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Dynamic and migration characteristics of soil free amino acids during the whole growth period of rice after application of milk vetch

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Abstract. Free amino acids (FAAs) in soil play an important role in the soil nitrogen cycle and plant nutrition. However, the attributing factors and migration characteristics of free amino acid pools in paddy soils after green manure application during the entire growth period of rice have not been elucidated. In this study, a single application of chemical fertilizer (CK) was used as a control under equal nitrogen, phosphorus and potassium conditions, and different application rates of milk vetch (15 000 kghm^{-2}(CL), 30 000 kghm^{-2}(CM) and 45 000 kg hm^{-2}(CH)) were selected to investigate the dynamic of FAAs concentration and composition in paddy soil. Soil FAAs concentration at different growth stages under the same fertilization treatments was highest at the seedling stage and lowest at the tillering stage. The concentration of threonine, alanine, valine, isoleucine, leucine, phenylalanine was most abundant under different fertilization treatments during the growth period, accounting for 59.42%-76.46% of the respective FAAs pool. The application of milk vetch was shown to increase the soil FAAs concentration, especially glutamic acid, which increased by 368.17%-680.78%, but excessive application had an inhibitory effect. Soil pH, organic matter, protease, bacterial biomass and community were critical factors affecting the concentration of soil FAAs. Bacteroidetes, Firmicutes and Nitrospirae significantly affected the dynamics of FAAs in bacterial communities, and their total contribution rate was 56.89%. FAAs displayed significant vertical profile characteristics, and the mobility of serine, glycine and proline was high. Conclusively, the application of milk vetch was able to significantly change the concentration and composition of soil FAAs, which may affect the capture of N by plants.

Keywords: Free Amino Acids; Composition; Paddy soil; Bacterial community; Structural equation model

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1 Introduction

More than 90% of total soil nitrogen (N) is organic and, within the organic forms of N, amino acids are the most abundant (Rovira et al., 2008). Free amino acids (FAAs) are usually present in soil solution and pores, which are less abundant but more readily absorbed by plant roots and microorganisms (Gao et al., 2020). FAAs are mainly generated by hydrolysis of soil protein and peptides using extracellular enzymes (Jan et al., 2009), exudation and death from plant roots (Feng et al., 2018), and microbial turnover and excretion (Hill et al., 2019). These amino acids cycle very rapidly in soil, can be selectively absorbed by plants and microorganisms (Harrison et al., 2007), and in addition, easily migrate and are subsequently lost (Nie et al., 2018). The concentration of FAAs in soil depends on the production and consumption of FAAs, and a higher concentration is probably conducive to FAAs capture by plants (Jones et al., 2005). Therefore, determining the bioavailable concentration of FAAs in soil is crucial to our understanding of plant nitrogen acquisition and the soil nitrogen cycle (Hill et al., 2019). It was reported that FAAs concentration varied greatly when comparing different ecosystems, and the concentrations of amino acids in boreal forest, agricultural soil and alpine meadow soils were 438.00-4867.00 ng N g^{-1} dry soil (Werdin-Pfisterer et al., 2009), 12.87-48.93 ng N g^{-1} dry soil (Gonzalez Perez et al., 2015) and under the detection limit (Feng et al., 2018), respectively. However, most of current studies focused on the size of the pool of FAAs in upland soils, while very limited information is currently available looking at the concentration and composition of FAAs in chronically flooded paddy soils with high anthropogenic disturbance.

Fertilizers are the key source of available nitrogen in farmland ecosystems, and different types of nitrogen fertilizers generate a different composition of soil amino acids. Addition of urea causes important microbial community changes and leads to cells lysis of some microbes and subsequently leading to the release of amino acids into soil (Gonzalez Perez et al., 2015). Plant residues applied to soil were shown to release amino acids and easily degradable proteins after decomposition, but different plant residues have different C/N ratios, and the way and time of release of amino acids to soil are different. In 10 days of aerobic culture of fresh alfalfa residue extracts, a large proportion (29-100%, depending on culture temperature) of soluble N containing a variety of amino acids can be released (El-Naggar et al., 2010). In contrast, the protein contained in poor quality residues, such as straw, must be digested by enzymes to release the amino acids it contains (Geisseler and Horwath, 2008). Although more and more attention has been paid to soluble organic nitrogen, the research on amino acids in agricultural soil is still lacking, and the existing research mainly focuses on the concentration of exchangeable amino acids. Chinese milk vetch (Astragalus sinicus L., CMV), a winter grown legume plant with

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high N_2-fixing ability, is commonly planted as an alternative N source for chemical fertilizer in rice cropping systems in Southern China (Chen et al., 2020). However, the effect of application of CMV on the concentration and composition of free amino acids in paddy soil is still unclear.

Soil microorganisms play a critical role in nitrogen cycling by breaking down organic matter to amino acids and other small molecules of organic nitrogen through the mineralization processes, which can be taken up by plants or microbes (Schimel and Bennett, 2004). Then, amino acids were mineralized via microbial ammonification, nitrification and denitrification processes to inorganic nitrogen, ammonium(NH_4^+) and nitrate (NO_3^-) (Schimel and Bennett, 2004). We still know considerably less about the metabolism of microorganisms and how this effects the presence and/or consumption of amino acids. In very general terms, the dynamic variation of FAAs may be due to temporal variations in soil microorganisms. This is because the concentration of FAAs in soil is affected by absorption (Gao et al., 2020), release (Hill et al., 2019) and release of extracellular enzymes by microorganisms (Jan et al., 2009). The temporal variation of microbial N may determine the soil reservoir in FAAs (Weintraub and Schimel, 2005). Lipson et al. (1999) reported that the decrease in microbial biomass in alpine soils is correlated to an increase in pools of FAAs. Microbial biomass is a strong competitor for amino acids, which can regulate FAAs content (Warren and Taranto, 2010). However, as far as we know, the specific microbial communities that cause the dynamic changes of soil amino acids and their roles are still poorly understood.

In this study, four different treatments (chemical fertilizer, low amount of CMV, medium amount of CMV and high amount of CMV) were chosen to test the following three hypotheses: (i) Soil FAAs could be affected by the application of CMV, and is correlated to the application rate. (ii) The concentration and composition of soil FAAs will vary with rice growth stages. (iii) The dynamics of FAAs in paddy soil are closely correlated to soil microbial community structure.

2 Materials and methods

2.1 Experimental site and design

The experimental area was located in Minhou County, Fujian Province (119°04 '10"E，26°13 '31"N，which is situated in the transition zone between the middle subtropical zone and the south subtropical zone. The annual average temperature is 19.5°C, the annual sunshine hours are 1812.5 h, the frost-free period is about 311 days, and the mean annual average precipitation is 1350 mm. The soil was composed of gray yellow paddy soil with loamy clay and the main initial chemical properties were as follow: pH 5.53, total organic matter17.65 g kg^{-1}, alkali-hydrolyzed nitrogen 29.95 mg kg^{-1}, available phosphorus 20.85 mg kg^{-1} and available potassium 96.65 mg kg^{-1}.

Four equal quantity of nitrogen treatments were set up in this experiment, including chemical fertilizer (CK), low amount of CMV (15000 kg hm^{-2}, CL), medium amount of CMV (30000 kg hm^{-2}, CM) and high amount of CMV (450 000 kg hm^{-2}, CH). Three replicate experimental plots (3 m ×4 m in size) per treatment were designed to be in a random block arrangement. The fertilizers applied in the experiment were urea 481.67 kghm^{-2}, superphosphate 900 kghm^{-2} and potassium chloride 300 kghm^{-2}, within which 50% of urea and potassium chloride were used as base fertilizer and 50% those fertilizers were applied at the tillering stage of rice growth, and all off the superphosphate was applied as the base fertilizer. The variety of CMV was Minzi No.7, with 90% water content, 752.75 gkg^{-1}of total organic matter, 30.94 gkg^{-1}of total nitrogen, 5.91 gkg^{-1}of total phosphorus, 32.47 gkg^{-1}of total potassium, 82.35 mg kg^{-1} of acid-hydrolyzed amino acid (acid-hydrolyzed amino acid compositions shown in Fig. S1), 193.40 g kg^{-1} of protein. The insufficient part of nitrogen, phosphorus and potassium in the treatment with CMV should be supplemented with chemical fertilizer when applying base fertilizer. Irrigation was carried out before rice planting and a certain flooded layer was maintained.

2.2 Soil sampling

In this experiment, a multi-point sampling method was used to collect mixed samples of topsoil (0-20 cm) in each experimental plot at 0d (background soil, B), 10d (seedling stage, S), 38d (tillering stage, T), 80d (flowering stage, F) and 122d (maturity stage, M) respectively after CMV application, according to the decomposition rule of rapid decomposition in early stage and gradually slowing down in late stage and rice growth stage. Soil samples of 0-20 cm, 20-40 cm and 40-60 cm in each plot were collected at the background and rice maturity stage. The mixed fresh soil samples were divided into three parts: one part was used for analysis of soil FAAs and relative biochemical properties, another part was stored in -80°C refrigerators for bacterial community analysis, and the other part air-dried and sieved for determination of physical and chemical properties.

2.3 Analysis of soil FAAs

Soil FAAs content and composition were analyzed using a water extraction- automatic amino acid analyzer method. In brief, 10g fresh soil was placed in a 100 mL triangular flask, 50 ml ultra-pure water was added and cultured in a 70 C constant temperature oscillator for 18 hours, then shaken for 5 minutes, and subsequently filtered through a 0.45 mm filter membrane. 10 ml filtrate and 0.25 g sulfosalicylic acid to was taken to deproteinate and the centrifuged at 5 000 r min^{-1} for 5 min. After adjusting the pH to 2.2, the filtrate was filtered with a 0.45 μm filter.1 ml filtrate was absorbed and determined by automatic amino acid analyzer. The FAA concentration was calculated from the peak areas with reference to amino acid standards (Fig. S2，Table S1).

2.4 Analysis of soil bacterial biomass and community

2.4.1 Quantitative real-time PCR

Soil DNA was extracted from 0.25 g freeze-dried soil using PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad) following the instructions in the manual. The purity and quality of DNA were checked on 0.8% agarose gels. The 16S rRNA gene of soil bacteria was amplified by real-time fluorescence, and the copy number of the 16S rRNA gene of the bacteria was determined. Fluorescence quantification was carried out by a two-step method. The specific conditions were 94°C pre-denaturation for 5 min, (94°C 30 s, 55°C 30 s, 72°C 30 s) × 30 cycles, and extension at 72°C for 10 min after the end of the cycle. The plasmids were extracted as the standard for the preparation of the standard curve and the quantitative standard curve was established.

2.4.2 Analysis of bacterial community structure

DNA was extracted by using MoBio Laboratories DNA kit. The V3-V4 region of 16S rRNA genes were amplified by PCR using the primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) (Peris et al., 2015). The PCR was carried out on a Mastercycler Gradient (Eppendorf, Germany) with the following program: 95 °C for 5 min, 32 cycles of 95 °C for 45 s, 55 °C for 50 s and 72 °C for 45 s. The PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN, Germany) before quantification and sequencing. After sequencing, image analysis, base calling and error estimation were performed using Illumina Analysis Pipeline (Version 2.6). The sequences were clustered into operational taxonomic units (OTUs) at a similarity level of 97% (Edgar et al., 2013) to generate rarefaction curves and calculate the richness and diversity indices. The Ribosomal Database Project (RDP) Classifier tool was used to classify all sequences into different taxonomic groups (Cole et al., 2009).

2.5 Statistical analysis

All statistical analysis and correlation analysis were performed using SPSS 19.0or Excel 2007, and data plots were obtained using SigmaPlot 12.0 and R 3.5.1 software. Analysis of variance (ANOVA) and Least significant difference (Duncan, p<0.05) analysis were used to separate the means with significant differences. The structural equation model (SEM) was used to study the impact path and effect of driving factors on FAAs dynamics by AMOS 21.0 (the structural equation analysis process is shown in S3), and the mantel test was used to explore the impact of the microbial community on FAAs dynamics by PASSaGE3.0 and the variance partition analysis (VPA) was used to quantify the contribution rate of the major bacterial communities to FAAs by the "Vegan" program in R 3.5.1 statistical software.

3 Results

3.1 Dynamics of FAAs concentration

The temporal variation in concentration of FAAs displayed a similar pattern under different fertilization treatments (Fig. 1), with the concentration increasing from background to the peak in seedling stage and then rapidly decreased to the lowest level in tillering stage, then increasing gradually to a second peak in tillering stage and decreased gradually. The FAAs concentration in maturity stage only accounts for 46.77%-55.30% of the background soil. The application of CMV can increase soil FAAs concentrations, but excessive application has a certain inhibitory effect. Compared to CK treatment, the FAAs concentration under CL, CM and CH treatments increased by 24.08%, 40.16% and 18.78%, respectively.

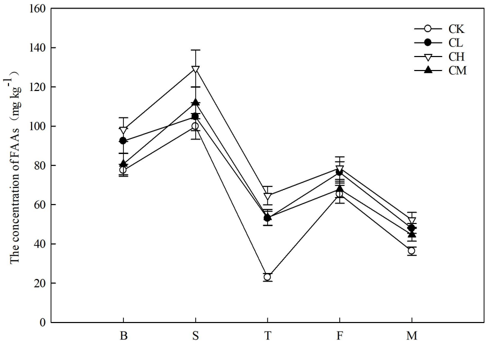

Figure 1. Dynamics of soil FAAs concentration under different fertilization treatments during different rice growth stages

Note: B: background soil, S: seedling stage, T: tillering stage, F: flowering stage, M: maturity stage. The error line indicates standard error (n = 3).

3.2 Dynamics of FAAs composition

The FAAs composition under different fertilization treatments in different rice growing periods was diverse, with 15 neutral amino acids and 2 acidic amino acids detected (Fig. 2). The concentration dynamics of neutral amino acids and acidic amino acids under different fertilization treatments followed a similar temporal pattern as that of the FAAs concentration, accounting for 95.74%-100% and 0%-4.26% of the FAAs pool respectively. Acidic amino acids were present only in the background, seedling stage and flowering stage. The concentration of individual FAAs followed a similar pattern to that of the amino acid composition. During the different rice growth periods, different fertilization treatments had similar FAAs pools, in which threonine, alanine, valine, isoleucine, leucine, phenylalanine were the most abundant, and accounted for 59.42%-76.46% of the amino acid pools. In general, CMV application could significantly increase the concentration of amino acids components, especially glutamic acid, which increasesd by 368.17%-680.78%.

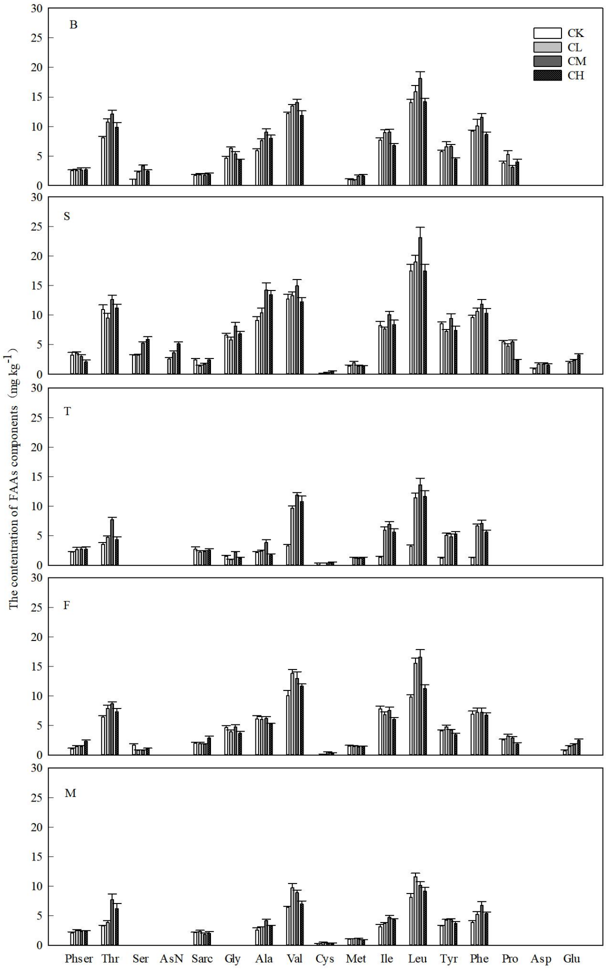

Figure 2. Dynamics of soil FAAs composition under different fertilization treatments during the rice growth stage

Note: B: background soil, S: seedling stage, T: tillering stage, F: flowering stage, M: maturity stage. The error line indicates standard error (n = 3).

3.3 Profile distribution of FAAs

The concentration and type of soil FAAs in the background and maturity stage decreased with increasing soil layer (Fig. 3). In the 0-20 cm soil layer, the FAAs concentration was background>maturity stage, while in the 20-40 and 40-60 cm soil layer, the FAAs concentration was maturity stage>background, indicating that FAAs displayed a downward migration trend. The application of CMV increased the accumulation of FAAs in the soil profile. Compared to CK, the application of CMV increased the FAAs concentration in 0-20 cm, 20-40 cm and 40-60cm soil layers by 9.21%-22.86%, 25.90%-44.19% and 7.05%-13.92%, respectively, during the different rice growth stages. There were more types of FAAs that migrated to the 20-40 cm soil layer. Compared to the background, the FAAs increased serine, glycine and proline in the 20-40 cm soil layer and increased proline in the 40-60 cm soil layer at the maturity stage, indicating that serine, glycine and proline displayed strong migration.

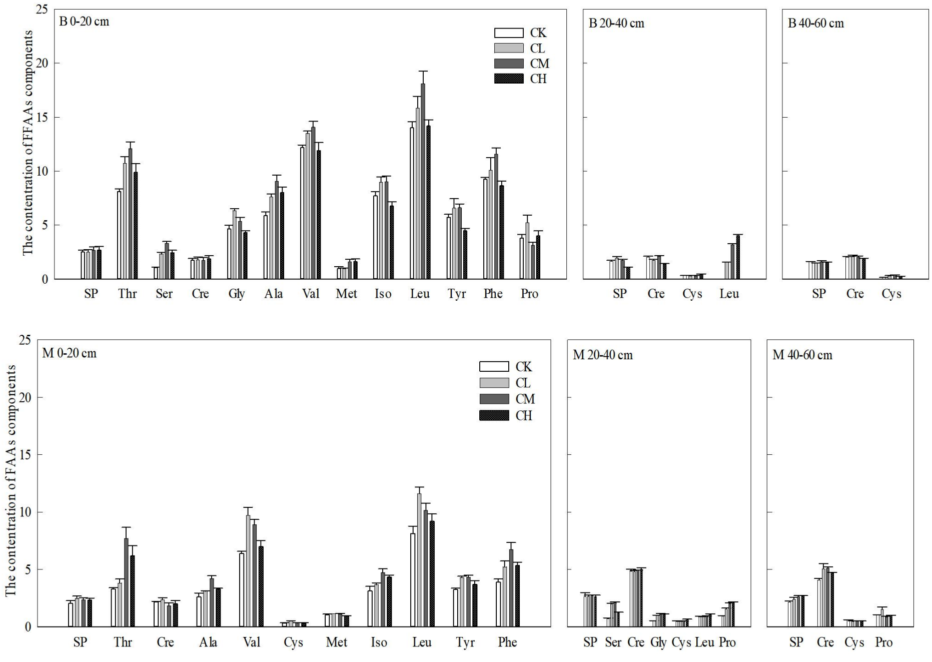

Figure 3. FAAs Profile distribution of different fertilization treatments in the rice growth stage

Note: B: background soil, M: maturity stage. The error line indicates standard error (n = 3).

3.4 Dynamics of soil bacterial community during the rice growth stage

3.4.1 Soil bacterial biomass and diversity

The copy number of soil bacteria and Chao1 index under different fertilization treatments in different rice growing stages increased from background, decreased after seedling stage and reached a low point in the tillering stage, then increased rapidly to the highest point in the flowering stage and then decreased rapidly (Fig. 4). The copy number of soil bacteria at maturity stage only accounted for 24.74%-28.68% of the background soil. The application of CMV can increase the copy number of soil bacteria and Chao1 index, but excessive application has a certain inhibitory effect. Compared to CK treatment, the copy number of soil bacteria and Chao1 index under CMV treatment increased by 13.95%-25.14% and 2.41%-4.97%, respectively. However, there was no significant difference in Shannon index between different fertilization treatments during the different rice growth periods.

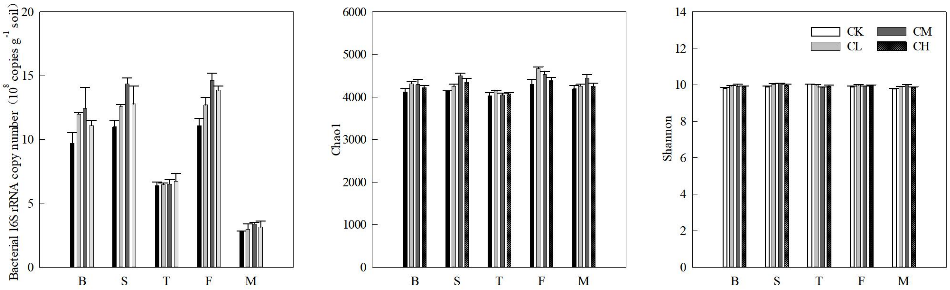

Figure 4. Dynamics of the copy number of soil bacteria and alpha diversity under different fertilization treatments during the different rice growth stages

Note: B: background soil, S: seedling stage, T: tillering stage, F: flowering stage, M: maturity stage. The error line indicates standard error (n = 3).

3.4.2 Soil bacterial community structure

The phyla Chloroflexi, Acidobacteria and Proteobacteria occupied 69.75%-73.96% of the bacterial sequences obtained from different fertilization treatments and were followed by Actinobacteria (2.98%-5.96%)，Planctomycetes (3.06%-4.47%), Bacteroidetes (2.18%-3.87%), Nitrospirae (1.60%-3.70%)， Verrucomicrobia (1.88%-3.36%), Latescibacteria (1.33%-2.62%) and Firmicutes (0.71%-3.97%). The composition and structure of the bacterial community was shown be significantly changed at different growth stages of rice (Fig. 5). The treatments were separated along the first axis reflecting the different growth periods of rice. Different treatments in the same growth stage were clustered together, and different growth periods were far apart, especially the tillering period and other growth periods were far apart, indicating that the bacterial community structure at the tillering stage was significantly different from other growth stages.

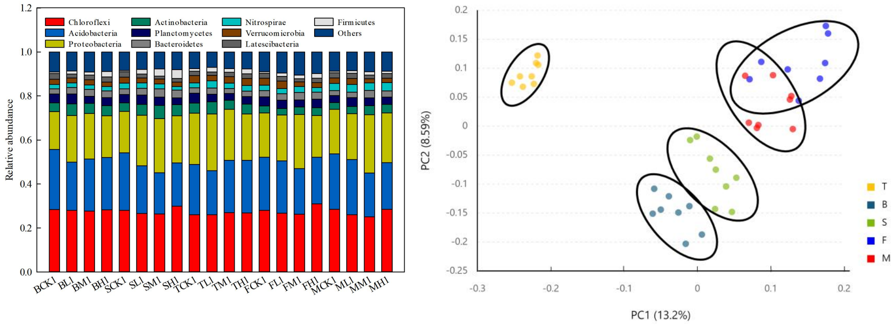

Figure 5. Relative abundance of bacterial taxa at the phylum level and PCA plot of soil bacterial community distribution under different fertilization treatments during the different rice growth stages

Note: B: background soil, S: seedling stage, T: tillering stage, F: flowering stage, M: maturity stage.

3.5 Impact factors of FAAs dynamics during the rice growth stage

3.5.1 Influence factors and paths of FAAs dynamics

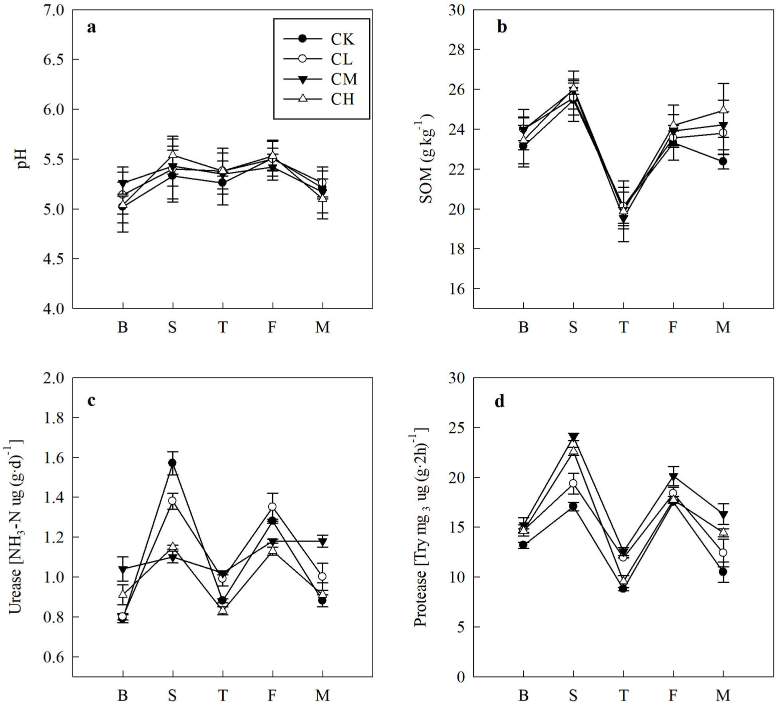

Figure 6. Soil pH, SOM, urease activities and protease activities under different fertilization treatments during different rice growth stages

Note: B: background soil, S: seedling stage, T: tillering stage, F: flowering stage, M: maturity stage. The error line indicates standard error (n = 3).

In this study, pH, SOM, urease, protease, bacterial biomass and microbial community indicators were measured (Fig. 6) as impact factors of FAAs. The structural equation model analysis showed that pH, SOM, protease, bacterial biomass and microbial community accounted for 83% of the variations in soil FAAs dynamics during the rice growth stage (Fig. 7, the structural equation analysis process is shown in S3). Soil pH, SOM, protease, bacterial biomass and microbial community had direct effects on FAAs, and their standard path coefficients were -0.43, 0.52, 0.71, 0.49 and -0.72, respectively. Meanwhile, pH and SOM could indirectly affects FAAs through protease, bacterial biomass and microbial community, and the standardized indirect effect was 0.65 and 0.12. Soil bacterial biomass and microbial community could indirectly affect FAAs through protease, and the standardized indirect effect both was 0.22.

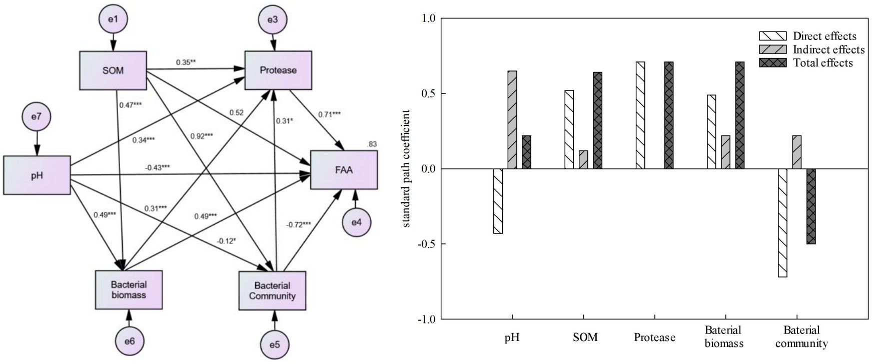

Figure 7. Structural equation model (a) and standard path coefficient (b) of impact factors on soil FAAs during the rice growth stage

Note： The values next to the arrow are standard path coefficients (also known as regression coefficients), ***Significant at P < 0.001, e1-e6: errors 1-6

3.5.2 Key microbial communities of FAAs dynamics

The results of Mantel test results showed that Bacteroidetes, Firmicutes and Nitrospirae had a significant influence on FAAs dynamics of different fertilization treatments during the rice growth stage, and the correlation coefficients were 0.40, 0.30 and -0.26 respectively. The VPA analysis showed that the relative abundance of Bacteroidetes, Firmicutes and Nitrospirae contributed 22.99%, 22.18% and -0.42% of the variation of SON content, respectively (Fig. 8). The contribution rate of the interaction of the three major effect to SON content variation was 17.27%, and the total contribution rate of each variable and its interaction to SON concentration variation was 56.89%.

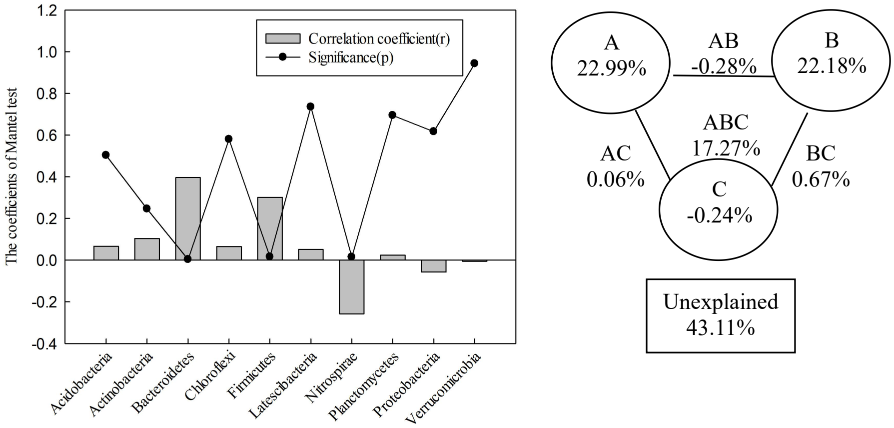

Figure 8. Mantel test (a) and variance partition analysis(b) between bacterial phyla and soil FAAs dynamics of different fertilization treatments during the rice growth stage

Note: A: Nitrospirae, B: Bacteroidetes, C: Firmicutes

4 DISCUSSION

4.1 Effect of CMV treatments on soil FAAs concentration dynamics

The production and degradation of FAAs in soil is a dynamic process, influenced by the presence of plants, soil microorganisms and enzyme activity. The content of FAAs in soil with CMV increased rapidly to peak value and then quickly decreased to the lowest value, thereafter gradually increasing to a second peak value at the flowering stage and then gradually decreasing (Fig. 1). This result is consistent with the following four stages of rapid decomposition, rapid decline of decomposition rate, accelerated decomposition and slow decomposition after CMV application (Zhu et al., 2013). The FAAs concentration in the soil reached a peak at the rice seedling stage, indicating that the turnover rate of CMV was extremely fast, which may be due to the low C/N value of CMV (14.11) (Zhang et al., 2017). Moreover, the rapid decomposition of CMV after overturning to was shown to produce a large amount of FAAs. Furthermore, the addition of fresh organic matter did not only directly provide a large amount of energy and nutrients for soil microorganisms, but also provided nutrients and energy for the reproduction of soil microorganisms through priming effect (Kuzyakov et al., 2000). In addition, the addition of fresh organic matter improved soil protease activity, microbial biomass and diversity (Fig. 4, Fig. 6), thereby promoting the decomposition of macromolecular organic matter into FAAs. The FAAs concentration in the soil quickly dropped to a minimum from seedling stage to tillering stage. The reason was partly due to this stage belonging to a period of rapid decline in decomposition of CMV and the source of FAAs decreasing (Zhu et al., 2013). On the other hand, this stage was a flourishing stage for rice growth, and the enhanced nutrient uptake ability of rice roots, which competed with microorganisms for inorganic nitrogen and small-molecule organic nitrogen (Ma et al., 2018), leading to a reduction in microbial biomass (Fig. 4) and protease activity (Fig. 6), which led to a decrease in soil FAAs content. Along with the above observations, we also found that the soil FAAs concentration at maturity stage under different fertilization treatments was lower than that at background soil (Fig. 1). This may be due to the fact that the background soil under aerobic condition, which is conducive to mineralization of organic nitrogen, releases more amino acids (Ma et al., 2017). At the same time, the maturity soil is flooded and the number of bacteria is therefore reduced (Fig. 4), which in turn is not conducive to the production of FAAs. Aerobic systems generally display a more metabolically active microbial community than anaerobic systems (El-Naggar et al., 2010). In addition, amino acids are highly mobile and tend to migrate to the bottom layer (Fig. 3), resulting in a decrease in the concentration of amino acids in surface soil at the maturity stage.

The application of CMV can continuously bring a large amount of nitrogen-containing organic matter into the soil, and significantly increase the soil FAAs content. The protein and amino acid concentrations of the tested CMV were 193.4 g kg^{-1} and 82.35 mg kg^{-1}, respectively, and the Fourier infrared spectroscopy results showed that the main components of the tested CMV were carbohydrates, phenolics, protein, lignin and aliphatic compounds, etc. (Fig. S1). These nitrogen-containing substances were depolymerized into small molecule FAAs by depolymerization catalyzed by microorganisms and proteases, so that the concentration of FAAs in the soil was higher than the concentration under CK treatment. In addition, the application of fresh green manure increased soil active organic carbon and nitrogen, and thereby changed the soil microbial activity and community composition, promoted the decomposition of CMV and the secretion of rice roots (Yang et al., 2020), thereby contributing to the increase of FAAs concentration. The results of this study showed that a high dose application of CMV was not conducive to the accumulation of FAAs, mainly because excessive application of CMV to the soil produced a large number of organic acids and inhibited the growth of microorganisms (Macias-Benitez et al., 2020). In addition, excessive application of CMV was shown to reduce soil Eh, produce a large amount of reducing substances such as Fe^{2+},Mn^{2+},H_2S, etc.(Liu et al., 2014), leading to a decrease in microbial biomass (Fig. 4), which is consistent of the experimental results of Cheng et al. 's (2020) studying the effect of applying differing concentrations of CMV is having on soluble organic nitrogen.

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4.2 Effects of CMV treatments on soil FAAs composition dynamics

The different concentrations of acidic amino acids and neutral amino acids displayed similar time dynamics as did different concentrations of FAAs in paddy soil, which was in part consistent with the results that amino acids have temporal dynamics in the temperate grassland (Warren & Taranto, 2010), temperate forest (Gao et al., 2020) and alpine meadow soil (Feng et al., 2018). In these studies, in addition to neutral amino acids, both acidic and basic amino acids also accounted for a distinct proportion of the soil amino acid pools. However, in our study, only neutral amino acids and acidic amino acids were detected under different fertilization treatments during the different rice growth periods. The different composition of individual amino acid pools in these studies may be ascribed to the difference between the free and the exchangeable pool of amino acids. The free amino acid pool consists mainly of acidic and neutral amino acids that have been extracted by water or weak salts, whereas the exchangeable amino acids pool has been shown to include a larger proportion of basic amino acids since these were absorbed to the soil sold phase and could only be extracted with strong salts (Warren and Taranto, 2010). The difference in soil pH also led to a difference in amino acid composition in different studies (Feng et al., 2018). The test site in our study was located in the subtropical zone, where desilication and iron-rich aluminization in the process of soil formation resulted in the acidity of the tested paddy soil (pH value is 5.02-5.51). Under acidic conditions, the chemical stability of basic amino acids was low and these was easily degradable, so no basic amino acids were detected in the tested soil (Guo et al., 2013).

The pool of FAAs was dominated by threonine, alanine, valine, isoleucine, leucine and phenylalanine under four fertilization treatments during different rice growth periods. The finding that similar amino acids dominated under four different fertilization treatments is predicted to be due to soil amino acids originating from similar biochemical processes (Werdin-Pfisterer et al., 2009). Hence, with the exception of valine, these amino acids have previously been shown to be among the most abundant amino acids in vegetable soil and alpine meadow soil (Feng et al., 2018; Gonzalez Perez et al., 2015). Alanine and leucine are abundant in microbial cell walls (Weintraub & Schimel, 2005, Feng et al., 2018), especially in fungal cell walls (Weintraub & Schimel, 2005), and threonine has been shown to be secreted into soil by rice roots (Rae & Castro, 1976). The presence of large amounts of tryptophan in paddy soil is somewhat unusual and may be related to the decomposition of organic matter. Increases in abundance of glycine, alanine and threonine have been found during organic matter decomposition and are thought to be linked to their abundance in recalcitrant structures (Jämtgård et al., 2010). Gonzalez Perez et al. (2015) showed that the production and mineralization of phenylalanine was correlated to the presence of isoleucine and leucine, and the variation trend was similar. Glycine was also shown to be one of

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the most abundant amino acids in agricultural soils, and its concentration increased during the process of organic decomposition (Rovira et al., 2008). Glycine is believed to be abundant in bacterial, fungal and plant cell walls (Jämtgård et al., 2010). However, in this study, the concentration of glycine was higher in the background soil and seedling stage, but was not detected in the maturity stage, which may be correlated to the strong migration of glycine (Fig. 3).

The decomposition of organic matter is one of the direct sources of the soil amino acids (Laudicina et al., 2013). This study showed that application of CMV was able to significantly increase the acidic FAAs in soil. This is due to the high amounts of acidic amino acids (aspartic acid and glutamic acid) in CMV (Fig. S1). After being applied to the soil, the amount of aspartic and glutamic acid in soil were significantly increased under the catalytic activity mediated by microorganisms and free proteases. Moreover, another reason for the increased presence might be that the addition of exogenous organic materials increased soil microbial biomass (Ma et al., 2020). Soil FAAs were secreted and autolyzed products of microorganisms, and their cell walls have been shown to be rich in glutamine, glutamate, aspartic acid and asparagine (Friedel & Scheller, 2002). In addition, a high concentration of acidic amino acids was found to correlate with the organic matter concentration, and improved the availability of nitrogen (Werdin-Pfisterer et al., 2009). Studies have shown that polar amino acids have a high degree of mineralization (Rothstein, 2010), and the concentration of polar glutamic acid and aspartic acid increased after applying CMV, which increased the availability of nitrogen.

4.3 Effects of CMV treatments on soil FAAs migration

FAAs displayed significant vertical distribution characteristics in the soil profile. CMV application was shown to significantly improve the concentration and composition of FAAs in all soil layers, especially in the 0-20 cm soil layer (Fig. 3). This finding is mainly caused by the distribution of CMV and rice roots in the 0-20 cm soil layer. The tested CMV contained a large amount of protein (193.4 g kg^{-1}) and acid-hydrolyzed amino acid (82.35 mg kg^{-1}), which were mainly accumulated in the surface soil after being applied to the soil, providing abundant nutrients and energy for microbes in the 0-20 cm soil layer, thus improving the mineralization rate of organic material (Yang et al., 2020). In addition, the application of CMV is beneficial to the growth of rice and promotes the increase of root exudates (Huang et al., 2016), which have been shown to be one of the important sources of soil FAAs (Yang et al., 2015). On the other hand, the vertical profile characteristics of FAAs is correlated to the adsorption of FAAs by soil. Fischer et al (2007) had previously shown that the mobility of FAAs in soil is affected by the process of soil adsorption. The test soil in this study was clay with an abundance of surface charges, which displayed a strong absorption of FAAs (Zhu et al., 2019), leading to a relatively weak infiltration of FAAS. Moreover, the plough pan of paddy soil is 20 cm below the surface, which has an intercept effect on water infiltration (Islam et al., 2014), so only part of FAAs migrated to the bottom.

Different types of soil FAAs have different properties, which make their migration characteristics in soil different. This study showed that glycine, serine and proline displayed strong mobility (Fig. 3), which may be correlated to the adsorbed amount of amino acids in soil. As neutral amino acids, glycine, serine and proline were not easily adsorbed by soil colloids and displayed strong mobility in the soil profiles (Hedges and Hare, 1987). Glycine is the amino acid with the smallest molecular weight and the simplest structure in soil, therefore making it easy to migrate in the soil profile (Dashman and Stotzky, 1982), while serine, as a hydrophilic amino acid, can easily be dissolved in the soil solution and migrate to the deep soil under the influence of gravity (Trevino et al., 2007). Proline, as one of the most water-soluble amino acids (Jensen et al., 2014), easily migrates downward with soil water, and can migrate to the 40-60 cm soil layer during the growth period of rice. In addition, the distribution of amino acids in the profile is closely correlated to their properties. The degree of mineralization of polar amino acids is higher than that of non-polar amino acids (Rothstein, 2010). Therefore, the degree of mineralization of proline in the tested soil as a non-polar amino acid is lower and its turnover rate is slower, thus becoming one of the main components of amino acid migration in the soil profile.

4.4 Factors driving soil FAAs dynamics

Previous studies have shown that pH, SOM, enzyme activities and microbial community were important factors affecting soil FAAs dynamics (Gonzalez Perez et al., 2015, Feng et al., 2018, Reeve et al., 2008). In this study, the results of structural equation model analysis showed that pH, SOM, protease, bacterial biomass and microbial community accounted for 83% of the variations in soil FAAs dynamics (Fig 7), which were the important factors affecting the variations in soil FAAs dynamics during rice growth period.

The pH value is one of the main factors affecting nitrogen conversion. The pH value was shown to affect the adsorption of amino acids by soil colloids and directly affect the concentration of free amino acids in soil (Fischer et al., 2007). The results of the structural equation model in this study showed that pH had a direct influence on the soil FAAs concentration, and the standardized path coefficient was -0.43, which may be correlated to the variable charge in amino acids (Laudicina et al., 2013). Amino acids are amphoteric electrolytes, which usually carry a positive charge in acidic soil. CMV contains high ash alkali, which can neutralize soil acidity and improve soil pH when applied to acidic soil (Wang et al., 2013). With an increase in pH (Fig 6), amino acids usually carry a net neutral charge or negative charge. They were shown to interact weakly with soil colloid and were easily lost from the soil, leading to a decrease in soil FAAs content. Fischer et al. (2007) found that about 10% of amino acids were adsorbed to soil solid phase, and this value strongly varied depending on the soil pH. Soil pH is considered to be a primary control of microbial activity and enzyme kinetics, as it strongly influences both nutrient availabilities and enzyme denaturation and folding (Chen et al., 2019; Sinsabaugh et al., 2008). The results of structural equation in this study also showed that the pH could indirectly affect FAAs concentrations through protease, bacterial biomass and bacterial community structure, which might be due to pH affecting the decomposition of soil soluble organic matter to produce FAAs by affecting soil bacterial enzyme activity (Min et al., 2014). Li et al. (2019) also showed that soil pH could indirectly affect the mineralization of soil organic nitrogen by affecting microbial biomass and enzyme activity.

Soil organic matter is an important source of soil FAAs and is closely correlated to FAAs concentration (Fischer et al., 2007). The results of the structural equation model in this study showed that organic matter is an important factor affecting the FAAs concentration after applying CMV with the total effect of 0.64. This is predicted to be due to an increase in soil organic matter content after CMV addition, which can increase soil microbial enzyme activities (Yang et al., 2020), and promote the decomposition and transformation of soil organic matter and increase the source of FAAs (Huang et al., 2009). This is consistent with Kieloaho et al. (2016) who found that after OM decomposition, released organic N forms, including proteinaceous material, can be degraded into smaller units that can be utilized by the majority of soil organisms and plants. In addition, the increase of soil organic matter content was shown to improve the adsorption of FAAs and reduce its migration (Fischer et al., 2007).

Soil enzymes activity are the main driving factors of organic matter decomposition and nitrogen transformation in soil, which can directly affect the availability of soil nitrogen (Cenini et al., 2016). The application of CMV has been shown to release enzymes into the soil, provide energy and nutrients for soil microorganisms, improve soil enzyme activity, and promote the decomposition of organic matter (Tang et al., 2014). Similarly, in our study, soil protease activity increased significantly after application of CMV (Fig 6), and protease had an important influence on the soil SON concentration, with the path coefficients of 0.71. This is mainly because protease plays a decisive role in the process of protein hydrolysis and conversion, and is an important enzyme in the process of hydrolysis of soil organic nitrogen into amino acids (Mishra et al., 2005; Reiskind et al., 2011). Generally, the complex organic polymers with large molecular weight have been shown to be released after CMV application into the soil, which were difficult to be utilized by microorganisms. Therefore, large molecular weight organic nitrogen needs to be depolymerized by enzymes to release small molecular weight organic nitrogen (e.g., amino acids and amino sugars) before they can be absorbed by plants (Yang et al., 2014).

Soil microbes play an important role in the nitrogen cycle by driving global soil nitrogen mineralization and availability (Li et al., 2019). Our study showed that soil bacterial biomass increased significantly after application of CMV, which is similar to a previous study that showed that turning over CMV can create good organic substrates and carbon resources for soil microorganisms to promote microbial growth and significantly increase soil bacterial biomass (Tao et al., 2017). Microbial decomposition of the soil SOM is considered to be a major factor controlling the amount of soluble OM retained in soil (Qualls et al., 2002). The results of the structural equation model in this study showed that the bacterial biomass could directly affect the soil FAAs content, and the path coefficient was 0.49, which was mainly due to the fact that bacteria were the producers of FAAs generated by decomposition of organic matter (Hossain and Sugiyama, 2020). Secondly, bacterial secretion and death were determined to be one of the sources of FAAs, with the most abundant component of bacterial cells being proteins consisting of amino acid (AA) chains (Miltner et al., 2009). The cell walls of microorganisms contain a large proportion of alanine, aspartate and glutamic acid (Friedel and Scheller, 2002), and a considerable amount of amino acids will be released after bacterial apoptosis. The amino acids in soil are mainly derived from the hydrolysis of proteins by extracellular enzymes. The results of the structural equation of this study also indicated that bacteria can indirectly affect the soil FAAs concentration by affecting the protease activity, with a path coefficient of 0.22. Similar findings were also reported by Hofmockel et al (2010) who found that extracellular enzymes (protease) produced by the microbial community were one of the important factors on the rate of proteolysis.

The composition of the microbial community was closely correlated to the decomposition of organic matter (Xu et al., 2015). Gao et al. (2020) showed that functional microorganisms that played an important role in soil nitrogen (N) cycle changed after years of application of Chinese milk vetch, thus affecting organic nitrogen mineralization. The results of the structural equation model in this study showed that the bacterial community structure could directly impact the soil FAAs concentration, and also indirectly influence the FAAs concentration through the catalytic activities of proteases. This was due to bacteria not only being the source of amino acids, but also absorbing amino acids to meet the needs of life. Therefore, the influence on the amino acid pool is a comprehensive action of the two processes (Philben et al., 2018). Interestingly, the direct influence path coefficient of bacterial community structure on FAAs was -0.72, indicating that the bacterial community structure was closely correlated to FAAs mineralization, and amino acid mineralization was an important amino acid consumption pathway (Moe, 2013). Different bacterial community composition had a direct influence on FAAs dynamics. The results of our studies showed that the phylum of Nitrospirae, Bacteroidetes and Firmicutes significantly affected FAAs dynamics, and their contribution rates were 22.99%, 22.18% and -0.42%, respectively. Nitrospirae phylum was one of the main bacteria affecting soil nitrification, which was shown to reduce the concentration of ammonium nitrogen in soil and promote the mineralization of amino acids (Daims and Wagner, 2018; Ma et al., 2017). The genus of *Cytophaga* and *Flavobacterium* in the Bacteroidetes phylum, and *Bacillus* genus in the Firmicutes phylum were reported to be the dominant proteolytic bacteria in many soils, and they all secreted metalloproteinases to promote the formation of FAAs (Bach and Munch, 2000). The results showed that neutral metallopeptidase secreted by *Bacillus subtilis* and alkaline serine protease secreted by *Bacillus subtilis* were shown to be proteases that control the degradation of peptides in the paddy soil (Watanabe and Hayano, 1994). Therefore, bacteria may directly affect the mineralization of FAAs through Nitrospirae, and indirectly affect the production of FAAs through Bacteroidetes and Firmicutes controlling protease activity.

5 CONCLUSIONS

The concentration and composition of FAAs in paddy soil were significantly affected by the input of green manure and the growth period of rice. Soil FAAs displayed temporal dynamics during the rice growth period, with the pool size increasing from background to the peak level in the seedling stage and then rapidly decreasing to the lowest level in the tillering stage, then increasing gradually to a second peak in the flowering stage and then decreasing gradually. The application of CMV was shown to increase soil FAAs concentrations and types, but excessive application was shown to have an inhibitory effect. Therefore, 30000 kg hm^2 was determined to be a more appropriate application amount in paddy soil. Four different treatments shared six dominant amino acids in the different rice growth periods. Neutral amino acids, especially serine, glycine and proline displayed a strong migration. The relationships between FAAs and environmental factors in farmland ecosystem is multifaceted and complex, mainly being affected by pH, SOM, protease, microbial biomass and microbial community. The phylum of Bacteroidetes, Firmicutes and Nitrospirae were the main bacteria that were shown to affect the dynamics of FAAs, and the total contribution rate of the three bacteria reached 56.89%.

Data availability. The data that support the findings of this study are available from the corresponding author upon request.

Author contributions. JY, WY and SX planned the campaign; JY, YL, performed the measurements; JY, YL, LZ, and BZ analyzed the data; JY wrote the manuscript draft; CR, WY and SX reviewed and edited the manuscript.

Competing interests. The authors declare that they have no conflict of interest.

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References

Bach, H.J., Munch, J.C., 2000. Identification of bacterial sources of soil peptidases. Biol. Fert. Soils 31(3), 219-224. https://doi.org/10.1007/s003740050648.

Cenini, V.L., Fornara, D.A., McMullan, G., Ternan, N., Carolan, R., Crawley, M.J., Clément, J.-C., Lavorel, S., 2016. Linkages between extracellular enzyme activities and the carbon and nitrogen content of grassland soils. Soil Biol. Biochem. 96, 198-206. https://doi.org/10.1016/j.soilbio.2016.02.015.

Chen, J., Wu, Q., Li, S., Ge, J., Liang, C., Qin, H., Xu, Q., Fuhrmann, J.J., 2019. Diversity and function of soil bacterial communities in response to long-term intensive management in a subtropical bamboo forest. Geoderma 354, 113894. https://doi.org/10.1016/j.geoderma.2019.113894.

Chen, J.R., Qin, W.J., Chen, X.F., Cao, W.D., Qian, G.M., Liu, J., Xu, C.X., 2020. Application of Chinese milk vetch affects rice yield and soil productivity in a subtropical double-rice cropping system. J. Integ. Agr. 19(8), 2116-2126. https://doi.org/10.1016/S2095-3119(19)62858-3.

Cheng, H.D., Lu, Y.H., Nie, J., Zhu, Q.J., Nie, X., Cao, W.D., Gao, Y.J., Liao, Y.L., 2020. Effects of reducing chemical fertilizer combined with Chinese milk vetch on soil carbon and nitrogen in paddy fields. J. Agro. Environ. Sci. 39(6), 1259-1270. https://doi.org/10.11654/jaes.2019-1356.

Daims, H., Wagner, M., 2018. Nitrospira. Trends Microbiol. 26(5), 462-463.

Dashman, T., Stotzky, G., 1982. Adsorption and binding of amino acids on homoionic montmorillonite and kaolinite. Soil Biol. Biochem. 14(5), 447-456. https://doi.org/10.1016/0038-0717(82)90103-1.

El-Naggar, A., Rasmussen, J., Neergaard, A., El-Araby, A., Høgh-Jensen, H., 2010. Effect of plant species and temperature on amino acid release from plant material. Agron. Sustain. Dev. 30(3), 679-688. https://doi.org/10.1051/agro/2009049.

Feng, Y.L., Wang, J., Yuan, K., Zong, W.Z., Guo, D., 2018. Vegetation affects pool size and composition of amino acids in Tibetan alpine meadow soils. Geoderma 310, 44-52. https://doi.org/10.1016/j.geoderma.2017.09.018.

Fischer, H., Meyer, A., Fischer, K., Kuzyakov, Y., 2007. Carbohydrate and amino acid composition of dissolved organic matter leached from soil. Soil Biol. Biochem. 39(11), 2926-2935. https://doi.org/10.1016/j.soilbio.2007.06.014.

Friedel, J., Scheller, E., 2002. Composition of hydrolysable amino acids in soil organic matter and soil microbial biomass. Soil Biol. Biochem. 34(3), 315-325. https://doi.org/10.1016/S0038-0717(01)00185-7.

Gao, L., Hill, P.W., Jones, D.L., Guo, Y.F., Gao, F., Cui, X.Y., 2020. Seasonality is more important than forest type in regulating the pool size and composition of soil soluble N in temperate forests. Biogeochemistry 150(3), 279-295. https://doi.org/10.1007/s10533-020-00698-z.

Geisseler, D., Horwath, W.R., 2008. Regulation of extracellular protease activity in soil in response to different sources and concentrations of nitrogen and carbon. Soil Biol. Biochem. 40(12), 3040-3048. https://doi.org/10.1016/j.soilbio.2008.09.001.

Gonzalez Perez, P., Zhang, R., Wang, X., Ye, J., Huang, D., 2015. Characterization of the amino acid composition of soils under organic and conventional management after addition of different fertilizers. J. Soil. Sediment. 15(4), 890-901. https://doi.org/10.1007/s11368-014-1049-3.

Guo, X.C., Cao, Y.S., Xing, S.H (2013) Composition of Soft Free Amino Acid and Its Difference in Three Plantations in Southern China. J. Jiangxi Norm. Univ. 37(03), 310-315. (in Chinese) https://doi.org/10.16357/j.cnki.issn1000-5862.2013.03.011.

Gao, S.J., Cao W.D., Zhou, G.P., Rees, R.M., 2020. Bacterial communities in paddy soils changed by milk vetch as green manure： A study conducted across six provinces in South China. Pedosphere 31(4), 521-530. https://doi.org/10.1016/s1002-016(21)60002-4.

Guo, X.C., Cao, Y.S., Xing, S.H., 2013. Composition of Soft Free Amino Acid and Its Difference in Three Plantations in Southern China. J. Jiangxi Norm. Univ. 37(03), 310-315. (in Chinese) https://doi.org/10.16357/j.cnki.issn1000-5862.2013.03.011.

Harrison, K.A., Bol, R., Bardgett, R.D., 2007. Preferences for different nitrogen forms by coexisting plant species and soil microbes. Ecology 88(4), 989-999. https://doi.org/10.1890/07-1579.1.

Hedges, J.I., Hare, P.E (1987) Amino acid adsorption by clay minerals in distilled water. Geochim. Cosmochim. Ac. 51(2), 255-259. https://doi.org/10.1016/0016-7037(87)90237-7.

Hill, E.J., Jones, D.L., Paterson, E., Hill, P.W., 2019. Hotspots and hot moments of amino acid N in soil: Real-time insights using continuous microdialysis sampling. Soil Biol. Biochem. 131, 40-43. https://doi.org/10.1016/j.soilbio.2018.12.026.

Hofmockel, K.S., Fierer, N., Colman, B.P., Jackson, R.B., 2010. Amino acid abundance and proteolytic potential in North American soils. Oecologia 163(4), 1069-1078. https://doi.org/10.1007/s00442-010-1601-9.

Hossain, M.Z., Sugiyama, S.i., 2020. Relative importance of soil microbes and litter quality on decomposition and nitrogen cycling in grasslands. Ecol. Res. 35(5), 912-924. https://doi.org/10.1111/1440-1703.12163.

Huang, C.C., Liu, S., Li, R.Z., Sun, F.S., Zhou, Y.Z., Yu, G.H (2016) Spectroscopic Evidence of the Improvement of Reactive Iron Mineral Content in Red Soil by Long-Term Application of Swine Manure. PLoS One 11(1), e0146364. https://doi.org/10.1371/journal.pone.0146364.

Huang, Q.R., Hu, F., Huang, S., Li, H.X., Yuan, Y.H., Pan, G.X., Zhang, W.J., 2009. Effect of Long-Term Fertilization on Organic Carbon and Nitrogen in a Subtropical Paddy Soil. Pedosphere 19(06), 727-734. https://doi.org/10.1016/S1002-0160(09)60168-5.

Islam, M.M., Saey, T., De Smedt, P., Van De Vijver, E., Delefortrie, S., Van Meirvenne, M (2014) Modeling within field variation of the compaction layer in a paddy rice field using a proximal soil sensing system. Soil Use Manage. 30(1), 99-108. https://doi.org/10.1111/sum.12098.

Jämtgård, S., Näsholm, T., Huss-Danell, K., 2010. Nitrogen compounds in soil solutions of agricultural land. Soil Biol. Biochem. 42(12), 2325-2330. https://doi.org/10.1016/j.soilbio.2010.09.011.

Jan, M.T., Roberts, P., Tonheim, S.K., Jones, D.L., 2009. Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. Soil Biol. Biochem. 41(11), 2272-2282. https://doi.org/10.1016/j.soilbio.2009.08.013.

Jensen, K.T., Lobmann, K., Rades, T., Grohganz, H (2014) Improving co-amorphous drug formulations by the addition of the highly water soluble amino Acid, proline. Pharmaceutics 6(3), 416-435. https://doi.org/10.3390/pharmaceutics6030416.

Jones, D.L., Shannon, D., Junvee-Fortune, T., Farrar, J.F., 2005. Plant capture of free amino acids is maximized under high soil amino acid concentrations. Soil Biol. Biochem. 37(1), 179-181. https://doi.org/10.1016/j.soilbio.2004.07.021.

Kieloaho, A.J., Pihlatie, M., Dominguez Carrasco, M., Kanerva, S., Parshintsev, J., Riekkola, M.L., Pumpanen, J., Heinonsalo, J., 2016. Stimulation of soil organic nitrogen pool: The effect of plant and soil organic matter degrading enzymes. Soil Biol. Biochem. 96, 97-106. https://doi.org/10.1016/j.soilbio.2016.01.013.

Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32(11), 1485-1498. https://doi.org/10.1016/S0038-0717(00)00084-5.

Laudicina, V., Palazzolo, E., Badalucco, L., 2013. Natural Organic Compounds in Soil Solution: Potential Role as Soil Quality Indicators. Curr. Org. Chem. 17(24), 2991-2997. https://doi.org/10.2174/13852728113179990120.

Li, Z.L., Tian, D.S., Wang, B.X., Wang, J.S., Wang, S., Chen, H.Y.H., Xu, X.F., Wang, C.h., He, N.p., Niu, S.L., 2019. Microbes drive global soil nitrogen mineralization and availability. Global Change Biol. 25(3), 1078-1088. https://doi.org/10.1111/gcb.14557.

Lipson, D.A., Schmidt, S.K., 1999. Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. Ecology 80(5), 1623-1631. https://doi.org/10.1890/0012-9658(1999)080[1623:LBMPDA]2.0.CO.

Liu, Y., Lou, J., Li, F.B., Xu, J.M., Yu, X.S., Zhu, L.A., Wang, F., 2014. Evaluating oxidation–reduction properties of dissolved organic matter from Chinese milk vetch (Astragalus sinicus L.): a comprehensive multi-parametric study. Environ. Technol. 35(15), 1916-1927. https://doi.org/10.1080/09593330.2014.885586.

Ma, H., Pei, G.T., Gao, R., Yin, Y.F., 2017. Mineralization of amino acids and its signs in nitrogen cycling of forest soil. Acta Ecol. Sinica 37(1), 60-63. https://doi.org/10.1016/j.chnaes.2016.09.001.

Ma, Q.X., Wu, L.H., Wang, J., Ma, J.Z., Zheng, N.G., Hill, P.W., Chadwick, D.R., Jones, D.L., 2018. Fertilizer regime changes the competitive uptake of organic nitrogen by wheat and soil microorganisms: An in-situ uptake test using 13C, 15N labelling, and 13C-PLFA analysis. Soil Biol. Biochem. 125, 319-327. https://doi.org/10.1016/j.soilbio.2018.08.009.

Miltner, A., Kindler, R., Knicker, H., Richnow, H.-H., Kästner, M., 2009. Fate of microbial biomass-derived amino acids in soil and their contribution to soil organic matter. Org. Geochem. 40(9), 978-985. https://doi.org/10.1016/j.orggeochem.2009.06.008.

Min, K., Lehmeier, C.A., Ballantyne, F., Tatarko, A., Billings, S.A., 2014. Differential effects of pH on temperature sensitivity of organic carbon and nitrogen decay. Soil Biol. Biochem. 76, 193-200. https://doi.org/10.1016/j.soilbio.2014.05.021.

Mishra, S., Di, H., Cameron, K., Monaghan, R., Carran, A., 2005. Gross Nitrogen Mineralisation Rates in Pastural Soils and Their Relationships with Organic Nitrogen Fractions, Microbial Biomass and Protease Activity under Glasshouse Conditions. Biol. Fert. Soils 42, 45-53. https://doi.org/10.1007/

s00374-005-0863-6.

Moe, L.A., 2013. Amino acids in the rhizosphere: from plants to microbes. Am. J. Bot. 100(9), 1692-1705. https://doi.org/10.3732/ajb.1300033.

Nie, S.A., Zhao, L.X., Lei, X.M., Sarfraz, R., Xing, S.H., 2018. Dissolved organic nitrogen distribution in differently fertilized paddy soil profiles: Implications for its potential loss. Agr. Ecosyst. Environ. 262, 58-64. https://doi.org/10.1016/j.agee.2018.04.015.

Philben, M., Billings, S.A., Edwards, K.A., Podrebarac, F.A., van Biesen, G., Ziegler, S.E., 2018. Amino acid δ15N indicates lack of N isotope fractionation during soil organic nitrogen decomposition. Biogeochemistry 138(1), 69-83. https://doi.org/10.1007/s10533-018-0429-y.

Qualls, R.G., Haines, B.L., Swank, W.T., Tyler, S.W., 2002. Retention of soluble organic nutrients by a forested ecosystem. Biogeochemistry 61(2):135–171. https://doi.org/10.1023/A:1020239112586

Reeve, J.R., Smith, J.L., Carpenter-Boggs, L., Reganold, J,P., 2008. Soil-based cycling and differential uptake of amino acids by three species of strawberry (Fragariaspp.) plants. Soil Biol. Biochem. 40(1), 2547-2552. https://doi.org/10.1016/ j.soilbio.2008.06.015.

Reiskind, J.B., Lavoie, M., Mack, M.C., 2011. Kinetic studies of proteolytic enzyme activity of arctic soils under varying toluene concentrations. Soil Biol. Biochem. 43(1), 70-77. https://doi.org/10.1016/ j.soilbio.2010.09.014.

Rothstein, D.E., 2010. Effects of amino-acid chemistry and soil properties on the behavior of free amino acids in acidic forest soils. Soil Biol. Biochem. 42(10), 1743-1750. https://doi.org/10.1016/j.soilbio.2010.06.011.

Rovira, P., Kurz-Besson, C., Hernàndez, P., Coûteaux, M.-M., Vallejo, V.R., 2008. Searching for an indicator of N evolution during organic matter decomposition based on amino acids behaviour: a study on litter layers of pine forests. Plant Soil 307(1-2), 149-166. https://doi.org/10.1016/j.ijmultiphaseflow.2006.06.004.

Schimel, J., Bennett, J., 2004. Nitrogen mineralization: Challenges of a changing paradigm [Review]. Ecology 85(3), 591-602. https://doi.org/10.1890/03-8002.

Sinsabaugh, R., Lauber, C., Weintraub, M., Ahmed, B., Allison, S., Crenshaw, C., Contosta, A., Cusack, D., Frey, S., Gallo, M., Gartner, T., Hobbie, S., Holland, K., Keeler, B., Powers, J., Stursova, M., Takacs-Vesbach, C., Waldrop, M., Wallenstein, M., Zeglin, L., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. lett. 11, 1252-1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x.

Tang, H.M., Xiao, X.P., Tang, W.G., Lin, Y.C., Wang, K., Yang, G.L., 2014. Effects of winter cover crops residue returning on soil enzyme activities and soil microbial community in double-cropping rice fields. PLoS One 9(6), e100443. https://doi.org/10.1371/journal.pone.0100443.

Tao, J.M., Liu, X.D., Liang, Y.L., Niu, J.J., Xiao, Y.H., Gu, Y.B., Ma, L.Y., Meng, D.L., Zhang, Y.G., Huang, W.K., 2014. Maize growth responses to soil microbes and soil properties after fertilization with different green manures. Appl. Microbiol. Biot 101(3), 1289-1299. https://doi.org/10.1007/s00253-016-7938-1.

Trevino, S.R., Scholtz, J.M., Pace, C.N (2007) Amino Acid Contribution to Protein Solubility: Asp, Glu, and Ser Contribute more Favorably than the other Hydrophilic Amino Acids in RNase Sa. J. Mol. Biol. 366(2), 449-460. https://doi.org/10.1016/j.jmb.2006.10.026.

Warren, C.R., Taranto, M.T., 2010. Temporal variation in pools of amino acids, inorganic and microbial N in a temperate grassland soil. Soil Biol. Biochem. 42(2), 353-359. https://doi.org/10.1016/j.soilbio.2009.11.017.

Watanabe, K., Hayano, K., 1994. Source of soil protease based on the splitting sites of a polypeptide. Soil Sci. Plant Nutr. 40(4), 697-701. https://doi.org/10.1080/00380768.1994.10414310.

Wang, Y.F., Liu, X.M., Butterly, C., Tang, C.X., Xu, J.M., 2013. pH change, carbon and nitrogen mineralization in paddy soils as affected by Chinese milk vetch addition and soil water regime. J Soil Sediment. 13, 654-663. https://doi.org/10.1007/s11368-012-0645-3.

Weintraub, M.N., Schimel, J.P., 2005. The seasonal dynamics of amino acids and other nutrients in Alaskan Arctic tundra soils. Biogeochemistry 73(2), 359-380. https://doi.org/10.1007/s10533-004-0363-z.

Werdin-Pfisterer, N.R., Kielland, K., Boone, R.D., 2009. Soil amino acid composition across a boreal forest successional sequence. Soil Biol. Biochem. 41(6), 1210-1220. https://doi.org/10.1007/s10533-004-0363-z.

Xu, Z.w., Yu, G.R., Zhang, X.Y., Ge, J.P., He, N.P., Wang, Q.F., Wang, D., 2015. The variations in soil microbial communities, enzyme activities and their relationships with soil organic matter decomposition along the northern slope of Changbai Mountain. Appl. Soil Ecol. 86, 19-29. https://doi.org/10.1016/j.apsoil.2014.09.015.

Yang, J., Yang, W., Wang, F., Zhang, L., Zhou, B., Sarfraz, R., Xing, S., 2020. Driving factors of soluble organic nitrogen dynamics in paddy soils: Structure equation modeling analysis. Pedosphere 30(6), 801-809. https://doi.org/10.1016/S1002-0160(18)60032-3.

Yang S H, Peng S Z, Z, X.J., Yan, D., 2015. Effects of water saving irrigation and controlled release nitrogen fertilizer managements on nitrogen losses from paddy fields. Paddy Water Environ. 1(3), 71-80. https://doi.org/10.1007/s10333-013-0408-9.

Yang, Z.P., Zheng, S.X., Nie, J., Liao, Y.L., Xie, J., 2014. Effects of Long-Term Winter Planted Green Manure on Distribution and Storage of Organic Carbon and Nitrogen in Water-Stable Aggregates of Reddish Paddy Soil Under a Double-Rice Cropping System. J. Integ. Agr. 13(8), 1772-1781. https://doi.org/10.1016/S2095-3119(13)60565-1.

Zhu, B., Yi, L.X., Hu, Y.G., Zeng, Z.H., Lin, C.W., Tang, H.M., Yang, G.L., Xiao, X.P., 2013. Nitrogen release from incorporated 15N-labelled Chinese milk vetch (Astragalus sinicus L.) residue and its dynamics in a double rice cropping system. Plant Soil 374(1-2), 331-344. https://doi.org/10.1007/ s11104-013-1808-8.

Zhu, C., Wang, Q., Huang, X.X., Yun, J.N., Hu, Q.L., Yang, G (2019) Adsorption of amino acids at clay surfaces and implication for biochemical reactions: Role and impact of surface charges. Colloids Surface B. 183, 1-8. https://doi.org/10.1016/j.colsurfb.2019.110458.