

Plant growth enhancement by elevated CO₂ eliminated by joint water and nitrogen limitation

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Rising atmospheric CO₂ concentrations can fertilize plant growth. The resulting increased plant uptake of CO₂ could, in turn, slow increases in atmospheric CO₂ levels and associated climate warming. CO₂ fertilization effects may be enhanced when water availability is low, because elevated CO₂ also leads to improved plant water-use efficiency. However, CO₂ fertilization effects may be weaker when plant growth is limited by nutrient availability. How variation in soil nutrients and water may act together to influence CO₂ fertilization is unresolved. Here we report plant biomass levels from a five-year, open-air experiment in a perennial grassland under two contrasting levels of atmospheric CO₂, soil nitrogen and summer rainfall, respectively. We find that the presence of a CO₂ fertilization effect depends on the amount of available nitrogen and water. Specifically, elevated CO₂ levels led to an increase in plant biomass of more than 33% when summer rainfall, nitrogen supply, or both were at the higher levels (ambient for rainfall and elevated for soil nitrogen). But elevated CO₂ concentrations did not increase plant biomass when both rainfall and nitrogen were at their lower level. We conclude that given widespread, simultaneous limitation by water and nutrients, large stimulation of biomass by rising atmospheric CO₂ concentrations may not be ubiquitous.

The CO₂ fertilization of plant growth by rising atmospheric CO₂ concentrations [CO₂] sequesters carbon in plant biomass and thus has the potential to slow the future rate of increase in [CO₂] and thus the pace of associated climate change¹, but how this fertilization varies with environmental conditions remains unclear. The CO₂ fertilization effect may be limited, for example, by low supply of soil resources^{2–6}. This hypothesis follows logically from multiple resource limitation theory^{2,7}, which suggests that responses to elevated [CO₂] (eCO₂) may depend on levels of other resources (Supplementary Fig. 1). There is some evidence of this for nitrogen (N; refs 2–6), but less for water^{8–14} (see below for details).

Moreover, eCO₂ can enhance or reduce the eCO₂ fertilization effect by influencing soil resource supply^{15–17}, and thereby altering the relative limitations imposed by different resources (that is, shifting position on the soil resource axes in Supplementary Fig. 1), or by favouring species that are more or less responsive to eCO₂ or that themselves alter soil resource supply¹⁸. For example, the N limitation feedback hypothesis^{4–6,16} suggests that negative impacts of eCO₂ on N cycling can constrain responses to eCO₂. In contrast, eCO₂ fertilization may be enhanced under conditions of low water supply given that eCO₂ reduces stomatal conductance, and thus plant water use, increasing soil moisture^{8,9,11–15,19–23}.

Soil resource constraints on eCO₂ fertilization

Past studies show that interactive effects among multiple global-change factors on ecosystem processes are common, although not ubiquitous^{4–6,8,10,24,25}, and models of global-change impacts on ecosystems point to the importance of such potential interactions²⁶. However, an understanding of the interactive effects of eCO₂ and multiple soil resources on ecosystems is limited by the low number of pertinent studies. To our knowledge, only four well-replicated studies longer than three years have been published quantifying

the effects of eCO₂ on biomass production at contrasting nutrient supply rates^{5,10,18,27} and only one experiment longer than three years reported effects of eCO₂ at contrasting water regimes¹⁰. The hypothesis that low nutrient availability will reduce the enhancement of biomass production by eCO₂ has been supported in short- to medium-term experiments^{4–6,18,25,27,28}. However, other studies showed no difference in eCO₂ response at contrasting N supply^{10,29}.

Hypotheses involving water × [CO₂] interactions have also received little testing. Growth at eCO₂ typically reduces the leaf conductance of water vapour, leading to higher leaf water-use efficiency and often higher soil water^{8,15,19–21,23}. Soil water savings under eCO₂ could have important impacts locally and globally. For example, an experimental study of [CO₂] × warming interactions found that eCO₂ eliminated desiccation caused by warming in a semi-arid perennial grassland⁸, and soil water savings purportedly explain why rising CO₂ increases maximum foliage cover across the world's arid ecosystems⁹. Thus, soil water savings under eCO₂ could partially or fully offset any constraints of low water supply on the eCO₂ effect. Consistent with this possibility, four experiments of eCO₂ effects on biomass under contrasting water availability showed no dependency on water availability level^{10–14}.

Understanding of the three-way interaction among [CO₂], N and water supply is even more limited, although their individual effects suggest the potential for combined effects. For example, low water supply might be expected to exacerbate N constraints on growth response to eCO₂ by limiting microbial activity or nutrient diffusion in soils. To our knowledge, only one study has evaluated growth responses to [CO₂] and N supply under ambient versus increased precipitation¹⁰: in a California annual grassland, [CO₂] had no effect on biomass over a five-year period, regardless of water or N supply, perhaps owing to the low availability of soil phosphorus¹⁰.

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Table 1 | Weather data for growing season (1 April–30 August) and rainfall removal (1 May–10 August) periods, 2007–2011.

Year	Mean daily maximum temperature (°C), 1 April–30 Aug.	Mean daily maximum temperature (°C), 1 May–10 Aug.	Rain (mm) 1 April–30 Aug.	Rain (mm) 1 May–10 Aug.	Rain (mm) removed 1 May–10 Aug.	Percentage of rain removed 1 April–30 Aug.	Percentage of rain removed 1 May–10 Aug.
2007	24.3	28.2	294	144	54	18.3	37.3
2008	23.3	25.5	437	235	110	25.0	46.6
2009	23.1	24.5	369	185	86	23.3	46.3
2010	24.5	25.4	498	445	217	43.6	48.7
2011	22.2	24.1	706	518	236	33.4	45.6

Interactions between $e\text{CO}_2$ and soil resources

Herein we present evidence of a three-way interaction among water, N, and $[\text{CO}_2]$ in a five-year open-air study of a temperate perennial grassland in Minnesota, USA. We tested the following (non-mutually exclusive) hypotheses.

H₁: Lower availabilities of CO_2 , N and water will each reduce biomass (H_{1a}), indicating multiple resource co-limitation. Strong co-limitation by water and/or N will reduce the biomass fertilization effect of $e\text{CO}_2$ (that is, response to $e\text{CO}_2$ will be smaller under lower soil resource levels, H_{1b}).

H₂: Elevated CO_2 will reduce net N mineralization (H_{2a}), strengthening multiple resource limitations and reducing the $e\text{CO}_2$ effect (that is, reducing the fertilization of biomass due to $e\text{CO}_2$, especially under the low N treatment (H_{2b})).

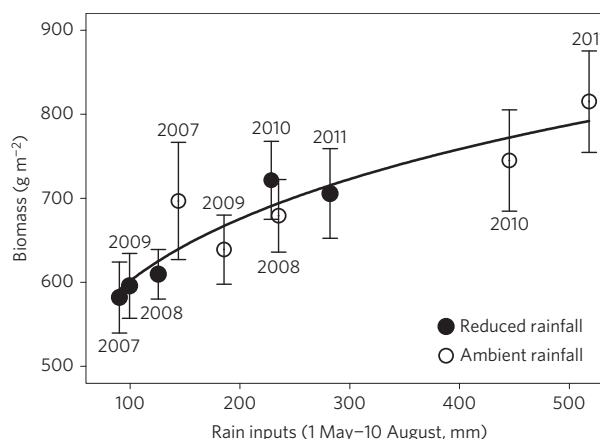
H₃: Elevated CO_2 will increase soil water availability (H_{3a}), weakening multiple resource limitations and increasing the $e\text{CO}_2$ effect size (that is, increasing the fertilization of biomass due to $e\text{CO}_2$ (H_{3b})).

H₄: Vegetation composition response to treatments (that is, increased abundance of C_4 grasses under low rainfall and low N conditions) will reduce ecosystem-scale response to $e\text{CO}_2$ under lower soil resource conditions¹⁹.

Experimental contrasts included ambient versus reduced summer rainfall, ambient versus $e\text{CO}_2$, and ambient versus elevated N inputs in factorial combination. Reduced summer rainfall was achieved by excluding roughly half of rain events during the 1 May–10 August period each year from 2007 to 2011 (Table 1). The rainfall exclusions removed an average of 140 mm of rain per year, which represented 45% of rain on average (over the five years) for that 102-day period and 29% of growing season rain (1 April–30 August; Table 1). The five years varied substantially in ambient rainfall amounts during the 102-day removal period (from 144 to 518 mm). The other experimental treatments were ambient versus enriched soil N availability [$+4 \text{ g N m}^{-2} \text{ yr}^{-1}$]; and ambient and elevated $[\text{CO}_2]$ ($+180 \mu\text{mol mol}^{-1}$, $e\text{CO}_2$; refs 6,21,27,30). The treatments were imposed in an open-air field experiment in factorial combination on 48 2 m \times 2 m perennial grassland mixtures planted in 1997 with random selections of 9 species out of a 16 species pool (containing 4 species of each of 4 functional groups, Supplementary Methods). From 2007 to 2011, C_3 grasses, legumes, C_4 grasses, and forbs averaged 33.7, 31.6, 25.5 and 8.8% of total above-ground biomass across all treatments, respectively. The 48 plots are a sub-experiment within the larger biodiversity, $[\text{CO}_2]$ and N (BioCON) experiment^{6,20,21,27,30}.

Biomass reduced by lower rainfall

Averaged across $e\text{CO}_2$ and N treatments, biomass varied nonlinearly with rainfall inputs, with highest sensitivity at lower inputs (Fig. 1). Response within years (to experimental manipulation) and across years (to natural rainfall variation) fell roughly on a single relationship, indicating that experimental treatments were effective,

**Figure 1 | Biomass tracks rainfall inputs across treatments and years.**

Mean biomass (above-ground + fine root, 0–20 cm) in relation to summer rainfall inputs (1 May–10 August) in ambient (open symbols) and reduced rainfall (filled symbols) treatments ($P < 0.0001$, $R^2 = 0.85$). Each symbol represents the mean (\pm one s.e.m.) of 24 plots (6 per $\text{CO}_2 \times \text{N}$ combination) per rainfall treatment per year. Tests of the slope and intercept of log-biomass versus log-summer rain (to linearize the relationship) showed no difference between the rain treatments. Thus, response to experimental treatments generally matched response to natural variation.

as responses to those treatments generally matched response to natural variation. Within and across years, average soil moisture content was reduced by rainfall manipulation (Supplementary Fig. 2 and Table 1) and tracked rainfall inputs, whether natural or manipulated (Supplementary Fig. 3a). Biomass was positively related to mean soil moisture availability (Supplementary Fig. 3b), indicating that the effects of rainfall variation (natural and experimental) were mediated via soil water availability.

Although strongly related to moisture inputs, the range of biomass across the wide range of rain input (across years and treatments) was moderate, with biomass at the lowest rainfall reduced by 37% compared to that at the highest rainfall (Fig. 1). This response is consistent with previous studies showing modest grassland production response to large inter-annual rainfall variability^{31–33} or to experimental rain manipulation within a growing season³⁴. For example, based on 27 years of data at Konza Prairie (Kansas, USA), above-ground biomass was 22% lower when growing season rainfall was at the 10th percentile compared to the 90th percentile, corresponding with 60% less rainfall³³. Furthermore, wetter summers at our site tend to be cooler (Supplementary Fig. 4) and, as biomass production was significantly suppressed by cooler summer temperatures and enhanced by rainfall under such conditions (Supplementary Discussion), the negative covariance of rainfall and temperature

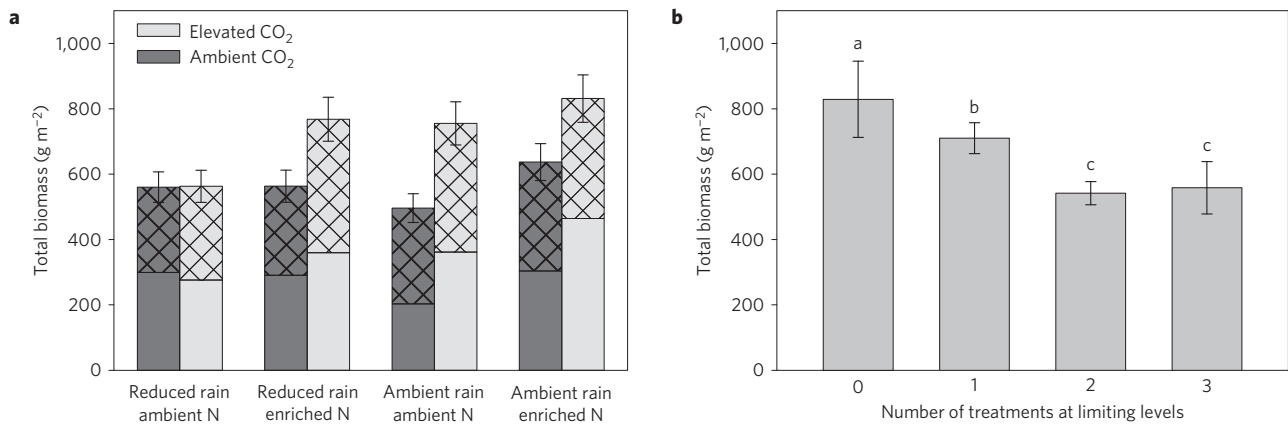


Figure 2 | Biomass in relation to treatments and number of limiting resources. **a**, Total biomass (above-ground plus fine root biomass, 0–20 cm) at all combinations of rainfall, N and [CO₂] treatments. Data are means \pm one SE after averaging across five years to eliminate pseudo-replication that would deflate SE. Root biomass shown in open, and above-ground biomass in cross-hatched, part of each bar. For statistical analyses, biomass was log-transformed to normalize the distribution for repeated-measures analysis of variance. **b**, Total biomass \pm one SE versus number of limiting resources, with each treatment considered limiting when at the lower of the two supply levels.

results in low temperatures dampening a positive response to wetter summers.

Low N and water supply jointly eliminate eCO₂ effect

On average across the five study years, plant biomass was markedly enhanced by eCO₂, but only when availability of either N or rainfall (or both) were at their higher levels (significant three-way interaction among [CO₂], rainfall and N; $P = 0.04$, Fig. 2a and Table 2). Under the higher level of either rainfall or N availability—or both—the eCO₂ treatment increased biomass by at least 33% (Fig. 2a). In contrast, under the lower rainfall and N treatments, the eCO₂ stimulation of biomass was completely eliminated (Fig. 2a), supporting H₁. The proportion of total biomass that was below ground (that is, fine roots) was $\approx 50\%$ on average across all plots and years. There was no significant main effect or interactions of CO₂, N or rainfall manipulations on this proportion ($P > 0.15$), indicating the observed treatment effects on total biomass did not result from shifts in biomass distribution. The magnitude of the main effects of CO₂, N and rainfall were, however, slightly larger for below-ground than above-ground biomass (Fig. 2a).

Biomass varied by year and was generally higher at higher availabilities of CO₂, N and water (Supplementary Fig. 5). Responses to treatments did not differ significantly over time, except that the strength of the stimulation of biomass by eCO₂ varied among years (Table 2, CO₂ \times year interaction, $P = 0.007$). When examined as individual main effects (averaged across years and all levels of other treatment factors), higher levels of [CO₂], N and rainfall all increased biomass—by 29%, 17% and 12%, respectively ($P = 0.04$, $P = 0.004$, and $P = 0.09$; Fig. 2 and Supplementary Fig. 5) and responses to CO₂ and N treatments for the plots and years of this study were roughly similar when compared with prior years (and other plots) of the experiment^{6,27} (see Supplementary Discussion for more information).

The results from 2007 to 2011 were modestly affected by including plot biomass before the onset of rain treatments (2004–2006) in analyses. In an analysis of covariance (Supplementary Table 2), the relative significance of the treatment main effects were $P = 0.06$, $P = 0.006$ and $P = 0.009$ for [eCO₂], N and rainfall respectively, and the three-way interaction between [CO₂], rainfall and N increased in significance (to $P = 0.009$). The analyses of variance and of covariance thus both supported the hypotheses that biomass was limited by multiple resources (H_{1a}) and that, as a result, responses to eCO₂ were suppressed at low soil resource supply (H_{1b}).

Table 2 | Summary of repeated-measures analysis of variance of CO₂, N and rainfall removal effects on total plant biomass (total above-ground plus fine roots from 0 to 20 cm depth).

Source	F ratio	Prob > F
CO ₂	9.62	0.0393
N	9.26	0.0043
Rainfall manipulation	3.03	0.0904
CO ₂ \times N	0.48	0.4914
CO ₂ \times rainfall manipulation	2.97	0.0933
N \times rainfall manipulation	0.02	0.8933
CO ₂ \times N \times rainfall manipulation	4.45	0.0417
Year	4.88	0.0010
CO ₂ \times year	3.67	0.0069
N \times year	0.96	0.4293
CO ₂ \times N \times year	0.58	0.6764
Rainfall manipulation \times year	0.51	0.7320
CO ₂ \times rainfall manipulation \times year	0.71	0.5845
N \times rainfall manipulation \times year	0.98	0.4214
CO ₂ \times N \times rainfall manipulation \times year	0.44	0.7822

Biomass data log₁₀-transformed before analysis. Whole model $R^2 = 0.55$, $P < 0.0001$, $n = 240$.

When cumulative summer (1 May–10 August) rainfall was substituted for ‘year’ in the statistical model, it was significantly ($P < 0.0001$) and positively related to biomass (Supplementary Table 3), consistent with the interpretation that biomass was co-limited by water supply. However, the interaction of CO₂, N and rainfall treatment did not vary with ambient summer rainfall ($P = 0.83$; Supplementary Table 3). Thus, the tendency of plots with lower rainfall and ambient N treatment to produce little additional biomass under elevated CO₂ did not differ in dry versus wet summers. This may have been due in part to wet summers being cool, and in part due to year-to-year differences in spring rainfall (Table 1), and thus in soil moisture reserves going into summer.

Low levels of any two resources limit response to a third

From a multiple resource limitation perspective, each of the three resources (CO₂, N, rainfall) was limiting on average to total biomass at its lower supply rate when the other two resources were at higher supply rates (for example, biomass averaged 890 g m⁻² when

all three resources were at higher supply versus 666–791 g m⁻² when one of the three was at lower supply; Fig. 2b). When any two resources were at their lower levels, biomass was even lower (averages from 528 to 598 g m⁻²; Fig. 2b). However, having all three resources at their lowest levels did not further reduce biomass (Fig. 2b), as the effects of any two of the three resources being in limited supply apparently eliminated response to variation in the third, consistent with multiple limitation theory^{2,7,35}.

This is further supported by mean separate tests (Student's *t*) that showed that none of the three treatments at their higher levels had a significant positive effect when the other two treatments were at their lower levels (mean response, -5%). In contrast, elevated levels of each of the three treatments always resulted in at least a 13% increase (mean + 27%) in biomass, when only one or neither of the other two treatments were at their lowest levels. Evidence of resource co-limitation in this grassland ecosystem also comes from a previous experiment (under ambient CO₂) at this site³³, which demonstrated no effect on biomass of three-month summer rainfall reduction (of 63%) under ambient N, whereas biomass was greater in the higher rainfall treatment with N addition.

The diminished CO₂ fertilization effect at low levels of two soil resources is consistent with H_{1b} and the multiple limitation hypothesis illustrated in Supplementary Fig. 1. When supplies of both N and water were at their higher levels, biomass was higher at both CO₂ levels than when one of those soil resources was at its lower supply level (Fig. 2a and Supplementary Fig. 1). In all cases where one or both soil resources were at their higher levels, the CO₂ effect was large and positive. In contrast, when both soil resources were at their lower supply rates, eCO₂ elicited no increase in biomass (Fig. 2). Although we found evidence for multiple resource limitation constraints on response to eCO₂ here, responses to CO₂, N and rainfall probably vary across the range of real-world possible conditions. For example, regardless of the level of CO₂ or soil fertility, a year of extreme drought might well drive the system to single resource limitation by water, resulting in lower biomass production.

Treatments acted directly and not through indirect paths

In this experiment, biomass was largely influenced by treatments as they interacted directly through multiple resource limitation, not through effects on soil resources or plant communities. The eCO₂ treatment had no significant main effect on mid-summer net N mineralization rates nor did it interact with rainfall or N treatments to influence N mineralization (refuting H_{2a}; Supplementary Tables 4 and 5 and Fig. 6). Although extrapolating from a single month-long assay to the full field season is difficult, the data cannot support the notion that interactive effects of eCO₂, rainfall manipulation and N treatment on N cycling eliminated the eCO₂ effect under the low N and low rainfall treatment combination (refuting H₂).

There was a modest but non-significant positive effect of eCO₂ on mean soil water content that did not differ (no significant interaction) with rainfall or N treatments (Supplementary Table 1). Thus, interactive effects of eCO₂, rainfall manipulation and N treatment on soil water content could not be responsible for the elimination of the eCO₂ fertilization effect under the low N and low rainfall treatment combination.

There was no evidence of compositional shifts favouring C₄ grasses at low N and low water, refuting the hypothesis (H₄) that such changes would reduce eCO₂ responsiveness at low soil resource levels. Species and functional group composition were neither markedly nor consistently different across rainfall, N and CO₂ combinations (Supplementary Table 5 and Figs 7 and 8). Moreover, the prediction that the relative abundance of C₄ grasses would be greatest in the reduced rainfall and ambient N treatments was not supported (Supplementary Table 5 and Figs 7 and 8), as

there were no significant main or interactive effects of treatments on C₄ relative abundance.

Rather, the responses observed in this study are consistent with a multiple resource limitation framework (H₁), where under the most limiting combination of soil resources (ambient N and reduced rainfall), [CO₂] at ambient levels was not limiting to biomass production and thus the biomass accumulation in response to eCO₂ was negligible^{2,7,35}. These constraints on plant response to eCO₂ would probably operate in non-experimental systems as well, as the lower levels of the experimental N and rainfall treatments were within the range of variability of rainfall and N cycling that occurs spatially and temporally for the tallgrass prairie biome. Hence, when conditions of both soil resources are low (in space or time), the CO₂ fertilization response is likely to be low (or even negligible). In contrast, given projected increases in N deposition in many temperate grassland regions³⁶, it is possible that these will fuel sustained CO₂ fertilization effects in this biome³⁷.

More broadly, these results suggest that caution is necessary in assuming strong [CO₂] fertilization effects in a world with widespread soil nutrient and water limitation, or in assuming proportionally increasing [CO₂] fertilization effects as water becomes more limiting (the opposite of the findings herein), at least in mesic ecosystems where co-limitation by other resources precludes plants from responding positively to the modest water savings arising under eCO₂ (refs 8,15,20,22). Strong CO₂ fertilization may be apparent in very arid ecosystems, where primary production is solely limited by lack of water⁹, but not elsewhere (as in our grassland study), where multiple limitations act jointly. Hence, accurate incorporation of potential interactions of changes in CO₂, N and rainfall into earth system models will require the development of a richer understanding of long-term [CO₂] fertilization impacts on a range of systems and under a range of nutrient supplies and soil moisture conditions.

Methods

This experiment used 48 2 m × 2 m plots from the BioCON experiment (Minnesota, USA; refs 6,30), chosen randomly from the 64 plots initially planted with nine species. We used a complete factorial design of 2 summer rainfall × 2 CO₂ × 2 N treatments. Plots were planted in 1997 with nine species randomly selected from a pool of 16 species: C₃ grasses *Agropyron repens*, *Bromus inermis*, *Koeleria cristata*, *Poa pratensis*; C₄ grasses *Andropogon gerardii*, *Bouteloua gracilis*, *Schizachyrium scoparium*, *Sorghastrum nutans*; herbaceous forbs *Achillea millefolium*, *Anemone cylindrica*, *Asclepias tuberosa*, *Solidago rigida*; and N-fixing legumes *Amorpha canescens*, *Lespedeza capitata*, *Lupinus perennis*, *Petalostemum villosum*.

[CO₂] and soil N treatments began in 1998. [CO₂] treatments were applied using free-air enrichment^{6,30}. The enriched nitrogen treatment (4 g N m⁻² yr⁻¹) was implemented as slow-release ammonium nitrate (NH₄NO₃) in equal fractions in early May, June and July. Beginning in 2007, portable (2 m × 2 m) rainout shelters were used to reduce both the number of precipitation events and total rainfall from 1 May to 10 August each year on half the plots (Table 1). Rainfall reduction during these periods averaged 45%. Intercepted rain was channelled using gutters well away from all plots. Shelters were in place <1% of the time (usually at night) and reduce the cumulative integrated photosynthetic photon flux density (May–August) by less than 0.1%.

Peak annual biomass in each plot was sampled annually in early August, with a 10 cm × 100 cm clipping of above-ground biomass and three 5 cm diameter (20 cm deep) cores for extracting root biomass. Above-ground biomass was sorted to species in each plot each year. Roots were separated from soil using gentle spray washing and sorted into fine roots (<1 mm diameter), coarse roots and crowns^{6,30}. Coarse roots and crowns were spatially much more variable than fine roots; for example, any one core could have considerable or few coarse root or crown biomass, regardless of the amount of fine roots. Even with three cores composited per plot, the coefficient of variation was more than twice as high for coarse roots and crowns as for fine roots, and the distributions of coarse root and crown biomass were strongly skewed, with many zero or very low values and a long tail of higher values (neither was the case for fine roots). Thus, as our sampling intensity and methodology was much better suited for measuring fine roots than coarse roots or crowns, and as fine roots are more involved in water and nutrient uptake, we report data only for fine root biomass in this paper. Soil net N mineralization rates were measured *in situ* each year in each plot by using a

semi-open core, during one-month incubations⁶ beginning in late June. Net N mineralization is the net transformation of N from organic to inorganic forms and is considered to represent the availability of N to plants in systems such as this, where plants obtain the vast majority of N from inorganic forms. Net N mineralization data from 2008 were compromised, probably by contamination, and were not used. Biomass and net N mineralization data were log₁₀-transformed before statistical analysis to normalize the distribution.

Soil moisture was measured²⁰ at four 17 cm-depth increments (0–17, 22–39, 42–59, 83–100 cm) using a Trime FM3 Time Domain Reflectometry system, version P3 with T3 tube-access probe (IMKO Micromodultechnik). The instrument's volumetric soil water content (VSWC) output was calibrated to soil-specific VSWC (cm³ cm⁻³ or %) using VSWC measurements calculated from coincident gravimetric soil moisture and bulk density measurements²⁰. Soil moisture was measured in all 48 plots at approximately two-week intervals across each growing season. Herein we report on data from the 0 to 17 cm soil depth from the measurements (number of samplings ranged from 7 to 11 among years) made during the rainfall removal period (1 May–10 August), because these best match the soil depths of fine root sampling (0–20 cm) and because the majority (60%) of fine root biomass is found in the top 20 cm of the soil (data not shown). Effects of rain removal treatments were similar across soil depths, although the proportional reduction in soil moisture decreased with depth.

The statistical test for treatment effects and interactions was a repeated-measures analysis of variance⁶. All analyses were performed using JMP PRO 10.0.0 (SAS Institute).

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Author contributions

This experiment was designed and implemented by all three authors; P.B.R. did the analyses and wrote the initial draft; all three authors contributed to editing and revising the manuscript.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Data reported in this paper are stored as part of the Cedar Creek Long-Term Ecological Research project www.cedarcreek.umn.edu/research/data. Correspondence and requests for materials should be addressed to P.B.R.

Competing financial interests

The authors declare no competing financial interests.