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Keywords

C4 grasses, carbon storage, inorganic carbon, invasive species, novel ecosystems, organic carbon, prairie, soil depth, tallgrass prairie

Disciplines

Ecology and Evolutionary Biology | Plant Sciences | Soil Science

Comments

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Lower soil carbon stocks in exotic vs. native grasslands are driven by carbonate losses

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Abstract. Global change includes invasion by exotic (nonnative) plant species and altered precipitation patterns, and these factors may affect terrestrial carbon (C) storage. We measured soil C changes in experimental mixtures of all exotic or all native grassland plant species under two levels of summer drought stress (0 and +128 mm). After 8 yr, soils were sampled in 10-cm increments to 100-cm depth to determine if soil C differed among treatments in deeper soils. Total soil C (organic + inorganic) content was significantly higher under native than exotic plantings, and differences increased with depth. Surprisingly, differences after 8 yr in C were due to carbonate and not organic C fractions, where carbonate was ~250 g C/m² lower to 1-m soil depth under exotic than native plantings. Our results indicate that soil carbonate is an active pool and can respond to differences in plant species traits over timescales of years. Significant losses of inorganic C might be avoided by conserving native grasslands in subhumid ecosystems.

Key words: *C₄ grasses; carbon storage; inorganic carbon; invasive species; novel ecosystems; organic carbon; prairie; soil depth; tallgrass prairie.*

INTRODUCTION

Globally, soils contain at least twice the amount of C as the atmosphere, and changes in fluxes between soils and the atmosphere can substantially affect the global C cycle (Schlesinger and Andrews 2000, Jörn et al. 2014, Köchy et al. 2015). The C flux from soils to the atmosphere is much greater than the release of carbon dioxide (CO₂) from anthropogenic activities (fossil fuel burning and deforestation; Raich and Schlesinger 1992). Thus, small changes in net primary productivity (NPP) and soil respiration due to vegetation differences can alter the balance between atmospheric CO₂ and soil C stocks (Raich and Tufekcioglu 2000, Hungate et al. 2017). Global change soil research has dominantly focused on the dynamics of organic C, yet inorganic C stored as carbonate minerals comprises as much as 40% of total global soil C and is generally much more abundant than organic C in semiarid and arid environments (Eswaran et al. 2000). Small rates of carbonate accretion and a large global carbonate stock imply a mean residence time of ~85,000 yr for this C pool (Schlesinger 1985). However, recent work highlighted the potential for anthropogenic activities to release soil inorganic C as

CO₂ as a consequence of fertilizer-mediated soil acidification (Zamanian and Kuzyakov 2018, Zamanian et al. 2018). The potential impacts of other environmental factors on the coupled dynamics of organic and inorganic C remain poorly understood.

Soil C is present in organic and inorganic forms (Eswaran et al. 2000, Reeder et al. 2004, Morgan et al. 2010, Zamanian et al. 2016). Soils contain approximately 1,500 Pg of organic C to a depth of 1 m, and this pool is known to be affected by land-use and plant species compositional changes (Batjes 1996, Schlesinger and Andrews 2000, Conant et al. 2001, 2017). Soils to 1-m depth also contain approximately 750 Pg of inorganic C in the form of carbonates (typically CaCO₃ and MgCO₃) and bicarbonate, and concentrations can be especially high in grassland soils (i.e., Mollisols and Vertisols, Batjes 1996, Fischer and Stocklin 1997). Many soils contain substantial carbonate derived from parent material (lithogenic carbonate) as well as pedogenic carbonate formed from exogenous inputs of Ca and Mg (e.g., via dust or mineral weathering) or from dissolution and reprecipitation of lithogenic carbonate. Climate and biological activity control the formation and losses of pedogenic carbonates over seasonal to millennial timescales (Emmerich 2003, Breecker et al. 2009, 2015, Zamanian et al. 2016). Researchers have long assumed that the carbonate C pool cycles slowly relative to organic C (Schlesinger 1985), although this assumption has seldom been tested. For example, Kuzyakov et al.

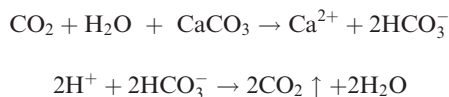
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(2006) stated that the contribution of carbonate dissolution to CO₂ fluxes was marginal over subannual to decadal timescales, and only became significant over geologic timescales. Complete exchange of root-derived CO₂ with extant carbonates was estimated to occur over timescales of hundreds to thousands of years (Gocke et al. 2012). A common method for measuring organic C is to acidify soils prior to analysis (Harris et al. 2001), or to combust samples at a low enough temperature to leave carbonates behind (Potter and Derner 2006). These measurement methods ignore inorganic C entirely, under the assumption that organic C is the major active fraction.

However, first principles suggest that carbonate dynamics may be relevant for ecosystem C balance over short timescales (Zamanian et al. 2018). Carbonate formation rates are highest, and loss through dissolution is lowest, when soil pCO₂ is low, when wet and dry cycles are present, and temperature is high (Schlesinger 1985, Breecker et al. 2015). Low-latitude grasslands are warm and, importantly, have periodic extended wet and dry periods (Isbell et al. 2015). CO₂ from microbial and rhizosphere respiration can produce large amounts of carbonic acid (H₂CO₃), which can promote carbonate dissolution to bicarbonate (Emmerich 2003, Breecker et al. 2015, Zamanian et al. 2018). Plants and associated microbes may also exude organic acids into the soil as a mechanism of nutrient acquisition and/or detrital decomposition, and often acidify the rhizosphere (Dakora and Phillips 2002). Microbial nitrification and plant uptake of ammonium also generate acidity (Zamanian et al. 2018). Thus, changes in plant species composition could affect carbonate content by (1) favoring species that produce more organic acids in root leachates (Pausch and Kuzyakov 2017, Boldt-Burisch et al. 2019), (2) altering CO₂ and carbonic acid inputs from autotrophic respiration, and/or (3) altering the depth distribution of soil moisture and acidity as a consequence of altered rooting depths and nitrogen demand. Carbonate loss from large changes in respiration and acids from plant roots can result from the following equations (Schlesinger 1997, Ramnarine et al. 2012):



CO₂ from respiration can dissolve in soil water, dissociating Ca from calcium carbonate. The reaction can reach equilibrium (i.e., no net C loss) at this point without additional H⁺ (Mongor et al. 2015). However, with additional H⁺, bicarbonate is released as gaseous CO₂.

Grasslands, which cover ~25% of Earth's ice-free land surface and harbor most of their C in soils due to the nonpersistent nature of their plant canopies, contain about 30% of global (SOC) stocks (Janzen 2005) and a substantial fraction of soil inorganic C (SIC) (Eswaran et al. 2000). Grasslands are important as a source of forage for grazing mammals, biofuels, green space in urban

and suburban areas, as wildlife and pollinator habitat, and potentially as areas for soil C storage (Wilsey 2018). One-half or more of NPP occurs belowground in grasslands (Hui and Jackson 2005), and soil respiration correlates significantly with NPP (Raich and Tufekcioglu 2000). Soil respiration responds rapidly to changes in canopy photosynthesis (e.g., Bremer et al. 1998), and thus, shifts in plant species composition toward more productive species can alter rates of soil C accumulation (Conant et al. 2001, 2017). In grassland and desert systems, inorganic C often dominates the total C stock (Eswaran et al. 2000), but its response to vegetation change has received very little attention (Zamanian et al. 2016).

Grassland species composition has been heavily impacted by the introduction and spread of nonnative ("exotic" or "introduced" or "alien" or "invasive") plant species, leading to a patchwork of native grasslands embedded in a nonnative dominated matrix (e.g., Wilsey et al. 2009, 2011). Novel ecosystems of newly arrived species are predicted to become more common in the future (Hobbs et al. 2006), with the largest increases in novelty in North America expected in the tallgrass prairie of the central United States, where our study was conducted (Martinuzzi et al. 2015). Rainfall outside the normal range in terms of amounts and intensity could also affect C accumulation (Kuebbing et al. 2013, Felton et al. 2018), and responses to rainfall could be interacting with exotic species spread. Nonnative dominated grasslands and savannahs may differ ecologically from native-dominated areas (Kuebbing et al. 2013, Fahey et al. 2018), especially with respect to soil C storage.

Perennial grasslands have a relatively deep distribution of soil C (Jobbágy and Jackson 2000), a characteristic that may be strongly altered following exotic species invasion due to the typically shallower root distribution of exotic than the native species that they replace (Wilsey and Polley 2006). Relatively few studies of plant impacts on soil C have been conducted at soil depths greater than 30 cm (Fisher et al. 1994, Conant et al. 2001, 2017, Ward et al. 2016, Hicks Pries et al. 2017, Ye and Hall 2019), although it has long been acknowledged that native prairie species root deeply (Weaver 1968). Jackson et al. (1996) found that the majority of roots in temperate grasslands were in the top 30 cm of soil, and close to 100% were in the top 100 cm. However, there was significant variation in rooting depth among studies. Ward et al. (2016) found that 60% of soil C was below 30 cm in European grasslands, echoing previous work at the global scale (Jobbágy and Jackson 2000). In a major literature review, it was noted that previous studies on grassland C had a median depth of 15 cm (mean 32 cm) as of 2001; this increased to only 20 cm (median; mean 44.5 cm) by 2017 (Conant et al. 2001, 2017). We know very little about impacts of nonnative plant species on soil C dynamics in deeper soil layers, and such mechanisms are missing in commonly used land ecosystem models (e.g., Community Land Model, and Century).

Roots and associated mycorrhizae in deeper soils could affect C pools, and this could be missed if only surface soils are considered.

We compared soil C contents between native and non-native (exotic) dominated grassland plots under two irrigation treatments in a long-term experiment in central Texas. With our experimental approach, we were able to compare native and nonnative grassland communities without the potentially confounding factors that can sometimes complicate observational comparisons. We established a factorial design of native vs. nonnative planting types \times two levels of rainfall (ambient and ambient + 128 mm per year applied during summer). Thus, we compared these plant community types under ambient and novel rainfall conditions under a common soil type, planting density, and management regime. We tested the hypotheses that (1) soil C differs between native and exotic plots, (2) summer irrigation during an annual dry period will alter soil C, and (3) organic and inorganic (carbonate) pools of C will respond differently to treatments.

METHODS

Experimental design and study site

We sampled the MEND (maintenance of exotic vs. native diversity) experiment to test our hypotheses. The experiment was conducted near Temple, Texas, USA in the southern part of the tallgrass prairie region. The site is a subhumid grassland that receives an average of 878 mm precipitation per year in a bimodal pattern with a peak in the spring and a smaller peak in fall. The soil type was Houston Black clay, a Vertisol (Udic Haplustert), which developed on calcareous shales from the Cretaceous age. Soil texture is 49–55% clay, and soils have a low leaching ability as a result (Fay et al. 2009). Potential evapotranspiration can greatly exceed precipitation during summer months. Soil pH averaged 7.5–7.8 and increased with depth (Upton et al. 2019). Carbonates in this soil were likely to be primarily derived from the parent material, with a very small contribution of pedogenic carbonate implied by $\delta^{13}\text{C}$ analyses (Nordt et al. 1998).

Treatments were randomly applied to plots in a factorial design that crossed native and exotic grassland plant treatments (origin) with summer irrigation treatments. A field was lightly disked to remove the standing vegetation (mostly the exotic grass King Ranch bluestem *Bothriochloa ischaemum*), and plot locations were established. The four treatments were established by planting 72 equal-sized transplants, of either all native plant species or all exotics, into 1-m² experimental plots within two blocks (one established in October 2007 and one in March 2008). Plots were populated with either nine native species or nine exotic species, with all functional groups (C₄ grasses, C₃ grasses, forbs, legumes) included in all plots (for details, see Wilsey et al. 2011, Xu et al.

2017). Half of the plots received summer irrigation of 128 mm per year applied each year between July 15 and August 15. Replication was achieved by including four random species draws within each block, with two replicates per treatment for a total of 2 origins (native or exotic) \times 2 irrigation levels (0 or 128 mm/yr) \times 8 draws \times 2 replicates = 64 plots. Species draws were produced by randomly selecting species from each functional group. Random draws ensured that differences would not be driven by a single species, and previous sampling found that any one of five grass species could be dominant in any given exotic plot (Wilsey et al. 2011, Xu et al. 2017). The plots were never fertilized, and were maintained by weeding volunteer plants when they were young through the eighth growing season, the sampling year of 2015. Alleyways between plots were dominated by seeded side oats gramma (*Bouteloua curtipendula*) and *Bothriochloa ischaemum*.

Soil carbon sampling

We sampled soils in 2008 at the beginning of the study (time 0, $n = 4$ cores) and after eight growing seasons (64 mixtures in October 2015) during the peak biomass period, which is October in this area. Sampling involved extracting 100-cm-deep soil cores (4.2-cm internal diameter) with a hydraulically driven steel coring tube with an acetate liner (Giddings Rig) from a random location within each plot. Cores were partitioned into 10-cm depth increments and samples were removed from each increment of each core. Thus, variables were estimated on 640 soil samples in total (64 mixture plots \times 10 depth increments). Total C and N were estimated with a combustion approach using a NA 1500 C/H/N Analyzer (Carlo Erba, Strumentazione, Milan, Italy) at the Stable Isotope Ecology Laboratory at the University of Georgia, Athens, Georgia, USA. Subsamples were frozen at -80°C for subsequent microbial analyses (Upton et al. 2019).

Organic C was calculated by subtracting carbonate C (determined as described below) from total C. Carbon stocks were expressed per square meter of surface area by multiplying C contents in cores by mean bulk density (g/cm³) in a given depth increment for the site, and then by scaling up the area of the core to square meters. Bulk density was estimated by collecting cores with a Giddings rig (4.2-cm internal diameter) from six randomly selected plots (three in each block), assuming that bulk density did not change during the study period. Cores were partitioned into 10-cm increments, dried at 105°C until dry, and were then weighed. Multiplication of bulk density by proportion C resulted in estimates of total C, SOC, and carbonate C per square meter.

$\delta^{13}\text{C}$ of CO₂ from incubated soil

To determine if contributions from C₃ or C₄ plant species to actively cycling soil C differed among treatments,

we measured $\delta^{13}\text{C}$ of soil respiration in vitro. Soil subsamples (~20 g dry mass equivalent) were thawed, and deionized water was added to achieve field capacity (i.e., moist but well-drained conditions; 0.37 g water/g soil) estimated from mean texture (11% sand, 50% clay) and organic matter content following Saxton and Rawls (2006). Samples were pre-incubated at 23°C for 7 d prior to gas measurements to avoid the period of transient $\delta^{13}\text{C}$ variation in soil respiration often observed following disturbance or wet-up of dry or frozen soils (Breecker et al. 2015, Hall et al. 2017, Ye and Hall 2019). Soils were incubated in 0.5-L glass jars capped with Viton gaskets and stainless steel lids equipped with two gas-tight Swagelok fittings connected to stainless steel tubing. These fittings provided an inlet and outlet for a carrier gas of humidified CO_2 -free air, which was produced by pumping ambient air through soda lime and bubbling through a gas-tight jar with deionized water. Prior to measurement, jars were flushed for 12 h at 80 mL/min to remove atmospheric CO_2 and achieve a steady-state diffusive flux of CO_2 produced from soil microbial respiration. Jars were connected to an automated 16-port sampling manifold in line with a tunable diode laser (TGA200A, Campbell Scientific, Logan, Utah, USA) for measurement of $\delta^{13}\text{C}$ of CO_2 as reported previously (Huang and Hall 2017). Individual samples were measured for 180 s, and $\delta^{13}\text{C}$ values were averaged over the last 120 s of measurement to remove transients following valve switching. Each soil was measured at least three times over a period of 144 min, or until consistent $\delta^{13}\text{C}$ values (within ~0.2‰) were obtained during consecutive analyses. The $\delta^{13}\text{C}$ values of CO_2 were calibrated following Hall et al. (2017), using three reference gases analyzed between every 12 soil samples, and expressed in ‰ notation relative to Vienna Pee Dee Belemnite.

Soil carbonate content and $\delta^{13}\text{C}$ values of carbonates

Soil carbonate C was measured directly from dried and ball mill ground soil subsamples by quantifying the CO_2 released following addition of 3M HCl to gas-tight vials (Amundson et al. 1988) which had previously been flushed with CO_2 -free air. The CO_2 produced after acidification and its $\delta^{13}\text{C}$ values were measured by direct injection to the TGA200A (Hall et al. 2017). Feasible contributions of different C sources to observed soil CO_2 production were calculated with a Bayesian mixing model analysis of $\delta^{13}\text{C}$ values using the *simmr* package in R (see SI for additional information; Parnell 2019).

Field soil CO_2 efflux

Soil CO_2 efflux (including respiration and possible contributions from inorganic C dissolution) was measured during the 2015 growing season with a LiCor 6400 infrared gas analyzer in a 10-cm-diameter ring placed near the center of each plot (Fay et al. 2009).

Temperature and soil moisture to 15-cm depth, which were measured with thermocouples and a handheld time domain reflectometry meter, respectively, did not differ among treatments. Soil respiration was measured in June and October in each plot, which corresponds to peak biomass for C_3 and C_4 plant species, respectively (Wilsey et al. 2011, Xu et al. 2017).

Statistical analyses

Data were analyzed as a split-plot mixed-model ANOVA, with the origin and irrigation treatments considered main plot fixed effect terms, and soil depth and interactions as subplot terms. Soil C variables were normally distributed after transformation (Appendix S1: Figs. S1–S4). Depth was considered as a continuous variable in all analyses, although results were similar when depth was considered categorical. Species draw and replication were random terms. Sequential (Type 1) sums of squares were used in *F* tests, and tests for interactions were made by comparing slopes. CO_2 efflux date was included in the model as a repeated-measures term. CO_2 efflux was ln transformed to achieve normality and low heteroscedasticity (Appendix S1: Fig. S5). To test for differences between time-zero cores ($n = 4 \times 10$ depths) and postexperiment cores ($n = 64 \times 10$ depths), a mixed model was conducted with before vs. after as a fixed effect, depth as a continuous variable, depth \times before vs. after interaction as a fixed effect, and rep and rep \times before vs. after as random terms. Analyses were done using SAS 9.4 (SAS, Cary, NC, USA).

RESULTS

Soil carbon

Soil carbon was higher at the beginning of the experiment than after eight growing seasons (Fig. 1). Time-zero soil C to 1-m depth was 112.3 kg/m² (SE = 2.0, $n = 4$), with 63.2 kg/m² as carbonate C (SE = 1.5) and 49.1 kg/m² as organic C (SE = 0.8). These values were all higher than they were at the end of the experiment, where soil C was 62.6 (SE = 0.6), carbonate C was 34.5 (SE = 0.5), and organic C was 28.1 kg/m² (SE = 0.4; all *P* values < 0.001).

After eight growing seasons, native plots had higher soil C stocks to 1-m depth (64.3 kg C/m², SE = 1.1) than did exotic plots (61.7 kg C/m², SE = 0.6), and these significant native–exotic differences (origin $F_{1,59} = 4.6$, $P = 0.036$) were consistent across irrigation treatments (Fig. 2, Table 1). Most of total C (78.3%) occurred below 20-cm depth in both native and exotic plots. Differences between native and exotic plots were greatest at deeper soil depths, particularly at 60–100-cm depth (native–exotic, depth interaction $F_{1,574} = 14.2$, $P < 0.0001$, Fig. 2). Total soil C differed negligibly between native and exotic plots in the top 10 cm, but was 10.9% higher under native than exotic plots in the

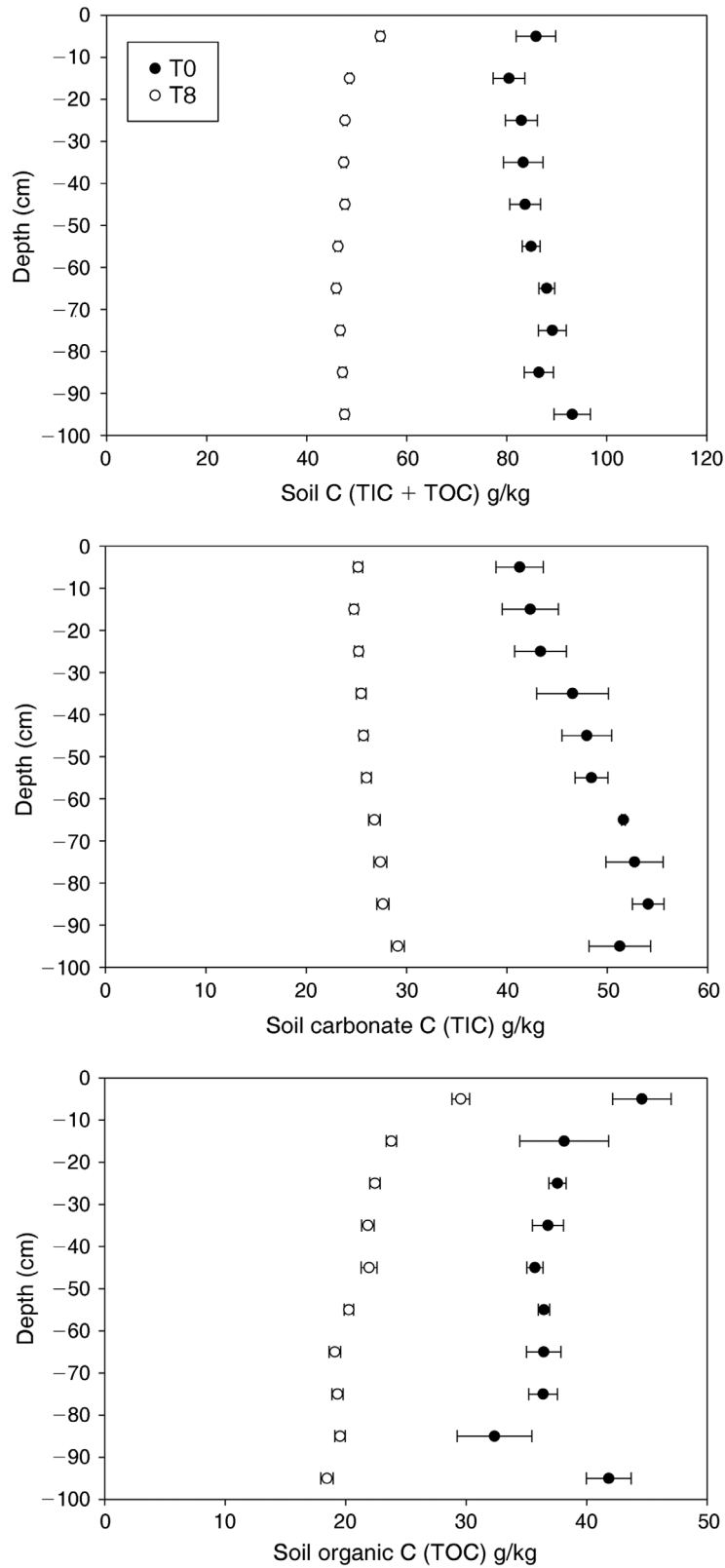


FIG. 1. Relationship between total soil C (TIC + TOC, top panel), carbonate C (TIC, middle panel), soil organic C (TOC, bottom panel) and depth (-100 cm) at the beginning (T0) and end (T8 growing seasons) of the study.

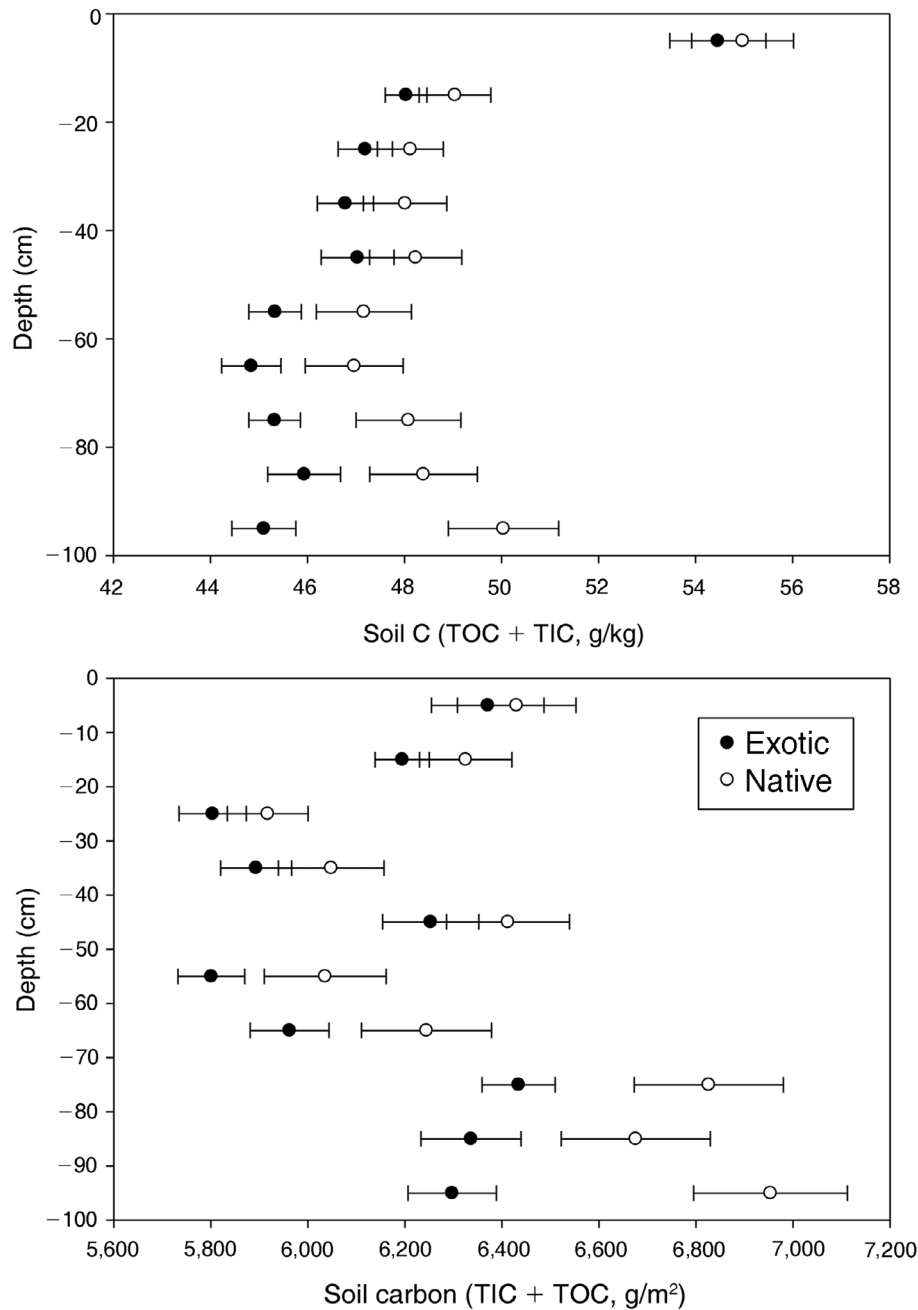


FIG. 2. Soil carbon (SIC + SOC in g/kg, means \pm SE in top panel, and g/m² in bottom panel) with depth under plantings of exotic or native plant species after eight growing seasons since establishment.

90–100-cm increment. Irrigation treatments had no significant effects on soil C (irrigated mean 62.5, SE = 0.90, nonirrigated mean 63.5, SE = 0.85, Table 1).

These differences in soil C were associated with a difference in the isotope composition ($\delta^{13}\text{C}$) of CO₂ emitted from incubated soils (Table 1). Soils from exotic plots had significantly greater $\delta^{13}\text{C}$ values of CO₂ than native plots ($\delta^{13}\text{C}$ values; native mean = -20.1‰ , exotic mean = -18.9‰ , $F_{1,52} = 13.6$, $P < 0.001$, Fig. 3).

Increased $\delta^{13}\text{C}$ values of CO₂ were consistent with greater contributions of C₄ biomass and/or carbonate C to CO₂ produced from the exotic soils (Appendix S1: Fig. S6). In both treatments, $\delta^{13}\text{C}$ of CO₂ declined significantly with depth ($F_{1,\ 565} = 44.9$, $P < 0.0001$, depth \times origin interaction $P > 0.1$).

Surprisingly, the change in soil C between native and exotic plots was due to changes in carbonates rather than organic C (Fig. 4). Greater than one-half of total

TABLE 1. Mixed-model ANOVA results for soil C, $\delta^{13}\text{C}$ of respired CO_2 , and carbonate C in response to the following planting treatments: plant composition (native origin or exotic origin), irrigation (128 mm added during July 15–August 15 yearly or 0 added as a control), and depth (1–100 cm in 10-cm depth increments)

	$\delta^{13}\text{C}$			Total C			Carbonate C		Organic C	
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Block	1			1						
Origin (Or.)	1	13.6	0.001	1	4.6	0.036	5.1	0.028	0.0	0.876
Irrig (Irr.)	1	1.4	0.249	1	0.9	0.353	0.1	0.905	1.5	0.219
Or. × Irr.	1	0.6	0.453	1	0.1	0.723	1.5	0.230	1.3	0.267
Error	53			59						
Depth (D)	1	44.9	<0.001	1	74.7	<0.001	600.7	<0.001	86.2	<0.001
D × Or.	1	2.7	0.100	1	14.5	<0.001	5.6	0.0186	1.8	0.183
D × Irr.	1	1.1	0.293	1						
D × Or. × Irr.	1	0.4	0.522	1						
Error	565			574						

Bold *P* values are significant at $P < 0.05$.

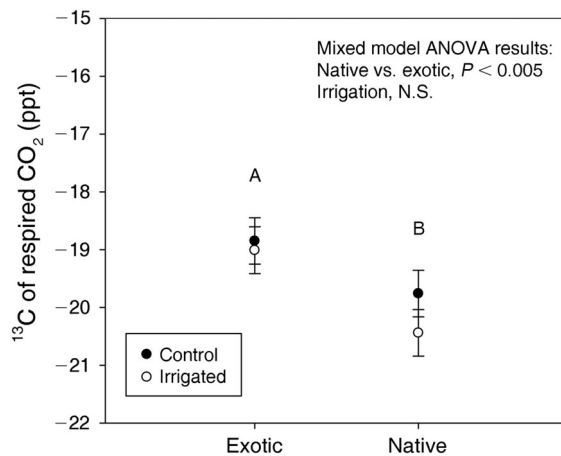


FIG. 3. $\delta^{13}\text{C}$ of CO_2 produced during laboratory incubations of soil sampled from plantings of exotic or native plant species after eight growing seasons since establishment (means + SE). Plant treatments were either irrigated during summer with 128 mm (Irrigated) or were nonirrigated (Control).

soil C was carbonate C. Carbonate C was significantly higher to 1-m depth in native (36.0 kg/m^2 , SE = 0.8) than exotic plots (33.5 kg/m^2 , SE = 0.7). Native–exotic differences in carbonates ($F_{1,59} = 5.1$, $P = 0.028$), grew larger with depth (depth × origin interaction: $F_{1,574} = 5.5$, $P < 0.019$; Fig. 4). Differences were largest from 50–100-cm depths (Fig. 4). Organic C did not differ significantly between native and exotic plots to 1-m depth (28.8 vs. 27.8 kg/m^2 , SE = 0.6 and 0.5 for native and exotic, respectively; Fig. 5). Neither carbonate C nor organic C were affected by the summer irrigation treatments (means [SE] for carbonate and organic C, respectively: irrigated: 34.8 [0.7] and 27.8 [0.6] kg/m^2 , nonirrigated: 34.7 [0.7] and 28.8 [0.6]). Across all samples, the $\delta^{13}\text{C}$ values of carbonate averaged $-0.7 \pm 0.6\text{‰}$ (mean and standard deviation), did not

differ between native and exotic plots, and showed minor variation with depth, decreasing from $-0.5 \pm 0.5\text{‰}$ at 0–10 cm to $-0.8 \pm 0.6\text{‰}$ at 90–100 cm.

SOIL CO_2 EFFLUX

Soil CO_2 efflux was 15 and 28% higher under exotic plots than under native plots during June and October sampling dates ($P < 0.01$, Table 2), respectively. On average, soil CO_2 efflux was higher in June ($5.47 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in exotic plantings vs. $4.98 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for natives) than in October ($2.45 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for exotic plantings vs. $1.92 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for natives; Fig. 6). Soil CO_2 efflux did not differ significantly between irrigated and nonirrigated plots, and there were no significant two- or three-way interactions in the model.

DISCUSSION

We found that differences in the geographic origin of plant species (native to the United States vs. introduced from other regions) led to differences in soil C contents under common environmental conditions in a replicated field experiment. Native-dominated grassland plots had significantly greater soil C to 1-m depth than exotic-dominated plots, after only eight growing seasons. Most significantly, the native vs. exotic difference in total C reflected a native vs. exotic difference in soil carbonate rather than organic C in a clay soil. Inorganic C is frequently ignored in soil C studies. Furthermore, many studies examining vegetation impacts on soil C are based on the surface layers of soil (Conant et al. 2011, 2017). The traditions of considering only organic C and focusing on surface soil layers are based, in part, on assumptions that carbonate C cycles very slowly, and that most soil C is in surface layers. Our results show that both of these assumptions were incorrect at our study site. This suggests the likelihood that they also are incorrect

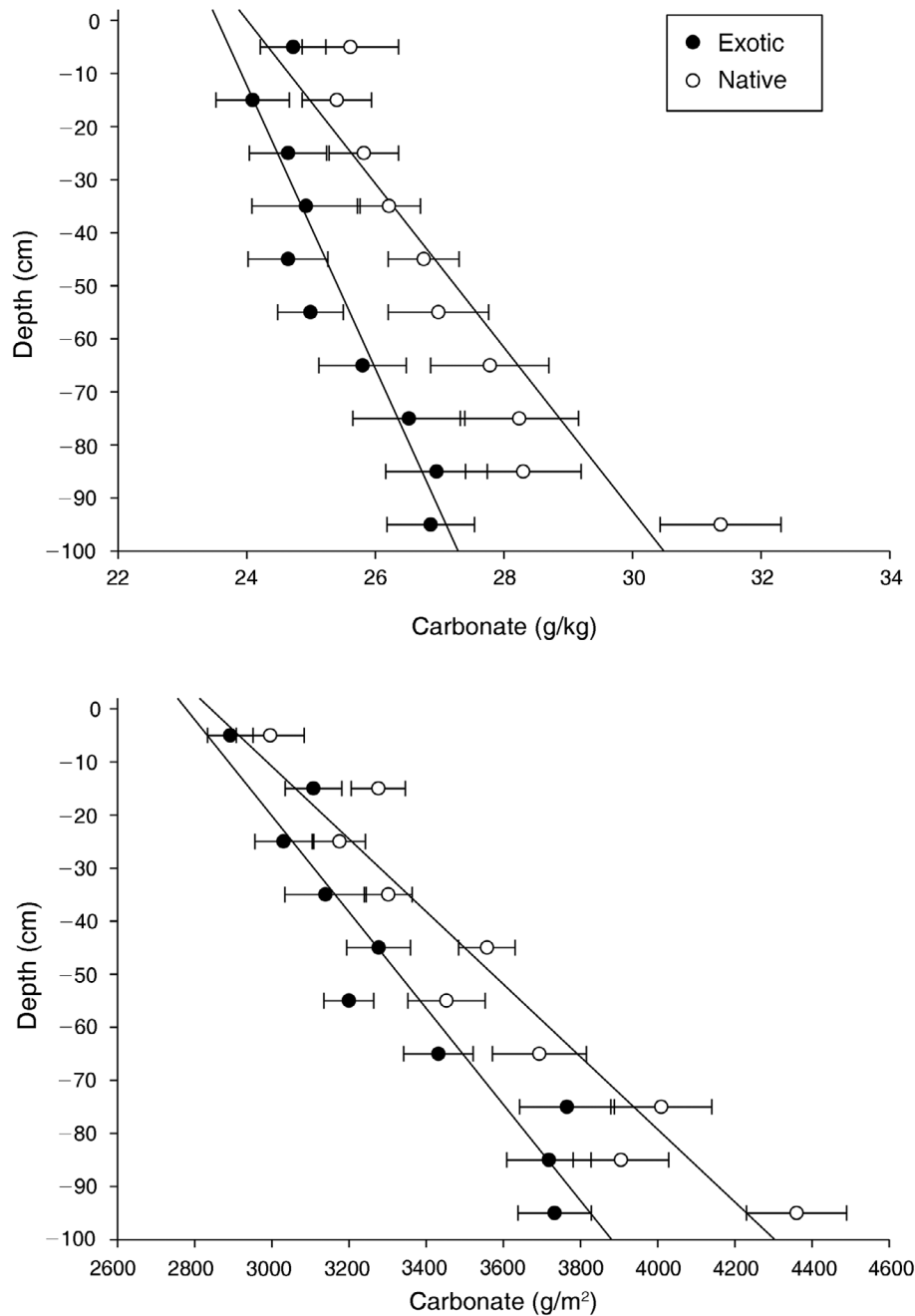


FIG. 4. Carbonate C (TIC g/kg, means \pm SE in top panel, and g/m^2 in bottom panel) with depth in experimental plots planted with all exotic or all native plant species in central Texas after eight growing seasons.

elsewhere, as carbonates are present in many surficial parent materials.

Earlier meta-analyses have found altered soil C contents in native/nonnative species comparisons (e.g., Liao et al. 2008), but, to our knowledge, our finding of a major change in inorganic C mediated by invasive plant species is new. Most of total C (78%) occurred below 20-cm depth in both native and exotic plots, which is typical

of deep prairie soils. Liao et al. (2008) developed a large meta-analysis of published studies and pointed out that most research sampled soils to a depth of 10 cm. Exotic species were found to increase soil C to 10-cm depth by approximately 7%, and most of these positive increases were found in woody species comparisons (Liao et al. 2008). The few studies on exotic grasses were inconsistent and depended on the rooting depth of the exotic

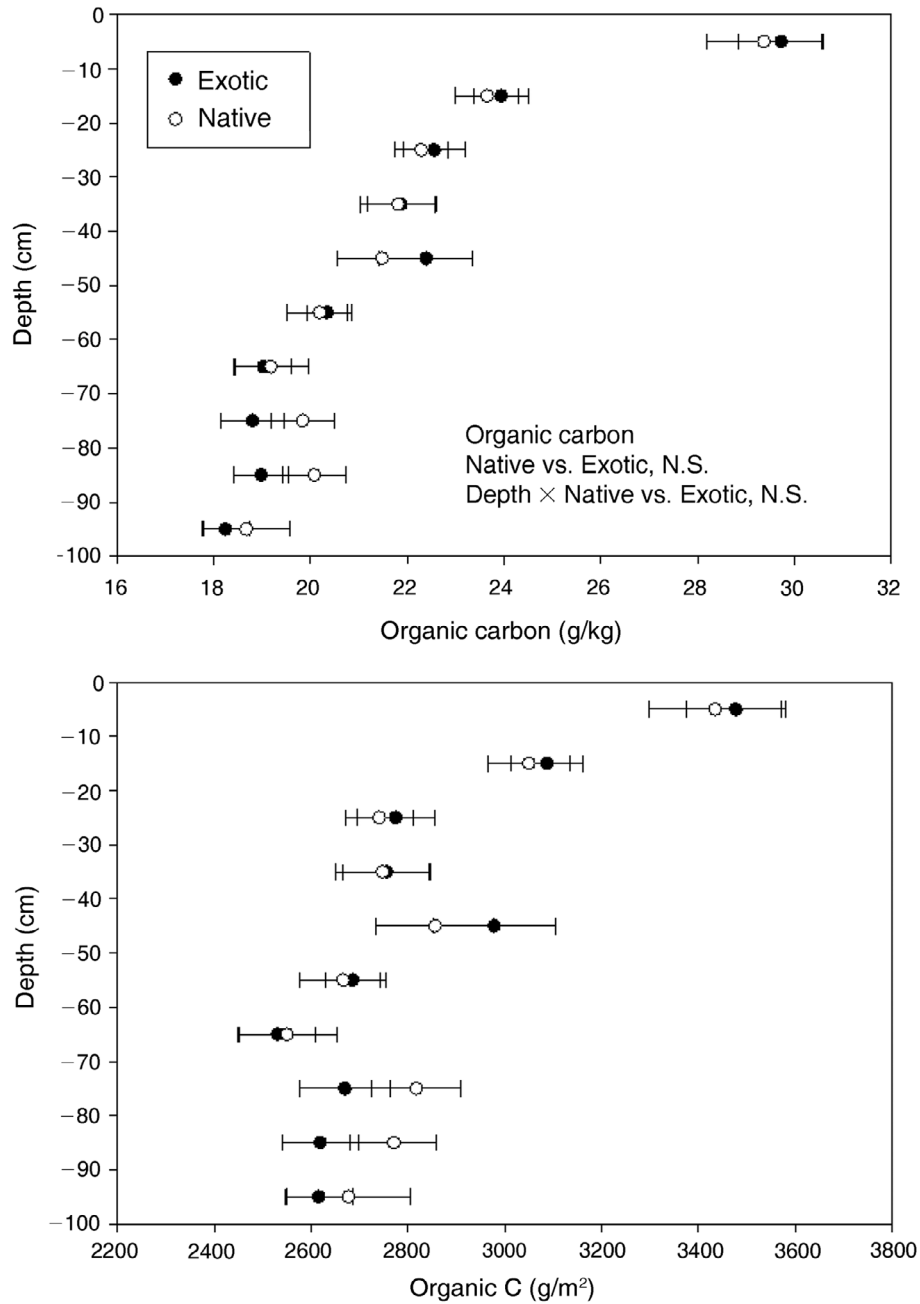


FIG. 5. Organic C (g/kg top panel and g/m² bottom panel) with depth in experimental plots planted with all exotic or all native plant species in central Texas after eight growing seasons.

species. Fisher et al. (1994) found that a deeply rooted grass invading Colombian savannah led to increased soil C accumulation. MacDougall and Wilson (2011) found that the shallowly rooted species *Agropyron cristatum* did not alter soil C even though it had higher root production than native species in mixed-grass prairie. Our exotic plots, which were strongly dominated by C₄ grasses (Martin et al. 2014), the common invaders in southern temperate and tropical regions (Sage and

Monson 1998, D’Antonio et al. 2001), had reduced soil C in deeper soil layers as a consequence of carbonate loss.

Total soil C and carbonate C decreased in both the native and exotic plots, likely as a consequence of soil disturbance when the experiment was established. This mimicked soil disturbances that commonly precede exotic plant establishment in grasslands (Hobbs and Huenneke 1992). Our results indicate that the impact of exotic

TABLE 2. Mixed model ANOVA results for soil CO₂ efflux ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}$) under plantings of all native or all exotic plant species (North American origin or exotic origin), that were irrigated yearly at 128 or 0 mm in summer.

	R _{soil}		
	df	F	P
Block	1		
Origin (Or.)	1	7.3	0.009
Irrig (Irr.)	1	0.3	0.58
Or. × Irr.	1	0.4	0.55
Error	59		
Date (D)	1	237.6	<0.0001
D × Or.	1	1.2	0.28
D × Irr.	1	2.1	0.15
D × Or. × Irr.	1	0.8	0.37
Error	60		

Note: Plots were sampled in June and October of 2015 (eighth growing season). Bold *P* values are significant at $P < 0.05$.

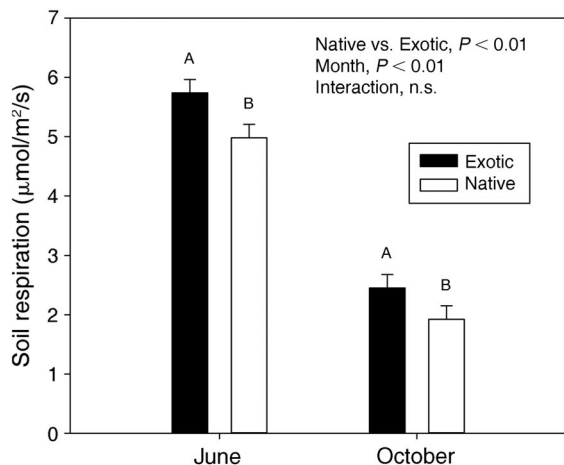


FIG. 6. Soil CO₂ efflux in June and October of 2015 during the eighth growing season of the study. Plots were planted with all exotic or all native plant species, with and without summer irrigation (0, 128 mm). Irrigation treatment and interactions were not significant, so data were averaged across irrigation treatments for presentation (letters above bars denote significant differences between native and exotic treatments).

species on inorganic C stocks was mediated by disproportionate losses. This interpretation is bolstered by the fact that $\delta^{13}\text{C}$ values of inorganic C did not vary between soils under native and exotic species and varied little with depth. If the soil carbonate pool was markedly influenced by exchange with contemporary CO₂ over the course of the experiment, we would expect variation in the $\delta^{13}\text{C}$ values of inorganic C as a function of treatments and depth. Specifically, recently formed carbonate originating from soil CO₂ should have a $\delta^{13}\text{C}$ value approximately 15‰ greater than soil-respired CO₂ (Cerling et al. 1989). Given that $\delta^{13}\text{C}$ of soil-respired CO₂ averaged -19.5‰ , we would expect $\delta^{13}\text{C}$ values of carbonate formed via reprecipitation of soil CO₂

(approximately -4.5‰) to be systematically lower than the observed values of bulk soil inorganic C ($-0.7 \pm 0.6\text{‰}$).

Impacts of soil inorganic C losses on regional C budgets hinge critically on the mechanism(s) of carbonate dissolution and the fate of dissolved inorganic C. We suggest a possible mechanism behind the change in carbonates in response to differences in native vs. exotic differences in plant species composition. Higher belowground productivity in exotic plots could have led to greater production of carbonic acid via rhizosphere respiration and of organic acids via root and microbial exudates (Boldt-Burisch et al. 2019), leading to the dissolution of carbonates to bicarbonate, and ultimately CO₂. We did find that carbonate mass was higher in all treatments at the beginning of our study, and that its mass was reduced after eight growing seasons, which is consistent with carbonate loss as CO₂. Soil CO₂ efflux (comprised of both soil respiration and inorganic C release) was significantly higher in soils from exotic plots, and C₄ grasses more strongly dominated the exotic plots (Martin et al. 2014), which could have led to greater carbonic acid production. Belowground productivity to 45 cm in root ingrowth cores was significantly higher in exotic than native plots (Xu et al., *unpublished data*), and root biomass production positively correlates with organic acid production (Pausch and Kuzyakov 2017, Boldt-Burisch et al. 2019). A shift to productive C₄ grasses, higher root production, and associated organic acid production could have dissolved carbonate at a greater rate under exotic than native plots.

An alternative explanation is that inorganic C was redistributed below the sampling depth (1 m), and we cannot completely rule this out at this time. A study in southern Spain found that approximately half of the soil inorganic C was present below 1-m depth, suggesting the possible importance of even deeper sampling (Diaz-Hernandez et al. 2003). The reaction of carbonic acid with carbonate to form two moles of bicarbonate can serve as a regional C sink when bicarbonate is exported to surface and/or groundwater (Raymon and Cole 2003, Lapeñis et al. 2008). However, this mechanism was not consistent with our results for two reasons. First, unless all carbonates were leached below 1-m depth, leaching of bicarbonate to deeper soil layers would result in exotic plots having a trend of *greater* carbonate than native plots at greater soil depths, which was opposite of our results. Second, the high clay soils and subhumid climate at our site tend to reduce leaching to soil depths beyond 1 m. Fay et al. (2009) found that drainage in our high clay soils was virtually zero, and concluded that “drainage is a negligible portion of the water budget.” A more plausible explanation is that differences in acid production between native and exotic species mixtures drove differential carbonate loss to the atmosphere as CO₂. Future studies should test these and other mechanisms behind carbonate formation and dissolution, especially in deeper soils.

Differences in total soil C were substantial in the context of total soil C stocks and the relatively short duration (8 yr) of this experiment, and even more significant if these processes apply to many deep Mollisols and Vertisols of the central United States, or other grassland soils of the world. Approximately 4% of the tallgrass prairie region remains as native prairie (Samson and Knopf 1994). Based on our results, preventing the conversion of the 26% of the former tallgrass prairie region that is now exotic-dominated pasture (Lubowski et al. 2006) might have prevented the loss of 48.18 Tg C (2,769 kg/ha \times 17.4 million ha). Carbonates average 1/3 of total soil C globally (Batjes 1996) and can be even higher in arid and subhumid environments. Our subhumid system had one-half of its total soil carbon as carbonates. High soil carbonate concentrations are typical of calcareous grasslands throughout Europe (Fischer and Stocklin 1997), the United States, and other locations (Huber et al. 2019). If inorganic carbon is an active pool, as our results indicate, then substantial stocks of soil C may be cycling unknowingly.

In conclusion, our results indicate that the carbonate fraction is an active soil C pool and can respond to differences in plant species composition over timescales of years. Significant soil C losses might be avoided by replacing exotic plant species with native species in the southern plains of the United States and on similar soils worldwide. Had we restricted our analyses to surface soil layers (e.g., top 30 cm) and only organic C, we would have failed to detect differences between natives and exotics.

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DATA AVAILABILITY

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.v6wwpzgrx>.