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GLOBAL CHANGE ECOLOGY – ORIGINAL RESEARCH

Nitrous oxide emissions decrease with plant diversity but increase with grassland primary productivity

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Abstract

Nitrous oxide (N_2O), a main greenhouse gas that contributes to ozone layer depletion, is released from soils. Even when it has been argued that agriculture is the main cause of its increase in the atmosphere, natural ecosystems are also an important source of N_2O. However, the impacts of human activities on N_2O emissions through biodiversity loss or primary productivity changes in natural ecosystems have rarely been assessed. Here, we analyzed the effects of vegetation attributes such as plant diversity and production, as drivers of N_2O emission rates, in addition to environmental factors. We measured N_2O emissions monthly during 1 year in 12 sites covering a large portion of the Rio de la Plata grasslands, Argentina, and related these emissions with climate, soil and vegetation attributes. We performed spatial and temporal models of N_2O emissions separately, to evaluate which drivers control N_2O in space and over time independently. Our results showed that in the spatial model, N_2O emissions decreased with increments in plant species richness, with concomitant reductions in soil NO_3^-, whereas N_2O emissions increased with primary productivity. By contrast, in the temporal model, monthly precipitation and monthly temperature were the main drivers of N_2O emissions, with positive correlations, showing important differences with the spatial model. Overall, our results show that biological drivers may exert substantial control of N_2O emissions at large spatial scales, together with climate and soil variables. Our results suggest that biodiversity conservation of natural grasslands may reduce regional greenhouse gas emissions, besides maintaining other important ecosystem services.

Keywords Ecosystem services · Greenhouse gases · Climate change · Biodiversity–ecosystem function relationship · Spatial and temporal drivers

Introduction

Nitrous oxide (N_2O) is one of the components in the atmosphere responsible for global warming and also causes ozone layer depletion (Ravishankara et al. 2009). N_2O is a long-lived, infrared absorbing trace gas, with a global warming potential 265 times larger than CO_2 that accounts for 6.2% of total atmospheric greenhouse gas (GHG) emissions. Its global atmospheric concentration has increased 40% since 1750 and continues increasing at a rate of 0.73 ppb year^{-1} (IPCC 2014). It has been argued that agriculture is one of the main causes of N_2O increments in the atmosphere, but unmanaged ecosystems are also an important source (Saggar et al. 2013). Natural grasslands are one of the major sources of N_2O emissions worldwide, accounting for approximately 18% of global N_2O emissions (Lee et al. 1997), and the current loss of biodiversity can have large impacts on the emission of this potent GHG from natural grasslands (Tilman et al. 2014). Whereas the Montreal Protocol has helped

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reverse the growth rate of the stratospheric ozone hole by regulating chlorofluorocarbon emissions, N_2O emissions are not regulated and, therefore, are expected to remain the dominant ozone-depleting source over the next decades (Ravishankara et al. 2009). Reducing nitrous oxide emissions would thus enhance the recovery rate of the ozone hole and simultaneously reduce the anthropogenic forcing of climate.

Nitrous oxide emissions from soils are mainly derived from denitrification (but see Butterbach-Bahl et al. 2013), a microbiological process controlled by several factors (Robertson 1989). Robertson's model (1989) classifies the factors that control denitrification in a continuum from proximal (more direct factors, with large variability in time or space, affecting the process) to distal (factors at a larger scale, with low variability in time or space, that indirectly affect the process). On this continuum, proximal factors include soil nitrate (NO_3^-) and ammonium contents (NH_4^+), soil oxygen concentration, soil moisture and soil temperature, which have been frequently studied (Conrad 1996; Davidson et al. 2000). In addition to these factors, ecological theory suggests that distal controls, such as soil texture, climate, plant diversity and aboveground net primary productivity (ANPP), could constrain or enhance N_2O emissions through the influence on proximal controls (Chapin et al. 2002; Niklaus et al. 2016). However, few studies have evaluated the impact of distal controls on N_2O emissions (but see Groffman et al. 2000). Distal controls could be potentially modified through management practices to contribute to GHG mitigation strategies (Saggar et al. 2013). In addition, some distal factors such as ANPP could be measured and monitored over large spatial scales, serving for national N_2O emissions inventories with feasible and affordable methodologies.

The relationship between biodiversity and ecosystem functioning has been deeply demonstrated through theoretical work and field experiments (Tilman et al. 2014), but they have rarely involved N_2O emissions. The strong stabilizing effect of biodiversity on several ecosystem processes and its relationship with ecosystem services and human well-being might include the mitigation of GHG emissions and consequent regulation of climate change (Balvanera et al. 2006). Plant species may regulate N_2O emissions through resource use, by reducing available substrates for denitrification (Niklaus et al. 2016). Highly diverse plant communities, such as those occurring in natural grasslands, constrain soil nitrogen (N) loss by niche complementarity that promotes elevated and continuous plant N uptake, decreasing soil inorganic N content (Tilman et al. 1996; Scherer-Lorenzen et al. 2013). However, the connection between biodiversity and N_2O emissions has not been assessed in natural field conditions, and the few mesocosm studies have shown contrasting results. Particularly, Niklaus et al. (2006, 2016) showed a negative relationship between plant species richness and N_2O emissions, while Abalos et al. (2014, 2017) showed that species identity or plant functional types determined N_2O emissions and not plant species richness.

Aboveground net primary productivity is a key ecosystem process (Sala 2001). Both ANPP and N_2O share similar drivers and usually increase with growing annual precipitation, mean annual temperature, and soil N availability (Sala, 2001; Groffman et al. 2000). Higher ANPP likely increases litter quantity and root exudates, increasing labile soil carbon for decomposition and mineralization, and enhancing N_2O emissions through nitrification and denitrification process (Firestone and Davidson 1989). On the other hand, plant N uptake may compete with denitrifiers for available soil N, potentially decreasing soil N_2O emissions (Niklaus et al. 2006). Previous studies analyzing the relationship between ANPP and N_2O emissions showed a positive correlation between them, both in grasslands and forest ecosystems (Groffman and Turner 1995; Wolf et al. 2011). Nevertheless, more studies are needed to generalize this pattern and to infer mechanistic relationships.

The relationships between ecosystem processes (such as N_2O emissions, ANPP or N mineralization) and its drivers may differ across space and time dimensions (Lauenroth and Sala 1992). For instance, Sala and colleagues (2012) discussed the variability of ANPP with annual precipitation considering space (from arid to sub-humid ecosystems, based on multi-year averages for each location) and time (based on year-to-year changes at each location) and argued that differences between these models are based on lag responses due to the inertial characteristic of the ecosystem structure. In addition, the same factors may differ in the range of variation comparing spatial and temporal dimensions. For instance, plant species composition and soil type vary more across space (comparing different sites) than over time, at least in short- to mid-term temporal scales (within a year or in a few years) (Perelman et al. 2001). Therefore, we do not expect that plant species composition or soil texture affects N_2O emissions in short- to mid-term time scales and, on the other hand, climate variables such as monthly precipitation and temperature may not vary within nearby sites (some kilometers away). So, these factors will not affect N_2O variability in spatial models of a climate-homogeneous region but are expected to influence in temporal models. Therefore, spatial and temporal models of ecosystem functioning, including N_2O emissions, may show differential drivers and should therefore be treated separately (Groffman et al. 2009).

Here, we present a regional field study that separately analyzes spatial and temporal models of N_2O emissions considering distal controls, such as climate variables, plant species richness, soil texture, and ANPP, and proximal controls such as soil nutrients, moisture and temperature. Our main objective was to assess the importance of plant attributes in determining N_2O emissions in both spatial and temporal models separately, in addition to abiotic factors. First, we expected that a negative relationship between species richness and N_2O emissions will be evident when comparing different sites (spatial model), but not when the same sites are analyzed over time (temporal model), since species richness has a small range of variation throughout the year. Second, we hypothesize that ANPP (distal control) will be positively associated with soil N contents and N_2O emissions because similar climate factors, such as precipitation and temperature, are distal controls of both processes (Robertson 1989; Groffman and Turner 1995; Groffman et al. 2000; Wolf et al. 2011). Therefore, we expected that the relationship between ANPP and N_2O will be strong in both temporal and spatial models. On the other hand, variations in climate variables are larger in time throughout the year than in nearby sites of relatively climate-homogeneous region; we thus expect that climate factors explain N_2O emissions in the temporal model. We evaluated these relationships across 12 sites in the Rio de la Plata grasslands region of Argentina (Fig. 1) and constructed spatial and temporal linear mixed-effects regressions models, based on our best knowledge of plant–soil interactions affecting N_2O emissions. We expected to find strong relationships between distal controls and N_2O emissions, in addition to proximal controls, and that these relationships will differ between temporal and spatial models.

Materials and methods

Site description and experimental design

The study was conducted at 12 field sites encompassing two sub-regions of the *Rio de la Plata* grasslands, the Inland Pampas and the Mesopotamic Pampas in Argentina (Table 1; Fig. 1; Soriano 1991). In both sub-regions, we selected 12 sites with relict grasslands (6 sites per sub-region) between 1 to 12 ha and installed one exclosure in each site (up to 50 m^2) to ensure lack of disturbances during the experiment. Sites were selected based on satellite images and landowners advice, to avoid relict grasslands that had disturbances such

Table 1 Summary of soil variables describing the Mesopotamic (MP) and the Inland Pampas (IP) sub-regions. CEC: cation exchange capacity. Values are average of six sites per sub-region ± 1 standard error. *F* and *P* values show differences between the two sub-regions

	MP	IP	*F*	*P*
Sand (%)	18.92 ± 3.3	46.90 ± 3.5	28.3	< 0.01
Silty (%)	58.30 ± 4.5	40.32 ± 2.4	11.7	< 0.01
Clay (%)	23.65 ± 2.6	12.80 ± 1.2	10.3	< 0.01
pH (1:2.5_H_2O)	5.47 ± 0.1	6.07 ± 0.1	17.2	< 0.01
C (g kg^{-1})	26.59 ± 2.4	23.23 ± 1.9	1.0	0.33
N (g kg^{-1})	2.27 ± 0.2	1.97 ± 0.2	0.9	0.36
P (mg kg^{-1})	21.25 ± 11.2	15.85 ± 1.7	0.2	0.67
CEC (cmolc kg^{-1})	24.50 ± 3.1	18.62 ± 1.9	2.1	0.17

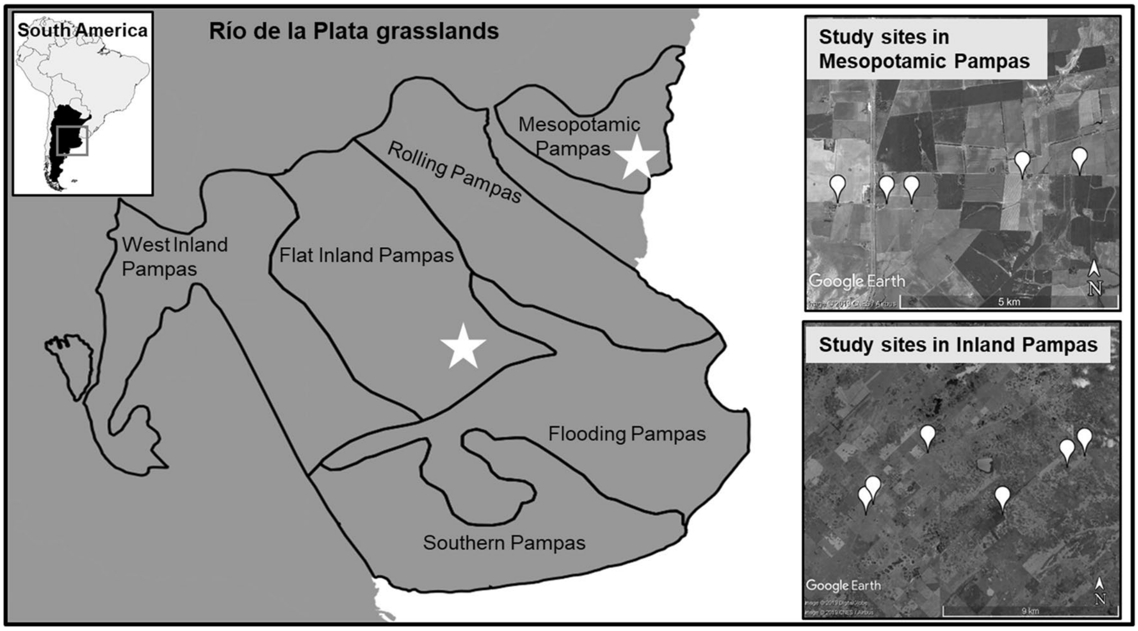

Fig. 1 Maps showing location of two sub-regions (stars) and location study sites (bubbles) in Rio de la Plata grasslands, Argentina (modified from: Soriano 1991). Insets on the right show site locations in each sub-region: Inland and Mesopotamic Pampas

as grazing, fertilization, cropping, etc. The climate at both sites is sub-humid, with long-term average annual rainfall around 960 mm (WorldClim global climate database version 1.4; Hijmans et al. 2005). Rainfall during our study period was higher than average, 1012 mm and 1507 mm, in the Inland and the Mesopotamic Pampas, respectively (Online Resource 3). Mean monthly temperatures during the study period ranged from 9 to 23 °C and from 13 to 25 °C in the Inland and Mesopotamic Pampas, respectively (Online Resource 3). Both sub-regions have a history of land-use change where livestock development and crop production replaced natural grasslands (Soriano 1991). Nevertheless, relicts of natural tussock grasslands remain in small dispersed and linear fragments (between 1 and 12 ha) along fences, roadsides and train rail lines (Burkart et al. 2011). Typically, relict grasslands are scarcely or never grazed, fertilized or sown, and are dominated by native perennial and annual grasses (*Poaceae*) and forbs (*Asteraceae*), and usually contain few exotic grasses (Table 2 and Online Resource 1).

N_2O fluxes measurements

Nitrous oxide emissions were measured near-monthly from November 2013 to October 2014 using two static chambers randomly located inside each exclosure (Parkin and Venterea 2010; De Klein and Harvey 2015). A total of eight sampling dates were considered in our analyses for the Mesopotamic Pampas sub-region and nine sampling dates for the Inland Pampas. Plastic chambers were 37 cm × 25.5 cm × 14 cm high (13.2 L), covered by a light-reflecting aluminum film and vented with a 10 cm long stainless-steel tube to equalize air pressure inside and outside. The chambers were placed on iron bases previously buried and sealed with water, following strictly USDA protocols (Parkin and Venterea 2010). Iron bases were buried 8 cm deep 1 month before measurements, to avoid effects derived from base installation (Parkin and Venterea 2010).

At each sampling date air samples were extracted from chambers using a manual vacuum pump and stored in 10 ml vials; this small volume of sample was collected so as to not generate any large pressure disturbances in the chamber. Each sample consisted of three gas extractions from chambers at approximately 0, 20, and 40 min from chamber deployment, following the USDA protocol (Parkin and Venterea 2010). Samples were taken from 10:00 AM to 12:30 AM, when temperature approaches the daily average (Parkin and Venterea 2010; Cosentino et al. 2012).

Gas samples were analyzed within 10 days of collection, using a Gas Chromatographer (GC) 7890A with automatic sampler 7697A Agilent Technologies (Agilent Technologies Network) in the Facultad de Agronomía de la Universidad de Buenos Aires, Argentina. The GC was fitted with a 63Ni micro electron capture detector. The columns used were G3591-81004 6Ft 1/8 2 mm HayeSep Q 80/100 UM and G3591-81121 12Ft 1/8 2 mm HayeSep Q 80/100 UM. Oven temperature was 60 °C, both Loop and Transfer temperatures were 75 °C and detector (µEDC) temperature was 300 °C. N_2 was used as carrier gas and Argon Methane 5% as make up gas (µECD). The N_2O fluxes were calculated based on changes in three sub-samples of N_2O concentrations over time (at approximately 0, 20, and 40 min from chamber deployment) using linear regression and taken the regression slope parameter as the flux of N_2O emission, following De Klein and Harvey (2015).

Soil characterization and microclimate measurements

At the beginning of the experiment, soil samples were taken at each of the 12 sites for initial site characterization (Table 1). Five soil sub-samples were taken at each site

Table 2 Average plant species composition and Normalized Difference Vegetation Index (NDVI) values of the two sub-regions, the Inland Pampas (IP) and the Mesopotamic Pampas (MP)

Sub-region	Species richness	Mean NDVI ± SE	Family	Family frequency (%)	Most frequent species	Average abundance (%)
MP	43	0.64 ± 0.03	Poaceae	29	*Cynodon dactylon*	27.7 ± 0.3
					Paspalum dilatatum	39.1 ± 0.5
					Setaria geniculata	12.2 ± 0.9
			Asteraceae	7	*Baccharis* sp.	2.4 ± 0.3
IP	56	0.47 ± 0.03	Poaceae	17	*Paspalum quadrifarium*	70.3 ± 0.6
					Festuca sp.	46.1 ± 0.8
					Bromus unioloides	38.2 ± 1.7
					Sorghum halepense	11.5 ± 2.0
					Cynodon dactylon	11.4 ± 0.2
			Asteraceae	9	*Picris* sp.	5.1 ± 0.0

Average abundance values are the mean cover of the most abundant families (Poaceae and Asteraceae) and species, ± 1 SE. See Online Resource 1 for the complete list of plant species inside the exclosures with a 2-cm-wide soil corer from 0 to 10 cm depth, and texture (sand, silt and clay percentage), total carbon, nitrogen and phosphorous content, soil pH and cation exchange capacity were measured from each composed soil sample, following standard laboratory procedures (Robertson et al. 1999). Soil texture (sand, silt, and clay percentage) was estimated by the Bouyoucos method (Bouyoucos 1962). Total organic carbon and nitrogen contents were estimated by Walkley–Black technique and Kjeldahl digestion, respectively (Robertson et al. 1999). Phosphorous content was estimated following Bray and Kurtz method (Bray and Kurtz 1945). Soil pH (soil:H_2O 1:2.5) and cation exchange capacity were estimated following Summer and Miller (1996). In addition, at each sampling date, after N_2O measurements, three 0–10 cm depth soil cores were extracted from within each iron base (two per exclosure) and analyzed for soil NO_3^- and NH_4^+ content, soil bulk density, pH, porosity, and soil moisture, to estimate soil water filled pore space (WFPS). Samples were extracted with 2 M KCl and analyzed for inorganic N (N-NO_3^- and N-NH_4^+) using the steam distillation method in the presence of MgO and Devarda's alloy (Keeney and Nelson 1982). Soil bulk density was estimated following Blake and Hartge (1986). Soil porosity was calculated, assuming particle density of 2.65 g cm^{-3} (Linn and Doran 1984), as:

$$\text{Soil porosity} = 1 - \left(\frac{\text{Bulk density}}{\text{Particle density}}\right).$$

From each soil sample, a 5 g sub-sample was dried at 105 °C for 48 h to calculate soil water content and then WFPS was calculated as:

$$\text{WFPS (\%)} = \frac{\text{Soil water content}\left(\frac{g}{g}\right)}{\text{Soil porosity}} \times \frac{\text{Bulk density}}{\text{Density of water}} \times 100.$$

Soil temperature (average of 0–10 cm depth) and air temperature were also measured at each sampling date with handheld thermometers. Also, for both sub-regions, monthly precipitation and monthly mean, maximum and minimum temperatures were retrieved from nearby weather stations (Table 1).

Plant community measurements

At each N_2O sampling date, plants growing inside the iron bases were identified to the species level and their relative abundance was estimated visually based on their above ground cover (Table 2). Species richness was estimated based on species census performed inside the two bases established at each site (24 census each month, Online Resource 1). We recorded 56 different plant species in the Inland Pampas sub-region and 43 species in the Mesopotamic Pampas sub-region (Online Resource 1). Poaceae and Asteraceae were the most frequent families in both sub-regions (see Table 2 and Online Resource 1). Aboveground net primary productivity from each site was assessed through the Normalized Difference Vegetation Index (NDVI), estimated using a manual radiation sensor (Skye-SpectroSense2®) or a handheld digital camera. We took at least three NDVI measures inside each base and then averaged them to obtain a proxy of aboveground net primary productivity of each site (Piñeiro et al. 2006; Paruelo et al. 1997). These measures were taken at each sampling date from 11:00 AM to 2:00 PM to avoid deviations from the solar zenith angle (Table 2).

Statistical analysis

To analyze N_2O emission drivers and the relationships between distal and proximal controls, we adjusted linear mixed-effects simple and multiple regression models, with sub-regions as a random factor, separately for a spatial dataset (spatial model) and a temporal dataset (temporal model) as explained below. The rationale for including the sub-regions as a random factor was the large variability in soil characteristics observed between sub-regions (Table 1). Linear effect models were fitted by maximum likelihood to compare among models but the reported values of the selected models were fitted by restricted maximum likelihood following standard statistical methods as proposed by Zuur et al. (2009) (see Online Resource 2 for details). N_2O emissions were log-transformed to approximate a normal distribution of residuals. Best spatial and temporal models were selected based on the lowest AICc (Aakike Index Criterion corrected by small samples) and when all selected variables were statistically significant with $P < 0.05$ (Zuur et al. 2009).

Using an automated stepwise procedure, we selected significant models for the spatial and the temporal models. We searched the best models that explain N_2O emissions but also species richness, NDVI, NO_3^- and NH_4^+, which are our main biological and soil variables measured to elucidate potential mechanisms that could explain N_2O emissions. To evaluate spatial changes in N_2O emissions (spatial model), we used a dataset constructed with the averaged values of N_2O emissions across the sampling period for each of the 12 sites (average of nine or eight temporal measurements, depending on the sub-region) resulting in 12 observations for each variable in the spatial model. For the temporal model, we constructed a dataset where we averaged measurements made in the six sites of each sub-region performed in the same month, to eliminate variations due to local spatial heterogeneity between sites. Therefore, we had two sub-regions over nine and eight sampling dates, making a dataset with 17 observations for each variable. On all models, we did not include variables with autocorrelation in the same model (see correlation matrices in the Online Resource 2). In spatial and temporal regression models, we analyzed our data considering the sub-regions (Inland and Mesopotamic Pampas) as a random factor in the mixed-effects models. These analyses were performed with package *nlme*, *MuMIn* and *lmerTest* in R software (Barton 2016; Kuznetsova et al. 2016; R core team 2017), which allows the use of mixed-effects models on the selection routine of the best models by *dredge* function in *MuMIn* package.

Finally, we also analyzed the effects of species composition on N_2O emissions, to detect effects associated with community assemblages. To analyze the relationship between N_2O emissions and species composition, we performed a correspondence analysis (CA), with the package *vegan* in R software (Oksanen et al. 2017). In each sampling date, plant species were determined inside the two iron bases (sub-samples), and these estimations were averaged considering a complete list of species richness between both bases. CA for spatial and temporal dimension was performed based on the same datasets as explained before. Thus, spatial values represent the mean species abundance throughout the year for each site and temporal values represent the mean abundance across sites of each sub-region. We related the first two CA axes (in the spatial dimension: first and second axes explained 27% and 23% of the total variance, respectively; in the temporal dimension first and second axes explained 47% and 13% of the total variance, respectively) with N_2O emissions for spatial and temporal dimensions separately considering the factor sub-region with two levels (Inland and Mesopotamic Pampas) as a random factor in the mixed-effects models.

Results

Considering the spatial dimension, N_2O emissions were mainly controlled by distal variables (species richness and ANPP) that significantly explained variations in emissions throughout the region (Table 3). Nitrous oxide emissions significantly decreased with species richness ($P < 0.01$, $R^2 = 0.39$, Fig. 2a, Table 3), whereas N_2O emissions increased with NDVI, our proxy of ANPP ($P < 0.01$, $R^2 = 0.31$, Fig. 2b, Table 3). As expected, N_2O emissions also significantly increased with soil NO_3^- content ($P < 0.01$, $R^2 = 0.46$, Fig. 2c, Table 3). In addition, species richness negatively affected soil NO_3^- contents ($P < 0.05$, $R^2 = 0.44$, Fig. 2d, Table 3) and soil NO_3^- was positively related with NDVI ($P < 0.01$, $R^2 = 0.14$, Fig. 2e, Table 3). Sand content negatively affected species richness ($P < 0.01$, $R^2 = 0.59$, Fig. 2f, Table 3), whereas other soil variables such as pH, total soil carbon, nitrogen, phosphorous content, and cation exchange capacity (CEC) were not included in the best spatial models (Table 3 and Table 1, Online Resource 2), due to their scarce variation in the region (Table 1, Fig. 2). Overall, the best spatial model, with two predictor variables, included the species richness and ANPP, estimated through NDVI, showing that NDVI explained a large portion of the variance not accounted for by species richness (Table 3, Fig. 2). The other variables tested with the selection procedure (soil NH_4^+ content, WFPS, soil gravimetric and volumetric humidity, field soil and air temperature, mean monthly precipitation, and mean monthly temperatures) did not show significant relationships with N_2O emissions in the spatial models (see Online Resource 2). Finally, plant species composition assessed through the correspondence analysis scores was not related with N_2O emissions across sites (CA axis 1: P value = 0.72 and CA axis 2: P value = 0.84) (see Online Resource 2).

Table 3 Best simple and multiple spatial linear mixed-effects regression models selected by AICc for N_2O emissions, species richness, NO_3^- and NDVI

Model	Standardized slope	*df*	AICc	R^2
logN_2O (simple regression)				
Species richness	−1.02	4	38.33	0.39
NO_3^-	0.77	4	37.54	0.46
logN_2O (multiple regression)				
Species richness	−0.80	5	32.93	0.74
NDVI	0.79			
Species richness				
Sand	−0.78	4	55.53	0.59
NO_3^-				
Species richness	−0.68	4	41.29	0.44
NDVI				
NO_3^-	0.55	4	−23.61	0.14

Tested variables were: carbon content, Normalized Difference Vegetation Index (NDVI), soil ammonium (NH_4^+) and nitrate content (NO_3^-), soil pH, Species richness, soil temperature and water filled pore space. Note that: NH_4^+ is not shown as response variable because no significant relationships were found

df Degrees of freedom, *AICc* Akaike index criterion value corrected for small samples

Temporal controls of N_2O emissions, which described intra-annual variations in N_2O emissions during the experimental period, differed from the controls revealed by spatial models. In the temporal models selection, only mean monthly precipitation ($P < 0.05$, $R^2 = 0.28$, Table 4, Fig. 3a) and mean monthly minimum temperature were associated with temporal variations in N_2O emissions throughout the year ($P < 0.05$, $R^2 = 0.27$, Table 4, Fig. 3b). These two distal controls showed the larger intra-annual variations and, therefore, were both selected as the best temporal models with one variable (Table 4), whereas none of the other variables measured (species richness, NDVI, soil NO_3^- and NH_4^+ content, WFPS, soil gravimetric

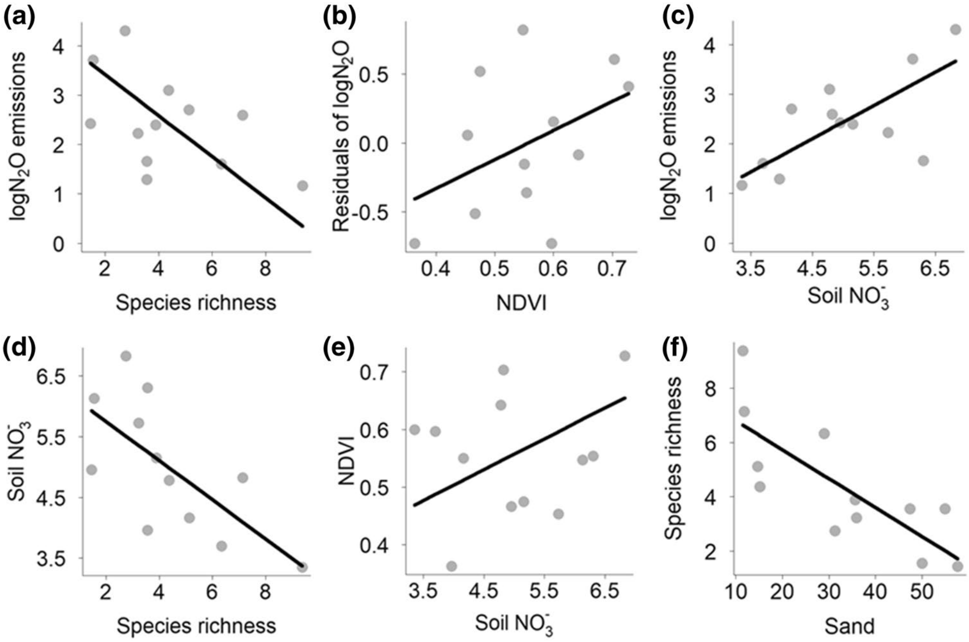

Fig. 2 Estimated spatial linear mixed-effects regression models for all $P < 0.05$. Panels show the relationship between **a** N$_2$O emissions (log N$_2$O μg N m^{-2} h^{-1}) from grassland soils and species richness; **b** N$_2$O emissions and Normalized Difference Vegetation Index (NDVI), considered as a second factor in a multiple regression model (response variable represents residuals of N$_2$O emissions, see Table 3); **c** N$_2$O emissions and soil NO$_3^-$ (μg N g soil^{-1}); **d** soil NO$_3^-$ (ug N g soil^{-1}) and species richness; **e** NDVI and soil NO$_3^-$ (μg N g soil^{-1}); and **f** species richness and soil sand percentage (see Table 3 for regression coefficients). Each point corresponds to an observation in the spatial dataset (see text for details)

Table 4 Best simple temporal linear mixed-effects regression models selected by AICc for N$_2$O emissions and Normalized Difference Vegetation Index (NDVI)

Model	Standardized slope	*df*	AICc	R^2
logN$_2$O				
Monthly precipitation	0.58	4	29.84	0.28
Mean monthly temperature	0.56	4	30.60	0.27
NDVI				
Monthly precipitation	0.70	4	− 30.52	0.48

Tested variables were: monthly precipitation, NDVI, soil ammonium (NH$_4^+$) and NO$_3^-$ content, species richness, mean monthly temperature, and water filled pore space

df Degrees of freedom, *AICc* Akaike index criterion value corrected for small samples

and volumetric humidity, soil temperature, and air temperature) were significantly associated with N$_2$O emissions through time (Table 4). Additionally, monthly precipitation was positively associated with NDVI ($P < 0.01$, $R^2 = 0.48$, Fig. 3c, Table 4). Plant species composition assessed through the scores of the correspondence analysis as predictor variables were not related with N$_2$O emissions over the year (CA axis 1: P value = 0.07 and CA axis 2: P value = 0.44) (see Online Resource 2).

Discussion

Our study showed for the first time that distal biological attributes, such as species richness and primary productivity, are as important as environmental controls in explaining spatial changes in N$_2$O emissions from natural unmanaged grasslands. Indeed, we only found sand content, in addition to biological variables, as an important distal driver of N$_2$O emissions at a regional scale, and its effect was indirect, through a decrease in plant species richness. By contrast, environmental distal controls, such as monthly precipitation and mean temperature, were the main drivers of N$_2$O emissions throughout the year, suggesting that different drivers explained variations of the emissions of this potent greenhouse gas in space and time (Fig. 4). To illustrate all results together, we performed two conceptual figures, synthesizing the best spatial and temporal linear mixed-effects regression models selected (Fig. 4), showing the significant relationships between all variables.

Our spatial analysis in the Pampas region showed that plant species richness was the main control of N$_2$O emissions at a regional scale (Table 3, Fig. 4a). This result supports the biodiversity–ecosystem function hypothesis based on niche complementarity, which states that higher plant species richness increases resource capture (such as soil NO$_3^-$, Fig. 4a) and consequently decreases N$_2$O emissions

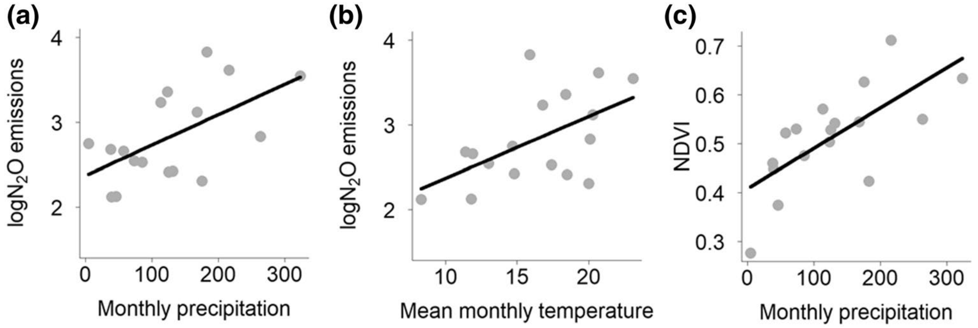

Fig. 3 Estimated temporal linear mixed effect regression models for all $P < 0.05$. Panels show the relationship between **a** N_2O emissions (log N_2O μg N m^{-2} h^{-1}) and monthly precipitation (mm); panel **b** N_2O emissions (log N_2O μg N m^{-2} h^{-1}) from grasslands and mean monthly temperature (°C); **c** Normalized Difference Vegetation Index (NDVI) and monthly precipitation (mm) (see Table 3 for regression coefficients). Each point corresponds to an observation in the temporal dataset (see text for details)

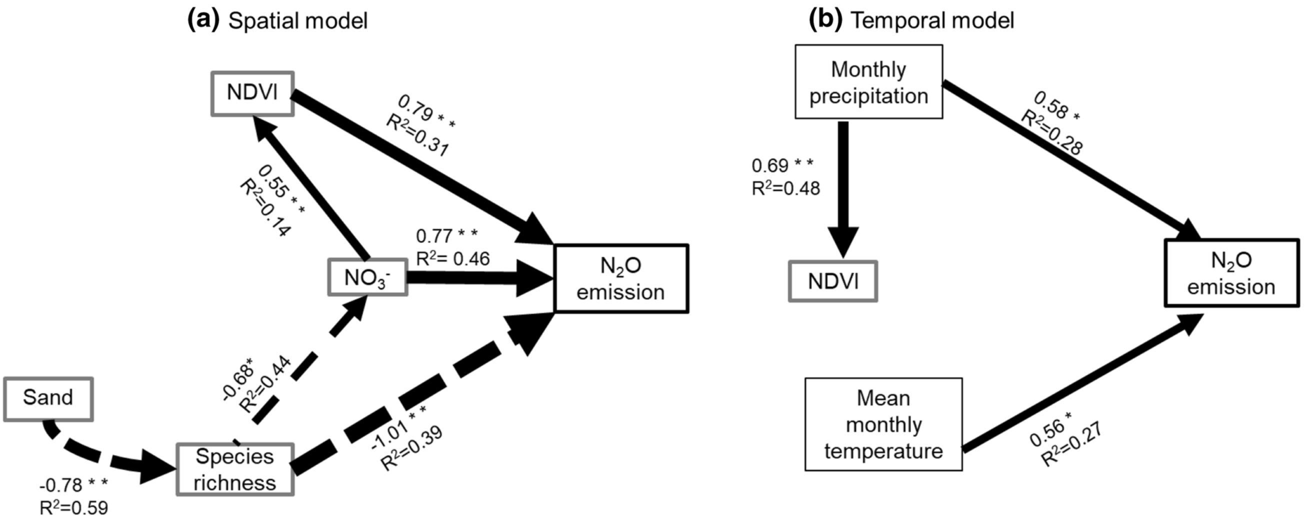

Fig. 4 Conceptual synthesis of linear mixed-effects regression models found for **a** spatial and **b** temporal models of N_2O emissions. Black arrows denote significant direct positive relationships between variables and dashed black lines show indirect relationships. Standardized slope coefficients and R^2 are shown near the arrows, together with their significance ($*P < 0.05$, $**P < 0.01$). Only significant variables are included in the diagram (see text and Online Resource 2 for details). This conceptual model synthesizes all the best models selected by AICc (see text for details)

(Tilman et al. 1996; Niklaus et al. 2016). In addition, we expected lower emissions from sandy soils, which were less likely to experience anoxia, known to stimulate N_2O emissions (Brentrup et al. 2000). However, soil sand contents at regional scales would increase N_2O emissions, instead of decreasing it, probably through its potential indirect effect of decreasing plant species richness (Fig. 4a), supporting findings obtained by Niklaus et al. (2006, 2016) in mesocosm studies.

ANPP estimated through NDVI in our study was positively correlated with regional N_2O emissions after accounting for species richness effects, in support of findings reported by Groffman and Turner (1995) and Wolf et al. (2011), although these studies had lower temporal sampling resolution (only 4 and 6 measurements per year). Primary productivity is usually associated with higher precipitation, temperature and soil N availability, all factors that increase N_2O emissions (Sala 2001; Groffman et al. 2000). Therefore, at large spatial scales NDVI can potentially be used to monitor N_2O emissions, creating maps and scaling up for reliable N_2O accounting in national greenhouse gas inventories. Other distal factors, in addition to ANPP, species richness or soil texture, can be explored in other regions and used to make spatial estimates of N_2O emissions based on available datasets. More research on these findings should be encouraged, specifically considering spatial vs temporal models separately.

We did not detect significant effects of plant species composition on N_2O emissions, neither in spatial nor in temporal models, as opposed to findings reported by Abalos et al. (2014, 2017) and Niklaus et al. (2006) in mesocosms. These opposite findings could be due to the absence of legumes in our unmanaged grasslands that were intentionally included in the synthetic plant assemblages performed in mesocosm studies. Legumes were almost completely absent in our field sites and, when present, were particularly small sized (see Online Resource 1). In addition, in our natural grasslands plant community assemblages are a result of long-lasting interactions, contrary to mesocosm's synthetic assemblages (Flombaum and Sala 2008).

As previously found for other ecosystem processes, separating spatial vs temporal models in our analyses enhanced the detection of species richness and NDVI as key controls of the regional variations in N_2O emissions (Lauenroth and Sala 1992; Sala et al. 2012). Our spatial models linked distal controls (as species richness, primary productivity, and soil texture) with proximal controls (NO_3^-) on denitrification processes, as proposed by Robertson (1989). As previously proposed by Sala et al. (2012) by relating ANPP with precipitation, our results suggest that we should not use the patterns obtained from repeated measures in one site, to make inferences about N_2O emissions at different sites. Considering this premise, further research with large datasets would considerably expand our current knowledge on N_2O emissions based on both the classic "hole in the pipe" model (Firestone and Davidson 1989; Davidson et al. 2000), and the continuum from distal to proximal controls proposed by Robertson (1989). We believe that our study represents the first showing spatial and temporal models of N_2O emissions from unmanaged grasslands, in addition to Groffman et al. (2000).

Also, according to Groffman et al. (2000), we found that distal climate factors such as precipitation and temperature were the main controls of intra-annual variations in N_2O emissions. However, our dataset included monthly measurements over 1 year, so we did not evaluate interannual controls of N_2O emissions. Sala (2001) suggested that structural constraints in grasslands, such as plant community or soil quality, may not show large temporal variations and, therefore, are not usually related to temporal changes in different ecosystem processes, such as N_2O emissions. In other words, intra-annual variations in species richness, or primary productivity were probably not large enough to significantly correlate with N_2O emissions over time in our study. In addition, previous studies showed that soil water content, soil inorganic nitrogen, and soil temperature are the main proximal controls of N_2O emissions in temporal models (Robertson 1989). The lack of significant relationships among these proximal controls and N_2O emissions in our dataset could be due to the short time frame of analysis. Longer temporal datasets are needed to confirm the temporal connections between distal and proximal controls, and their effects on N_2O emissions (Groffman et al. 2000).

Although usually not explicitly separated, spatial and temporal dimensions have different implications for policy and decision-making. In this study, our proposed conceptual spatial model (Fig. 4) suggests two key policy strategies for climate change mitigation. First, conserving grassland biodiversity will reduce soil NO_3^- contents and consequently, N_2O emissions. In fact, this would generate a tighter and closed N cycle, with additional benefits in terms of other kinds of environmental pollution like nitrate leaching (Tilman et al. 1996; Erisman et al. 2013; Smith 2017). Second, grasslands conservation programs may focus mainly on sandy soils, where lower species richness produces higher N_2O emissions. In the temporal dimension, by contrast, current climate change models forecast global warming increases in the frequency of extreme events (IPCC, 2014), which could enhance denitrification rates. In addition, global change involves biodiversity loss, which along with climate change predictions may hamper ecosystem stability and promote higher temperature increases through a feedback mechanism (Sala et al. 2000; IPBES see Díaz et al. 2015). As a consequence, biodiversity conservation of natural grasslands may reduce greenhouse gas emissions, in addition to maintaining other important ecosystem functions.

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Author contribution statement JMPG, LY, TDC and GP conceived the ideas and designed the methodology, JMPG and TDC collected and analyzed the data. JMPG led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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