

OPINION

Nitrogen deposition and greenhouse gas emissions from grasslands: uncertainties and future directions

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Abstract

Increases in atmospheric nitrogen deposition (N_{dep}) can strongly affect the greenhouse gas (GHG; CO_2 , CH_4 , and N_2O) sink capacity of grasslands as well as other terrestrial ecosystems. Robust predictions of the net GHG sink strength of grasslands depend on how experimental N loads compare to projected N_{dep} rates, and how accurately the relationship between GHG fluxes and N_{dep} is characterized. A literature review revealed that the vast majority of experimental N loads were higher than levels these ecosystems are predicted to experience in the future. Using a process-based biogeochemical model, we predicted that low levels of N_{dep} either enhanced or reduced the net GHG sink strength of most grasslands, but as experimental N loads continued to increase, grasslands transitioned to a N saturation-decline stage, where the sensitivity of GHG exchange to further increases in N_{dep} declined. Most published studies represented treatments well into the N saturation-decline stage. Our model results predict that the responses of GHG fluxes to N are highly nonlinear and that the N saturation thresholds for GHGs varied greatly among grasslands and with fire management. We predict that during the 21st century some grasslands will be in the N limitation stage where others will transition into the N saturation-decline stage. The linear relationship between GHG sink strength and N load assumed by most studies can overestimate or underestimate predictions of the net GHG sink strength of grasslands depending on their N baseline status. The next generation of global change experiments should be designed at multiple N loads consistent with future N_{dep} rates to improve our empirical understanding and predictive ability.

Keywords: CH_4 , grassland, methane, N_2O , net ecosystem CO_2 exchange, net ecosystem productivity, nitrogen deposition, nitrogen fertilization, nitrous oxide, uncertainty

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Introduction

Increases in atmospheric nitrogen deposition (N_{dep}) from human activities can strongly affect the exchange of greenhouse gases (GHG; CO_2 , CH_4 , and N_2O) between terrestrial ecosystems and the atmosphere (Vitousek *et al.*, 1997; Reay *et al.*, 2008). Since the Industrial Revolution, increased N_{dep} (from 17.4 TgN yr⁻¹ in late 1860s to 60 TgN yr⁻¹ in the 1990s; Galloway *et al.*, 2008) has enhanced terrestrial net ecosystem productivity (NEP) globally by ~175 Pg C (Bala *et al.*, 2013). However, corresponding increases in N_2O and CH_4 emissions in response to increased N_{dep} could potentially offset the effect of greater terrestrial carbon

storage on the atmosphere (Liu & Greaver, 2009; Zaehle *et al.*, 2011; Templer *et al.*, 2012). Thus, characterizing the response of GHG fluxes to N_{dep} and determining the thresholds above which the net GHG sink strength declines as N increases are crucial for predicting how terrestrial ecosystems will feedback to climate (Liu & Greaver, 2009).

Emission scenarios for the main gaseous forcing agents causing climate change over the 21st century, referred to as representative concentration pathways (RCPs; Ciais *et al.*, 2013), differ from previous scenarios (e.g., Special Report on Emissions Scenarios, SRES; Lamarque *et al.*, 2013) in that they include regulation of air pollutants such as nitrogen oxides that cause N_{dep} (Moss *et al.*, 2010). The RCP predicts that over the coming decades, N_{dep} will increase in most regions of the world (Lamarque *et al.*, 2013; Ciais *et al.*, 2013).

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However, the predicted emission of reactive nitrogen to the atmosphere and corresponding rates of N_{dep} are lower in the RCPs compared to SRES scenarios because they include climate policies to reduce their emissions (van Vuuren *et al.*, 2011; Lamarque *et al.*, 2011).

Responses of GHG fluxes to N addition are nonlinear and characterized by biological thresholds (Fig. 1a, b; Aber *et al.*, 1998; Lu *et al.*, 2011; Zaehle *et al.*, 2011; Lu & Tian, 2013; Fleischer *et al.*, 2013; Shcherbak *et al.*, 2014). Under N limitation, N addition will increase the net GHG sink capacity of ecosystems (Fig. 1b; Liu & Greaver, 2009; Zaehle *et al.*, 2011). At this limiting stage, additional N increases NEP more than it stimulates losses of N_2O and CH_4 (Fig. 1a; Liu & Greaver, 2009). As N continues to increase, ecosystems transition into the N saturation-decline stage, and after a critical threshold, their net GHG sink strength will likely decrease (Aber *et al.*, 1998; Liu & Greaver, 2009). During the intermediate and saturation-decline stages, the sensitivity of NEP to N additions declines at the same time as the emissions of N_2O and CH_4 increase (Fleischer *et al.*, 2013; Shcherbak *et al.*, 2014). Increased emissions of N_2O and CH_4 could potentially offset the net CO_2 sink capacity of terrestrial ecosystems including grasslands by 53–76% (Liu & Greaver, 2009).

Grasslands are important determinants of the concentration of GHGs in the atmosphere. They contribute ~10% of terrestrial net productivity and store up to 30% of the world's organic C in their soils (Scurlock & Hall, 1998; Lal, 2004). Plant productivity in many grasslands is N limited (LeBauer & Treseder, 2008; Lee *et al.*, 2010) suggesting that further increases in N_{dep} will increase productivity in these ecosystems into the future, leveling off only when the critical threshold of N saturation is reached.

Considerable effort has been made to understand the impact of N_{dep} on ecosystem C and N dynamics, but loads commonly used in experimental N additions may have been too high to realistically predict the net effect on GHG emissions. Liu & Greaver (2009) concluded that most studies of GHG fluxes in grassland ecosystems applied high N loads and very few used multiple N loads. Data on GHG fluxes at low experimental N loads are necessary to accurately predict the net GHG sink strength of grasslands, especially if the responses of GHG fluxes to N_{dep} are nonlinear. It remains unclear if experimental N loads used by most studies are sufficiently low to mimic the loads these ecosystems are predicted to experience in the future. The rarity of GHG data at N_{dep} rates consistent with RCP projections is likely limiting our mechanistic understanding of the relationship between GHG fluxes and N_{dep} , rendering highly uncertain predictions of the net GHG sink strength of grasslands in the future.

Accurate estimates of the net GHG sink strength of grasslands in response to future N_{dep} rates depend on a robust characterization of the relationship between GHG fluxes and N loads, and on the identification of the critical N saturation-decline thresholds (Liu & Greaver, 2009). To predict how grasslands will perform in the future, it is important that N loads applied in experiments compare favorably to future rates of N_{dep} projected by RCPs. This is particularly important if grasslands during the 21st century were at the N limitation stage transitioning to the saturation-decline stage (Fig. 1a, b).

Many studies predicting the net GHG sink strength of grasslands during the 21st century have assumed a linear relationship between GHG fluxes and N_{dep} (e.g., Schindler & Bayley, 1993; Liu & Greaver, 2009; Templer *et al.*, 2012; Pinder *et al.*, 2012). Because the relationship between GHG fluxes and N_{dep} rates is nonlinear (Fig. 1a), it is likely that predictions of the net GHG sink strength assuming linearity are inaccurate. Compared to approaches using a nonlinear relationship, predictions of their net GHG sink strength based on a linear relationship would likely underestimate their net GHG sink capacity if grasslands were at N limitation and intermediate stages during the 21st century and would likely overestimate their net GHG sink strength if grasslands were at the N saturation-decline stage (Fig. 1b, nonlinear and linear responses of the net GHG balance).

The objective of this study was to determine whether experimental applications of N and corresponding GHG exchange from grasslands are consistent with RCP projections for future rates of N_{dep} and to predict the relationship between GHG emissions and N_{dep} . Using the process-based biogeochemical model DayCent (Parton *et al.*, 1998; Del Grosso *et al.*, 2009, 2011), parameterized with data from grassland ecosystems located in the major climate zones, we characterize the relationship between N and GHGs fluxes, other N-trace gases fluxes, and N leaching of these grasslands. We used this biogeochemical model to investigate the shapes of the responses of GHG fluxes to changes in N_{dep} (e.g., linear vs. nonlinear) and to determine whether grasslands located in the major climate zones will be at the N limitation and intermediate stages, or at the saturation-decline stage over this century. DayCent has been extensively used to simulate GHG fluxes under different environmental scenarios such as changes in N addition in natural and managed grasslands, providing accurate predictions of ecosystem fluxes of CO_2 , N_2O , and CH_4 (Del Grosso *et al.*, 2000, 2005; Stehfest and Müller, 2004). In addition, DayCent, as other mechanistic-based models, is able to capture the nonlinearity of ecosystem responses to N addition (Zaehle *et al.*, 2011).

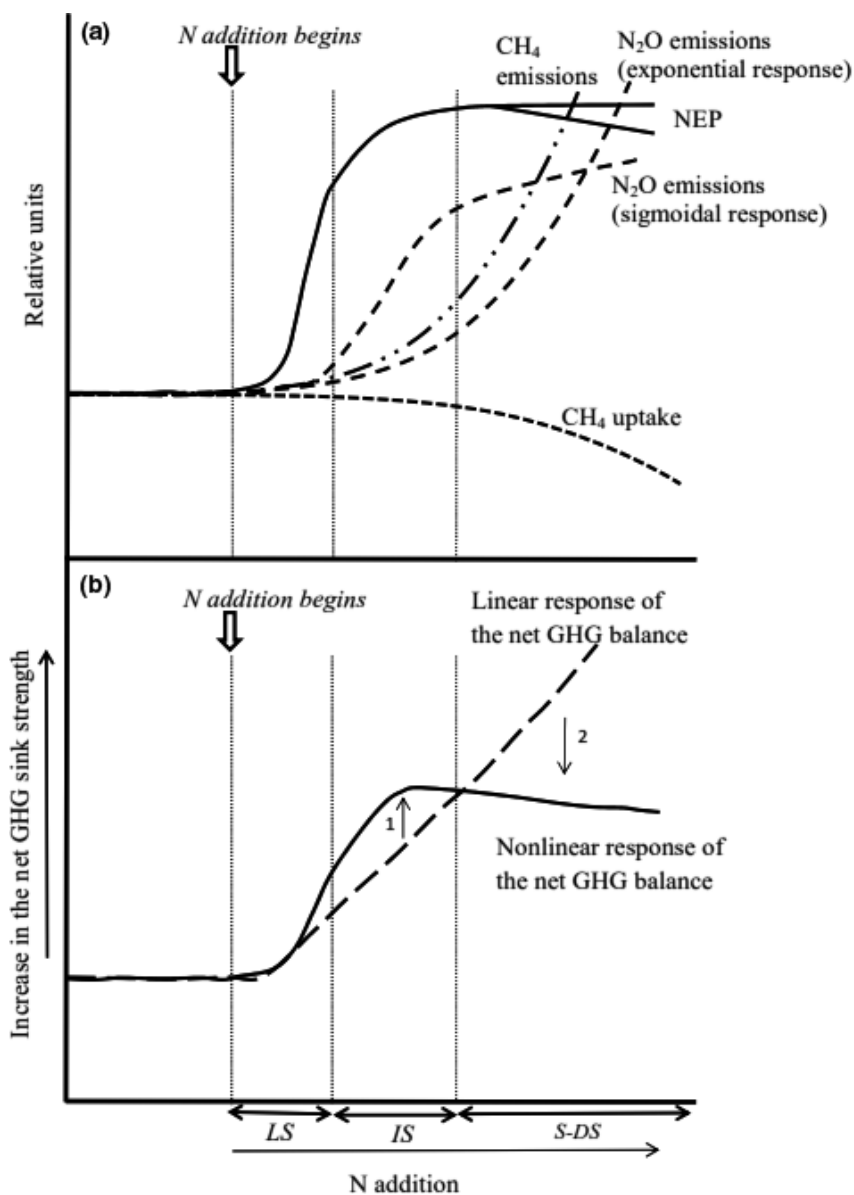


Fig. 1 Hypothetical effect of increased N on NEP, and N₂O and CH₄ fluxes (a), and the net GHG balance (b) in relative units for terrestrial ecosystems. Ecosystem GHG responses are nonlinear, with NEP either saturating or declining as N increases above the saturation threshold (Aber *et al.*, 1998; Fleischer *et al.*, 2013), N₂O emissions following exponential or sigmoidal responses as shown in many agro-ecosystems including perennial grasslands (Shcherbak *et al.*, 2014), and net CH₄ fluxes an exponential-type response (Aronson & Helliker, 2010; Banger *et al.*, 2012). Responses are characterized by stages referred to as the N limited (LS), the intermediate stage (IS), and the N saturation-decline stage (S-DS; a & b nonlinear response). During the N limitation stage, the net GHG sink strength of an ecosystem increases with N addition as increases in NEP and corresponding CO₂ uptake is greater than increases in the emissions of N₂O and net CH₄. As N addition continues, the ecosystem transitions to the N saturation-decline stage in which C and N losses outbalance C uptake and the net GHG sink strength of an ecosystem declines progressively as N increases. We are also comparing the effect of N addition on the net GHG balance for nonlinear and linear response types (a, nonlinear and linear GHG response). The commonly used linear response in global GHG balance estimates (e.g., Liu & Greaver, 2009) likely underestimates GHG predictions if ecosystems are in the limitation or intermediate stages (b, 1), but overestimates projections if ecosystems are in the saturation-decline stage compared to nonlinear response approaches (b, 2).

Material and methods

A literature search was conducted using Science Citation Index Expanded database (ISI Web of Knowledge, Web of

Science) to determine whether experimental N loading rates are consistent with future scenarios. We screened for publications using the search terms: net ecosystem CO₂ exchange (NEE) or NEP, N₂O, or CH₄ emission or uptake in native,

semi-native, or managed grasslands. To be included in this analysis, published studies had to meet selection criteria similar to Liu & Greaver (2009): (i) GHG fluxes had to be reported for N addition and ambient treatments; and (ii) N addition and ambient treatments should have experienced the same climatic, edaphic, and vegetation conditions to avoid possible confounding factors caused by site conditions. Therefore, studies examining N enrichment effects along N deposition gradients were not included. In addition, studies examining organic fertilization (i.e., animal manures) were excluded as this fertilization affects C input, and the effects of N and C on GHG fluxes cannot be disentangled. Measurements in native, semi-native, and managed grasslands at different N addition levels and forms within each study were considered independent observations. Measurements through several years from a specific study were considered a single observation. The reported N additions or N_{dep} represented the annual sum of the total N load in each study. For studies in which ambient N_{dep} rate was not reported, we used the global N_{dep} dataset used by the National Center for Atmospheric Research (NCAR; <http://www.cesm.ucar.edu/models/cesm1.0/>). For studies with measurements through several years in which ambient N_{dep} rate was not reported, this value was averaged to provide one value for each observation. The dataset contains historical and predicted N_{dep} values at 0.5 degree resolution for the years 1890–2100.

We also used the NCAR database to obtain N_{dep} rates projected for the RCP 4.5 and RCP8.5 scenarios ($N_{\text{projected}}$). We calculated the ratio between the N_{dep} rate applied in each study and the site-specific N_{dep} rate projected for 2100 using the RCP 4.5 and RCP 8.5, referred to as the 'N applied:N projected Ratio'. Uncertainty for the NCAR modeled ambient and projected N_{dep} was quantified in Lamarque *et al.* (2005). Using these estimates, we calculated the uncertainty in the ratio from the propagated sum of errors in both ambient and projected N_{dep} rates for each study site. Error in either ambient or projected N_{dep} was assumed $\pm 17\%$ for American sites, $\pm 70\%$ for European sites, and $\pm 33\%$ for Asian sites as reported in Lamarque *et al.* (2005).

We modeled the relationship between variable rates of N_{dep} and GHGs, other N-trace gases fluxes, and N leaching of grasslands using a biogeochemical model and existing data from grassland ecosystems located in major climate zones (Table 1). Model simulations were performed using DayCent (v. 4.5; Parton *et al.*, 1998), the most recent daily time step version of CENTURY. DayCent has been used to simulate the effects of climate and land use change on C and nutrient cycling in terrestrial ecosystems including grasslands across the globe (Stehfest & Müller, 2004, Parton *et al.*, 2007; Ryals *et al.*, 2015).

To capture variation among sites, model inputs included vegetation cover, daily precipitation and temperature, soil texture, and current and historical land use practices (Appendix S2, Table S2). DayCent calculates potential plant growth as a function of water, light, and soil temperature and limits actual plant growth based on soil nutrient availability. Soil organic C (SOC) is estimated from the turnover of soil organic matter pools, which change with the decomposition

rate of dead plant material. For this study, DayCent was parameterized to model SOC dynamics to a depth of 30 cm. European and South American study sites reported SOC. For American study sites in which SOC was not reported, we used SSURGO soil carbon data (NRCS, 2010). For the individual site simulations (Table 1), we used 30-year daily climate records from the Daymet database (USA sites; www.daymet.org; Thornton *et al.*, 2012) and the European Climate Assessment Historical database (non-USA sites; www.ecad.eu; Klein Tank *et al.*, 2002). We ran 80 model simulations which included the known land use history (Table 1) and current species composition for each site. Using the reported data for each site, ambient and N treatment simulations were run for the corresponding years of data, duplicating the site treatments.

The model was calibrated using plant functional-type parameters appropriate for each site (Appendix S2, Table S2), and the potential plant productivity value (PRDX) was optimized based on observed NPP and known soil N availability. For example, at a US tallgrass prairie site (Seadstedt *et al.*, 1991), there was a response of total biomass to N fertilization in burned plots but not in unburned plots. We were able to duplicate this response in DayCent by removing N when the site was historically annually burned to simulate N limitation (Appendix S2, Fig. S1a). Likewise for a water-limited site (Mediterranean grassland; Harpole *et al.*, 2007), we were able to optimize the PRDX so that when the actual climate was used, there was no N fertilization response, but when water was increased during the growing season, the observed response to N fertilization was duplicated (Appendix S2, Fig. S1b). We ran 30-year simulations for each of the sites (Table 1) for N deposition rates ranging from 0 to 150 kg N ha⁻¹ yr⁻¹.

Following calibration, the model was validated against above- and belowground plant productivity and SOC data reported for each site to improve our confidence in model predictions (Table 1; Appendix S2, Figs S2–S4; Medlyn *et al.*, 2005). Simple linear regression was used to compare observed and modeled data using MATLAB® v. 7.8.0 (Mathworks Inc., Natick, MA, USA).

The following criteria were adopted to select the studies used for modeling purposes (Table 1): (i) several years of aboveground and belowground NPP observations needed to be reported in both N addition and ambient treatments; (ii) baseline SOC needed to be reported or retrieved from reliable soil databases (e.g., SSURGO soil carbon data); (iii) N addition and ambient treatments should have experienced the same climatic, edaphic, and vegetation conditions to avoid possible confounding factors caused by site conditions, and hence, studies examining N enrichment effects along N deposition gradients were not included; and (iv) information on vegetation cover, current and historical land use practices information needed to be reported as these are crucial inputs for our model.

Disentangling the effects of soil moisture and N addition on GHG fluxes is challenging as water and increased N_{dep} often covary through wet deposition, and both factors limit plant growth and exert a strong control on the exchange of GHG fluxes between the ecosystem and the atmosphere (Gomez-

Table 1 Site description, land use history, location, mean annual precipitation (MAP), N treatments, current N deposition (N_{dep}), number of observations, and the reference citation for each study site used to calibrate DayCent v4.5. Plant and root productivity observations refer to annual plant productivity data from each study for both control and N treatments. Asterisk on observation reflects that annual root productivity was also reported in addition to plant productivity in that specific study with that specific number of observations. We also retrieved 7 baseline SOC observations for each site. Data needed for DayCent parameterization for each study site can be found at Appendix S2, Table S1

Site name	Description and land use history	Location	MAP (cm)	Experiments	N_{dep} (kg N ha ⁻¹ yr ⁻¹)	Plant and root productivity observations	Source
CPER	Temperate shortgrass steppe, uncultivated	Colorado, USA (40.83, -104.72)	31.2	Control, 100 kg N ha ⁻¹ yr ⁻¹	12	8	Paschke <i>et al.</i> (2000)
Konza	Temperate tallgrass prairie, uncultivated, portions burned annually for 16 years,	Kansas, USA (39.08, -96.58)	83.5	Unburned and burned control, 100 kg N ha ⁻¹ in both burned and unburned plots	10	16	Seadstedt <i>et al.</i> (1991)
Irvine Ranch	no grazing for past 12 years Mediterranean grassland, dominated by spring annuals, site was mowed and an herbicide was applied to eliminate invasive weeds	California, USA (33.62, -117.76)	32.5	Control, 100 kg N ha ⁻¹ , 30% increased water supply, combined N and water additions	5	4*	Harpole <i>et al.</i> (2007)
Amerongse	Temperate floodplain <i>Arrhenatherion</i> grassland of the river Rhine, no human disturbance since 1870, flooded in December 1993 and January 1995, severe drought in 1999	Amerongse Bovenpolder, Netherlands (51.98, 5.43)	85.5	Control, 100 kg N ha ⁻¹ yr ⁻¹	25	42	Beltman <i>et al.</i> (2007)
Fabriano	Mediterranean species-poor grassland, early succession old field in central Italy, rotation crops until 1980	Fabriano, Italy (43.33, 13.00)	94.5	Control, 35 kg N ha ⁻¹ yr ⁻¹	15	6	Bonanomi <i>et al.</i> (2006)
Flooding Pampa	Subtropical grassland, native grassland, burned at 2 to 4 years of intervals, not burned during the year of study	Flooding Pampa, Buenos Aires, Argentina (-34.6, -58.4)	90	Control, 250 kg N ha ⁻¹ yr ⁻¹	4	2*	Semmartin <i>et al.</i> (2007)
Hato El Frio	Tropical savanna, burned annually	Llanos, Venezuela (6.9, -68.5)	147	Control, 50 kg N ha ⁻¹ yr ⁻¹	7	2	Sarmiento <i>et al.</i> (2006)

Casanovas *et al.*, 2012, 2013; DeLucia *et al.*, 2014). DayCent is well equipped to model the single and combined effects of water and N limitation on growth (Del Grosso *et al.*, 2009). The model includes dynamic representation of water and nitrogen availability for calculations of actual NPP and dynamic allocation of NPP to above- and belowground components depending on water and N availability (i.e., root:shoot ratio can increase with less N or water; Parton *et al.*, 1998). To disentangle the effect of soil moisture from the impact of N addition on GHG fluxes, especially in water-limited ecosystems, we modeled GHG fluxes from a study site (Irvine Ranch, Mediterranean grassland, Harpole *et al.*, 2007; Table 1) with observations for the single and combined factors of N addition and water. We found, as expected, that DayCent was able to duplicate the observed NPP for each condition without modifying the model (Appendix S2, Fig. S1).

Validation based on plant productivity for grasslands reliably predicts NEP, N₂O, and CH₄ fluxes (Del Grosso *et al.*, 2000; Stehfest and Müller, 2004; Del Grosso *et al.*, 2005; Cheng *et al.*, 2013). However, to increase confidence in the model, predicted NEP, N₂O, and CH₄ fluxes were validated with reported data from sites at both control and N additions treatments and from sites at ambient N_{dep} with similar land use history, mean annual precipitation and temperature, and soil and vegetation type as our modeled sites. Details for the literature search criteria, number of total publications, and observations retrieved are provided in Appendix S2, Figs S5–S7.

Total GHG fluxes from each site were expressed as CO₂ equivalents (Bridgman *et al.*, 2014; Neubauer & Magonigal, 2015). The GWP for N₂O and CH₄ fluxes were 298 and 28, respectively (according to the Fifth Assessment Report, Ciais *et al.*, 2013; on a time horizon of 100 years). Total uncertainty in GHG estimates was computed from the propagated sum of the error in N_{dep}, and the error in GHG fluxes obtained from the model as in Peichl *et al.* (2010). The error in NEP estimates derived from the model for each grassland site and N_{dep} rate was calculated by comparing observed and modeled aboveground NPP, assuming that uncertainties in aboveground and belowground NPP were similar and that the uncertainty in heterotrophic respiration was 1.5× larger than in total NPP (Hudiburg *et al.*, 2014). Uncertainty in N₂O and CH₄ estimates derived from the model was assumed to be constant for all grasslands sites and N_{dep} rates and to be equal to the upper uncertainty estimate derived from Del Grosso *et al.* (2009). The uncertainty in N_{dep} rates was calculated as explained above. The propagated sum of these error terms resulted in an estimate of the total uncertainty in GHG fluxes.

A segmented regression between NEP and N_{dep} rates was employed as in Fleischer *et al.* (2013) to determine the N saturation threshold at which NEP levels off for each study site. The slope of this relationship at the N limitation stage represents the CO₂ response of these ecosystems to N_{dep} (Fleischer *et al.*, 2013). These tests were implemented using MATLAB® v. 7.8.0.

Results

Our literature search found a total of 1177 references (148 for NEE, 735 for N₂O, and 294 for CH₄), and 28

articles matched inclusion criteria. Among the selected references, we retrieved a total of 90 observations that reported NEP (10 observations), CH₄ (37 observations), and N₂O (43 observations) fluxes at both ambient and N addition treatments (Appendix S1, Table S1).

The rates of N_{dep} predicted by RCP 4.5 for the end of this century ranged from 1 to 15 kg N ha⁻¹ yr⁻¹, and predictions from RCP 8.5 for this same time period ranged from 3 to 18 kg N ha⁻¹ yr⁻¹. Most studies of N_{dep} impacts on NEP, N₂O, and CH₄ fluxes used loads at least 5–10 times the projected N_{dep} rates under RCP 4.5 and 8.5 when the uncertainty in the N applied: N projected ratio was calculated for both the lower and upper range (Fig. 2a–d).

Few studies used experimental low N loads (Appendix S1, Table S1). For NEP, we found two observations from studies using loads between 20 and 60 kg N ha⁻¹ yr⁻¹ and 8 observations with experimental loads above 90 kg N ha⁻¹ yr⁻¹. For CH₄ fluxes, there were only 2 and 5 observations with loads below 20 kg N ha⁻¹ yr⁻¹, and between 20 and 60 kg N ha⁻¹ yr⁻¹, respectively, and most experimental loads were above 60 kg N ha⁻¹ yr⁻¹ (30 observations; Appendix S1, Table S1). For N₂O fluxes, we found only 1 and 7 observations from studies using loads below 20 kg N ha⁻¹ yr⁻¹ and between 20 and 60 kg N ha⁻¹ yr⁻¹, respectively, and most experimental loads were above 60 kg N ha⁻¹ yr⁻¹ (37 observations; Appendix S1, Table S1).

The DayCent biogeochemical model provided robust predictions of carbon cycling in grasslands. Predicted aboveground NPP (ANPP; $r^2 = 0.79$; 80 experimental observations; Appendix S2, Fig. S2), belowground NPP (BNPP; $r^2 = 0.99$; eight experimental observations; Appendix S2, Fig. S3), and SOC ($r^2 = 0.97$; seven baseline observations; Appendix S2, Fig. S4) agreed well with observed values from each site. The predicted responses of ANPP to burning, increased precipitation and N, and their associated interactive effects fell within one standard deviation of the observations (Appendix S2, Fig. S1a, b).

Predicted NEP, N₂O, and CH₄ fluxes agreed well with observed values from sites at both ambient and N addition treatments (Appendix S2, Figs S5–S7). For each GHG, there were positive and negative model biases, but predicted values fell within one standard deviation for each grassland site at both ambient and N addition treatments (Appendix S2, Figs S5–S7).

Modeled NEP increased with N_{dep}, but it leveled off at rates of N_{dep} below 50 kg N ha⁻¹ yr⁻¹ (Fig. 3). The critical saturation point for NEP varied among grasslands (Fig. 3). NEP reached a critical saturation point between 20 and 50 kg N ha⁻¹ yr⁻¹, except for the temperate shortgrass (T-SG), unburned temperate tallgrass

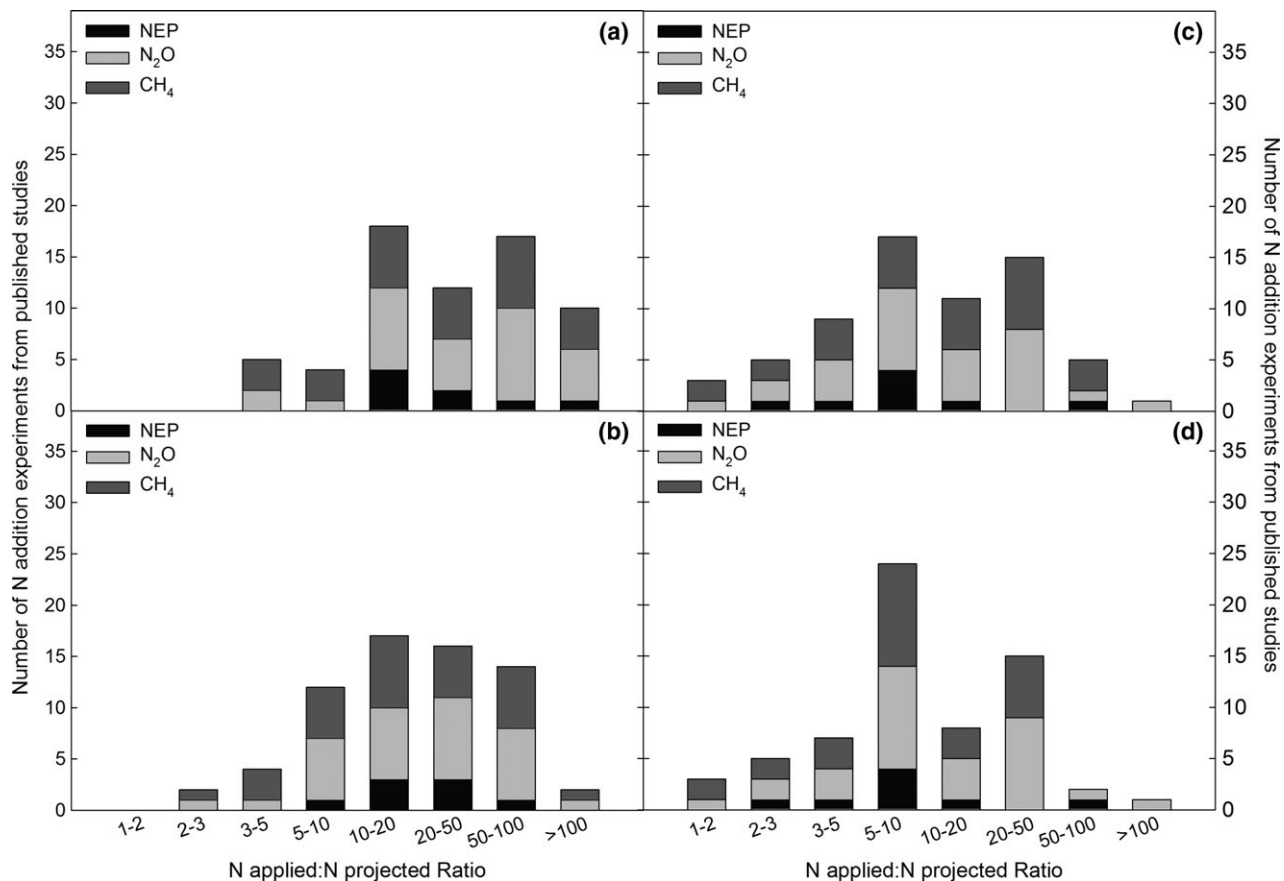


Fig. 2 Ratio between N applied and N_{dep} rate predicted using the RCP 4.5 (a & c) and RCP 8.5 (b & d) for net ecosystem productivity (NEP; 10 observations), CH_4 (37 observations), and N_2O (41 observations) (Appendix S2). The 'N applied:N projected Ratio' refers to the ratio between the N_{dep} rate applied in that study and the site-specific N_{dep} rate projected for 2100 using the RCP 4.5 and RCP 8.5. For each study site, the uncertainty in each ratio was calculated considering uncertainties in ambient and projected N_{dep} rates of $\pm 17\%$ for American sites, $\pm 70\%$ for European sites, and $\pm 33\%$ for Asian sites as reported in Lamarque *et al.* (2005). For each RCP scenario, the upper range of the uncertainty in the ratio is presented in panels a & b, and the lower in panels c & d. Ratios > 1 indicate that N additions were larger than site-specific projected N_{dep} .

grasslands (T-TG), and the Mediterranean grassland with or without water addition (No-water M-G and Water M-G) that saturated at N loads at or below $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Fig. 3). Combining N_{dep} and irrigation did not affect the critical NEP saturation point (Fig. 3), but increased NPP (Appendix S2, Fig. S1). The response of NEP to N_{dep} varied between -0.2 ± 0.1 and $167 \pm 35 \text{ kg C uptake per kg N}$, and these grasslands reached maximum NEP values from -0.5 ± 0.1 to $530 \pm 90 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Table 2). Fire increased both the sensitivity of NEP to increased N_{dep} before the N threshold and the critical NEP saturation point in the temperate tallgrass prairie (Fig. 3; Table 2), and the burned grassland consistently was a stronger sink of CO_2 than the unburned one (Fig. 3).

Increased N_{dep} enhanced the production of N_2O , NO, and N leaching (Fig. 4). In most grasslands, emissions of N_2O and NO increased with N_{dep} up to a rate of

$100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, then increased at a lesser extent at higher N loads (Fig. 4a, b). In most grasslands, N leaching increased with N_{dep} at rates above $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Combining fire and N_{dep} reduced N_2O and N leaching losses from the temperate tallgrass prairie compared to the unburned one, particularly at N additions between 1 and $60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

According to the model, increased N_{dep} did not affect CH_4 uptake (Appendix S2, Fig. S8a). However, CH_4 emissions in temporarily flooded grasslands in tropical, subtropical, and temperate climates increased with N_{dep} until these fluxes plateaued at N_{dep} rates below $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Appendix S2, Fig. S8b).

In most grasslands, N_{dep} enhanced the net GHG sink capacity or reduced the net GHG source strength, particularly at N_{dep} rates at which N stimulated NEP (Figs 3 and 5). As N_{dep} continued to increase, however, grasslands transitioned to a N saturation-decline stage

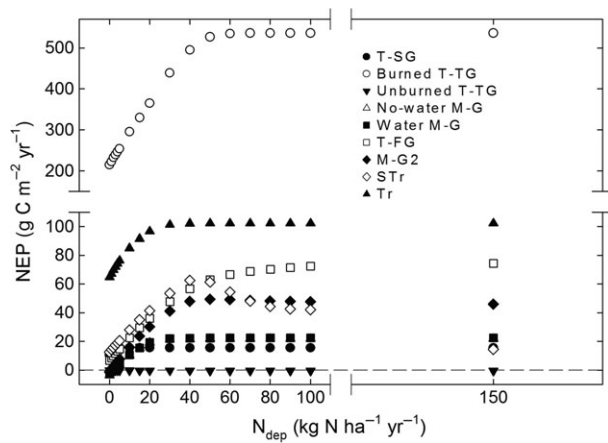


Fig. 3 Modeled relationship between N_{dep} rates and net ecosystem productivity (NEP) for grassland ecosystems located at major climate zones using DayCent 4.5. Positive values indicate that the ecosystem is a net CO_2 sink. Negative values indicate that the ecosystem is a net CO_2 source. T-SG refers to temperate shortgrass steppe (Paschke *et al.*, 2000), burned and unburned T-TG refers to temperate tallgrass prairie (Seadstedt *et al.*, 1991), M-G refers to Mediterranean grassland with or without water addition (Harpole *et al.*, 2007), T-FG refers to temperate floodplain grassland (Beltman *et al.*, 2007), M-G2 refers to Mediterranean grassland (Bonanomi *et al.*, 2006), STr refers to subtropical grassland (Semmartin *et al.*, 2007), and Tr refers to tropical grassland (Sarmiento *et al.*, 2006). Site description, land use history, location, and mean annual precipitation for each study are described in Table 1.

(Fig. 5). The critical N threshold occurred at rates at or below $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in some grasslands (unburned T-TG, no-water, and water M-G, T-SG, and Tr), and between >20 and $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in others (burned T-TG, T-FG, M-G2, and STr; Fig. 5). In all grasslands except in the burned temperate tallgrass prairie, after the critical N threshold for GHGs was reached, the sensitivity of their net GHG sink or source strength to N_{dep} declined, and further increases in N lead to reductions in net GHG sink strength or increases in net GHG source capacity. At the N saturation-decline stage, the reduction in the net GHG sink strength or increases in the net GHG source capacity of grasslands concurred with decreased stimulation of NEP and enhanced losses of N_2O and net CH_4 emissions (Figs 3–5). At all N_{dep} rates, the burned temperate grassland was a stronger net GHG sink than the unburned one at all N_{dep} rates (Fig. 5). Even at very low N rates, temperate flooded, tropical, and subtropical grasslands were net sources for GHGs (Appendix S2, Figs S8B and 5). For loads lower than $40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, increased N_{dep} decreased the net GHG source strength of temperate flooded and subtropical pastures, but the N addition did not decrease

Table 2 Slope of the relationship between net ecosystem productivity (NEP) and N_{dep} ($\text{kg C sequestered}:\text{kg N applied}$), and N saturation threshold for NEP for grassland ecosystems located at major climate zones. T-SG refers to temperate shortgrass steppe (Paschke *et al.*, 2000), burned and unburned T-TG refers to temperate tallgrass prairie (Seadstedt *et al.*, 1991), M-G refers to Mediterranean grassland with or without water addition (Harpole *et al.*, 2007), T-FG refers to temperate floodplain grassland (Beltman *et al.*, 2007), M-G2 refers to Mediterranean grassland (Bonanomi *et al.*, 2006), STr refers to subtropical grassland (Semmartin *et al.*, 2007), and Tr refers to tropical grassland (Sarmiento *et al.*, 2006). For each study site, the uncertainty in each N saturation threshold for NEP was calculated considering uncertainties in N_{dep} rates of $\pm 17\%$ for American sites, $\pm 70\%$ for European sites, and $\pm 33\%$ for Asian sites as reported in Lamarque *et al.* (2005). For each study site, total uncertainty in each ratio was computed from the propagated sum of error in N_{dep} and the error in GHG estimates obtained from the model. Site description, land use history, location, and mean annual precipitation for each study are described in Table 1

Grassland	Ratio $\text{kg C sequestered}:\text{kg N applied}$	N saturation threshold for NEP ($\text{g C m}^{-2} \text{ yr}^{-1}$)
T-SG	64 ± 17	16 ± 3
Burned T-TG	167 ± 35	530 ± 90
Unburned T-TG	-0.2 ± 0.1	-0.5 ± 0.1
Water M-G	34 ± 6	22 ± 4
No-water M-G	34 ± 6	22 ± 4
T-FG	35 ± 25	70 ± 49
MG2	40 ± 30	50 ± 35
STr	49 ± 9	62 ± 10
Tr	18 ± 5	102 ± 17

the net GHG source strength of tropical pasture (T-FG, STr, and Tr; Fig. 5). However, as N_{dep} continued to increase, these systems transitioned to stronger net sources (Fig. 5).

Averaged across all grasslands, total uncertainty in the net GHG balance was slightly lower at N_{dep} rates between 0 and $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ than between 50 and $150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (data not shown). At N_{dep} rates between 0 and $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, the highest uncertainty in CO_2 eq was observed in the burned T-TG and in the temperate flooded, subtropical, and tropical grasslands (average over 0 and $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ N_{dep} rates of $-1158 \pm 554 \text{ g CO}_2 \text{ eq m}^{-2} \text{ yr}^{-1}$, $155 \pm 134 \text{ g CO}_2 \text{ eq m}^{-2} \text{ yr}^{-1}$, $243 \pm 131 \text{ g CO}_2 \text{ eq m}^{-2} \text{ yr}^{-1}$ and $118 \pm 53 \text{ g CO}_2 \text{ eq m}^{-2} \text{ yr}^{-1}$ in burned T-TG, T-FG, STr, and Tr, respectively). In contrast, at N_{dep} rates between 0 and $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, the lowest uncertainty in CO_2 eq was observed in the Mediterranean grassland with and without water addition (average over 0 and $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ N_{dep} rates of

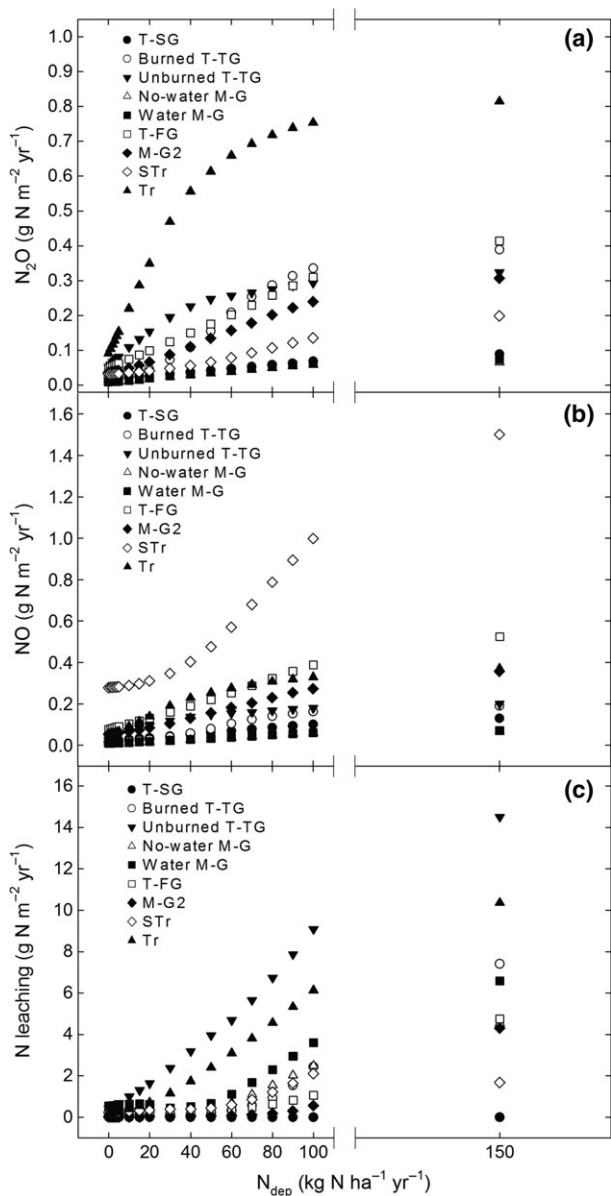


Fig. 4 Modeled relationship between N_{dep} rates and annual N_2O (a) and NO (b) fluxes, and N leaching (c) for grassland ecosystems located at major climate zones using DayCent 4.5. Positive values of N_2O and NO fluxes indicate efflux. T-SG refers to temperate shortgrass steppe (Paschke *et al.*, 2000), burned and unburned T-TG refers to temperate tallgrass prairie (Seadstedt *et al.*, 1991), M-G refers to Mediterranean grassland with or without water addition (Harpole *et al.*, 2007), T-FG refers to temperate floodplain grassland (Beltman *et al.*, 2007), M-G2 refers to Mediterranean grassland (Bonanomi *et al.*, 2006), STr refers to subtropical grassland (Semmartin *et al.*, 2007), and Tr refers to tropical grassland (Sarmiento *et al.*, 2006). Site description, land use history, location, and mean annual precipitation for each study are described in Table 1.

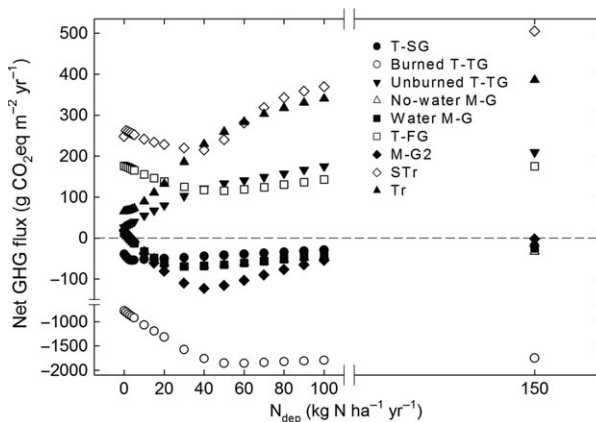


Fig. 5 Modeled relationship between N_{dep} rates and annual net GHG flux for grassland ecosystems located at major climate zones using DayCent 4.5. Annual net GHG flux is reported as CO_2 equivalents converted to account for differences in warming potential. Positive values indicate that the ecosystem is a net CO_2 source. Negative values indicate that the ecosystem is a net CO_2 sink. T-SG refers to temperate shortgrass steppe (Paschke *et al.*, 2000), burned and unburned T-TG refers to temperate tallgrass prairie (Seadstedt *et al.*, 1991), M-G refers to Mediterranean grassland with or without water addition (Harpole *et al.*, 2007), T-FG refers to temperate floodplain grassland (Beltman *et al.*, 2007), M-G2 refers to Mediterranean grassland (Bonanomi *et al.*, 2006), STr refers to subtropical grassland (Semmartin *et al.*, 2007), and Tr refers to tropical grassland (Sarmiento *et al.*, 2006). Site description, land use history, location, and mean annual precipitation for each study are described in Table 1.

$-28 \pm 14 \text{ g } CO_2 \text{ eq m}^{-2} \text{ yr}^{-1}$). For all grasslands, the percentage uncertainty in N_2O fluxes contributed the most to total uncertainty (percentage uncertainty for averaged annual N_2O fluxes, CH_4 fluxes, and NEP across all sites and N_{dep} rates was 60%, 50%, and 49%, respectively).

Discussion

In this study, we surveyed the available literature of GHG fluxes of grasslands to determine whether experimental N loads were consistent with predicted future N_{dep} rates and used DayCent to model the relationship between GHG fluxes and N additions. The vast majority of studies used N loads higher than levels these ecosystems will experience in the future and represented treatments well into the N saturation-decline stage. In most grasslands, increased N_{dep} enhanced the net GHG sink strength or reduced the net GHG source strength, but as it continued to increase, grasslands transitioned to a N saturation-decline stage in which the sensitivity of the net GHG sink or source strength to

N_{dep} declined. The N saturation thresholds for GHGs varied greatly among grasslands and with fire management. Fire increased the net GHG sink strength of the temperate grassland and this grassland reached the critical N saturation threshold for GHGs at higher N_{dep} rates than the unburned grassland. The results presented here indicate that the relationship between GHG fluxes and N rates is nonlinear. Assuming that the relationship is linear can either overestimate or underestimate predicted net GHG sink strength depending on the baseline status of the grasslands. Our findings suggest that some grasslands during this century will likely be at the N limitation stage and others will transition into the N saturation-decline stage. Overall, our results indicate that to improve our capacity for accurately predicting responses of GHG fluxes from grasslands, future experiments should be designed at multiple N loads consistent with projected N_{dep} rates.

Model predictions of NEP, CH_4 , N_2O , and NO fluxes were consistent with published literature values at both ambient and N addition treatments from grasslands, suggesting that DayCent accurately predicted ecosystem C and N fluxes in these ecosystems (Appendix S2, Figs S5–S7; Bouwman *et al.*, 2002). Annual CH_4 uptake rates from nonflooded grasslands were consistent with other similar studies including fertilized grasslands and agricultural land located in temperate and Mediterranean regions (Appendix S2, Fig. S5; Del Grosso *et al.*, 2000). Modeled annual CH_4 emissions from flooded grasslands were similar to estimates from temperate flooded, subtropical, and tropical savannahs and grasslands (Appendix S2, Fig. S7).

Increased N_{dep} enhanced NEP, but it plateaued at N loads between 20 and 50 kg N $\text{ha}^{-1} \text{yr}^{-1}$ in most grasslands, whereas others were already saturated at low N_{dep} rates ($<20 \text{ kg N ha}^{-1} \text{yr}^{-1}$; Fig. 3). Low critical N saturation thresholds for NEP found in this study were consistent with the observation that photosynthesis in some temperate and boreal forests levels out at low N_{dep} rates, whereas others are already saturated at ambient N_{dep} (Fleischer *et al.*, 2013). The response of NEP to increased N_{dep} in grasslands (-0.2 ± 0.1 and $167 \pm 35 \text{ kg C sequestered per kg N}$; Table 2) fell within the range of 5 to 225 kg C per kg N reported for forests and heathlands (Magnani *et al.*, 2007; De Vries *et al.*, 2009). It is likely that high variability of the sensitivity of NEP to N_{dep} is the result of how climate shapes the response of this C component to N additions, and how vegetation, soil microbial communities, soil properties, and management practices in grasslands across the globe respond to N addition (Matson *et al.*, 2002).

Increased N_{dep} did not affect CH_4 consumption in agreement with recent findings that CH_4 oxidizers in grasslands are not sensitive to changes in N applications, and thus, CH_4 consumption did not alter the net GHG sink strength of temperate and Mediterranean grasslands (Appendix S2, Fig. S8a; Liu & Greaver, 2009; Teh *et al.*, 2011). However, increased N_{dep} dramatically enhanced CH_4 emissions from temperate flooded, subtropical, and tropical grasslands (Appendix S2, Fig. S8b), increasing the net GHG source strength of these systems at relatively low N loads (Fig. 5). Given the predicted increase in anthropogenic N_{dep} in tropical and subtropical regions (Galloway *et al.*, 2008; Hietz *et al.*, 2011), it is likely that increased N_{dep} will stimulate net GHG losses in these ecosystems in the future.

In agreement with results from De Schrijver *et al.* (2008), for many agro-ecosystems including perennial grasslands, predicted emissions of N_2O and NO increased with N_{dep} , but increases in fluxes were less pronounced at higher N loads (Fig. 4). Less pronounced fluxes at higher N loads were likely related with increased losses of N leaching (Fig. 4c; McSwiney & Robertson, 2005). Given that N losses at these grasslands occurred even at low N loads, our results highlight the importance of quantifying ecosystem C gain and losses in tandem with N losses and support the argument that NEP cannot be solely used as an indicator of N saturation (Matson *et al.*, 2002; De Schrijver *et al.*, 2008).

There was considerable variability in the threshold at which different grasslands transitioned to the N saturation-decline stage (Fig. 5). This variability is likely explained by how multiple interacting factors at various climate zones drive the responses of GHGs to changes in N_{dep} such as environmental conditions, plant and soil microbial compositions, soil properties, and land management practices (Clark & Tilman, 2008; De Schrijver *et al.*, 2008). Disentangling the effects of these variables from those of N_{dep} is critical to improve predictions of the net GHG sink strength of grasslands to future N_{dep} rates.

Consistent with the view that fire generally stimulates NPP and accelerates plant N uptake from soils, reducing soil N losses as N-trace gases or leachates, the temperate grassland subjected to fire management transitioned to the N saturation-decline stage at higher N_{dep} rates than the unburned grassland (Fig. 5; Briggs & Knapp, 1995; Ansley *et al.*, 2006; Beringer *et al.*, 2007). This suggests that land management practices are important determinants of the N status of grasslands.

During the 21st century, N_{dep} is projected to increase over many regions across the world, especially for RCP8.5 predictions (Lamarque *et al.*, 2011, 2013). Only

in North America and Western Europe are N_{dep} rates projected to decrease (Lamarque *et al.*, 2011, 2013). In regions where N_{dep} is expected to increase, rates are projected to range from 0.5 to 22 kg N ha⁻¹ yr⁻¹ by 2100, whereas in those where N_{dep} is expected to decrease, rates are predicted to be below 7 kg N ha⁻¹ yr⁻¹ (Lamarque *et al.*, 2011, 2013). Grasslands are among the largest ecosystems in the world and occur naturally in all continents except in the Antarctica (Dixon *et al.*, 2014). Thus, most areas where grasslands dominate will likely experience increases in N_{dep} during this century.

In this study, we showed that the majority of published experimental loads in grassland ecosystems were higher than predicted future N_{dep} rates and corresponded to the N saturation-decline stage. Our results suggest that grasslands during the 21st century will likely be in the N limitation and intermediate stages, although it is likely that some will transition into the N saturation-decline stage and that these conditions were not mimicked in most previous experiments. These findings have important implications for constructing robust predictions of the net GHG sink strength of grasslands. First, global GHG estimates using linear relationships between GHGs and N are likely under- or overestimating predictions of the net GHG sink or source strength of grasslands to changes in N_{dep} (e.g., Schindler & Bayley, 1993; Liu & Greaver, 2009; Templer *et al.*, 2012; Pinder *et al.*, 2012). It is likely that linear-based estimates resulted in lower predictions of net GHG sink strength or higher predictions of source capacity of grasslands at the limitation and intermediate stages, whereas they overestimated predictions of net GHG sink strength or underestimated the source capacity of grasslands at the N saturation-decline stage compared to nonlinear-based approaches (Figs 1b and 5). Second, our ability to predict the net GHG sink strength of grasslands in response to changes in N_{dep} could be greatly improved by designing studies with N loads comparable with future N_{dep} rates. Prior studies that used high N loads provided foundational knowledge on how C and N processes in grasslands respond to N addition and provided fundamental mechanistic understanding for improving model predictions. However, the validity of model predictions depends on experimental data and improved mechanistic understanding of the relationship between GHG fluxes and N addition (Zaehle *et al.*, 2011; Lu and Tian, 2013). It is critical that the new generation of environmental change experiments is designed at multiple N loads consistent with future N_{dep} rates to advance future predictive efforts, assessments, and policy decisions regulating anthropogenic reactive N emissions.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Published studies used to determine if experimental N loading rates are consistent with future N_{dep} projected by RCPs.

Table S1. Published studies that investigated the effect of N addition on NEP, N_2O and CH_4 in grassland ecosystems.

Appendix S2. DayCent parameterization for site soil attributes, and published studies used to validate predicted above- and below-ground productivity, SOC, Net Ecosystem Productivity (NEP), N_2O and CH_4 fluxes.

Table S2. DayCent parameterization for site soil attributes. Site description, land use history, location, mean annual precipitation (MAP), and N treatments, current N deposition (N_{dep}), and the reference citation for each study site are described in Table 1.

Table S3. Published studies reporting Net Ecosystem Productivity (NEP) in temperate shortgrass and tallgrass prairies, Mediterranean grasslands, temperate floodplain grasslands, and tropical and subtropical grasslands used to validate modeled NEP.

Table S4. Published studies reporting N_2O and CH_4 in temperate floodplain grasslands, and tropical and subtropical grasslands used to validate modeled N_2O and CH_4 .

Figure S1. Observed and modeled aboveground NPP (ANPP) for the Konza (a; Seadstedt *et al.*, 1991) and the Irvine Ranch (b; Harpole *et al.*, 2007) sites.

Figure S2. Observed and modeled aboveground NPP (ANPP) values using the reported data for each site described in Table 1.

Figure S3. Observed and modeled belowground NPP (BNPP) values using the reported data for each site described in Table 1.

Figure S4. Observed and modeled Soil Organic Carbon (SOC) values using the reported data for each site described in Table 1.

Figure S5. Observed and modeled NEP (a), and N_2O (b) and CH_4 (c) fluxes for ambient and N addition treatments.

Figure S6. Observed and modeled Net Ecosystem Productivity (NEP).

Figure S7. Observed and modeled N_2O (a) and CH_4 (b) fluxes.

Figure S8. Modeled dose-response relationship between N_{dep} rates and annual net CH_4 uptake (a) and emission (b) for grassland ecosystems located at major climate zones using DayCent 4.5.