12-21-2020

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Recommended Citation

Multiple constraints cause positive and negative feedbacks limiting grassland soil CO2 efflux under CO2 enrichment Philip A. Fay, Dafeng Hui, Robert B. Jackson, Harold P. Collins, Lara G. Reichmann, Michael J. Aspinwall, Virginia L. Jin, Albina R. Khasanova, Robert W. Heckman, H. Wayne Polley Proceedings of the National Academy of Sciences Jan 2021, 118 (2) e2008284117; DOI: 10.1073/pnas.2008284117

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Multiple constraints cause positive and negative feedbacks limiting grassland soil CO_2 efflux under CO_2 enrichment

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Edited by James J. Elser, University of Montana, Polson, MT, and approved November 6, 2020 (received for review May 7, 2020)

Terrestrial ecosystems are increasingly enriched with resources such as atmospheric CO_2 that limit ecosystem processes. The consequences for ecosystem carbon cycling depend on the feedbacks from other limiting resources and plant community change, which remain poorly understood for soil CO_2 efflux, J_{CO2}, a primary carbon flux from the biosphere to the atmosphere. We applied a unique CO_2 enrichment gradient (250 to 500 $\mu L\ L^{-1}$) for eight years to grassland plant communities on soils from different landscape positions. We identified the trajectory of J_{CO2} responses and feedbacks from other resources, plant diversity [effective species richness, exp(H)], and community change (plant species turnover). We found linear increases in J_{CO2} on an alluvial sandy loam and a lowland clay soil, and an asymptotic increase on an upland silty clay soil. Structural equation modeling identified CO_2 as the dominant limitation on J_{CO2} on the clay soil. In contrast with theory predicting limitation from a single limiting factor, the linear J_{CO2} response on the sandy loam was reinforced by positive feedbacks from aboveground net primary productivity and exp(H), while the asymptotic J_{CO2} response on the silty clay arose from a net negative feedback among exp(H), species turnover, and soil water potential. These findings support a multiple resource limitation view of the effects of global change drivers on grassland ecosystem carbon cycling and highlight a crucial role for positive or negative feedbacks between limiting resources and plant community structure. Incorporating these feedbacks will improve models of terrestrial carbon sequestration and ecosystem services.

tallgrass prairie | soil respiration | productivity | biodiversity | CO_2 enrichment

Terrestrial ecosystems are increasingly enriched with resources that limit ecosystem function and carbon cycling, such as atmospheric carbon dioxide (CO_2) from fossil fuel combustion and land use conversion (1, 2). Soil CO_2 efflux (J_{CO2}), the diffusion of CO_2 from soil to the atmosphere, is a large and increasing feedback on the atmospheric carbon balance (3–5) closely linked to primary productivity and expected to increase with CO_2 enrichment (6). The trajectory of the CO_2 response of J_{CO2} remains poorly understood but is crucial to ecosystem carbon cycling in a changing climate. CO_2 meets the classic operational definition of a limiting resource (7): experimental enrichment with CO_2 often increases rates of ecosystem processes involved in carbon cycling, including J_{CO2} (6). Theory suggests a linear increase in J_{CO2} with CO_2 enrichment if other constraints remain constant. In contrast, diminishing increases in J_{CO2} with CO_2 enrichment, yielding an asymptotic J_{CO2} response, may occur if the next most limiting factor constrains the CO_2 effect by imposing a negative feedback on the J_{CO2} increase (8–10). However, few long-term studies implement enough CO_2 levels to resolve the shape of the J_{CO2} response.

The factors that may impose a negative feedback on the response of J_{CO2} to CO_2 enrichment fall into two classes. First, the CO_2 response of J_{CO2} may be constrained by other resources: water, mineral nutrients, or light. For example, CO_2 effects on J_{CO2} may diminish if plant productivity becomes limited by water or nutrients (11, 12) or may be reinforced if enrichment reduces water limitation by increasing soil moisture, resulting in higher aboveground net primary productivity (ANPP), vegetative cover, and light interception (13, 14). Second, changes in resource availability can drive changes in plant community composition (15). Species turnover is a broad descriptor of compositional change encompassing changes in species richness due to immigration and extinction and reordering of species abundances within a community. Many studies link turnover or its elements to ANPP (16–20), and turnover commonly occurs when resources are added, especially in communities limited by multiple nutrients (e.g., ref. 21). Productivity, resources, and community composition affect each other through feedback mechanisms (22, 23). Multiple constraints and feedbacks on the CO_2 response of J_{CO2} have received little attention (24, 25) but are crucial to forecast the consequences for ecosystem carbon cycling and related ecosystem services.

Significance

Understanding ecosystem carbon-cycling responses to atmospheric CO_2 enrichment is critical to preserve biodiversity and maintain vital ecosystem services in grasslands impacted by global change. We conducted an 8-y experiment enriching CO_2 concentrations from preindustrial to midtwenty-first century levels on grassland plant communities on upland, lowland, and alluvial soils. CO_2 enrichment increased the CO_2 efflux from soils to atmosphere in amounts depending on whether the dominant limitation was CO_2 or feedbacks (net positive or negative) from soil moisture and plant species turnover. These findings highlight how multiple concurrent limitations, not single limitations in sequence, regulate the impacts of global change drivers in diverse grasslands. Incorporating multiple limitations will improve forecasts of terrestrial carbon sequestration and ecosystem services.

Author contributions: P.A.F., R.B.J., and H.W.P. designed research; P.A.F., D.H., H.P.C., L.G.R., M.J.A., V.L.J., A.R.K., and H.W.P. performed research; P.A.F. and R.W.H. analyzed data; P.A.F. wrote the paper; and all coauthors contributed to manuscript writing.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2008284117/-/DCSupplemental.

Published December 21, 2020.

Feedbacks on the J_{CO2} response to CO_2 are likely to vary with edaphic factors that influence resource availability, productivity, plant community structure, and decomposition. Soils along catenas share the same climate but vary in texture, water holding capacity, organic matter content, ANPP, and plant community structure (26, 27); thus, they may vary in both autotrophic and heterotrophic contributions to J_{CO2}. For example, coarse-textured soils have lower nutrient and water holding capacity (28), less soil organic matter (27, 29), and larger pore spaces permitting easier diffusion of gases (30). Fine-textured soils tend to have higher labile C pools and faster rates of root growth and root litter decomposition (31, 32). Therefore, studying soils of varying properties from different positions in landscapes is necessary to delineate variation in the constraints and feedbacks on the J_{CO2} response to CO_2 enrichment and to predict ecosystem responses across landscapes, where the aggregate response across multiple soil types may differ from that of any one soil type.

Here, we examined the trajectory of the J_{CO2} response to CO_2 using a unique continuous CO_2 enrichment gradient with CO_2 levels spanning preindustrial ($250\ \mu L\ L^{-1}$) to late twenty-first century levels ($500\ \mu L\ L^{-1}$). Specifically, we asked the following questions. 1) What is the trajectory of J_{CO2} in response to CO_2 enrichment, and does it correspond to that of ANPP? 2) Is the J_{CO2} trajectory mediated by CO_2-related changes in other resources or the plant community? 3) Does the J_{CO2} trajectory and its mediation differ among soils of contrasting properties representing differing landscape positions? We addressed these questions in grassland plant communities established on soil series from upland, lowland, and alluvial landscape positions that differed in texture, water holding capacity, and nitrogen mineralization rates among other properties (Table 1). These soils are from the orders Alfisols, Mollisols, and Vertisols, which are dominant soils in grassland biomes across large areas of North America and Eurasia (28). The Mollisol we studied is typical of soils common in arid and semiarid biomes (33).

Plant communities were experimentally established on these soils and maintained on a CO_2 concentration gradient spanning preindustrial to anticipated midtwenty-first century values (34, 35). Gradient designs are the preferred approach for identifying trajectories in responses to continuous environmental drivers (36). Previous studies revealed that a decade of CO_2 enrichment resulted in soil-specific increases in ANPP and C_4 grass dominance (37), accompanied by increases in soil moisture, fast-cycling soil organic carbon pools (38), decomposition rates, microbial biomass, fungal richness and abundance, and microbial enzyme activities (39, 40). Based on these findings, we hypothesize increased J_{CO2} in response to CO_2 enrichment and stronger increases in J_{CO2} when CO_2 enrichment is reinforced by positive feedbacks from other limiting resources, increases in ANPP, or species turnover toward more productive dominant grass species (41–44). However, gains in J_{CO2} may also be offset by negative feedbacks from decreased species richness (45–47).

Results

J_{CO2}. As hypothesized, CO_2 enrichment resulted in J_{CO2} increases differing in both shape and magnitude among the three soil series (soil × CO_2 $P = 0.01$) (Table 2). J_{CO2} was a linear increasing function of CO_2 on the alluvial sandy loam and lowland clay soils (Fig. 1*A*). However, J_{CO2} was an asymptotic function of CO_2 on the upland silty clay soil ($P = 0.0013$). As a result, mean J_{CO2} was lower on the silty clay compared with the other soils ($P = 0.0009$) (Fig. 1 *A*, *Inset* and Table 2). J_{CO2} was unrelated to CO_2 ($P = 0.73$) (Table 2) for the soils combined. The J_{CO2} response to CO_2 was consistent among years (year effects $P = 0.41$ to 0.69) (Table 2 and *SI Appendix*, Fig. S1). These results held whether the soil effect was modeled with soil texture as a covariate or with soil as a categorical variable, with the latter yielding better model fit (*SI Appendix*, Table S2).

CO_2 Responses of ANPP, Resources, and Community Change. CO_2 enrichment caused a distinct set of linear responses in the hypothesized drivers of the J_{CO2} response on each soil (soil × CO_2 $P < 0.02$) (Table 2). For the two soils with linear J_{CO2}–CO_2 responses, the alluvial sandy loam and lowland clay, ANPP was high, and photosynthetic photon flux density (PPFD) at the soil surface was low (soil $P < 0.04$) (Fig. 1 *A*, *Inset* and *D*, *Inset* and Table 2). However, soil water potential (Ψsoil) was higher in the sandy loam than clay soil (soil $P < 0.0001$) (Fig. 1 *C*, *Inset* and Table 2). On the sandy loam, CO_2 enrichment increased ANPP (Fig. 1*B*), modestly increased Ψsoil (Fig. 1*C*), and increased species turnover (Fig. 2*A*) while decreasing effective species richness, exp(H) (Fig. 2*B*). On the lowland clay, CO_2 enrichment resulted in a weaker increase in ANPP and a stronger increase in Ψsoil (Fig. 1 *B* and *C*). Species turnover and exp(H) were not correlated with CO_2 on the clay soil (Fig. 2).

On the upland silty clay soil where the J_{CO2}–CO_2 response was asymptotic, mean ANPP was the lowest of the three soils despite high mean Ψsoil (Fig. 1 *B*, *Inset* and *C*, *Inset*). CO_2 enrichment caused increases in ANPP, Ψsoil, and species turnover comparable

Table 1. Classification and physical properties of the three soils in the LYCOG facility

	Soil series		
Soil property	Austin	Bastsil	Houston Black
Order	Mollisol	Alfisol	Vertisol
Texture class	Silty clay	Sandy loam	Clay
Sand, %	12	67	10
Silt, %	45	24	38
Clay, %	43	9	52
Organic carbon, %	1.4	0.4	2.0
Organic matter, %	2.1	3.2	3.5
Bulk density, $Mg\ m^{-3}$	1.24	1.48	1.21
Field capacity (Θ_{33}), $m^3\ m^{-3}$	0.41	0.17	0.43
Permanent wilting point ($\Theta_{1,500}$), $m^3\ m^{-3}$	0.26	0.07	0.30
NRCS/National Map Unit symbol	AsC/2vtgk	BaA/2vtj2	HoB/2ssh0
Collection site			
Latitude	31.045297	31.245306	31.457284
Longitude	−97.349303	−97.467884	−96.877026

NRCS, Natural Resources Conservation Service.

Table 2. Results of linear mixed models

	$\ln(J_{CO2})$		ANPP		Ψsoil		PPFD		Exp(H)	
Effect	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Soil (S)	$9.0_{2,28}$	0.0010	$6.1_{2,28}$	0.0063	$92.5_{2,28}$	<0.0001	$3.8_{2,28}$	0.0337	$26.2_{2,28}$	<0.0001
CO_2 (C)	$0.1_{1,171}$	0.7280	$90.6_{1,172}$	<0.0001	$47.1_{1,172}$	<0.0001	$21.7_{1,152}$	<0.0001	$4.9_{1,172}$	0.0282
C × S	$4.6_{2,171}$	0.0113	$4.0_{2,172}$	0.0195	$25.2_{2,172}$	<0.0001	$2.2_{2,152}$	0.1150	$27.7_{2,172}$	<0.0001
Year (Y)	$0.8_{7,171}$	0.5479	$2.3_{7,172}$	0.0265	$6.9_{7,172}$	<0.0001	$5.1_{7,152}$	<0.0001	$0.5_{7,172}$	0.8665
S × Y	$1.1_{14,171}$	0.4054	$0.9_{14,172}$	0.5623	$6.4_{14,172}$	<0.0001	$0.4_{14,152}$	0.9772	$0.5_{14,172}$	0.9027
C × Y	$0.9_{7,171}$	0.5118	$4.0_{7,172}$	0.0005	$3.4_{7,172}$	0.0018	$2.9_{7,152}$	0.0067	$0.5_{7,172}$	0.8126
C × S × Y	$0.8_{14,171}$	0.6869	$1.1_{14,172}$	0.3519	$3.6_{14,172}$	<0.0001	$0.4_{14,152}$	0.9697	$0.3_{14,172}$	0.9866

Results of linear mixed models analysis of the effects of soil, CO_2 enrichment, year, and their interactions on J_{CO2}, ANPP, Ψsoil, and exp(H) for the three soil series along the CO_2 gradient during 2006 to 2014. Subscripts of *F* statistics are degrees of freedom for numerator,denominator.

with those of the sandy loam (Figs. 1 *B* and *C* and 2*A*) but increased exp(H), the opposite response of the sandy loam (Fig. 2*B*).

For the three soils in aggregate, CO_2 increased ANPP, Ψsoil, and species turnover while decreasing PPFD (Fig. 1*D*). The mixed models analysis indicated a CO_2 response in exp(H) (P = 0.028) (Table 2), but the slope was small (0.0009) (*SI Appendix*, Table S1).Species turnover in response to CO_2 enrichment was largely explained ($R^2 = 0.79$, $P < 0.0001$) by the increase in abundance of *Sorghastrum nutans* relative to the abundance of *Bouteloua curtipendula* (Fig. 3*A*), but was not correlated with change in exp(H) ($P > 0.74$, Fig. 3*B*).

Combined Effects. Structural equation models resolved how the simultaneous CO_2 effects on ANPP, Ψsoil, turnover, and exp(H) combined on each soil to jointly predict the CO_2 responses of J_{CO2}. Structural equation model fit was adequate on each soil ($P > 0.58$) (Table 3). On the alluvial sandy loam and the lowland clay, the structural equation models resolved the CO_2 response of ANPP as the largest single predictor of the J_{CO2}–CO_2 response (Fig. 4 and *SI Appendix*, Table S3), consistent with the shared linear CO_2 responses of J_{CO2} and ANPP (Fig. 1 *A* and *B*). On the sandy loam, total effects of species turnover and exp(H) on J_{CO2} were positive (Fig. 5). The species turnover effect was mediated by ANPP, reinforcing the ANPP–J_{CO2} relationship (Fig. 4). The direct exp(H)–J_{CO2} path (0.87) was nearly as large as the direct ANPP–J_{CO2} path (0.90), but exp(H) concurrently caused a negative feedback on J_{CO2} through a negative exp(H)–ANPP effect (−0.40) (Fig. 4). The structural equation model for the clay soil was notable for a lack of effects, leaving turnover mediated by ANPP as drivers of J_{CO2} responses to CO_2.

The silty clay structural equation model differed from the other structural equation models in several respects. Standardized total effects and individual path coefficients were generally

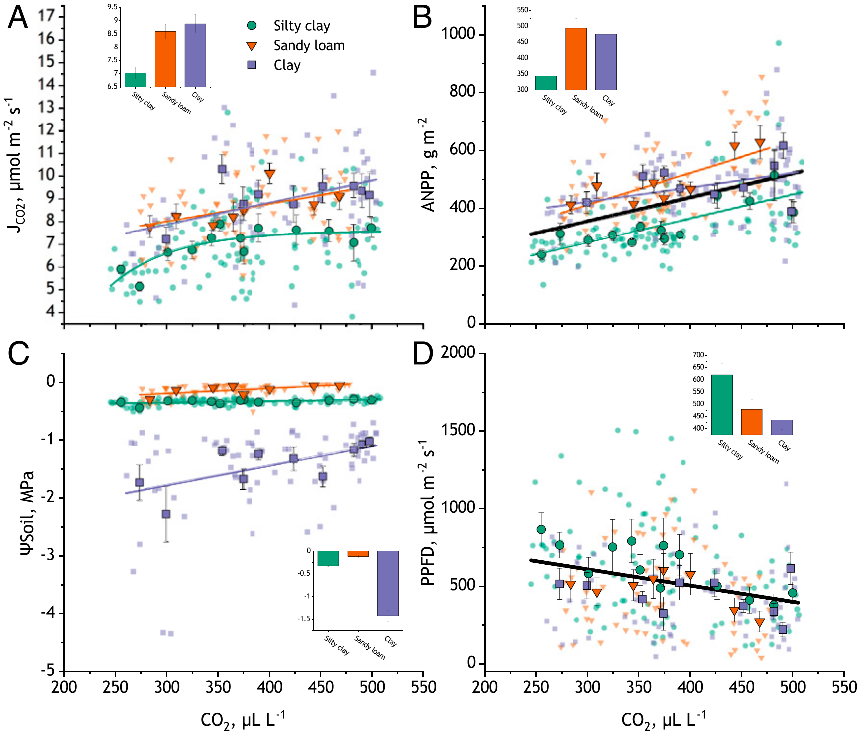

Fig. 1. (*A*) J_{CO2}, (*B*) ANPP, (*C*) Ψsoil, and (*D*) PPFD in relation to atmospheric CO_2 concentration on silty clay, sandy loam, and clay soil series. Large symbols represent the mean ± SE across 8 y of CO_2 treatments. Small symbols represent values for individual years. Lines denote significant regression relationships for individual soils (color) or for all soils combined (black). *Insets* depict means ±1 SE across years and CO_2 levels. Table 2 shows linear mixed model statistics, and *SI Appendix*, Table S1 shows regression parameters and statistics.

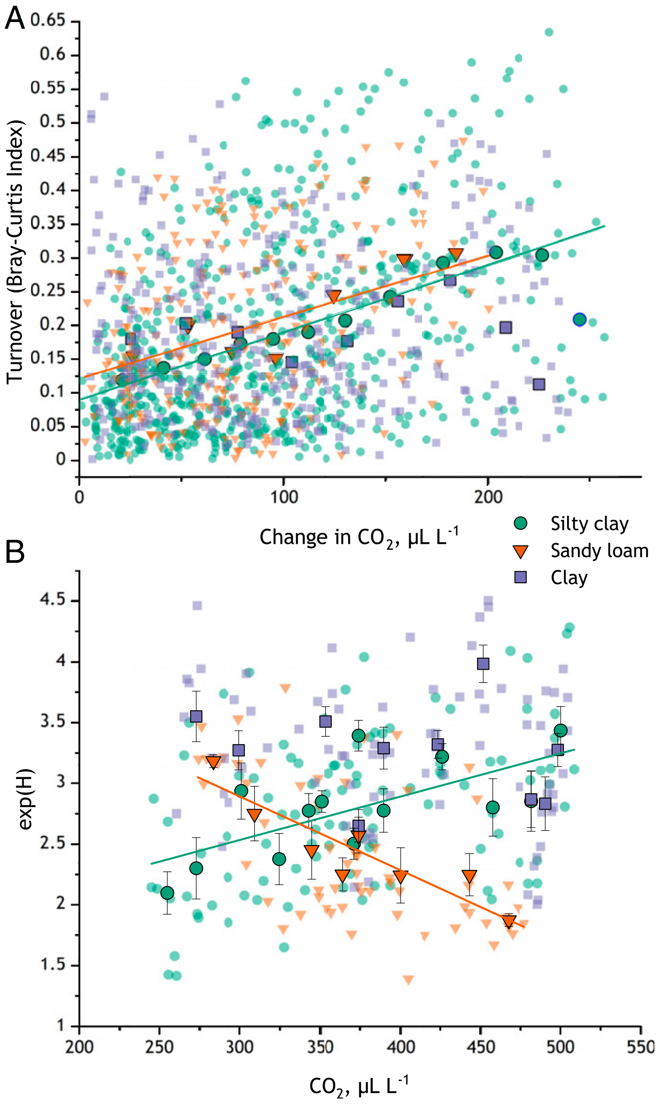

Fig. 2. Community diversity and composition as functions of atmospheric CO_2 concentration on silty clay, sandy loam, and clay soils. (*A*) Plant species turnover (Bray–Curtis index) per unit change in CO_2 in relation to the difference in CO_2 between all pairwise combinations of monoliths in each soil series. Linear mixed models soil effect $P = 0.0012$. (*B*) exp(H) in relation to CO_2 concentration. Large symbols with error bars represent means ±1 SE over 8 y of CO_2 treatments. Small symbols represent data for individual years. *SI Appendix*, Table S1 shows regression parameters and statistics.

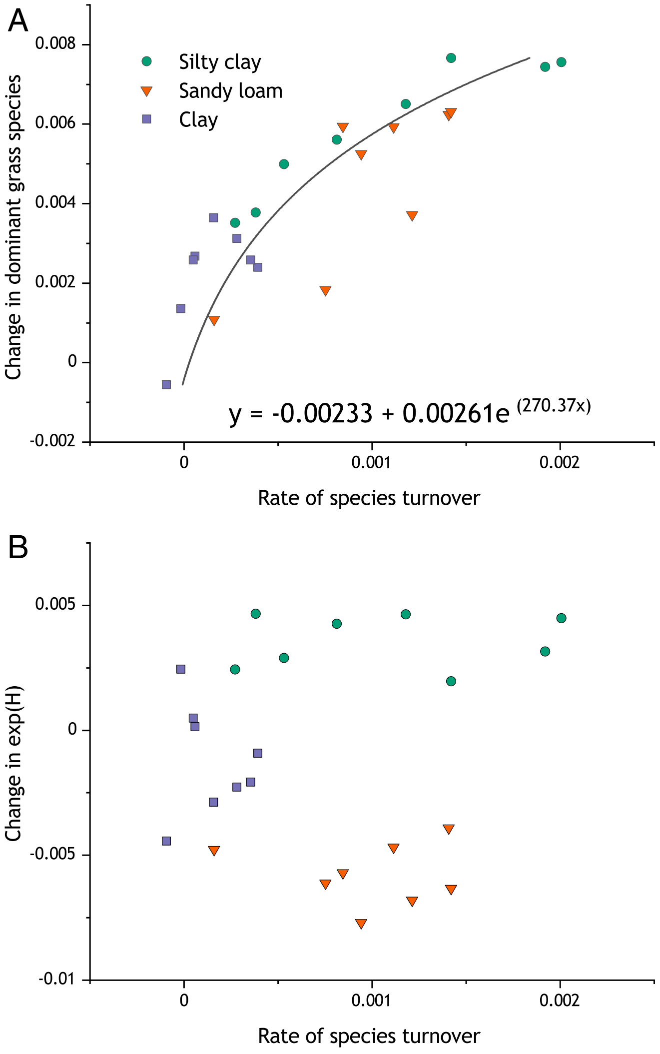

Fig. 3. Rates of change in response to CO_2 enrichment in (*A*) dominant grass species and (*B*) exp(H) as a function of the rate of plant species turnover in response to CO_2 enrichment. Each datum represents the slope of the CO_2 relationship for a single year.

larger (Fig. 5 and *SI Appendix*, Table S3), although no direct ANPP–J_{CO2} path was resolved (Fig. 4). Positive turnover–J_{CO2} and Ψsoil–J_{CO2} paths suggest that species turnover and soil moisture reinforced CO_2 effects on J_{CO2}. However, a negative exp(H)–J_{CO2} path suggests a concurrent negative feedback acting directly on the CO_2 response of J_{CO2}, not indirectly as for the sandy loam.

Discussion

The effects of atmospheric CO_2 enrichment on terrestrial carbon cycles depend on the concurrent effects of CO_2 on ecosystem function, the availability of other limiting resources, and changes in plant community diversity and composition. Our findings show how CO_2 effects on J_{CO2} depend on concurrent responses in multiple limiting factors that combined to yield either positive or negative feedbacks on J_{CO2}. This fundamentally challenges the current paradigm that ecosystem functions in grassland plant communities are constrained by sequential limitation from single resources (8–10). Importantly, the findings demonstrate how CO_2-mediated feedbacks on J_{CO2} varied in magnitude and direction among soils representing upland, lowland, and alluvial landscape positions and soil orders commonly supporting grassland biomes. J_{CO2} is the main avenue of C loss to the atmosphere in this temperate perennial grassland. Understanding the controls on the response of J_{CO2} to CO_2 enrichment is crucial to more

Table 3. Structural equation model (Fig. 4) fit statistics for each soil series

	Model fit		
	χ^2 (*P* value)	RMSEA	CFI
Soil series	$P > 0.05$	$P < 0.06$	$P > 0.95$
Silty clay	0.2177 (0.6408)	0.0000	1.0000
Sandy loam	1.0937 (0.5788)	0.0000	1.0000
Clay	0.1751 (0.9162)	0.0000	1.0000

Bentler CFI (1).

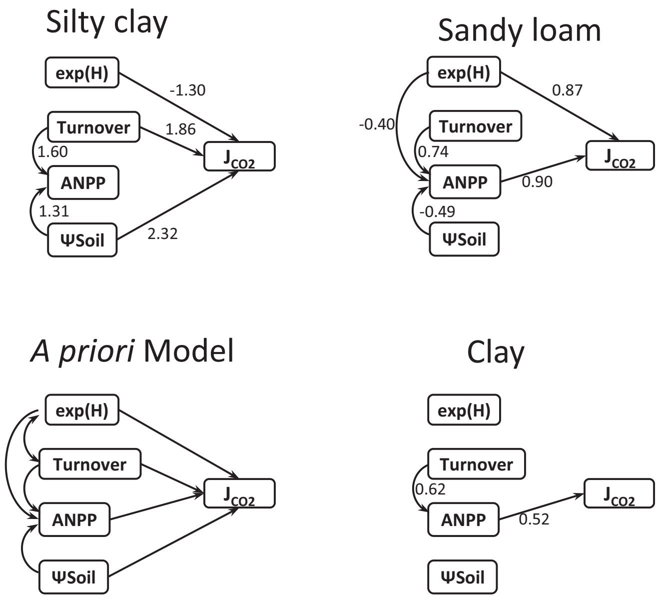

Fig. 4. Structural equation models relating the CO_2 responses of 0- to 40-cm soil water potential (Ψsoil), aboveground net primary productivity (ANPP), turnover in community composition, and effective species richness, exp(H) to the CO_2 response of soil CO_2 efflux, J_{CO2}. The a priori model was fit separately to the individual soil series. Depicted paths indicate significant direct effects. Nonsignificant paths are omitted in the fitted models. See Table 3 for model fit statistics, Fig. 5 for visualization of total effects, and *SI Appendix*, Table S3 for partitioning of direct and indirect effects.

accurately forecast changes in critical pools and fluxes of C in terrestrial carbon cycling, a core process connecting ecosystem productivity, biological diversity, and the provision of services.

Resource limitation theory (8–10) predicts a linear increase in ecosystem processes when a limiting resource is added and an asymptotic response when a second constraint adds a negative feedback limiting further response. However, as demonstrated here, this view is not well suited to ecosystems composed of diverse plant communities (7). The J_{CO2} responses to CO_2 enrichment nominally matched expectations from resource limitation theory for two of the three soils. J_{CO2} increased linearly with CO_2 on the lowland clay soil. Indeed, we found no evidence that Ψsoil, exp(H), or species turnover provided feedbacks on the J_{CO2}–CO_2 relationship on the clay soil, as expected if CO_2 was the dominant limitation on J_{CO2}. On the upland silty clay soil, the asymptotic J_{CO2} response to CO_2 was also nominally consistent with a negative feedback from a single second limiting factor. Instead, the asymptotic J_{CO2} response arose from concurrent negative feedback from exp(H) and positive feedbacks from species turnover and Ψsoil, not from changes in the next single limiting factor. Unexpectedly, the linear J_{CO2}–CO_2 response on the alluvial sandy loam soil also supports a multiple concurrent constraints paradigm because the linear J_{CO2}–CO_2 response depended on a positive feedback from increased species turnover as *S. nutans* became dominant and species richness declined. Together, these findings experimentally show that responses to added limiting resources depend on how multiple constraints change in concert to create positive or negative feedbacks, casting doubt on a fundamental assumption of many resource manipulation experiments.

Our results corroborate previous studies indicating that CO_2 enrichment increased J_{CO2} by 20 to 30% over ambient CO_2 levels (6). We found smaller increases from ambient to enriched, with up to 15% higher J_{CO2} depending on the soil series, but comparable increases in J_{CO2} over our full range of CO_2 concentrations. Inclusion of subambient CO_2 concentrations was crucial for resolving the asymptotic J_{CO2} response to CO_2 enrichment on the silty clay soil. The decreasing gains in J_{CO2} on the silty clay imply that past increases in CO_2 had larger effects on J_{CO2} than will near-future increases through 500 $\mu L\ L^{-1}$, while the sandy loam and clay soils are more likely to experience continued increases in J_{CO2}. The contribution of exp(H) to the J_{CO2} response in the silty clay reinforces findings of Burri et al. (48), showing that increased species richness stabilized the effect of drought on soil respiration across 19 European grasslands. Similarly, functional composition of plant communities predicted soil respiration responses to warming in a North American tallgrass prairie (49). Community structure thus provides general value in understanding ecosystem responses to global change drivers particularly when, as here, community change was marked by shifts in dominant species that differed in functional traits related to the rate and efficiency of carbon cycling (15, 24, 50).

Our gradient approach identified variation in the trajectory of the J_{CO2} responses not discernable from experiments with only elevated and ambient CO_2 concentrations. For example, an elevated/ambient experiment would not have identified the CO_2 concentration at which J_{CO2} began to diverge among soils. The shape of the response of J_{CO2} to CO_2 enrichment has implications for carbon cycling. If the trajectory of J_{CO2} matches that of ANPP, the ratio of carbon gain to carbon loss may remain constant. In contrast, if the ratio of ANPP to J_{CO2} increases as CO_2 concentration rises, as found on the silty clay soil, we might expect a greater fraction of primary productivity to accumulate in the system despite it being the least productive of the three soils. Moreover, the differing J_{CO2} responses to CO_2 enrichment on these soils are consistent with analogies from economic theory applied to ecosystems in suggesting differing optimization solutions to multiple limiting constraints (8).

The responses of J_{CO2} to CO_2 enrichment are generally consistent with the known mechanistic linkages of J_{CO2} to ANPP and soil carbon dynamics. CO_2 enrichment increases allocation to autotrophic and heterotrophic sources of respired CO_2,

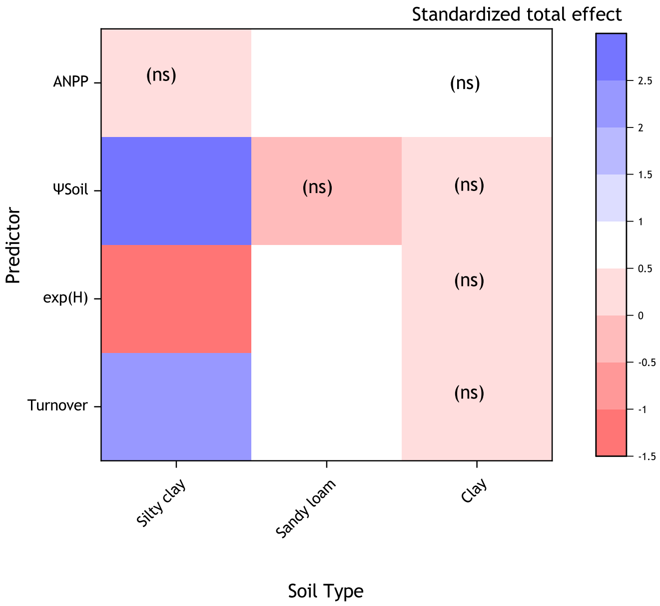

Fig. 5. Total effects of predictors of soil CO_2 efflux on each soil series from structural equation models (Fig. 4). See *SI Appendix*, Table S3 for partitioning of total effects into direct and indirect components. ns, not statistically significant.

including increased litterfall, fine root production, and root exudation (51), implying increased root mass, soil organic matter, and microbial biomass. Furthermore, the greater increase in ANPP than in J_{CO2} with CO_2 enrichment implies increasing net carbon uptake in general for these soils. The weaker J_{CO2} response combined with lower ANPP on the silty clay is consistent with previous studies showing decreased old soil organic matter pools at elevated CO_2 as labile pools were exhausted and carbon cycling became more tied to recent carbon inputs (52). The contributions of autotrophic and heterotrophic respiration sources and old and new carbon pools likely shifted on each soil (48, 49, 53–56). For example, on the clay soil CO_2 enrichment resulted in a weaker increase in ANPP, with stronger increases in microbial biomass, labile C fraction, and diversity and relative abundance of saprophytic fungi (38, 40). In contrast, on the sandy loam stronger increases in ANPP combined with weaker increases in soil carbon (38, 40). Our experiment included only three soils, limiting our ability to attribute responses to specific quantitative properties, such as texture, or water holding capacity. However, these soils represent a cross-section of landscape positions and dominant soil orders in grassland biomes and thus, highlight a key source of spatial variation in the controls on grassland J_{CO2}.

These results reveal how soil water availability, a key limiting resource in most grasslands, and plant community change can combine in different ways to shape the response of J_{CO2} to rising atmospheric CO_2 concentration. However, we cannot rule out potential contributions from other factors. Although there was little relationship of resin-available N to ANPP in this experiment (57), other evidence suggests possible N limitation of ANPP. For example, Kelley et al. (39) reported increased activity of nitrogen-cycling enzymes under CO_2 enrichment on the clay soil, and Jin et al. (58) reported decreased C:N of *Bouteloua curtipendula* litter but concluded that soil moisture was more important for N mineralization rates. CO_2 enrichment also increased alkaline phosphatase activity and abundance of Glomeromycota fungi on the sandy loam soil (38, 39), where Polley et al. (59) reported decreased tiller P in the dominant grasses, suggesting possible P limitation of ANPP. However, limitations on J_{CO2} from N and P or other macro- or micronutrients remain unresolved. Light availability could limit J_{CO2} by limiting photosynthetic carbon assimilation; however, it likely did not contribute to the asymptotic J_{CO2} response to CO_2 on the silty clay because similar light levels yielded high rates of J_{CO2} on the clay and sandy loam soils.

This experiment focused on a C_4-dominated community, but our key finding—that ecosystem response to CO_2 depends on the net outcome of responses in multiple constraints—is not an artifact of C_4 dominance. For example, we argued (44) that changes in community composition predicted at least 80% of the productivity response in both a C_3-dominated grassland and a C_4-dominated grassland. Our experimental system contained summer-active C_3 species, notably *Solidago canadensis*, *Salvia azurea*, and the legume *Desmanthus illinoensis*, yet they did not dominate at high CO_2 levels, likely because our C_4 grasses were generally favored by our hot summer climate (60). Our experimental design excluded species immigration as a source of species turnover. This was a necessary limitation because the experimental site was surrounded by urban and agricultural landscapes, not tallgrass prairie. Also, the linear, interconnected chamber design means propagules entering the system would be highly nonrandomly distributed along the gradient. Although immigration may have affected levels of diversity and species turnover, we consider it unlikely that immigration would have lessened the importance of diversity and species turnover as regulators of the JCO_2_CO_2 response.

Conclusions

The core finding of this study is that the effects of CO_2 enrichment on J_{CO2} depend on how constraints from other limiting resources or plant community change combine to impose positive or negative feedbacks on the CO_2 enrichment response. Our findings emphasize that effects of global change drivers on ecosystem processes may be constrained by multiple, potentially interacting feedbacks. This finding is of practical relevance, especially for temperate grasslands, because it highlights the degree to which these constraints may vary across landscapes to define the likely trajectories of past and future soil C losses related to atmospheric CO_2 enrichment. Less productive soils may contribute more to carbon sequestration than their productivity response might suggest. Correctly accounting for spatial variation in the mechanisms controlling this flux, particularly biodiversity change, is important for refining terrestrial carbon cycle models.

Materials and Methods

Study Site and Experimental Design.

Site description. The study was conducted in the Lysimeter CO_2 Gradient (LYCOG) facility, located in Temple, TX (31°05′N, 97°20′W) in the southern US Central Plains. Mean annual precipitation is 917 mm (1981 to 2010), with growing season wet periods in May–June and September–October and a pronounced July–August dry period. Temperatures range from a July–August mean maximum of 35 °C to a December mean minimum of 2.9 °C. The mean frost-free period is ~250 d, from mid-March to late November (61).

CO_2 chambers. The CO_2 gradient experiment was conducted in two outdoor linear chambers. The design and operation of these chambers are detailed elsewhere (34, 35, 62). Each chamber consisted of ten 5-m-long × 1.2-m-wide sections. Each 5-m section was enclosed with clear polyethylene (0.006-inch/0.15-mm thickness) (61). This film transmits >90% of incident light with minimal effects on spectral quality and is similar to polyethylene films used in other global change experiments [e.g., Dermody et al. (63)].

The sections contained intact soil monoliths (1-m^2 area × 1.5-m deep) collected from three soil series common to the Texas Blackland Prairie Region: a silty clay Mollisol (fine-silty, carbonatic, thermic Udorthentic Haplustolls, Austin series; $n = 32$), a sandy loam Alfisol (fine-loamy, siliceous, active, thermic Udic Paleustalfs, Bastsil series; $n = 16$), and a clay Vertisol (very-fine, smectitic, thermic Udic Haplusterts, Houston Black series; $n = 32$) (61). Soils series names and soil texture classifications were identified for the monolith collection locations using soil series maps in the United States Department of Agriculture - Natural Resources Conservation Service Web Soil Survey (https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm) (Table 1). Pretreatment texture and organic carbon for the top 50 cm of the profile were measured as described in ref. 35. Organic matter, bulk density, field capacity, and permanent wilting point were estimated from pedotransfer functions (64). The silty clay and clay soils have higher inorganic carbon content than the sandy loam (58), but this potential source of CO_2 efflux was not considered here (65). Monoliths were excavated and encased in steel boxes in 2002, and they were used for the duration of the experiment. Each 5-m section contained two of the three soil series in duplicate, in random order within the section. The sandy loam was included in alternate sections.

Experimental communities. Experimental communities were planted in the monoliths in spring 2003. Seedlings of four C_4 grasses, two C_3 forbs, and one herbaceous legume were planted in a Latin Square design (61). All were perennials and native to Texas Blackland Prairie, the original natural vegetation at this location. The C_4 grasses were *S. nutans* (L.) Nash, *B. curtipendula* (Michx.) Torr., *Schizachyrium scoparium* (Michx.) Nash, and *Tridens albescens* (Vasey) Wooton & Standl. The C_3 forbs were *S. canadensis* L., *S. azurea* Michx. ex Lam., and the legume *D. illinoensis* (Michx.) MacMill. ex B. L. Rob. & Fernald. All are widespread and common in the Central Plains grasslands of North America. *Sorghastrum*, *Solidago*, and *Tridens* are typically found in more mesic locations with deeper soils, while *Bouteloua* and *Schizachyrium* more often occupy drier locations. In 2007, 20 monoliths (8 silty clay, 12 clay) were replanted to switchgrass (*Panicum virgatum*) to improve CO_2 control by increasing photosynthetic sink strength (66). This left 60 monoliths (silty clay $n = 24$, sandy loam $n = 16$, and heavy clay $n = 20$) in the grassland experiment.

Plant species composition was maintained during the experiment by removing other species as they appeared by hand weeding or selective glyphosate application. Thus, community change reflected changes in abundance of members of the planted community without immigration from the regional species pool. We judged the reduced realism from constraining immigration preferable to several problems likely to arise if new species were allowed. Immigrant species would likely be unrepresentative of native tallgrass prairie because the experimental site is in a highly impacted mixed urban/agroecosystem landscape dominated by exotic and invasive species. Propagules entering during the growing season when the chambers are closed would be highly nonrandomly distributed along the gradient because they would enter through the air intake and likely fall out in the first chambers, confounding the CO_2 enrichment effect.

When we applied glyphosate (*SI Appendix*, Fig. S2) to remove new species, we minimized the amount of glyphosate used by carefully painting it on individuals to be removed. We took care to avoid touching neighboring species or drip glyphosate solution on the soil.

CO_2 treatments. A daytime linear CO_2 gradient of 500 to 250 $\mu L\ L^{-1}$ was maintained during April to October of each growing season from 2006 to 2014 (61). The gradient was initiated by introducing air enriched to 500 $\mu L\ L^{-1}$ CO_2 into the first section of the chamber. Fans advected this air through successive sections, and photosynthesis by the enclosed vegetation progressively depleted the air of CO_2. The airflow rate was controlled so that air exited the last section of the first chamber at 380 $\mu L\ L^{-1}$ CO_2. Similarly, ambient air was introduced into the first section of the second chamber and exited at 250 $\mu L\ L^{-1}$. Air temperature in the chambers was controlled to match outside ambient temperature. Each monolith was watered twice a week in events summing to the average growing season rainfall amount for this locale when the treatments were initiated (1971 to 2000: 560 mm). This amount is near the current (1981 to 2010) value of 578 mm. The seasonal pattern of irrigation was varied among years to introduce realistic variation in spring and summer rainfall, by shifting between wetter springs/drier summers, the typical ambient pattern, and the opposite on 1- or 2-y cycles. The plant communities were exposed to ambient conditions during winter (approximately November through April).

Field Measurements.

Soil CO_2 efflux. J_{CO2} was measured monthly in the growing season (May through October) during years 2007 through 2014 of CO_2 manipulation. J_{CO2} was measured with an infrared gas analyzer fitted with a soil chamber (LI-6400 photosynthesis system and LI-6400-09 soil chamber; LI-COR Biosciences). To perform a measurement, the chamber was placed on the sample point, and after a stable rate of $[CO_2]$ increase was established (usually within ∼30 s), the chamber CO_2 concentration was logged as it increased over a span of 10 to 20 ppm centered on the mean CO_2 concentration for that location along the CO_2 gradient. The logged increase measurement typically lasted 20 to 30 s. J_{CO2} was measured at two sample points in each monolith defined by poly vinyl chloride collars placed 4 cm into the soil at the start of each growing season to reduce potential CO_2 pulses arising from soil disturbance during placement of the chamber. Plants emerging within the collars were clipped prior to each measurement. Soil temperature in the top 10 cm of soil was measured concurrently with handheld probes. J_{CO2} measurements from June to August of each year were retained for this analysis. Peak rates of J_{CO2} consistently occurred during these months.

Aboveground biomass. All aboveground biomass was clipped by species at 5-cm height each November after plant senescence, dried for 72 h at 60 °C, and weighed.

Soil moisture. Volumetric soil water content at 0- to 20-cm and 20- to 40-cm depths was measured weekly each growing season, except biweekly in 2006, with a calibrated neutron attenuation probe (503DR Hydroprobe; CPN International) at a permanent access tube in each monolith. The two depths were then averaged to estimate the 0- to 40-cm soil water content, which was converted to Ψ_{soil} using previously established soil water release curves (57).

Canopy light at ground level. PPFD at 10 cm above the soil surface in the plant canopy of each monolith was measured with the chambers opened once each July using a ceptometer (SunScan; Delta-T Devices Ltd.). PPFD was measured across both diagonals of each monolith. Boundary conditions for measurements were solar elevation angle greater than 30° and above-canopy PPFD greater than 600 $\mu mol\ m^{-2}\ s^{-1}$. For this analysis, we further excluded readings with ambient light <1,000 $\mu mol\ m^{-2}\ s^{-1}$.

Plant diversity and composition indices. We measured three aspects of CO_2 effects on the experimental plant communities, all derived from the aboveground biomass sampling in each monolith. 1) exp(H), which is the exponential of the Shannon diversity index (H) (Eq. 1), where p_i represents the relative abundance of each species calculated from its fraction of total ANPP. exp(H) is interpreted as the number of equally abundant species required to give the observed H (67):

$$\exp(\mathrm{H}) = \exp\Big(-\sum p_i \times \log(p_i)\Big). \qquad [1]$$

2) The dominant grass species *Sorghastrum* and *Bouteloua* trade off in dominance along the CO_2 gradient, and *Sorghastrum* abundance is a key predictor of ANPP gains with CO_2 enrichment (41, 42). This balance was quantified from the biomass of each species (Eq. 2):

$$Dominantgrass = \frac{(Mass(Sorghastrum) - Mass(Bouteloua))}{(Mass(Sorghastrum) + Mass(Bouteloua))}. \qquad [2]$$

3) Community composition change ("turnover") along the gradient is quantified by the Bray–Curtis dissimilarity (d_{BC}) metric (68), with X_{ij} *and* X_{ik} denoting the biomass of species *i* in monolith *j* and in monolith *k* (Eq. 3):

$$d_{BC} = \frac{\sum \left|X_{ij} - X_{ik}\right|}{\sum \left(X_{ij} + X_{ik}\right)}. \qquad [3]$$

We estimated the rate of turnover as a function of CO_2 enrichment from "distance-decay" curves (69) constructed for each soil series in each year of the study. Distance-decay curves relate d_{BC} for all pairwise combinations of monoliths to the corresponding difference in CO_2 concentration (δ_{CO2}). Turnover was represented by the slope of a linear regression fit to each decay curve.

Data Processing and Analysis.

Data preparation. Individual J_{CO2} and soil water content measurements more extreme than 1.5× the interquartile range were considered outliers. This removed ∼3% of ∼7,600 J_{CO2} measurements and of ∼8,500 soil water content measurements. Then, for J_{CO2}, Ψsoil, PPFD, and exp(H), duplicate spatial or multiple temporal measurements were reduced in three steps: first, by averaging duplicate spatial measures within monoliths; second, by averaging repeated measurements within growing seasons, yielding a single value per monolith; and third, by averaging across the duplicate monoliths of each soil series within each 5-m section, yielding a single yearly value per soil in each section.

Statistical methods. We applied linear mixed models in SAS/STAT 13.1 (Proc MIXED; SAS Institute) to test the effects of CO_2 enrichment and whether responses to CO_2 enrichment varied among the soil series. We fit the following model (Eq. 4) to J_{CO2}, ANPP, Ψsoil, PPFD, and exp(H). J_{CO2} was natural log transformed for analysis to meet assumptions of normality but was graphed in the untransformed scale:

$$\begin{aligned} \mathrm{y_{ijkl}} = {} & \mathrm{intercept + soil_i + monolith_j(soil_i) + \alpha(CO_2) + \beta_i(CO_2 \times soil_i) + year_k} \\ & \mathrm{+\, year \times soil_{ik} + \gamma_k\big(CO_2 \times year_k\big) + \delta_{ik}\big(CO_2 \times soil_{\mathit{i}} \times year_k\big) + e_{ijkl}}. \end{aligned} \qquad [4]$$

Monolith nested within soil series [$\mathrm{monolith_j(soil_i)}$] was fit as a random effect, and year was fit as a repeated effect with an autoregressive covariance structure. Soil effects on the rate of species turnover with change in CO_2 were tested by fitting a reduced model including soil and random effect of year.

For variables where these analyses returned significant CO_2 or CO_2 × soil series effects, we plotted the significant linear relationships with CO_2 for each soil series using OriginPro 9.7. For $\ln J_{CO2}$, significant CO_2, or soil × CO_2 interactions using a linear model imply a nonlinear response in untransformed J_{CO2}, so we also fit exponential functions to untransformed J_{CO2} vs. CO_2 on each soil series. The exponential function was retained on the silty clay soil because the Bayesian Information Criteria decreased by at least two, indicating an improved fit compared with a linear function. However, this criterion was not met for exponential functions fit to the sandy loam and clay soils, so for them, the linear regressions were retained.

Informed by the mixed model analyses, we developed a structural equation model to resolve how the CO_2 responses of Ψsoil, exp(H), turnover, and ANPP combined to predict the CO_2 response of J_{CO2}. The dataset consisted of the linear slopes of CO_2 responses for each variable calculated for each year. Expressing Ψsoil, exp(H), ANPP, and J_{CO2} as functions of CO_2 expresses all variables in the same form as turnover. Direct effects describe how the CO_2 response of one variable affects that of the second variable, and indirect effects describe how an effect may be mediated by the CO_2 response of a third variable. All variables were standardized to mean = 0 and SD = 1.

We devised an a priori path model representing 1) how the CO_2 responses of ANPP, Ψsoil, exp(H), and turnover were related to the CO_2 response of J_{CO2} and 2) how the CO_2 response of ANPP may have mediated the effects of the other variables on J_{CO2}. The a priori model was fit separately to each soil using Proc Calis (SAS Institute). The a priori model was modified when suggested by LaGrange statistics for paths to add or Wald statistics for paths to remove. Model fit (Table 3) was evaluated using indices indicating absolute fit (χ^2), parsimony (root mean square error of approximation [RMSEA]), and accounting for sample size (comparative fit index [CFI]) following Hooper et al. (70).

Data Availability. The datasets are available from the Dryad Data Repository, http://doi.org/10.5061/dryad.fbg79cnt0 (71).

ACKNOWLEDGMENTS. Research was supported by in-house funds from US Department of Agriculture (USDA)-Agricultural Research Service (ARS). L.G.R. was supported by USDA-National Institute of Food and Agriculture (2010-65615-20632), A.R.K. and M.J.A. by NSF - Plant Genome Research Program (PGRP) (IOS-0922457), R.W.H. by NSF PGRP (IOS-1444533), and D.H. by NSF (DBI-1919897, DEB-2000058). USDA-ARS is an equal opportunity employer.

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