Supplementary Information for

Progressive nitrogen limitation across the Tibetan alpine permafrost region

Kou et al.

Supplementary Notes

Supplementary Note 1. Analyses for changes in vegetation community between the two sampling periods

Vegetation communities were investigated for both periods of our resampling campaign. During the investigation, all the occurred plant species were identified, and the relative cover of each species was virtually estimated. Based on these information, two indexes including species richness and Shannon-Wiener index were calculated to characterize the vegetation community composition in each of the two sampling periods. Of them, species richness refers to the species number per quadrat, and Shannon-Wiener index (H) was calculated as: $H = -\sum P_i \ln P_i$, where P_i is the relative cover of species i[1]. With the calculated species richness and Shannon-Wiener index in the 2000s and the 2010s, we examined changes in vegetation composition between the two sampling periods based on linear mixed-effects models, in which the fixed effect was sampling years and the random effects were the investigated sites as well as replicates within each site[2]. Results showed that neither of the two indicators exhibited any significant changes over the detection period (Supplementary Fig. 5), indicating the minimal effect of vegetation community shifts on the dynamics of plant $\delta^{15}N$ observed in this study. Considering the potential effects of mycorrhizal plants on the average community $\delta^{15}N$, we further detected changes in vegetation composition at the family and species levels that are associated with mycorrhizae. Of them, changes in vegetation composition at the family level were analyzed based on two indicators (species richness and the relative cover) for five families that can be heavily colonized by mycorrhizae on the Tibetan Plateau, including *Gramineae*, *Leguminosae*, *Asteraceae*, *Cyperaceae* and *Rosaceae*[3]. The species-level analyses were conducted to detect changes in the relative cover for the dominant species of the five families mentioned above. With linear mixed-effect models, results also indicated that the vegetation composition was unchanged either at the family or species level between the 2000s and the 2010s (Supplementary Figs. 6-7). Taken together, these analyses eliminated the potential confounding effects of vegetation community shifts on the plant $\delta^{15}N$ dynamics observed in this study.

Supplementary Note 2. Meta-analyses of CO_2 enrichment and warming effects on mycorrhizal symbiosis

Meta-analysis was conducted to explore the potential effects of two major environmental changes on the Tibetan Plateau, i.e., elevated CO_2 (eCO_2) and climate warming on mycorrhizal symbiosis. To be specific, by searching with mycorrhiza* and CO_2 or mycorrhiza* and carbon dioxide in the Web of Science, we collected studies that reported the responses of mycorrhizal symbiosis to eCO_2. Likewise, we synthesized publications that investigated the effects of experimental warming on mycorrhizal symbiosis with terms mycorrhiza* and warming or mycorrhiza* and temperature. All collected publications dated to February 25th, 2020. To avoid biases, the following criteria were adopted during the searching process: (i) variables that represented mycorrhizal symbiosis (e.g., percent of root length colonized and extraradical mycorrhizal hyphal density) were reported; (ii) mean value, sample size and error (standard error, standard deviation or confidence interval) for eCO_2 or warming were recorded or could be calculated from the individual studies; (iii) treatment (eCO_2 or warming) and control plots had same ecosystem types, soil types and dominant plant species. Finally, two datasets were compiled including 100 studies that reported eCO_2 effects on mycorrhizal symbiosis and 22 studies that recorded warming effects on mycorrhizal symbiosis.

As commonly used in meta-analysis, we adopted the response ratio (RR) to quantify the responses of mycorrhizal symbiosis to eCO_2 or experimental warming. The RR refers to the natural-logarithm-transformed ratio of target variables associated with mycorrhizal symbiosis between experimental treatment and control[4]. Typically, the collected data are weighted either by variance or sample size, and data with a lower variance or larger sample size are assumed to contribute more to the mean effect size. Because the collected studies did not always report the sampling variance (e.g., the standard error or standard deviation), the number of replicates was used as the weighting factor, i.e., $weight_n = n_c n_t/(n_c+n_t)$, where n_c and n_t are the number of replicates for control and treatment groups, respectively[5]. A random-effect model with bootstrap approach was applied to estimate the mean effect size and its 95% confidence interval (CI) with MetaWin 2.1 (Sinauer Associates Inc., Sunderland, MA, USA). The eCO_2 or warming effect was considered significant if the 95% CI did not overlap with zero. Based on the meta-analyses, we observed that both eCO_2 and climate warming significantly enhanced the degree of mycorrhizal colonization ($P < 0.05$, Supplementary Fig. 9).

Supplementary Note 3. Changes in soil moisture on the Tibetan Plateau during 2000s-2010s and its potential effects on gaseous N loss

We collected four independent data sources to examine soil moisture dynamics in the Tibetan alpine permafrost region during 2000s-2010s. The first data source was derived from our resampling investigations. Based on this dataset, we analyzed changes in the gravimetric soil moisture in the top 10 cm with the linear mixed-effects model. Results showed that surface soil moisture did not exhibit any significant differences between the two sampling periods (Supplementary Fig. 11). The second data source was purchased from China Meteorological Administration (http://data.cma.cn/). Using field observations recorded by 7 meteorological stations across the study area, we found that the average surface (0-10 cm) soil moisture did not exhibit any significant trend over 2000-2010 (Supplementary Fig. 12a). The third data source was derived from a recent study exploring permafrost changes and their effects on hydrological processes on the Tibetan Plateau, which provided the observed dataset for soil moisture dynamics at five monitoring sites in the hinterland of the Tibetan alpine permafrost region[6]. By averaging the monitored data, we observed that the surface soil moisture remained stable over the detection period (Supplementary Fig. 12b). The fourth data source was obtained from a global dataset, CFSV2 (NCEP Climate Forecast System Version 2)[7]. From the CFSV2 dataset, we extracted the surface (5 cm) volumetric soil moisture for the 107 resampling sites, and found that the surface soil moisture on the Tibetan Plateau experienced no significant changes over 2000-2015 (Supplementary Fig. 12c). Collectively, results from these four independent data sources consistently demonstrated that the relatively stable surface soil moisture on the Tibetan Plateau from the 2000s to the 2010s. The stability of soil moisture indicated its limited effect on the increase in gaseous N loss observed across the study area. To further demonstrate this point, we explored the relationship between each year's total gaseous N loss from denitrification with the corresponding annual average soil water-filled pore space over the detection period. Results showed that this relationship was insignificant (Supplementary Fig. 14), which provided additional evidence for the argument that soil moisture dynamics could not be responsible for the increased gaseous N loss across our study area.

Supplementary Methods

Supplementary Methods: References used to synthesize N_2O observations for DNDC model validation

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Supplementary Methods: References used to analyze the effects of experimental warming on mycorrhiza symbiosis

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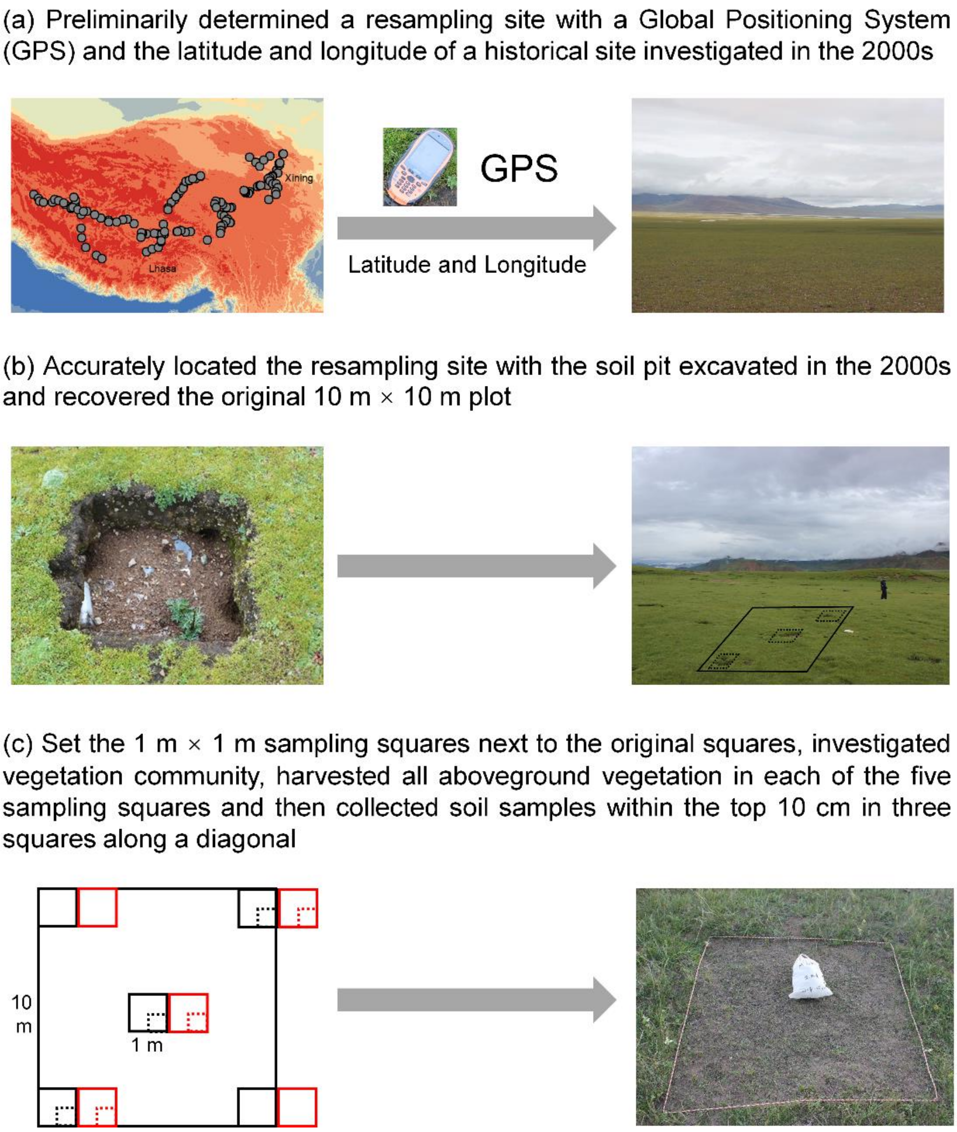
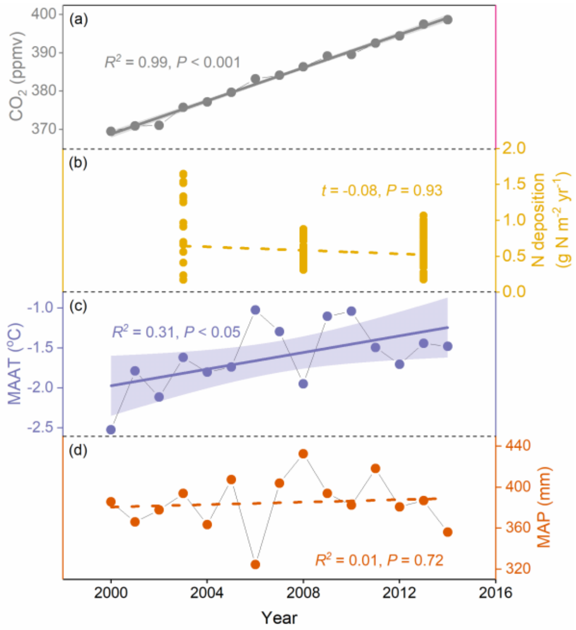
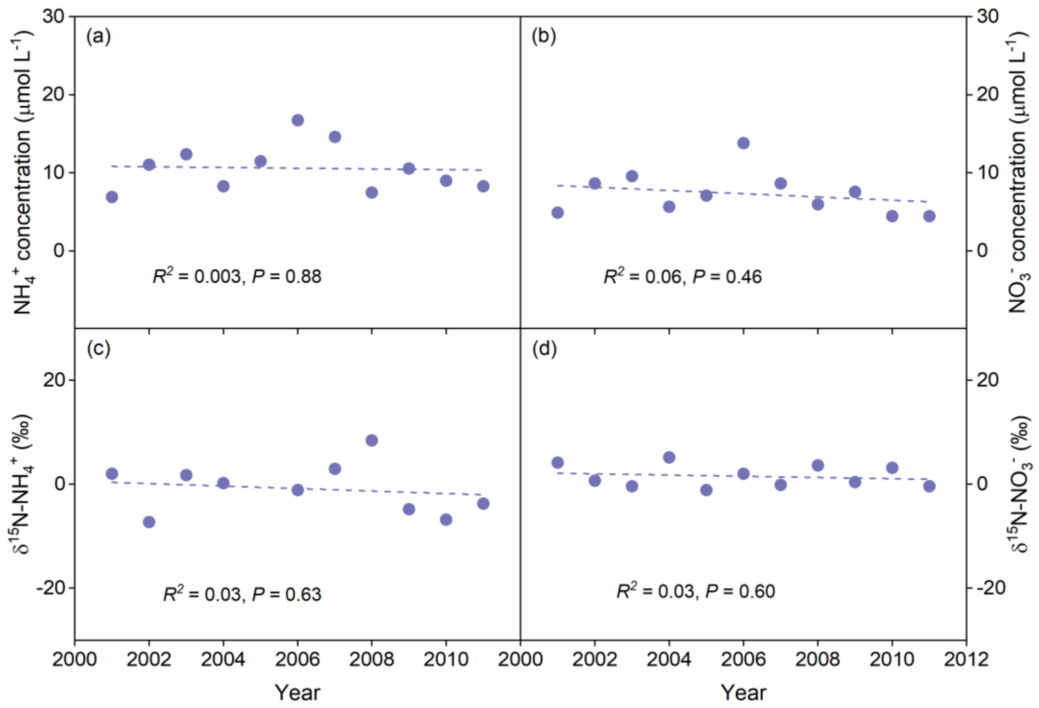
Supplementary Figures



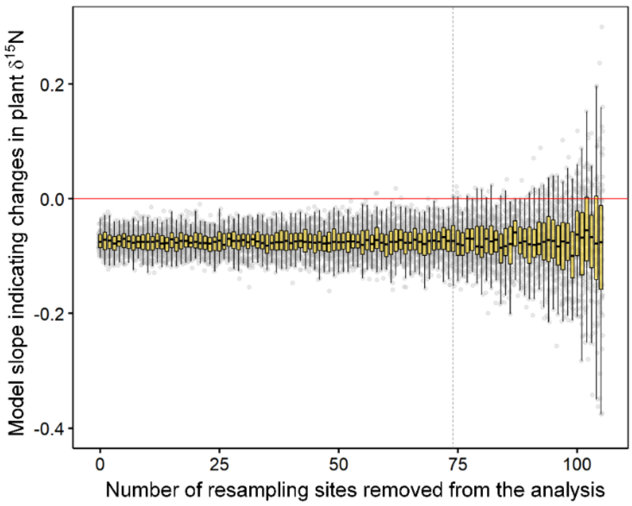
Supplementary Fig. 1. The schematic diagram for the Tibetan resampling campaign.



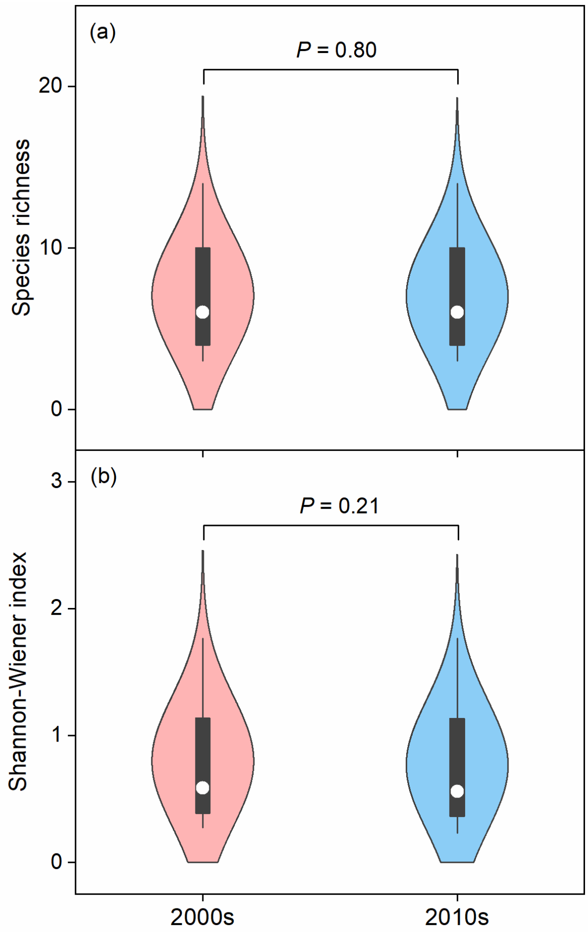
Supplementary Fig. 2. Environmental changes on the Tibetan Plateau. Panels (a-d) represent changes in atmospheric carbon dioxide (CO_2) concentration, nitrogen (N) deposition, mean annual air temperature (MAAT) and mean annual precipitation (MAP) over 2000s~2010s, respectively. Data for atmospheric CO_2 concentration, N deposition and climate (MAAT and MAP) are derived from Chinese Research Network or Special Environment and Disaster (http://www.crensed.ac.cn), Science Data Bank (http://www.csdata.org/p/199/) and China Meteorological Administration (http://data.cma.cn/), respectively. The changes in CO_2 concentration and climate (MAAT and MAP) were examined with Ordinary Least Square regressions, and the change in N deposition was detected with linear mixed-effects model, in which the fixed effect was year and the random effect was site. The solid fitted lines indicate significant environmental changes, while the dashed fitted lines denote insignificant dynamics. The shade accompanying with the solid fitted line represents 95% confidential interval.



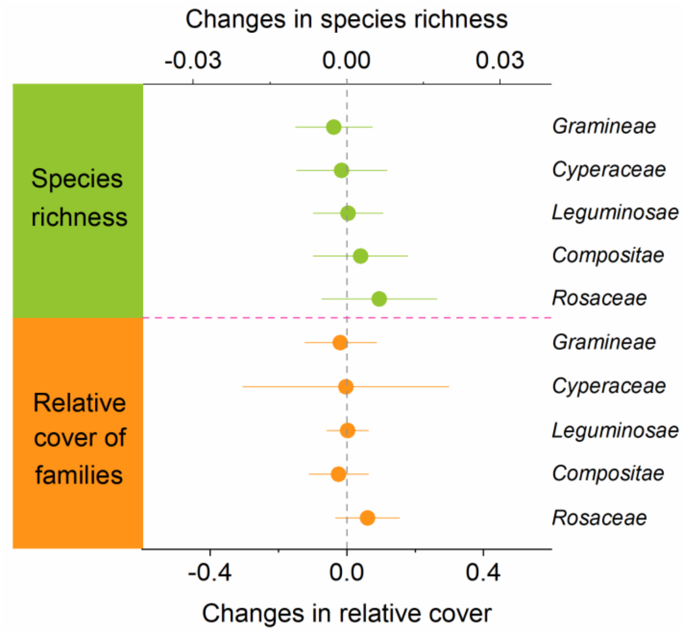
Supplementary Fig. 3. Changes in atmospheric N deposition and its δ^{15}N. Panels (a-d) represent ammonium (NH_4^+) concentration, nitrate (NO_3^-) concentration, δ^{15}N-NH_4^+, and δ^{15}N-NO_3^- derived from a representative ice core on the Tibetan Plateau during the period from 2001 to 2011. The N concentration and δ^{15}N in ice cores have widely used to reflect the dynamics of atmospheric nitrogen (N) deposition and its isotopic characteristics[8,9,10]. In our case, to characterize changes in δ^{15}N values of atmospheric N deposition, we obtained isotopic observations based on an ice core from our co-author (Dr. Yunting Fang)[11]. The changes in N concentration and δ^{15}N were examined with Ordinary Least Square regressions, and the dashed fitted lines denote insignificant changes.



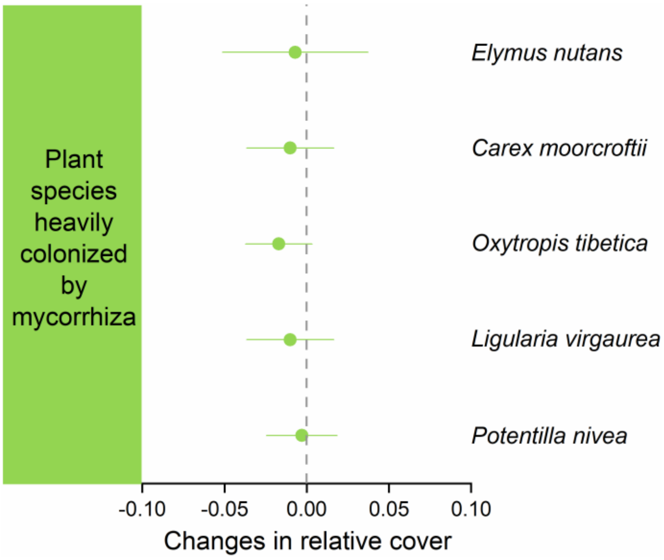
Supplementary Fig. 4. Changes in plant $\delta^{15}N$ dynamics with the resampling size. Plant $\delta^{15}N$ dynamics was detected by the bootstrapped estimate of model slope after a specific number of resampling sites were randomly removed, of which the model refers to the linear mixed-effects model used to statistically analyze changes in plant $\delta^{15}N$ on the Tibetan Plateau over the period from the 2000s to the 2010s. This analysis was used to estimate the threshold that made the findings of changes in plant $\delta^{15}N$ lose statistical significance once we randomly removed sites from the original dataset. In the analyses, the bootstrap was iterated for 10,000 times, in each of which the resampling sites were removed randomly and the slope of the linear mixed-effects model used to test changes in plant $\delta^{15}N$ was estimated based on the remaining sites[12]. The estimated slopes were then used to calculate interquartile range for each number of resampling sites that were removed from the dataset[12]. The average slope was considered non-significant if the interquartile range of the slope overlapped with the horizontal zero line[12]. The dashed line represents the first interquartile range which overlapped with the horizontal zero line. Each point in the diagram represents a bootstrapped estimate of model slope, and each box-plot presents the interquartile range and median slope for each number of resampling sites removed from the analysis.



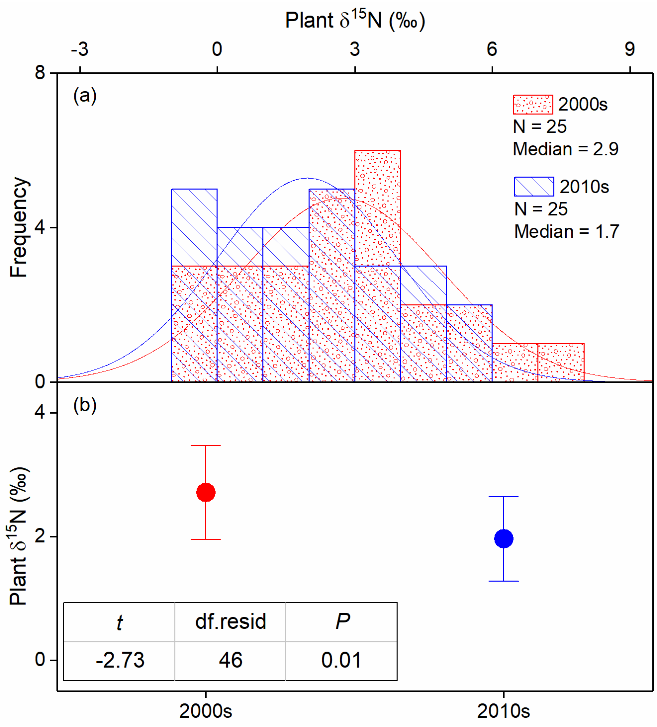
Supplementary Fig. 5. Changes in vegetation composition at the community level. Panels (a-b) represent changes in species richness and Shannon-Wiener index across the resampling sites on the Tibetan Plateau during the period from the 2000s to the 2010s, respectively. Species richness refers to species number per quadrat, and Shannon-Wiener index (*H*) was calculated as: $H = -\sum P_i \ln P_i$, where P_i is the relative cover of species *i*[1]. The change in vegetation composition was examined with linear mixed-effects model, in which the fixed effect was sampling years and the random effects were the investigated sites as well as replicates within each site. Numbers near each box diagram denote the percentile of 95%, 75%, 50%, 25% and 5%, respectively.



Supplementary Fig. 6. Changes in vegetation composition at the family level. During this analysis, changes in species richness and the relative cover of five families that can be heavily colonized by mycorrhizae were detected on the Tibetan Plateau over the period from the 2000s to the 2010s. The species richness here refers to species number per quadrat for each of the five families, and the relative cover was calculated as the sum of the relative cover of each species within a specific family[1]. To conduct the family-level analysis, we selected five families that are heavily colonized by mycorrhizae consisted of *Gramineae*, *Cyperaceae*, *Leguminosae*, *Compositae* and *Rosaceae*[3]. The changes in species richness and the relative cover were examined with linear mixed-effects models, in which the fixed effect was sampling years and the random effects were the investigated sites as well as replicates within each site. Points in the plot indicate the estimated model slopes, and error bars denote 95% confidence intervals. The change in species richness or the relative cover is considered non-significant if the 95% confidence interval overlaps with the vertical zero line.

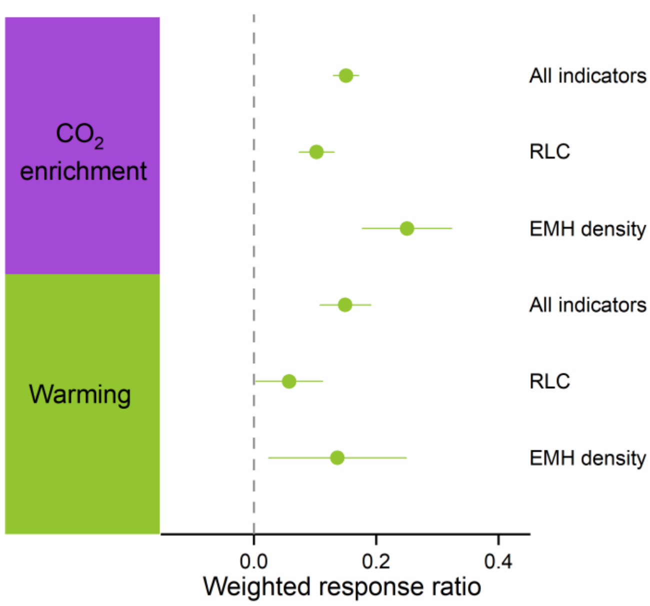


Supplementary Fig. 7. Changes in vegetation composition at the species level. During this analysis, changes in the relative cover of host plant species that can be heavily colonized by mycorrhizae were detected on the Tibetan Plateau over the period from the 2000s to the 2010s. The host plant species *Elymus nutans*, *Carex moorcroftii*, *Oxytropis tibetica*, *Ligularia virgaurea* and *Potentilla nivea* belong to the plant family *Gramineae*, *Cyperaceae*, *Leguminosae*, *Compositae* and *Rosaceae*, respectively[3]. The change in species richness and the relative cover was examined with linear mixed-effects models, in which the fixed effect was sampling years and the random effects were the investigated sites as well as replicates within each site. Points in the plot indicate the estimates of model slopes, and the error bars denote 95% confidence intervals. The change in the relative cover is considered non-significant if the 95% confidence interval overlaps with the vertical zero line.

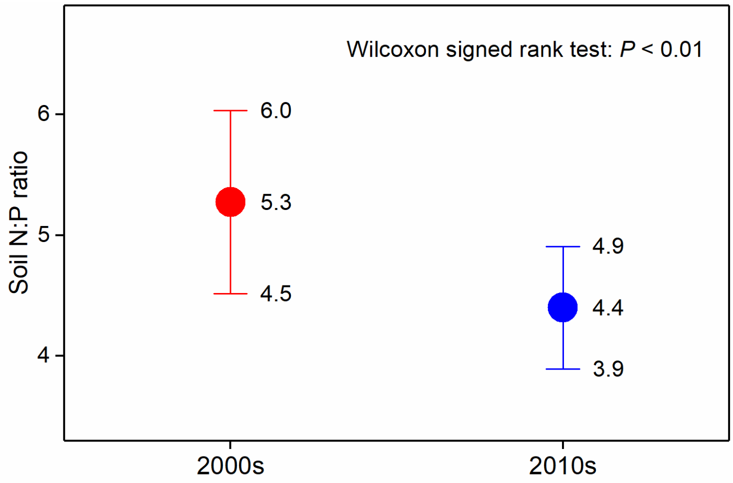


t	df.resid	*P*
-2.73	46	0.01

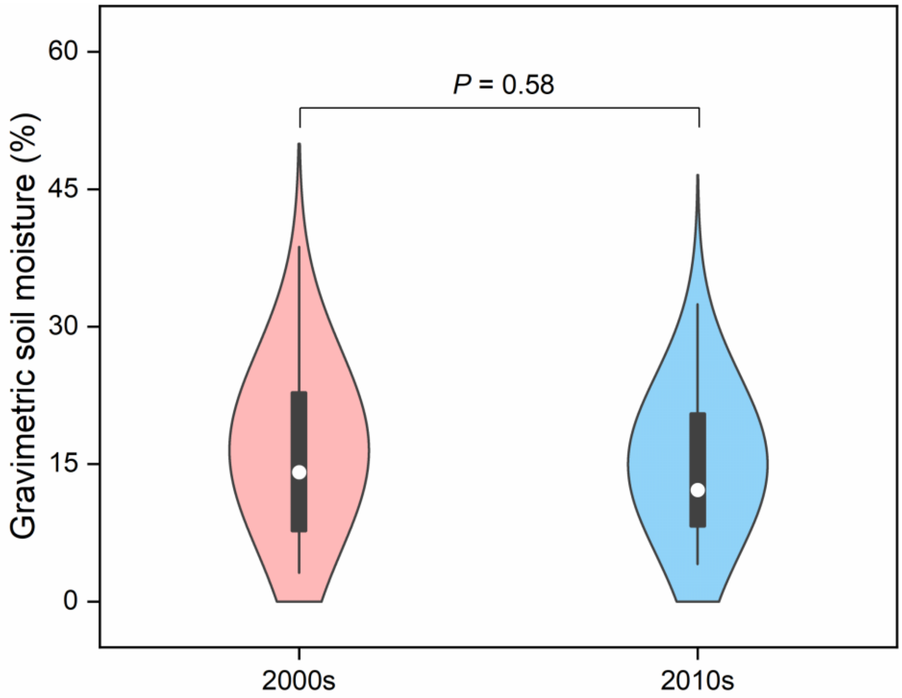
Supplementary Fig. 8. Changes in plant $\delta^{15}N$ among sites without *Leguminosae*. Panels (a-b) represent frequency distributions of plant $\delta^{15}N$ among those resampling sites without the *Leguminosae* during the two sampling periods, and changes in the corresponding plant $\delta^{15}N$ over the detection period, respectively. The change in plant $\delta^{15}N$ was examined with linear mixed-effects model, in which the fixed effect was year and the random effect was sampling site. Points in panel (b) denote mean values and error bars represent 95% confidence intervals. N, the number of sites used for analyzing plant $\delta^{15}N$; Median, median value of plant $\delta^{15}N$; df.resid, residual degrees of freedom.



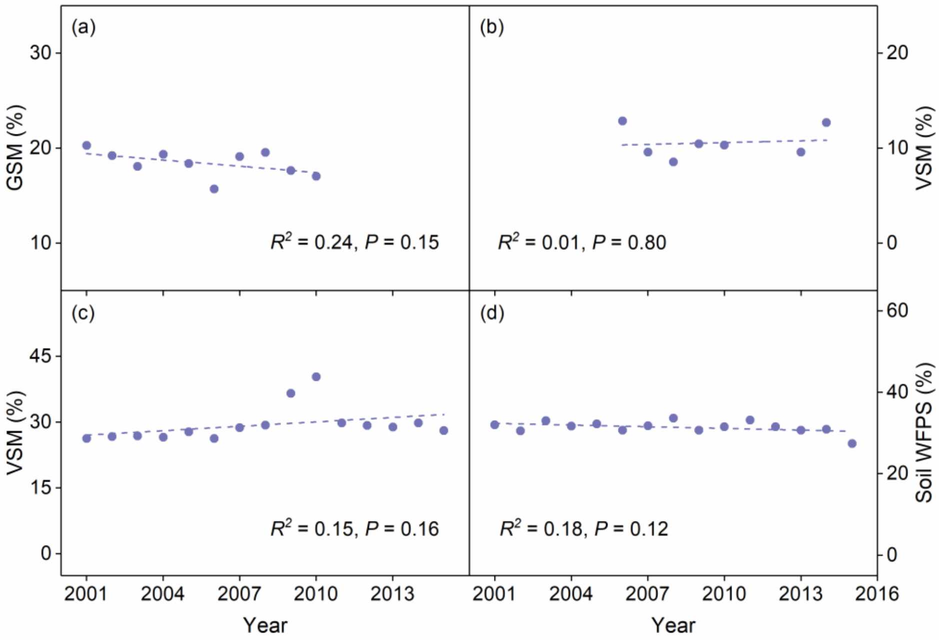
Supplementary Fig. 9. Effects of environmental change on mycorrhizal symbiosis. During this analysis, the weighted response ratios reflecting CO_2 enrichment or warming effects on mycorrhizal symbiosis were estimated based on meta-analyses of published literature (Supplementary Note 2; Supplementary Methods). The all indicators mean all the collected variables associated with mycorrhizal symbiosis. RLC represents the percentage of root length colonized. EMH denotes extraradical mycorrhizal hyphal. Points in the plot denote ln-transformed weighted response ratios, and error bars represent 95% confidence intervals. The vertical dashed line is drawn at the weighted response ratio = 0. The CO_2 enrichment or warming effect on mycorrhizal symbiosis was considered non-significant if the 95% confidence interval overlapped with the dashed vertical line.



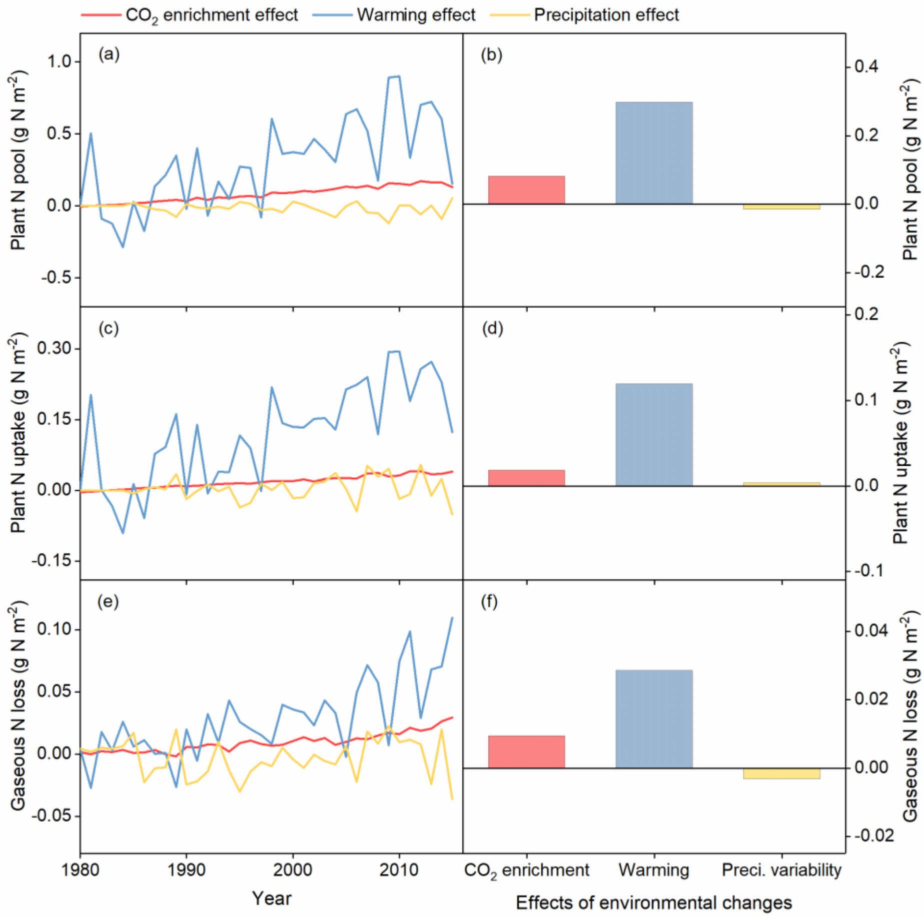
Supplementary Fig. 10. Changes in topsoil N:P ratio over 2000s~2010s. Soil N:P ratio refers to the ratio of soil nitrogen (N) content to phosphorus (P) content in the top 10 cm on the Tibetan Plateau. Soil N and P contents were measured based on samples collected across the resampling sites. The change in soil N:P ratio was examined with Wilcoxon signed rank test due to skewed distributions[13]. Points in the plot denote mean values and error bars represent 95% confidence intervals.



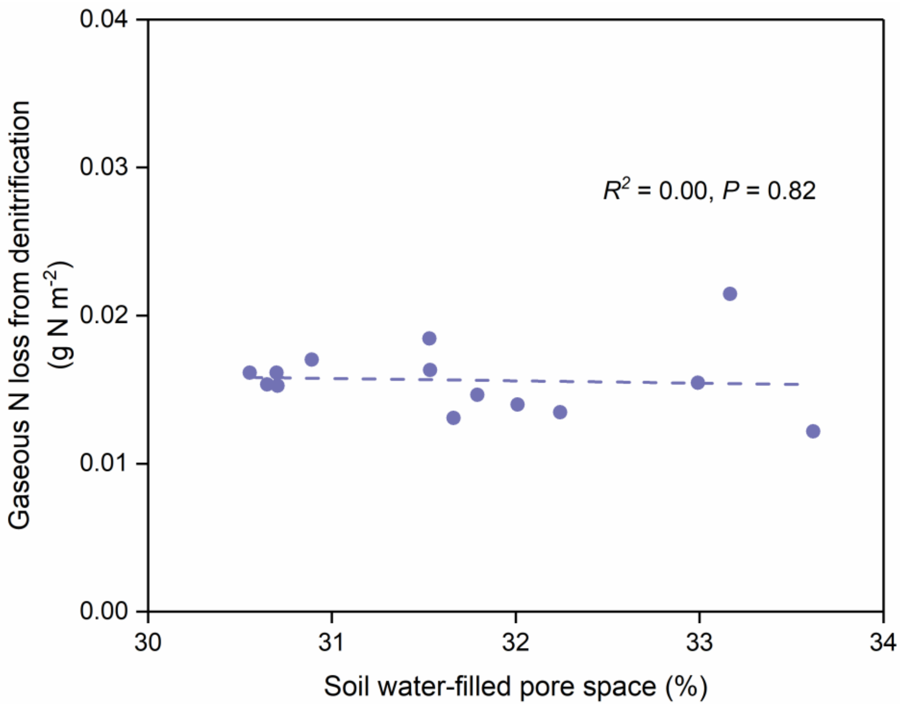
Supplementary Fig. 11. Comparison of soil water status during the two sampling periods. The soil water status refers to gravimetric soil moisture in the top 10 cm on the Tibetan Plateau. Soil moisture data were derived from the resampling investigations conducted in this study. Soil moisture dynamics was examined with linear mixed-effects model, in which the fixed effect was year and the random effect was sampling site. Numbers near each box diagram denote the percentile of 95%, 75%, 50%, 25% and 5%, respectively.



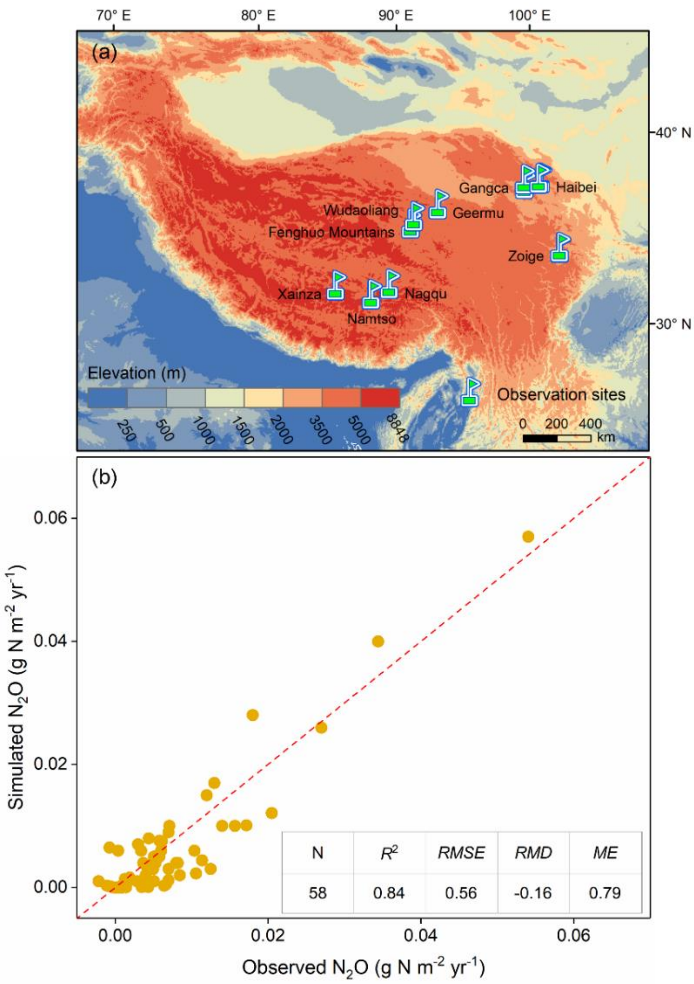
Supplementary Fig. 12. Changes in topsoil water status over 2000s~2010s. Panels (a-d) represent changes in soil water status within the top 10 cm depth derived from China Meteorological Administration (http://data.cma.cn/, a), Zhao et al. (2019)[6] (b), CFSV2 (NCEP Climate Forecast System Version 2, https://rda.ucar.edu/datasets/ds094.0/, c) and the output of DeNitrification-DeComposition (DNDC) model (d) on the Tibetan Plateau from the 2000s to the 2010s, respectively. Based on these datasets, changes in soil water status were examined with Ordinary Least Square regressions, and the dashed fitted lines in the diagram denoted insignificant trends. GSM: gravimetric soil moisture; VSM, volumetric soil moisture; WFPS, water-filled pore space.



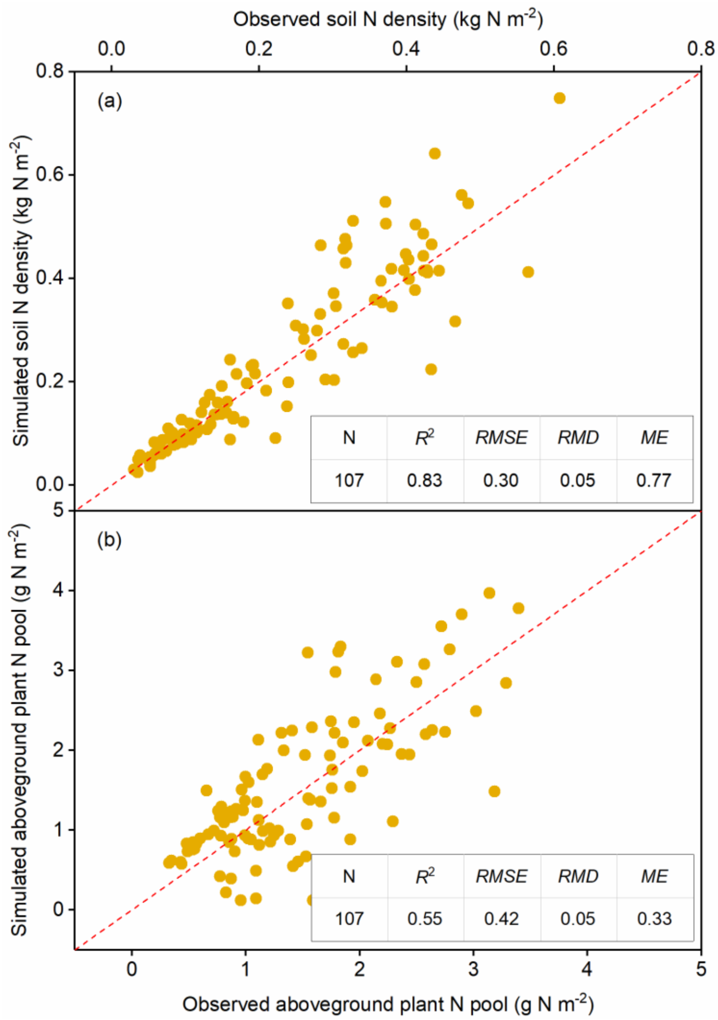
Supplementary Fig. 13. Effects of environmental change on Tibetan N cycle. Panels (a-f) represent effects of environmental changes, including enrichment of atmospheric carbon dioxide (CO_2) concentration, climate warming and precipitation variability, on plant nitrogen (N) pool (a-b), annual plant N uptake rate (c-d) and annual gaseous N loss rate (e-f) during the past decades, respectively. Effects of environmental changes on the related N pools or N cycling processes were analyzed with the simulation experiments performed by the DeNitrification-DeComposition (DNDC) model. Preci. refers to precipitation.



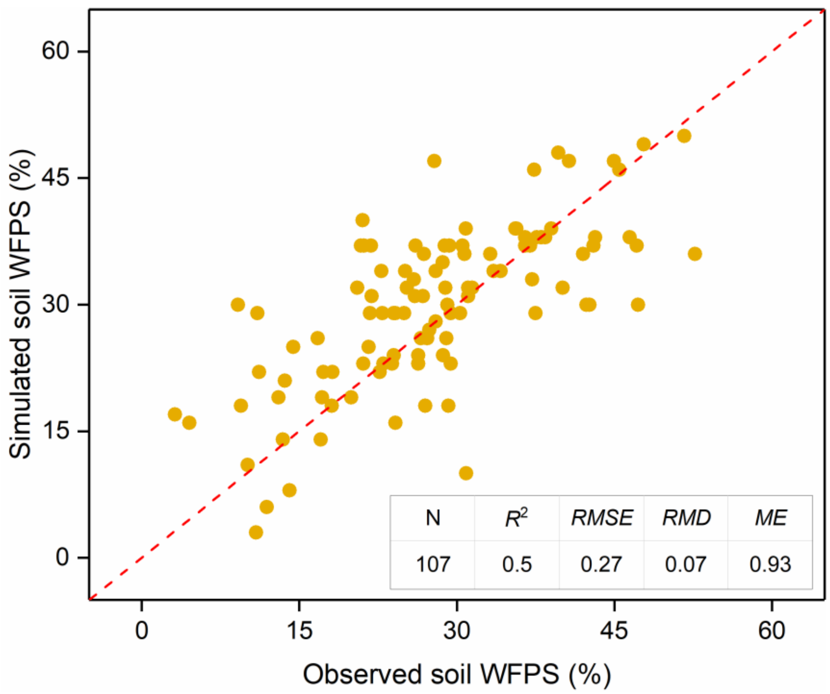
Supplementary Fig. 14. Effects of soil water status on gaseous N loss. Gaseous N losses are each year's total gaseous N loss from denitrification over the period from 2001 to 2014, and soil water status refers to the corresponding annual average soil water-filled pore space. Both gaseous N loss and soil water-filled pore space are derived from the simulation output of the DeNitrification-DeComposition (DNDC) model, and their relationship is examined with Least Ordinary Square regression. The dashed fitted line denotes an insignificant relationship.



Supplementary Fig. 15. Validation of the DNDC model based on flux measurements. Panels (a-b) represent location of the observation sites used to validate the DNDC model, and performance of the validated model detected by its simulation in ecosystem nitrous oxide (N_2O) emissions, respectively. The N_2O observations were derived from publications across the study area (Supplementary Methods). The background map in panel (a) represents the elevation across the study area. The dashed line in panel (b) denotes the 1:1 line. Four indicators including R^2 (coefficient of determination), *RMSE* (root mean square error), *RMD* (relative mean deviation), and *ME* (model efficiency), were used to evaluate the model performance. N, the number of observation-simulation pairs.



Supplementary Fig. 16. Validation of the DNDC model based on pool measurements. Panels (a-b) represent validation of the DNDC model for its simulations in soil N density within the top 10 cm and aboveground plant N pool, respectively. The observed soil N density and plant N pool were derived from the field campaign in this study. The dashed line denotes the 1:1 line. Four indicators, including R^2 (coefficient of determination), *RMSE* (root mean square error), *RMD* (relative mean deviation), and *ME* (model efficiency), were used to evaluate the model performance. N, the number of observation-simulation pairs.



Supplementary Fig. 17. Validation of the DNDC model based on soil water status. During this analysis, the model performance was validated through its simulation in soil water-filled pore space (WFPS) across the 107 resampling sites on the Tibetan Plateau. The observed soil WFPS was calculated based on gravimetric soil moisture and bulk density[14] in the top 10 cm detected through the repeated field sampling campaign. The dashed line denotes the 1:1 line. Four indicators, including R^2 (coefficient of determination), *RMSE* (root mean square error), *RMD* (relative mean deviation), and *ME* (model efficiency), were used to evaluate the model performance. N, the number of observation-simulation pairs.

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