

## LETTERS

# Sensitivity of global soil carbon stocks to combined nutrient enrichment

T. W. Crowther,<sup>1,\*</sup>  C. Riggs,<sup>2</sup>  
 E. M. Lind,<sup>2</sup> E. T. Borer,<sup>2</sup>   
 E. W. Seabloom,<sup>2</sup>   
 S. E. Hobbie,<sup>2</sup> J. Wubs,<sup>3</sup>   
 P. B. Adler,<sup>4</sup>  J. Firn,<sup>5</sup>   
 L. Gherardi,<sup>6</sup> N. Hagenah,<sup>7</sup>  
 K. S. Hofmocker,<sup>8,9</sup> J. M. H.  
 Knops,<sup>10</sup>  R. L. McCulley,<sup>11</sup>  
 A. S. MacDougall,<sup>12</sup> P. L. Peri,<sup>13</sup>  
 S. M. Prober,<sup>14</sup> C. J. Stevens<sup>15</sup> and  
 D. Routh<sup>1</sup>

### Abstract

Soil stores approximately twice as much carbon as the atmosphere and fluctuations in the size of the soil carbon pool directly influence climate conditions. We used the Nutrient Network global change experiment to examine how anthropogenic nutrient enrichment might influence grassland soil carbon storage at a global scale. In isolation, enrichment of nitrogen and phosphorous had minimal impacts on soil carbon storage. However, when these nutrients were added in combination with potassium and micronutrients, soil carbon stocks changed considerably, with an average increase of  $0.04 \text{ KgCm}^{-2} \text{ year}^{-1}$  (standard deviation  $0.18 \text{ KgCm}^{-2} \text{ year}^{-1}$ ). These effects did not correlate with changes in primary productivity, suggesting that soil carbon decomposition may have been restricted. Although nutrient enrichment caused soil carbon gains most dry, sandy regions, considerable absolute losses of soil carbon may occur in high-latitude regions that store the majority of the world's soil carbon. These mechanistic insights into the sensitivity of grassland carbon stocks to nutrient enrichment can facilitate biochemical modelling efforts to project carbon cycling under future climate scenarios.

### Keywords

Global change, nutrient enrichment, nutrient Network (NutNet), soil carbon.

Ecology Letters (2019) 22: 936–945

## INTRODUCTION

The majority of terrestrial carbon is stored in the soil. A massive flux of carbon enters the soil each year via the photosynthetic activity of plants. An approximately equivalent flux is then released from the soil via decomposition and respiration by soil organisms. If human activity alters the balance between carbon uptake and release, then it could drastically alter atmospheric carbon concentration and climate (Todd-Brown *et al.* 2013; Crowther *et al.* 2016). Understanding how global change might alter this soil carbon balance (i.e. the difference in respiration vs. photosynthesis) is critical for constraining uncertainty in future climate projections (Bradford *et al.* 2016). Along with anthropogenic changes to land use and climate, global nutrient enrichment of terrestrial soils is expected to be among the most important factors affecting this balance between respiratory losses and photosynthetic

gains at a global scale (Knorr *et al.* 2005; Janssens *et al.* 2010; IPCC 2013; Todd-Brown *et al.* 2013; Crowther *et al.* 2015). Yet, the response of terrestrial carbon storage to increased nutrient enrichment remains unclear and this translates to considerable uncertainty in Earth System Model projections of future climate scenarios (Wieder *et al.* 2015a; Zaehle *et al.* 2015; Devaraju *et al.* 2016).

The use of fertilizers and fossil fuels has drastically enhanced the supply of biologically reactive nitrogen (N) and phosphorus (P) in the soil over the past century (IPCC 2013). Although effects of macronutrient deposition are highly variable across regions, large-scale synthesis efforts suggest that N and P enrichment generally stimulates primary productivity by alleviating nutrient limitations of plant growth (Fay *et al.* 2015; Yue *et al.* 2016) and broad-scale meta-analyses suggest that elevated nutrient supply tends to reduce the activity of microbial decomposers in soil (Knorr *et al.* 2005; Treseder

<sup>1</sup>Institute of Integrative Biology, ETH Zurich, Universitätsstrasse 16, 8092 Zurich, Switzerland

<sup>2</sup>Department of Ecology, Evolution, and Behavior, University of Minnesota, 1479 Gortner Ave. St. Paul, MN 55108, USA

<sup>3</sup>Department of Terrestrial Ecology, Netherlands Institute of Ecology, Droevendaalsesteeg 10, 6708 PB Wageningen, Netherlands

<sup>4</sup>Department of Wildland Resources and the Ecology Center, Utah State University, Logan, UT 84322, USA

<sup>5</sup>Queensland University of Technology (QUT), Gardens Point Campus, Brisbane Queensland 4000, Australia

<sup>6</sup>School of Life Sciences and Global Drylands Center, Arizona State University, Tempe, USA

<sup>7</sup>Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

<sup>8</sup>Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA 99352, USA

<sup>9</sup>Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50010, USA

<sup>10</sup>School of Biological Sciences, University of Nebraska, Lincoln, NE 68588, USA

<sup>11</sup>Department of Plant & Soil Sciences, University of Kentucky, Lexington, KY 40546, USA

<sup>12</sup>Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

<sup>13</sup>Department of Forestry, Agriculture and Water, National University-INTA-CONICET, Rio Gallegos, Santa Cruz, Patagonia, Argentina

<sup>14</sup>CSIRO Land and Water, Private Bag 5, Wembley, Western Australia 6913

<sup>15</sup>Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK

\*Correspondence: E-mail: Tom.crowther@usys.ethz.ch

2008; Yue *et al.* 2016). In combination, stimulated plant uptake and limited decomposition are expected to lead to additional carbon accumulation in soil over the rest of the century (Liu & Greaver 2010; Wieder *et al.* 2015b; Devaraju *et al.* 2016). However, these effects are highly idiosyncratic across different regions and we lack consensus on the direction or magnitude of these nutrient effects at a global scale. As such the changes in net soil carbon balance under nutrient enrichment remain untested.

Nitrogen and phosphorous are by far the main focus of most nutrient enrichment experiments and biogeochemical models (Wieder *et al.* 2015b; Devaraju *et al.* 2016), but they are not the only macronutrients that are increasing under global change. A growing body of evidence highlights the importance of less-studied elemental nutrients such as potassium (K) (Fay *et al.* 2015), the deposition of which is also increasing under global change (Wetherbee & Mast 2016). Enhanced rates of desertification and weathering under changing climate and land use scenarios can lead to considerable increases in K enrichment through dust deposition across grassland biomes. Recent evidence suggests that K enrichment can enhance primary productivity across global grasslands, as plant growth is limited by the availability of multiple nutrients (Fay *et al.* 2015). Yet, it remains unclear whether these increases in plant growth translate to enhanced carbon accumulation in soil or if concurrent changes in belowground decomposition will mediate the ecosystem-scale responses. If soil carbon accumulation is constrained by the availability of multiple nutrients, then global change models that represent only the supply of individual elements (e.g., only N or P) are likely to underestimate the magnitude of future soil carbon changes.

Here, we use an internationally distributed global change experiment – NutNet (Borer *et al.* 2014) – to examine how enrichment by multiple nutrients affects soil carbon storage across a wide variety of biotic and abiotic conditions. For this analysis, we focus on grasslands, which are a critically endangered biome that accounts for approximately one-third of Earth's terrestrial net primary production (Fay *et al.* 2015). Three nutrients (N, P and K plus micronutrients added alone and in combination) were added annually to 25 m<sup>2</sup> replicated plots in 25 field-scale nutrient enrichment experiments conducted across North America, Europe, Africa and Australasia (ranging from sub-Arctic to Mediterranean grasslands; Supporting Information). The K treatment included a micronutrient mix only in the first treatment year, to avoid micronutrient toxicity (thus K<sub>+μ</sub>, hereafter). Nutrient addition rates in our experiment were similar to those added to many agricultural fields worldwide (see Methods). As such, these addition rates are higher than expected atmospheric deposition rates in many non-agricultural areas. This standardized nutrient enrichment has proved essential for isolating the effect of nutrient enrichment on grassland ecosystem functioning (Fay *et al.* 2015; Harpole *et al.* 2016), allowing the identification of general patterns across ecosystems that vary considerably in other environmental conditions (Borer *et al.* 2014). By measuring soil carbon stocks in the topsoils of these treatment plots and comparing them to standing carbon stocks in the ambient control plots at each site, we could

evaluate how high-intensity nutrient enrichment changes affect soil carbon stocks across a wide range of grasslands.

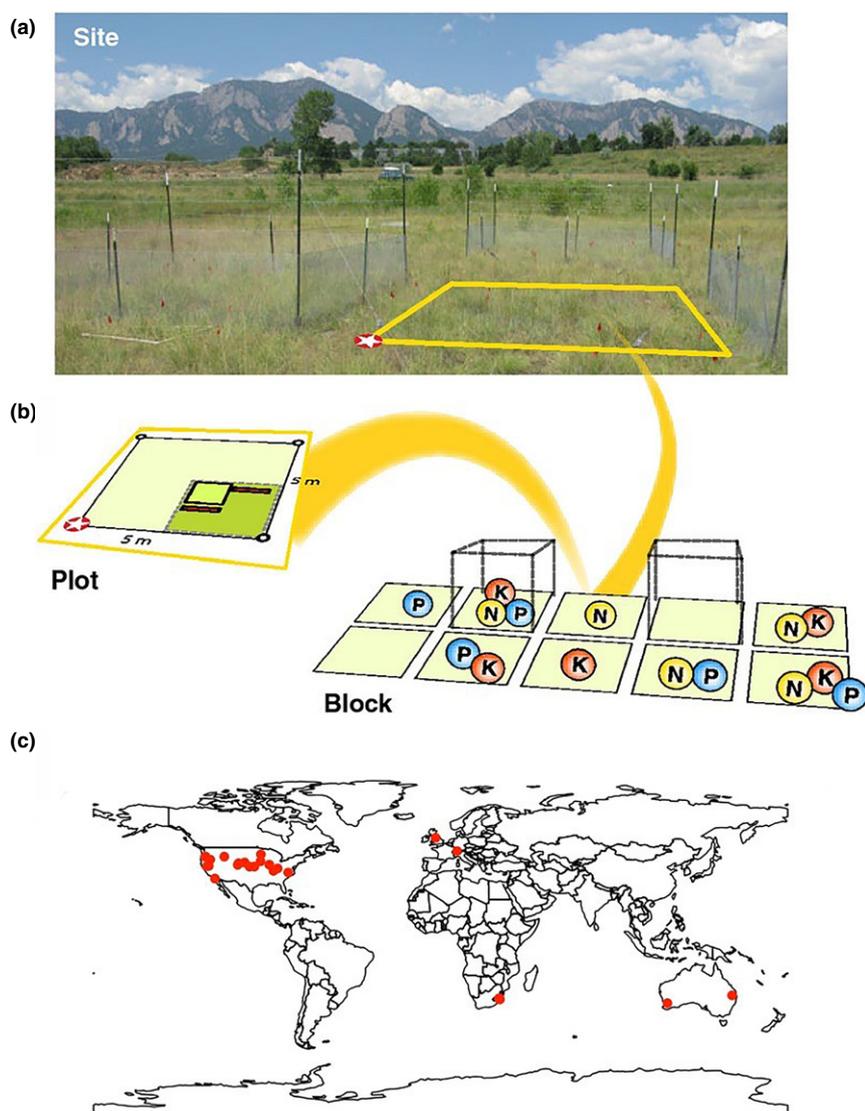
## MATERIALS AND METHODS

### Overview and study design

Soils were collected from sites in the Nutrient Network (Fig. 1), a distributed, coordinated research network designed to experimentally evaluate the effects of nutrient addition and herbivory on ecosystem processes in grasslands worldwide (Borer *et al.* 2014). Participating sites are located across a range of climate and soil types and follow identical methods for experimental set-up, sampling and analysis. The Nutrient Network experimental set-up, sampling and sample analyses are described in detail in Borer *et al.* (2014). Briefly, each site consists of 30 – 5 × 5 m plots, with treatments replicated across three blocks (randomized block design) at most sites. The experimental nutrient addition treatments – N, P and K plus micronutrients (Fig. 1) – are applied at the plot level in full factorial for a total of eight treatment combinations per block. At all sites, N, P and K are applied annually (10 g m<sup>-2</sup> year<sup>-1</sup>) as time-released urea [(NH<sub>2</sub>)<sub>2</sub>CO], triple-super phosphate [Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>] and potassium sulphate [K<sub>2</sub>SO<sub>4</sub>], respectively. Micronutrients (6 g Ca, 3 g Mg, 12 g S, 0.1 g B, 1 g Cu, 17 g Fe, 2.5 g Mn, 0.05 g Mo and 1 g Zn) were added with K only in year 1 to avoid possible micronutrient toxicity (Borer *et al.* 2014).

For the present analysis, we used 25 sites spanning all continents except Antarctica. Each site contained replicated plots representing eight treatments (Control (C), Nitrogen (N), Phosphorous (P), Potassium and micronutrients (K<sub>+μ</sub>), Nitrogen + Phosphorous (NP), Nitrogen + Potassium (NK<sub>+μ</sub>), Phosphorous + Potassium (PK<sub>+μ</sub>) and the combination of all nutrients (NP K<sub>+μ</sub>)). Consequently, the low number of replicates for any individual treatment at each site ( $n = 3$ ) does not provide enough statistical power to detect treatment effects at any individual site(s). However, the entire Nutrient Network (with 560 plots that remained undisturbed after the entire study duration) provides a unique opportunity to explore the generalizable trends in grassland soil C responses to nutrient enrichment across broad spatial scales.

We measured soil carbon stocks in all plots. This enabled us to calculate the total difference in soil carbon, as well as the response ratio, which reflects the proportional changes in soil carbon stock in response to each experimental treatment (compared to the controls) over time. These differences in soil carbon stock represent the net annual changes in soil carbon uptake (through primary production) and release (decomposition) per year. We test the hypothesis that nutrient enrichment will stimulate soil carbon accumulation by comparing soil carbon storage in the upper soil horizon (top 10 cm) of control and treatment plots. Carbon accumulation can still be substantial below these depths, so our results do not reflect the total nutrient effects on total soil carbon stocks. However, given that this upper soil horizon represents the most biologically active component of soil and stores by far the greatest concentrations of soil carbon (Crowther *et al.* 2016), nutrient-induced changes in soil carbon accumulation in the upper soil



**Figure 1** The NutNet experimental design and distribution of plots used in our study. Panel 'a' shows an example of a NutNet site and plate 'b' describes the design of blocks and plots within each study site (both adapted from Borer *et al.* (2014)). Eight nutrient treatments (Nitrogen (N), Phosphorous (P), Potassium (K), Nitrogen+Phosphorous (NP), Nitrogen+Potassium (NK), Phosphorous+Potassium (PK), Nitrogen+Phosphorous+Potassium (NPK) and Control (C) plots were replicated across each site. Panel 'c' reveals the 25 study locations where soil data were provided for this study.

horizon can be indicative of the direction and magnitude of the net ecosystem response. We then explored the spatial variation in the sensitivity of soil carbon stocks to multiple nutrient enrichment in order to comprehend the net global sensitivity of grassland soil carbon stocks to nutrient enrichment over the short-term.

#### Sampling collection and analysis

After 2–4 years of experimental nutrient addition at least two 2.5 cm diameter and 10 cm deep soil cores were collected from random locations in each plot. Surficial plant litter was removed from the top of each core and the samples were homogenized, air-dried and analysed for total C and N by combustion (Costech ESC 4010 Elemental Analyzer, Valencia, CA, USA). At sites where  $\text{pH} > 7.5$ , soil samples were pre-

treated with 0.1 M hydrochloric acid (HCl) to remove carbonates (relevant sites: Cedar Point Biological Station, Hart Mountain, Saline Experimental Range and Sheep Experimental Station).

Total ANPP ( $\text{gm}^{-2} \text{year}^{-1}$ ) was estimated annually from clip samples of current year peak aboveground biomass from two 0.1 m<sup>2</sup> quadrats per plot. Live roots were collected from soil cores. Samples were washed with deionized water to remove soil. Samples were dried to constant mass at 60 °C and weighed to approximate total plant biomass. Plant biomass sampling methods are detailed in Borer *et al.* (2014).

#### Other covariates

We supplemented the measurement data with site-level climate metrics (mean annual temperature and annual precipitation)

extracted from the WorldClim 2 database (Fick & Hijmans 2017), potential evapotranspiration was extracted from the CGIARCSI database (Zomer *et al.* 2008) and edaphic characteristics (pH, soil texture and organic carbon stock) from the SoilGrids database (Hengl *et al.* 2014). By correlating the proportional (response ratio) and total (absolute differences) changes in soil carbon stocks with these covariates, we could explore the environmental determinants of the general vitiation in soil carbon sensitivity to nutrient enrichment.

### Data analysis

All statistical analyses were performed using R (R version 3.3.1; R Foundation for Statistical Computing 2013) aside from the spatial extrapolation of the results across the Northern Hemisphere, which was conducted using Google Earth Engine (Gorelick *et al.* 2017). In order to evaluate the effects of experimental nutrient addition on soil C stocks, we tested the effects of N, P and K addition using ANOVA models, in which K and NPK<sub>+μ</sub> were the only treatments that demonstrated statistical significance when models applied weights via the varPower() function (nlme package). Individual custom contrasts were then performed between each treatment effect and the control. The NPK<sub>+μ</sub> treatment effect was the only treatment to show statistical significance during contrast assessment ( $z = 3.31$ ;  $P < 0.001$ ), though the K treatment showed a nearly significant response ( $z = 1.93$ ;  $P = 0.05332$ ).

We used mixed effects models to evaluate which environmental conditions were the best predictors of the variation in nutrient-induced changes in soil carbon stocks across landscapes. Separate models were constructed to explore the best predictors of changes in proportional (response ratios) changes and total (absolute differences) changes in soil carbon stocks. These models included predictors that address the hypothesized effects of climatic, biotic and edaphic factors on soil C stocks. Consequently, the following predictors were evaluated: mean annual temperature (MAT), mean annual precipitation (MAP), root biomass standing stock, ANPP, soil texture (% sand), soil pH, potential evapotranspiration and aboveground net primary productivity (ANPP). Predictors were included as fixed effects, while site identity was included as a random effect (mixed effects model; lme() function from the nlme package). The response variable (i.e., ratio of soil C content between treatment plots and control plots) was log-transformed to meet the normality assumptions of linear regression. Before running the models, Spearman correlation between variables was assessed using a heatmap. Stepwise refinement of predictor variables included only soil carbon, mean annual precipitation and sand content, which showed no collinearity across our sites (see Supplementary Information).

Refinement of the linear models was performed using the stepAIC() function (MASS package), wherein various iterations of variable inclusion were performed while simultaneously assessing AIC values. For the soil carbon response ratio model, the final linear models with the lowest AIC values included sand content and mean annual precipitation as covariates. For the total soil carbon response model, the only remaining covariates were soil carbon stock and sand content.

Model validation was performed following Zuur *et al.* (2009) and none of the residual plots showed heteroscedasticity. By extrapolating these linear models across the Northern Hemisphere (where the vast majority of our data originate from), using global models of soil texture, carbon content (Hengl *et al.* 2017) and precipitation (Fick & Hijmans 2017), we are able to visualize the general trends in these relationships across space.

All detailed methods and code can be found in supplementary material.

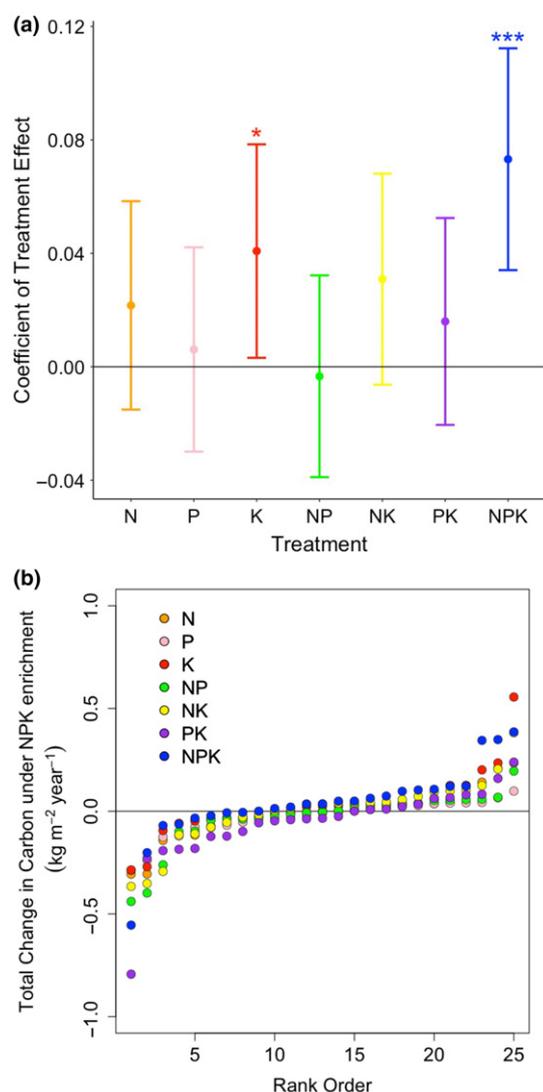
## RESULTS

### Treatment effects in experimental plots

The effects of nutrient enrichment on soil carbon storage were highly variable, with positive, negative and neutral impacts of all nutrient treatments observed in different regions (Fig. 2). However, the study 'site' strongly influenced the directional effects of the different treatments (there was a strong effect of study 'site' on the effect of nutrient enrichment). As such, in regions where N enrichment caused increases in soil carbon content, P enrichment was also likely to drive the same direction of effects. Given that each site was only represented by 2–3 replicates, we cannot have statistical confidence in the nutrient effects at any individual site. However, by combining data across all experimental sites ( $n = 560$ ), we could identify consistent trends. As such, despite the strong effect of site, our overall analysis revealed that soil carbon storage varied considerably ( $P < 0.05$ ) between the different nutrient treatments (see supplementary material for breakdown of all statistical results).

We found no consistent changes in grassland soil carbon storage under N ( $z = 1.04$ ;  $P = 0.30$ ) or P ( $z = 0.08$ ;  $P = 0.94$ ) enrichment alone or in combination ( $z = -0.32$ ;  $P = 0.75$ , Fig. 2) after 2–4 years. For each of these treatments, effects varied considerably across sites, but there were no consistent directional changes. Although primary productivity in most of these sites was enhanced by N and P availability (Fay *et al.* 2015; Yue *et al.* 2016), the observed increases in plant growth did not translate to consistent increases in soil carbon storage over this time period.

In contrast to N and P, the addition of K and micronutrients (alone and in combination with the macronutrients) caused notable increases in soil carbon storage across our sites. In isolation, K<sub>+μ</sub> enrichment trended towards an overall positive ( $z = 1.93$ ;  $P = 0.05$ , Fig. 2) effect on soil carbon storage, with a slightly higher probability of increased soil carbon storage relative to the controls. But when K<sub>+μ</sub> was added in combination with N and P, the full combination of nutrients significantly ( $z = 3.31$ ;  $P < 0.001$  – the 95% CI for the difference in soil carbon between control and NPK plots did not span zero) increased soil carbon storage across our sites. The mean increase in soil carbon across our sites was  $0.04 \text{ KgCm}^{-2} \text{ year}^{-1}$  ( $\pm 0.18 \text{ KgCm}^{-2} \text{ year}^{-1}$  Standard Deviation). However, this effect was characterized by considerable variability, with strong positive and negative effects at individual sites (Fig. 2b). To minimize the possible influence of this intersite variability, we conducted a secondary validation



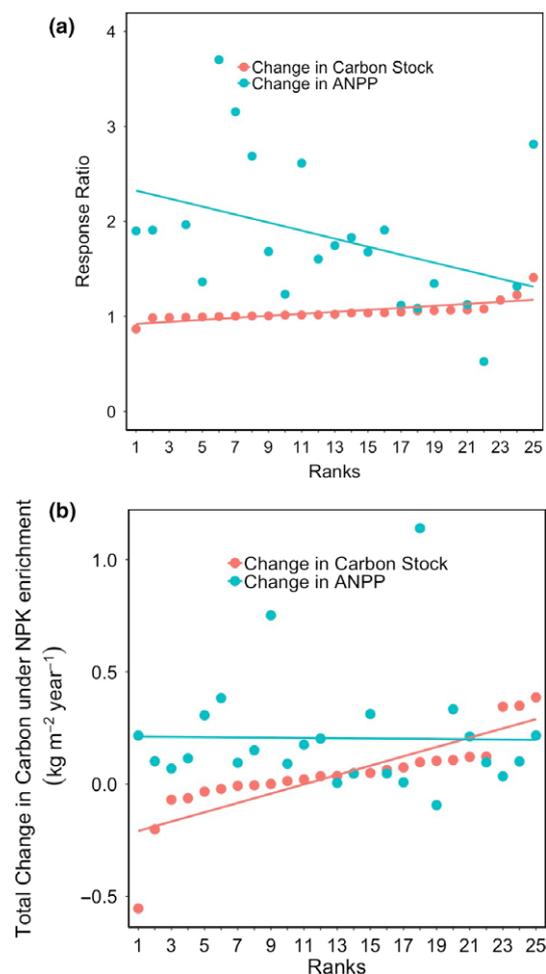
**Figure 2** Overall changes in soil carbon stocks in response to nutrient enrichment. Plate 'a' shows the modelled mean differences in carbon stocks (derived from coefficients in the linear mixed effects model) in each treatment, compared to the control (the horizontal black line). Points indicate mean treatment effects across all sites and error bars indicate 95% confidence intervals. Stars indicate significance values (\*\*\*)  $> 0.001$ ; \*  $> 0.05$ ). Plate 'B' shows the mean site-level average changes in total soil carbon compared to the control for each nutrient treatment (indicated by different colours). This reveals the spread of effects across sites. Although the addition of  $K_{+\mu}$  and  $NPK_{+\mu}$ , generally increased soil carbon stocks, relative to the control, the range of effects varied from soil carbon gains to losses across sites.

model, excluding the sites with the three biggest soil C losses and the three biggest soil C gains (i.e. removing possible outliers at either extreme of the effects across sites). The overall effect of NPK enrichment across these sites remained significantly ( $z = 1.96$ ,  $P = 0.05$ ) positive. The overall mean effect remained unchanged, with NPK enrichment increasing soil C stocks by  $0.04 \text{ KgCm}^{-2} \text{ year}^{-1}$ , with a standard deviation of  $0.05 \text{ KgCm}^{-2} \text{ year}^{-1}$ . Yet, although sites were significantly ( $P < 0.05$ ) more likely to experience slight gains in soil carbon under combined nutrient enrichment (i.e. the slope of the relationship between NPK and control treatments was positive;

Fig. 1a), this effect was still characterized by huge variation, with some regions experiencing losses of soil carbon under NPK enrichment. Exploring the environmental drivers of these differences can provide insights into which regions are more or less responsive to changes in combined nutrient enrichment.

#### Predicting the changes in soil carbon stocks under NPK+ $\mu$ enrichment across sites

To test whether the increases in soil carbon storage under combined nutrient treatment were driven by increases in plant productivity, we examined the relationship between the 'changes in soil carbon stocks' and the equivalent 'change in aboveground net primary productivity' in response to combined nutrient enrichment across the sites. Although both productivity and soil carbon storage generally increased across most sites, we found no correlation between the proportional ( $t = 1.61$ ;  $P = 0.11$ ; Fig. 3) or absolute ( $t = 0.51$ ;  $P = 0.61$ ;



**Figure 3** The change in soil carbon stocks across sites was not correlated with the mean change in net primary productivity. Given that combined nutrient enrichment generally increased primary productivity across these sites (Fay *et al.* 2015), we expected that the magnitude of the change in NPP might translate to the extent of the increase in soil carbon storage. However, there was no significant ( $P > 0.05$ ) relationship between the proportional (a) or absolute (b) 'change in aboveground primary productivity' or the 'change in soil carbon stock' under combined nutrient enrichment across Nutrient Network sites.

Fig. 3) changes in primary productivity and soil carbon stocks after 2–4 years of treatments. In fact, visual inspection of these relationships suggests that the changes in soil carbon stocks tended to be higher in regions where the vegetation response was minimal, although this negative relationship was not significant ( $P < 0.05$ ; Fig. 3).

Linear models were used to test which climate variables (mean annual temperature, mean annual precipitation and evapotranspiration) and soil characteristics (soil texture, pH and organic carbon stock) might explain the magnitude and direction of the changes in soil carbon stocks under NPK<sub>+μ</sub> enrichment. Initial models were constructed to test the controls on the proportional (response ratio) changes in soil carbon stocks. Only soil texture (% sand) and Mean Annual Precipitation (MAP) were retained within the final model after our variable selection (See supplementary Information for full statistical details and code). Both had a similar directional influence on the nutrient effect on soil carbon stocks: the impacts of combined nutrient enrichment were greatest in regions with low levels of Mean Annual Precipitation ( $t = -1.37$ ,  $P = 0.15$ ) and a high percentage of sand in the soil ( $t = 2.08$ ,  $P = 0.02$ ) (Fig. 4).

We then used a second model to examine if these patterns in the proportional changes in soil carbon reflect the total changes in soil carbon stocks. Following model refinement, the signal of soil texture was still apparent across our sites, as % sand was retained in the final model ( $t = 1.46$ ,  $P = 0.15$ ). However, the initial size of the standing soil carbon stock in that location emerges as by far the strongest predictor. Specifically, the effect of nutrient enrichment becomes negatively correlated with increases in soil carbon stocks ( $t = -2.07$ ,  $P = 0.04$ ; Fig. 4). Even minor proportional changes caused considerable absolute changes in regions with larger standing soil carbon. As such, although most of the sites in our analysis generally experience increases in soil carbon under combined nutrient enrichment, total absolute losses were consistently observed in regions with high standing soil carbon stocks (Fig. 4).

By extrapolating these linear relationships across the northern Hemisphere (where the vast majority of our data comes from), we can visualize the general linear trends in total and proportional changes in soil carbon stocks under strong nutrient enrichment. Generally, there was no clear latitudinal trend in the proportional soil carbon sensitivity to combined nutrient enrichment. The greatest changes detected were the considerable proportional increases in soil carbon in dry, sandy regions. However, the dominant role of standing soil carbon stock in determining the absolute carbon changes resulted in a strong latitudinal trend, with the largest carbon losses tending to occur in high-latitude regions (i.e. regions which are dominated by huge standing soil carbon stocks; Hengl *et al.* 2017; See Fig. 4).

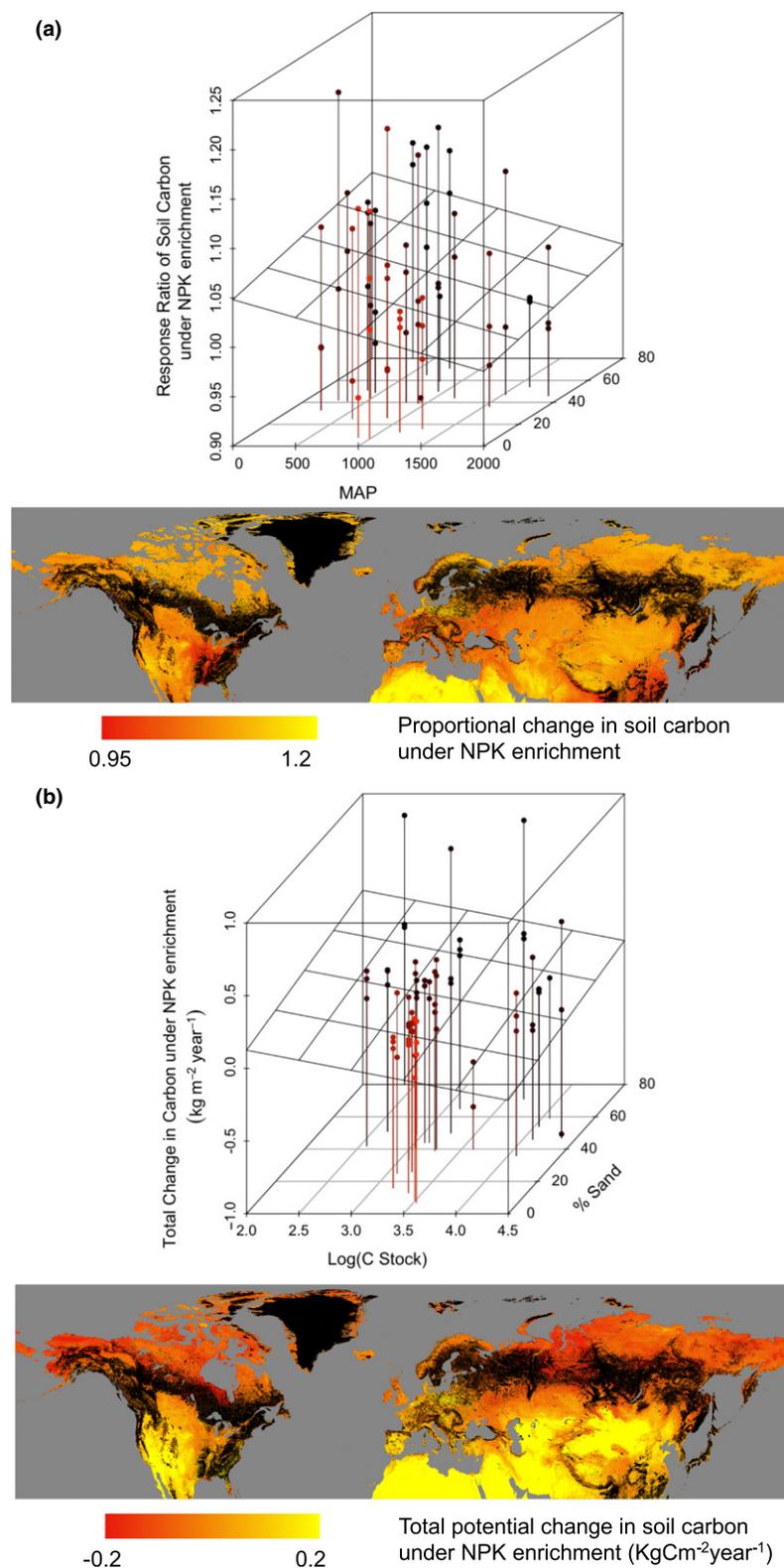
## DISCUSSION

Our study represents the first large-scale analysis of the initial (2–4 years) changes in soil carbon storage under combined nutrient enrichment. By focusing on differences in soil carbon stocks, we are able to detect the net changes in carbon storage that are a product of the differences in soil carbon inputs (net

primary production) and release (decomposition). Although nutrient enrichment generally tended to increase soil carbon our grassland sites, these effects were highly variable across biogeographic regions, with considerable soil carbon losses occurring in regions with large standing soil carbon stocks. Our broad-scale approach enables us to see past the contrasting effects in specific regions so that we can identify the unifying mechanisms that drive the patterns in the sensitivity of grassland soil carbon stocks to nutrient enrichment.

In contrast to expectations from empirical (Knorr *et al.* 2005; Treseder 2008; Yue *et al.* 2016) and theoretical (Wieder *et al.* 2015b; Devaraju *et al.* 2016) research, we found no consistent changes in grassland soil carbon storage under N or P enrichment, alone or in combination. Although the growth of plants in most ecosystems – including these specific study locations – is generally limited by N and P availability (Fay *et al.* 2015; Yue *et al.* 2016), increased primary production did not translate to consistent increases in soil carbon storage. The limited effects of N and P enrichment compared to previous analyses highlight the value of using total soil carbon stock as an indicator of the total soil carbon balance. Although previous analyses have generally focused on changes in short-term fluxes (photosynthesis and respiration) which can be highly responsive over short time periods, the total soil carbon balance is far less sensitive to such anthropogenic effects. It is possible that longer-term analyses may be necessary to detect the effects of these macronutrients, but the limited effects observed here suggest that total soil C stocks are less responsive to N and P enrichment than previously expected.

In contrast to the major macronutrients, the addition of K and micronutrients caused a marginal increase in soil carbon storage across our sites (Fig. 2a). The generally positive effect of K<sub>+μ</sub> on soil carbon accumulation is in keeping with the understanding that productivity in most soils (in particular arid soils) is strongly limited by the availability of K (Sardans & Peñuelas 2015). Indeed, these results lend support to the findings of local scale studies highlighting that K enrichment has a stronger impact on soil carbon dynamics than the macronutrients N and P (Ochoa-Hueso *et al.* 2019). However, it was the full combination of nutrients (N, P and K<sub>+μ</sub>) that drove the most consistent changes, driving both increases and decreases in soil carbon across different sites (Fig. 1b). Although gains and losses of soil carbon were observed, the mean site-level change was a gain of  $0.04 \text{ KgCm}^{-2} \text{ year}^{-1}$  (Standard Deviation =  $0.18 \text{ KgCm}^{-2} \text{ year}^{-1}$ ). By definition, co-limitation is synergistic when the ecosystem response to multiple nutrients is greater than the sum of the response to each nutrient added individually (Harpole *et al.* 2011). Here, the positive and negative impacts of combined nutrient enrichment far outweighed the additive effects of the nutrients added in isolation, suggesting synergistic effects on soil carbon storage (Fig. 1b). Thus, even if nutrient enrichment alleviates the N and P limitations on grassland productivity, changes in soil carbon accumulation are still likely to be constrained by the availability of K and micronutrients in many regions. This will have direct implications for biogeochemical model simulations of future soil carbon storage, most of which assume strong global impacts of N enrichment with little consideration of micronutrients (Wieder *et al.* 2015b).



**Figure 4** General patterns in the sensitivity of soil carbon stocks to NPK enrichment. 3-D plots show the overall effects of the dominant environmental predictors of the direction and magnitude of the proportional (a) and total (b) changes in soil carbon under combined NPK enrichment. Associated maps reveal the general trends in these effects, as described in each of the linear models. Soil texture (% sand) and mean annual precipitation (mm) were the strongest predictors of changes in the proportional soil carbon stock, with the greatest increases occurring in dry, sandy regions (a). In contrast, soil carbon content and texture were the dominant drivers of the total soil carbon change, with considerable carbon losses occurring in regions with large standing soil carbon stocks (b).

The detected changes in soil carbon stocks are likely to be driven by changes in plant carbon inputs to soil, reductions in soil carbon decomposition or both. Identifying the relative importance of these processes can provide a mechanistic understanding of nutrient-induced soil carbon accumulation. To address this, we examined the extent to which changes in soil carbon storage could be explained by increases in plant productivity nutrient enrichment. Although primary productivity generally increased in response to combined nutrient enrichment (Fig 3b; Fay *et al.* 2015), the 'change in net primary productivity' was not correlated with changes in soil carbon stocks (Fig. 3). This lack of correlation is surprising, given the expectation that increased soil carbon accumulation would be strongly driven by increases in plant carbon inputs. However, increased plant growth under nutrient enrichment does not necessarily translate to increased carbon losses into soil. These results suggest that the increases in soil carbon accumulation might be driven, at least in part, by reductions in soil carbon decomposition, as observed directly in many litter decomposition studies (Knorr *et al.* 2005). Although increases in nutrient-induced changes in plant growth can enhance soil carbon inputs in some regions, a considerable proportion of the initial soil carbon increase under nutrient enrichment is likely to be driven by the suppressed activity of the soil decomposer community.

In order to generate a mechanistic understanding of which ecosystems are most sensitive to nutrient enrichment, we explored which environmental variables could explain the changes in soil carbon stocks to NPK<sub>+μ</sub> enrichment across sites. Scaling theory in ecology describes how emergent patterns can arise from distinct and causative relationships operating at finer-scales (Levin 1992). Therefore, based on the results from previous soil carbon analyses (Knorr *et al.* 2005; Treseder 2008; Crowther *et al.* 2015), we expected that the soil carbon response to nutrient enrichment would be contingent upon climate and soil characteristics. Linear models revealed that soil texture (% sand) and Mean Annual Precipitation (MAP) had strong directional effects on the proportional changes in soil carbon stocks under nutrient enrichment. The proportional increases in soil carbon stocks were greatest in grasslands that are dominated by dry and coarse-textured soils (Fig. 4a). These soils are often associated with long-term weathering and nutrient depletion. Increasing the availability of macro and micronutrients in these regions might be necessary for alleviating the nutrient limitations on plant growth and driving the build-up of soil organic carbon. In addition, increased plant growth might also increase the moisture limitations on the soil microbial community in dry soils, potentially reducing carbon losses through decomposition.

Although dry, sandy regions were proportionally the most sensitive to nutrient enrichment, the absolute annual changes were dominated by the size of the initial standing soil carbon stocks. Even tiny proportional carbon losses in regions with huge standing soil carbon stocks translate to considerable carbon changes under combined nutrient enrichment (Fig. 4). Specifically, the total soil carbon response to combined nutrient enrichment correlated negatively with the size soil carbon stock. Therefore, although nutrient enrichment led to increased soil carbon storage in the majority of our study sites, carbon losses consistently occurred in regions with relatively high standing

soil carbon. Visual extrapolation of this general relationship highlights that nutrient enrichment has the potential to drive soil carbon losses in many high-latitude Arctic and sub-Arctic regions, which are characterized by huge standing carbon stocks (Hengl *et al.* 2014) (Fig. 4b). Given that the greatest proportion of the world's soil carbon exists in these high-latitude regions, these general trends reinforce the sensitivity of Arctic and sub-Arctic carbon storage to global change. Even though the majority of terrestrial soils is likely to experience proportional increases in soil carbon under combined nutrient enrichment, it is possible that these high-latitude regions might experience losses of soil carbon that could potentially outweigh the gains, leading to global soil carbon losses under nutrient enrichment. Validating this trend across a wider geographic range, including more high-latitude sites with high standing carbon stocks, will be critical for quantifying the contribution of these high-latitude carbon losses, relative to the carbon gains observed across the rest of the Northern Hemisphere. These preliminary global patterns of soil carbon sensitivity to combined nutrient enrichment provide valuable insights that improve our capacity to understand and predict the potential changes in the terrestrial carbon balance under global change.

Our analysis has a number of limitations that should be considered when interpreting the results. The relatively short time-scale over which our analysis was conducted (2–4 years) means that these responses only reflect initial, short-term soil carbon responses to nutrient enrichment. Although soil carbon uptake and release are both highly dynamic processes (Carey *et al.* 2016), the full extent of changes in soil carbon stocks in response to global change drivers may require decades to be realized (Bradford *et al.* 2016). Yet, this level of nutrient enrichment has been shown to induce strong changes in soil carbon dynamics in short-term global change experimental studies (Liu & Greaver 2010; Crowther *et al.* 2015; Yue *et al.* 2016) and the magnitude of the NPK<sub>+μ</sub> responses we present clearly highlights that nutrient enrichment has the capacity to alter the balance between grassland soil carbon uptake and release at a global scale. Most importantly, we stress that our results cannot accurately represent the magnitude of the potential changes in global soil carbon because the standardized nutrient addition rates do not reflect the highly variable deposition rates occurring across the globe. Instead, they provide mechanistic insights by highlighting that soil carbon changes are not likely to be driven by one or few macronutrients, but by a combination of nutrients that will act synergistically under global change scenarios. Finally, as with all global experiments, the limited geographic distribution of study sites, restricts our confidence in the spatial patterns in the effects of nutrients across terrestrial grasslands. Further research over longer-timescales will be necessary to evaluate the initial spatial patterns in soil carbon sensitivity that we describe here.

## CONCLUSIONS

Our analysis provides a global perspective on the sensitivity of grassland soil carbon stocks to nutrient enrichment. In contrast to expectations, N and P enrichment alone had minimal impacts on soil carbon storage across global grasslands after 2–4 years. Yet, as with primary productivity (Fay *et al.* 2015),

the accumulation of carbon in soil appears to be constrained by multiple nutrients; the addition of K and micronutrients in combination with N and P had considerable effects on soil carbon storage, with proportional increases in soil carbon storage across most sites in this study. Thus, even if nutrient enrichment alleviates the N and P limitation of grasslands, the accumulation of carbon in soil may still be limited by the availability of micronutrients in many regions. The large-scale collaborative empirical approach enables us to elucidate the regional patterns in this soil carbon response, revealing that nutrient enrichment might lead to the largest proportional increases of carbon in the world's dry, sandy soils. However, soil carbon losses were found in high-latitude sites with large standing soil carbon. Given that the majority of the world's soil carbon exists in high-latitude Arctic and sub-Arctic regions, absolute losses of soil carbon in these regions might potentially outweigh the gains at lower latitudes. This highlights the vulnerability of global carbon stocks to combined nutrient enrichment at a global scale. Given the devastating impacts that this nutrient enrichment generally has on local biodiversity and ecosystem functioning, these results reinforce the negative effects of fertilizers on terrestrial ecosystems around the world, as global carbon losses could accelerate the rate of climate change. But this information can help us to improve our mechanistic understanding of the terrestrial carbon balance as anthropogenic nutrient enrichment increases under future global change scenarios. Evaluating and building on this spatially explicit understanding will be necessary to comprehend the net global soil carbon response to nutrient enrichment, which will ultimately be valuable for benchmarking and parameterizing modeling efforts that aim to predict the changes in carbon cycling under global change scenarios.

#### ACKNOWLEDGEMENTS

This work was funded by grants to TWC from DOB Ecology, Plant-for-the-Planet and the German Federal Ministry for Economic Cooperation and Development. The 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 to Cedar Creek LTER) programs and the University of Minnesota 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.

#### DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.0dt27vb>.

#### AUTHORSHIP

This specific study was designed by TWC and CR, with help from EL, ETB, SEH and EWS. The NutNet experiment was

coordinated by EL, ETB, SEH and EWS. Statistical analyses were performed by DR, EL, JW and CR. Data collection was performed by PBA, JF, LG, NH, KSH, JMHK, RLM, AM, PLP, SMP and CJS. TWC wrote the first draft of the manuscript and all authors contributed substantially to revisions.

#### REFERENCES

- Borer, E.T., Harpole, W.S., Adler, P.B., Lind, E.M., Orrock, J.L., Seabloom, E.W. *et al.* (2014). Finding generality in ecology: a model for globally distributed experiments. *Methods Ecol. Evol.*, *5*, 65–73.
- Bradford, M.A., Wieder, W.R., Bonan, G.B., Fierer, N., Raymond, P.A. & Crowther, T.W. (2016). Managing uncertainty in soil carbon feedbacks to climate change. *Nat. Clim. Chang.*, *6*, 751–758.
- Carey, J.C., Tang, J., Templer, P.H., Kroeger, K.D., Crowther, T.W., Burton, A.J. *et al.* (2016). Temperature response of soil respiration largely unaltered with experimental warming. *Proc. Natl Acad. Sci.*, *113*, 13797–13802.
- Crowther, T.W., Thomas, S.M., Maynard, D.S., Baldrian, P., Covey, K., Frey, S.D. *et al.* (2015). Biotic interactions mediate soil microbial feedbacks to climate change. *Proc. Natl Acad. Sci.*, *112*, 7033–7038.
- Crowther, T., Todd-Brown, K., Rowe, C., Wieder, W., Carey, J., Machmuller, M. *et al.* (2016). Quantifying global soil C losses in response to warming. *Nature*, *540*, 104–108.
- Devaraju, N., Bala, G., Caldiera, K. & Nemani, R. (2016). A model based investigation of the relative importance of CO<sub>2</sub>-fertilization, climate warming, nitrogen deposition and land use change on the global terrestrial carbon uptake in the historical period. *Clim. Dyn.*, *47*, 173–190.
- Fay, P.A., Prober, S.M., Harpole, W.S., Knops, J.M.H., Bakker, J.D., Borer, E.T. *et al.* (2015). Grassland productivity limited by multiple nutrients. *Nat. Plants*, *1*, 15080.
- Fick, S.E. & Hijmans, R.J. (2017). Worldclim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.*, *37*, 4302–4315.
- Gorelick, N., Hancher, M., Dixon, M., Ilyushchenko, S., Thau, D. & Moore, R. (2017). Google Earth Engine: planetary-scale geospatial analysis for everyone. *Remote Sens. Environ.*, *202*, 18–27.
- Harpole, W.S., Ngai, J.T., Cleland, E.E., Seabloom, E.W., Borer, E.T., Bracken, M.E.S. *et al.* (2011). Nutrient co-limitation of primary producer communities. *Ecol. Lett.*, *14*, 852–862.
- Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T. *et al.* (2016). Addition of multiple limiting resources reduces grassland diversity. *Nature*, *537*, 93–96.
- Hengl, T., de Jesus, J.M., MacMillan, R.A., Batjes, N.H., Heuvelink, G.B., Ribeiro, E. *et al.* (2014). SoilGrids1 km–global soil information based on automated mapping. *PLoS ONE*, *9*, e105992.
- Hengl, T., De Jesus, J.M., Heuvelink, G.B.M., Gonzalez, M.R., Kilibarda, M., Blagotić, A. *et al.* (2017). SoilGrids250 m: global gridded soil information based on machine learning. *PLoS ONE*, *12*, 1–40.
- IPCC. (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J., Reichstein, M., Ceulemans, R. *et al.* (2010). Reduction of forest soil respiration in response to nitrogen deposition. *Nat. Geosci.*, *3*, 315–322.
- Knorr, M., Frey, S.D. & Curtis, P.S. (2005). Nitrogen additions and litter decomposition: a meta-analysis. *Ecology*, *89*, 3252–3257.
- Levin, S.A. (1992). The problem of pattern and scale in ecology. *Ecology*, *73*, 1943–1967.
- Liu, L. & Greaver, T.L. (2010). A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecol. Lett.*, *13*, 819–828.

- Ochoa-Hueso, R., Delgado-Baquerizo, M., An King, P.T., Benham, M., Arca, V. & Power, S.A. (2019). Ecosystem type and resource quality are more important than global change drivers in regulating early stages of litter decomposition. *Soil Biol. Biochem.*, 129, 144–152.
- Sardans, J. & Peñuelas, J. (2015). Potassium: a neglected nutrient in global change. *Glob. Ecol. Biogeogr.*, 24, 261–275.
- Todd-Brown, K.E.O., Randerson, J.T., Post, W.M., Hoffman, F.M., Tarnocai, C., Schuur, E.A.G. *et al.* (2013). Causes of variation in soil carbon simulations from CMIP5 Earth system models and comparison with observations. *Biogeosciences*, 10, 1717–1736.
- Treseder, K.K. (2008). Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecol. Lett.*, 11, 1111–1120.
- Wetherbee, G.A. & Mast, M.A. (2016). Annual variations in wet-deposition chemistry related to changes in climate. *Clim. Dyn.*, 47, 3141–3155.
- Wieder, W., Cory, C., Smith, W. & Todd-Brown, K. (2015a). Future productivity and carbon storage limited by terrestrial nutrient availability. *Nat. Geosci.*, 8, 441–444.
- Wieder, W.R., Cleveland, C.C., Smith, W.K. & Todd-Brown, K. (2015b). Future productivity and carbon storage limited by terrestrial nutrient availability. *Nat. Geosci.*, 8, 441–444.
- 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.
- Zaehle, S., Jones, C., Houlton, B., Lamarque, J.-F. & Robertson, E. (2015). Nitrogen availability reduces CMIP5 projections of twenty-first-century land carbon uptake. *J. Clim.*, 28, 2494–2511.
- Zomer, R.J., Trabucco, A., Bossio, D.A., van Straaten, O. & Verchot, L.V. (2008). Climate change mitigation: a spatial analysis of global land suitability for clean development mechanism afforestation and reforestation. *Agric. Ecosyst. Environ.*, 126, 67–80.
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. Springer Science and Business Media, New York, NY.

#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Editor, Josep Penuelas

Manuscript received 16 October 2018

First decision made 20 November 2018

Second decision made 20 February 2019

Manuscript accepted 25 February 2019