Soil & Tillage Research 197 (2020) 104463

Contents lists available at ScienceDirect

Soil & Tillage Research

journal homepage: www.elsevier.com/locate/still

Ecoenzymatic stoichiometry reveals microbial phosphorus limitation decreases the nitrogen cycling potential of soils in semi-arid agricultural ecosystems

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ARTICLE INFO

Keywords:
Extracellular enzyme activity
Microbial metabolic limitation
Functional genes

ABSTRACT

Variations in soil microbial metabolism currently represent one of the greatest areas of uncertainty with regard to soil nutrient cycles and the control of terrestrial carbon (C) and nitrogen (N) loss and are poorly understood in agricultural ecosystems with intensive farming practices. In this study, extracellular enzymatic stoichiometry models and quantitative PCR techniques were used to examine microbial metabolic limitation and its relationship with N-cycling gene expression in semi-arid agricultural ecosystems considering four N fertilization levels (N 0, N 100, N 250, and N 400 kg N ha^{-1}) and two agronomic strategies (film mulching and no mulching). Film mulching increased microbial C limitation (reflecting microbial C metabolism size; 0.189 of the total effects), while very small effects on microbial phosphorus (P) limitation were observed (-0.007 of the total effects). N fertilization increased the microbial demand for P (microbial P limitation; 0.504 of the total effects). Increased microbial C metabolism was mainly attributed to increased soil moisture content after film mulching, which enhanced microbial decomposition of organic C (high C-acquiring enzyme activities). Changes in nutrient stoichiometry and the increase in N availability due to N fertilization were largely responsible for increased microbial P limitation. Furthermore, microbial P limitation negatively affected the abundance of AOA *amoA*, AOB *amoA* (involved in nitrification), *nirK*, *nirS*, *nosZ* (involved in denitrification) genes, strongly inhibiting nitrification and denitrification potential (-0.743 and -0.761 of the total effects, respectively). The present results suggest that agricultural ecosystems with film mulching are conducive to organic residue decomposition, while appropriate P limitation under N fertilization could reduce the loss of N due to nitrification and denitrification in soil. This study highlights the importance of elemental stoichiometry-driven microbial metabolic variation in understanding soil nutrient cycles and optimizing agricultural practices.

1. Introduction

Nitrogen (N) cycling is the most important process in agricultural systems and N loss is a widespread problem (Weier, 1994; Mmm et al., 2018). The loss not only increases agricultural costs, but also causes serious ecological problems, such as drinking water pollution, climate warming, and air pollution (Stark and Richards, 2008; Li et al., 2017). As a result, the strategy to control N cycling and reduce N loss from agricultural ecosystems are urgently needed (Stark and Richards, 2008; Mmm et al., 2018). Understanding the impacts of different agricultural managements and practices on N cycling will help solve these problems.

Attempting to increase grain yields through agricultural practices can profoundly affect soil nutrient levels, thereby affecting soil fertility and productivity (Wei et al., 2017). The use of N fertilizer is a common management practice that alters the availability of soil N, and the widespread use of N fertilizer could change nutrient cycles on a global

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https://doi.org/10.1016/j.still.2019.104463
Received 27 June 2019; Received in revised form 20 September 2019; Accepted 9 October 2019
Available online 15 November 2019
0167-1987/

scale (Sistla and Schimel, 2012; Marklein and Houlton, 2012; Wrage-Mönnig et al., 2018) given that nitrification and denitrification systems are linked and play the key roles in N loss and the emission of nitrous oxide from soils (Ishii et al., 2011). However, previous studies have reported that N fertilization had dramatic but inconsistent influences on the soil N cycling and N loss. Studies have found that N fertilization enhances nitrification and denitrification processes (Mori et al., 2010) and increases N loss (Mori et al., 2010; He and Dijkstra, 2015). N fertilization has also been shown to increase the abundances of AOB (Fan et al., 2011) and denitrifiers (Chen et al., 2012; Zhang et al., 2019), whereas other studies have reported that N fertilization had little impacts on the abundance of *nifH* and *nirS*-containing microbes (Sun et al., 2015) or AOB (Shen et al., 2008). The negative effects of N fertilization on the abundance of AOA (Fan et al., 2011; Sun et al., 2015) and AOB (He et al., 2007) have also been found. Furthermore, Zhang et al. (2019) reported that inorganic N levels primarily affected microbial N cycling, while Sun et al. (2015) found that available P and total N in soil were the most important factors influencing the abundances of microbial community involved in the N cycling. Similarly, others have reported that nitrification and denitrification were strongly affected by soil P availability in a wide range of ecosystems (Wang et al., 2014; Ford et al., 2016). These findings suggest that the effects of N fertilization on N cycles in soil may depend on the supply of multiple nutrients, in which microbial metabolic balances may be involved. Therefore, identifying microbial metabolic responses to the nutrient status of soils might be the key to revealing the effects of N fertilization on N cycles in soil.

In addition, film mulching techniques have proven to be effective in improving grain yields, and have been widely used in arid and semiarid agricultural ecosystems (Ramakrishna et al., 2006; Zhao et al., 2012). Film mulching mainly increases soil water retention, potentially affecting soil microbial metabolism. For instance, soil water availability suppresses litter decomposition and nutrient release through its effects on the activities of microorganisms (Cui et al., 2018). As a result, soil moisture is an important limiting factor related to microbial nutrient requirements in the arid and semi-arid regions. Also, the stoichiometric flexibility of a biological system reflects its ability to maintain function through the modification of its elemental balance (Sistla and Schimel, 2012). Hence, understanding how microbial metabolism responds to changes in the soil nutrients and moisture conditions could improve predictions of the ecological consequences stemming from agricultural practices and conducive to the development of better management strategies.

Variations in soil microbial metabolism currently represent one of the greatest uncertainties in understanding soil nutrient cycles and predicting terrestrial C sinks (Exbrayat et al., 2013; Noah and Bradford, 2019). C, N, and P metabolisms are the most important metabolic processes for soil microorganisms and are strongly affected by nutrient dynamics and physicochemical properties in soil. Nutrient stoichiometry is the key driver controlling microbial metabolism (Sinsabaugh et al., 2009; Wei et al., 2017; Cui et al., 2018). Changes in C, N and P stoichiometry are critical for the regulation of major soil ecosystem processes, such as SOM decomposition and elemental cycling (Zechmeister-Boltenstern et al., 2015; Spohn, 2016; Cui et al., 2019a). Soil moisture is an important physical property and a key factor affecting microbial metabolism, especially in the arid and semi-arid ecosystems (Smith, 2011; Ru et al., 2018). Generally, microbial metabolism is inhibited by low water conditions in soil, resulting in reduced microbiological activities and nutrient turnover rates (Borken et al., 2006). Low soil nutrient content also strongly limits the microbial metabolism. Previous studies have consistently indicated that soil microbial metabolism under different vegetation types was limited by soil P in the arid and semi-arid regions (Zhao et al., 2012; Cui et al., 2018, 2019a). Consequently, soil functions mediated by microorganisms could prove particularly sensitive to soil nutrient and moisture variations, which may be more pronounced in soils limited by the availability of nutrients and water (e.g., arid and semi-arid ecosystems). However, the responses of microbial metabolism to variations in soil nutrient and water availability are still poorly understood, especially in agricultural ecosystems with intensive farming practices.

Extracellular enzymes play the key roles in microbial metabolism and nutrient cycling (Jones et al., 2009; Cui et al., 2018, 2019b). Ecoenzymatic stoichiometry can reflect the relationships of microbial metabolic demand with soil nutrient supply (Sinsabaugh et al., 2009, 2012; Cui et al., 2018) and thus, is widely used to reveal the characteristics of microbial metabolic limitation represented by C, N or P (Jones et al., 2009; Sinsabaugh et al., 2009; Cui et al., 2018, 2019a). To illuminate the characteristics of microbial metabolism, Moorhead et al. (2016) proposed calculating the 'lengths' and 'angles' of vectors in a plot of proportional activities of enzyme C:N vs C:P acquisition to quantify the relative investments in C vs nutrient acquisition (vector lengths) or P vs N acquisition (vector angles). Converting these ratios into vector lengths and angles identified the simultaneous and relative nutrient demands of the microorganisms, independent of the variations in total enzymatic activities, and provided a clear metrics of relative C limitation and P vs N limitation (Moorhead et al., 2016; Cui et al., 2019a). Therefore, this method can help elucidate microbial metabolism responses to changes in soil nutrient and moisture availability in arid and semi-arid agricultural ecosystems.

In the present study, we investigated the abundances of soil N-cycling genes (including *nifH*, AOA *amoA*, AOB *amoA*, *narG*, *nirK*, *nirS* and *nosZ*) and extracellular enzymatic activities (involved in C-, N-, and P-acquisition) in different N-fertilization and film mulching treatments. Specifically, we quantified the characteristics of microbial metabolic limitation in these treatments with extracellular enzymatic stoichiometry models. In this manner, the relationships between microbial metabolic limitation and N-cycling gene expression were clarified further. We hypothesized that microbial metabolism could be limited to a greater extent by P in soil with added N because changes in nutrient stoichiometry lead to relative low P availability (Zhao et al., 2012; Cui et al., 2018, 2019b). On the contrary, P limitation may be mitigated by film mulching because improved soil moisture availability and microbial activities promote organic matter decomposition and P activation. Therefore, the main objectives of this study were to (1) determine the characteristics of microbial metabolic limitation under N fertilization and film mulching, (2) reveal the responses of N-cycling genes to nutrient and moisture variations, and (3) decipher the relationships of microbial metabolic limitation with the changes of soil nutrients and moisture as well as N-cycling genes expression.

2. Materials and methods

2.1. Site description

Field research during the 2016 and 2017 growing seasons was conducted at the Changwu Research Station of Agriculture and Ecology on the Loess Plateau in China (35.28 °N, 107.88 °E; 1200 m elevation). The station is located in a region with a typical semiarid climate, a mean annual temperature of 10.1 °C, and 556 mm precipitation from 1993-2012. Approximately 73% of precipitation occurs during the maize growing season (May-September). Rain-fed cropping systems, in which maize (*Zea mays* L.) or wheat (*Triticum aestivum* L.) are grown as continuous monocrops, are predominant in the study site. The soil is classified as Cumuli-Ustic Isohumosols (sand, 4%; silt, 59%; clay, 37%) according to Chinese soil taxonomy. The chemical properties of the soil (0–20 cm) before planting in 2016 were as follows: pH (8.4), bulk density (1.3 g cm^{-3}), organic matter (13.92 g kg^{-1}), total N (0.97 g kg^{-1}), Olsen-P (10.95 mg kg^{-1}), mineral N (12.93 mg kg^{-1}), 22.4% field capacity by weight (g g^{-1}), and a 9% wilting point by weight (g g^{-1}) (Li et al., 2018).

2.2. Experimental design and treatments

The study was arranged as a randomized complete block design with three replicates. The plot size was 30 m^2 (5 m × 6 m). Before planting, all plots were laid out with ridge furrows; that is, large (60 cm) and small (40 cm) ridges with ridge heights of 10 and 15 cm, respectively, were alternated. Adjacent ridges were separated by furrows in which the maize seeds were planted. The two treatment factors were mulch practices and N fertilization. A total of 24 treatments were examined.

Two mulch practices were established each year: (i) ridge furrow mulched with plastic film (FM), which has been widely adopted in the semiarid areas of northwestern China (Eldoma et al., 2016); and (ii) ridge-furrow with no mulching (NM), which was the control. In FM, both ridges and furrows were mulched with sections of transparent plastic film with widths measuring 120–130 cm before planting. The midline of the large ridge was the joint between the two sections of film and the location of where the soil was placed on top of the film. The plastic film was used throughout the entire maize growing season. It was removed at harvest and the field was re-mulched before seeding in the second year. Four different N fertilization treatments were employed in 2016: 0, 100, 250, and 400 kg N ha^{-1} (hereafter referred to as N0, N100, N250, and N400, respectively). In both years, the planting density was 65,000 plants ha^{-1} in all treatments.

Nitrogen fertilizer was applied as urea (N 46%) at three points during field work: 40% was applied before planting as a basal N fertilizer, 30% was applied at the jointing stage, and 30% was applied at the silking stage as a topdressing using a hole-sowing machine. After ridging the treatment plots, base fertilizer was applied consisting of basal N fertilizer, 40 kg P ha^{-1} (calcium superphosphate, P_2O_5 12%), and 80 kg K ha^{-1} (potassium sulphate, K_2O 45%) and was manually spread over the soil surface and then ploughed into the subsurface before planting each plot. All other agronomic practices were standard and uniform for all treatments. Natural rainfall was the only water resource for maize growth as no additional irrigation was supplied during the maize-growing season. Monthly weather data were provided by the Changwu Meteorological Monitoring Station situated approximately 50 m from the experimental field.

Maize was cropped (2015 season) without fertilization in the study site preceding our experiment. The high-yielding maize hybrid 'Pioneer 335' (~1510 growing degree-days) was planted using a hand-powered hole-drilling machine on April 2016 and 2017. The plots were harvested at ripeness in September 2016 and 2017.

2.3. Soil sample collection

Soil samples were collected from the top 20 cm of the soil profile after removing the litter in September 2017. Ten cores were collected at intervals along an "S" shape pattern in each plot and mixed into one composite sample. After removing the roots, litter, debris, and stones, each composite sample was divided into three parts for future analysis. The first two parts were placed in an ice box and then transported to the laboratory. The first part was immediately stored at −80 °C for future DNA extraction. The second part was passed through a 2.0 mm sieve and stored at 4 °C for the analysis of extracellular enzymatic activities within 2 weeks. The third part was air-dried for analyzing physicochemical properties.

2.4. Soil physiochemical analysis

Soil-moisture content was determined by oven-drying 15 g of fresh soil at 105 °C for 48 h. Soil organic C content was determined using dichromate oxidation; approximately 0.10 g of air-dried soils was digested with 5 ml of 0.8 M $K_2Cr_2O_7$ and 5 ml of H_2SO_4 at 170–180 °C for 5 min, and the digestate was then titrated with 0.2 M $FeSO_4$. Total N content was measured by the Kjeldahl method (2300 FOSS™) (Bremner and Mulvaney, 1982). Briefly, approximately 0.70 g of air-dried soil was digested with 1.85 g mixed catalyst (K_2SO_4:$CuSO_4$:Se = 100:10:1) and 5 ml of H_2SO_4 at 385 °C for 45 min, and the digestate was then titrated with 0.02 M HCl. The contents of NO_3^--N and NH_4^+-N were measured using a Seal Auto Analyzer after extraction with 2 M KCl with a 1:5 ratio (AutAnalyel AAA™). Total P and available P (Olsen-P) were extracted with $H_2SO_4 - HClO_4$ and 0.5 M $NaHCO_3$ (Olsen and Sommers, 1982), respectively. The extracts of Olsen-P were filtered through a Millipore 0.45-μm filter. The contents of TP and Olsen-P were then determined by the molybdenum blue method using an ultraviolet spectrophotometer (Hitachi UV2300).

2.5. DNA extraction and quantitative PCR

Total soil DNA was extracted from about 0.5 g soil samples using a PowerSoil DNA Isolation Kit (MoBio Laboratories, CA, USA), following the manufacturer protocol. Extracted DNA was checked on a 1% (w/v) agarose gel, quantified using UV-VIS spectrophotometry (ND-1000, NanoDrop Technologies, Wilmington, DE, USA), and then stored at −20 °C until further analysis. The experimental procedure of quantitative PCR (qPCR) is consistent with Wei et al. (2017). The primer sequences were showed in Table S1.

2.6. Assays of extracellular enzymatic activities (EEA)

The activities of C-acquiring enzymes (β-1,4-glucosidase (BG) and β-D-cellobiosidase (CBH)), N-acquiring enzymes (β-1,4-*N*-acetylglucosaminidase (NAG) and L-leucine aminopeptidase (LAP)), and P-acquiring enzyme (alkaline phosphatase (AP)) were measured using the method of Saiya-Cork et al. (2002) and German et al. (2011). The experimental procedure has been described in our previous study in detail (Cui et al., 2019a). Finally, the enzyme activities were expressed as nanomoles of substrate released per hour per gram of dry soil (nmol g^{-1} h^{-1}).

2.7. Quantification of microbial metabolic limitation

The microbial metabolic limitation was quantified by calculating the vector length and angle of enzyme activities for all data based on untransformed proportional activities (e.g. [BG + CBH]/[BG + CBH + AP]) (Moorhead et al., 2013, 2016). Considering organic matter is the main substrate involved in hydrolysis of enzyme, in the enzymatic stoichiometry models, the values of enzyme activities were transformed as nanomoles of substrate released per hour per gram of soil organic matter (i.e., nmol g SOM^{-1} h^{-1}; SOM = 1.724 × SOC). Vector lengths were calculated via Eq. 1, and vector angles were calculated via Eq. 2. Microbial C limitation increases with the vector lengths. Vector angles more than 45° represent microbial P limitation, while vector angles less than 45° represent microbial N limitation. Microbial P limitation increases with the vector angles, and microbial N limitation decreases with the vector angles. For a more detailed analysis and interpretation of the models, see Cui et al. (2019a).

$$Length = \sqrt{(x^2 + y^2)} \quad (1)$$

$$Angle \text{ (degree)} = \text{DEGREES (ATAN2 } (x, y)) \quad (2)$$

2.8. Statistical analysis

The two-way ANOVA was used to identify the effects of N fertilizer, treatment (film mulching and no mulching), and fertilizer-treatment interactions on soil physicochemical properties, copy numbers of functional genes, enzyme activities, and microbial metabolic limitation, after which, mean comparisons were determined with Tukey's multiple comparison post-hoc test ($P < 0.05$). Correlations among soil physicochemical variables, copy numbers of functional genes and microbial

Table 1
Results of two-way ANOVAs showing the effects of N fertilizer, treatments (film mulching and no mulching), and fertilizer-treatment interactions on soil moisture, available nutrients and nutrient stoichiometry.

Fertilizer	Treat	Soil moisture (%)	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	Olsen-P (mg kg^{-1})	SOC:TN ratio	SOC:TP ratio	TN:TP ratio
N0	FM	0.164 ± 0.011 Aa	3.95 ± 0.62 Ab	0.853 ± 0.048 Ac	12.6 ± 0.42 Aa	9.25 ± 0.29 Aab	9.08 ± 0.12 Ab	0.98 ± 0.031 Ab
	NM	0.167 ± 0.012 Aa	4.98 ± 0.51 Ab	0.763 ± 0.052 Ac	4.46 ± 0.34 Bab	8.91 ± 0.40 Aa	9.30 ± 0.19 Ab	1.04 ± 0.027 Ac
N100	FM	0.134 ± 0.009 Ab	4.32 ± 0.43 Aab	1.121 ± 0.125 Ab	9.98 ± 0.60 Ab	9.34 ± 0.04 Aa	9.77 ± 0.06 Ba	1.05 ± 0.004 Bb
	NM	0.127 ± 0.006 Ab	4.39 ± 0.20 Ab	0.773 ± 0.050 Bc	4.32 ± 0.34 Bb	8.59 ± 0.28 Ba	10.5 ± 0.43 Aa	1.22 ± 0.011 Aa
N250	FM	0.164 ± 0.010 Aa	5.59 ± 0.59 Ba	1.391 ± 0.082 Ba	9.02 ± 0.35 Ab	8.55 ± 0.33 Ab	7.65 ± 0.17 Bc	0.90 ± 0.039 Bc
	NM	0.137 ± 0.008 Bb	7.06 ± 0.47 Aa	1.734 ± 0.138 Aa	5.51 ± 0.48 Ba	8.47 ± 0.15 Aa	9.14 ± 0.59 Ab	1.08 ± 0.067 Abc
N400	FM	0.158 ± 0.006 Aa	5.15 ± 0.23 Aab	0.905 ± 0.072 Bbc	5.79 ± 0.15 Ac	7.72 ± 0.31 Bc	9.84 ± 0.38 Aa	1.27 ± 0.008 Aa
	NM	0.144 ± 0.005 Bb	5.57 ± 0.89 Aab	1.104 ± 0.042 Ab	4.49 ± 0.50 Bab	8.57 ± 0.30 Aa	10.0 ± 0.24 Aab	1.17 ± 0.014 Bab
Factor (Df)		*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Fertilizer (3)		*16.3*	***	*17.7*	***	*95.0*	***	*67.1*	***	*13.9*	***	*36.4*	***	*69.9*	***
Treat (1)		*9.15*	**	*11.8*	**	*0.595*	*0.452*	*744*	***	*0.459*	*0.508*	*24.4*	***	*35.9*	***
*Fertilizer * Treat (3)*		*3.22*	*0.051*	*2.06*	*0.146*	*20.4*	***	*73.4*	***	*8.60*	**	*5.55*	**	*27.0*	***

Note: Values are the means (± standard errors) of three replicate soil cores. N0, N100, N250 and N400 represent N fertilizer 0, 100, 250, and 400 kg N ha^{-1}, respectively. FM and NM represent film mulching and no mulching. Different uppercase letters within a column indicate significant differences ($P < 0.05$) between the film mulching and no mulching at each fertilizer level, the same lowercase letters within a column indicate no significant differences ($P > 0.05$) amongst the fertilizer level in the film mulching treat or no mulching treat. ***, $P < 0.001$; **, $P < 0.01$.

metabolic limitation were calculated using the non-parametric, bivariate, two-tailed Spearman rank-order correlation test. Generalized linear models were adopted to determine the relationships of the microbial metabolic limitation with functional genes. Partial least squares path modelling (PLS-PM) was used to further identify the possible pathways controlling microbial metabolic limitation and functional gene expression. The model was constructed using the "innerplot" function from the "plspm" package. All statistical analyses were performed using the R software package v.3.5.2.

3. Results

3.1. Effects of N fertilization and film mulching on soil physicochemical properties

Nitrogen fertilization and film mulching had significant main and interactive effects on soil moisture, available nutrients, and nutrient stoichiometry ($P < 0.05$; Table 1). Soil moisture and Olsen-P content were significantly higher in the FM than the NM treatment ($P < 0.05$). In the FM treatment, Olsen-P content was significantly reduced with increased N levels ($P < 0.05$). The NO_3^--N and NH_4^+-N contents were higher in N250 and N400 than N0 and N100 treatments. The SOC:TN was higher in N0 and N100 than N250 and N400 treatments. The SOC:TP was lower in the FM than the NM treatment. The correlation analysis showed that the soil moisture was negatively correlated with SOC, TN, and nutrient stoichiometry (SOC:TP and TN:TP; $P < 0.05$; Fig. 6b).

3.2. Effects of N addition and film mulching on N-cycling gene abundance

No significant changes were observed in the copy numbers of 16S genes among different treatments ($P > 0.05$; Fig. 1 a). Whereas the application of N fertilizer and film mulching had significant main and interactive effects on N cycling gene abundance (including *nifH*, AOA *amoA*, AOB *amoA*, *narG*, *nirK*, *nirS*, *nosZ*; $P < 0.05$; Fig. 1(b–h)). Film mulching significantly affected the abundance of seven N-cycling genes under the high N treatment (N400; $P < 0.05$). Correlation analysis showed that there were significant positive correlations among N-cycling genes ($P < 0.05$; Fig. 6 a).

The correlation heat map showed that *16S* and *nifH* gene abundance was not significantly correlated with soil moisture, available nutrients, or nutrient stoichiometry (Fig. 2). N-cycling gene abundance (including AOA *amoA*, AOB *amoA*, *narG*, *nirK*, *nirS* and *nosZ*) were significantly and positively correlated with available nutrients, such as the contents of NO_3^--N and NH_4^+-N, and negatively correlated with SOC:TP and TN:TP ratios ($P < 0.05$). In addition, *nirK* gene abundance was positively correlated with soil moisture ($P < 0.001$).

3.3. Influence of N fertilization and film mulching on extracellular enzyme activities (EEA)

The application of N fertilizer and film mulching had significant main and interactive effects on C-acquisition enzyme activities (including BG and CBH; $P < 0.05$; Fig. 3 a). C-acquisition enzyme activities were higher in the FM than the NM treatment. N fertilization significantly affected N-acquisition enzyme activities (including NAG and LAP) in the FM treatment but not in the NM treatment ($P < 0.05$; Fig. 3 b). N fertilization also significantly affected P-acquisition enzyme activities (AP; $P < 0.05$; Fig. 3 c). P-acquisition enzyme activities increased significantly after N fertilization ($P < 0.05$).

The linear regression analysis showed that N-cycling gene abundance (including AOA *amoA*, AOB *amoA*, *nirK*, *nirS* and *nosZ*) was positively correlated with C-acquisition enzyme activities (BG and CBH; $P < 0.05$; Fig. S1). The abundance of AOB *amoA*, *nirS*, and *nosZ* genes was positively correlated with N-acquisition enzyme activities (NAG and LAP; $P < 0.05$; Fig. S2), whereas the abundance of *nirK* and *nirS*

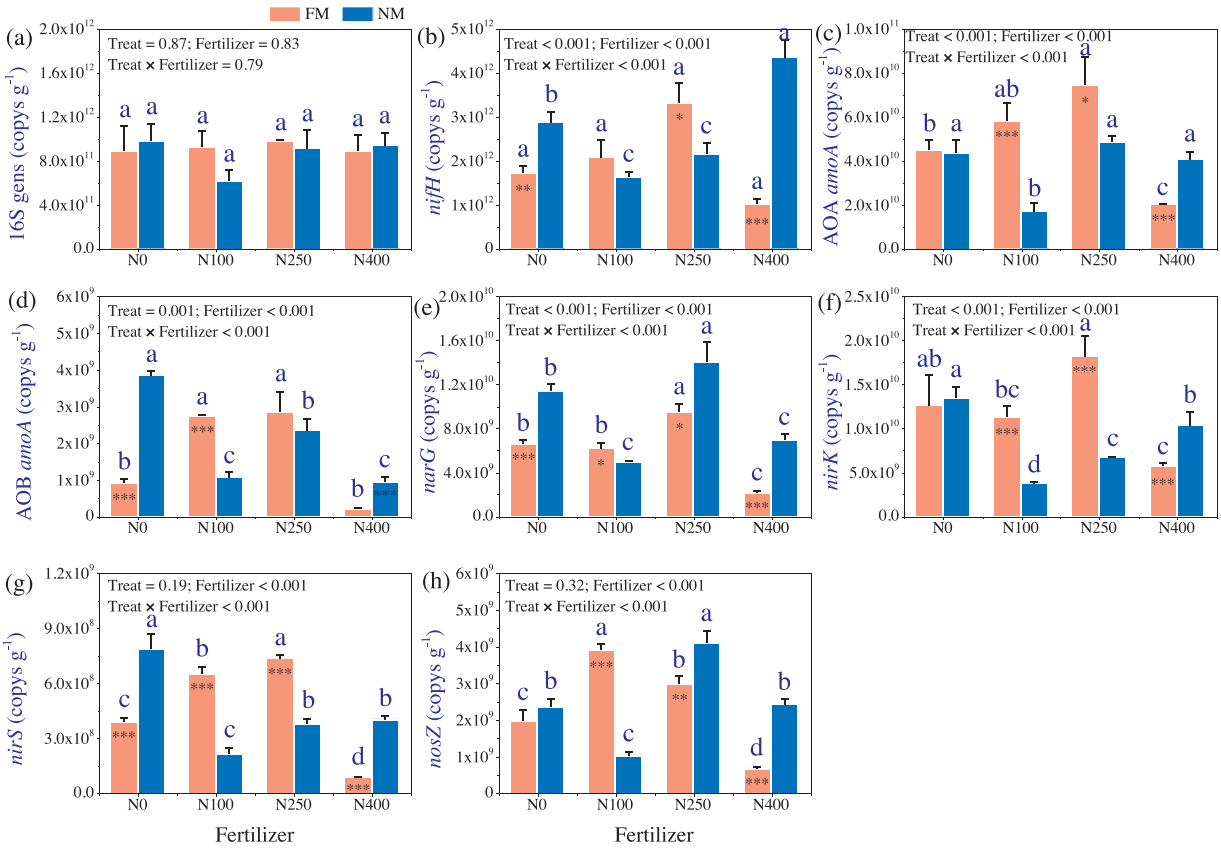

Fig. 1. Changes in the copy numbers of functional genes between treatments (film mulching and no mulching) with different N fertilizer levels.

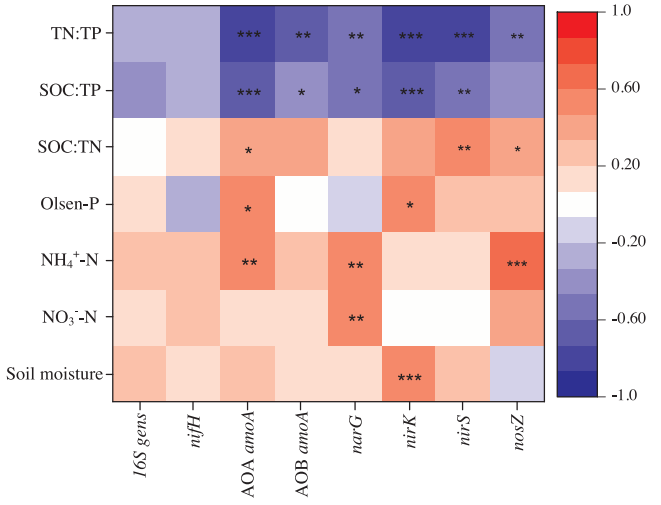

Fig. 2. Correlation heat map between soil physicochemical properties and the copy numbers of functional genes.

genes was negatively correlated with P-acquisition enzyme activities (AP; $P < 0.05$; Fig. S3).

3.4. Influence of N fertilization and film mulching on EEA vector characteristics

The characteristics of ecoenzymatic stoichiometry differed among N fertilization and film mulching treatments (Fig. 4 a). All data points were above the 1:1 line, indicating strong P limitation in the microbial community in our study area. Further, the relative C and P limitation of microbes was quantified by calculating the vector lengths and angles (Fig. 4 b and c). Vector lengths and angles (ranging from 0.639 to 0.864 and 62.7 to 71.1°, respectively) changed significantly with N fertilization and film mulching ($P < 0.001$). Vector lengths were higher in the FM than the NM treatment (Fig. 4 b), whereas vector angles were higher in the NM than FM treatment, with the exception of the N400 treatment (Fig. 4 c). In addition, the linear-regression analysis identified significant negative correlations between vector lengths and angles ($P < 0.001$; Fig. 4 d).

3.5. The relationships between microbial metabolic limitation with functional gene abundance and soil properties

Vector lengths (microbial C limitation) were positively correlated with soil moisture and Olsen-P content, and negatively correlated with SOC:TP and TN:TP ratios ($P < 0.01$; Fig. 6 c). Whereas vector angles (microbial P limitation) showed the opposite pattern and were negatively correlated with soil moisture, Olsen-P content, and the SOC:TN ratio, and positively correlated with SOC:TP and TN:TP ratios ($P < 0.05$; Fig. 6 c). The linear regression analysis showed that vector angles were significantly and negatively correlated with the abundance of AOA *amoA*, AOB *amoA*, *nirK*, *nirS*, and *nosZ* genes ($P < 0.05$; Fig. 5).

The PLS-PM analysis identified direct and indirect effects of N fertilization and the FM treatments on soil physicochemical properties, microbial metabolic limitation, and nitrification and denitrification potentials (Fig. 7 a). Nitrogen fertilization positively affected soil available N content (0.502 of the direct effects) and the soil N:P ratio (0.441 of the direct effects). Film mulching positively affected the soil moisture content (0.331 of the direct effects). Soil moisture further mediated microbial C limitation (0.571 of the direct effects), and microbial C limitation positively affected Olsen-P content (0.610 of the direct effects) while negatively affecting the soil C:P ratio (-0.711 of the direct effects). Together, available N and Olsen-P content (0.250 and -0.672 of the direct effects, respectively) and C:P and N:P ratios (0.634

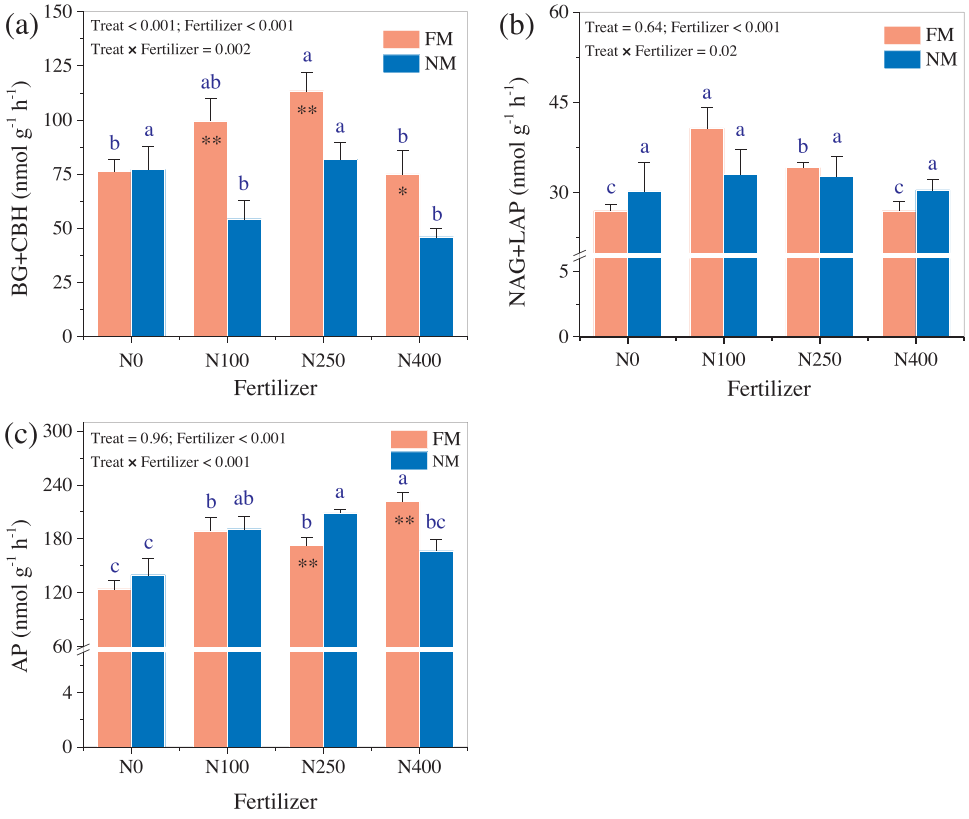

Fig. 3. Changes in the extracellular enzyme activity between treatments (film mulching and no mulching) with different N fertilizer levels.

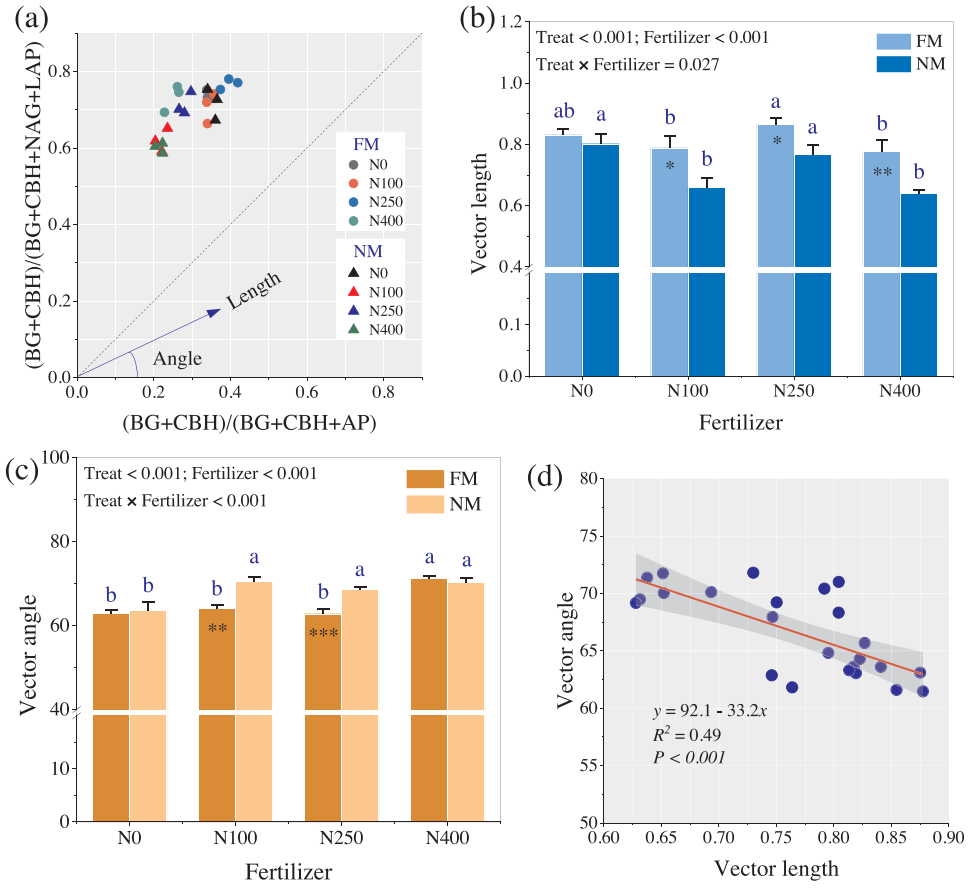

Fig. 4. Extracellular enzyme stoichiometry of the relative proportions of C to N acquisition versus C to P acquisition (A), the variation of vector length and angle (B and C), and their relationships (D).

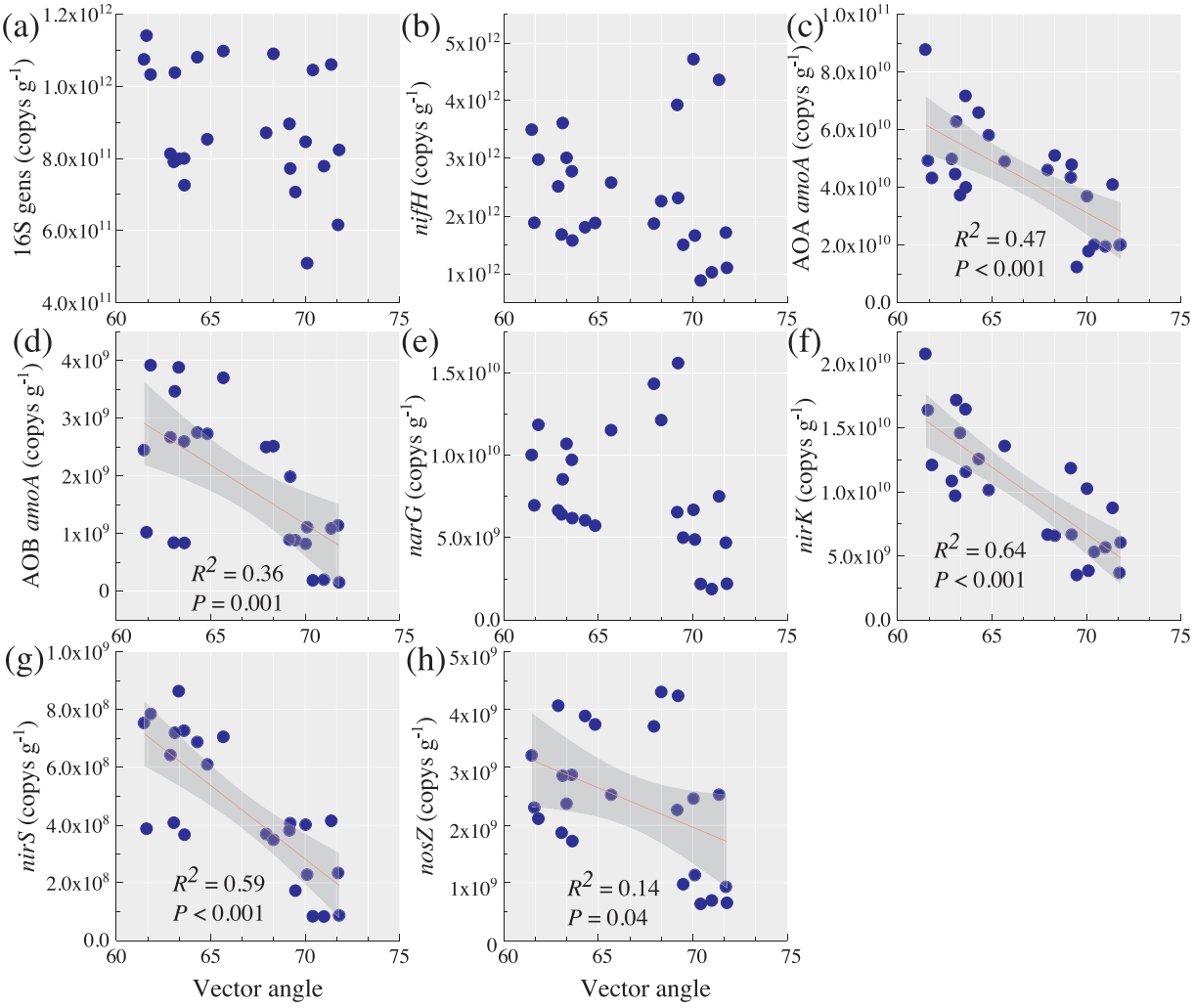

Fig. 5. Relationships between microbial P limitation (vector angles) and the copy numbers of functional genes.

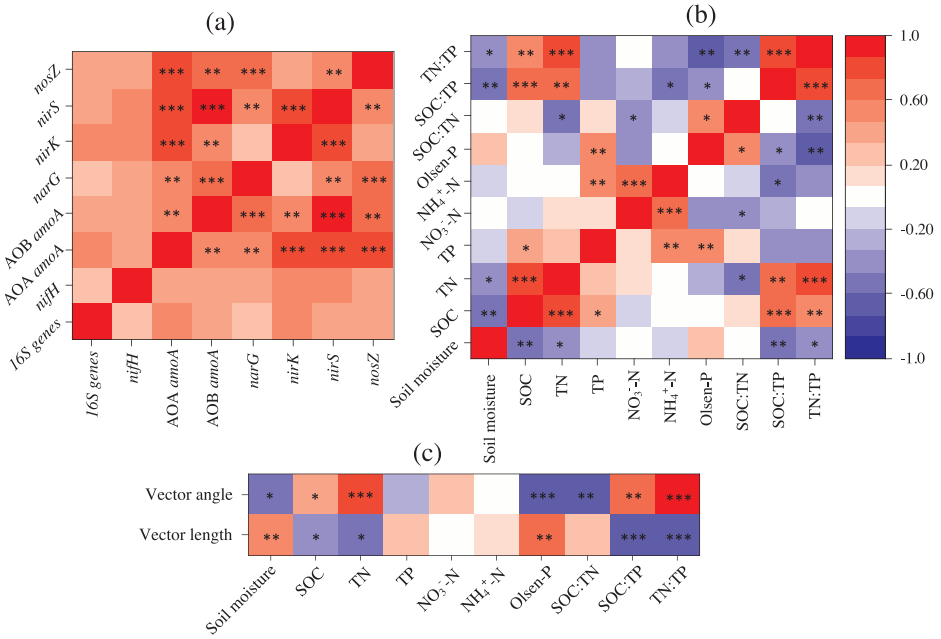

Fig. 6. Correlation heat map of the copy numbers of functional genes, soil physicochemical properties, and between microbial nutrient limitation and soil physicochemical properties.

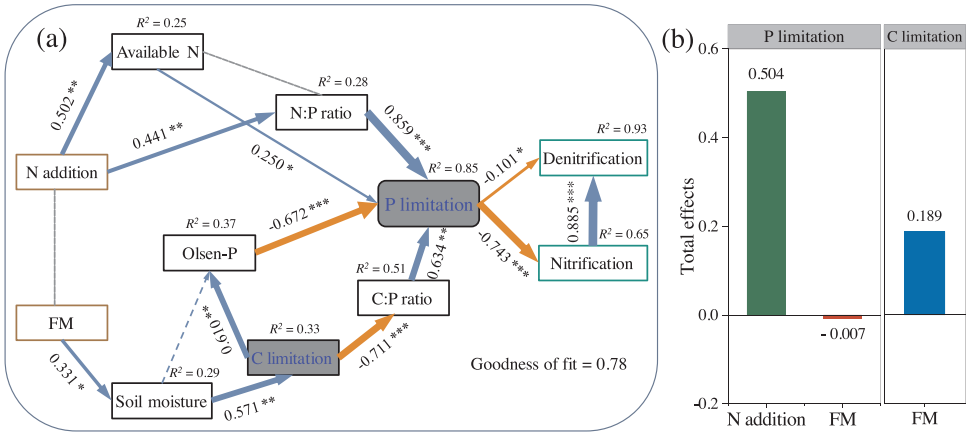

Fig. 7. Cascading relationships of N cycle potentials with soil physicochemical properties and microbial metabolic limitation.

and 0.859 of the direct effects, respectively) determined the variations in microbial P limitation. Overall, N fertilization positively affected microbial P limitation (0.504 of the total effects) and the FM positively affected microbial C limitation (0.189 of the total effects; Fig. 7 b). Furthermore, microbial P limitation negatively affected soil nitrification and denitrification potentials (-0.743 and -0.761 of the total effects, respectively), which were represented by the gene abundance involved in the reactions (Fig. 7 b).

4. Discussion

4.1. The distinct effects of N fertilization and film mulching on microbial metabolic limitation in semi-arid agricultural ecosystems

Extracellular enzymatic stoichiometry revealed that soil microorganisms were strongly limited by P in our study area (Fig. 4). Furthermore, N fertilization (0–400 kg N ha^{-1}) positively affected microbial P limitation (0.504 of the total effects; Fig. 7 b), which supports our first hypothesis. Heuck et al. (2018) also reported that increases in N, such as atmospheric N deposition, may induce P limitation in forest ecosystems on a global scale. Changes in nutrient stoichiometry after N fertilization were the main reason behind increasing microbial P limitation (Figs. 6 c and 7 a) due to microbial biomass homeostasis (Sinsabaugh et al., 2009; Cui et al., 2018). The C:P and N:P ratios were positively correlated with microbial P limitation ($P < 0.01$; Fig. 6 c). This result suggests that microorganisms tend to increase their acquisition of the most limiting P to maintain stoichiometric homeostasis (Elser et al., 2003; Cleveland and Liptzin, 2007) under shifting element stoichiometry (Sistla et al., 2015; Cui et al., 2018). In addition, N fertilization improved N availability in soils, such as NO_3^--N content (Table 1), which further increased the demand for P in microbial N metabolism. The results of PLS-PM also indicated that increased N availability induced microbial P limitation (0.250 of the direct effects; Fig. 7 a).

In contrast to the effects of N fertilization on microbial P limitation, the FM treatment significantly increased the relative C limitation of microorganisms (0.189 of the total effects; Fig. 7 b). The effect was mainly due to soil moisture variations (Fig. 7 a). Soil in the FM treatment had higher moisture content and C-acquiring enzyme activities than the NM treatment (Table 1 and Fig. 3), which suggests that high soil moisture content could promote microbial metabolism, thereby increasing the microbial demand for C sources (i.e., relative C limitation of microorganisms). Also, the FM treatment rarely affected microbial P limitation (only -0.007 of the total effects; Fig. 7 b), which refutes our second hypothesis. Previous studies have indicated that changes in soil moisture had large impacts on SOM turnover and the microorganism-mediated release of CO_2 from soils (Ru et al., 2018; Cui et al., 2019a). The increased C metabolism of microorganisms in the FM treatment could increase C release from soils due to the decomposition of organic matter, negatively affecting the C:P ratio (Fig. 7 a) and leading to lower C:P ratios in the FM than the NM treatment (Table 1). In addition, the high microbial C metabolism observed in the FM treatment simultaneously increased P availability in soils (Table 1; Fig. 7 a), possibly because increased microbial C metabolism can hydrolyze more P from organic residues (Tarafdar and Claassen, 1988; Ru et al., 2018). Therefore, the FM treatment with its high moisture content, reduced the C:P ratio and increased the P availability by promoting microbial C metabolism processes in the soil. Furthermore, the available P in soil negatively affected microbial P limitation (-0.672 of the direct effects), while the soil C:P ratio positively affected microbial P limitation (0.634 of the direct effects; Fig. 7 a). This suggests that high soil P availability lessens P limitation while high soil C:P ratios enhance P limitation due to the stoichiometric homeostasis of microbial biomass (Cleveland and Liptzin, 2007). Ultimately, microbial P limitation was barely affected by the FM treatment with increased soil P availability and decreased soil C:P ratio (Fig. 7 b).

4.2. Microbial P limitation decreases soil N cycling potential

The microbial P limitation can greatly affect microbial growth and metabolism because decomposer cells need to maintain a balanced composition of C, N, and P and the homeostasis of the microbial biomass (Sinsabaugh and Shah, 2012; Manzoni et al., 2012; Cui et al., 2018). Microbial P limitation was negatively correlated with the abundance of genes involved in nitrification and denitrification processes (i.e., AOA *amoA*, AOB *amoA*, *nirK*, *nirS* and *nosZ*; $P < 0.05$; Fig. 5). The PLS-PM analysis further identified that the expression of N-cycling genes was negatively affected by microbial P limitation (-0.743 of the total effects in nitrification and -0.761 of the total effects in denitrification; Fig. 7 a). This result suggests that increased microbial P limitation could inhibit soil nitrification and denitrification, which is consistent with the principle of nutrient stoichiometry in soil and microbial biomass (Sinsabaugh et al., 2009). The principle of nutrient stoichiometry indicates that microbial nutrient demand is determined by the elemental stoichiometry of microbial biomass in relation to environmental nutrient availability, and microbial metabolism of one nutrient can also limited by another. Bernhardt (2013) also showed that low P levels can inhibit microbial N metabolism; specially, that lakes of low P and high N with weak 'biological pump' lead to a lot of N to go downstream, whereas lakes with high P and high N could release more N into atmosphere via denitrification. Furthermore, Ullah et al. (2016) found that P fertilization markedly increases N_2O emissions in soil under both low and high soil moisture content, with or without N fertilization. Therefore, soil with relative high N:P ratios (microbial P

limitation status) could decrease soil N cycling potential, which is not conducive to soil N cycling in agricultural ecosystems.

Microbial P limitation in soil ecosystems may have significant ecological effects. Nitrogen fertilization and FM greatly improve soil fertility and crop yields in dryland farming and represent the most common agricultural strategy in arid and semi-arid agricultural ecosystems (Zhang et al., 2011; Luo et al., 2015). However, N fertilization and FM also greatly increase relative P limitation and C metabolism in soil microorganisms, respectively (Fig. 7). Our study suggests that N fertilization strongly induces microbial P limitation by changing soil nutrient stoichiometry and suppressing nitrification and denitrification processes. Consequently, relative high soil N:P ratios may be also conducive to decreasing N loss due to denitrification and risk of nitrate leaching due to nitrification. In addition, FM greatly promoted microbial C metabolism by increasing soil moisture, facilitating the decomposition of soil organic residues, which thus is not conducive to soil C storage in semi-arid agricultural ecosystems (Liu et al., 2014).

5. Conclusions

The present study revealed that N fertilization increases the metabolic demand of microorganisms for P (i.e., microbial P limitation), whereas the film mulching increases microbial C metabolism in semi-arid agricultural ecosystems. The increase of C metabolism in microorganism is attributed to increased soil moisture in the film mulching treatment, which promoted the decomposition of organic matter. The variations in nutrient stoichiometry and the increase in soil N availability were mainly responsible for increased microbial P limitation. Furthermore, microbial P limitation inhibited soil nitrification and denitrification, impeding N cycles and potentially reducing N loss due to denitrification and risk of nitrate leaching due to nitrification. Our study suggests that agricultural ecosystems with film mulching could be not conducive to soil C storage due to increased organic C decomposition, while appropriate soil P limitation under N fertilizer application could reduce the loss of soil N due to nitrification and denitrification. This study provides important insights linking microbial metabolism theory to functional gene expression in agricultural ecosystems and improves our understanding of the mechanisms by which agricultural practices drive soil nutrient cycling.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (41977031), CAS "Light of West China" Program (XAB2016A03), Program of State Key Laboratory of Loess and Quaternary Geology CAS (SKLLQGZR1803), and State Key Research & Development Plan Project (2017YFC0504504), and Education Department Foundation of Zhejiang Province (Y201738652).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.still.2019.104463.

References

Bernhardt, E.S., 2013. Cleaner lakes are dirtier lakes. Science 342, 205–206. https://doi.org/10.1126/science.1245279.

Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen-total. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbial Properties. Agronomy Society of America, Madison, pp. 595–624.

Borken, W., Savage, K., Davidson, E., Trumbore, S., 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. Glob. Change Biol. 12 (2), 177–193. https://doi.org/10.1111/j.1365-2486.2005.001058.x.

Chen, Z., Liu, J., Wu, M., Xie, X., Wu, J., Wei, W., 2012. Differentiated response of denitrifying communities to fertilization regime in paddy soil. Microb. Ecol. 63, 446–459. https://doi.org/10.1007/s00248-011-9909-5.

Cui, Y.X., Fang, L.C., Guo, X.B., Wang, X., Zhang, Y.J., Li, P.F., Zhang, X.C., 2018. Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the northern Loess Plateau. China. Soil Biol. Biochem. 116, 11–21. https://doi.org/10.1016/j.soilbio.2017.09.025.

Cui, Y.X., Fang, L.C., Deng, L., Guo, X.B., Han, F., Ju, W.L., Wang, X., Chen, H.S., Tan, W.F., Zhang, X.C., 2019a. Patterns of soil microbial nutrient limitations and their roles in the variation of soil organic carbon across a precipitation gradient in an arid and semi-arid region. Sci. Total Environ. 658, 1440–1451. https://doi.org/10.1016/j.scitotenv.2018.12.289.

Cui, Y.X., Bing, H.J., Fang, L.C., Wu, Y.H., Yu, J.L., Shen, G.T., Jiang, M., Wang, X., Zhang, X.C., 2019b. Diversity patterns of the rhizosphere and bulk soil microbial communities along an altitudinal gradient in an alpine ecosystem of the eastern Tibetan Plateau. Geoderma 338, 118–127. https://doi.org/10.1016/j.geoderma.2018.11.047.

Cleveland, C.C., Liptzin, D., 2007. C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85, 235–252. https://doi.org/10.2307/20456544.

Eldoma, I.M., Li, M., Zhang, F., Li, F.M., 2016. Alternate or equal ridge–furrow pattern: which is better for maize production in the rain-fed semi-arid Loess Plateau of China? Field Crop. Res. 191, 131–138. https://doi.org/10.1016/j.fcr.2016.02.024.

Elser, J.J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., Watts, T., Hobbie, S., Fagan, W., Schade, J., Hood, J., Sterner, R.W., 2003. Growth rate-stoichiometry couplings in diverse biota. Ecol. Lett. 6, 936–943. https://doi.org/10.1046/j.1461-0248.2003.00518.x.

Exbrayat, J.F., Pitman, A.J., Zhang, Q., Abramowitz, G., Wang, Y.P., 2013. Examining soil carbon uncertainty in a global model: response of microbial decomposition to temperature, moisture and nutrient limitation. Biogeosciences 10, 7095–7108. https://doi.org/10.5194/bg-10-7095-2013.

Fan, F., Yang, Q., Li, Z., Wei, D., Cui, X., Liang, Y., 2011. Impacts of organic and inorganic fertilizers on nitrification in a cold climate soil are linked to the bacterial ammonia oxidizer community. Microb. Ecol. 62, 982–990. https://doi.org/10.1007/s00248-011-9897-5.

Ford, H., Roberts, A., Jones, L., 2016. Nitrogen and phosphorus colimitation and grazing moderate nitrogen impacts on plant growth and nutrient cycling in sand dune grassland. Sci. Total Environ. 542, 203–209. https://doi.org/10.1016/j.scitotenv.2015.10.089.

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 Biol. Biochem. 43, 1387–1397. https://doi.org/10.1016/j.soilbio.2011.03.017.

He, J.Z., Shen, J.P., Zhang, L.M., Zhu, Y.G., Zheng, Y.M., Xu, M.G., Di, H., 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environ. Microbiol. 9, 2364–2374. https://doi.org/10.1111/j.1462-2920.2007.01358.x.

He, M.Z., Dijkstra, F.A., 2015. Phosphorus addition enhances loss of nitrogen in a phosphorus-poor soil. Soil Biol. Biochem. 82, 99–106. https://doi.org/10.1016/j.soilbio.2014.12.015.

Heuck, C., Smolka, G., Whalen, E.D., Frey, S., Gundersen, P., Moldan, F., Fernandez, I.J., Spohn, M., 2018. Effects of long-term nitrogen addition on phosphorus cycling in organic soil horizons of temperate forests. Biogeochemistry 141, 167–181. https://doi.org/10.1007/s10533-018-0511-5.

Ishii, S., Ikeda, S., Minamisawa, K., Senoo, K., 2011. Nitrogen cycling in rice paddy environments: past achievements and future challenges. Microbes Environ. 26, 282–292. https://doi.org/10.1264/jsme2.me11293.

Jones, D.L., Kielland, K., Sinclair, F.L., Dahlgren, R.A., Newsham, K.K., Farrar, J.F., Murphy, D.V., 2009. Soil organic nitrogen mineralization across a global latitudinal gradient. Global Biogeochem. Cy. 23, 1–5. https://doi.org/10.1029/2008GB003250.

Li, Y.Y., Chapman, S.J., Nicol, G.W., Yao, H.Y., 2017. Nitrification and nitrifiers in acidic soils. Soil Biol. Biochem. 116, 290–301. https://doi.org/10.1016/j.soilbio.2017.10.023.

Li, T., Liu, J.L., Wang, S.J., Zhang, Y., Zhan, A., Li, S.Q., 2018. Maize yield response to nitrogen rate and plant density under film mulching. Agron. J. 110, 996–1007.

Liu, J.L., Zhu, L., Luo, S.S., Bu, L.D., Chen, X.P., Yue, S.H., Li, S.Q., 2014. Response of nitrous oxide emission to soil mulching and nitrogen fertilization in semi-arid farmland. Agric., Ecosyst. Environ. 188, 20–28. https://doi.org/10.1016/j.agee.2014.02.010.

Luo, S.S., Zhu, L., Liu, J.L., Bu, L.D., Yue, S.C., Shen, Y.F., Li, S.Q., 2015. Sensitivity of soil organic carbon stocks and fractions to soil surface mulching in semiarid farmland. Eur. J. Soil Biol. 67, 35–42. https://doi.org/10.1016/j.ejsobi.2015.01.004.

Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol. 196, 79–91. https://doi.org/10.1111/j.1469-8137.2012.04225.x.

Marklein, A.R., Houlton, B.Z., 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. New Phytol. 193, 696–704. https://doi.org/10.1111/j.1469-8137.2011.03967.x.

Mmm, K., Marchant, H.K., Kartal, B., 2018. The microbial nitrogen-cycling network. Nat. Rev. Microbiol. 16, 263–267. https://doi.org/10.1038/nrmicro.2018.9.

Moorhead, D.L., Rinkes, Z.L., Sinsabaugh, R.L., Weintraub, M.N., 2013. Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: informing enzyme-based decomposition models. Front. Microbiol. 4, 223. https://doi.org/10.3389/fmicb.2013.00223.

Moorhead, D.L., Sinsabaugh, R.L., Hill, B.H., Weintraub, M.N., 2016. Vector analysis of ecoenzyme activities reveal constraints on coupled C, N and P dynamics. Soil Biol. Biochem. 93, 1–7. https://doi.org/10.1016/j.soilbio.2015.10.019.

Mori, T., Ohta, S., Ishizuka, S., Konda, R., Wicaksono, A., Heriyanto, J., Hardjono, A., 2010. Effects of phosphorus addition on N_2O and NO emissions from soils of an

Acacia mangium plantation. Soil Sci. Plant Nutr. 56, 782–788. https://doi.org/10.1111/j.1747-0765.2010.00501.x.
Noah, W.S., Bradford, M.A., 2019. Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. Nat. Geosci. 12, 46–53. https://doi.org/10.1038/s41561-018-0258-6.
Olsen, S.R., Sommers, L.E., 1982. Phosphorous. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbial Properties. Agronomy Society of America, Madison, pp. 403–430.
Ramakrishna, A., Tam, H.M., Wani, S.P., Long, T.D., 2006. Effect of mulch on soil temperature, moisture, weed infestation and yield of groundnut in northern Vietnam. Field Crop. Res. 95, 115–125. https://doi.org/10.1016/j.fcr.2005.01.030.
Ru, J., Zhou, Y., Hui, D., Zheng, M., Wan, S., 2018. Shifts of growing-season precipitation peaks decrease soil respiration in a semiarid grassland. Glob. Change Biol. 24, 1001–1011. https://doi.org/10.1111/gcb.13941.
Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an acer saccharum, forest soil. Soil Biol. Biochem. 34, 1309–1315. https://doi.org/10.1016/s0038-0717(02)00074-3.
Shen, J.P., Zhang, L.M., Zhu, Y.G., Zhang, J.B., He, J.Z., 2008. Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. Environ. Microbiol. 10, 1601–1611. https://doi.org/10.1111/j.1462-2920.2008.01578.x.
Sistla, S.A., Schimel, J.P., 2012. Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. New Phytol. 196, 68–78. https://doi.org/10.1111/j.1469-8137.2012.04234.x.
Sistla, S.A., Appling, A.P., Lewandowska, A.M., Taylor, B.N., Wolf, A.A., 2015. Stoichiometric flexibility in response to fertilization along gradients of environmental and organismal nutrient richness. Oikos 124, 949–959. https://doi.org/10.1111/oik.02385.
Sinsabaugh, R.L., Hill, B.H., Shah, J.J.F., 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462, 795–798. https://doi.org/10.1038/nature09548.
Sinsabaugh, R.L., Shah, J.J.F., 2012. Ecoenzymatic stoichiometry and ecological theory. Annu. Rev. Ecol. Evol. Syst. 43, 313–343. https://doi.org/10.1146/annurev-ecolsys-071112-124414.
Smith, M.D., 2011. The ecological role of climate extremes: current understanding and future prospects. J. Ecol. 99, 651–655. https://doi.org/10.1111/j.1365-2745.2011.01833.x.
Spohn, M., 2016. Element cycling as driven by stoichiometric homeostasis of soil microorganisms. Basic Appl. Ecol. 17, 471–478. https://doi.org/10.1016/j.baae.2016.05.003.
Stark, C.H., Richards, K.G., 2008. The continuing challenge of nitrogen loss to the environment-environmental consequences and mitigation strategies. Dyn. Soil Dyn. Plant 2, 41–55.
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. Appl. Soil Ecol. 95, 171–178. https://doi.org/10.1016/j.apsoil.2015.06.010.
Tarafdar, J.C., Claassen, N., 1988. Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. Biol. Fertil. Soils 5, 308–312. https://doi.org/10.1007/bf00262137.
Ullah, B., Shaaban, M., Hu, R.G., Zhao, J.S., Lin, S., 2016. Assessing soil nitrous oxide emission as affected by phosphorus and nitrogen addition under two moisture levels. J. Integr. Agr. 15, 2865–2872. https://doi.org/10.1016/s2095-3119(16)61353-9.
Wei, X., Hu, Y., Peng, P., Zhu, Z., Atere, C.T., O'Donnell, A.G., Wu, J.S., Ge, T.D., 2017. Effect of P stoichiometry on the abundance of nitrogen-cycle genes in phosphorus-limited paddy soil. Biol. Fertil. Soils 53, 767–776. https://doi.org/10.1007/s00374-017-1221-1.
Weier, K.L., 1994. Nitrogen use and losses in agriculture in subtropical Australia. Fertil. Res. 39, 245–257. https://doi.org/10.1007/bf00750253.
Wang, F.M., Li, J., Wang, X.L., Zhang, W., Zou, B., Neher, D.A., Li, Z.A., 2014. Nitrogen and phosphorus addition impact soil N2O emission in a secondary tropical forest of South China. Sci. Rep. 4, 5615. https://doi.org/10.1038/srep05615.
Wrage-Mönnig, N., Horn, M.A., Well, R., Müller, C., Velthof, G., Oenema, O., 2018. The role of nitrifier denitrification in the production of nitrous oxide revisited. Soil Biol. Biochem. 123, A3–A16. https://doi.org/10.1016/j.soilbio.2018.03.020.
Zechmeister-Boltenstern, S., KeiblingerK, M., Mooshammer, M., Penuelas, J., Richter, A., Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plant-microbial-soil organic matter transformations. Ecol. Monogr. 85, 133–155. https://doi.org/10.1890/14-0777.1.
Zhang, S., Li, P., Yang, X., Wang, Z., Chen, X., 2011. Effects of tillage and plastic mulch on soil water, growth and yield of spring-sown maize. Soil Tillage Res. 112, 92–97. https://doi.org/10.1016/j.still.2010.11.006.
Zhang, C., Song, Z.L., Zhuang, D.H., Wang, J., Xie, S.S., Liu, G.B., 2019. Urea fertilization decreases soil bacterial diversity, but improves microbial biomass, respiration, and N-cycling potential in a semiarid grassland. Biol. Fertil. Soils 55, 229–242. https://doi.org/10.1007/s00374-019-01344-z.
Zhao, H., Xiong, Y.C., Li, F.M., Wang, R.Y., Qiang, S.C., Yao, T.F., Mo, F., 2012. Plastic film mulch for half growing-season maximized WUE and yield of potato via moisture-temperature improvement in a semi-arid agroecosystem. Agric. Water Manage 104, 68–78. https://doi.org/10.1016/j.agwat.2011.11.016.