



# Impacts of nutrient addition on soil carbon and nitrogen stoichiometry and stability in globally-distributed grasslands

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Received: 27 November 2021 / Accepted: 11 April 2022 / Published online: 15 May 2022  
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**Abstract** Global changes will modify future nutrient availability with implications for grassland biogeochemistry. Soil organic matter (SOM) is central to grasslands for both provision of nutrients and climate mitigation through carbon (C) storage. While we know that C and nitrogen (N) in SOM can be influenced by greater nutrient availability, we lack understanding of nutrient effects on C and N coupling and

stability in soil. Different SOM fractions have different functional relevance and mean residence times, i.e., mineral-associated organic matter (MAOM) has a higher mean residence time than particulate organic matter (POM). By separating effects of nutrient supply on the different SOM fractions, we can better evaluate changes in soil C and N coupling and stability and associated mechanisms. To this end, we studied responses of C and N ratios and distributions across POM and MAOM to 6–10 years of N, phosphorus (P), potassium and micronutrients ( $K_{+μ}$ ), and combined NPK $_{+μ}$  additions at 11 grassland sites spanning 3 continents and globally relevant environmental

Responsible Editor: Myrna Simpson.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10533-022-00932-w>.

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gradients in climate, plant growth, soil texture, and nutrient availability. We found addition of N and NPK<sub>+μ</sub> generally reduced C:N in MAOM and POM. However, at low fertility and at warm, sandy sites, nutrient addition promoted higher MAOM and POM C:N, respectively. Addition of NPK<sub>+μ</sub> also promoted C storage in POM relative to MAOM, and this was consistent across sites. Our results suggest that addition of macro- and micronutrients consistently decrease SOM stabilization, whereas responses of soil C:N stoichiometry were contingent on SOM fraction and environmental conditions.

**Keywords** Nutrient network (NutNet) · Soil organic matter · Nutrient addition · Mineral-associated organic matter · Particulate organic matter · Grasslands · Nitrogen · Phosphorous · Potassium

## Introduction

Global environmental change is expected to greatly change nutrient availability to ecosystems, through warming induced mineralization of nitrogen (N) and phosphorus (P) (Bai et al. 2013; Shaw and Cleveland 2020), anthropogenically-enhanced atmospheric deposition of N, P, and potassium (K; Van Langenhove et al. 2020), and direct fertilizer application to managed systems. These changes to nutrient supply may alter soil organic matter (SOM), a key component of ecosystems which provides nutrients for plant growth and climate mitigation by storing carbon (C; Bossio et al. 2020). Grasslands are crucial areas for SOM storage, covering 30–40% of the global land surface (Reynolds 2005; Petri et al. 2009) and storing 16% of global soil C (Anderson 1991). Previous research has revealed that grassland SOM C is affected by nutrient availability, depending on environmental conditions (Yue et al. 2017; Crowther et al. 2019; Keller et al. 2021). However, investigating SOM C and N coupling and distribution between functionally different pools of SOM can provide new insight for soil responses to global change, such as model constraints for microbial metabolism or vulnerability of soil C to warming (Buchkowski et al. 2019; Rocci et al. 2021).

By investigating SOM C and N stoichiometry and the distribution of C and N between SOM fractions differing in their stability, we can better understand how changes in nutrient availability may modify future grassland biogeochemistry.

Experiments that investigate responses of SOM to changes in nutrient availability are often carried out as nutrient addition experiments. Nutrient addition has been shown to influence total soil C and N in grassland systems, with directionality dependent on nutrient identity, climate and edaphic characteristics, and land management (e.g., increased with N: Ziter and MacDougall 2013; Cenini et al. 2015; Seabloom et al. 2021b; no change with N or P: Song et al. 2014; Chen et al. 2019; Crowther et al. 2019; Luo et al. 2020; Mazzorato et al. 2022; reduced with N and P: Luo et al. 2020; increased with NPK: Cenini et al. 2015; Crowther et al. 2019; Seabloom et al. 2021b). However, understanding whether soil C and N responses are coupled can provide unique information beyond the independent response of C or N. Variation in SOM C:N represents changes in the nutrient requirement for C storage (Cotrufo et al. 2019), can be indicative of mechanistic changes, and its inclusion in soil biogeochemistry models reduces uncertainty in element cycling (Buchkowski et al. 2019). There is limited work explicitly investigating response of SOM C:N to nutrient addition, but both global and continental scale studies of chronic nutrient addition in grasslands suggest C and N are coupled, with no change in C:N in response to N and P addition (Keller et al. 2021), or their combined addition with K (Seabloom et al. 2021a). However, SOM is a highly complex and heterogeneous pool (Christensen 2001), and separating it into components may provide more mechanistic insight regarding soil C:N responses to nutrient additions.

Soil organic matter is made of a high diversity of molecules and physical structures, but one way to reduce this complexity is to characterize SOM into a small number of distinct pools with contrasting physical–chemical characteristics and functionalities. One example of such a separation is to distinguish particulate organic matter (POM) and mineral-associated organic matter (MAOM), which are two fractions whose formation and persistence are thought to be controlled by different mechanisms (Cambardella and Elliott 1992; Cotrufo et al. 2019; Lavalley et al. 2020; Heckman, Hicks-Pries et al. 2022). Particulate

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organic matter is thought to form from the fragmentation of structural plant and microbial compounds (Six et al. 2001; Cotrufo et al. 2015), and unless it is occluded in stable aggregates, it lacks physical protection. MAOM is thought to form from microbial necromass and dissolved organic matter (DOM) sorption to minerals or organic matter occlusion in very fine (< 53  $\mu\text{m}$ ) aggregates (Kleber et al. 2015; Liang et al. 2017; Wagai et al. 2020). Therefore, it is thought to be less accessible to further microbial decomposition. POM is lost through enzymatic degradation, the rate of which depends on POM chemistry and microbial community and activity, which may be inhibited or limited by temperature, moisture, or nutrient availability (Keiluweit et al. 2017; Bailey et al. 2019; Vaughn and Torn 2019; Ochoa-Hueso et al. 2020). In contrast, MAOM is expected to be lost via desorption (Bailey et al. 2019), leading to a higher mean residence time for MAOM than POM (von Lützow et al. 2007; Kögel-Knabner et al. 2008), when POM is not occluded in aggregates (Heckman, Hicks-Pries et al. 2022).

Because of these different formation and loss processes, changes in C:N stoichiometry of POM and MAOM may be indicative of changes in the mechanisms underlying SOM C:N change. For example, POM C:N may vary due to changes in plant input C:N stoichiometry (Buchkowski et al. 2019). In contrast, MAOM C:N may vary due to changes in C:N stoichiometry of DOM, given the direct sorption pathway of MAOM formation (i.e., *ex vivo*, sensu Liang et al. 2017), or through changes in the microbial community, given the microbial anabolism pathway of MAOM formation (i.e., *in vivo*, sensu Liang et al. 2017). There is limited work on drivers of MAOM desorption but recent work contrasts, suggesting organic N may be preferentially desorbed in the rhizosphere (Jilling et al. 2018) and that N-rich molecules are strongly sorbed to minerals (Possigner et al. 2020), making it difficult to hypothesize about desorption-driven changes in MAOM C:N. Because of these more specific controls of MAOM and POM, investigating their C:N stoichiometry can inform the overall response of SOM C:N to nutrient addition. For example, it has been shown that N addition reduces plant C:N (e.g., Rueth and Baron 2002; Han et al. 2014) and P addition desorbs C compounds (Spohn and Schleuss 2019), potentially resulting in decreased POM C:N and MAOM C:N, respectively.

Thus, C:N stoichiometry of SOM fractions could be a powerful tool for better characterizing grassland soil biogeochemistry in response to greater nutrient availability.

While changes in C:N can indicate changes in the mechanisms driving nutrient addition responses, investigating the distribution of C and N between SOM fractions can provide additional characterization of SOM responses. Specifically, changes in the distribution of C and N between MAOM and POM can indicate changes in SOM stability/vulnerability (i.e., more MAOM = greater resistance to disturbance and global change; Grandy and Robertson 2007; Rocci et al. 2021), turnover time (i.e., more MAOM = longer turnover time; Kögel-Knabner et al. 2008), N requirement for C storage (i.e., more MAOM = higher N requirement; Cotrufo et al. 2019) and dominant drivers of formation and loss. Thus, we interpret greater C and N storage in POM, relative to MAOM, as less stable total C and N pools. Previous work supports disparate responses of POM and MAOM to nutrient addition, and, importantly, that these responses are modified by environmental properties. For example, at both a temperate grassland with sandy soil (Cenini et al. 2015) and an alpine grassland with clay loam soil (Chen et al. 2019), N addition promoted relative C storage in MAOM. In contrast, at a semiarid grassland with sandy soil, N addition promoted increased POM C and N (Song et al. 2014). These results point to the likelihood of climatic and edaphic control of SOM responses to nutrient addition, which is expected to align with the formation and loss pathways associated with POM and MAOM. For example, addition of a specific nutrient in climates where that nutrient is generally limiting (e.g., cool climates have greater N limitation; Fay et al. 2015) or a combination of nutrients (NPK) in a variety of climates (Fay et al. 2015), may more effectively release plant growth limitation, and consequently increase structural input into the POM pool. In contrast, wetter climates may support loss of POM C and N with addition of multiple nutrients (NPK), due to concurrent release of water and nutrient limitation to decomposition (Ochoa-Hueso et al. 2020). In clay-rich soils, greater availability of sorption sites may allow for sorption of increased DOM (Lu et al. 2011b; Schleuss et al. 2019) and microbial necromass (Averill and Waring 2018) following nutrient addition, promoting increased MAOM-C and

-N. However, it is difficult to compare responses of MAOM and POM in studies from different sites with variable methodologies that measure different covariates, preventing clear attribution of variability to environmental, rather than methodological, differences.

The Nutrient Network [NutNet (Borer et al. 2014)] is a global experiment with identical nutrient addition treatments replicated in grasslands experiencing many different edaphic and climatic conditions, allowing for assessment of the influence of site conditions on the response of grassland SOM to addition of N, P, and K plus micronutrients ( $K_{+\mu}$ ). Previous NutNet studies suggest  $K_{+\mu}$  availability is important in determining soil C and N, finding increased soil C under  $K_{+\mu}$  and NPK $_{+\mu}$  additions (Crowther et al. 2019; Seabloom et al. 2021a) and increased C in the light fraction (conceptually similar to POM) under NPK $_{+\mu}$  addition (Lin et al. 2019). However, studies that examine soil C fractions at NutNet sites that did not include the  $K_{+\mu}$  treatments (e.g., Riggs et al. 2015; Keller et al. 2021) have not detected effects of nutrient addition on total soil C. For example, a short term (3–5 years of nutrient addition) study of NutNet sites in the central plains of the US found increased C in macroaggregates under N addition, with no change in total soil C (Riggs et al. 2015). In contrast, a longer-term continental-scale study (9–10 years) of N and P addition, inclusive of sites involved in the short-term study, found no effect of nutrient addition on C or N in SOM or its fractions (Keller et al. 2021). Thus, there is uncertainty around fraction responses to nutrient addition.

To this end, we investigated C and N stoichiometry and distribution in SOM, MAOM, and POM in response to six or more years of N, P,  $K_{+\mu}$ , and combined (NPK $_{+\mu}$ ) nutrient addition at 11 NutNet sites on three continents, varying in climate, soil texture, plant productivity, and soil fertility (Table 1). We tested the following hypotheses about responses of SOM-C and -N to greater nutrient availability, and variation in these responses across a wide range of environmental characteristics: (1) Nitrogen in SOM, MAOM, and POM will be more responsive to the addition of N and NPK $_{+\mu}$  than C, reducing C:N. We expect POM C:N stoichiometry to be more responsive to nutrient addition than MAOM C:N stoichiometry. This is because POM C:N will more strongly mirror responses of plant C:N, which has been shown to be sensitive to nutrient addition. Consequently,

C and N will be preferentially stored in POM with added nutrients, due to release of plant growth limitation, preferentially increasing POM C and N (N,  $K_{+\mu}$ , NPK $_{+\mu}$  addition), and desorption of MAOM-C and -N (P addition; Spohn and Schleuss 2019). (2) We expect decoupling of C:N stoichiometry of SOM, MAOM, and POM in productive systems with non-limiting nutrients and climate (high temperature and precipitation). These systems may allow for luxurious N use by plants, likely reducing plant and, subsequently, POM C:N. Additionally, excess N in DOM may be directly sorbed to MAOM, reducing MAOM C:N. In contrast, in nutrient-limited grasslands, we expect nutrients to increase plant productivity, leading to preferential storage of C and N in POM, with no change in stoichiometry. We expect this to be moderated by soil texture, as sandy soils are limited in their ability to store MAOM C and N. Our study provides insight on differing responses of soil C and N, and SOM fractions, to increased nutrient supply in grasslands spanning gradients of climate, plant productivity, and soil conditions, that can help inform how nutrient availability will regulate soil C and N coupling and stability across grassland environments.

## Methods

### Study sites and soil collection

This study used archived soil samples from 11 NutNet (Borer et al. 2014) sites spanning three continents (Table 1). NutNet is a coordinated, globally-distributed experiment with identical treatments of synthetic nutrient addition in more than 90 grassland sites around the world. NutNet treatments include a control with no nutrients added, and a full factorial combination of N [timed-release urea;  $(\text{NH}_2)_2\text{CO}$ ], P [triple-super phosphate;  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ], and  $K_{+\mu}$  [potassium sulfate;  $\text{K}_2\text{SO}_4$ ], each applied annually at  $10 \text{ g m}^{-2} \text{ yr}^{-1}$ . A micronutrient mix (6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo, and 1% Zn) was added at a rate of  $100 \text{ g m}^{-2}$  to plots fertilized with  $K_{+\mu}$  in the first treatment year only, to prevent potential toxicity. For each treatment and the control,  $5 \times 5 \text{ m}$  plots replicated three times were established in a randomized complete block design (Borer et al. 2014).

At each NutNet site, two to three 2.5 cm diameter and 10 cm deep soil cores were collected from

**Table 1** Nutrient Network sites used in analysis of soil organic matter (SOM) responses to nutrient addition

Site name	Country, continent	Years of treatment (YT)	Soil pH (Y0)	MAT (°C; Y0)	MAP (mm; Y0)	WRB classification, % sand (Y0)	Aboveground biomass (g m <sup>-2</sup> ; Y0-YT)	Soil N (%; Y0)	Soil P (ppm; Y0)	Soil K (ppm; Y0)
Burrawan	Australia, Australia	8	5.6	18.4	682	VR, 82.2%	238.4	0.084	17.1	72.5
Bogong	Australia, Australia	7	4.5	6.5	1507	LC, 63.7%	524.9	0.602	46.0	188.0
Cereep	France, Europe	6	6.1	11	643	LC, 73.1%	N/A	0.134	126.0	181.0
Lancaster	England, Europe	8	4.8	8.1	1297	GL, 50.2%	120.2	1.070	35.3	106.0
Val Mustair	Switzerland, Europe	8	5.7	0.5	1093	LP, 57.6%	219.9	0.576	44.4	108.9
Cedar point biological station	USA, North America	9	6.6	9.2	457	KS, 70.2%	177.9	0.113	33.1	285.0
Chichaqua bot-toms	USA, North America	10	6.1	9.1	850	PH, 88.3%	395.3	0.061	62.3	93.3
Cowichan	Canada, North America	9	5.6	9.7	807	PH, 32.7%	507.8	0.399	38.7	88.3
Elliot Chap- paral	USA, North America	8	5.7	16.7	242	LC, 54.2%	285.8	0.152	16.8	261.0
Smith Prairie	USA, North America	9	6.1	9.8	614	AN, 78.0%	373.7	0.522	75.6	179.0
Spindletop	USA, North America	9	6.4	12.5	1136	LC, 29.3%	375.3	0.255	230.0	79.6

Soil data represent ambient conditions (pre-treatment sampling detailed in Supplementary Material A). Climate data were extracted from the WorldClim dataset using R statistical software based on latitude and longitude of sites. WRB soil classifications were extracted from the FAO digital coverage using ArcGIS based on latitude and longitude of sites. Years of treatment (YT) pertains to the time the soil used for SOM measurements was collected

MAT mean annual temperature, MAP mean annual precipitation, WRB World Reference Base soil classification, aboveground biomass, mean peak annual biomass in the control plots, N nitrogen, P phosphorus, and K potassium, Y0 pre-treatment measurements made in year 0 of the treatment. For control plot aboveground biomass, values were averaged over Y0–YT. For WRB, VR Vertisols, LC Luvisols, Cambisols, GL Gleysols, Histosols, Fluvisols, LP Leptosols, Regosols, KS Kastanozems, Solonetz, PH Phaeozems, AN Andosols

each plot before treatments were applied (pre-treatment soils). Soils were collected from the surface with vegetation removed prior to sampling and roots were removed by 2 mm sieving. A depth of 10 cm was chosen to capture the most biologically and chemically active area of the soil. Once treatments were initiated, soils were collected and handled with the same method every 3–5 years and sent to the University of Minnesota where they were air-dried and archived (post-treatment soils). Because SOM C and N has been shown to be unaltered when air-dried and stored for long periods (Blake et al. 2000), we used post-treatment archived soils for measurement of SOM fractions and used pre-treatment soil data to characterize the ambient environment of each grassland. We analyzed archived post-treatment soil samples from sites that had received a minimum of 6 years of nutrient addition, with the assumption that the treatment had sufficient time to affect SOM pools (Hagedorn et al. 2003; Ferreira et al. 2018), leaving us with soil samples from 25 NutNet sites. We conducted the SOM fractionation (see [Soil Analyses](#)) on a set of 11 of those sites that represented the widest range of climate, productivity, and soil texture and fertility (e.g., concentrations of soil N, P, and K) of all NutNet grassland sites (Supplementary Fig. 1; Table 1). Soil texture (% sand) and nutrients (ambient soil N, P, and K) were measured on pre-treatment soils (detailed in the Supplementary Material A). Only % sand was used as it was the direct complement of % silt + clay. Live plant biomass was estimated annually (except at Cereep, which did not separate live and dead biomass) from clipped samples of live peak aboveground biomass from two 0.1 m<sup>2</sup> quadrats per plot. Samples were dried to constant mass at 60 °C.

We assessed the effects on SOM fractions of addition of N, P, and K<sub>+μ</sub> individually, and in combination (NPK<sub>+μ</sub>), along with control plots, giving a total of 5 treatments. We did not include pairwise factorial combinations of N, P, and K<sub>+μ</sub> (e.g., NP, NK<sub>+μ</sub>, etc.) in our analysis. Treatments were replicated in 3 blocks at each site providing a total of 163 observations at the plot scale, because Elliot Chapparral and Cowichan were each missing archived soils for a single control plot.

## Soil analyses

To separate MAOM and POM, we performed a physical fractionation procedure, modified from Cotrufo et al. (2019). In brief, 10 g of archived soil for each replicate, treatment, and site (n=163), were dispersed in DI water with glass beads (Balesdent 1987) to break up aggregates by shaking at low speed on a reciprocal shaker for 18 h. For the Lancaster and Cedar Point soils, 5.5 g soil was used, because they were resistant to dispersion and in low availability, respectively. Lancaster soils were also shaken at fast speed for 2 h and at low speed for 16 h to increase dispersion. Any remaining aggregates were manually disrupted during wet sieving. Following shaking, the slurry was then rinsed onto a 53 μm sieve with the fraction passing through (<53 μm) representing MAOM and the fraction remaining on the sieve (>53 μm) representing POM. We ensured the sum of POM and MAOM mass following fractionation was 100 ± 5% of the starting weight of the soil sample (mean; range = 99.5%; 96.3–104.1%). There are many fractionation approaches to characterize SOM pools but this was chosen as the most appropriate to separate SOM into two meaningful fractions (POM and MAOM) with distinct controls while being an efficient approach for our large sample number (Poeplau et al. 2018; Cotrufo et al. 2019). However, we acknowledge that all SOM fractionation schemes have limitations, and that this method may allow for inclusion of small-sized structural material (conceptually POM) in our MAOM pool (<53 μm), as well as some large-sized protected material (conceptually MAOM) in our POM pool (>53 μm). Nevertheless, we contend that the inclusion of conceptually incorrect material in these pools is small and that our physical pools have average properties expected of their conceptual matches (e.g., higher C and N and lower C:N in MAOM), as has also been shown by Poeplau et al. (2018) for this fractionation method. Following physical fractionation, we measured total C and N individually in SOM (bulk soil), MAOM, and POM (n=489) on either a LECO Tru-SPEC elemental analyzer (Leco Corp., St. Joseph, MI) or a Costech elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA), as for ambient soil N, above. Samples and standards were crosschecked between instruments to ensure values were comparable. All soils were free of carbonates, as evidenced

by effervescence class assessment with 1 M HCl (Soil Survey Staff 2014), so total soil C is defined as organic.

### Data analyses

The response variables of interest for this study were C:N stoichiometry in SOM, MAOM, and POM, and the proportion of C and N in MAOM ( $fMAOM-C = MAOM-C/SOM-C$ ;  $fMAOM-N = MAOM-N/SOM-N$ ; Supplementary Table 1). These responses are informed by C and N concentration (g of C or N  $kg^{-1}$  soil) data, which can be found in the Supplemental Material. Because plot-resolved bulk density values were not available for all sites, we were unable to evaluate C and N stocks. We evaluated (1) how SOM C and N stoichiometry and distribution responded to nutrient treatment, (2) how SOM responses to nutrient treatments were modified by site-specific environmental variables, and (3) the relative importance of each predictor variable (combining treatment and environmental variables as predictors in the same model). At the plot level, we analyzed ambient soil N (%), P (ppm), and K (ppm) concentrations, measured at the start of the experiment. At the site-level, we analyzed mean annual precipitation (MAP;  $mm\ yr^{-1}$ ), mean annual temperature (MAT;  $^{\circ}C$ ), average peak season aboveground biomass at the control plots ( $g\ m^{-2}$ ), and sand content (% sand), which each had one value per site. Climate data were extracted from the WorldClim dataset using R statistical software based on latitude and longitude of sites. Separate models were fit for site and plot level variables, as these strongly covaried (Supplementary Fig. 2), such that variance inflation factors reached concerning values between 4 and 5 (up to 4.8; O'Brien 2007; Zuur et al. 2007) when these were analyzed together. We were also interested in unique effects of ambient soil nutrients (plot-level) as compared to biophysical effects (site-level).

Statistical analyses were carried out using RStudio in R version 4.0.2 (R Core Team 2019). We followed the data exploration procedures outlined in Zuur et al. (2010) and found that many of our response variables and some of our predictor variables had outliers that led to non-homogeneous variances. Data for SOM, MAOM and POM-C and -N concentration, C:N, and ambient soil P concentration were all natural log transformed to mitigate outliers and meet the

assumptions of the linear model. Additionally, environmental variables were standardized to a mean of 0 and standard deviation of 0.5, as the variables differed widely in scale, allowing for direct comparison of response estimates (Grueber et al. 2011; Rasmussen 2018). The responses of fMAOM-C and -N were modeled with beta regression (*glmmTMB* package; Brooks et al. 2017) because they are continuous proportion data (Douma and Weedon 2019). SOM, MAOM, and POM C and N concentrations and stoichiometry (C:N) were modeled with the normal distribution. Our analyses follow our hypotheses and the layout of data analyses presented in the above paragraph: (1) To assess overall nutrient effects, we created mixed models (*lme4* package; Bates et al. 2015) with SOM, MAOM, or POM C:N or fMAOM-C or -N as response variables, nutrient treatment as the predictor variable and block nested within site as the random variable, to account for the blocking structure. We used analysis of variance (ANOVA; *car* package; Fox and Weisberg 2019) and pairwise comparisons between all nutrient treatments and the control (*emmeans* package; Lenth 2020) to determine overall effect of treatment and individual nutrient effects, respectively. (2) We determined how nutrient addition effects varied with environmental factors by using the same model structure as for (1) but with the mean percent change in a SOM component under nutrient addition (Eq. 1) as the response variable and environmental variables as the predictors. Mean percent changes were calculated as a back-transformed log response ratio for ease of interpretation using the equation:

Mean Percent Change

$$= \left( \exp \left( \ln \left( \bar{X}_{\text{trt}} \right) - \ln \left( \bar{X}_{\text{ctrl}} \right) \right) - 1 \right) \times 100 \quad (1)$$

where  $\bar{X}_{\text{trt}}$  is the mean value under the nutrient treatment (i.e. N, P,  $K_{+\mu}$ , or  $NPK_{+\mu}$ ) and  $\bar{X}_{\text{ctrl}}$  is the mean value under the control, with no nutrients added. For this analysis we used the normal distribution, since mean percent change approximates a normal distribution (Hedges et al. 1999). (3) Finally, we determined the relative importance of the treatment and environmental factors by carrying out multi-model averaging with all environmental variables and the treatment following Grueber et al. (2011) using the *MuMIn* package (Barton 2020). We used the natural

averaging method to avoid shrinkage towards zero of predictors that only appear in low-weighted models (Grueber et al. 2011) and a model inclusion criterion of 2AICc (Burnham and Anderson 2002). Data for Cereep could not be included in multi-model averaging because of missing aboveground biomass data. For all hypothesis tests and pairwise comparisons, significance was determined at  $p < 0.05$ .

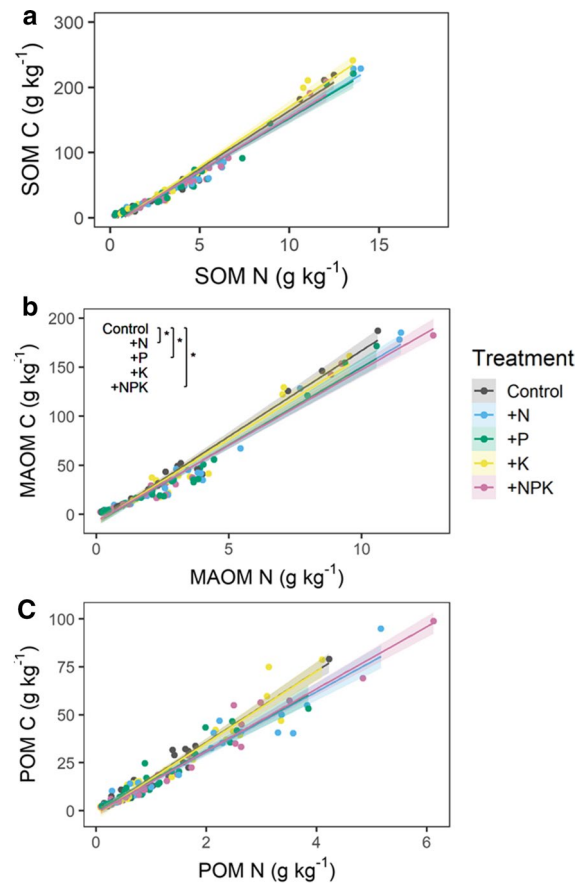
## Results

### Data distribution

Our sites spanned a variety of soil textures and nutrient concentrations, climate factors, and productivities (Table 1), leading to a diversity of SOM-C and -N stoichiometries, concentrations, and distributions between SOM components. Carbon stored per unit N (median; range), i.e., C:N stoichiometry, was highest in POM (14.3; 10.3–36.1) and lowest in MAOM (10.6; 7.4–18.3), with SOM falling between (12.0; 7.1–22.4). SOM-C had a median (range) value of 26.6 g OC kg<sup>-1</sup> soil (3.5–278.0 g OC kg<sup>-1</sup> soil), with most of that C stored in MAOM (17.0; 1.9–187.3 g OC kg<sup>-1</sup> soil) as opposed to POM (9.9; 1.6–99.0 g OC kg<sup>-1</sup> soil). Similarly, SOM-N (2.7; 0.2–18.3 g N kg<sup>-1</sup> soil) was mostly found in MAOM (1.7; 0.2–12.7 g N kg<sup>-1</sup> soil) as opposed to POM (0.7; 0.1–6.12 g N kg<sup>-1</sup> soil). Consequently, proportions of C and N in MAOM (fMAOM-C and -N) had median values of 0.62 (0.18–0.93) and 0.65 (0.23–1.06), respectively.

### Overall responses to nutrient addition

The effect of nutrient treatment on C stored per unit N was subtle and only significant for MAOM [ $\chi^2$  (4, N=163)=18.0,  $p=0.001$ ] and POM [ $\chi^2$  (4, N=163)=10.4,  $p=0.035$ ] but not for total SOM [ $\chi^2$  (4, N=163)=5.9,  $p=0.210$ ; Fig. 1]. Overall SOM C:N was likely unaffected by treatment, because the nutrient treatments caused proportional increases in both SOM-C and -N (Table 2; Supplementary Material B2). For SOM fractions, addition of N ( $p=0.002$ ), P ( $p=0.028$ ), and NPK<sub>+μ</sub> ( $p=0.048$ ) decreased MAOM C:N. Similarly, addition of NPK<sub>+μ</sub> ( $p=0.059$ ) or of N ( $p=0.055$ ) decreased POM C:N, but not significantly. Response



**Fig. 1** Soil (a), mineral-associated (b), and particulate (c) organic matter (SOM, MAOM, and POM) carbon (C) versus nitrogen (N) concentrations under nutrient addition treatments (+N = nitrogen, +P = phosphorus, +K = potassium + micronutrients, +NPK = combined) at 11 globally-distributed grasslands (Table 1). There were only significant pairwise comparisons for MAOM at  $p < 0.05$ , which are denoted with a “\*\*\*” in the upper left of the MAOM plot. The main effect of nutrient treatment was not significant for SOM C:N [ $\chi^2$  (4, N=163)=5.9,  $p=0.210$ ], but was for MAOM [ $\chi^2$  (4, N=163)=18.3,  $p=0.001$ ] and POM [ $\chi^2$  (4, N=163)=10.4,  $p=0.035$ ] C:N

of POM C:N to nutrient treatment was likely driven by changes in soil N concentration rather than soil C concentration, as POM-N was significantly increased by N and NPK addition. MAOM-N was also significantly affected by nutrient treatment but was only higher under N addition as compared to K addition, indicating a more subtle response of MAOM C:N (Table 2; Supplementary Material B2). The nutrient treatment significantly affected fMAOM-C and -N [C:  $\chi^2$  (4, N=163)=10.1,

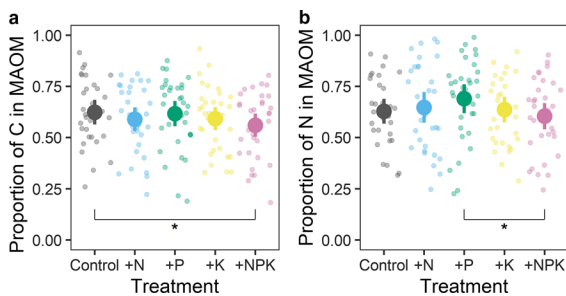


**Table 2** Means and standard errors (in parentheses) of soil total, mineral, and particulate organic matter (SOM, MAOM, and POM) carbon (C) and nitrogen (N) concentrations and

ratios in response to nutrient treatments at 11 globally-distributed grasslands (Table 1)

Treatment	Control	Nitrogen (N)	Phosphorus (P)	Potassium + micronutrients (K <sub>+μ</sub> )	Combined (NPK <sub>+μ</sub> )
<i>SOM component</i>					
SOM C:N	12.1 (0.5)	12.0 (0.5)	12.6 (0.5)	12.5 (0.5)	12.1 (0.4)
SOM-C*	45.3 (10.1)	51.6 (10.7)	45.5 (9.1)	46.1 (10.2)	50.5 (10.8)
SOM-N*	3.3 (0.6)	3.9 (0.6)	3.4 (0.6)	3.3 (0.5)	3.8 (0.7)
MAOM C:N*	12.1 (0.6)	11.1 (0.4)	11.2 (0.4)	11.7 (0.5)	11.4 (0.5)
MAOM-C	31.5 (7.9)	33.0 (7.9)	30.8 (7.2)	28.1 (6.6)	29.9 (7.6)
MAOM-N*	2.25 (0.4)	2.6 (0.5)	2.5 (0.4)	2.1 (0.4)	2.4 (0.5)
POM C:N*	16.7 (0.8)	15.2 (0.9)	15.8 (0.7)	16.0 (0.8)	15.0 (0.6)
POM-C	15.7 (2.8)	20.1 (3.7)	15.7 (2.4)	19.7 (3.7)	21.1 (4.0)
POM-N*	0.9 (0.1)	1.3 (0.2)	1.0 (0.1)	1.2 (0.2)	1.4 (0.2)
fMAOM-C*	0.62 (0.03)	0.59 (0.03)	0.62 (0.03)	0.59 (0.03)	0.56 (0.03)
fMAOM-N*	0.63 (0.3)	0.65 (0.04)	0.69 (0.03)	0.64 (0.03)	0.60 (0.03)

Proportions of C and N in MAOM are denoted as fMAOM-C and fMAOM-N, respectively. An asterisk (\*) indicates a significant treatment effect at  $p < 0.05$



**Fig. 2** Proportion of carbon and nitrogen in MAOM (fMAOM-C and -N) under nutrient addition treatments (+ N = nitrogen, + P = phosphorus, + K = potassium + micronutrients, + NPK = combined) at 11 globally-distributed grasslands (Table 1). Means with 95% confidence intervals ( $n = 33$  for treatments and  $n = 31$  for control) are shown as large dots with whiskers and individual observations are behind the large dots. Significantly different pairwise comparisons, at  $p < 0.05$ , are denoted with a “\*\*”

$p = 0.039$  and N:  $\chi^2$  (4,  $N = 163$ ) = 12.7,  $p = 0.013$ , respectively; Fig. 2]. Addition of NPK<sub>+μ</sub> decreased the proportion of C in MAOM ( $p = 0.029$ ), relative to the control, and the proportion of N in MAOM, relative to P addition ( $p = 0.011$ ; Fig. 2).

### Responses to nutrient addition as modified by environmental factors

Generally, soil C:N tended to increase with nutrient addition at sites with low ambient soil nutrients, and tended to decrease at sites with high ambient soil nutrients. Specifically, SOM C:N responses to N, P, and K<sub>+μ</sub> addition were all negatively related to ambient soil P (Table 3; Fig. 3). In addition, SOM C:N responses to N and NPK<sub>+μ</sub> addition were negatively related to ambient soil K concentration (Table 3) and SOM C:N responses to P addition were negatively related to ambient soil N concentration (Table 3; Fig. 3). Similarly, MAOM C:N responses to P and NPK<sub>+μ</sub> addition were negatively related to ambient soil N (Table 3; Fig. 3). MAOM C:N response to NPK<sub>+μ</sub> was also negatively related to ambient soil K concentration (Table 3; Fig. 3). Nitrogen was likely responsible for some SOM C:N responses, as the SOM-N response to K<sub>+μ</sub> was positively related to ambient soil P, whereas other nutrient relationships with C and N concentrations were more subtle and not directly connected to SOM and MAOM C:N responses (Supplementary Material B3). In contrast, the response of POM C:N to nutrient addition was not related to ambient soil nutrient concentrations (Fig. 3). Both POM-C and -N responses to N and P additions were negatively related to ambient soil P,

**Table 3** Chi-square estimate and (p-value) from type 3 Wald chi-squared tests for models of the response of total soil, mineral-associated, and particulate organic matter (SOM, MAOM, and POM) carbon-to-nitrogen ratio (C:N) to nutrient addition, as modified by environmental variables at 11 globally-distributed grasslands (Table 1)

	SOM C:N			MAOM C:N			POM C:N					
	N	P	K	NPK	P	K	NPK	N	P	K	NPK	
Ambient soil N (%)	2.7 (0.097)	<b>4.1 (0.042)</b>	0.3 (0.615)	2.0 (0.158)	2.2 (0.138)	<b>4.1 (0.043)</b>	0.4 (0.520)	<b>8.0 (0.005)</b>	0.2 (0.660)	0.1 (0.722)	0.0 (0.831)	0.2 (0.680)
Ambient soil P (ppm)	<b>7.7 (0.006)</b>	<b>6.4 (0.011)</b>	<b>4.6 (0.032)</b>	1.6 (0.204)	0.4 (0.528)	0.0 (0.877)	0.0 (0.914)	1.6 (0.211)	1.4 (0.231)	0.1 (0.774)	0.2 (0.624)	2.0 (0.153)
Ambient soil K (ppm)	<b>9.5 (0.002)</b>	1.8 (0.186)	0.7 (0.394)	<b>4.4 (0.036)</b>	0.2 (0.682)	0.2 (0.634)	0.6 (0.444)	<b>6.2 (0.013)</b>	0.1 (0.752)	0.2 (0.644)	3.4 (0.066)	0.1 (0.803)
MAT (°C)	<b>4.3 (0.038)</b>	3.8 (0.052)	0.2 (0.639)	0.4 (0.522)	0.2 (0.655)	2.0 (0.156)	0.4 (0.527)	1.4 (0.243)	<b>5.1 (0.024)</b>	1.0 (0.310)	0.4 (0.505)	1.2 (0.280)
MAP (mm yr <sup>-1</sup> )	1.5 (0.215)	0.3 (0.580)	0.0 (0.901)	0.5 (0.465)	0.7 (0.390)	0.3 (0.613)	0.1 (0.744)	0.0 (0.877)	0.0 (0.937)	0.5 (0.466)	1.1 (0.305)	0.0 (0.922)
Sand content (%)	1.6 (0.206)	2.0 (0.157)	0.1 (0.780)	2.2 (0.137)	0.5 (0.472)	0.3 (0.567)	0.0 (0.982)	0.9 (0.341)	0.2 (0.659)	0.0 (0.994)	0.4 (0.535)	<b>5.0 (0.025)</b>
Aboveground biomass (g m <sup>-2</sup> )	0.0 (0.950)	0.1 (0.752)	0.0 (0.883)	0.0 (0.904)	0.2 (0.645)	1.9 (0.164)	0.6 (0.432)	0.7 (0.408)	1.0 (0.329)	2.7 (0.097)	1.3 (0.263)	0.0 (0.922)

Columns for N, P, K, and NPK represent responses to nitrogen, phosphorus, potassium + micronutrients and their combined addition. Bolded relationships are significant at  $p < 0.05$

likely leading to no change in POM C:N (Supplementary Material B3). Instead, addition of NPK<sub>+μ</sub> tended to decrease POM C:N at sites with low sand content (Table 3). Climate also modified this response. In particular, SOM and POM C:N increased more with N addition with increasing MAT (Table 3).

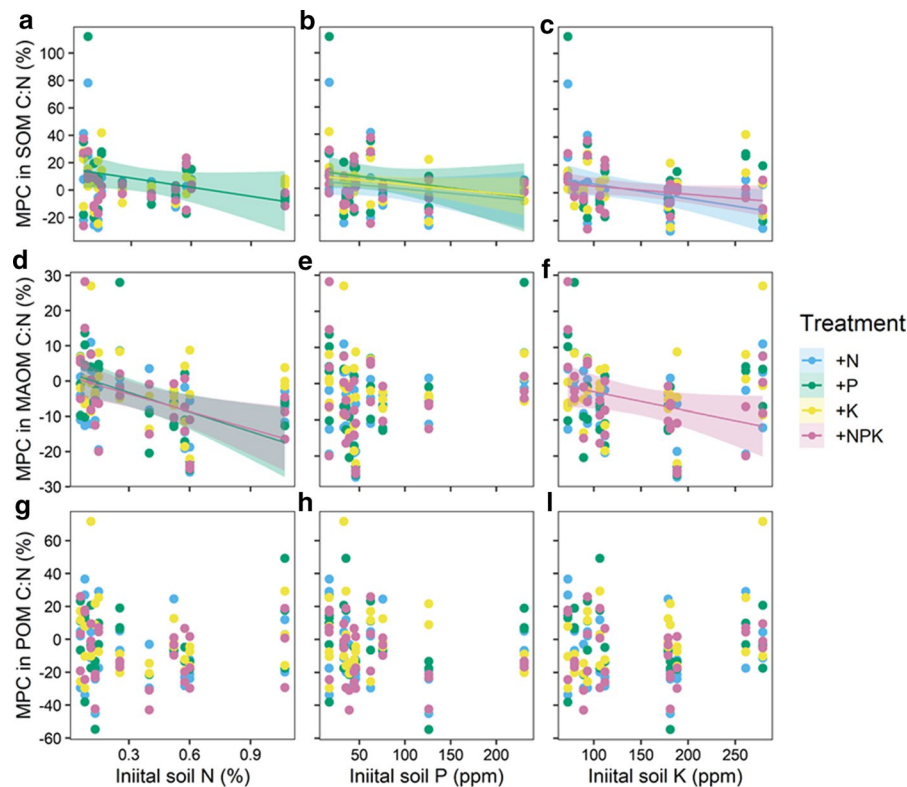
Addition of all nutrients decreased the proportion of C and N in MAOM relatively consistently across environmental variables. The response of fMAOM-C to nutrient addition was not related to any environmental factor. The response of fMAOM-N to N addition was positively related to MAT and the response to N and K<sub>+μ</sub> addition were negatively related to ambient soil P (Supplementary Table 6). These responses were subtle and could not be directly connected to MAOM and POM-C and -N responses (Supplementary Material B3).

#### Relative importance of explanatory variables

To indicate the overall strongest drivers of SOM, MAOM, and POM C:N stoichiometry and C and N distribution (fMAOM), we performed multi-model averaging using all environmental variables and the treatment variables. Soil N was included in the averaged model for all soil components except POM C:N. Soil texture and MAT also emerged as consistently strong (high estimate) predictors across our response variables, whereas other predictor variables were only related to changes in a subset of SOM components or had relatively low estimates (Table 4). Multi-model averaging for SOM, MAOM, and POM C and N concentrations followed similar patterns as above (Supplementary Table 5). Treatment was only included in models of the distribution of C and N between MAOM and POM (fMAOM-C and -N).

## Discussion

We found that six or more years of experimental nutrient addition, particularly of N and NPK<sub>+μ</sub>, promoted N accrual more strongly than C accrual in MAOM and POM and reduced average stability of SOM-C, as evidenced by reduced fMAOM-C. However, responses of C:N stoichiometry to chronic nutrient addition were subtle—total SOM C:N was unaffected by nutrient treatment—and depended on environmental context. Generally, for SOM and



**Fig. 3** Relationships between ambient soil nitrogen (N; **a**, **d**, **g**), phosphorus (P; **b**, **e**, **h**), and potassium (K; **c**, **f**, **i**) concentrations and mean percent change (MPC) in soil, mineral-associated, and particulate organic matter [SOM (**a–c**), MAOM (**d–f**), and POM (**g–i**)] carbon to nitrogen ratios (C:N) under +N (blue), +P (green), +K +micronutrients (yellow) and +NPK +micronutrients (pink) addition at 11 globally-distributed grasslands (Table 1). Linear trendlines are reported only for significant relationships from ANOVA. Statistical information for linear trendlines are reported here. For SOM C:N fertilized with each individual nutrient, soil P estimates, p-values, and  $R^2$  were, +N:  $-11.7$ ,  $p=0.010$ ,

and  $r^2=0.18$ ; +P:  $-13.7$ ,  $p=0.017$ , and  $r^2=0.16$ ; +K:  $-7.4$ ,  $p=0.083$ , and  $r^2=0.14$ , for SOM C:N fertilized with N and NPK + $\mu$ , soil K estimates, p-values, and  $R^2$  were, +N:  $-0.14$ ,  $p=0.005$ , and  $r^2=0.22$ , +NPK + $\mu$ :  $-0.08$ ,  $p=0.046$ , and  $r^2=0.02$ , and for SOM C:N fertilized with P, soil N estimate, p-value, and  $R^2$  were,  $-26.8$ ,  $p=0.052$ , and  $r^2=0.10$ . For MAOM C:N fertilized with P and NPK + $\mu$ , soil N estimates, p-values, and  $R^2$  were, +P:  $-19.5$ ,  $p=0.085$ , and  $r^2=0.21$ ; +NPK + $\mu$ :  $-19.5$ ,  $p=0.028$ , and  $r^2=0.26$  and for NPK + $\mu$ , soil K estimate, p-value, and  $R^2$  were  $-0.08$ ,  $p=0.043$ , and  $r^2=0.21$ . Chi-squared estimates and p-values from ANOVA analysis are in Table 3

MAOM, addition of specific nutrients increased C relative to N at sites with low ambient soil fertility; whereas for POM this occurred at warm sites with sandy soils following N and NPK + $\mu$  addition. In contrast, responses of fMAOM-C were consistent across the wide range of climatic and edaphic conditions represented in this study. These results suggest that increased availability of NPK + $\mu$  could consistently promote C storage in the less stable POM pool but that the response of soil C per unit N is dependent on SOM fraction, nutrient identity, and local environmental conditions.

### Responses to increased nutrient availability

Effects of nutrient addition on soil stoichiometry were subtle, with SOM C:N unaffected by increased nutrient availability. This lack of change was driven by similar increases in SOM-C and -N in response to nutrient addition (Table 2) and aligns with previous NutNet work (Crowther et al. 2019; Keller et al. 2021; Seabloom et al. 2021a) and with meta-analyses (Lu et al. 2011a, b; Yue et al. 2016, 2017; Rocci et al. 2021), which all find increased soil C and N with nutrient addition. While there are certainly

**Table 4** Top model (< 2 AICc) estimates and standard errors (in parentheses) from multi-model averaging for bulk soil, mineral-associated, and particulate organic matter (SOM, MAOM, and POM) carbon (C) and nitrogen (N) stoichiometries (C:N), and distributions (fMAOM) with all environmental variables and the treatment at 11 globally-distributed grassland nutrient addition experiments (Table 1)

Soil component	Number of models	Percent sand	Above-ground biomass	MAT	MAP	Ambient soil N	Ambient soil P	Ambient soil K	Nutrient addition
SOM C:N	3					0.26 (0.08)	-0.21 (0.06)		
MAOM C:N	8	0.24 (0.09)		0.22 (0.09)	0.29 (0.12)	0.34 (0.12)	-0.24 (0.12)		
POM C:N	2						-0.26 (0.10)	-0.28 (0.10)	
fMAOM-C	3	-0.23 (0.09)	0.31 (0.09)	1.14 (0.10)	-0.26 (0.13)	1.04 (0.13)	0.28 (0.10)	0.25 (0.09)	N: -0.19 (0.12) P: -0.05 (0.12) K: -0.03 (0.12) NPK: -0.31 (0.12)
fMAOM-N	3	-0.70 (0.12)	0.14 (0.11)	1.16 (0.13)		0.59 (0.14)	0.39 (0.13)	0.22 (0.10)	N: 0.20 (0.15) P: 0.44 (0.16) K: 0.11 (0.16) NPK: -0.12 (0.15)

MAT mean annual temperature, MAP mean annual precipitation

exceptions to this general finding of increased soil C and N with nutrient addition (e.g., Mack et al. 2004; Boot et al. 2016), these findings might be particularly expected given the relatively long period of treatment (6–10 years) and relatively high nutrient addition rate for this study, as soil C responds more positively at higher N addition durations and amounts (Xu et al. 2020). We might expect individual C and N responses to be stronger on decadal time scales, potentially leading to altered C:N ratios, as has been found for fertilized retired farmland (Seabloom et al. 2021b). However, given the heterogeneity of the total SOM pool, it is important to unpack these findings by analyzing distinct fractions of SOM. Because POM and MAOM are thought to have distinct pathways of formation and loss, they can indicate both potential mechanisms underlying responses and the stability of SOM following global change.

In contrast to the total SOM pool, and aligned with our hypotheses, we found that greater nutrient (specifically N, P, and NPK<sub>+μ</sub>) availability reduced MAOM and POM C:N. Since these findings were likely driven by increases in MAOM and POM N, our results suggest nutrient addition promotes MAOM and POM formation or loss pathways that increase N, relative to C. MAOM formation is thought to occur through two main pathways; efficient microbial resource use efficiency and subsequent necromass sorption (i.e., *in vivo*) and exo-enzymatic breakdown of polymeric residues followed by direct DOM sorption (i.e., *ex vivo*; *sensu* Liang et al. 2017). Both of these pathways could reduce MAOM C:N with N addition. Addition of N tended to reduce plant C:N at our sites (Supplementary Fig. 3), which aligns with previous NutNet work (Anderson et al. 2018), and is expected to favor MAOM formation via the *in vivo* pathway (Cotrufo et al. 2013). Since microbial necromass is more N-rich than plant inputs (Cleveland and Liptzin 2007), this may decrease MAOM C:N. Direct addition of labile N may also form MAOM through the *ex vivo* pathway. A previous NutNet study of 6 globally-distributed grasslands, including one of our sites, found that N addition increases dissolved N concentrations (Schleuss et al. 2021), and N compounds are thought to sorb strongly to mineral surfaces (Possinger et al. 2020), thus potentially increasing MAOM-N. Carbon stored per unit N in MAOM was also reduced under P addition, which aligns strongly with our expectations, given findings of C desorption with

P addition (Spohn and Schleuss 2019). The drivers for MAOM C:N responses to N and P addition can likely be extended to explain reduced MAOM C:N under NPK<sub>+μ</sub> addition. Responses of POM C:N were more variable than MAOM C:N but nevertheless tended to decline with N and NPK<sub>+μ</sub> addition. At our sites, addition of N and NPK<sub>+μ</sub> increased standing aboveground biomass and NPK<sub>+μ</sub> addition also reduced live aboveground plant C:N (Supplementary Fig. 3), in line with other NutNet studies (Fay et al. 2015; Anderson et al. 2018). Thus, reduced POM C:N is likely due to greater inputs of lower C:N plant material with N and NPK<sub>+μ</sub> addition, although responses of live plant biomass may be different than plant litter (Seabloom et al. 2021a). We lack root data for all of our sites but a study at one of our sites (Cowichan) suggested root biomass and C:N responses would mirror aboveground plant responses (Ziter and MacDougall 2013), potentially adding greater low quality plant input belowground, also potentially reducing POM C:N. Thus, our results suggest increasing nutrient—especially N—availability in grasslands will likely store soil C at a higher N cost, which could be a benefit in areas experiencing rapidly increasing N deposition (Ackerman et al. 2019) but may be problematic for management that depends on fertilization.

Beyond studying C relative to N, we also studied content of C and N in MAOM relative to POM, to assess how the average stability of soil C and N may change with greater nutrient availability. Addition of NPK<sub>+μ</sub> reduced the relative distribution of C and N in MAOM versus POM (fMAOM-C and -N) as compared to the control and P, respectively. These findings align with previous NutNet work that suggests grassland biomass production is co-limited by N, P, and K, as adding these together increases aboveground plant growth (Fay et al. 2015; Carroll et al. 2021), as we also found in our sites (Supplementary Fig. 3). This is expected to favor POM, rather than MAOM, formation. Addition of NPK<sub>+μ</sub> may be more effective at promoting N storage in POM than P addition because P addition alone did not increase average aboveground biomass in our sites (Supplementary Fig. 3). Overall, our findings of lower fMAOM-C with NPK<sub>+μ</sub> addition suggest that providing limiting nutrients to grasslands will shift distributions of soil C towards storage in POM, potentially making them more susceptible to loss due to warming (Rocci

et al. 2021) and management (Grandy and Robertson 2007).

### Responses to nutrient addition along environmental gradients

While C:N stoichiometry responses indicated that, on average, soil N, and C to a lesser extent, increased with greater nutrient availability, we found that nutrient addition in sites with low ambient nutrients may allow for more C stored per unit N added. Some of these relationships (Fig. 3b, d, f) were aligned with our expectation that adding a nutrient when it has low ambient availability may promote more coupled C and N responses, but only for SOM and MAOM. Thus, these relationships were likely mediated through, for example, increased microbial necromass inputs to MAOM with N addition (Fig. 3d; Averill and Waring 2018) rather than through plant productivity, as we would expect for POM. However, many of these relationships were not expected and indicated interactions between different nutrients. For example, positive responses of SOM C:N to N addition at low ambient soil P and K suggest that adding a non-limiting nutrient may preserve existing soil C and N through reduced microbial acquisition of those elements (Schleuss et al. 2019). In contrast, responses of POM C:N to the addition of N and NPK<sub>+μ</sub> did not vary with soil fertility but rather with MAT and soil texture, respectively, which is likely related to the formation pathway of POM. Warmer temperatures are generally associated with higher plant tissue C:N (Reich and Oleksyn 2004; Anderson et al. 2018). Since aboveground biomass increased with N addition at our sites (Supplementary Fig. 3), N addition in warm grasslands may promote more structural (C-rich, nutrient poor) plant inputs to the POM pool (Cotrufo et al. 2015). Our finding of increased POM C:N with NPK<sub>+μ</sub> addition in sandy soils is strongly in line with findings for total soil C in a previous NutNet study (Crowther et al. 2019) and for fertilized retired farmland (Seabloom et al. 2021b). Notably, we corroborate this finding for the POM pool only, which may be due to relatively smaller MAOM pool size in sandy soils (Supplementary Fig. 4). Because our fractionation method defines POM C as the C in the sand-sized fraction, this is not a surprising finding, but, nevertheless,

suggests that increased soil C with fertilization of sandy soils may be occurring in the less stable POM pool. Overall, findings of environmental influence on responses of SOM, MAOM, and POM C:N align with relatively high estimates for ambient soil N, MAT, and sand content for top models of SOM components C and N (Table 4). In addition, they emphasize the importance of site edaphic and climatic conditions, as found in other NutNet studies (Keller et al. 2021), in determining the strength and direction of soil responses to nutrient addition (Supplementary Table 7, Supplementary Fig. 5). These results confirm and extend previous NutNet results to show that findings of increased soil C in sandy versus low nutrient sites may be realized in POM and MAOM, respectively, with implications for the stability of soil C.

In contrast to C:N stoichiometry responses to environmental gradients, NPK<sub>+μ</sub> addition consistently reduced C content in MAOM, relative to POM, across a wide range of climatic and edaphic conditions. This may be because plant growth is often co-limited by multiple nutrients (Fay et al. 2015), so addition of NPK<sub>+μ</sub> is able to reduce plant growth limitation under a wide range of baseline conditions, thus increasing C content preferentially in POM. Responses of fMAOM-N were slightly less consistent but were subtle and could not be directly related to POM or MAOM-N responses (Supplementary Table 3). It is possible that increased fMAOM-N with N addition at warmer sites could be due to greater microbial anabolism and decomposition in these conditions (Lu et al. 2013; Averill and Waring 2018; Li et al. 2019), increasing MAOM-N and decreasing POM-N, respectively. However, a study at 21 globally-distributed NutNet sites found increased decomposition rate with N addition in cool, not warm, sites (Ochoa-Hueso et al. 2020), suggesting reduced POM-N from heightened decomposition is not likely to drive the fMAOM-N response. The generally uniform response of increased C content in POM relative to MAOM under nutrient addition may represent a consistent soil response that could be considered in management decisions and soil modeling.

## Conclusions

Because nutrient availability is expected to increase with global environmental change, it is important we evaluate not just the total soil C responses to nutrient addition, but also the coupling of C and N and the distribution of C and N between soil components, to improve our understanding of future grassland biogeochemistry. While C and N were coupled in the total SOM pool, evaluating MAOM and POM allowed us to find more subtle decreases in C:N after N, P, and NPK<sub>+μ</sub> addition, suggesting that increased nutrient supplies may cause C and N to decouple in MAOM and POM. Furthermore, MAOM and POM responded differently to nutrient addition across environmental gradients. This revealed that nutrient addition-driven increases in soil C may be driven by increases in MAOM C:N in low fertility soils and by POM C:N in sandy soils. By evaluating distribution of C and N between MAOM and POM, we were able to show that addition of macro- and micronutrients in combination (NPK<sub>+μ</sub>) drives C accumulation preferentially in POM, suggesting reduced soil C stability. Our results highlight that more mechanistic and practical insight can be gained by investigating coupled C and N and SOM heterogeneity in addition to bulk soil C.

**Acknowledgements** We thank Aaron Prairie for his assistance in the lab and the Nutrient Network organizers and participants for being willing to share their data, soils, and ideas. We especially thank Brooke Osbourne, Sumanta Bagchi, Sally Power, and Adrienne Keller for their helpful comments on earlier versions of this manuscript. This work was generated using data from the Nutrient Network (<http://www.nutnet.org>) experiment, funded at the site-scale by individual researchers. Coordination and data management have been supported by funding to E. Borer and E. Seabloom from the National Science Foundation Research Coordination Network (NSF-DEB-1042132) and Long Term Ecological Research (NSF-DEB-1234162 and NSF-DEB-1831944 to Cedar Creek LTER) programs, and the Institute on the Environment (DG-0001-13). We also thank the Minnesota Supercomputer Institute for hosting project data and the Institute on the Environment for hosting Network meetings. Soil analyses were supported, in part, by USDA-ARS Grant 58-3098-7-007 to ETB.

**Author contributions** KSR and MFC developed and framed the research questions. KSR analyzed the data and KSB contributed to data analyses. EWS and ETB coordinate the Nutrient Network and their lab funded and generated the pre-treatment soil dataset. SEH manages the permits for the archived soils used in this manuscript. RLM, ASM, CJS, XR, JLM, and JDB were site coordinators for sites whose data are used in this

manuscript. KSR wrote the manuscript. All authors contributed to writing and approved the final manuscript.

**Funding** This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. #1650114. This work has benefited from technical and human resources provided by CEREEP-Ecotron IleDeFrance (CNRS/ENS UMS 3194) as well as financial support from the Regional Council of Ile-de-France under the DIM Program R2DS bearing the reference I-05-098/R. It has received support under the program “Investissements d’Avenir” launched by the French government and implemented by ANR with the reference ANR-11-INBS-0001 AnaEE France and ANR-10-IDEX-0001-02 PSL.

**Data availability** The data for this study are available at the Environmental Data Initiative (<https://doi.org/10.6073/pasta/b8cdf07124285fa2b511ef27882c421e>).

**Code availability** Analyses were conducted in R Studio (<https://rstudio.com/>) and code is available from the corresponding author upon request.

## Declarations

**Conflict of interest** The authors report no conflict of interest.

## References

- Ackerman D, Millet DB, Chen X (2019) Global estimates of inorganic nitrogen deposition across four decades. *Global Biogeochem Cycles* 33:100–107
- Anderson J (1991) The effects of climate change on decomposition processes in grassland and coniferous forests. *Ecol Appl* 1:326–347
- Anderson TM, Griffith DM, Grace JB, Lind EM, Adler PB, Biederman LA, Blumenthal DM, Daleo P, Firm J, Hagenah N (2018) Herbivory and eutrophication mediate grassland plant nutrient responses across a global climatic gradient. *Ecology* 99:822–831
- Averill C, Waring B (2018) Nitrogen limitation of decomposition and decay: how can it occur? *Glob Change Biol* 24:1417–1427
- Bai E, Li S, Xu W, Li W, Dai W, Jiang P (2013) A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. *New Phytol* 199:431–440
- Bailey VL, Pries CH, Lajtha K (2019) What do we know about soil carbon destabilization? *Environ Res Lett* 14:083004
- Balesdent J (1987) The turnover of soil organic fractions estimated by radiocarbon dating. *Sci Total Environ* 62:405–408
- Barton K (2020) MuMIn: multi-model inference. R package version 1.43.17. Accessed from <https://CRAN.R-project.org/package=MuumIn>
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67(1):1–48. <https://doi.org/10.18637/jss.v067.i01>

- Blake L, Goulding K, Mott C, Poulton P (2000) Temporal changes in chemical properties of air-dried stored soils and their interpretation for long-term experiments. *Eur J Soil Sci* 51:345–353
- Boot CM, Hall EK, Deneff K, Baron JS (2016) Long-term reactive nitrogen loading alters soil carbon and microbial community properties in a subalpine forest ecosystem. *Soil Biol Biochem* 92:211–220
- Borer ET, Harpole WS, Adler PB, Lind EM, Orrock JL, Seabloom EW, Smith MD (2014) Finding generality in ecology: a model for globally distributed experiments. *Methods Ecol Evol* 5:65–73
- Bossio D, Cook-Patton S, Ellis P, Fargione J, Sanderman J, Smith P, Wood S, Zomer R, von Unger M, Emmer I (2020) The role of soil carbon in natural climate solutions. *Nat Sustain* 3:391–398
- Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM (2017) *glmmTMB* balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* 9(2):378–400
- Buchkowski RW, Shaw AN, Sihi D, Smith GR, Keiser AD (2019) Constraining carbon and nutrient flows in soil with ecological stoichiometry. *Front Ecol Evol* 7(382):1–17
- Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York
- Cambardella CA, Elliott ET (1992) Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Sci Soc Am J* 56:777–783
- Carroll O et al (2021) Nutrient identity modifies the destabilizing effects of eutrophication in grasslands. *Ecol Lett* 00:1–12
- Cenini VL, Fornara DA, McMullan G, Ternan N, Lajtha K, Crawley MJ (2015) Chronic nitrogen fertilization and carbon sequestration in grassland soils: evidence of a microbial enzyme link. *Biogeochemistry* 126:301–313
- Chen Y, Liu X, Hou Y, Zhou S, Zhu B (2019) Particulate organic carbon is more vulnerable to nitrogen addition than mineral-associated organic carbon in soil of an alpine meadow. *Plant Soil* 458:93–103
- Christensen BT (2001) Physical fractionation of soil and structural and functional complexity in organic matter turnover. *Eur J Soil Sci* 52:345–353
- Cleveland CC, Liptzin D (2007) C: N: P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry* 85:235–252
- Cotrufo MF, Wallenstein MD, Boot CM, Deneff 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? *Glob Change Biol* 19:988–995
- Cotrufo MF, Soong JL, Horton AJ, Campbell EE, Haddix ML, Wall DH, Parton WJ (2015) Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nat Geosci* 8:776
- Cotrufo MF, Ranalli MG, Haddix ML, Six J, Lugato E (2019) Soil carbon storage informed by particulate and mineral-associated organic matter. *Nat Geosci* 12:989–994. <https://doi.org/10.1038/s41561-019-0484-6>
- Crowther TW, Riggs C, Lind EM, Borer ET, Seabloom EW, Hobbie SE, Wubs J, Adler PB, Firn J, Gherardi L (2019) Sensitivity of global soil carbon stocks to combined nutrient enrichment. *Ecol Lett* 22:936–945
- Douma JC, Weedon JT (2019) Analysing continuous proportions in ecology and evolution: a practical introduction to beta and Dirichlet regression. *Methods Ecol Evol* 10:1412–1430
- Fay PA et al (2015) Grassland productivity limited by multiple nutrients. *Nat Plants* 1:1–5
- Ferreira GWD, Oliveira FCC, Silva LOG, Souza J, Soares EMB, Araujo EF, Silva IR (2018) Nitrogen alters initial growth, fine-root biomass and soil organic matter properties of a eucalyptus dunnii maiden plantation in a recently afforested grassland in Southern Brazil. *Forests* 9:62
- Fox J, Weisberg S (2019) *An R companion to applied regression*, 3rd edn. Sage, Thousand Oaks
- Grandy AS, Robertson GP (2007) Land-use intensity effects on soil organic carbon accumulation rates and mechanisms. *Ecosystems* 10:58–73
- Grueber CE, Nakagawa S, Laws RJ, Jamieson IG (2011) Multimodel inference in ecology and evolution: challenges and solutions. *J Evol Biol* 24:699–711
- Hagedorn F, Spinnler D, Siegwolf R (2003) Increased N deposition retards mineralization of old soil organic matter. *Soil Biol Biochem* 35:1683–1692
- Han X, Sistla SA, Zhang Y-H, Lü X-T, Han X-G (2014) Hierarchical responses of plant stoichiometry to nitrogen deposition and mowing in a temperate steppe. *Plant Soil* 382:175–187
- Heckman K, Hicks Pries CE, Lawrence CR, Rasmussen C, Crow SE, Hoyt AM, von Fromm SF, Shi Z, Stoner S, McGrath C (2022) Beyond bulk: density fractions explain heterogeneity in global soil carbon abundance and persistence. *Glob Change Biol* 28:1178–1196
- Hedges LV, Gurevitch J, Curtis PS (1999) The meta-analysis of response ratios in experimental ecology. *Ecology* 80:1150–1156
- Jilling A, Keiluweit M, Contosta AR, Frey S, Smith RG, Tiemann L, Jo JS, Grandy AS (2018) Minerals in the rhizosphere: overlooked mediators of soil nitrogen availability to plants and microbes. *Biogeochemistry* 139:103–122
- Keiluweit M, Wanzek T, Kleber M, Nico P, Fendorf S (2017) Anaerobic microsites have an unaccounted role in soil carbon stabilization. *Nat Commun* 8(1771):1–10
- Keller AB, Borer ET, Collins SL, DeLancey LC, Fay PA, Hofmockel KS, Leakey AD, Mayes MA, Seabloom EW, Walter CA (2021) Soil carbon stocks in temperate grasslands differ strongly across sites but are insensitive to decade-long fertilization. *Glob Change Biol* 28(4):1659–1677
- Kleber M, Eusterhues K, Keiluweit M, Mikutta C, Mikutta R, Nico PS (2015) Mineral–organic associations: formation, properties, and relevance in soil environments. *Advances in agronomy*, vol 130. Elsevier, Amsterdam, pp 1–140
- Kögel-Knabner I, Guggenberger G, Kleber M, Kandeler E, Kalbitz K, Scheu S, Eusterhues K, Leinweber P (2008) Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. *J Plant Nutr Soil Sci* 171:61–82



- Lavallee J, Soong J, Cotrufo MF (2020) Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Glob Change Biol* 26:261–273
- Lenth R (2020) Emmeans estimated: marginal means, aka least-squares means. R package version 1.5.1. Accessed from <https://CRAN.R-project.org/package=emmeans>
- Li J, Wang G, Mayes MA, Allison SD, Frey SD, Shi Z, Hu XM, Luo Y, Melillo JM (2019) Reduced carbon use efficiency and increased microbial turnover with soil warming. *Glob Change Biol* 25:900–910
- Liang C, Schimel JP, Jastrow JD (2017) The importance of anabolism in microbial control over soil carbon storage. *Nat Microbiol* 2:17105
- Lin Y, Slessarev EW, Yehl ST, D'Antonio CM, King JY (2019) Long-term nutrient fertilization increased soil carbon storage in California grasslands. *Ecosystems* 22:754–766
- Lu M, Yang Y, Luo Y, Fang C, Zhou X, Chen J, Yang X, Li B (2011a) Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytol* 189:1040–1050
- Lu M, Zhou X, Luo Y, Yang Y, Fang C, Chen J, Li B (2011b) Minor stimulation of soil carbon storage by nitrogen addition: a meta-analysis. *Agr Ecosyst Environ* 140:234–244
- Lu M, Zhou X, Yang Q, Li H, Luo Y, Fang C, Chen J, Yang X, Li B (2013) Responses of ecosystem carbon cycle to experimental warming: a meta-analysis. *Ecology* 94:726–738
- Luo R, Kuzyakov Y, Liu D, Fan J, Luo J, Lindsey S, He J-S, Ding W (2020) Nutrient addition reduces carbon sequestration in a Tibetan grassland soil: disentangling microbial and physical controls. *Soil Biol Biochem* 144:107764
- Mack MC, Schuur EAG, Bret-Harte MS (2004) Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature* 431:658–661
- Mazzorato AC, Esch EH, MacDougall AS (2022) Prospects for soil carbon storage on recently retired marginal farmland. *Sci Total Environ* 806:150738
- O'Brien RM (2007) A caution regarding rules of thumb for variance inflation factors. *Qual Quan* 41:673–690
- Ochoa-Hueso R, Borer ET, Seabloom EW, Hobbie SE, Risch AC, Collins SL, Alberti J, Bahamonde HA, Brown CS, Caldeira MC (2020) Microbial processing of plant remains is co-limited by multiple nutrients in global grasslands. *Glob Change Biol* 26(8):4572–4582
- Peri M, Batello C, Villani R, Nachtergaele F (2009) Carbon status and carbon sequestration potential in the world's grasslands. In: Abberton M, Conant R, Batello C (eds) *Grassland carbon sequestration: management, policy and economics*. Food and Agricultural Organization of the United Nations, Rome
- Poeplau C, Don A, Six J, Kaiser M, Benbi D, Chenu C, Cotrufo MF, Derrien D, Gioacchini P, Grand S (2018) Isolating organic carbon fractions with varying turnover rates in temperate agricultural soils—a comprehensive method comparison. *Soil Biol Biochem* 125:10–26
- Possinger AR, Zachman MJ, Enders A, Levin BD, Muller DA, Kourkoutis LF, Lehmann J (2020) Organo-organic and organo-mineral interfaces in soil at the nanometer scale. *Nat Commun* 11:1–11
- R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing. Accessed from <https://www.R-project.org/>.
- Rasmussen C et al (2018) Beyond clay: towards an improved set of variables for predicting soil organic matter content. *Biogeochemistry* 137:297–306
- Reich PB, Oleksyn J (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. *Proc Natl Acad Sci USA* 101:11001–11006
- Reynolds SG (2005) Introduction. In: Suttie JM, Reynolds SG, Batello C (eds) *Grasslands of the world*. Food and Agricultural Organization of the United Nations, Rome
- Riggs CE, Hobbie SE, Bach EM, Hofmockel KS, Kazanski CE (2015) Nitrogen addition changes grassland soil organic matter decomposition. *Biogeochemistry* 125:203–219
- Rocci KS, Lavallee JM, Stewart CE, Cotrufo MF (2021) Soil organic carbon response to global environmental change depends on its distribution between mineral-associated and particulate organic matter: a meta-analysis. *Sci Total Environ* 793:1–12
- Rueth HM, Baron JS (2002) Differences in Englemann spruce forest biogeochemistry east and west of the continental divide in Colorado, USA. *Ecosystems* 5:45–57
- Schleuss P-M, Widdig M, Heintz-Buschart A, Guhr A, Martin S, Kirkman K, Spohn M (2019) Stoichiometric controls of soil carbon and nitrogen cycling after long-term nitrogen and phosphorus addition in a mesic grassland in South Africa. *Soil Biol Biochem* 135:294–303
- Schleuss P-M, Widdig M, Biederman LA, Borer E, Crawley M, Kirkman K, Seabloom E, Wragg P, Spohn M (2021) Microbial substrate stoichiometry governs nutrient effects on nitrogen cycling in grassland soils. *Soil Biol Biochem* 155:1–11
- Seabloom EW, Adler PB, Alberti J, Biederman L, Buckley YM, Cadotte MW, Collins SL, Dee L, Fay PA, Firn J (2021a) Increasing effects of chronic nutrient enrichment on plant diversity loss and ecosystem productivity over time. *Ecology* 102(2):1–11
- Seabloom EW, Borer ET, Hobbie SE, MacDougall AS (2021b) Soil nutrients increase long-term soil carbon gains threefold on retired farmland. *Glob Change Biol* 27(19):4909–4920
- Shaw AN, Cleveland CC (2020) The effects of temperature on soil phosphorus availability and phosphatase enzyme activities: a cross-ecosystem study from the tropics to the Arctic. *Biogeochemistry* 151:113–125
- Six J, Guggenberger G, Paustian K, Haumaier L, Elliott ET, Zech W (2001) Sources and composition of soil organic matter fractions between and within soil aggregates. *Eur J Soil Sci* 52:607–618
- Soil Survey Staff (2014) *Soil survey field and laboratory methods manual*. Soil survey investigations report No. 51, Version 2.0. U.S. Department of Agriculture, Lincoln
- Song B, Niu SL, Li LH, Zhang LX, Yu GR (2014) Soil carbon fractions in grasslands respond differently to various levels of nitrogen enrichments. *Plant Soil* 384:401–412
- Spohn M, Schleuss P-M (2019) Addition of inorganic phosphorus to soil leads to desorption of organic compounds and thus to increased soil respiration. *Soil Biol Biochem* 130:220–226

- Van Langenhove L, Verryckt LT, Bréchet L, Courtois EA, Stahl C, Hofhansl F, Bauters M, Sardans J, Boeckx P, Fransen E (2020) Atmospheric deposition of elements and its relevance for nutrient budgets of tropical forests. *Biogeochemistry* 149:175–193
- Vaughn LJ, Torn MS (2019)  $^{14}\text{C}$  evidence that millennial and fast-cycling soil carbon are equally sensitive to warming. *Nat Clim Change* 9:467–471
- von Lutzow M, Kogel-Knaber I, Ekschmitt K, Flessa H, Guggenberger G, Matzner E, Marschner B (2007) SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. *Soil Biol Biochem* 39:2183–2207
- Wagai R, Kajiura M, Asano M (2020) Iron and aluminum association with microbially processed organic matter via meso-density aggregate formation across soils: organo-metallic glue hypothesis. *Soil* 6:597–627
- Xu C, Xu X, Ju C, Chen HY, Wilsey BJ, Luo Y, Fan W (2020) Long-term, amplified responses of soil organic carbon to nitrogen addition worldwide. *Glob Change Biol* 27(6):1170–1180
- Yue K, Peng Y, Peng C, Yang W, Peng X, Wu F (2016) Stimulation of terrestrial ecosystem carbon storage by nitrogen addition: a meta-analysis. *Sci Rep* 6:1–10
- Yue K, Fornara DA, Yang W, Peng Y, Peng C, Liu Z, Wu F (2017) Influence of multiple global change drivers on terrestrial carbon storage: additive effects are common. *Ecol Lett* 20:663–672
- Ziter C, MacDougall AS (2013) Nutrients and defoliation increase soil carbon inputs in grassland. *Ecology* 94(1):106–116
- Zuur A, Ieno EN, Smith GM (2007) *Analyzing ecological data*. Springer, New York
- Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. *Methods Ecol Evol* 1:3–14

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