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# Effect of peanut straw mulching on the soil nitrogen change and functional genes in the *Camellia oleifera* intercropping system

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## Abstract

**Purpose** This study aims to evaluate the impact of peanut straw mulching on the N change and the functional genes in *Camellia oleifera* intercropping systems.

**Methods** A field experiment with different types of straw mulch treatments (conventional tillage, whole, and crushed) and timing was (50 d and 150 d) established between 2018–2022; the soil N fractions, N transformation rates, the abundance and dominant species compositions of ammonia-oxidizing archaea (*AOA*), *nirK*, and *nirS*-harboring genes were investigated.

**Results** The whole peanut straw mulching of 150 d significantly improved ($P < 0.05$) the content of soil microbial biomass N (MBN), ammonia N ($NH_4^+$), and nitrate N ($NO_3^-$). The soil nitrification and ammonification rates increased by 96.8% and 132% in the 150 d of peanut crushed and whole straw mulching, respectively. Notably, the peanut straw mulching of 50 d mainly affects the diversity and relative abundance of *AOA* while the soil *nirK* and *nirS*-harboring genes were affected by 150 d crushed and whole peanut straw mulching, respectively. Redundancy analysis showed that crushed and whole peanut straw mulching affects nitrate reductase as the primary factor in regulating the soil N cycle via functional genes and soil variables.

**Conclusions** Long-term whole peanut straw or whole and crushed mixed straw mulching could hence be recommended to dryland farming communities to increase the soil N cycle and crop productivity in the *C.oleifera*-peanut intercropping system.

**Keywords** Straw mulch · N fractions · Nitrification · Ammonification · *AOA* · *nirK* · *nirS*

## 1 Introduction

*Camellia oleifera (C.oleifera),* an essential agricultural and forestry cash crop, is extensively cultivated across 18 provinces (3.7 million ha) in Southern China (Ye et al. 2021). The oil extracted from mature fruits has wide application in food and industrial processes and together with palm oil, olive oil and coconut oil is one of the world's four major edible tree oils. The high content of unsaturated fatty acids in *C.oleifera* oil is believed to be beneficial to human health (Ma et al. 2011) and may protect the liver from oxidative damage (Lee et al. 2007). However, substantial challenges pervade its cultivation, such as land resource wastage and soil nutrient loss caused by excessive row spacing (Cao et al. 2022; Quan et al. 2022). Established studies suggest that intercropping *C.oleifera* with annual crops such as peanuts, ryegrass, and soybean is an effective management practice for the utilization of soil resources and the promotion of nutrient cycling (Farooq et al. 2021; Wang et al. 2023). However, the information needed to optimize nutrient cycling and water use in such cropping systems remains

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limited, especially for soil nitrogen (N) cycling. For other cropping systems such as maize and orchard system (Wang et al. 2020; Xie et al. 2022), the incorporation of straw has been demonstrated to help sustain yields and to be beneficial in maintaining soil organic carbon and nutrient cycling (Tao et al. 2021). It is therefore critical to improve our understanding of soil N cycling and driving factors in *C.oleifera* intercropping system.

Mulching practices, especially straw mulch, are increasingly used globally as the major protective agricultural measure (Wang et al. 2020). This methodology is considered effective in mitigating soil erosion and increasing soil quality (Marinari et al. 2015; Daryanto et al. 2018). The benefits of straw amendment encompass the improvement of soil physicochemical properties (Zheng et al. 2021; Gao et al. 2022) and the maintenance of root surface area (Yang et al. 2020) which ultimately helps maintain crop yields.

With the introduction of new agronomic practices and cropping systems it has become increasingly important to understand how straw return affects the cycling and availability of essential plant nutrients such as N. Plant available N is closely linked to the inputs of soil organic matter through mineralization and immobilization reactions and is directly affected by the processes governing the transformation of both organic and inorganic N (ammonification, nitrification and denitrification) (Elser et al. 2007; Frouz 2018; Yu et al. 2022). Notably, the genes of soil microbial such as ammonia-oxidizing bacteria (*AOB*), ammonia-oxidizing archaea (*AOA*), nitrifying bacteria (*nirS*), and denitrifying bacteria (*nirK*) are important in N fixation, nitrification, denitrification, mineralization, and other processes of soil N conversion (Tu et al. 2017; Dai et al. 2020; Yang et al. 2023). However, straw mulch has been shown to affect the soil N cycle by influencing microbial and soil variables in many ecosystems, while the research of straw mulch on the N cycling in *C.oleifera* intercropping system remains unknown. Specifically, straw mulch alters the structure and diversity of microbes to regulate the soil N cycling and affects soil aeration, thereby promoting or inhibiting N transformation processes. Understanding these complex interactions and how they are affected by the species used for intercropping and the rate and timing of straw return, is integral to the development of reliable and sustainable cropping systems.

In the work reported here, we used a combination of N fractionation analysis and in situ rate measurements of organic N mineralization, ammonification and nitrification to determine the effects of different forms of peanut straw on soil N transformation processes in a *C.oleifera*-peanut intercropping system. Changes in microbial community structure driving these transformations were investigated using functional gene analysis of *AOA*, *nirS* and *nirK*. The specific aims of this study were to (i) compare the effects of different forms of peanut straw mulch (whole straw and crushed straw) and the duration of mulch application (50 days and 150 days) on organic N mineralization and transformation (ammonification and nitrification rates) and (ii) to model the extent to which straw amendment, N cycle process and N cycle functional genes might be causally connected to each other and to the prevailing environmental variables. We hypothesized that long-term peanut straw mulching benefits for soil N cycle by affecting functional genes and soil variables in *C.oleifera* system.

## 2 Materials and methods

### 2.1 Site description

Field experiments were conducted on a farm, located at the *C.oleifera* experimental station at Central South University of Forestry & Technology (113° 12′ 51″ E, 28° 35′ 14″ N; elevation = 70 m) in Changsha, Hunan Province, China. Based on the United States Department of Agriculture textural classification system, the soils were red sandy loams with 18% sand, 72% silt, and 7% clay. The study area was dominated by a subtropical monsoon climate, with a mean annual precipitation and temperature of 1454.4 mm and 17.9 °C, respectively. Approximately 68% of the precipitation occurs between April to June.

### 2.2 Experiment design

Trees of *C.oleifera* (Theaceae cv 'Huajin') were planted in 2018 at a density of 3 m × 3 m (1,110 plants·ha$^{-1}$). In March 2021, the straw mulch experiment was established using a randomized complete block design with five replications. Between April and October 2021, peanuts were intercropped, with a spacing between plants and rows of 3 m × 2.5 m. At the end of their growth cycle (about 5 months), the peanut crop was harvested and the straw retained. Three experimental treatments were applied: (1) conventional management (CK, control) where plots received no peanut mulching and were weeded monthly by farmers with plant residues regularly removed to ensure soils were bare and uncovered throughout the experiment; (2) with whole peanut straw (WS) with plant residues applied to the soil surface and weeded monthly, the whole peanut straw is peanut straw that is left untreated after harvesting; and (3) crushed peanut straw mulching (CS) where the peanut straw was crushed and left on the soil surface as mulch and weeded monthly, the crushed peanut straw is peanut straw that has been crushed to 1–3 cm with a pulverizer. In total, 18 plots of two rows each were established with six *C. oleifera* trees per row to give a total of 216 trees (Fig. 1). The height, growth morphology, and canopy cover of each

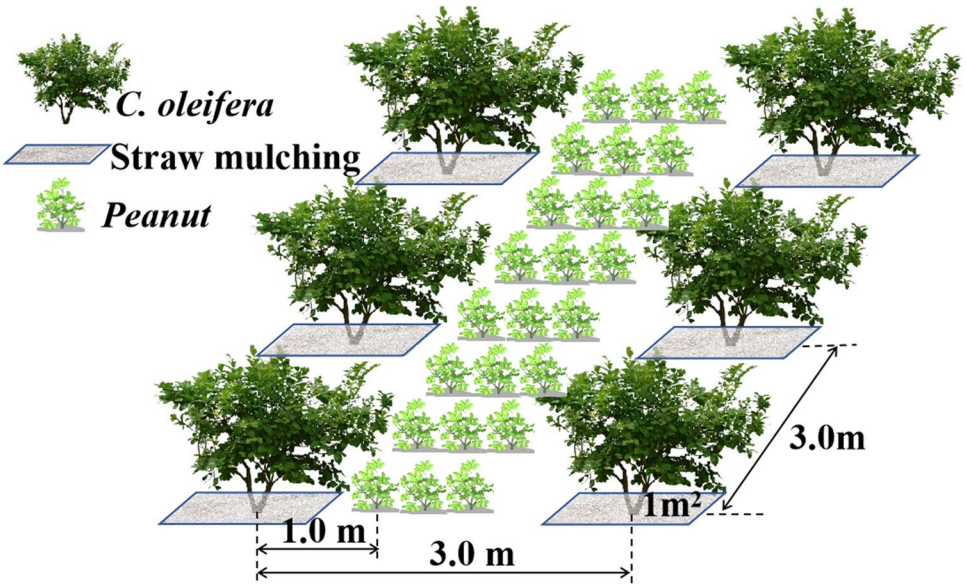


**Fig. 1** The experiment design of *C.oleifera*-Peanut intercropping ecosystem

of the *C.oleifera* trees was consistent between plots, none of the plots received mineral fertilizers during our experiment. To investigate the length of the mulching period on soil N transformation and considering the actual farming practices and the idle period for peanut planting (the period between peanut harvest and replanting peanuts the following year), peanut mulch (rate equivalent to 4000 kg·ha$^{-1}$) was left for 50 days and (CK-1, WS-1 and CS-1) and 150 days (CK-2, WS-2 and CS-2) prior to soil sampling and analysis.

## 2.3 Soil sampling and determination

### 2.3.1 Soil sampling

In both mid-November 2021 and mid-April 2022, five soil cores were randomly collected using a stainless steel corer (5 cm diameter × 20 cm depth) from the mulched areas on each plot using an "S-shaped" sampling method then pooled into one composite sample. Sampling locations were recorded to ensure that different parts of the plot could be randomly sampled at the second sampling. Each plot was sampled six times resulting in total of 180 samples (3 treatments × 5 replicates × 6 samples × 2 time periods). Visible stones and roots residues were removed by hand prior to subsequent analysis. Each of the 30 soil samples were subsampled for chemical and molecular analyses as follows: (1) immediately after sampling and prior to air-drying, soils were mixed thoroughly and weighed to determine their fresh weight; (2) soils were then immediately sieved to 2 mm and stored in constant temperature insulated box at -4 ℃ for subsequent soil enzyme and microbial biomass analysis; (3) soils for molecular analysis were sampled fresh and transferred immediately to sterile centrifuge tubes and kept at -4 ℃ in a constant temperature insulated box with ice cubes for transportation to the laboratory, then stored at -80 ℃ prior to DNA extraction and analysis; (4) soil physic-chemical properties were measured in the laboratory on soils that had been air-dried and sieved to 2 mm and 0.149 mm for further analysis.

### 2.3.2 Physic-chemical and N transformation analyses

Soil organic carbon (SOC) was measured using the $K_2CrO_7$-$H_2SO_4$ oxidation method.Total N (TN) and total phosphorus (TP) contents were determined using Kjeldhal digestion (Jing et al. 2017) and the acid digestion ($H_2SO_4 + HClO_4$ solution) (Hu et al. 2016), respectively. Soil pH values was measured using a Starter-2100 pH probe (Ohaus, Brooklyn, USA) in a 1:2.5 soil solution (0.01 M $CaCl_2$). Inorganic N ($NH_4^+$ and $NO_3^-$) contents were measured in an auto flow analyzer after extraction with KCl as described by (Xiao et al. 2021). Available N (AN) and available potassium (AK) were assessed using the alkali-hydrolyzed reduction diffusion (Xu et al. 2021). Soil moisture content (SMC) was measured by oven-drying the samples at 105 °C for 8 h. Microbial biomass N (MBN) on freshly collected 5.0 g soils was determined using chloroform fumigation-extraction. Protease, nitrate and nitrite reductase were determined using a commercial available kit according to the manufacturer's instructions (Solarbio, http//:www.solarbio.com. Beijing, China).

Mineralization, nitrification, and ammonification rates were determined directly in soil using plastic pipes. Briefly, when the experimental plots were established in October 2021, polyvinyl chloride pipes (5 cm in diameter, 20 cm depth) were inserted into the soil on each plot. Control samples (CK) for each plot were then analyzed for $NH_4^+$ and $NO_3^-$ content. For each plot and at the end of each mulching

period (50 days and 150 days), the PVC columns were removed and the soils analyzed to quantify the mineralization, nitrification, and ammonification rates. Calculate using the following methods:

$$R_I = \frac{I_{2-}I_1}{t} \quad (1)$$

$$R_A = \frac{A_{2-}A_1}{t} \quad (2)$$

$$R_N = \frac{N_{2-}N_1}{t} \quad (3)$$

where the $R_I$, $R_A$ and $R_N$ represent mineralization, ammonification and nitrification rates ($mg \cdot kg^{-1} \cdot d^{-1}$); $I_1$, $I_2$, $A_1$, $A_2$, $N_1$ and $N_2$ is inorganic N, $NH_4^+$ and $NO_3^-$ content before and after mulching, t is the length (days) of the mulching period.

#### 2.3.3 DNA extraction and high-throughput sequencing

Total DNA was extracted from 0.5 g soil samples by the OMEGA soil DNA kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacture's protocols. For amplification, the *AOA*, *nirS*, and *nirK* genes were subjected to polymerase chain reaction (PCR) under the following conditions: initial denaturation at 95 °C for 5 min, followed by 25 cycles at 98 °C for 30 s, 53 °C for 30 s, and 72 °C for 45 s. The final extension step was conducted at 72 °C for 5 min. The barcode consisted of a unique seven-base sequence assigned to each sample. Importantly, the primers utilized for amplifying the *AOA*, *nirS*, and *nirK* genes were distinct from one another. In brief, the forward and reverse primers of *AOA* was Arch-amoA 26F (*5′~GACTACATMTTCTAYACWGAY TGGGC~3′*) and Arch-amoA 417R (*5′~GGKGTCATRTAT GGWGGYAAYGTTGG~3′*) respectively; the forward and reverse primers of *nirS* was cd3aF (*5′~GTSAACGTSAAG GARACSGG~3′*) and R3cdR (*5′~GASTTCGGRTGSGTC TTGA~3′*) respectively; the forward and reverse primers of *nirK* was *nirK* F1aCuF (*5′~ATCATGGTSCTGCCGCG~3′*) and *nirK* R3CuR (*5′~GCCTCGATCAGRTTGTGGTT~3′*) respectively. Amplicons were extracted from 2% agarosegels and purified using the Vazyme VAHTSTM DNA (Nanjing, China) and quantified using Quant-iT PicoGreen dsDNA (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. For quantification, individual amplicons were collected in equal quantities. Subsequently, sequencing was performed using the Illumina NovaSeq platform and NovaSeq 6000 SP kit (500 cycles) at https://www.genescloud.cn/home (Shanghai, China). The sequencing involved a terminal detection of 2 × 250 bp for the sequencing process.

Microbiome bioinformatics adjustments were carried out using QIIME2 version 2019.4. The process involved utilizing the Demux plugin to demultiplex the original sequence data and employing the cutadapt plugin to trim the primers from the sequences. To ensure data quality, the DADA2 plugin was utilized for tasks including quality filtering, denoising, merging, and removal of chimeric sequences from the sequence dataset. Non-monomeric amplicon sequence variants (ASVs) were aligned using Mafft, and the resulting alignments were used to construct a phylogenetic tree using FastTree 2. Additionally, classification of ASVs was achieved using the sklearn naive Bayes classifier within the feature classifier plugin, enabling the assignment of classifications to the ASVs. Following the identification of chimeric sequences and their removal, the retained high-quality sequences were subjected to clustering using UCLUST. A sequence consistency of 97% was employed as the criterion for grouping these sequences into operational taxonomic units (OTUs). The classification of OTU was accomplished through a BLAST search targeting the widely used Greengenes database. This process generated an OTU table, which documented the abundance and taxonomic classification of each in every sample. The OTU that contained less than 0.001% of the total sequence across all samples were excluded. To minimize variations in sequencing depth among samples, an average sparse OTU table was generated by averaging 100 uniformly resampled OTU subsets, ensuring a minimum sequencing depth of 90%. The resulting rounded sparse OTU table was prepared for subsequent analysis. The DNA sequences of *AOA*, *nirS*, and *nirK*-harboring genes from the soil samples in the study have been submitted to the NCBI Sequence Read Archive (SRA) database. The data can be accessed through the following accession numbers: NCBI: PRJNA985852 and SRA: SUB13560277. The NCBI database link is https://ncbi.nlm.nih.gov/.

### 2.4 Statistical Data analysis

A test for normality and homogeneity of variance showed that the data was normally distributed and could be compared using analysis of variance (one-way and two-way ANOVA) with Least Significant Difference (LSD). Tukey' test was used to determine whether the differences between the treatments were significant ($P < 0.05$) at a confidence level of 95%. Pearson's correlation coefficients were used to establish the relationships between soil biotic and abiotic variables. All the statistical analyses were conducted with SPSS 24.0 (SPSS, Inc., Chicago, IL, USA) and the data were presented as the mean value ± standard deviation. Furthermore, redundancy analysis (RDA) and Nonmetric multi-dimensional scaling (NMDS) analysis were performed by the meta-MDS function and in the 'vegan' package. The heatmap of soil *AOA*, *nirK*, and *nirS* relative abundance in

the mulching treatments was constructed using the 'pheatmap' package in R (R Development Core Team, 2023, https://www.r-project.org/).

## 3 Results

### 3.1 Response of straw mulching to soil variables

In comparison to the CK, both AK, TP, TN, SMC, and SOC contents exhibited an increase after addition peanut straw mulching (Table S1). Furthermore, peanut straw mulching had a significant effect on soil stoichiometry ($P < 0.05$), particularly on the C/N and N/P, which consistently remained below 12 and 14, respectively (Table S1). Notably, straw mulching of 50 d led to a significant reduction in the N/P, while 150 d resulted in an improvement in the N/P (Table S1). Peanut straw mulching of 150 d was associated with decreased protease activity compared to 50 d, especially the mulching of 50 d inhibited nitrate reductase activity, while 150 d stimulated it (Fig. S1). However, peanut straw mulching enhanced nitrite reductase activity but no significant difference was observed in relation to straw mulching duration (Fig. S1).

### 3.2 Response of straw mulching on soil N fractions and transformation rates

In WS-1 and WS-2, soil AN was 36.39 $mg \cdot kg^{-1}$ and 19.68 $mg \cdot kg^{-1}$, respectively (Table 1). Mulching increased soil AN by 17.97% and 5.0% compared to the CK. Furthermore, the contents of AN was 29.88 $mg \cdot kg^{-1}$ and 20.99 $mg \cdot kg^{-1}$ in CS-1 and CS-2, respectively. Both CS and WS mulching led to a significant improvement ($P < 0.05$) on soil MBN, $NH_4^+$ and $NO_3^-$ (Table 1). Notably, soil MBN (8.73 $mg \cdot kg^{-1}$) and $NH_4^+$ (11.79 $mg \cdot kg^{-1}$) in WS-2 significantly increased and enhanced by 93.0% and 95.8% compared to WS-1, this effect becomes increasingly evident with the increased duration of mulch application (Table 1). Compared to CK-2 and WS-1, soil MBN in WS-2 improved by 13.74% and 93.01%, respectively. Additionally, the $NH_4^+$ content in WS-2 improved by 57.68% and 95.84%, respectively (Table 1).

Soil mineralization rate in CK-1, WS-1 and CS-1 was 3.63 $mg \cdot kg^{-1} \cdot d^{-1}$, -0.06 $mg \cdot kg^{-1} \cdot d^{-1}$ and -0.02 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively (Table 1). Straw mulch of 50 d led to a significant decline in the soil mineralization rate ($P < 0.05$). However, both the ammonification and nitrification rates were significantly improved in WS-2 and CS-2. Specifically, the ammonification rate in CK-1 was 0.05 $mg \cdot kg^{-1} \cdot d^{-1}$, while the nitrification rate was 0.02 $mg \cdot kg^{-1} \cdot d^{-1}$ (Table 1). In contrast, the ammonification rate in WS-2 increased to 0.11 $mg \cdot kg^{-1} \cdot d^{-1}$, and the nitrification rate in CS-2 was elevated to 0.04 $mg \cdot kg^{-1} \cdot d^{-1}$ (Table 1).

### 3.3 Response of straw mulching on soil AOA, nirK, and nirS-harboring genes

Straw mulching significantly impact the dominant species composition of soil *AOA*, *nirS*, and *nirK* at order level (Fig. 2). When comparing WS-1 and CS-1, both Chao-1 and Shannon index of soil *AOA* decreased in WS-2 and CS-2 (Fig. 3a-b). However, straw mulch of 150 d exhibited an increasing trend in the Chao-1 and Shannon index of soil *nirK* and *nirS* (Fig. 3c-f). Overall, stronger impact of peanut straw mulching time on the α diversity of soil functional genes (Fig. 3).

**Table 1** Soil nitrogen fractions and transformation rates in different peanut straw mulching and times. Different capital and lowercase letters represent that there are significant differences between the same treatment at different times and different treatments at the same time ($P < 0.05$), respectively (n = 5). Significant effects of peanut straw mulching types ($F_S$), times ($F_T$), and co-influence ($F_T \times F_S$) on soil nitrogen fractions and transformation rates were detected by two-way ANOVA ($^{*}$ $P < 0.05$ $^{**}$ $P < 0.01$ $^{***}$ $P < 0.001$ ns $P > 0.05$)

| | CK-1 | WS-1 | CS-1 | CK-2 | WS-2 | CS-2 | $F_S$ ($df = 2$) | $F_T$ ($df = 1$) | $F_T \times F_S$ ($df = 2$) |
|---|---|---|---|---|---|---|---|---|---|
| AN ($mg \cdot kg^{-1}$) | 29.85 ± 3.49Ab | 36.39 ± 3.43Aa | 29.88 ± 5.30Ab | 18.69 ± 1.95Ba | 19.68 ± 2.17Ba | 20.99 ± 2.28Ba | 3.71$^{*}$ | 3.37$^{ns}$ | 102.59$^{***}$ |
| MBN ($mg \cdot kg^{-1}$) | 0.58 ± 0.08Bb | 0.61 ± 0.11Bb | 1.20 ± 0.12Ba | 7.53 ± 0.58Ab | 8.73 ± 0.29Aa | 7.60 ± 0.73Ab | 11.53$^{***}$ | 5.74$^{***}$ | 230.23$^{***}$ |
| $NO_3^-$ ($mg \cdot kg^{-1}$) | 3.22 ± 0.44Bb | 4.93 ± 0.62Ba | 3.40 ± 0.30Bb | 5.41 ± 0.63Ab | 8.10 ± 0.45Aa | 7.68 ± 1.05Aa | 6.97$^{**}$ | 31.47$^{***}$ | 198.03$^{***}$ |
| $NH_4^+$ ($mg \cdot kg^{-1}$) | 0.14 ± 0.01Ba | 0.49 ± 0.10Ba | 1.19 ± 0.08Ba | 4.99 ± 1.09Ac | 11.79 ± 0.83Aa | 8.64 ± 1.63Ab | 26.31$^{***}$ | 33.06$^{***}$ | 454.19$^{***}$ |
| M-rate ($mg \cdot kg^{-1} \cdot d^{-1}$) | 3.63 ± 0.57Aa | -0.06 ± 0.01Ab | -0.02 ± 0.01Ab | 0.07 ± 0.02Ba | 0.15 ± 0.01Aa | 0.12 ± 0.02Aa | 212.79$^{***}$ | 199.07$^{***}$ | 157.71$^{***}$ |
| A-rate ($mg \cdot kg^{-1} \cdot d^{-1}$) | 0.02 ± 0.01Ba | 0.03 ± 0.01Ba | -0.001 ± 0.01Ba | 0.05 ± 0.01Ac | 0.11 ± 0.01Aa | 0.07 ± 0.02Ab | 28.56$^{***}$ | 43.06$^{***}$ | 592.76$^{***}$ |
| N-rate ($mg \cdot kg^{-1} \cdot d^{-1}$) | 0.02 ± 0.01Ba | -0.07 ± 0.01Bc | -0.02 ± 0.01Bb | 0.02 ± 0.01Ab | 0.03 ± 0.01Aab | 0.04 ± 0.01 | 31.78$^{***}$ | 22.88$^{***}$ | 195.07$^{***}$ |

AN, available nitrogen; $NO_3^-$, nitrate nitrogen; $NH_4^+$, ammonia nitrogen; MBN, microbial biomass nitrogen; M-rate, mineralization rate; A-rate, ammonification rate; N-rate, nitrification rate. CK-1, no peanut straw mulching 50 d; CK-2, no peanut straw mulching 150 d; CS-1, crushed peanut straw mulching 50 d; CS-2, crushed peanut straw mulching 150 d; WS-1, whole peanut straw mulching 50 d; WS-2, whole peanut straw mulching 150 d

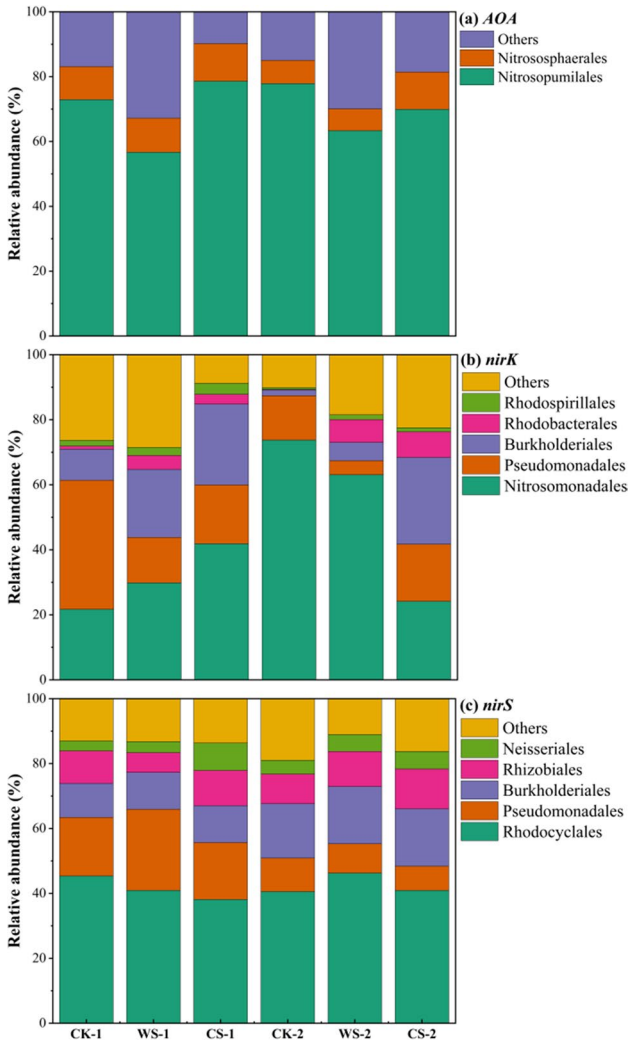


**Fig. 2** Relative abundance of soil *AOA* (**a**), *nirK* (**b**), and *nirS* (**c**) at the order level. CK-1, no peanut straw mulching 50 d; CK-2, no peanut straw mulching 150 d; CS-1, crushed peanut straw mulching 50 d; CS-2, crushed peanut straw mulching 150 d; WS-1, whole peanut straw mulching 50 d; WS-2, whole peanut straw mulching 150 d

The β diversity of soil functional genes was significantly difference (stress value < 0.2), especially in CS (Fig. 4). Response of soil *AOA* functional gene was more sensitive to straw mulch, the 150 d resulted in a change from a "random" to a "conservative" distribution for *AOA* (Fig. 5a). However, mulching of 150 d changed the expression of soil *nirK* and *nirS* functional gene while consistently maintaining the "conservative" distribution pattern (Fig. 5b-c). *Nitrososphaera*, *Nitrosopumilus*, and *Candidatus Nitrososocosmicus* are the dominant species compositions of *AOA,* and CS-1 significantly increased their relative abundance (Fig. 5a). Furthermore, mulching of 150 d improved the expression of dominant species compositions expression for soil *nirK* and *nirS* (Fig. 5b-c). Especially in CS-2 had a significant impact on *nirK* (*Pleomorphomonas, Devosia, Paracoccus, Achromobacter, Rhizobium,* and *Mesorhizobium*) and WS-2 on *nirS* (*Thaurea, Acidovoras, Pseudogulbenkia, Herbaspirllum, Brachymonas, Dechloromonas, and Zoogloea*) (Fig. 5b-c). The overall 50 d and 150 d peanut crushed mulching is beneficial for *AOA* and *nirK*, respectively while 150 d peanut whole mulching is conducive to *nirS* (Fig. 5).

### 3.4 Redundancy analysis of soil variables, N fractions, and functional genes in response to straw mulching

Redundancy analysis (RDA) showed that increasing the duration of straw mulch enhanced the positive relationship between the soil inorganic N and *AOA*, especially $NH_4^+$ (explain = 45%, F = 5.7, and $P = 0.002$) under WS conditions (Fig. 6a-b and Table S2). The interaction between soil variables, N fractions, and N cycle functional genes (*nirK*) was stronger in CS-1 than in WS-1. However, higher community compositions of soil *nirK*-harboring genes involved in interactions in WS-2 than CS-2. Overall, soil N cycle regulated by microorganisms and variables has shifted from being dominated by CS to WS with the increasing duration of straw mulch (Fig. 6c-d). WS and CS both had a positive effect on the interactions between soil variables, the *nirS*, and N fractions, but more community of soil *nirS*-harboring genes with the increasing mulching time. Soil nitrate was the primary factor to affect soil N cycle by crushed and whole peanut straw mulching in *C.oleifera* intercropping system (Fig. 6e-f and Table S2).

## 4 Discussion

### 4.1 Effects of straw mulching on soil variables

Our results demonstrated that the peanut straw mulching of 150 d improved SOC, TP, TN, protease, nitrate reductase, and nitrite reductase (Fig. S1 and Table S1). The result is consistent with the findings that straw mulch as an organic mulch usually contributes SOC and TN to soil and enhance the nutrient cycle and quality (Wang et al. 2016; Chenu et al. 2019). Straw mulch increases soil water availability, regulates pH, and alters the surface temperature and humidity (He et al. 2023; Huang et al. 2021). Furthermore, it enhances the absorption and transportation of nutrient elements by plant roots in intercropping systems (Li et al., 2022). As a result, soil organic matter accelerates decomposition, both directly and indirectly, leading to an increase in soil nutrient content (Wang et al. 2020; Tejada and Benitez 2014).

Whole straw mulching was more beneficial for increasing soil nutrients (Table S1) in our study is likely due to its lower ground runoff loss compared to crushed (Wang et al. 2020). Crushed straw mulching is more favourable for

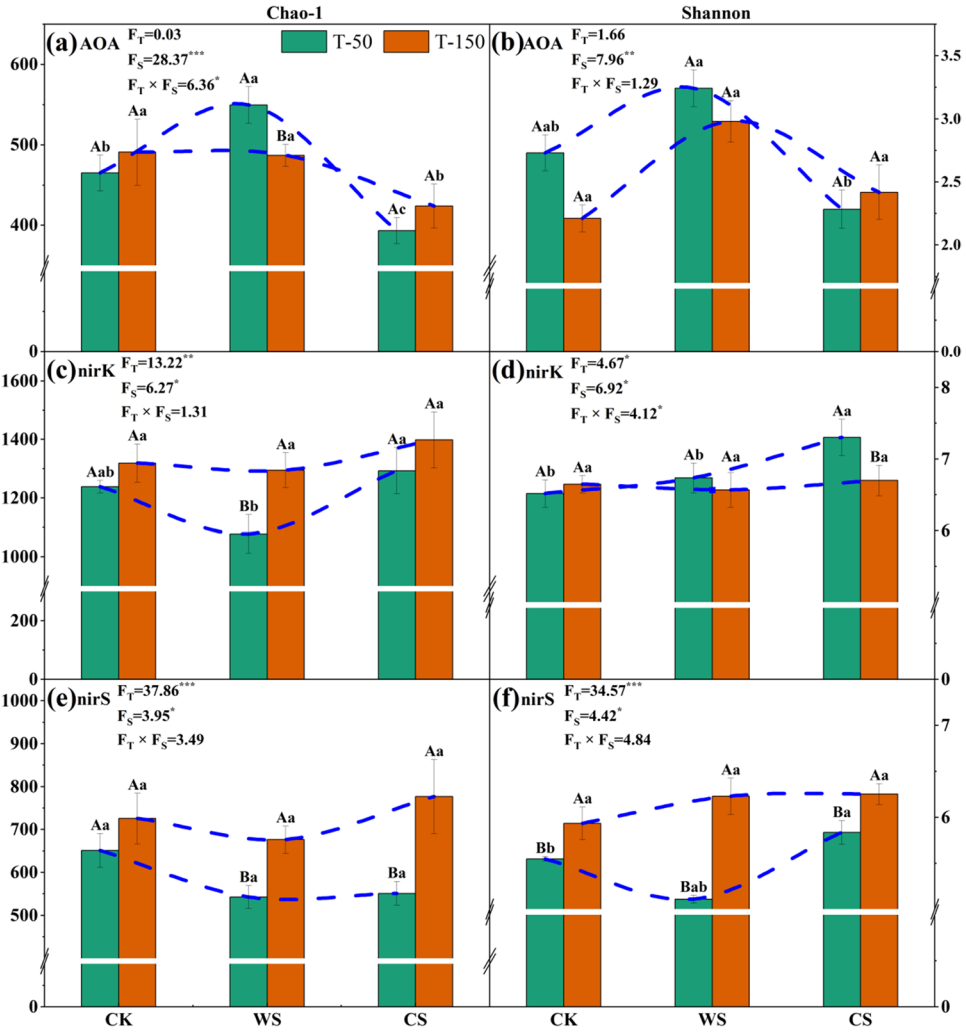


**Fig. 3** Barplots showing the α diversity of soil *AOA*, *nirS*, and *nirK* at different peanut straw mulching types and times. Different capital letters represent significant differences between the same treatments at different mulching times, different lowercase letters represent significant differences between different treatments at the same mulching times. Significant effects of straw mulch types ($F_S$), times ($F_T$), and co-influence ($F_T \times F_S$) on the α diversity were detected by two-way ANOVA ($^{*}$ $P<0.05$ $^{**}$ $P<0.01$ $^{***}$ $P<0.001$.$^{ns}$ $P>0.05$)

increasing soil enzyme activities compared to whole peanut straw mulching (Fig. S1). This is attributed to its ability to enhance the contact area between straw and soil microbes and enzymes, leading to the formation of a greater amount of humus in the soil than whole straw mulching (Huang et al. 2021). To be specific, crushed peanut straw mulching particularly provides a conducive environment for microorganisms, thereby promoting the release and formation of both extracellular and intracellular enzymes.

## 4.2 Effects of straw mulching on soil N fractions and transformation rate

Soil inorganic N ($NO_3^-$ and $NH_4^+$), AN, and MBN serve as essential N sources for crop productivity in terrestrial ecosystem (Zhong et al. 2015). Our study found that soil N fraction contents improved with 150 d straw mulch except for AN (Table 1). This is mainly because of the contribution of peanut straw mulching to soil N pool through crop residue decomposition and the reduction of soil $NO_3^-$ and $NH_4^+$ leaching (Tejada & Benitez 2014; Zhang et al. 2016). In addition, straw mulch directly affects soil aeration to promote microbial activity leading to the sequestration of N sources in the anaerobic habitat (Biswal et al. 2022; He et al. 2023), which is beneficial for enhancing the content of MBN, $NO_3^-$ and $NH_4^+$. However, soil AN can be directly absorbed by plants (Huang et al. 2021), decreases with the growth cycle of *C.oleifera* and the reduction of exogenous N input, which results in an imbalance between input and output. Similarly, the effects of ground runoff loss on $NO_3^-$, $NH_4^+$, AN, and MBN likely explain why whole straw mulching is more beneficial for increasing N fractions (Huang et al. 2021).

Soil nitrification and ammonification rates increased with the time of peanut straw mulching in our study (Table 1). Nitration and ammonification are critical processes in soil N cycle, and their rates are essential for regulating N sequestration, particularly regarding soil $NO_3^-$ and $NH_4^+$ contents. Aerobic and anaerobic ammonia oxidation processes are important for returning N to the atmosphere from terrestrial

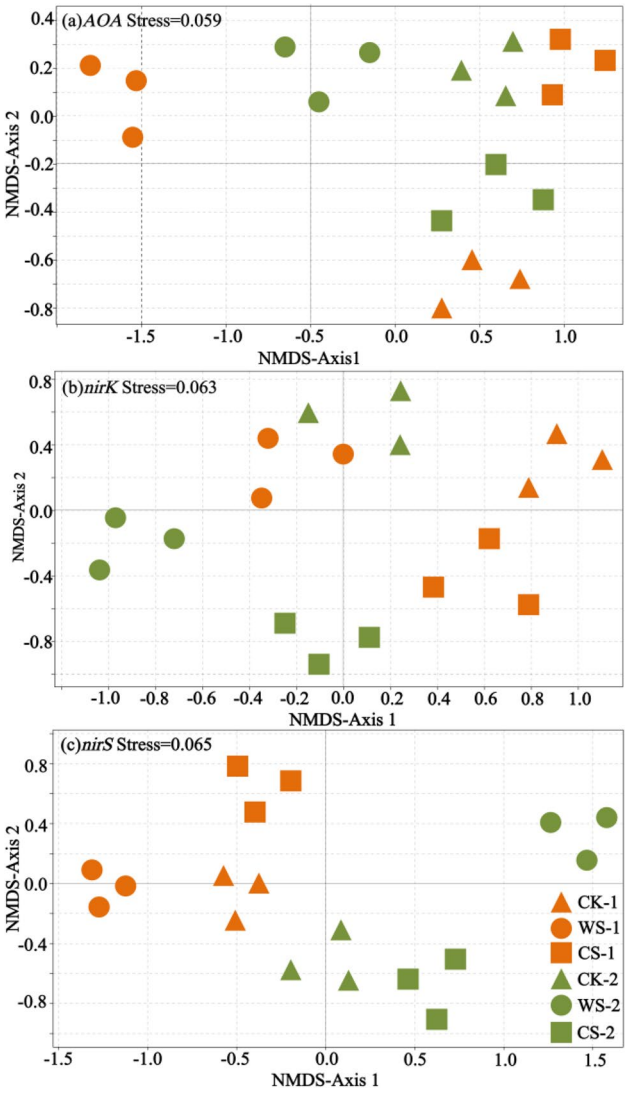


**Fig. 4** Nonmetric multi-dimensional scaling (NMDS) analysis of soil *AOA* (**a**), *nirK* (**b**), and *nirS* (**c**) based on Bray-curits in different peanut straw mulching types and times (n = 3). CK-1, no peanut straw mulching 50 d; CK-2, no peanut straw mulching 150 d; CS-1, crushed peanut straw mulching 50 d; CS-2, crushed peanut straw mulching 150 d; WS-1, whole peanut straw mulching 50 d; WS-2, whole peanut straw mulching 150 d

ecosystems that have accumulated N from biological N fixation, deposition, and fertilizers. Whole and crushed straw mulching can enhance anaerobic conditions (Biswal et al. 2022) while maintaining soil temperature and humidity (Huang et al. 2021). Compared to nitrification, which can only be carried out under aerobic conditions, ammonification can occur through the participation of relevant microorganisms under both anaerobic and aerobic conditions. In the early stage of straw mulch, due to the undecomposed residue, soil anaerobic conditions became more pronounced, and with the increase of mulching time, anaerobic conditions weakened. This explains why the nitrification rate decreases in 50 d mulching and increases in 150 d in this study (Table 1).

### 4.3 Effects of straw mulching on AOA, nirS, and nirK-harboring genes

Our results indicated an increasing trend in both diversity and relative abundance of soil *AOA, nirS,* and *nirK*-harboring genes with peanut straw mulching and led to a change in the composition of dominant species (Fig. 2–4). Straw mulch, as an effective practice of straw return, plays a pivotal role in reducing the burning of straw waste and increasing nutrient input while maintaining soil temperature and humidity (Zhang et al. 2023). Nitrification is believed to be driven by ammonia-oxidizing bacteria (*AOB*) and ammonia-oxidizing archaea (*AOA*). Denitrification is driven by different groups, particularly nitrite-reducers harboring a nitrite reductase encoded by either the *nirS* or *nirK* genes (Kandeler et al. 2006). In agricultural ecosystems, agricultural residue removal tends to reduce the surface soil temperature, moisture availability, and soil organic matter content. This can lead to anaerobic microbial dormancy and even the transformation of microorganisms into microbial residues (Frouz 2018; Kooch et al. 2020). However, straw mulch has the opposite effect, improving surface soil temperature, moisture availability, and introducing more organic matter, SOC, and TN input (Huang et al. 2021). As a result, the relative abundance of dominant species in soil N cycle functional genes, including *AOA*, *nirK*, and *nirS*, tends to be higher with straw mulching.

The abundance and expression of soil *AOA*, *nirK*, and *nirS*-harboring genes exhibited significant differences based on peanut straw mulching types and timing (Fig. 5). Numerous studies showed that short-term straw mulching appears to regulate soil pH and aeration, facilitating the rapid introduction of organic matter and nutrients from crushed straw, thus rise to a more pronounced relative abundance of *AOA* (Kandeler et al. 2006; Zhang et al. 2023). In contrast, *nirK* and *nirS*-harboring genes which are key microorganisms involved in the denitrification process, demonstrate a higher relative abundance in the case of long-term mulching, as indicated in Fig. 5. This phenomenon can likely be attributed to the fact that long-term mulching is more effective in creating an oxygen-isolated environment, with precipitation accelerating the decomposition of straw. This process not only preserves reaction substrates but also provides an ideal anaerobic setting for denitrification processes to take place (Zhong et al. 2015; Huang et al. 2021). Additionally, the extended use of straw mulch appears to enhance soil nutrient cycle, leading to increased release and absorption of extracellular enzymes and nutrients by plant roots, constitutes a significant factor contributing to the elevated relative abundance of *nirK* and *nirS* (Ma et al. 2021, Fracetto et al. 2017).

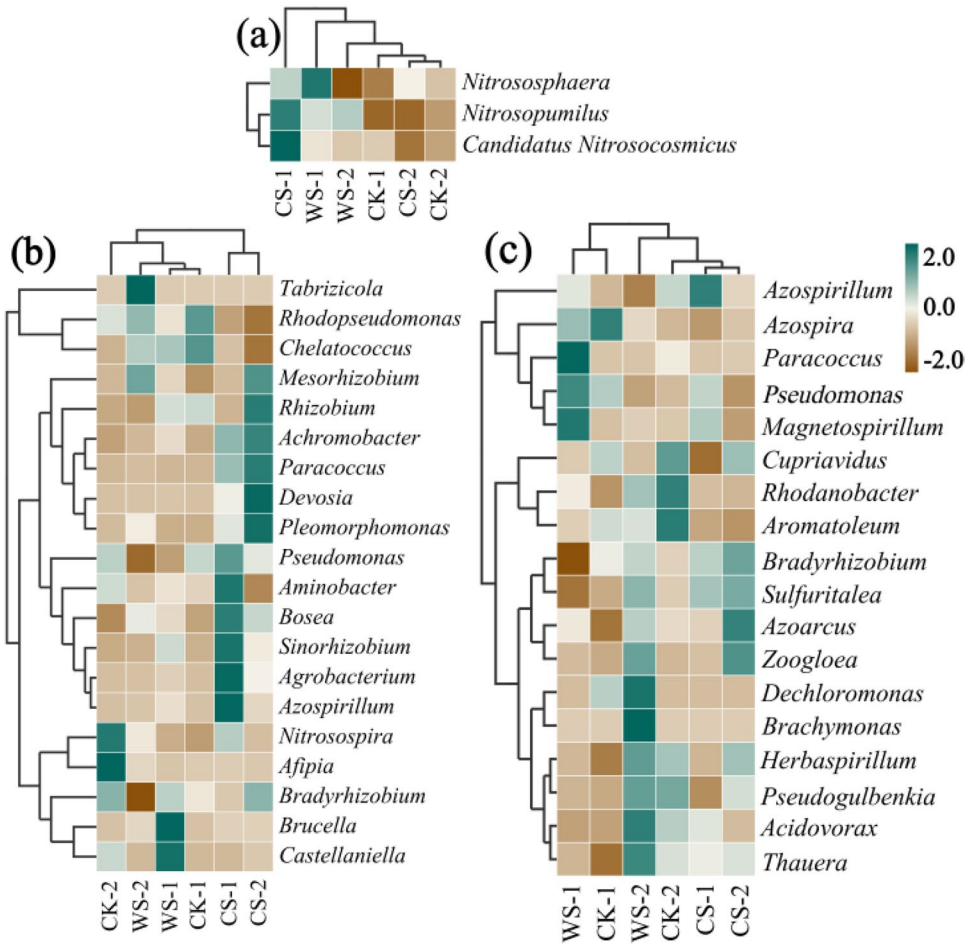


**Fig. 5** Species compositions of soil *AOA* (**a**), *nirK* (**b**), and *nirS* (**c**) at the order level in different straw mulch types and times, the color of dark green (high) and brown (low) represent the relative abundance expression of soil nitrogen cycle functional genes. CK-1, no peanut straw mulching 50 d; CK-2, no peanut straw mulching 150 d; CS-1, crushed peanut straw mulching 50 d; CS-2, crushed peanut straw mulching 150 d; WS-1, whole peanut straw mulching 50 d; WS-2, whole peanut straw mulching 150 d

### 4.4 The Mechanism of straw mulching on soil variables, N transformation rates, and N cycle functional genes

Our results found that the soil N fractions and N cycle become increasingly pronounced with the duration of mulching, primarily driven by changes in nitrate reductase (Fig. 6). This is because the agricultural soils are subject to frequent tillage practices and subsequent depletion of soil organic matter, leading to the loss of various nutrients and the instability of microbial communities. However, the increasing of straw mulch time has been shown to reduce the frequency of non-tillage activities, thereby enhancing the stability of soil properties (Qiu et al. 2020). In addition, SMC exhibits a rapid response to peanut straw mulching and the sensitivity of microorganisms to alterations in their habitat has emerged as a prominent factor influencing changes in other factors (Qiu et al. 2020; Ma et al. 2021; Zhao et al. 2023). As an example, microbial diversity has both direct and indirect effects on the release of soil enzymes and the retention of MBN. Simultaneously, SMC has both direct and indirect impacts on the conversion and loss of $NH_4^+$ and $NO_3^-$ (Srivastava et al. 2016; Zhang et al. 2022). However, we believe that soil aeration, especially in anaerobism habitat, resulting from straw mulch may also play a crucial role in influencing soil N cycle in the *C.oleifera* intercropping system (Fig. 6). Overall, peanut straw mulching affects nitrate reductase as a key factor driving soil N cycle through changes in functional genes and soil variables.

## 5 Conclusions

In conclusion, this study demonstrated that peanut straw mulching as a rate of 4000 kg·ha$^{-1}$ significantly enhances the soil nutrient levels, N fractions, ammonification, and nitrification rates. The effect is particularly pronounced with whole peanut straw mulching after addition 150 d. Peanut straw mulching drives significant differences in the diversity and relative abundance of soil N cycle functional genes, particularly the dominant species of *nirK* and *nirS*-harboring genes. Furthermore, interactions between soil variables, N fractions, *AOA*, *nirK*, and *nirS* exhibit a significant strengthening phenomenon with peanut straw mulching. Our study provides a clear empirical evidence supporting the crucial role of agricultural residues on soil

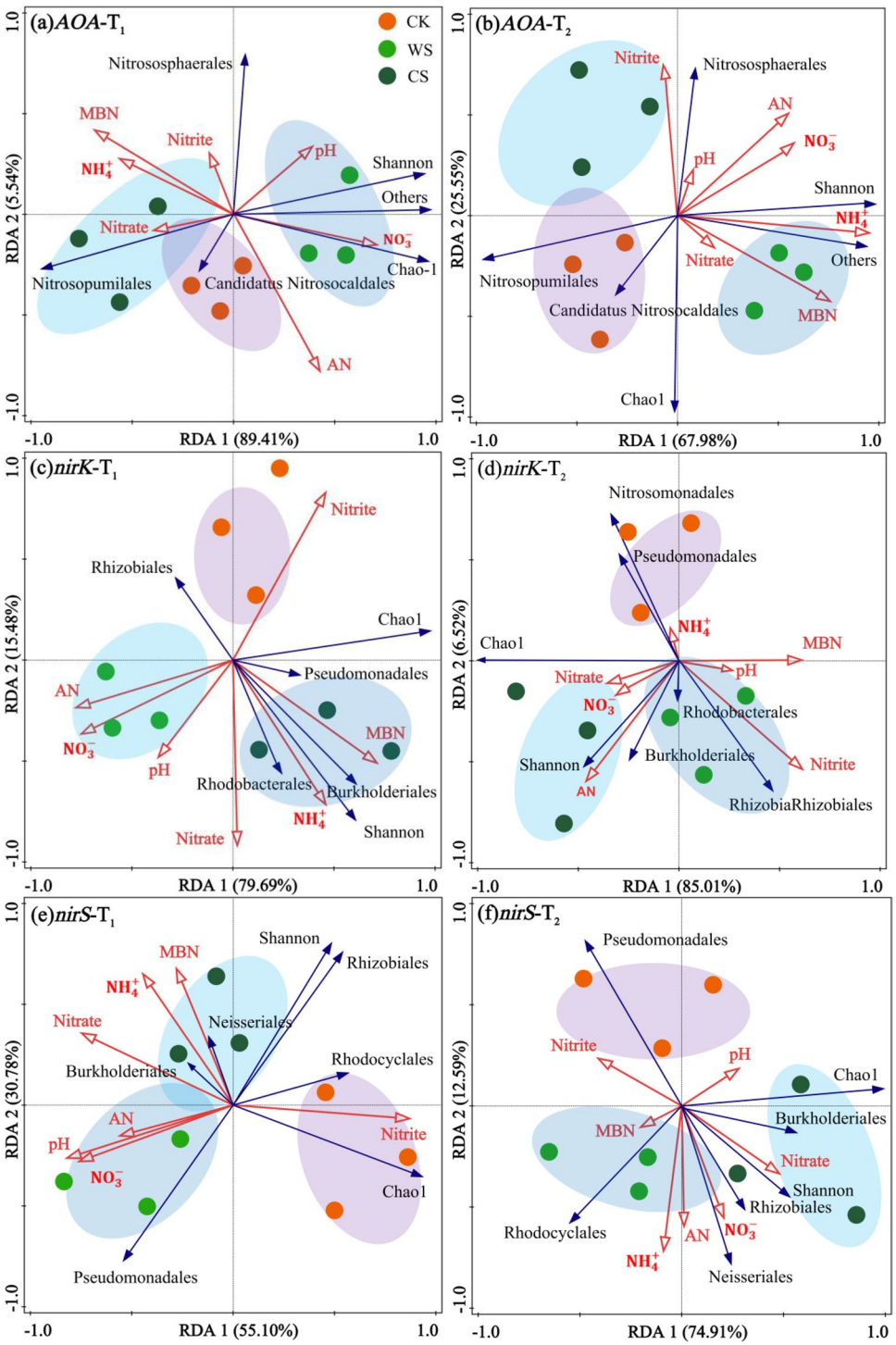


**Fig. 6** Redundancy analysis (RDA) of soil nitrogen fractions ($NO_3^-$, $NH_4^+$, AN, and MBN), variables (pH, nitrate, and nitrite reductase), and nitrogen cycle functional genes (*AOA*, *nirK*, and *nirS*) in different peanut straw mulching types and times. CK, no peanut straw mulching; CS, crushed peanut straw mulching; WS, whole peanut straw mulching. $T_1$, 50 d; $T_2$, 150 d

nutrient cycle in the agroecosystem, and potential practices include short-term crushed and whole peanut straw mulching blends as well as long-term whole peanut straw mulching to enhance the soil N cycle.

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**Data availability** Data will be made available on request.

## Declarations

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

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