Received: 28 June 2021 | Revised: 24 April 2022 | Accepted: 13 May 2022

DOI: 10.1111/ejss.13257

ORIGINAL ARTICLE

European Journal of Soil Science WILEY

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

Qiqi Wang[1,2,3] | Wei Gao[4] | Roland Bol[2,3] | Qiong Xiao[1] | Lei Wu[1] | Wenju Zhang[1]

[1]Key Laboratory of Arable Land Quality Monitoring and Evaluation, Ministry of Agriculture and Rural Affairs, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China

[2]Institute of Bio- and Geosciences, Agrosphere (IBG-3), Forschungszentrum Jülich GmbH, Jülich, Germany

[3]Department of Ecosystem and Landscape Dynamics (ELD), Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam, The Netherlands

[4]Tianjin Institute of Agricultural Resources and Environment, Tianjin Academy of Agricultural Sciences (TAAS), Tianjin, China

Correspondence
Wenju Zhang, Zhongguan South Str. 12, Haidian District, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China.
Email: zhangwenju01@caas.cn

Funding information
National Natural Science Foundation of China, Grant/Award Number: 41877105

Abstract

Soil net nitrogen mineralisation (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 fluvo-aquic soil under wheat–maize rotation. Biotic properties, for example, soil microbial community, were determined based on phospholipid fatty acid (PLFA) together with high-throughput sequencing technologies. Abiotic properties were characterised 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 Gram-negative bacteria (G-, 29.3 nmol g^{-1}), arbuscular mycorrhizal fungi (AMF, 4.57 nmol g^{-1}) abundance, which was also significantly higher than that of the NG and NS. Structural Equation Modelling 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, and 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 contents were top-rated 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.

Highlights

- Animal manure significantly enhanced Nt rather than plant residue incorporation
- Nt is negatively correlated with the AN:Olsen-P ratio and Dothideomycetes abundance

- Stoichiometric ratios of nutrients associated with enzymes activities regulate Nt
- SOC and Olsen-P affected Nt as key factors under N enrichment conditions

KEYWORDS

enzyme activities, microbial community, net nitrogen mineralisation, phosphorus availability

1 | INTRODUCTION

Soil nitrogen availability is a crucial factor that limits the primary productivity of ecosystem. Soil net nitrogen mineralisation (Nt), an indicator of the quantity of soil organic nitrogen mineralisation, is regarded as an effective index of nitrogen availability (Liu et al., 2017; Schimel & Bennett, 2004). Fertilisation is widely recommended to improve soil nitrogen mineralisation potential and availability, which in turn enables sustainable crop production (Marzi et al., 2019). The effect of different fertilisation on soil nitrogen mineralisation has been intensively studied in agricultural ecosystems (Ashraf et al., 2020; Li, Wang, et al., 2020; 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 fertilisers (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 mineralisation 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 & Marschner, 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, Zhao, et al., 2019; Li et al., 2019; Liu et al., 2017), a comprehensive understanding of the regulation of Nt under fertilisation, especially organic amendments with different C:N ratios in agro-ecosystems, is still lacking. Both soil abiotic and biotic properties regulate nitrogen mineralisation under different fertilisation (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, Zeng, et al., 2020). Microbial activity stimulates mineralisation processes directly by increasing enzyme activities (Xu et al., 2020). A more diverse community was found towards a greater capacity to mineralise N under organic management (Berthrong et al., 2013). Additionally, based on phospholipid fatty acid (PLFA) analysis together with high-throughput sequencing technologies, which are believed to be effective to provide information on microbial community quantifying (Chen, Xing, et al., 2019; Orwin et al., 2018), soil nitrogen transformation processes are found to be linked with some functional bacterial or fungal taxa (Chen, Zhao, et al., 2019; 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 short-term 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 fertilisers, 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): (i) 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: (ii) The content of nutrient could affect Nt directly. (iii) 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 is located in Tianjin, northern China (117°60E, 39°10N) and was initialled in 1979. The cropping system was winter wheat and summer maize rotation at this site since 1979. This region is characterised 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 SOC, total nitrogen (TN), phosphorus (TP), and potassium were 10.96, 1.00, 1.59, and 16.14 g kg^{-1}, respectively. Soil available N, phosphorus (Olsen-P), and K were 75.10, 15.80, and 176.6 mg kg^{-1}, respectively (Yang et al., 2015).

The 11 treatments analysed in this study were grouped as follows: (a) control, no fertiliser (CK), and pure fallow (F); (b) mineral fertilisers, mineral nitrogen (N) alone, mineral phosphorus with potassium (PK), and mineral N with P and K (NPK); (c) animal manure incorporation, animal 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 m^2 was randomly replicated and isolated using cement baffle plates. The specific amounts of fertilisers that were annually applied were as follows (Tables S1 and S2): no fertilisers or manure was applied in the CK and fallow treatments. Mineral N fertiliser for the N, NPK, NG, NS, and NM treatments was 285 kg N ha^{-1} for winter wheat and 210 kg N ha^{-1} for maize. The mineral fertilisers used were in urea (N 40%), calcium superphosphate (16% P_2O_5), and potassium chloride (K_2O 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

Soil samples were collected in October 2017 after maize harvest. Five cores were randomly collected from the plough 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 analyses. The second sub-sample was stored at 4°C for the microbial biomass, enzyme activity, and nitrogen mineralisation determination. The last sub-sample was air-dried to determine the basic soil properties.

2.3 | Soil abiotic properties

SOC was determined using the Walkley–Black dichromate oxidation method (Walkley & Black, 1934). The contents of TN and TP were determined using the Kjeldahl digestion–distillation and molybdenum-blue colorimetry methods, respectively (Pansu & Gautheyrou, 2007). And Olsen-P was measured via extraction with 0.5 M $NaHCO_3$ (Olsen et al., 1954). Available N (AN, the sum of NH_4^+ and NO_3^-) was measured using the $CaCl_2$ extraction method (Joergensen & Potthoff, 2005). Dissolved organic carbon (DOC) and nitrogen (DON) were quantified as total organic carbon and nitrogen in the extractions of non-fumigated soil (Wu 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.

2.4 | Determination of net soil nitrogen mineralisation

Net nitrogen mineralisation (Nt) was determined using the aerobic incubation method (Agbenin et al., 1999; Stanford & Smith, 1972). 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} $CaCl_2$ solution, followed by 25 ml of a minus-N nutrient solution consisting of 0.002 mol L^{-1} $CaSO_4{\cdot}2H_2O$, 0.002 mol L^{-1} $MgSO_4$, 0.005 mol L^{-1} Ca $(H_2PO_4)_2{\cdot}2H_2O$ and 0.0025 mol L^{-1} K_2SO_4. 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} $CaCl_2$ with a 60 cm Hg suction for 2 h. The leachate was then analysed 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 μM solution of 4-methylumbelliferone (MUB)-labelled 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 homogenising 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):

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

2.6 | Soil microbial community

Soil microbial biomass carbon (SMBC) and nitrogen (SMBN) were measured via the $CHCl_3$ fumigation–extraction method (Wu et al., 1990). PLFAs were extracted from the soil using Frostegård et al. (1991) procedure. Total PLFA was calculated by adding all PLFAs detected in the soil. The *i*14:0, *i*15:0, *a*15:0, *i*16:0, *i*17:0, and *a*17:0 PLFAs were analysed as a proxy of Gram-positive bacteria (G+) (Frostegård & Bååth, 1996), while 16:1ω7c, 16:1ω9c, cy17:0, 18:1ω5c, 18:1ω7c, cy19:0ω8c, and 17:1ω8c were determined as proxies of Gram-negative bacteria (G−) (García-Orenes et al., 2013; Olsson et al., 1995). The sum of 14:0, 15:0, 16:0, 17:0, G+, and G− was considered as the bacterial content (Frostegård & Bååth, 1996). Additionally, 10Me16:0, 10Me17:0, and 10Me18:0 were used as markers of actinomycetes (Zelles, 1997), 16:1ω5c, marked as the presence of arbuscular mycorrhizal fungi (AMF) (Olsson et al., 1995). 18:1ω9c, 18:2ω6,9c, and 18:3ω6c (6,9,12) were used to indicate fungi (Frostegård & 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.

$$H = -\sum \mathrm{Pi} \cdot \ln(\mathrm{Pi}) \quad (2)$$

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

2.7 | Bacterial and fungal community assessment: DNA extraction and pyrosequencing

2.7.1 | DNA extraction and PCR amplification

Microbial DNA was extracted from 0.5 g of each soil sample using an E.Z.N.A. soil DNA kit (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 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) using the primers 338F 5′-ACTCCTACGGGAGGCAGCAG-3′ and 806R 5′-GGACTACHVGGGTWTCTAAT-3′. The ITS ribosomal RNA gene of the fungi was amplified via 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 a final extension at 72°C for 10 min) using the primers ITS3F 5′-GCATCGATG AAGAACGCAGC-3′ and ITS4R 5′-TCCTCCGCTTATTG ATATGC-3′. The produced barcode was an eight-base sequence unique to each sample. PCR reactions were performed in triplicate in a 20 μl mixture containing 4 μl of 5 × FastPfu Buffer, 2 μl of 2.5 mM dNTPs, 0.8 μl of each primer (5 μM), 0.4 μl of FastPfu Polymerase, and 10 ng of template DNA.

2.7.2 | Illumina MiSeq sequencing

Amplicons were extracted from 2% agarose gels and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions, and quantified using QuantiFluor™ -ST (Promega Corporation, Madison, WI, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina MiSeq platform (Majorbio, Shanghai, China) according to standard protocols.

2.7.3 | Processing of sequencing data

Raw FASTQ files were demultiplexed and quality-filtered via QIIME (version 1.17) using the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window, while truncated reads that were shorter than 50 bp were discarded; (ii) exact barcode matching, wherein two nucleotide mismatches during primer matching as well as reads containing ambiguous characters were removed; and (iii) only sequences that overlapped for lengths over 10 bp were assembled according to their overlapped sequences. Reads that could not be assembled were discarded.

Operational taxonomic units (OTUs) were clustered using a 97% similarity cut-off via UPARSE (version 7.1; http://drive5.com/uparse), while chimeric sequences were identified and removed using UCHIME. The taxonomy of 16S rRNA and ITS rRNA gene sequences was analysed using the RDP Classifier (http://rdp.cme.msu.edu) against the silva (SSU115) 16S rRNA database and the Unite ITS rRNA database, respectively, with a confidence threshold of 70%.

All sequenced data were uploaded to the NCBI Sequence Read Archive (SAR) database (https://www.ncbi.nlm.nih.gov/sra). The bacterial accession number is PRJNA669222 and the fungal accession number is PRJNA669221.

2.8 | Statistical analysis

Differences between treatments were analysed using the one-way ANOVA (Duncan; $p < 0.05$). Spearman correlation was analysed using SPSS Statistics 22 software, and the random forest model (RFM) was constructed using R (3.6.3). Structural equation modelling (SEM) analysis was performed using R (3.6.3) and AMOS 21. Bar plots were mapped using Sigmaplot 12.5. The analysis of sequencing data was performed and relevant figures were calculated via R from the Majorbio I-Sanger Cloud Platform (https://www.i-sanger.com).

TABLE 1 Soil abiotic properties under different long-term management practices

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

Note: Different lower letters indicate significant difference among various treatments ($p < 0.05$).

Abbreviations: AN, available nitrogen; CK, no fertiliser; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; F, pure fallow; FM, fallow with animal manure; HNM, half application rate of NM; M, animal manure only; N, mineral nitrogen only; NG, mineral N with green manure; NM, mineral N with animal manure; NPK, mineral N with P, K; NS, mineral N with straw; PK, mineral phosphorus with potassium; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus.

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

Note: Different lower letters indicate significant difference among various treatments ($p < 0.05$).

Abbreviations: AN:Olsen-P, available nitrogen-to-available phosphorus ratio; C:N, soil organic carbon-to-total nitrogen ratio; C:P, soil organic carbon-to-total phosphorus ratio; CK, no fertiliser; DOC:AN, dissolved organic carbon-to-available nitrogen ratio; DOC:Olsen-P, dissolved organic carbon-to-available phosphorus ratio; F, pure fallow; FM, fallow with animal manure; HNM, half application rate of NM; M, animal manure only; N, mineral nitrogen only; N:P, soil total nitrogen-to-total phosphorus ratio; NG, mineral N with green manure; NM, mineral N with animal manure; NPK, mineral N with P, K; NS, mineral N with straw; PK, mineral phosphorus with potassium; SMBC:SMBN, soil microbial biomass carbon-to-biomass nitrogen ratio.

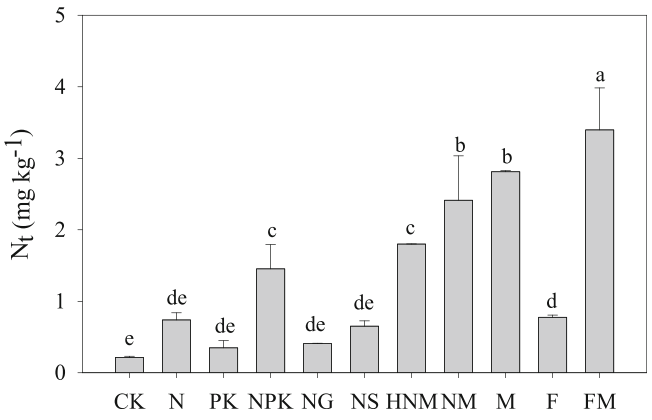

FIGURE 1 Soil net nitrogen mineralisation (Nt) under different long-term management practices. CK, no fertiliser; N, mineral nitrogen only; PK, mineral phosphorus with potassium; NPK, mineral N with P, K; NG, mineral N with green manure; NS, mineral N with straw; NM, mineral N with animal manure; HNM, 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 ($p < 0.05$).

3 | RESULTS

3.1 | Soil abiotic properties and net nitrogen mineralisation under management practices

Compared with the CK control, the NPK, fallow, and organic amendments (NS, NG, HNM, NM, M, FM) significantly increased the SOC and TN contents (Table 1), especially the FM treatment (SOC 25.2 $g \cdot kg^{-1}$; TN 2.43 $g \cdot kg^{-1}$). Treatments with animal manure (HNM, NM, M, FM) had better improvement for SOC and TN (increased by 81% and 86% on average, respectively) relative to the NS, NG, NPK, and fallow (increased by 35% and 27% on average). Treatments with mineral P fertiliser 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 $mg \cdot kg^{-1}$) 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 (Figure 1). The FM had the highest Nt (3.40 $mg \cdot kg^{-1}$) relative to the CK (0.22 $mg \cdot kg^{-1}$), followed by the M (2.81 $mg \cdot kg^{-1}$) and NM (2.41 $mg \cdot kg^{-1}$) 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

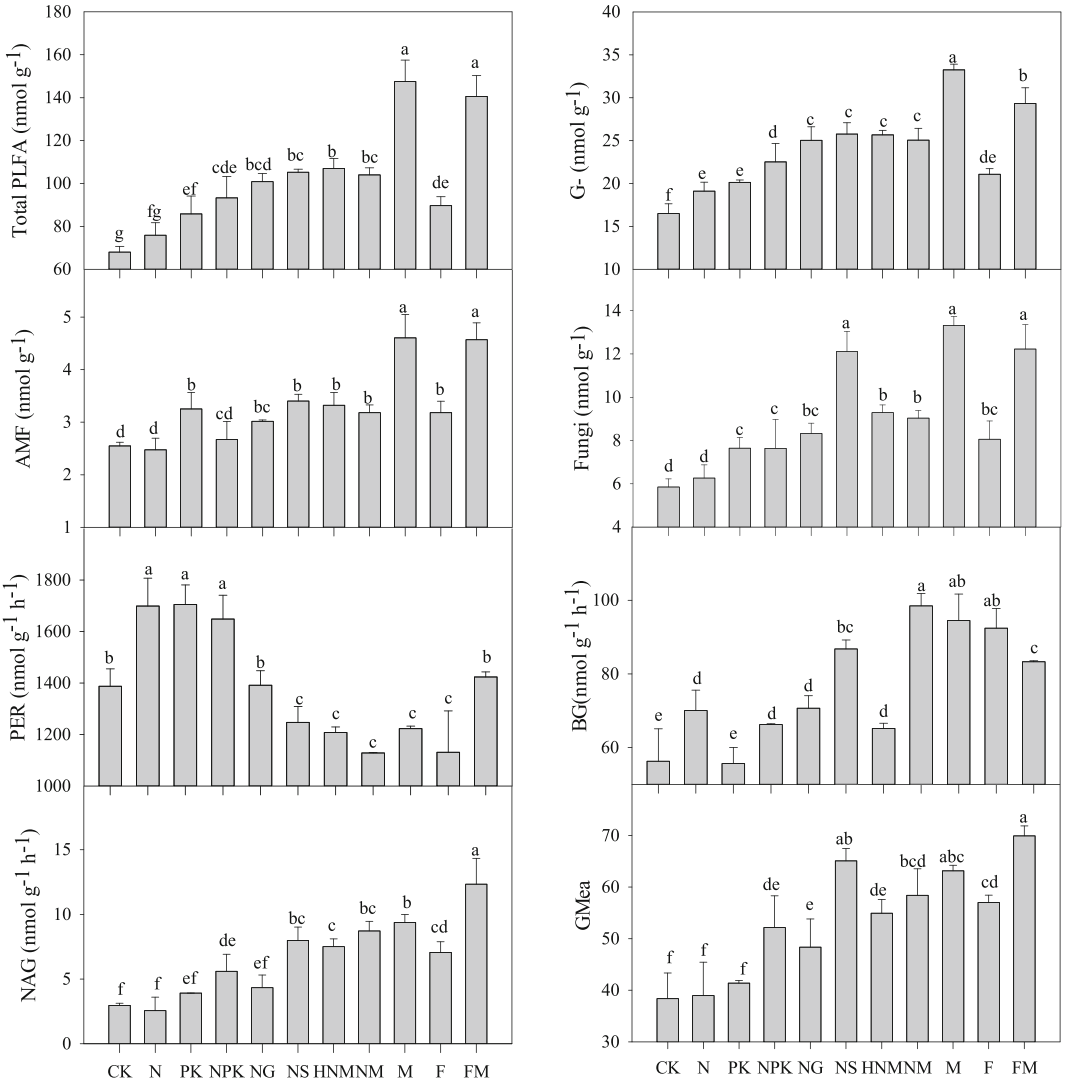

FIGURE 2 Soil microbial PLFA groups abundances and soil extracellular enzyme activities under different long-term management practices. AMF, arbuscular mycorrhizal fungi; G−, Gram-negative bacteria; PER, peroxidase; BG, β-1,4-glucosidase; NAG, β-1,4-*N*-acetyl-glucosaminidase; GMea, geometric mean of soil hydrolytic enzymes; CK, no fertiliser; 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 ($p < 0.05$).

amendments (FM, M, NM, HNM, NS, NG, 330.7–441.4 mg kg^{-1}) had a better effect on SMBC than treatments with mineral fertilisers (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 (Figure 2). Furthermore, the M and FM treatments showed a remarkably higher total PLFA (147.5 and 140.6 nmol g^{-1}), G- (33.3, 29.3 nmol g^{-1}), and AMF (4.61, 4.57 nmol g^{-1}) than other treatments. All treatments significantly increased the G- abundance compared with the CK. Moreover, G- abundance for the NG, NS treatments (25.1 and 25.8 nmol g^{-1}) were relatively higher than that of mineral fertilisers (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 nmol g^{-1}) and N (2.48 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 (Figure 2). The NAG activity under treatments with animal manure (e.g., the HNM, NM, M, FM) tended

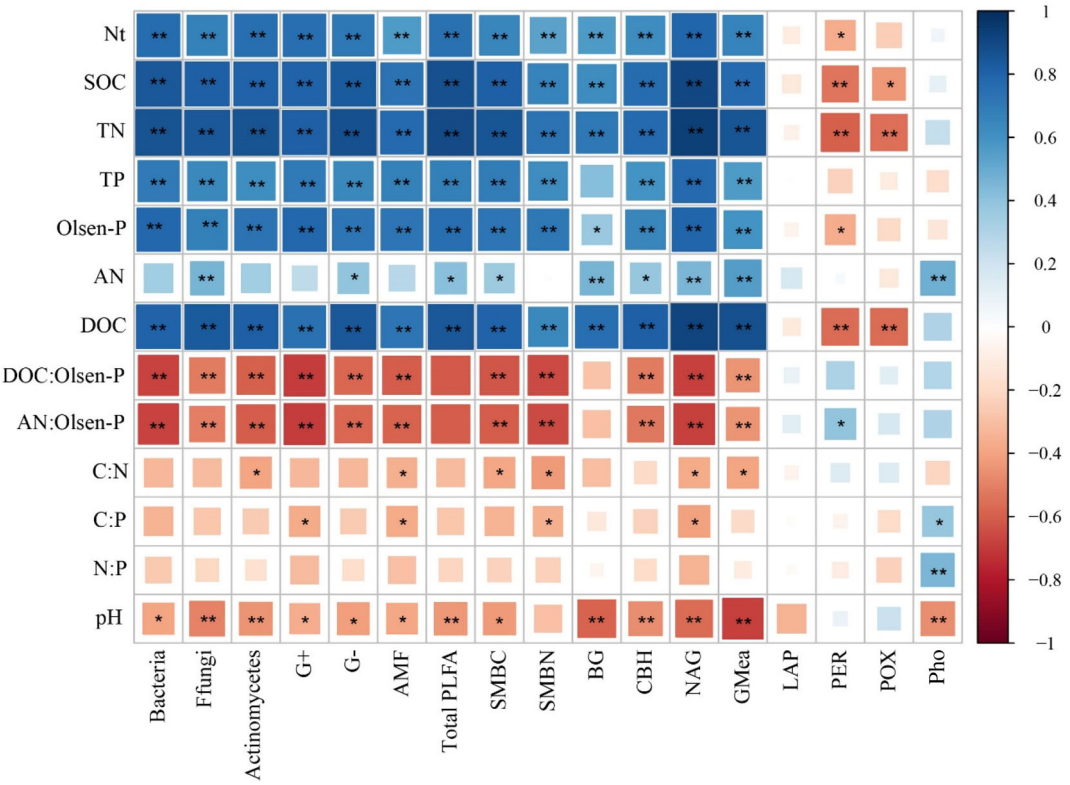

FIGURE 3 Spearman's correlation relationship between soil biotic properties (microbial biomass, PLFA groups, enzymes activities) and soil abiotic properties and net nitrogen mineralisation (Nt). SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; Olsen-P, available phosphorus; AN, available 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 colour gradient in heatmap indicate the Spearman's correlation coefficients, asterisks denote for different significance levels at $p < 0.05$ (*), $p < 0.01$ (**).

to be higher than that in mineral fertilisers (N, PK, NPK). The FM had the highest NAG activity (12.3 nmol g^{-1} h^{-1}). However, mineral fertilisers (N, PK, NPK) increased PER significantly, while organic amendments (NG, NS, HNM, NM, M) decreased that (Figure 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 (Figure 2). The NS significantly increased Pho activity (Figure S2).

High-throughput sequencing results showed that over 10 bacterial phyla were detected in all these treatments (Figure S3). *Proteobacteria*, *Actinobacteria, Acidobacteria, and Chloroflexi*, which were detected in all fertilisation treatments, displayed abundances ranging from 18% to 31%, 18% to 28%, 11% to 26%, and 8% to 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% to 71% and 4% to 31%, respectively (Figure S3). Moreover, NM, M, F, and FM treatments led to a relatively lower amount of *Dothideomycetes* than the other treatments.

3.3 | Net nitrogen mineralisation linked with soil biotic and abiotic properties

Results from Spearman's correlation analysis indicated that the Nt, SOC, TN, Olsen-P, and DOC were positively correlated with all PLFA groups as well as SMBC, SMBN, CBH, NAG, and GMea, but negatively correlated with PER activity. In contrast, the stoichiometry of Olsen-P (DOC:Olsen-P, AN:Olsen-P) showed a negative correlation with these above microbial variables (Figure 3). In

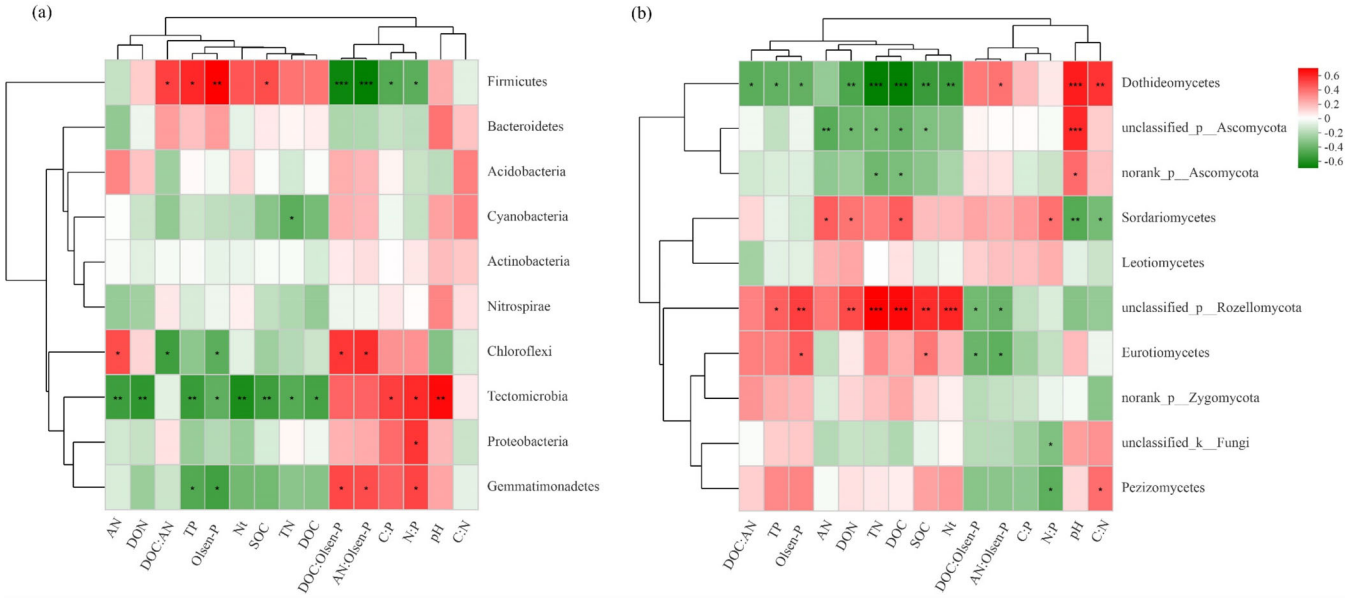

FIGURE 4 Spearman's correlation heatmap of bacteria on phylum level (a) and fungi on class level (b) with soil abiotic properties and net soil nitrogen mineralisation (Nt). SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; Olsen-P, available phosphorus; AN, available nitrogen (ammonium 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 carbonto available phosphorus ratio; AN:Olsen-P, available nitrogen to available phosphorus ratio. Different colour gradient in heatmap indicates the Spearman's correlation coefficients. Asterisks in the square denote different significance levels at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

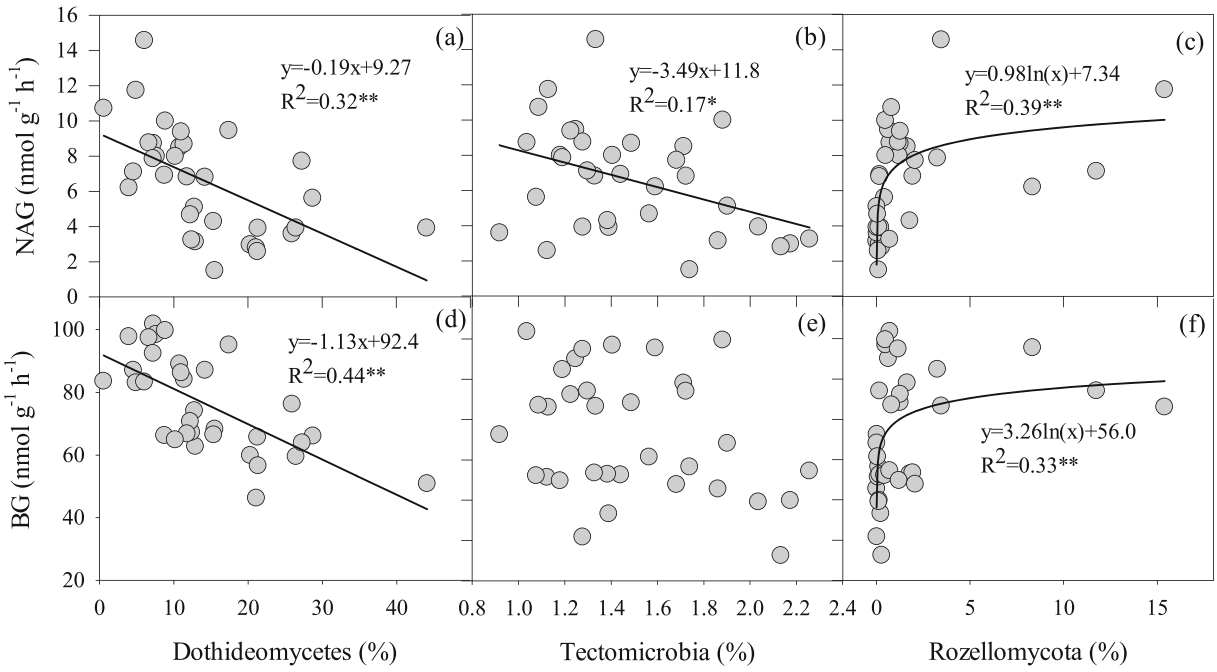

FIGURE 5 The relationships between BG, NAG activities and key taxa (significantly correlated with the net nitrogen mineralisation). Asterisks denote different significance levels at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$(***).

general, there were no significant relationships between fungi, total PLFA, BG, and the stoichiometric ratios of total nutrients (C:N, C:P, N:P).

A negative relationship was found between the Nt and *Tectomicrobia and Dothideomycetes*. Moreover, *Tectomicrobia* and *Dothideomycetes* were negatively

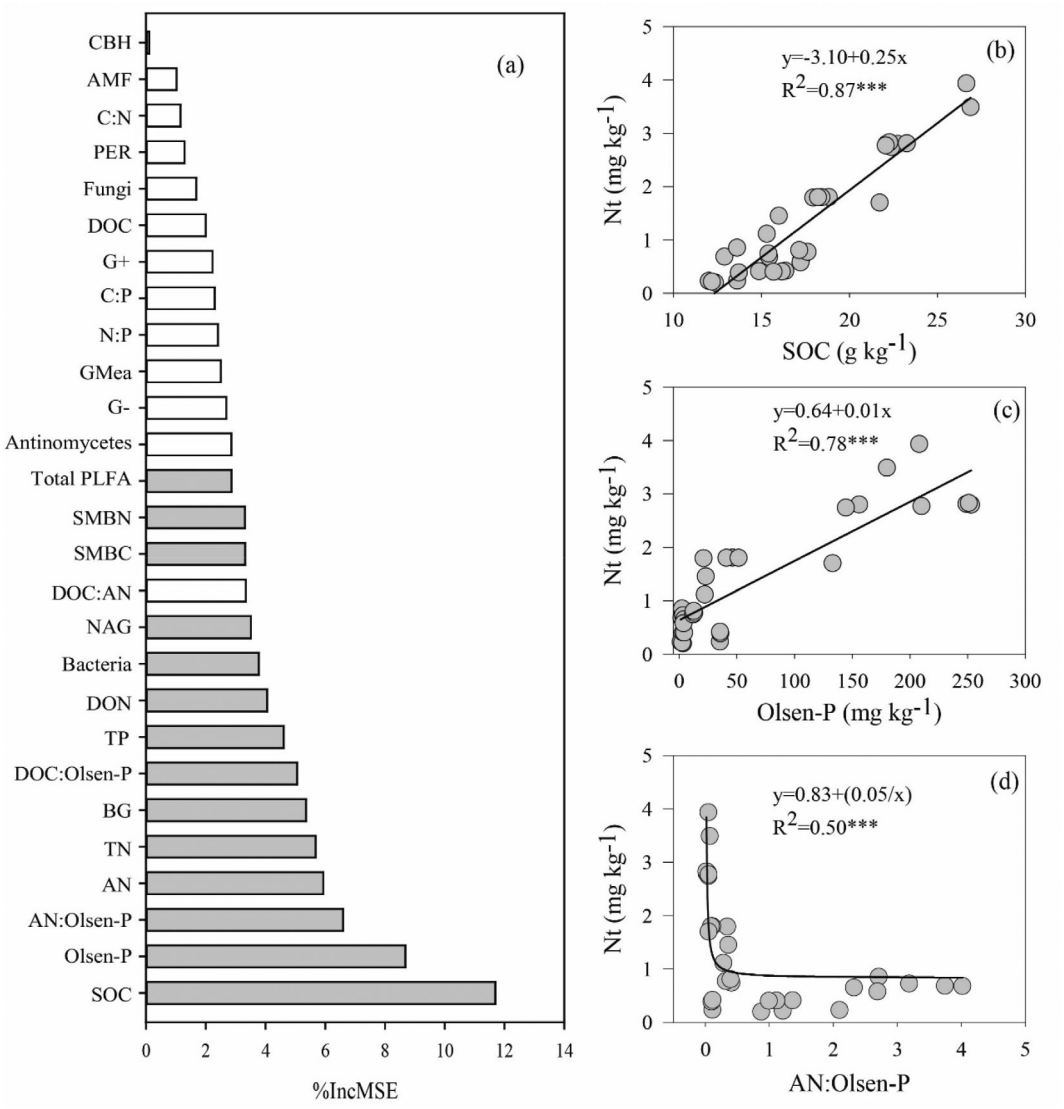

FIGURE 6 The importance of abiotic and biotic properties on the net nitrogen mineralisation (Nt) estimated by random Forest model (a), and the relationship between the Nt and top-rated three variables (b, c, d). Abiotic properties include soil nutrients (SOC, TN, TP, Olsen-P, AN, DOC, DON) and their 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 significance at $p < 0.05$, white bars were not significant. Asterisks denote different significance levels at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

correlated with TP, Olsen-P, SOC, TN, DOC, DON (Figure 4). *Unclassified p_Rozellomycota* was negatively correlated with DOC:Olsen-P and AN:Olsen-P, and positively correlated with Nt, SOC, Olsen-P (Figure 4b). *Tectomicrobia, Dothideomycetes* and *Unclassified p_Rozellomycota* were also significantly linked with NAG or BG enzyme activities (Figure 5). Specifically, *Dothideomycetes* negatively correlated with BG and NAG activities, while *Unclassified p_Rozellomycota* positively linked with these two enzymes.

The RFM showed that, of all the factors significantly corrected with Nt, SOC was the most important factor influencing the Nt, explaining 20.1% of the variations, followed by Olsen-P (14.9%) (Figure 6a). Additionally, the AN:Olsen-P and DOC:Olsen-P ratios also showed significant effects than soil C:N, C:P, and N:P ratios in regulating Nt. BG activity and bacteria abundance were top-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; Figure 6b,c). Initially, the Nt decreased dramatically, but stabilised with the increase in AN:Olsen-P ($R^2 = 0.50$, $p < 0.001$, Figure 6d). Similarly, the predominant contribution of soil abiotic properties for the Nt was observed from SEM results (Figure 7). The content of total and available nutrients ($\lambda = 0.85$, 0.38, respectively) rather than biotic properties, such as microbial community ($\lambda = -0.26$) and enzyme

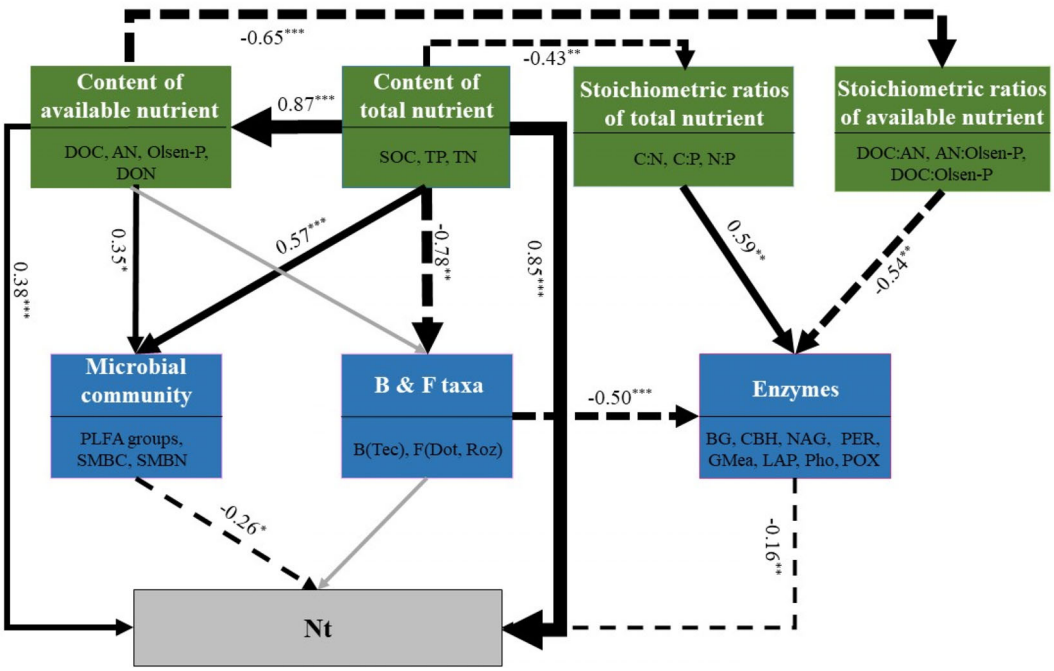

FIGURE 7 Direct and indirect effects of microbial community, enzyme activities, soil (total and available) nutrients content, and their stoichiometric ratios on soil net nitrogen mineralisation (Nt) by structural equation modelling. Content of total nutrient include soil organic carbon (SOC); total nitrogen (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). Contents 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 includes 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.

activities ($\lambda = -0.16$), had the strongest direct effect on Nt (Figure 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 the 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 ($\lambda = 0.59, -0.54$; Figure 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, characterised by the content and stoichiometric ratios of total and available nutrient, played a dominant role over soil biotic properties in determining the Nt. Specifically, the content of total and available nutrients directly control soil Nt (Figure 7), high Nt corrected with high SOC, Olsen-P content, but low AN:Olsen-P (Figure 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 mineralisation (Zhang et al., 2019). Our findings were consistent with these results, indicating that abiotic properties were dominants of N mineralisation. 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 fertilisers with or without plant residues (Meng et al., 2017; Ye et al., 2019). Our results indicated that balanced mineral fertiliser (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, which 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 a notable lower C:N and C:P ratio than plant residues (Table S2). With the input of animal manure combined with mineral N fertiliser, the N and P nutrient availability was 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 immobilisation 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

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, Zeng, et al., 2020). 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 (Figure 3). This study indicated that the application of organic amendments promoted soil nutrient status, further favouring microbial growth, especially microbes such as G- and AMF, preferably growing under conditions of nutrient sufficiency and enhancing nitrogen mineralisation from organic residues (Atul-Nayyar et al., 2009; Balasooriya et al., 2016). On the contrary, long-term application of mineral N fertiliser alone caused the imbalanced nutrient condition, thereby restricting the growth of G- and AMF (Eo & 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 (Figure 7). Specifically, *Dothideomycetes* was negatively correlated with Nt, BG, NAG, and Olsen-P (Figure 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 & Kema, 2009). The *Dothideomycetes* abundance was found to decrease under long-term fertilisation (Zhou et al., 2016). Our findings indicated that organic amendments input inhibited the growth of *Dothideomycetes*, which tends to grow in low-nutrient environments and further promotes N mineralisation.

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 (Figure 6a). In general, extracellular enzymes degrade soil organic matter into smaller units, which can also be directly utilised 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 (Figures 3 and 6). Accordingly, BG and NAG activities increase with elevated N availability (Ekenler & 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 utilised 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 negative 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 fertilisation would limit the growth of several bacterial groups by P unavailability (Zhou et al., 2017). Our study thus indicated that with long-term 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 abiotic properties (Figure 7). The importance of soil abiotic properties not only represented the direct control of Nt, but also indirectly regulated Nt through complex microbial responses.

5 | CONCLUSION

Our study provided further comprehensive evidence that soil abiotic properties (i.e., nutrient content and their stoichiometric ratios) determined the Nt. The direct microbial regulation of Nt depends on soil abiotic properties, and the complex interactions of abiotic and biotic properties were also important determinants of Nt. Also, with mineral N fertiliser application, Nt was controlled by SOC and P nutrient status. Therefore, relative to plant residues incorporation with higher C:N and C:P ratios, animal manure showing better performance on SOC and P nutrient balance is recommended for improving soil N availability in fluvo-aquic soil.

AUTHOR CONTRIBUTIONS

Qiqi Wang: Data curation (lead); formal analysis (equal); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Wei Gao:** Data curation (supporting); resources (lead); writing – review and editing (equal). **Roland Bol:** Visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). **Qiong Xiao:** Data curation (supporting); formal analysis (equal); visualization (supporting); writing – review and editing (equal). **Lei Wu:** Data curation (supporting); formal analysis (supporting); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). **Wenju Zhang:** Conceptualization (lead); data curation (supporting); funding acquisition (lead); project administration (lead); supervision (lead); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal).

ACKNOWLEDGEMENTS

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.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

ORCID

Wenju Zhang https://orcid.org/0000-0002-4602-1072

REFERENCES

Agbenin, J. O., Agbaji, E. B., Suleiman, I., & Agbaji, A. S. (1999). Assessment of nitrogen mineralization potential and availability from neem seed residue in a savanna soil. *Biology and Fertility of Soils*, *29*(4), 408–412.

Ashraf, M. N., Hu, C., Wu, L., Duan, Y.-H., Zhang, W.-J., Aziz, T., … Xu, M.-G. (2020). Soil and microbial biomass stoichiometry regulate soil organic carbon and nitrogen mineralization in rice-wheat rotation subjected to long-term fertilization. *Journal of Soils and Sediments*, *20*, 3103–3113.

Atul-Nayyar, A., Hamel, C., Hanson, K., & Germida, J. (2009). The arbuscular mycorrhizal symbiosis links N mineralization to plant demand. *Mycorrhiza*, *19*(4), 239–246.

Balasooriya, W. K., Huygens, D., Rajapaksha, R. M. C. P., & Boeckx, P. (2016). Effect of rice variety and fertilizer type on the active microbial community structure in tropical paddy fields in Sri Lanka. *Geoderma*, *265*, 87–95.

Berthrong, S. T., Buckley, D. H., & Drinkwater, L. E. (2013). Agricultural management and labile carbon additions affect soil microbial community structure and interact with carbon and nitrogen cycling. *Microbial Ecology*, *66*, 158–170.

Bol, R., Kandeler, E., Amelung, W., Glaser, B., Marx, M. C., Preedy, N., & Lorenz, K. (2003). Short-term effects of dairy slurry amendment on carbon sequestration and enzyme activities in a temperate grassland. *Soil Biology and Biochemistry*, *35*, 1411–1421.

Börjesson, G., Menichetti, L., Kirchmann, H., & Kätterer, T. (2011). Soil microbial community structure affected by 53 years of nitrogen fertilisation and different organic amendments. *Biology and Fertility of Soils*, *48*, 245–257.

Chen, D., Xing, W., Lan, Z.-C., Saleem, M., Wu, Y., Hu, S.-J., & Bai, Y.-F. (2019). Direct and indirect effects of nitrogen enrichment on soil organisms and carbon and nitrogen mineralization in a semi-arid grassland. *Functional Ecology*, *33*(1), 175–187.

Chen, H., Zhao, X.-R., Lin, Q.-M., Li, G.-T., & Kong, W.-D. (2019). Using a combination of PLFA and DNA-based sequencing analyses to detect shifts in the soil microbial community composition after a simulated spring precipitation in a semi-arid grassland in China. *Science of the Total Environment*, *657*, 1237–1245.

Cui, Y.-X., Zhang, Y.-L., Duan, C.-J., Wang, X., Zhang, X.-C., Ju, W.-L., … Fang, L.-C. (2020). Ecoenzymatic stoichiometry reveals microbial phosphorus limitation decreases the nitrogen cycling potential of soils in semi-arid agricultural ecosystems. *Soil and Tillage Research*, *197*, 104463.

DeForest, J. L. (2009). The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic

forest soils using MUB-linked substrates and l-DOPA. *Soil Biology and Biochemistry*, *41*, 1180–1186.

Ekenler, M., & Tabatabai, M. A. (2002). β-Glucosaminidase activity of soils: Effect of cropping systems and its relationship to nitrogen mineralization. *Biology and Fertility of Soils*, *36*, 367–376.

Eo, J., & Park, K. C. (2016). Long-term effects of imbalanced fertilization on the composition and diversity of soil bacterial community. *Agriculture, Ecosystems and Environment*, *231*, 176–182.

Francioli, D., Schulz, E., Lentendu, G., Wubet, T., Buscot, F., & Reitz, T. (2016). Mineral vs. organic amendments: Microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term fertilization strategies. *Frontiers in Microbiology*, *7*, 1446. https://doi.org/10.3389/fmicb.2016.01446

Frostegård, Å., Tunlid, A., & Bååth, E. (1991). Microbial biomass measured as total lipid phosphate in soils of different organic content. *Journal of Microbiological Methods*, *14*, 151–163.

Frostegård, E., & Bååth, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils*, *22*, 59–65.

Gai, X.-P., Liu, H.-B., Liu, J., Zhai, L.-M., Wang, H.-Y., Yang, B., … Lei, Q.-L. (2019). Contrasting impacts of long-term application of manure and crop straw on residual nitrate-N along the soil profile in the North China plain. *Science of the Total Environment*, *650*, 2251–2259.

Gan, H.-Y., Schöning, I., Schall, P., Ammer, C., & Schrumpf, M. (2020). Soil organic matter mineralization as driven by nutrient stoichiometry in soils under differently managed forest stands. *Frontiers in Forests and Global Change*, *3*, 99.

Gao, W., Yang, J., Ren, S.-R., & Liu, H.-L. (2015). The trend of soil organic carbon, total nitrogen, and wheat and maize productivity under different long-term fertilizations in the upland fluvo-aquic soil of North China. *Nutrient Cycling in Agroecosystems*, *103*, 61–73.

García-Orenes, F., Morugán-Coronado, A., Zornoza, R., & Scow, K. (2013). Changes in soil microbial community structure influenced by agricultural management practices in a Mediterranean agro-ecosystem. *PLoS One*, *8*, e80522.

García-Ruiz, R., Ochoa, V., Hinojosa, M. B., & Carreira, J. A. (2008). Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. *Soil Biology and Biochemistry*, *40*, 2137–2145.

Geisseler, D., Horwath, W. R., Joergensen, R. G., & Ludwig, B. (2010). Pathways of nitrogen utilization by soil microorganisms – A review. *Soil Biology and Biochemistry*, *42*, 2058–2067.

German, D. P., Weintraub, M. N., Grandy, A. S., Lauber, C. L., Rinkes, Z. L., & Allison, S. D. (2011). Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biology and Biochemistry*, *43*, 1387–1397.

Goodwin, S. B., & Kema, G. H. (2009). Gearing up for comparative genomics: Analyses of the fungal class Dothideomycetes. *The New Phytologist*, *183*(2), 250–253.

Guo, Z.-B., Wan, S.-X., Hua, K.-K., Yin, Y., Chu, H.-Y., Wang, D.-Z., & Guo, X.-S. (2020). Fertilization regime has a greater effect on soil microbial community structure than crop rotation and growth stage in an agroecosystem. *Applied Soil Ecology*, *149*, 103510. https://doi.org/10.1016/j.apsoil.2020.103510

Guo, Z.-C., Zhang, Z.-B., Zhou, H., Rahman, M. T., Wang, D.-Z., Guo, X.-S., … Peng, X.-H. (2018). Long-term animal manure application promoted biological binding agents but not soil aggregation in a vertisol. *Soil and Tillage Research*, *180*, 232–237.

Huang, Y.-P., Wang, Q.-Q., Zhang, W.-J., Zhu, P., Xiao, Q., Wang, C.-J., … Gunina, A. (2021). Stoichiometric imbalance of soil carbon and nutrients drives microbial community structure under long-term fertilization. *Applied Soil Ecology*, *168*, 104119.

Joergensen, R. G., & Potthoff, M. (2005). Microbial reaction in activity, biomass, and community structure after long-term continuous mixing of a grassland soil. *Soil Biology and Biochemistry*, *37*, 1249–1258.

Li, J., Cooper, J. M., Lin, Z.-A., Li, Y., Yang, X., & Zhao, B. (2015). Soil microbial community structure and function are significantly affected by long-term organic and mineral fertilization regimes in the North China plain. *Applied Soil Ecology*, *96*, 75–87.

Li, W.-X., Wang, C., Zheng, M.-M., Cai, Z.-J., Wang, B.-R., & Shen, R.-F. (2020). Fertilization strategies affect soil properties and abundance of N-cycling functional genes in an acidic agricultural soil. *Applied Soil Ecology*, *156*, 103704. https://doi.org/10.1016/j.apsoil.2020.103704

Li, Z.-L., Tian, D.-S., Wang, B.-X., Wang, J.-S., Wang, S., Chen, H.-Y.-H., … Niu, S.-L. (2019). Microbes drive global soil nitrogen mineralization and availability. *Global Change Biology*, *25*(3), 1078–1088.

Li, Z.-L., Zeng, Z.-Q., Tian, D.-S., Wang, J.-S., Fu, Z., Wang, B.-X., … Niu, S.-L. (2020). The stoichiometry of soil microbial biomass determines metabolic quotient of nitrogen mineralization. *Environmental Research Letters*, *15*(3), 034005. https://doi.org/10.1088/1748-9326/ab6a26

Liu, Y., Wang, C.-H., He, N.-P., Wen, X.-F., Gao, Y., Li, S.-G., … Yu, G.-R. (2017). A global synthesis of the rate and temperature sensitivity of soil nitrogen mineralization: Latitudinal patterns and mechanisms. *Global Change Biology*, *23*, 455–464.

Luo, G.-W., Xue, C., Jiang, Q.-H., Xiao, Y., Zhang, F.-G., Guo, S.-W., … Ling, N. (2020). Soil carbon, nitrogen, and phosphorus cycling microbial populations and their resistance to global change depend on soil C:N:P stoichiometry. *American Society for Microbiology*, *5*, e00162–e00120. https://doi.org/10.1128/mSystems.00162-20

Marzi, M., Shahbazi, K., Kharazi, N., & Rezaei, M. (2019). The influence of organic amendment source on carbon and nitrogen mineralization in different soils. *Journal of Soil Science and Plant Nutrition*, *20*, 177–191.

Meng, F., Dungait, J. A., Xu, X., Bol, R., Zhang, X., & Wu, W. (2017). Coupled incorporation of maize (*Zea mays* L.) straw with nitrogen fertilizer increased soil organic carbon in Fluvic Cambisol. *Geoderma*, *304*, 19–27.

Miller, K., Aegerter, B. J., Clark, N. E., Leinfelder-Miles, M., Miyao, E. M., Smith, R., … Geisseler, D. (2018). Relationship between soil properties and nitrogen mineralization in undisturbed soil cores from California agroecosystems. *Communications in Soil Science and Plant Analysis*, *50*, 77–92.

Olsen, S. R., Code, C. V., Watanabe, F. S., & Alan, L. A. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate* (p. 939). United States Department of Agriculture (USDA) Circular.

Olsson, P. A., Baath, E., Jakobsen, I., & Soderstrom, B. (1995). The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. *Mycological Research*, *99*, 623–629.

Orwin, K. H., Dickie, I. A., Holdaway, R., & Wood, J. R. (2018). A comparison of the ability of PLFA and 16S rRNA gene metabarcoding to resolve soil community change and predict ecosystem functions. *Soil Biology and Biochemistry*, *117*, 27–35.

Pansu, M., & Gautheyrou, J. (2007). Chapter ten - organic and total C, N (H, O, S) analysis. In *Handbook of soil analysis: Mineralogical, organic and inorganic methods* (pp. 335–349). Springer-Verlag.

Pascault, N., Ranjard, L., Kaisermann, A., Bachar, D., Christen, R., Terrat, S., … Maron, P. A. (2013). Stimulation of different functional groups of bacteria by various plant residues as a driver of soil priming effect. *Ecosystems*, *16*, 810–822.

Pereg, L., Morugán-Coronado, A., McMillan, M., & García-Orenes, F. (2018). Restoration of nitrogen cycling community in grapevine soil by a decade of organic fertilization. *Soil and Tillage Research*, *179*, 11–19. https://doi.org/10.1016/j.still.2018.01.007

Risch, A. C., Zimmermann, S., Ochoa-Hueso, R., Schutz, M., Frey, B., Firn, J. L., … Moser, B. (2019). Soil net nitrogen mineralisation across global grasslands. *Nature Communications*, *10*, 4981. https://doi.org/10.1038/s41467-019-12948-2

Salazar, O., Casanova, M., Nájera, F., Contreras, A., & Tapia, Y. (2020). Net nitrogen mineralisation in maize-cover crop rotations in Mediterranean Central Chile. *Journal of Soil Science and Plant Nutrition*, *20*, 1042–1050.

Schimel, J. P., & Bennett, J. (2004). Nitrogen mineralization: Challenges of a changing paradigm. *Ecology*, *85*(3), 591–602.

Schleuss, P. M., Widdig, M., Heintz-Buschart, A., Guhr, A., Martin, S., Kirkman, K., & Spohn, M. (2019). Stoichiometric controls of soil carbon and nitrogen cycling after long-term nitrogen and phosphorus addition in a Mesic grassland in South Africa. *Soil Biology and Biochemistry*, *135*, 294–303.

Soinne, H., Keskinen, R., Räty, M., Kanerva, S., Turtola, E., Kaseva, J., … Salo, T. (2020). Soil organic carbon and clay content as deciding factors for net nitrogen mineralization and cereal yields in boreal mineral soils. *European Journal of Soil Science*, *72*(4), 1497–1512. https://doi.org/10.1002/ejss.13003

Stanford, G., & Smith, S. J. (1972). Nitrogen mineralization potentials of soils. *Soil Science Society of America Journal*, *36*, 465–472.

Sun, R.-B., Guo, X.-S., Wang, D.-Z., & Chu, H.-Y. (2015). Effects of long-term application of chemical and organic fertilizers on the abundance of microbial communities involved in the nitrogen cycle. *Applied Soil Ecology*, *95*, 171–178.

Tang, Y.-Q., Yu, G.-R., Zhang, X.-Y., Wang, Q.-F., Tian, D.-S., Tian, J., … Ge, J.-P. (2019). Environmental variables better explain changes in potential nitrification and denitrification activities than microbial properties in fertilized forest soils. *Science of the Total Environment*, *647*, 653–662.

Truong, T. H. H., & Marschner, P. (2018). Respiration, available N and microbial biomass N in soil amended with mixes of organic materials differing in C/N ratio and decomposition stage. *Geoderma*, *319*, 167–174. https://doi.org/10.1016/j.geoderma.2018.01.012

Walkley, A., & Black, I. A. (1934). An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, *37*, 29–38.

Wang, Q.-Q., Liu, L.-L., Li, Y., Qin, S., Wang, C.-J., Cai, A.-D., … Zhang, W.-J. (2020). Long-term fertilization leads to specific PLFA finger-prints in Chinese Hapludults soil. *Journal of Integrative Agriculture*, *19*(5), 1354–1362.

Wei, X.-M., Hu, Y.-J., Peng, P.-Q., Zhu, Z.-K., Atere, C. T., O'Donnell, A. G., … Ge, T.-D. (2017). Effect of P stoichiometry on the abundance of nitrogen-cycle genes in phosphorus-limited paddy soil. *Biology and Fertility of Soils*, *53*, 767–776.

Wei, X. M., Zhu, Z. K., Liu, Y., Luo, Y., Deng, Y. W., Xu, X. L., … Ge, T. D. (2020). C:N:P stoichiometry regulates soil organic carbon mineralization and concomitant shifts in microbial shifts in microbial community composition in paddy soil. *Biology and Fertify of Soils*, *56*, 1093–1107. https://doi.org/10.1007/s00374-020-01468-7

Wu, J., Joergensen, R. G., Pommerening, B., Chaussod, R., & Brookes, P. C. (1990). Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. *Soil Biology and Biochemistry*, *22*(8), 1167–1169.

Wu, L., Zhang, W.-J., Wei, W.-J., He, Z.-L., Kuzyakov, Y., Bol, R., & Hu, R.-G. (2019). Soil organic matter priming and carbon balance after straw addition is regulated by long-term fertilization. *Soil Biology and Biochemistry*, *135*, 383–391.

Xiao, D., He, X.-Y., Wang, G.-H., Xu, X.-C., Hu, Y.-Y., Chen, X.-B., Zhang, W., Su, Y.-R., Wang, K.-L., Soromotin, A. V., Alharbi, H. A., & Kuzyakov, Y. (2022). Network analysis reveals bacterial and fungal keystone taxa involved in straw and soil organic matter mineralization. *Applied Soil Ecology*, *173*, 104395.

Xu, P.-D., Liu, Y.-R., Zhu, J., Shi, L., Fu, Q.-L., Chen, J.-Z., … Huang, Q.-Y. (2020). Influence mechanisms of long-term fertilizations on the mineralization of organic matter in Ultisol. *Soil and Tillage Research*, *201*, 104594.

Yang, J., Gao, W., & Ren, S.-R. (2015). Long-term effects of combined application of chemical nitrogen with organic materials on crop yields, soil organic carbon and total nitrogen in fluvo-aquic soil. *Soil and Tillage Research*, *151*, 67–74.

Ye, G.-P., Lin, Y.-X., Liu, D.-Y., Chen, Z.-M., Luo, J.-F., Bolan, N., … Ding, W.-X. (2019). Long-term application of manure over plant residues mitigates acidification, builds soil organic carbon and shifts prokaryotic diversity in acidic Ultisols. *Applied Soil Ecology*, *133*, 24–33.

Zelles, L. (1997). Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere*, *35*, 275–294.

Zhang, K.-R., Li, X.-S., Cheng, X.-L., Zhang, Z.-H., & Zhang, Q.-F. (2019). Changes in soil properties rather than functional gene abundance control carbon and nitrogen mineralization rates during long-term natural revegetation. *Plant and Soil*, *443*, 293–306.

Zhang, X., Bol, R., Rahn, C., Xiao, G.-M., Meng, F.-Q., & Wu, W.-L. (2017). Agricultural sustainable intensification improved nitrogen use efficiency and maintained high crop yield during 1980-2014 in northern China. *Science of the Total Environment*, *596–597*, 61–68.

Zhong, W.-H., Gu, T., Wang, W., Zhang, B., Lin, X.-G., Huang, Q.-R., & Shen, W.-S. (2010). The effects of mineral fertilizer and organic manure on soil microbial community and diversity. *Plant and Soil*, *326*, 511–522.

Zhou, J., Jiang, X., Wei, D., Zhao, B.-S., Ma, M.-C., Chen, S.-F., … Li, J. (2017). Consistent effects of nitrogen fertilization on soil bacterial communities in black soils for two crop seasons in China. *Scientific Reports*, *7*, 3267.

Zhou, J., Jiang, X., Zhou, B.-K., Zhao, B.-S., Ma, M.-C., Guan, D.-W., … Qin, J. (2016). Thirty four years of nitrogen fertilization decreases fungal diversity and alters fungal community composition in black soil in Northeast China. *Soil Biology and Biochemistry*, *95*, 135–143.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Wang, Q., Gao, W., Bol, R., Xiao, Q., Wu, L., & Zhang, W. (2022). Microbial regulation of net N mineralisation is driven by C, N, P content and stoichiometry. *European Journal of Soil Science*, *73*(3), e13257. https://doi.org/10.1111/ejss.13257