Received: 6 December 2022 | Accepted: 14 April 2023

DOI: 10.1111/1365-2745.14111

Journal of Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Soil nutrients cause threefold increase in pathogen and herbivore impacts on grassland plant biomass

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Funding information
US National Science Foundation Long-Term Ecological Research Program (LTER), Grant/Award Number: DEB-1831944 and DEB-1234162; UMN EEB; Cedar Creek

Handling Editor: Anne Kempel

Abstract

1. A combination of theory and experiments predicts that increasing soil nutrients will modify herbivore and microbial impacts on ecosystem carbon cycling.
2. However, few studies of herbivores and soil nutrients have measured both ecosystem carbon fluxes and carbon pools. Even more rare are studies manipulating microbes and nutrients that look at ecosystem carbon cycling responses.
3. We added nutrients to a long-term, experiment manipulating foliar fungi, soil fungi, mammalian herbivores and arthropods in a low fertility grassland. We measured gross primary production (GPP), ecosystem respiration (ER), net ecosystem exchange (NEE) and plant biomass throughout the growing season to determine how nutrients modify consumer impacts on ecosystem carbon cycling.
4. Nutrient addition increased above-ground biomass and GPP, but not ER, resulting in an increase in ecosystem carbon uptake rate. Reducing foliar fungi and arthropods increased plant biomass. Nutrients amplified consumer effects on plant biomass, such that arthropods and foliar fungi had a threefold larger impact on above-ground biomass in fertilized plots.
5. *Synthesis*. Our work demonstrates that throughout the growing season soil resources modify carbon uptake rates as well as animal and fungal impacts on plant biomass production. Taken together, ongoing nutrient pollution may increase ecosystem carbon uptake and drive fungi and herbivores to have larger impacts on plant biomass production.

KEYWORDS
consumer-nutrient interaction, ecosystem C fluxes, fertilization, fungi, insects, mammalian herbivores

1 | INTRODUCTION

Grasslands play a critical role in the global carbon (C) cycle, covering a quarter of the Earth's land surface and contributing up to 20% to the total terrestrial C sink (Xia et al., 2014). As with most ecosystems, C accumulation in grasslands reflects the balance and seasonal variation of C inputs via photosynthesis (gross primary production, GPP) and C emissions from respiration by microbes, plants and animals (ecosystem respiration, ER). Observational studies and field experiments manipulating food webs have shown that plant consumers ranging from microbes to mammals can regulate grassland GPP and ER (Kohli et al., 2021; Risch & Frank, 2006; Yan et al., 2017). GPP and ER also depend on nutrients such as nitrogen and phosphorus, which are increasing in many terrestrial systems due to human activities (Ackerman et al., 2019; Galloway et al., 2004; Mackenzie et al., 2002; Wilcots et al., 2022). Notably, consumer and nutrient impacts on GPP, ER and vegetation C pools (plant biomass) do not occur in isolation. For example, theory and data show that higher nutrient inputs often increase herbivore and disease impacts on plant biomass (Borer et al., 2021; Ebeling et al., 2021; Oksanen et al., 1981; Sandin & Ngai, 2008). Increased nutrient supply also can shift plant composition towards fast-growing plant species that are more palatable to herbivores or susceptible to microbial pathogens (Cappelli et al., 2020, 2022; Heckman et al., 2019; Lind et al., 2013). With recognition that herbivores, microbes and nutrients all play important roles in grassland ecosystems, an active area of investigation now focuses on quantifying the interactive effects of a broad range of consumers (mammals, arthropods and fungi) and nutrients on key aspects of ecosystem C cycling.

In a food web, primary consumers (defined here as heterotrophs including mammals, insects, pathogens or mutualists that rely directly on living plants for energy) can influence C cycling via their impacts on plant biomass, tissue chemistry, leaf-level photosynthesis, respiration and soil respiration (Bilgin et al., 2010; Borer et al., 2015; Mitchell, 2003; van der Heijden et al., 2008). While many studies have examined the effects of mammalian and insect herbivores on plant biomass (Borer et al., 2020; Chase et al., 2000; Coupe & Cahill, 2003; Sandin & Ngai, 2008), few have directly measured herbivore effects on both GPP and ER at the same time (Morgan et al., 2016; Yan et al., 2017) and herbivore impacts on ecosystem C fluxes can be hard to detect under heterogenous climate regimes and across large landscapes (Risch & Frank, 2006). Studies of fungal effects on GPP and ER are almost nonexistent, but in a recent study Kohli et al. (2021) found that foliar fungi reduced GPP and ER more than insect herbivores and fungal effects on C flux were mediated by changes in plant composition. As primary consumers that rely on plant biomass, fungi and herbivores may have similar roles in controlling GPP and ER; however they vary greatly in energy use efficiency and growth rates (Lafferty & Kuris, 2002) that may result in distinct impacts of herbivores versus fungi on GPP or ER via their consumption of plant biomass. While past studies have collectively quantified the role of herbivores and fungi in influencing GPP, ER or plant biomass (Allan et al., 2010; Kohli et al., 2019, 2021; Seabloom et al., 2017), they have not accounted for the role of nutrients in modifying consumer effects on C cycling.

In terrestrial ecosystems, addition of biologically limiting nutrients such as nitrogen, phosphorus and potassium often increases above-ground plant biomass (Fay et al., 2015). Ecological theory predicts that such fertilization effects may regulate how consumers influence ecosystem C cycling. For instance, simple food-chain models are characterized by strong top-down control of plant biomass by herbivores (Borer et al., 2020; Chase et al., 2000; Oksanen et al., 1981; Sandin & Ngai, 2008), predicting that herbivores increase their offtake of plant biomass as ecosystem productivity increases (e.g. with added nutrients). In cases when herbivores do not consume the additional plant growth (e.g. allocation or turnover favouring plant defence), an alternative prediction is that even in the presence of consumers, plant biomass will continue to increase with increasing nutrient supply (resource-controlled models, Borer et al., 2020; DeAngelis & Huston, 1993). For plant–microbe interactions, increased nutrient supply can concurrently modify host physiology, pathogen physiology, plant population densities and plant community composition, which all potentially can shape the dynamics of disease, determining rates of ecosystem C and nutrient cycling (Borer et al., 2022; Cappelli et al., 2020; Ebeling et al., 2021; Mitchell et al., 2003; Strengbom & Reich, 2006). In general, theoretical approaches describing herbivore mediation of ecosystem C and nutrient cycling can be extended by analogy to make predictions about the role of other consumer groups, including microbes (Atkinson et al., 2017; Borer et al., 2022; Sterner & Elser, 2002). While theory and individual experiments suggest that nutrients may modify herbivore and microbial impacts on ecosystem C cycling in similar ways, no experiments have measured directly how varying nutrient supply affects consumer impacts on grassland GPP, ER and plant biomass across a broad range of consumer types.

Here we quantify the interactive effects of nutrient supply and three very different consumer types on ecosystem C fluxes and plant biomass in a grassland ecosystem. We use a long-term experiment that reduced foliar fungi, soil fungi, arthropods and large mammalian herbivores via pesticides and fencing and increased elemental nutrient supply via fertilization at rates that overcame plant nutrient limitation at this grassland site (Fay et al., 2015). Using these combined treatments, we determine the independent and joint effects of nutrients and each consumer group on grassland carbon cycling. Given the within-season variation of ecosystem C fluxes and plant biomass production (Xiao et al., 2008; Zaret et al., 2022), we also look at a different time scales and test for within-season variation of consumer and nutrient controls on grassland C cycling using monthly Normalized Difference Vegetation Index (NDVI, a proxy for plant biomass production) and C flux measurements from the same experiment. Using these data, we tested the following hypotheses:

H1. *Consumer reductions and nutrient addition independently lead to increases in GPP, ER and plant biomass.* Past studies of consumer reduction and fertilization effects on plant biomass and composition (Borer et al., 2020; Fay et al., 2015; Kohli et al., 2021; Seabloom et al., 2017; Wilcots et al., 2021) suggest that these treatments will have stronger effects on GPP than on plant or soil respiration, resulting in an increased rate of net C uptake (more negative rate of net ecosystem exchange, NEE).

H2. *Environmental nutrient supply determines the impacts of reducing different consumer groups on carbon fluxes and plant biomass.* A range of studies suggests that fertilization can amplify both herbivore and pathogen impacts on plants (Borer et al., 2020; Ebeling et al., 2021; Heckman et al., 2016; Mitchell et al., 2003; Sandin & Ngai, 2008), which may result in increased consumer impacts on GPP, ER and plant biomass under higher nutrient supply.

H3. *The effects of consumer reductions and nutrient addition on carbon flux and biomass increase over the growing season*. Nutrient impacts should increase later in the growing season, as finite resources become depleted in unfertilized plots (Klaus et al., 2016). Previous work in our study system suggests that consumer impacts on plant biomass can also be greatest in the later parts of the growing season as more plant C becomes available for offtake by herbivores and pathogens (Zaret et al., 2022). Taken together, these results suggest that nutrient and consumer impacts on ecosystem C flux also will be greatest later in the growing season.

2 | MATERIALS AND METHODS

2.1 | Study system

The work described here was conducted at Cedar Creek Ecosystem Science Reserve, located in East Bethel, Minnesota, USA. This work was approved by field station leadership after a review process and was carried out following Cedar Creek's conduct, sampling and land management policies. The site of the experiment is an old field, abandoned from agriculture in 1956 (Seabloom et al., 2018). The sandy soils are very nutrient limited with a nitrogen content of 0.05% resulting in a low mean above-ground biomass of $138\,g\,m^{-2}$ (Fay et al., 2015). Average annual precipitation of the site is 750 mm and average annual temperature is 6°C.

In 2008, a consumer manipulation experiment was established (Seabloom et al., 2018) with six food web manipulation treatments randomly assigned to $3\,m \times 3\,m$ plots, replicated in eight blocks ($N=48$). The treatments were as followed: Control, Soil Fungicide, Foliar Fungicide, Insecticide, Fenced and All Removal (fencing plus all the pesticide treatments). Pesticides were applied throughout the growing season from mid-April to the end of September each year. The treatments are maintained as follows: Foliar Fungicide as biweekly application of Quilt (Syngenta Crop Protection, Inc.), Soil Fungicide as monthly applications of Ridomil Gold SL (Syngenta Crop Protection, Inc.) and Insecticide as biweekly applications of Marathon II (OHP, Inc.). Two times each growing season, Malathion was applied instead of Marathon II to prevent development of insecticide resistance in the arthropod community. For the Fenced and All Removal plots, a 2 m wire fence surrounded the plot (mesh size $5\,cm \times 10\,cm$) that excluded large herbivores. In a given sampling year, Foliar Fungicide and Insecticide treatments received $2\,L\,m^{-2}$ of pesticides per an experimental plot (0.06% more water relative to annual rainfall) while Soil Fungicide received a larger amount of $12\,L\,m^{-2}$ of pesticide (0.36% more water relative to annual rainfall) due to the higher dilution required for application.

Results from a greenhouse experiment showed that none of the pesticides have effects on plant growth in the absence of consumers (Seabloom et al., 2017), but they do reduce foliar pathogen and insect herbivory incidence in the field (Borer et al., 2015). The fungicides and insecticides used here can reduce both plant enemies (herbivores or pathogens) and mutualists (pollinators or mycorrhizae), and so the measured effect represents the balance of both positive and negative effects. Thus, the measured effect is a conservative estimate of how arthropod herbivores or fungal pathogens impact ecosystem functioning (Coupe & Cahill, 2003; Seabloom et al., 2017).

At the start of the 2019 growing season, a nutrient addition treatment was added within each experimental plot of the consumer manipulation experiment. Each plot was divided into two subplots ($N=96$) and the plot on the left (facing more West) was assigned to a nutrient treatment (nitrogen, phosphorous, potassium and micronutrients). To avoid nutrient leakage plots were trenched to a depth of 30 cm and sampling of all plant and ecosystem C measurements occurred at least 0.25 m away from the trenched area. Nutrient addition rates and sources were: $10\,g\,N\,m^{-2}\,yea^{-1}$ as timed-release urea $[(NH_2)_2CO]$, $10\,g\,P\,m^{-2}\,year^{-1}$ as triple-super phosphate $[Ca(H_2PO_4)_2]$, $10\,g\,K\,m^{-2}\,year^{-1}$ as potassium sulphate $[K_2SO_4]$ and $100\,g\,m^{-2}\,year^{-1}$ of a micronutrient mix of Ca (6%), Fe (17%), S (12%), Mg (3%), Mn (2.5%), Cu (1%), Zn (1%), B (0.1%) and Mo (0.05%). Nutrients were applied once a year every spring except the micronutrient mix which was only applied in the first year to avoid toxicity.

2.2 | Ecosystem carbon flux

Once a month, from May to October in 2020, we used a LI-COR 850 infrared gas analyser (IRGA, LI-COR, Lincoln, Nebraska) that can be operated in the field via a ‘Flux Puppy’ setup (Carbone et al., 2019) to measure CO_2 concentration within a $1\,m^3$ cube chamber that was made from a polyvinyl chloride (PVC) frame and transparent 2-mil plastic sheeting. At the time of sampling, the chamber was moved and placed over individual experimental plots and a total of 60 plots were sampled (five blocks) over the course of 3–5 days in each month of sampling. The chamber was sealed to the ground with heavy steel chains which held the plastic sheeting to the ground. Fans were placed inside the chamber to mix the air as measurements were taken, however, there were no other systems used to control temperature or humidity inside the chamber. Fluxes were measured for 2 minutes at a sampling frequency of 0.5 Hz conducted in full light (clear skies with no cloud cover) between the hours of 10:00 and 14:00. Photosynthetically active radiation (PAR) also was measured during the time of flux measurement using a MQ-100 Apogee PAR metre (Apogee Instruments) that was mounted within the cube on top of the IRGA. Full-light measurements were recorded at a PAR ranging between 800 and $2000\,\mu mol\,m^{-2}\,s^{-1}$ dependent on the light intensity on a given day of sampling. Each full-light measurement was used to calculate net ecosystem exchange (NEE, rate of gas exchange between the atmosphere, plants and soil) using the following relationship:

$$\mathrm{NEE} = \left(p^{*}\, V^{*}\, (dC/dt)/A\right), \tag{1}$$

in which p is the air density (mol air m^{-3}) calculated as P/RT where P is the atmospheric pressure inside the chamber, R is the ideal gas constant (8.314 J mol^{-1} air K^{-1}) and T is the mean temperature in Kelvin. V is the chamber volume (1 m^3), dC/dt is the slope of CO_2 concentration against time (μmol CO_2 s^{-1}) and A is the surface area of the ground being sampled (1 m^2). In this study we define NEE as the flux from the atmosphere to the system, where negative NEE values indicate net capture of C by plots and positive NEE values indicate net C release into the atmosphere.

To standardize NEE values across space and time at a fixed light level, and to partition NEE fluxes into gross primary production (GPP), and ecosystem respiration (ER), we placed consecutive layers of shade cloth over the chamber to reduce chamber light levels and create a NEE light response curve (Lasslop et al., 2010). We measured CO_2 fluxes in consecutive 2-min intervals with a single shade cloth (incoming light reduced on average by 64%), two shade cloths (incoming light reduced on average by 85%) and finally an opaque tarp (0% light penetrating chamber, i.e. no photosynthesis) which measured only ER. Using the four light measurements (full light, one shade cloth, two shade cloths and opaque tarp) we fit both linear and hyperbolic functions using field measured PAR values to predict NEE at a standardized PAR of 800 (NEE_{800}) following methods from previous experimental grassland C flux studies (Kohli et al., 2021; Wilcots et al., 2022). Specifically for the hyperbolic function, NEE_{800} was predicted using the following relationship:

$$\mathrm{NEE}_{800} = \mathrm{Amax}^{*}\ \mathrm{PAR}/(k + \mathrm{PAR}), \tag{2}$$

where Amax is the maximum rate of CO_2, k is the light level at half saturation and PAR is equal to 800. The R^2 values for the hyperbolic function were higher than those from the linear fits, so we used the hyperbolic functions in our final calculation of NEE_{800}. Finally, we calculated GPP using NEE_{800} and field measurements of ER (flux in dark under the opaque tarp) with the following relationship:

$$\mathrm{GPP} = \mathrm{ER} - \mathrm{NEE}_{800}. \tag{3}$$

2.3 | Plant biomass

In early August of 2020, we collected above- and below-ground biomass in each subplot (with and without added nutrients) of the consumer manipulation experiment. Above-ground biomass was harvested from two 1 m × 0.1 m strips in each plot using a handheld clipper. The above-ground biomass was dried to a constant mass and weighed to the nearest 0.001 g. Root biomass was collected from four cores (two cores from each strip location where the above-ground mass was harvested) using a 5 cm diameter by 30 cm depth soil corer. Soil was washed from the samples using water, leaving behind only root mass which was then dried to a constant mass and weighed to the nearest 0.001 g. Above- and below-ground mass were converted to units of $\mathrm{g}^{-1}\,\mathrm{m}^{-2}$.

2.4 | Normalized difference vegetation index (NDVI)

Normalized difference vegetation index (NDVI) was used as a non-destructive index of above-ground plant biomass, following methods from other studies at this site (Zaret et al., 2022). Every 2 weeks, from May to August in 2020, we used a MSR5 multispectral radiometer (Cropscan, Inc.) to measure reflected radiation (reflectance) in all experimental plots of the consumer manipulation experiment. Measurements were taken in a 1.5 m^2 area above the vegetation canopy in the centre of each plot. NDVI was derived from the red: near-infrared reflectance ratio (Running, 1990) using 830 mm (near-infrared) and 660 mm (red) reflectance readings. NDVI values were calculated for each plot on each day of measurement resulting in 768 NDVI estimates across the entire study.

2.5 | Plant community composition

To determine potential drivers of change in C flux, we looked at plant composition response to consumer reduction and fertilization treatments. We specifically used the abundance of plant functional groups (C3 grasses, C4 grasses, legumes and forbs) in the experimental plant communities. In early August 2020, species per cent cover data were visually estimated where total cover was constrained to 100% in a 1 m × 1 m quadrat in the centre of all subplots. Species cover data were aggregated by plant functional type yielding a proportion of each functional group abundance within each plant community of the experiment.

2.6 | Statistical analysis

All statistical analyses were done in R Version 4.0.0 (R Core Team, 2020). To determine the effects of consumer reduction, nutrient addition and their interactions on ecosystem carbon fluxes we constructed mixed effects models (NLME package, Pinheiro et al., 2014) that included month of sampling (to characterize carbon flux dynamics across the season), consumer reduction treatment and nutrient addition as fixed effects, while experimental block, the 3 m × 3 m consumer reduction plots, and 1.5 m × 3 m nutrient addition plots were included as random effects. To test for effects of treatments on above-ground biomass, below-ground biomass and the relative abundance of plant functional groups, we constructed linear mixed effect models using the same fixed and random effects as the ecosystem carbon flux models (excluding Month of sampling, since biomass/cover sampling was done at peak season, in August). For our NDVI analysis, we constructed the same mixed effects models as for the ecosystem carbon flux models; however, Julian day of year of sampling was used instead of month of sampling to characterize NDVI dynamics across the season. To ensure there was no temporal autocorrelation, we included sampling month (for NEE, GPP and ER models) and sampling day (NDVI model) as a correlation term in the mixed effect models, but this did not improve models (diagnosed with a likelihood ratio test). All model specifications are included in each table of results (Tables S1–S6).

For each mixed effect model, we ran an ANOVA to determine if consumer reduction or nutrient addition impacted the response variable and to determine interactions among the fixed terms (interactions between consumer reduction and nutrient addition as well as interactions of consumer reduction or nutrient addition with time of sampling). The LSMEANS R package was then used to look at pairwise differences between individual consumer reduction treatments and control (Tukey's honest significant difference test) to determine which consumer reduction treatment(s) accounted for the overall effect of consumer reduction in the ANOVA models. We log transformed NEE, above- and below-ground biomass to meet assumptions of normality. If consumers had a significant effect on these log transformed responses but there was no significant interaction with nutrient addition, this indicated a constant proportional effect by consumers across the nutrient treatment (Borer et al., 2020) and reflected an increased effect in back transformed units (e.g. grams of biomass). We also looked at correlations between ecosystem carbon fluxes in August and plant biomass, as well as correlations between above-ground biomass and NDVI.

3 | RESULTS

3.1 | Effects of nutrient addition on ecosystem carbon fluxes and plant biomass

Nutrient addition increased net C uptake (reduced NEE) on average by 45% (Table S1, Figure 1). This effect was driven by increased GPP (23% increase, Table S2), but no overall effect of nutrient addition on ER (Table S3). Nutrient addition increased above-ground biomass by 76% on average (Table S4, Figure 2). In contrast, below-ground biomass did not change with nutrient addition (Table S5), which reduced the root to shoot ratio by 12% ($F=19.44$, $p=0.0001$).

3.2 | Effects of consumer reduction on ecosystem carbon fluxes and plant biomass

For C flux measurements taken across the growing season, consumer reduction did not impact NEE (Table S1, Figure 1), GPP (Table S2) or ER (Table S3). However, multiple consumer reduction treatments increased above-ground biomass (Table S4, Figure 2). On average the All Removal treatment increased above-ground biomass by 63% ($t=-3.94$, $p=0.01$), the Insecticide treatment by 45% ($t=-3.225$, $p=0.03$) and the Foliar Fungicide by 25% ($t=-2.919$, $p=0.04$) relative to Control plots. The Soil Fungicide and Fenced treatments did not increase above-ground biomass ($t=-0.401$, $p=0.48$, $t=-0.327$, $p=0.30$ respectively). Consumer reduction also increased below-ground biomass (Table S5, Figure 2). Specifically, the Insecticide treatment increased below-ground biomass by 50% ($t=-3.266$,

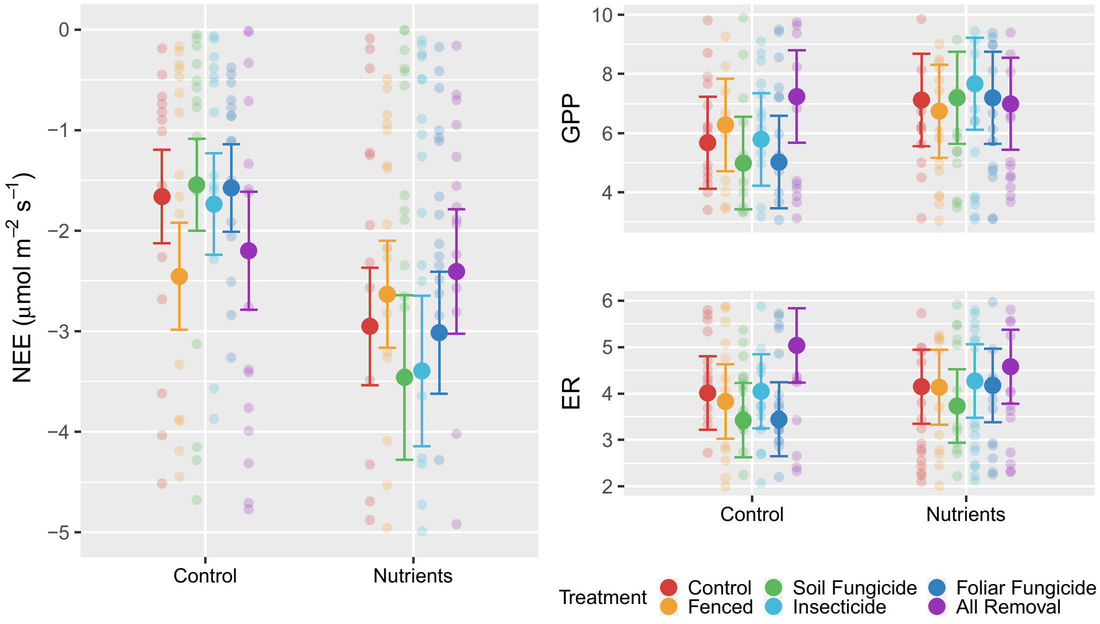

FIGURE 1 Ecosystem carbon flux response to experimental reduction of consumers and nutrient addition. Light points show data and darker points and bars show mean and standard error. Units of GPP and ER are the same as NEE. NEE and GPP values are based on modelling at standardized PAR of 800 and ER values are from field measures (PAR 0), see Section 2 for more detail.

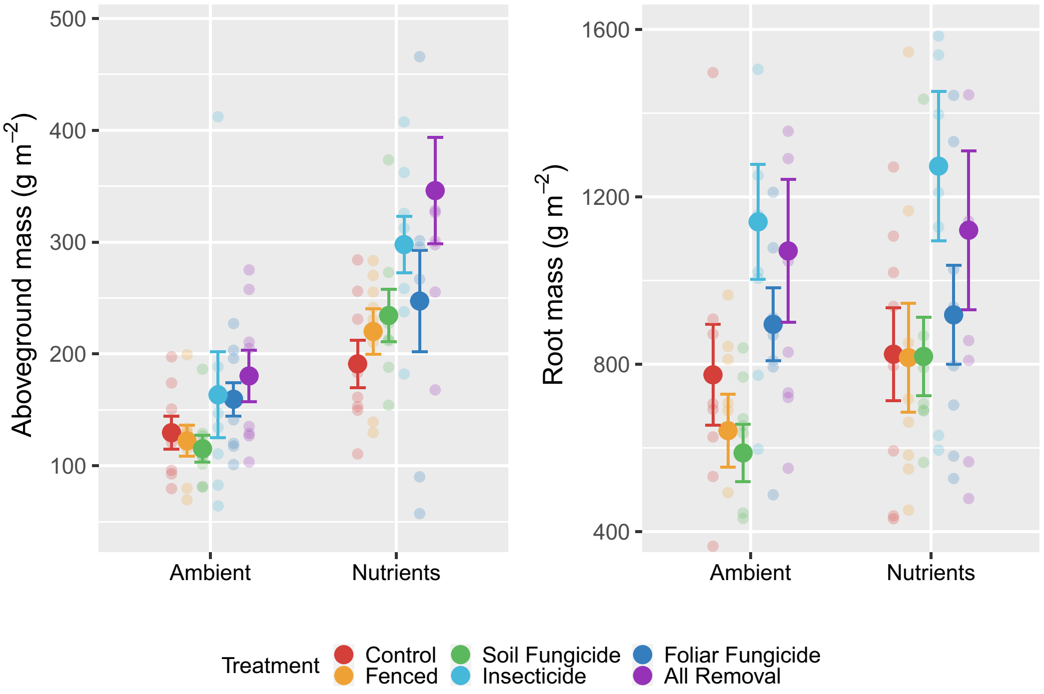

FIGURE 2 Foliar fungicide, Insecticide, All Removal and nutrient addition increased above-ground biomass, Insecticide increased below-ground biomass (root mass). Light points show data and darker points and bars show mean and standard error.

$p = 0.01$) relative to control, but no other consumer group had any significant effects on below-ground biomass ($p > 0.05$).

3.3 | Interactions between consumer reduction and nutrient addition on C fluxes and plant biomass

Nutrient addition did not significantly modify the effect of consumer reduction treatments on NEE (Table S1, Figure 1), GPP (Table S2) or ER (Table S3). In contrast, reducing consumers caused a greater impact on plant biomass in fertilized plots compared to ambient nutrients, reflecting a constant proportional effect of consumers on above- and below-ground biomass (nonsignificant interaction in log transformed biomass models, Tables S4 and S5, Figure 2). The All Removal treatment increased above-ground biomass by 155 g m^{-2} in fertilized plots compared to an increase of 50 g m^{-2} under ambient nutrient conditions, a 310% increase in absolute magnitude of the All Removal treatment on above-ground biomass in fertilized plots (Figure 2). Below-ground, the Insecticide treatment increased root biomass by 450 g m^{-2} in fertilized plots compared to an increase of 365 g m^{-2} under ambient conditions, reflecting a 23% increase in absolute magnitude of the Insecticide treatment on below-ground biomass in fertilized plots (Figure 2).

3.4 | Relationship between plant composition and C fluxes

Nutrient addition increased the abundance of C3 grasses and decreased C4 grasses (Table S6, Figure S1). Consumer reduction had no impact on any plant functional group abundances, and there were also no significant interactions between nutrient addition and consumer reduction on the proportion of plant functional groups (Table S6). C4 grass abundance was negatively related to GPP ($t = -2.575$, $p = 0.0133$, $R^2 = 0.13$, Figure S2), while there was no relationship between C4 grasses and ER ($t = -0.818$, $p = 0.417$).

3.5 | Seasonal variation in nutrient and consumer impacts on C fluxes and plant biomass

As the growing season progressed, nutrient effects on GPP increased (significant interaction between nutrient addition and month of sampling, Table S2, Figure 3), whereas the effect of nutrient addition on NEE and ER remained constant across the season. Despite no overall effect of consumer reduction on ER, consumer reduction did interact with month of sampling (Table S3, Figure 4). Specifically, ER increased in the All Removal treatment late in the growing season relative to Control plots ($t = -3.141$, $p = 0.004$) but none of the other consumer reduction treatments interacted with month of sampling ($p > 0.05$).

NDVI measurements correlated significantly with above-ground biomass ($R^2 = 0.52$, $p < 0.001$, Figure S3). Nutrient addition increased NDVI across the season but had the greatest impacts on NDVI later in the growing season (Table S7, Figure 5). Consumers impacts on NDVI increased over the course of the growing season (Table S7, Figure 5), with the All Removal treatment increasing NDVI later in the growing season ($t = -4.020$, $p = 0.003$) but none of the other consumer reduction treatments interacted with time of sampling ($p > 0.05$).

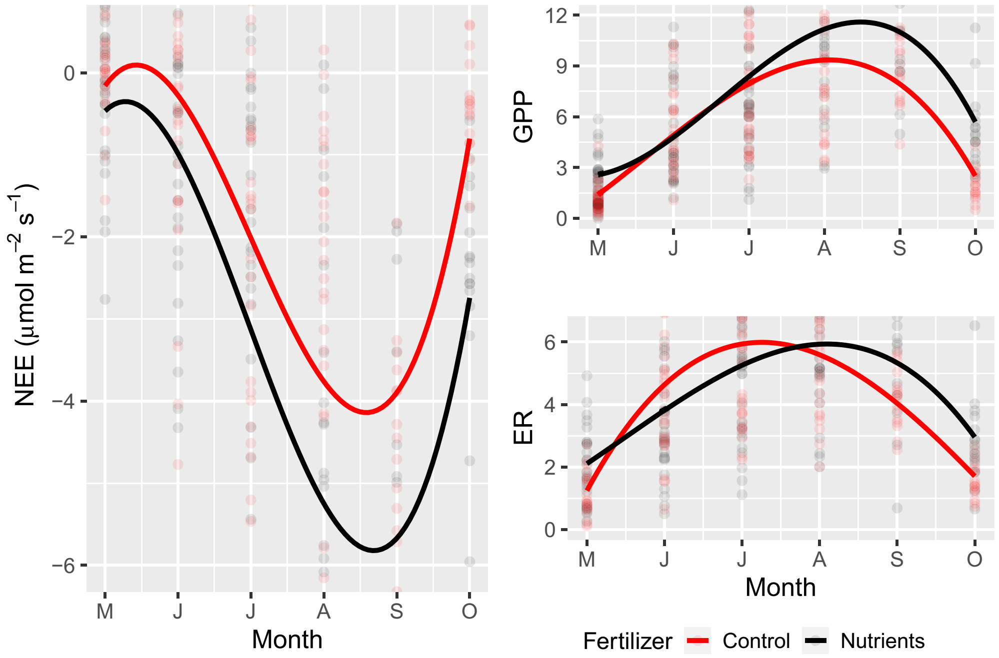

FIGURE 3 Seasonal effects of nutrient addition on ecosystem carbon flux. Nutrients impact GPP in the later months of the growing season (NEE and ER interactions with month of sampling were nonsignificant). GPP and ER are same units of measurement as NEE. NEE and GPP values are based on modelling at standardized PAR of 800 and ER values are from field measures (PAR 0), see Section 2 for more detail. Data are pooled across all consumer reduction treatment plots.

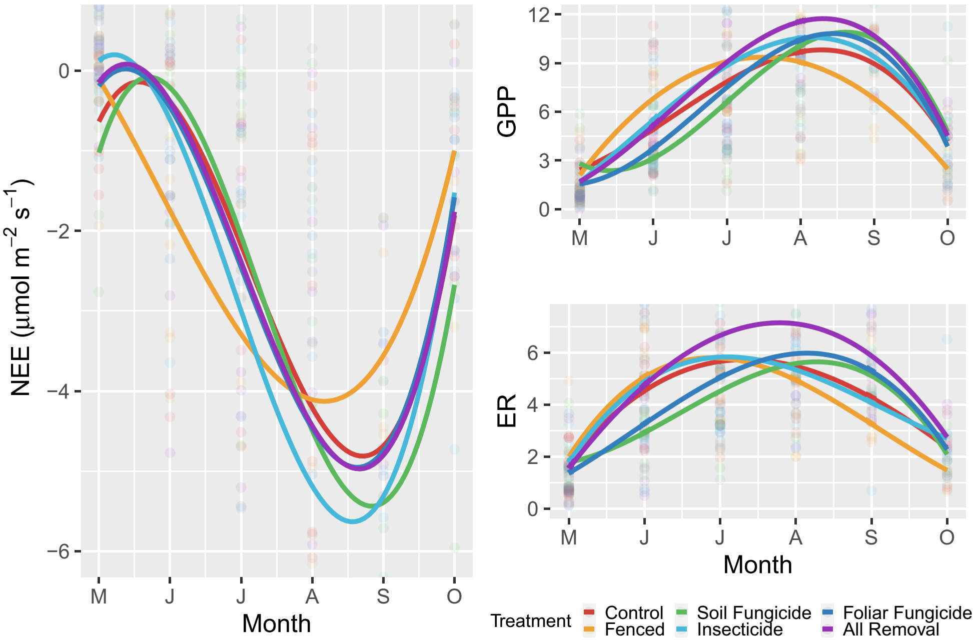

FIGURE 4 Seasonal effects of consumer reduction on ecosystem carbon flux. All Removal treatment impacts ER in later months of the growing season (NEE and GPP interactions with month of sampling were nonsignificant). GPP and ER are same units of measurement as NEE. NEE and GPP values are based on modelling at standardized PAR of 800 and ER values are from field measures (PAR 0), see Methods for more detail. Data are pooled across nutrient addition treatment plots.

4 | DISCUSSION

We found strong impacts of nutrient addition on ecosystem C fluxes, while responses to consumer reduction treatments were weaker and strongest late in the growing season (partial support for H1). Specifically, the Foliar Fungicide and Insecticide treatments increased plant biomass, but none of the consumer reduction treatments consistently impacted grassland GPP or ER. In contrast,

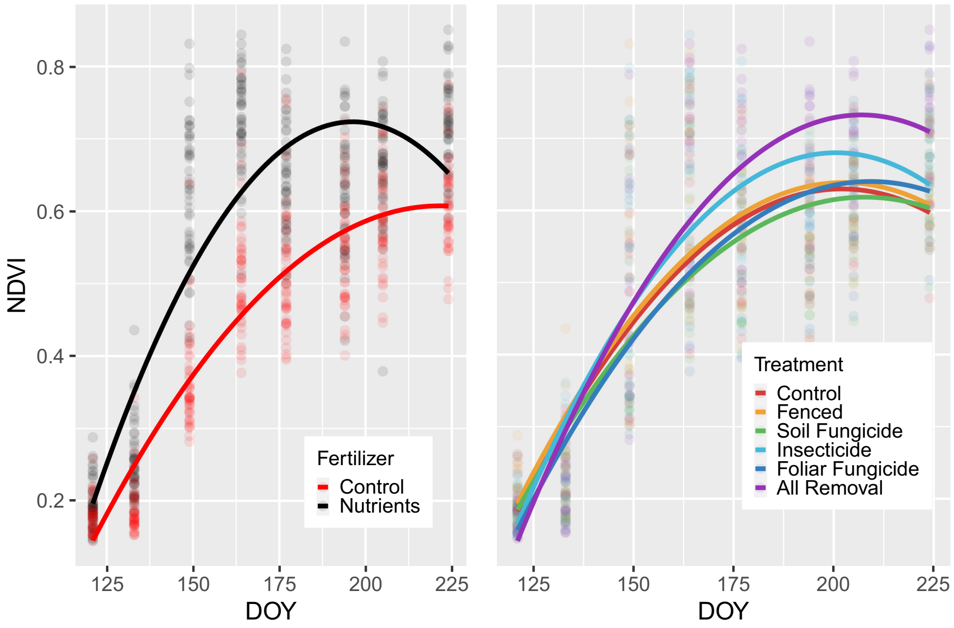

FIGURE 5 Effects of nutrient addition and consumer reduction on NDVI (measure of above-ground biomass) across the growing season. NDVI is the Normalized Difference Vegetation Index. Both nutrient and consumer effects (All Removal treatment only) were greater late in the growing season. DOY = Julian day of year, with '1' being 1 January, etc. On left, data are pooled across consumer reduction treatments and on right, data are pooled across nutrient addition plots.

nutrient addition increased GPP and net C uptake (more negative NEE), resulting in greater plant biomass at the end of the season. Nutrient addition led to a threefold greater effect of herbivores and fungi on above-ground biomass (155 vs. 50 g m^{-2}), which reflects a constant proportional impact of consumers on plants. However, consumer impacts on ecosystem C fluxes did not depend on nutrient supply (no support for H2 for C fluxes). Both nutrient and consumer impacts on plant biomass and ecosystem C fluxes were strongest towards the later parts of the growing season (in support of H3). Taken together, results suggest that the absolute impacts of microbes and herbivores on plant biomass, but not ecosystem C fluxes, will be modified as grasslands experience increased nutrient inputs.

A large body of work in grasslands has shown that mammalian herbivores, insect herbivores and microbes such as fungal pathogens can mediate plant community composition and biomass production (Allan et al., 2010; Bever et al., 2015; Borer et al., 2014, 2020; Coupe & Cahill, 2003; Maron et al., 2011; Mitchell, 2003; Seabloom et al., 2017), and the impact of consumers on plant composition and function is likely to alter ecosystem C fluxes (Kohli et al., 2021; Morgan et al., 2016; Risch & Frank, 2006; Yan et al., 2017). For example, previous work by Kohli et al. (2021) found strong effects of foliar fungi on GPP and ER that were mediated by fungicide induced changes to the abundance of C4 grasses. We similarly found that C4 grass abundance is related to GPP in our system, however, none of the consumer reduction treatments altered plant composition in this study year (but see Seabloom et al., 2018). Reduction of plant consumers is predicted to shift plant composition towards species that are investing less in defence against consumers and investing more in high growth rates (Heckman et al., 2019; Lind et al., 2013; Seabloom et al., 2018) and such compositional changes would likely increase rates of C flux (e.g. an increased abundance of faster growing and poorly defended species may drive higher rates of GPP). Contrasting results between our study and previous studies of consumer impacts on ecosystem C cycling point to a potential role of plant defence and plant composition as mediators of C flux response to consumer groups, however, this remains a promising knowledge gap to fill.

While we did not detect effects on C fluxes (i.e. GPP and ER), reduction of foliar fungi or arthropods increased above-ground plant biomass. For the Insecticide treatment, one explanation for this apparent disconnect between fluxes and biomass response may be the large effects on below-ground biomass; when arthropods were removed, plants invested more in below-ground biomass, but this would not necessarily impact rates of GPP which are dependent on rates of carbon fixation by above-ground plant tissues (Chapin III et al., 2006). Overall, the finding that consumers impacted plant C pools and not fluxes suggests that grassland consumers may influence C pools by removing plant biomass through consumption (e.g. offtake of plant biomass to the food web and secondary production), but such biomass effects may not alter instantaneous rates of photosynthesis or respiration at the scale of aggregate plant community responses. Future studies relating disease or herbivory incidence or consumer densities to C fluxes and plant biomass would help clarify consumer roles in ecosystems and enhance our mechanistic understanding of biotic controls on ecosystem processes.

The positive effect of nutrient addition on C fluxes and pools in this study was not surprising given the strong nutrient limitation of plant growth at this study site (Fay et al., 2015; Isbell et al., 2013;

Seabloom et al., 2021), however, these previously observed effects could have arising through a variety pathways (e.g. increased GPP or decreased ER). Here we demonstrate that the biomass increases arose because of increased C fixation; GPP increased with nutrient addition while ER remained unchanged. The lack of an effect of nutrients on ER suggests that plant respiration as well as below-ground microbe and animal respiration (all components of ER) are less limited or stimulated by nutrients in comparison to rates of plant C uptake at this site. Therefore, fertilization drove greater rates of carbon fixation while carbon losses from respiration remained unchanged, leading to greater net rates of C uptake, more negative NEE. Greater GPP was likely linked to the observed increase in above-ground biomass in fertilized plots, and fertilization effects on GPP and biomass were strongly related to a decrease in C4 grasses under nutrient addition. Previous work has found that NEE and above-ground plant biomass were not changed by nitrogen addition alone (Harpole et al., 2007; Wilcots et al., 2022). Harpole et al. (2007) and Wilcots et al. (2022) only added nitrogen while we added nitrogen, phosphorus, potassium and micronutrients. The contrasting results between our work and these previous studies for both NEE and above-ground biomass suggest nutrient colimitation not only for plant productivity but also for ecosystem C fluxes (Fay et al., 2015; Harpole et al., 2011).

Previous studies of consumer–resource interactions suggest that nutrients can amplify pathogen and herbivory incidence in plant communities (Ebeling et al., 2021; Heckman et al., 2016; but see Cappelli et al., 2020) suggesting that nutrients may modify consumer impacts on ecosystem C fluxes. We found that, regardless of the type of consumer, nutrients did not modify the relative impact of consumers on C fluxes. For plant biomass, when log transformed, we found no interaction between nutrient addition and consumer reduction. This result indicates that consumers have constant proportional effects on plant biomass (Borer et al., 2020; Seabloom et al., 2017). Thus, when fertilization increases plant biomass, the relative proportion of biomass offtake by consumers remains unchanged, but the absolute amount of plant biomass being provisioned to the food web is greater (310% absolute increase above-ground and 23% increase below-ground in our study). A previous study, which had no fertilization treatment, found constant proportional impacts of consumers on plant biomass across a gradient of plant diversity resulting in more plant mass being lost in high diversity plots compared to monocultures (Seabloom et al., 2017). A study by Borer et al. (2020) also found proportional off take by vertebrate herbivores in response to nutrients in a wide range of grasslands world-wide. The similar result of constant proportional offtake by consumers in our work and these previous studies demonstrates the generality of the hypothesis that in settings where consumer densities are controlled by the environment (e.g. amount of plant biomass), we should expect constant proportional offtake by both herbivores and pathogens. Additionally, our findings suggest that increased plant biomass in grasslands arising from human driven nutrient pollution (Ackerman et al., 2019; Galloway et al., 2004) will likely be increased provisioning to a broad range of groups in the food web including soil fungi, foliar fungi, arthropods and mammalian herbivores.

Both nutrient and consumer impacts on grassland C fluxes and NDVI increased as the growing season progressed. Specifically, reducing all consumer types (All Removal treatment) caused a temporary increase in ER late in the growing season, suggesting that the combined effect of multiple consumer groups on C flux can be additive. The late-season impact of consumers on ER documented in this study is concordant with studies looking at grazing impacts in tropical and alpine grasslands (Cao et al., 2004; Wilsey et al., 2002), however, future tests on the temporal dynamics of consumer control on ecosystem C fluxes across gradients of climate would help clarify the generality of these patterns. These late growing season interactions between consumers, resources and ecosystem C flux suggest that nutrient limitation, herbivory and fungal impacts on plants may be related to the size of the plant biomass C pool—as plants accumulate more C through the growing season, resource limitation and biomass offtake to the food web increased in magnitude driving larger effects on C flux. The lack of plant consumer effects on ER early in the growing season may also reflect the smaller plant biomass in early months that contribute relatively less to ER rates such that consumer effects on biomass do not alter ER in the early season. In general, inclusion of these temporal dynamics improves our understanding of abiotic and biotic controls on terrestrial carbon cycling. Studies measuring C fluxes only in earlier parts of the growing season likely underestimated the impacts that animals, fungi or limiting resources have on ecosystem processes like C fluxes.

Using a unique experimental study that integrates nutrient supply and a broad range of plant consumer types, we directly measured the joint consumer and resource control of GPP, ER, NEE and plant biomass. We found that increased nutrient supply did not modify the relative impacts of consumers on GPP or ER regardless of whether the consumer group was fungi, arthropods or large mammalian herbivores. Importantly, our results demonstrated that foliar fungi and arthropods have a constant proportional but larger absolute effect on plant biomass in fertilized plots compared to ambient conditions. By comparing mammal, insect and fungal controls on C cycling, we build from previous work to clarify the generality of consumer–resource relationships in the context of plant communities and ecosystem C fluxes. In addition, we found that consumer and nutrient effects on grassland C cycling in terms of both fluxes and pools were dynamic across the growing season, increasing in magnitude late in the season. This work enhances a broad understanding of nutrient and consumer controls of temperate grassland carbon cycling and suggests that ongoing eutrophication will increase rates of ecosystem C uptake and modify fungal and herbivore interactions with plant communities.

AUTHOR CONTRIBUTIONS

Elizabeth T. Borer, Eric W. Seabloom and Linda Kinkel coordinated the consumer reduction experiment. Max Zaret, Eric Seabloom and Linda Kinkel conceived this study. Max Zaret collected the data. Max Zaret analysed data with inputs from Elizabeth T. Borer, Eric W. Seabloom and Linda Kinkel. Max Zaret wrote the first draft of the manuscript. All authors contributed substantially to manuscript revisions.

ACKNOWLEDGEMENTS

Special thanks to Sara de Sobrino, Megan Wilcots and Jeremiah Henning for assistance and guidance with carbon flux sampling and to Sarah Hobbie for comments on the manuscript. We also thank Jon Anderson, Troy Mielke, Anita Krause, Dan Bahauddin, Susan Barrott and Cedar Creek summer interns who all aided in the logistics, maintenance and data collection of this experiment. Finally, we thank two anonymous reviewers whose comments greatly improved this manuscript. This work was supported by grants from the US National Science Foundation Long-Term Ecological Research Program (LTER) including DEB-1234162 and DEB-1831944. Funding from UMN EEB and Cedar Creek supported M.Z.

CONFLICT OF INTEREST STATEMENT

None of the authors have a conflict of interest to declare.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14111.

DATA AVAILABILITY STATEMENT

Data are publicly available on the Environmental Data Initiative platform at: https://doi.org/10.6073/pasta/6642a7601ddc7e24f44aea39684d8ab4 (Kinkel, 2022) and https://doi.org/10.6073/pasta/b746e13f00ca73b8b3b57adc549419f7 (Zaret, 2022).

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SUPPORTING INFORMATION

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

Table S1. Net ecosystem exchange (NEE) response to nutrient addition and consumer removal across the growing season. Absolute NEE values were log transformed for analysis. Effect size of individual treatments and standard error is reported in main text. This table is linked with Figures 1, 3 and 4 in the main text.

Table S2. Gross primary production (GPP) response to nutrient addition and consumer removal across the growing season. Effect size of individual treatments and standard error is reported in main text. This table is linked with Figures 1, 3 and 4 in the main text.

Table S3. Ecosystem respiration (ER) response to nutrient addition and consumer removal across the growing season. ER values were square root transformed for analysis. Effect size of individual treatments and standard error is reported in main text. This table is linked with Figures 1, 3 and 4 in the main text.

Table S4. Aboveground biomass response to nutrient addition and consumer removal. Aboveground biomass values were log transformed for analysis. Effect size of individual treatments and standard error is reported in main text. This table is linked with Figure 2 in the main text.

Table S5. Belowground biomass response to nutrient addition and consumer removal. Belowground biomass values were log transformed for analysis. Effect size of individual treatments and standard error is reported in main text. This table is linked with Figure 2 in the main text.

Table S6. Plant functional group response to nutrient addition (Nutrient) and consumer reduction (Removal Trt) treatments. Linear mixed effect models were run for C3 grasses, C4 grasses, non-leguminous forbs (Forbs), and legumes.

Table S7. Normalized difference vegetation index (NDVI) response to nutrient addition and consumer removal across the growing season. DOY = day of year when sampling occurred. Effect size of individual treatments and standard error is reported in main text. This table is linked with Figure 5 in the main text.

Figure S1. Nutrient addition increased C3 grass and decreased C4 grass abundances. Model outputs for plant functional group response to nutrient addition and consumer reduction treatments are in Table S6.

Figure S2. Rates of gross primary production (GPP) decrease with increasing abundance of C4 grasses ($t = -2.575$, $df = 46$, $p = 0.0133$, $R^2 = 0.13$). Fertilizer.f denotes nutrient addition treatment with 0 being ambient nutrients and 1 being nutrient addition treatment. GPP values are from August 2020 only (same time of sampling as plant cover).

Figure S3. NDVI correlates with aboveground biomass ($R^2 = 0.52$, $p < 0.0001$). Both NDVI and Aboveground biomass are log transformed.

How to cite this article: Zaret, M., Kinkel, L., Borer, E. T., & Seabloom, E. W. (2023). Soil nutrients cause threefold increase in pathogen and herbivore impacts on grassland plant biomass. *Journal of Ecology*, *00*, 1–12. https://doi.org/10.1111/1365-2745.14111