# PLFA Analysis of the Effects of Continuous Cropping on Microbial Community Structure in Processing Tomato Rhizosphere Soil (Postprint)

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## Abstract

A fixed-point microplot experiment of processing tomato continuous cropping was conducted at the experimental station of the College of Agriculture, Shihezi University. Using a combination of chloroform fumigation and phospholipid fatty acid (PLFA) analysis, the effects of different continuous cropping treatments (1-year planting, 3-year, 5-year, and 7-year continuous cropping) on rhizosphere soil microbial community structure and soil microbial biomass at the flowering-fruiting and maturity stages of processing tomatoes in Xinjiang were investigated. The results showed that continuous cropping led to decreases in soil microbial biomass carbon (SMBC), microbial biomass nitrogen (SMBN), and microbial quotient (qMB), while the SMBC/SMBN ratio increased. Microbial biomass phosphorus (SMBP) varied differently with continuous cropping years and growth stages. Continuous cropping significantly increased fungal PLFAs content, decreased bacterial PLFAs content, total soil PLFAs, and the bacteria/fungi PLFAs ratio, while actinomycetal PLFAs content showed no consistent pattern. Under 7-year continuous cropping, bacterial PLFAs content and total soil PLFAs at the maturity stage decreased by 62.9% and 50.3% ($P<0.05$), respectively, compared with the control, whereas fungal PLFAs content increased by 60.2% ($P<0.05$). Analysis of diversity indices revealed that Shannon-Wiener index, Simpson index, Brillouin index, and Pielou index all exhibited an initial increase followed by a decrease with prolonging continuous cropping years, reaching maximum values under 3-year continuous cropping and minimum values under 7-year continuous cropping, indicating that within the experimental time frame, continuous cropping reduced microbial community diversity and evenness to some extent. Correlation analysis demonstrated relationships among various soil microbial group PLFAs, microbial biomass, and soil fertility, suggesting that soil microbial biomass is correlated with soil fertility level and can serve as a biological indicator for evaluating soil fertility. These findings indicate that processing tomato continuous cropping altered soil

microbial community structure, reduced soil microbial biomass, and ultimately led to continuous cropping obstacles under the combined effects of rhizosphere soil microecosystem and environmental factors.

## Full Text

## Preamble

### Microbial Community Structure in Rhizosphere Soils of Long-Term Continuously Cropped Processing Tomato Based on PLFA Method

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## Abstract

A fixed microplot experiment was conducted at the experimental station of the College of Agriculture, Shihezi University to investigate the effects of continuous cropping on rhizosphere soil microbial community structure and soil microbial biomass in processing tomato. Using a combination of chloroform fumigation and phospholipid fatty acid (PLFA) analysis, we examined soils at the flowering-fruit bearing stage and maturity stage under different continuous cropping treatments (1-year planting, 3-year, 5-year, and 7-year continuous cropping). The results demonstrated that continuous cropping led to decreases in soil microbial biomass carbon (SMBC), microbial biomass nitrogen (SMBN), and microbial biomass entropy (qMB), while the SMBC/SMBN ratio increased. Microbial biomass phosphorus (SMBP) showed variable responses depending on cropping duration and growth stage. Continuous cropping significantly increased fungal PLFA content while decreasing bacterial PLFA content, total soil PLFA content, and the ratio of bacterial to fungal PLFAs. Actinomycete PLFA content exhibited no consistent pattern. After 7 years of continuous cropping, bacterial PLFA content and total soil PLFA content at the maturity stage decreased by 62.9% and 50.3%, respectively ($P<0.05$), compared to the control, whereas fungal PLFA content increased by 60.2% ($P<0.05$). Diversity index analysis revealed that Shannon-Wiener, Simpson, Brillouin, and Pielou indices all initially increased then decreased with prolonged continuous cropping, reaching maximum values at 3 years and minimum values at 7 years. This indicates that within the experimental timeframe, continuous cropping reduced both microbial community diversity and evenness to some extent. Correlation analysis showed significant relationships among various microbial group PLFAs, microbial biomass, and soil fertility, suggesting that soil microbial biomass is correlated with soil fertility and can serve as a biological indicator for evaluating soil fertility. In conclusion, continuous cropping of processing tomato altered

soil microbial community structure, reduced soil microbial biomass, and ultimately induced continuous cropping obstacles through the combined effects of rhizosphere soil microecosystem and environmental factors.

**Keywords:** Processing tomato; Continuous cropping; Soil microbial biomass; Microbial community structure; Phospholipid fatty acid analysis

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## Methods

Principal components analysis (PCA) and cluster analysis were employed for data analysis, with Duncan' s multiple range test used for inter-treatment comparisons.

### 2.1 Effects of Continuous Cropping on Soil Microbial Biomass Carbon, Nitrogen, and Phosphorus

Within 3 years of continuous cropping, SMBC, SMBN, SMBP, and qMB at the maturity stage were greater than those at the flowering-fruit bearing stage, but this pattern reversed after 5 years of continuous cropping (Table 2 ). At 3 years of continuous cropping, SMBC, SMBN, SMBP, and qMB at the maturity stage were 1.11, 1.18, 1.81, and 1.11 times greater than at the flowering-fruit bearing stage, respectively, with differences being highly significant (P<0.01).

Continuous cropping of processing tomato caused decreases in SMBC, SMBN, and qMB, while increasing the SMBC/SMBN ratio (Table 2). After 7 years of continuous cropping, SMBC, SMBN, and qMB at the maturity stage decreased by 52.3%, 78.8%, and 48.2%, respectively, compared to the control, while SMBC/SMBN was 2.25 times that of the control, with highly significant differences (P<0.01). At the flowering-fruit bearing stage, SMBP showed an increasing trend with continuous cropping duration, whereas at the maturity stage, SMBP first increased then decreased. After 7 years of continuous cropping, SMBP content decreased by 54.9% compared to the control, and SMBP content at the flowering-fruit bearing stage was 4.77 times that at the maturity stage, with highly significant differences (P<0.01).

### 2.3 Effects of Continuous Cropping on Total Soil PLFAs

A total of 16 PLFAs were detected in the soil samples (Figure 1 [Figure 1: see original paper]). Among these, 14 PLFAs served as bacterial markers: six for Gram-positive bacteria (12Me13:0, 12Me14:0, 14Me15:0, 14Me16:0, i16Me17:0, 17Me18:0), one for Gram-negative bacteria $(16{:}1\$\ 11c), two for aerobic bacteria (16:1\ 7, 16:1\ 9c), and five for other bacteria (14:0, 15:0, 16:0, 17:0, 20:0). Additionally, one PLFA marker for actinomycetes (10Me18:0) and one for fungi (18:1\ \$9c)$ were identified.

Total PLFA content in processing tomato rhizosphere soil varied with

growth stage and continuous cropping duration (Figure 1). At the flowering-fruit bearing stage, 14Me15:0 showed the highest content after 3 years of continuous cropping, followed by 16:1$ 9$c. At the maturity stage, 18 : 1 9$c exhibited the highest content after 7 years of continuous cropping. Overall, the most abundant PLFA markers$ 0, 16 : 0, $i16Me17 : 0, $and 18 : 1 $9c.

Total microbial PLFA content in processing tomato rhizosphere soil showed a significant decreasing trend with continuous cropping duration, but followed a pattern of initial increase then decrease across growth stages (Table 3 ). After 7 years of continuous cropping, total microbial PLFA content decreased by 41.8% and 50.3% at the flowering-fruit bearing and maturity stages, respectively, compared to the control, with highly significant differences ($P<0.01$). Within 5 years of continuous cropping, total microbial PLFA content was greater at the maturity stage than at the flowering-fruit bearing stage, but this pattern reversed after 7 years (Table 3). Specifically, after 7 years of continuous cropping, total microbial PLFA content at the maturity stage was 10% lower than at the flowering-fruit bearing stage. These results demonstrate that total PLFA content in processing tomato rhizosphere soil varied substantially depending on both growth stage and continuous cropping duration.

### 2.4 Effects of Continuous Cropping on Major Microbial Groups (PLFAs)

Based on different PLFA marker types, soil microbial communities can be broadly classified into four major groups: bacteria, fungi, actinomycetes, and protozoa [19]. As shown in Table 3, microbial communities in continuously cropped soils comprised bacteria, actinomycetes, and fungi, with no protozoa detected. At both growth stages, the relative abundance of microbial PLFAs followed the order: bacteria > fungi > actinomycetes, with bacterial PLFAs accounting for 69–93% of total microbial PLFAs, fungal PLFAs for 5–28%, and actinomycete PLFAs for 2–26%.

Continuous cropping of processing tomato significantly decreased bacterial PLFA content while increasing fungal PLFA content (Table 3). After 7 years of continuous cropping, bacterial PLFA content at the maturity stage decreased by 62.9% compared to the control, whereas fungal PLFA content increased by 151.4%, with significant differences ($P<0.05$). Within 3 years of continuous cropping, bacterial PLFA content was greater at the maturity stage than at the flowering-fruit bearing stage, but this trend reversed after 5 years. Under the same continuous cropping duration, fungal PLFA content was higher at the maturity stage than at the flowering-fruit bearing stage, showing the opposite pattern to actinomycete PLFA content (Table 3). At the maturity stage, actinomycete PLFA content reached its maximum after 3 years of continuous cropping, being 1.29 times that of the control, with highly significant differences ($P<0.01$).

### 2.5 Effects of Continuous Cropping on PLFA Ratios

The ratio of Gram-positive to Gram-negative bacterial PLFAs (G+/G-) in processing tomato rhizosphere soil showed an initial increase then decrease with continuous cropping duration at the flowering-fruit bearing stage, while the opposite pattern occurred at the maturity stage (Figure 2 [Figure 2: see original paper]). At the flowering-fruit bearing stage, the G+/G-ratio reached its maximum value of 12.65 after 3 years of continuous cropping, which was 1.6 times that of the control, with highly significant differences (P<0.01). At the maturity stage, the G+/G-ratio reached its maximum value of 22.38 after 7 years of continuous cropping, which was 3.3 times that of the control, with highly significant differences (P<0.01).

The ratio of bacterial to fungal PLFAs (B/F) serves as an important indicator of buffering capacity in soil ecosystems [20]. Continuous cropping of processing tomato significantly decreased the B/F ratio, which was consistently higher at the flowering-fruit bearing stage than at the maturity stage (Figure 2). After 7 years of continuous cropping, the B/F ratio decreased by 71.1% and 93.5% at the flowering-fruit bearing and maturity stages, respectively, compared to the control, with highly significant differences (P<0.01). These results indicate that the buffering capacity of the soil ecosystem against continuous cropping stress was weakest during the maturity stage of processing tomato.

### 2.6 Principal Component Analysis of Microbial PLFAs

As shown in Figure 3 [Figure 3: see original paper], PC1 explained 64.92% of the variation in continuous cropping years, while PC2 explained 23.55%. Together, PC1 and PC2 effectively distinguished rhizosphere soil microbial community PLFAs under different continuous cropping treatments. Further analysis revealed that the projection points for 1-year planting (CK) were most concentrated and closely spaced in the PCA plot, indicating very similar microbial community structures between growth stages. After 3 years of continuous cropping, the projection points became more dispersed and widely separated, suggesting that continuous cropping created substantial differences in rhizosphere soil microbial community composition between growth stages.

Principal component analysis of the 16 individual PLFAs (Figure 4 [Figure 4: see original paper]) showed that PC1 and PC2 explained 82.51% of the total variation, with PC1 accounting for 58.46% and PC2 for 24.05%. Varimax orthogonal rotation analysis identified five representative variables for PC1 (12Me14:0, 17Me18:0, 14Me15:0, 16:1$ 7, $12Me13 : 0 with loading values > 0.800) and six for PC2 (20 : 0, 14 : 0, 16 : 1 9c, i16Me17 : 0, 10Me18 : 0, 17 : 0 with loading values > 0.800). Continuous cropping reduced the content of bacterial marker PLFAs 14Me15 : 0 and 16 : 0 while increasing the content of the fungal characteristic PLFA 18 : 1 $9c, indicating a trend toward fungal-dominated soils under continuous cropping.

### 2.7 Cluster Analysis of Microbial Community PLFA Markers

At a Euclidean distance of 10, soil PLFA biomarkers clustered into three major groups (Figure 5 [Figure 5: see original paper]). Type I included PLFA biomarkers 12Me13:0, 14:0, 12Me14:0, 15:0, 16:1$ 7, 16 : 1 9$c$, 16 : 1 11$c$, 14$Me$16 : 0, 17 : 0, $i$16$Me$17 : 0, 10$Me$18 : 0, 17$Me$18 : 0, $and$20 : 0, $characterizedbyunevendistributionandrelativelylowabundancewithin7yearsofcontinuouscropping.TypeI1$ 0$and$18 : 1 $9c, showing complete distribution and relatively high abundance. Only 16:0 belonged to Type III, characterized by complete distribution and the highest abundance. These results indicate that while continuous cropping did not substantially alter the clustering patterns of dominant PLFA biomarker groups, it significantly reduced the distribution abundance of these dominant groups.

### 2.8 Analysis of Microbial Community Diversity in Continuously Cropped Processing Tomato

Shannon-Wiener, Brillouin, and Simpson indices all showed an initial increase then decrease with prolonged continuous cropping duration (Table 4 ), reaching maximum values after 3 years and minimum values after 7 years. This suggests that within 3 years of continuous cropping, rhizosphere soil microbial species richness and community diversity were enhanced, but diversity declined when continuous cropping exceeded 3 years, with decreasing individual microbial populations.

From the perspective of growth stages, Shannon-Wiener and Brillouin indices were higher at the flowering-fruit bearing stage than at the maturity stage, while the Simpson dominance index showed the opposite pattern. This may be explained by the long, cold winter in Xinjiang following tomato harvest, during which residual roots, leaves, and stems decompose minimally. When soil temperature rises rapidly in early summer, microbial activity intensifies, accelerating decomposition of residues. Concurrently, as plants enter their peak growth period, increased root exudates provide more available carbon and nitrogen sources for microorganisms, stimulating microbial metabolism and enhancing soil microbial diversity while promoting certain microbial groups to become dominant. However, during the maturity stage, although temperatures remain high with minimal soil temperature variation, reduced root exudates lead to decreased microbial diversity.

The Pielou evenness index represents the relative density of species within a community, with higher values indicating greater relative density [20]. As shown in Table 4, the evenness index reached its maximum after 3 years of continuous cropping and its minimum after 7 years, though differences between treatments were not significant. This indicates that continuous cropping beyond 3 years caused some decline in evenness, but the relative density of microbial groups within the community did not change substantially.

### 2.9 Relationships Between Soil Physicochemical Properties and Rhizosphere Soil Microbial PLFAs

Correlation analysis revealed that available potassium and available phosphorus contents were extremely significantly positively correlated with actinomycete PLFAs (Table 5 ). These results demonstrate that PLFAs of various microbial groups in processing tomato soils can serve as important indicators of soil fertility, and that soil nutrient levels directly influence microbial abundance and diversity.

**2.9.1 Correlations Between Soil Physicochemical Properties and Microbial Biomass** Correlation analysis indicated that pH significantly inhibited soil microbial biomass ($P<0.05$), which is unfavorable for microbial reproduction and survival. Major soil nutrients showed significant or extremely significant positive correlations with microbial biomass and qMB. Bacterial PLFAs and total soil PLFAs were extremely significantly positively correlated with soil organic matter, total nitrogen, total phosphorus, and available potassium contents ($P<0.01$). Fungal PLFAs were extremely significantly negatively correlated with major soil nutrients ($P<0.01$). Total PLFAs measured by PLFA analysis were correlated with microbial biomass measured by chloroform fumigation (Table 6 ). Fungal PLFAs were significantly or extremely significantly negatively correlated with SMBC, SMBN, SMBP, and qMB ($P<0.01$). Total bacterial PLFAs, the bacterial/fungal PLFA ratio, and total soil PLFAs were extremely significantly positively correlated with SMBC, SMBN, and qMB, but extremely significantly negatively correlated with SMBC/SMBN ($P<0.01$). Actinomycete PLFAs were extremely significantly positively correlated with SMBP ($R=0.963$, $P<0.01$). These findings demonstrate that both methods yielded consistent results regarding the impact of continuous cropping on soil microorganisms, showing that continuous cropping significantly reduced soil microbial biomass. Therefore, combining the fumigation method with phospholipid fatty acid analysis can more effectively and accurately reveal the mechanisms of continuous cropping obstacles from the perspective of changes in soil microbial biomass.

## Discussion

### 3.1 Effects of Continuous Cropping on Soil Microbial Biomass in Processing Tomato

Microbial biomass reflects soil nutrient availability, biological activity, and energy cycling [20]. Our results demonstrate that long-term continuous cropping of processing tomato significantly reduced soil microbial biomass, consistent with previous studies on cucumber, cotton, and soybean [3,6]. This reduction can be attributed to two main factors. First, long-term intensive tillage practices caused severe topsoil erosion and accelerated mineralization of organic matter. Additionally, removal of tomato residues from fields after harvest directly reduced humus formation, decreasing available carbon and nitrogen sources for

microorganisms and hindering their reproduction and activity. Second, long-term continuous cropping led to accumulation of autotoxic substances in root exudates, increased soil pH, and promoted soil fungalization, altering the soil microbial flora [1-3] and ultimately reducing total rhizosphere microbial biomass.

Microbial biomass entropy (qMB) reflects the efficiency of microbial carbon and nitrogen utilization and can reveal changes in the biological properties of rhizosphere soil under continuous cropping [16]. Our results showed that continuous cropping decreased qMB, indicating that long-term continuous cropping of processing tomato reduced soil labile organic carbon content and slowed organic carbon turnover, which is unfavorable for soil mineral fixation of organic matter and leads to soil carbon and nitrogen losses. Additionally, long-term continuous cropping caused imbalances in microbial carbon-nitrogen composition ratios, resulting in differential microbial energy demands that collectively affected qMB values [16] and ultimately reflected changes in soil microbial flora [2-3].

The SMBC/SMBN ratio is related to changes in soil microbial community structure [21]. Our results showed that SMBC/SMBN increased under long-term continuous cropping, ranging from 4.84 to 10.88. Typically, fungi have C/N ratios of 7–12, while bacteria have ratios of 3–6 [22]. These results indicate that longer continuous cropping durations caused a shift from bacterial- to fungal-dominated soils, leading to soil fertility depletion and obvious continuous cropping obstacles, consistent with previous findings [2-3]. Other studies have suggested that changes in the type and quantity of crop root exudates alter rhizosphere microbial flora composition, disrupting microbial population balance, promoting harmful microorganisms, and reducing beneficial microorganisms, ultimately manifesting as continuous cropping obstacles [23-24].

### 3.2 Changes in Rhizosphere Soil Microbial Community Structure Under Continuous Cropping of Processing Tomato

Phospholipid fatty acid profiling provides an accurate and objective method for determining changes in soil microbial biomass and revealing differences in microbial community structure, playing an important role in studying soil environmental quality changes. Our study detected the same types of PLFAs across different continuous cropping durations, identifying 13 PLFA biomarkers with significantly different contents. The total soil PLFA content followed the trend: control > 3-year > 5-year > 7-year continuous cropping, indicating that long-term continuous cropping caused a dramatic decline in soil microbial biomass, consistent with previous studies on soybean [25] and maize [10] continuous cropping. This decline occurred because long-term intensive agricultural practices destroyed stable soil structure, reduced substrate abundance and uniformity, and hindered microbial metabolism, leading to decreased microbial diversity and reduced soil microbial biomass.

Our study also revealed significant differential effects of continuous cropping on PLFA contents indicating different microbial groups. Long-term continuous

cropping decreased bacterial and actinomycete PLFA contents while increasing fungal PLFA content, indicating a shift from bacterial- to fungal-dominated rhizosphere soils, consistent with previous research [2-3]. Some studies have suggested that increased root exudates after continuous cropping induce higher fungal populations [23]. The decreased bacterial/fungal PLFA ratio under continuous cropping indicates reduced ecosystem stability and weakened buffering capacity against environmental stress, manifesting as continuous cropping obstacles. Li et al. [26] proposed that changes in soybean rhizosphere microbial community structure under continuous cropping were caused by allelochemicals in root exudates, with flavonoid content positively correlated with soil microbial communities [10]. Previous studies have identified obvious allelopathic effects in processing tomato, with major allelochemicals including phthalic acid, benzoic acid, and cinnamic acid [27]. However, whether the content of these allelochemicals correlates with soil microbial community structure requires further investigation.

### 3.3 Correlations Between Soil Microbial Biomass and Physicochemical Properties

This study combined the fumigation method for measuring total microbial biomass with PLFA analysis for specific microbial groups, providing a more comprehensive understanding of soil microbial biomass. Fungal PLFAs were significantly or extremely significantly negatively correlated with SMBC, SMBN, SMBP, and qMB ($P<0.01$), which differs from Zhao et al. [28] due to differences in soil samples and experimental treatments. However, we can infer that changes in soil microbial biomass primarily resulted from changes in bacterial and fungal populations, leading to significantly correlated trends. Total bacterial PLFAs and total soil PLFAs were extremely significantly positively correlated with soil microbial biomass carbon and nitrogen ($P<0.01$), while actinomycete PLFA content was extremely significantly positively correlated with soil microbial biomass phosphorus ($R=0.963$, $P<0.01$). These findings align with previous research showing significant correlations between soil microbial diversity and microbial biomass in agricultural ecosystems [3,25].

The three major microbial groups—bacteria, fungi, and actinomycetes—constitute the main soil microbial biomass, with their community composition and abundance reflecting soil biological activity. Our correlation analysis showed that fungal PLFAs were significantly or extremely significantly negatively correlated with major soil nutrients, while bacterial PLFAs and total soil PLFAs were extremely significantly positively correlated with organic matter and total nitrogen contents ($P<0.01$). On one hand, this suggests that organic matter and total nitrogen may be primary factors affecting soil microbial growth, and that total PLFAs, bacterial PLFAs, and fungal PLFAs can reflect soil carbon and nitrogen levels. On the other hand, long-term continuous cropping of processing tomato led to soil impoverishment with decreased organic matter and total nitrogen contents, which inhibited bacterial and actinomycete growth and

reduced their biomass, demonstrating that soil microbial biomass is related to soil fertility [29]. Conversely, fungal growth was not inhibited by soil impoverishment but instead increased, as fungi are known to be better adapted to nutrient-poor soils than bacteria [30].

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*Note: Figure translations are in progress. See original paper for figures.*