

Soil carbon stocks in temperate grasslands differ strongly across sites but are insensitive to decade-long fertilization

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Abstract

Enhancing soil carbon (C) storage has the potential to offset human-caused increases in atmospheric CO₂. Rising CO₂ has occurred concurrently with increasing supply rates of biologically limiting nutrients such as nitrogen (N) and phosphorus (P). However, it is unclear how increased supplies of N and P will alter soil C sequestration, particularly in grasslands, which make up nearly a third of non-agricultural land worldwide. Here, we leverage a globally distributed nutrient addition experiment (the Nutrient Network) to examine how a decade of N and P fertilization (alone and in combination) influenced soil C and N stocks at nine grassland sites spanning the continental United States. We measured changes in bulk soil C and N stocks and in three soil C fractions (light and heavy particulate organic matter, and mineral-associated organic matter fractions). Nutrient amendment had variable effects on soil C and N pools that ranged from strongly positive to strongly negative, while soil C and N pool sizes varied by more than an order of magnitude across sites. Piecewise SEM clarified that small increases in plant C inputs with fertilization did not translate to greater soil C storage. Nevertheless, peak season aboveground plant biomass (but not root biomass or production) was strongly positively related to soil C storage at seven of the nine

sites, and across all nine sites, soil C covaried with moisture index and soil mineralogy, regardless of fertilization. Overall, we show that site factors such as moisture index, plant productivity, soil texture, and mineralogy were key predictors of cross-site soil C, while nutrient amendment had weaker and site-specific effects on C sequestration. This suggests that prioritizing the protection of highly productive temperate grasslands is critical for reducing future greenhouse gas losses arising from land use change.

KEYWORDS

carbon cycling, carbon sequestration, fertilization, nutrient network, piecewise structural equation model, soil carbon fractions

1 | INTRODUCTION

One potential strategy for mitigating human-caused increases in atmospheric CO₂ is to enhance long-term soil carbon (C) storage. Soils store more C than the atmosphere and vegetation combined, and consequently serve a critical role in regulating ecosystem C-climate feedbacks. Rising CO₂ has occurred concurrently with increasing supply rates of biologically limiting nutrients such as nitrogen (N) and phosphorus (P). Atmospheric N deposition, and to a lesser extent anthropogenic P inputs, has increased globally in recent decades, with greater spatial variability compared to rising atmospheric CO₂ (Ackerman et al., 2019; Peñuelas et al., 2013). Given that ecosystem C cycling is coupled to that of nitrogen (N) and phosphorus (P), understanding how concurrent anthropogenic perturbations of C, N, and P cycles drive fundamental ecosystem processes such as soil organic matter (SOM) storage is essential for predicting feedbacks between nutrient supply and climate.

Much of the work exploring consequences of simultaneous changes in biogeochemical cycles for soil C pools and fluxes has relied on fertilization experiments in forested ecosystems. However, grasslands sequester more C in their soils than forests (Schlesinger, 1977, FAOSTAT 2009) and make up nearly one-third of non-agricultural land area globally (Chapin et al., 2002). Importantly, previous work suggests grasslands are highly sensitive to nutrient amendment with respect to plant biomass (LeBauer & Treseder, 2008; Lee et al., 2010; Stevens et al., 2015; Suding et al., 2005), diversity (Borer et al., 2014b; Isbell et al., 2013; Midolo et al., 2019), and species composition (Suding et al., 2005). While several cross-site studies have examined short-term nutrient effects on soil C stocks (Crowther et al., 2019; Riggs et al., 2015; Sitters et al., 2020), how long-term (i.e., decadal scale) nutrient addition affects soil C dynamics in grasslands remains unresolved (but see Fornara & Tilman, 2012; Seabloom et al., 2021b).

Soil organic C (hereafter, soil C) responses to fertilization depend on the relative responses of inputs (from net primary productivity; NPP) and losses (mostly from litter and SOM decomposition; Figure 1). In forests, nutrient addition commonly increases soil C storage, with strongest effects in the organic horizon (e.g., Frey et al., 2014; Zak, 2008). This has been shown to largely result from shifts in microbial

community structure and concomitant suppression of lignin-degrading enzymatic activity (Edwards et al., 2011; Eisenlord et al., 2013; Frey et al., 2014; Waldrop et al., 2004), which together reduce overall soil C losses. However, grasslands lack lignin-rich litter and are host to distinct decomposer communities (dominated by *Glomeromycota*, *Ascomycota*, and *Actinobacteria*) compared to forests (dominated by *Basidiomycota*) (Prober et al., 2015; Sinsabaugh, 2010). Thus, grassland soils may not experience the same effects of nutrient addition as do forests. Indeed, studies of nutrient addition effects on soil C in grasslands (albeit relatively rare compared to those in forests, and mostly short term) indicate that such effects are highly context dependent (e.g., Crowther et al., 2019), with increases (e.g., Fornara et al., 2013; He et al., 2013; Riggs et al., 2015) or no change (e.g., Lu et al., 2011; Riggs et al., 2015) in bulk soil C stocks commonly reported. Results from five U.S. grasslands suggest that sites with low percent sand are more likely to exhibit increased soil C stocks with added N (Riggs et al., 2015), while across 25 sites, percent sand was positively related to increased soil C with multi-nutrient addition Crowther et al. (2019). These results highlight that soil physicochemical and environmental factors are important mediators of fertilization effects on soil C pools in grasslands, yet the drivers of these patterns remain unclear (but see Fornara & Tilman, 2012).

Fertilization is expected to stimulate plant growth and consequently increase C inputs to soil. Indeed, nutrient addition to grasslands has been shown to increase aboveground biomass (Elser et al., 2007; Fay et al., 2015; LeBauer & Treseder, 2008). Belowground plant responses are more variable but total plant allocation belowground has been found to increase in response to increased resource availability (Adair et al., 2009; Cleland et al., 2019; Fornara & Tilman, 2012), even though relative allocation belowground may decline (Li et al., 2016). Given the importance of root-derived inputs to SOM formation (Jackson et al., 2017; Keller et al., 2021; Rasse et al., 2005), this leads to the expectation that soil C stocks should increase with nutrient amendment (as seen in Huang et al., 2020), although associated increased SOM priming could offset soil C sequestration.

Fertilization also might affect C losses from grasslands by altering litter and SOM decomposition patterns, through potentially different mechanisms depending on the SOM pool in question (Riggs et al., 2015). First, fertilization might alter litter decomposition with

subsequent effects on SOM pools by altering litter chemistry; for example, N fertilization often decreases litter C:N, potentially stimulating initial litter decay while suppressing late-stage decay (Berg & Matzner, 1997; Knorr et al., 2005). For four perennial grasses, root decomposability increased with elevated N supply (Van Der Krift et al., 2001). Given that labile plant substrates fuel microbial growth, and microbial products are considered the dominant precursors to stable SOM (Cotrufo et al., 2013), fertilization effects on litter decomposition could be expected to increase soil C accumulation. Such effects may be particularly pronounced in temperate grasslands,

where microbial necromass may make up ~62% of soil C compared to only ~30% in temperate forests (Liang et al., 2019). More rapid early-stage litter decomposition could contribute to greater stabilization of microbial products in mineral-associated organic matter. At the same time, slower late-stage litter decomposition could result in accumulation of organic matter in the unprotected light particulate organic matter (POM) fraction, composed of partially decomposed plant material (Cotrufo et al., 2015). Effects of fertilization on microbial community structure and enzymatic activity could also influence decomposition patterns (Ochoa-Hueso et al., 2020), although

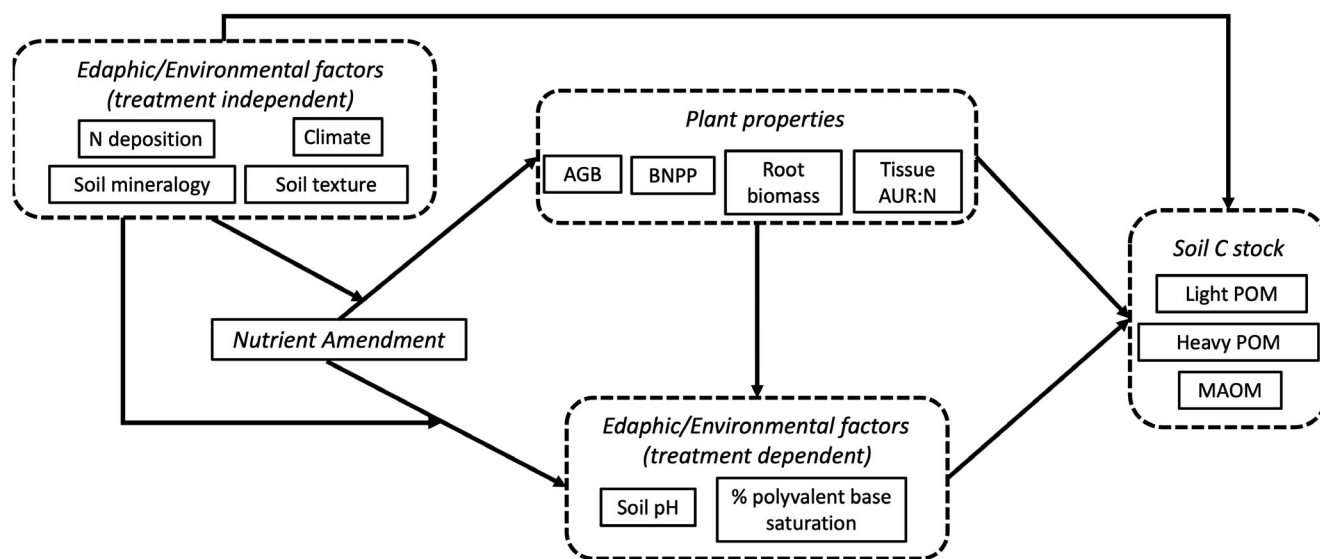


FIGURE 1 Conceptual meta-model of proposed relationships between soil C stocks and plant, soil, and environmental factors in control and fertilized plots. Arrows connecting to arrows indicate interactive effects (e.g., of edaphic/environmental factors and nutrient amendment on plant properties). Edaphic/Environmental factors are separated into those hypothesized to be unaffected by fertilization effects over the duration of the experiment (i.e., treatment independent) and those hypothesized to change due to fertilization (i.e., treatment dependent). AGB, aboveground peak biomass; BNPP, belowground net primary productivity; AUR:N, acid unhydrolyzable residue:nitrogen; POM, particulate organic matter; MAOM, mineral-associated organic matter

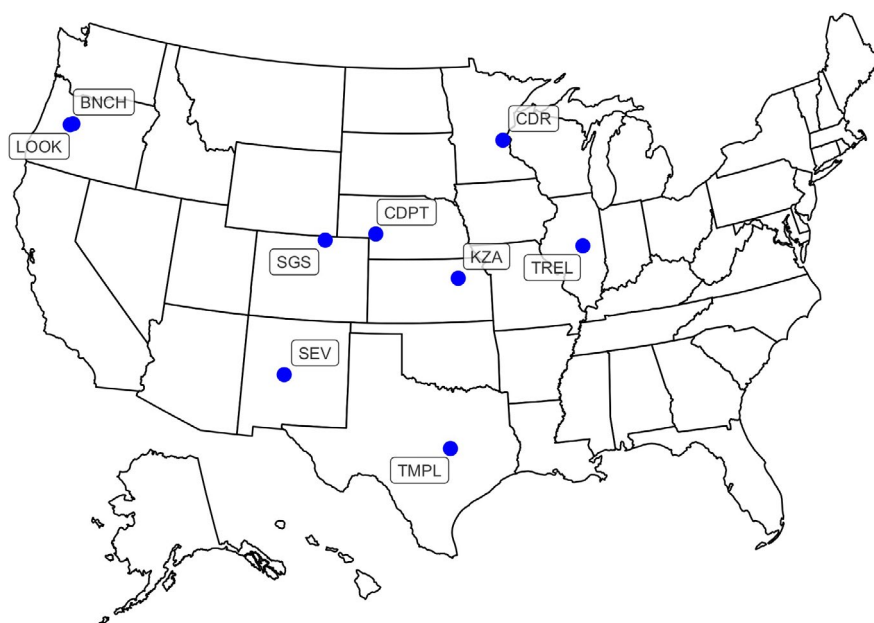


FIGURE 2 Map of the United States showing location of the nine study sites. Site abbreviations as in Table 1 [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

TABLE 1 Site characteristics for the nine nutrient network sites included in this study

Site	Latitude, Longitude (°)	MAT (°C)	MAP (mm)	Aridity index	Elevation (m)	Atmospheric N deposition (kg N ha ⁻¹ yr ⁻¹)
Bunchgrass, OR (BNCH)	44.28, -121.97	6.77	1618	1.93	1318	2.62
Cedar Creek LTER, MN (CDR)	45.43, -93.21	6.34	740	0.83	270	7.8
Cedar Point Bio. Station, NE (CDPT)	41.20, -101.63	9.64	456	0.40	965	5.48
Konza LTER, KS (KZA)	39.07, -96.58	11.6	971	0.89	440	8.29
Lookout Ridge, OR (LOOK)	44.21, -122.13	6.9	1877	2.31	1500	2.62
Sevilleta LTER, NM (SEV)	34.36, -106.69	13.1	252	0.17	1600	2.91
Shortgrass Steppe LTER, CO (SGS)	40.82, -104.77	8.95	369	0.32	1650	5.31
Temple, TX (TMPL)	31.04, -97.35	19.4	877	0.60	184	7.77
Trelease Prairie, IL (TREL)	40.08, -88.83	11.1	992	0.89	200	8.58

Note: Additional soil and plant characteristics are found in Table S5.

Abbreviations: MAT, mean annual temperature; MAP, mean annual precipitation; Soil percent C is organic C.

functional redundancy and edaphic constraints may limit such effects (Widdig et al., 2020; Zeglin et al., 2007).

Second, fertilization may indirectly influence soil C accumulation and decomposition by affecting soil physicochemical stabilization pathways. For example, enhanced belowground root biomass and productivity under fertilization may promote physical aggregation and C storage, particularly in the macroaggregate-rich heavy POM pool (Amezketta, 1999). Chemically, N addition can reduce soil pH, depleting concentrations of polyvalent base cations while increasing solubility of acid-hydrolyzing cations. As polyvalent cations are effective bridging agents that form organomineral complexes and protect organic matter from microbial attack (Baldock & Skjemstad, 2000; Lützwow et al., 2006), net effects of fertilization on chemical stabilization of organic matter likely depend on underlying soil chemical properties such as initial pH, clay mineralogy, and cation chemistry. For example, low-charge clays are more sensitive to base cation depletion (Jastrow et al., 2007), and acidic soils with minimal buffering capacity should be more sensitive to the indirect acidification effects of N addition on soil C stabilization potential. Nutrient addition can also alter soil C chemistry by changing soil mineral-protein interactions, consequently modifying organic matter interactions with mineral surfaces (Zhao et al., 2020). In sum, fertilization effects are expected to interact with soil physicochemical properties to drive differing C storage responses across different SOM pools.

Across biogeographic scales, the potential for soil C storage varies with soil texture and mineralogy. Fine-textured soils and relatively less weathered soils with poorly crystalline mineral structure generally exhibit greater aggregation (Barto et al., 2010; Duiker et al., 2003; Wick et al., 2009) and provide greater mineral surface area for cation-bridging and direct adsorption of organic matter onto clay particles compared to coarse-textured soils (Six et al., 2002; Torn et al., 1997a). Fine-textured and poorly crystalline soils are thus expected to promote relatively greater soil C stabilization while also being more resistant to losses via microbial decomposition, dissolved organic C leaching, and mineralization. As such, fertilization should stimulate greater soil C gains in younger and fine-textured,

compared to coarse-textured, soils, and such responses should be strongest in mineral-associated and macroaggregate C fractions. However, nutrient limitation of plant growth may be stronger in coarse-textured soils due to low organic matter storage, which could result in greater positive soil C responses to fertilization as found by Crowther et al. (2019). Taken together, soil texture may mediate the dominant pathway by which fertilization increases soil C, with increased inputs being relatively more important in low fertility, coarse-textured soils and altered soil physicochemical properties affecting soil C responses to fertilization more strongly in high fertility, fine-textured soils.

Adding a layer of complexity, environmental factors such as atmospheric N deposition rates and climate are expected to interact with plant and edaphic characteristics to influence the response of soil C sequestration to fertilization. For example, Knorr et al. (2005) found that N fertilization effects on litter decay were dependent on both litter quality (i.e., lignin content) and ambient N deposition, with decay rates inhibited by low litter quality and high N deposition but stimulated by high litter quality and low N deposition. In contrast, fertilization effects on SOM dynamics may be weak where factors other than nutrient availability, such as water availability, more strongly constrain plant and microbial activities.

Previous research showing idiosyncratic responses of SOM to nutrient addition highlights the importance of replicated, long-term field experiments across sites that vary in key abiotic properties such as climate, soil texture, and soil mineralogy. Moreover, examining nutrient addition effects on distinct SOM pools in addition to bulk soil C and N stocks can help reveal the mechanisms by which nutrient effects may occur. To this end, we examined how 10 years of N and P fertilization, alone and in combination, affected soil C and N storage using a long-term nutrient addition experiment replicated in nine temperate grassland sites spanning climatic and edaphic gradients.

We hypothesized that soil C and N storage would increase with N and P fertilization regardless of environmental conditions due to a combination of increased plant inputs and early-stage litter

Grassland type	Management regime	Soil order	Soil texture (% sand, silt, clay)	Soil % C (0–15 cm)	Total plant biomass (g m ⁻²)
Montane grassland	N/A	Inceptisol	70.4, 26.5, 2.9	9.98 (0.4)	1216 (65)
Tallgrass prairie	N/A	Entisol	89.3, 21.6, 9.5	1.20 (0.1)	783 (66)
Shortgrass prairie	N/A	Mollisol	68.8, 21.6, 9.5	1.44 (0.1)	1170 (78)
Tallgrass prairie	Irregularly burned	Mollisol	31.9, 49.8, 18.3	3.54 (0.2)	1424 (99)
Montane grassland	N/A	Andisol	69.0, 30.1, 0.9	16.2 (0.5)	1152 (178)
Desert grassland	N/A	Aridisol	83.5, 10.6, 5.8	0.335 (0.01)	369 (23)
Shortgrass prairie	N/A	Entisol	71.3, 15.1, 13.6	1.28 (0.06)	431 (42)
Tallgrass prairie	Mowed	Mollisol	26.4, 34.2, 39.2	4.62 (0.1)	1138 (102)
Tallgrass prairie	N/A	Mollisol	22.2, 62.1, 15.6	3.37 (0.1)	587 (47)

decomposition and suppressed late-stage litter and SOM decomposition at all sites. However, we expected to find variation in the magnitude of site-specific responses to fertilization due to differences in plant and soil physicochemical properties (e.g., soil texture, mineralogy, pH, and cation exchange capacity), climate, and atmospheric N deposition (Figure 1). Specifically, we hypothesized that highly productive sites with wetter climates should have larger gains in soil C under elevated nutrient supply compared to other sites. Site-specific nutrient effects on soil C should reflect N and/or P nutrient limitation of aboveground net primary productivity at these sites (Fay et al., 2015). Finally, we hypothesized that fertilization would increase C storage in macroaggregate-rich and mineral-associated SOM pools, with the greatest absolute increases at wetter sites with fine-textured soils due to greater potential for aggregation, cation-bridging, and direct adsorption to mineral surfaces.

2 | METHODS

2.1 | Study sites

Soil and plant samples were collected from nine temperate U.S. grassland sites that are part of the Nutrient Network (<https://nutnet.org/>), a distributed and coordinated research network designed to experimentally test the effects of nutrient addition on ecosystem processes (Borer et al., 2014a). For site locations and properties, see Figure 2 and Table 1. Details of the experimental design are available in Borer et al. (2014). Briefly, at each of nine sites, we selected plots amended with elevated supply of N and P (alone and in combination), as well as control plots receiving no fertilizer. Plots were replicated in 3 or 4 (depending on the site) completely randomized blocks at each site. While within-site replication was relatively low ($n = 3$ or 4), the replicated experimental design across multiple sites and conditions provided a unique opportunity to test the generality of treatment responses across sites, as well as how plant, edaphic, and climate factors may mediate such responses (total # of plots = 120).

N and P were applied annually ($10 \text{ g m}^{-2} \text{ yr}^{-1}$) for 9–10 years prior to sampling. At most sites, N fertilizer was applied as ammonium nitrate (NH_4NO_3) in treatment year 1 and time-released urea [$(\text{NH}_2)_2\text{CO}$] in subsequent years; exceptions were SEV and TMPL, which received ammonium nitrate in all years, and TREL, which received urea in all years. Previous work found that different sources of N had no effect on plant biomass or richness across Nutrient Network sites (Seabloom et al., 2013). A separate study on multiple soil types found microbial respiration rates were not significantly affected by the form of N supply, suggesting microbial processes may also be insensitive to N source (Ramirez et al., 2010). All sites received P fertilizer annually as triple-super-phosphate [$\text{Ca}(\text{H}_2\text{PO}_4)_2$].

To test hypotheses of how N and P amendment influence soil C dynamics broadly, we selected sites spanning a wide climate and soil texture gradient. Site-level metrics of mean annual temperature (MAT), mean annual precipitation (MAP), potential evapotranspiration (PET), and a moisture index (calculated as MAP/PET) were obtained from BIOCLIM (Karger et al., 2017). Sites also varied in exposure to atmospheric N deposition, based on modeled output from Ackerman et al. (2019). For a time-integrated metric of N deposition spanning the duration of this study, we averaged annual estimates of N deposition from 2014 to 2016. N deposition was not correlated with MAP.

2.2 | Soil sampling and analysis

Soils were sampled once in the fall at each site, in either 2017 or 2018, with multiple cores per plot (5 cores in 2017, 3 cores in 2018) composited together. Two soil depths were sampled in each plot, with the exception of KZA where a shallow bedrock prevented deep soil sampling (sample depth at KZA varied from 10 to 15 cm). Upper soil horizons (0–15 cm) were sampled with a 5 cm diameter soil core and deeper soils (15–30 cm) were sampled with a 2.54 cm diameter core. Due to challenges we encountered in accurately measuring bulk density in the deeper soils, we focus our analysis and

presentation on the surface soil C stock data. In the field, soils were passed through a 2 mm sieve and all visible roots were removed. Bulk density was measured on these soils both before and after sieving. Pre- and post-sieved bulk density estimates were tightly correlated, although pre-sieved estimates were reduced for sites with high root biomass and consequently low bulk density. Given that soil C was measured on sieved soils, data in the main text are calculated from post-sieved bulk density, with pre-sieved bulk density presented in supplementary materials. Soils were transported to the University of Minnesota laboratory for processing within 24 h. Soils were refrigerated prior to shipping and after receiving; for overnight shipping, soils were packed in coolers with ice packs.

In the laboratory, soils were then air-dried, and subsamples were ground to a fine powder using steel BBs packed with samples into vials and shaken in paint cans on a paint shaker. Ground samples were packed into aluminum tins and analyzed for total percent C and percent N by combustion (COSTECH ESC 4010 Elemental Analyzer, Valencia, CA, USA). Soils from sites known or suspected to have significant levels of inorganic C in the form of carbonates (i.e., CDPT, SEV, SGS, TMPL) were fumigated with 12 M HCl prior to percent C analysis following Harris et al. (2001). For the samples that contained carbonates, we report percent C from fumigated samples and percent N from non-fumigated samples, as acid fumigation has been shown to reduce total N values by up to 50% (Harris et al., 2001). Sieved, air-dried soil (0–15 cm) was used for soil pH analysis (1:1 soil:water slurry) and measurement of exchangeable base cation concentrations (K^+ , Ca^{2+} , Na^+ , and Mg^{2+}). For cation measurements, soils were extracted with 10 mM NH_4HCO_3 (1:5 w:v of soil:acid), shaken for 2 h, and centrifuged at 4000g for 10 min. The supernatant of each sample was decanted onto 0.2 μ m filters pre-rinsed with extractant. The extract was subsequently acidified with 5% nitric acid for storage at 4°C. Extracts were then measured by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Thermo-Scientific iCAP® 7400 IC spectrometer at the University of Tennessee Water Quality Core Facility. Percent polyvalent base saturation was calculated as:

$$\frac{[Ca^{2+}] + [Mg^{2+}]}{\text{Cation exchange capacity}} \times 100\%$$

where concentrations of cations and cation exchange capacity were expressed in units of meq/100 g soil. Oxalate-extractable Al and Fe, a measure of poorly crystalline Al- and Fe-bearing minerals, were measured on the surface soils by first shaking 0.5 g air-dried soil with 30 ml of an oxalate solution (4:3 ratio by volume of 0.2 M ammonium oxalate: 0.2 M oxalic acid) for 16 h, centrifuging, and then filtering to 0.22 μ m. Extracts were analyzed for poorly crystalline Al and Fe using an ICP-OES. Soil texture (percent sand, silt, clay) was directly measured in control plots prior to fertilization; where these data were not available at the block-level, site-level means were used.

To examine how nutrient addition affected distinct SOM pools in surface soils, a composite density and size fractionation procedure was used to separate bulk soil into three pools: light particulate

matter (light POM, not associated with soil minerals), heavy POM (POM contained in aggregates >53 μ m), and mineral-associated organic matter (MAOM, OM contained in aggregates <53 μ m) (Soong & Cotrufo, 2015). Briefly, ~6 g of sieved and air-dried surface soils was first shaken in ~30 ml of DI water for 15 min and then centrifuged at 3400 Rpm for 15 min. After decanting the supernatant (dissolved organic matter), each soil sample was mixed with enough sodium polytungstate (1.85 g cm^{-3}) to bring the total solution to 25 ml, shaken lightly for 18 h, and then centrifuged at 3400 rpm for 30 min. The suspended light POM was aspirated and washed with DI water until visibly clear, collected on a 20 μ m nylon mesh filter, and dried at 60°C. The remaining sample was then washed with DI water iteratively, vortexed to suspend, centrifuged at 3400 Rpm for 20 min, and decanted three times, or until the supernatant was clear. Finally, each sample was passed through a 53 μ m sieve to separate into heavy POM (>53 μ m) and MAOM (<53 μ m). Each fraction was dried at 60°C, ground to a fine powder, and analyzed for percent C and percent N as above. Small sample mass prohibited accurate measurements of percent C and percent N for many light fraction samples. Therefore, across all plots, we estimated light POM fraction percent C and percent N by mass balance (i.e., as the difference between bulk soil and heavy POM + MAOM C and N content). This approach includes dissolved organic C and N in the light POM fractions.

2.3 | Plant biomass, primary production, and turnover

Given the importance of plant inputs to SOM dynamics, we related soil C stocks and fractions to root standing biomass (hereafter, root biomass), root production, and root turnover in each plot and root and leaf tissue chemistry (i.e., acid un-hydrolysable residue [AUR], percent N, Keller et al., 2017–2018, unpublished), and aboveground peak biomass (hereafter aboveground biomass). Root measurements were taken from three soil cores (5 cm diameter, 15 cm depth) per plot. In Fall of 2016, root biomass was calculated as the average root dry mass per plot on an areal basis. Root production was then measured by placing one plastic mesh root ingrowth core filled with 2:1 root-free native soil:sand in each of the holes from the soil cores. Roots were allowed to grow into the plastic core for 1 year, and ingrowth cores were harvested in Fall 2017. Roots were removed using forceps, washed with DI water, dried at 60°C for 48 h, and weighed to 0.001 g. Root production was calculated as net root mass within each core at the time of harvesting and expressed on an areal basis. Root turnover was estimated as the ratio of root production to root standing biomass. While we recognize interannual variation is likely high for belowground and aboveground plant biomass and turnover, and capturing such variation could be important for revealing relationships between plant and soil C pools, the significant time and effort required for measuring belowground plant properties limited our belowground data to a single time point. However,

measurements of aboveground biomass were made annually since fertilization began at each site by clipping two 0.1 m² quadrats of peak aboveground biomass per plot each year. We calculated the mean annual aboveground biomass in each plot for all years with available data since fertilization started. Data are archived with the Environmental Data Initiative (Hobbie, 2021).

2.4 | Data analysis

All data analysis was performed in R (R version 3.6.1, R Foundation for Statistical Computing 2013). We used a series of statistical models to understand how soil C stocks varied across fertilization treatments and sites. In brief, we first examined direct N x P fertilization effects on soil C stocks using two-way ANOVA and then accounted for variation across sites using linear mixed modeling (site as a random effect). Second, we assessed fertilization by site interactions with nutrient treatments and site as fixed effects. Third, to understand the drivers of any site effect and its interaction with nutrient treatments, we constructed a series of linear mixed models that included nutrient treatments and biotic, environmental, and edaphic covariates as fixed effects and sites as random effects. This allowed us to determine whether any site effects could be explained by variation in measured plant, soil chemistry, or climate factors. Finally, we constructed a piecewise structural equation model (SEM) to fully explore direct and indirect effects of these covariates and nutrient amendment on soil C stocks.

More specifically, the two-way ANOVA analyses included nutrient treatment (N or P) as the grouping factors and soil C stocks or concentrations as the response variable. In our linear mixed models to test for fertilization effects on soil C stocks, fertilization treatments were fixed factors and block nested within site was a random intercept. Random slopes were not included due to sample size limitations within sites. Models were fit with the *nlme* package (*lme()*), with weights applied using *varIdent(form = ~1|site)* where different variances per site are allowed. In our linear model including only fixed effects, soil C stocks were predicted by fertilization treatment, site, and their interactions.

The covariate linear mixed models align with our conceptual meta-model (Figure 1), and we fit separate models for each construct of the meta-model. Prior to model fitting, we examined distributions of all univariate predictor variables and performed log transformations on predictor variables as needed to normalize variable distributions. Variance inflation factors were all <3, indicating low collinearity. We then used both *a priori* hypotheses and pairwise correlation analysis to inform variable selection (highly correlated variables, i.e., $r > 0.70$, were not included together in linear models). All models followed a similar structure; nutrient treatment (+N, +P, +N+P, control), additional covariates, and interactions between nutrient treatment and covariates were included as fixed factors, and blocks nested within sites were included as random intercepts. As above, models were fit with the *nlme* package, with weights applied using *varIdent()*. Interaction terms were dropped from models that

were not significant ($\alpha < 0.05$). The first set of models tested the degree to which plant biomass, plant productivity, and litter chemistry predicted soil C stocks. Specifically, the initial full model included aboveground biomass, root production, root biomass, leaf and root AUR:N, nutrient treatment (in factorial), and interactions between nutrient treatments and plant covariates. Using this same linear mixed modeling framework, we then tested the effects of nutrient amendment and soil chemistry (pH and % polyvalent base saturation) on soil C stocks. Finally, we tested the effects of nutrient amendment and edaphic and environmental factors (oxalate-extractable Al + Fe, soil percent silt + clay, moisture index, and N deposition) on soil C stocks. Due to the high collinearity between moisture index and soil Al + Fe oxide content (log-transformed oxalate-extractable Al + Fe; $r = 0.96$), a parallel statistical model was built substituting moisture index for soil mineralogy. While the two Oregon sites (i.e., BNCH and LOOK) were by far the wettest and had the highest values for oxalate-extractable Al + Fe, a strong correlation between moisture index and oxalate-extractable Al + Fe persisted after excluding these two sites ($r = 0.84$). To further examine cross-site patterns of soil C stocks, we performed simple and multiple linear regressions relating plant, soil physicochemical, and environmental variables to soil C stocks across sites.

To assess how plant, edaphic, and environmental factors together, in combination with fertilization, affect soil C stocks, we constructed two parallel piecewise SEMs, one including soil mineralogy and one including moisture index. The strong correlation between moisture index and oxalate-extractable Al + Fe prevented inclusion of both variables simultaneously. This dataset is well-suited for analysis via piecewise SEM, which allows for nested experimental designs and is not limited by some of the constraints of standard SEM (e.g., small sample sizes). SEM model analysis was carried out with the *piecewiseSEM* 2.1 R package (Lefcheck, 2016). Piecewise SEM uses tests of directed separation to evaluate if there is justification for excluding specific pathways in an underspecified model, as omission of a missing path could change the interpretation of the overall model. Overall model fit is evaluated using Fisher's C statistic which simultaneously evaluates all tests of directed separation for the given model. For all missing paths identified in our analyses, we added the pathway into the model but transitioned to exploratory data analysis at this point, as we did not have *a priori* hypotheses for these pathways. Linear mixed models were fit for each component of the SEM, with block nested within site treated as a random effect in all cases. A composite variable was constructed to represent the effects of plant biomass and productivity. Specifically, we used the *stepAIC()* function in the MASS R package to determine which metrics of plant biomass and productivity best predicted each soil C fraction in a multiple linear regression model. Initial variable selection was based on *a priori* hypotheses and pairwise correlation coefficients, as described above, and AIC model selection determined that our initial full model, including root biomass, root production, and aboveground biomass, best predicted soil C for all three fractions. Multiple linear regression coefficients were then used to create a composite variable for plant biomass and productivity. Plant

tissue chemistry was represented as a weighted average (by relative root biomass) of leaf and root AUR:N. Soil mineralogy was represented as the sum of oxalate-extractable Al and Fe.

3 | RESULTS

3.1 | Treatment effects

Across nine temperate grassland sites varying widely in vegetation (including productivity, species composition, and diversity), edaphic, and climatic properties, a decade of N and P fertilization (alone and in combination) had no effect on soil C and N pools ($p > .10$; Figure 3), concentrations ($p > .10$; Table S1, Figure S1), or fractions ($p > .10$; Figure 4). Soil C and N were tightly correlated across treatments and sites. Hereafter we focus on soil C responses, noting that soil N responded similarly.

After controlling for differences among sites and blocks in a linear mixed model, nutrient amendment had no consistent effect on bulk soil C stocks ($p > .10$; Figure 3d). While positive, negative, and null responses of C stocks to fertilization were observed at individual

sites, there was no consistent directional response across sites, sites receiving N as urea or ammonium nitrate were similarly insensitive (Table S5), and variation due to fertilization was small compared to cross-site variation (Figure S2). The fixed effects only model showed significant site, P amendment, and site by nutrient amendment effects (Table S2).

3.2 | Factors influencing C stock responses to nutrient addition

Metrics of C supply to soil (i.e., aboveground biomass, root production, root biomass, aboveground litter AUR:N, root AUR:N) did not predict soil C stocks, as analyzed with the covariate linear mixed models (Table S3). Soil chemistry (pH and percent polyvalent base saturation) also did not predict soil C stock response to nutrient addition (Table S4). Large random effects variance in these covariate linear mixed models suggests there were strong site-specific factors that influenced soil C stocks and fractions. Indeed, there were large differences between marginal and conditional R^2 values in the models of plant effects ($R^2_m = 0.0010$,

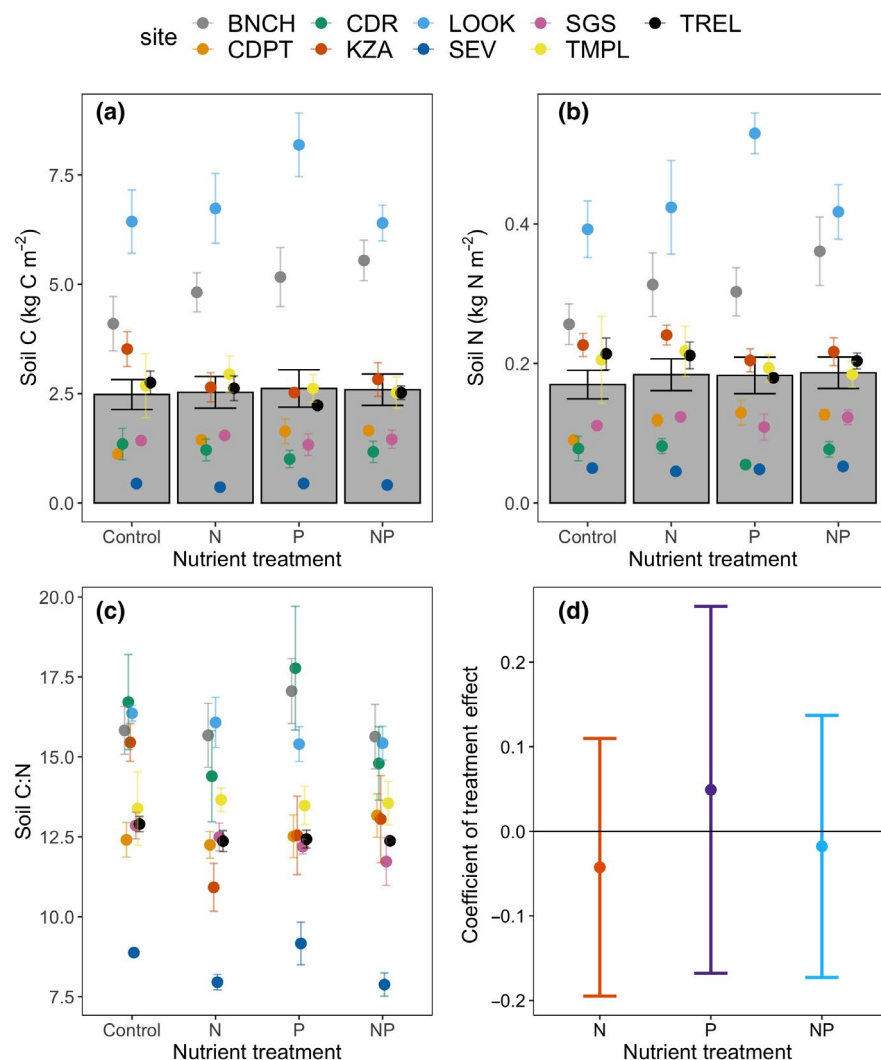


FIGURE 3 Nutrient treatment effects on bulk soil C and N pools. Following long-term nutrient amendment and measured from 0 to 15 cm depth, (a) soil C stocks, (b) soil N stocks, and (c) soil C:N ratios in control, +N, +P, and +NP plots. Across sites, there were no significant effects of factorial N × P addition on soil C and N stocks. Mean differences in soil C stocks between nutrient amended and control plots shown in (d) as coefficients from linear mixed model (nutrient treatments = fixed effects, site/block = random effect), with points representing mean treatment effects across all sites and error bars representing 95% confidence intervals. Confidence intervals spanning either side of zero indicate no significant differences in soil C stocks between control and nutrient amended plots. Site abbreviations as in Table 1. Note that one +NP sample at CDPT and one +N sample at KZA were missing, resulting in $n = 118$ [Colour figure can be viewed at wileyonlinelibrary.com]

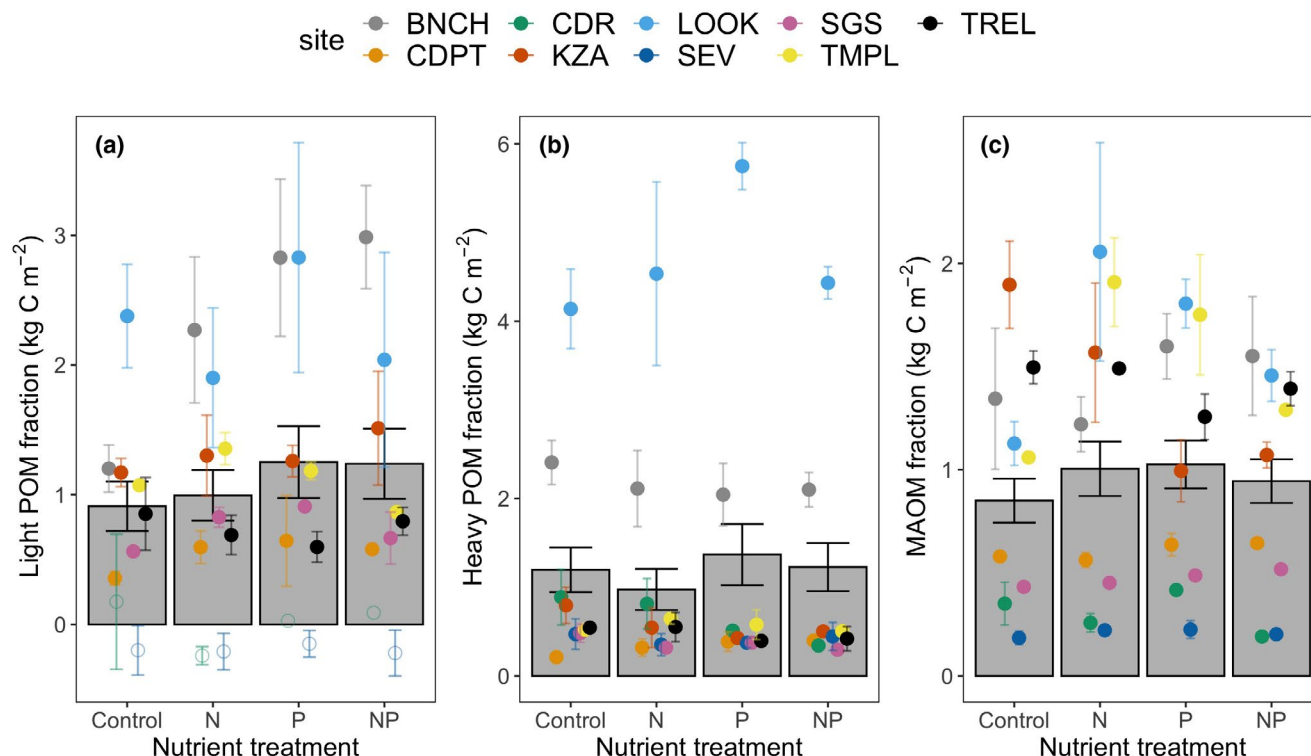


FIGURE 4 Nutrient treatment effects on soil C fractions. Following long-term nutrient amendment and measured from 0 to 15 cm depth, (a) light POM-C, (b) heavy POM-C, and (c) MAOM-C pools in control, +N, +P, and +NP. Across sites, there were no significant effects of factorial N $\times$ P addition on soil C pools for any soil fractions. Light POM pool size was estimated by mass balance as difference between bulk soil C and heavy POM-C + MAOM-C pools. Due to low C pools at SEV and CDR, estimates of light POM were negative; these points are shown as hollow circles and not included in calculations of overall treatment means and SE. Site abbreviations as in Table 1. Note, due to poor recovery of some samples, sample sizes per treatment for all fractions were reduced to: Control ($n = 22$), +N ($n = 26$), +P ($n = 22$), +NP (21) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Covariate linear mixed model predicting how nutrient amendment and environmental and edaphic factors (N dep = atmospheric N deposition, silt + clay = percent silt + percent clay, Al + Fe = oxalate-extractable Al + Fe) affect soil C stocks

Parameter	Estimate	Std error	t-value	p-value
+N	-0.10	1.40	-1.64	.10
+P	0.10	0.06	0.90	.37
+NP	0.13	0.11	1.45	.15
N dep	0.00	0.09	-1.00	.35
Silt + clay	0.00	0.01	0.43	.67
Log (Al + Fe)	0.35	0.16	2.17	.03
P $\times$ N dep	0.00	0.00	-1.94	.05
Random effects				
Site		1.51		
Block		<0.001		

Note: $R^2_{\text{marginal}} = 0.062$; $R^2_{\text{conditional}} = 0.75$.

$R^2_c = 0.80$) and soil chemical effects ($R^2_m < 0.001$, $R^2_c = 0.87$) on soil C, indicating that the predictors in the model accounted for very little of the among-site variation in the random effects, while other unmeasured site factors explained most of the

TABLE 3 Covariate linear mixed model predicting how nutrient amendment and environmental and edaphic factors (N dep = atmospheric N deposition, silt + clay = percent silt + percent clay) affect soil C stocks

Parameter	Estimate	Std error	t-value	p-value
+N	-0.05	0.06	-0.86	.39
+P	0.07	0.07	0.98	.33
+NP	0.05	0.08	0.54	.59
N dep	-0.07	0.04	-1.77	.13
Silt + clay	0.02	0.01	3.28	.00
Moisture index	2.59	0.22	11.72	.00
P $\times$ N dep	-0.03	0.01	-1.99	.05
Random effects				
Site		0.24		
Block		0.05		

Note: $R^2_{\text{marginal}} = 0.74$; $R^2_{\text{conditional}} = 0.77$.

Analogous to Table 2 but substituting moisture index for oxalate-extract Al + Fe as a predictive variable.

variation. In the covariate linear mixed model with site factors and fertilization effects predicting soil C stocks, soil oxalate-extractable Al + Fe was a significant fixed effect and there was

a weak interactive effect of atmospheric N deposition and P fertilization, while soil texture (percent silt + percent clay) and N amendment were not statistically significant; regardless, the explanatory power of the random site effects was much greater than that of the fixed effects (Table 2, $R^2_m = 0.062$, $R^2_c = 0.75$). Given the strong correlation between moisture index and oxalate-extractable Al + Fe ($r = 0.96$), a separate and parallel covariate linear mixed model was constructed with moisture index substituted for oxalate-extractable Al + Fe; while these two statistical models were largely similar, two notable differences arose. In the model including moisture index (but not in the model including soil Al + Fe oxides), soil texture (percent silt + clay) was a significant predictor of soil C and the fixed effects explained a large amount of the total variation (Table 3, $R^2_m = 0.74$, $R^2_c = 0.77$).

3.3 | Piecewise SEM examining soil C

While our linear mixed modeling approach revealed few consistent direct effects of nutrient amendment or plant, edaphic, or environmental factors on bulk soil C stocks, we expected many of these effects to be indirect. Thus, we conducted piecewise SEM analysis to examine direct and indirect controls on soil C storage. Analysis of our initial SEM including soil oxalate-extractable Al + Fe showed we were missing significant pathways between soil oxalate-extractable Al + Fe and plant biomass and productivity. Here, we present our revised model structure, noting that by revising our initial model structure the analysis becomes strictly exploratory in nature. Overall, the soil mineralogy SEM explained 85% of variation in soil C (Fisher's $C = 19.831$, $df = 20$, $p = .489$), yet most of this variation was due to the random variance structure ($R^2_m = 0.02$, $R^2_c = 0.85$) and there were no significant direct effects of any exogenous or endogenous variables on soil C (Figure 5a). However, N fertilization (and the interaction between N fertilization and N deposition) and soil oxalate-extractable Al + Fe significantly affected plant biomass. Given the strong correlation between soil oxalate-extractable Al + Fe and moisture index (preventing inclusion of both variables in a single SEM), the SEM analysis cannot determine whether the apparent effect of soil oxalate-extractable Al + Fe on plant biomass is mechanistic or reflects the effect of moisture on plant biomass.

Our SEM including moisture index (instead of soil oxalate-extractable Al + Fe and with an added path between moisture index and soil pH) explained 86% of variation in soil C (Figure 5b, Fisher's $C = 21.765$, $df = 18$, $p = .243$). In contrast to the SEM with soil oxalate-extractable Al + Fe, most of this variation was accounted for with the fixed effects ($R^2_m = 0.79$, $R^2_c = 0.85$). There were significant direct positive effects of moisture index on soil C. Moisture index had a direct negative effect on soil pH, and similar to the SEM with soil Al + Fe oxides, N fertilization and the interaction between N fertilization and N deposition affected plant biomass.

3.4 | Site factors driving soil C

Nutrient treatment effects on soil C pools were small compared to strong site differences in soil C storage (Table S5). Site effects were particularly strong when comparing the two Oregon sites (i.e., BNCH, LOOK), which have young soils dominated by poorly crystalline minerals, with the remaining seven sites. On average, the Oregon sites had 2.5 times more soil C compared to the other seven sites. Excluding the Oregon sites, bulk soil C stocks were strongly predicted by aboveground biomass ($F_{1,92} = 62.42$, $R^2 = 0.40$, $p < .001$), but not by root production (Figure 6a,b). Soil texture (percent silt + percent clay) was also positively related to bulk soil C stocks ($F_{1,92} = 190.3$, $R^2 = 0.67$, $p < .001$) and MAOM-C ($F_{1,67} = 315.1$, $R^2 = 0.82$, $p < .001$) across these seven sites (Figure 7c,f). While oxalate-extractable Al + Fe was positively related to MAOM-C across the seven sites outside Oregon ($F_{1,67} = 110.5$, $R^2 = 0.62$, $p < .001$; Figure 7d), Al + Fe also strongly predicted bulk soil C across all nine sites ($F_{1,115} = 404.2$, $R^2 = 0.78$, $p < .001$; Figure 7a). Similarly, moisture index was positively related to bulk soil C across all nine sites ($F_{1,116} = 470.1$, $R^2 = 0.80$, $p < .001$; Figure 7b), and positively related to MAOM-C after excluding the two Oregon sites ($F_{1,67} = 55.0$, $R^2 = 0.44$, $p < .001$; Figure 7e). Additionally, across all nine sites, MAOM-C stocks were positively related to aboveground biomass ($F_{1,89} = 38.39$, $R^2 = 0.29$, $p < .001$) but not root production (Figure 6c,d).

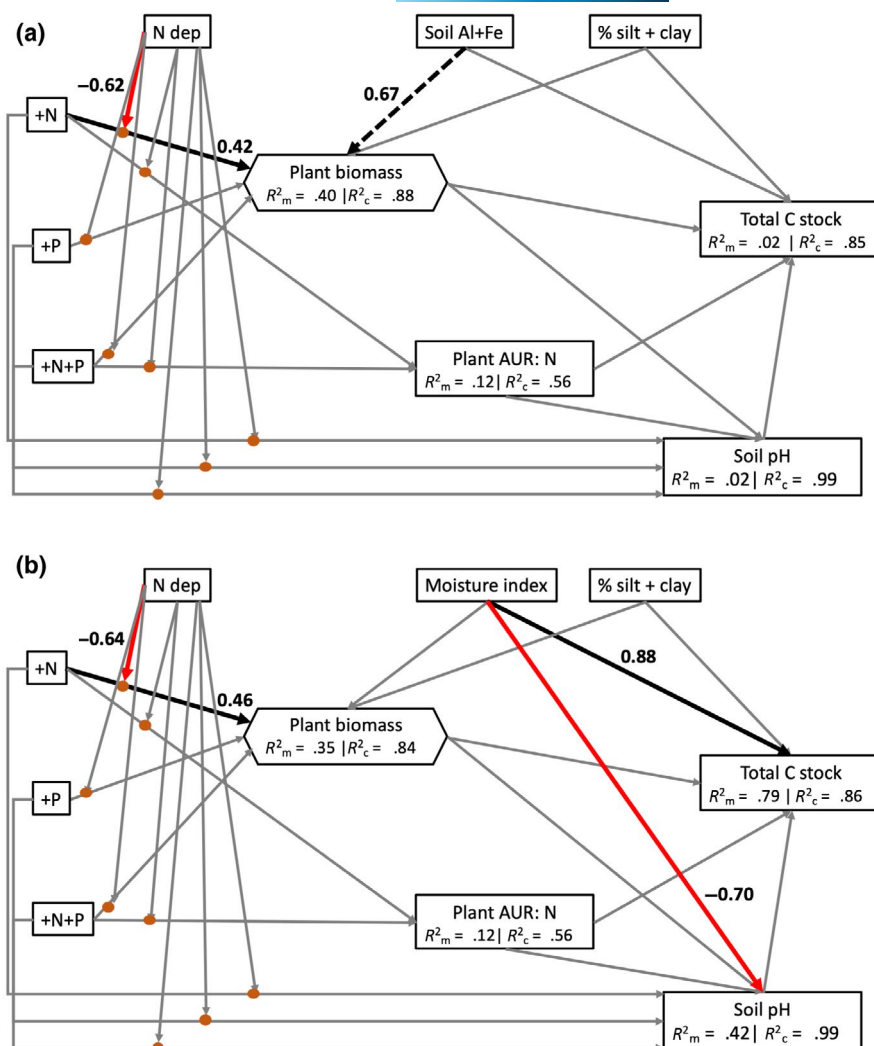
4 | DISCUSSION

4.1 | Fertilization effects on soil C pools

We examined the responses of soil C and N pools and fractions to a decade of N and P fertilization across nine temperate U.S. grasslands and assessed how plant, soil, and environmental factors mediated such responses (given the tight correlation between soil C and N patterns, hereafter we refer only to soil C). In contrast to our initial hypothesis, N and P inputs had highly variable effects on bulk soil C with site-specific nutrient effects ranging from a ~40% decrease (+P at KZA) to a 48% increase (+NP at CDPT) in soil C. While nutrient effects could be large, among-site variability in soil C was much larger. Soil C stocks varied nearly 40-fold across sites. Our sites represent a subset of sites previously reported to show minimal change in bulk soil C following short-term (2–4 years) N or P fertilization (Crowther et al., 2019). The results presented here indicate that soil C pools responded inconsistently to fertilization even after a decade. Our results contradict both theoretical expectations (Wieder et al., 2015) and empirical patterns observed in forested ecosystems (e.g., Frey et al., 2014) that suggest long-term nutrient amendment should promote soil C sequestration (Huang et al., 2020, but see Liu & Greaver, 2010).

Nutrient addition did increase total plant inputs (Fay et al., 2015; Seabloom et al., 2021a), yet this increase did not translate to greater soil C storage in fertilization treatments across sites

FIGURE 5 Structural equation model representing direct and mediating effects on bulk soil C storage (kg C m^{-2}). Soil oxalate-extractable Al + Fe is included in (a) and moisture index is included in (b). Composite variables are represented by hexagons while measured variables are presented in rectangles. Arrows ending in an orange dot indicate an interaction term. Statistically significant positive pathways are shown in black, significant negative pathways are shown in red, and pathways not originally included in the model (but deemed necessary by tests of directed separation) are shown with a dashed line. Non-significant pathways are shown in gray. Standardized coefficients are shown next to significant pathways. Marginal R^2 (R^2_m) values and conditional R^2 (R^2_c) values are shown for each component of the piecewise SEM, indicating the variability explained by the fixed effects or the fixed and random effects, respectively [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]



(Figures 3 and 5, Figure S3). Notably, one site, CDPT, exhibited strong aboveground biomass responses to short term (Fay et al., 2015) and decadal (this study) nutrient addition, and experienced the greatest relative increases in soil C storage in fertilized plots when compared to the other eight sites. However, across these sites, there was no direct relationship between fertilization responses of plant biomass and soil C (analysis not shown). Thus, while the overall lack of a consistent soil C response to fertilization was surprising, these results corroborate previous work in forests, grasslands, and agricultural systems concluding that aboveground inputs (which were responsive to fertilization in our study) are not the primary drivers of SOC formation and stabilization (Jackson et al., 2017; Rasse et al., 2005; Sokol & Bradford, 2019). Indeed, after 20 years of aboveground litter removal in a forest neighboring our Oregon grassland sites, soil C stocks did not change, while root removal increased MAOM but decreased POM pools (Pierson et al., 2021). While we did not directly measure decomposition rates of litter inputs, increases in aboveground biomass may have been offset by increased litter decomposition and C losses in response to the experimental treatments (Ochoa-Hueso et al., 2020). Previous single-site studies found that aboveground plant productivity increased in response to nutrient amendment but did not translate

into effects on SOM pools in grasslands (Fornara et al., 2013) and forests (Lajtha & Bowden, 2014).

In contrast to aboveground inputs, previous work suggests that the quantity and quality of root-derived inputs are stronger drivers of SOC. For example, root mass was positively related to SOC accrual following 27 years of N addition at Cedar Creek in Minnesota, USA (CDR) (Fornara & Tilman, 2012), while lignin-rich root litter promoted soil C stabilization under N fertilization (Dijkstra et al., 2004). Meanwhile, in a dryland site, belowground inputs were the primary contributor to SOM, yet the high lignin content of roots appeared to constrain SOM formation (Berenstecher et al., 2021). In a companion study to this one (at the same NutNet sites), N and P stimulated aboveground biomass but productivity belowground was insensitive to N addition and increased less with P addition (Keller et al., 2017–2018, unpublished). Thus, given weak fertilization effects on belowground plant productivity, root tissue chemistry, and total plant inputs, it is not surprising that SOC pools were insensitive to nutrient addition across our sites. Overall, the SEM illustrates that plants were directly influenced by nutrient inputs (e.g., fertilization treatment, atmospheric N deposition), soil factors such as mineralogy, and climate (given the strong correlation between soil mineralogy and moisture index, the relative importance of these two

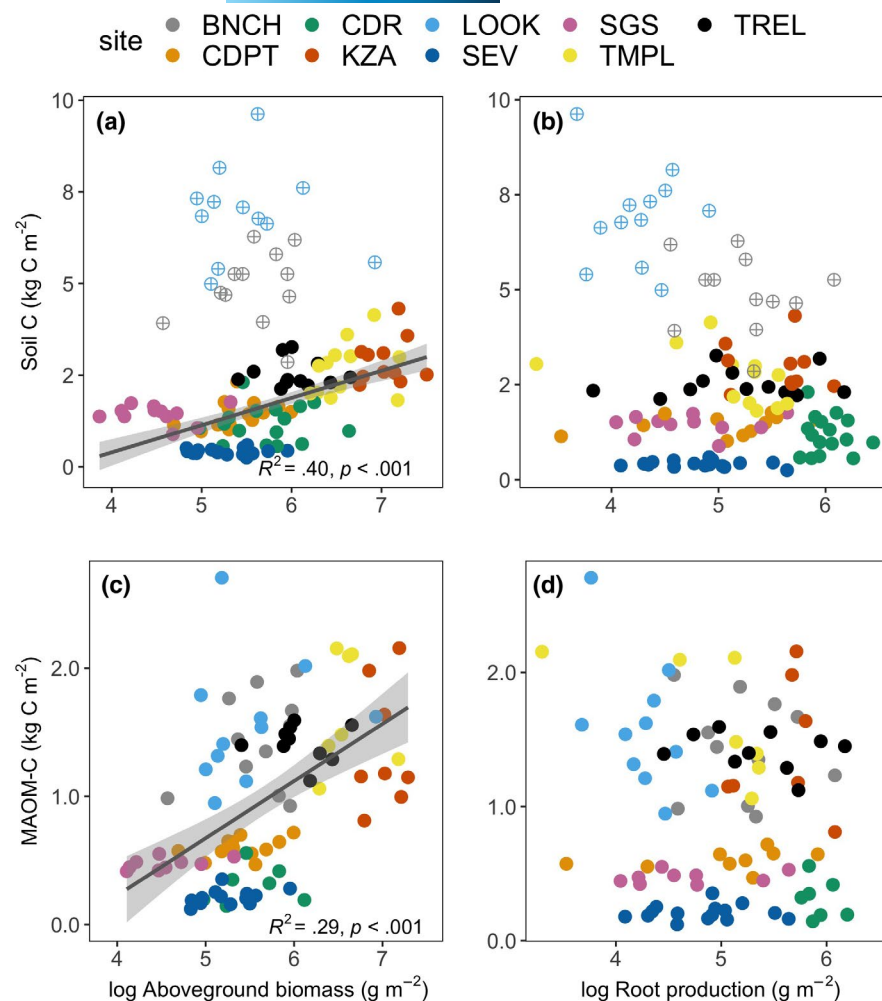


FIGURE 6 Bivariate relationships between bulk soil C stocks and (a) aboveground biomass and (b) root production and between MAOM-C and (c) aboveground biomass and (d) root production across sites. The significant linear regression between soil C and aboveground biomass across seven of the nine sites is shown, with the Oregon data points shown as hollow cross-hatched points for bulk soil C. For consistency, the Oregon data are also shown as cross-hatched points in (b) although no significant relationship was observed. The significant relationship across all nine sites for MAOM-C and aboveground biomass is shown, while no significant relationship was observed between MAOM-C and root production [Colour figure can be viewed at wileyonlinelibrary.com]

factors could not be teased apart), yet these effects did not translate to enhanced soil C storage in response to plot-scale nutrient supply (Figure 5). This result suggests that microbial activity—the critical link between C inputs and soil C formation—was either minimally affected by fertilization or increased in ways that offset increased C supply (Hargreaves & Hofmockel, 2014; Kaskanke et al., 2021), although direct measures of microbial decomposition are needed (Kallenbach et al., 2016).

We also expected nutrient amendment to drive soil acidification and alter cation concentrations, with consequences for SOC dynamics (Figure 1). Although soil acidification is commonly reported following experimental N addition (Li et al., 2018), treatments in this study did not consistently affect soil pH (Table S1, Figure 5; (Moore et al., 2021)). Meanwhile, exchangeable concentrations of polyvalent base cations (i.e., Ca + Mg) decreased with N addition (Table S2), but without apparent effects on soil C storage (Figure 5). While this could indicate more time is needed for nutrient effects on soil chemistry to alter soil C storage, or that changes in base cation concentrations were too small to have measurable effects on soil C stabilization, base cation concentrations and soil C pools were unrelated regardless of treatment.

Given that bulk soil C stocks represent a large C pool composed of distinct fractions, each with unique factors driving soil C cycling,

we expected that nutrient amendment effects would be more pronounced for specific soil fractions. Again, contrary to our hypotheses, we found no fertilization effects on sizes of the light POM, heavy POM, or MAOM-C fractions (Figure 4). This may reflect the difficulty in detecting small changes in relatively large pools, even for distinct C fractions. Indeed, there was a non-significant trend toward greater light POM pools in the nutrient addition treatments, likely a result of greater aboveground plant inputs in fertilized plots. More time may be needed to detect changes in intermediate-aged and old C fractions (Neff et al., 2002). Sampling immediately following the growing season may have captured seasonal increases in light POM (due to fresh inputs of detritus) along with short-term decreases in MAOM (due to priming of older SOC) (Blagodatskaya & Kuzyakov, 2008). Alternatively, soil fractions may be resistant to fertilization effects. For example, belowground plant inputs promote aggregate formation and thus influence heavy POM pool size (Rillig, 2004). Therefore, heavy POM-C storage may not vary significantly across treatments if there are not strong fertilization effects on belowground biomass, as observed in a companion study (Keller et al., 2017–2018, unpublished). MAOM-C pools may also show minimal treatment effects if, for example, mineral exchange sites are saturated, or fertilization effects on pH and cation chemistry are not sufficient to alter SOM adsorption onto mineral surfaces.

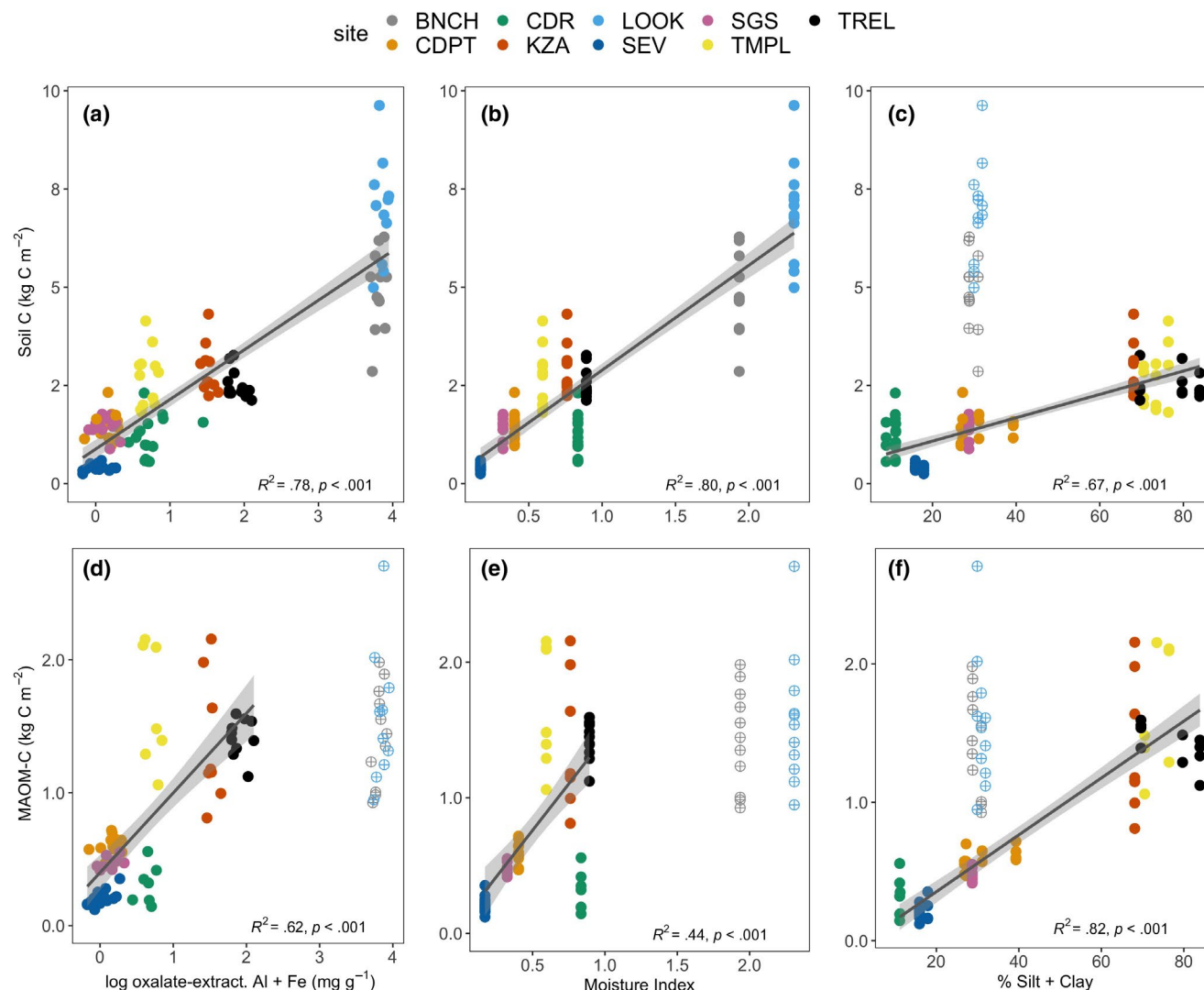


FIGURE 7 Bivariate relationships between bulk soil C stocks and (a) poorly crystalline oxalate-extractable Al and Fe, (b) moisture index, and (c) percent silt + clay and between MAOM-C and (d) oxalate-extractable Al + Fe, (e) moisture index, and (f) percent silt + clay across sites. Simple linear regressions are shown either across all nine sites (a, b) or across seven sites excluding the Oregon sites (c–f), dependent on which regression was strongest. Where regressions exclude the Oregon sites, the Oregon site data are shown as hollow cross-hatched points [Colour figure can be viewed at wileyonlinelibrary.com]

4.2 | Site-specific effects on soil C pools

While fertilization did not consistently and strongly alter soil C storage, soil C pools varied by more than an order of magnitude among sites (Figures 6 and 7). In general, these among-site differences were strongly driven by the two Oregon sites, which are in close proximity to one another and are characterized by a high moisture index and young soils of volcanic origin, enriched with poorly crystalline, amorphous colloidal materials with high surface area. Consequently, these soils had low percent clay but high percent C and percent ferrihydrite (Zhao et al., 2020). Ferrihydrite, a highly reactive short-range ordered iron oxide that is important for C stabilization (Lv et al., 2016), was previously shown to relate strongly to organic matter stabilization across a subset of our sites (Zhao et al., 2020). Similarly, here we report a strong positive relationship between

poorly crystalline Al + Fe and soil C stocks; importantly, this pattern holds after excluding the young Oregon sites, suggesting oxalate-extractable Al + Fe, with high reactive surfaces and sorption affinity, may be useful in predicting soil C across sites with disparate mineralogies (Coward et al., 2018; Zhao et al., 2020). Our results also add to the growing evidence that percent clay does not consistently predict soil C storage across a broad range of mineralogies (Coward et al., 2017; Rasmussen et al., 2018; Torn et al., 1997b). Meanwhile, both soil texture and oxalate-extractable Al + Fe were good predictors of MAOM-C only after excluding the Oregon sites (Figure 7c,d). Along with the large heavy POM-C pools in the Oregon soils (Figure 4b), this emphasizes that poorly crystalline metal oxides may be important facilitators of aggregation in addition to promoting chemical stabilization (Chesters et al., 1957). Thus, while soil texture may reasonably predict soil C at many sites, our results highlight that

oxalate-extractable Al + Fe should also be considered for accurate modeling of soil C at broad scales (Figure 7a,b).

In addition to soil mineralogy, water availability, as measured by moisture index, strongly predicted soil C across sites (Figure 7b,e). Notably, the Oregon sites characterized by unique soil Al + Fe oxide contents were also the wettest sites (cool, wet winters and summer drought conditions are common at these sites), making it difficult to tease apart the relative importance of these two factors in driving soil C patterns (oxalate-extractable Al + Fe and moisture index were strongly correlated, $r = 0.96$, data not shown). Indeed, there may be a direct link between moisture and soil Al + Fe oxide content. Sites with high soil moisture likely experience frequent rain events resulting in higher weathering rates and redox fluctuations of soil. Redox fluctuations may increase reducible poorly crystalline Fe hydroxide pools (determined by oxalate-extractable Al + Fe), as organic C co-precipitation and cation substitutions inhibit mineral ripening (Chen et al., 2015; Ginn et al., 2017; Thompson et al., 2011). Independent of soil mineralogy, we expect water availability to predominantly affect soil C storage indirectly via effects on plant inputs, decomposition rates (Zhao et al., 2021), and pH and polyvalent base cation effects on soil organic C chemical stabilization. Soil C storage may also be suppressed in arid sites with high soil pH if phenol oxidase and peroxidase enzyme activities are high and stimulate SOM decomposition (Sinsabaugh, 2010, but see Hou et al., 2020).

Aboveground biomass was positively related to soil C storage across sites with the exception of the Oregon sites (Figure 6a,c). At most sites, this relationship likely reflects a positive feedback between plant productivity and soil fertility such that C- and nutrient-rich soils promote plant productivity and the return of this plant C to the soil enhances soil C (Seabloom et al., 2021a). Previous work showed that soil C storage was driven more by new C inputs rather than the turnover of existing soil C (Lange et al., 2015), and our observed positive relationship between aboveground biomass and soil C across seven of our nine sites supports this interpretation. Distinct C stabilization pathways in the Oregon sites driven by their poorly crystalline, Al + Fe oxide-rich soils may explain why those sites have higher soil C pools that might be expected based on their aboveground biomass and do not show the relationship between soil C and aboveground biomass seen across sites outside of Oregon. Indeed, the Oregon sites have much greater heavy POM-C pools compared to the other sites, suggesting macro-aggregation as well as direct sorption may be a dominant C stabilization pathway at these sites (Kong et al., 2005; Wagai et al., 2018).

Meanwhile, we found no positive relationship between root production and soil C (with or without the Oregon sites; Figure 6b,d). Several factors may explain this. For one, our measures of belowground inputs do not include rhizodeposition and mycorrhizal fungal inputs. While root detritus, rhizodeposition, and mycorrhizal fungal biomass all influence soil C formation and stabilization, these three components of belowground inputs can vary independently from one another and across sites (Frey, 2019). This may act to decouple root production and soil C. Second, in contrast to aboveground plant tissues, roots respond to a highly heterogeneous soil environment. Consequently, the broader suite of factors controlling belowground (compared to aboveground) productivity may preclude clear relationships between root

productivity and single environmental factors such as soil C. Third, it is possible that a relationship between root production and soil C does exist, but methodological challenges prevented the detection of such a pattern. While aboveground biomass turns over annually and is relatively easy to measure in these grasslands, root phenology may not match aboveground phenology and can vary considerably across sites making consistent cross-site measures of root productivity methodologically challenging (Finzi & Abramoff, 2015).

4.3 | Outstanding questions and future directions

Why did fertilization not consistently and significantly increase soil C storage? Across nine grassland sites, the weak and variable soil C responses to fertilization were similar at 2–4 years (Crowther et al., 2019) and 10 years (this study) after the initiation of nutrient treatments. On one hand, significant treatment effects may take multiple decades to manifest, especially in slow cycling soil pools (Fornara & Tilman, 2012; Seabloom et al., 2021b). This may be particularly relevant here, where NPP inputs only slightly increased with fertilization. On the other hand, increased inputs may be offset by enhanced SOM decomposition resulting in no net change in soil C storage. However, while we did not directly measure SOM decomposition rates in this study, we have no evidence that fertilization stimulated SOM decomposition. To the contrary, related studies at these same sites find evidence that fertilization suppressed SOM decomposition rates (Q. Zhao, personal communication, July 28, 2021; L. DeLancey, personal communication, July 28, 2021), as has been reported elsewhere (Ramirez et al., 2012). However, these studies of nutrient effects on SOM decomposition were done in the absence of rhizosphere effects. Whether nutrients influence rhizosphere priming of SOM decomposition to offset increased SOM inputs is relatively unstudied (Kuzyakov & Xu, 2013). Long-term studies of fertilization effects on soil C pools and fluxes are thus critical to assessing the sensitivity of soil C to fertilization over multiple decades.

Regardless of mechanism, our study adds to growing evidence that grassland soils may be highly resistant to changes in soil C storage in response to nutrient enrichment, with minimal effects of single element fertilization on soil C stocks. We also show that soil C responses were not sensitive to the form of N supply (i.e., urea or ammonium nitrate). Enhanced soil C storage may occur only when macro- and micronutrients are applied in concert (Crowther et al., 2019; Fornara et al., 2013; Kaspari, 2021; Pastore et al., 2021; Seabloom et al., 2021b), consistent with the notion that nutrient co-limitation is common across grasslands (Fay et al., 2015; Harpole et al., 2011). Moreover, fertilization effects on soil C storage may be particularly weak in mineral soils in both forests and grasslands. In forests, nutrient effects were predominantly observed in the O-horizon while deeper soils were generally insensitive to treatments (Frey et al., 2014; Zak, 2008). Unique properties of the O-horizon, such as high lignin-rich organic matter content, high soil C:N, and distinct decomposer communities, may make these horizons particularly sensitive to nutrient addition. Nutrient addition can alter organic matter chemistry, reduce fungal decomposer biomass, and shift microbial metabolism, factors which can reduce

decomposition and lead to accrual of soil C, particularly in organic-rich soil layers. Therefore, the lack of an O-horizon in grassland systems may explain the weak response to nutrient treatments we observed.

Inconsistent fertilization responses across sites in this study also highlight the importance of site-specific factors and context dependence in driving soil C dynamics, while cautioning against generalizing individual site patterns to broader scales. While soil C responses to fertilization ranged from negative to null to positive, plant and edaphic factors were not strong predictors of such site context dependency. To improve our mechanistic understanding of SOM dynamics in grasslands, additional cross-site research teasing apart the effects of mineralogy and climate on SOM pools and fluxes is needed. Rates of anthropogenic nutrient deposition are highly variable across space and time, and such variation can affect ecosystem C responses to nutrient addition (Wang et al., 2019). Interactive effects of additional global change factors, such as rising atmospheric CO₂ and altered precipitation regimes, are also expected to influence ecosystem responses to nutrients (Reich et al., 2020). Thus, it remains challenging to predict how long-term nutrient deposition may influence grassland soil C sequestration into the future.

Soil C has been shown to generally accumulate over time where highly productive perennial grass crops are grown (Anderson-Teixeira et al., 2009). This provides evidence for a positive relationship between NPP and soil C storage. However, it is not clear whether experimental outcomes differ between agricultural sites with legacies of forming under grassland versus forest vegetation. Whatever the answer, the site-specific variation in soil C dynamics described in the current study reinforces that the benefits of deploying crops to sequester C will vary considerably across soil types. Improved understanding of the interaction between soil, vegetation, and management is needed if C sequestration and climate mitigation are to be maximized (Lemus & Lal, 2005; Smith, 2004).

Here we show that while grasslands are important reservoirs of soil C globally, the capacity of these ecosystems to sequester additional C is unlikely to increase consistently in response to N and P fertilization. Thus, our work demonstrates that the effects of anthropogenic N or P addition to temperate grasslands may do little to mitigate rising atmospheric CO₂ concentrations, and could even contribute to rising atmospheric CO₂ if emissions associated with fertilizer production are considered (Schlesinger, 1999). However, the variation among sites and the cross-site positive relationship between aboveground biomass and soil C storage (especially in slow-cycling C pools) observed in this study suggest that prioritizing the protection of highly productive temperate grasslands with C-rich soils could be an effective tool for limiting future greenhouse gas losses arising from land use change.

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DATA AVAILABILITY STATEMENT

Data can be accessed at: <https://doi.org/10.6073/pasta/7f984c2ed9e63754577ee711e6d74a6e>.

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REFERENCES

- Ackerman, D., Millet, D. B., & Chen, X. (2019). Global estimates of inorganic nitrogen deposition across four decades. *Global Biogeochemical Cycles*, 33, 100–107. <https://doi.org/10.1029/2018GB005990>
- Adair, E. C., Reich, P. B., Hobbie, S. E., & Knops, J. M. H. (2009). Interactive effects of time, CO₂, N, and diversity on total belowground carbon allocation and ecosystem carbon storage in a grassland community. *Ecosystems*, 12, 1037–1052. <https://doi.org/10.1007/s10021-009-9278-9>
- Amezketta, E. (1999). Soil aggregate stability: A review. *Journal of Sustainable Agriculture*, 14, 83–151. https://doi.org/10.1300/J064v14n02_08
- Anderson-Teixeira, K. J., Davis, S. C., Masters, M. D., & Delucia, E. H. (2009). Changes in soil organic carbon under biofuel crops. *GCB Bioenergy*, 1, 75–96. <https://doi.org/10.1111/j.1757-1707.2008.01001.x>

- Baldock, J. A., & Skjemstad, J. O. (2000). Role of the soil matrix and minerals in protecting natural organic materials against biological attack. *Organic Geochemistry*, 31, 697–710. [https://doi.org/10.1016/S0146-6380\(00\)00049-8](https://doi.org/10.1016/S0146-6380(00)00049-8)
- Barto, E. K., Alt, F., Oelmann, Y., Wilcke, W., & Rillig, M. C. (2010). Contributions of biotic and abiotic factors to soil aggregation across a land use gradient. *Soil Biology & Biochemistry*, 42, 2316–2324. <https://doi.org/10.1016/j.soilbio.2010.09.008>
- Berenstecher, P., Araujo, P. I., & Austin, A. T. (2021). Worlds apart: Location above- or below-ground determines plant litter decomposition in a semi-arid Patagonian steppe. *Journal of Ecology*, 1–12. <https://doi.org/10.1111/1365-2745.13688>
- Berg, B., & Matzner, E. (1997). Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. *Environmental Reviews*, 5, 1–25. <https://doi.org/10.1139/a96-017>
- Blagodatskaya, E., & Kuzyakov, Y. (2008). Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: Critical review. *Biology and Fertility of Soils*, 45, 115–131. <https://doi.org/10.1007/s00374-008-0334-y>
- Borer, E. T., Harpole, W. S., Adler, P. B., Lind, E. M., Orrock, J. L., Seabloom, E. W., & Smith, M. D. (2014a). Finding generality in ecology: A model for globally distributed experiments. *Methods in Ecology and Evolution*, 5, 65–73. <https://doi.org/10.1111/2041-210X.12125>
- Borer, E. T., Seabloom, E. W., Gruner, D. S., Harpole, W. S., Hillebrand, H., Lind, E. M., Adler, P. B., Alberti, J., Anderson, T. M., Bakker, J. D., Biederman, L., Blumenthal, D., Brown, C. S., Brudvig, L. A., Buckley, Y. M., Cadotte, M., Chu, C., Cleland, E. E., Crawley, M. J., Yang, L. H. (2014b). Herbivores and nutrients control grassland plant diversity via light limitation. *Nature*, 508, 517–520. <https://doi.org/10.1038/nature13144>
- Chapin, F. S., Matson, P. A., & Mooney, H. A. (2002). *Principles of terrestrial ecosystem ecology*. Springer-Verlag.
- Chen, C., Kukkadapu, R., & Sparks, D. L. (2015). Influence of coprecipitated organic matter on Fe²⁺(aq)-catalyzed transformation of ferrihydrite: Implications for carbon dynamics. *Environmental Science and Technology*, 49, 10927–10936.
- Chesters, G., Attoe, O. J., & Allen, O. N. (1957). Soil aggregation in relation to various soil constituents. *Soil Science Society of America Journal*, 21, 272–277. <https://doi.org/10.2136/sssaj1957.03615995002100030007x>
- Cleland, E. E., Lind, E. M., DeCraeppeo, N. M., DeLorenze, E., Wilkins, R. A., Adler, P. B., Bakker, J. D., Brown, C. S., Davies, K. F., Esch, E., Firn, J., Gressard, S., Gruner, D. S., Hagenah, N., Harpole, W. S., Hautier, Y., Hobbie, S. E., Hofmockel, K. S., Kirkman, K., ... Seabloom, E. W. (2019). Belowground biomass response to nutrient enrichment depends on light limitation across globally distributed grasslands. *Ecosystems*, 22, 1466–1477. <https://doi.org/10.1007/s10021-019-00350-4>
- Cotrufo, M. F., Soong, J. L., Horton, A. J., Campbell, E. E., Haddix, M. L., Wall, D. H., & Parton, W. J. (2015). Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nature Geoscience*, 8, 776–779. <https://doi.org/10.1038/ngeo2520>
- Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The microbial efficiency-matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology*, 19, 988–995. <https://doi.org/10.1111/gcb.12113>
- Coward, E. K., Ohno, T., & Plante, A. F. (2018). Adsorption and molecular fractionation of dissolved organic matter on iron-bearing mineral matrices of varying crystallinity. *Environmental Science and Technology*, 52, 1036–1044. <https://doi.org/10.1021/acs.est.7b04953>
- Coward, E. K., Thompson, A. T., & Plante, A. F. (2017). Iron-mediated mineralogical control of organic matter accumulation in tropical soils. *Geoderma*, 306, 206–216. <https://doi.org/10.1016/j.geoderma.2017.07.026>
- Crowther, T. W., Riggs, C., Lind, E. M., Borer, E. T., Seabloom, E. W., Hobbie, S. E., Wubs, J., Adler, P. B., Firn, J., Gherardi, L., Hagenah, N., Hofmockel, K. S., Knops, J. M. H., McCulley, R. L., MacDougall, A. S., Peri, P. L., Prober, S. M., Stevens, C. J., & Routh, D. (2019). Sensitivity of global soil carbon stocks to combined nutrient enrichment. *Ecology Letters*, 22, 936–945. <https://doi.org/10.1111/ele.13258>
- Dijkstra, F. A., Hobbie, S. E., Knops, J. M. H., & Reich, P. B. (2004). Nitrogen deposition and plant species interact to influence soil carbon stabilization. *Ecology Letters*, 7, 1192–1198. <https://doi.org/10.1111/j.1461-0248.2004.00679.x>
- Duiker, S. W., Rhoton, F. E., Torrent, J., Smeck, N. E., & Lal, R. (2003). Iron (Hydr)Oxide crystallinity effects on soil aggregation. *Soil Science Society of America Journal*, 67, 606–611. <https://doi.org/10.2136/sssaj2003.6060>
- Edwards, I. P., Zak, D. R., Kellner, H., Eisenlord, S. D., & Pregitzer, K. S. (2011). Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a Northern Hardwood forest. *PLoS One*, 6, 1–10. <https://doi.org/10.1371/journal.pone.0020421>
- Eisenlord, S. D., Freedman, Z., Zak, D. R., Xue, K., He, Z., & Zhou, J. (2013). Microbial mechanisms mediating increased soil C storage under elevated atmospheric N deposition. *Applied and Environmental Microbiology*, 79, 1191–1199. <https://doi.org/10.1128/AEM.03156-12>
- Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., & Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, 10, 1135–1142. <https://doi.org/10.1111/j.1461-0248.2007.01113.x>
- Fay, P. A., Prober, S. M., Harpole, W. S., Knops, J. M. H., Bakker, J. D., Borer, E. T., Lind, E. M., MacDougall, A. S., Seabloom, E. W., Wragg, P. D., Adler, P. B., Blumenthal, D. M., Buckley, Y. M., Chu, C., Cleland, E. E., Collins, S. L., Davies, K. F., Du, G., Feng, X., ... Yang, L. H. (2015). Grassland productivity limited by multiple nutrients. *Nature Plants*, 1(7), <https://doi.org/10.1038/nplants.2015.80>
- Finzi, A. C., & Abramoff, R. Z. (2015). Are above-and below-ground phenology in sync? *New Phytologist*, 205, 1054–1061.
- Fornara, D. A., Banin, L., & Crawley, M. J. (2013). Multi-nutrient vs. nitrogen-only effects on carbon sequestration in grassland soils. *Global Change Biology*, 19, 3848–3857. <https://doi.org/10.1111/gcb.12323>
- Fornara, D. A., & Tilman, D. (2012). Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition. *Ecology*, 93, 2030–2036. <https://doi.org/10.1890/12-0292.1>
- Frey, S. D. (2019). Mycorrhizal fungi as mediators of soil organic matter dynamics. *Annual Review of Ecology, Evolution, and Systematics*, 50(1), 237–259.
- Frey, S. D., Ollinger, S., Nadelhoffer, K., Bowden, R., Brzostek, E., Burton, A., Caldwell, B. A., Crow, S., Goodale, C. L., Grandy, A. S., Finzi, A., Kramer, M. G., Lajtha, K., LeMoine, J., Martin, M., McDowell, W. H., Minocha, R., Sadowsky, J. J., Templer, P. H., & Wickings, K. (2014). Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. *Biogeochemistry*, 121, 305–316. <https://doi.org/10.1007/s10533-014-0004-0>
- Ginn, B., Meile, C., Wilmoth, J., Tang, Y., & Thompson, A. (2017). Rapid iron reduction rates are stimulated by high-amplitude redox fluctuations in a tropical forest soil. *Environmental Science and Technology*, 51, 3250–3259. <https://doi.org/10.1021/acs.est.6b05709>
- Hargreaves, S. K., & Hofmockel, K. S. (2014). Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. *Biogeochemistry*, 117, 67–79. <https://doi.org/10.1007/s10533-013-9893-6>
- Harpole, W. S., Ngai, J. T., Cleland, E. E., Seabloom, E. W., Borer, E. T., Bracken, M. E. S., Elser, J. J., Gruner, D. S., Hillebrand, H., Shurin, J. B., & Smith, J. E. (2011). Nutrient co-limitation of primary

- producer communities. *Ecology Letters*, 14, 852–862. <https://doi.org/10.1111/j.1461-0248.2011.01651.x>
- Harris, D., Horwa, W. R., & Kessel, C. V. (2001). Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. *Soil Science Society of America Journal*, 1856, 1853–1856. <https://doi.org/10.2136/sssaj2001.1853>
- He, N., Yu, Q., Wang, R., Zhang, Y., Gao, Y., & Yu, G. (2013). Enhancement of carbon sequestration in soil in the temperate grasslands of Northern China by addition of nitrogen and phosphorus. *PLoS One*, 8, 1–11. <https://doi.org/10.1371/journal.pone.0077241>
- Hobbie, S. E. (2021). *Plant and soil organic matter responses to ten years of nutrient enrichment in the Nutrient Network. A cross-site investigation of bottom-up control over herbaceous plant community dynamics and ecosystem function ver 1*. Environmental Data Initiative. <https://doi.org/10.6073/pasta/7f984c2ed9e63754577ee711e6d74a6e>
- Hou, S., Hättenschwiler, S., Yang, J., Sistla, S., Wei, H., Zhang, Z., Hu, Y.-Y., Ru-Zhen, W., Shu-Yan, C., Xiao-Tao, L., & Xing-Guo, H. (2020). Increasing rates of long-term nitrogen deposition consistently increased litter decomposition in a semi-arid grassland. *New Phytologist*, 229, 296–307.
- Huang, X., Terrer, C., Dijkstra, F. A., Hungate, B. A., Zhang, W., & van Groenigen, K. J. (2020). New soil carbon sequestration with nitrogen enrichment: A meta-analysis. *Plant and Soil*, 299–310. <https://doi.org/10.1007/s11104-020-04617-x>
- Isbell, F., Reich, P. B., Tilman, D., Hobbie, S. E., Polasky, S., & Binder, S. (2013). Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proceedings of the National Academy of Sciences U S A*, 110, 11911–11916. <https://doi.org/10.1073/pnas.1310880110>
- Jackson, R. B., Lajtha, K., Crow, S. E., Hugelius, G., Kramer, M. G., & Piñeiro, G. (2017). The ecology of soil carbon: Pools, vulnerabilities, and biotic and abiotic controls. *Annual Review of Ecology Evolution and Systematics*, 48, 419–445. <https://doi.org/10.1146/annurev-ecolsys-112414-054234>
- Jastrow, J. D., Amonette, J. E., & Bailey, V. L. (2007). Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. *Climatic Change*, 80, 5–23. <https://doi.org/10.1007/s10584-006-9178-3>
- Kallenbach, C. M., Grandy, A., & Frey, S. D. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications*, 7, 1–10. <https://doi.org/10.1038/ncomms13630>
- Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., Zimmermann, N. E., Linder, H. P., & Kessler, M. (2017). Climatologies at high resolution for the earth's land surface areas. *Scientific Data*, 4, 1–20. <https://doi.org/10.1038/sdata.2017.122>
- Kasanke, C. P., Zhao, Q., Bell, S., Thompson, A. M., & Hofmocker, K. S. (2021). Can switchgrass increase carbon accrual in marginal soils? The importance of site selection. *GCB Bioenergy*, 13, 320–335. <https://doi.org/10.1111/gcbb.12777>
- Kaspari, M. (2021). The invisible hand of the periodic table: How micro-nutrients shape ecology. *Annual Review of Ecology Evolution and Systematics*, 52, 199–219. <https://doi.org/10.1146/annurev-ecolsys-012021-090118>
- Keller, A. B., Brzostek, E. R., Craig, M. E., Fisher, J. B., & Phillips, R. P. (2021). Root-derived inputs are major contributors to soil carbon in temperate forests, but vary by mycorrhizal type. *Ecology Letters*, 24, 626–635. <https://doi.org/10.1111/ele.13651>
- Knorr, M., Frey, S. D., & Curtis, P. S. (2005). Nitrogen additions and litter decomposition: A meta-analysis. *Ecology*, 86, 3252–3257. <https://doi.org/10.1890/05-0150>
- Kong, A. Y. Y., Six, J., Bryant, D. C., Denison, R. F., & van Kessel, C. (2005). The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. *Soil Science Society of America Journal*, 69, 1078–1085. <https://doi.org/10.2136/sssaj2004.0215>
- Kuzyakov, Y., & Xu, X. (2013). Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytologist*, 198, 656–669. <https://doi.org/10.1111/nph.12235>
- Lajtha, K., & Bowden, R. D. (2014). Litter and root manipulations provide insights into soil organic matter dynamics and stability. *Soil Science Society of America Journal*, 78(S1). <https://doi.org/10.2136/sssaj2013.08.0370nafsc>
- Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., Mellado-Vázquez, P. G., Malik, A. A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B. C., Trumbore, S. E., & Gleixner, G. (2015). Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications*, 6(1). <https://doi.org/10.1038/ncomms7707>
- LeBauer, D., & Treseder, K. K. (2008). Nitrogen limitation of net primary productivity. *Ecology*, 89, 371–379.
- Lee, M., Manning, P., Rist, J., Power, S. A., & Marsh, C. (2010). A global comparison of grassland biomass responses to CO₂ and nitrogen enrichment. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 2047–2056.
- Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, 7, 573–579.
- Lemus, R., & Lal, R. (2005). Bioenergy crops and carbon sequestration. *Critical Reviews in Plant Sciences*, 24, 1–21. <https://doi.org/10.1080/07352680590910393>
- Li, Y., Niu, S., & Yu, G. (2016). Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: A meta-analysis. *Global Change Biology*, 22, 934–943. <https://doi.org/10.1111/gcb.13125>
- Li, Y., Sun, J., Tian, D., Wang, J., Ha, D., Qu, Y., Jing, G., & Niu, S. (2018). Soil acid cations induced reduction in soil respiration under nitrogen enrichment and soil acidification. *Science of the Total Environment*, 615, 1535–1546. <https://doi.org/10.1016/j.scitotenv.2017.09.131>
- Liang, C., Amelung, W., Lehmann, J., & Kästner, M. (2019). Quantitative assessment of microbial necromass contribution to soil organic matter. *Global Change Biology*, 25, 3578–3590. <https://doi.org/10.1111/gcb.14781>
- Liu, L., & Greaver, T. L. (2010). A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters*, 13, 819–828. <https://doi.org/10.1111/j.1461-0248.2010.01482.x>
- Lu, M., Zhou, X., Luo, Y., Yang, Y., Fang, C., Chen, J., & Li, B. O. (2011). Minor stimulation of soil carbon storage by nitrogen addition: A meta-analysis. *Agriculture, Ecosystems & Environment*, 140, 234–244. <https://doi.org/10.1016/j.agee.2010.12.010>
- Lützow, M. V., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., & Flessa, H. (2006). Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions—A review. *European Journal of Soil Science*, 57, 426–445. <https://doi.org/10.1111/j.1365-2389.2006.00809.x>
- Lv, J., Zhang, S., Wang, S., Luo, L., Cao, D., & Christie, P. (2016). Molecular-scale investigation with ESI-FT-ICR-MS on fractionation of dissolved organic matter induced by adsorption on iron oxyhydroxides. *Environmental Science and Technology*, 50, 2328–2336. <https://doi.org/10.1021/acs.est.5b04996>
- Midolo, G., Alkemade, R., Schipper, A. M., Benítez-López, A., Perring, M. P., & De Vries, W. (2019). Impacts of nitrogen addition on plant species richness and abundance: A global meta-analysis. *Global Ecology and Biogeography*, 28, 398–413. <https://doi.org/10.1111/geb.12856>
- Moore, J. A. M., Anthony, M. A., Pec, G. J., Trocha, L. K., Trzebny, A., Geyer, K. M., Diepen, L. T. A., & Frey, S. D. (2021). Fungal community structure and function shifts with atmospheric nitrogen deposition. *Global Change Biology*, 27, 1349–1364. <https://doi.org/10.1111/gcb.15444>
- Neff, J. C., Townsend, A. R., Gleixner, G., Lehman, S. J., Turnbull, J., & Bowman, W. D. (2002). Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature*, 419, 915–917. <https://doi.org/10.1038/nature01136>

- Ochoa-Hueso, R., Borer, E. T., Seabloom, E. W., Hobbie, S. E., Risch, A. C., Collins, S. L., Alberti, J., Bahamonde, H. A., Brown, C. S., Caldeira, M. C., Daleo, P., Dickman, C. R., Ebeling, A., Eisenhauer, N., Esch, E. H., Eskelinen, A., Fernández, V., Güsewell, S., Gutierrez-Larruga, B., ... Zamin, T. (2020). Microbial processing of plant remains is co-limited by multiple nutrients in global grasslands. *Global Change Biology*, 26(8), 4572–4582. <https://doi.org/10.1111/gcb.15146>
- Pastore, M. A., Hobbie, S. E., & Reich, P. B. (2021). Sensitivity of grassland carbon pools to plant diversity, elevated CO₂, and soil nitrogen addition over 19 years. *Proceedings of the National Academy of Sciences USA*, 118, 1–10.
- Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., & Janssens, I. A. (2013). Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. *Nature Communications*, 4(1), <https://doi.org/10.1038/ncomms3934>
- Pierson, D., Evans, L., Kayhani, K., Bowden, R. D., Nadelhoffer, K., Simpson, M., & Lajtha, K. (2021). Mineral stabilization of soil carbon is suppressed by live roots, outweighing influences from litter quality or quantity. *Biogeochemistry*, 154, 433–449. <https://doi.org/10.1007/s10533-021-00804-9>
- Prober, S. M., Leff, J. W., Bates, S. T., Borer, E. T., Firn, J., Harpole, W. S., Lind, E. M., Seabloom, E. W., Adler, P. B., Bakker, J. D., Cleland, E. E., DeCrappeo, N. M., DeLorenze, E., Hagenah, N., Hautier, Y., Hofmockel, K. S., Kirkman, K. P., Knops, J. M. H., La Pierre, K. J., ... Fierer, N. (2015). Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecology Letters*, 18, 85–95. <https://doi.org/10.1111/ele.12381>
- Ramirez, K. S., Craine, J. M., & Fierer, N. (2010). Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied. *Soil Biology & Biochemistry*, 42, 2336–2338. <https://doi.org/10.1016/j.soilbio.2010.08.032>
- Ramirez, K. S., Craine, J. M., & Fierer, N. (2012). Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, 18, 1918–1927. <https://doi.org/10.1111/j.1365-2486.2012.02639.x>
- Rasmussen, C., Heckman, K., Wieder, W. R., Keiluweit, M., Lawrence, C. R., Berhe, A. A., Blankinship, J. C., Crow, S. E., Druhan, J. L., Hicks Pries, C. E., Marin-Spiotta, E., Plante, A. F., Schädel, C., Schimel, J. P., Sierra, C. A., Thompson, A., & Wagai, R. (2018). Beyond clay: Towards an improved set of variables for predicting soil organic matter content. *Biogeochemistry*, 137, 297–306. <https://doi.org/10.1007/s10533-018-0424-3>
- Rasse, D. P., Rumpel, C., & Dignac, M. F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil*, 269, 341–356. <https://doi.org/10.1007/s11104-004-0907-y>
- Reich, P. B., Hobbie, S. E., Lee, T. D., Rich, R., Pastore, M. A., & Worm, K. (2020). Synergistic effects of four climate change drivers on terrestrial carbon cycling. *Nature Geoscience*, 13, 787–793. <https://doi.org/10.1038/s41561-020-00657-1>
- Riggs, C. E., Hobbie, S. E., Bach, E. M., Hofmockel, K. S., & Kazanski, C. E. (2015). Nitrogen addition changes grassland soil organic matter decomposition. *Biogeochemistry*, 125, 203–219. <https://doi.org/10.1007/s10533-015-0123-2>
- Rillig, M. C. (2004). Arbuscular mycorrhizae, glomalin, and soil aggregation. *Canadian Journal of Soil Science*, 84, 355–363. <https://doi.org/10.4141/S04-003>
- Schlesinger, W. H. (1977). Carbon balance in terrestrial detritus. *Annual Review of Ecology and Systematics*, 8, 51–81. <https://doi.org/10.1146/annurev.es.08.110177.000411>
- Schlesinger, W. H. (1999). Carbon sequestration in soils. *Science*, 284(5423), 2095.
- Seabloom, E. W., Adler, P. B., Alberti, J., Biederman, L., Buckley, Y. M., Cadotte, M. W., Collins, S. L., Dee, L., Fay, P. A., Firn, J., Hagenah, N., Harpole, W. S., Hautier, Y., Hector, A., Hobbie, S. E., Isbell, F., Knops, J. M. H., Komatsu, K. J., Laungani, R., ... Borer, E. T. (2021a). Increasing effects of chronic nutrient enrichment on plant diversity loss and ecosystem productivity over time. *Ecology*, 102, 1–11. <https://doi.org/10.1002/ecy.3218>
- Seabloom, E. W., Borer, E. T., Buckley, Y., Cleland, E. E., Davies, K., Firn, J., Harpole, W. S., Hautier, Y., Lind, E., MacDougall, A., Orrock, J. L., Prober, S. M., Adler, P., Alberti, J., Michael Anderson, T., Bakker, J. D., Biederman, L. A., Blumenthal, D., Brown, C. S., ... Yang, L. (2013). Predicting invasion in grassland ecosystems: Is exotic dominance the real embarrassment of richness? *Global Change Biology*, 19, 3677–3687. <https://doi.org/10.1111/gcb.12370>
- Seabloom, E. W., Borer, E. T., Hobbie, S. E., & MacDougall, A. S. (2021b). Soil nutrients increase long-term soil carbon gains threefold on retired farmland. *Global Change Biology*, 27(19), 4909–4920. <https://doi.org/10.1111/gcb.15778>
- Sinsabaugh, R. L. (2010). Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology & Biochemistry*, 42, 391–404. <https://doi.org/10.1016/j.soilbio.2009.10.014>
- Sitters, J., Wubs, E. R. J., Bakker, E. S., Crowther, T. W., Adler, P. B., Bagchi, S., Bakker, J. D., Biederman, L., Borer, E. T., Cleland, E. E., Eisenhauer, N., Firn, J., Gherardi, L., Hagenah, N., Hautier, Y., Hobbie, S. E., Knops, J. M. H., MacDougall, A. S., McCulley, R. L., ... Veen, G. F. (2020). Nutrient availability controls the impact of mammalian herbivores on soil carbon and nitrogen pools in grasslands. *Global Change Biology*, 26, 2060–2071. <https://doi.org/10.1111/gcb.15023>
- Six, J., Conant, R. T., Paul, E. A., & Paustian, K. (2002). Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil*, 241, 155–176.
- Smith, P. (2004). Carbon sequestration in croplands: The potential in Europe and the global context. *European Journal of Agronomy*, 20, 229–236. <https://doi.org/10.1016/j.eja.2003.08.002>
- Sokol, N. W., & Bradford, M. A. (2019). Microbial formation of stable carbon is more efficient from belowground than aboveground input. *Nature Geoscience*, 12.
- Soong, J. L., & Cotrufo, M. F. (2015). Annual burning of a tallgrass prairie inhibits C and N cycling in soil, increasing recalcitrant pyrogenic organic matter storage while reducing N availability. *Global Change Biology*, 21, 2321–2333. <https://doi.org/10.1111/gcb.12832>
- Stevens, C. J., Lind, E. M., Hautier, Y., Harpole, W. S., Borer, E. T., Hobbie, S., Seabloom, E. W., Ladwig, L., Bakker, J. D., Chu, C., Collins, S., Davies, K. F., Firn, J., Hillebrand, H., Pierre, K. J. L., MacDougall, A., Melbourne, B., McCulley, R. L., Morgan, J., ... Wrang, P. D. (2015). Anthropogenic nitrogen deposition predicts local grassland primary production worldwide. *Ecology*, 96, 1459–1465. <https://doi.org/10.1890/14-1902.1>
- Suding, K. N., Collins, S. L., Gough, L., Clark, C., Cleland, E. E., Gross, K. L., Milchunas, D. G., & Pennings, S. (2005). Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences USA*, 102, 4387–4392. <https://doi.org/10.1073/pnas.0408648102>
- Thompson, A., Rancourt, D. G., Chadwick, O. A., & Chorover, J. (2011). Iron solid-phase differentiation along a redox gradient in basaltic soils. *Geochimica Et Cosmochimica Acta*, 75, 119–133. <https://doi.org/10.1016/j.gca.2010.10.005>
- Torn, M. S., Trumbore, S. E., Chadwick, O. A., Vitousek, P. M., & Hendricks, D. M. (1997a). Mineral control of soil organic carbon storage and turnover. *Nature*, 389, 170–173. <https://doi.org/10.1038/38260>
- Torn, M. S., Trumbore, S. E., Chadwick, O. A., Vitousek, P. M., & Hendricks, D. M. (1997b). Mineral control of soil organic carbon storage and turnover content were measured by horizon down to the depth at which. *Nature*, 389, 3601–3603. <https://doi.org/10.1038/38260>
- Van Der Krift, T. A. J., Kuikman, P. J., Möller, F., & Berendse, F. (2001). Plant species and nutritional-mediated control over rhizodeposition and root decomposition. *Plant and Soil*, 228, 191–200.

- Wagai, R., Kajiura, M., Uchida, M., & Asano, M. (2018). Distinctive roles of two aggregate binding agents in allophanic andisols: Young carbon and poorly-crystalline metal phases with old carbon. *Soil Systems*, 2, 1–23. <https://doi.org/10.3390/soilsystems2020029>
- Waldrop, M. P., Zak, D. R., Sinsabaugh, R. L., Gallo, M., & Lauber, C. (2004). Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecological Applications*, 14, 1172–1177. <https://doi.org/10.1890/03-5120>
- Wang, J., Gao, Y., Zhang, Y., Yang, J., Smith, M. D., Knapp, A. K., Eissenstat, D. M., & Han, X. (2019). Asymmetry in above- and belowground productivity responses to N addition in a semi-arid temperate steppe. *Global Change Biology*, 25, 2958–2969. <https://doi.org/10.1111/gcb.14719>
- Wick, A. F., Huzurbazar, S. V., & Stahl, P. D. (2009). Use of Bayesian methods to model soil aggregation in undisturbed semiarid grasslands. *Soil Science Society of America Journal*, 73, 1707–1714. <https://doi.org/10.2136/sssaj2008.0185>
- Widdig, M., Heintz-Buschart, A., Schleuss, P.-M., Guhr, A., Borer, E. T., Seabloom, E. W., & Spohn, M. (2020). Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil. *Soil Biology & Biochemistry*, 151, 108041. <https://doi.org/10.1016/j.soilbio.2020.108041>
- Wieder, W. R., Cleveland, C. C., Smith, W. K., & Todd-Brown, K. (2015). Future productivity and carbon storage limited by terrestrial nutrient availability. *Nature Geoscience*, 8, 1–15. <https://doi.org/10.1038/ngeo2413>
- Zak, D. R. (2008). Simulated atmospheric NO₃ deposition increases soil organic matter by slowing decomposition. *Ecological Applications*, 18.
- Zeglin, L. H., Stursova, M., Sinsabaugh, R. L., & Collins, S. L. (2007). Microbial responses to nitrogen addition in three contrasting grassland ecosystems. *Oecologia*, 154, 349–359. <https://doi.org/10.1007/s00442-007-0836-6>
- Zhao, Q., Callister, S. J., Thompson, A. M., Kukkadapu, R. K., Tfaily, M. M., Bramer, L. M., Qafokuc, N. P., Bella, S. L., Hobbied, S. E., Seabloom, E. W., Borer, E. T., & Hofmockel, K. S. (2020). Strong mineralogic control of soil organic matter composition in response to nutrient addition across diverse grassland sites. *Science of the Total Environment*, 736, 137839.
- Zhao, Q., Thompson, A. M., Callister, S. J., Tfaily, M. M., Bell, S. L., Hobbie, S. E., & Hofmockel, K. S. (2021). Dynamics of organic matter molecular composition under aerobic decomposition and their response to the nitrogen addition in grassland soils. *Science of the Total Environment*, 806, 150514.

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