Phosphorus Alleviation of Nitrogen-Suppressed Methane Sink in Global Grasslands

Lihua Zhang[1], Fenghui Yuan[2], Junhong Bai[3], Hongtao Duan[4], Xueying Gu[4], Longyu Hou[4], Yao Huang[5], Mingan Yang[2], Jin-Sheng He[6], Zhenhua Zhang[7], Lijun Yu[4], Changchun Song[8], David Lipson[2], Donatella Zona[2], Walter Oechel[2], Ivan Janssens[9], and Xiaofeng Xu[2]

[1]Minzu University of China
[2]San Diego State University
[3]Beijing Normal University
[4]Chinese Academy of Sciences
[5]Institute of Botany, Chinese Academy of Sciences
[6]Peking University
[7]Chinese Academy of Sciences, Northwest Institute of Plateau Biology
[8]Chinese Academy of Sciences Changchun Branch
[9]University of Antwerp

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Abstract

Grassland ecosystems account for more than 10% of the global CH4 sink in soils. A 4-year field experiment found that addition of P alone did not affect CH4 uptake and experimental addition of N alone significantly suppressed CH4 uptake, while concurrent N and P additions suppressed CH4 uptake to a lesser degree. A meta-analysis including 382 data points in global grasslands corroborated these findings. Global extrapolation with an empirical modeling approach estimated that contemporary N addition suppresses CH4 sink in global grassland by 11% and concurrent N and P deposition alleviates this suppression by 6%. The P alleviation of N-suppressed CH4 sink is primarily attributed to substrate competition, defined as the competition between ammonium and CH4 for the methane monooxygenase enzyme. The N and P impacts on CH4 uptake indicate that projected increases in N and P depositions might substantially affect CH4 uptake and alter the global CH4 cycle.

Introduction

Methane (CH_4) is an important greenhouse gas, contributing approximately 20% to the anthropogenic climate warming (IPCC 2013). Grasslands cover more than 20% of the Earth's land surface and typically serve as a net sink for atmospheric CH_4(Aronson & Helliker 2010; Chen *et al.* 2011; Dijkstra *et al.* 2013). However, this CH_4 sink may be altered by the growing input of nutrients, particularly nitrogen (N) (Galloway *et al.* 2008), due to anthropogenic activities. While previous studies have reported various impacts of N (particularly NH_4^+) addition on the oxidation of atmospheric CH_4 in soil (Bollag & Czlonkowski 1973; Liebig *et al.* 2008), it has become clear that N addition typically suppresses this oxidation process, with few exceptions (Aronson & Helliker 2010; Liu *et al.* 2017). This N-induced suppression of CH_4 oxidation has been attributed to two mechanisms. First, CH_4 and NH_4^+ compete for the same methane monooxygenase (MMO) enzyme (Gulledge *et al.* 2004), which can oxidize both CH_4 (to CH_3OH) and NH_4^+ (to NO_2^-)

due to the similar molecular structure of CH_4 and NH_4^+ (Dunfield & Knowles 1995; Gulledge *et al.* 2004). Second, the intermediates and end products (primarily nitrite) of methanotrophic NH_4^+ oxidation can be toxic to methanotrophic bacteria, explaining the inhibition of CH_4 consumption under increased N inputs (Schnell & King 1994; Bodelier & Laanbroek 2004). According to the substrate competition mechanism, added NH_4^+reduces the amount of CH_4 consumed by methanotrophic bacteria, based on enzyme kinetics (Davidson & Schimel 1995; Singh & Strong 2016), and inhibits the growth of methanotrophic bacteria as oxidizing NH_4^+ does not support CO_2 fixation for cell growth (Bédard & Knowles 1989; Carlsen *et al.* 1991; Gulledge & Schimel 1998), further suppressing the CH_4 oxidation (Gulledge & Schimel 1998).

While the negative impact of N addition is thus well understood, phosphorus (P) addition may alter this N-induced suppression on CH_4 oxidation. In a recent study, P addition alone was reported to enhance CH_4 oxidation (Veraart *et al.*2015). Given that P-fertilizer inputs and atmospheric P deposition are also increasing globally (Mahowald *et al.* 2008; Penuelas *et al.* 2012; Penuelas *et al.* 2013), investigating P impacts on CH_4 uptake rate, and especially its interactions with the increasing N inputs, is important for better understanding the role that aerobic soils play in the global atmospheric CH_4cycle.

Atmospheric deposition and fertilizer inputs of N and P have increased across the globe since the industrial revolution (Mahowald *et al.*2008; Blankinship *et al.* 2010; Penuelas *et al.* 2013; Brahney *et al.* 2015; Wang *et al.* 2017). Increased inputs of N may reduce the CH_4 sink strength of grassland ecosystems, with potentially important feedbacks to the global climate system (Templer *et al.* 2012; Tian *et al.* 2015). The interactive effect of N + P additions on CH_4 oxidation has been reported as neutral (Lund *et al.* 2009) or positive (Zhang *et al.* 2014), and P has been found to alleviate the N suppression on CH_4 oxidation (Zhang *et al.* 2014); however, the mechanisms by which P alters the N suppression of atmospheric CH_4 oxidation in soils remain elusive. We therefore integrated a 4-year field manipulation experiment, a meta-analysis, and an empirical modeling approach to investigate the effects of N + P additions on CH_4 flux in grasslands and the underlying mechanisms.

Materials and Methods

Field Experiment

The field experiment was conducted in Duolun County (42°02'N, 116°70'E, 1324 m a.s.l.), a semiarid temperate steppe in Inner Mongolia, northern China. The multiple-year mean annual precipitation and mean annual temperature are approximately 385 mm and 2.1°C, respectively. The topography is characterized by low foothills at elevations of 1150-1800 m. Soil is Calcis-orthic Aridisol (the US Soil Taxonomy classification), with 62.75 ± 0.04% sand, 20.30 ± 0.01% silt and 16.95 ± 0.01% clay (Wu *et al.* 2010). The dominant plant species include *Stipa krylovii* , *Artimesia frigida* , *Potentilla acaulis* ,*Cleistogenes squarrosa* , *Allium bidentatum* and*Agropyron cristattum* .

To assess the nitrogen (N) and phosphorus (P) impacts on CH_4 flux, a block design experiment with different combinations of N and P additions was established in early 2013 and run to 2016. A complete random design, with three block replicates, was adopted to address the high spatial heterogeneity. There were four experimental treatments (i.e., control (CK), N addition (N), P addition (P), both N and P additions (N+P)), which were randomly assigned to four 6 m × 6 m plots in each block. Two chambers for greenhouse gas measurement were set up in each plot. All blocks were separated with a 3-m buffer zone. The N and P were applied twice per month from May to September during 2013-2016; we sprayed the fertilizer solution to ensure that the application was evenly distributed in the plots. The dose of N addition was 100 kg N ha^{-1} y^{-1} as NH_4NO_3 solution; this dose was selected because it meets the N required to sustain the local maximum vegetation productivity (Bai *et al.* 2010). We monitored the atmospheric N deposition and estimated it to be 20.4 kg N $ha^{-1}y^{-1}$ in our experimental site in 2012. The dose of P was 100 kg P ha^{-1} y^{-1}, equivalent to previous nutrient addition experiments in grasslands (Phoenix *et al.* 2003). The plots with both N and P additions received the same amounts of N and P as in the N-only or P-only addition treatments. Control

plots were not fertilized, but rather watered with the same amount of water as used in the fertilizer solutions; the water used to dissolve N and P was approximately 800 ml, which is equivalent to 0.8 mm for a 1 m^2 plot, a pre-treatment field experiment found that this small amount of water addition did not cause any significant changes in water supply, thus unlikely to have altered the ecosystem functions.

Over the study period, soil and plant properties were measured (Supplementary Online Materials). Soil samples were collected to a depth of 10 cm once per year during 2013-2016. Six soil cores were randomly taken in each plot and mixed completely. The measured soil variables included ammonium (NH_4^+) and nitrate (NO_3^-), soil pH, soil organic carbon (SOC), soil total nitrogen (STN), soil total phosphorus (STP), soil microbial biomass carbon (MBC) and soil microbial biomass nitrogen (MBN). It should be noted that no nitrite was detected at our field site. The measured vegetation variables included aboveground biomass, plant total carbon (PTC) and total nitrogen (PTN).

Field Measurements of CH_4Flux

A static chamber technique was used to measure the CH_4flux (Song *et al.* 2009; Zhang *et al.* 2017). Stainless steel permanent bases (50 × 50 × 12 cm) with a 3-cm-deep groove for water sealing were inserted into soil down to a depth of 12 cm in the plots approximately one month before the experiment started in the first year. The chamber base was left in the field for one month before any flux measurement; this was to let the system evolve into an equilibrium state, avoiding any potential disturbances to methanogens and methanotrophs and thus the CH_4 flux. An opaque stainless-steel top chamber (50 cm height) with heat-isolation was installed over the base, with bottom in the groove. The grooves were filled with water for sealing the chamber. Two electric fans were installed inside the chamber to mix the air in the headspace continuously and thoroughly during the measurements (Fan *et al.*2007). Sixty-ml gas samples were collected at 10-min intervals for 30 min using sixty-ml syringes with airtight stopcocks. Simultaneously, the air temperature and pressure in the chamber were measured, and the soil temperature and moisture were measured at a 5 to 10-cm depth using a thermometer (Spectrum Technologies, Inc. East - Plainfield, Illinois) and a portable soil moisture measuring kit ML2x (ThetaKit, Delta-T Devices, Cambridge, UK), respectively. The CH_4concentrations of the gas samples were analyzed using a gas chromatograph (Agilent 7890A, Agilent Technologies Limited Co., USA) equipped with flame ionization detector (FID) using 60–80 mesh 13 XMS column (2 mm inner diameter and 2 m long), with an oven temperature of 55°C. Pure nitrogen was used as carrier gas at a flow speed of 30 ml min^{-1}, and the CH_4 flux was determined from the linear slope of the mixing ratio changes in four samples taken at 0, 10, 20 and 30 min after chamber closure. The CH_4flux was measured weekly from May to September in 2013, 2014, 2015 and 2016, respectively.

The CH_4 fluxes were linearly interpolated and sequentially cumulated to estimate the total flux over growing seasons (Zhang *et al.* 2017). The linear interpolation was adopted due to two reasons: (1) it has been widely used in previous studies and has been suggested to be effective for seasonal interpolation of ecological variables (Song *et al.* 2009; Nikiema *et al.* 2011); (2) the auxiliary data to assist annually interpolation was lacking. Throughout this analysis, positive fluxes represent gas uptake by the grassland ecosystems.

Meta-analysis

A meta-analysis was conducted to investigate the N and P impacts on CH_4 across global grasslands. We collected publications by searching for "nitrogen and phosphorus", "methane", "grassland", and "upland" in Google Scholar in July 2016, and later updated in September 2018. The search returned 6230 publications. A few criteria were then used to screen these publications for our purpose. The criteria applied to determine whether or not to use the data were: (1) it must be manipulation experiments with either external N and/or P addition; (2) the field measurements must cover at least one full growing season, which makes the estimation of annual budget more reliable; (3) the studies report clear information for the field site that is useful when extracting edaphic and meteorological data from global datasets. For sites without clear latitude and longitude, we Googled and found their geographic coordinates with country and site

names. Finally, 35 papers were selected for data extraction. When the data were presented in figures, we extracted mean values and standard errors using GraphClick (http://www.arizona-software.ch/graphclick/). For studies with measurements from different N and P addition levels in one paper, they were extracted and treated as independent data points.

Finally, we compiled a comprehensive dataset of 382 data points for meta-analysis (**Figs. S4 and S5**). The dataset covers the major grassland types across the globe and all experiments were carried out between 1980-2017. For all field experiments, the atmospheric depositions of N and P from the global dataset were treated as background rate and were added to the reported N and/or P addition rates. Since a majority of the data points from Yu et al. did not contain N or P deposition (Yu *et al.* 2017), we used the extracted contemporary nutrient deposition (Mahowald *et al.* 2008), based on latitudes and longitudes, as nutrient inputs. Every CH_4flux rate corresponds to one site with auxiliary information including latitude, longitude, and factors such as N deposition, P deposition, soil temperature (ST), soil moisture (SM), soil pH, soil organic carbon (SOC), bulk density (BD), and clay content (CL). Across the studies in our data set, the N and P treatments fell within the ranges of 0~200 kg N ha^{-1} and 0~50 kg P ha^{-1}, respectively. The non-growing season CH_4 flux is normally not available for most field experiments, therefore, the annual rate of CH_4 uptake (kg C-$CH_4$$ha^{-1}$ yr^{-1}) thereafter was calculated by the ratio of the growing season to non-growing season CH_4 uptake as reported by a few studies (Li *et al.* 2012; Yue *et al.* 2016).

The soil properties for each point data site were extracted from global datasets according to their latitude and longitude. Specifically, the soil pH, SOC, BD and clay were retrieved from the Re-gridded Harmonized World Soil Database v1.2 in the Oak Ridge National Laboratory Distributed Active Archive Center for Biogeochemical Dynamics (available online: https://daac.ornl.gov/SOILS/guides/HWSD.html); soil temperature and moisture for the top 10 cm were from the NCEP/DOE AMIP-II Reanalysis (Reanalysis-2): http://www.esrl.noaa.gov/psd/data/gridded/data.ncep.reanalysis.derived.surfaceflux.html).

Global Extrapolation

The data for the grassland distribution were derived from the Global Mosaics of the standard MODIS land cover type data product (MCD12Q1) from http://glcf.umd.edu/data/lc/index.shtml. The spatial coverage is -180.0° ~ 180.0° in longitude, and -64.0° ~ 84.0° in latitude. The global land cover data sets are available as ESRI ASCII Grid format files and was re-projected to be consistent with soil and climate datasets (**Fig. S4**). The global simulations were carried out at a spatial resolution of 0.5° × 0.5°. According to the definition of Food and Agriculture Organization of the United Nations (FAO), any geographic area dominated by natural herbaceous plants including grasslands, prairies, steppes, and savannahs with coverage of at least 10% was designated as grassland (Latham*et al.* 2014). The annual CH_4 uptake was determined by summing up all grassland grids (equation 1). For the model simulation, we set N and P depositions to pre-industrial level to represent ambient condition, N set to pre-industrial level and P set to contemporary level represent P addition, P set to pre-industrial and N set to contemporary represent N addition, both N and P were set to contemporary level to represent N and P concurrent treatment.

Both climate and soil data were resampled and re-projected to be consistent through NCL (NCAR Command Language, current version 6.4.0). The data with higher resolution were uniformly transformed to the lowest resolution at 0.5° × 0.5°. Based on the calculated CH_4uptake rate in every grid and grid area of grassland, we scaled up the results from this analysis by multiplying them for target CH_4 uptake with the corresponding grid areas currently summarized:

$$F(CH4) = \sum_{k=0}^{n} \left(\frac{n}{k}\right) \mathrm{CH}_{4k} A_k (1)$$

where F(CH_4) is the sum of the global grassland CH_4 uptake expressed as Tg C-$CH_4$$year^{-1}$, and CH_{4k} is the CH_4 uptake rate in kth grid as kg C-$CH_4$$ha^{-1}$ y^{-1}, and A_k is the area of the kth grid (**Fig. S8**).

Statistical Analysis and Uncertainty Analysis

We used a one-way ANOVA analysis (ANOVA; R package) followed by Duncan's post-hoc test to examine the N and P impacts on annual CH_4 uptake rate, soil NH_4^+ and NO_3^-, and plant N content. A stepwise multivariate analysis was adopted to evaluate the response of the soil plant and microbial communities to environmental factors as well as their correlations with each other. A number of key variables, including N input rate, P input rate, soil temperature, soil pH, soil organic carbon density, bulk density, and clay content, are kept in the multiple linear equation to quantify their impact on CH_4 flux (Equation 2).

A structure equation model (SEM) was used to explore how nutrient (N, P and N + P)-induced changes in CH_4 uptake considering meteorological, plant, edaphic factors, and microbial biomass. The "lavaan" package (https://cran.r-project.org/web/packages/lavaan/lavaan.pdf) for R program (version 3.4) was used for the structural equation modeling. Data used in the SEM and linear regression analyses were calculated as the mean of every year during the four years (**Figs. S2 and 3**). Prior to the SEM procedure, we conducted principal component analysis (PCA) to remove the redundant variables for meteorological factors, soil edaphic parameters, plant and microbial parameters. Meanwhile, due to the strong multicollinearity among other variables, the variance inflation factor (VIF) was used to quantitatively select the right factor for SEM model (VIF < 5). One SEM model (Fig. S3) was used to disentangle the direct and indirect environmental impacts on the CH_4 uptake, and the other (Fig. 3) was used to analyze the N and P impacts on CH_4 uptake. A conceptual model was created to represent the key linkages and connecters between different parameters. The fitted conceptual model was further modified with "mod_ind" (within the R package "lavaan") to yield a reliable multivariate causal network. The SEM results were evaluated with the comparative fit index (CFI), the normed fit index (NFI), and the chi-square test (χ^2). The chi-square value is the traditional measure for evaluating the overall model fit and accessing the magnitude of discrepancy between the sample and fitted covariance matrices; NFI is an incremental fit index that assesses the model by comparing the chi-square value of the model to the null model that assumes that all variables are uncorrelated; CFI is a revised form of the NFI that takes into account sample size; the model fits with NFI > 0.95 and CFI > 0.95, indicating a good SEM (Hooper *et al.* 2008).

The Latin Hypercube Sampling based Monte Carlo method was adopted to quantify the uncertainties of the global grassland CH_4uptake under different treatments (Control, N, P and N+P conditions) estimated by the empirical models. The LHS approach has been used in our previous study (Xu 2010); detailed procedure can be found in**Supplementary Online Material** .

Results

A Field Experiment for N and P Impacts on CH_4Uptake

The field experiment showed that the N-only and concurrent N + P additions treatments significantly suppressed CH_4 uptake over the entire duration of the experiment (2013-2016, **Fig. 1a**). Suppression of CH_4 oxidation by N addition and N + P additions amounted to -16.3% ($p < 0.01$) and -7.9% ($p = 0.04$) relative to the control plots in 2013, -7.8% ($p<0.01$) and -1.8% ($p=0.10$) in 2014, -12.3% ($p<0.01$) and -6.6% ($p<0.01$) in 2015, and -18.3% ($p<0.01$) and -12.5% ($p<0.01$) in 2016, respectively (**Fig. 1d**). Over the study period, the average annual CH_4 uptake rates were 2.94 ± 0.07 kg C-CH_4 ha^{-1} in control plots, 2.54 ± 0.04 kg C-CH_4 ha^{-1} in N addition plots, and 2.73 ± 0.06 kg C-$CH_4$$ha^{-1}$ in N + P addition plots (**Fig. 1a**). In comparison with the ambient treatment, the P-only treatment slightly and not statistically significantly stimulated CH_4 uptake throughout the experiment: +7.4% ($p = 0.12$) in 2013, +4.0% ($p = 0.09$) in 2014, +1.2% ($p = 0.2$) in 2015, and +2.3% ($p = 0.16$) in 2016, respectively (**Fig. 1d**). Overall, P addition alone did thus not affect CH_4 uptake, N addition alone suppressed CH_4 uptake, and concurrent N + P additions suppressed CH_4 uptake to a lower degree, suggesting that P addition alleviates the N suppression of CH_4 uptake in semiarid grasslands (**Figs. 1a and 1d**).

[Insert Figure 1 here]

A Meta-analysis of N and P Impacts on CH_4 Uptake in Grasslands

To investigate the universality of these N and P effects on CH_4 uptake in grassland soils, we carried out a global meta-analysis to quantify the responses of CH_4 uptake rates in grassland ecosystems to nutrient addition treatments (N-alone, P-alone, and concurrent N + P additions) (**Fig. 2**). This global meta-analysis showed that, relative to the control treatments, N addition and concurrent N + P additions cause significant declines in annual CH_4 uptake: -27% ($p < 0.01$) and -14% ($p < 0.01$), respectively (**Fig. 2**). The smaller inhibition effect of concurrent N + P additions than the N-only addition on CH_4 uptake ($p = 0.02$) (**Fig. 2**) is consistent with the results of our field experiment (**Figs. 1a and2**).

[Insert Figure 2 here]

Mechanisms of N and P impacts on CH_4uptake

Based on various statistical analysis, we hypothesize that the following mechanisms underpin the N and P impacts on CH_4 uptake. Whereas experimental N addition leads to an accumulation of mineral N (NH_4^+ and NO_3^-) in the soil (N-only compared with control), P addition enhances plant N uptake and N accumulation in vegetation biomass (N+P treatment compared with N-only addition) (**Figs. 1b, 1c, S1, 3a, 3b, 3c, and 3d**). In our field experiment, P-only addition significantly decreased soil NH_4^+ content ($p < 0.01$,**Fig. 1b**), but did not significantly affect plant N content (P-only addition compared with control) ($p = 0.35$, **Fig. 1c**). The concurrent N + P additions, in contrast, did stimulate N accumulation in vegetation to a much higher degree than N addition alone ($p < 0.01$, **Fig. 1c**). This increase in vegetation N accumulation significantly reduced soil NH_4^+ content under the N + P additions treatment compared to the N-only treatment ($p < 0.01$,**Fig 1b**). Therefore, the P addition substantially stimulates plant growth and plant uptake of NH_4^+(Dijkstra et al., 2012), which reduces the NH_4^+ concentration in soils and thereby alleviates the N suppression of CH_4 uptake (**Fig. S1**).

[Insert Figure 3 here]

To disentangle the contribution of the various potential drivers, we applied structural equation models (SEM) to quantify the environmental controls of CH_4 uptake (all factors were classified into four groups: meteorology, microbes, plant, and edaphic factors,**Figs. S2 and S3**) using the field experiment's data (**Figs. 3a, 3b, 3c, and 3d**). Our models considered how nutrient additions directly or indirectly affects CH_4 uptake (**Figs. 3 and S3**). The SEM results suggest that under control (non-fertilized) conditions, the impact of meteorology on CH_4 uptake was directly (β = -0.33, standardized coefficient) or indirectly mediated through microbes (β = -0.8, standardized coefficient) and plants (β = -0.21, standardized coefficient) (**Fig. S3a**). N and P additions affected CH_4 uptake indirectly through edaphic factors (N treatment: $\beta = 0.5$, standardized coefficient, **Fig. S3b** ; N+P treatment: $\beta = 0.37$, standardized coefficient, **Fig. S3d**). Soil NH_4^+ had a direct negative effect and plant N content had a positive effect on the CH_4 uptake under control conditions and in all nutrient addition treatments (**Figs. 3a, 3b, 3c, and 3d**). Meanwhile, added N stimulated the accumulation of soil NH_4^+ (N treatment: $\beta = 0.45$), added P had a positive impact on plant P and soil P, but had no impact on soil NH_4^+; while the N + P treatment had a negative impact on soil NH_4^+ (β = -0.18). Compared to the control, the N addition strengthened the negative effect of NH_4^+ on CH_4 uptake (β ranging from -0.57 to -0.77) (**Figs. 3a and 3b**); P addition did not change the negative effect of NH_4^+(β = -0.57) and the positive effect of plant N ($\beta = 0.34$) (**Figs. 3a and 3c**); N + P additions strengthened the suppression effect of soil NH_4^+ (β changing from -0.57 to -0.72) (**Figs. 3a and 3d**). These results showed that CH_4 uptake is highly associated with soil N and P contents in semiarid grasslands.

Global Estimation of P alleviation of N-suppressed CH_4 Sink in Grasslands

We further developed an empirical model to quantify the N and P impacts on soil oxidation of atmospheric CH_4 across global grasslands, using existing global datasets of soil properties and meteorology (Methods). Two thirds of the compiled data were used for model fitting, while the remaining one third of the data was used for model validation (**Fig. S7**). The best fitting equation obtained with the stepwise regression procedure was:

$$F_{CH4} = m + a \times N + b \times P + c \times \ln(N) \times \ln(P) + d \times ST + e \times pH + f \times SOC + g \times BD + h \times CL \quad (2)$$

where F_{CH4} is the annual CH_4 uptake rate; N is the nitrogen input in g $ha^{-1}y^{-1}$; P is the phosphorus input rate in g ha^{-1} y^{-1}; ln represents the natural logarithm; ST is soil temperature (K); pH is the soil pH value; SOC is soil organic carbon content (in %); BD is bulk density (g cm^{-3}); CL is clay content (in %); m is the incept of the function; and a, b, c, d, e, f, g, and h are coefficients. The coefficients and key parameters for the regression are listed in**Table S1** . The model explained more than 37% of the variation in CH_4 uptake rate across the globe (**Fig. S7**).

[Insert Figure 4 here]

We then applied the empirical model to estimate the CH_4sink strength of global grasslands (**Fig. S8**), under four treatments: ambient (*i.e.* pre-industrial) levels of N and P depositions, elevated N deposition (contemporary N deposition and pre-industrial P deposition), elevated P deposition (contemporary P deposition and pre-industrial N deposition), and concurrently elevated N + P depositions (contemporary N and P depositions) (**Fig. 4**). Simulated global grassland CH_4 sinks amounted to 4.43 ± 0.20 Tg C-CH_4 y^{-1} for the ambient scenario, 3.92 ± 0.16 Tg C-CH_4 y^{-1} for the elevated N scenario, 4.60 ± 0.22 Tg C-CH_4y^{-1} for the elevated P scenario, and 4.18 ± 0.18 Tg C-CH_4 y^{-1}, for the N + P scenario (**Fig. 4**). Addition of N-only thus suppressed the global grassland CH_4 sink by ˜0.50 Tg C (˜11.4%), while concurrent P deposition alleviated this suppression by more than half (˜5.8%).

Conceptual Model for P Alleviation of N-suppressed CH_4Sink

Based on our field experiments, the global meta-analysis, and the empirically-derived insights of N and P impacts on CH_4uptake, we here propose a conceptual framework that summarizes the possible mechanisms underlying the interactive impacts of N and P additions on CH_4 uptake (**Fig. 5**). In this conceptual model, MMO represents the whole group of enzymes responsible for CH_4 oxidation under aerobic conditions (Dunfield & Knowles 1995) (**Fig. 5a**). Under ambient conditions (e.g. pre-industrial N and P deposition), grassland productivity is generally limited by low soil N availability (Ladwig *et al.* 2012) and by low soil P availability in more than half of the grasslands (Fay*et al.* 2015) and grassland plant species have therefore optimized their N uptake and allocation processes during ecological succession (Bai *et al.* 2004). Soil N does not leach under these low-N conditions and available N is either assimilated by plants or immobilized by soil microbes. Because soil mineral N (particularly NH_4^+) is maintained at a low level, competition with CH_4 for the MMO enzyme is weak (**Fig. 5a**). In contrast, sustained or high N addition will push the system out of N limitation and result in NH_4^+ accumulation in the soil (**Fig.5b**). This in turn strengthens the competition with CH_4 for the MMO enzyme, thereby suppressing the oxidation of atmospheric CH_4 in grassland soils (**Fig. 5b**). If N and P are concurrently added, the added P stimulates vegetation growth and uptake of mineral N, especially NH_4^+, and thus alleviates the N-induced suppression of CH_4 oxidation. (**Fig. 5c**). This theoretical framework emphasizes the substrate competition theory when explaining the P alleviation of N-suppression on CH_4 uptake.

[Insert Figure 5 here]

Discussion

Results from both the field experiments and the global meta-analysis support the substrate competition theory as a predominant mechanism explaining P alleviation of N-suppression of CH_4 uptake in global grassland. A number of studies have concluded that soil NH_4^+ content is a major driver of CH_4 uptake suppression in drylands (Dunfield & Knowles 1995; Gulledge & Schimel 1998). The toxic impact of nitrite on methanotrophic activity might be another reason for this suppression (Dunfield & Knowles 1995). In our field site, however, the toxic impact of nitrite on methanotrophic bacteria (Schnell & King 1994) most likely did not contribute to the inhibited CH_4 uptake because no nitrite was detected in the soil, a widespread phenomenon in semi-arid grasslands (Holst *et al.* 2007; Giese *et al.*2013; Zhang *et al.* 2017). Therefore, we conclude that substrate competition remains the best theory to explain the P alleviation of N-suppression of CH_4 oxidation.

A recent study in tropical forest postulated that P mitigation of the N-suppressed CH_4 oxidation might due to P stimulation of methanotrophic activities, although it further concluded it might be a minor contribution due to the trivial fraction of microbial biomass taken in by CH_4 oxidation bacteria (Zhang *et al.*2011). This mechanism was postulated, but not supported by empirical data (Zhang *et al.* 2011). Enhanced methanotrophic activity by P-enrichment is thus one possible mechanism, yet it remains to be empirically confirmed as a predominant mechanism. More molecular and genomic analysis of the linkages between microbial mechanisms and ecosystem function remain needed to mechanistically elucidate and predict the CH_4 oxidation in a changing environment (Xu*et al.* 2016). Combined, the field experiment, meta-analysis, and global empirical modeling analysis unambiguously demonstrate that P-stimulated soil N depletion mitigate the N-induced reduction of CH_4 uptake.

Both the global meta-analysis and modeling results indicate a widespread impact of N and P on CH_4 uptake in grassland. Summarizing all grids with treatment impacts compared with ambient grids, we found that N suppression of CH_4 uptake occurs in over 90% of global grasslands; the pervasive N suppression is consistent with reported N suppression of CH_4 uptake in many ecosystems (Mosier *et al.* 2003; Chen *et al.* 2013; Liu*et al.* 2017; Zhang *et al.* 2017). Model results suggest that P alleviation of N-suppression of CH_4 uptake occurs in over 89% of the global grasslands (**Fig. 4**). Considering the limited studies on P impacts on CH_4 uptake (Veraart*et al.* 2015), more field experiments on this aspect are needed. Estimates of the global grassland sink for CH_4 have been made in a large number of studies, yielding a broad range of 1.9 - 9.3 Tg CH_4 yr^{-1} (Potter *et al.*1996; Ridgwell *et al.* 1999; Curry 2007; Zhuang *et al.*2013; Yu *et al.* 2017). Our result (5.9 Tg CH_4y^{-1}) falls within the range of previous studies. According to our empirical model, increased N deposition reduced the grassland CH_4 sink by 11%, which was similar to another modeling study (10%) (Zhuang *et al.* 2013), but lower than the value reported in a data synthesis study (38%) (Liu & Greaver 2009).

The effects of N and P depositions on CH_4 uptake were quantified, yet a few issues should be paid attention to when interpreting the results. First, we quantified the potential CH_4 patterns under four N and P deposition scenarios, but did not consider the contributions of other changing environmental factors, such as increasing atmospheric CO_2concentrations, and shifting land management practices, which have been shown to be important determinants of CH_4 flux (Xu*et al.* 2010). Second, our global-scale estimates of CH_4 uptake were based on a simple empirical model. Intensive data-model integration and model- model inter-comparisons are still needed to better quantify the uncertainties in these CH_4 budgets. Third, the meta-analysis was carried based on the most comprehensive dataset for the impacts of N and P additions on CH_4 flux in global grassland; however, the uneven distribution of the field observational dataset might cause biases in the global analysis of nutrient impacts. This unequal spatial distribution of field observations exist for eddy covariance towers (Baldocchi *et al.* 2001), vegetation data (Kattge *et al.*2011), and soil data (Xu *et al.* 2013). This problem needs to be resolved for reaching a more accurate global extrapolation and increasing our understanding of the functioning of terrestrial ecosystems. Deposition and addition of N and P take place in many forms, including inorganic and organic, dry and wet deposition. Due to limited data availability (Mahowald *et al.* 2008) and field experiments (Wang *et al.* 2015), the impacts of various N and P forms in atmospheric depositions could not be fully investigated in the present study. Understanding the impacts of

different N and P forms on CH_4 oxidation still remains to be fully investigated.

Conclusion

With a combination of a field experiment, a meta-analysis, and an empirical modeling approach we found that the substrate competition theory is most likely the predominant mechanism explaining the P alleviation of the N-suppression of CH_4 uptake, and we further estimated that contemporary atmospheric N deposition suppresses the CH_4 sink in global grasslands by 11.4% while P deposition alleviates this suppression by 5.8%.

This study is among the first attempts to quantify the P impacts on the N suppression of soil oxidation of atmospheric CH_4 in global grasslands. It advocates model development to incorporate P mechanisms not only for the nutrient impacts on carbon cycling, but also for a more accurate projection of the CH_4 budget of terrestrial ecosystems.

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Additional information

Supplementary Information accompanies this paper at https://doi.org/...

Fig. S1 Correlation between NP-induced changes in soil total N content and NP-induced changes in plant N content. ΔPTN equals plant total nitrogen under concurrent N+P treatment minus plant total nitrogen under N only condition. Additions; ΔSTN equals soil total nitrogen under concurrent N+P treatment minus soil total nitrogen under N only condition. The log-transformation was used to producing variables following normal distribution.

Fig. S2 Bivariate correlations between variables used in structure equation models (observational dataset)

Fig. S3 Structural equation models of meteorology, plant, edaphic and microbial biomass as predictors of CH_4 uptake for (a) control (n=96, χ^2=2.041, DF=2, CFI=1, NFI=0.99), (b) N addition only (n=96, χ^2=0.667, DF=2, CFI=1, NFI=0.997), (c) P addition only (n=96, χ^2=1.946, DF=2, CFI=1, NFI=0.991) and (d) concurrent N+P additions (n=96, χ^2=2.079, DF=2, CFI=1, NFI=0.99) (black lines represent positive paths; red lines represent negative paths; solid lines indicate significant impacts [$p<0.05$]; dotted lines indicate non-significant impacts [$p>0.05$]).

Fig. S4. Spatial distribution of the data points for the whole dataset used for meta-analysis; 382 data points distributed across the global grasslands, covering major grassland types, spanning major climate zones

Fig. S5. The correlation matrix of the key variables in the dataset used for meta-analysis (noted most variables are not in normal distribution, indicating the needs of log-transformation in further analysis and model development)

Fig. S6. Correlation between NH_4^+ and total inorganic N (NH_4^++NO_3^-).

Fig. S7. Comparison of modeled CH_4 with the compiled CH_4 uptake.

Fig. S8. Frequency distribution of the best estimates of CH_4 oxidation across global grasslands (CK: pre-industrial N and P; N: the model simulation with pre-industrial P and contemporary N inputs; P: N: the model simulation with contemporary P and pre-industrial N inputs; NP: model simulations with contemporary N and P depositions).

Table S1 Correlation coefficients for the regression between independent variables and CH_4 uptake rates based on the comprehensive data ($R^2 = 0.44$; $P < 0.001$); it is used as the empirical model for global estimation of N and P impacts on CH_4 uptake in grasslands

Competing interests: The authors declare no competing interests.

Figure Legends

Fig. 1 Differential effects of N, P, and concurrent N and P additions on surface CH_4 flux, soil NH_4^+ and plant N content in semiarid grassland from 2013 to 2016. (a) Net nutrient effects on mean CH_4 uptake; (b) Net nutrient effects on soil NH_4^+ content in top 50 cm; and (c) Net nutrient effects on plant N content. The error bars indicate the standard error of means ($n = 3 \times 2$). Different letters represent significant differences among treatments ($p<0.05$) by the one-way ANOVA. (d) N and P effects on CH_4 uptake as normalized to the control treatment in semiarid grassland from 2013 to 2016; error bars indicate the standard error (note that the N + P all have suppression impact but in a lesser degree)

Fig. 2 A meta-analysis of N and P effects on CH_4 uptake in grassland ecosystems (percentage is used to report the N and P impacts)

Fig. 3 Structural equation modeling (SEM) for the relative controls of soil NH_4^+ content, soil P content, plant N and plant P on CH_4 flux under various treatments; (a) ambient (n=96, χ^2=1.389, DF=0, CFI=0.986, NFI=1), (b) N addition (n=96, χ^2=5.671, DF=0, CFI=0.968, NFI=1), (c) P addition (n=96, χ^2=1.644, DF=0, CFI=0.993, NFI=1) and (d) concomitant N + P additions (n=96, χ^2=10.347, DF=0, CFI=0.943, NFI=1) (black lines represent positive paths; red lines represent negative paths; the solid lines represent the path is statistically significant [$p<0.05$]; the dotted lines represent non-significant effects [$p>0.05$]).

Fig. 4 The model simulated contemporary (2005-2014) CH_4 oxidation and N and P impacts across global grasslands; (a) absolute CH_4 flux (Kg C ha^{-1} y^{-1}) under ambient condition (pre-industrial N + P deposition); (b) relative impact of N deposition (contemporary N deposition and pre-industrial P deposition) compared to the ambient condition; (c) relative impact of P deposition (pre-industrial N deposition and contemporary P deposition) compared to the ambient condition; (d) relative impact of N + P depositions (contemporary N + P depositions) compared to the ambient condition.

Fig. 5 Graphic diagram showing substrate competition between CH_4 and NH_4^+ for methane monooxygenase (MMO); under ambient condition (left panel), N addition condition (middle panel), and concomitant N + P additions (right panel); the trivial soil $NH_4^+$is in weak competitive equilibrium with CH_4 for MMO under ambient condition; and the added NH_4^+ shifts the competitive equilibrium and thus reduces the CH_4 oxidation; the P addition pushes the shifted equilibrium back and alleviates the N suppression on CH_4 oxidation by stimulating N cumulation in vegetation biomass.

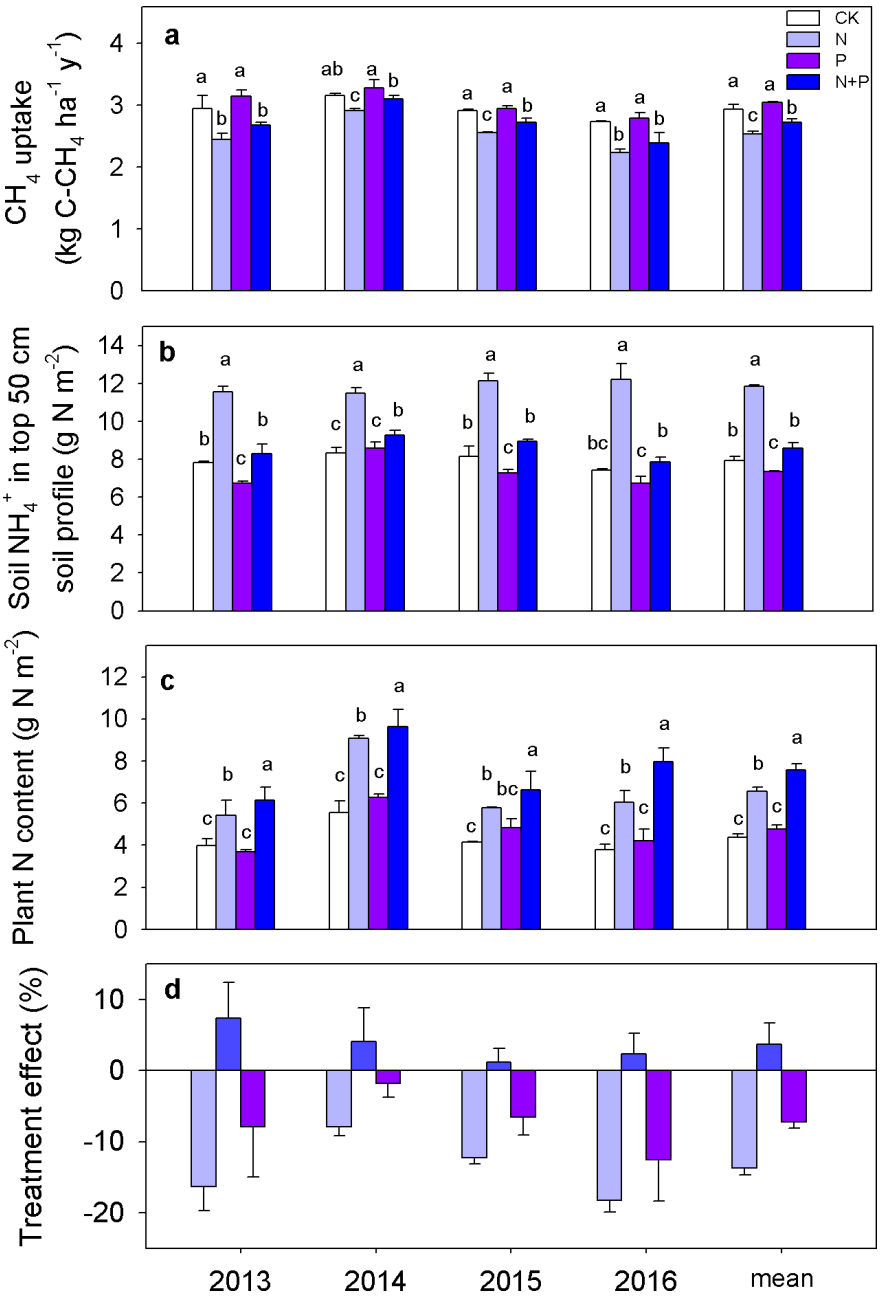

Figure 1

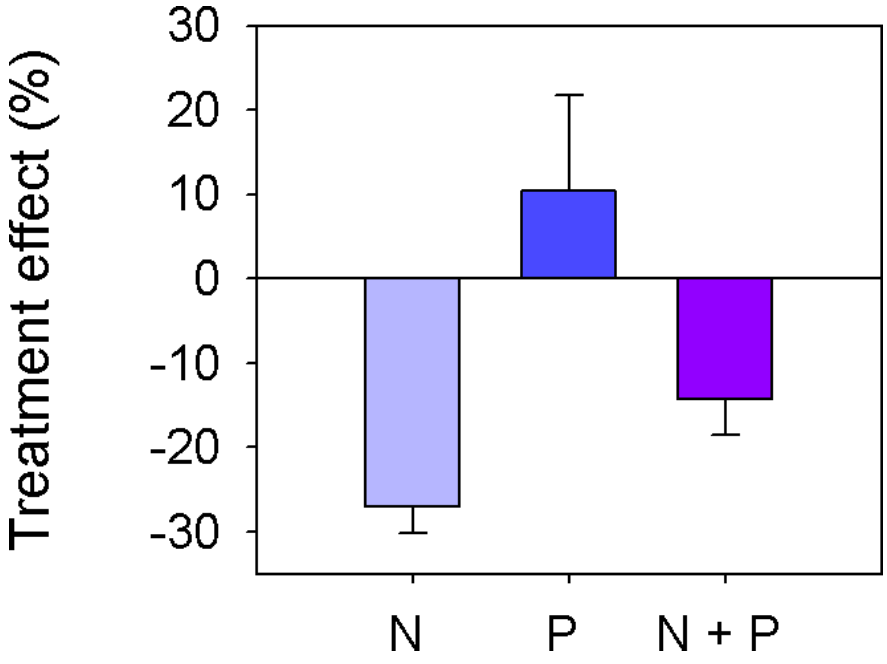

Figure 2

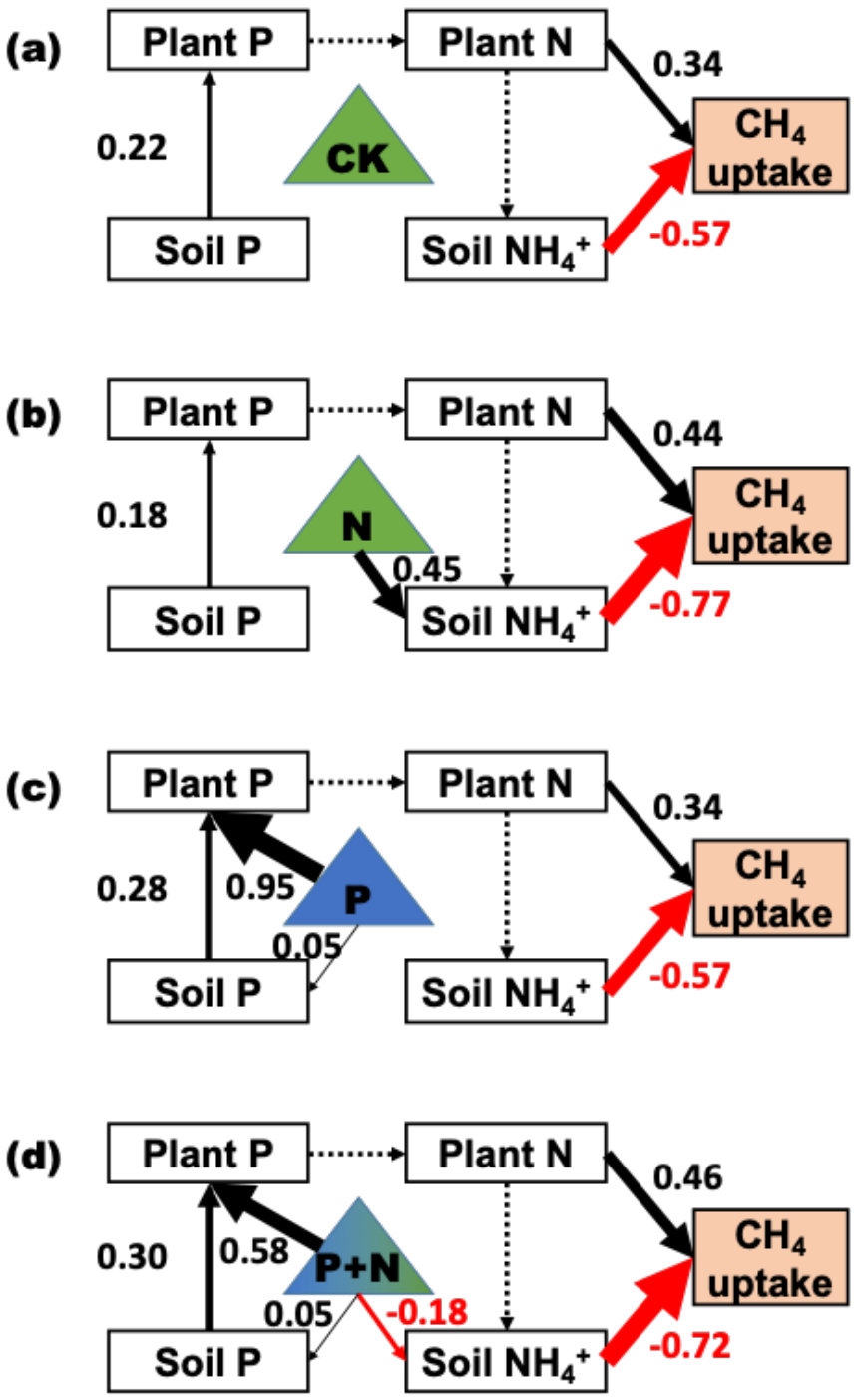

Figure 3

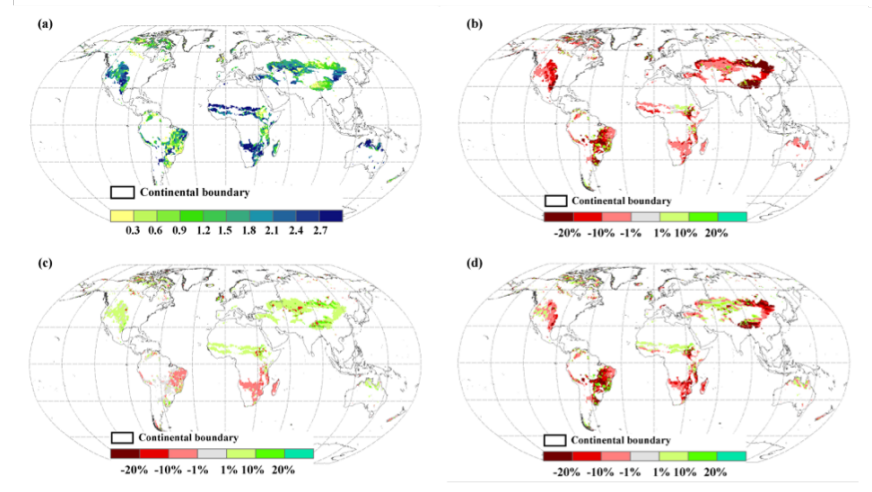

Figure 4

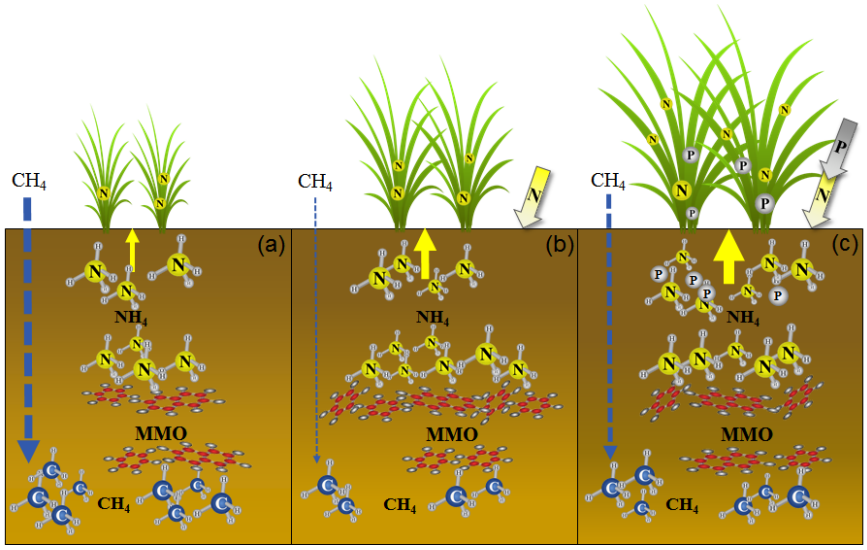

Figure 5

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