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Dynamic Response of Soil Enzymes and Microbial Diversity to Continuous Application of Atrazine in Black Soil of a Cornfield without Rotation in Northeast China

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Abstract: Atrazine has been extensively used in China's agricultural production for a long time and the potential risks to the environment have received widespread attention. The purpose of this study was to examine the effect of the continuous application of atrazine on soil herbicide residues, soil enzyme activity, and microbial community structure, as well as to provide a theoretical reference for the appropriate application of atrazine and the improvement of soil. Previous studies have focused on the effects of atrazine on soil microorganisms, but the experiments used higher doses than recommended. To reveal the actual effects of atrazine on soil microorganisms, the recommended dose of atrazine was used for 0, 1, and 2 years. We studied atrazine residues and enzyme activity in the soil, and phospholipid fatty acids (PLFA) were measured to study the structure of the soil microbiome. The results show that the rate of atrazine degradation occurred rapidly after the initial administration, and with the increase in continuous administration, its half-life decreased from 24.6 d in the first year to 14.1 d in the second year. The application of atrazine significantly affected soil urease activity and cellulase activity, but it had no significant effect on saccharase activity. The continuous application of atrazine had a significant effect on the biomass of cultured bacteria in soil, but not on the biomass of culturable fungi and actinomycetes in the soil. Furthermore, the results of PLFA analysis show that the application of atrazine had a significant effect on the microbial structure of the soil. These results indicate that the significant increase in the degradation rate of atrazine during continuous application is related to the high adaptability of the soil microbiome.

Keywords: atrazine; urease; saccharase; cellulase; PLFA; soil microbial communities

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1. Introduction

Atrazine is an endosynthetic herbicide that is applied before and after selective seeding [1,2]. It is mainly absorbed by plant roots and is transmitted upward to inhibit the photosynthesis of weeds. Atrazine kills a wide spectrum of undesired plants and can control a variety of annual grasses and broad-leaved weeds. It is suitable for controlling weeds in dry fields with crops such as sorghum, sugarcane, fruit trees, and especially maize, and it can be applied in nurseries and forest land. It targets the detoxification mechanism in plants and has an effective duration of 2 to 3 months [3]. Atrazine is also effective for some deep-rooted grass species. However, this herbicide has harmful effects on sensitive crops such as wheat, soybean, and rice [4]. Atrazine is easily retained in the soil and readily leaches into the deeper soil with rain due to its long half-life and low soil

absorption rate [5–7]. Jablonowski et al. (2009) reported that atrazine and its degradation products were still detected in soils 22 years after its application [8]. Atrazine was produced in China in the 1970s, and it remains one of the most widely used herbicides in the country. Northeast China is an important agricultural production base in the country, and it is also the area where atrazine is most widely used.

Atrazine is applied to inhibit the growth of target plants, but its effects can often be extended to nontarget soil microorganisms as has been reported in the literature [9,10]. Studies have shown that atrazine has a toxic effect on microorganisms in the soil [10,11]. However, some studies have found that the degradation of atrazine in soil is enhanced after long-term repeated applications [12]. Additionally, studies have shown that the improvement of the degradation rate is closely related to the activities of microorganisms in the soil [13]. Further research revealed that microorganisms could use herbicides as substrates for growth. A variety of atrazine degrading bacterial strains have been isolated from sediments in water bodies, and microorganisms have been observed to utilize atrazine as the only carbon source [14]. Crop rotation is usually implemented in agriculture, and the same herbicide is not typically applied over time. Therefore, the study of changes in the soil microbial community after short-term continuous use of atrazine is more relevant for agricultural production and environmental remediation. However, little is known about the relationship between atrazine degradation and the soil bacterial community structure after short-term application. Therefore, it is necessary to study the degradation behavior of atrazine and its relationship with soil microbial functional diversity and community structure [15–18].

Most of the above studies were pot experiments that used higher or lower doses of atrazine than are used in practice. Soil microbial community structure has not been adequately studied with the continuous application of atrazine at doses that are used in the field. In this study, the dynamic responses of herbicide residues, soil enzyme activities, and microbial diversity to the application of atrazine in black soil (without rotation) in corn fields in Northeast China during the tillage period were evaluated based on the actual tillage conditions in this region. The aim of this study was to reveal the true effects of a field application of atrazine on soil microorganisms and to provide a reference for further research on the appropriate use of this herbicide and environmental governance.

2. Materials and Methods

2.1. Research Site and Soil

The research site is located in Hulan Experimental Field, Harbin City, Heilongjiang Province, China (126°11′−127°19′ E, 45°49′−46°25′ N), at an altitude of 150 m. The region has a northern temperate continental monsoon climate with four distinct seasons, long winters, and short summers. The average annual temperature is 4.5 °C, the average annual precipitation is 507.7 mm, and the average annual sunshine duration is 2491.7 h. The characteristics of soil samples were as follows: organic matter 31.2 g kg^{-1}, total potassium content 18.77 g kg^{-1}, total nitrogen content 1.67 g kg^{-1}, total phosphorus content 0.54 g kg^{-1}, maximum field capacity 18.31%, clay 34.26%, silt 27.31%, sand 38.43%, and pH 6.27. The soil has been used in agricultural practice for many years. In the five years prior to the trial, no atrazine had been used at the site. Since then, continuous corn cropping was carried out in the field for two consecutive years. Atrazine was applied according to the agricultural recommendation (3.15 kg herbicide/ha) at the 3 to 5-leaf stage of maize growth. The first year of application is designated Treat 1, and the second year is designated Treat 2. At the same time, test sites without atrazine were used as a control check (CK). All test areas were subject to local conventional field management practices; atrazine application was the only difference. Three replicate plots were set for each treatment, with 10 m × 10 m = 100 m^2 for each plot, and the interval between plots was 10 m. A 5 m-wide protective line was established around the experimental plot.

A five-point sampling method was used to collect soil samples at 0 to 10 cm depth from each standard plot. Soils were sieved (2 mm), and any remaining visible plant material

and stone were removed by hand. Then, each sample was divided into two subsamples: the air-dried sample for soil atrazine residue and enzyme activity analyses and the fresh sample for PLFA tests. Soil samples were collected at the following time-points: soil samples before atrazine application (year 1: May 8; year 2: May 15), defined as 0 d; the day of atrazine application (year 1: May 9; year 2: May 16) defined as 1 d. Days 21 (year 1: May 30; year 2: Jun 6), 58 (year 1: July 6; year 2: July 13), 89 (year 1: Aug 6; year 2: Aug 13), 122 (year 1: Sep 8; year 2: Sep 15), and 150 (year 1: Oct 6; year 2: Oct 13) after the application of atrazine are defined as 21 d, 58 d, 89 d, 122 d, and 150 d, respectively.

2.2. Determination of Atrazine Residues in the Soil

The contents of atrazine and its metabolites in the soil samples were measured following the procedure described by Liu et al. (2020) with some modifications [18]. The air-dried soil was screened with a 60 mesh sieve. Ten grams of the screened air-dried soil was added to 4 g of diatomite and 20 mL of 1:1 (*v/v*) n-hexane/acetone mixed solvent. The samples were ultrasonically extracted for 5 min and then centrifuged for 5 min at 5000 r. The supernatant was blown dry with nitrogen, dissolved in a constant volume of 2 mL of methanol, and passed through a 0.22 µm filter membrane. Atrazine soil residues were determined by high-performance liquid chromatography (HPLC). The HPLC chromatographic conditions included a mobile phase with 4:1 (*v/v*) chromatographic methanol/water, sample quantity of 20 µL, column temperature of 25 °C, flow rate of 0.8 mL min^{-1}, and detection wavelength of 220 nm. The calibration curves for HPLC analyses were linear from 25 to 200 ng mL^{-1} ($r \geq 0.99$, five points in triplicate). The average recovery of atrazine was 91.28% to 92.52%. The detection limit of this method was 0.02 mg kg^{-1}.

2.3. Determination of Soil Enzyme Activity

The activities of urease, saccharase, and cellulase were determined within 15 days of soil sample collections. The soil urease activity was determined via indigophenol blue colorimetry [18]; soil saccharase and cellulase activities were measured via 3, 5-dinitrosalicylic acid colorimetry [18,19]. All samples were tested in three replicates, and the average value of the three replicates was taken as the enzyme activity of the samples.

2.4. Culturable Soil Microbial Biomass

Culturable bacteria, fungi, and actinomycetes were detected with the traditional dilution plate method. A 10 g soil sample was suspended and vortexed in 90 mL of sterile water and serially diluted 10-fold to 10^8-fold. Diluted samples (0.2 mL) were then spread on agar plates containing beef extract peptone medium for bacteria, Rose Bengal medium for fungi, and Gauze No. 1 medium for actinomycetes. Three replicates were prepared for each dilution series. The biomass of colony-forming units (CFU) was measured after incubation for 36 h at 30 °C for bacteria, 48 h at 28 °C for fungi, and 120 h at 30 °C for actinomycetes. The CFU of each microorganism was then calculated per kilogram of soil.

2.5. Soil Phospholipid Fatty Acid Analysis

The microbial community composition was studied using the phospholipid fatty acid (PLFA) method, according to Xiao Chen and Baihui Hao [20], with a few modifications. The freeze-dried and ground samples were extracted twice using an extractant that contained chloroform, methanol, and phosphate buffer with a ratio of 1:2:0.8. The extracted phospholipids in the organic phase were then separated by a 3 mL silica column. The collected fatty acids were identified using gas chromatography (Agilent 6850, Agilent Technologies, Inc., Wilmington, DE, USA) equipped with a flame ionization detector. The contents of individual PLFAs were determined based on the added internal standard 19:0 and the total amount of individual PLFAs (nmol g^{-1}). PLFA concentration is expressed as nmol PLFA g^{-1} dry soil. Phospholipid fatty acids were named, as described by Wilkinson [21]. The fungal PLFA (Fun) biomarkers used were *16:1w5c*, *18:1w9c*, *18:2w6*, *9c*. Gram-positive (G+) bacteria biomass was estimated from the summed concentrations of the following

PLFAs: *i14:0*, *i15:0*, *a15:0*, *i16:0*, *a16:0*, *i17:0*, *a17:0*; Gram-negative (G−) bacteria biomass was estimated from the summed concentrations of the following PLFAs: *16:1w5c*, *16:1w7c*, *16:1w9c*, *17:1w8c*, *18:1w5c*, *18:1w7c*, *cy17:0*, *cy19:0*. The biomarkers of G+ bacteria, G− bacteria, and *14:0, 17:0*, *2OH16:0* were summed to represent bacteria (Bac). All microbial PLFAs were summed as an index of microbial biomass. Moreover, the ratios of G+/G− and fungal/bacterial (Fun/Bac) PLFAs were calculated to reveal changes in microbial community structure under various experimental treatments. Anaerobic biomass was assessed by quantifying *cy17:0* and *cy19:0*, and aerobic biomass was assessed by quantifying *16:1ω7c* and *18:1ω7c*. The stress indicator for the microbial community was calculated from the anaerobic biomass/aerobic biomass ratio. The sum of all identified PLFAs was used as total PLFAs. Shannon's diversity index (H) was calculated using the following formula [22]:

$$H = -\sum_{i=i}^{n} P_i ln P_i \quad (1)$$

Note: n is the number of PLFAs, and P_i is the proportion of the PLFA of "i" in total PLFA.

2.6. Statistical Analysis

A linear hybrid model of repeated measurements was performed to test the importance of the continuous application of atrazine on the primary and interactional effects of soil enzyme activity and microbial community characteristics. Principal component analysis (PCA) was used to detect differences in microbiome composition indicated by the PLFA profile. Correlation analysis was carried out, combined with redundant analysis (RDA), to calculate the significance level of the relationship between environmental factors and the composition of microbial structures in the soil. The linear hybrid model of repeated measurements and related analyses were performed in IBM SPSS Statistics 22. The principal component analysis (PCA) was performed using R software. CANOCO 5 was used for RDA analysis.

3. Results

3.1. The Residue of Atrazine in the Soil

After the application of atrazine, the residue concentration decreased gradually with time (Figure 1). Atrazine residues were highest on the first day after application (Treat 1: 4.90 ± 0.63 mg kg^{-1}; Treat 2: 6.50 ± 0.52 mg kg^{-1}), and decreased rapidly within a short time after application. Residues of atrazine in the treatment groups decreased by 53.12% (Treat 1) and 78.76% (Treat 2) within 1 to 21 d. (Treat 1: 2.30 ± 1.42 mg kg^{-1}; Treat 2: 1.54 ± 1.24 mg kg^{-1}). Residual levels of atrazine in the two treatment groups were reduced to 3.23% and 1.62% of the initial concentration on 122 d, respectively, and returned to the pre-application level (Treat 1: 0.11 ± 0.03 mg kg^{-1}; Treat 2: 0.08 ± 0.01 mg kg^{-1}). After 150 days of recovery, atrazine residue in the soil was reduced to 2.25% (Treat 1) and 1.45% (Treat 2). In the second year, the residue concentration of atrazine in the soil before application was 0.08 ± 0.01 mg kg^{-1}. After one year of natural growth, the residues accounted for 1.65% of the original spray concentration.

As shown in Figure 1, there was a non-linear reduction in atrazine residues in the soil under the treatment conditions. The soil residues were highest immediately after the initial application of atrazine, and they decreased rapidly within a short time after application. The content reached a stable state 93 d after application. According to the fitted regression equation (Figure 1), the variation in the atrazine residues in the soil within 150 d after use was consistent with the first-order exponential model ($R \geq 0.9$), and the half-life of atrazine was 24.6 d (Treat 1) and 14.1 d (Treat 2), respectively. The decay model indicated that the continuous application of atrazine increased the rate of degradation.

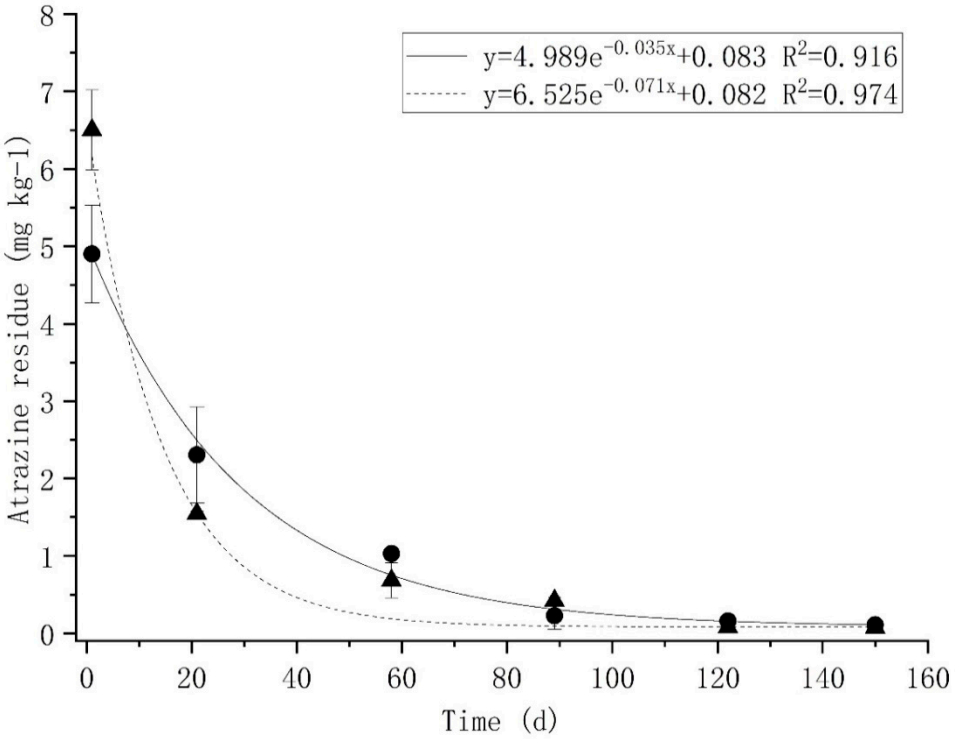

Figure 1. Atrazine dynamic curve in soil after atrazine application. Data represent the mean ± SD of each soil sample selected at each time-point. Treat 1 and Treat 2: soil spiked with atrazine at 3.15 kg km^{-2} in year 1 and year 2, respectively. ●: Treat 1; ▲: Treat 2.

3.2. Effect of Atrazine on Soil Enzyme Activity

The linear mixed model (Table 1) showed that soil urease activity was significantly affected by atrazine ($p < 0.001$) and changed significantly with time ($p < 0.001$). The application of atrazine had a significant effect on cellulase in soil ($p < 0.001$), and the interaction between application and time also had a significant effect on cellulase activity ($p < 0.01$). In contrast to urease and cellulase, the saccharase activity in the soil was not significantly affected by the application of atrazine or the interaction between application and time ($p > 0.05$). However, the activity of saccharase in the soil showed significant differences over time ($p < 0.01$).

Table 1. Results of the repeated measures analysis of variances, the significance of atrazine treatment and recovery time on soil enzyme activity, and microbial community properties.

Index	Treat	Time	Treat × Time
Urease	15.713 ***	361.418 ***	14.037 ***
Cellulase	35.158 ***	2.139	4.351 **
Saccharase	1.927	17.320 ***	1.168
Bacterial biomass	7.794 **	9.196 ***	5.174 **
Fungal biomass	0.288	5.722 **	0.727
Actinomycetes biomass	2.522	39.643 ***	5.552 **
G+	4.995 ***	249.166 ***	54.991 ***
G−	5.476 **	245.163 ***	22.981 ***
Bac	25.272 ***	306.662 ***	38.948 ***
Fun	7.023 **	153.387 ***	14.321 ***
PLFA	15.625 ***	148.743 ***	20.877 ***
Fun/Bac	19.690 ***	483.814 ***	9.383 ***
G+/G−	8.416 **	10.623 ***	4.113 *
Shannon	25.765 ***	172.668 ***	28.879 ***
Stress indicator	101.162 ***	24.608 ***	25.229 ***

Note: ***, **and *: The correlation was significant at the level of 0.001, 0.01, and 0.05 (double-tailed), respectively.

The urease activity in the control group without atrazine showed a slowly decreasing trend (Figure 2A). Significant differences in urease activity between the control group (CK) and treatment group (Treat 1 and Treat 2) were observed on the 1 d of treatment ($p < 0.001$). During the two years of atrazine application, the change in soil urease activity, which rapidly decreased on the day of treatment, was similar between Treat 1 and Treat 2. Treat 1 and Treat 2 had the lowest enzyme activity on the 58 d after treatment (65% and 67% of the original value, respectively). As time increased, the residues of atrazine gradually decreased, and the urease activity also showed a trend towards recovery. Enzyme activity in Treat 1 and Treat 2 returned to the initial value 150 d after treatment.

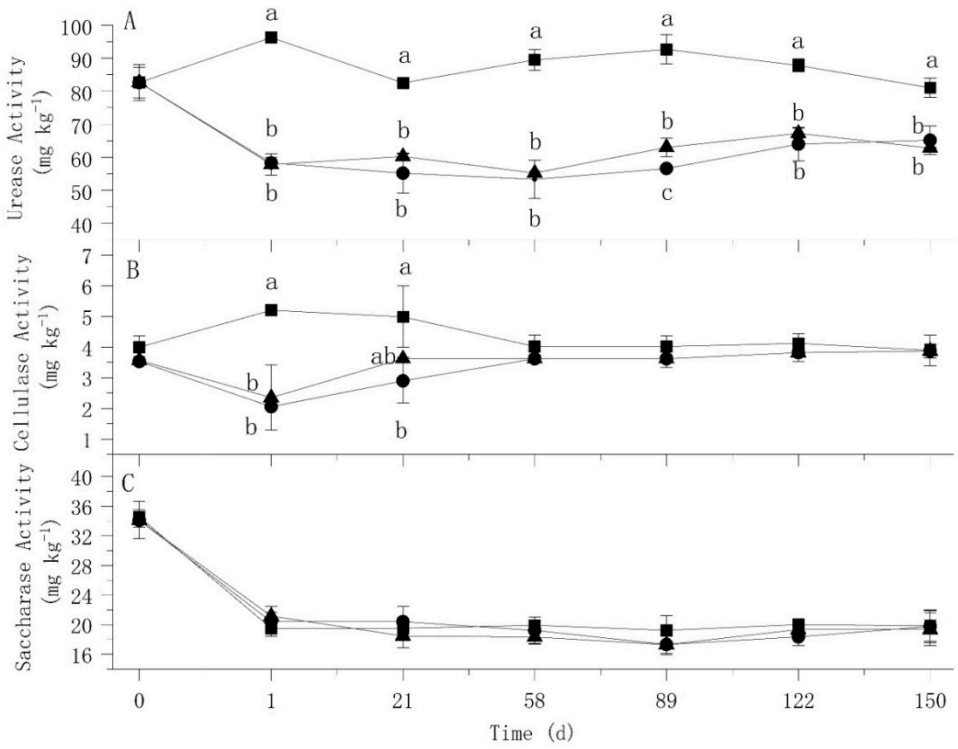

Figure 2. Soil enzyme activities under the different atrazine application treatments. Data represent the mean ± SD of each soil sample selected at each time-point. (**A**) Urease; (**B**) cellulase; (**C**) saccharase. ■: CK; ●: Treat 1; ▲: Treat 2.

Soil cellulase activity in the non-atrazine group first increased and then decreased, and it returned to the initial level 58 days after application (Figure 2B), while the 2-year atrazine treatment group showed a decreasing and then increasing pattern. Cellulase activity decreased rapidly on the first day after treatment, decreasing by 41.69% (Treat 1) and 34.19% (Treat 2). Treat 1 and Treat 2 had the lowest enzyme activities on the first day after treatment. Treat 1 and Treat 2 returned to their initial levels 58 d and 21 d after treatment, respectively. The difference between the treatment groups and the control group was statistically significant 1 to 58 d after treatment. Compared with the first year, cellulase activity in the 2-year treatment group was less inhibited, and the recovery time was shorter.

The soil saccharase activity was measured and was found to significantly differ between the treatment groups and the control group at all sampling time points (Figure 2C). Soil saccharase activity was 34 mg kg^{-1} in the three groups before application; it rapidly decreased to 19.52 to 21.17 mg kg^{-1} on the first day after application, and remained at this level in all treatments.

3.3. Effects of Atrazine on Soil Culturable Microorganisms

In this study, bacteria, fungi, and actinomycetes in the soil were artificially cultured. A linear mixed model analysis of culturable microbe data from all sampling times (Table 1) showed that the continuous application of atrazine had a very significant effect on the

biomass of culturable bacteria in the soil ($p < 0.01$). However, continuous application of atrazine had no significant effect on the biomass of culturable fungi or actinomycetes in the soil ($p > 0.05$). With the progression of soil recovery time after atrazine application, the effect of time on culturable soil microbial biomass was significant ($p < 0.01$), and the effect of time on bacteria and actinomycetes was higher than that on fungi. The interaction between the continuous application of atrazine and the change in recovery time resulted in significant differences in the biomass of bacteria and actinomyces among different treatment groups ($p = 0.001$), while the biomass of fungi showed no significant differences among different treatment groups ($p = 0.704$).

The biomass of culturable bacteria in plots without atrazine application first increased and then decreased (Figure 3A). The biomass of culturable bacteria in Treat 1 increased and reached its maximum after 21 d. Then, the biomass decreased rapidly and reached the lowest level 89 d after application. Treat 2 biomass began to decrease after 21 d and remained stable after 58 d. Interestingly, the results of one-way ANOVA showed that there was a significant difference between the treatments only 89 d after atrazine application, contradicting the results of the linear mixed model. Compared with CK, the fungal biomass in Treat 1 and Treat 2 was slightly increased or decreased after the use of atrazine (Figure 3B), but there was no significant difference in the biomass at each sampling time point (Table 1, $p > 0.05$). The biomass of actinomycetes decreased slowly under different atrazine treatments but increased after 122 d in the control group and showed a significant difference after 150 d (Figure 3C). Interestingly, the actinomycete biomass did not recover in the first year, but in the second year of the experiment, the initial actinomycete biomass returned to the same level as that in the control group.

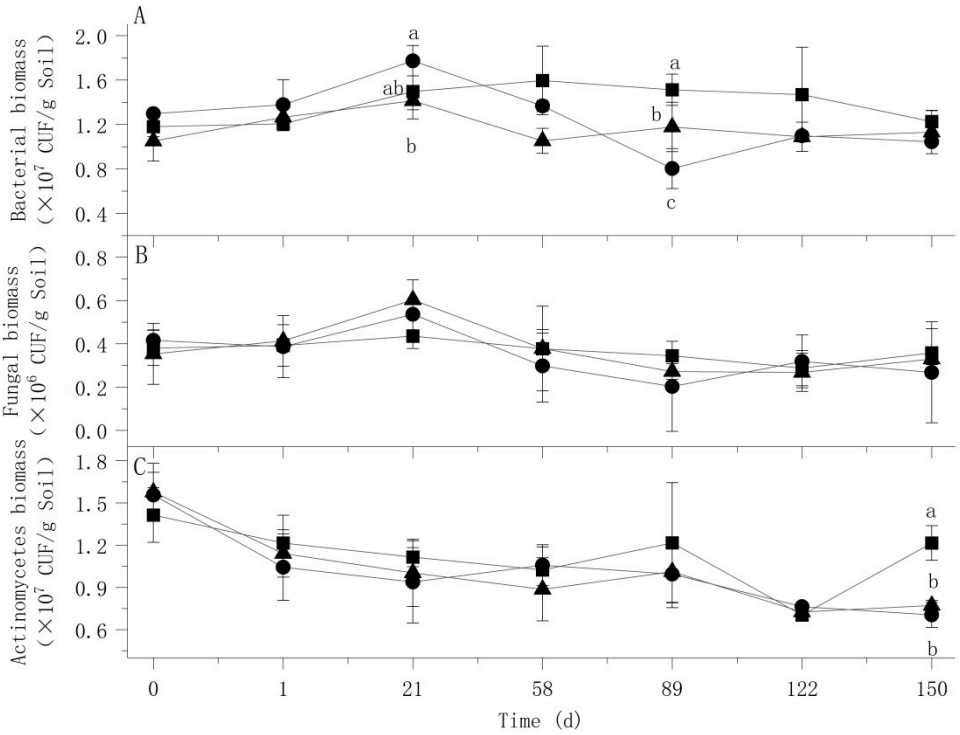

Figure 3. Effects of different atrazine treatments on the biomass of microorganisms that can be cultured in soil. Data represent the mean ± SD of each soil sample selected at each time-point. (**A**) Bacterial biomass; (**B**) fungal biomass; (**C**) actinomycetes biomass. ■: Control; ●: Treat 1; ▲: Treat 2.

3.4. Soil PLFA Analysis and Changes in Microbial Community Composition

The principal component analysis results for the characteristics of the microbial community structure are shown in Figure 4. In this study, the changes in microbial community

composition were mainly caused by the application of atrazine, but not by the recovery time. Among the principal components, the first and second axes contributed to 47.01% and 27.63% of the variation, respectively, and the cumulative contribution rate of the two axes was 74.64%. CK is located in the third quadrant, Treat 1 is located in the fourth quadrant, and Treat 2 is located in the first and second quadrants. There is no intersection between the three treatments (Figure 4).

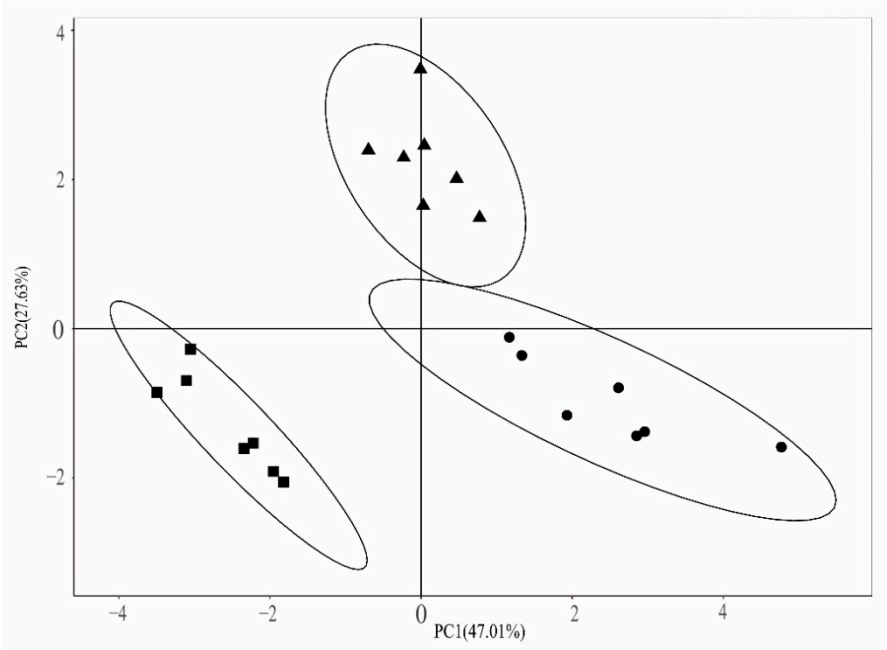

Figure 4. Principal component analysis on microbial community composition identified by the phospholipid fatty acid method. ■: Control; ●: Treat 1; ▲: Treat 2.

A total of 93 PLFAs, including saturated straight-chain, saturated branched-chain, monounsaturated, and saturated fatty acids, were detected after three successive treatments of atrazine. Continuous use of atrazine, post-application recovery time, and their interaction were associated with statistically significant differences in total PLFA between treatments (Table 1, $p < 0.001$) when all PLFAs were combined. The total PLFA of the control group without atrazine increased slightly in the first 58 d of the trial, decreased rapidly on days 58 to 89, and then slowly recovered (Figure 5). In contrast, in the first year of atrazine administration (Treat 1), the total PLFA increased rapidly after application and remained significantly higher than that in the control group throughout the trial. Soil PLFA in Treat 2 decreased rapidly and then increased slowly in the second year of continuous application of atrazine. From 1 to 58 d after treatment, soil PLFA in Treat 2 was lower than that in the control group, and the total PLFA level remained basically unchanged after 58 d.

The continuous application of atrazine, prolonged recovery time, and their interaction had statistically significant effects on the abundance of different microbial functional characteristics (G+, G−, total fungal, and total bacterial PLFA contents) (Table 1, $p < 0.01$). G+ PLFA in the soil of the control group increased slightly between days 1 and 58 (1 to 58 d) of the experiment, decreased rapidly between days 58 and 89 (58 to 89 d), and then largely remained stable (Figure 6A). The G+ PLFA level in Treat 1 rose rapidly and remained higher than that in the control group ($p < 0.001$). Before atrazine was administered in the second year of the trial, the G+ PLFA level was higher than that in CK and Treat 1 ($p < 0.001$), but it was lower than the G+ PLFA level 150 d after the first year of application. These results indicate that soil G+ PLFA content may have decreased after the first year of application but did not recover to the level of the control group. Interestingly, the changing trend of G+ PLFA content in Treat 2 soil was the opposite of that of Treat 1. After applying atrazine, the atrazine content decreased rapidly and was significantly lower than that in CK ($p < 0.001$).

The content returned to the level in the control group on day 89 (89 d) after treatment. The content of G− PLFA in the soil without atrazine application (CK) was similar to that of G+ (Figure 6B). The G− PLFA level in Treat 1 increased rapidly and was higher than that in CK during the whole observation period. Treat 2 levels of G− PLFA first increased and then decreased after atrazine treatment. The G− PLFA level in Treat 2 was always higher than that in CK, and after 58 d, it was higher than that in CK but lower than that in Treat 1 ($p < 0.001$). The PLFA of bacteria and fungi in the soil of CK and Treat 2 showed similar change patterns (Figure 6C,D), and significant differences were found on days 21 and 89 (21 d and 89 d, $p < 0.001$). Soil bacterial and fungal PLFA content in Treat 1 increased rapidly after atrazine treatment, and were significantly higher than that in CK throughout the whole observation period ($p < 0.01$).

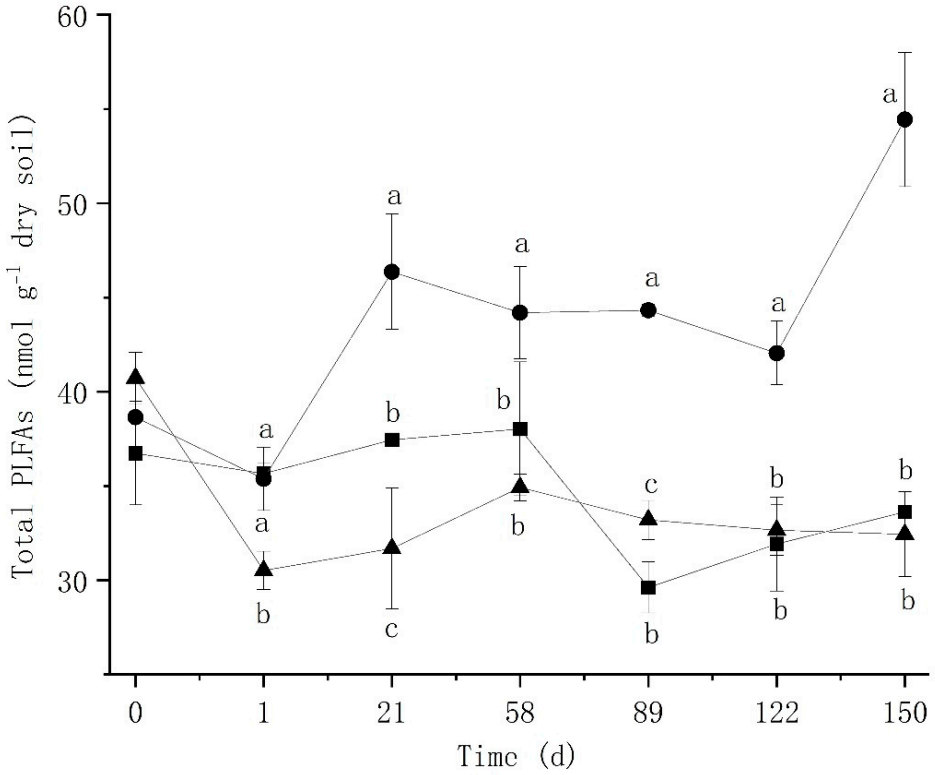

Figure 5. Total PLFA content of the soil under different atrazine treatment conditions. Data represent the mean ± SD of each soil sample selected at each time-point. ■: Control; ●: Treat 1; ▲: Treat 2.

The effects of the continuous application of atrazine, prolonged recovery time, and their interaction on soil microbial community structure characteristics (G+/G−, Fun/Bac, Shannon index, stress index) were statistically significant (Table 1, $p < 0.01$). The G+/G− ratio in Treat 1 and Treat 2 rapidly decreased on the day after atrazine application compared with pre-treatment levels (0 d). The G+/G− ratio in Treat 1 and Treat 2 slowly increased after treatment with atrazine, and it then continued to decrease, and remained significantly lower than that in CK (Figure 7A, $p < 0.001$). In the second year of atrazine application, the ratio of G+/G− in the pre-application soil was significantly lower than that in the first year of the experiment ($p < 0.001$), indicating that the G+/G− ratio did not recover after the observation period. Under different treatment conditions, the overall change patterns of the Fun/Bac ratio in the soil were similar, showing a trend of first decreasing and then increasing (Figure 7B). The Fun/Bac ratio in Treat 1 first decreased and reached a minimum value on day 21 (21 d), after which it increased and returned to the CK level on day 150 (150 d). The Fun/Bac ratio in Treat 1 was lower than that in the control group from days 21 to 122 (except for day 58, $p < 0.05$). In the second year of treatment, the ratio of Fun/Bac was higher than that in CK at the beginning of treatment. From days 58 to 89, the Fun/Bac

ratio decreased rapidly and was significantly lower than that in the control group ($p < 0.05$), and it rose synchronously in Treat 1 and Treat 2 on day 89. The relative change between aerobes and anaerobes in the soil was mainly manifested in the stress index. In the soil without atrazine application, the stress index first increased and then decreased (Figure 7C). Atrazine rapidly decreased 1 to 21 d after treatment (Treat 1 and 2), and it was significantly lower on day 21 than in the control group ($p < 0.01$). The stress index rose rapidly on days 21 to 58, with Treat 1 and Treat 2 being significantly higher than CK on day 58 ($p < 0.001$). On day 58 of treatment, the stress index values of both treatment groups and the control group began to decrease.

The Shannon index is an important measure of microbial diversity in the soil. During the observation period of the experiment, the Shannon index in the soil without atrazine application slightly changed (Figure 7D). In the first year of atrazine application (Treat 1), Shannon indices of the soil microbiome were significantly higher after application than in the control group, except for day 21 ($p < 0.01$). Similarly, except for day 58, the Shannon index of the soil microbiome in Treat 2 was significantly higher than that in the control group.

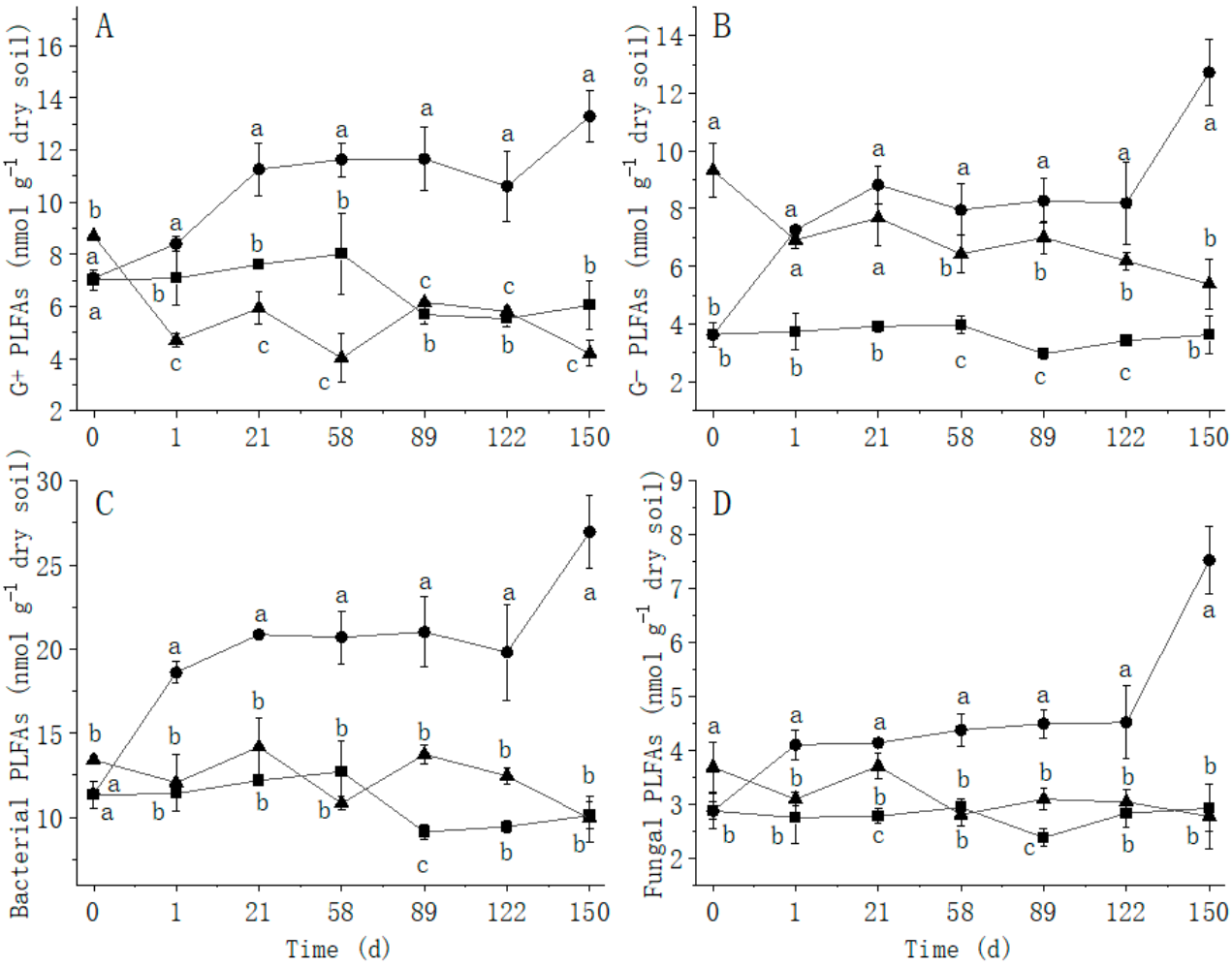

Figure 6. Soil PLFAs under different atrazine application treatments. Data represent the mean ± SD of each soil sample selected at each time-point. (**A**) Gram-positive bacterium (G+); (**B**) Gram-negative bacterium (G−,); (**C**) bacterial; (**D**) fungal. ■: Control; ●: Treat 1; ▲: Treat 2.

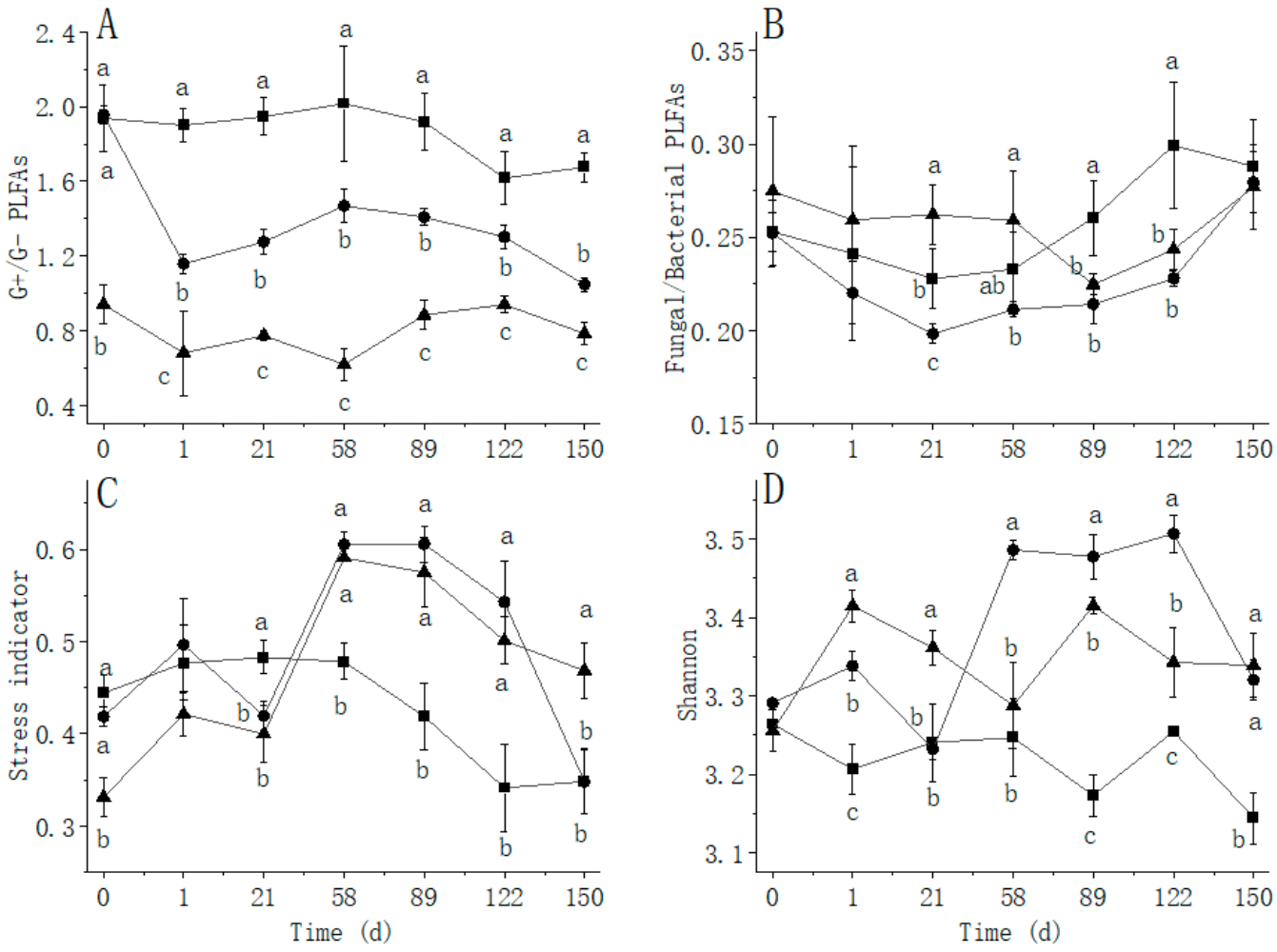

Figure 7. Ratios of Gram-positive to Gram-negative bacterial PLFAs (G+/G−,) and fungal to bacterial PLFAs, stress indicator, and Shannon index. Data represent the mean ± SD of each soil sample selected at each time-point. (**A**) Ratios of fungal to bacterial PLFAs; (**B**) ratios of Gram-positive to Gram-negative bacterial PLFAs (G+/G−,); (**C**) stress indicator; (**D**) Shannon index was calculated based on PLFA. ■: Control; ●: Treat 1; ▲: Treat 2.

3.5. Correlation between Soil Microbial Community Composition and Environmental Factors

Redundancy analysis (RDA) showed that the two axes accounted for 93.88% of the cumulative variation in the microbial community (Figure 8). Axes 1 and 2 accounted for 57.03% and 36.85% of soil microbial changes, respectively. The positive selection results show that the soil urease content and continuous years of atrazine application were the main explanatory variables, which played an important role in the change in soil microbial structure. The application amount of atrazine was the main factor, which explained 20.6% of the variation, followed by soil urease activity, which explained 18.2%. There was no significant correlation between other environmental factors and soil microbial structure (Table 2).

Table 2. Pearson correlation coefficient between soil PLFA index and environmental factors.

Index	Time	Years	Atrazine Residue	Urease	Cellulase	Saccharase	Bacterial Biomass	Fungal Biomass	Actinomycetes Biomass	G+	G−	Bacteria	Fungi	Total PLFA	Fun/Bac	G+/G−	Shannon	Stress Indicator
Time	1.000																	
Years	0.000	1.000																
Atrazine Residue	−0.417	0.316 *	1.000															
Urease	−0.095	−0.671 **	−0.480 **	1.000														
Cellulase	0.185	−0.439 **	−0.719 **	0.547 **	1.000													
Saccharase	−0.501 **	−0.048	−0.112	0.336 **	−0.047	1.000												
Bacterial Biomass	−0.235	−0.326 **	0.176	0.168	−0.029	−0.082	1.000											
Fungal Biomass	−0.350 **	0.019	0.206	−0.070	−0.085	0.048	0.226	1.000										
Actinomycetes Biomass	−0.570 **	−0.156	0.006	0.378 **	−0.010	0.602 **	0.081	0.080	1.000									
G+	0.035	−0.163	−0.077	−0.236	−0.069	−0.014	−0.054	0.043	−0.074	1.000								
G−	0.106	0.540 **	0.230	−0.645 **	−0.374 **	−0.124	−0.292 *	0.096	−0.263 *	0.652 **	1.000							
Bacteria	0.109	0.122	0.152	−0.541 **	−0.267 *	−0.194	−0.144	0.107	−0.280 *	0.898 **	0.837 **	1.000						
Fungi	0.239	0.138	0.071	−0.432 **	−0.193	−0.114	−0.222	0.077	−0.271 *	0.803 **	0.853 **	0.910 **	1.000					
Total PLFA	0.069	−0.064	−0.159	−0.254 *	−0.014	0.078	−0.113	0.102	−0.054	0.899 **	0.682 **	0.846 **	0.817 **	1.000				
Fun/Bac	0.273 *	0.000	−0.170	0.283 *	0.144	0.205	−0.075	−0.095	0.057	−0.356 **	−0.133	−0.394 **	−0.002	−0.248 *	1.000			
G+/G−	−0.152	−0.899 **	−0.403 **	0.712 **	0.459 **	0.239	0.331 **	−0.034	0.339 **	0.162	−0.617 **	−0.191	−0.253 *	0.057	−0.104	1.000		
Shannon	0.097	0.506 **	0.257 *	−0.669 **	−0.320 *	−0.226	−0.330 **	−0.217	−0.252 *	0.329 **	0.510 **	0.495 **	0.378 **	0.238	−0.328 **	−0.434 **	1.000	
Stress Indicator	−0.001	0.208	0.026	−0.448 **	−0.005	−0.385 **	−0.224	−0.237	−0.188	0.091	0.063	0.186	−0.064	0.050	−0.591 **	−0.130	0.590 **	1.000

Note: ** and *: The correlation was significant at the level of 0.01 and 0.05 (double-tailed), respectively.

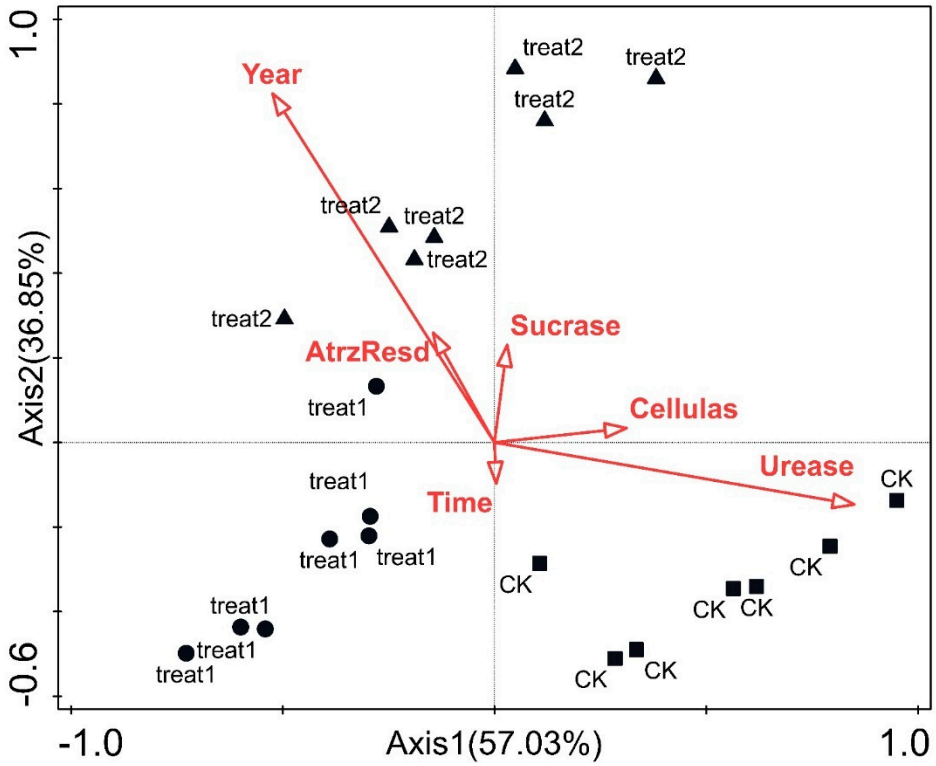

Figure 8. Redundancy analysis (RDA) of the PLFA data as explained by environmental variables. ■: Control; ●: Treat 1; ▲: Treat 2.

Pearson correlation analysis (Table 2) showed that continuous application of atrazine had a strong negative correlation with urease, cellulase, culturable bacterial biomass, and the G+/G− ratio and a positive correlation with soil atrazine residues, G− PLFA content, and the Shannon index. In addition to the continuous application time of atrazine, the change in atrazine residues in the soil was negatively correlated with the contents of urease and cellulase in the soil and the G+/G− ratio, and positively correlated with the Shannon index of the soil microbial community. According to the analysis of the soil microbial community and soil enzyme activity, soil urease activity was significantly negatively correlated with the microbial community composition (including G−, Bac, Fun, and total PLFA), stress index, and microbial Shannon index and significantly positively correlated with culturable soil actinomycetes biomass, G+/G−, and the Fun/Bac ratio. The cellulase activity in the soil was significantly negatively correlated with the content of G−, bacterial PLFA and the Shannon index in the soil and positively correlated with G+/G−. However, there was a weak correlation between the activity of glycosylase and microbial community composition in the soil, which was positively correlated with culturable actinomycetes and negatively correlated with the microbial stress index.

4. Discussion

In this study, the application of atrazine resulted in a high initial content of atrazine residues in the soil, after which the residues were continuously degraded. At the initial stage of application, the rate of atrazine degradation was rapid. With the progression of time, the amount and degradation rate of atrazine residues in the soil gradually decreased and finally reached a stable state. The results of Liu et al. showed that the degradation of atrazine in soil was mainly completed through hydrolysis and catabolism by microorganisms in the soil [23]. The hydrolysis theory explains the contradiction between the low activity of microorganisms at the initial stage of atrazine application and the high rate of atrazine degradation. However, this theory does not account for the increase in the degradation rate of atrazine in the second year of continuous application. Atrazine is

chemically hydrolyzed in the soil to desethylatrazine and deisopropylatrazine, and the hydrolyzed products can be directly utilized by microorganisms [9,24]. This may be one of the reasons for the increase in the number and species diversity of microorganisms in the soil.

Since the 1980s, many atrazine-degrading microorganisms have been isolated from the soil. Zhao et al., Sebaï et al., Dougglass et al., and others have isolated atrazine-degrading bacteria from farmland soils in which maize had been planted for many years [25–27]. Fan and Song (2014) also showed that the long-term application of atrazine enhanced the ability of soil microorganisms to degrade atrazine [7]. In this study, the degradation rate of atrazine in the soil increased in the second year of continuous application. This finding may provide new insight into the treatment of atrazine-contaminated soil. These data indicate that the soil microbial community structure can develop a high degradation capacity of atrazine after its application. Therefore, the increase in the degradation rate of atrazine caused by the continuous application of atrazine can be attributed to the adaptation of soil microorganisms to environmental stress, which is consistent with the research results of Li. et al. [28]. Furthermore, it was found that atrazine was not completely degraded in winter after 150 days of application, and the atrazine remained in the frozen soil until the next year; whether this would have adverse effects on the growth of the next crop requires further study. In addition, whether atrazine enters the surface water or groundwater with surface runoff or leaching in spring to cause nonpoint-source pollution remains to be further explored [7].

Soil enzyme activity is considered to be an early sensitive indicator of microbial responses to natural and agroecosystem stresses [29]. Any pesticide application that can affect the microbial community and its biochemical activities in the soil will lead to changes in soil enzyme activities. Moreover, enzyme activities in the soil are closely related to soil fertility, so they are a very important factor to study [30]. Soil urease, saccharase, and cellulase are related to soil nitrogen and carbon cycling [31–33]. In this study, these three soil enzymes showed different responses to the application of atrazine, which may be due to the unique chemical structure of each enzyme [34,35]. In addition, there was a significant correlation between the activities of urease and cellulase in the soil and the structure of the microbial community, indicating that the change in the microbial community may also lead to changes in enzyme function in the soil. This result is similar to the findings of Thapa et al. [36].

In this study, the contents of urease and cellulase in the soil decreased rapidly with the application of atrazine. Interestingly, the application of atrazine had no significant effect on the content of saccharase in the soil, which may be due to the fact that saccharase is an extracellular enzyme of microorganisms in the plant root stubble [37], and its activity reflects the ability of the microbial community to utilize carbohydrates [38], which is almost entirely unaffected by the growth and increase in soil microorganisms.

With the alleviation of the environmental stress and the progression of the recovery time, the cellulase recovered, but the activity of urease did not improve and remained low. This indicates that atrazine had an inhibitory effect on urease and made it difficult for this enzyme to quickly recover in the soil. Urease can promote the hydrolysis of the amide peptide bonds of urea in the soil, and ammonia is one of the sources of N nutrients in plants [39]. The results indicate that atrazine can affect nitrogen conversion in the soil, and related studies have shown that soil urease activity was positively correlated with soil fertility, indicating that atrazine application could reduce the level of soil fertility [40].

Microbial community composition is important for the maintenance of soil ecosystem function because it usually controls the transformation and utilization of nutrients and the decomposition rate of organic matter in the soil [41,42]. The effect of pesticides on the soil microbial community is variable and determined not only by the response of microorganisms and active substances but also by the development of and changes in specific microbial species [43–45]. In a study on the culturable microbial biomass of the soil, we found that the bacteria in the soil were significantly affected by the application of

atrazine. Bacterial biomass increased rapidly, especially early in the first year of application, indicating that atrazine application caused the rapid reproduction of some resistant bacteria in the soil. Studies have shown that the application of atrazine can inhibit the abundance of microorganisms in the soil, and some soil microorganisms can use their biological ability to compensate for this adverse effect under pesticide stress. Microorganisms that are not sensitive to pesticides can use the nutrients released by dead cells to increase the PLFA content in the soil [46]. The results of PCA in this study show that the application of atrazine resulted in differences in the microbial community composition; it significantly reduced the ratio of fungi to bacteria, but there was no significant change in fungal PLFA content, indicating that atrazine could be utilized by bacteria, resulting in an increase in the number of bacteria. Previous studies have shown that the application of atrazine can induce bacterial resistance so that some bacteria have the ability to degrade atrazine [47]; an atrazine application will thus select for bacterial strains that degrade it [7,28,48].

PLFA content is an important index of microbial biomass in soil [49]. The response of PLFA to soil environmental changes reflects differences in the tolerance of microorganisms to such changes. Bacterial biomass in Treat 1 increased significantly during the two years of continuous application of atrazine, while there was no significant difference in bacterial biomass between Treat 2 and the control group. Atrazine is chemically hydrolyzed in the soil to desethylatrazine and deisopropylatrazine, and the hydrolyzed products can be directly utilized by microorganisms [9]. This directly leads to an increase in microbial biomass in the soil, and the response of microorganisms to environmental changes leads to an increase in atrazine-degrading bacteria in the soil. In the second year after the application of atrazine, the PLFAs of microorganisms returned to the control level, but the diversity of the microbial community increased significantly, the biomass of G+ was significantly decreased compared with that of G−, and the biomass of anaerobic bacteria was increased compared with that of aerobic bacteria. This change in the composition of the microbial community may be the root cause of the increased rate of atrazine degradation.

5. Conclusions

As the atrazine application time increased, the concentration of the atrazine residues in the soil showed non-linear changes. At the initial stage of atrazine application, the soil residues decreased rapidly, and the degradation rate was faster in the second year. The activities of urease and cellulase in soil were significantly inhibited by atrazine, but there was no significant effect on saccharase. In addition, the microbial community structure in the soil was changed by the application of atrazine; the content of PLFA in the soil increased in the first year of continuous application and tended to return to the normal level in the second year.

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References

1. Gysin, H.; Knüsli, E. Chemistry and the herbicidal properties of triazine derivatives. *Adv. Pest Control Res.* **1960**, *3*, 615–623.
2. Szewczyk, R.; Kuśmierska, A.; Bernat, P. Ametryn removal by *Metarhizium brunneum*: Biodegradation pathway proposal and metabolic background revealed. *Chemosphere* **2018**, *190*, 174–183. [CrossRef] [PubMed]
3. Metzger, B.A.; Soltani, N.; Raeder, A.J.; Hooker, D.C.; Robinson, D.E.; Sikkema, P.H. Effect of hybrid varieties, application timing, and herbicide rate on field corn tolerance to tolpyralate plus atrazine. *Weed Sci.* **2019**, *67*, 475–484. [CrossRef]
4. Barchanska, H.; Sajdak, M.; Szczypka, K.; Swientek, A.; Tworek, M.; Kurek, M. Atrazine, triketone herbicides, and their degradation products in sediment, soil and surface water samples in Poland. *Environ. Sci. Pollut. Res.* **2016**, *24*, 644–658. [CrossRef] [PubMed]
5. Barrios, R.E.; Akbariyeh, S.; Liu, C.; Gani, K.M.; Kovalchuk, M.T.; Li, X.; Li, Y.; Snow, D.; Tang, Z.; Gates, J.; et al. Climate change impacts the subsurface transport of atrazine and estrone originating from agricultural production activities. *Environ. Pollut.* **2020**, *265 Pt A*, 115024. [CrossRef]
6. Ghirardelli, A.; Otto, S.; Masin, R.; Bano, C.; Altissimo, L.; Russo, S.; Zanin, G. Thirty-year monitoring of s-triazine herbicide contamination in the aquifer north of Vicenza (north-east Italy). *Sci. Total. Environ.* **2021**, *752*, 141647. [CrossRef]
7. Fan, X.; Song, F. Bioremediation of atrazine: Recent advances and promises. *J. Soils Sediments* **2014**, *14*, 1727–1737. [CrossRef]
8. Jablonowski, N.D.; Köppchen, S.; Hofmann, D.; Schäffer, A.; Burauel, P. Persistence of 14C-labeled atrazine and its residues in a field lysimeter soil after 22years. *Environ. Pollut.* **2009**, *157*, 2126–2131. [CrossRef]
9. Huang, H.; Zhang, C.; Rong, Q.; Li, C.; Mao, J.; Liu, Y.; Chen, J.; Liu, X. Effect of two organic amendments on atrazine degradation and microorganisms in soil. *Appl. Soil Ecol.* **2020**, *152*, 103564. [CrossRef]
10. Imfeld, G.; Vuilleumier, S. Measuring the effects of pesticides on bacterial communities in soil: A critical review. *Eur. J. Soil Biol.* **2012**, *49*, 22–30. [CrossRef]
11. Muñoz-Leoz, B.; Ruiz-Romera, E.; Antigüedad, I.; Garbisu, C. Tebuconazole application decreases soil microbial biomass and activity. *Soil Biol. Biochem.* **2011**, *43*, 2176–2183. [CrossRef]
12. Fang, H.; Lian, J.; Wang, H.; Cai, L.; Yu, Y. Exploring bacterial community structure and function associated with atrazine biodegradation in repeatedly treated soils. *J. Hazard. Mater.* **2015**, *286*, 457–465. [CrossRef] [PubMed]
13. Howell, C.C.; Semple, K.T.; Bending, G.D. Isolation and characterisation of azoxystrobin degrading bacteria from soil. *Chemosphere* **2014**, *95*, 370–378. [CrossRef]
14. Vargha, M.; Takats, Z.; Márialigeti, K. Degradation of atrazine in a laboratory scale model system with Danube river sediment. *Water Res.* **2005**, *39*, 1560–1568. [CrossRef]
15. Bhardwaj, P.; Singh, K.R.; Jadeja, N.B.; Phale, P.S.; Kapley, A. Atrazine Bioremediation and Its Influence on Soil Microbial Diversity by Metagenomics Analysis. *Indian J. Microbiol.* **2020**, *60*, 388–391. [CrossRef]
16. Douglass, J.F.; Radosevich, M.; Tuovinen, O.H. Microbial attenuation of atrazine in agricultural soils: Biometer assays, bacterial taxonomic diversity, and catabolic genes. *Chemosphere* **2017**, *176*, 352–360. [CrossRef]
17. Fan, X.; Chang, W.; Sui, X.; Liu, Y.; Song, G.; Song, F.; Feng, F. Changes in rhizobacterial community mediating atrazine dissipation by arbuscular mycorrhiza. *Chemosphere* **2020**, *256*, 127046. [CrossRef]
18. Liu, Y.; Fan, X.; Zhang, T.; He, W.; Song, F. Effects of the long-term application of atrazine on soil enzyme activity and bacterial community structure in farmlands in China. *Environ. Pollut.* **2020**, *262*, 114264. [CrossRef] [PubMed]
19. Sun, J.; Yang, L.; Wei, J.; Quan, J.; Yang, X. The responses of soil bacterial communities and enzyme activities to the edaphic properties of coal mining areas in Central China. *PLoS ONE* **2020**, *15*, e0231198. [CrossRef] [PubMed]
20. Chen, X.; Hao, B.; Jing, X.; He, J.S.; Ma, W.; Zhu, B. Minor responses of soil microbial biomass, community structure and enzyme activities to nitrogen and phos-phorus addition in three grassland ecosystems. *Plant Soil* **2019**, *444*, 21–37. [CrossRef]
21. Wilkinson, S.; Anderson, J.; Scardelis, S.; Tisiafouli, M.; Taylor, A.; Wolters, V. PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. *Soil Biol. Biochem.* **2002**, *34*, 189–200. [CrossRef]
22. Ganguly, R.K.; Chakraborty, S.K. Qualitative assessment of paper mill waste valorisation through combinatorial PLFA markers and spectroscopical analysis: An ecotechnology towards waste to resource transformation. *Environ. Technol. Innov.* **2021**, *23*, 101532. [CrossRef]
23. Liu, J.; Zhou, J.H.; Guo, Q.N.; Ma, L.Y.; Yang, H. Physiochemical assessment of environmental behaviors of herbicide atrazine in soils associated with its degrada-tion and bioavailability to weeds. *Chemosphere* **2020**, *262*, 127830. [CrossRef] [PubMed]
24. Graymore, M.; Stagnitti, F.; Allinson, G. Impacts of atrazine in aquatic ecosystems. *Environ. Int.* **2001**, *26*, 483–495. [CrossRef]
25. Douglass, J.F.; Radosevich, M.; Tuovinen, O.H. Molecular analysis of atrazine-degrading bacteria and catabolic genes in the water column and sediment of a created wetland in an agricultural/urban watershed. *Ecol. Eng.* **2015**, *83*, 405–412. [CrossRef]
26. El Sebaï, T.; Devers-Lamrani, M.; Changey, F.; Rouard, N.; Martin-Laurent, F. Evidence of atrazine mineralization in a soil from the Nile Delta: Isolation of Arthrobacter sp TES6, an at-razine-degrading strain. *Int. Biodeterior. Biodegrad.* **2011**, *65*, 1249–1255. [CrossRef]
27. Zhao, X.; Ma, F.; Feng, C.; Bai, S.; Yang, J.; Wang, L. Complete genome sequence of Arthrobacter sp. ZXY-2 associated with effective atrazine degradation and salt adaptation. *J. Biotechnol.* **2017**, *248*, 43–47. [CrossRef]
28. Li, K.-B.; Cheng, J.-T.; Wang, X.-F.; Zhou, Y.; Liu, W.-P. Degradation of Herbicides Atrazine and Bentazone Applied Alone and in Combination in Soils. *Pedosphere* **2008**, *18*, 265–272. [CrossRef]

29. Gil-Sotres, F.; Trasar-Cepeda, C.; Leirós, M.; Seoane, S. Different approaches to evaluating soil quality using biochemical properties. *Soil Biol. Biochem.* **2005**, *37*, 877–887. [CrossRef]
30. Zhang, Q.; Zhu, L.; Wang, J.; Xie, H.; Wang, J.; Wang, F.; Sun, F. Effects of fomesafen on soil enzyme activity, microbial population, and bacterial community composition. *Environ. Monit. Assess.* **2014**, *186*, 2801–2812. [CrossRef]
31. Chen, F.-S.; Fahey, T.J.; Yu, M.-Y.; Gan, L. Key nitrogen cycling processes in pine plantations along a short urban–rural gradient in Nanchang, China. *For. Ecol. Manag.* **2010**, *259*, 477–486. [CrossRef]
32. A Lockington, R.; Rodbourn, L.; Barnett, S.; Carter, C.J.; Kelly, J.M. Regulation by carbon and nitrogen sources of a family of cellulases in *Aspergillus nidulans*. *Fungal Genet. Biol.* **2002**, *37*, 190–196. [CrossRef]
33. Yan, B.; Wang, X.; Sun, Y.; Fan, B.; Shi, L.; Liu, G. Vegetation rehabilitation increases soil enzyme activities in degraded land via carbon supply and nitrogen retention. *Eur. J. Soil Biol.* **2020**, *98*, 103186. [CrossRef]
34. Xiong, D.; Gao, Z.; Fu, B.; Sun, H.; Tian, S.; Xiao, Y.; Qin, Z. Effect of pyrimorph on soil enzymatic activities and respiration. *Eur. J. Soil Biol.* **2013**, *56*, 44–48. [CrossRef]
35. Zhang, W.; Zhang, M.; An, S.; Lin, K.; Li, H.; Cui, C.; Fu, R.; Zhu, J. The combined effect of decabromodiphenyl ether (BDE-209) and copper (Cu) on soil enzyme activities and microbial community structure. *Environ. Toxicol. Pharmacol.* **2012**, *34*, 358–369. [CrossRef]
36. Thapa, V.R.; Ghimire, R.; Acosta-Martínez, V.; Marsalis, M.A.; Schipanski, M.E. Cover crop biomass and species composition affect soil microbial community structure and enzyme activities in semiarid cropping systems. *Appl. Soil Ecol.* **2021**, *157*, 103735. [CrossRef]
37. Zhang, W.; Zhu, J.; Zhou, X.; Li, F. Effects of shallow groundwater table and fertilization level on soil physico-chemical properties, enzyme activities, and winter wheat yield. *Agric. Water Manag.* **2018**, *208*, 307–317. [CrossRef]
38. Li, H.; Yao, J.; Gu, J.; Duran, R.; Roha, B.; Jordan, G.; Liu, J.; Min, N.; Lu, C. Microcalorimetry and enzyme activity to determine the effect of nickel and sodium butyl xanthate on soil microbial community. *Ecotoxicol. Environ. Saf.* **2018**, *163*, 577–584. [CrossRef]
39. Nim, Y.S.; Wong, K.-B. The Maturation Pathway of Nickel Urease. *Inorganics* **2019**, *7*, 85. [CrossRef]
40. Cheng, H.; Zhang, D.; Huang, B.; Song, Z.; Ren, L.; Hao, B.; Liu, J.; Zhu, J.; Fang, W.; Yan, D.; et al. Organic fertilizer improves soil fertility and restores the bacterial community after 1,3-dichloropropene fumigation. *Sci. Total. Environ.* **2020**, *738*, 140345. [CrossRef] [PubMed]
41. Nsabimana, D.; Haynes, R.; Wallis, F. Size, activity and catabolic diversity of the soil microbial biomass as affected by land use. *Appl. Soil Ecol.* **2004**, *26*, 81–92. [CrossRef]
42. Wang, C.; Xiao, R.; Guo, Y.; Wang, Q.; Cui, Y.; Xiu, Y.; Ma, Z.; Zhang, M. Changes in soil microbial community composition during Phragmites australis straw decomposition in salt marshes with freshwater pumping. *Sci. Total. Environ.* **2021**, *762*, 143996. [CrossRef]
43. Cheng, J.; Lee, X.; Gao, W.; Chen, Y.; Pan, W.; Tang, Y. Effect of biochar on the bioavailability of difenoconazole and microbial community composition in a pesti-cide-contaminated soil. *Appl. Soil Ecol.* **2017**, *121*, 185–192. [CrossRef]
44. Díaz-López, M.; García, C.; Garrido, I.; Navarro, S.; Vela, N.; Nicolás, E.; Fenoll, J.; Bastida, F. Solarization-based pesticide degradation results in decreased activity and biomass of the soil microbial community. *Geoderma* **2019**, *354*, 113893. [CrossRef]
45. Fernández-Gómez, M.J.; Nogales, R.; Insam, H.; Romero, E.; Goberna, M. Role of vermicompost chemical composition, microbial functional diversity, and fungal com-munity structure in their microbial respiratory response to three pesticides. *Bioresour. Technol.* **2011**, *102*, 9638–9645. [CrossRef] [PubMed]
46. Sun, G.; Du, Y.; Yin, J.; Jiang, Y.; Zhang, D.; Jiang, B.; Li, G.; Wang, H.; Kong, F.; Su, L.; et al. Response of microbial communities to different organochlorine pesticides (OCPs) contamination levels in contaminated soils. *Chemosphere* **2019**, *215*, 461–469. [CrossRef] [PubMed]
47. Udiković-Kolić, N.; Scott, C.; Martin-Laurent, F. Evolution of atrazine-degrading capabilities in the environment. *Appl. Microbiol. Biotechnol.* **2012**, *96*, 1175–1189. [CrossRef] [PubMed]
48. Omotayo, A.E.; Ilori, M.; Radosevich, M.; Amund, O.O. Metabolism of Atrazine in Liquid Cultures and Soil Microcosms byNocardioidesStrains Isolated from a Contaminated Nigerian Agricultural Soil. *Soil Sediment Contam. Int. J.* **2013**, *22*, 365–375. [CrossRef]
49. Miura, T.; Makoto, K.; Niwa, S.; Kaneko, N.; Sakamoto, K. Comparison of fatty acid methyl ester methods for characterization of microbial communities in forest and arable soil: Phospholipid fraction (PLFA) versus total ester linked fatty acids (EL-FAME). *Pedobiologia* **2017**, *63*, 14–18. [CrossRef]