



# Discrepant long-term nitrogen mineralization in soil at early and later period after fertilization

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**Abstract.** Soil mineralization, the process of organic to inorganic N which balances the N uptake by crop and N loss to environment, was always quantified by short-term (one day to thirty weeks) incubation experiment. However, the residuary effect of fertilization for N mineralization, especially manure application, is long-term existed, which is important and thought to be shaped by fertilization, soil properties, and climate. Here, we defined and examined dynamic shifts in long-term N mineralization (LT-N<sub>min</sub>) between the first decade and later period after treated with no-fertilizer, conventional fertilizer with/without manure, fertilizer with stover return, in six typical agricultural zones over multiple decades. Soil total N (TN) and available N (AN) increased at rates of 10.1–58.2 mg·kg<sup>-1</sup>·yr<sup>-1</sup> and 1.41–4.13 mg·kg<sup>-1</sup>·yr<sup>-1</sup>, respectively, by manure application at five sites, suggesting that manure enhanced both soil N storage and availability. The LT-N<sub>min</sub> rate, defined as slope of the correlation between soil AN and TN indicated that AN increased by 70–81 mg per gram of TN increase, regardless of fertilization. Considering the fertilization period, the LT-N<sub>min</sub> rate with manure application were higher in the first decade (42–181 mg·g<sup>-1</sup>) than those in later period (33–92 mg·g<sup>-1</sup>) at all sites. Variance partitioning analysis showed that soil properties contributed 35% to LT-N<sub>min</sub> in the first decade and increased to 45% in the later period, while climatic conditions contributed 19% first, but 8% in the later period. Structural equation modeling suggested that LT-N<sub>min</sub> was directly affected by annual temperature, with a standardized path coefficient of 0.86 in the first decade and 0.45 in the later period. These results showed that the residual N in soil was mineralized with a high rate in the first decade after fertilization and then slowed down, and the interactions between climate and soil had an enhanced impact on LT-N<sub>min</sub> in later years of fertilization. This context-dependent understanding of interactions between soil properties, N cycling, and climate can thus inform soil management strategies to improve N availability and reduce the N loss to environment.

**Keywords.** Nitrogen mineralization · Climatic effect · Soil properties · Long-term fertilization · Manure application

## 1 Introduction

Nitrogen (N) is an essential soil nutrient required for plant growth and crop production. Although more than 90% of N in soil is present as organic N (Schulten and Schnitzer, 1997), plants primarily take up inorganic N and only absorb a small proportion



30 of low molecular weight organic N compounds (e.g., glycine), typically under extreme conditions (Nasholm et al., 1998). Enhanced soil N mineralization provided the opportunity to increase the N fertilizer use efficiency, enabling sufficient crop yields with reduced risk in N loss (Giacomo et al., 2012). Therefore, soil N mineralization ( $N_{\min}$ ) from organic forms to labile or biologically available ammonia or nitrates can greatly impact crop production and agroecosystem sustainability.

35 Soil  $N_{\min}$  rates were usually determined by short-term intermittent leaching or isotopically labeled laboratory incubation experiments (Stanford and Smith, 1972; Zhang et al., 2019), which represented the soil capacity of N mineralization at a certain state in the short term. Actually, soil N-supplying capacity increased with the continuous fertilization, and fertilizer-N retained in the soil may contribute substantially to crop N uptake in subsequent years (Proffenbarger et al., 2018; Vonk et al., 2022), indicated that the effects of fertilization are long-lasting. Therefore, estimating site-specific  $N_{\min}$  rates by incubation experiment could pose several challenges (Wade et al., 2018). For instance, estimation of soil  $N_{\min}$  performed in the laboratory  
40 (Beesigamukama et al., 2021) might fail to account for the differences in the effects of long-term fertilization that occur under field conditions (Risch et al., 2019). Moreover, climatic conditions and fertilization regimes shaped soil properties over long time scales (Doetterl et al., 2015; Wang et al., 2018), and thus understanding soil long-term  $N_{\min}$  (LT- $N_{\min}$ ) under various climatic conditions, soil types, and fertilization practices is crucial for reliable and robust estimates of soil N availability, and ultimately, productivity in terrestrial ecosystems.

45 Climatic conditions and soil properties have been identified as primary factors controlling  $N_{\min}$  on a global scale (Liu et al., 2017; Risch et al., 2020). The rate of  $N_{\min}$  increased with mean annual temperature (Dawes et al., 2017) and precipitation (Burke et al., 1997). Soil total N (TN) content could be an informative predictor of  $N_{\min}$  since the inorganic mineralization substrates were included in this pool (Dessureault-Romppe et al., 2015; Matar et al., 2008). However, other studies have shown that  $N_{\min}$  may share only a weak correlation with TN (Vigil et al., 2002). Soil properties could mutually influence each other  
50 (Kleber et al., 2015) and thus an integrative understanding of the impacts of various climate and soil properties on  $N_{\min}$  is essential for predicting crop performance under different climatic conditions and management practices, especially in the context of global warming.

In addition to climate and soil properties, fertilizer resources could directly affect soil N mineralization rates. For example, various fertilizer applications were well-known to profoundly impact soil microbial biomass and bacterial ureolytic and  
55 chitinolytic communities, which in turn drive N mineralization (Lee and Jose, 2003; Treseder, 2010; Yang et al., 2019). Manure application and stover return have also been shown to improve both  $N_{\min}$  potential and rates by providing carbon and nutrient substrates that enhance enzyme activity, such as that of urease (Qin et al., 2013). Some studies have found that manure application increase the proportion of easily degraded soil organic matter and thus the content of potentially mineralized soil N (Nett et al., 2009). Fertilization not only affected soil  $N_{\min}$  directly by providing metabolic resources, but also altered  $N_{\min}$   
60 by influencing other soil properties. For instance, manure application promoted soil N mineralization by increasing the contents of soil organic carbon (SOC), soil micro-biomass nitrogen (SMBN), and by enriching for bacteria (*Gemmatimonadetes* and *Latescibacteria*), while conventional urea application had no effect on  $N_{\min}$  (Wei et al., 2011; Guo et al., 2019). However, the different roles of fertilization, climate and soil-related factors in N mineralization are not well understood.



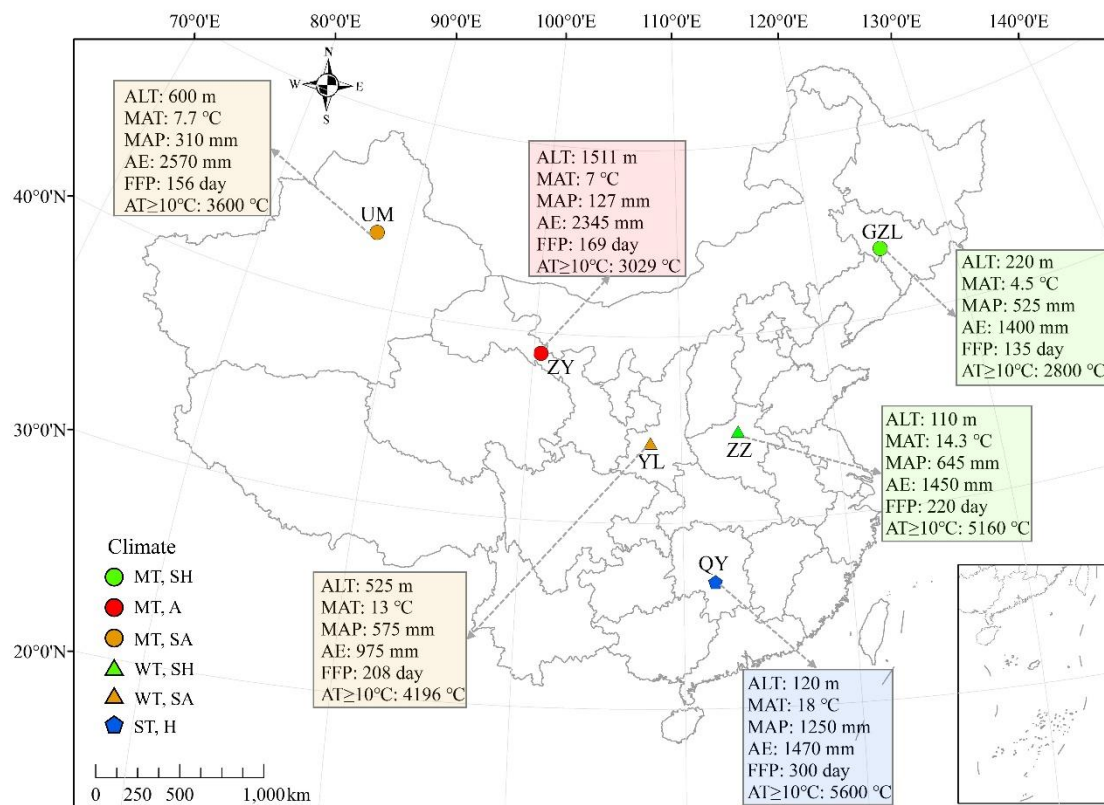
This study was designed to identify the long-term effects of fertilization and climate conditions on soil N availability, and to  
65 guide development of the best N management practices under diverse agricultural conditions. Using six 21–29 years long-term  
agricultural experiment sites in China, dynamic shifts in soil TN and available N (AN) were quantified to determine the long-  
term N mineralization (LT- $N_{\min}$ ) rate, defined as the ratio of AN change rate to TN change rate. The LT- $N_{\min}$  rate thus largely  
reflects soil  $N_{\min}$  during the study period, since it can show how much of the TN accumulated in soil is activated to AN annually.  
The specific objectives of this work were to: (1) investigate the soil LT- $N_{\min}$  under fertilization with manure or stover return;  
70 (2) examine the soil LT- $N_{\min}$  over different durations of fertilizer application; and, (3) evaluate the effects of fertilizer treatment,  
climate, and soil properties on soil LT- $N_{\min}$ .

## 2 Materials and methods

### 2.1 Site descriptions

Six long-term experiments were studied at Gongzhuling (GZL), Zhengzhou (ZZ), Urumqi (UM), Yangling (YL), Zhangye  
75 (ZY), and Qiyang (QY). The GZL is located in the Northeast; ZZ is in central China; UM, YL, and ZY are in the Northwest;  
and QY is in the south of China (Fig. 1). All the experiments were set up in 1990 except ZY, which was initiated in 1982. The  
climates in the locations ranged from arid, temperate zones to humid subtropical zones (Fig. 1). Annual mean temperature  
varied from 4.5 °C at GZL to 18.0 °C at QY. Annual precipitation ranged from 127 mm to 1 250 mm, and annual evaporation  
ranged from 975 mm to 2570 mm. Frost-free period varied from 135 days at GZL to 300 days at QY. The annual active  
80 accumulated temperature (daily temperature > 10°C), ranged from 2 800 °C at GZL to 5 600 °C at QY (data from China  
meteorological sharing service system, <http://cdc.cma.gov.cn/>).

All experimental fields were used for agriculture for more than ten years before the experiments were initiated. Soil  
classifications and properties before the treatments (1982 or 1990) were presented in Table S1. The initial SOC content was  
higher at GZL and ZY sites (11.5–13.2 g·kg<sup>-1</sup>) than other sites (6.7–8.8 g·kg<sup>-1</sup>). The highest total N content was detected at  
85 GZL (1.40 g·kg<sup>-1</sup>), follow by QY (1.07 g·kg<sup>-1</sup>), and other sites (0.67–0.87 g·kg<sup>-1</sup>). The soil C/N ratio was the highest at ZY  
(13.4), and the lowest at QY (7.4). The soil pH was 5.7 at QY and 7.6–8.6 at other sites.



**Figure 1: Locations and climatic conditions of the six long-term experimental sites.** GZL, Gongzhuling; ZZ, Zhengzhou; UM, Urumqi; YL, Yangling; ZY, Zhangye; QY, Qiyang. MT, mild-temperate; WT, warm-temperate; ST, subtropical-temperature; A, arid; SA, semi-arid; SH, semi-humid; H, humid; ALT, altitude; MAT, mean annual temperature; MAP, mean annual precipitation; AE, annual evaporation; FFP, frost free period; AT  $\geq 10^{\circ}\text{C}$ , accumulated temperature  $\geq 10^{\circ}\text{C}$ .

## 2.2 Cropping systems

Wheat (*Triticum Aestivium* L.) or maize (*Zea mays* L.) were grown alone or in rotation at all sites. The GZL, UM, and ZY sites were used for mono-cropping, while rotating crops were grown at ZZ, YL, and QY sites (Table S1). Maize was sown during late April to early May, and harvested during late September to early October under mono-cropping practices. Wheat (spring wheat) was seeded in late March and harvested in late July at ZY. At UM, spring wheat was sown during late March to early April and harvested in mid-July, winter wheat was sown in late September in the same year and harvested during late June to early July in the next year. In double cropping sites, maize was sown in mid-June and harvested between late September and early October at ZZ and YL, or sown in late March to early April and harvested in mid-June at QY. Wheat (winter wheat) was sown in mid-October and harvested during mid- to early June at the ZZ and YL sites, whereas it was sown in early November and harvested in mid-May at QY. Each field was under the same rotation for 2–3 years prior to initiating the experiment at each site.



### 2.3 Field design and treatment

There were five treatments at each study site: unfertilized control (CK); inorganic N, phosphorus and potassium (NPK);  
105 inorganic NPK plus manure (NPKM); 1.5- or 2-times application rate of NPKM (hNPKM); and inorganic NPK with stover  
return (NPKS); except ZY where only CK, NPK, and NPKM treatments were available (Table S2).

At GZL, ZZ, YL and QY, the total amount of N applied was the same for all fertilizer treatments (except CK and hNPKM),  
*i.e.*, 30% of the total N applied was inorganic while the rest was derived from composted manure. Both the inorganic N fertilizer  
and manure application rates were 1.5 times higher in hNPKM treatments than in NPKM at the above four sites. At UM, the  
110 manure supplied in NPKM was 123% more N than that present in the NPK component, while the inorganic N and manure  
application rates for hNPKM were two times that in the NPKM treatment. At ZY, the amount of inorganic N applied was same  
for all fertilizer treatments (except CK), and the N provided by the manure component of NPKM was equivalent to the N rate  
present in NPK. For NPKS treatments, crop stover was chopped and incorporated into the soil *in situ* immediately following  
harvest, annually. All the stover harvested from each NPKS plot was returned to the same plot. Nutrients (N, P, and K) in the  
115 stover, however, were not counted towards N applications because changes in their availability with time were not recorded.

The inorganic N, P and K fertilizers applied were urea, calcium superphosphate, and potassium sulfate, respectively. The source  
of manure was from household livestock such as pig, cattle, horse, and goat. Manure was applied before seeding once a year  
for all sites with mono-cropping systems. At sites with double cropping, manure was applied before wheat seeding. The  
fertilizer treatments were arranged in a completely randomized design in the field. There were three replicates at ZY and ZZ  
120 with a plot size of 33 m<sup>2</sup> and 43 m<sup>2</sup>, respectively. Due to the relatively large size of each plot (196–468 m<sup>2</sup>), replications were  
not included when the experiment was established for demonstration/outreach purposes at the GZL, UM, YL and QY sites.  
These plots were isolated by 100 cm cement baffle plates.

To ensure high maize yields, the target fertilizer application rates were slightly different at the six sites based on the common  
practices of local growers, which were specific to local cultivars, climate, and soil conditions (Table S2). The annual target  
125 nutrient application rates ranged from 120 kg· N ha<sup>-1</sup>–300 kg· N ha<sup>-1</sup> for the mono-cropping systems (NPK at GZL, UM, and  
ZY). The N application rates were 188–210 kg· N ha<sup>-1</sup> for maize and 90–165 kg· N ha<sup>-1</sup> for wheat in the double cropping  
rotation systems (ZZ, YL, and QY). The P and K fertilizer application rates were 36–150 kg· P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 61–150 kg· K<sub>2</sub>O  
ha<sup>-1</sup> for the mono-cropping systems, respectively, every crop season. The P and K fertilizer application rates were 36–198  
kg· P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 36–124 kg· K<sub>2</sub>O ha<sup>-1</sup> for the double cropping systems, respectively. One-third of the N fertilizer was applied  
130 as basal fertilizer before seeding and the rest applied as topdressing at the jointing stage at GZL. For ZZ and UM sites, 60%  
of the N fertilizer was applied as basal fertilizer before seeding and 40% as topdressing at the jointing stage. At ZY, YL and  
QY, half of the N fertilizer was applied as basal fertilizer before seedling and the remaining was applied as a topdressing during  
the growing season. All the P and K fertilizers were applied as basal fertilizers before seeding at all sites.



## 2.4 Soil sampling and analysis

135 The data in this study was collected from 1982–2002 at ZY, and from 1990 to 2011, or 2012, or 2014, or 2018 in other sites  
(Table S2). While soil samples were not available for all sites or for every year; the quantity of available data are shown in  
Table 1. Soil samples were collected from the topsoil (0–20 cm) after harvest in early October. At ZY and ZZ, five core (2.5  
cm i.d.) samples were randomly collected and mixed as one composite sample for each replicate plot. For the other sites, a  
total of 20 soil core samples (0–20 cm depth), were collected from each treatment plot using a 2.5 cm diameter auger and five  
140 core samples were mixed as one composite sample, totaling four samples for determining soil properties. The soil samples  
were then air-dried, sieved through a 2 mm screen to determine pH (1:2.5 w/v water) and 0.25 mm mesh for soil nutrient  
contents. The average values were used for statistical analysis.

Soil organic carbon (SOC) content was determined by vitriol acid-potassium dichromate wet oxidation (Walkley, 1935). Soil  
TN was determined by the Kjeldahl digestion-distillation method (Black, 1965). Soil total P was determined by the  
145 molybdenum blue colorimetric method using an  $\text{HClO}_4\text{--H}_2\text{SO}_4$  solution for digestion (Murphy and Riley, 1962). Soil total  
potassium and available potassium was determined by flame photometer (Lu, 2000). Soil AN was determined by alkaline  
solution diffusion (Lu 2000), and available P was quantified by the Olsen method (Olsen, 1954).

Climatic data, including mean annual precipitation (MAP), mean annual temperature (MAT), mean annual humidity and mean  
annual evaporation, were obtained from the China Meteorological Data Service Center (<http://cdc.cma.gov.cn/>).

## 150 2.5 Estimation of soil TN and AN rate of change and $\text{LT-N}_{\min}$

To analyze the cumulative effect of the fertilizer regime on soil TN, AN and long-term N mineralization ( $\text{LT-N}_{\min}$ ), the annual  
rates of change for soil TN ( $\text{g}\cdot\text{kg}^{-1}\cdot\text{yr}^{-1}$ ) and AN ( $\text{mg}\cdot\text{kg}^{-1}\cdot\text{yr}^{-1}$ ) were determined using the least square linear regression (Tang  
et al., 2008).

$$TN = a_1 + b_1X \quad (1)$$

155  $AN = a_2 + b_2X \quad (2)$

Where  $TN$  was total N content ( $\text{g}\cdot\text{kg}^{-1}$ ),  $AN$  was available N content ( $\text{mg}\cdot\text{kg}^{-1}$ ),  $a_1$  and  $a_2$  are the intercepts (*i.e.*, the initial TN  
and AN),  $b_1$  and  $b_2$  were the slopes (annual rates of change for TN and AN) and  $X$  is the year.

The soil TN and AN content were positively correlated, and the slope of the linear relationship between AN and TN was used  
to calculate the annual increase in AN per 1  $\text{g}\cdot\text{TN ha}^{-1}$  increase annually. This slope was defined as the soil long-term N  
160 mineralization rate ( $\text{LT-N}_{\min}$ ) here.

$$AN = a_3 + \text{LT-N}_{\min} * TN \quad (3)$$

The soil C/N, C/P and N/P ratios were calculated from the ratio of SOC, soil TN, and total P contents (Qaswar et al., 2019).



## 2.6 Data analysis

Linear regression analyses were conducted using the Sigmaplot 10.0 system (Systat Software, Inc. SigmaPlot for Windows  
165 2006). Variation partitioning analysis (VPA), to partition the variance shared by all factors, was used to quantify the unique  
contribution of each group of factors including soil properties, fertilizer treatments, and climatic conditions (Legendre, 2007).  
A negative value in the variance explained for group factors was interpreted as zero, which indicated that the explanatory  
variables explained less of the variation than random normal variables (Delgado-Baquerizo et al., 2017). The VPA was  
conducted with the R package vegan v.3.2.4 (R Development Core Team, 2016).

170 Structural equation modelling (SEM) was further used to evaluate the direct and indirect relationships between long-term N  
mineralization rate and climatic conditions, soil properties, and/or fertilizer treatments. This approach could partition isolate  
the direct and indirect effects that one variable may have on another (Grace, 2006; Hershberger, 2001) and was therefore  
helpful for exploring complex relationships in natural ecosystems. Owing to strong correlations among the factors within each  
group, we conducted principal component analysis (PCA) to create a multivariate functional index before construction of  
175 structural equation models (Chen et al., 2019). The first component (PC1), which explained 40.93–98.67% of the total variance  
for these three groups, was then introduced as a new variable to represent the combined group properties into the subsequent  
analysis (Table S3). The fitness of the final model was evaluated using the model  $\chi^2$  test ( $p > 0.05$ ) and the root mean-squared  
error (RMSEA,  $< 0.05$ ) of approximation. The structural equation modelling analyses were conducted using AMOS 21.0  
(Amos Development Corporation, Chicago, IL, USA).

## 180 3 Results

### 3.1 Increased soil TN and AN by long-term manure application

To better understand the effects of long-term diverse fertilization on soil nitrogen (N) storage and availability, annual rates of  
change (ARC) for soil total N (TN) and available N (AN) were examined at six sites with long-term organic or inorganic  
fertilizer treatments (Table 1). Linear regression analyses showed that NPK manure (NPKM) or high manure (hNPKM)  
185 applications resulted in significantly increased soil TN contents over time at all sites except QY. The ARC of TN was higher  
in hNPKM treatments at different sites (range = 28.8–58.2  $\text{mg}\cdot\text{kg}^{-1}\cdot\text{yr}^{-1}$ ) compared with that in NPKM treatments at the same  
sites (range = 10.1–48.3  $\text{mg}\cdot\text{kg}^{-1}\cdot\text{yr}^{-1}$ ). Positive ARC values were also observed in sites treated with stover return (NPKS) at  
the GZL, ZZ, and YL sites (range = at 8.50–18.0  $\text{mg}\cdot\text{kg}^{-1}\cdot\text{yr}^{-1}$ ). By contrast, significant increases in TN were only observed at  
ZZ and YL sites under inorganic fertilizer (NPK) treatments, while TN remained constant at other sites. Notably, TN declined  
190 in the unfertilized control (CK) treatment ( $-18.0\text{ mg}\cdot\text{kg}^{-1}\cdot\text{yr}^{-1}$ ) at the UM site, but remained stable at other sites. At QY, no  
significant changes in TN were observed over the 23-year treatment period. Comparison of sites with positive ARC values  
under NPKM and hNPKM treatments indicated that the increase was highest at GZL, followed by UM and YL, and lower at  
the ZZ and ZY sites.



The dynamics of soil AN followed similar pattern as those observed for TN (Table 1). Specifically, ARC values for soil AN increased in both NPKM and hNPKM treatments at all sites except QY (range = 1.41–4.13 mg·kg<sup>-1</sup>·yr<sup>-1</sup>) over the experimental treatment period. Similarly, soil AN also increased in NPKS treatments at the ZZ and YL sites (ARC = 1.52 mg·kg<sup>-1</sup>·yr<sup>-1</sup> and 2.28 mg·kg<sup>-1</sup>·yr<sup>-1</sup>, respectively), but decreased at QY (ARC = 1.32 mg·kg<sup>-1</sup>·yr<sup>-1</sup>), and kept constant at the GZL and UM sites. Similar to soil TN, AN also significantly increased in NPK treatment plots at ZZ and YL. In the unfertilized CK plots, AN decreased at UM (ARC = -0.97 mg·kg<sup>-1</sup>·yr<sup>-1</sup>) and QY (ARC = -0.91 mg·kg<sup>-1</sup>·yr<sup>-1</sup>). These results indicated that long-term application of manure led to higher levels of soil N compared to treatments with inorganic fertilizer.

**Table 1: Annual rates of change (ARC, mg·kg<sup>-1</sup>·yr<sup>-1</sup>) of soil total nitrogen (TN) and available nitrogen (AN) under various long-term treatments at the six study sites.**

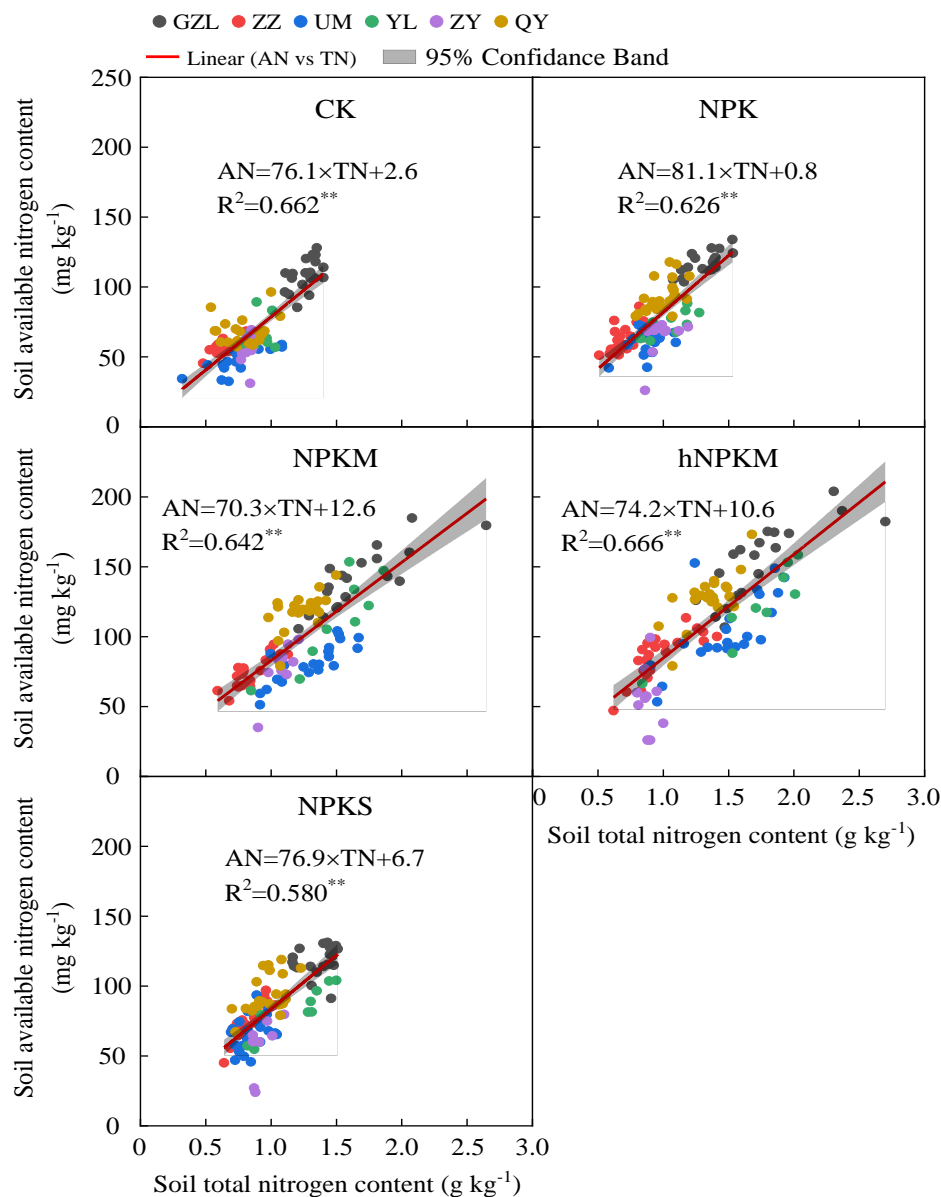
Sites	Experimental periods	n <sub>1</sub>	CK	NPK	NPKM	hNPKM	NPKS
Annual rates of change of soil TN							
GZL	1990–2018	27	0.00	0.70	48.3**	58.2**	8.50**
ZZ	1990–2011	21	0.43	6.73*	17.9**	28.8**	13.4**
UM	1990–2011	22	-18.0**	-5.74	23.8**	32.3**	-11.6
YL	1990–2014	20	3.19	15.3**	30.4**	33.7**	18.0**
ZY	1982–2002	11	-3.16	4.86	10.1**	NA	NA
QY	1990–2012	22	2.13	-3.65	2.82	10.5	-6.72
Annual rates of change of soil AN							
		n <sub>2</sub>	CK	NPK	NPKM	hNPKM	NPKS
GZL	1990–2012	18	-0.61	-0.50	2.32**	3.02**	0.47
ZZ	1990–2011	21	0.27	0.81**	1.41**	2.23**	1.52**
UM	1990–2011	22	-0.97**	0.52	1.75**	3.63**	0.68
YL	1990–2009	9	0.56	1.05*	4.13**	3.65**	2.28**
ZY	1982–2002	11	0.18	0.72	2.01**	NA	NA
QY	1990–2012	22	-0.91**	-0.70	0.56	1.07	-1.32**

Notes: The change rate was determined using a least squares linear regression:  $Y = a + bX$ , where Y is the soil TN or AN; X is the year; b is the slope (*i.e.*, the change rate). GZL, Gongzhuling; ZZ, Zhengzhou; UM, Urumqi; YL, Yangling; ZY, Zhangye; QY, Qiyang. CK, unfertilized control; NPK, inorganic N, phosphorus and potassium; NPKM, inorganic NPK plus manure; hNPKM, high application rate of NPKM; NPKS, inorganic NPK with stover return. n<sub>1</sub> and n<sub>2</sub>, quantity of the available annual data of soil TN and AN content, respectively. NA, data not available because the treatments are not included. \*, significant correlation at  $P < 0.05$ . \*\*, significant correlation at  $P < 0.01$ .

### 3.2 Responses of soil long-term N mineralization to fertilization treatments

In order to characterize differences in the factors contributing to shifts from TN to AN under organic and inorganic fertilizers, we next examined the long-term N mineralization (LT-N<sub>min</sub>) rate, *i.e.* the slope of the linear correlation between AN and TN, at each site. We found a highly significant positive correlation ( $p < 0.01$ ) between soil AN and TN under all treatments across sites (Fig. 2). The LT-N<sub>min</sub> rates for the CK, NPK, NPKM, hNPKM, and NPKS treatments were 76.1, 81.1, 70.3, 74.2, and 76.9 mg·g<sup>-1</sup>, respectively, which suggested that around 70–80 mg N could be transformed to available N for every 1 g·kg<sup>-1</sup> increase in TN, and the LT-N<sub>min</sub> was generally similar among these strategies over long-term application.





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**Figure 2: The linear relationship between soil AN and TN content under various long-term treatments (n = 104).** The slope of the linear relationship between AN and TN was used to calculate the annual increase in AN per 1 gram·TN ha<sup>-1</sup> increase annually. GZL, Gongzhuling; ZZ, Zhengzhou; UM, Urumqi; YL, Yangling; ZY, Zhangye; QY, Qiyang. \*\*, significant correlation between the soil AN and TN at P < 0.01. CK, unfertilized control; NPK, inorganic N, phosphorus and potassium; NPKM, inorganic NPK plus manure; hNPKM, high application rate of NPKM; NPKS, inorganic NPK with stover return.

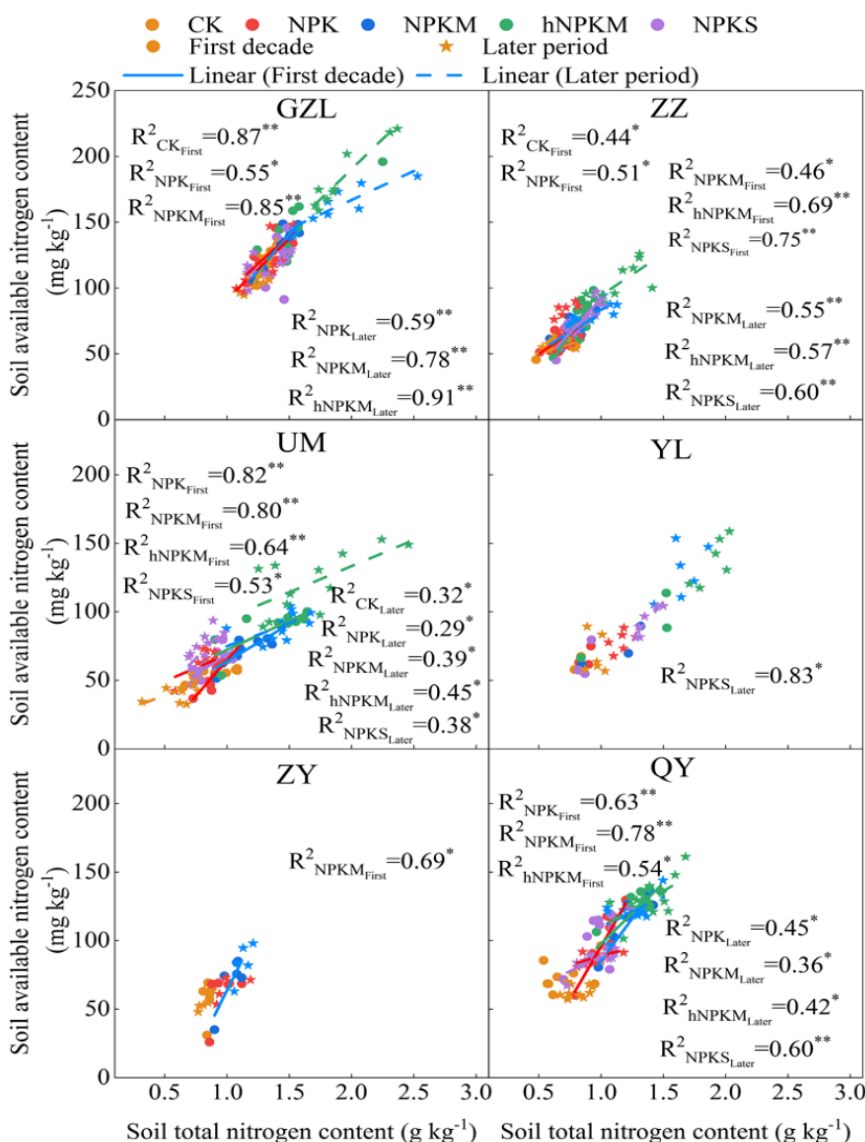
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### 3.3 Differences in soil LT-N<sub>min</sub> between the first decade and later period of fertilizer application and the factors influencing these changes

We next sought to determine whether the effects of manure or conventional fertilizer changed over time by comparing LT-N<sub>min</sub> rates between the first decade and the later period of treatment at each site (Fig. 3). Linear regression analyses indicated that



225 both the NPKM and hNPKM treatments resulted in significantly ( $P < 0.05$  or  $0.01$ ) increased soil AN, concurrent with significantly enhanced soil TN, at all sites except YL and ZY. Notably,  $LT-N_{min}$  rates in soils treated with manure were higher in the first decade (range =  $42\text{--}181\text{ mg}\cdot\text{g}^{-1}$ ) than in the later period (range =  $33\text{--}92\text{ mg}\cdot\text{g}^{-1}$ ), suggesting that the effect of these treatments on soil AN decreased over long-term manure application (Table 2). Moreover, the  $LT-N_{min}$  rates differed between sites, fertilization treatments and time periods, suggesting that  $LT-N_{min}$  was influenced by those diverse factors.



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**Figure 3: The linear relationship between soil AN and TN content in the first decade and later period under various long-term treatments at the six study sites.** The slope of the linear relationship between AN and TN was used to calculate the annual increase in AN per  $1\text{ gram}\cdot\text{TN ha}^{-1}$  increase annually. GZL, Gongzhuling; ZZ, Zhengzhou; UM, Urumqi; YL, Yangling; ZY, Zhangye; QY, Qiyang. \*\*, significant correlation between the soil AN and TN at  $P < 0.01$ . CK, unfertilized control; NPK, inorganic N, phosphorus and potassium; NPKM, inorganic NPK plus manure; hNPKM, high application rate of NPKM; NPKS, inorganic NPK with stover return.

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**Table 2. Soil  $LT-N_{min}$  rates ( $mg \cdot g^{-1}$ ) in the first decade and later period under various long-term treatments at the six study sites.**

Sites	Years	n	CK	NPK	NPKM	hNPKM	NPKS
GZL	1–10	9	190**	86.9*	110**	72.6	65.1
	11–23	9	51.9	92.1**	44.0**	91.9**	42.0
ZZ	1–10	10	26.6*	62.9*	58.2*	142 **	105**
	11–22	11	22.2	38.0	44.1**	60.1**	100**
UM	1–10	10	14.8	105**	54.4**	42.4**	58.7*
	11–22	12	33.3*	39.0*	33.3*	38.3*	97.9*
YL	1–10	3	132	85.1	49.6	50.1	212
	11–20	6	-36.8	44.4	68.9	104	108*
ZY	1–10	6	109	134	181 *	NA	NA
	11–21	5	99.7	40.2	163	NA	NA
QY	1–10	9	22.2	157**	122**	88.7*	75.2
	11–23	13	10.0	25.6*	43.5*	53.2*	42.3**

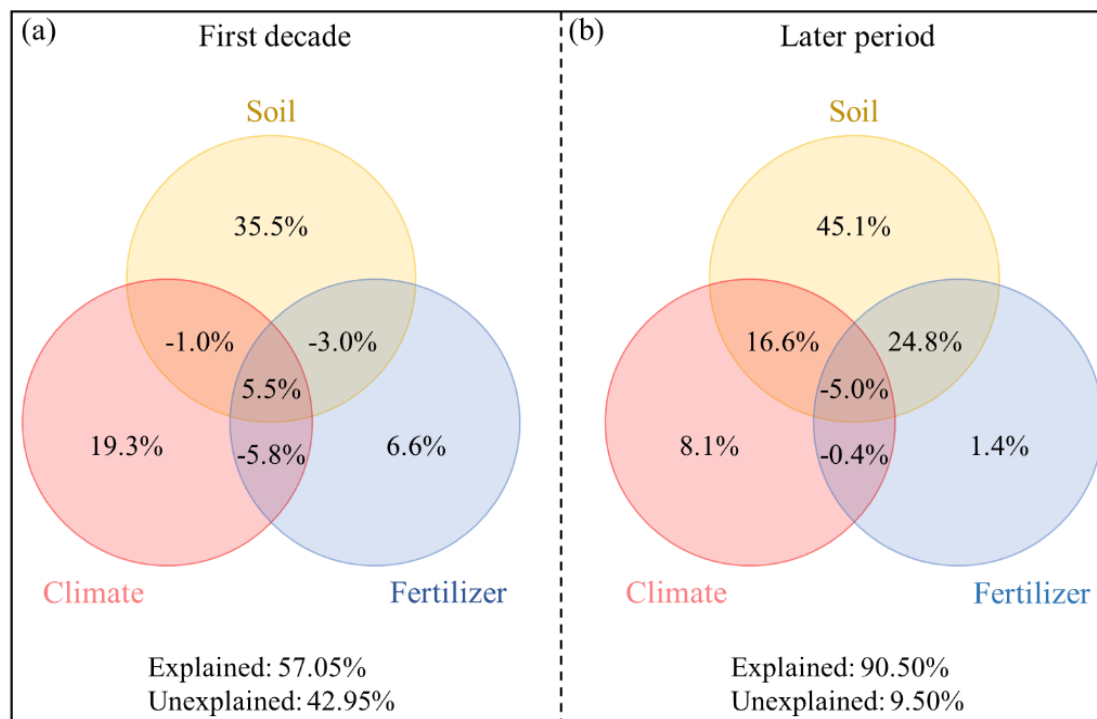
Notes: The  $LT-N_{min}$  value was determined using a least squares linear regression:  $Y = a + bX$ , where Y is the soil AN content; X is the TN content; b is the slope (i.e., the change rate, reflects the increased AN every TN increased). GZL, Gongzhuling; ZZ, Zhengzhou; UM, Urumqi; YL, Yangling; ZY, Zhangye; QY: Qiyang. CK, unfertilized control; NPK, inorganic N, phosphorus and potassium; NPKM, inorganic NPK plus manure; hNPKM, high application rate of NPKM; NPKS, inorganic NPK with stover return. n, quantity of the available annual data during the long-term treatment period. NA, data not available because the treatments are not included. \*, significant correlation at  $P < 0.05$ ; \*\*, significant correlation at  $P < 0.01$ .

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In light of the above findings showing a gradual change in the dynamics of long-term N mineralization, we next sought to determine which factors drive the higher  $LT-N_{min}$  in the two period of treatments. Variance partitioning analysis (VPA) revealed that differences in soil properties, fertilizer regimes, and climatic conditions could explain the majority of variation (57%) between treatments in the first decade (Fig. 4a) and accounted for almost all of the variation (90%) in the later period (Fig. 4b). More specifically, soil properties had the highest influence, while fertilizer regime and climatic conditions contributed relatively less effect (i.e., accounting for 36%, 6.6% and 19% of the total variation, respectively), which indicated that soil properties comprised the dominant determining factor in  $LT-N_{min}$  dynamics. In the later period, the influence of soil properties on  $LT-N_{min}$  increased to 45.1%, while that of climatic conditions and fertilizer treatment decreased to 8% and 1%, respectively. The interpretation rate for soil properties combined with fertilizer treatment was 24.8%, while soil properties and climatic conditions together accounted for 16.6% of the total variation in the later period. Collectively, these results suggested that the influence of climate on  $LT-N_{min}$  was attenuated with increasing duration of fertilization, while fertilizer regime enhanced, indirect contribution on by altering soil properties.



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**Figure 4: Relative contributions of soil properties, climatic conditions and fertilization managements to  $LT-N_{min}$  in the first decade (a) and later period (b).** Soil properties include pH, soil organic carbon (SOC), ratio of soil organic carbon and total N (C/N), total and available N, P and K nutrients contents. Fertilization managements include the unfertilized control (CK), inorganic N, phosphorus and potassium (NPK); inorganic NPK plus manure (NPKM), high application rate of NPKM (hNPKM), inorganic NPK with stover return (NPKS). Climatic conditions include mean annual temperature (MAT), mean annual precipitation (MAP), mean annual humidity and mean annual evaporation (AE). The total interpretation of soil  $LT-N_{min}$  by were showed at bottom of the figures by soil properties, climatic conditions and fertilization managements.

### 3.4 Climate and long-term fertilization impact $LT-N_{min}$ via soil organic carbon, pH, nutrients and stoichiometry

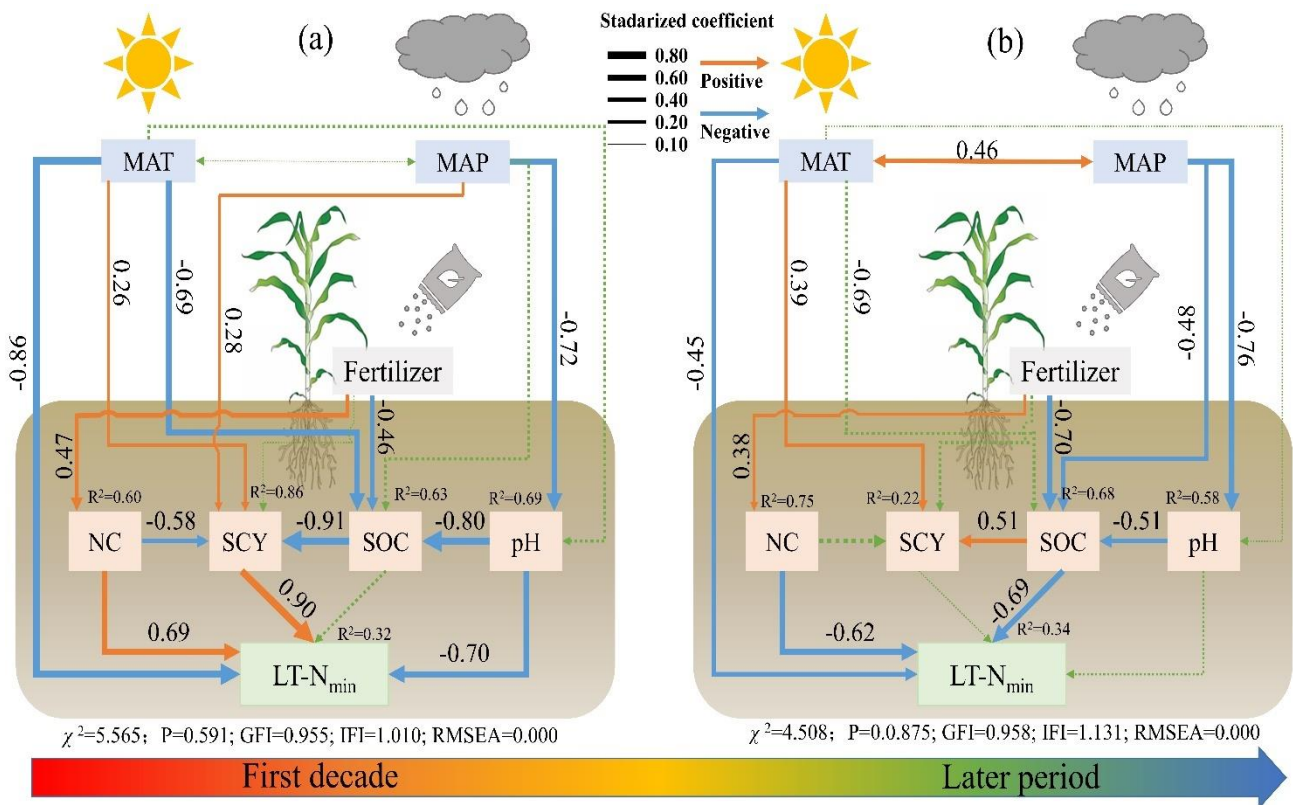
We carried out structural equation modeling (SEM) to investigate the potentially contribution of soil properties, fertilizer treatments and climatic conditions to shaping  $LT-N_{min}$  at these cereal production fields (Fig. 5a). In the first decade, the established SEM explained 32% of the total variation in  $LT-N_{min}$  ( $\chi^2 = 5.565$ ; Fisher's C statistic  $P = 0.591$ , GFI = 0.955, IFI = 1.010,  $RMSEA = 0.000$ ). In this model, soil nutrient content and stoichiometry both exhibited strong positive correlations with  $LT-N_{min}$  (path coefficients = 0.69 and 0.90, respectively). In addition, mean annual temperature (MAT) and soil pH shared strong negative correlations with  $LT-N_{min}$  (path coefficients = -0.86 and -0.70 respectively). Mean annual precipitation (MAP) indirectly affected  $LT-N_{min}$  by impacting soil pH and nutrient stoichiometry, while fertilizer treatments shared an indirect positive correlation with  $LT-N_{min}$  through its impacts on nutrient content, soil organic carbon (SOC) content, and nutrient stoichiometry. The high path coefficients for MAT and nutrient stoichiometry indicated that these factors provided the greatest direct influence on soil  $LT-N_{min}$  in the first decade of treatment.

In the later period of fertilizer applications, the established SEM explained 34% of the total variation in  $LT-N_{min}$  ( $\chi^2 = 4.508$ ; Fisher's C statistic  $P = 0.875$ , GFI = 0.958, IFI = 1.131,  $RMSEA = 0.000$ , Fig. 4b). In particular, SOC, nutrient content, and

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MAT were negatively correlated with  $LT-N_{min}$  (path coefficients = 0.69, 0.62, and 0.45, respectively). These high coefficient values indicated that SOC and nutrient content provided the strongest direct influence on soil  $LT-N_{min}$  in the later period of the fertilizer applications. MAP was correlated with soil pH, but pH was not significantly correlated with  $LT-N_{min}$ . However, MAP indirectly affected  $LT-N_{min}$  through impacts on SOC content. Similar with modeling results of data collected in the first decade, fertilizer treatments showed no direct relationship with  $LT-N_{min}$ , but indirectly affected  $LT-N_{min}$  by altering SOC and nutrient content. Overall, climatic effects (*i.e.*, MAP and MAT) led to decreases in  $LT-N_{min}$  in the later period with fertilization. These cumulative data showed that the contributions of climatic conditions on  $LT-N_{min}$  were alleviated over a decade by decreasing both direct influence and also its impact on soil  $LT-N_{min}$  via pH.



285 **Figure 5: Structural equation model (SEM) illustrating the direct and indirect effects of soil properties, fertilization managements and climatic conditions on soil  $LT-N_{min}$ : at the first decade (a) and the later period (b) of fertilizer applications.** Single-headed arrows indicate the hypothesized direction of causation and two-headed arrows indicate related correlations. Continuous and dashed arrows represent the significant and non-significant relationships, respectively. Orange and blue arrows indicate positive and negative relationships, respectively. The numbers adjacent to the arrows are the standardized path coefficients and the width of the arrow is in proportion to the degree of standardized path coefficients.  $R^2$  values indicate the proportion of variance explained by each variable. The nutrient content (NC), stoichiometry (SCY) and fertilizer represent the first component from PCA conducted for soil total and available nutrient of N, P and K; C/N, C/P, N/P; and the treatments of CK, NPK, NPKM, hNPKM and NPKS, respectively. The chi-square ( $\chi^2$ ), nonsignificant probability ( $P > 0.05$ ), high goodness-of fit index ( $GFI > 0.90$ ), high incremental fit index ( $IFI > 0.90$ ), and low root-mean-square errors of approximation ( $RMSEA < 0.05$ ) listed below the SEMs indicate our data matches the hypothetical models. MAT, mean annual temperature; MAP, mean annual precipitation.

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#### 4 Discussion

We found that manure application enhanced both TN and AN accumulation in soil (Table 1). The effects of manure on improving soil TN retention have been confirmed in previous studies (Li and Shi, 2007; Gao et al., 2005). However, the role of manure in N availability was less clear or AN data were often inconsistent due to its high variability under different environmental conditions. To our knowledge, this study represented the first estimation of soil long-term N mineralization (LT- $N_{\min}$ ), and showed that enhanced N availability was consistently coupled with increased TN, 7–8% of which could be transformed into available N (Fig. 2). It should be noted that LT- $N_{\min}$  values at all sites were higher in the first decade than those in the later period in plots treated with manure (Fig. 3 and Table 2). This finding indicated that soil organic N tends to be mineralized in the early stages of manure fertilization, and thus the mineralization rate declined over prolonged manure application. One explanation for this difference in LT- $N_{\min}$  between decades was that manure application significantly increased soil C and N contents in the first decade, but slowed down in later stages (Gong et al., 2011; Spargo et al., 2011). Other studies have shown that soil carbon adsorption reaches saturation with increasing experimental time due to the decreased soil-specific surface area and adsorption sites by manure application (Klaus and Georg, 2000). This decrease in adsorption could result in limited microbial growth, which may have ultimately alleviated N mineralization in the later period (Mohanty et al., 2013).

Another explanation for the observed decrease in LT- $N_{\min}$  was that repeated fertilizer application led to high  $\text{NH}_4^+$  content, which was widely known to repress the activity of enzymes responsible for breaking down organic polymers (Padhan et al., 2020), and thereby decreasing LT- $N_{\min}$  in the later period. Furthermore, repetitive manure application could potentially provide direct substrate to stimulate autotrophic nitrification, consequently enriching the size and activity of ammonia-oxidizing bacterial and archaeal population in these soils; the enhanced autotrophic nitrification may have led to higher N loss (Marchant et al., 2016). These results thus provided a framework for fertilizer management to maximize N use efficiency and N retention in agricultural soils.

Variance partitioning analysis showed that soil properties were the primary contributing factor to LT- $N_{\min}$  rates (Fig. 4). Among the relevant soil parameters, pH, SOC, nutrient stoichiometry, and nutrient content influenced directly or indirectly LT- $N_{\min}$  in both decades (Fig. 5). However, the modes by which these soil properties contributed to soil LT- $N_{\min}$  differed between the first decade and later period. Soil pH was widely considered a dominant selection factor for soil microbes, as well as for metabolic and enzymatic activities in soil, which was directly correlated with LT- $N_{\min}$  in the first decade but indirectly affected LT- $N_{\min}$  via SOC in the later period (Fig. 5). Other studies have proposed that a narrow pH range in later stages of treatment indirectly affect soil microbial community composition (DeForest et al., 2012). Nutrient content was positively correlated with LT- $N_{\min}$  in the first decade, but negatively correlated in the later period, suggesting that the mechanisms mediating its effects on LT- $N_{\min}$  changed in the later stage. In low concentrations, soil nutrients supported microbial growth and promoted LT- $N_{\min}$ . However, high nutrient accumulation promoted soil N immobilization, which resulted in decreasing N mineralization (Choi et al., 2004), and provided an alternative source of N which consequently lowered crop requirements for mineralization of soil N (Meyer et al., 2018). Therefore, it was important to clarify the direct and indirect influences of various soil parameters on LT-



$N_{\min}$  to better understand the process of soil N transformation and regulation of available N for crops.

330 Our models showed that temperature and precipitation also provided a stronger contribution to  $LT-N_{\min}$  in the first decade (19%) than in the later period (8%) (Fig. 4). This finding suggested that climatic impacts on soil  $LT-N_{\min}$  could be attenuated by long-term fertilization practices. This possibility was supported by our results showing that MAT directly affected  $LT-N_{\min}$  with a higher standardized path coefficient (0.86) in the first decade than in the later period (0.45) (Fig. 5). Notably, annual temperatures were higher in the later period than in the first decade at all sites except GZL, increasing by 0.7°C on average

335 across the six sites (Table S4). There are three primary modes through which climate can affect  $LT-N_{\min}$ . First, temperature and moisture significantly affected the soil microbial biomass and activity. In a previous study, Liu and colleagues (2016) calculated Q10 values to assess the temperature sensitivity of soil N mineralization and found that higher Q10 was associated with increased soil mineralization rates. Second, precipitation significantly and positively influenced N deposition on a global scale (Serna-Chavez et al. 2013), and detection of microbial carbon suggested that N deposition could decrease soil microbial

340 biomass production by 15% (Treseder, 2010) which in turn inhibiting  $LT-N_{\min}$ . Third, climatic conditions affected  $LT-N_{\min}$  by altering soil properties, especially pH and SOC in our study.

In both periods with fertilization, annual precipitation was correlated with pH, while pH was negatively correlated with  $LT-N_{\min}$  in the first decade but had no relationship with  $LT-N_{\min}$  in the later period (Fig. 5). Soil pH has been described as the second most important factor affecting  $N_{\min}$  on a global scale (Li et al., 2020), although the relationship between soil pH and

345 N mineralization was inconsistent among published studies. Increasing soil pH from 3.8 to 6.8 was shown to influence both soil gross N mineralization and immobilization rates, resulting in declining trend in  $N_{\min}$  rates (Cheng et al., 2013). By contrast, Fu et al. (1987) reported that  $N_{\min}$  increased with the increase of soil pH due to the promotion of substrate availability by high pH conditions. Kemmitt et al. (2006) found that both soil N mineralization and nitrification rates were positively correlated with pH, since soil acidity limited soil microbial activity. The contrasting effects of pH on  $N_{\min}$  may be related to differences

350 in the effects of soil pH on gross N mineralization and gross N immobilization, because microbial populations were responsible for both the release and immobilization of soil nutrients. It is worth noting that variation in pH was higher in first decade than in later period for most treatments in this study due to pH had a negative relation with MAP (Fig. 5 and Table S5), which could also contribute to the variability of climatic influence on  $LT-N_{\min}$  under long-term soil management regimes.

Temperature and precipitation were both independently correlated with SOC content, which was correlated with  $LT-N_{\min}$  in

355 both decades, suggesting that the SOC response to climatic conditions was a determining factor in  $LT-N_{\min}$ . The increase in annual temperature recorded in the later period could potentially drive the transfer of labile C into soil by increasing the decomposition rate of SOC, which subsequently enhanced microbial N demand and decreases  $LT-N_{\min}$  (Rustad et al., 2001). In our study, trends in SOC content differed between the first decade and later period potentially due to different treatments (Table S6). For example, SOC significantly increased under manure application at the YL and QY sites during the first decade,

360 but did not significantly change during the later period, which could lead to variability in  $LT-N_{\min}$ . Furthermore, SOC content indirectly affected  $LT-N_{\min}$  by shaping soil nutrient stoichiometry in the first decade, but directly impacted  $LT-N_{\min}$  in the later period (Fig. 5), suggesting that the mechanisms by which SOC affected  $LT-N_{\min}$  changed over time. These results thus



illustrated how climate directly modulate soil  $LT-N_{min}$  and shape its response to various soil conditions. Li et al. (2019) reported that 30% of the unexplained variations in  $N_{min}$  may be attributable to shifts in microbial community structure. However, it remained unclear how microbial communities impact  $N_{min}$  due to the complexity and diversity of metabolic processes and environmental conditions at play in soils. A comprehensive understanding of the regulatory mechanisms by which climatic conditions and soil properties modulate  $LT-N_{min}$  at different times could quantify the long-term residuary effects and mechanism of fertilization on soil N availability, which would facilitate the establishment of management strategies appropriate for specific soils and growing conditions to maximize crop N utilization.

## 370 5 Conclusions

Evaluation and SEM modeling of soil N under conventional fertilizer or conventional fertilizer plus manure treatments in 6 soils > 20-year field research sites showed that both total soil N and available N contents were significantly increased by application of manure except in one acidic soil. Total soil N content shared a positive linear correlation available N content, and the slope of the linear regression (*i.e.*,  $LT-N_{min}$ , the long-term N mineralization rate) indicated that available N increased by 70–81 mg per gram total N increased in soil. The  $LT-N_{min}$  rate was higher in the first decade of fertilizer treatments than in the later period. Mean annual temperature, soil pH, nutrient contents, and stoichiometry were shown to be determining factors that affect  $LT-N_{min}$  rate, and may drive major differences over long time periods. Climatic conditions also strongly contributed to  $LT-N_{min}$  rates in the first decade, but decreased the effect in the later period. However, the effects of climate interactions with soil properties on  $LT-N_{min}$  rates were enhanced in the later period. Collectively, these results suggested that manure incorporation with conventional N fertilizers could be a highly effective strategy to improve soil fertility/productivity for high crop yield while also alleviating the effects of climate variation on soil N availability.

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### Author contributions

Y.H.D, and M.G.X designed the study, Y.H.D, M.G.X, and H.Q.Z developed the overall research idea and wrote the first draft, Y.H.D, M.G.X, W.J.Z, and C.A.L revised, discussed, and finalized the manuscript. H.Q.Z, K.Y.R, and D.J.L helped in data analysis. All authors contributed to the manuscript writing, discussion, and revision.

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## 390 Data availability

Data generated in this study can be made available upon request from the corresponding author.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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