EFFECTS OF ELEVATED ATMOSPHERIC CO_2, O_3 AND SOIL PHENANTHRENE ON SOIL ENZYME ACTIVITIES AND MICROBIAL BIOMASS

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Abstract. This paper aimed to investigate the effects of elevated [CO_2], [O_3] or [$CO_2 + O_3$] on soil physicochemical properties, microbial biomass and soil enzyme activities in phenanthrene-added and -unadded soils. The results showed that elevated [CO_2] increased the dissolved organic carbon (DOC), total phosphorus (TP), available phosphorus (AP), total potassium (TK), total carbon (TC), and nitric nitrogen (NN) contents to some extent in phenanthrene-untreated soils, which contrary to the elevated [O_3], and thus differentially promoted the contents of microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) as well as activities of soil protease (PRA), sucrase (SA), acid phosphatase (APA) and neutral phosphatase (NPA) in the soils. The elevated [O_3] increased ratios of MBC/MBN, and led to the decrease of the activities of PRA, APA and NPA to some extent. However, to some extent, [$CO_2 + O_3$] alleviated the inhibition of the soil microbial biomass and soil enzyme activities by [O_3] elevation and basically did not change the tendency of the soil physicochemical properties, microbial biomass and enzyme activities, but their contents in phenanthrene-added soils compared with the phenanthrene-untreated soils. Therefore, the alteration of soil microbial biomass and soil enzyme activities can be used for assessment of the effects of elevated atmospheric CO_2, O_3 or phenanthrene-polluted soil on the soil microbial systems.
Keywords: *open top chamber (OTC), phenanthrene-added soils, elevated [CO_2], elevated [O_3], soil physicochemical properties*

Introduction

For the past few years, the continuous increase of CO_2 and O_3 concentration in the atmosphere and global climate change have attracted wide attention of the whole society (Wang et al., 2014). The research results show that elevated atmospheric CO_2 concentration significantly affects the land ecological system through improving the plant photosynthesis changes of plant root secretion and soil microbial community composition and the quantity, flow and distribution of carbon and nitrogen in soil, and the size of soluble carbohydrate pool indirectly changes the physical and chemical properties of soil, so as to affect the alteration of soil microbial ecosystem (Xu et al., 2013; Sulman et al., 2014; Manna et al., 2012).

O_3 is a naturally occurring photochemical oxidant in the troposphere of the Earth, and a major component of greenhouse gases and photochemical smog. Higher O_3 levels can have negative effects on plants. O_3 enters plant tissue thorough stomata, and can reduce stomatal conductance, and then inhibits photosynthesis and nutrient absorption, and finally reduces plant biomass and crop yield (Díaz-de-Quijano et al., 2012; Zhang et al., 2011). Under the stress of O_3, changes in plant root secretions might also change the physical and chemical properties of soil, and reduce soil microbial biomass carbon and nitrogen, and potentially change the rhizosphere microbial community, nutritional status

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and soil enzyme activity (Wu et al., 2012; Haesler et al., 2014; Cheng et al., 2014; Veeraragavan et al., 2018).

Soil enzymes are involved in the material circulation, energy flow and degradation of organic pollutants in soil, and changes in their activity can reflect the strength and direction of various biochemical processes in soil. The microbial biomass is very sensitive to environmental factors, and is always applied to monitor the scale and activity of soil microbial community (Lou et al., 2011). Therefore, soil microbial biomass and soil enzyme activity have been developed into ecological indicators for evaluating soil environmental quality (Han et al., 2016; Tripathi et al., 2007; Kumar and Jaafar, 2018), and have been applied to evaluate the alteration of soil microecological environment under atmospheric CO_2 and O_3 pollution conditions (Li et al., 2010; Rozuki et al., 2018).

People usually adopt free-air CO_2 enrichment (FACE) or Open top chamber (OTC) system to investigate the elevated atmospheric CO_2 and O_3 concentration influence on plant and soil microbial system (Wu et al., 2012; Formánek et al., 2014). The study found that elevated atmospheric CO_2 and O_3 concentration can change the plant population composition, and then affect plant chemistry (Booker et al., 2005; Johnson and Pregitzer, 2007), and increases the content of phenolic substances (Li et al., 2008; Sen et al., 2018), and finally indirectly change soil chemical characteristics and induce changes in soil microbial functional diversity, biomass and enzyme activity (Xu et al., 2013; Huang et al., 2013; Wu et al., 2015). As a representative of polycyclic aromatic hydrocarbons pollutants, the phenanthrene (PHE) is gradually accumulated in the soil with a low-dose superposition. Its potential ecological risk of soil pollution has been widely concerned (Zhang et al., 2018).

At present, most researches about the change of soil–plant system under elevated CO_2 and O_3 conditions, are to simulate impact and evaluation of a single elevated CO_2 and O_3 on ecosystem safety, while there are few researches on the combined conditions of the two (Tian et al., 2011; Ren et al., 2014), especially on the effect of soil phenanthrene on soil enzyme activity under the condition of atmospheric CO_2 and O_3 rising. Based on OTC platform, under the condition of CO_2, O_3, CO_2 + O_3 increase, the research build micro universe soil - green vegetable potted simulation system to discuss the change of soil protease, polyphenol oxidase, phosphatase, urease and invertase as well as the change of soil microbial biomass with or without adding phenanthrene (López-Hernández, 2018), which was aimed to obtain the future elevated atmospheric CO_2 and/or O_3 micro ecological impact on the soil.

Materials and methods

Test platform and processing mode

The test platform is located in Xianlin Campus of Nanjing University (118°57′36.15″E, 31°7′23.99″N). The platform includes 4 air chambers. One is connected to the normal atmosphere. One has 200 ppm more CO_2 concentration than normal atmosphere. One has 50 ppb O_3 concentration than normal atmosphere. One control the compound increase of CO_2 and O_3 concentration. The air chamber is octagon with 2 m of diameter and 2.8 m of height. CO_2 was provided by Du Wa Guan (Q/JB-THB002, Beijing Tianhai Industry Co., Ltd). CO_2 was purchased by Nanjing Tianze Gas Co., Ltd with 99.9% of purity. O_3 is produced by O_3 generator (NPF 10/W, Shandong Tianze). CO_2 and O_3 (gas was controlled by valves and flow meter) mixed

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with normal atmosphere by axial flow fan (SFG-2, Shanghai Jia Bao) into the bottom of the chamber. And then the gas entered the chamber through holes (0.5 cm of diameter) on the bottom of the chamber and the stainless steel plate in the middle of the chamber. The gas then entered that outer atmosphere through the opening at the top of the gas chamber. The gas replacement frequency should ensure than the gas will be replaced 3~4 times per minute in the chamber. CO_2 concentration was monitored by CO_2 detector (Li-7000, Li-Cor, USA), and O_3 concentration was monitored by O_3 detector (Model 205, 2B Co., USA). CO_2 was injected into the gas chamber all the times, and the injection time of O_3 was from 9 am to 5 pm (except rainy days).

In July 2014, the soil with 100 ppm of exo-phenanthrene and the soil without adding phenanthrene were prepared. In June 2017, the concentration of phenanthrene in the contaminated soil was measured at about 30 ppm. Meanwhile, these aged soils were divided into flowerpots (10 kg/pot). Fertilizer (2 g/pot, N:P:K = 1:1:1) was added, and then turned over, and mixed well. Then 10 green vegetable seeds were planted. Finally the flowerpots without adding phenanthrene were transferred to 4 chambers in OTC which were ambient[Amb], [CO_2], [O_3] and [CO_2 + O_3] representing the normal atmosphere, high CO_2, high O_3 and mixed increase of CO_2 and O_2.

At the same time, the soil polluted by phenanthrene was also transferred to the 4 air chambers represented by [Amb] + [PHE], [CO_2] + [PHE], [O_3] + [PHE] and [CO_2 + O_3] + [PHE]. 3 pots were prepared for each treatment. After germination, the seeding began to grow for another week. Each flowerpot kept 3 seedlings with same size. They were replenished regularly with the same amount of deionized water. Soil samples were collected after 30 days for the detection and analysis of the following indicators.

Determination of physical and chemical properties of soil

The method of Shi Cuie and teammates (Shi et al., 2015) was referenced to determine the physical and chemical properties.

Determination of soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN)

Soil MBC and MBN were measured by chloroform fumigation - K2SO4 extraction (Shi et al., 2015). The content of organic carbon and total N in the extraction solution (Multi C/N 3100, Jena) were determined by TOC analyzer. The calculation methods of MBC and MBN are as follows. MBC = EC/0.45, MBN = EN/0.45, EC, EN were the differences of organic carbon or total nitrogen between the fumigated and non-fumigated soil leaching solution (Liu et al., 2014), and 0.45 is the proportion coefficient of the extracted biomass after chloroform fumigation (Iqbal et al., 2010). The carbon nitrogen ratio of microorganisms was expressed as MBC/MBN.

Determination of soil enzyme activity

Soil enzyme activity was determined by indanone colorimetry in accordance with the method of Guan (1986). 1 mg NH_2-N produced by 1 g soil after 24 h was defined as 1 mg NH_2-N$\cdot g^{-1}\cdot 24\ h^{-1}$ for soil protease (PRA) activity. Sucrose enzyme (SA) was determined by 3,5-dinitrosalicylic acid colorimetry. 1 g glucose produced by 1 g soil after 24 h was defined as 1 mg glucose$\cdot g^{-1}\cdot 24\ h^{-1}$. Urease activity (UA) was determined by sodium phenol - sodium hypochlorite colorimetric method. 1 μg NH_3-N produced by 1 g soil after

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24 h under 37 °C was defined as 1 μg NH_3-N·g^{-1}·24 h^{-1}. The activity of polyphenol oxidase (POA) was determined by using purple gallic acid colorimetry. 1 g purple gallic acid colorimetry produced by 1 g soil after 2 h was defined as 1 mg PG·g^{-1}·2 h^{-1}. The activity of soil acid phosphatase (APA) and neutral phosphatase (NPA) were determined by disodium phenyl phosphate. 1 mg P_2O_5 produced by 100 g soil after 2 h under 37 °C was defined as 1 mg P_2O_5·100 g^{-1}·2 h^{-1}.

Data processing

Raw data were sorted out by Excel 2003. SPSS 20.0 was applied for statistical analysis. Variance analysis was used to test the differences between the factors, and SNK method was used for multiple comparisons between the two factors.

Results and analysis

Effects of the rising of CO_2 and O_3 in atmosphere and phenanthrene in soil on physical and chemical properties of soil

With the increase of CO_2 or/and O_3 in OTC, the content alteration of NN, TP, TK and TC in the soil without adding phenanthrene soil presents a trend of [CO_2] > [Amb] > [CO_2 + O_3] > [O_3]. The trend of content alteration of AN, DON, TN and DOC was [CO_2] > [CO_2 + O_3] > [Amb] > [O_3]. The trend of content alteration SWC and AP was [CO_2] > [CO_2 + O_3] > [O_3] > [Amb], while the decline of pH value was as the trend of [O_3] > [CO_2 + O_3] > [Amb] > [CO_2] (*Table 1*).

Table 1. *Effects of elevated atmospheric CO_2 or/and O_3 on soil physicochemical properties of phenanthrene-added and unadded soils*

Treatments	NN/mg·g^{-1}	TP/mg·kg^{-1}	TK/mg·g^{-1}	TC/mg·g^{-1}	AN/mg·g^{-1}	TN/mg·g^{-1}
Amb	9.98±1.12ab	404.65±45.90bc	10.78±1.89ab	13.14±1.20a	0.77±0.10 bc	1.28±0.20a
CO_2	13.72±2.61b	463.33±47.09c	11.40±1.31b	14.70±2.32ab	0.93±0.12bc	1.50±0.21a
O_3	5.87±1.57a	293.50±23.07a	6.78±0.70a	12.30±1.30a	0.56±0.12a	1.23±0.19a
CO_2+O_3	8.43±1.13a	370.60±50.00abc	9.71±1.02ab	12.89±1.31a	0.83±0.12bc	1.40±0.18a
Amb+phenanthrene	10.07±2.01ab	435.40±50.00bc	10.51±1.91ab	15.90±2.43ab	0.89±0.13bc	1.37±0.22a
CO_2+phenanthrene	13.84±2.02b	583.70±42.95d	11.01±2.01ab	16.70±1.02b	0.97±0.14c	1.60±0.15a
O_3+phenanthrene	8.08±1.45a	332.90±50.00ab	8.95±1.03ab	12.50±2.13a	0.70±0.11ab	1.24±0.20a
CO_2+O_3+phenanthrene	8.61±1.12a	429.25±44.99bc	10.87±2.06b	13.61±1.34ab	0.81±0.11bc	1.45±0.18a

Treatments	DON/mg·g^{-1}	DOC/mg·g^{-1}	AP/mg·g^{-1}	SWC/%	pH
Amb	16.45±2.40ab	33.54±4.00b	7.76±1.21a	21.77±3.06a	6.11±1.50a
CO_2	18.25±2.05ab	72.36±10.34d	20.81±3.97c	28.36±3.51a	5.78±1.00a
O_3	14.23±1.50a	20.70±2.56a	12.27±1.96ab	24.49±4.00a	6.35±1.65a
CO_2+O_3	16.57±2.21ab	44.74±4.67c	14.12±1.98b	24.90±3.50a	6.17±1.11a
Amb+phenanthrene	17.56±1.92ab	49.07±5.35c	18.77±1.94c	25.35±4.00a	6.02±1.03a
CO_2+phenanthrene	21.70±4.13b	82.97±10.12e	13.91±2.11b	27.20±3.55a	5.85±0.76a
O_3+phenanthrene	16.26±1.50ab	27.07±4.01ab	10.82±0.88ab	25.03±2.62a	6.33±1.13a
CO_2+O_3+phenanthrene	16.97±2.32ab	49.92±5.05c	10.52±1.97ab	25.40±1.92a	6.19±1.10a

SWC: Soil water content; TP: Total phosphorus; TC: Total carbon; TN: Total nitrogen; TK: Total K; AN: NH_4^+-N: NN: NO_3—N; AP: Available P; DOC: Dissolved organic carbon; DON: Dissolved organic nitrogen. Values represent mean ± SD, n = 3. Different small letters in the same column represent significant differences at 0.05 level. The same as follows

The increase of CO_2 or/and O_3 also induced the change of physical and chemical properties of the soil with adding phenanthrene. In the soil with adding phenanthrene,

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the content trend of NN, TP, TK, TC, AN and DON was [CO_2] + [PHE] > [Amb] + [PHE] > [CO_2 + O_3] + [PHE] > [O_3] + [PHE]. The content alteration of TN, SWC and DOC was [CO_2] + [PHE] >[CO_2 + O_3] + [PHE] > [Amb] + [PHE] > [O_3] + [PHE], while the soil pH alteration was [O_3] + [PHE] > [CO_2 + O_3] + [PHE] > [Amb] + [PHE] > [CO_2] + [PHE].

The results also indicated that the contents of NN, TP, AN, DON, TK, TC and TN in the soil with adding phenanthrene were slightly higher than that of the soil without adding phenanthrene (*Table 1*). Therefore, the increase of atmospheric CO_2 or/and O_3, as well as the pollution of phenanthrene in soil, induced the change of physical and chemical properties of the experimental soil to some extent.

Effects of rising of CO_2, O_3 in atmosphere and phenanthrene in soil on soil microbial biomass

The change of soil microbial biomass carbon (MBC) and nitrogen (MBN) in the soil without adding phenanthrene was [CO_2] > [CO_2 + O_3] > [Amb] > [O_3]. Multiple comparison results showed that compared with [Amp], CO_2 injection induced a significant increase in MBC and MBN content ($P < 0.05$), while only O_3 injection induced a significant decrease in MBN content ($P < 0.05$). The carbon nitrogen ratio (MBC/MBN) of microbial biomass showed a trend of [O_3] > [CO_2 + O_3]> [CO_2] > [Amb]. Multiple comparison results showed that only [O_3] induced significant increase in MBC/MBN compared with [Amb] ($P < 0.05$).

In the soil with adding phenanthrene, the content change of MBC and MBN was [CO_2] + [PHE] > [Amb] + [PHE] > [CO_2 + O_3] + [PHE] > [O_3] + [PHE]. Multiple comparison results suggested that compared with [Amb] + [PHE], [O_3] + [PHE] induced a significant decrease in MBC and MBN ($P < 0.05$), while no significant change was observed in other treatment groups. Under normal atmospheric conditions, MBC and MBN in soil with adding phenanthrene increased by 47% and 45% (P < 0.05) respectively compared with that of the soil without adding phenanthrene (*Table 2*). MBC/MBN presented an alteration trend of [O_3] + [PHE] > [CO_2] + [PHE] > [Amb] + [PHE] > [CO_2 + O_3] + [PHE], while the changes within the treatment groups was not significant ($P > 0.05$).

Table 2. *Effect of elevated atmospheric CO_2 and/or O_3 on contents of carbon and nitrogen of microbial biomass and ratio of microbial biomass carbon/nitrogen in phenanthrene-added and -unadded soils*

Treatments	MBC/mg·kg^{-1}	MBN/mg·kg^{-1}	MBC/MBN
Amb	50.42±7.8a	7.08±1.02b	7.11±0.10ab
CO_2	83.36±7.39c	10.69±1.12c	7.82±0.54b
O_3	46.52±4.18a	4.81±1.03a	9.68±0.19c
CO_2+O_3	57.69±10.01ab	7.3±1.02b	7.88±0.29b
Amb+phenanthrene	73.9±10.06bc	10.29±1.02c	7.16±0.38ab
CO_2+phenanthrene	86.55±7.2c	11.52±1.01c	7.56±1.00b
O_3+phenanthrene	54.19±5.04a	7.08±1.04b	7.88±0.46b
CO_2+O_3+phenanthrene	60.52±11ab	9.78±1.01c	6.16±0.40ab

MBC: Microbial biomass carbon; MBN: Microbial biomass nitrogen; MBC/MBN: ratio of microbial biomass carbon/nitrogen

The results of multivariate analysis of variance showed suggested that the effects of different treatment groups [CO_2], [CO_2 + O_3], [Amb] and [O_3], (Pascual-Córdova, 2018)

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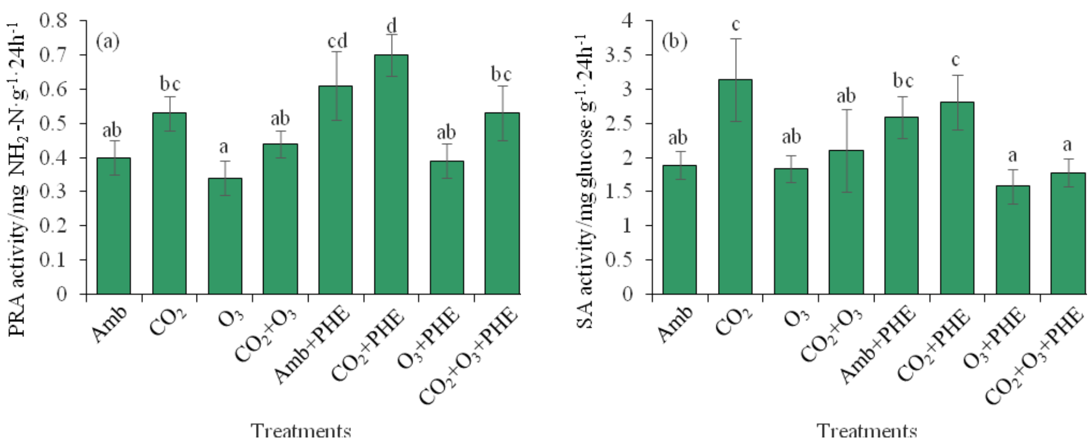
as well as the soil treatment model with adding phenanthrene and the model without adding phenanthrene on soil microbial biomass carbon and nitrogen were extremely significant ($P < 0.05$). At the same time, the interaction between different treatment groups and two soil treatment models had no significant influence on soil microbial biomass carbon and nitrogen.

Effects of rising of CO_2, O_3 in atmosphere and phenanthrene on soil enzyme activity

The alteration trends in the activity of soil protease (PRA), invertase (SA), urease (UA), (Espinoza, 2017) polyphenol oxidase (POA) and acidic phosphatase (APA) under the condition of increase of atmospheric CO_2 or and O_3 without adding phenanthrene was like $[CO_2] > [CO_2 + O_3] > [Amb] > [O_3]$ that was consistent with the alteration trends of MBC and MBN in soil. Multiple comparison results showed that, compared with [Amb], only the injection of O_3 induced significant decrease of APA ($P < 0.05$), and only the injection of CO_2 induced significant increase of SA ($P < 0.05$). However, there was no significant difference between UA or POA in each treatment group ($P > 0.05$). Meanwhile, NPA showed a trend of $[CO_2] > [Amb] > [CO_2 + O_3] > [O_3]$, and CO_2 injection induced significant increase of NPA ($P < 0.05$) (*Fig. 1*).

In the soil with adding phenanthrene, with the increase of CO_2 or/and O_3, the alteration trend of PRA, SA, UA, POA, APA and NPA was all $[CO_2] + [$ phenanthrene$] > [Amb] + [PHE] > [CO_2 + O_3] + [PHE] > [O_3] + [PHE]$ which was same as the trend of MBC and MBN. Multiple comparison results showed that, compared with [Amb] + [PHE], $[O_3]$ + [PHE] induced significant decline of PRA ($P < 0.05$), and significant increase of NPA ($P < 0.05$), while the $[O_3]$ + [PHE] and $[CO_2 + O_3]$ + [PHE] induced significant decrease of SA ($P < 0.05$). In addition, under normal atmospheric conditions, the PRA, SA and APA activity in soil with adding phenanthrene increased by 53%, 37% and 30% respectively compared with that in soil without adding phenanthrene. The activity of UA and POA increased in soil with adding phenanthrene, but the trend was not significant ($P > 0.05$).

The results of multifactorial variance analysis showed that the effects of $[CO_2]$, $[CO_2 + O_3]$, [Amb] and $[O_3]$ had significant influence on PRA, SA, UA, APA, NPA ($P < 0.05$), while had few influences on POA ($P > 0.05$) (Jaimes, 2017). The two soil groups with and without adding phenanthrene had significant influences on PRA, APA and NPA ($P < 0.05$), while had few influences on SA, UA and POA ($P > 0.05$). Meanwhile, interaction within different treatment groups and two soil treatment models had bare influence on PRA, SA, UA, POA, APA and NPA.



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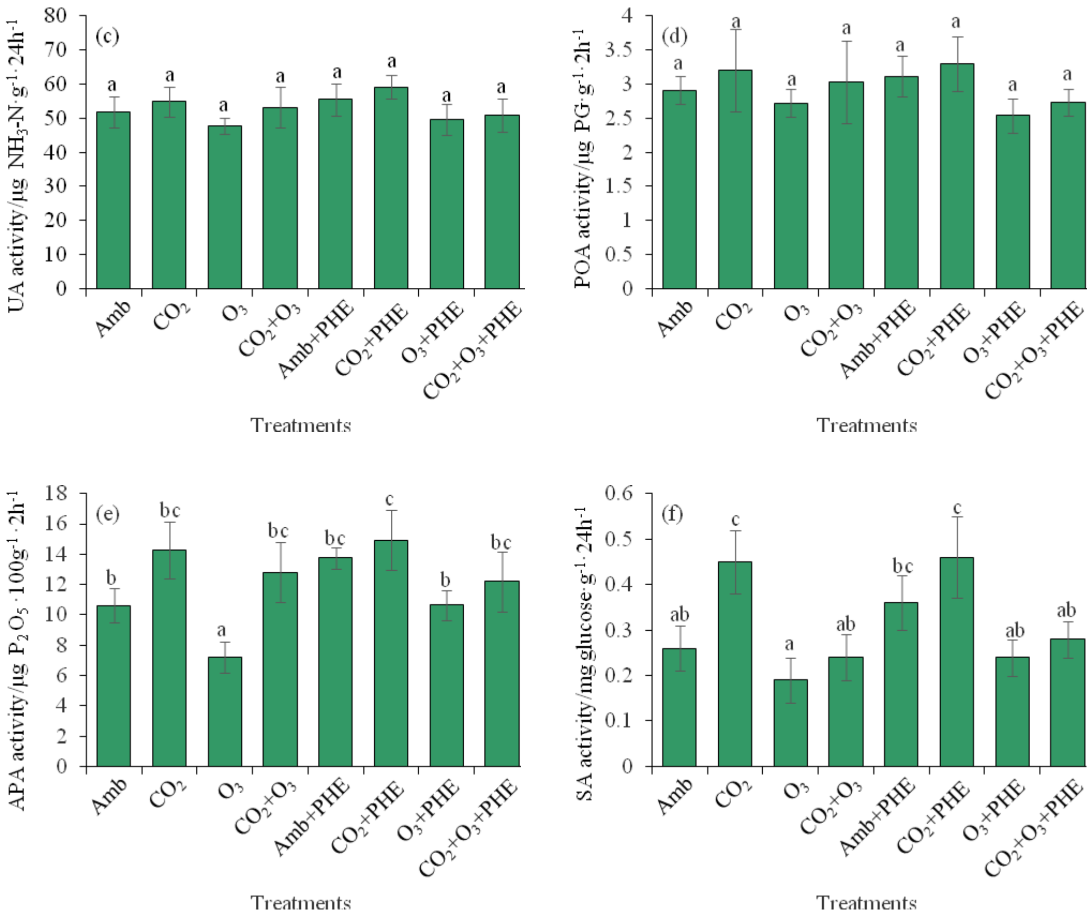

Figure 1. *Effect of elevated atmospheric CO_2 and/or O_3 on soil enzymatic activities in phenanthrene-added and -unadded soils. (PRA: Protease; SA: Sucrase; UA: Urease; POA: Phenol oxidase; APA: Acid phosphatase; NPA: neutral phosphatase)*

Discussion

Elevated atmospheric CO_2 or/and O_3 induce changes in the physical and chemical properties and enzyme activity of soil without adding phenanthrene

In this experiment, and OTC platform was used to build a soil-vegetable pot simulation system, and the changes of soil physical and chemical properties and soil enzyme activities under the elevated environment of CO_2 or/and O_3 were studied (Dager, 2017). The results showed that the increase of [CO_2] induced the increase of physical and chemical indicators such as NN, TP, TK, TC, DOC, AP, and MBC and MBN in the soil without adding phenanthrene, and the alteration trend was [CO_2] > [Amb]. Meanwhile, it was also found that soil enzyme activities such as NPA, PRA, SA and APA showed similar alteration trend. It was speculated that the increase of [CO_2] promoted the above soil physical and chemical indicators and soil enzyme activity. Studies have reported that the increase of atmospheric CO_2 concentration can promote the photosynthesis of plants, and improve the biomass and secretion of roots, and change the material composition of roots into soil (Xu et al., 2013; Sulman et al., 2014; Sunny et al., 2018). The increase of atmospheric CO_2 can also improve total organic carbon content and rhizospheric carbon availability through the metabolism of

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plants, which lead to the increase of the soil microbial biomass C, N and microbial activity (Tian et al., 2011; Ren et al., 2014; Shrestha and Baral, 2018). Changes in soil microorganism amount induced changes in soil enzyme activities such as PRA, SA, UA, POA, APA and NPA (Ren et al., 2014; Larson et al., 2002; Mahmood et al., 2018). Therefore, in this experiment, the increase of [CO_2] improved soil NN, TP, TK, TC, DOC, AP and other physical and chemical indicators, and MBC and MBN probably through promoting photosynthesis of vegetables and the synthesis and output of root secretions, and then induced the increase the soil enzyme activity of NPA, PRA, SA and APA (*Tables 1* and *2*).

It was also found that the increase of [O_3] decreased the physical and chemical indicators and enzyme activity of the above soil, and they showed a decline trend of [O_3] < [Amb]. Meanwhile, the increase of [O_3] also led to the increase of MBC/MBN ratio of microorganisms (*Table 2*). Studies have reported that the increase of atmospheric O_3 concentration can close the stomata of leaves and restrict the entering of CO_2 into the leaf tissue, thus reducing the photosynthetic rate and the carbohydrate content in plants (Chen et al., 2012; Anan, 2019). Increased O_3 concentration can reduce root secretions and change the types of secretions (Díaz-de-Quijano et al., 2012; Hu et al., 2011). It can also reduce the metabolism of soil microorganism C and the content of N, and increase the ratio of MBC/MBN (Li et al., 2014), which indirectly interferes with soil nutrients, rhizosphere microecological environment and soil enzyme activity (Johnson and Pregitzer, 2007; Huang et al., 2013; Cheng et al., 2011; Xu et al., 2013; Qiao, 2018). It was speculated that the rise of [O_3] reduces and changes the vegetables root secretion through inhibiting photosynthesis of green vegetables, and changed the soil C and N metabolism to some extent, and leads to abnormal changes in physical and chemical indicators such as NN, AN, TP, AP, TK, DOC, DON and MBC, MBN, which indirectly inhibits the activity of soil enzymes such as NPA, PRA, APA and NPA (*Tables 1* and *2*).

The results also showed that the soil enzyme activity of NN, AN, TN, AP, TP, TK, TC, DOC, DON and MBC, MBN, and NPA, PRA, SA, UA, POA, APA showed a trend of [$CO_2 + O_3$] > [O_3] (*Tables 1* and *2*). [CO_2] might reduce the damage of [O_3] to the leaf tissue of vegetables and the interference of physiological and biochemical process such as photosynthesis on the leaf tissue of vegetables, thus indirectly inducing the increase of soil physical and chemical indicators such as TP, TK, AN, AP, DOC, DON, and MBC, MBN, as well as enzyme activities such as APA, NPA and PRA. The correlation mechanism remains to be further discussed.

Elevated atmospheric CO_2 or/and O_3 induce changes in the physical and chemical properties and enzyme activities of the soil with adding phenanthrene

Phenanthrene is one of the typical polycyclic aromatic hydrocarbon pollutants in soil. Studies on the effects of atmospheric CO_2 or/and O_3 increase on the microecological environment of the phenanthrene polluted soil have not been reported (Olivares et al., 2017). The results of this study showed that [CO_2] increased the physical and chemical indicators (NN, AN, TP, AP, TC, DOC, DON, etc.), and MBC, MBN, and the soil enzyme activities of NPA, PRA, SA and APA. The alteration trend of above indicators was [CO_2] + [PHE] > [Amb] + [phenanthrene]. It was also found that the increase of [O_3] decreased the above soil physical and chemical indicators and enzyme activity, and showed the trend of [O3] + [PHE] < [Amb] + [PHE]. Therefore, the alteration trend of [CO_2] + [PHE] > [Amb] + [PHE] > [O_3] + [PHE] showed by the above indicators was

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consistent with the soil with adding phenanthrene. The direct effects of CO_2 and O_3 on the leaf tissue of vegetables might promote or interfere with the secretion of root substances through affecting the photosynthesis of leaves, thus causing the change of coil microecological environment (Wang et al., 2014; Cheng et al., 2011; Gautam et al., 2019). Soil phenanthrene affect directly on the root system of vegetables, and its target organs are different from CO_2 and O_3. Therefore, in the same CO_2 and O_3 exposure environment, the addition of phenanthrene to soil only changed the size of physical and chemical indicators but did not change its alteration trend (*Table 1*).

It was also found that [CO_2 + O_3] could alleviate the inhibition of [O_3] on the physical and chemical indicators and enzyme activity of the above mentioned soil, and its alteration trend of [CO_2 + O_3] + [PHE] > [O_3] + [PHE] was consistent with the that in soil without adding phenanthrene. The potential mechanism might be related to the alleviation of CO_2 on damage of O_3 to vegetables or the reduction of inhibition effect of O_3 on photosynthesis of vegetables. The interaction between soil pollution and atmospheric CO_2 or/and O_3 increase is a complex process, and its microscopic mechanism remains to be further studied.

Conclusion

(1) In the soil without adding phenanthrene, the increase of [CO_2] induced the increase of physical and chemical indicators, NN, TP, TK, TC, DOC and AP, and MBC, MBN, as well as soil enzyme activities of NPA, PRA, SA, APA with a trend of [CO_2] > [Amb]. The increase of [O_3] decreased the above soil physical and chemical indicators and enzyme activity ([Amb] > [O_3]), while increased the ratio of soil microorganism MBC/MBN. [CO_2 + O_3], to some extent, decreased the inhibition effect of [O_3] on soil microbial biomass and enzyme activity, and also released the stimulating effect of [CO2] increase on soil microbial biomass and enzyme activity.

(2) In the soil with adding phenanthrene, [CO_2] increased physical and chemical indicators, NN, AN, TP, AP, TC, DOC, DON and the ratio MBC/MBN with a rising trend of [CO_2] + [PHE] > [Amb] + [PHE]. [O_3] exposure decreased the above indicators and enzyme activities ([Amb] + [PHE] > [O_3] + [PHE]). [CO_2 + O_3] + [PHE] could alleviate the inhibition effect of [O_3] + [PHE] on those indicators and enzyme activities. These trends were basically consistent with the alteration trend in soil without adding phenanthrene. Moreover, the interaction within [CO_2], [CO_2 + O_3], [Amb], [O_3] and the two soil treatment models groups had no significant impact on MBC, MBN, PRA, SA, UA, POA, APA, NPA, the reason of which might be that CO_2 and O_3 directly affect of leaf tissue of cabbage, while the soil phenanthrene directly affect the root system of cabbage. The increase of CO2 or/and O2 might play an indirect role with soil phenanthrene through affecting photosynthesis of leaves and secretion of root system of cabbage, and only changed the value of physical and medical while did not change its trend. Its microscopic mechanism remains to be further studied.

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