Crowther, T.W.[1], Riggs, C.[2], Lind, E.[2], Wubs, J.[3], Borer, E. T.[2], Seabloom, E. W.[2], Hobbie, S. E.[2], Adler, P. B.[4], Firn, J.[5], Gherardi, L.[6], Hagenah, N.[7], Hofmockel, K.S.[8,9], Knops, J. M. H.[10], McCulley, R. L.[11], McDougall, A.[12], Peri, P. L.[13], Prober, S. M.[14], Stevens, C. J.[15], , Routh, D.[1]

Affiliations:

1. Institute of Integrative Biology, ETH Zurich, Univeritätstrasse 16, 8092 Zurich, Switzerland
2. Department of Ecology, Evolution, and Behavior, University of Minnesota, 1479 Gortner Ave. St. Paul, MN 55108 USA
3. Department of Terrestrial Ecology, Netherlands Institute of Ecology, Droevendaalsesteeg 10, 6708 PB Wageningen, Netherlands
4. Department of Wildland Resources and the Ecology Center, Utah State University, Logan, UT 84103 USA
5. Queensland University of Technology (QUT), Gardens Point Campus, Brisbane Queensland, 40000
6. School of Life Sciences and Global Drylands Center, Arizona State University
7. Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa
8. Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA 99352, United States
9. Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50010, United States
10. School of Biological Sciences, University of Nebraska, Lincoln, NE 68588, USA
11. Department of Plant & Soil Sciences, University of Kentucky, Lexington, KY, 40546, USA
12. Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1
    519-824-4120
13. Instituto Nacional de Teconología Agropecuaria (INTA)-UNPA-CONICET
14. CSIRO Land and Water, Private Bag 5, Wembley, Western Australia, 6913
15. Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK

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Soil stores approximately twice as much carbon as the atmosphere, and fluctuations in the size of the soil carbon pool directly influence climate conditions. Understanding how soil carbon storage will respond to global change is critical if we are to project future climate scenarios. Here, we used the Nutrient Network coordinated global change experiment to examine how anthropogenic nutrient enrichment (nitrogen [N] phosphorous [P], and potassium [K]) might influence grassland soil carbon storage at 26 sites spanning four continents. When applied in isolation, enrichment of N and P had minimal impacts on soil carbon storage. However, when these nutrients were added in combination with K and micronutrients, soil carbon increased considerably, revealing that the accumulation of soil carbon is constrained by multiple nutrients in most grassland regions. Combined nutrient enrichment increased soil carbon storage by an average of 8.4% across these experimental sites. The soil carbon response was contingent on regional factors, with the largest proportional increases in dry, sandy soils that are prominent in the Southern Hemisphere. However, given that these regions have the smallest standing carbon stocks, the absolute changes are likely to be minor relative to high-latitude regions. These biogeographic patterns provide unique insights into the global responses of carbon stocks to nutrient enrichment and will facilitate mechanistic modeling efforts that aim to project carbon cycling under future global change scenarios.

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 concentrations and climate[1,2]. 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[3]. Along with anthropogenic changes to land use and climate, global nutrient enrichment of terrestrial soils is expected to be among the greatest threats to this balance between respiratory losses and photosynthetic gains at a global scale[2,4–7]. 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[8–10].

The use of fertilizers and fossil fuels have enhanced the supply of biologically reactive nitrogen (N) and phosphorus (P) in the soil over the past century[4]. Globally, concentrations of these macronutrients are expected to double by 2050[11]. Although effects of nutrient 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[12,13] and meta-analyses across large spatial scales suggest that elevated nutrient supply tends to reduce the activity of microbial decomposers in soil[5,13,14]. In combination, stimulated plant uptake and limited decomposition are expected to lead to additional carbon accumulation in soil over the rest of the century[9,15]. However, we lack empirical data on the existence or magnitude of these effects at a global scale, and the changes in net soil carbon balance remain untested. In addition, a growing body of evidence highlights the importance of less-studied elemental nutrients such as potassium (K)[12], the concentrations of which are also expected to increase as a result of enhanced weathering under global change[16]. If soil carbon accumulation is constrained by the availability of multiple nutrients, then global change studies that manipulate the supply of individual elements (e.g., only N or P) are likely to under-estimate the magnitude of future soil carbon changes.

Here, we use an internationally distributed global change experiment (NutNet[17]) 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[12]. Three nutrients (N, P, and K plus micronutrients added alone and in combination) were added to 25 m^2 replicated plots in 26 field-scale nutrient enrichment experiments conducted across North America, Europe, Africa and Australasia (ranging from subarctic to medeterranian grasslands; Suplementary Information). The K treatment included a micronutrient mix only in the first treatment year, to avoid micronutrient toxicity (thus $K_{+\mu}$, hereafter). Nutrient addition rates in our experiment were at the high end of expected deposition rates associated with global change in the coming decades, and are similar to rates added to many agricultural fields worldwide. As such, they represent an ideal opportunity to examine how soil carbon is influenced by nutrient supply[12].

We measured soil carbon stocks in all plots to calculate the log response ratio, which reflects the proportional changes in soil carbon stock in response to each experimental treatment. This change in soil carbon stock represents the net change in soil carbon uptake (through primary production) and release (decomposition) after 3 years. 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, where biological soil activity (including nutrient cycling and rates of carbon accumilation) are generally highest. Given that carbon accumulation can still be substantial below these depths[17], our results are likely to be a conservative representation of the total effects of nutrient effects on soil carbon stocks. 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.

Results

Treatment effects in experimental plots

In contrast to expectations from empirical[5,13,14] and theoretical[9,15] research, we found no consistent changes in grassland soil carbon storage under N (z = 1.04; P = 0.30, Figure 1) or P (z = 0.08; P = 0.94, Figure 1) enrichment alone or in combination (z = -0.32; P = 0.75, Figure 1) after 3 years. Although the growth of plants in most ecosystems – including these specific study locations – is limited by N and P availability[12,13], increased primary production did not translate to consistent increases in soil carbon storage, suggesting that soil carbon losses (decomposition rates) increased in concert with plant productivity. There was marked variability across sites: although soil carbon stocks increased in some regions, there were equivalent decreases in others, and there were no consistent effects across sites (Figure 1). However, despite the limited effects of macronutrients N and P, the addition of K and micronutrients (alone and in combination with the macronutrients) caused considerable increases in soil carbon storage across our sites. In isolation, our models revealed that $K_{+\mu}$ enrichment was the only nutrient treatment to exert a strong and consistent effect on soil carbon storage across sites (z = 1.93; P = 0.05, Figure 1), and

when applied alongside N and P, carbon stocks increased by approximately 8.35% compared to the unenriched controls (see supplementary material).

Despite considerable variability in the effects of nutrient enrichment on soil carbon stocks across sites (Figure 1), the overall direction of the combined (N, P and $K_{+\mu}$) nutrient effect was surprisingly consistent, with increases in soil carbon occurring in 68% of the sites (Figure 2). This impact is consistent with the idea that grassland NPP is constrained by multiple nutrients and suggests that the increased soil carbon arises, in part, from increased soil carbon inputs[12]. 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[18]. Here, the impacts of combined nutrient enrichment far outweighed the additive effects of the nutrients added in isolation, suggesting synergistic effects on soil carbon storage (Figure 1b). Thus, even if nutrient enrichment alleviates the N and P limitations on grassland productivity, increased soil carbon accumulation is still likely to be constrained by the availability of micronutrients in many regions. This will have direct implications for Earth System Model simulations of future soil carbon storage, most of which assume strong global impacts of N enrichment with little consideration of micronutrients[15].

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 of roots and shoots) and release (decomposition). But it remains challenging to identify whether increased soil carbon accumulation is driven by nutrient-induced increases in primary productivity or decreases in decomposition. These possibilities are not mutually exclusive; identifying the relative importance of these processes is necessary for a mechanistic understanding of nutrient-induced soil carbon accumulation. To address this, we examined the extent to which changes in soil carbon storage were governed by increases in plant carbon uptake under nutrient enrichment. However, across our sites, the 'change in net primary productivity' was not correlated with changes in soil carbon stocks ($t = 1.17$; $P = 0.24$), and it explained a negligible proportion of the variation in soil carbon accumulation across our sites (Figure 2). This suggests that, although increases in nutrient-induced plant growth can enhance soil carbon

inputs to soil, a considerable proportion of the overall soil carbon response to nutrient enrichment is likely to be driven by simultaneous reductions in decomposer activity[1].

Spatial distribution of soil carbon sensitivity to NPK enrichment

In order to generate a global perspective of the soil carbon sensitivity to nutrient enrichment, we explored the spatial patterns in the sensitivity of soil carbon to the $NPK_{+\mu}$ treatment. Scaling theory in ecology describes how emergent patterns can arise from distinct and causative relationships operating at finer-scales[19]. Therefore, based on the results from previous local-scale analyses[5,7,14], we expected that the soil carbon response to nutrient enrichment would be contingent upon climate and soil characteristics. To test this, we used linear models to explore whether a range of climate (mean annual temperature, mean annual precipitation and evapotranspration) and soil characteristics (soil texture, pH, and organic carbon stock) might explain the magnitude and direction of the $NPK_{+\mu}$ effects on soil carbon stocks across our sites (see methods). In line with this expectation soil texture (% sand) and Mean Annual Precipitation (MAP) were both retained within the final model after our variable selection (See supplementary Information for full statistical details and code). Both had a strong directional influence on the nutrient effect on soil carbon stocks: nutrient enrichment had the greatest effects on the proportional soil carbon in regions with lower Mean Annual Precipitation (t = -2.33, P = 0.02) and increased with the percentage of sand in the soil (t = 1.96, P = 0.05) (Figure 3). Thus, combined nutrient enrichment caused the greatest proportional increases in soil carbon storage in sandy sites with low levels of annual precipitation (Figure 3a).

Given that precipitation and soil texture strongly influenced the magnitude of the nutrient effect, we used these variables to visualize of the spatial patterns in the potential $NPK_{+\mu}$ response across the world's grasslands. Using a Random Forest machine learning approach that was trained using spatially-explicit global models of % Sand[20] and Mean Annual Precipitation[21], we could explain ~65% of the variation in the proportional $NPK_{+\mu}$ effect on soil carbon storage across our sites (See Supplementary Information Figure 9). Given that the variation in these two variables across our sites was equivalent to that observed globally (Figure 3 A and B), we could approximate the spatial patterns in the soil carbon response across grassland ecosystems. By applying this model

to every pixel throughout the world's grassland soil, we could visualize the patterns in the soil carbon sensitivity to nutrient enrichment (Figure 4). The limited number of samples in this analysis means that these global-scale models are highly uncertain and cannot represent viable products that capture fine-scale biogeographic patterns in the nutrient effect. However, given the high predictive strength of our machine-learning model ($R^2 = 0.65$), and the fact that the variation in these two variables across our sites was equivalent to that observed globally (see Figure 3), these global maps can provide a useful approximation of the broad-scale patterns of soil carbon change that may be induced under future scenarios of nutrient enrichment (Figure 4).

The spatial model of soil carbon sensitivity reveal broad-scale patterns, as well as regional contingencies in the response to combined nutrient enrichment. The greatest proportional increases in soil carbon under combined nutrient enrichment are likely to occur in grasslands that are dominated by dry and coarse-textured soils that are widespread across the Southern Hemisphere (Africa and Australasia in particular) (Figure 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. However, these effects are only proportional to the size of the soil carbon stocks in those regions. When we scale these effect sizes based on the total initial carbon storage in each location, the greatest total effects are observed in the high-latitude Northern Hemisphere regions, which are characterized by larger standing carbon stocks[20] (Figure 4B). 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. These initial trends now require additional evaluation and testing across a far broader range of bioclimatic conditions and ecosystem types in order to generate a thorough global-scale predictions.

Limitations

The relatively short timescale over which our analysis was conducted (3 years) represents a major limitation in our capacity to generate long-term soil carbon stock predictions. Although soil carbon uptake and release are both highly dynamic processes[22], the full extent of changes in soil carbon stocks in response to global change drivers may require decades to be realized[23]. This might explain why negligible effects were observed in N and P treatments, as the full extent of effects might take several years to be detected. However, this level of nutrient enrichment has been shown to induce strong changes in soil carbon dynamics in short-term experimental studies[5,7,13,14], and the magnitude of the NPK responses we present clearly highlight that nutrient enrichment has the capacity to alter the balance between grassland soil carbon uptake and release at a global scale. Yet, longer-term analyses may be necessary to comprehend the full magnitude of nutrient-driven changes in soil carbon storage under global change. In addition, our micronutrient treatment included trace amounts of carbon ($\sim$0.003kg m^{-2}), which were subtracted from final carbon stock estimates prior to our statistical analyses. Although this negligible carbon addition is unlikely to have directly altered the carbon accumulation in the soil, it is possible that it initiated some sort of priming effect that stimulated microbial activity in the soil[24]. However, any potential priming effects of this minor carbon addition would likely enhance the respiratory loss of carbon from the soil[24], so it is unlikely to explain the considerable increases in soil carbon storage detected across sites under NPK$_{+\mu}$ enrichment. Nevertheless, as with all global change experiments, it is worth noting that these results only highlight the possible ecosystem-level responses, and further research over longer-timescales will be necessary to elucidate the full magnitude of the global soil carbon response to combined nutrient enrichment.

Discussion and Conclusion

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 3 years. Yet, as with primary productivity[12], 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 increased soil carbon storage by an average of 8.35% across the 26 sites included 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 contingencies of this soil carbon response, revealing that nutrient enrichment might lead to the largest proportional increases of carbon in the world's dry, sandy soils. Yet, when converted into total changes in soil carbon, our spatial visualization of these effects suggests that the greatest total changes might occur in high-latitude regions with large standing soil carbon stocks. 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.

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Materials and Methods

All detailed methods and code can be found in supplementary material

Experimental design

Soils were collected from sites contributing to the Nutrient Network (Table 1), a distributed, coordinated research network designed to experimentally evaluate the effects of nutrient addition and herbivory on ecosystem processes in grasslands worldwide[17]. Participating sites are located across a range of climate and soil types, and follow identical methods for experimental set-up, sampling, and analysis. Consequently, the Nutrient Network provides a unique opportunity to examine how soil C responses to nutrient addition vary, or are generalizable, across environmental conditions and grassland ecosystems.

Nutrient Network experimental set-up, sampling and sample analyses are described in detail in Borer et al.[17]. Briefly, each site consists of 30 – 5 x 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 – are applied at the plot level in full factorial for a total of eight treatment combinations per block. At some sites, the control and +NPK$_{+\mu}$ plots are also crossed with a fencing treatment; that treatment is the not the focus of this study. At all sites receiving the nutrient treatment, N, P and K are applied annually (10 g m^{-2} yr^{-1}) as time-released urea [(NH$_2$)$_2$CO], triple-super phosphate [Ca(H$_2$PO$_4$)$_2$], and potassium sulfate [K$_2$SO$_4$], respectively. Micronutrients (6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo and 1% Zn) were added with K only in year 1 to avoid possible micronutrient toxicity.

Soil sampling and analysis

After two to four years of experimental nutrient addition (Table 1), 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 cores were homogenized, air dried, and analyzed for total C and N by combustion (Costech ESC 4010 Elemental Analyzer, Valencia, California, USA). At sites where pH > 7.5, soil samples were pre-treated with acid to remove carbonates (relevant sites: Cedar Point Biological Station, Hart Mountain, Saline Experimental Range, and Sheep Experimental Station).

Other covariates

We supplemented the belowground data with measurements of aboveground live plant biomass sampled at each plot the same year soil samples were collected. Plant biomass sampling methods are detailed in Borer et al.[17]. Additionally, site-level climate metrics (mean annual temperature

and annual precipitation) were extracted from the WorldClim 2 database[21], potential evapotranspiration was extracted from the CGIARCSI database[25], and edaphic characteristics (pH, soil texture, and organic carbon stock) from the SoilGrids database[20]. These environmental variables were used to explore the mechanisms underlying the spatial patterns in the sensitivity of soil carbon to nutrient enrichment (see the "Data Analysis" section below).

Data analysis

All statistical analyses were performed using R (R version 3.3.1; R Foundation for Statistical Computing 2013) aside from the final global map and model. 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 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 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).

The global model 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, and potential evapotranspiration. 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. High correlation values were noted between potential evapotranspiration and mean annual temperature as well as mean annual precipitation and pH, though later stepwise refinement of predictor variables included only mean annual precipitation and sand content (i.e., collinearity between the final linear model covariates was not present).

Refinement of the linear models was performed using the stepAIC() function (MASS package), wherein various iterations of variable inclusion was performed while simultaneously assessing AIC values. The final linear model with the lowest AIC values included sand content and mean annual precipitation as covariates. Model validation was performed following Zuur et. al[26], and none of the residual plots showed heteroscedasticity.

Map Generation and Validation

In order to maximize the quality of the final global maps showing log-response ratio and projected carbon stock changes, the linear model covariates (% sand[20] and Mean Annual Precipitation[21]) were used as the predictor variables in a random forests machine learning model implemented within Google Earth Engine[27] at 1-km^2 resolution parameterized with 2000 trees, 2 variables per node split, and a bag-fraction of 0.632. The map produced using the random forests algorithm was then convolved using a 5-pixel square kernel, and each original plot's value was compared against the predicted value. The calculated coefficient of determination for the predicted versus observed data was 0.6523 (hence the "65% of variation explained" in the dataset mentioned above). Log-response ratio map values were transformed into raw carbon stock values by taking the exponent of the logarithmic values, then multiplying the ratios by the carbon stock spatial layer from the SoilGrids database to estimate the resulting soil carbon stock from nutrient enrichment. To best illustrate major global patterns and upper latitude variation, final maps were exported from Google Earth Engine at 10-km^2 resolution and were presented using EPSG:3857 (Web Mercator WGS 84). QGIS version 2.18 was used for map formatting.
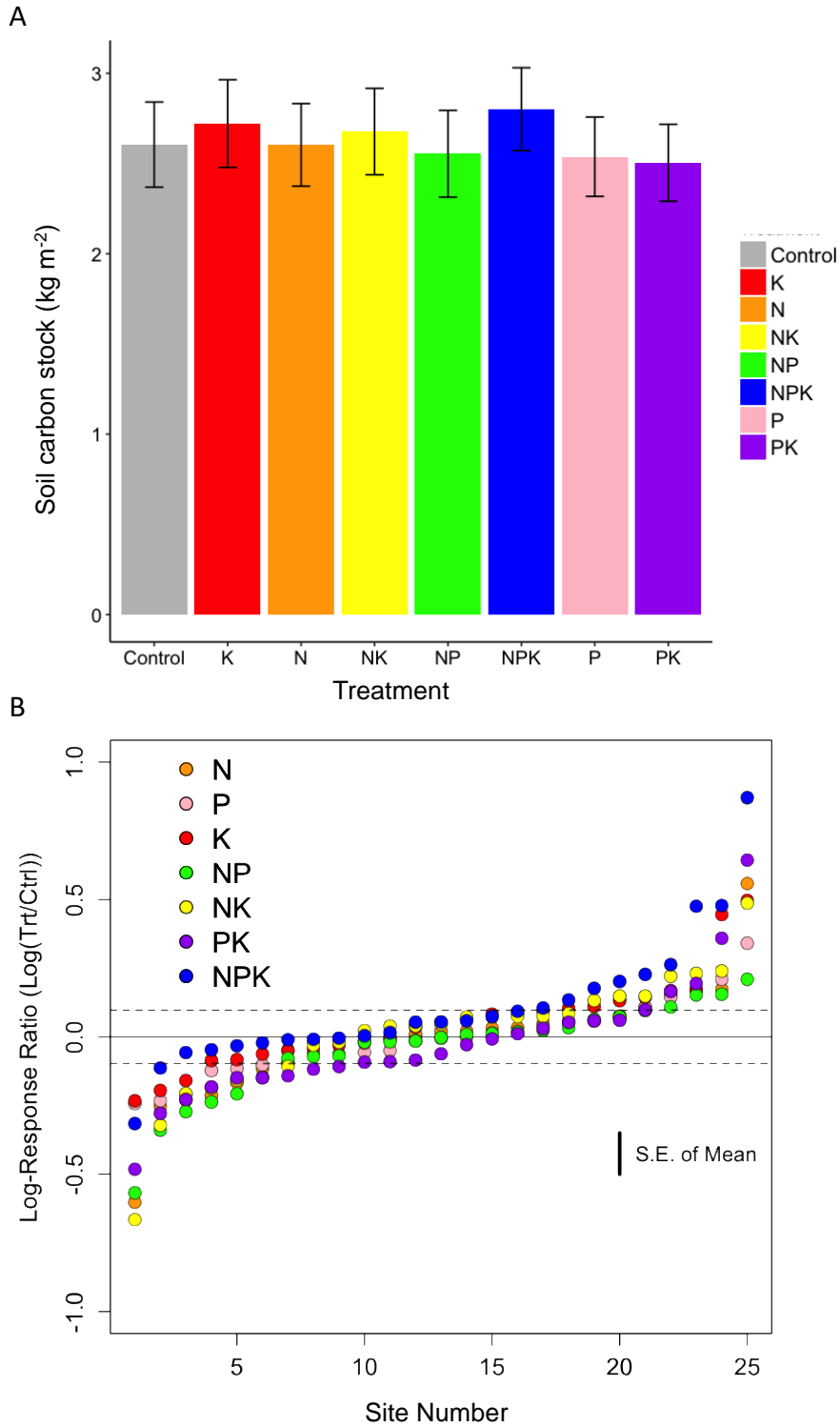
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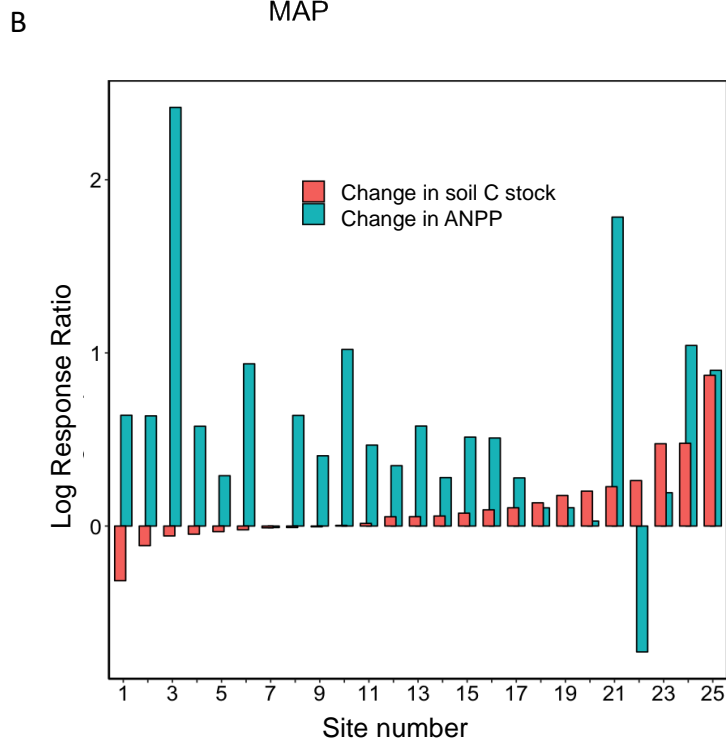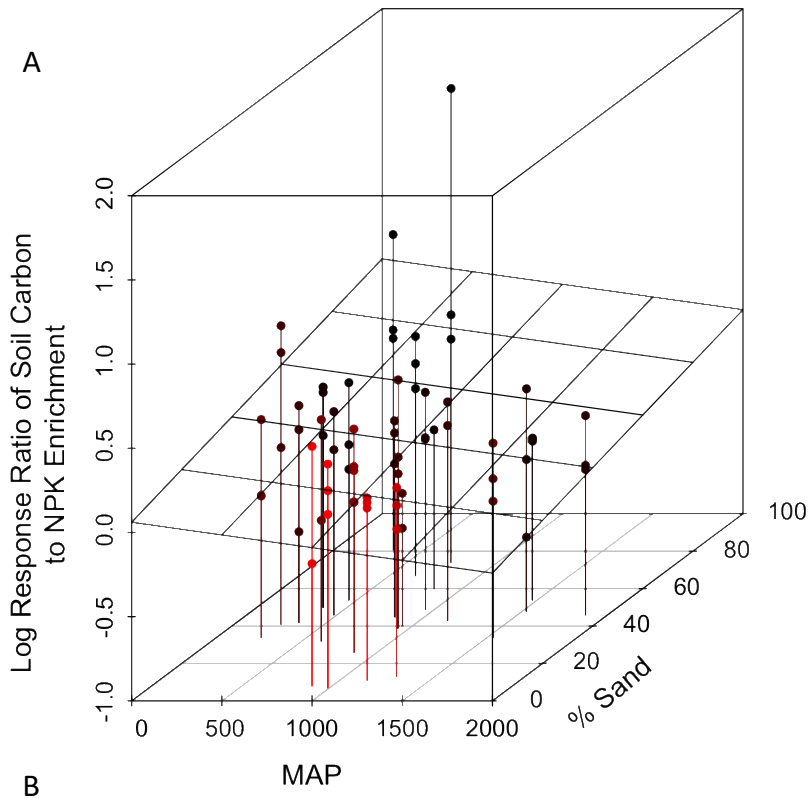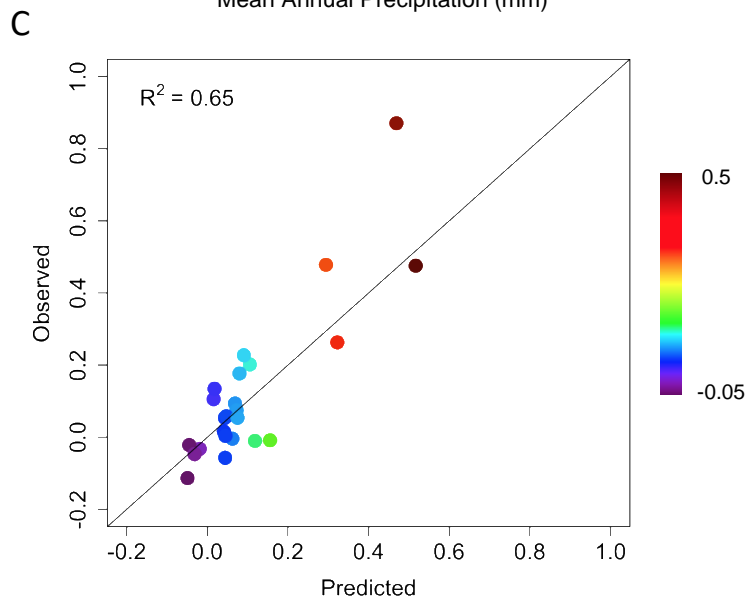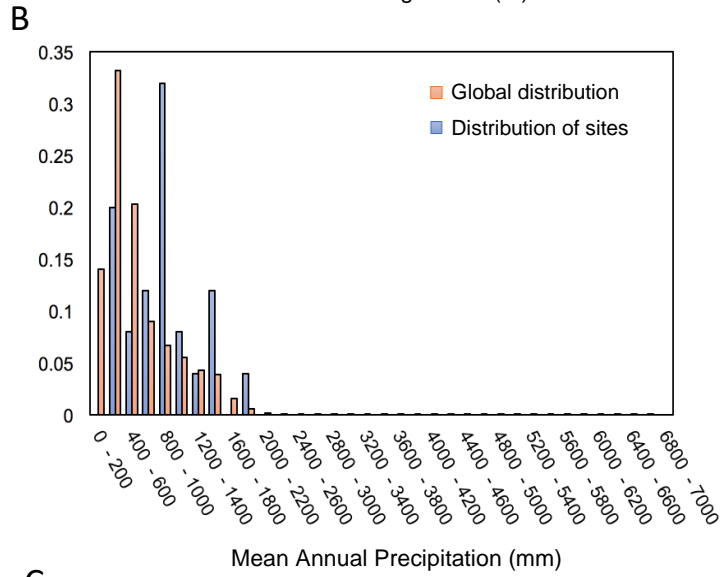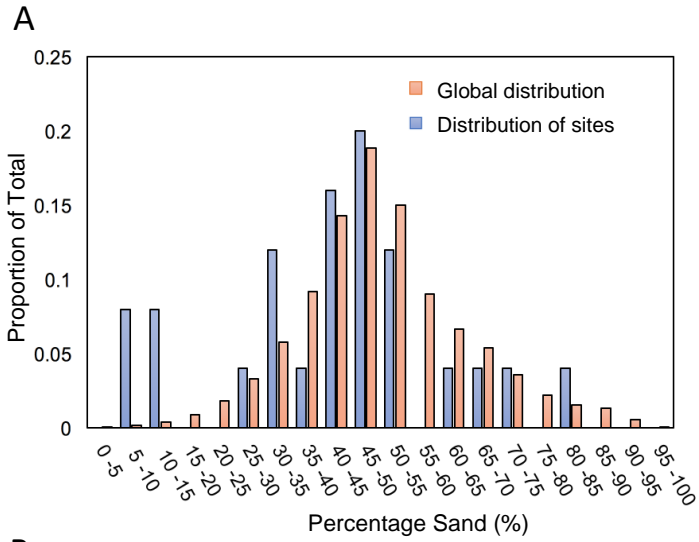
Figures

Figure 1: Overall changes in soil carbon stocks in response to nutrient enrichment. Plate

'A' shows the overall carbon stocks in each treatment, averaged across all sites (Error bars

indicate 95% CI). Plate 'B' shows the site-level average change in soil carbon (log response ratio compared to the control) for each nutrient treatment (indicated by different colours). This reveals the spread of effects across sites, and shows that, for the combined $NPK_{+\mu}$, soil carbon stocks increased in 68% of the sites, an effect that did not occur in other nutrient treatments.
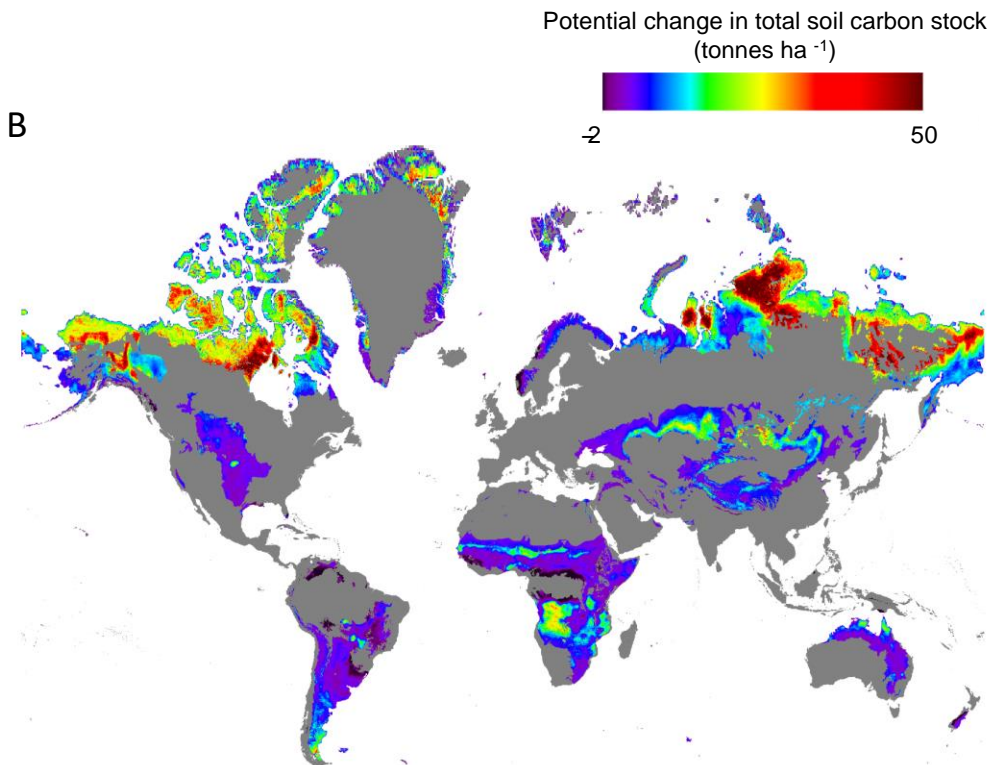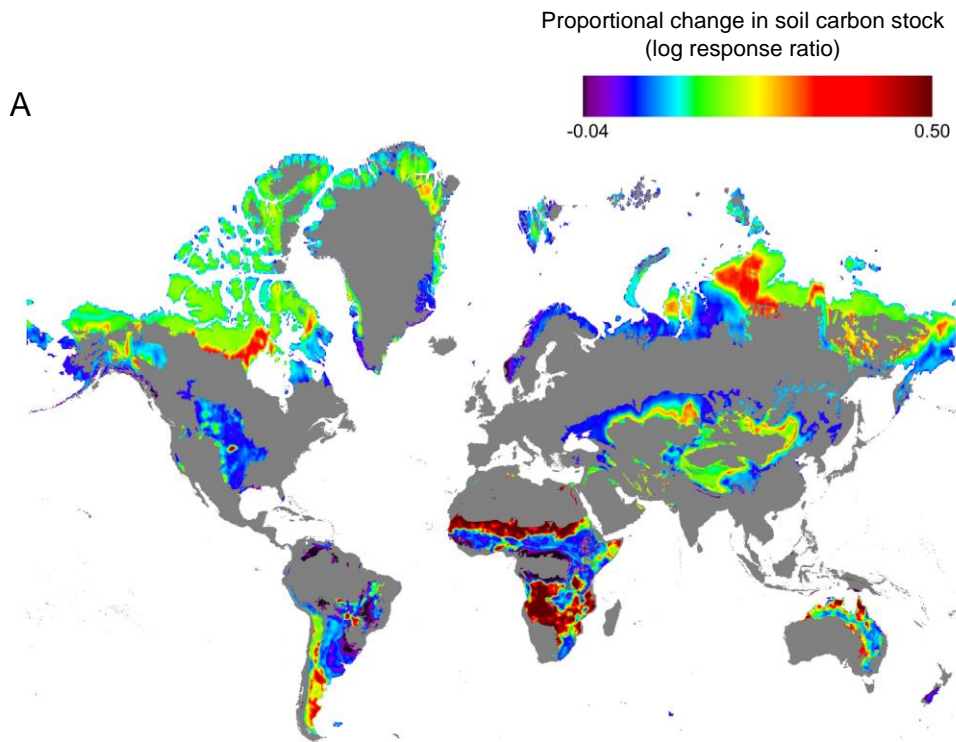
A

B

Figure 2: Predictors of the change in soil carbon stock (log response ratio between control and NPK plots) in response to combined nutrient enrichment. Plate 'A' shows a 3D plot of the two strongest predictors of the proportional change in soil carbon stock (log response ratio) under combined (NPK) nutrient enrichment: Mean Annual Precipitation (MAP), and soil texture (% sand). The colours indicate the size of the measured log response ratio, with red colours indicating minimal changes and black dots representing large increases in soil carbon under combined nutrient enrichment. Plate 'B' shows the negligible relationship between the 'change in aboveground primary productivity' and the 'change in soil carbon stock' under combined nutrient enrichment across Nutrient Network sites (linear model: t = 1.23; P = 0.23).

A

B

C

Figure 3: Validation of spatial patterns in the NPK treatment effect on soil carbon stocks.
Plate A shows the frequency of Percent Sand values experienced globally across the world's grasslands vs the frequency observed in our experimental sites. Plate B shows the frequency of Mean Annual Precipitation values experienced globally across the world's grasslands vs the frequency observed in our experimental sites. Our sites show good distribution across both environmental variables that were used to predict soil carbon changes in our spatial extrapolation. Panel C shows the predicted vs observed for the Log response ratio of soil carbon change under combined nutrient enrichment. The 1:1 trend line indicates high accuracy in the magnitude of our predictions, while the high R^2 value suggests that our machine learning model captures 65% of the variation across our 26 sites. The colour scale reflects the predicted values, and it is exactly the same as the ramp used in Figure 4A.

Proportional change in soil carbon stock
(log response ratio)

-0.04   0.50

A

Potential change in total soil carbon stock
(tonnes ha $^{-1}$)

-2   50

B

Figure 4: Spatially extrapolated map of soil carbon sensitivity to multiple nutrient enrichment across the world's grasslands. Panel A shows the expected proportional (log response ratio) changes in soil carbon stocks under combined N, P and K enrichment. Red colours highlight regions that are highly sensitive to proportional changes in soil carbon under nutrient enrichment, with considerable proportional changes occurring in dry, sandy regions that are prevalent throughout the southern hemisphere. Panel B shows the total change in soil carbon that might be expected based on this model, accounting for the total amount of soil carbon in those locations around the world. Even though the proportional changes of soil carbon are lower in the high-latitude regions, these are the areas with the greatest standing carbon stocks, and so total soil carbon changes are likely to be greater than in the lower latitudes. In both maps black indicates regions with negative soil carbon changes, where soil carbon stocks are depleted under nutrient enrichment.