Effects of atrazine on the black community land function and role of microbial ecology

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Abstract. By measuring the concentration of atrazine stress and different culture days, the abundance of key genes in soil nitrogen transformation was studied by means of real-time PCR with the increase of atrazine stress time. The abundance of soil nitrogen-fixing function gene (nif-H) increased significantly with the increase of time in the middle and late culture period (15-30 d), indicating that the abundance of soil nitrogen-fixing function gene was promoted; soil ammonia oxidation. The abundance of functional gene (amo A) was reduced at the low concentration of atrazine (0 mg/kg, 20 mg/kg), and then decreased with increasing stress time, while at high concentration of atrazine. Under the treatment of (100mg/kg), with the increase of time, the abundance of soil ammonia oxidation function increased first and then decreased, indicating that the abundance of soil ammonia oxidation function gene was affected in the process of culture. II Based on high-throughput sequencing technology, the results of soil nitrogen fixation and ammonia oxidation community diversity showed that the functional gene diversity of soil samples was affected by the concentration of atrazine and stress time. According to the analysis of soil diversity index, nitrogen-fixing functional genes of soil samples treated with atrazine at 0 mg/kg, 20 mg/kg and 100 mg/kg were tested at the late stage (30 d). The diversity index of (nif-H) was higher than the mid-test (15 d). At the beginning of the experiment (0 d), the ammonia functional gene (amo A) diversity index of soil samples treated with atrazine at 0 mg/kg, 20 mg/kg and 100 mg/kg were tested at the late stage (30 d). The diversity index of (nif-H) was higher than the mid-test (15 d). At the beginning of the experiment (0 d), the ammonia functional gene (amo A) diversity index of soil samples treated with atrazine at 0 mg/kg, 20 mg/kg and 100 mg/kg were tested at the late stage (30 d). 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1. Introduction
Black soil is a natural resource that is indispensable for ensuring grain yield in the black soil region of
Northeast China[1]. It pays attention to the key process of nitrogen stress in the black soil of Atrazine-the disturbance law of nitrogen fixation and nitrification, and the response law of microbial functional gene abundance and diversity[2]. The effect of atrazine on the nitrogen fixation and nitrification of black soil and the related mechanism from the molecular level have important research value[3]. Fluorescence quantitative PCR was used to further study the dynamic changes of key functional gene abundance in black soil nitrogen fixation and nitrification under different concentrations of atrazine stress and stress time[4-5]. The soil nitrogen cycle, especially nitrogen fixation and nitrification, was to maintain soil[6-7]. The important process of crop nutrient supply capacity and level, so attention to the nitrogen fixation and nitrification of farmland black soil and its bacterial community structure, quantity and activity can accurately understand the nutrient cycling and nitrogen supply potential of farmland black soil in the main grain-producing areas of Northeast China[8]. It is also possible to indirectly grasp the health status of farmland black soil. The effect of the use of atrazine in the black soil region of Northeast China on the nitrogen cycle of black soil especially the nitrogen fixation process and the nitrification process and its driving microorganisms, is still unclear[9]. In view of the above research hotspots and research gaps, the project uses farmland black soil as the test soil, and takes the central link of farmland black soil nitrogen cycle as the entry point to investigate the widely applied corn farmland herbicide Atrazine to the farmland black soil in the northeast black soil area. The main driver of the nitrogen-fixing process and the ammoxidation process is the bacterial microbial community, quantity and functional activity[10]. In order to reveal the response of the ammonia fixing process and the ammoxidation process to the herbicide atrazine from the perspective of functional microbial community structure[11], quantity and functional activity, the herbicide Atrazine was used for the nitrogen cycle of farmland black soil, especially It is the possibility of interference caused by nitrogen fixation and ammonia oxidation[12]. The research results can also provide necessary basic information for black soil nutrient management and regulation, black soil ecological environment protection and restoration[13], food security and food safety, and have extremely important theoretical and practical significance for the protection of scarce black soil resources.

2. Materials and methods

2.1 Test soil and its cultivation method
The soil samples of this experiment were collected in Jiamusi City, Heilongjiang Province. The soil of the cornfield surface (0-20cm) was used. This soil sample is a typical northeast black soil Baoquanling farm, China. The debris in the collected soil samples was removed, air-dried at room temperature, ground, and passed through a 2 mm sieve for use.

2.2 Soil culture test
The designed soil atrazine concentration was: 0 mg/kg, 10 mg/kg, 20 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg, using corn soil topsoil, this soil was sieved. After the test soil, each treatment was set to three parallels. Based on the basis of the previous research, it is planned to simulate the repair experiment for 30 consecutive days. The above-treated soil was placed in a 25°C incubator for continuous culture. Distilled water was added every other day, and the soil moisture content was maintained at 20%. During the culture process, samples were taken at 0, 15 and 30 days, each time taking fresh soil weight of 10 soil samples and 30g. The soil samples were packed in polyethylene ziplock bags and stored at ultra-low temperature -80°C for determination of gene abundance and community diversity.

2.3 Soil nitrogen fixation and ammonia oxidation key gene abundance determination
The nucleotide sequence of the absolute content of nif-H (nitrogen-fixing gene), amo A (ammonia monoxygenase gene) and 16S gene was obtained from the Genbank database. Primer Premier 5.0
software was used to perform primers according to the principle of fluorescent quantitative PCR primer design (in Table 1).

| gene  | Primer  | Sequence (5′-3′)                                      | Fragment size(bp) |
|-------|---------|-----------------------------------------------------|-------------------|
| nif-H | nif-H 1F | AAAGGYGGWATCCGGYAARTCCACCAC                       | 458               |
|       | nif-H 2R | TTGTTSGCSCGRTACATSGCCATCAT                         |                   |
| amoA  | amoA-1F-B | GGGGTTCCTACTGGTGTT                              | 491               |
| A     | amoA-2R-B | CCCCTCKGSAAGCCTTCTTC                             |                   |

2.4 Data processing

Significant differences were analyzed for different differences (P<0.05). The results were plotted using Origin 9.0 software and differences between treatments were analyzed by one-way ANOVA. High-throughput sequencing images were drawn using software such as Pheat-map, Venn Diagram, and igraph. The data were analyzed using Qiime software, and the Greens database was used to compare 16S rRNA gene sequences to determine the taxonomic status of the corresponding microbes. Among them, in high-throughput data, Chao1: an index used to estimate the number of OTUs in a community, commonly used to estimate the total number of species in the ecology. Shannon: estimated microbial diversity index in the sample, used to reflect the alpha diversity index. The larger the Shannon value, the higher the diversity of the community. Simpson: an estimate of microbial diversity indicators used to quantitatively describe the biodiversity of a region. The smaller the index, the higher the diversity of the community. Calculated as follows:

\[
S_{\text{chao}} = S_{\text{obs}} + \frac{n_1(n_1 - 1)}{2(n_2 + 1)} \]  
\[
H_{\text{Shannon}} = -\sum_{i=1}^{S_{\text{obs}}} \frac{n_i}{N} \ln \frac{n_i}{N} \]  
\[
D_{\text{Simpson}} = \frac{\sum_{i=1}^{S_{\text{obs}}} N_i(n_i - 1)}{N(N - 1)} \]

3. Results and discussion

3.1 Effect of Atrazine on Microbial Biomass Carbon Content in Black Soil

Changes of microbial biomass carbon content in tested soils with different levels of atrazine stress over time. The results showed that on the first day 0, there was no significant difference in the microbial biomass carbon content in the soil samples treated with atrazine stress (P<0.05), but in the middle and late culture period (15-30 days). There was no significant difference in the microbial biomass carbon content in the soil samples treated with atrazine stress compared with the blank treatment (P<0.05). With the increase of culture time, the content of carbon in each treatment increased first and then decreased. On the 0-15th day of the whole culture, the increase of microbial carbon content in blank treatment was not significant (P<0.05), while the abundance of nif-H gene in the other contaminated soil samples showed a downward trend, but the degree of decline reached a significant level (P<0.05), while the abundance of nif-H gene in the other contaminated soil samples showed Increase the trend. At the end of the experiment (day 30), the abundance of nif-H gene in the blank treatment was higher than that in the...
initial stage of culture (0-15d), and the degree of increase reached a significant level (P<0.05), while the remaining treatment samples were processed. The abundance of nif-H gene was slowed down, and the difference in nif-H gene abundance in soil samples was no longer significant (P<0.05) (in Figures 1 and 2).

3.3 Soil dilution curve and diversity index analysis of OTU population and community diversity

The results of this study showed that the Chaol, Ace and Shannon diversity indices of the nitrogen-fixing functional genes (nif-H) of soil samples treated with atrazine at 0 mg/kg, 20 mg/kg and 100 mg/kg were high for 30 days. The gene parameters in soil samples from the treatment of 15 mg of atrazine at 0mg/kg, 20 mg/kg and 100 mg/kg for 15 days, indicating that the concentration of atrazine was 0mg/kg for 30 days. Soil samples treated with 20 mg/kg and 100 mg/kg have higher genetic diversity and species richness. Studies have confirmed that the nitrogen-fixing gene diversity expressed by the Chaol, Ace, and Shannon indices is consistent with the trend of dilution analysis (in Table 2).

| Samp T | Stress C | Seqnum | OTU num | Chaol | Ace | Shannon | Simpson | Coverage |
|--------|----------|--------|---------|-------|-----|---------|---------|----------|
| 0      | 0        | 35732  | 1314    | 1529.87 | 1599.74 | 4.47 | 0.06 | 0.99     |
| 0      | 25       | 33673  | 1231    | 1601.04 | 1587.55 | 4.47 | 0.06 | 0.99     |
| 0      | 100      | 38824  | 1343    | 1618.73 | 1630.12 | 4.76 | 0.05 | 0.99     |
| 15     | 0        | 33135  | 1006    | 1324.82 | 1322.95 | 4.94 | 0.03 | 0.99     |
| 15     | 25       | 35026  | 1086    | 1400.55 | 1418.51 | 4.65 | 0.05 | 0.99     |
| 15     | 100      | 35007  | 1033    | 1310.24 | 1300.95 | 4.86 | 0.06 | 0.99     |
| 30     | 0        | 40115  | 1552    | 1862.75 | 1907.73 | 4.83 | 0.05 | 0.99     |
| 30     | 25       | 53963  | 2414    | 2576.13 | 2663.16 | 4.56 | 0.12 | 0.99     |
| 30     | 100      | 30572  | 1325    | 1649.24 | 1642.74 | 4.68 | 0.07 | 0.99     |

4. Conclusions

The content of microbial biomass nitrogen in soil samples increased gradually and then decreased. The content of microbial biomass carbon in soil samples increased gradually and then decreased. At the end of the experiment (30d), the microbial biomass nitrogen content in the blank treatment decreased to a significant level, and the microbial biomass nitrogen content in the soil samples with the atrazine concentration of 100 mg/kg was similar to the blank treatment. Soil nitrogen fixation function gene (nif-H) abundance on the first day of experiment, atrazine stress can inhibit the nif-H gene abundance in soil, but the dose-effect relationship of inhibition is not obvious. Among them, on the 0th-15th day of the whole culture, the abundance of nif-H gene in the blank treatment showed a significant decrease. During the whole experimental period (0-30d), the abundance of soil ammonia oxidation function gene (amoA) was reduced at a low concentration of atrazine (0 mg/kg, 20 mg/kg), and then decreased with time. It can be seen that under different concentrations of atrazine stress and time, the abundance of soil ammonia oxidation function has been affected in the cultivation process. According to the analysis of soil Chaol, Ace and Shannon index, the total amount of nitrogen-fixing functional genes in
blank treatment was higher than 20 mg/kg and 200 mg/kg in the whole experiment (0-30d). At 0-30 days, the total amount of ammoxidation function genes treated with blanks increased with the prolongation of culture time, and the number of Vibrio nitrobacteria increased first and reached a stable amount. The results of principal component analysis further confirmed that atrazine with different culture days and different stress concentrations had certain effects on the composition of soil nif-H and amoA gene communities.

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