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RESEARCH ARTICLE

Effects of imidacloprid on soil microbial communities in different saline soils

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Abstract The effects of imidacloprid in the soil environment are a worldwide concern. However, the impact of imidacloprid on soil microorganisms under salt stress is almost unknown. Therefore, an indoor incubation test was performed, and the denaturing gradient gel electrophoresis (DGGE) approach was used to determine the response of different saline soil bacterial and fungal community structures to the presence of imidacloprid (0.4, 2, 10 mg kg^{-1}). The results showed that the soil bacterial diversity slightly declined with increasing imidacloprid concentration in soils with low salinity. In moderately saline soils, a new band in the DGGE profile suggested that imidacloprid could improve the soil bacterial diversity to some degree. An analysis of variance indicated that the measured soil bacterial diversity parameters were significantly affected by dose and incubation time. Compared with the control, the soil fungal community structure showed no obvious changes in low and moderately saline soils treated with imidacloprid. The results of these observations provide a basic understanding of the potential ecological effects of imidacloprid on different microorganisms in saline soils.

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Introduction

Soil salinity is of broad concern worldwide due to its negative effects on soil texture, soil microbial community, and plant growth (Rietz and Haynes 2003; Porcel et al. 2012; Estrada et al. 2013). Inputs of xenobiotic pollutants probably result in different effects in saline soils than in non-saline soils. Although previous studies have shown that some pollutants such as pesticides, fertilizers, and other substances impact the non-saline soil environment to some degree (Gómez-Sagasti et al. 2012; Zhang et al. 2012, 2014a, b), little is known about the effects of xenobiotic pollutants on saline soils. In China, the Yellow River Delta, located in Shandong Province (from 117° 48′ to 119° 45′ E and 36° 52′ to 38° 12′ N), is one of the largest river deltas in China with an area of 12,000 km^2 (Zhang and Sun 2005). Rapid economic development has made this area an important agricultural production base. Cotton, wheat, corn, and vegetables are the major food products grown here to support the growing Chinese population (Yang et al. 2013). However, soil salinity (mainly low and moderate salinity) is a major obstacle to the development of agriculture in this area (Wang et al. 2014b). To encourage sustainable development in this region, it is necessary to investigate the response of xenobiotic pollutants on the soil environment.

Imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine) is the most extensively used neonicotinoid insecticide in worldwide agriculture for the control of sucking insects, termites, soil insects, and some chewing insects (Tomizawa and Casida 2005; Muhammad Ashraf et al. 2012). It is also one of the principal pesticides used in the Yellow River Delta (Dong et al. 2010). The half-life for the dissipation

of imidacloprid in soils ranged from a few days to dozens of days (Sarkar et al. 2001; Wu et al. 2012). Previous studies have shown that the soil physicochemical and biological parameters such as the soil type, organic matter, pH, temperature, and microbial activity can affect the behavior of imidacloprid in soils (Oi 1999; Flores-Céspedes et al. 2002; Wu et al. 2012). The effects of imidacloprid on soil microorganisms had also been widely investigated. Cycoń et al. (2013; Cycoń and Piotrowska-Seget 2015a, 2015b) and Wang et al. (2014a) reported that imidacloprid can cause changes in the community structure of soil bacteria, ammonia-oxidizing archaea, and ammonia-oxidizing bacteria and decrease the biomass and the activity of soil respiration, dehydrogenase, acid, and alkaline phosphatase, indicating that imidacloprid has a potential risk to the soil biochemical characteristics and microbial activity. However, little is known about the effects of imidacloprid on the saline soil microbial community structure.

Since denaturing gradient gel electrophoresis (DGGE) was introduced for analyzing the genetic diversity of complex microbial populations by Muyzer et al. (1993), it has been one of the most popular molecular methods to monitor microbial community structure in a large number of samples, including soils (Cycoń et al. 2013), fermented products (Kim et al. 2010), and decaying wood (Rajala et al. 2010). Therefore, we used DGGE to study the microcosm after application of imidacloprid to various saline soils. The aim of this study was to assess the potential ecological risk of imidacloprid to the soil microbial community in the Yellow River Delta.

Materials and methods

Soil

An alluvial soil that had not been previously treated with imidacloprid was collected from the top layer (0–20 cm) of a cotton field located in the Yellow River Delta. According to the USDA Soil Taxonomy System, it is classified as fluvo-aquic soil, and its physicochemical properties are as follows: pH (in water) 7.8, salt content 1.4 g kg^{-1}, electrical conductivity 4.32 ms cm^{-1}, organic matter 11.3 mg kg^{-1}, organic nitrogen 86.3 mg kg^{-1}, available phosphorus 11.2 mg kg^{-1}, and available potassium 123.4 mg kg^{-1}. The soil was classified as a low saline soil according to the saline soil classification standard (Szabolcs 1989; Wang et al. 1993).

Experimental design

Imidacloprid (CAS No. 138261-41-3, purity 98.5 %) purchased from Ehrenstorfer GmbH (Augsburg, Germany) was used in this study. The concentration of imidacloprid in soil was established according the experimental scheme of Cycoń et al. (2013) with some modifications, and the treatments were control, 0.4, 2, and 10 mg kg^{-1} dry soil. Additionally, for determining the effects of imidacloprid on soil microorganisms under moderate salt stress, a portion of soil was pulsed with 0.3 % NaCl. A complete list of soil treatments is provided in Table 1. For each treatment, 3000 g non-sterile soils were thoroughly mixed with an acetone solution of imidacloprid and/or NaCl to achieve the required concentration, and 1000 g soil (three replicates) was placed into a 1.5 L brown plastic pot. The same volume of acetone was also added to the control soil. The water content of the soil was adjusted to 60 % of the maximum water holding capacity by adding deionized water after the acetone solvent was evaporated. The pots were sealed with perforated polypropylene sheets and incubated at 25 °C in the dark for 28 days. Deionized water was added to the soil every 3 days by weight to compensate for water loss. Soil samples were collected on days 7, 14, 21, and 28 from each plot at each sampling time and stored in a refrigerator at −20 °C for DNA extraction.

DNA extraction, PCR amplification, and DGGE analysis

Total DNA was extracted from each soil sample (0.5 g) using the E.Z.N.A^{TM} Soil DNA Kit (Omega Bio-tek, Doraville, GA, USA) as described in the manufacturer's instructions. The extracted DNA was subjected to electrophoresis in a 1.0 % agarose gel and quantified using a spectrophotometer (Biophotomether, Eppendorf, Germany). For soil bacteria, the primer pair GC-338F (5′-CGCCCGCCGCGCGCGGCG-GGCGGGGCGGGGGCACGGGGGGCCTACG-GGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGG-CTGCTGG) were used for direct amplification of the variable V3 region of the 16S ribosomal DNA (rDNA) (Muyzer et al. 1993). For soil fungi, the primer pair GC-Fung (5′-CGCCCGCCGCGCCCCGCGCCC GGCCCG-CCGCCCCCGCCCCATTCCCCG TTACCCG-TTG-3′) and NS1 (5′-GTAGTCATATGCTFGTCTC-3′) were used for direct amplification of the 18S rDNA gene sequences (May et al. 2001). All PCR amplifications were performed on a PTC-220 Thermal Cycler (Bio-Rad, USA) using a 50 μL reaction volume. The reaction mixture contained 5 μL of 10× PCR buffers (TaKaRa, Japan), 3.2 μL of dNTP mix (2.5 mM), 0.4 μL of Taq DNA polymerase (5 U μL^{-1}), 1 μL of each primer (20 mM), 1 μL of template DNA (100 ng), and sterile-filtered milli-Q water to a final volume of 50 μL. The PCR amplification conditions were initial denaturation for 5 min at

Table 1 The design of different soil treatments

Treatments	I_0N_0	I_1N_0	I_2N_0	I_3N_0	I_0N_1	I_1N_1	I_2N_1	I_3N_1
Imidacloprid (mg kg^{-1})	0	0.4	2	10	0	0.4	2	10
NaCl (%)	0	0	0	0	0.3	0.3	0.3	0.3

94 °C, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. The final extension was 10 min at 72 °C. The PCR products were purified using the DNA Gel Extraction Kit (Axygen, USA) according to the manufacturer's instruction.

DGGE analysis was performed with a DCode Mutation Detection System (Bio-Rad, USA). For soil bacteria, PCR products (10 μL) were loaded into each lane an 8 % (*w*/*v*) polyacrylamide gel (37.5:1 acrylamide/bis-acrylamide) with a 35 to 55 % denaturing gradient. Denaturing solutions were prepared by mixing the appropriate volumes of two (0–100 %) stock solutions (7 mol L^{-1} urea and 40 % *v*/*v* formamide). The gel was run at 150 V for 4 h at 60 °C in a 1× TAE buffer (40 mM Tris–acetate, 1 mM EDTA). For soil fungi, an 8 % polyacrylamide gel with 25 to 40 % denaturing gradient was used for PCR products, and the gel was run in a 1× TAE buffer with a constant voltage of 150 V for 8 h at 60 °C. After electrophoresis, the gel was stained with silver nitrate (Bassam et al. 1991), photographed, and analyzed.

The pictures of the gels were analyzed using Quantity One software (Bio-Rad, USA) to calculate the similarity values of the bacterial and fungal community. On the basis of a band's presence or absence and band density analyses, phylogenic dendrograms were constructed using the Dice coefficient and the unweighted pair-group method with arithmetic averages (UPGMA). Richness (*S*) values were calculated according to the number of DNA bands detected in the respective lines of the DGGE profile. The Shannon–Wiener index (*H*) and evenness (E_H) values were calculated by the equations $H = -\sum pi\ (lnpi)$ and $EH = H/H_{max} = -H/lnS$, respectively, where *pi* is the ratio between the intensity of a specific band and the total intensity of all bands and *S* is the total number of bands in each sample (Cycoń et al. 2013).

Special bands from the DGGE profile of soil bacteria were excised and eluted in 50 μL sterile water overnight at 4 °C. The eluent (2 μL) was used for reamplification with primers 338F and 518R. DNA sequences were analyzed by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., China.

Statistical analyses

On the basis of the data obtained from DGGE, the percentage of variation attributable to the treatment factors and incubation time was determined with a two-way analysis of variance (ANOVA) using SPSS 18.0 software. The statistical significance of differences in data was evaluated by a post hoc comparison of the means utilizing the least significant differences (LSD) test.

Results

Effects of imidacloprid on bacterial communities in soils with different salinities

As shown in Fig. 1a, the position and intensity of the DGGE bands were different in the low saline soil treated with imidacloprid compared to the control at different incubation times, indicating that imidacloprid (0–10 mg kg^{-1}) affected the structure of the soil bacterial community during the 28-day experiment. However, cluster analysis showed no obvious trend, and the similarities between different treatments were greater than 50 %, suggesting that imidacloprid (0–10 mg kg^{-1}) did not have a serious effect on the soil bacterial community structure (Fig. 1b). From the values of Shannon–Wiener index (*H*), the richness (*S*), and the evenness (E_H) (Table 2), the data generally declined with increasing imidacloprid concentration on the seventh day. It is worth noting that the values of *H* and *S* in the I_3N_0 treatment were higher than those in I_1N_0 and I_2N_0 treatments on the 14th, 21st, and 28th day. As shown by the two-way ANOVA analysis (Table 3), the *H* index and the *S* value were significantly affected by the dose ($P<0.001$) and the incubation time ($P<0.001$), as well as by the interaction between these factors ($P=0.032$ for *H*, $P=0.007$ for *S*). The dose effect explained 36 and 28 % of the variance, whereas incubation time accounted for 28 and 32 %, and the interactions between these factors explained a further 17 and 13 % for *H* index and *S* value, respectively. However, the value of E_H was not significantly affected by the dose, incubation, and interaction between both factors during the course of the experiment (Table 3).

As shown in Fig. 2a, the densities and number of some bands in moderately saline soil were significantly weaker than those in low saline soil (Fig. 1a). The *H* index and *S* value in moderately saline soil were also significantly less than those in the low saline soil (Table 2). These indicated that salt can significantly inhibit the diversity indices of the soil bacterial community structure. Compared to Fig. 1a, bands 1, 2, 3, 4, and 5 disappeared in Fig. 2a. A BLAST search revealed that bands 1–5 had close relationships with *Pseudomonadaceae*, *Methylocystaceae*, *Phyllobacteriaceae*, and *Sphingobacteriales*, with sequence homologies ranging from 98 to 100 % similarity. The result of a cluster analysis (Fig. 2b) was similar to Fig. 1b, indicating that imidacloprid (0–10 mg kg^{-1}) also did not induce a very serious effect on bacterial community structure in the moderately saline soil. However, the results in Fig. 2b and Tables 2 and 3 indicated that imidacloprid could alter/improve the bacterial community structure to a certain extent. Band 6 (similar to *Acidobacteria*) appeared in soil contaminated with imidacloprid (Fig. 2b), and the *H* index and *S* value in the I_3N_1 treatment were higher than that in the control group on the 28th day (Table 2). These results indicated that imidacloprid could improve the bacterial diversity under the salt stress over the course of the

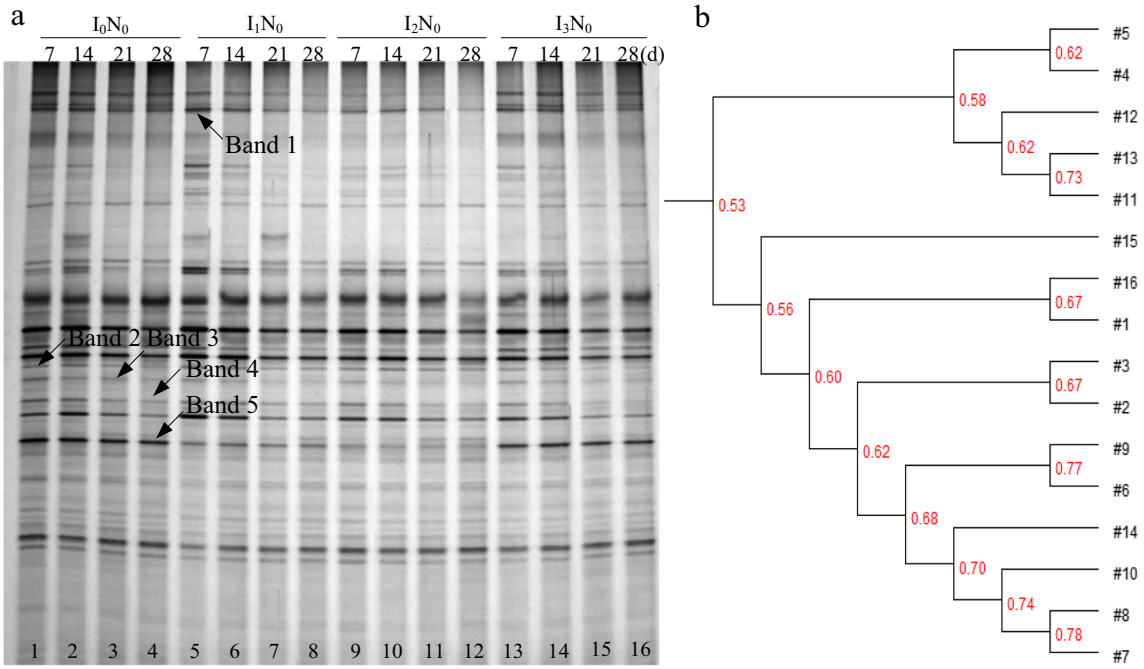

Fig. 1 DGGE profile and phylogenic dendrogram of the bacterial communities in soil treated with imidacloprid. The *lanes* from *1* to *16* are the treatments presented in the Table 1 (I_0N_0, I_1N_0, I_2N_0, I_3N_0) on days 7, 14, 21, and 28

experiment. The results of the two-way ANOVA (Table 3) showed that the *H* index and *S* value were also significantly affected by the dose ($P<0.001$), incubation time ($P<0.001$), and the interaction between dose and incubation ($P=0.014$ for *H*, $P=0.013$ for *S*). The dose effect accounted for 40 and 25 % of the variance, whereas incubation time explained 35 and 30 %, and the interactions between the dose and the incubation time accounted for a further 21 and 27 % of the variance for *H* index

Table 2 DGGE analysis results of soil bacterial and fungal diversity using the Shannon–Wiener index (*H*), species richness (*S*), and evenness (E_H) under different treatments and exposure time

Microorganism	Treatments	Shannon–Wiener index (*H*)				Species richness (*S*)				Evenness (E_H)			
		7	14	21	28	7	14	21	28	7	14	21	28 d
Bacteria	I_0N_0	3.42a	3.47a	3.31a	3.24a	34a	32a	29a	28a	0.99a	0.98a	0.98a	0.97a
	I_1N_0	3.35a	3.25b	3.02c	3.04c	32b	29b	27b	26b	0.96b	0.96b	0.96b	0.96b
	I_2N_0	3.28ab	3.14c	3.13b	3.01c	31b	27c	27b	25b	0.96b	0.95b	0.95b	0.96b
	I_3N_0	3.17c	3.23b	3.15b	3.17b	28c	29b	28a	27a	0.95b	0.96b	0.95b	0.96b
	I_0N_1	3.18a	3.18a	2.91a	2.80b	26a	27a	21a	19b	0.97a	0.96a	0.95a	0.95a
	I_1N_1	2.71c	2.89b	2.78b	2.74b	21b	23b	21a	20b	0.92b	0.92b	0.91b	0.91b
	I_2N_1	2.84b	2.79b	2.60b	2.78b	22b	21b	19a	20b	0.92b	0.92b	0.92b	0.93a
	I_3N_1	2.89b	3.09a	2.89a	3.01a	22b	26a	22a	23a	0.93b	0.95a	0.95a	0.96a
Fungi	I_0N_0	2.75a	2.68a	2.87a	2.94a	16a	15a	18a	20a	0.99a	0.99a	0.99a	0.98a
	I_1N_0	2.76a	2.93b	2.88a	2.88a	16a	19b	18a	18a	0.99a	0.99a	0.99a	0.99a
	I_2N_0	2.82ab	2.82ab	2.88a	2.94a	17ab	17ab	18a	19a	0.99a	0.99a	0.99a	0.99a
	I_3N_0	2.92b	2.93b	2.93a	2.93a	19b	19b	19a	19a	0.99a	0.99a	0.99a	0.99a
	I_0N_1	2.89a	2.90a	2.85a	2.81a	19a	19a	18a	17a	0.98a	0.98a	0.98a	0.99a
	I_1N_1	2.79a	2.87a	2.87a	2.87a	17a	18a	18a	18a	0.98a	0.99a	0.99a	0.99a
	I_2N_1	2.78a	2.74a	2.81a	2.81a	17a	16a	17a	17a	0.98a	0.99a	0.99a	0.99a
	I_3N_1	2.79a	2.86a	2.81a	2.86a	17a	18a	17a	18a	0.98a	0.99a	0.99a	0.99a

The data presented are the means of three replicates. The *different letters* indicate significant difference ($P<0.05$) between control and imidacloprid treatments at the same exposure time

Table 3 Two-way ANOVA results for the effects of treatment, time, and their interaction on the DGGE measured parameters

Treatment	Parameter	Variation	df	Sum of squares	Mean squares	Variance explained (%)	*F*	*P*
Bacteria I_0N_0-I_3N_0	*H*	Dose	3	43.21	14.40	36	16.74	***P*<0.001**
		Time	3	21.65	7.22	28	8.40	***P*<0.001**
		Dose × time	9	9.45	1.05	17	1.22	***P*=0.032**
	S	Dose	3	12.73	4.24	28	12.92	***P*<0.001**
		Time	3	31.57	10.52	32	32.07	***P*<0.001**
		Dose × time	9	20.38	2.26	13	6.89	***P*=0.007**
	E_H	Dose	3	0.0382	0.0127	16	1.21	*P*=0.153
		Time	3	0.023	0.0077	8	0.73	*P*=0.325
		Dose × time	9	0.0186	0.0021	2	0.20	*P*=0.718
Bacteria I_0N_1-I_3N_1	*H*	Dose	3	15.32	5.11	40	15.73	***P*<0.001**
		Time	3	10.83	3.61	35	11.11	***P*<0.001**
		Dose × time	9	17.42	1.94	21	5.97	***P*=0.014**
	S	Dose	3	49.03	16.34	25	14.74	***P*<0.001**
		Time	3	84.33	28.11	30	25.35	***P*<0.001**
		Dose × time	9	31.40	3.49	27	3.15	***P*=0.013**
	E_H	Dose	3	1.43	0.48	40	5.39	***P*=0.008**
		Time	3	0.89	0.30	13	3.37	*P*=0.061
		Dose × time	9	0.47	0.052	9	0.58	*P*=0.472
Fungi I_0N_0-I_3N_0	*H*	Dose	3	31.23	10.41	23	3.36	*P*=0.059
		Time	3	24.65	8.22	17	2.73	*P*=0.135
		Dose × time	9	38.04	4.23	21	1.36	*P*=0.286
	S	Dose	3	12.46	4.15	12	2.13	*P*=0.074
		Time	3	23.54	7.85	8	3.02	*P*=0.052
		Dose × time	9	14.05	1.56	24	0.80	***P*=0.041**
	E_H	Dose	3	1.92	0.64	31	1.09	*P*=0.306
		Time	3	0.89	0.30	25	0.51	*P*=0.582
		Dose × time	9	1.04	0.12	20	0.20	*P*=0.741
Fungi I_0N_1-I_3N_1	*H*	Dose	3	10.43	3.48	17	1.11	*P*=0.294
		Time	3	21.38	7.13	21	2.28	*P*=0.066
		Dose × time	9	17.46	1.94	15	0.62	*P*=0.602
	S	Dose	3	8.45	2.82	8	0.96	*P*=0.302
		Time	3	10.33	3.44	13	1.18	*P*=0.214
		Dose × time	9	12.68	1.41	20	0.39	*P*=0.701
	E_H	Dose	3	1.65	0.55	15	1.08	*P*=0.243
		Time	3	1.44	0.48	21	0.95	*P*=0.288
		Dose × time	9	1.69	0.19	18	0.37	*P*=0.708

H Shannon–Wiener index, *S* richness, E_H evenness, *df* degrees of freedom, *F* *F*max of Hartley. The effects (bold) are significant at $P<0.05$

and *S* value, respectively. The ANOVA also indicated that the dose significantly ($P=0.008$) affected the E_H value, and it accounted for most of the variance (40 %). However, incubation time and the dose × time interaction did not significantly affect the E_H value (Table 3).

Effects of imidacloprid on fungal communities in soils with different salinities

In the low saline soil groups, the position and intensity of the DGGE bands were basically similar in treatments with different doses of imidacloprid (Fig. 3a). The cluster analysis indicated that the similarities for the different treatments were all above 50 % (Fig. 3b). At the same time, the *H* index, the *S* value, and the E_H value for the different imidacloprid doses were not obviously different from the control (Table 2). These results confirmed that imidacloprid (0–10 mg kg^{-1}) did not significantly affect the fungal community structure in the low saline soil. As shown by the ANOVA analysis, dose, incubation time, and the dose × time interaction did not significantly affect the *H* index or the E_H value (Table 3). However, the dose ×

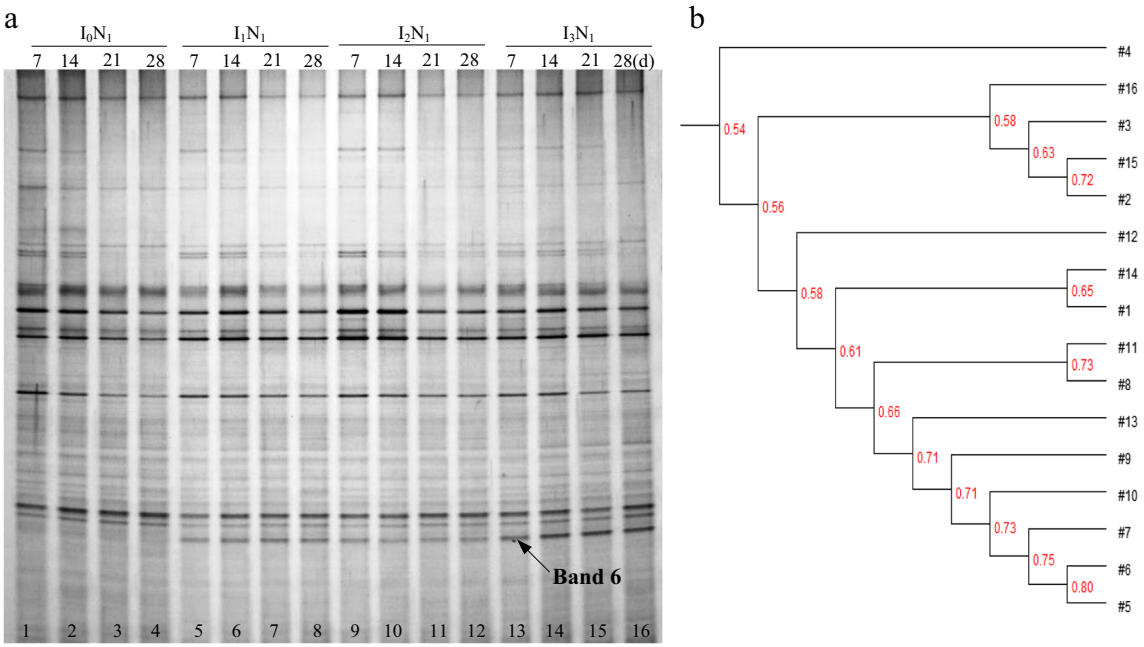

Fig. 2 DGGE profile and phylogenic dendrogram of the bacterial communities in soil treated with imidacloprid and 0.3 % NaCl. The *lanes* from *1* to *16* are the treatments presented in the Table 1 (I_0N_1, I_1N_1, I_2N_1, I_3N_1) on days 7, 14, 21, and 28

time interaction significantly (P=0.041) affected the S value accounting for 24 % of the variance (Table 3).

In moderately saline soil, neither the position nor the intensity of the DGGE bands nor the calculated H index, S value, and E_H value were different from those in low saline soil (Fig. 4a and Table 2), indicating that moderate levels of salt did not induce obvious changes in the diversity of soil fungal community structure. As shown in Fig. 4 and Table 2, the position and intensity of DGGE bands and the calculated parameters showed no significant difference from the control, and the results of cluster analysis showed no obvious trend, which indicated that imidacloprid (0–10 mg kg^{-1}) did not significantly affect the fungal community structure in the moderately saline soil. The two-way ANOVA analysis showed that imidacloprid dose, the incubation time, and the dose × time interaction did not cause obvious changes in the H index, the S value, or the E_H value (Table 3). This result further confirmed that imidacloprid (0–10 mg kg^{-1}) did not affect the fungal community structure in moderately saline soil.

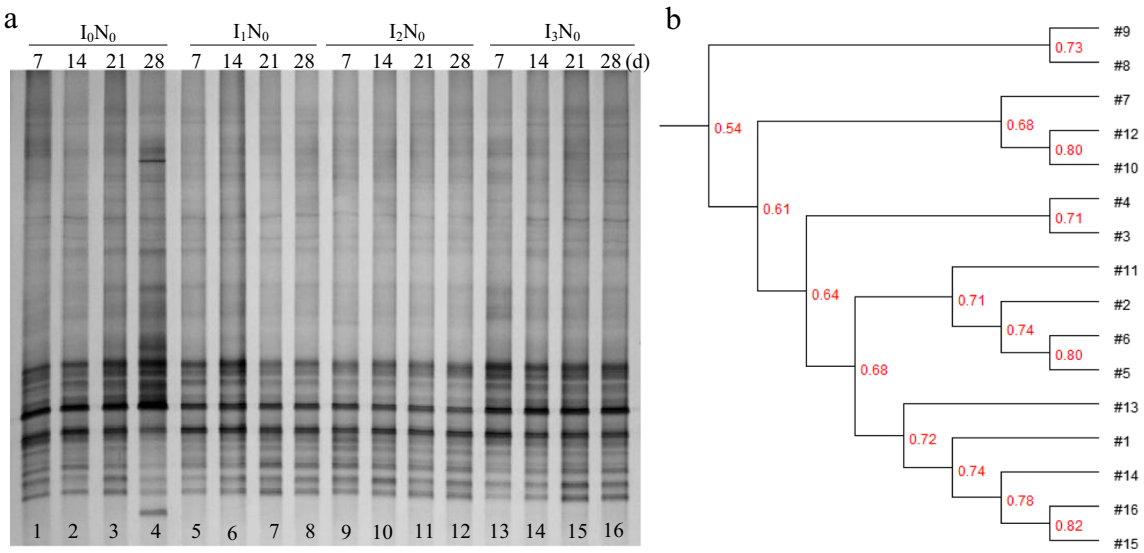

Fig. 3 DGGE profile and phylogenic dendrogram of the fungal communities in soil treated with imidacloprid. The *lanes* from *1* to *16* are the treatments presented in the Table 1 (I_0N_0, I_1N_0, I_2N_0, I_3N_0) on days 7, 14, 21, and 28

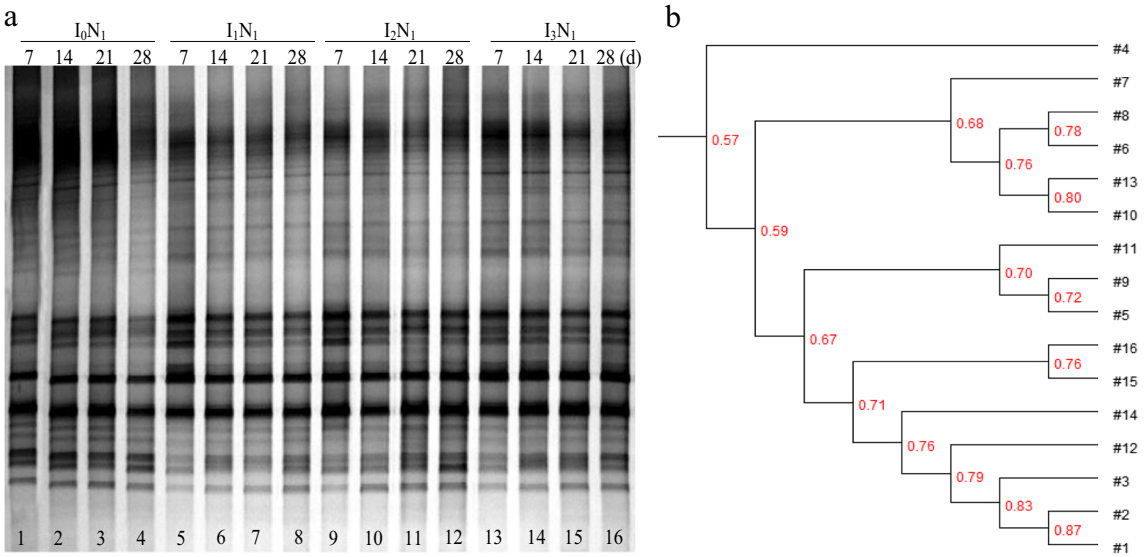

Fig. 4 DGGE profile and phylogenic dendrogram of the fungal communities in soil treated with imidacloprid and 0.3 % NaCl. The *lanes* from *1* to *16* are the treatments presented in the Table 1 (I_0N_1, I_1N_1, I_2N_1, I_3N_1) on days 7, 14, 21, and 28

Discussion

In this study, we aimed to assess the effects of imidacloprid on soil bacterial and fungal biodiversity and community structure in low and moderately saline soils under laboratory conditions using DGGE. The results indicated that imidacloprid slightly affected the bacterial community structure in the low saline soil, and this effect was affected by the dose, the incubation time, and the dose × time interaction. These findings were similar to the results of Cycoń et al. (2013), who reported that imidacloprid (1 and 10 mg kg^{-1}) affected soil bacterial biodiversity and community structure. However, the magnitude of the effect 10 mg kg^{-1} imidacloprid on soil bacteria was more obvious in the results of Cycoń et al. (2013) than in our study. The reason may be difference in soil type; the soil used in this study was weakly alkaline soil (pH, 7.8), whereas in the study of Cycoń et al. (2013), the soil was weakly acidic (pH, 6.6). Hsiao et al. (2013) found that an alteration in microbial diversity in an acidic loamy soil was greater than in an alkaline sandy loam. In moderately saline soil, salt is a stronger inhibitor of the soil bacterial community structure compared to imidacloprid. Previous studies have confirmed that salinity could stress the soil microbial community and result in an alteration of soil microbial activity (Morrissey et al. 2014; Rietz and Haynes 2003). Band 6 was absent in Fig. 1a but appeared in Fig. 2a, indicating that imidacloprid could alter or improve the bacterial community in the moderately saline soil to some degree. Our previous study had confirmed that the number of bacteria can be enhanced by imidacloprid in a moderately saline soil (Zhang et al. 2014a, b), which is consistent with the findings in this study. A possible reason is that imidacloprid can be a source for the growth of bacteria band 6 under the stress of salt. Many previous studies have also reported that the size of specific bacterial populations increase in response to the application of various insecticides (Das et al. 2005; Cycoń et al. 2010, 2013) because some bacteria might use insecticides as a source of energy and nutrients to multiply, which results in an increase in band intensity or the appearance of new bands in the DGGE profile (Cycoń et al. 2013; Zhang et al. 2014a, b). In this study, the newly appeared bacterium (band 6) was identified as *Acidobacteria* by BLAST algorithms from the National Center for Biotechnology (NCBI), which provides the possibility of screening for a highly effective imidacloprid-degrading bacterium.

Soil fungi had a stronger tolerance to salt stress in this study. Previous studies have reported that some soil fungi, especially the arbuscular mycorrhizal fungi, showed some tolerance to salt stress and alleviated the detrimental effect of salinity on plants (Giri et al. 2003; Latef and He 2011; Porcel et al. 2012). Interestingly, the diversity of the soil fungal community structure showed no obvious differences in the low or moderately saline soils treated with different concentrations (0–10 mg kg^{-1}) of imidacloprid, indicating that imidacloprid did not produce negative effects on soils with different salinities. Our previous study (Zhang et al. 2014a, b) and Devashree et al. (2014) also found that the effect of imidacloprid was insignificant in the different soils regarding fungal numbers and activity. From the results pertaining to the soil microbial community structure, we can conclude that the recommended doses of imidacloprid are safe in field soil environments of different salinity.

Conclusion

This study is the first to examine the effects of the insecticide imidacloprid on different saline soil bacterial and fungal

communities. The results obtained with DGGE indicated that imidacloprid (0–10 mg kg^{-1}) slightly affected the bacterial community structure in low and moderately saline soils. Imidacloprid altered or improved the soil bacterial community under salt stress to some degree. The soil fungal community structure showed no obvious changes in low and moderately saline soils contaminated with 0–10 mg kg^{-1} of imidacloprid. Our results demonstrated that imidacloprid is safe in the Yellow River Delta if the instructions for pesticide application in agricultural practice are followed. Because soil physicochemical properties have a strong effect on the behavior and fate of pesticides, further studies should be conducted to assess the impact of imidacloprid in other different soil environments.

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References

Bassam BJ, Caetano-Anollés G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196: 80–83

Cycoń M, Piotrowska-Seget Z (2015a) Biochemical and microbial soil functioning after application of the insecticide imidacloprid. J Environ Sci 27:147–158

Cycoń M, Piotrowska-Seget Z (2015b) Community structure of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in soil treated with the insecticide imidacloprid. Biomed Res Int (Article ID 582938)

Cycoń M, Piotrowska-Seget Z, Kozdrój J (2010) Microbial characteristics of sandy soils exposed to diazinon under laboratory conditions. World J Microbiol Biotechnol 26:409–418

Cycoń M, Markowicz A, Borymski S, Wójcik M, Piotrowska-Seget Z (2013) Imidacloprid induces changes in the structure, genetic diversity and catabolic activity of soil microbial communities. J Environ Manag 131:55–65

Das AC, Chakravarty A, Sen G, Sukul P, Mukherjee D (2005) A comparative study on the dissipation and microbial metabolism of organophosphate and carbamate insecticides in orchaqualf and fluvaquent soils of West Bengal. Chemosphere 58:579–584

Devashree Y, Dutta BK, Paul SB, Choudhury S (2014) Effect of imidacloprid on the soil and rhizosphere microflora of tea agro-ecosystem. Glob J Biotechnol Biochem 9:35–40

Dong HZ, Li WJ, Xin CS, Tang W, Zhang DM (2010) Late planting of short-season cotton in saline fields of the yellow river delta. Crop Sci 50:292–300

Estrada B, Barea JM, Aroca R, Ruiz-Lozano JM (2013) A native *Glomus intraradices* strain from a Mediterranean saline area exhibits salt tolerance and enhanced symbiotic efficiency with maize plants under salt stress conditions. Plant Soil 366:333–349

Flores-Céspedes F, González-Pradas E, Fernández-Pérez M, Villafranca-Sánchez M, Socías-Viciana M, Ureña-Amate MD (2002) Effects of dissolved organic carbon on sorption and mobility of imidacloprid in soil. J Environ Qual 31:880–888

Giri B, Kapoor R, Mukerji KG (2003) Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis*. Biol Fertil Soils 38:170–175

Gómez-Sagasti MT, Alkorta I, Becerril JM, Epelde L, Anza M, Garbisu C (2012) Microbial monitoring of the recovery of soil quality during heavy metal phytoremediation. Water Air Soil Pollut 223:3249–3262

Hsiao YL, Ho WH, Yen JH (2013) Vertical distribution in soil column and dissipation in soil of benzoylurea insecticides diflubenzuron, flufenoxuron and novaluron and effect on the bacterial community. Chemosphere 90:380–386

Kim TW, Lee JH, Park MH, Kim HY (2010) Analysis of bacterial and fungal communities in Japanese- and Chinese-fermented soybean pastes using nested PCR–DGGE. Curr Microbiol 60:315–320

Latef AAHA, He CX (2011) Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. Sci Hortic 127:228–233

May LA, Smiley B, Schmidt MG (2001) Comparative denaturing gradient gel electrophoresis analysis of fungal communities associated with whole plant corn silage. Can J Microbiol 47:829–841

Morrissey EM, Gillespie JL, Morina JC, Franklin RB (2014) Salinity affects microbial activity and soil organic matter content in tidal wetlands. Global Change Biol 20:1351–1362

Muhammad Ashraf B, Shafi Muhammad N, Syed Tufail Hussain S, Muhammad Iqbal B (2012) Adsorption and leaching potential of imidacloprid pesticide through Alluvial soil. Am J Anal Chem 3: 604–611

Muyzer G, De Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700

Oi M (1999) Time-dependent sorption of imidacloprid in two different soils. J Agric Food Chem 47:327–332

Porcel R, Aroca R, Ruiz-Lozano JM (2012) Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. Agron Sustain Dev 32:181–200

Rajala T, Peltoniemi M, Pennanen T, Mäkipää R (2010) Relationship between wood-inhabiting fungi determined by molecular analysis (denaturing gradient gel electrophoresis) and quality of decaying logs. Can J For Res 40:2384–2397

Rietz DN, Haynes RJ (2003) Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biol Biochem 35:845–854

Sarkar MA, Roy S, Kole RK, Chowdhury A (2001) Persistence and metabolism of imidacloprid in different soils of West Bengal. Pest Manag Sci 57:598–602

Szabolcs I (1989) Salt-affected soils. CRC Press, Boca Raton

Tomizawa M, Casida J (2005) Neonicotinoid insecticide toxicology: mechanisms for selective action. Annu Rev Pharmacol Toxicol 45: 247–268

Wang ZQ, Zhu SQ, Yu RP (1993) Saline-alkali soil of China. Science Publishing, Beijing, pp 312–390, **in Chinese**

Wang F, Yao J, Chen HL, Yi ZJ, Choi MMF (2014a) Influence of short-time imidacloprid and acetamiprid application on soil microbial metabolic activity and enzymatic activity. Environ Sci Pollut Res 21:10129–10138

Wang LL, Sun XY, Li SY, Zhang T, Zhang W, Zhai PH (2014b) Application of organic amendments to a coastal saline soil in north China: effects on soil physical and chemical properties and tree growth. PLoS ONE 9:e89185. doi:10.1371/journal.pone.0089185

Wu JL, Wei HD, Xue J (2012) Degradation of imidacloprid in chrysanthemi flos and soil. Bull Environ Contam Toxicol 88:776–780

Yang HJ, Xie WJ, Liu Q, Liu JT, Yu HW, Lu ZH (2013) Distribution of phthalate esters in topsoil: a case study in the yellow river delta, China. Environ Monit Assess 185:8489–8500

Zhang JF, Sun QX (2005) Causes of wetland degradation and ecological restoration in the yellow river delta region. For Stud China 7:15–18

Zhang QC, Shamsi IH, Xu DT, Wang GH, Lin XY, Jilani G, Hussain N, Chaudhry AN (2012) Chemical fertilizer and organic manure inputs

in soil exhibit a vice versa pattern of microbial community structure. Appl Soil Ecol 57:1–8

Zhang QM, Feng RZ, Zhang BH, Liu XG, Wang Z (2014a) Effects of imidacloprid on soil microbes and enzyme activities under the salinity stress. Res Soil Water Conserv 21:25–30 **(in Chinese)**

Zhang QM, Zhu LS, Wang J, Xie H, Wang JH, Wang FH, Sun FX (2014b) Effects of fomesafen on soil enzyme activity, microbial population, and bacterial community composition. Environ Monit Assess 186:2801–2812