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Microbial regulation of net N mineralization is driven by C, N, P content and stoichiometry

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Complete List of Authors:	Wang, Qiqi; CAAS IARRP; Research Centre Julich Institute of Bio- and Geosciences; University of Amsterdam Institute for Biodiversity and Ecosystem Dynamics Gao, Wei; Tianjin Institute of Agricultural Resources and Environment Bol, Roland; Forschungszentrum Juelich, IBG3; University of Amsterdam Institute for Biodiversity and Ecosystem Dynamics Xiao, Qiong; CAAS IARRP Wu, Lei; CAAS IARRP Zhang, Wenju; Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences
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- 1 Soil nutrients drive net nitrogen mineralization
- 2 Microbial regulation of net N mineralization is driven by C, N, P
- 3 content and stoichiometry

5 Qiqi Wang^{a, c, d}, Wei Gao^b, Roland Bol^{c, d}, Qiong Xiao^a, Lei Wu^a, Wenju Zhang^{a*}

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- 7 ^aKey Laboratory of Arable Land Quality Monitoring and Evaluation, Ministry of
- 8 Agriculture and Rural Affairs, Institute of Agricultural Resources and Regional
- 9 Planning, Chinese Academy of Agricultural Sciences (CAAS), Beijing, 100081, P. R.
- 10 China
- 11 bTianjin Institute of Agricultural Resources and Environment, Tianjin Academy of
- 12 Agricultural Sciences (TAAS), Tianjin, 300192, P. R. China
- 13 °Institute of Bio- and Geosciences, Agrosphere (IBG-3), Forschungszentrum Jülich
- 14 *GmbH*, 52425, Jülich, Germany
- ⁴Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam,
- 16 P.O. Box 94240, 1090 GE Amsterdam, The Netherlands

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- *Corresponding author: Wenju Zhang
- 19 Address: Zhongguan South Str. 12, Haidian District, Institute of Agricultural Resources and
- 20 Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, 100081, P. R. China; Tel:
- 21 +86-10-82108661; Fax: +86-10-82106225; E-mail: zhangwenju01@caas.cn

Abstract

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Soil net nitrogen mineralization (Nt) is crucial for nitrogen availability and ecosystem productivity. However, the patterns and drivers of Nt remain unclear under different management practices. We examined the biotic and abiotic determinants of Nt, using 11 treatments in Northern China Fluvoaquic soil under wheat-maize rotation. Biotic properties, e.g., soil microbial community were determined based on phospholipid fatty acid (PLFA) together with high-throughput sequencing technologies. Abiotic properties were characterized by the content and stoichiometric ratios of soil nutrients. Animal manure applications (HNM, NM, M, FM) significantly increased the Nt (1.80-3.40 mg kg⁻¹) and available phosphorus (Olsen-P) (46.3-199.3 mg kg⁻¹), compared with treatments with plant residues (NG, NS) incorporation. Fallow with animal manure (FM) had the highest gramnegative bacteria (G-, 29.3 nmol g⁻¹), arbuscular mycorrhizal fungi (AMF, 4.57 nmol g⁻¹) abundance, which also significantly higher than that of the NG and NS. Structural Equation Modeling revealed that the content of total nutrient, including soil organic carbon (SOC), total nitrogen (TN) and total phosphorus (TP) rather than biotic properties, such as microbial community ($\lambda = -0.26$) and enzyme activities ($\lambda = -0.16$) had the strongest direct effect on Nt ($\lambda = 0.85$). Stoichiometric ratios of C, N, P controlled Nt indirectly by mediating enzyme activities. Specifically, high Nt was associated with low Dothideomycetes, Tectomicrobia abundance that negatively correlated with SOC, TN, TP, Olsen-P, and BG activity. Random Forest Model indicated that SOC and Olsen-P content were toprated determinants of Nt. Our result indicated that the content and stoichiometric ratios of SOC and N, P directly drive Nt or via microbial ways. Our study highlighted the importance of P to improve Nt, animal manure was thus recommended for nitrogen availability.

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- 44 **Keywords:** net nitrogen mineralization; phosphorous availability; microbial community; enzyme
- 45 activities



1. INTRODUCTION

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Soil nitrogen availability is a crucial factor that limits the primary productivity of ecosystem. Soil net nitrogen mineralization (Nt), an indicator of the quantity of soil organic nitrogen mineralization, is regarded as an effective index of nitrogen availability (Liu et al., 2017; Schimel and Bennett, 2004). Fertilization is widely recommended to improve soil nitrogen mineralization potential and availability, which in turn enables sustainable crop production (Marzi et al., 2019). The effect of different fertilization on soil nitrogen mineralization has been intensively studied in agricultural ecosystems (Ashraf et al., 2020; Li et al., 2020a; Pereg et al., 2018). Organic amendments are easily decomposed by soil microorganisms (Pascault et al., 2013), tend to exert a better effect relative to mineral fertilizers (Francioli et al., 2016) by increasing microbial biomass (Börjesson et al., 2011) and enzyme activities (Bol et al., 2003), as well as shifting microbial community structure (Li et al., 2015). However, the effects exerted on nitrogen mineralization by various organic amendments, such as animal manure and plant residues, tend to vary according to the substrate carbon to nitrogen (C:N) ratios (Truong et al., 2018). Knowledge concerning the driving factors of Nt is crucial for soil N availability, and further agricultural productivity. However, the mechanisms of factors in regulating Nt remain unclear. Climate and soil properties are regarded as the primary drivers of Nt on a global scale (Liu et al., 2017). Simultaneously, the importance of soil microbial properties in determining Nt is highlighted across the globe (Li et al., 2019). Although the regulation of soil Nt by climate, soil properties, or microbial properties have been reported based on meta-analysis or grassland field experiment (Chen et al., 2019b; Li et al., 2019; Liu et al., 2017), a comprehensive understanding of the regulation of Nt under fertilization, especially organic amendments with different C:N ratios in

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agro-ecosystems is still lacking. Both soil abiotic and biotic properties regulate nitrogen mineralization under different fertilization (Miller et al., 2018; Risch et al., 2019). Soil nutrient content, like soil organic carbon (SOC), is believed as the deciding factor for the Nt (Soinne et al., 2020). Furthermore, the stoichiometry of nutrient content influences the decomposer community structure and activities which are believed as the regulator of nitrogen cycling (Gan et al., 2020; Luo et al., 2020; Wei et al., 2017), However, how soil nutrient content together with their stoichiometric ratios regulated Nt is rarely discussed. Soil biotic properties (microbial biomass, community, taxa, enzyme activities) are believed as the crucial indicators in soil nitrogen cycle (Ashraf et al., 2020; Geisseler et al., 2010; Li et al., 2020b). Microbial activity stimulates mineralization processes directly by increasing enzyme activities (Xu et al., 2020). A more diverse community was found towards a greater capacity to mineralize N under organic management (Berthrong et al., 2013). Additionally, based on phospholipid fatty acid (PLFA) together with high-throughput sequencing technologies, which are believed effective to provide information on microbial community quantifying (Chen et al., 2019a; Orwin et al., 2018), soil nitrogen transformation processes are found to be linked with some functional bacterial or fungal taxa (Chen et al., 2019b; Xiao et al., 2022). The complex interactions of soil abiotic and biotic properties on the Nt have so far been neglected when they were studied individually. Long-term experiments could provide a much stronger and more convincing way than shortterm experiments to uncover the underlying mechanism of soil nutrients cycling (Guo et al., 2018; Guo et al., 2020). Based on a 38-year field experiment, 11 treatments including mineral fertilizers, animal manure, plant residues, and fallow were selected to explore the effect of soil abiotic

properties (total, available nutrient content and their stoichiometric ratios) and biotic properties (soil microbial community, bacterial and fungal taxa, extracellular enzyme activities) on Nt. We made our hypothesis (Figure S1) according to the conceptual framework for Nt from Li et al. (2019): (1) Overall, both soil abiotic and biotic properties might directly regulate Nt, while abiotic properties might play a more important role than biotic properties. Specifically, soil abiotic properties might regulate Nt in two ways: (2) The content of nutrient could affect Nt directly. (3) The content and stoichiometric ratios of soil nutrient would regulate Nt indirectly via biotic response.

2. MATERIALS AND METHODS

2.1 Experimental site and design

The long-term experimental site located in Tianjin, northern China (117°60E, 39°10N) and initialed in 1979. the cropping system was winter wheat and summer maize rotation at this site since 1979. This region is characterized by a warm and semi-humid continental monsoon climate with an annual average temperature and evaporation of 11.6 °C and 1736 mm, respectively. The active accumulated temperature (the sum of the daily temperature over 10 °C) is approximately 4200 °C. The annual precipitation is 607 mm, with approximately 80% occurring from June to September. The soil type is heavy loamy fluvo-aquic soil with pH 8.1. The initial soil organic carbon (SOC), total nitrogen (TN), phosphorus (TP), and potassium was 10.96, 1.00, 1.59, and 16.14 g kg⁻¹, respectively. Soil available N, phosphorus (Olsen-P), and K was 75.10, 15.80, and 176.6 mg kg⁻¹, respectively (Yang et al., 2015).

The eleven treatments analyzed in this study were grouped as follows: (a) control, no fertilizer (CK) and pure fallow (F); (b) mineral fertilizers, mineral nitrogen (N) alone, mineral phosphorus with potassium (PK), and mineral N with P and K (NPK); (c) animal manure incorporation, animal

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manure alone (M), mineral N with animal manure (NM), half application rate of NM (HNM), fallow with animal manure (FM); (d) plant residues incorporation, mineral N with straw (NS), mineral N with green manure (silage maize, NG). Each plot with 16.7 m² was randomly replicated and isolated using cement baffle plates. The specific amounts of fertilizers were annually applied were as follows (Table S1, S2): No fertilizers or manure was applied in the CK and fallow treatments. mineral N fertilizer for the N, NPK, NG, NS, and NM treatments was 285 kg N ha⁻¹ winter wheat and 210 kg N ha⁻¹ for maize. The mineral fertilizers used were in urea (N 40%), calcium superphosphate (16%) P₂O₅), and potassium chloride (K₂O 60%). About 11,535 kg ha⁻¹ of animal manure (air-dried weight) was applied to the NM, FM, and M treatments. For the HNM treatment, the amount of mineral and animal manure was half of that for the NM. For the 1979-1988 period, the animal manure used for the NM, HNM, and M treatments was dung (raw manure mixed with garbage soil, average C 367.75 g kg⁻¹, N 63.82 g kg⁻¹, P 13.22 g kg⁻¹, K 16.04 g kg⁻¹, C:N, 5.76; C:P, 27.82). After 1998, chicken manure was used (average C 301.46 g kg⁻¹, N 23.38 g kg⁻¹, P 9.29 g kg⁻¹, K 16.06 g kg⁻¹, C:N, 12.89; C:P, 32.45). Moreover, wheat (average C 323.00 g kg⁻¹, N 3.40 g kg⁻¹, P 0.46 g kg⁻¹, K 7.20 g kg⁻¹, C:N, 95.0; C:P, 702.2) and maize straw (average C 137.00 g kg⁻¹, N 3.20 g kg⁻¹, P 0.49 g kg⁻¹, K 4.20 g kg⁻¹, C:N, 42.8; C:P, 279.6) left after harvest was added to the NS treatment, 30,600 kg ha⁻¹ of fresh maize silage was applied in the NG treatment. Management and cropping practices at the study site are detailed by Gao et al. (2015) and Yang et al. (2015). 2.2 Soil sampling

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Soil samples were collected in October 2017 after maize harvest. Five cores were randomly collected from the plow layer (0–20 cm) of each plot and mixed thoroughly. Fresh soil samples were brought back to the laboratory immediately. After removing all stones and roots, these samples were

- passed through a 2 mm sieve. Fresh soil was separated into three sub-samples. One sub-sample was stored at -80 °C for microbial community analyzing. The second sub-sample was stored at 4 °C for the microbial biomass, enzyme activity, and nitrogen mineralization determination. The last subsample was air-dried to determine the basic soil properties.
- 2.3 Soil abiotic properties

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- 139 Soil organic carbon (SOC) was determined using the Walkley-Black dichromate oxidation method 140 (Walkley and Black, 1934). The content of TN and TP were determined using the Kjeldahl digestion-distillation and molybdenum-blue colorimetry methods, respectively (Pansu and 142 Gautheyrou, 2007). And Olsen-P was measured via extraction with 0.5 M NaHCO₃ (Olsen et al. 143 1954). Available N (AN, the sum of NH₄⁺ and NO₃⁻) was measured using the CaCl₂ extraction 144 method (Joergensen and Potthoff, 2005). Dissolved organic carbon (DOC) and nitrogen (DON) 145 were quantified as total organic carbon and nitrogen in the extractions of non-fumigated soil (Wu 146 et al., 2019). Soil pH was measured with a glass electrode using a soil to water ratio of 1:2.5. Soil moisture was determined after drying for 24 h at 105 °C. 147
- 148 2.4 Determination of net soil nitrogen mineralization
 - Net nitrogen mineralization (Nt) was determined using the aerobic incubation method (Stanford and Smith, 1972; Agbenin et al., 1999). According to this method, 15 g quartz sand was pre-built into 60 mL plastic filter funnels and covered with a glass wool pad. Next, 15 g air-dried soil samples and 15 g quartz sand (1 mm < d < 2 mm) were mixed with distilled water and placed in these funnels. Initial mineral N was removed by leaching four times with 100 mL of a 0.01 mol L-1 CaCl₂ solution, followed by 25 mL of a minus-N nutrient solution consisting of 0.002 mol L⁻¹ CaSO₄·2H₂O, 0.002 mol L⁻¹ MgSO₄, 0.005 mol L⁻¹ Ca (H₂PO₄)₂·2H₂O and 0.0025 mol L⁻¹ K₂SO₄. The gravimetric

method was used to maintain soil moisture during cultivation. All soil samples were cultured at 25 °C in quad-replicates. On the second week leachate was collected using 0.01 mol L⁻¹ CaCl₂ with

a 60 cm Hg suction for 2 h. The leachate was then analyzed for NH_4^+ and NO_3^- .

2.5 Extracellular enzyme activities

We measured the activity of acid phosphatase (Pho), β -glucosidase (BG), β -cellobiosidase (CBH), and β -1,4-N-acetyl-glucosaminidase (NAG) using a 200 μ M solution of 4-methylumbelliferone (MUB)-labeled substrates. L-leucine aminopeptidase (LAP) was measured using L-Leucine-7-amino-4-methylcoumarin, while phenol oxidase (POX) and peroxidase (PER) were measured using L-3,4-dihydroxyphenylalanine (L-DOPA) (DeForest 2009). Soil suspensions were prepared by adding 1 g of fresh soil to 100 mL of 50 mM acetate buffer and homogenizing for 1 min. The pH of the buffer was adjusted to the mean soil pH of each treatment (DeForest. 2009). Pho, BG, CBH, LAP, and NAG microplates were incubated in the dark at 25 °C for 4 h, and fluorescence was assessed using a microplate fluorometer (SymergyH1M, BioTek Instruments Inc., Winooski, VT, USA) with 365 nm excitations and 450 nm emission filters. POX and PER microplates were incubated in the dark at 25 °C for 20 h and fluorescence was detected using the visible spectrum (460 nm). Absolute activities of the soil enzymes were calculated and expressed in nmol g⁻¹ h⁻¹ (German et al., 2011). The geometric mean of the hydrolytic enzyme activities (GMea) was calculated as follows (García-Ruiz et al., 2008):

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$$GMea = (Pho \times BG \times CBH \times LAP \times NAG)^{1/5}$$
 (1)

2.6 Soil microbial community

Soil microbial biomass carbon (SMBC) and nitrogen (SMBN) were measured via the CHCl₃ fumigation-extraction method (Wu et al., 1990). PLFAs were extracted from the soil using

Frostegård's (1991) procedure. Total PLFA was calculated by adding all PLFAs detected in the soil. The i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0 PLFAs were analyzed as a proxy of gram-positive bacteria (G+) (Frostegård and Baath. 1996), while 16:1ω7c, 16:1ω9c, cy17:0, 18:1ω5c, 18:1ω7c, cy19:0w8c, and 17:1w8c were determined as proxies of gram-negative bacteria (G-); (Olsson et al., 1995; García-Orenes et al., 2013). The sum of 14:0, 15:0, 16:0, 17:0, G+, and G- was considered as the bacterial content (Frostegård and Bååth. 1996). Additionally, 10Me16:0, 10Me17:0, and 10Me18:0 were used as markers of actinomycetes (Zelles. 1997), 16:1 w5c, marked as the presence of arbuscular mycorrhizal fungi (AMF) (Olsson et al., 1995). 18:1 w9c, 18:2 w6,9c, and 18:3 w6c (6,9,12) were used to indicate fungi (Frostegård and Bååth. 1996). Shannon index (H) and Simpson index (D) were calculated according to Zhong et al. (2010): where Pi is the ratio of each fatty acid to the sum of all detected PLFAs.

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$$H = -\sum \text{Pi} \cdot \ln(\text{Pi})$$

$$D = 1 - \sum (\text{Pi})^2$$
(3)

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$$D = 1 - \sum (Pi)^2$$
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- 2.7 Bacterial and fungal community assessment: DNA extraction and pyrosequencing
- 193 2.7.1 DNA extraction and PCR amplification

194 Microbial DNA was extracted from 0.5 g of each soil sample using an E.Z.N.A. soil DNA kit 195 (Omega Biotek, Norcross, GA, USA) according to the manufacturer's protocol. The V3-V4 region of the bacterial 16S ribosomal RNA gene was amplified via PCR (95 °C for 3 min, followed by 27 196 197 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min) 5'-ACTCCTACGGGAGGCAGCAG-3' 5′-198 using the primers 338F and 806R

GGACTACHVGGGTWTCTAAT-3'. The ITS ribosomal RNA gene of the fungi was amplified via

200 PCR (95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and 201 final extension 72 °C for ITS3F at 10 min) using the primers 5′-202 GCATCGATGAAGAACGCAGC-3' and ITS4R 5'-TCCTCCGCTTATTGATATGC-3'. The 203 produced barcode was an eight-base sequence unique to each sample. PCR reactions were 204 performed in triplicate in a 20 µL mixture containing 4 µL of 5 × FastPfu Buffer, 2 µL of 2.5 mM 205 dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. 206 2.7.2 Illumina MiSeq sequencing 207 Amplicons were extracted from 2% agarose gels and purified using an AxyPrep DNA Gel 208 Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's 209 instructions, and quantified using QuantiFluorTM -ST (Promega Corporation, Madison, WI, USA). 210 Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina 211 MiSeq platform (Majorbio, Shanghai, China) according to standard protocols. 212 2.7.3 Processing of sequencing data Raw FASTQ files were demultiplexed and quality-filtered via QIIME (version 1.17) using the 213 214 following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score 215 < 20 over a 50 bp sliding window, while truncated reads that were shorter than 50 bp were discarded; 216 (ii) exact barcode matching, wherein 2 nucleotide mismatches during primer matching as well as 217 reads containing ambiguous characters were removed; and (iii) only sequences that overlapped for 218 lengths over 10 bp were assembled according to their overlapped sequences. Reads that could not 219 be assembled were discarded. 220 Operational taxonomic units (OTUs) were clustered using a 97% similarity cutoff via UPARSE (version 7.1; http://drive5.com/uparse), while chimeric sequences were identified and removed 221

222	using UCHIME. The taxonomy of 16S rRNA and ITS rRNA gene sequences was analyzed using
223	the RDP Classifier (http://rdp.cme.msu.edu) against the silva (SSU115) 16S rRNA database and the
224	Unite ITS rRNA database, respectively, with a confidence threshold of 70%.
225	All sequenced data were uploaded to the NCBI Sequence Read Archive (SAR) database
226	(https://www.ncbi.nlm.nih.gov/sra). The bacterial accession number is PRJNA669222 and the
227	fungal accession number is PRJNA669221.
228	2.8 Statistical analysis
229	Differences between treatments were analyzed using the one-way ANOVA (Duncan; $P < 0.05$).
230	Spearman correlation was analyzed using SPSS Statistics 22 software, the Random Forest Model
231	(RFM) was constructed using R (3.6.3). Structural Equation Modeling (SEM) analysis was
232	performed using R (3.6.3) and AMOS 21. Bar plots were mapped using Sigmaplot 12.5. The
233	analysis of sequencing data was performed and relevant figures were calculated via R from the
234	Majorbio I-Sanger Cloud Platform (https://www.i-sanger.com).
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236	3. RESULTS
237	3.1 Soil abiotic properties and net nitrogen mineralization under management
238	practices
239	Compared with the CK control, the NPK, fallow, and organic amendments (NS, NG, HNM, NM,
240	M, FM) significantly increased the SOC and TN contents (Table 1), especially the FM treatment
241	(SOC 25.2 g·kg ⁻¹ ; TN 2.43 g·kg ⁻¹). Treatments with animal manure (HNM, NM, M, FM) had better
242	improvement for SOC and TN (increased by 81% and 86% on average, respectively) relative to the
243	NS, NG, NPK, and fallow (increased by 35% and 27% on average). Treatments with mineral P

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fertilizer and animal manure (PK, NPK, HNM, NM, M, FM) significantly increased TP and Olsen-P contents, compared with the CK. On average, the Olsen-P content in the PK, NPK, and HNM treatments (22.15–46.25 mg·kg⁻¹) increased by 14-fold, while that in the NM, M, and FM treatments (144.50–251.00 mg kg⁻¹) increased by 80-fold. The DOC to Olsen-P ratio (DOC:Olsen-P) and the AN to Olsen-P ratio (AN:Olsen-P) were notably higher for the CK, N, NG, and NS treatments (Table 2). The Nt changed significantly under different management practices (Fig. 1). The FM had the highest Nt (3.40 mg·kg⁻¹) relative to the CK (0.22 mg·kg⁻¹), followed by the M (2.81 mg·kg⁻¹) and NM (2.41 mg·kg⁻¹) treatments. The Nt of the NM, M, and FM treatments increased considerably by 15.5, 12.8, and 11.0 times, respectively. The NM, M, FM, NPK, and HNM treatments exerted a greater effect on Nt than the F treatment. It was noteworthy that the incorporation of plant residues (NG, NS) exerted little effect on the Nt. 3.2 Soil biotic properties under fertilizations All treatments, except for the N and NPK, increased SMBN and SMBC significantly (Table S3). Organic amendments (FM, M, NM, HNM, NS, NG, 330.7-441.4 mg kg⁻¹) had a better effect on SMBC than treatments with mineral fertilizers (N, PK, NPK, 264.4-314.9 mg kg⁻¹). Relative to the CK, SMBC in the M (439.7 mg kg⁻¹) and FM (441.4 mg kg⁻¹) treatments increased by an average of 73%, and were considerably higher than that of other treatments. Total PLFA and fungi abundance in all treatments, except N, were notably increased (Fig. 2). Furthermore, the M and FM treatments showed a remarkable higher total PLFA (147.5 nmol g⁻¹ and 140.6 nmol g⁻¹), G- (33.3 nmol g⁻¹, 29.3 nmol g⁻¹), and AMF (4.61 nmol g⁻¹, 4.57 nmol g⁻¹) than other treatments. All treatments significantly increased the G- abundance compared with the CK.

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Moreover, G- abundance for the NG, NS treatments (25.1 and 25.8 nmol g ⁻¹) were relatively higher
than that of mineral fertilizers (N, PK, NPK, 19.1-22.5 nmol g ⁻¹). AMF abundance in the PK, NG,
NS, HNM, NM, and F treatments (3.02–3.40 nmol g ⁻¹) was considerably higher than that in the CK
$(2.55 \text{ nmol g}^{-1})$ and N $(2.48 \text{ nmol g}^{-1})$ treatments. The abundance of bacteria, G+, and actinomycetes
in the various treatments showed similar trends as total PLFA (Table S3). The Shannon and Simpson
indexes among different treatments showed no significant differences. Moreover, the G- to G+
(G-:G+) and F:B ratios of these treatments showed no significant differences, except for the NS
treatment (Table S4).
Organic amendments increased the GMea significantly (Fig. 2). The NAG activity under
treatments with animal manure (e.g., the HNM, NM, M, FM) tended to be higher than that in mineral
fertilizers (N, PK, NPK). The FM had the highest NAG activity (12.3 nmol g ⁻¹ h ⁻¹). However,
mineral fertilizers (N, PK, NPK) increased PER significantly, while organic amendments (NG, NS,
HNM, NM, M) decreased that (Fig. 2). Relatively higher BG activities were observed for the NM,
M, and F treatments (98.5, 94.5, 92.4 nmol g ⁻¹ h ⁻¹), followed by the NS, FM, N, NG, NPK, and
HNM, all of which recorded higher activities than the CK, PK. NPK, F treatments (Fig. 2). The NS
significantly increased Pho activity (Fig. S2).
High-throughput sequencing results showed that over ten bacterial phyla were detected in all
these treatments (Fig. S3). Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi, which
were detected in all fertilization treatments, displayed abundances ranging from 18%-31%, 18%-
28%, 11%-26%, and 8%-14%, respectively. However, the proportion of fungal species found in
different treatments showed high variability. Sordariomycetes and Dothideomycetes were the
dominant classes, with relative abundances ranging from 21%–71% and 4%–31%, respectively (Fig.

288 S3). Moreover, NM, M, F, and FM treatments led to a relatively lower amount of *Dothideomycetes* 289 than the other treatments. 290 3.3 Net nitrogen mineralization linked with soil biotic and abiotic properties 291 Results from Spearman's correlation analysis indicated that the Nt, SOC, TN, Olsen-P, and DOC 292 were positively correlated with all PLFA groups as well as SMBC, SMBN, CBH, NAG, and GMea, 293 but negatively correlated with PER activity. In contrast, the stoichiometry of Olsen-P (DOC:Olsen-P, 294 AN:Olsen-P) showed a negative correlation with these above microbial variables (Fig. 3). In general, 295 there were no significant relationships between fungi, total PLFA, BG and the stoichiometric ratios 296 of total nutrients (C:N, C:P, N:P). A negative relationship was found between the Nt and Tectomicrobia and Dothideomycetes, 297 298 Moreover, Tectomicrobia and Dothideomycetes were negatively correlated with TP, Olsen-P, SOC, 299 TN, DOC, DON (Fig. 4). Unclassified p Rozellomycota was negatively correlated with 300 DOC:Olsen-P and AN:Olsen-P, and positively correlated with Nt, SOC, Olsen-P (Fig. 4b). 301 Tectomicrobia, Dothideomycetes, and Unclassified p Rozellomycota were also significantly linked 302 with NAG or BG enzyme activities (Fig. 5). Specifically, Dothideomycetes negatively correlated 303 with BG and NAG activities, while *Unclassified p Rozellomycota* positively linked with these two 304 enzymes. 305 The RFM showed that, of all the factors significantly corrected with Nt, SOC was the most 306 important factor influencing the Nt, explaining 20.1% of the variations, followed by Olsen-P (14.9%) 307 (Fig. 6a). Additionally, the AN:Olsen-P and DOC:Olsen-P ratios also showed significant effects 308 than soil C:N, C:P, and N:P ratios in regulating Nt. BG activity and bacteria abundance were top-309 rated biotic properties that significantly affected the Nt. The Nt linearly increased with increase in

SOC, Olsen-P content ($R^2 = 0.87$, 0.78; Fig. 6b, c). Initially the Nt decreased dramatically, but stabilized with the increase in AN:Olsen-P ($R^2 = 0.50$, P < 0.001, Fig. 6d). Similarly, the predominant contribution of soil abiotic properties for the Nt was observed from SEM results (Fig. 7). The content of total and available nutrient ($\lambda = 0.85$, 0.38, respectively) rather than biotic properties, such as microbial community ($\lambda = -0.26$) and enzyme activities ($\lambda = -0.16$) had the strongest direct effect on Nt (Fig. 7). Furthermore, the content of (total and available) nutrient also indirectly affected the Nt by complex microbial responses. Specifically, the content of (total and available) nutrient shifted microbial community to regulate the Nt, or influenced the enzyme activities of some key taxa, which further affected Nt. Differently from the content of nutrient, the stoichiometric ratios of (total and available) nutrients regulated Nt indirectly by changing microbial enzyme activities ($\lambda = 0.59$, -0.54; Fig. 7).

4. DISCUSSION

4.1 Soil abiotic properties directly regulate Nt

Based on the SEM and RFM statistical approaches, our hypotheses were confirmed that soil abiotic properties, characterized by the content and stoichiometric ratios of total and available nutrient, played a dominant role than soil biotic properties in determining the Nt. Specifically, the content of total and available nutrient directly control soil Nt (Fig.7), high Nt corrected with high SOC, Olsen-P content, but low AN:Olsen-P (Fig. 6). Soil chemical properties are crucial environmental factors for soil N cycling (Tang et al., 2019), Liu et al. (2017) found that Nt was primarily affected by SOC content, soil C:N ratio, and clay content. Moreover, mineral N and soil available P are essential factors associated with N mineralization (Zhang et al. 2019). Our findings were consistent with

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these results, indicating that abiotic properties were dominants of N mineralization. The regulation of soil abiotic properties, especially SOC, Olsen-P on Nt could be attributed to the effects of

different management practices.

On the one hand, our results were consistent with previous studies that organic amendments input all exerts a positive effect on SOC, but animal manure improves SOC better than mineral fertilizers with or without plant residues (Ye et al., 2019; Meng et al., 2017). Our results indicated that balanced mineral fertilizer (NPK) and animal manure application (HNM, NM, M, FM) increased Nt significantly by increasing SOC, which was consistent with previous studies (Gai et al., 2019; Zhang et al., 2017), indicating that manure application enhanced nitrogen availability for plants. On the other hand, Nt was improved by Olsen-P accumulation under animal manure application. It was noteworthy that, in our study, although plant residues return (NG, NS) significantly increased SOC content, showing no effect on Nt. That might be due to the low soil TP and Olsen- P contents (Table 2). Sun et al. (2015) found that the manure had a stronger impact on N-cycling gene abundance than wheat straw, and available P was the most important factor affecting N-cycling communities. We observed that animal (dung or chicken) manure has notable lower C:N and C:P ratio than plant residues (Table S2). With the input of animal manure combined with mineral N fertilizer, the N and P nutrient availability were improved comprehensively. However, the application of plant residues with mineral N largely decreased soil TP and Olsen-P content relative to the initial value. Plant residues are known to cause nitrogen immobilization due to the presence of more C and a higher N decomposition rate, resulting in a suppressed N content (Marzi et al., 2019; Salazar et al., 2020). Microbes store surplus C as biomass or shift microbial community to adjust soil element stoichiometry as the C:N or C:P imbalance increases, indicating N or P

constraint for microorganisms (Huang et al., 2021). Moreover, in our study, mineral nitrogen applied with plant residues compensates for microbial N requirement, while without P input, microbes consume soil P to compensate for P deficiencies leading to a lower soil P content. In summary, the low Nt under treatments N, PK, NS, and NG attributed to either low SOC or Olsen-P content.

4.2 Soil abiotic properties affect Nt indirectly via microbial response

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Nitrogen cycling processes significantly depend on the microbial community, biomass, and enzyme activities occurring in the soil (Cui et al., 2020; Geisseler et al., 2010; Li et al., 2020b). On the one hand, total and available nutrient contents directly shifted soil microbial community, thus indirectly regulating the Nt. Particularly, bacterial, fungi, G-, total PLFA abundance, and SMBC were positively correlated with SOC, TN, TP, Olsen-P, and DOC (Fig. 3). This study indicated that the application of organic amendments promoted soil nutrient status, further favoring microbial growth. Especially microbes such as G- and AMF, preferably growing under conditions of nutrient sufficiency and enhancing nitrogen mineralization from organic residues (Balasooriya et al., 2016; Atul-Nayyar et al., 2009). On the contrary, long-term application of mineral N fertilizer alone caused the imbalanced nutrient condition, thereby restricting the growth of G- and AMF (Eo and Park., 2016; Wang et al., 2020), which negatively affected Nt consequently. Furthermore, the total nutrient content indirectly influenced the Nt via combined responses from bacterial and fungal taxa as well as enzyme activities (Fig. 7). Specifically, Dothideomycetes was negatively correlated with Nt, BG, NAG, and Olsen-P (Fig. 5). As decomposers of saprophytes and plant litter, these fungi are essential for the overall health of the ecosystem as well as for the global carbon cycle (Goodwin et al., 2009). The Dothideomycetes abundance was found to decrease under long-term fertilization

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(Zhou et al., 2016). Our findings indicated that organic amendments input inhibited the growth of *Dothideomycetes*, which tended to grow in low-nutrient environments, and further promotes N mineralization.

On the other hand, different from soil nutrient content, the stoichiometric ratios of soil nutrients (total and available) content regulated Nt indirectly by altering soil enzyme activities (Fig. 6a). In general, extracellular enzymes degrade soil organic matter into smaller units, which can also be directly utilized by microorganisms and plants. Particularly, BG and NAG activities played more important role than other biotic properties in regulating Nt, and were positively correlated with Nt (Fig. 3, 6). Accordingly, BG and NAG activities increase with elevated N availability (Ekenler and Tabatabai 2002; Schleuss et al., 2019). In addition, all transformations related to soil N processes are mediated by enzymatic systems that require C, N, and energy (Geisseler et al., 2010). Available nutrients that are mobile may be directly utilized by soil microbes and thereby play a significant role in microbial growth. In our study, the importance of AN:Olsen-P for Nt was highlighted from the RFM results. Our findings that the negatively correlations between AN:Olsen-P ratio and Nt were consistent with Wei et al. (2020). Increase in microbial P limitation could decrease the soil nitrogen cycling potential (Cui et al., 2020). Long-term N fertilization would limit the growth of several bacterial groups by P unavailability (Zhou et al. 2017). Our study thus indicated that longterm application of mineral N, soil microorganisms would compete with plants for P nutrient to maintain a constant biomass stoichiometry. Overall, the regulation of Nt by biotic properties all depends on the soil nutrient condition and their stoichiometric ratios, which were significantly affected by long-term management practices. Our hypotheses were confirmed, microbial community and enzyme activities directly affected soil Nt, but the effects were lower than soil

398	abiotic properties (Fig. 7). The importance of soil abiotic properties not only represented as the
399	direct control on Nt, but also indirectly regulated Nt through complex microbial responses.
400	CONCLUSION
401	Our study provided further comprehensive evidence that soil abiotic properties (i.e. nutrient content
402	and their stoichiometric ratios) determined the Nt. The direct microbial regulation of Nt depends on
403	soil abiotic properties, and the complex interactions of abiotic and biotic properties were also
404	important determinants of Nt. Also, with mineral N fertilizer application, Nt was controlled by SOC
405	and P nutrient status. Therefore, relative to plant residues incorporation with higher C:N and C:P
406	ratios, animal manure showing better performance on SOC and P nutrient balance is recommended
407	for improving soil N availability in Fluvo-aquic soil.
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411 412 413 414	We are thankful to the staff who were involved in maintaining the long-term trial. This work is financially supported by the National Natural Science Foundation of China (Grant Number: 41877105) and the Innovation Talent Promotion Program of the Ministry of Science and Technology. AUTHOR CONTRIBUTIONS Study concept and design: Wenju Zhang. Laboratory and statistical analysis: Qiqi Wang, Qiong Xiao,
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The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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Table 1 Soil abiotic properties under different long-term management practices.

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Treatment s	SOC (mg kg ⁻ 1)	TN (mg kg ⁻¹)	TP (mg kg ⁻¹)	Olsen-P (mg kg ⁻ 1)	AN (mg kg ⁻ ¹)	рН	DOC (mg kg ⁻ 1)	DON (mg kg ⁻ 1)
CK	12.2 ± 0.17 g	$1.07 \pm 0.02 f$	$0.55 \pm 0.10f$	$2.47 \pm 1.01f$	$3.01 \pm 0.11d$	$8.70 \pm 0.08a$	$32.5 \pm 3.63 \text{ f}$	5.17 ± 0.89 d
N	$14.0 \pm 1.31 \text{fg}$	1.15 ± 0.03 ef	0.69 ± 0.01 ef	$2.20 \pm 0.26 f$	7.56 ± 0.69 b	$8.30 \pm 0.18c$	42.3 ± 3.31 ef	11.4 ± 2.26 cd
PK	14.6 ± 1.55 ef	1.15 ± 0.06 ef	$1.33 \pm 0.04c$	35.5 ± 0.20 d	3.76 ± 0.32 cd	$8.58 \pm 0.15a$	36.7 ± 5.30 f	6.83 ± 1.76 cd
NPK	16.4 ± 1.37cde	1.30 ± 0.05 de	$1.25 \pm 0.24c$	$22.2 \pm 0.95e$	$7.23 \pm 1.01b$	$8.28 \pm 0.19c$	44.8 ± 4.30 ef	17.9 ± 7.98 bc
NG	15.6 ± 0.66def	1.30 ± 0.06 de	0.71 ± 0.01ef	3.70 ± 0.56 f	$4.24 \pm 0.68c$	8.52 ± 0.05ab	47.1 ± 2.63 ef	11.2 ± 1.00 cd
NS	17.3 ± 0.03 cd	$1.42 \pm 0.06d$	$0.78 \pm 0.05e$	$3.85 \pm 0.45 f$	10.38 ± 0.42a	$8.28 \pm 0.14c$	64.5 ± 1.63 d	$26.4 \pm 1.29ab$
HNM	$18.5 \pm 0.31c$	$1.49 \pm 0.08d$	$1.03 \pm 0.10d$	$46.3 \pm 5.25d$	$4.55 \pm 0.12c$	$8.61 \pm 0.05a$	58.3 ± 6.67 de	12.3 ± 1.14 cd
NM	22.3 ± 0.53 b	$1.86 \pm 0.14c$	1.58 ± 0.16 b	$144.5 \pm 11.50c$	6.66 ± 0.35 b	8.35 ± 0.08bc	$82.1 \pm 8.53c$	28.7 ± 16.54 ab
M	$22.5 \pm 0.62b$	$2.17 \pm 0.09b$	$2.02 \pm 0.08a$	$251.0 \pm 2.00a$	$4.52 \pm 0.55c$	8.33 ± 0.07bc	$100.3 \pm 20.42b$	17.8 ± 4.26 bc
F	16.7 ± 1.17cde	$1.43 \pm 0.08d$	1.01 ± 0.05 d	12.6 ± 0.50 ef	$4.69 \pm 0.45c$	$8.31 \pm 0.02c$	55.8 ± 6.20 de	11.6 ± 2.57 cd
FM	$25.2 \pm 2.72a$	$2.43 \pm 0.27a$	$1.78 \pm 0.17b$	199.3 ± 16.77 b	10.86 ± 1.27a	$8.08 \pm 0.02d$	117.3 ± 17.59a	$32.0 \pm 7.87a$

SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; AN, available nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen. CK, no fertilizer; N, mineral nitrogen only; PK, mineral phosphorus with potassium; NPK, mineral N with P, K; NG, mineral N

with green manure; NS, mineral N with straw; NM, mineral N with animal manure; HNM, half application rate of NM; M, animal manure only; F, pure fallow; FM, fallow with animal manure. Different lower letters indicate significant difference among various treatments (P< 0.05).



Table 2 Stoichiometric ratios of soil (total and available) nutrients under different long-term management practices.

Treatments	C:N	C:P	N:P	DOC:AN	DOC:Olsen-P	AN:Olsen-P
CK	$11.4 \pm 0.08ab$	$22.5 \pm 4.17a$	$1.98 \pm 0.38a$	10.8 ± 1.50 b	$15.6 \pm 9.28ab$	$1.39 \pm 0.63c$
N	$12.2 \pm 1.25a$	$20.2 \pm 1.83ab$	$1.66 \pm 0.08 bc$	$5.60 \pm 0.12c$	$19.5 \pm 3.66a$	$3.49 \pm 0.69a$
PK	$12.7 \pm 1.05a$	$10.9 \pm 0.86e$	$0.86 \pm 0.05g$	$9.74 \pm 0.79b$	$1.03 \pm 0.15c$	$0.11 \pm 0.01d$
NPK	$12.6 \pm 0.57a$	$13.3 \pm 1.32e$	1.06 ± 0.15 fg	$6.34 \pm 1.48c$	$2.03 \pm 0.25c$	$0.33 \pm 0.04d$
NG	$12.0 \pm 0.87a$	$21.9 \pm 0.88a$	$1.82 \pm 0.06ab$	11.3 ± 1.54 b	$12.9 \pm 2.15b$	$1.15 \pm 0.19c$
NS	$12.2 \pm 0.46a$	$22.2 \pm 1.29a$	$1.82 \pm 0.15ab$	$6.22 \pm 0.38c$	16.9 ± 1.66 ab	$2.73 \pm 0.43b$
HNM	$12.4 \pm 0.73a$	18.1 ± 2.10 bc	1.45 ± 0.14 cd	12.9 ± 1.79 b	$1.28 \pm 0.27c$	$0.10 \pm 0.01d$
NM	$12.1 \pm 1.08a$	14.2 ± 1.55 de	1.18 ± 0.04 ef	$12.4 \pm 1.37b$	$0.57 \pm 0.10c$	$0.05 \pm 0.01d$
M	10.4 ± 0.70 b	$11.2 \pm 0.63e$	1.07 ± 0.03 fg	$22.3 \pm 4.16a$	$0.40 \pm 0.08c$	$0.02 \pm 0.00d$
F	11.7 ± 0.64 ab	16.5 ± 1.12 cd	1.42 ± 0.03 cde	11.9 ± 0.83 b	$4.44 \pm 0.67c$	$0.37 \pm 0.05d$
FM	10.4 ± 0.62 b	14.2 ± 0.25 de	1.37 ± 0.06 de	10.8 ± 0.59 b	$0.60 \pm 0.13c$	$0.06 \pm 0.01d$

C:N, soil organic carbon to total nitrogen ratio; C:P, soil organic carbon to total phosphorus ratio; N:P, soil total nitrogen to total phosphorus ratio; DOC:AN, dissolved organic carbon to available nitrogen ratio; DOC:Olsen-P, dissolved organic carbon to available phosphorus ratio; AN:Olsen-P, available nitrogen to available phosphorus ratio; SMBC:SMBN, soil microbial biomass carbon to biomass nitrogen ratio. CK, no fertilizer; N, mineral nitrogen only; PK, mineral phosphorus with potassium; NPK, mineral N with P, K; NG, mineral N with green manure; NS, mineral N with straw; NM, mineral N with animal manure; HNM, half application rate of NM; M, animal manure only; F, pure fallow; FM, fallow with animal manure. Different lower letters indicate significant difference among various treatments (P < 0.05).

FIGURE CAPTIONS

Figure 1 Soil net nitrogen mineralization (Nt) under different long-term management practices. CK, no fertilizer; N, mineral nitrogen only; PK, mineral phosphorus with potassium; NPK, mineral N with P, K; NG, mineral N with green manure; NS, mineral N with straw; NM, mineral N with animal manure; HNM, half application rate of NM; M, animal manure only; F, pure fallow; FM, fallow with animal manure. Different lower letters indicate significant difference among various treatments (p < 0.05).

Figure 2 Soil microbial PLFA groups abundances and soil extracellular enzyme activities under different long-term management practices. AMF, arbuscular mycorrhizal fungi; G-, gram-negative bacteria; PER, peroxidase; BG, β-1,4-glucosidase; NAG, β-1, 4-N-acetyl-glucosaminidase; GMea, geometric mean of soil hydrolytic enzymes; CK, no fertilizer; N, mineral nitrogen only; PK, mineral phosphorus with potassium; NPK, mineral N with P, K; NG, mineral N with green manure; NS, mineral N with straw; NM, mineral N with animal manure; HNM, half application rate of NM; M, animal manure only; F, pure fallow; FM, fallow with animal manure. Different lower letters indicate significant difference among various treatments (p < 0.05).

Figure 3 Spearman's correlation relationship between soil biotic properties (microbial biomass, PLFA groups, enzymes activities) and soil abiotic properties and net nitrogen mineralization (Nt). SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; Olsen-P, available phosphorus; AN, available nitrogen (ammonium nitrogen and nitrate nitrogen); DOC, dissolved organic carbon, DON, dissolved organic nitrogen; C:N, soil organic carbon to total nitrogen ratio; C:P, soil organic carbon to total phosphorus ratio; N:P, soil total nitrogen to total phosphorus ratio; DOC:AN, dissolved organic carbon to available nitrogen ratio, DOC:Olsen-P, dissolved organic carbon to available phosphorus ratio; AN:Olsen-P, available nitrogen to available phosphorus ratio. G+: gram-positive bacteria; G-: gram-negative bacteria; AMF: arbuscular mycorrhizal fungi;

SMBC: soil microbial biomass carbon; SMBN: soil microbial biomass nitrogen; BG, β -1,4-glucosidase; CBH, cellobiohydrolase; NAG, β -1, 4-N-acetyl-glucosaminidase; LAP, L-leucine aminopeptidase; GMea, geometric mean of soil hydrolytic enzymes; PER, peroxidase; POX, polyphenol oxidase; Pho, acid phosphatase; Different color gradient in heatmap indicate the Spearman's correlation coefficients, asterisks denote for different significance levels at p < 0.05 (*), p < 0.01 (**).

Figure 4 Spearman's correlation heatmap of bacteria on phylum level (a) and fungi on class level (b) with soil abiotic properties and net soil nitrogen mineralization (Nt). SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; Olsen-P, available phosphorus; AN, available nitrogen (ammonium nitrogen and nitrate nitrogen); DOC, dissolved organic carbon; DON, dissolved organic nitrogen; C:N, soil organic carbon to total nitrogen ratio; C:P, soil organic carbon to total phosphorus ratio; N:P, soil total nitrogen to total phosphorus ratio; DOC:AN, dissolved organic carbon to available nitrogen ratio; DOC:Olsen-P, dissolved organic carbon to available phosphorus ratio; AN:Olsen-P, available nitrogen to available phosphorus ratio. Different color gradient in heatmap indicate the Spearman's correlation coefficients. Asterisks in the square denote for different significance levels at p < 0.05 (*), p < 0.01 (**) and p < 0.001(***).

Figure 5 The relationships between BG, NAG activities and key taxa (significantly correlated with the net nitrogen mineralization). Asterisks denote for different significance levels at p < 0.05 (*), p < 0.01 (**) and p < 0.001(***).

Figure 6 The importance of abiotic and biotic properties on the net nitrogen mineralization (Nt) estimated by Random Forest Model (a), and the relationship between the Nt and top-rated three variables (b, c, d). Abiotic properties include soil nutrients (SOC, TN, TP, Olsen-P, AN, DOC, DON) and their stoichiometric ratios (C:N, C:P, N:P, DOC:AN, DOC:Olsen-P, AN:Olsen-P). Biotic properties include PLFA groups (SMBC, SMBN, bacteria, fungi, G-, G+, total PLFA, actinomycetes, AMF) and enzymes (BG, NAG, PER, GMea, CBH). The grey bars indicated significant at p < 0.05,

white bars were not significant. Asterisks denote for different significance levels at p < 0.05 (*), p < 0.01 (**) and p < 0.001(***).

Figure 7 Direct and indirect effects of microbial community, enzyme activities, soil (total and available) nutrients content and their stoichiometric ratios on soil net nitrogen mineralization (Nt) by Structural Equation Modelling. Content of total nutrient include soil organic carbon (SOC); total nitrogen (TN); total phosphorus (TP). Stoichiometric ratios of total nutrient include soil organic carbon to total nitrogen ratio (C:N), soil organic carbon to total phosphorus ratio (C:P), soil total nitrogen to total phosphorus ratio (N:P). Content of available nutrient include available nitrogen (AN), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), Olsen-P. Stoichiometric ratios of available nutrient include DOC:AN ratio, DOC:Olsen-P ratio, AN:Olsen-P ratio. Microbial community include soil microbial biomass carbon (SMBC), microbial biomass nitrogen (SMBN) and PLFA groups. Enzymes include L-leucine aminopeptidase (LAP), β-1,4-glucosidase (BG), peroxidase (PER), cellobiohydrolase (CBH), β-1,4-N-acetyl-glucosaminidase (NAG), polyphenol oxidase (POX), acid phosphatase (Pho), geometric mean of soil hydrolytic enzymes (GMea). B & F taxa include Tectomicrobia (Tec), Dothideomycetes (Dot), Unclassified p Rozellomycota (Roz). Multiple-layer rectangles represent the first component from the PCA conducted for the abiotic and biotic properties. Solid and dash arrows indicated positive and negative relationships, respectively. Grey arrows indicate insignificant relationship. The arrow width is proportional to the strength of the relationship. The numbers adjacent to the arrows are the correlation coefficients. (*), (**), (***) represent significant at p < 0.05, p < 0.01, and p < 0.001, respectively.

Fig. 1

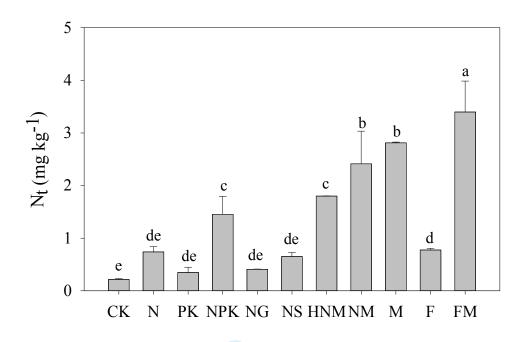


Fig. 2

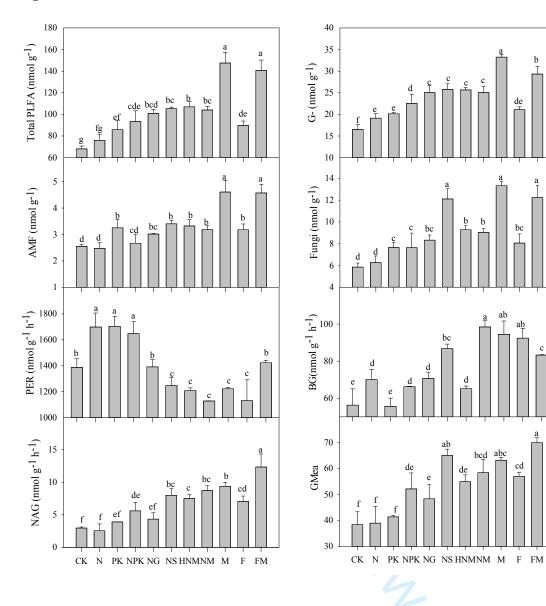
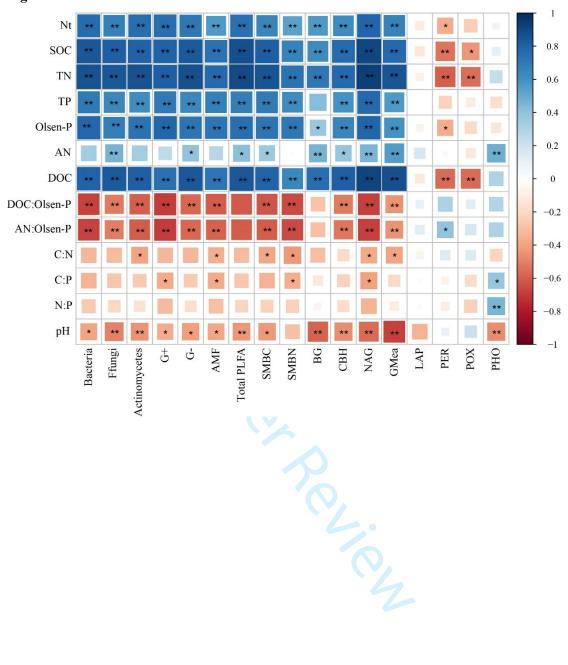


Fig. 3



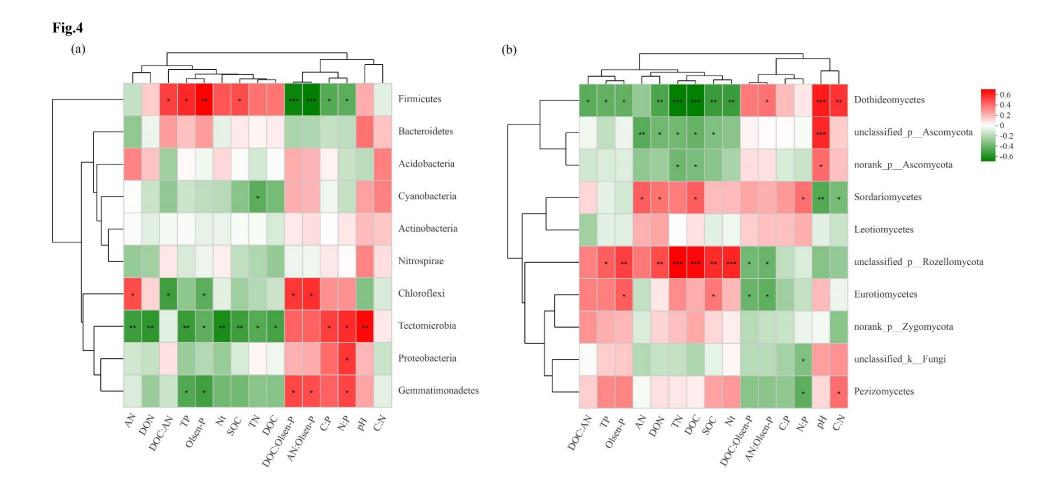


Fig.5

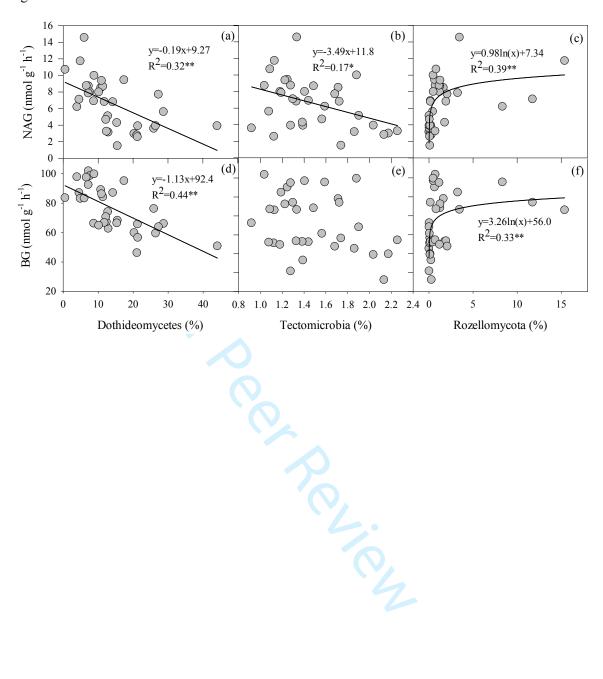


Fig.6

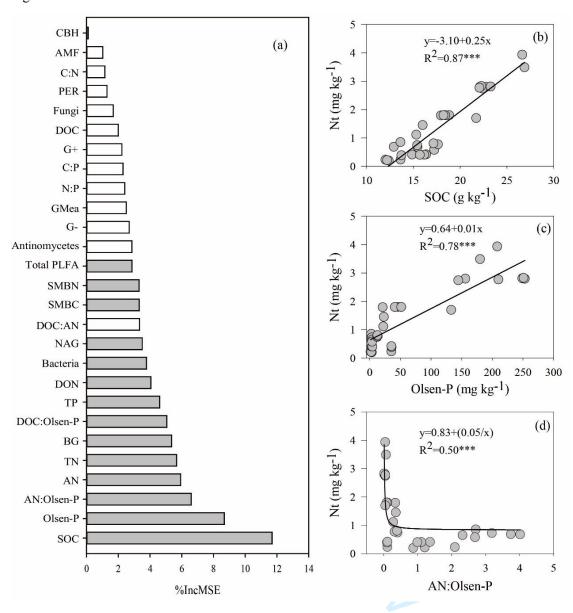


Fig. 7

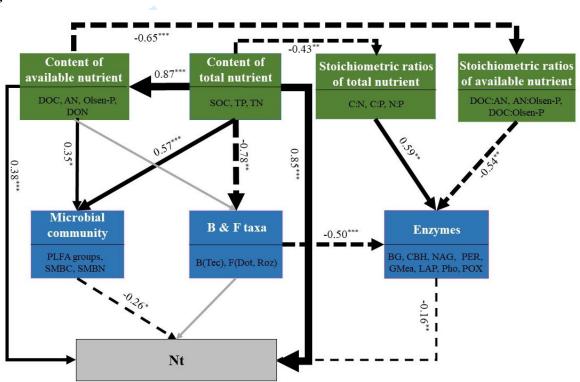


Table S1 Annual application amount of chemical N, P, K fertilizer and organic amendments.

		Whe	at (kg ha ⁻¹)		Corn (kg ha ⁻¹)			
Treatments	Chemical N	P ₂ O ₅	K ₂ O	Organic amendments ^a	Chemical N	P ₂ O ₅	K ₂ O	Organic amendments ^a
CK	0	0	0	0	0	0	0	0
N	285	0	0	0	210	0	0	0
PK ^b	0	142.5	71.3	0	0	0	0	0
NPK	285	142.5	71.3	0	210	0	0	0
NG	285	0	0	0	210	0	0	30600°
NS	285	0	0	straw return ^d	210	0	0	straw return ^d
HNM	142.5	0	0_	5768	105	0	0	0
NM	285	0	0	11535	210	0	0	0
M^e	0	0	0	11535	0	0	0	0
$\mathbf{F^f}$	0	0	0	0	0	0	0	0
FM	0	0	0	11535	0	0	0	0

a In air-dried dung or chicken manure weight; b start from 1995; c In fresh maize straw weight; d All wheat and maize straw were returned to the soil each year; e start form 2011; f start from 2011; CK, no fertilizer; N, mineral nitrogen only; PK, mineral phosphorus with potassium; NPK, mineral N with P, K; NG, mineral N with green manure; NS, mineral N with straw; NM, mineral N with animal manure; HNM, half application rate of NM; M, animal manure only; F, pure fallow; FM, fallow with animal manure. Different lower letters indicate significant difference among various treatments (p < 0.05).

Table S2 The annual application amount of organic C, N, P, K from animal manure and the stoichiometric ratios of soil organic C, N, and P.

*7	Treatments	Organic	C and nutrient	s input from	Stoichiometric ratios				
Year		Type	Organic C	N	P	K	C:N	C:P	N:P
1979-1998	HNM	dung	2121	368	76	93	5.76	27.82	4.83
	NM	dung	4242	736	152	185	5.76	27.82	4.83
	M	dung	4242	736	152	185	5.76	27.82	4.83
	FM	dung	4242	736	152	185	5.76	27.82	4.83
	HNM	chicken	1739	135	54	93	12.89	32.45	2.52
1000 2017	NM	chicken	3477	270	107	185	12.89	32.45	2.52
1999-2017	M	chicken	3477	270	107	185	12.89	32.45	2.52
	FM	chicken	3477	270	107	185	12.89	32.45	2.52

Table S3 Soil microbial biomass and PLFA groups abundances under different long-term management practices.

Treatments	Bacteria (nmol g ⁻¹)	Actinomycetes (nmol g ⁻¹)	G+ (nmol g ⁻¹)	SMBC (mg kg ⁻¹)	SMBN (mg kg ⁻¹)
CK	$36.0 \pm 1.21h$	$9.92 \pm 0.31e$	11.9 ± 0.65 g	$254.5 \pm 26.7e$	$29.2 \pm 4.66e$
N	$40.5 \pm 3.14 gh$	11.0 ± 0.81 de	$13.0 \pm 1.20 fg$	264.4 ± 28.9 de	$26.2 \pm 3.62e$
PK	$45.2 \pm 3.50 fg$	11.1 ± 0.25 de	14.6 ± 2.03 efg	314.9 ± 5.9 cd	40.9 ± 0.87 cd
NPK	$50.0 \pm 5.01 def$	13.1 ± 1.86 cd	16.8 ± 1.83 cde	308.5 ± 32.4 cd	34.5 ± 4.45 de
NG	55.0 ± 2.60 cd	15.1 ± 0.53 bc	18.8 ± 0.54 cd	$340.3 \pm 7.8bc$	$46.1 \pm 3.39c$
NS	52.0 ± 1.54 cde	14.3 ± 0.63 bc	15.5 ± 0.92 ef	344.7 ± 16.6 bc	39.1 ± 2.35 cd
HNM	$56.4 \pm 2.16c$	$15.8 \pm 0.14b$	$19.1 \pm 0.95c$	330.7 ± 17.7 bc	42.3 ± 1.98 cd
NM	55.5 ± 1.95 cd	14.8 ± 0.33 bc	18.4 ± 0.50 cd	$372.4 \pm 38.4b$	44.0 ± 9.26 cd
M	$78.9 \pm 4.35a$	$20.3 \pm 0.83a$	$28.7 \pm 2.23a$	$439.7 \pm 30.3a$	$59.7 \pm 6.34b$
F	46.9 ± 2.01 ef	14.0 ± 0.10 bc	16.1 ± 0.97 de	310.5 ± 11.3 cd	44.1 ± 1.35 cd
FM	71.2 ± 5.85 b	$20.9 \pm 3.67a$	25.5 ± 2.97 b	$441.4 \pm 58.7a$	$69.4 \pm 10.2a$

G+: gram-positive bacteria; SMBC: soil microbial biomass carbon; SMBN: soil microbial biomass nitrogen; CK, no fertilizer; N, mineral nitrogen only; PK, mineral phosphorus with potassium; NPK, mineral N with P, K; NG, mineral N with green manure; NS, mineral N with straw; NM, mineral N with animal manure; HNM, half application rate of NM; M, animal manure only; F, pure fallow; FM, fallow with animal manure. Different lower letters indicate significant difference among various treatments (p < 0.05).

Table S4 The PLFA α -diversity indexes and G-/G+, F/B ratios under different long-term management practices.

Treatments	Shannon	Simpson	G-:G+	F:B
CK	$3.09 \pm 0.02a$	$0.94 \pm 0.002a$	1.33 ± 0.08 bc	0.16 ± 0.010 bc
N	$3.08 \pm 0.01a$	$0.94 \pm 0.001a$	$1.47 \pm 0.06b$	0.15 ± 0.003 bc
PK	$3.04 \pm 0.17a$	$0.94 \pm 0.007a$	$1.40\pm0.19b$	$0.17 \pm 0.007b$
NPK	$2.98 \pm 0.10a$	$0.94 \pm 0.008a$	1.34 ± 0.02 bc	$0.15 \pm 0.011c$
NG	$3.06 \pm 0.01a$	$0.93 \pm 0.001a$	1.33 ± 0.05 bc	$0.15 \pm 0.001c$
NS	$3.04 \pm 0.02a$	0.93 ± 0.004 a	$1.67 \pm 0.17a$	$0.23 \pm 0.021a$
HNM	$3.06 \pm 0.05a$	$0.94 \pm 0.001a$	$1.35 \pm 0.04b$	0.16 ± 0.000 bc
NM	$3.05 \pm 0.03a$	$0.94 \pm 0.001a$	$1.36 \pm 0.07b$	0.16 ± 0.006 bc
M	2.95 ± 0.06 ab	0.94 ± 0.006 a	1.16 ± 0.07 cd	$0.17 \pm 0.004b$
F	$3.03 \pm 0.02a$	0.94 ± 0.001 a	1.32 ± 0.04 bcd	$0.17 \pm 0.011b$
FM	$2.84 \pm 0.16b$	0.94 ± 0.004 a	$1.16 \pm 0.06d$	$0.17 \pm 0.012b$

G-:G+: gram-negative bacteria to gram-positive bacteria ratio; F:B, fungi to bacteria ratio; CK, no fertilizer; N, mineral nitrogen only; PK, mineral phosphorus with potassium; NPK, mineral N with P, K; NG, mineral N with green manure; NS, mineral N with straw; NM, mineral N with animal manure; HNM, half application rate of NM; M, animal manure only; F, pure fallow; FM, fallow with animal manure. Different lower letters indicate significant difference among various treatments (p < 0.05).

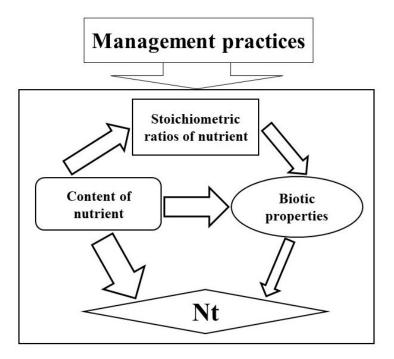


Figure S1 A proposed conceptual framework for net nitrogen mineralization (Nt) under different management practices. The arrow width indicates the relative strength of the relationship between the two.

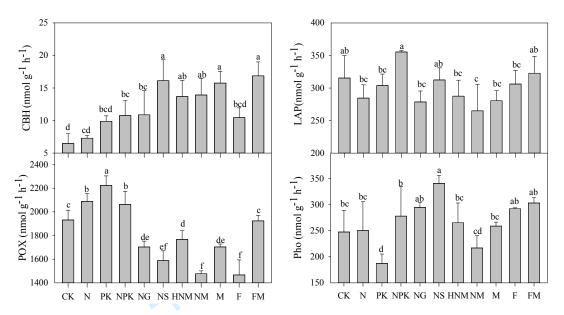


Figure S2 Soil extracellular enzymes activities under different long-term management practices.

CBH, cellobiohydrolase; LAP, L-leucine aminopeptidase; POX, polyphenol oxidase; Pho, acid phosphatase; CK, no fertilizer; N, mineral nitrogen only; PK, mineral phosphorus with potassium; NPK, mineral N with P, K; NG, mineral N with green manure; NS, mineral N with straw; NM, mineral N with animal manure; HNM, half application rate of NM; M, animal manure only; F, pure fallow; FM, fallow with animal manure. Different lower letters indicate significant difference among various treatments (p < 0.05).

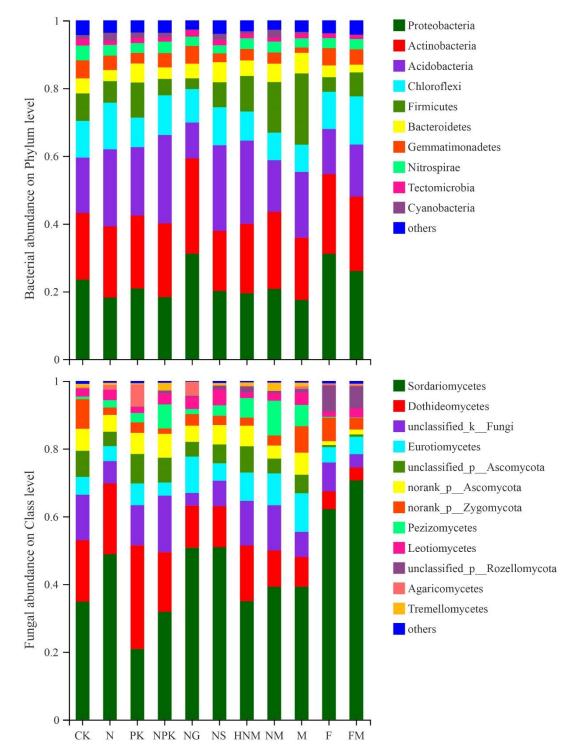


Figure S3 Relative abundances of dominant bacteria (relative abundance > 0.02%) on phylum and fungi (relative abundance > 0.01%) on class level under different long-term management practices. CK, no fertilizer; N, mineral nitrogen only; PK, mineral phosphorus with potassium; NPK, mineral N with P, K; NG, mineral N with green manure; NS, mineral N with straw; NM, mineral N with animal manure; HNM, half application rate of NM; M, animal manure only; F, pure fallow; FM, fallow with animal manure.