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Effect of elevated O_3 associated with Bt cotton on the abundance, diversity and community structure of soil Collembola

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ABSTRACT

A strain of cotton that was modified to express a gene derived from the bacterium *Bacillus thuringiensis* (Bt) to combat cotton bollworm was extensively planted in China. Meanwhile, tropospheric ozone (O_3) concentrations have increased alongside rapid industrialization and urbanization. Four treatments including two cotton varieties (Bt vs. non-Bt) and two concentrations of O_3 (current vs. doubled) were designed to investigate the abundance and community structure of soil Collembola in open-top chambers (OTCs) in 2009.

The above- and below-ground plant biomass and abundance of Collembola strongly decreased under elevated O_3 compared with ambient atmospheric O_3 in the OTCs. The abundance of Collembola from the genus *Onychiurus* and the diversity of Collembola were significantly lower in the Bt cotton OTCs compared with the non-Bt cotton OTCs. Under the elevated O_3 level, the abundance and diversity of Collembola were significantly reduced in the non-Bt cotton fields but not in the Bt cotton fields at some cotton stages. In addition, the Bt cotton cultivation strongly decreased the diversity of Collembola under atmospheric conditions but not with elevated O_3 levels.

Our results suggest that Bt cotton can buffer the effect of elevated O_3 on soil collembolans through the root-derived ways. In addition, elevated O_3 had an extensive adverse effect on soil Collembola, but the effect of the Bt crop on Collembola was species-specific.

1. Introduction

Due to rapid industrialization and urbanization as well as emissions from fossil fuel consumption, ground-level ozone (O_3) concentrations have increased in China, especially in the megacities (Chen et al., 2008; He et al., 2002; Wang et al., 2007). O_3 may reduce vegetation growth, photosynthesis, carbohydrate allocation to roots and the abundance of soil fauna by altering stomatal conductance, decreasing the activity and concentration of Rubisco and reducing leaf longevity (Andersen, 2003; Loranger et al., 2004; Schrader et al., 2009; Tingey et al., 2006).

Collembola, one of the most abundant and ubiquitous soil arthropods, can mobilize C and N by grazing on microorganisms that readily release nutrients from litter and soil for their own production, and they can affect plant growth and performance by depositing fecal pellets for root proliferation (Endlweber et al., 2009; Filser, 2002). Thus, collembolans are regarded as an important index of the soil community to indicate the alteration of C and N turnover caused by environmental factors.

Cotton is one of the most important cultivated crops in China, and it is adversely impacted by elevated O_3 (Grantz, 2003). Bt cotton is genetically modified to express a gene derived from the bacterium *Bacillus thuringiensis* (Bt) to combat cotton bollworms, which are the most significant insect pests. Its use was greatly extended in China because its benefits could significantly reduce the quantity of pesticides used and the level of pesticide expenditures (Huang et al., 2003; Wang et al., 2009). Most of the research on Bt plants has shown that they have no adverse effects on non-target soil organisms due to the specificity of the Bt proteins (Hönemann and Zurbrügg, 2008). However, some experiments have indicated otherwise, demonstrating a direct negative effect on standard soil animals, such as *Folsomia candida* and *Caenorhabditis elegans* (Broza et al., 2001; Höss et al., 2008). Moreover, Bakonyi et al. (2006) found that *F. candida* preferred non-Bt maize residues to Bt ones. This preference could be related to different lignin or carbohydrate contents between the Bt and non-Bt varieties (Wang et al., 1998; Breen et al., 1999; Chen et al., 2005). Moreover, elevated O_3 can increase the Bt Cry1Ac concentrations in the leaves of Bt oilseed rape and reduce the allocation of carbohydrates to the roots (Andersen, 2003; Himanen et al., 2009). The introduction of elevated O_3 could complicate the relationships between collembolans and cotton varieties. Therefore, it is worthwhile to ascertain

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whether and how soil Collembola are impacted by Bt cotton and elevated O_3.

Our study was conducted to investigate the individual and combined effects of elevated O_3 and Bt cotton on soil Collembola. We hypothesized that (i) elevated O_3 would have a harmful effect on soil Collembola due to the decreased allocation of carbohydrates to the roots; (ii) considering the specificity of Bt proteins, Bt cotton cultivation would have no effect on soil Collembola; and (iii) elevated O_3 could deteriorate the effect of Bt cotton cultivation on soil Collembola due to increased concentrations of Bt proteins.

2. Materials and methods

2.1. Open-top chamber

The experiment was conducted in eight octagonal, field open-top chambers (OTCs) (2.2 m high and 2 m in diameter, following Zheng et al., 2007) at the Observation Station for Global Change Biology at the Institute of Zoology, Chinese Academy of Science, in Xiaotangshan County, Beijing, China (40°11′N, 116°24′E). Four OTCs were used for each O_3 concentration treatment. In the elevated O_3 treatment, O_3 was generated from ambient air by an O_3 generator (3S-A15, Tonglin Technology Beijing, China) and then moved to the OTC entries using a fan (HB-429, 4.1 m^3 min^{-1}, Ruiyong Mechanical and Electrical Equipment Company). Mixed air (O_3 and ambient air) was ventilated to each OTC through columniform polyvinyl chloride pipes (diameter (outer, inner) = 16 cm, 11 cm). O_3 concentrations were monitored both from the fan outlet (Shenzhen Yiyuntian Electronic Co. Ltd.) and within OTCs (AQL-200, Aeroqual). In the current ambient atmospheric O_3 treatment, ambient air was ventilated to each OTC as described in "elevated O_3 treatment" except for O_3 ventilation. From June 19 to August 30, 2009, except on 12 rainy days, air was blown from 9:00 to 15:00 through a hemispherical stainless steel sprayer (diameter = 30 cm) situated 0.5 m above the canopy at a rate corresponding to approximately 15 m^3 min^{-1}, resulting in approximately two air changes per minute in each OTC. Before the experiment, the number and direction of holes in the hemispherical sprayer were adjusted to make a homogenous distribution of treated gas throughout each OTC. Gas concentrations were measured once every hour in each chamber receiving O_3 treatment.

The atmospheric O_3 concentration treatments were the following: 1) current ambient atmospheric O_3 levels ("AA" = 37.3 nmol mol^{-1}, average value from 9:00 to 15:00 of all air-treated days), and 2) double the current ambient O_3 levels ("EO" = 72.2 nmol mol^{-1}, average value from 9:00 to 15:00 of all air-treated days).

2.2. Site description and experimental design

In the OTCs, cotton had been planted in the three years prior to the study without insecticide application for the purposes of scientific research. To eliminate the effect of different cotton varieties and concentrations of O_3 caused by cotton planting, the top 15 cm of soil was removed from the surface and then mixed and refilled into each OTC. The soil properties of the field are summarized as follows: pH = 7.43 ± 0.01; soil organic matter = 17.36 ± 0.94 g/kg; total nitrogen = 1.72 ± 0.11 g/kg; plant available phosphorus = 35.79 ± 3.12 mg/kg. Two cotton varieties, a Bt cotton GK (GK12, producing the fused Cry1Ac toxic protein) and its near-isoline of the Bt variety SM (Simian 3), were separated by polyvinyl chloride barriers (15 cm buried in the soil, 25 cm above ground) in the middle of each OTC. Cotton was planted in each OTC on May 9 2009. Based on the horizontal distances to the polyvinyl chloride barriers, either 5, 4 or 3 plants of a single variety were planted in three different ridges in each OTC. Cotton plants emerged on May 15 2009. Two O_3 treatments (current ambient atmospheric and elevated O_3 levels) were applied to each variety for a total of four OTCs. The farms were managed according to ordinary cultivation but without the application of insecticides.

2.3. Sampling and analysis

On October 15 2009, four stems per cotton variety in each OTC (64 stems in total) were randomly selected. Cotton stems were cut off at the surface of the soil then washed and dried at 80 °C for 72 h, and the dry biomass of the root and shoot were measured. Then, cotton leaves from another four plants per cotton variety in each OTC (64 plants in total) were randomly selected and stored at −20 °C until being subjected to chemical analysis.

Soil Collembola sampling started on June 8, 2009, and continued until October 8, 2009. During this period, four undisturbed soil cores (height: 52 mm, diameter: 70 mm) were taken from each O_3 treatment of the two cotton varieties, in every field, once per month. The five sampling times included four growing stages of cotton: seeding (from emergence to bud), budding (from bud to flower), flower and boll-1 (earlier stage of flower to boll), flower and boll-2 (latter stage of flower to boll) and boll-opening (after boll opened). Soil Collembola were extracted using the Macfadyen method (Macfadyen, 1961). The extracted organisms were preserved in 75% ethanol for subsequent identification. Collembolans were identified by genus using the keys of Yin (1998) and Christiansen and Bellinger (1980). On the 8 of June, July, August, and October, one undisturbed soil core was taken for analysis of the soil properties. Soil pH was measured in 0.01 M $CaCl_2$, total soil oxidizable organic matter (SOM) was determined by wet oxidation with $K_2Cr_2O_7$ and soil nitrogen (SN) content was assayed using Kjeltec nitrogen analysis (Foss automated Kjeltec™ instruments, Model 2100). During the experiment, soil organic matter ranged from 15.1 ± 0.55 to 17.0 ± 1.00 g/kg, and soil nitrogen ranged from 1.41 ± 0.05 to 1.58 ± 0.09 g/kg.

2.4. Data analyses

The diversity (H') of Collembola was calculated using the Shannon–Weaver index. The Shannon–Weaver diversity index H′ (Shannon and Weaver, 1949),

$$H = -\sum p_i \log_e p_i$$

where p_i denotes the proportion of the individuals of the ith genus in the total sample, was calculated to measure the community diversity of collembolans. For data analyses, SPSS 13.0 for Windows and CANOCO Version 4.5 were used. Individual numbers of Collembola were log (n + 1) transformed to obtain a normal distribution. The differences in the Collembola composition between the cotton varieties and the different concentrations of O_3 were compared using a canonical correspondence analysis (CCA) with CANOCO. Only genera of collembolans that were present in at least three independent samples were included in the analysis.

A repeated measurement ANOVA was performed using SPSS to analyze the impact of Bt and non-Bt cotton varieties and different concentrations of O_3 on the soil properties and the abundance and diversity of Collembola. The sampling times were included as repetition levels; the cotton varieties and concentrations of O_3 were used as "between subject" factors. A two-way ANOVA was performed using SPSS 13.0 to analyze the abundance and diversity of Collembola in each sampling time of different cotton stages. The abundance and diversity of Collembola in Bt or non-Bt cotton varieties under two concentrations of O_3 in each sampling time were analyzed by one-way ANOVA with SPSS.

Table 1
Root and shoot biomass of cotton in different cotton varieties (Bt cotton, GK12 vs. Simian 3, the near-isoline of the Bt cotton GK12) and different concentrations of O_3 (ambient air vs. doubled concentration of current O_3 level).

Plant properties	Ambient air		Elevated O_3	
	SM (non-Bt)	GK (Bt)	SM (non-Bt)	GK (Bt)
Root biomass (g)	54.7 ± 7.58 a,A	60.6 ± 10.4 a,A	33.7 ± 5.53 a,B	34.8 ± 5.53 a,B
Shoot biomass (g)	309 ± 53.4 a,A	248 ± 38.3 a,A	167 ± 34.5 a,B	167 ± 30.0 a,B
Root/shoot	0.19 ± 0.02 b,A	0.24 ± 0.02 a,A	0.21 ± 0.01 b,A	0.22 ± 0.02 a,A

Each value represents the average (±SE) of four replicates (one replicate = one open-top chamber). Different lowercase letters within a row indicate a significant effect between two cotton varieties (LSD test: d.f. = 1, 12; $p < 0.05$). Different uppercase letters indicate significant differences between two O_3 concentrations (LSD test: d.f. = 1, 12; $p < 0.05$).

3. Results

3.1. Plant properties of Bt and non-Bt cotton varieties under two concentrations of O_3

The elevated concentrations of O_3 significantly reduced the biomass of the shoots ($F_{1,12} = 44.355$, $p < 0.0001$) and the roots ($F_{1,12} = 88.820$, $p < 0.0001$), but elevated O_3 did not change the root/shoot ratio. The root/shoot ratio of GK-12 was significantly higher than that of Simian 3 ($F_{1,12} = 4.732$, $p = 0.050$; Table 1).

3.2. Community structure

A total of 10,055 collembolans, including 22 genera of Collembola (Table 2), were extracted from the four treatments (AASM, AAGK, EOSM and EOGK) in the OTCs. The Collembola community included *Hypogastrura* (69.3%), *Onychiurus* (7.8%), *Entomobrya* (4.3%), *Isotoma* (4.2%) and *Arrhopalites* (4.7%), comprising more than 90% of the collembolans.

The Collembola community significantly differed between different cotton stages (see Fig. 1, $n = 320$, CCA, $p = 0.002$) and different O_3 levels ($n = 320$, CCA, $p = 0.004$) but not in the cotton varieties ($n = 320$, CCA, $p = 0.066$). Many rare genera of collembolans preferentially occurred in the seeding and budding stages and were adversely impacted by elevated O_3 in the stages of budding to flower and boll-1. Most genera of collembolans, including three common genera (*Hypogastrura*, *Isotoma* and *Entomobrya*), were adversely affected by elevated O_3; however, the collembolans from Onychiuridae (ON.pr, ON.bi, ON.on and On.cr) were not impacted by elevated O_3. Bt cotton cultivation had a positive effect on collembolans from genera *Hypogastrura*, *Isotoma* and *Entomobrya*.

3.3. Abundance and diversity of Collembola under O_3 treatments

Elevated O_3 significantly decreased the abundance of Collembola ($F_{1,60} = 9.737$, $p < 0.001$, Table 3) in the OTCs. However, diversity (Shannon–Weaver index) of Collembola did not significantly differ between the two concentrations of O_3 in the OTCs. In addition, the abundance of Collembola from the genus *Hypogastrura* (69.3% of individuals) strongly decreased ($F_{1,60} = 18.621$, $p < 0.0001$, Table 4) under elevated O_3 compared with the ambient air in the OTCs.

Elevated O_3 significantly reduced the abundance of Collembola in the stages of flower and boll-1 ($F_{1,60} = 4.345$, $p = 0.041$, Table 3) as well as flower and boll-2 ($F_{1,60} = 8.073$, $p = 0.006$) in the OTCs; the diversity (Shannon–Weaver index) of Collembola also significantly decreased under elevated O_3 in the flower and boll-2 ($F_{1,60} = 4.890$, $p = 0.031$) and boll-opening ($F_{1,60} = 8.388$, $p = 0.005$) stages in the OTCs.

Table 2
The number of collembolans by genus for the four treatments (Simian 3 and GK12 under current and doubled O_3 levels) in open-top chambers in 2009.

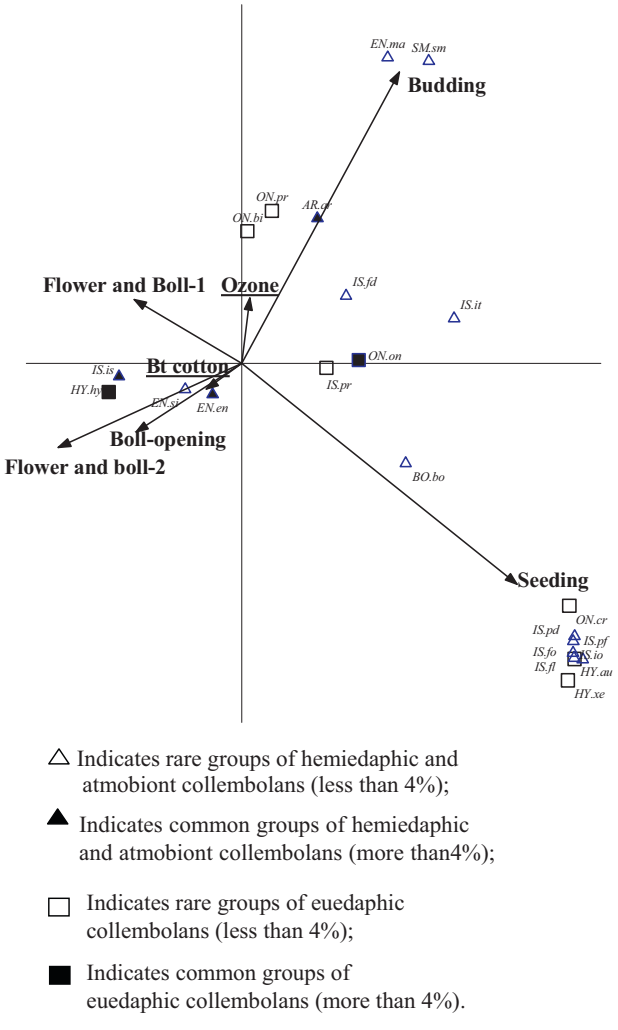
Collembola (10^3 individuals/m^{-2})		Ambient air (AA)		Elevated O_3 (EO)	
		SM[a]	GK[b]	SM	GK
Family	Genus (abbreviation)	Non-Bt	Bt	Non-Bt	Bt
Arrhopalitidae	*Arrhopalites* (AR.ar)	51	15	19.8	36
Bourletiellidae	*Bourletiella* (BO.bo)	11	9	12	8.6
Entomobryidae	*Entomobrya* (EN.en)	31	27	15.9	40
	Marginobrya (EN.ma)	3.1	1	2.34	1.6
	Sinella (EN.si)	2.3	3	1.82	2.3
Hypogastruridae	*Hypogastrura* (HY.hy)	583	619.1	157	452
	Xenylla (HY.xe)	0	1	0	0
	Austrogastrura (HY.au)	0.8	1	0	0.3
Isotomidae	*Folsomia* (IS.fa)	5.5	2	0	0.5
	Folsomides (IS.fd)	24	15	12.5	16
	Folsomina (IS.fo)	3.1	0	0	0
	Isotoma (IS.is)	31	20	35.9	23
	Isotomiella (IS.io)	0.8	1	0.52	0.3
	Isotomodes (IS.it)	0.8	1	1.56	0
	Proisotoma (IS.pr)	15	18	5.2	14
	Pseudisotoma (IS.pd)	0.8	0	0	0.8
	Pseudofolsomia (IS.pf)	0.5	5	1.04	0
Onychiuridae	*Bionychiurus* (ON.bi)	5.7	3	4.68	4.9
	Cribrochiurus (ON.cr)	0.5	0	2.08	2.6
	Onychiurus (ON.on)	75	41	57.7	31
	Probolaphorura (ON.pr)	5.7	3	8.32	7.8
Sminthuridae	*Sminthurus* (SM.sm)	1	0	0	0
Total		852	783	339	641

[a] Simian 3, near-isoline of the Bt cotton GK12.
[b] GK12, Bt cotton producing the fused Cry1Ac toxic protein.

Table 3
p-Values from ANOVAs of the effects of O_3 level and cotton variety on the abundance and diversity (Shannon–Weaver index) of Collembola in open-top chambers.

Sampling time	Variables	O_3 (contrast)	Variety (contrast)	O × V[a]
Total	AoC[b]	0.003** (AA > EO)	0.952 (GK > SM)	0.007**
In OTCs	SoC[c]	0.687 (AA > EO)	0.037* (GK < SM)	0.027*
Seeding	AoC	0.517 (AA < EO)	0.368 (GK < SM)	0.468
	SoC	0.981 (AA > EO)	0.955 (GK < SM)	0.071
Budding	AoC	0.333 (AA > EO)	0.041* (GK < SM)	0.126
	SoC	0.459 (AA < EO)	0.036* (GK < SM)	0.300
Flower and boll-1	AoC	0.006** (AA > EO)	0.236 (GK > SM)	0.150
	SoC	0.003** (AA < EO)	0.107 (GK < SM)	0.393
Flower and boll-2	AoC	0.014* (AA > EO)	0.552 (GK > SM)	0.088
	SoC	0.031* (AA > EO)	0.150 (GK > SM)	0.150
Boll-Opening	AoC	0.548 (AA > EO)	0.597 (GK > SM)	0.373
	SoC	0.005** (AA > EO)	0.147 (GK < SM)	0.185

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
[a] The interaction effect between O_3 × cotton varieties.
[b] Abundance of Collembola.
[c] Shannon–Weaver index of Collembola.



△ Indicates rare groups of hemiedaphic and atmobiont collembolans (less than 4%);

▲ Indicates common groups of hemiedaphic and atmobiont collembolans (more than4%);

□ Indicates rare groups of euedaphic collembolans (less than 4%);

■ Indicates common groups of euedaphic collembolans (more than 4%).

Fig. 1. Ordination biplot of the canonical correspondence analysis (CCA) with mean abundances of Collembola genus in different cotton varieties (Bt cotton, GK12 vs. Simian 3, near-isoline of the Bt cotton GK12), O_3 level (ambient air vs. doubled concentration of current O_3 level) treatments and cotton stages (seeding, budding, flower and boll-1, flower and boll-1 and boll-opening) in open-top chambers (OTCs). The full names of Collembola taxa are given in Table 2.

Table 4
p-Values from ANOVAs of the effect of O_3 levels (ambient air vs. doubled concentration of current O_3 level) and cotton varieties (Bt cotton, GK12 vs. Simian 3, near-isoline of the Bt cotton GK12) on the abundance of the main genera of Collembola in open-top chambers.

Main genus	O_3 (contrast)	Variety (contrast)	O × V[a]
Arrhopalites	0.237 (AA > EO)	0.505 (GK < SM)	0.081
Entomobrya	0.260 (AA > EO)	0.355 (GK > SM)	0.004**
Hypogastrura	0.000*** (AA > EO)	0.088 (GK > SM)	0.006**
Isotoma	0.329 (AA < EO)	0.433 (GK > SM)	0.314
Onychiurus	0.181 (AA > EO)	0.003** (GK < SM)	0.747

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
[a] The interaction effect between O_3 × cotton varieties.

The diversity of Collembola was not significantly affected by the cotton varieties ($F_{1,30} = 0.033$, $p = 0.856$, data not shown) under elevated O_3. In the non-Bt cotton field, the abundance of Collembola was significantly reduced in the flower and boll-1 ($F_{1,30} = 9.672$, $p = 0.004$) and flower and boll-2 ($F_{1,30} = 9.258$, $p = 0.005$) stages under elevated O_3, and the diversity of Collembola significantly diminished in the flower and boll-1 ($F_{1,30} = 6.961$, $p = 0.013$), flower and boll-2 ($F_{1,30} = 8.723$, $p = 0.006$) and boll-opening ($F_{1,30} = 10.885$, $p = 0.003$) stages under elevated O_3 in OTCs.

3.4. Abundance and diversity of Collembola in Bt and non-Bt cotton fields

The diversity of Collembola from the Bt cotton field was significantly diminished compared with the non-Bt cotton field in the OTCs ($F_{1,60} = 4.587$, $p = 0.037$, Table 3). Under atmospheric conditions, Bt cotton cultivation strongly decreased the diversity of Collembola ($F_{1,30} = 10.981$, $p = 0.002$), and the abundance of Collembola from the genus *Onychiurus* (7.8% of individuals) was highly reduced ($F_{1,60} = 9.280$, $p = 0.003$, Table 4) in the Bt cotton field.

The abundance (budding, $F_{1,60} = 4.345$, $p = 0.041$) and diversity (Shannon–Weaver index, budding, $F_{1,60} = 4.621$, $p = 0.036$) of Collembola from the Bt cotton field was significantly lower than the non-Bt cotton field during the budding stage.

3.5. Abundance and diversity of Collembola in the O_3 × cotton variety treatments

The abundance ($F_{1,60} = 7.907$, $p = 0.007$) and diversity ($F_{1,60} = 5.119$, $p = 0.027$) of Collembola was significantly impacted by the interaction effect of the O_3 × cotton varieties in the OTCs.

Additionally, the interaction between O_3 and cotton varieties had a significant effect on the abundance of Collembola from the genera *Hypogastrura* ($F_{1,60} = 7.977$, $p = 0.006$) and *Onychiurus* ($F_{1,60} = 8.803$, $p = 0.004$) in the OTCs.

4. Discussion

Our study has definitively shown that elevated O_3 reduced the abundance of Collembola in the OTCs. Elevated O_3 diminished carbon allocation to the roots and, therefore, decreased soil microbial biomass C (McCool and Menge, 1983; Cooley and Manning, 1987; Rennenberg et al., 1996; Andersen, 2003; Islam et al., 2000). In addition, the cotton biomass of the roots and shoots significantly decreased in the elevated O_3 treatments in this study. The results suggest that decreased food resources suppress the abundance of soil Collembola in the OTCs. This finding is in accordance with Schrader et al. (2009) who found that above- and belowground plant biomass and the individual density of collembolans decreased significantly in winter wheat under elevated O_3. Moreover, we found that the abundance and diversity of Collembola were significantly impacted by the elevated O_3 after the budding stage. CCA analysis also showed that the most adverse effect of O_3 occurred between the stages of budding and flower and boll-1. Gelang et al. (2001) reported that the O_3 sensitivity of wheat was higher during and after anthesis than before anthesis, which probably resulted from a reduced source/sink ratio in these periods. The decreased abundance and diversity of soil Collembola might be due to the decreased source/sink ratio and carbohydrate allocation to the roots during and after budding. However, the diversity of Collembola did not significantly differ between the two concentrations of O_3. Our findings indicate that elevated O_3 would affect the abundance rather than the genera richness of soil Collembolas in cotton fields. It is in accordance with our hypothesis that elevated O_3 had a harmful effect on soil Collembola due to the decreased allocation of carbohydrates to roots.

Crop variety also influenced soil animals. The diversity of Collembola, but not their abundance, was impacted by the cotton variety (Bt < non-Bt). Bt cotton cultivars utilize more nutrients for stem and branch growth due to greater leaf N, free amino acids and soluble proteins than their non-Bt near-isolines (Wang et al., 1998; Breen et al., 1999; Chen et al., 2005). Furthermore, Fang et al. (2007) found that the structure of microbial communities significantly differed between the corn residue from Bt and the non-Bt near-isoline due to a different lignin contents and lignin/N ratio. Bakonyi et al. (2006) found that Collembola (*F. candida*) prefer non-Bt maize residues to Bt ones. Consequently, Collembola diversity might decrease in the Bt cotton fields compared with non-Bt fields. Furthermore, at the stage of budding, the abundance and diversity of Collembola strongly decreased in the Bt cotton field compared with the non-Bt cotton field. This finding indicates that the effect of crop variety had a larger impact during the budding stage than in other stages.

In the OTCs, the abundance of Collembola from the genus *Hypogastrura* was remarkably decreased in the elevated O_3 treatment compared with the ambient air O_3 condition. Additionally, the abundance of Collembola from the genus *Onychiurus* was significantly reduced in the Bt cotton field compared to the non-Bt field. According to former studies, collembolans from the genera *Hypogastrura* and *Onychiurus* belong to the same functional group, euedaphic collembolans, which predominantly feed on microorganisms and particularly fungi (Addison et al., 2003; Chahartaghi et al., 2005; Salamon et al., 2008; Sticht et al., 2008). The abundances of Collembola from the genera *Arrhopalites* (hemiedaphic collembolans), *Entomobrya* (atmobiont collembolans) and *Isotoma* (hemiedaphic collembolans) were not significantly impacted by cotton variety and concentrations of O_3. In these previous studies, euedaphic collembolans but not hemiedaphic or atmobiont collembolans were impacted by cotton varieties and concentrations of O_3. This finding is because euedaphic collembolans had poorer mobility (furcula are absent or degraded) and simpler food resources than hemiedaphic or atmobiont collembolans. The elevated O_3 had an extensive and adverse effect on the abundance of Collembola. The abundances of Collembola from the genera *Onychiurus*, *Arrhopalites* and *Entomobrya* were also decreased, but not significantly, in elevated O_3. The elevated O_3 could decrease carbohydrate allocation both above and below ground (Andersen, 2003); thus, the effect of elevated O_3 did not vary between the euedaphic, hemiedaphic and atmobiont collembolans. However, the Bt cotton variety did not have a universal effect on Collembola like elevated O_3 treatment: two of the five main genera of Collembola decreased in the Bt cotton field, while the other three increased. Bakonyi et al. (2006) also found that collembolans preferred non-Bt corns to Bt ones, and the effect of the Bt-toxin producing corn on Collembola was species-specific.

In the non-Bt cotton field, the abundance ("flower and boll-1 and flower and boll-2") and diversity (flower and boll-1, flower and boll-2 and boll-opening) of Collembola were significantly reduced in the elevated O_3 treatments; however, in the Bt cotton field, elevated O_3 did not significantly affect the abundance or diversity of Collembola at any cotton growing stage. Furthermore, Bt cotton cultivation strongly decreased diversity of Collembola under atmospheric conditions but not elevated O_3 levels. This finding suggests that Bt cotton can buffer the effect of elevated O_3 on soil collembolans through the root-derived ways. This result conflicts with our hypothesis that elevated O_3 could deteriorate the effect of Bt cotton cultivation on soil Collembola due to the increasing concentrations of Bt proteins. It is reasonable to assume that the Bt cotton had a higher carbohydrate allocation to the roots, and we also found that the root/shoot ratio of GK-12 was significantly higher than that of Simian 3. To ascertain these mechanisms, we need to research the effects of elevated O_3 on detailed chemical components of Bt and non-Bt cottons in the future.

5. Conclusion

Our study has clearly shown that elevated concentrations of O_3 have a remarkably adverse effect on the abundance of Collembola in the open-top chambers. The diversity of Collembola was significantly suppressed in the Bt crop open-top chambers. Moreover, Bt cotton had a negative effect on the abundance of collembolans from the genus *Onychiurus* in the open-top chambers. Elevated O_3 had an extensive adverse effect on Collembola, but the effect of Bt crop on Collembola was species-specific. Our study also indicated that Bt cotton can buffer the effect of elevated O_3 on soil collembolans through the root-derived ways.

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