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Key Points:

- Both the rate and duration of N addition impact soil mineral nutrient contents
- Short-term N addition induced different responses in micronutrients, while longer-term treatments decreased total mineral nutrients
- Plant uptake impacted soil available micronutrients at early stage, while soil pH showed stronger effects at later stages

Supporting Information:

Supporting Information may be found in the online version of this article.

Correspondence to:

Q. Ning and J. Huang,
ningqiushi@ibcas.ac.cn;
jhhuang@ibcas.ac.cn

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Author Contributions:

Conceptualization: Guoxiang Niu, Qiushi Ning, Xiankai Lu, Xingguo Han, Jianhui Huang
Data curation: Guoxiang Niu, Yinliu Wang, Ruzhen Wang, Qiushi Ning, Junjie Yang, Xingguo Han, Jianhui Huang
Formal analysis: Guoxiang Niu, Yinliu Wang, Ruzhen Wang, Qiushi Ning, Huiling Guan, Junjie Yang, Xiankai Lu, Xingguo Han, Jianhui Huang
Funding acquisition: Xingguo Han, Jianhui Huang
Investigation: Guoxiang Niu, Yinliu Wang, Ruzhen Wang, Qiushi Ning, Junjie Yang, Xiankai Lu, Xingguo Han, Jianhui Huang
Methodology: Guoxiang Niu, Junjie Yang, Jianhui Huang

Intensity and Duration of Nitrogen Addition Jointly Alter Soil Nutrient Availability in a Temperate Grassland

Guoxiang Niu[1,2,3], Yinliu Wang[1,3], Ruzhen Wang[4], Qiushi Ning[1,3], Huiling Guan[2,3], Junjie Yang[1,3], Xiankai Lu[2,3], Xingguo Han[1,3], and Jianhui Huang[1,3]

[1]State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing, China, [2]Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China, [3]College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, China, [4]Erguna Forest-Steppe Ecotone Ecosystem Research Station, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China

Abstract Increasing N input can alter soil nutrient availability and influence plant growth. Previous studies focused on N addition effects on N and P availability, while less on other mineral nutrients. Besides, how N addition duration affects nutrient availability has remained unclear. Based on a simulative N deposition experiment in a typical steppe with four N addition levels (0, 2, 10, and 50 g m^{-2} yr^{-1}) under three N addition duration (2, 5, and 10 years), we determined contents of 10 mineral nutrients in surface soils. In the 0–10 cm soil, short-term N addition (2-year) significantly increased exchangeable Ca (+7.2%) and decreased exchangeable Mg (−22.5%) as compared with the control, while decreased available Fe, Cu, and Zn, but increased Mn remarkably (+80.4%). Medium-term N addition (5-year) significantly raised soil total N and available Fe, Mn and Cu, while decreased total P and exchangeable Ca, Na and Mg. The response patterns of these nutrients were largely similar in the 10–20 cm soil, but were weaker and significant only at high N inputs (50 g m^{-2} yr^{-1}). Long-term N addition (10-year) significantly decreased contents of total base cations (K, Ca, Na, Mg) and micronutrients (Fe, Mn, Cu, Zn) by an average of 32.1% and 20.4%, respectively, across the two soil depths. Influences of pH and plant growth on micronutrients showed remarkable differences among different duration of N addition. These findings indicate that intensity and duration of N addition jointly alter soil nutrient availability and this should be considered in soil nutrient-cycling modeling.

Plain Language Summary Multiple soil mineral nutrients support plant growth and help plants to complete their life cycles, but human activities have perturbed global N cycle, which may cause imbalance in the availability of different soil nutrients in ecosystems. In this report we thoroughly assessed the dynamics of essential mineral nutrients for plant growth under different rates and duration of N addition. Short-term N addition (by 2 years) increased availability of Ca and Mn, but decreased the availability of Mg, Fe, Cu, and Zn. Longer-term N addition (by 5 or 10 years) raised soil total N and the availability of Fe, Mn, and Cu, while decreased soil total P and the availability of Ca, Na, and Mg. Changes in soil pH and plant production after N addition were the main reasons to cause abovementioned changes in the availability of those mineral nutrients. These results revealed the existence of accumulative effects of N addition.

1. Introduction

Multiple elements sustain plant growth and help plants to complete their life cycles (Kao et al., 2020; Marschner, 2012; Vitousek et al., 2009). These elements include not only carbon (C), hydrogen and oxygen acquired primarily from air and water, but also include nitrogen (N), phosphorus (P), base cations and micronutrients, which are obtained primarily from mineral soils (Watanabe et al., 2007). All those mineral elements play a unique role in plant health and ecosystem functioning, and are also regarded as limiting factors of plant productivity (Fang et al., 2017; Kao et al., 2020; Waldron et al., 2009). For example, the primary productivity of terrestrial ecosystems is widely limited by either N or P, or by both (Du et al., 2020; Hou et al., 2021). Calcium (Ca) and magnesium (Mg) can not only regulate soil pH especially in alkaline soil, but also play important roles in processes of cell signal transduction and C assimilation (Bowman et al., 2008; X. K. Lu et al., 2018; Verbruggen & Hermans, 2013). Deficiency of some micronutrients, such as iron (Fe) and manganese (Mn), could often induce negative effects on plant enzyme synthesis, but their high concentrations could also cause toxicity for growth of plants and microorganisms (Kao et al., 2020; Tian et al., 2020; Waldron et al., 2009). Currently, high

Project Administration: Xingguo Han, Jianhui Huang
Resources: Guoxiang Niu, Yinliu Wang, Ruzhen Wang, Xiankai Lu, Xingguo Han
Software: Guoxiang Niu, Ruzhen Wang, Qishi Ning, Huiling Guan
Supervision: Xingguo Han, Jianhui Huang
Validation: Guoxiang Niu, Huiling Guan
Visualization: Guoxiang Niu, Ruzhen Wang, Qishi Ning, Huiling Guan, Junjie Yang, Xiankai Lu
Writing – original draft: Guoxiang Niu
Writing – review & editing: Guoxiang Niu, Ruzhen Wang, Qishi Ning, Junjie Yang, Xiankai Lu, Xingguo Han, Jianhui Huang

reactive N inputs to soils induced by human activities might cause some unpredictable changes in the availability of those mineral nutrients (Galloway et al., 2008; Wen et al., 2020). However, dynamics of the full set of these mineral nutrients under increasing and long-lasting N inputs to date remain largely unknown, especially for those soil base cations and micronutrients.

In N enriched soils, the base cations (K, Ca, Na, and Mg) binding to soil surface can be replaced by the NH_4^+ ions from the N fertilizer, thereby leached out from soils (Cameron et al., 2013). Following the continuous N addition and depletion of base cations, the micronutrients (Fe, Mn, Cu, and Zn) are mobilized for acid buffering, with toxic effects on plant and microorganisms (Bowman et al., 2008; Keiblinger et al., 2018; Lucas et al., 2011). The effects of N inputs on the contents of mineral elements may depend on both intensity and duration of N inputs (Chen et al., 2020; Lucas et al., 2011; Meng et al., 2019). Generally, N addition has increasing effects on contents of soil available N and some micronutrients, and decreasing effects on contents of P and base cations, and all those effects were usually amplified with the rates and duration of N inputs (Deng et al., 2017; Hou et al., 2018; M. Lu et al., 2011; Lucas et al., 2011). However, there is no consistency towards the N effects on the above-mentioned findings. Taking soil P as an example, Chen et al. (2020) reported that N addition had no significant effects on the contents of soil available and total P whether in short- or in long-term studies, while soil phosphatase activity was diminished over time by N addition. Results from a meta-analysis also showed that the decreasing effects of N fertilization on base cations in terrestrial ecosystems only occurred in the time periods of first-5 years (Lucas et al., 2011). Collectively, the above inconsistency may result from that (a) the studies include soils with heterogeneous properties and different vegetation types and (b) the studies have been conducted with one or two N addition rates in short-term. Hence, studies that carried out with soils from the exact same origin and with varied rates and durations of N addition are needed to systematically test the effects of N addition on soil mineral nutrients.

Increased use of fertilizers in agricultural activities, and excessive consumption of fossil fuel in industries have led to high reactive N emission to the atmosphere over the last century, which further results in enhanced anthropogenic N deposition to terrestrial ecosystems (Fowler et al., 2013; Galloway et al., 2008; Wen et al., 2020). Increase in N deposition would lead to a series of consequences, causing changes in plant biomass, microbial activities and mineral elements contents in grassland ecosystems (Bengtsson et al., 2019; Greaver et al., 2016; Vitousek, 2015). Importantly, N addition might also indirectly affect soil mineral nutrients through altering plant properties and soil pH (Kao et al., 2020; Liu et al., 2021; Meng et al., 2019; Tian et al., 2019). For example, Stevens (2015) reported that the aboveground net primary production (ANPP) was positively correlated with dose of atmospheric N deposition in herbaceous ecosystems, and the increase by 1 g N m^{-2} yr^{-1} could raise ANPP up by 3% globally. Therefore, the plant uptake of mineral nutrients for growth under N enrichment might profoundly influence dynamics of mineral nutrients in soils. Nevertheless, the N addition, especially at high rates, can also lower soil pH which may further affect the contents of base cations and micronutrients (Liu et al., 2021; Meng et al., 2019). The N-addition effects on plant growth and soil pH also vary with different duration time. In a dosage accumulation analysis, Phoenix et al. (2012) demonstrated that even very low N deposition rates can result in significant ecological changes of plant and soil properties if the N loading continued long enough. Thus, the contents of mineral elements are expected to undergo cumulative effects over longer term N addition. However, the regulatory roles of plant and soil pH in changes of soil mineral nutrients under scenarios of increasing N deposition remain unclear. In addition, although ecologists can predict effects of N addition with different intensities and durations on terrestrial ecosystems based on results of meta-analysis, evidence from the long-term field experiments still remains scarce (Clark & Tilman, 2008; Lucas, et al., 2011; Meng et al., 2019).

China's grassland covers 12.5% of the global grassland area, or ~41% of terrestrial China, and they have played important roles in provisions of ecosystem service and functioning (Bengtsson et al., 2019). However, they are also confronted some environmental issues, such as increasing N deposition (Wen et al., 2020). Most previous studies addressed effects of multiple N addition rates on the species composition and biomass production of vegetation (Bai et al., 2010; Clark & Tilman, 2008; Niu et al., 2018), yet the data is still scarce on the soil properties and nutrient availability, especially under different duration of N application. Furthermore, although huge progresses have been made in understanding the dynamics of mineral elements in grassland region, most of them mainly focused on some single mineral element with only snapshot sampling, which might only provide us some fragmentary images (Fang et al., 2017; Horswill et al., 2008; Liu et al., 2021; Lucas et al., 2011). Therefore, evidences from the long-term field experiments across different N addition rates are required for accurately predict the response of primary soil mineral nutrients to N enrichment in grassland. In this study, we assessed the dynamics of 10 mineral elements (including N, P, base cations K, Ca, Na, and Mg, and micronutrients Fe, Mn, Cu, and Zn) in surface soils (0–10 and 10–20 cm) using a long-term field N addition experiment with four N addition levels (0, 2, 10, and 50 g N m^{-2} yr^{-1}) under three N addition durations (2, 5, and 10 years). Based on the results of previous studies (Liu et al., 2021; Lucas et al., 2011; 5R. Wang et al., 2018), we hypothesized that, (a) N addition would increase contents of total N and exchangeable base cations, but decrease contents of total P and available micronutrients in this N-deficient grassland; (b) N addition effects would be strengthened and accumulated with increasing rates and prolonged durations of N addition; (c) The effects of soil pH on changes of mineral elements would become stronger with increasing duration of N addition as the progressive soil acidification.

2. Material and Methods

2.1. Study Site and Experimental Set Up

The field experiment was conducted in a fenced temperate semiarid grassland site of the Inner Mongolia Grassland Ecosystem Research Station (IMGERS; 116°40′E, 43°13′N; elevation 1,200 m). The mean annual temperature was 0.9°C (1985–2017 of IMGERS) and the mean annual precipitation was 321.8 mm with ∼70% of the precipitation falling in the growing season from May to August. The soil type is a Haplic Calcisols according to the FAO Soil Taxonomy classification with percent contents of clay, silt and sand in the 0–10 cm soil being 5.2%, 44.2%, and 50.6%, respectively (Niu, Wang, et al., 2021). *Leymus chinensis* and *Stipa grandis* are the two dominant plant species in the vegetation, and they account for over 60% of total community ANPP which generally peaks in late August. The background amount of atmospheric N deposition in the study area is less than 2 g N m^{-2} yr^{-1} (G. R. Yu et al., 2019).

The N addition experiment was established in September 2008 and N addition treatment has been implemented yearly since then. To exclude disturbance from large livestock (i.e., cattle and sheep) grazing, the experimental site has been fenced since 1999. The whole experiment was set up as a completely randomized block design with 10 blocks as replicates. In each block, the treatment included 9 levels of N addition (0, 1, 2, 3, 5, 10, 15, 20, and 50 g N m^{-2} yr^{-1}) crossed with two addition frequency (half-yearly and monthly) and two levels of mowing (mown and unmown). The detailed experimental design was described in Niu, Wang, et al. (2021). In this study, only four N addition rates (0, 2, 10, and 50 g N m^{-2} yr^{-1} with half-yearly addition and unmown treatments, representing control, low-N, mid-N, and high-N addition, respectively) in 6 out of 10 blocks with three durations (2-, 5-, and 10-year, representing short-term, medium-term, and long-term N addition treatment, respectively) were selected to assess the effects of intensity and duration of N addition on abovementioned mineral nutrients. The atmospheric N deposition at the rate of 2 g N m^{-2} yr^{-1} was close to the background atmospheric N deposition and the 10 g N m^{-2} yr^{-1} was considered as the critical N saturation threshold of ecosystem functioning in this region (Bai et al., 2010; G. R. Yu et al., 2019). The level of 50 g N m^{-2} yr^{-1} was also applied to simulate extremely high N fertilization scenario in this agro-pastoral region (Niu, Hasi, et al., 2021). We selected three durations of time based on a previous study with similar vegetation by Tian et al. (2020), who divided the dynamics of ecosystem structure and functioning under N enrichment into three stages, that is, early (∼1–3 years), middle (∼4–9 years) and late stage (∼10–15 years). The N was added in the form of NH_4NO_3 equally on the first day of June (in solution by spraying) and November (spread by hand). It should be noted that no N and other nutrients were added prior to our experiment, and we did not add any other nutrients (e.g., K and Fe) except for N in our system, thus limitation by other nutrients may occur over time.

2.2. Plant and Soil Sampling

In August of 2010, 2013 and 2018 (after consecutive N addition of 2, 5, and 10 years, respectively), ANPP of each plant species in a 0.5 × 2 m quadrat was harvested by clipping vegetation above the ground, while roots or belowground biomass (BGB) (by depth of 0–10 cm and 10–20 cm) were sampled by collecting soil samples using core with diameter of 6.8 cm. The composite soil samples by soil depth (0–10 and 10–20 cm) were randomly collected at five locations and mixed in each plot using a core (diameter in 5 cm). To remove rocks and visible roots, the fresh soil samples were passed through a 2 mm sieve, and air-dried for determining contents of soil exchangeable or available mineral nutrients, physicochemical properties, and contents of total soil mineral nutrients.

2.3. Laboratory Analyses

All plant materials were oven-dried at 65°C for 48 hr and weighed to calculate ANPP and BGB. Soil pH was measured with a pH meter (sartorius PB-10; Goettingen, Germany) in a 1:2.5 (wt/vol) ratio of soil-to-water extract. The content of total N (TN) was determined with an elemental analyzer (Analytik-jena multi N/C3100; Jena, Germany). An inductively coupled plasma optical emission spectrometer (ICP-OES, Thermo Electron Corporation, USA) was used to measure the contents of total amount of P (TP), potassium (TK), calcium (TCa), sodium T (Na), magnesium (Mg), ferrum (TFe), manganese (TMn), cuprum (TCu) and zinc (TZn) in soils digested with strong acid mixture (HCl + HF + HClO). The exchangeable amount of K, Ca, Na and Mg were determined by extracting soil samples with 1 mol L^{-1} ammonium acetate solution in a 1:10 (wt/vol) ratio of soil to solution (Ochoa-Hueso et al., 2014). Briefly, a 5 g air-dried soil sample was extracted with 50 ml ammonium acetate (pH = 7.0) and shaken at 200 rpm for half-hour. The mixture was filtered and the concentrations of aforementioned exchangeable base cations in filtrates were measured by ICP-OES. Soil available Fe, Mn, Cu, and Zn were extracted with a mixture solution (including 0.005 mol L^{-1} diethylenetriaminepentadcetic acid, 0.01 mol L^{-1} $CaCl_2$ and 0.1 mol L^{-1} triethanolamine) in a 1:3 (wt/vol) ratio of soil to solution (Haddad & Evans, 1993; Lindsay & Norvell, 1978). The mixed soil solution was also shaken and filtered to pass through filter paper, and the concentrations of available micronutrients were also determined by ICP-OES.

2.4. Statistical Analyses

All statistical analyses were executed in R4.0.5 (https://www.r-project.org/2021-3-31) and all data were tested whether to meet the requirement of statistical analyses in "Car" package. Then, two-way ANOVA were conducted to assess the effects of the amount and duration of N addition on soil nutrients respectively in 0–10 and 10–20 cm soil layers. One-way ANOVA with Tukey HSD post-hoc test was used to test the effects of different N addition rates or different N addition duration on mineral nutrients. Soil pH controls the transfer processes (hydrolysis and sorption) of soil mineral nutrients, while plant biomass can reflect the amount of plant uptake to these nutrients (Bowman et al., 2008; Cai et al., 2017; Meng et al., 2019). Thus, they both are important factors in regulating the availability of soil nutrients. To determine the relative influence of soil pH and plant biomass (including ANPP and BGB) on the changes of mineral nutrients and indicate the specific pathways of the two variables, we run variation partitioning analysis (VPA) in the "Vegan" package and structure equation model (SEM) in the "piecewiseSEM" package under different N addition duration, respectively (Fang et al., 2019; Liu et al., 2021; B. Wang et al., 2021). Because soil mineral nutrients in the SEM include 10 nutrients, we further show the significant responses ($P \leq 0.05$) of specific nutrients by combing the results of ANOVA. Pearson correlation analyses were conducted to explore the potential correlations of mineral nutrients with ANPP, BGB, and pH in both soil layers.

3. Results

3.1. Soil Total N and P

The duration of N addition significantly affected the contents of TN and TP in both 0–10 and 10–20 cm soil layers. Long-term N addition (10 years) increased TN content by 15.6% and decreased TP content by 16.6% across the two soil layers as compared with those of short-term (2-year) N addition (Figure 1). In the 0–10 cm soil, high-N addition significantly increased TN content by 32.4% after 10-year N addition as compared with the control (Figure 1a), while significant decrease of TP content were observed in all N addition levels especially after 5- and 10-year N addition (Figure 1c). In the 10–20 cm soil, TN content increased along with N addition in all N addition durations and was accumulative with increasing durations (Figure 1b), while high-N addition significantly decreased TP content only after 5- and 10-year N addition (Figure 1d).

3.2. Soil Base Cations

Both the amount and duration of N addition (either singly or interactively) significantly affected the contents of exchangeable Ca, Na, and Mg in the both soil layers (Figure 2). The high N addition rates had strong negative effects on contents of soil exchangeable Ca with all the N addition durations in the 0–10 cm soil layer, and with 10-year N addition duration in the 10–20 cm soil layer (Figures 2c and 2d). Comparing with the control, mid-N addition significantly increased the exchangeable Ca content after 2-year N addition in both soil layers. In the

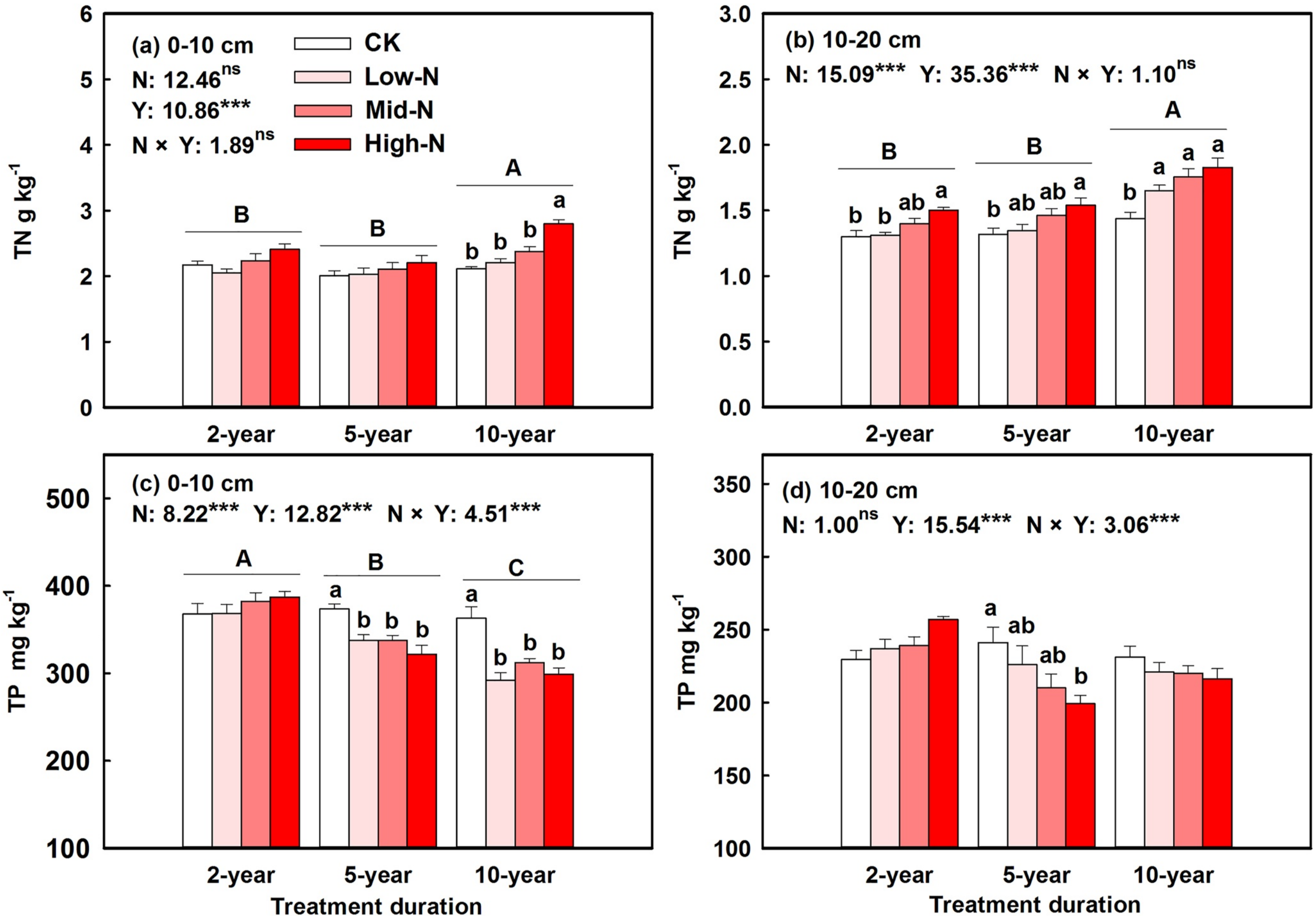

Figure 1. Effects of rates and duration of nitrogen addition on soil total N (TN) and P (TP) contents. Notes: Different capital letters denote significant differences ($P \leq 0.05$) among different durations, and different lowercase letters mean significant differences ($P \leq 0.05$) among different treatments in a given year. The results of two-way ANOVA were shown at the top of each figure. The letter ns and symbols *, **, *** indicate insignificant or significant differences at $P \leq 0.05$, 0.01, 0.001, respectively (F values, $n = 6$).

0–10 cm soil layers, high N addition rate with 10-year duration remarkably reduced soil exchangeable Na content, and the combination of longer duration and high rates of N addition tended to result in lower soil exchangeable Na content in the 10–20 cm soil (Figures 2e and 2f). The high-N addition significantly decreased the exchangeable Mg content in the 0–10 cm soil after 2- and 10-year N addition, and in the 10–20 cm soil after 10-year N addition (Figures 2g and 2h).

The 5- and 10-year N addition negatively affected total K, Ca, Na, and Mg in both soil layers, which was opposite with those after 2-year N addition (FigureS1 in Supporting Information S1). In the 0–10 cm soil layers, the total contents of K, Ca, Na, and Mg increased significantly with N addition rates after 2-year N addition, but they decreased significantly after 5- and 10-year N addition (FigureS1in Supporting Information S1). When comparing with the results of 2-year N addition across the two soil depths, the contents of total K, Ca, Na, and Mg after 10-year N addition significantly decreased by 9.3%, 41.8%, 23.8%, and 53.5%, respectively (Figure S1 in Supporting Information S1).

3.3. Soil Micronutrients

The rates and duration of N addition, either singly or interactively, significantly increased the concentrations of available micronutrients in both soil layers (Figure 3). After 2-year N addition, low- and mid-N addition generally decreased the contents of available Fe, Mn, Cu, and Zn in the 0–10 cm soil as compared with the control, but showed an increasing tendency with high-N addition (Figure 3). This is especially true for Mn, the content of

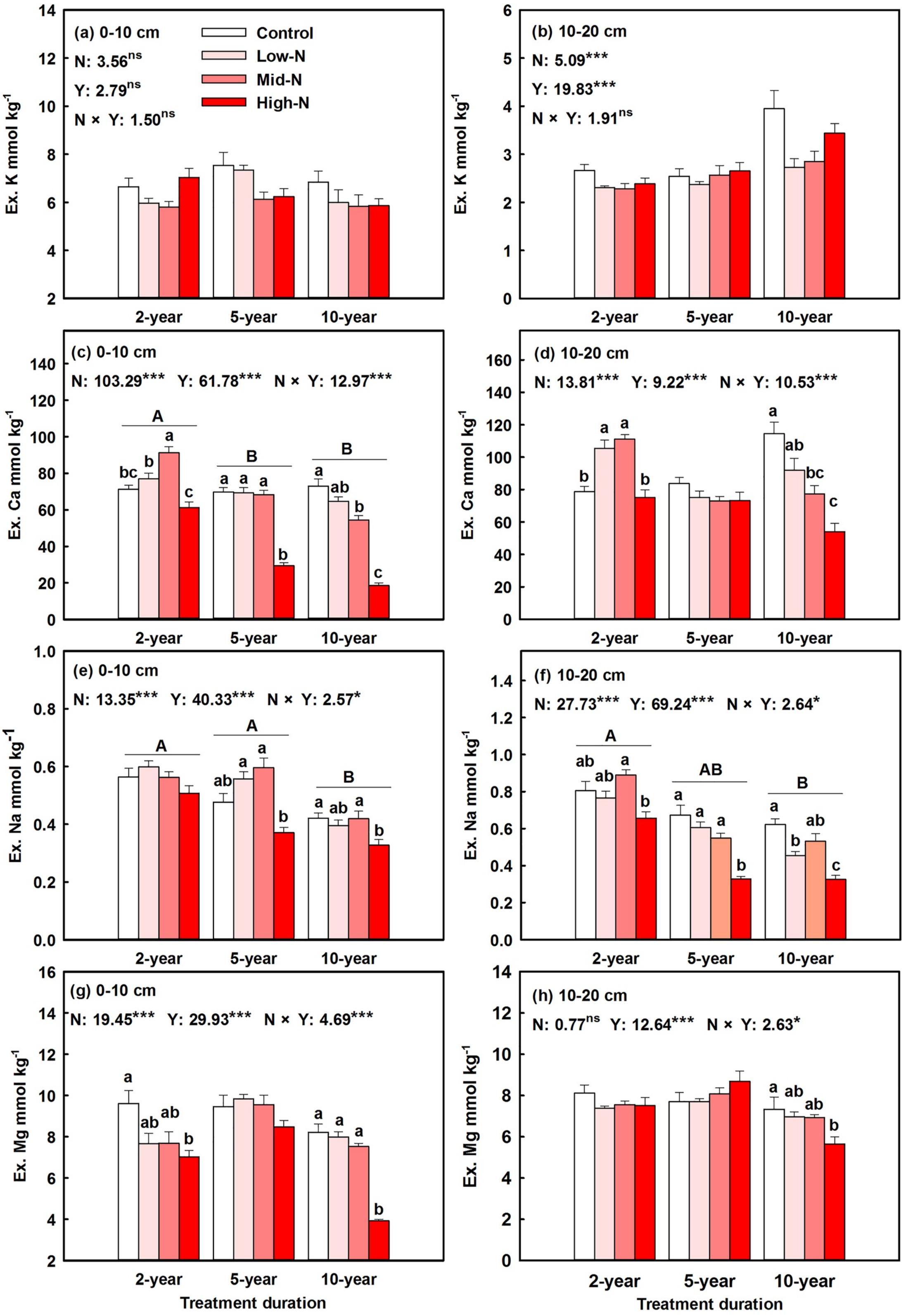

Figure 2. Effects of duration and amounts of nitrogen addition on soil exchangeable K (Ex. K), Ca (Ex. Ca), Na (Ex. Na), and Mg (Ex. Mg) contents in a typical steppe. See other notes in Figure 1.

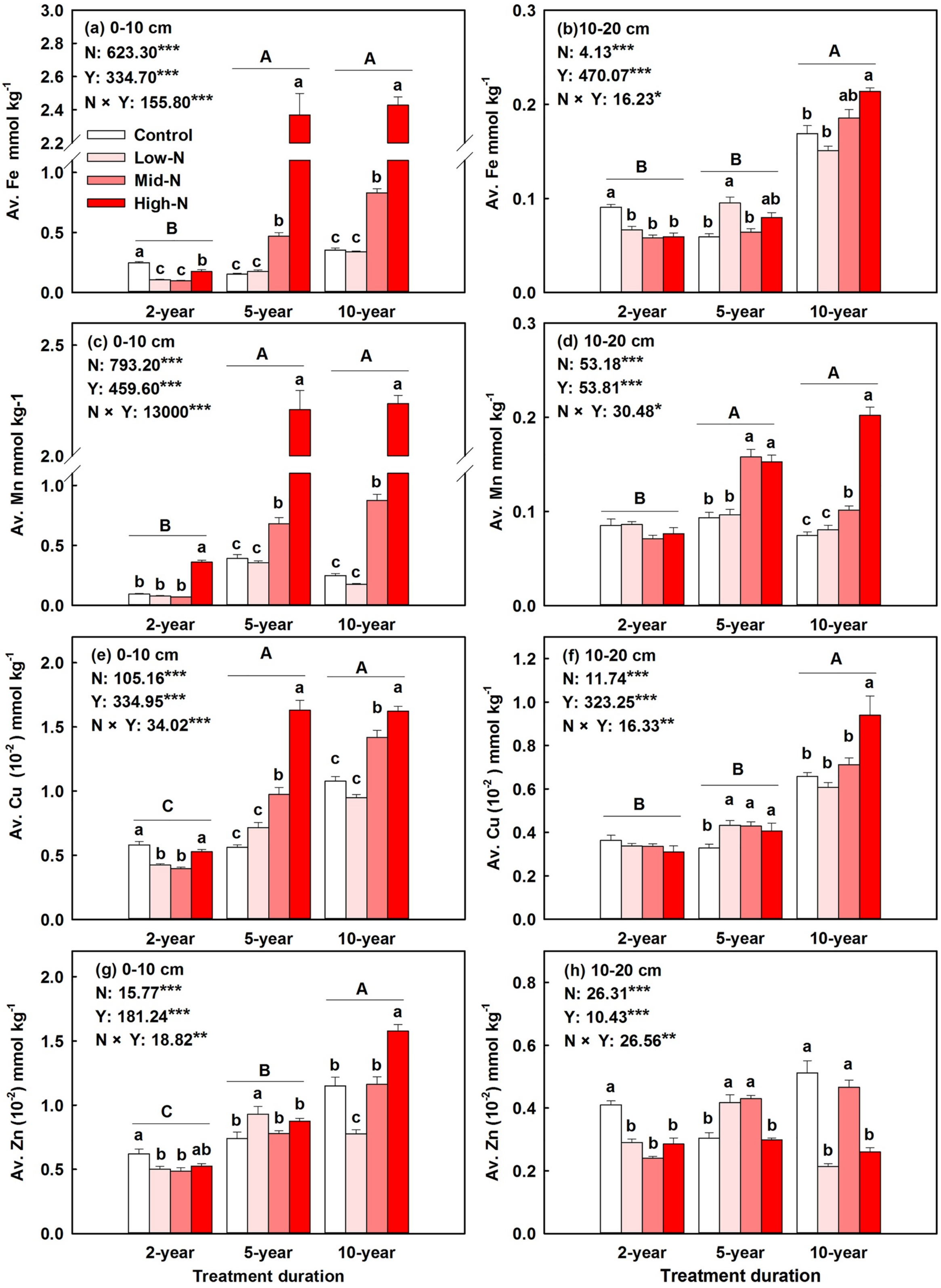

Figure 3. Effects of duration and amounts of nitrogen addition on the concentrations of soil available Fe (Av. Fe), Mn (Av. Mn), Cu (Av. Cu), and Zn (Av. Zn). See other notes in Figure 1.

which increased remarkably (+285.4%) (Figure 3c). After 5- and 10-year N addition, the contents of available Fe, Mn, and Cu increased strongly with mid- and high-N addition (Figures 3a, 3c, and 3e). Significant increase for Zn was also found with low-N addition (5-year) and high-N addition (10-year) in the 0–10 cm soil (Figure 3g). The pattern was largely similar for the behavior of these micronutrients in the 10–20 cm soil, but the increasing amplitude was smaller with high rates of and under long-term N addition especially for Fe and Mn (Figures 3b and 3d).

The rates and duration of N addition showed inconsistent effects on the total amount of these micronutrients (Figure S2 in Supporting Information S1). The total contents of Fe and Mn declined overall in both soil layers after 5- and 10-year N addition (Figure S2b, d in Supporting Information S1), while both insensitive responses were found for Cu and Zn to the rates of N addition (Figure S2f, h in Supporting Information S1). The 10-year N addition decreased the contents of total Fe, Mn, Cu, and Zn, respectively, by 20.5%, 33.9%, 14.8%, 12.4% overall in comparison with those under 2-year N addition in both soil layers (Figure S2 in Supporting Information S1).

3.4. Correlations of Mineral Elements With ANPP, BGB, and Soil pH

Results of VPA showed that the relative contribution of soil pH on total variation of the 10 mineral nutrients in the 0–10 cm soil increased with duration of N addition, and the relative contribution of soil pH was all significant after 5- and 10-year N addition, which accounted for 45.7% and 42.7% of total variation, respectively (Figures 4a–4c). The relative contribution of plant biomass (including both ANPP and BGB) was smaller than soil pH in regulating the response of soil mineral nutrients to 5- and 10-year N addition (Figures 4a–4c). The subsequent SEM analysis also suggested that soil pH was the main pathway affecting soil nutrient availability after 5- and 10-year N addition, and the exchangeable Ca and Mg, and the available Fe, Mn, and Cu were more sensitive to N addition regardless of N addition duration (Figures 4d–4f). Overall, the relative contribution of soil pH was higher than plant biomass (Figure 4d) for the variation of soil mineral nutrients, and the unexplained part decreased over time (Figure 4).

When considering all data of the three sampling years and based on results of Pearson correlation analyses, we further explored the potential correlations of each mineral nutrient with ANPP, BGB, and pH over time. Soil TN was positively correlated with BGB and negatively correlated with pH in the 0–10 cm soil (all $P \leq 0.05$, Table 1), while significantly negative correlations of TN with ANPP, BGB and pH, and TP with ANPP were detected in the 10–20 cm soil (Table 1). When considering data by sampling year, soil TN was positively correlated with ANPP in the 0–10 cm soil after 2-year N addition and negatively correlated with pH in both 0–10 and 10–20 cm soils (all $P \leq 0.05$, Table 1). Soil TP correlated positively with BGB in the 0–10 cm soil and negatively with pH in the 10–20 cm soil (all $P \leq 0.05$, Table 1). After 5-year N addition, soil TN correlated positively with BGB in the 0–10 cm soil and positively with ANPP in the 10–20 cm soil, while significantly negative correlations of pH with TN and positive correlations of pH with TP were also found in both soil layers (Table 1). Soil TP was negatively correlated with ANPP and BGB in the 0–10 cm soils (Table 1). After 10-year N addition, soil pH correlated negatively with TP in both soil layers (all $P \leq 0.05$, Table 1).

Generally, ANPP was positively correlated with the content of available Mn, and negatively with available Zn in the 0–10 cm soil (all $P \leq 0.05$, Table 2). Soil pH was also positively correlated with contents of exchangeable Mg, Ca and Na, and negatively with available Fe, Mn and Cu in both soil layers (all $P \leq 0.05$, Table 2). In the 0–10 cm soil, ANPP was positively correlated with Mn content after 2-year N addition, and soil pH also correlated negatively with Mn content (all $P \leq 0.05$, Table 2). The ANPP and BGB was positively correlated with available Fe, Mn, and Cu after 5-year N addition, while soil pH was negatively correlated with these micronutrients (all $P \leq 0.05$, Table 2). The BGB was positively correlated with the contents of available Zn, Mn, and Cu under 10-year N addition, while pH was positively correlated with exchangeable Ca and Mg, and negatively correlated with available Fe, Mn, and Zn in the 0–10 cm soil (Table 2).

4. Discussion

4.1. Effects of Intensity and Duration of N Addition on Soil TN and TP Contents

As we hypothesized, the intensifying effects of high N addition rate and cumulative effects of long-term N addition were shown for both soil TN (increase) in both soil layers and TP (decrease) in the 0–10 cm soil. It has been well established that the N addition could affect soil N pools by both plant uptake and soil N cycling processes

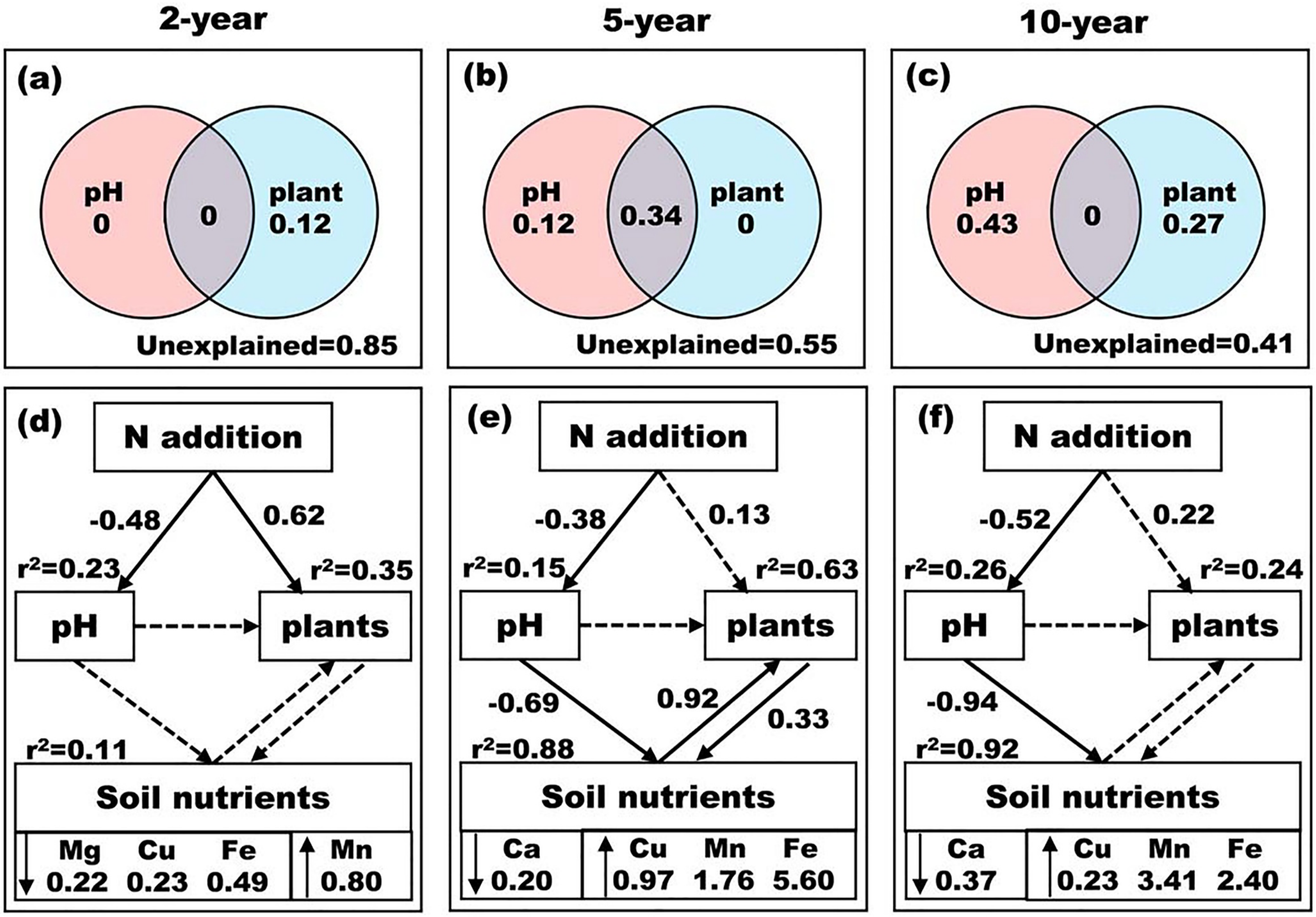

Figure 4. Variation partitioning analysis (VPA, a–c) and structural equation model (SEM) analysis (d–f) of the effects of N addition on soil nutrient availability in 0–10 cm soil under different N addition duration (2-year, 5-year, and 10-year). Results of VPA can indicate the individual relative contribution of soil pH, plant biomass and their intersection on total variation of 10 mineral element, while SEM analysis can further indicate the specific pathways. Negative values in the VPA were assigned as zero. Results of SEM model fitting by using "dsep" and "fisherC" test indicate a good fit of the model to data. The solid and dashed arrows indicate significant ($P \leq 0.1$) and insignificant effects, respectively, while r^2 values indicate the proportion of variation explained by relationships with other variables. Values associateds with arrows indicate standardized path coefficients. The symbols "↓" and "↑," respectively, indicate a significant decrease or increase ($P \leq 0.05$) in response to N addition, and the number in each square box indicates the response ratio of soil nutrients to N addition.

(M. Lu et al., 2011; Tian et al., 2020). Previous studies showed that N addition significantly increased the N stocks of plant and litter, but no significant increase was observed in topsoil (M. Lu et al., 2011; Ren et al., 2021). In this study, our results showed that only high-N addition could significantly increase soil TN content in both surface soil layers, with more pronounced responses being observed under long-term N addition (Figure 1). These results suggested that the effects of N addition on the soil N stocks are determined not only by the intensity of additional N input but also by the accumulation of the effects through years. The fact that soil TN contents lacked response to lower rates and relative short-term N addition probably resulted from enhanced plant N uptake in this N-limiting system (Bai et al., 2010; Ren et al., 2021) and gaseous losses from soils via denitrification (Boy-Roura et al., 2015; Cameron et al., 2013; Zhang et al., 2018; Zia et al., 2020). In this study, we also found that high-N addition significantly increased TN content in the 10–20 cm soil in all sampling years, indicating more N leached down especially under high N addition rate. Our previous study also reported that the contents of NO_3^--N, NH_4^+-N and DON in N addition plots were significantly higher as compared with those in control plots even down to the 70–100 cm depth of soil profiles (Niu, Hasi, et al., 2021).

The significant reduction of TP content in the 0–10 cm soil may suggest that P limitation will occur under continuous N enrichment scenario. Our results are consistent with the main findings of previous meta-analysis (Deng et al., 2017; Hou et al., 2018; Li et al., 2016). Two biogeochemical processes linking P cycling may help to explain our results. First, N addition can enhance plant production and consequently stimulate plant P uptake

Table 1
Pearson Correlations Among Soil Total N (TN) and P (TP) Contents With Above-Ground Net Primary Production (ANPP), Below-Ground Biomass (BGB) and pH

Factors	Duration	TN	TP	Factors	Duration	TN	TP
		(a) 0–10 cm				(b) 10–20 cm	
ANPP	2-year	0.36	0.35	ANPP	2-year	**0.60****	0.33
	5-year	0.20	**−0.44***		5-year	**0.50***	**−0.44***
	10-year	0.20	−0.32		10-year	0.35	−0.25
	Overall	−0.21	0.19		Overall	**−0.23***	**−0.53*****
BGB	2-year	−0.23	**0.44***	BGB	2-year	0.51	−0.09
	5-year	**0.40***	**−0.40***		5-year	0.27	0.14
	10-year	0.31	0.15		10-year	0.05	0.03
	Overall	**0.24***	0.16		Overall	**−0.27***	−0.01
pH	2-year	**−0.42***	−0.25	pH	2-year	**−0.28****	**−0.57****
	5-year	**−0.50***	**0.60****		5-year	**−0.45***	**0.64*****
	10-year	**−0.87*****	0.37		10-year	**−0.63*****	0.30
	Overall	**−0.60*****	**0.36****		Overall	**−0.66*****	−0.01

Note. Symbols *, **, and *** mean significance at $P \leq 0.05$, 0.01 and 0.001. Significant ($P \leq 0.05$) data were presented in bold.

(Deng et al., 2017; Marklein & Houlton, 2012), which will potentially increase P limitation in this grassland region. This is also supported by the negative correlations of ANPP with TP especially under longer-term (5- and 10-year) N addition (Table 2), indicating the exhaustion of P for plant growth. Second, N addition can cause soil acidification especially with high-N inputs and under long-term N addition, which further activates some metal ions, such as available Fe and Al (Barrow, 2017; Bowman et al., 2008; Fang et al., 2019; Spohn, 2020). Thus, the binding process of phosphate with Fe and Al ions may be accelerated by soil acidification and thereby contribute to the decreased P availability (Barrow, 2017; Spohn, 2020).

4.2. The Intensity and Duration of N Addition Jointly Affect Soil Base Cations

The longer-term (i.e., 5- and 10-year) N addition significantly decreased the contents of exchangeable Ca, Na, and Mg especially at mid- and high-N addition (Figure 2), which partly supported our second hypothesis. However, an unexpected finding was also observed that short-term N addition significantly increased the total contents of base cations and exchangeable Ca (Figure 2). Such finding is mainly because the impacts of external disturbance in this natural ecosystem, such as the excessive N supply in the closed system, may accelerate weathering process and physical fragmentation of rocks (Doetterl et al., 2018; Oliveira Garcia et al., 2020; Rafiei & Kennedy, 2020). Nevertheless, some other processes may also cause changes in the total and exchangeable contents of base cations, including plant uptake, binding and dissolution with soil organic materials, and leaching out from the soil system (Bünemann et al., 2018; Cameron et al., 2013; R. Wang et al., 2018). In our study area, the soil is weakly alkaline with relatively high exchangeable contents of Ca, Na, and Mg (Niu, Wang, et al., 2021) and thus has high capacity to buffer N-induced acidification. Besides, uptake of these cations by plants is relatively low, as indicated by low ANPP under either short-term N addition or low N inputs. Therefore, the sensitive responses of these cations to either short-term N addition or lower N inputs hardly occur. Moreover, with combination of dynamics of soil NO_3^--N, the amounts of base cations leaching out from upper to deeper soil layers would also increase with increasing rates and duration of N addition (Cameron et al., 2013; Doetterl et al., 2018; Niu, Hasi, et al., 2021). Our previous study in the same region also found that 13-year N addition only decreased the contents of base cations in surface soils while had insignificant effects on those in subsoils (Niu, Hasi, et al., 2021). The significant decrease in soil total and exchangeable contents of base cations under long-term N addition demonstrated that the decrease in supply potential of soil base cations to plants, which may cause nutrient imbalance and impoverishment under chronic N and incremental N enrichment (Fang et al., 2017; Lucas et al., 2011; R. Wang et al., 2018).

Table 2
Pearson Correlations Among the Contents of Soil Exchangeable K (Ex K), Ca (Ex. Ca), Na (Ex. Na), and Mg (Ex. Mg) and Available Fe (Av. Fe), Mn (Av. Mn), Cu (Av. Cu), and Zn (Av. Zn) With Above-Ground Net Primary Production (ANPP), Belowground Biomass (BGB), and pH

Factors	Duration	Ex. Ca	Ex. K	Ex. Mg	Ex. Na	Av. Cu	Av. Fe	Av. Mn	Av. Zn
(a) 0–10 cm									
ANPP	2-year	−0.33	0.34	**−0.41***	−0.22	0.02	−0.08	**0.73*****	−0.16
	5-year	**−0.73*****	−0.27	−0.34	**−0.41***	**0.85*****	**0.80*****	**0.80*****	0.20
	10-year	−0.23	−0.36	−0.07	0.04	0.48*	0.14	0.24	0.16
	all	−0.11	0.12	**0.30****	0.22	0.01	0.18	**0.27***	**−0.35****
BGB	2-year	0.01	0.01	0.22	0.01	0.05	−0.10	0.04	0.30
	5-year	**−0.49***	−0.37	−0.19	−0.13	**0.46***	**0.40***	**0.45***	−0.13
	10-year	−0.29	0.09	−0.28	−0.05	**0.59****	0.35	**0.42***	**0.45***
	all	−0.10	−0.17	−0.22	−0.02	0.10	0.12	0.10	0.05
pH	2-year	0.39	−0.32	0.37	0.38	−0.06	0.05	**−0.74*****	0.01
	5-year	**0.85*****	0.28	0.32	**0.61****	**−0.82*****	**−0.85*****	**−0.88*****	−0.18
	10-year	**0.96*****	0.16	**0.86*****	**0.43***	−0.86	**−0.95*****	**−0.96*****	**−0.77*****
	all	**0.81*****	0.11	**0.49*****	**0.48*****	**−0.64*****	**−0.81*****	**−0.85*****	**−0.45*****
(b) 10–20 cm									
ANPP	2-year	−0.32	−0.06	0.07	**−0.50***	−0.38	**−0.41***	0.14	−0.20
	5-year	**−0.48***	0.30	**0.59****	**−0.66*****	0.30	0.15	**0.78*****	−0.14
	10-year	−0.38	−0.31	−0.09	−0.02	0.10	0.24	0.08	0.27
	all	**−0.26***	**−0.35****	**0.55*****	−0.06	**−0.52*****	**−0.64*****	**0.26***	−0.05
BGB	2-year	0.20	−0.31	0.18	0.22	−0.11	−0.04	−0.06	−0.24
	5-year	−0.02	0.19	0.33	−0.13	−0.14	−0.35	0.33	−0.08
	10-year	−0.18	0.36	0.16	0.13	0.26	0.29	0.19	**0.56****
	all	0.21	**−0.31****	**0.25***	**0.53*****	**−0.48*****	**−0.47*****	**−0.24***	−0.19
pH	2-year	0.28	0.23	0.06	0.09	0.20	0.29	0.15	0.34
	5-year	**0.51***	−0.25	**−0.60****	**0.75*****	−0.32	−0.22	**−0.80*****	0.15
	10-year	**0.88*****	−0.19	**0.45***	**0.68*****	**−0.84*****	**−0.74*****	**−0.93*****	0.34
	all	**0.63*****	−0.40	**0.36****	**0.59*****	**−0.74*****	**−0.64*****	**−0.76*****	0.16

Note. See note in Table 1.

4.3. The Intensity and Duration of N Addition Jointly Affect Soil Micronutrients

Contrary to our second and third hypothesis, our results showed that the available contents of micronutrients increased only under relatively higher rates combined with longer duration of N addition while generally decreased under low- and mid-N rates and with short term N addition (Figure 3). These results might indicate that the relative importance of the effects of pH and plant uptake on these available micronutrients changed with prolonged N addition duration. Based on our results (Figure 4 and Table 2), effects of plant uptake of available micronutrients as indicated by significant increase in ANPP was stronger than that of soil pH at the early stage of N addition, while tended to be weaker as the ANPP was less sensitive to longer N addition durations (Table S1 in Supporting Information S1). Hence, under higher rate and long duration of N addition, the sharp increase of micronutrient contents was determined mainly by the increasing severity of soil acidification, which was also supported by our SEM analysis. Moreover, the notably high content of available Mn with high N addition and negative relationship between available Mn and soil pH after short-term N addition (Figure 2 and Table 2) demonstrated that response of available Mn was more sensitive to soil pH than those of Fe, Cu, and Zn (Alejandro et al., 2020; Andresen et al., 2018).

Although we lack the data of plant micronutrients, there are good evidences to show that plants are more likely to uptake more micronutrients (i.e., Mn) in leaves under chronic N addition especially in N-limited grassland ecosystems (Cai et al., 2017; Kao et al., 2020; Tian et al., 2020; Verbruggen & Hermans, 2013). Our analysis also suggested that soil pH is an important factor in affecting soil micronutrient availability, and its influence increased with increasing duration of N addition (Figures 4d–4f). This is because soil pH largely governs the sorption-desorption and oxidation-reduction processes of micronutrients, and further influences their availability (Kao et al., 2020). For instance, Fe and Mn generally tend to present as more soluble forms, that is, Fe/Mn oxides/hydroxide when soil pH decreases (Kao et al., 2020). However, their sorption with clay minerals and organic matter will become stronger when pH increases (Kao et al., 2020). In this study, long-term N addition significantly decreased soil pH by 0.84 unit (mean value across all N addition rates) in the 0–10 cm soil and by 0.48 unit in the 10–20 cm soil after long-term N addition (Table S1 in Supporting Information S1). Moreover, the deceasing effects on soil pH were magnified with increasing intensity and duration of N addition (Table S1 in Supporting Information S1). No obvious changes in micronutrients were found in low-N addition even under long-term N addition. This is possibly because the decreasing effects on these elements induced by plant uptake offset the increasing effects induced by changes in soil pH (Alejandro et al., 2020; Cai et al., 2017; Liu et al., 2021). The consistent increase of available Fe, Mn and Cu to mid- and long-term N addition reflect the increment of soil pH effects over time (Andresen et al., 2018; Fang et al., 2019), which is supported by our VPA and SEM analysis (Figure 4). B. Wang et al. (2017) also showed that the 9-year N addition in a field experiment increased the soil available Fe, Mn and Cu, and they were negatively correlated with soil pH. Overall, our results suggest that the relative influences of pH and plant growth on nutrients, especially on the micronutrients showed remarkable differences among different N addition durations.

4.4. Implications and Perspectives

Collectively, based on a long-term continuous field N addition experiment in temperate grasslands, we determined how intensity and duration of N addition individually and interactively affected the contents of mineral nutrients, and explored roles of plant uptake and soil pH. Our results indicated that micronutrients had quick responses, and the excessive accumulation of some specific elements such as Mn in plants may lead to lower nutritive value of forage and livestock as well (Kao et al., 2020; B. Wang et al., 2021). These results can also help to explain the inconsistent findings among meta-analysis studies and may be helpful in improving the predictability of biogeochemical models (Lucas et al., 2011; Meng et al., 2019). However, more studies are still needed to further verify these findings. For instance, it will be more convincing when the time period of study is long enough and include at least years with remarkably different size of precipitation (Ren et al., 2021). Additionally, we did not explore the difference in responses to N addition between the two soil layers (i.e., 0–10 cm vs. 10–20 cm) as their responses were generally similar, but deep soils are deserved more attention (Koarashi et al., 2012; Z. P. Yu et al., 2020). Finally, we only assessed the relative influence of pH and plant growth on the dynamics of mineral nutrients (Greaver et al., 2016; Horswill et al., 2008; Phoenix et al., 2020). Therefore, further studies should incorporate more explainable factors (such as plant nutrient status) into the statistical models and combine soil acidification experiment or liming experiments to explore the underlying mechanisms.

5. Conclusions

Our results demonstrated that N addition significantly altered the contents of 10 mineral nutrients in surface soils (0–20 cm) of a temperate grassland, and these effects varied with intensity and duration of N addition. Experiments with only short-term N addition could not accurately forecast the dynamics of mineral nutrients in grassland ecosystems since the decreasing effects of N addition on TP and base cations, or the increasing positive effects on TN and micronutrients generally occur under longer term N addition. The response of micronutrients may reflect that the effects of plant uptake on micronutrients were stronger than that of pH at the early stage of N addition, while the role of soil pH is increasingly important with prolonged duration of N addition. These results addressed the importance of accumulative N-addition effect and suggest that it is essential to consider effects of treatment duration when building soil biogeochemical models.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

Associated data are listed in Supporting Information S1. And the data are also archived in Figshare (https://figshare.com/s/9b5e4210d36d16ff470a). The DOI becomes active when the item is published (DOI: 10.6084/m9.figshare.17351771).

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