



# OPEN Effects of nitrogen deposition on soil nitrogen fractions and enzyme activities in wet meadow of the Qinghai-Tibet Plateau

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Soil nitrogen (N) transformation is an essential portion of the N cycle in wetland ecosystems, governing the retention status of soil N by controlling the effective soil N content. N deposition produced by human activities changes the physical characteristics of soil, affecting N fractions and enzyme activities. To characterize these influences, three different N addition levels (N5, 5 g/m<sup>2</sup>; N10, 10 g/m<sup>2</sup>; N15, 15 g/m<sup>2</sup>) were established using a wet meadow on the Qinghai-Tibet Plateau (QTP) as a control treatment (0 g/m<sup>2</sup>). We investigated the features of soil physical property alterations, N fractions contents, and enzyme activities under N addition conditions throughout the peak plant growth season. Our findings indicated that N addition significantly enhanced soil aeration, porosity, total nitrogen (TN), ammonium nitrogen (NH<sub>4</sub><sup>+</sup>), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>) content, and urease activity. At the same time, it decreased soil dissolved organic nitrogen (DON) content and bulk density (BD). Additionally, N addition treatment exerted a significant seasonal impact on soil nitrogen component content. The nitrogen component content within the surface soil (0–10 cm) under four treatments is more sensitive to N addition, whereas the nitrogen component in the deep soil is relatively stable. Principal component analysis demonstrated that soil aeration and porosity were the primary factors affecting soil N fractions and enzyme activities. The findings suggested that lower levels of N addition promoted the transformation process of soil N pools in wet meadows and exacerbated the loss of N in wetland ecosystems. Our findings indicate that sustained increases in N deposition will accelerate soil microbial N cycling, potentially overcoming N limitation in alpine wetland ecosystems and exacerbating the risk of N loss and greenhouse gas emissions from alpine wetland surface soils.

**Keywords** Nitrogen addition, Nitrogen fractions, Enzymatic activity, Qinghai-Tibet Plateau, Soil physical properties

Increased atmospheric nitrogen (N) deposition is a critical feature of global climate change and an essential indicator of changes in atmospheric environmental quality conditions, with implications for food production, carbon and nitrogen cycling, and environmental quality globally<sup>1</sup>. Worldwide atmospheric N deposition has increased from 31.6 Tg N yr<sup>-1</sup> in 1860 to 102.5 Tg N yr<sup>-1</sup> in 1993, and is anticipated to further increase to 194.5 Tg N yr<sup>-1</sup> in 2050<sup>2</sup>. As the world's largest developing country, China has been recognized as a significant region of global N deposition. N deposition in China may continue to increase rapidly over a prolonged period<sup>3</sup>. Studies have demonstrated that N addition can disrupt the usual balance of elemental cycling in the soil environment<sup>4</sup>, seriously influencing the N cycling in terrestrial ecosystems.

Soil is the material foundation for plant survival, and its physical properties and water characteristics characterize the soil texture and water-holding function of regional ecosystems<sup>5</sup>. As important markers of physical properties, bulk density (BD) and porosity represent essential channels and activity sites for soil water, nutrients, and microorganisms<sup>6</sup>. The larger the BD and lower the porosity, the more compact the soil is, the more apparent the degradation tendency being more prone to erosion. In contrast, the looser the soil is, the more permeable it is<sup>7</sup>. Soil amylase reflects the metabolic status of soil biota and the efficiency of soil carbon conversion, promoting the normal progress of soil carbon cycling<sup>8</sup>. Urease can promote the hydrolysis of urea into ammonia in soil,

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and its activity level is often used to characterize the intensity of soil nitrogen transformation<sup>9</sup>. The activities of soil amylase and urease play an essential role in carbon and nitrogen cycling in wet grassland soil systems. N is a critical element for plant growth and development, and it is also the primary limiting nutrient in soil, and the soil inorganic N form governs the migration transformation and effectiveness of soil N<sup>10</sup>. N deposition is a critical environmental factor affecting soil N fractions and enzyme activities, and indirectly influencing soil nutrient cycling processes by changing plant growth conditions and microbial activities impacting soil N fraction content and enzyme activities<sup>11</sup>. Soil is the largest N pool in wetland ecosystems, and its small changes can directly affect wetland soil fertility and plant growth, thereby altering the biogeochemical cycle processes of wetland ecosystems<sup>12</sup>.

Research has shown that changes in soil N fractions directly affect soil N availability, indirectly controlling wetland plant growth and ecosystem productivity<sup>13</sup>. The N elements in soil mainly exist in the forms of total nitrogen (TN), nitrate nitrogen ( $\text{NO}_3^-$ ), ammonium nitrogen ( $\text{NH}_4^+$ ), dissolved organic nitrogen (DON), and microbial biomass nitrogen (MBN)<sup>14</sup>, and the N fractions content and distribution are mainly regulated by a combination of biotic and abiotic factors, such as aboveground biomass, soil nutrients, and enzyme activities<sup>15,16</sup>. Most studies on soil N component fractions content and transformation processes in terrestrial ecosystems have focused on shallow soil layers (0–40 cm), where plant root density typically decreases with increasing soil depth<sup>17</sup>, the more active microorganisms in shallow soil layers promote N transformation and cycling, and nutrient leaching may change the distribution pattern of soil N fractions content and enzyme activities<sup>18</sup>. Previous studies have demonstrated that in N-saturated ecosystems, the addition of N inhibits soil organic N mineralization, exacerbates soil  $\text{NO}_3^-$  loss, and limits the amount of stored soil N<sup>19</sup>. Other research has shown that N addition has no significant effect on soil TN in saline reed wetland, while significantly increases  $\text{NO}_3^-$  and  $\text{NH}_4^+$  content<sup>20</sup>. Song et al.<sup>21</sup> found that N addition reduced DON content and increased soil  $\text{NH}_4^+$  content in peat wetland. However, Meng et al.<sup>22</sup> found through evaluating data from 206 papers related to N cycling that N addition increased soil DON content by 21.1%, and the degree of increase in soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  content varied depending on the ecosystem. Moreover, excessive N addition can directly lead to soil acidification and reduced biodiversity<sup>23,24</sup>, indirectly altering soil enzyme activity<sup>25,26</sup>, and ultimately affect soil nitrogen cycling processes. Therefore, studying the effects of N addition levels on soil N fractions and enzyme activities to uncover the impact of N addition levels on soil N cycling processes is critical in the context of global climate change.

The Qinghai-Tibet Plateau (QTP), an ecological security barrier in Asia and a sensitive area to global climate change, possesses a wetland area of about 133 km<sup>2</sup><sup>27</sup>, with more than 50% in wet meadow<sup>28</sup>. It is one of the most sensitive areas to global climate change and human activities<sup>29</sup>. Alpine wetlands typically have lower levels of N deposition, and vegetation is primarily influenced by the limitation of soil N. Increasing N deposition in this area<sup>30</sup> has been observed; for instance, the N deposition in the alpine wetlands of the QTP has reached 10–15 kg N hm<sup>-2</sup> a<sup>-1</sup><sup>31</sup>. N deposition influences the N cycle process in wetland ecosystems by modulating plant biomass and soil characteristics, which impact soil nutrients and enzyme activities<sup>32</sup>. Recent studies on the alpine meadow ecosystems of the QTP have primarily focused on altitude gradients<sup>33</sup>, rainfall<sup>34</sup>, and grazing<sup>35</sup>. In contrast, there has been limited research on the responses of soil N fractions and enzyme activities in the QTP wet meadows to atmospheric N deposition. Therefore, in this study, the wet meadows in the Gahai nature reserve were employed as the research location in this study. Different gradients of N addition experiments were executed to examine the responses of wetland soil physical properties, N fractions, and enzyme activities to N deposition by simulating N deposition<sup>31,36</sup>. This study addresses two questions: (1) How does N addition impact soil N fractions and enzyme activities in the wet meadows of the QTP? (2) What is the association between soil physical characteristics, N fractions, and enzyme activities under N addition? We put forth two hypotheses: (1) N addition elevates the soil N fraction contents and enzyme activity in wet meadows; (2) Relative to the subsoil, the influence of N addition on the surface soil N fractions and enzyme activities are more sensitive. Via this study, we aim to provide fundamental data for predicting soil nutrient balance and alterations in N fractions in alpine wetland ecosystems under future situations of increased N deposition.

## Materials and methods

### Site description and experimental design

The study area was chosen in the Gahai wetland, a national nature reserve in Gansu Province (33° 58′ 12″–34° 32′ 16″ N, 102° 05′ 00″–102° 47′ 39″ E), at an elevation of 3430–4300 m. This area's soil type is mainly peat, meadow, and swamp soil. It has a continental monsoon climate in the Tibetan Plateau, with a multi-year average temperature of 1.2 °C and an average precipitation of 781.8 mm from 1981 to 2022 (<http://data.cma.cn/data/wetherBk.html>), abundant herbaceous plants, and seasonal waterlogging.

In March 2020, in the Gahai nature reserve, an area with gentle terrain and consistent slope direction was selected to establish four N deposition treatment levels. This was accomplished according to the background value of N deposition in the alpine wetland of QTP, alongside the trend of future increases in N deposition<sup>31,37</sup>: CK (0 g/m<sup>2</sup>), N5 (5 g/m<sup>2</sup>), N10 (10 g/m<sup>2</sup>), and N15 (15 g/m<sup>2</sup>). A field randomized block design was employed, and each treatment was performed in triplicate, for a total of 12 plots, each with an area of 2 m × 2 m, and a plot spacing of 4 m. N was supplemented as urea ( $\text{CH}_4\text{N}_2\text{O}$ , containing 46% N). In May 2020, the urea was dissolved in 2 L of water and evenly sprayed on each treatment plot, while the CK plot received the same amount of water.

### Soil collection and measurement methods

Soil N fractions samples were obtained from May to October 2020. The early growth period (EG) of alpine wet meadow plants was from May to June, the peak growth period (MG) was from July to August, and the late growth period (LG) was from September to October. In the middle of each month, soil samples at 0–10, 10–20, and 20–40 cm were harvested in each sample plot using an auger, placed in sterile self-sealing bags, and the N fractions contents were determined. In addition, in the middle of August 2020, surface plants were excluded, and

100 cm<sup>3</sup> ring knives were employed to gather in situ soil samples in the middle of the 0–10, 10–20, and 20–40 cm soil layers, which underwent BD, porosity (soil total porosity, STP; soil capillary porosity, SCP), soil aeration (SA), and enzyme activity characterization.

BD, porosity and SA were determined by the ring knife method<sup>38</sup>; soil TN was determined by the Kjeldahl method<sup>39</sup>; soil NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and DON were determined according to the methods of Saha et al.<sup>40</sup> and Yu et al.<sup>41</sup>; soil MBN was determined by the K<sub>2</sub>SO<sub>4</sub> leaching method after chloroform fumigation<sup>42</sup>; soil amylase was determined by 3,5-dinitrosalicylic acid method<sup>43</sup>; soil urease was determined by indophenol blue colorimetric method<sup>44</sup>.

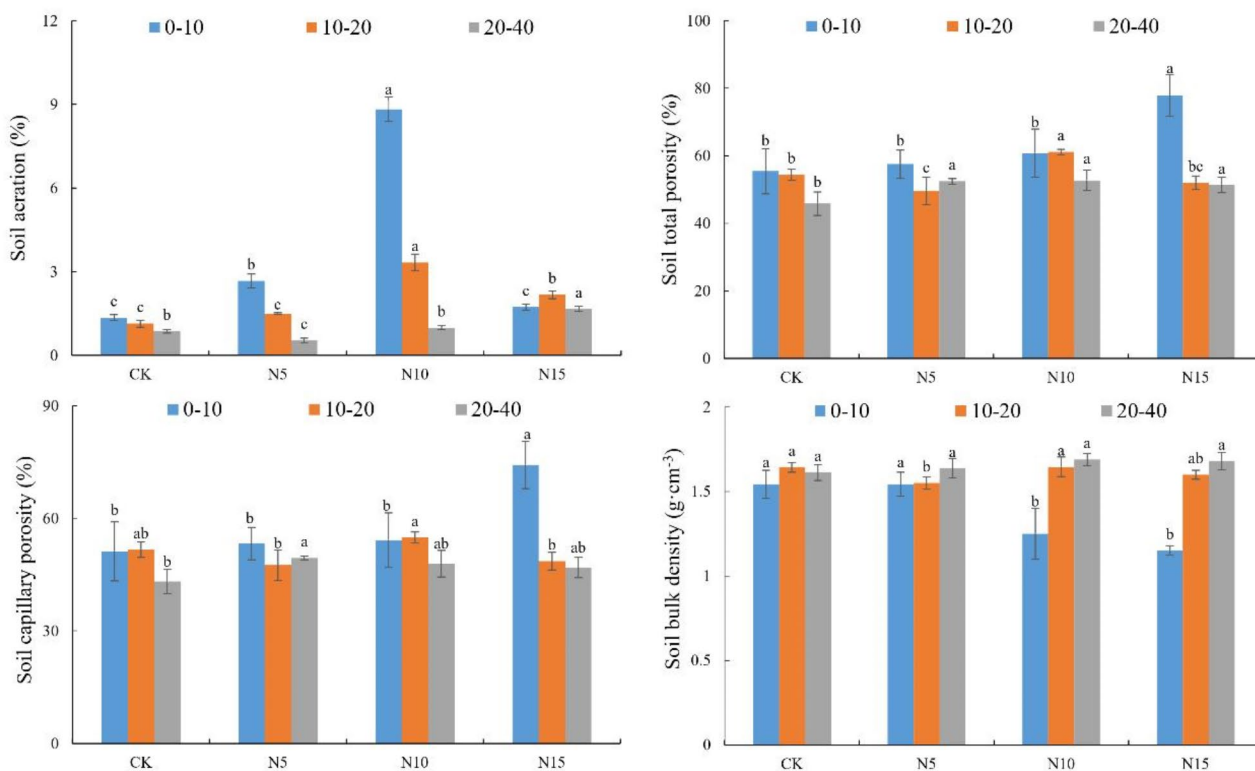
### Data analysis

The significance of the differences in soil physical properties (SA; BD; STP; SCP), N fractions (i.e., TN; NH<sub>4</sub><sup>+</sup>; NO<sub>3</sub><sup>-</sup>; MBN; and DON) and enzyme activities (amylase and urease) among four treatments and three soil layers were analyzed using LSD of variance in SPSS 26.0 (significant level of 95%,  $P < 0.05$ ). A repeated-measures ANOVA was employed to determine differences in N fractions (i.e., TN; NH<sub>4</sub><sup>+</sup>; NO<sub>3</sub><sup>-</sup>; MBN; and DON) among N addition using the season as the repeated variable. In addition, Principal Component Analysis (PCA) was used to study the relationships between soil physical properties and N fractions and enzyme activity variables. All graphical data are mean  $\pm$  standard error.

## Results

### Effects of N addition on the physical properties of wet meadow soil on the QTP

Compared to the CK treatment, SA exhibited an increasing then decreasing trend alongside increased N addition (Fig. 1); with its maximum value appearing in the N10 treatment, the soil BD in the 0–10 cm layer decreased significantly along with increased N addition ( $P < 0.05$ ). No significant differences in soil BD between treatments were observed in the 10–20 and 20–40 cm layers ( $P > 0.05$ ). Moreover, STP and SCP in the 0–10 cm layer exhibited an increasing trend along with increasing N addition, while STP (77.91%) and SCP (74.26%) in the N15 treatment were significantly higher than those in the other treatments ( $P < 0.05$ ). Larger values of STP and SCP in the 10–20 cm layer appeared in the N10 treatment (61.14% and 54.90%). The range of STP and SCP variability in the 20–40 cm layer is relatively stable. On the vertical soil profile, under the four treatments, soil BD gradually increased alongside increased soil depth, while SA, STP, and SCP gradually decreased with increased soil depth.



**Fig. 1.** Soil physical properties in the 0–10, 10–20, and 20–40 cm soil layers at different levels of N addition in August 2020. CK, 0 g/m<sup>2</sup>; N5, 5 g/m<sup>2</sup>; N10, 10 g/m<sup>2</sup>; N15, 15 g/m<sup>2</sup>. Lowercase letters represent significant differences between treatments ( $P < 0.05$ ).

### Effects of N addition on N fractions of wet meadow soils on the QTP

Nitrogen addition significantly affected TN content in QTP wet meadow soil ( $P < 0.05$ , Fig. 2). The TN content in the 0–40 cm layer showed a trend of first increasing and then decreasing with the increase of N addition during the EG period and showed a significant increase trend in MG and LG periods ( $P < 0.05$ ). The soil TN content under the N10 treatment gradually decreased as the season progressed (the maximum value appeared in the EG period). In contrast, the soil TN content under the other three treatments increased and then decreased with season extension, and the minimum value of soil TN content appeared in the LG period. Moreover, N addition reduced the soil N storage in the 0–10 cm layer and increased the soil N storage in the 20–40 cm layer (Fig. 3). Repeated analysis of variance demonstrated that nitrogen addition amount, season, and soil depth significantly influenced the TN content in wet meadow soil (Table 1).

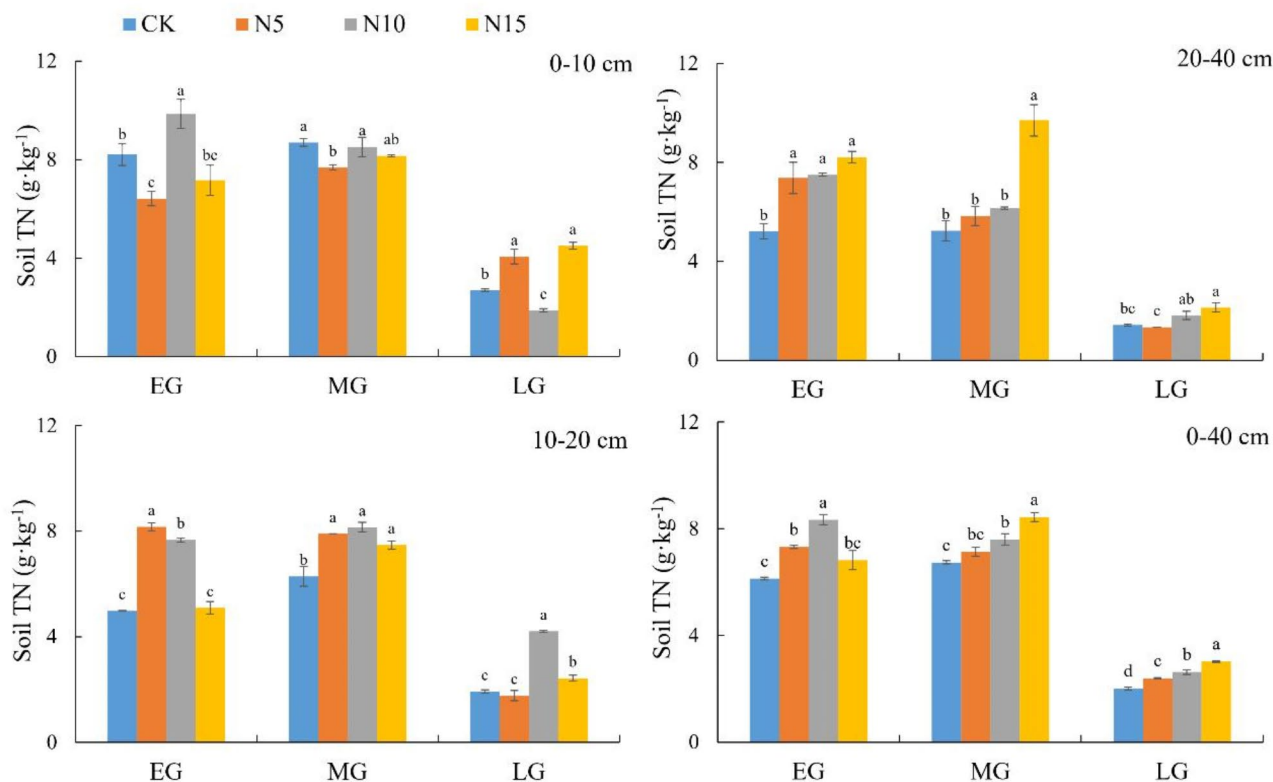
In the 0–40 cm layer, nitrogen addition significantly elevated the average levels of  $\text{NH}_4^+$  and MBN in QTP wet meadow soil, while it significantly decreased the average content of DON in soil ( $P < 0.05$ ). However, no significant effect on the average content of  $\text{NO}_3^-$  was identified (Fig. 4;  $P > 0.05$ ). The levels of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , MBN and DON in the 0–40 cm layer under the four treatments exhibited a trend of initially increasing and then decreasing as the season progressed, and the maximum value appeared in the MG period. Across different soil profiles, except for the 0–10 cm  $\text{NH}_4^+$  and 20–40 cm MBN content under CK treatment, which gradually decreased with the extension of the season, the nitrogen component content of each soil layer under various treatments exhibited a trend of first increasing and then decreasing as the season progressed (Figs. 5 and 6). Repeated analysis of variance demonstrated that nitrogen addition amount, season, and soil depth had significant impacts on nitrogen component content in wet meadow soil (Table 1).

### Effects of N addition on soil enzyme activities in wet meadows on the QTP

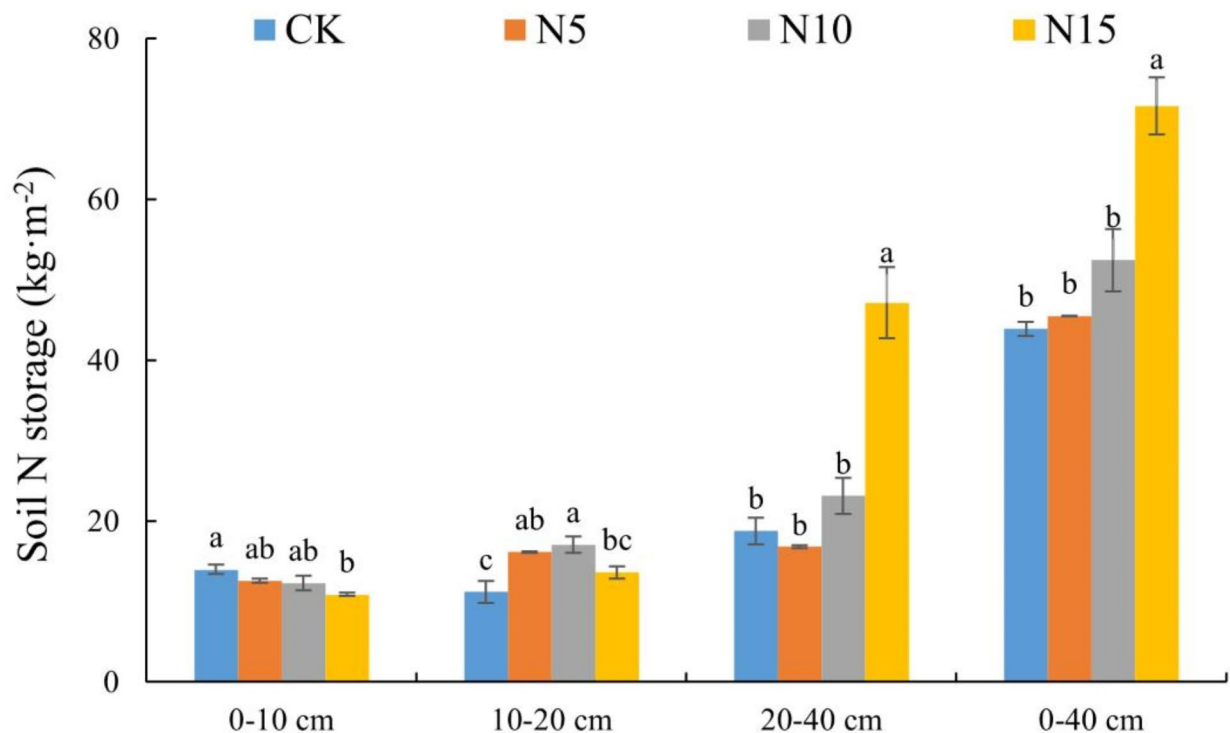
N addition significantly affected soil enzyme activities in wet meadows on the QTP ( $P < 0.05$ , Fig. 7; Table 2). Compared to the CK treatment, the N5 treatment significantly elevated soil amylase and urease activities in the 0–10 cm layer, whereas the N15 treatment inhibited soil amylase and urease activities ( $P < 0.05$ ). The soil amylase and urease activities under all four treatments were primarily identified in the 0–20 cm layer, significantly higher than those in the 20–40 cm layer. In addition, N addition and soil depth produced a significant interactive effect on amylase and urease activity of wet meadow soil (Table 2).

### Effects of soil physical properties on N fractions and enzyme activities

PCA analysis showed that the physical properties of wet meadow soil significantly affected N fractions and enzyme activities (Fig. 8). SA and STP showed strong positive correlations with DON, MBN,  $\text{NH}_4^+$ , amylase,



**Fig. 2.** Seasonal variation of soil TN content under different levels of N addition. CK, 0 g/m<sup>2</sup>; N5, 5 g/m<sup>2</sup>; N10, 10 g/m<sup>2</sup>; N15, 15 g/m<sup>2</sup>. EG early growth period, MG the peak growth period, LG the late growth period. Lowercase letters represent significant differences between treatments ( $P < 0.05$ ).



**Fig. 3.** Soil N storage in the 0–10, 10–20, and 20–40 cm soil layers at different levels of N addition. CK, 0 g/m<sup>2</sup>; N5, 5 g/m<sup>2</sup>; N10, 10 g/m<sup>2</sup>; N15, 15 g/m<sup>2</sup>. Lowercase letters represent significant differences between treatments ( $P < 0.05$ ).

Source of variation	df	TN		NO <sub>3</sub> <sup>-</sup>		NH <sub>4</sub> <sup>+</sup>		MBN		DON	
		F	P	F	P	F	P	F	P	F	P
T	3	34.194	0.000	4.592	0.005	278.694	0.000	34.943	0.000	94.780	0.000
S	2	1111.506	0.000	532.390	0.000	4578.495	0.000	1245.854	0.000	396.978	0.000
D	2	67.938	0.000	68.366	0.000	487.313	0.000	160.435	0.000	121.797	0.000
T×S	6	11.293	0.000	6.258	0.000	226.580	0.000	24.087	0.000	56.655	0.000
T×D	6	26.026	0.000	14.716	0.000	26.285	0.000	19.843	0.000	14.375	0.000

**Table 1.** Results of a repeated-measures ANOVA testing for differences in soil N fractions (TN, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, MBN, DON) among N additions using depth as the repeated variable. *T* treatment, *S* season, *D* depth.

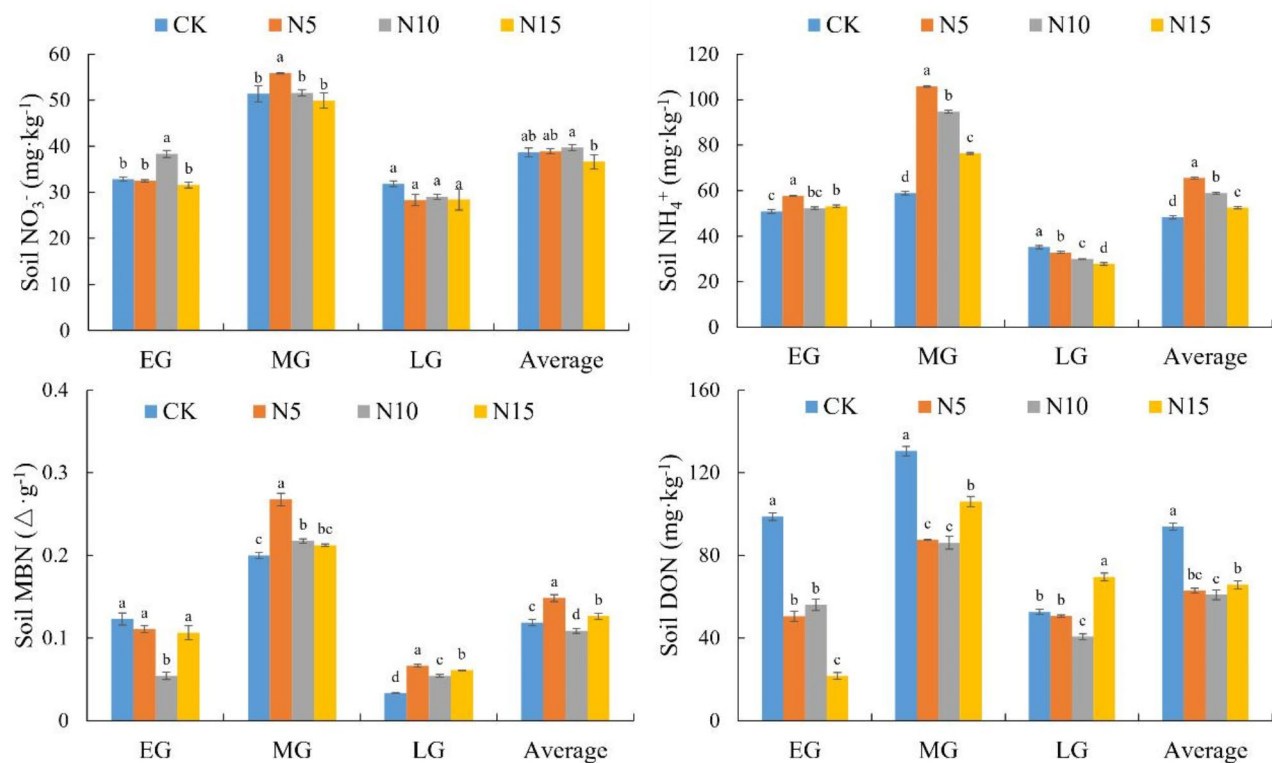
and urease, while BD showed strong negative correlations with DON, MBN, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, amylase, and urease ( $P < 0.05$ ). In addition, amylase and urease activities significantly affected soil DON, MBN, NH<sub>4</sub><sup>+</sup>, and NO<sub>3</sub><sup>-</sup> content ( $P < 0.05$ ), and their effects on soil TN content were at a non-significant level ( $P > 0.05$ ).

## Discussion

### N addition affects soil physical properties in wet meadows

Soil physical properties reflect the soil structure condition and influence vegetation growth, playing a crucial role in soil N cycling<sup>45</sup>. This study demonstrated that soil aeration increased and then decreased alongside increased N addition, with the maximum value appearing in the N10 treatment. N addition alleviated the N limitation of wet meadow soil in the QTP area, increased the effective N content in the soil, and promoted the rapid growth of fine roots of wetland plants<sup>46</sup>, favored soil granular structure formation, and improved soil aeration. However, excessive N addition (N15) exacerbates the limitation of soil phosphorus resources, reducing plants aboveground and root biomass<sup>47</sup>, leading to decreased soil aeration in the N15 treatment. We identified that N addition increased total soil porosity and capillary porosity, and that total soil porosity and capillary porosity decreased gradually alongside soil deepening. This is because wetland plants are primarily shallow-rooted<sup>48</sup>, and adding N promotes microbial mineralization of soil carbon and N, triggering more inorganic N uptake by plant roots for growth. A large number of plant roots lead to the loosening of surface soil, elevating the porosity of the surface soil and reducing the soil BD in the root zone.

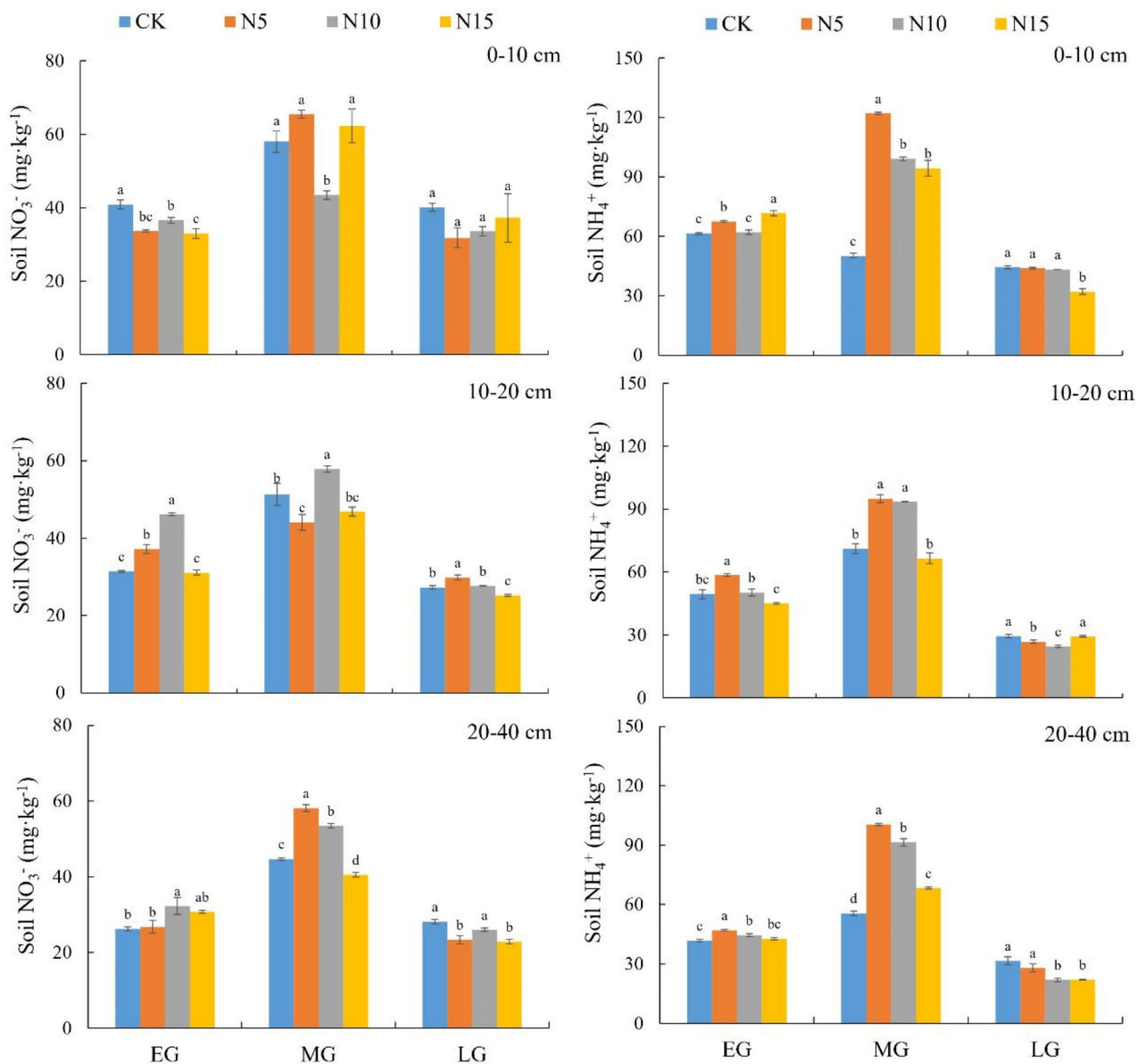




**Fig. 4.** Seasonal variation of average  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , MBN, and DON content in soil layer 0–40 cm under different levels of N addition. CK, 0 g/m<sup>2</sup>; N5, 5 g/m<sup>2</sup>; N10, 10 g/m<sup>2</sup>; N15, 15 g/m<sup>2</sup>. EG early growth period, MG the peak growth period, LG the late growth period. Lowercase letters represent significant differences between treatments ( $P < 0.05$ ).

#### N addition affects the content of soil N fractions in wet meadows

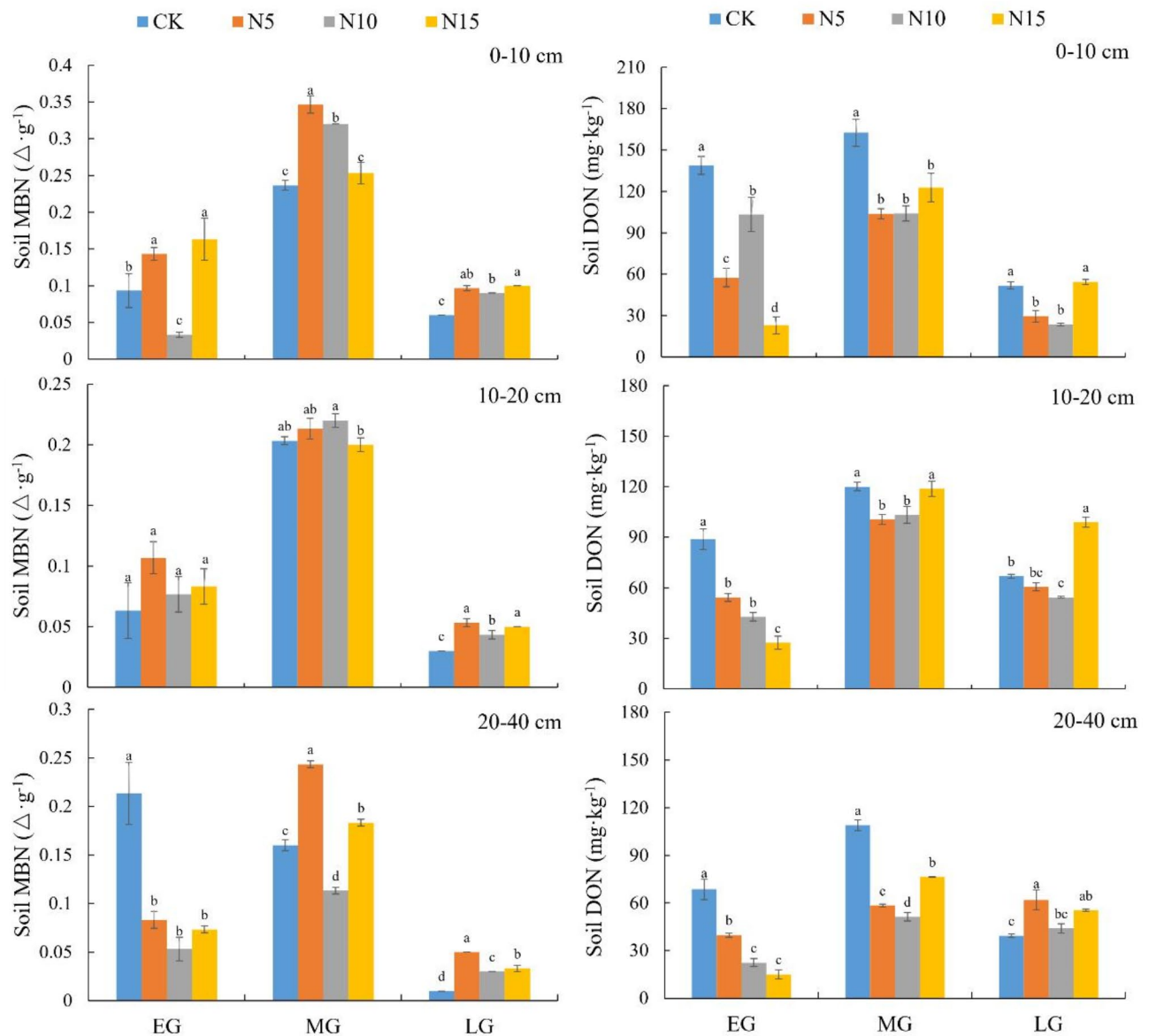
Soil N is a vital soil nutrient that influences the productivity of terrestrial ecosystems and global climate change, limiting plant growth<sup>49</sup>. In this study, we determined that the N addition treatment increased the average TN,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , MBN, and N storage in the 0–40 cm layer and reduced the DON content compared to CK. This is because N addition elevates the photosynthetic rate of plants, increases the growth of above- and below-ground biomass, and large amounts of litter and root secretions enhance the effective soil N<sup>50</sup>. Inconsistent with our initial hypothesis was the finding that N addition decreased soil DON content, as N addition promotes the leaching loss of soil DON content<sup>51</sup>. Moreover, plants can directly absorb soil DON<sup>52</sup>, and N addition enhances plant growth and the absorption of a large amount of DON, leading to decreased soil DON content. In this study, with the gradual increase in the amount of N added, soil TN and N storage increased gradually, whereas the content of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and MBN tended to increase first and then decrease, and soil DON tended to decrease first and then increase. This is because, alongside continuous increases in the amount of N added, the excessive N input to the soil produced a decrease in the amount of humus-degrading enzymes generated by soil microorganisms. Alongside this impact, microbial activity is affected, soil N mineralization is weakened<sup>53</sup>, and N storage increases. Moreover, when ecosystems reach N saturation, excess inorganic N undergoes nitrification and denitrification, with increased N inhibiting soil organic N mineralization, exacerbating the loss of soil  $\text{NO}_3^-$  content and reduced soil  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and MBN content<sup>19</sup>. These impacts increase the likelihood of N loss and greenhouse gas emissions<sup>37</sup>. The distribution patterns of soil TN,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$  levels in the soil layer under each treatment were relatively consistent, primarily concentrated in the 0–10 cm layer, with gradual decreases and increased soil depth, consistent with our second hypothesis. This is because the N in the soil predominantly originated from the decomposition of above-ground vegetation, apoplastic matter, and root secretions<sup>54</sup>, and the plants in the QTP area are shallow-rooted alpine plants with root systems are mainly concentrated in the 0–10 cm layer. With increased soil depth, the input of litter and vegetation root system decreased, and the microbial activity and N mineralization rate gradually decreased<sup>55</sup>, producing decreased soil N content. In this study, the contents of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , MBN and DON in the 0–40 cm layer of the QTP wet meadow first increased and then decreased with the extension of the season, with the maximum value appearing in the MG stage and the minimum value appearing in LG stage. As the growing season extended, the temperature and rainfall gradually increased (the MG period was highest<sup>56</sup>, and the more active microorganisms promoted the release of soil-available nutrients and the decomposition of litter<sup>57</sup>. After plant growth, plants absorb abundant nutrients and store them in their roots to resist cold winter conditions<sup>58</sup>. Lower soil temperature limits the decomposition of litter by microorganisms, causing the lowest nitrogen content in soil at the end of plant growth.



**Fig. 5.** Seasonal variation of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  content in soil layers 0-10, 10-20, and 20-40 cm under different levels of N addition. CK, 0 g/m<sup>2</sup>; N5, 5 g/m<sup>2</sup>; N10, 10 g/m<sup>2</sup>; N15, 15 g/m<sup>2</sup>. EG early growth period, MG the peak growth period, LG the late growth period. Lowercase letters represent significant differences between treatments ( $P < 0.05$ ).

### N addition affects wet meadow soil enzyme activities

Soil enzymes are the primary participants in material cycling and energy flow, reflecting the demand of soil microorganisms for nutrients and playing a critical role in soil nutrient transformation and fixation<sup>59</sup>. Hydrolytic enzyme activities such as urease and amylase characterize the cycling status of soil N fractions. Compared to natural wet meadows in the QTP area, low nitrogen (N5) addition significantly elevated soil amylase and urease activities in the 0–10 cm layer, whereas high nitrogen (N15) addition suppressed soil amylase and urease activities. This is not entirely consistent with our initial hypothesis because the QTP wet meadow area is primarily subject to the limiting effect of N. Low nitrogen addition can enhance soil fertility, overcome the limiting effect of N, promote alpine plant growth, and enhance plant root secretions, which promotes soil enzyme activities<sup>60</sup>. In contrast, N addition limits the competition between plants and soil microbes for N, enhances soil microbial activity, promotes nutrient mineralization by soil microbes<sup>61</sup>, and increases soil enzyme activity. However, high N supplementation causes soil sloughing and reduced permeability (Fig. 1), limiting microbial reproduction and growth, inhibiting mineralization and decomposition of loamy organic N<sup>62</sup>, and reducing soil pH. These features reduce plant biomass, inhibit QTP wet meadow soil amylase and urease activities, and increase soil N storage. The findings of PCA analysis between soil physical properties and enzyme activities and N fractions further



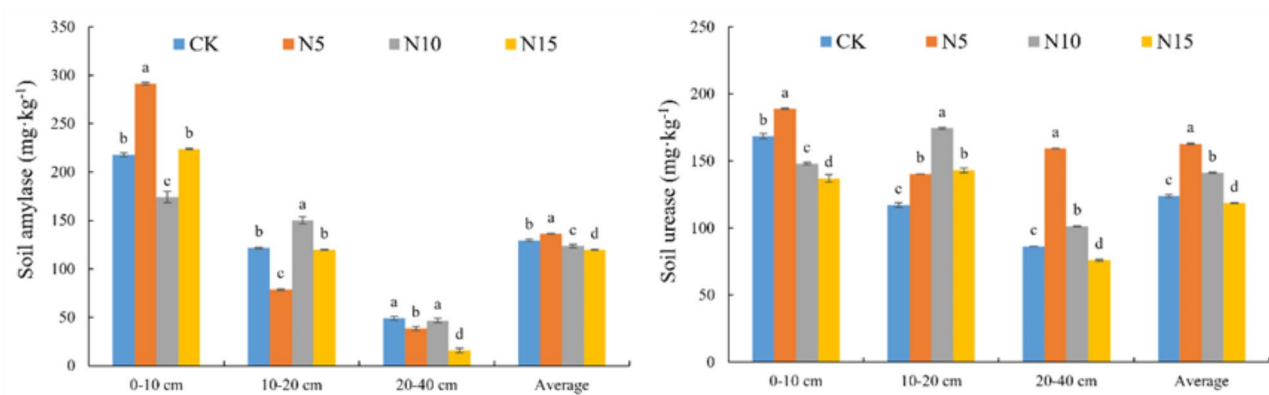
**Fig. 6.** Seasonal variation of MBN and DON content in soil layers 0-10, 10-20, and 20-40 cm under different levels of N addition. CK, 0 g/m<sup>2</sup>; N5, 5 g/m<sup>2</sup>; N10, 10 g/m<sup>2</sup>; N15, 15 g/m<sup>2</sup>. EG early growth period, MG the peak growth period, LG the late growth period. Lowercase letters represent significant differences between treatments ( $P < 0.05$ ).

confirmed this outcome. Soil amylase and urease activities gradually decreased along with increasing soil depth. Because effective soil nutrients are the main factor impacting enzyme activity, the aeration and nutrient content of QTP wet meadow soils (Fig. 2) gradually decreased with increasing soil depth, limiting the capacity of soil microorganisms to decompose nutrients and reducing soil enzyme activity.

## Conclusion

Experiments utilizing different concentrations of N addition during the peak plant growth season in the QTP area demonstrated that N addition increased soil TN, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, MBN, SON, and urease activities while it decreased soil DON and BD. The content of soil nitrogen components under the four treatments exhibited a trend of initially increasing and decreasing with season duration extension. The treatments and seasons had a significant interactive effect on soil nitrogen components. With the gradual increase of N addition level, soil TN and SON levels gradually increased. Low levels of N addition (N5 or N10) elevated soil N fractions and enzyme activities, whereas excessive N addition (N15) decreased soil N fractions and enzyme activities. Correlation analysis demonstrated that soil N fractions and enzyme activities influenced soil aeration and porosity. Overall, with increased N deposition, the soil N transformation process would be accelerated, and effective soil N content would increase, exacerbating the loss of N in the wet meadow ecosystem. The study's findings will assist in



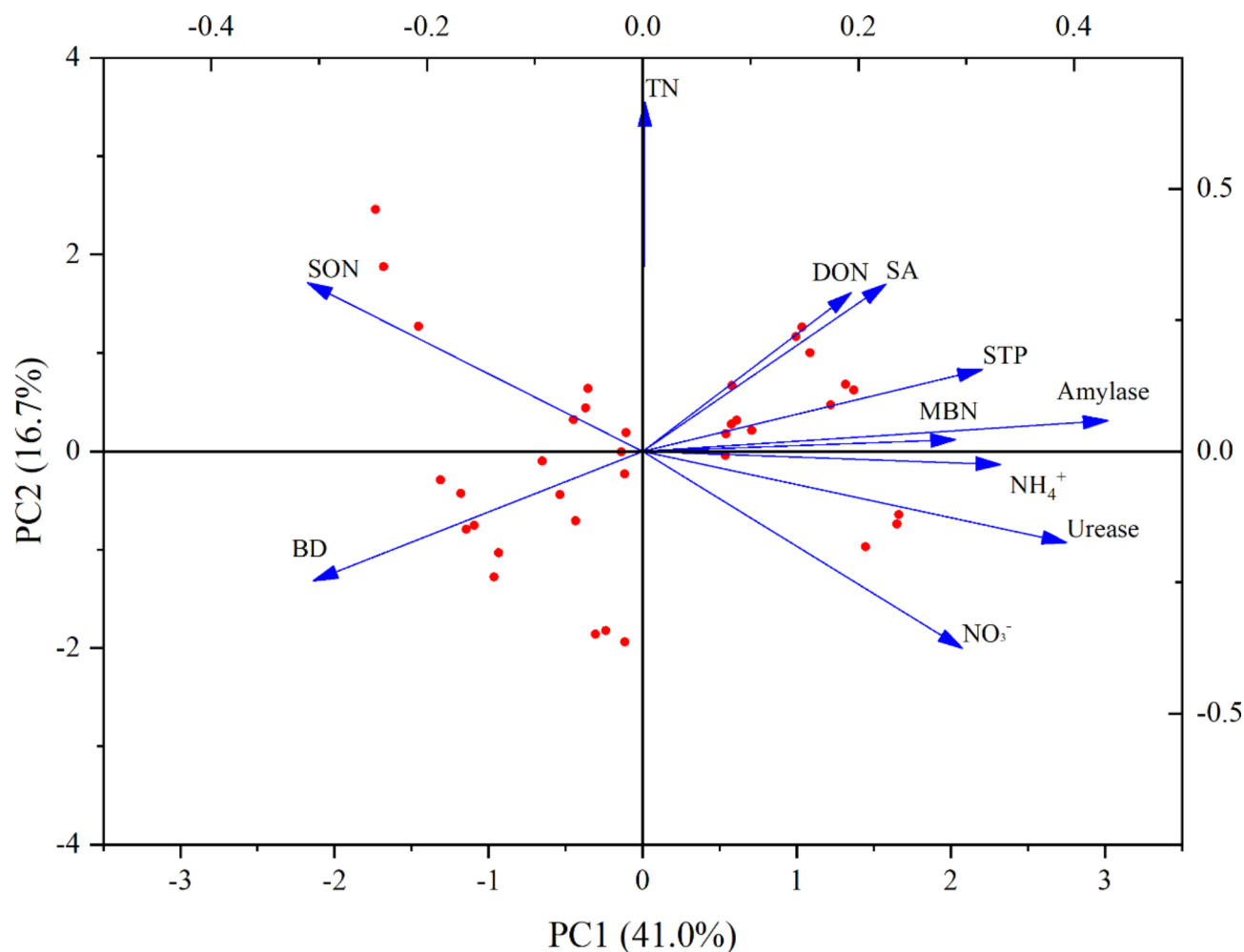


**Fig. 7.** Soil amylase and urease activities in the 0–10, 10–20, and 20–40 cm soil layers at different levels of N addition in August 2020. CK, 0 g/m<sup>2</sup>; N5, 5 g/m<sup>2</sup>; N10, 10 g/m<sup>2</sup>; N15, 15 g/m<sup>2</sup>. Lowercase letters represent significant differences between treatments ( $P < 0.05$ ).

Source of variation	df	Amylase		Urease	
		F	P	F	P
T	3	24.459	0.000	714.976	0.000
D	2	5677.837	0.000	1893.142	0.000
T×D	6	257.865	0.000	382.220	0.000

**Table 2.** Results of a repeated-measures ANOVA testing for differences in soil amylase and urease activity among N additions using depth as the repeated variable. *T* treatment, *D* depth.

further explaining the changes in the fractions of the soil N pool in wet meadows under the background of N deposition and offer a scientific foundation for predicting the soil N dynamics in alpine wetland ecosystems.



**Fig. 8.** The PCA analysis of soil physical properties, nitrogen components, and enzyme activity. *TN* total nitrogen, *NH<sub>4</sub><sup>+</sup>* ammonium nitrogen, *NO<sub>3</sub><sup>-</sup>* nitrate nitrogen, *MBN* microbial biomass nitrogen, *DON* dissolved organic nitrogen, *SON* soil nitrogen storage, *SA* soil aeration, *BD* bulk density, *STP* soil total porosity, *SCP* soil capillary porosity.

### Data availability

All data generated or analysed during this study are included in this published article.

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### References

1. Liu, L. et al. Exploring global changes in agricultural ammonia emissions and their contribution to nitrogen deposition since 1980. *Proc. Natl. Acad. Sci. USA*. **119**, e2121998119 (2022).
2. Wang, W. et al. Characteristics of atmospheric reactive nitrogen deposition in Nyingchi City. *Sci. Rep.-Uk*. **9**, 4645 (2019).
3. Yu, G. et al. Stabilization of atmospheric nitrogen deposition in China over the past decade. *Nat. Geosci.* **12**, 424–429 (2019).
4. Widdig, M. et al. Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil. *Soil. Biol. Biochem.* **151**, 108041 (2020).
5. Rudd, A. C., Kay, A. L. & Bell, V. A. National-scale analysis of future river flow and soil moisture droughts: potential changes in drought characteristics. *Clim. Change*. **156**, 323–340 (2019).
6. Guo, R. et al. Interactive effects of maize straw-derived biochar and n fertilization on soil bulk density and porosity, maize productivity and nitrogen use efficiency in arid areas. *J. Soil. Sci. Plant. Nut.* **22**, 4566–4586 (2022).
7. Tian, M., Whalley, W. R., Zhou, H., Ren, T. & Gao, W. Does no-tillage mitigate the negative effects of harvest compaction on soil pore characteristics in Northeast China? *Soil. Till Res.* **233**, 105787 (2023).
8. Wang, A., Zhang, Y., Wang, G. & Zhang, Z. Soil physicochemical properties and microorganisms jointly regulate the variations of soil carbon and nitrogen cycles along vegetation restoration on the Loess Plateau, China. *Plant. Soil*. **494**, 413–436 (2024).
9. Lan, T. et al. Synergistic effects of biological nitrification inhibitor, urease inhibitor, and biochar on NH<sub>3</sub> volatilization, N leaching, and nitrogen use efficiency in a calcareous soil-wheat system. *Appl. Soil. Ecol.* **174**, 104412 (2022).
10. Soldatova, E. et al. Nitrogen transformation and pathways in the shallow groundwater-soil system within agricultural landscapes. *Environ. Geochem. Hlth.* **43**, 441–459 (2021).

11. Zuccarini, P. et al. Effects of nitrogen deposition on soil enzymatic activity and soil microbial community in a Mediterranean holm oak forest. *Geoderma* **430**, 116354 (2023).
12. Feng, H. et al. Nitrogen cycling in plant and soil subsystems is driven by changes in soil salinity following coastal embankment in typical coastal saltmarsh ecosystems of eastern china. *Ecol. Eng.* **174**, 106467 (2022).
13. Niu, S. L. et al. Global patterns and substrate-based mechanisms of the terrestrial nitrogen cycle. *Ecol. Lett.* **19**, 697–709 (2016).
14. Yang, J. et al. Global positive effects of litter inputs on soil nitrogen pools and fluxes. *Ecosystems* **26**, 860–872 (2023).
15. Jia, X. et al. Effects of nitrogen enrichment on soil microbial characteristics: From biomass to enzyme activities. *Geoderma* **366**, 114256 (2020).
16. Jian, S. et al. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. *Soil. Biol. Biochem.* **101**, 32–43 (2016).
17. Zhang, T. et al. Effects of coastal wetland reclamation on soil organic carbon, total nitrogen, and total phosphorus in China: a meta-analysis. *Land. Degrad. Dev.* **34**, 3340–3349 (2023).
18. Piotrowska-Długosz, A., Długosz, J., Gryta, A. & Frąc, M. Responses of N-cycling enzyme activities and functional diversity of soil microorganisms to soil depth, pedogenic processes and cultivated plants. *Agronomy* **12**, 264 (2022).
19. Cheng, Y. et al. Nitrogen deposition affects both net and gross soil nitrogen transformations in forest ecosystems: A review. *Environ. Pollut.* **244**, 608–616 (2019).
20. Guan, B. et al. Effects of five years' nitrogen deposition on soil properties and plant growth in a salinized reed wetland of the Yellow River Delta. *Ecol. Eng.* **136**, 160–166 (2019).
21. Song, Y. et al. Nitrogen additions affect litter quality and soil biochemical properties in a peatland of Northeast China. *Ecol. Eng.* **100**, 175–185 (2017).
22. Meng, L. et al. Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytol.* **189** (4), 1040–1050 (2011).
23. Lin, B. L. et al. Increased nitrogen deposition contributes to plant biodiversity loss in Japan: Insights from long-term historical monitoring data. *Environ. Pollut.* **290**, 118033 (2021).
24. Chen, C., Xiao, W. & Chen, H. Mapping global soil acidification under N deposition. *Global Change Biol.* **29**, 4652–4661 (2023).
25. Liu, X. & Zhang, S. Nitrogen addition shapes soil enzyme activity patterns by changing pH rather than the composition of the plant and microbial communities in an alpine meadow soil. *Plant. Soil.* **440**, 11–24 (2019).
26. Chen, H., Li, D., Zhao, J., Xiao, K. & Wang, K. Effects of nitrogen addition on activities of soil nitrogen acquisition enzymes: A meta-analysis. *Agr. Ecosyst. Environ.* **252**, 126–131 (2018).
27. Ouyang, J. X. et al. Elevation, but not phosphorus, shapes arbuscular mycorrhizal fungal colonization of plateau wetland plants: A case study of the Qinghai-Tibet Plateau. *Glob. Ecol. Conserv.* **46**, e02611 (2023).
28. Li, H. et al. Degradation of wetlands on the Qinghai-Tibetan Plateau causing a loss in soil organic carbon in 1966–2016. *Plant. Soil.* **467**, 253–265 (2021).
29. Wei, D., Tarchen, T., Dai, D., Wang, Y. & Wang, Y. Revisiting the role of CH<sub>4</sub> emissions from alpine wetlands on the Tibetan Plateau: evidence from two in situ measurements at 4758 and 4320 m above sea level. *J. Geophys. Res.-Biogeo.* **120**, 1741–1750 (2015).
30. Shen, H., Dong, S. & DiTommaso, A. Nitrogen deposition shifts grassland communities through directly increasing dominance of graminoids: a 3-year case study from the Qinghai-Tibetan Plateau. *Front. Plant. Sci.* **13**, 811970 (2022).
31. Wang, H. et al. Effects of simulated nitrogen deposition on soil active carbon fractions in a wet meadow in the Qinghai-Tibet Plateau. *J. Soil. Sci. Plant. Nut.* **22**, 2943–2954 (2022).
32. Xu, H. et al. Impact of nitrogen addition on plant-soil-enzyme C-N-P stoichiometry and microbial nutrient limitation. *Soil. Biol. Biochem.* **170**, 108714 (2022).
33. Li, L. et al. Increasing sensitivity of alpine grasslands to climate variability along an elevational gradient on the Qinghai-Tibet Plateau. *Sci. Total Environ.* **678**, 21–29 (2019).
34. Shi, L. et al. Precipitation increase counteracts warming effects on plant and soil C:N:P stoichiometry in an alpine meadow. *Front. Plant. Sci.* **13**, 1044173 (2022).
35. Liu, C. et al. Response of soil nutrients and stoichiometry to grazing management in alpine grassland on the Qinghai-Tibet Plateau. *Soil. Till. Res.* **206**, 104822 (2021).
36. Wu, J., Wang, H. & Li, G. Effects of nitrogen deposition on N<sub>2</sub>O emission in a wet meadow on the Qinghai-Tibet Plateau. *Appl. Soil. Ecol.* **191**, 105049 (2023).
37. Peng, Y. et al. Soil temperature dynamics modulate N<sub>2</sub>O flux response to multiple nitrogen additions in an alpine steppe. *J. Geophys. Research: Biogeosciences.* **123**, 3308–3319 (2018).
38. Lu, Y., Si, B., Li, H. & Biswas, A. Elucidating controls of the variability of deep soil bulk density. *Geoderma* **348**, 146–157 (2019).
39. Thuc, L. V. et al. Effects of nitrogen fertilization and nitrogen fixing endophytic bacteria supplementation on soil fertility, N uptake, growth, and yield of sesame (*Sesamum indicum* L.) cultivated on alluvial soil in dykes. *Appl. Environ. Soil Sci.* 1972585 (2022).
40. Saha, U. K., Sonon, L. & Biswas, B. K. A comparison of diffusion-conductimetric and distillation-titration methods in analyzing ammonium- and nitrate-nitrogen in the KCl-extracts of Georgia soils. *Commun. Soil. Sci. Plan.* **49**, 63–75 (2018).
41. Yu, C. Q., Shen, Z. X., Zhang, X. Z., Sun, W. & Fu, G. Response of soil C and N, dissolved organic C and N, and inorganic N to short-term experimental warming in an alpine meadow on the Tibetan Plateau. *The Scientific World Journal.* 152576 (2014).
42. Wu, J. et al. Unimodal response of N<sub>2</sub>O flux to changing rainfall amount and frequency in a wet meadow in the Tibetan Plateau. *Ecol. Eng.* **174**, 106461 (2022).
43. Xie, X. et al. Response of soil physicochemical properties and enzyme activities to long-term reclamation of coastal saline soil, Eastern China. *Sci. Total Environ.* **607**, 1419–1427 (2017).
44. Yin, R., Deng, H., Wang, H. L. & Zhang, B. Vegetation type affects soil enzyme activities and microbial functional diversity following re-vegetation of a severely eroded red soil in sub-tropical China. *Catena* **115**, 96–103 (2014).
45. Li, J., Nie, M., Powell, J. R., Bissett, A. & Pendall, E. Soil physico-chemical properties are critical for predicting carbon storage and nutrient availability across Australia. *Environ. Res. Lett.* **15**, 094088 (2020).
46. Yan, G. et al. Nitrogen deposition and decreased precipitation altered nutrient foraging strategies of three temperate trees by affecting root and mycorrhizal traits. *Catena* **181**, 104094 (2019).
47. Li, Y., Niu, S. & Yu, G. Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: a meta-analysis. *Global Change Biol.* **22**, 934–943 (2016).
48. Granse, D., Titschack, J., Ainouche, M., Jensen, K. & Koop-Jakobsen, K. Subsurface aeration of tidal wetland soils: Root-system structure and aerenchyma connectivity in *Spartina* (Poaceae). *Sci. Total Environ.* **802**, 149771 (2022).
49. Gou, X. et al. Leguminous plants significantly increase soil nitrogen cycling across global climates and ecosystem types. *Global Change Biol.* **29**, 4028–4043 (2023).
50. Liao, L. et al. Nitrogen enrichment stimulates rhizosphere multi-element cycling genes via mediating plant biomass and root exudates. *Soil. Biol. Biochem.* **190**, 109306 (2024).
51. Fang, Y. et al. Large loss of dissolved organic nitrogen from nitrogen-saturated forests in subtropical China. *Ecosystems* **12**, 33–45 (2009).
52. Lei, Z. et al. Biochar mitigates dissolved organic carbon loss but does not affect dissolved organic nitrogen leaching loss caused by nitrogen deposition in Moso bamboo plantations. *Glob. Ecol. Conserv.* **16**, e00494 (2018).

53. Song, L., Wang, J., Pan, J., Yan, Y. & Niu, S. Chronic nitrogen enrichment decreases soil gross nitrogen mineralization by acidification in topsoil but by carbon limitation in subsoil. *Geoderma* **428**, 116159 (2022).
54. Lange, M., Eisenhauer, N., Chen, H. & Gleixner, G. Increased soil carbon storage through plant diversity strengthens with time and extends into the subsoil. *Global Change Biol.* **29**, 2627–2639 (2023).
55. Zhang, X., Zhu, B., Yu, F. & Cheng, W. Plant inputs mediate the linkage between soil carbon and net nitrogen mineralization. *Sci. Total Environ.* **790**, 148208 (2021).
56. Wu, J., Lu, Y., Wang, H. & Li, G. Effects of nitrogen and phosphorus additions on CH<sub>4</sub> flux in wet meadow of Qinghai-Tibet Plateau. *Sci. Total Environ.* **887**, 163448 (2023).
57. Wang, L. et al. Environmental conditions and litter nutrients are key determinants of soluble C, N, and P release during litter mixture decomposition. *Soil. Till Res.* **209**, 104928 (2021).
58. Jinfei, Y., Xiaobing, Z., Benfeng, Y., Yonggang, L. & Yuanming, Z. Species-dependent responses of root growth of herbaceous plants to snow cover changes in a temperate desert, Northwest China. *Plant. Soil.* **459**, 249–260 (2021).
59. López-Aizpún, M. et al. Atmospheric ammonia concentration modulates soil enzyme and microbial activity in an oak forest affecting soil microbial biomass. *Soil. Biol. Biochem.* **116**, 378–387 (2018).
60. Liu, Y., Evans, S. E., Friesen, M. L. & Tiemann, L. K. Root exudates shift how N mineralization and N fixation contribute to the plant-available N supply in low fertility soils. *Soil. Biol. Biochem.* **165**, 108541 (2022).
61. Jones, D. L. et al. Microbial competition for nitrogen and carbon is as intense in the subsoil as in the topsoil. *Soil. Biol. Biochem.* **117**, 72–82 (2018).
62. Zhang, S., Zheng, Q., Noll, L., Hu, Y. & Wanek, W. Environmental effects on soil microbial nitrogen use efficiency are controlled by allocation of organic nitrogen to microbial growth and regulate gross N mineralization. *Soil. Biol. Biochem.* **135**, 304–315 (2019).

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## Author contributions

J.Q.W. and H.Y.W. wrote the main manuscript text and prepared Figs. 1, 2, 3, 4, 5, 6 and 7, and 8. G.L. design of the work. N.C. prepared Table. All authors reviewed the manuscript.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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