and year were selected so that the grass composition spanned a gradient in the ratio of C_4 to C_3 grass cover. Although we did not experimentally manipulate the functional composition of grasses within plots, our plots likely represented functioning in restored and managed systems better than a contrived and freshly sown experimental assemblage. Within each 10 × 10-m plot we measured soil quality and grass species cover and functional composition. We also estimated net ecosystem production (NEP) as a measure of annual potential soil C storage within each plot. NEP estimates were based on measured annual ANPP, BNPP, and soil respiration (R_S).

Soil quality

Soil samples were taken near the center of each plot. Being mindful to minimize compaction, we used a soil core sampler to extract one 5 × 15-cm soil core per plot. Soil texture (i.e., percent sand, silt, and clay) was quantified in each plot in 2007 using the Buyocous hydrometer method (Robertson et al. 1999). Bulk density and soil chemical properties were calculated in each plot in 2007 and 2008. Bulk density (BD) was calculated as oven-dried mass of dry soil per unit volume of soil. We discounted the weight and volume of pebbles that were > 2 mm diameter. Volume was calculated using volumetric displacement in water (Elliot et al. 1999). Core samples were sent to the University of Wisconsin Soil and Plant Analysis Lab to determine soil total C, total N, potassium (K), phosphorus (P), and organic matter concentration (OM). Total C and total N were determined by dry combustion using a Leco CNS-2000 analyzer (organic carbon dry combustion method, Leco CN-2000, FP 2000, or CNS-2000). Plant available P was estimated using the Bray P1 method (Bray and Kurtz 1945) and OM was estimated using the loss-on-ignition method (Heiri et al. 2001). Plant available K was extracted using the Bray P1 reagent (0.03 N NH4F, 0.025 N HCl) and analyzed using an atomic absorption spectrophotometer.

Plant sampling

The cover of each grass species was estimated using the line-point method (Heady et al. 1959). Three 1-m^2 areas were sampled within each plot corresponding to the areas where soil cores, root ingrowth cores, and plant biomass sampling. The three within-plot sampling areas were each overlaid with a grid forming 25 intersections, and at each intersection the first intercept of a rod with any part of herbaceous vegetation was recorded. During the months when two distinctly vertical layers of different vegetation were observed, we recorded the first hit with the taller herbaceous vegetation and the second hit with the herbaceous vegetation in the layer below the first one totaling 50 hits per quadrat.

For each sampling event, species cover was calculated as total species hits divided by the total possible hits for each quadrat. Grasses were identified to species and grouped by their photosynthetic pathway (C_4 or C_3). Plant sampling was conducted four times during the season: in June (early-season), July (mid-season), August (peak of standing biomass) and late October or early November (Fall). Within each plot, cover estimates were averaged across the three sample areas within a sample period and then across sample periods to characterize the annual percent cover of C_4 and C_3 grasses. The ratio of C_4 to C_3 cover was used as a measure of functional composition.

Above- and below-ground net primary production

ANPP was estimated within each plot once in each of the months of June, July, August, and October by sampling biomass from randomly placed 50 × 50-cm quadrats. Biomass within quadrats was clipped to ~ 3 cm residual stubble height to preclude injury to crowns. Collected biomass was dried at 60 °C to a constant weight (minimum of 48 h) and then weighed. Annual ANPP of each plot was estimated by calculating the difference in the mass of biomass samples between sampling rounds (i.e., monthly production) and then summing those differences (Brye et al. 2002; Cahill et al. 2009). This approach allowed us to account for the simultaneous growth and senescence of plant matter (Vogt et al. 1986) that was not accounted for by simply assessing peak standing biomass, which may underestimate ANPP (Gill et al. 2002; Scurlock et al. 2002; Cahill et al. 2009).

It was not possible to measure the fall 2007 biomass produced in BRR plots after the 31 August harvest. We therefore estimated fall biomass values for these plots using the relationship between leaf area index (LAI) and biomass weights across sites and years ($R^2 = 0.69$). LAI of vegetation was measured in each quadrat prior to each clipping event. LAI readings were taken above and below the leaf canopy at four points within each quadrat using an AccuPAR LP-80 (Decagon, Inc., Pullman, WA, USA).

BNPP was estimated in each study plot using root ingrowth cores. We took four samples per plot in 2007 and six per plot in 2008. Soil cores were removed prior to the growing season (late May or early June) with a 5-cm soil sampler and sieved to remove rocks, roots, and debris (Fahey et al. 1999). Ingrowth cores were made of 2-mm plastic mesh (5 cm diameter × 15 cm long) and were filled with a 1:1 mixture of the sieved soil and sand and inserted into the original holes. In 2007, two cores were harvested in each of August and October. In 2008, two cores were harvested in each of July, August, and October. The harvested cores were bagged and refrigerated until washing. Roots were washed free of soil over a 1-mm sieve, dried at 60 °C to a constant weight (minimum of 48 h), and then weighed. We did not attempt to separate live from dead roots, assuming that only a very small fraction was dead (Fahey and Hughes 1994). For each collection period, we averaged the root biomass weights in each plot to estimate biomass per unit area. We calculated belowground biomass production as the difference in root biomass harvested at one time from the root biomass of an earlier harvest. The root net production was calculated as the sum of positive production values. Biomass estimates from root ingrowth cores do not account for roots that were produced and died during the period when the cores were deployed [i.e., fine root turnover (FRT)]. We therefore adjusted values of belowground biomass production using an exponential function of mean annual temperatures (MAT, °C) at each site ($\mathrm{FRT} = 0.2884\ e^{(0.046 \times \mathrm{MAT})}$; Gill et al. 2002).

We extrapolated root biomass collected from the 15 cm cores to a depth of 60 cm based on the relationship between 15- and 60-cm deep soil cores in our plots (Fahey et al. 1999). In April, August, and November of 2008, 60-cm soil cores were taken immediately above six randomly chosen C_4 and six C_3 individuals per plot. The 60-cm soil cores were divided into four 15-cm sections; sieved in 2-mm mesh; washed of debris, rocks, and soil; dried to constant weight at 60 °C (minimum of 48 h); and weighed. As above, no attempt was made to separate living from dead roots. For each grass functional group, the six root weight values for each segment were averaged across samples to access the vertical distribution of root stock biomass. On average, 84% of root biomass was found in the first 15 cm. Thus, there was strong correlation between root biomass at depths of 15 and 60 cm ($r = 0.99$, $P < 0.001$).

Soil respiration

Soil respiration (R_S) measurements were taken once each month from May to October each year between 0900 and 1500 h with a Li-Cor 6400 portable CO_2 IRGA (Li-Cor Biosciences, Lincoln, NE, USA) equipped with a LI-6400-09 R_S chamber (Norman et al. 1992). The efflux chamber was used in conjunction with polyvinyl chloride (PVC) thin-walled collars that were inserted 2 cm into the soil surface at least 30 min prior to conducting measurements. Measurements were made using three collars within a 1-m^2 quadrat that was randomly repositioned within study plots for each monthly sample. We estimated R_S, the soil CO_2 efflux (μmol CO_2 m^{-2} s^{-1}), in each plot by averaging the measurements from the three collars. Because shoots were clipped prior to measuring R_S, the efflux of soil CO_2 measured excludes shoot respiration. Linear mixed-effects models (*nlme* package; Pinheiro et al. 2017) were used to determine the relationship between measured values of R_S and soil temperature measured at a depth of 10 cm. Soil temperature and year were included as fixed effects and plot was included as a random effect because multiple measurements were taken within each plot over the course of a year. R_S was best predicted by a polynomial relationship with soil temperature and this relationship was then used to predict plot-specific daily values of R_S for each day of the years 2007 and 2008. Negative estimates of R_S, which generally occurred when soil temperatures were below 0 °C were given a value of zero. Annual R_S, the mass of C per unit area respired annually, was then estimated for each plot by summing the estimated daily values (Brye et al. 2002; Chou et al. 2008).

Net ecosystem production

Net ecosystem production (NEP) is the difference between C inputs from NPP and C outputs from heterotrophic respiration (R_H). We calculated NEP within each plot as an estimate of annual potential soil C storage. C makes up 40–50% of shoot and root tissues in restored and remnant prairies (Brye et al. 2002; Matamala et al. 2008). Measures of percent C in above- and below-ground grass tissue at WICST and other Wisconsin grassland sites indicated that C comprises 43.6% ± 0.38 (mean ± SE) of aboveground tissue and 40.6% ± 0.94 of belowground plant tissue (L. G. Oates, unpublished data). C inputs from ANPP and BNPP (i.e., ANPP and BNPP were calculated by multiplying our estimates of ANPP and BNPP by a constant 0.436 and 0.406, respectively.

Soil respiration (R_S) is the sum of heterotrophic respiration (i.e., microbial respiration, R_H) and autotrophic (i.e., root respiration, R_A). Although measuring R_S is relatively straightforward, separating the component autotrophic and heterotrophic forms of respiration is challenging (Kuzyakov and Larionova 2006). We therefore used data from *A Global Database of Soil Respiration* (Bond-Lamberty and Thomson 2014) to determine an empirical relationship between R_H and R_S and thus estimate R_H from the measured values of R_S in our study plots (von Haden and Dornbush 2017). We limited our search of the database to include only data from temperate grasslands in which empirical measures of R_H and R_S are both present, and then removed any quality-flagged data. This resulted in 10 different studies with 21 paired measurements of R_H and R_S, which had a strong linear relationship (Pearson's $r = 0.91$), taking the form: $\ln\left(R_H\right) = \ln\left(R_S\right) \times 0.905 + 0.114$. Using our empirically-based estimates of R_H and of % C inputs from ANPP and BNPP, we estimated NEP in units of g C m^{-2} year^{-1} as: NEP = (ANPP) + (BNPP) − R_H (Cahill et al. 2009). Because our intention was to assess potential C sequestration, we did not estimate C loss from harvesting or fire.

Statistical analysis

Root ingrowth data were not available at BRR from one plot in 2007 and four plots in 2008, nor from three plots at WICST in 2008. Because BNPP and NEP could not be calculated for these plots, we excluded them from all analyses. Therefore, $N = 25$ at BRR and $N = 27$ at WICST.

Plots at the two sites (BRR and WICST) were selected to span a similar gradient in the ratio of C_4 to C_3 grass cover. However, other components of the vegetation community and soil may have differed between sites. We, therefore, used *t* tests to examine differences in vegetation (i.e., grass species richness and total grass, forb, and litter cover) and soil characteristics (i.e., texture, OM, K, P, BD, N, and C).

Linear models were used to analyze the effect of grass functional composition (i.e., the $\log_{10}$-transformed ratio of C_4 and C_3 grass cover; C_4:C_3) on NEP. Our statistical model included C_4:C_3 as a main effect. Site was included as a main effect and as an interaction with C_4:C_3 to account for differences between sites in, for example, soil quality, vegetation composition, and management history. Year was also included as a main effect to account for variability between years in factors such as the weather and the difference in the number of root ingrowth cores taken in 2007 and 2008. The same model structure (i.e., $Y \sim$ year + site + C_4:C_3 + site × C_4:C_3) was used to separately analyze the relationship between C_4:C_3 and the individual components of NEP: ANPP, BNPP, and R_H. Because there were significant interactions between site and C_4:C_3 in their effects on NEP, ANPP, and BNPP (but not R_H; see "Results"), we performed separate analyses by site of all four response variables (including R_H). We checked to ensure multicollinearity among predictor variables was low and that residuals were normally distributed.

The components of NEP, ANPP, BNPP, and R_H, were each estimated from other factors. Thus, the variance of each of these estimates may propagate in our estimate of NEP. We, therefore, used Monte Carlo methods to examine how the known variance in the components of NEP affected the relationship between C_4:C_3 and NEP. Our estimate of BNPP was based on the relationship between root ingrowth to 15- and 60-cm soil cores with variance of 1.2×10^{-2}. Our estimates of ANPP and BNPP were based on an empirical relationship between ANPP or BNPP and percent C with variances of 8.2×10^{-5} and 4.4×10^{-4} respectively. Similarly, R_H was estimated from the empirical relationship between R_S and R_H with variance 1.8×10^{-1}. We used component estimates along with their variance to form distributions from which values of each component could be randomly drawn so that NEP could be re-calculated for 1000 replicate regression analyses. We then used model averaging to assess how results that incorporate uncertainty in NEP compare to those that do not. Model averaging was performed using the *MuMIn* package v1.15.6 (Barton 2016).

Although C_4:C_3 spanned a similar range at the two sites, some other aspects of grassland vegetation and soil quality were significantly different between sites (see "Results"). These differences may have contributed to a significant interaction between site and C_4:C_3 in their effect on NEP. We therefore examined which of these grass community or soil factors best explained the site effect by comparing separate statistical models that included each factor with the model that included site. Each statistical model was similar to those described above, but we substituted each grass community or soil descriptor (X) for site (i.e., NEP ~ year + X + $X \times C_4$:C_3). Model comparison was based on the Akaike information criterion, corrected for small sample sizes (AIC_C) and AIC_C weights (Burnham and Anderson 2002). We compared separate models that included either grass species richness, the percent cover of grass, forb, or litter as metrics of grassland vegetation communities. As a metric of soil quality, we used the first axis of a principal components analysis of the edaphic factors OM, K, P, BD, N, and C (i.e., soil PC1). The analyses described above were all performed in R v3.4.1 (R Development Core Team 2017).

It is possible that the site-dependent relationships between C_4:C_3 and NEP (and its components) are a result of inadvertent within-site correlations between soil quality and C_4:C_3. We therefore used structural equation modeling (SEM) to examine the direct and indirect effects of soil quality (i.e., soil PC1) on NEP and its components, and whether soil quality can mediate the effect of C_4:C_3 on NEP and its components. For each site, we assumed that soil PC1 had direct effects on C_4:C_3 and NEP, that C_4:C_3 had a direct effect on NEP, and that year had a direct effect on NEP. Because soil PC1 was significantly greater in 2008 compared to 2007 at WICST ($t_{16.5} = 2.4$, $P = 0.028$), we also assumed correlated errors of year and soil PC1 for WICST models (but not BRR). Models were fit using maximum likelihood. χ^2 tests and root mean square error of approximation (RMSEA) were used to assess model fit. Separate analyses of ANPP, BNPP, and R_H were performed using the same model structure. SEM was performed using Amos v24 (Arbuckle 2014).

Results

We selected study plots that spanned a similar range of C_4 to C_3 grass cover ratios between sites: 0–11.2 at BRR and 0–10.4 at WICST, thus there was no significant difference in functional composition between sites ($t_{48.2} = 0.9$, $P = 0.384$). Increasing the cover of C_4 grasses resulted in a linear decrease in C_3 cover ($r = -0.89$), so an increase in the ratio of C_4 to C_3 grass cover resulted in a simultaneous increase in C_4 cover and a decrease in C_3 cover.

Although the relative cover of each functional group was not different between sites, total grass cover was significantly greater in WICST plots ($t_{41.2} = 6.6$, $P < 0.001$). *Poa pratensis* L. (Kentucky bluegrass) and *Elymus repens* (L.) Gould (quackgrass) were the dominant C_3 grass species at both sites. The two dominant C_4 grass species at both sites were *Andropogon gerardii* Vitman (big bluestem) and *Sorghastrum nutans* (L.) Nash (indiangrass). However, BRR also had three other species of C_4 grasses present: *Panicum virgatum* L. (switchgrass), *Schizachyrium scoparium* (Michx.) Nash (little bluestem), and *Bouteloua curtipendula* (Michx.) Torr. (side oats grama). The difference in C_4 grass species composition between sites arose from differences in the seed mixture used in the initial restorations of these sites. Thus, grass species richness was significantly greater at BRR ($t_{48.2} = 3.3$, $P = 0.002$). There were also significant differences in total forb ($t_{42.6} = 3.4$, $P = 0.002$) and litter cover ($t_{39.8} = 5.6$, $P < 0.001$), which were greater at BRR. Thus, BRR and WICST plots spanned similar ranges of functional composition but grass species richness and total grass, forb, and litter cover differed between sites (Fig. 1).

The physical and chemical properties of soil varied greatly between sites. Soils at BRR were composed mainly of sand (89.2% ± 0.7; mean ± SE) with relatively little silt (5.1% ± 0.5) and clay content (5.7% ± 0.4), whereas WICST soils were a more even mix of sand (31.7% ± 2.2), silt (37.6% ± 2.3), and clay (30.7% ± 0.7). Soil texture was therefore significantly different between sites (sand: $t_{17.1} = 24.4$, $P < 0.001$; silt: $t_{15.6} = -13.6$, $P < 0.001$; clay: $t_{24.0} = -32.3$, $P < 0.001$; Fig. 1). Sand-dominated soils, such as those at BRR, generally have lower water-holding capacity and cation exchange capacity relative to soils with a more even mix of sand, silt, and clay (Jenny 1980), such as at WICST. Consequently, WICST soils had significantly greater total C ($t_{44.5} = 31.2$, $P < 0.001$), total N ($t_{35.3} = 25.6$, $P < 0.001$), K ($t_{31.2} = 9.6$, $P < 0.001$), and OM ($t_{41.4} = 34.1$, $P < 0.001$), and significantly lower bulk density ($t_{48.5} = -9.8$, $P < 0.001$) and P ($t_{27.6} = -3.0$, $P = 0.006$; Fig. A-1). The first axis of a principal components analysis of total C, total N, K, OM, bulk density, and P explained 77% of the variation in soil composition and was significantly different between sites ($t_{47.0} = 25.6$, $P < 0.001$; Fig. 1). Together, these results suggest that WICST soils have the potential to be more productive than BRR soils.

Incorporating variance in our estimates of NEP into our analyses had no qualitative effect and only a minor quantitative effect on the relationship between C_4:C_3 and NEP (< 1% difference; Tables B-1, B-2, and B-3). Therefore, we hereafter focus on the results of statistical models that utilized unadjusted estimates of NEP. Grass functional composition (C_4:C_3) had a strong positive correlation with NEP, but only at WICST, the site with higher potential soil productivity. Thus, we found a significant interaction between site and C_4:C_3 in their effect on NEP ($t_{1,47} = 4.04$, $P < 0.001$; Appendix B Table B-4). We, therefore, performed separated analyses of each site, finding a significant relationship between C_4:C_3 and NEP at WICST ($t_{1,25} = 4.7$, $P < 0.001$), but not BRR ($t_{1,22} = -0.7$, $P = 0.497$; Fig. 2; Table B-5). There were variable relationships between C_4:C_3 and the individual components of NEP (Fig. 3). There was a significant positive relationship between C_4:C_3 and ANPP at WICST ($t_{1,25} = 4.0$, $P < 0.001$) but not BRR ($t_{1,22} = -0.37$, $P = 0.719$; Fig. 3a; Table B-6). Similarly, there was a significant positive relationship between C_4:C_3 and BNPP at WICST ($t_{1,25} = 2.5$, $P = 0.020$) but not BRR ($t_{1,22} = -1.27$, $P = 0.217$; Fig. 3b; Table B-7), although the positive relationship was weaker than that between C_4:C_3 and ANPP. There was a significant negative relationship between C_4:C_3 and R_H at both WICST ($t_{1,25} = -3.1$, $P = 0.005$) and BRR ($t_{1,22} = -4.3$, $P < 0.001$; Fig. 3e; Table B-8). Reduced R_H with greater C_4:C_3 contributes to higher NEP but the difference between sites in their relationships between C_4:C_3 and NEP may be a result of differences in NPP rather than R_H.

In addition to soil quality, grass species richness, and the % cover of grass, forbs, and litter, also varied between sites. AIC_C-based model comparison showed that soil quality (soil PC1) is much more likely to explain the differences between sites than these four factors related to the vegetation community ($\Delta AIC_C > 11$; Table B-9). Although the model that included site was the best model, a model that included soil PC1 was not substantially worse ($\Delta AIC_C = 0.4$). These results suggest that the difference in soil quality between sites is likely to explain the site-dependent relationships between C_4:C_3 and NEP.

Structural equation models each had a non-significant lack fit (BRR models: $\chi^2 = 0.21$, $df = 2$, $P = 0.902$; WICST models: $\chi^2 = 0.43$, $df = 1$, $P = 0.511$) and very low RMSEA

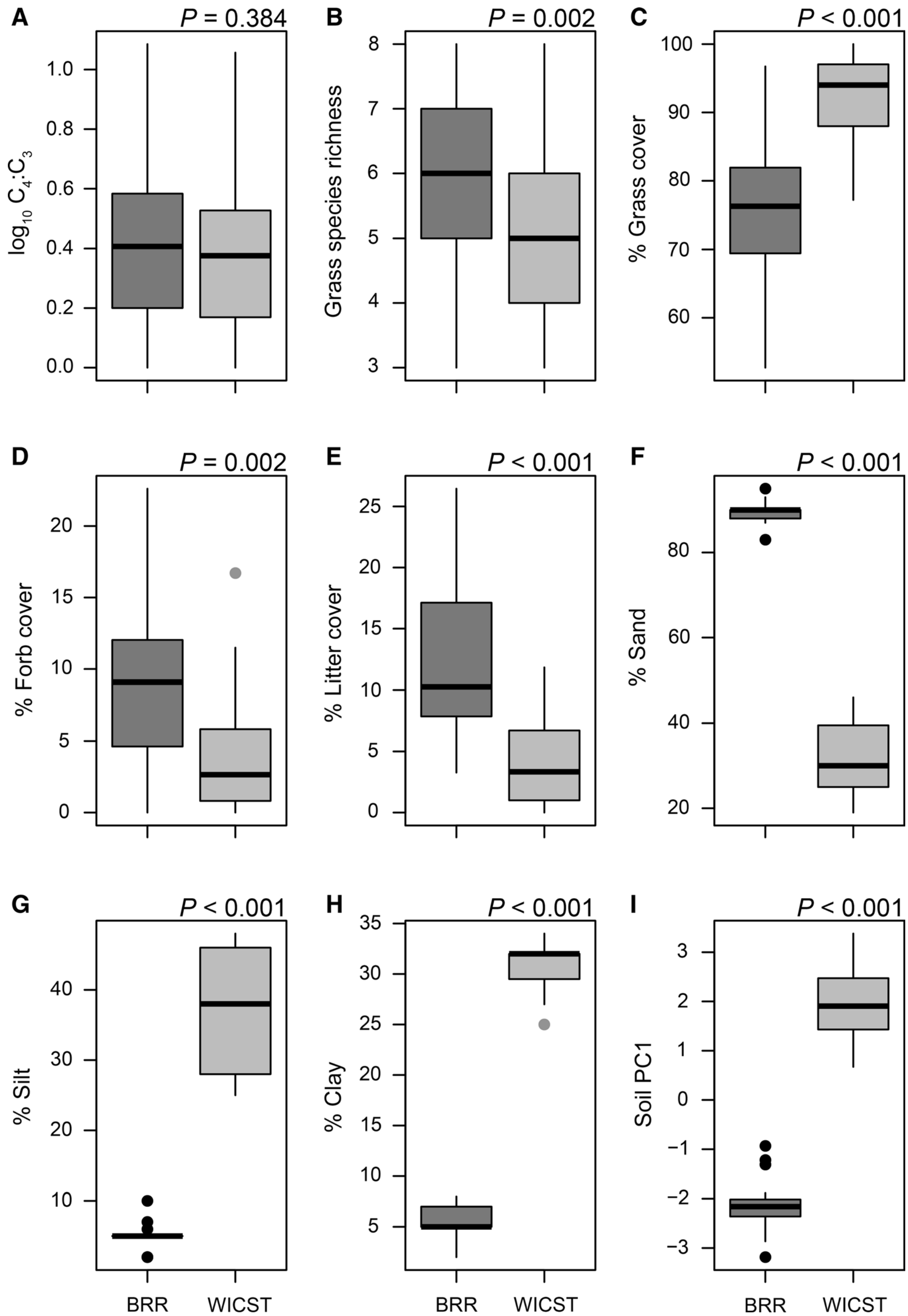

Fig. 1 Differences between BRR (dark gray) and WICST (light gray) in **a** $\log_{10}$ C_4:C_3, **b** grass species richness, **c** % grass cover, **d** % forb cover, **e** % litter cover, **f** % sand in soil, **g** % silt in soil, **h** % clay in soil, and **i** soil PC1. Boxes indicate the first and third quartiles, black bars indicate the median, and whiskers extend above or below boxes to 1.5 × the interquartile range

(all models < 0.001). Analysis of the relationships between C_4:C_3 and NEP and its components using SEM supported the results of our linear regression models: in each case, the sign and statistical significance of the relationships were the same (compare SEM results shown in Fig. 4 and C-1 with regression results in Tables B-5-8). Although there was a significant relationship between C_4:C_3 and soil PC1 at BRR but not WISC, this did not qualitatively affect the relationships of C_4:C_3 with NEP or its components compared to linear regressions. Moreover, permutation tests indicate that there was no significant indirect effect of soil PC1 on NEP or its components at either site. Structural equation models thus provide evidence that the site-dependent relationships between C_4:C_3 and NEP were not a result of different within-site responses to local soil gradients. Therefore, SEM results are consistent with the idea that the large

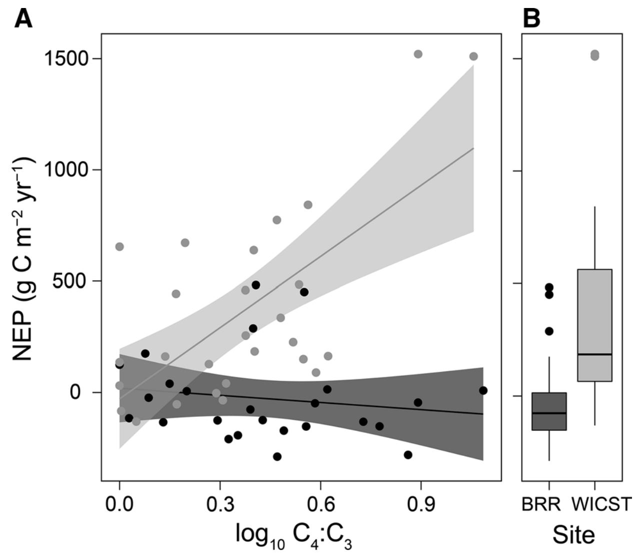

Fig. 2 **a** Relationship between grass functional composition ($\log_{10}$ C_4:C_3) and NEP. BRR values are indicated by black points and dark gray fit confidence interval. WICST values are indicated by light gray points and fit confidence interval. **b** Boxplots illustrates the difference between sites in NEP. Boxes indicate the first and third quartiles, black bars indicate the median, and whiskers extend above or below boxes to 1.5 × the interquartile range

between-site differences in soil properties are a likely cause of the different C_4:C_3-NEP relationships.

Discussion

Grassland NEP, a metric of potential soil C storage, may depend on both the functional composition of the grass community (i.e., the ratio of C_4 to C_3 grass cover) and the potential productivity of the soil. We found a positive relationship between NEP and functional composition, but only in the more potentially productive soils at WICST. There was no relationship between NEP and functional composition in the sandy BRR soils. Although differences between sites in grass cover or species richness may have contributed to the different relationships between NEP and functional composition, differences in soil quality were much more likely to explain the different relationships. Because C_4 and C_3 plants may allocate resources to growth differently (e.g., White et al. 2012; Atkinson et al. 2016), separately examining the components of NEP (i.e., ANPP, BNPP, and R_H) could help elucidate potential mechanisms of a soil-dependent effect of functional composition on NEP.

Similar to overall NEP, ANPP increased with greater C_4:C_3 cover, but only in the potentially productive WICST soils. This suggests that greater potential soil productivity allows plants to utilize the more abundant resources for aboveground growth (and thus NEP), but that higher

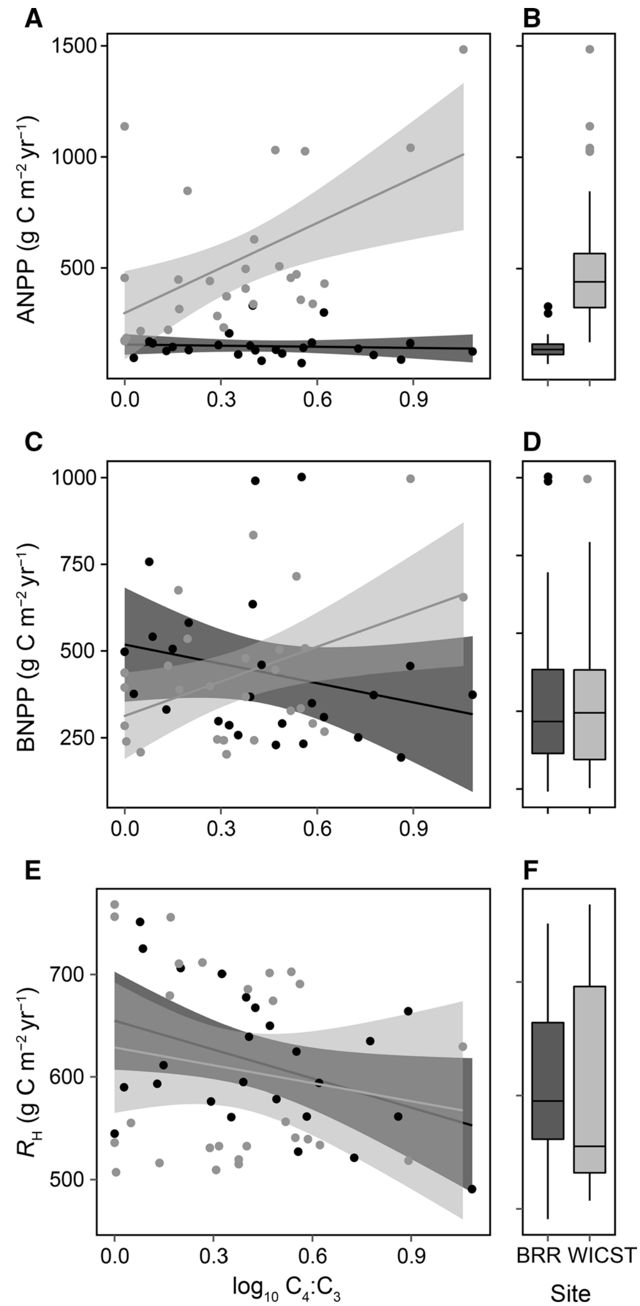

Fig. 3 Relationships between grass functional composition ($\log_{10}$ C_4:C_3) and the components of NEP: **a** ANPP, **c** BNPP, **e** R_H. BRR values are indicated by black points and dark gray fit confidence interval. WICST values are indicated by light gray points and fit confidence interval. Boxplots illustrate differences between sites in **b** ANPP, **d** BNPP, and **f** R_H. Boxes indicate the first and third quartiles, black bars indicate the median, and whiskers extend above or below boxes to 1.5 × the interquartile range

ANPP is only achieved when communities are increasingly composed of C_4 relative to C_3 grasses. C_4 plants have several physiological and anatomical features associated with their photosynthetic pathway that allow them to function more efficiently and be more productive in higher-resource

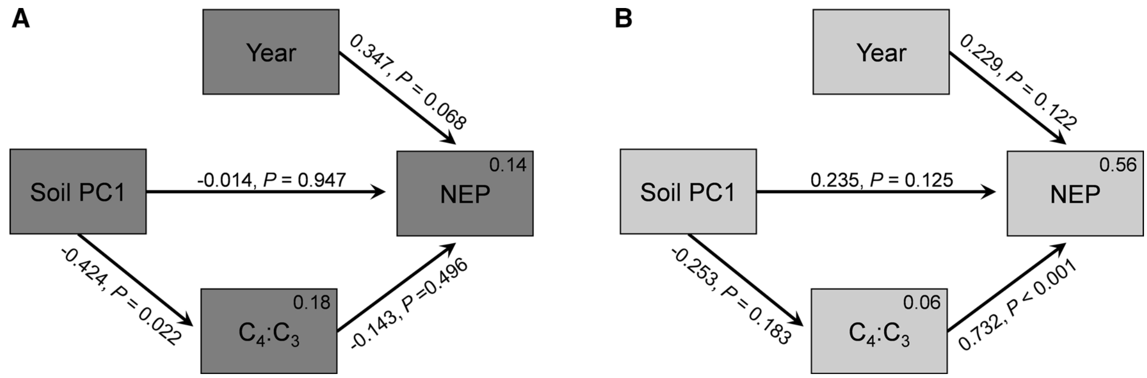

Fig. 4 Structural equation models relating effects of grass functional composition ($\log_{10}$ C_4:C_3, abbreviated C_4:C_3) on NEP at BRR (**a**) and WICST (**b**). Standardized path coefficients and *P* values are shown near arrows. For endogenous variables, R^2 values are given in the upper right corner of boxes

environments compared to C_3 plants. For example, the lower leaf density of C_4 compared to C_3 plants allows for greater C_4 ANPP because more leaves can be produced at the same C cost (Atkinson et al. 2016). Moreover, the different xylem structure of C_4 plants provides an advantage in hydraulic conductivity, allowing them to support greater leaf area per unit xylem (Kocacinar and Sage 2003).

Greater water- and N-use efficiencies of C_4 compared to C_3 plants can allow for greater ANPP when resources are limiting (Sage and Pearcy 1987; Long 1999). That ANPP was uncorrelated with functional composition at BRR, where resources for growth are in shorter supply, suggests that resources may not have been sufficiently limiting for different resource-use efficiencies to affect growth. Rather, different resource-use efficiencies combined with anatomical differences may have contributed to the difference in ANPP at WICST by allowing C_4 plants to take greater advantage of more plentiful resources. These use-efficiency and anatomical advantages of C_4 plants may also help overcome the increased energetic cost of C_4 relative to C_3 photosynthesis.

Although mean BNPP did not vary between sites, BNPP and ANPP had similar relationships with functional composition. That is, BNPP increased with a greater proportion of C_4 grass cover, but only in the more potentially productive soils at WICST. The benefit of higher C_4 cover for BNPP at WICST may have resulted from the more efficient growth of leaves, allowing for greater allocation of resources to root growth (Atkinson et al. 2016). As Atkinson et al. (2016) point out, this suggests that for C_4 plants, there is no inherent trade-off between allocation for above- and belowground growth (Wedin and Tilman 1993). Thus, BNPP contributes to the positive relationship between NEP and the proportion of C_4 grass cover in potentially productive soils, but the greater magnitude of NEP is much more dependent on ANPP. This indicates that differences in grass functional composition can have a much stronger effect on ANPP than on BNPP, which has implications for biomass production.

In contrast to NPP, R_H reduces C storage by transferring fixed C from soils back to the atmosphere. At broad spatial scales, NPP and R_S (which is directly correlated with R_H in our study) are positively correlated (Raich and Tufekciogul 2000). However, at the relatively fine-grained scale of our study, we found that R_H was negatively correlated with C_4 grass cover at both sites. Cahill et al. (2009) found a similar negative effect of C_4 cover on R_S in a set of nearby prairie restorations. Different vegetation types can affect the rate of soil respiration through effects on the microclimate (Raich and Tufekciogul 2000). It is therefore possible that the generally higher ANPP in the plots at WICST increased shading and/or detritus, thereby reducing soil temperatures and microbial activity. But the relationship between R_H and C_4:C_3 was similar between sites, suggesting that an effect of functional composition on R_H was not strongly associated with ANPP or its potential indirect effects on soil microclimate.

The negative correlation between R_H and C_4:C_3 may be better explained by belowground mechanisms. Plant roots can compete with soil microbes for N (Kuzyakov and Xu 2013) so it may be that C_4 grasses perform better than C_3 grasses in competition with soil microbes for N or other limiting nutrients. Whatever the mechanism, our result suggests that increasing the proportion of C_4 grass cover can enhance C storage by reducing R_H and, depending on soil quality, increasing NPP. Our results further suggest that any benefit for NPP from increased C_4 cover would not contribute to atmospheric C through increased R_H.

It is important to note here that our structural equation modeling results showed that differences between sites in the relationships between grass functional composition and NEP and its components was not an artifact of unintended within-site correlations with soil quality. The direct effects of C_4:C_3 on NEP and its components were qualitatively the same as in our linear model results.

Conclusions

Our results indicate that, depending on soil quality, a greater proportion of C_4 grasses, relative to C_3 grasses, can enhance C storage in Midwestern grasslands by both increasing NPP and decreasing R_H. Increases in NPP, especially ANPP, with greater C_4 cover, may have partly stemmed from higher efficiencies in N- and water-use associated with C_4 photosynthesis. In bioenergy production systems, these greater efficiencies can have additional environmental and economic benefits. For instance, greater N-use efficiency means that the application of fertilizers can be reduced, thus reducing production costs, pollution, and associated greenhouse emissions (Vitousek et al. 1997; Carpenter et al. 1998). Greater water-use efficiency means that C_4 grasses are well-adapted to drought stress (Barney et al. 2009), which is predicted to increase with climate change (IPCC 2007).

Much of the benefit of greater C_4 cover for C storage appears to be dependent on soil quality. Conversion of marginal, less productive, agricultural lands to native C_4-dominated grasslands for sustainable bioenergy production is a promising alternative to high-input corn production. The benefits for biodiversity are clear (Werling et al. 2014; Landis 2017; Spiesman et al. 2018), however, our results suggest that enhanced C sequestration may not be realized on relatively unproductive soils by utilizing greater proportions of C_4 relative to C_3 grasses. Studies conducted across a broader range of soil conditions would provide a clearer picture of how soil productivity affects C storage and whether the effect is non-linear. Rainfall was normal over the course of our two-year study. It is possible that the more efficient water-use of C_4 grasses may result in greater C storage in C_4-dominated grasslands on relatively unproductive soils over longer periods of time that include drought years. There is some evidence that an increase in atmospheric CO_2 concentration may itself trigger feedback mechanisms of resistance to climate change by plants in relatively low-productivity soils. Carbon fertilization of trees on N-limited soils can increase the C to N ratio in their structural roots (Dybzinski et al. 2015). If perennial grasses function similarly, grasses on N-limited soils may be able to increase allocation of C to longer-lived roots as atmospheric C increases. This would suggest that N-limited grasslands have great potential to sequester C without additional N input in the form of fertilizers.

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Author contribution statement HK and RDJ conceived and designed the study. HK performed the field and lab work. BJS analyzed the data. BJS wrote the manuscript with input and edits from HK and RDJ.

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