



Impact of nitrogen and phosphorus additions on soil gross nitrogen transformations in a temperate desert steppe

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Abstract

Nutrient addition has a significant impact on plant growth and nutrient cycling. Yet, the understanding of how the addition of nitrogen (N) or phosphorus (P) significantly affects soil gross N transformations and N availability in temperate desert steppes is still limited. Therefore, a ¹⁵N tracing experiment was conducted to study these processes and their underlying mechanism in a desert steppe soil that had been supplemented with N and P for 4 years in north-western China. Soil N mineralization was increased significantly by P addition, and N and P additions significantly promoted soil autotrophic nitrification, rather than NH₄⁺-N immobilization. The addition of N promoted dissimilatory NO₃⁻ reduction to NH₄⁺, while that of P inhibited it. Soil NO₃⁻-N production was greatly increased by N added alone and by that of N and P combined, while net NH₄⁺-N production was decreased by these treatments. Soil N mineralization was primarily mediated by pH, P content or organic carbon, while soil NH₄⁺-N content regulated autotrophic nitrification mainly, and this process was mainly controlled by ammonia-oxidizing bacteria rather than archaea and comammox. NH₄⁺-N immobilization was mainly affected by functional microorganisms, the abundance of *narG* gene and comammox Ntsp-*amoA*. In conclusion, gross N transformations in the temperate desert steppe largely depended

on soil inorganic N, P contents and related functional microorganisms. Soil acidification plays a more key role in N mineralization than other environmental factors or functional microorganisms.

KEYWORDS

¹⁵N diffusion technique, desert grassland, functional genes, soil gross N transformations

1 | INTRODUCTION

In recent decades, atmospheric nitrogen (N) and phosphorus (P) depositions have continued to increase due to human activities and dust input (Liu et al., 2013; Pan et al., 2021). Increased atmospheric N and P depositions influence soil N transformations and N availability, microbial composition and ecosystem stability (Cheng et al., 2019; Hao et al., 2020). In general, increased N and P depositions effectively alleviate soil N and P limitation, promoting plant growth and ecosystem productivity (Chen et al., 2016), and the increase in N deposition exacerbates soil P limitation (Zhu et al., 2014). Desert grasslands are sensitive to N and P depositions, especially, and they are significantly limited by N after precipitation (Yue et al., 2021). Therefore, it is critical and important to improve the availability of soil N after P deposition.

Nitrogen availability in soil is regulated by numerous gross N transformation processes (Elrys, Wang, et al., 2021). The advancement of ¹⁵N isotope tracing technologies has led to an improved understanding mechanisms of N transformations (Jansen-Willems et al., 2022; Zaman et al., 2021). Soil gross N transformations to N addition differ among ecosystems (Dai et al., 2017; Kwak et al., 2018). For example, N input was shown to significantly increase gross N mineralization or nitrification in grassland soils (Hao et al., 2020), while it did not produce any significant effect in forest soils (Barracough & Smith, 1987; Kwak et al., 2018). In particular, the addition of N can lead to increased soil organic carbon, NH₄⁺-N, NO₃⁻-N contents and lower pH, which in turn will affect soil microbial activity and composition (Hao et al., 2020). In carbon-rich soils, N addition significantly enhances N mineralization, nitrification and NH₄⁺-N immobilization, while in carbon-limited soils, it does not significantly affect N transformations, except for nitrification (Cheng et al., 2020). Gross N transformations have been mainly studied in farmland, grassland, yet less so in desert steppe soils (Wang, Cheng, et al., 2016; Wang, Wang, et al., 2016; Wang, Zhang, et al., 2016).

One third of the soil on land is P limited, a condition that is further aggravated by N deposition and that significantly affects plant growth and ecosystem productivity (Elser et al., 2007; Vitousek et al., 2010). Increasing P deposition

Highlights

- Phosphorus (P) additions significantly promoted soil nitrogen (N) mineralization and autotrophic nitrification.
- Autotrophic nitrification was mainly controlled by ammonia-oxidizing bacteria rather than ammonia-oxidizing archaea or comammox in desert steppes.
- N or P addition did not significantly affect NH₄⁺-N immobilization.
- Soil acidification was the most important factor affecting soil N mineralization.

can effectively alleviate the restriction on soil biological processes. For example, soil N mineralization, immobilization rate, denitrification and N₂O emission were largely enhanced by P input (Cheng et al., 2018). In contrast, the addition of P can influence gross N transformations and nitrification, although no significant impacts of P addition on N transformations were observed in grassland soils (O'Neill et al., 2021). The effects of the combined addition of N and P differ across soil types, but only a few studies have investigated this subject, especially in arid regions (Cheng et al., 2019).

Soil N transformations are driven by functional microorganisms (Daims et al., 2015) such as ammonia-oxidizing microorganisms, which specifically affect the soil nitrification process (Yue et al., 2021, 2022). Complete ammonia oxidizers (comammox) can oxidize NH₄⁺-N directly to NO₃⁻-N, rather than via two steps (oxidation of ammonia to nitrite and nitrite to nitrate) which is the traditional view of nitrification (Daims et al., 2015). P addition has been shown to significantly increase the abundance of bacteria (Wu et al., 2022), modifying soil microorganisms (Wu et al., 2022), which consequently affects the soil gross N transformation dynamics. In arid areas, soil microbial activity was significantly limited by P, especially with increasing N input (Wakelin et al. 2017). In contrast, the impact of functional microorganisms on soil nitrogen transformations under the influence of N and P additions has not been thoroughly investigated.

In addition, a suitable C/N ratio is also a key index affecting N mineralization, which mainly regulates the microbial biomass (Cheng et al., 2020), and changes in soil nutrient and pH have significantly impacted N transformations and its availability (Hao et al., 2020). Desert steppe is characterized by low soil organic carbon content, high pH and better aeration conditions, which will significantly affect soil N transformations (Zuo et al., 2022). The desert steppe is both limited by N and P that are key life elements that affect the growth of plants and microorganisms. Increased N and P depositions can significantly affect soil properties and microbial activity, which will significantly affect the N transformations. Yet, it is unclear how N and P additions and their interactive effects affect gross N transformations and N availability in temperate desert steppes. In this study, to address this knowledge gap, ^{15}N tracing technology was used to: (1) quantify the response of soil gross N transformations to N and P additions in a temperate desert steppe, in particular any interaction between the two nutrients; (2) identify the key N transformation processes and its drivers. We hypothesized that (1) P addition and N–P interaction, but not N addition, promoted N mineralization; (2) N addition significantly promoted soil autotrophic nitrification and N immobilization; and (3) these processes were mainly regulated by soil N functional microorganisms.

2 | MATERIALS AND METHODS

2.1 | Soil sampling

The Nutrient Network was conducted more than 130 sites worldwide using the same experimental treatment (<https://nutnet.org/>). Soil samples of this study were collected from the Urat desert steppe in northwest China, which joined the Nutrient Network in 2018. Over the past 38 years, the average rainfall was 151 mm, the average air temperature was 5.6°C. The soil is grey-brown desert soil composed of 40% sand, 43.2% silt and 6% clay. The Nutrient Network experiment included 16 treatments, four of which were examined in the present study: control (CK, i.e., no added N or P), N addition alone, P addition alone and their interaction. Slow-release $\text{CO}(\text{NH}_2)_2$ or $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ was used to simulate N or P additions, respectively. Each treatment was replicated six times. In mid-May each year, N ($10 \text{ g N m}^{-2} \text{ a}^{-1}$) and P ($10 \text{ g P m}^{-2} \text{ a}^{-1}$) were evenly added to each plot (36 m^2). Topsoil samples (10 cm deep) were taken in August 2021, and plant fine roots were removed, and the soils were stored at 4°C until use in culture experiments. Soil samples were used for ^{15}N tracing, and to measure soil properties.

2.2 | ^{15}N tracing experiment in the laboratory

Soil gross N transformation was evaluated by ^{15}N tracing using the Ntrace tool (Müller et al., 2011). Soil samples from each of the four treatments were labelled with $^{15}\text{NH}_4\text{NO}_3$ and $\text{NH}_4^{15}\text{NO}_3$ as sub-treatments and, for each of them, three replicates were conducted at four extraction times (i.e., 0.5, 12, 24 and 48 h). Fresh soil (20 g of oven-dried soil equivalent) was used for culture experiments, to add 2 mL of labelled solution of $^{15}\text{NH}_4\text{NO}_3$ or $\text{NH}_4^{15}\text{NO}_3$, equivalent to 50 mg of $\text{NH}_4^+\text{-N}$ or $\text{NO}_3^-\text{-N}$ per g of soil. Soil moisture content was set at 60% of the water holding capacity (WHC), as this percentage is thought to (1) promote microbial activity to obtain potential maximum N conversion under aerobic conditions, and it was used in other studies (Wang, Cheng, et al., 2016; Wang, Wang, et al., 2016; Wang, Zhang, et al., 2016); (2) 80% of the precipitation was mainly concentrated during the growth season, which resulted in relatively high soil moisture (Zuo et al., 2022). Each flask was sealed with Parafilm, which was punctured by seven holes to maintain humidity, while allowing air exchange. The soil samples were incubated at 18.6°C (the mean temperature recorded in the growing season) for up to 48 h. Soil samples were extracted using KCl (2 mol L^{-1}). This was used to determine the $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and the abundance of ^{15}N in the sample.

2.3 | Measurements

The C, N, Olsen-P, pH of the soil were measured through the method of Murphy and Riley (1962) and Yue et al. (2021). The soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ contents were analysed by flow analysis (AA3 flow analyser, Seal Inc.). The ^{15}N abundance of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ was determined by the method of Zhu et al. (2019). The method reported in Bremner (1996) was used to isolate $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the samples. Firstly, 0.3 g of magnesium oxide was added to 20 mL of the extract to convert the NH_4^+ contained in it to NH_3 , which was collected through the film with oxalic acid and converted to ammonium oxalate. Subsequently, Devarda's alloy was used to convert NO_3^- into NH_4^+ , and further converted into NH_3 and ammonium oxalate. Finally, the film was completely dried in a dryer containing anhydrous copper sulfate, was wrapped in tin foil and ^{15}N abundance was determined. For additional details of the procedure, refer to Zaman et al. (2021).

2.4 | ^{15}N tracing analysis

Gross N transformation was evaluated by the Ntrace_{Basic} tool, which calculates the following 10 transformations

TABLE 1 The impact of nitrogen (N) and phosphorus (P) additions on soil properties (0–10 cm, mean \pm SE, $n = 3$). Different letters indicate significant differences ($p < 0.05$) between treatments.

Treatment	Soil pH	SOC (g C kg ⁻¹)	TN (g N kg ⁻¹)	C/N	TP (g P kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ N (mg kg ⁻¹)	Olsen-P (mg kg ⁻¹)	WHC (%)
CK	8.67 \pm 0.04 a	7.64 \pm 0.49 b	0.32 \pm 0.01	17.98	0.29 \pm 0.00 c	0.46 \pm 0.02 d	3.42 \pm 0.02 d	6.13 \pm 0.19 c	28.41 \pm 0.39
N	8.55 \pm 0.01 b	8.15 \pm 0.78 b	0.32 \pm 0.02	18.71	0.27 \pm 0.01 c	30.15 \pm 0.06 b	37.83 \pm 0.25 b	4.53 \pm 0.32 c	29.79 \pm 1.17
P	8.20 \pm 0.03 c	9.33 \pm 0.67 a	0.33 \pm 0.05	19.34	0.90 \pm 0.06 a	1.68 \pm 0.11 c	3.84 \pm 0.10 c	187.32 \pm 2.77 a	29.68 \pm 0.59
NP	8.30 \pm 0.04 c	8.70 \pm 0.56 b	0.32 \pm 0.04	18.90	0.77 \pm 0.01 b	31.92 \pm 0.14 a	42.53 \pm 0.39 a	108.46 \pm 2.11 b	30.95 \pm 1.71

simultaneously, including mineralization, nitrification, assimilation and other nitrogen conversion processes (Table S1; Müller et al., 2007, 2011). The ratios of nitrification to ammonia immobilization, N mineralization activity, net NH₄⁺-N production and net NO₃⁻-N production were evaluated based on Hao et al. (2020).

2.5 | Determination of functional gene abundance

The abundance of functional genes for nitrogen mineralization (*ureC*), nitrification (comammox Ntsp-*amoA*, *amoA* (ammonia oxidizing bacteria, AOB), *amoA* (AOB), *hao*) and soil dissimilatory NO₃⁻ reduction to NH₄⁺ (DNRA) (*nxrA*, *nxrB*, *narG*, *napA*, *nrfA*) was measured. The primer sequences of functional gene are shown in Table S2. For each fresh soil sample, 0.25 g was weighed and the corresponding DNA was extracted. The measurements were made in triplicate for each sample. All DNA samples were amplified using a Real-time PCR system: 10 μ L of 2 \times Master Mix and 0.5 μ L of each PCR-specific primer (F and R) at a concentration of 10 μ M, and it was adjusted to a total volume of 18 μ L by adding water. The solution was mixed by flicking the bottom of the tube and centrifuging briefly at 5000 rpm, and was then added to a 96-well PCR plate. Subsequently, 2 μ L of extracted DNA was added to each well, the plate was sealed, briefly centrifuged and placed on ice before setting up the PCR program. The 96-well PCR plates were then placed in the Real-time PCR apparatus and the reactions were carried out (Hou et al., 2010).

2.6 | Statistical analyses

One-way ANOVA test was used to assess differences in the main nutrient contents of the soil associated with the treatments. Differences in soil gross N transformation rates were assessed using methods described in Hao et al. (2020). The relationships between N transformations (i.e., N mineralization, nitrification, NH₄⁺-N immobilization and DNRA) and environmental factors, substrate concentrations and functional genes were analysed. All statistical analyses were conducted in SPSS20 and each figure was plotted using Sigmaplot (12.5).

3 | RESULTS

3.1 | Soil properties and abundance of key functional genes

For all treatments (i.e., N, P and their interaction), no significant differences in soil maximum WHC, N content

and soil C/N ratio (Table 1) were detected between the experimental samples and the control. However, the nutrient additions significantly increased soil NH_4^+ -N and NO_3^- -N contents, especially in N treatment (Table 1). The NP treatment had a synergistic effect on

soil inorganic N content compared to the addition of either N or P alone (Table 1). Soil pH was significantly reduced by P and NP treatments. Under these two treatments, soil total P and Olsen-P content significantly increased, which was not observed with N

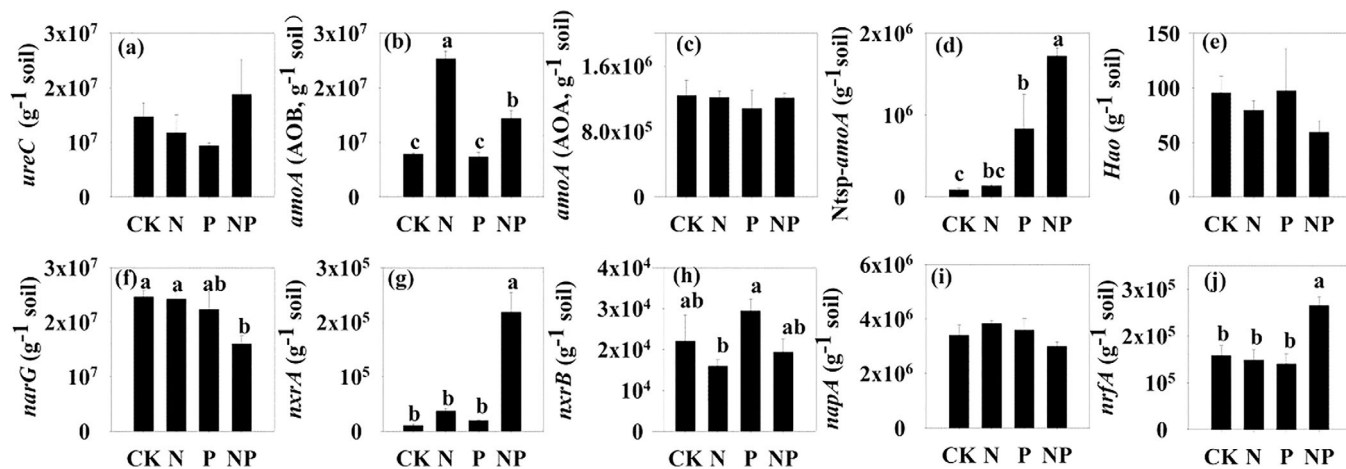


FIGURE 1 Changes in the abundance of functional gene *ureC* (a), *amoA* (AOB, b), *amoA* (AOA, c), comammox *Ntsp-amoA* (d), *Hao* (e), *narG* (f), *nxrA* (g), *nxrB* (h), *napA* (i), *nrfA* (j) and between treatments. Different letters indicate significant differences.

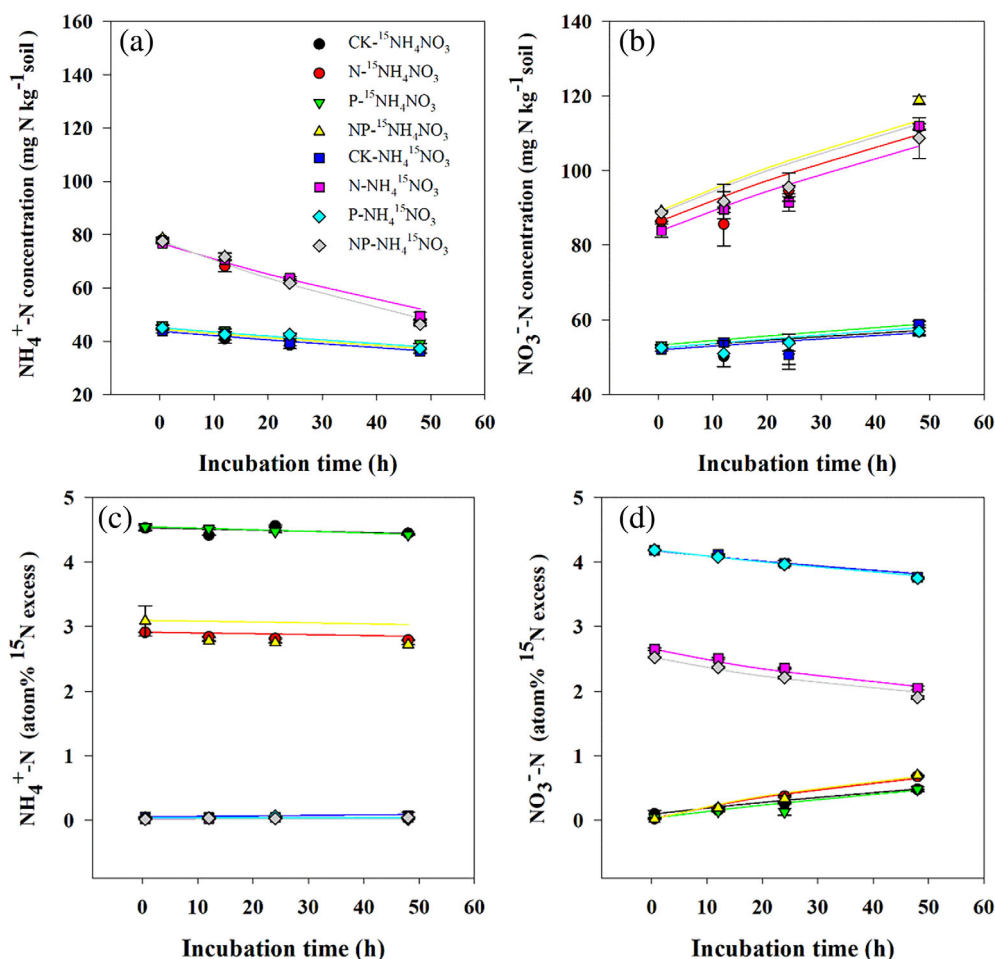


FIGURE 2 Soil concentrations of NH_4^+ -N (a), NO_3^- -N (b) and ¹⁵N enrichments of NH_4^+ -N (c) and NO_3^- -N (d) as measured (point) and modelled (line).

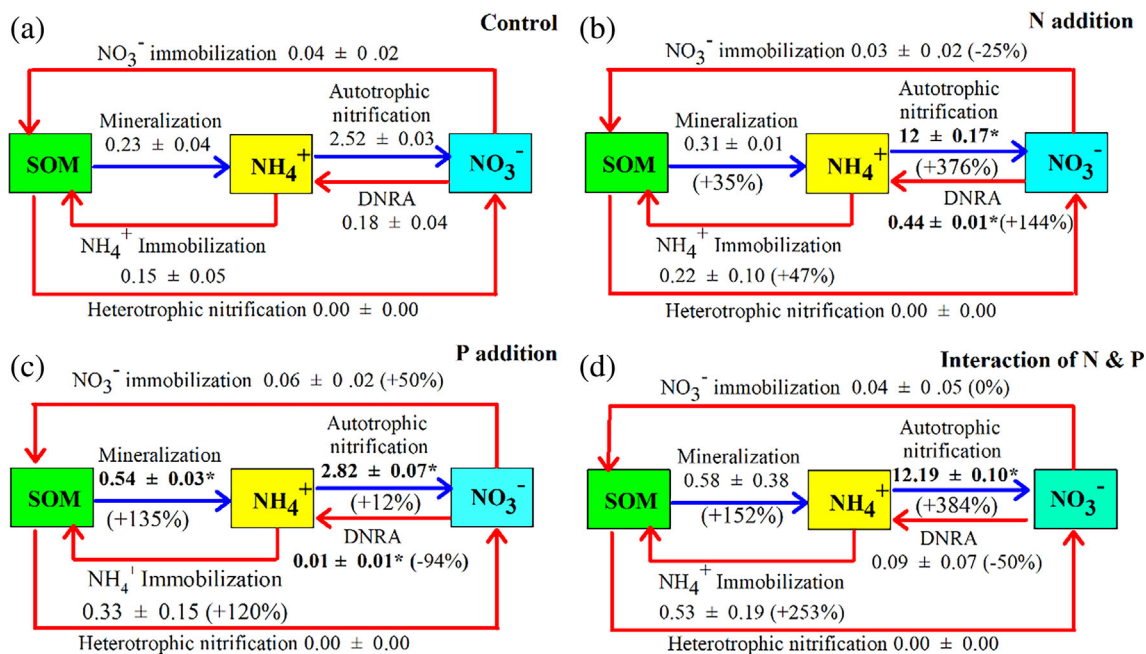


FIGURE 3 Gross N transformation rates (mean ± SD) and rate of change (%; + sign means an increase and - sign means a decrease) in control plots (a), nitrogen (N) addition alone treatment (b), phosphorus (P) addition alone treatment (c), combined N and P addition (d). Bold font and * indicate significant effects ($p < 0.05$). Note: mineralization rates are the sum of M_{Nrec} and M_{Nlab} .

addition (Table 1). Soil organic C content significantly increased only under the P treatment (Table 1).

No significant differences were detected in the number of *ureC*, *amoA* (ammonia oxidising archaea, AOA), *napA* and *hao* genes among treatments (Figure 1). In contrast, *amoA* (AOB) was significantly more abundant under the N and NP treatments (Figure 1b). In addition, *Ntsp-amoA* (complete ammonia oxidizer, comammox) was significantly increased by P and NP treatments (Figure 1). In contrast, the abundance of the *nrfA* and *nxrA* genes significantly increased only under the NP treatment (Figure 1). The number of *nxB* and *narG* genes significantly reduced under the N and NP treatments (Figure 1).

3.2 | ¹⁵N abundance of soil inorganic nitrogen

Soil NH₄⁺-N content decreased as the incubation time increased, and this occurred more rapidly under the N and NP treatments (Figure 2a). In contrast, the opposite was observed for soil NO₃⁻-N content, which increased sharply with incubation time (also more rapidly under the N and NP treatments; Figure 2b).

A slight increase in the ¹⁵N abundance of NH₄⁺-N was observed for the NH₄¹⁵NO₃-labelled treatment (Figure 2c), while this parameter remained unaltered under the ¹⁵NH₄NO₃-labelled treatment (Figure 2c). The ¹⁵N abundance of NO₃⁻-N in this treatment increased

with incubation time (Figure 2d), whereas it decreased over time under the NH₄¹⁵NO₃-labelled treatment (Figure 2d). The inorganic N concentrations and ¹⁵N abundance for each treatment were accurately simulated using Ntrace_{Basic} (Figure 2).

3.3 | Impact of nitrogen or phosphorus input on soil N transformation

Nitrogen transformation rates in soil were low in the study area (Table 1). The N and P additions significantly promoted soil N mineralization and autotrophic nitrification (Figure 3). The two nutrients, applied alone, significantly promoted and inhibited DNRA, respectively (Figure 3). Nitrogen mineralization was mainly regulated though soil pH, P content and organic C (Figure 4). However, autotrophic nitrification was mainly controlled by soil NH₄⁺-N and the number of *amoA* (AOB) rather than *amoA* (AOA) or *Ntsp-amoA* (Figure 5). In addition, NH₄⁺-N immobilization was primarily influenced by the abundance of *narG* and *Ntsp-amoA* (Figure S1).

4 | DISCUSSION

The N and P input affects plant diversity, productivity, soil nutrient cycling and ecological functions (Pan et al., 2021; Zhou et al., 2018), and has a significant impact on gross N

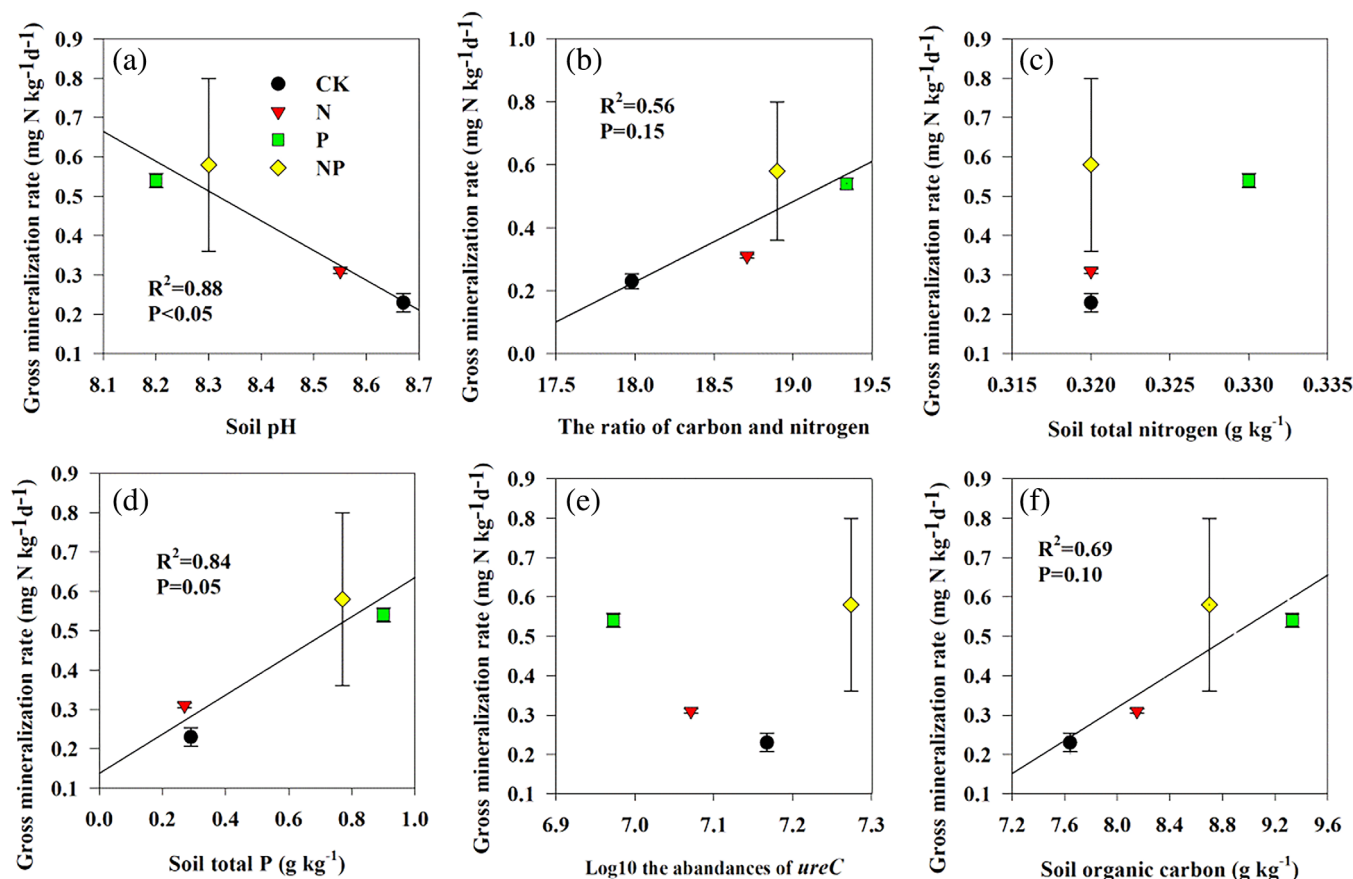


FIGURE 4 Relationship between soil pH (a), the ratio of soil carbon to nitrogen (N) (b), soil total nitrogen content (c), soil total phosphorus content (d), the abundance of *ureC* (e), soil organic carbon content (f) and soil gross N mineralization rate.

transformation rates, with direct effects on soil N availability (Cheng et al., 2019). N and P additions significantly affected soil N mineralization, autotrophic nitrification, NH₄⁺-N immobilization, DNRA, but the effect on other N transformation rates was negligible (Figure 3; Table S2), a result that is consistent with previous findings reported for temperate grasslands (Hao et al., 2020). The values obtained were mainly driven by N addition, soil properties and microorganisms, but the effects of P limitation on nitrogen transformations were also highlighted (Chen et al., 2017; Mehnaz et al., 2018). Moreover, N addition increased the risk of soil N loss (N/I > 1), while P addition largely reduced it (Table S2).

4.1 | Effect of N and P additions on N mineralization

Soil N mineralization rate in this study was significantly lower than that reported for temperate grasslands and other ecosystems (Hao et al., 2020). Furthermore, this process was mainly derived from recalcitrant organic N rather than labile one (Table S2), which is also in

contrast with previous results obtained for temperate grasslands (Wang, Cheng, et al., 2016; Wang, Wang, et al., 2016; Wang, Zhang, et al., 2016) and may be because of the lower organic N content available in desert steppe soils compared to other ecosystems (Yue et al. 2021). Nitrogen addition increased soil N mineralization by 35% (Figure 2), a result that was slightly higher than the average value (25%) obtained from a previous meta-analysis (Cheng et al., 2019). There may be two reasons for this: (1) soil pH, induced by N input, was the key controlling factor for affecting soil N mineralization in our study (Table 1); and (2) with N addition, an increasing trend for soil nutrient content, which are key factors affecting soil N mineralization, was observed (Table 1; Figure 4). Moreover, P addition also significantly promoted soil N mineralization, supporting our hypothesis, in line with previous results obtained for P-limited arable soils (Cheng et al., 2018), but not with those obtained for P-limited grassland soils (Mehnaz et al., 2018). This may be because, in our study, P addition significantly increased P content (a key factor affecting soil N mineralization) and, at the same time, it significantly reduced soil pH, thereby enhancing N mineralization (Table 1).

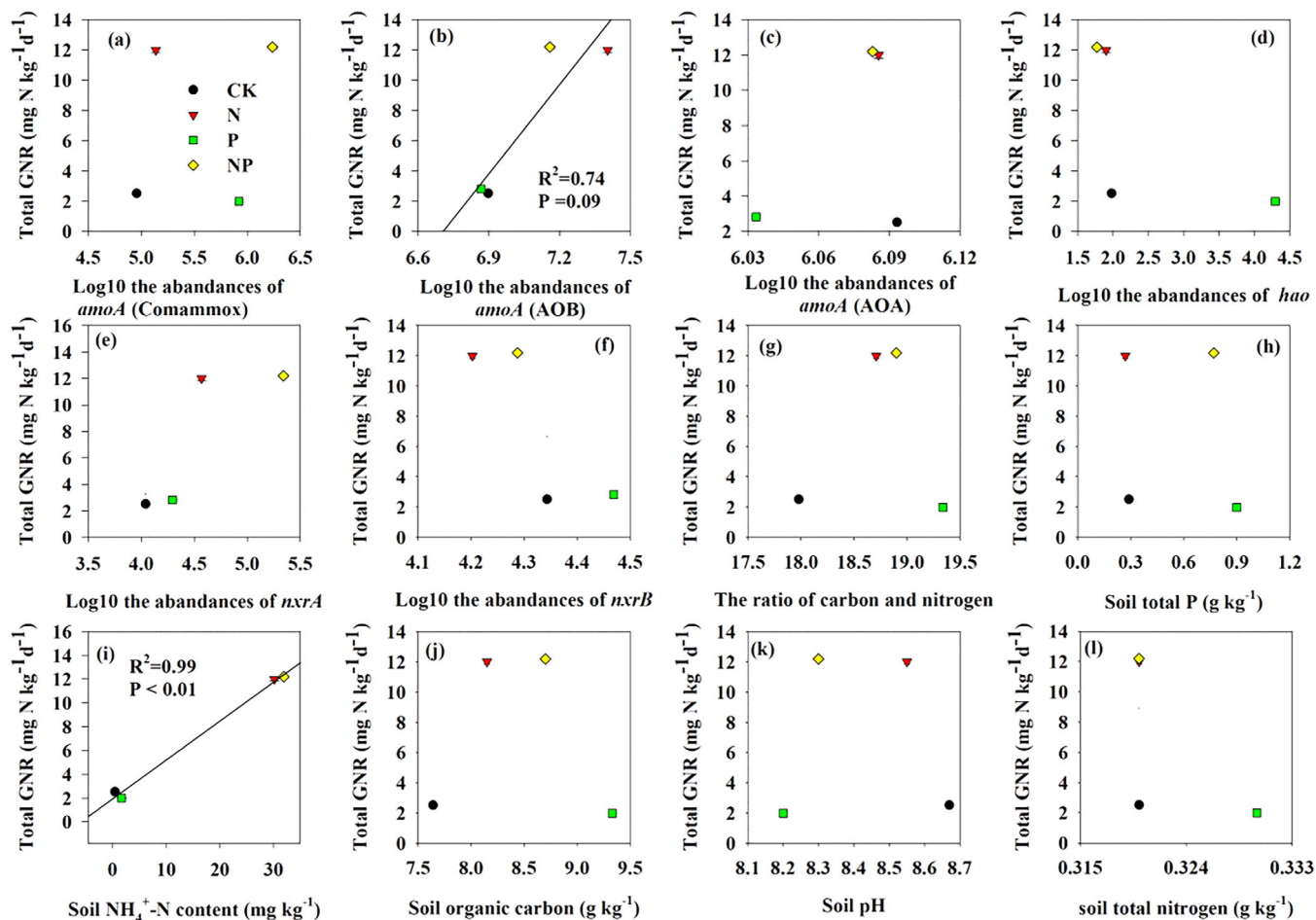


FIGURE 5 Relationship between the abundance of *amoA* (comammox) (a), the abundance of *amoA* (AOB) (b), the abundance of *amoA* (AOA) (c), the abundance of *hao* (d), the abundance of *nxrA* (e), the abundance of *nxrB* (f), the ratio of soil carbon to nitrogen (N) content (g), soil total phosphorus content (h), soil NH_4^+ -N content (i), soil organic carbon content (j), soil pH (k), soil total N content (l) and soil total gross N nitrification rate.

Furthermore, soil pH and P content had greater impacts on N mineralization than soil organic C or functional microorganisms (Figure 4), which supported previous observations (Cheng et al., 2013, 2019).

4.2 | Effect of N and P additions on nitrification

Nitrification consists of both autotrophic and heterotrophic processes (Cheng et al., 2019). The autotrophic nitrification rate in this study was similar to that previously reported for a temperate grassland in Inner Mongolia (Hao et al., 2020). This rate was much higher than the heterotrophic nitrification rate (Figure 3), also in line with results obtained for temperate grasslands in China (Hao et al., 2020). Nitrogen addition significantly enhanced autotrophic nitrification by 376% (Figure 3b), supporting our hypothesis, a rate that was considerably

higher than the average value obtained from a previous meta-analysis and research conducted in the temperate grassland (Cheng et al., 2019; Hao et al., 2020). This was because (1) desert soils, which consequently have a high oxygen content, and stronger nitrification in the presence of sufficient substrate; (2) an increase in soil NH_4^+ -N (the substrate of autotrophic nitrification) following N addition, which was the most important factor affecting autotrophic nitrification (Figure 5i); and (3) the *amoA* (AOB), another essential influencing factor during this process, which was sensitive to N addition (Figure S1b and 4b, Chen et al., 2016).

Autotrophic nitrification was significantly increased (12%) by P input (Figure 2c), which is consistent with the results obtained from a fertilization test on P deficiency (Cheng et al., 2018). This supported the fact that P addition increased the content of ammonium nitrogen by enhancing the mineralization of organic N (Table 1 and Figure 3), and it may be also associated with the

abundance of *amoA* (AOB, Figure 5; Chen et al., 2016). Autotrophic nitrification was also significantly increased by the NP treatment (Figure 2d) because of the increase in the soil NH_4^+ -N content (Table 1).

4.3 | Effect of treatments on soil N immobilization and DNRA

Soil microbial N immobilization includes the immobilization of inorganic N (Müller et al., 2011). In this study, the immobilization of the NH_4^+ -N was dominant, while that of the NO_3^- -N was extremely limited (Figure 3), which supports previous results (Hao et al., 2020). However, soil NH_4^+ -N immobilization in this study was still considerably lower than that previously reported for a temperate steppe in China (Hao et al., 2020). The process was increased by N and P additions, and particularly by the NP treatment (Figure 3), supporting our hypothesis, in line with results obtained for arable soils (Cheng et al., 2018). Nitrifying microorganisms (*narG* gene) was significantly associated with NH_4^+ -N immobilization, which may indicate that more NH_4^+ -N was used as a substrate for nitrification, thereby reducing the immobilization process (Figure S1). In contrast, the abundance of *Ntsp-amoA* (comammox) could explain the change in NH_4^+ -N immobilization (Figure S1), indicating that complete ammonia oxidation is more important for the immobilization of NH_4^+ -N than for its oxidation.

Recent studies have focused on the DNRA process (Cheng et al., 2022). DNRA ($0.18 \pm 0.04 \text{ mg N kg}^{-1} \text{ d}^{-1}$) can occur in this temperate desert steppe soils, which is lower than the DNRA rate reported globally ($0.31 \pm 0.05 \text{ mg N kg}^{-1} \text{ d}^{-1}$; Figure 3; Cheng et al., 2022). Nitrogen addition significantly increased the DNRA rate (Figure 3), possibly due to the resultant increase in NO_3^- -N (Cheng et al., 2022). In contrast, P addition significantly reduced DNRA, which may be attributed to the reduced microbial activity under P-limited conditions (Cheng et al., 2022). No significant relationships between DNRA and functional microbial abundance, substrate concentration or environmental factors were found (Figure S2). Sample replicates in our study were likely too small to allow the identification of key driving factors, therefore, the DNRA process in desert steppe soils should be further investigated in future studies.

5 | CONCLUSION

Nitrogen availability in the examined desert steppe soil was mainly regulated by soil N mineralization, autotrophic nitrification, DNRA and NH_4^+ -N immobilization.

These key processes were significantly affected by N and P additions, except for NH_4^+ -N adsorption. Soil N mineralization was mainly regulated by soil pH. In contrast, autotrophic nitrification and NH_4^+ -N immobilization were regulated by soil NH_4^+ -N concentration and AOB rather than AOA or comammox, respectively. Nitrogen addition significantly promoted DNRA, while this was significantly inhibited by P addition. Nitrogen addition also increased soil N loss, while P addition reduced it. Therefore, appropriate additions of these nutrients are necessary to decrease soil N loss and increase N availability in arid soils.

AUTHOR CONTRIBUTIONS

Ping Yue: Writing – original draft; data curation; investigation. **Jinbo Zhang:** Software; methodology. **Gaodi Zhu:** Methodology. **Xiaoyue Yin:** Data curation. **Xiaoxue Zhang:** Data curation. **Shaokun Wang:** Data curation; investigation. **Christoph Müller:** Software; methodology. **Tom Misselbrook:** Writing – review and editing. **Xiaoan Zuo:** Funding acquisition; conceptualization.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

- Barracough, D., & Smith, M. (1987). The estimation of mineralization, immobilization and nitrification in nitrogen-15 field experiments using computer simulation. *Journal of Soil Science*, 38, 519–530. <https://doi.org/10.1111/j.1365-2389.1987.tb02287.x>
- Bremner, J. M. (1996). Nitrogen-total. In D. L. Sparks (Ed.), *Methods of soil analysis. Part 3. Chemical methods* (pp. 1085–1121). Soil Science Society of America.
- Chen, H., Zhang, W., Gurmesa, G. A., Zhu, X., Li, D., & Mo, J. (2017). Phosphorus addition affects soil nitrogen dynamics in a nitrogen-saturated and two nitrogen-limited forests. *European Journal of Soil Biology*, 68, 472–479. <https://doi.org/10.1111/ejss.12428>
- Chen, Y., Sun, T. T., Qian, H. Y., Fan, J. B., He, Y. Q., & Sun, B. (2016). Nitrogen mineralization as a result of phosphorus

- supplementation in long term phosphate deficient soil. *Applied Soil Ecology*, 106, 24–32. <https://doi.org/10.1016/j.apsoil.2016.04.019>
- Cheng, Y., Elrys, A. S., Merwad, A. R. M., Zhang, H., Chen, Z., Zhang, J., & Müller, C. (2022). Global patterns and drivers of soil dissimilatory nitrate reduction to ammonium. *Environmental Science and Technology*, 56(6), 3791–3800. <https://doi.org/10.1021/acs.est.1c07997>
- Cheng, Y., Wang, J., Chang, S. X., Cai, Z., Muller, C., & Zhang, J. (2019). Nitrogen deposition affects both net and gross soil nitrogen transformations in forest ecosystems: A review. *Environmental Pollution*, 244, 608–616. <https://doi.org/10.1016/j.envpol.2018.10.054>
- Cheng, Y., Wang, J., Mary, B., Zhang, J., & Cai, Z. (2013). Soil pH has contrasting effects on gross and net nitrogen mineralizations in adjacent forest and grassland soils in Central Alberta, Canada. *Soil Biology and Biochemistry*, 57, 848–857. <https://doi.org/10.1016/j.soilbio.2012.08.021>
- Cheng, Y., Wang, J., Sun, N., Xu, M., Zhang, J., & Cai, Z. C. (2018). Phosphorus addition enhances gross microbial N cycling in phosphorus-poor soils: A ¹⁵N study from two long-term fertilization experiments. *Biology and Fertility of Soils*, 54(6), 783–789. <https://doi.org/10.1007/s00374-018-1294-5>
- Cheng, Y., Wang, J., Wang, J., Wang, S., Chang, S. X., Cai, Z., Zhang, J., Niu, S., & Hu, S. (2020). Nitrogen deposition differentially affects soil gross nitrogen transformations in organic and mineral horizons. *Earth-Science Reviews*, 201, 103033. <https://doi.org/10.1016/j.earscirev.2019.103033>
- Dai, S. Y., Wang, J., Cheng, Y., Zhang, J. B., & Cai, Z. C. (2017). Effects of long-term fertilization on soil gross N transformation rates and their implications. *Journal of Integrative Agriculture*, 16(12), 2863–2870. [https://doi.org/10.1016/S2095-3119\(17\)61673-3](https://doi.org/10.1016/S2095-3119(17)61673-3)
- Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R. H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P. H., & Wagner, M. (2015). Complete nitrification by *Nitrospira* bacteria. *Nature*, 528, 504–509. <https://doi.org/10.1038/nature16461>
- Elrys, A. S., Wang, J., Metwally, M. A. S., Cheng, Y., Zhang, J. B., Cai, Z. C., Chang, S. X., & Muller, C. (2021). Global gross nitrification rates are dominantly driven by soil carbon-to-nitrogen stoichiometry and total nitrogen. *Global Change Biology*, 27, 6512–6524. <https://doi.org/10.1111/gcb.15883>
- Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., & Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, 10, 1135–1142. <https://doi.org/10.1111/j.1461-0248.2007.01113.x>
- Hao, T., Zhang, Y. Y., Zhang, J. B., Müller, C., Li, K., Zhang, K., Chu, H., Stevens, C., & Liu, X. J. (2020). Chronic nitrogen addition differentially affects gross nitrogen transformations in alpine and temperate grassland soils. *Soil Biology and Biochemistry*, 149, 107962. <https://doi.org/10.1016/j.soilbio.2020.107962>
- Hou, Y., Zhang, H., Miranda, L., & Lin, S. (2010). Serious overestimation in quantitative PCR by circular (supercoiled) plasmid standard: Microalgal *pcna* as the model gene. *PLoS One*, 5, e9545. <https://doi.org/10.1371/journal.pone.0009545>
- Jansen-Willems, A. B., Zawallich, J., & Müller, C. (2022). Advanced tool for analysing ¹⁵N tracing data. *Soil Biology and Biochemistry*, 165, 108532. <https://doi.org/10.1016/j.soilbio.2021.108532>
- Kwak, J. H., Naeth, M. A., & Chang, S. X. (2018). Microbial activities and gross nitrogen transformation unaffected by ten-year nitrogen and sulfur addition. *Soil Science Society of America Journal*, 82, 362–370. <https://doi.org/10.2136/sssaj2017.11.0384>
- Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z., Vitousek, P., Erisman, J. W., Goulding, K., Christie, P., Fangmeier, A., & Zhang, F. (2013). Enhanced nitrogen deposition over China. *Nature*, 494(7438), 459–462. <https://doi.org/10.1038/nature11917>
- Mehnaz, K. R., Keitel, C., & Dijkstra, F. A. (2018). Effects of carbon and phosphorus addition on microbial respiration, N₂O emission, and gross nitrogen mineralization in a phosphorus-limited grassland soil. *Biology and Fertility of Soils*, 54, 481–493. <https://doi.org/10.1007/s00374-018-1274-9>
- Müller, C., Laughlin, R. J., Christie, P., & Watson, C. J. (2011). Effects of repeated fertilizer and cattle slurry applications over 38 years on N dynamics in a temperate grassland soil. *Soil Biology and Biochemistry*, 43(6), 1362–1371. <https://doi.org/10.1016/j.soilbio.2011.03.014>
- Müller, C., Rütting, T., Kattge, J., Laughlin, R. J., & Stevens, R. J. (2007). Estimation of parameters in complex ¹⁵N tracing models by Monte Carlo sampling. *Soil Biology and Biochemistry*, 39(3), 715–726. <https://doi.org/10.1016/j.soilbio.2006.09.021>
- Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, 31–36. [https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/10.1016/S0003-2670(00)88444-5)
- O'Neill, R. M., Krol, D. J., Wall, D., Lanigan, G. J., Renou-Wilson, F., Richards, K. G., Jansen-Willems, A. B., & Müller, C. (2021). Assessing the impact of long-term soil phosphorus on N-transformation pathways using ¹⁵N tracing. *Soil Biology and Biochemistry*, 152, 108066. <https://doi.org/10.1016/j.soilbio.2020.108066>
- Pan, Y., Liu, B., Cao, J., Liu, J., Tian, S., & Du, E. (2021). Enhanced atmospheric phosphorus deposition in Asia and Europe in the past two decades. *Atmospheric and Oceanic Science Letters*, 14(5), 100051. <https://doi.org/10.1016/j.aosl.2021.100051>
- Vitousek, P. M., Porder, S., Houlton, B. Z., & Chadwick, O. A. (2010). Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications*, 20, 5–15. <https://doi.org/10.1890/08-0127.1>
- Wakelin, S. A., Condron, L. M., Gerard, E., Dignam, B. E. A., Black, A., & O'Callaghan, M. (2017). Long-term P fertilisation of pasture soil did not increase soil organic matter stocks but increased microbial biomass and activity. *Biology and Fertility of Soils*, 53, 511–521.
- Wang, J., Cheng, Y., Zhang, J. B., Müller, C., & Cai, Z. C. (2016). Soil gross nitrogen transformations along a secondary succession transect in the north subtropical forest ecosystem of Southwest China. *Geoderma*, 280, 88–95. <https://doi.org/10.1016/j.geoderma.2016.06.016>

- Wang, J., Wang, L., Feng, X., Hu, H., Cai, Z., Müller, C., & Zhang, J. (2016). Soil N transformations and its controlling factors in temperate grasslands in China: A study from ^{15}N tracing experiment to literature synthesis. *Journal of geophysical research*, *121*(12), 2949–2959. <https://doi.org/10.1002/2016JG003533>
- Wang, J., Zhang, J., Müller, C., & Cai, Z. (2016). Temperature sensitivity of gross N transformation rates in an alpine meadow on the Qinghai-Tibetan Plateau. *Journal of Soils and Sediments*, *17*(2), 423–431. <https://doi.org/10.1007/s11368-016-1530-2>
- Wu, W., Wang, F., Xia, A., Zhang, Z., Wang, Z., Wang, K., Dong, J., Li, T., Wu, Y., Che, R., Li, L., Niu, S., Hao, Y., Wang, Y., & Cui, X. (2022). Meta-analysis of the impacts of phosphorus addition on soil microbes. *Agriculture, Ecosystems & Environment*, *340*, 108180.
- Yue, P., Cui, X. Q., Wu, W. C., Gong, Y. M., Li, K. H., Misselbrook, T., & Liu, X. J. (2021). Are annual nitrous oxide fluxes sensitive to warming and increasing precipitation in the Gurbantunggut Desert? *Land Degradation & Development*, *32*(3), 1213–1223.
- Yue, P., Zuo, X., Li, K., Li, X., Wang, S., & Misselbrook, T. (2022). No significant change noted in annual nitrous oxide flux under precipitation changes in a temperate desert steppe. *Land Degradation & Development*, *33*(1), 94–103.
- Zaman, M., Kleineidam, K., Bakken, L., Berendt, J., Bracken, C., Butterbach-Bahl, K., Cai, Z., Chang, S. X., Clough, T., Dawar, K., Ding, W. X., Dörsch, P., Eckhardt, C., Fiedler, S., Frosch, T., Goopy, J., Görres, C.-M., Gupta, A., Heng, L. K., ... Müller, C. (2021). Isotopic techniques to measure N_2O , N_2 and their sources. In M. Zaman, L. Heng, & C. Müller (Eds.), *Measuring emission of agricultural greenhouse gases and developing mitigation options using nuclear and related techniques* (pp. 213–301). Springer.
- Zhou, X., Bowker, M. A., Tao, Y., Wu, L., & Zhang, Y. (2018). Chronic nitrogen addition induces a cascade of plant community responses with both seasonal and progressive dynamics. *Science of the Total Environment*, *626*, 99–108. <https://doi.org/10.1016/j.scitotenv.2018.01.025>
- Zhu, F. F., Lu, X. K., & Mo, J. M. (2014). Phosphorus limitation on photosynthesis of two dominant understory species in a lowland tropical forest. *Journal of Plant Ecology*, *7*(6), 526–534. <https://doi.org/10.1093/jpe/rtu001>
- Zhu, G., Song, X., Ju, X., Zhang, J., Müller, C., Sylvester-Bradley, R., Thorman, R. E., Bingham, I., & Rees, R. M. (2019). Gross N transformation rates and related N_2O emissions in Chinese and UK agricultural soils. *Science of the Total Environment*, *666*, 176–186. <https://doi.org/10.1016/j.scitotenv.2019.02.241>
- Zuo, X., Li, X., Yue, P., Guo, A., Yue, X., Xu, C., Knapp, A. K., Smith, M. D., Luo, W., Allington, G. R. H., & Yu, Q. (2022). Drought-driven shifts in relationships between plant biodiversity and productivity in temperate steppes. *Functional Ecology*, *36*(12), 2917–2928.

SUPPORTING INFORMATION

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

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