Response of C, N and P Ecological Stoichiometry in Plants and Soils During a Soybean Growth Season to O3 Stress and Straw Return in Northeast China

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Abstract

Aims

C, N and P ecological stoichiometry plays important roles on biogeochemical cycles in ecosystems, yet the relationship between plant and soil stoichiometry and stoichiometric effects on the growth of soybean root in response to the O_3 stress and straw return remain poorly understood.

Methods

Here, a pot experiment was conducted in open top chambers to monitor the response of C, N and P ecological stoichiometry of leaves, shoots, roots and soils during a growing season (branching, flowering and podding stages) of soybean (Glycine max; a highly sensitive species to O_3) to background O_3 concentration (45 ± 10 ppb), O_3 stress (80 ±10 ppb) and straw treatment (no straw return and straw return).

Results

The O_3 stress significantly decreased root biomass. The straw return significantly increased root biomass under the O_3 stress at branching and flowering stages. Generally, the O_3 stress and straw return showed significant effects on the C, N, P concentrations of leaves and soils, and stoichiometric ratios of leaves, shoots and microbial biomass. C, N, P concentrations and stoichiometric ratios of leaves, shoots, roots and soils responses to the O_3 stress and straw return at branching stage were inconsistent with changes observed at the flowering and podding stages. The P conversion efficiency showed significant relationship with root P concentration under the combined effects of O_3 stress and straw return.

Conclusions

C, N, and P concentrations of soybean might be more important than stoichiometric ratios as a driver of defense against the O_3 stress in the case of straw return.

Introduction

Tropospheric O_3 (Ozone), which is an important greenhouse gas and a secondary air pollutant (Sitch et al. 2007; Saikawa et al. 2017), has a direct negative influence on ecosystem (Vingarzan 2004; Monks et al. 2009). The concentration of O_3 has doubled in the northern hemisphere since the 19th century (Mills et al. 2018). Results from coupled Chemical Transport Models (CTMs) and CTM simulations driven by the General Circulation Model (GCM) suggest that tropospheric O_3 will rise between 1 and 10 ppb by the year 2050 (Jacob and Winner 2009). In China, O_3 had exceed the WHO (the world health organization) standard of 100 μg m^{-3} on more than 30% of the days in 2013–2015 in Beijing, Chengdu, Guangzhou, and Shanghai because of urban extension and industrial development (Gong et al. 2018; Wang et al. 2017a). Exposed to the elevated O_3 doses, plants undergo a responsive process which involves a series of physiological and biological adjustments ranging from single cell to the whole plant level (Ashmore 2005; Bussotti et al. 2007; Fiscus et al. 2005; Jolivet et al. 2016; Munne-Bosch et al. 2013).

The elevated O_3 showed directly effects on aboveground ecological processes. For instance, the leaves were directly damaged (Feng et al. 2014), photosynthesis was significantly decreased (e.g. Wittig et al. 2007; Zhang et al. 2012), antioxidant capacity was significantly changed and aboveground biomass was significantly reduced by the O_3 stress (e.g. Hu et al. 2015; Shang et al. 2017; Wittig et al. 2009). Meanwhile, fewer studies found that the belowground ecological processes (such as the roots growth and soil processes) were indirectly influenced by the O_3 stress (Nikolova et al. 2010; Pregitzer et al. 2008).

There is closely linked between the above- and below-ground ecosystem due to the circulation and feedback of nutrients (Peichl et al. 2012; Wang et al. 2009). Plant roots could provide substrates to the soils and microbes, and therefore changes in the C, N, P concentrations and stoichiometric ratios of the plants might affect soil C, N, P concentrations and stoichiometric ratios (Peichl et al. 2012; Wang et al. 2009; Mulder and Elser 2009). Many studies have demonstrated strong relationships among C, N and P in soils or in plants (McGroddy et al. 2004; Yang and Luo 2011; Liao et al. 2014), and few have shown how these chemical traits in soil relate to these in plants (Jobbágy and Jackson 2001; Yu et al. 2010; Fan et al. 2015). C, N and P of ecological stoichiometry are critically important for prediction stress consequences in biochemical cycles and other ecological process. A handful of studies are about the effects of O_3 stress on leaf stoichiometry and nutrient resorption efficiency (Cao et al. 2016; Nakaji et al. 2004; Oksanen et al. 2005; Shang et al. 2018; Shi et al. 2016, 2017; Zhuang et al. 2017).

For example, Agathokleous et al. (2018) found that O_3 stress (68.84 ± 1.12 nmol mol^{-1}) altered the leaf stoichiometry, including impacted in nutrient resorption efficiency. Fewer studies focus on the effects of O_3 stress on stoichiometry of soil and root (Cao et al. 2016; Shang et al. 2018; Li et al. 2021). Cao et al. (2016) found that O_3 stress (100 ppb and 150 ppb) significantly decreased the C/N ratios in individual tissues (foliar, stem, and root) of both of Yang (*P. bournei*) and *Phoebe zhennan* S. Lee et F. N. Wei (*P. zhennan*), and C/P and N/P ratios in individual tissues of *P. bournei*, and the individual tissues of *P. zhennan* did not show consistent variation tendency under the O_3 stress. Shang et al. (2018) showed that the O_3 stress (60 ppb) significantly increased both N and P concentrations of individual organs (leaves, stems and roots) of poplar while a reduction in the C/N ratio was observed. Much-limited studies were concerned with the cycling of ecological stoichiometry between above- and below-ground ecosystem exposed to elevated O_3 stress.

Straw return is a common tillage practice for crops cultivation (such as rice, wheat, maize and soybean) in China. The annual output of straw in China is about 750 million tons. More than 1/3 of the straw is discarded or burned, resulting in resource waste and environmental pollution (Ma et al. 2019). According to the results of meta-analysis of 142 experiments, Zhao et al. (2015a) found that crop yields in 92% of 131 experiments increased with increasing straw incorporation. Straw returning to the field not only can improve the soil structure, reduce the soil bulk density, but also activate the soil organic phosphorus, increase the soil nitrogen and improve yields (Tan et al. 2007; Zhao et al. 2015a, b). The addition of organic matter might directly affect the soil C, N and P (Liu et al. 2014; Zhao et al. 2016b), and plants might adjust their growth rates with the changed stoichiometric ratio of soil C, N and P (Daufresne and Loreau 2001; Moe et al. 2005). However, much-limited information is available on the cycling of ecological stoichiometry between above- and below-ground ecosystem under straw return and/or O_3 stress. Tropospheric O_3 is currently the most important secondary air pollutant, and O_3 is posing serious threats to forest ecosystems, agriculture, and human health (Ainsworth 2017; Feng et al. 2019). Considering that the straw return is a common tillage practice for crops cultivation in China, it is necessary to study the combined influence of straw return and O_3 stress on the cycling of ecological stoichiometry between above- and below-ground in the cropland ecosystem, in order to be able to predict the impacts of elevated O_3 on crop yield and nutrient utilization of cropland ecosystem in an actual tillage practice conditions.

Soybean (*Glycine max* (L.) *Merr.*), one of the most widely grown legume in the world, is most susceptible to O_3 among food crops (Burkey and Carter 2009; Mills et al. 2006). Researches had found that O_3 stress reduced plant growth and seed yield and accelerated leaf senescence of soybean (Dermody et al. 2006; Mao et al. 2017; Morgan et al. 2006). Furthermore, straw return, which is a common tillage practice for soybean cultivation in China, might increase C and nutrient input through both root and aboveground biomass. Such an increase might be relatively great, and subsequent changes in stoichiometric relationship between the plants and soils might be significant. However, whether straw return could alleviate the damage from O_3 stress and whether the mitigation effect of straw return is due to the changes of stoichiometry between above- and below-ground in soybean cropland is still rarely evaluated. Thus, the objective of this study is to examine the effects of O_3 stress and/or straw return on C, N and P stoichiometry dynamic in a plant-soil system during a growing season of soybean. Furthermore, soybeans have naturally higher levels of floral and pod loss (Liu et al. 2003). Previous studies showed that soybean yield is mainly determined during the post-flowering phase (R1 stage onwards; Fehr and Caviness 1977), throughout flowering and pod setting (Board et al. 1995; Egli 2010). The subsequently changes in C, N and P stoichiometry caused by the O_3 stress and/or straw return might are greater during flowering and podding development. Thus, it is necessary to evaluate the responses of nutrient utilization and C distribution at different developmental stages (such as branching stage, flowering stage and podding stages) to the effects of O_3 stress and/or straw return.

We hypothesized that (1) significant effects of the O_3 stress and straw return on C, N, P concentrations and stoichiometric ratios of leaf, shoot, root and soils depended on the growth stage of soybean; and (2) straw return alleviated the damage from O_3 stress due to the changes of C, N and P stoichiometry between above- and below-ground in the soybean cropland. To test these hypotheses, branching, flowering and podding stages of soybean were chosen as sampling times to evaluate the temporal variations in C, N, P concentrations and stoichiometric ratios of leaf, shoot, root and soils in response to the O_3 stress and/or straw return using a pot experiment in open top chambers (OTC).

Materials And Methods

Experimental site

The experimental site located in Shenyang Experimental Station of Ecology, Chinese Academy of Sciences (41°310'N, 123°220'E). This region has a continental monsoon climate with a mean annual temperature of 7.0–8.0°C, annual precipitation of 650–700 mm, and an annual non-frost period of 147–164 days. The soil at the study site is classified as an Alfisol (US Soil Taxonomy) with 11.28 g kg^{-1} organic C, 1.20 g kg^{-1} total N, 0.41 g kg^{-1} total P, pH (H_2O) 6.7 at 0–15 cm depth.

Experimental design and sampling

The study was conducted on soybean plants grown in OTCs from June 16 to August 30 in 2017. The OTCs, which were established in 2008, had a diameter of 1.15 m and a height of 2.4 m with a 45° sloping frustum; the minimum distance between any two chambers was 4 m. Each chamber was made with an iron framework, clad with standard horticultural glass, with a plenum incorporated just below the mouth of the chamber (at 2.4 m from ground-level) to reduce the entrance of ambient air (Bao et al. 2014). From June to September, mean temperature of the day was about 25.6 ± 3.7 °C and mean relative air humidity in the OTCs throughout the day was 50.6 ± 19.9%. The mean value of O_3 concentration was about 45 ± 5 ppb and the average value of AOT40 (the O_3 concentration accumulated over a threshold O_3 concentration of 40 ppb during daylight hours) was 3.5 ppm h^{-1} during clear sky conditions from June to September in the OTCs.

The soybean cultivation was fumigated in the OTCs by O_3 for 2.5 months (from June 16 to August 30 in 2017). The experimental design was based on completely randomized plots including two O_3 treatments and tree replicates per O_3 treatment (overall 6 OTCs). Two O_3 treatments were carried out: (1) non-filtered air treatment (control, hereinafter referred to as CK, O_3 concentration 45 ± 10 ppb); (2) O_3 stress treatment, non-filtered air with addition of O_3 35 ppb (hereinafter referred to as O_3, 80 ± 10 ppb). The O_3 was produced from pure oxygen with an O_3 generator (GP-5J Guolin Ltd., Qingdao, China) and then it was mixed with ambient air to achieve the target O_3 concentration, and the mixture regulated by flow controllers in each OTC. The top of the OTCs is open. The O_3 concentrations were continuously monitored by O_3 analyzers (S-900 Aeroqual Ltd., Auckland, New Zealand) every day during the whole day from June 16 to August 30 in 2017, and controlled by computers using a professional program for O_3 dispensing and monitoring (Bao et al. 2014).

Meanwhile, there were two straw treatments (tree replicates per straw treatment) for each O_3 treatments. Two straw treatments were carried out: (1) no straw return (hereinafter referred to as S-); (2) The total amount of straw is returned to each pot (hereinafter referred to as S+). There were 18 pots per OTC, 3 collected periods (branching stage, flowering stage and podding stage) × 3 replications × 2 straw treatments. According to the average soybean yield and potted area, soybean straw (20 g), which was subjected to O_3 fumigation in 2016, was crushed and applied in situ to 20cm depth per pot. Soil, used in each pot experiment, was collected from a cropland (at 0–15 cm layer) at the study site before crops were planted. The previous crop was soybean (Tiefeng 29) and the field did not receive any N fertilization because of the fallow management before the beginning of the pot. After sieving (<2 mm), the soil was immediately used to prepare the pot experiment. The potted soybean cultivar was Tiefeng 29, which was seeded in each pot (26 cm long ×36 cm wide × 45 cm deep) on May 09 in 2017. Before sowing, $NH_4H_2PO_4$ at 300 kg ha^{-1} was applied to each plot. The plants were irrigated daily to avoid water stress and appropriate measures were taken to keep the plants free from any stresses of biotic, disease and grass. Five plants were planted in each pot.

Soil samples (containing rhizosphere and bulk soil) was collected at branching stage (July 10, 2017), flowering stage (August 03, 2017) and podding stage (August 30, 2017), respectively. Five soil samples from each pot were randomly collected at 0–10 cm depth by using soil-corer with an inner diameter of 4.5 cm and then they were pooled together to give one composite sample. The collected soil samples were immediately sieved (<2mm) to remove visible stones, roots and plant materials, and then divided into two sub-samples. One sub-sample was air-dried at 25°C for chemical analysis, one sub-sample was stored at 4°C for microbial biomass analysis. Furthermore, at harvest time, all plants were carefully removed from soil of each pot; plant sampling times were: branching stage (July 10, 2017), flowering stage (August 03, 2017) and podding stage (August 30, 2017); soil particles attached to roots were removed by water washing. After harvest, the root biomass was weighed after drying in the oven at 65 °C for 48 h and then were chemical analyzed. Meanwhile, the leaf samples and shoot samples were oven-dried at 65°C for chemical analysis.

Leaf, shoot, root and soil analysis

Total N and P concentration of leaf, shoot, root and soil were determined by elemental analyzer (Vario MAX CNS, Elementar Analysensysteme GmbH, Hanau, Germany) (Zhao et al. 2017). Soil organic carbon (SOC) and total C concentration of leaf, shoot, root and soil were analyzed by the $K_2Cr_2O_7$-H_2SO_4 calefaction and titration method (Nelson and Sommers 1982). The C/N and C/P ratios of leaf, shoot, root and soil were calculated from the values of SOC concentration, total C concentration, total N concentration and total P concentration.

Microbial biomass carbon (MBC) and Microbial biomass nitrogen (MBN) of soil cropped to soybean were analyzed by the chloroform fumigation-extraction method as described by Vance et al. (1987). Briefly, soil samples (25 g dry base) were fumigated with ethanol-free chloroform for 24 h at 25 °C. After removal of the chloroform, soluble C and N were extracted from fumigated and non-fumigated samples in 100 mL of 0.5M K_2SO_4 for 30 min on an orbital shaker. Total organic C in the filtered extract was determined by the $K_2Cr_2O_7$-H_2SO_4 calefaction and titration method. Total organic N in the filtered extract was determined using the elemental analyzer (Vario MAX CNS, Elementar Analysensysteme GmbH, Hanau, Germany). We converted microbial C flush (the difference in extractable C between fumigated and non-fumigated samples) to MBC using a factor of 0.45 (Vance et al. 1987) and microbial N flush to MBN using a factor of 0.54 (Brookes et al. 1985). Microbial biomass phosphorus (MBP) of soil cropped to soybean was determined using a fumigation extraction method as described by Brookes et al. (1982). The pre-treatment was in accordance with MBC and MBN. Soluble P in fumigated and non-fumigated soil samples (5 g dry base) was extracted in 100 mL of 0.5M $NaHCO_3$ (pH 8.5) for 30 min on an orbital shaker. We converted microbial P flush to MBP using a factor of 0.40 (Brookes et al. 1982).

The efficiency of conversion of nutrients taken up by the plant into crop biomass was calculated as follows (Tittonella et al. 2008):

Conversion efficiency of nutrient X = total aboveground biomass/total uptake of nutrient X,

where, the total aboveground biomass is the sum of the leaf biomass and shoot biomass at different stages, expressed on a dry weight basis. The conversion efficiencies for N and P have the units: g DM mg N^{-1}, g DM mg P^{-1} taken up by the soybean per plant, respectively. The uptake of nutrients was calculated from measurements of N and P concentrations in leaf and shoot biomass.

Statistical analysis

The differences in C, N, P concentrations and stoichiometric ratios, root biomass, aboveground biomass and conversion efficiency of N and P between the two straw treatments and between the two O_3 stress were evaluated by one-way analysis of variance (ANOVA) according to Tukey's test ($P < 0.05$). Then, a multivariate analysis of variance (general linear model (GLM)) was used to evaluate the effects of the growth stage, O_3 stress, straw return as well as their possible interactions on the C, N, P concentrations, stoichiometric ratios, root biomass, aboveground biomass and conversion efficiency of N and P (SPSS 16.0).

Path analyses were conducted to explore the direct and indirect influences of the O_3 stress and straw return on soybean root growth. The values of C, N, P concentrations and stoichiometric ratios, soil microbial biomass and root biomass were log-transformed before the path analyses. The path analyses in straw return and no straw return were carried out separately. We started the path analyses procedure with the specification of a conceptual model of hypothetical relationships, based on a priori and theoretical knowledge (Carrillo et al. 2017; Wang et al. 2017b). Data were fitted to the models using the maximum likelihood estimation method (Boldea and Magnus 2009). The best-fitting model was selected by step-wise removal of non-significant paths ($P \geq 0.05$). The chi-squared tests (χ^2; the model has a good fit when χ^2 is low and the P-value ≥ 0.05) were used to test the overall goodness of fit for the model (Grace and Keeley 2006). Standardized regression coefficients and significant level of each path were calculated. All the path analyses were conducted using Amos 22.0 (IBM, SPSS, New York, USA).

Results

The root biomass of soybean

The O_3 stress significantly decreased the root biomass and aboveground biomass (Tables 1 and 2). Furthermore, the straw return showed significant positive effects on the root biomass at branching and flowering stages and on the aboveground biomass at branching stage under the O_3 stress (Table 1). According to the results of general linear model (GLM), the growth stage × straw return, the growth stage × O_3 stress and the O_3 stress × straw return showed significant effects on the root biomass and aboveground biomass (all at $P < 0.05$; Table 3)

Table 1
Root biomass, aboveground biomass, N conversion efficiency and P conversion efficiency (mean ± SE) under the O_3 stress and the straw treatment at branching, flowering and podding stages

Stages	O_3 stress	Root biomass		Aboveground biomass		Conversion efficiency of N		Conversion efficiency of P	
		(g plant^{-1})		(g plant^{-1})		(g DM mg N^{-1})		(g DM mg P^{-1})	
		S+	S-	S+	S-	S+	S-	S+	S-
Branching	CK	4.8(0.2)^{aA}	4.9(0.1)^{aA}	21.9(0.5)^{aA}	18.6(0.6)^{bA}	1.00(0.06)^{aA}	0.63(0.04)^{bA}	6.5(0.3)^{aA}	4.7(0.1)^{bA}
	O_3	2.3(0.1)^{aB}	2.0(0.1)^{bB}	15.2(0.7)^{aB}	10.6(0.5)^{bB}	0.41(0.02)^{aB}	0.28(0.01)^{bB}	3.4(0.1)^{aB}	2.4(0.1)^{bB}
Flowering	CK	9.2(0.2)^{aA}	9.3(0.1)^{aA}	30.9(1.1)^{aA}	29.8(0.3)^{aA}	1.00(0.04)^{aA}	1.08(0.03)^{aA}	8.7(0.4)^{bA}	9.1(0.1)^{aA}
	O_3	4.1(0.1)^{aB}	3.0(0.2)^{bB}	13.8(0.2)^{aB}	13.6(0.5)^{aB}	0.40(0.01)^{aB}	0.34(0.01)^{bB}	2.9(0.1)^{aB}	2.9(0.1)^{aB}
Podding	CK	10.1(0.5)^{aA}	9.8(0.7)^{aA}	75.8(0.5)^{aA}	64.1(1.7)^{bA}	2.51(0.05)^{aA}	1.79(0.05)^{bA}	21.7(1.1)^{aA}	16.5(0.5)^{bA}
	O_3	5.2(0.2)^{aB}	5.2(0.1)^{aB}	28.0(0.9)^{aB}	28.6(0.5)^{aB}	0.77(0.02)^{bB}	0.86(0.03)^{aB}	6.8(0.2)^{bB}	7.6(0.2)^{aB}

Lowercase letters in rows indicate statistical difference between the two straw treatments according to Tukey's test ($P < 0.05$).

Uppercase letters in lines indicate statistical difference between the two O_3 stress according to Tukey's test ($P < 0.05$).

Stage, growth stage; CK, non-filtered air treatment (45 ± 10 ppb); O_3, O_3 stress (80 ± 10 ppb); S+, straw returning; S-, no straw returning

Table 2
Differences of root biomass, aboveground biomass, N conversion efficiency and P conversion efficiency among the developmental stage

Stages	**Treatments**	**Root biomass**		**Aboveground biomass**		**N conversion efficiency**		**P conversion efficiency**	
		(g plant^{-1})		**(g plant^{-1})**		**(g DM mg N^{-1})**		**(g DM mg P^{-1})**	
		S+	**S-**	**S+**	**S-**	**S+**	**S-**	**S+**	**S-**
Branching	CK	a	a	a	a	a	a	a	a
Flowering	CK	b	b	b	b	a	b	b	b
Podding	CK	c	b	c	c	b	c	c	c
Branching	O_3	A	A	B	A	A	A	B	A
Flowering	O_3	B	B	A	B	A	B	A	A
Podding	O_3	C	C	C	C	B	C	C	B

Lowercase letters in rows indicate statistical difference among the developmental stage under non-filtered air treatment (CK) according to Tukey's test ($P < 0.05$).

Uppercase letters in lines indicate statistical difference among the developmental stage under O_3 stress (80 ± 10 ppb) according to Tukey's test ($P < 0.05$).

Stage, growth stage; CK, non-filtered air treatment; O_3, O_3 stress (80 ± 10 ppb); S+, straw returning; S-, no straw returning;

Table 3
P values of the results of general linear model (GLM) testing for the effects of the growth stage, O_3 stress and straw return as well as their possible interactions on the root biomass, aboveground biomass, conversion efficiency of N and P

	Root biomass	**Aboveground biomass**	**N conversion efficiency**	**P conversion efficiency**
	(g plant^{-1})	(g plant^{-1})	(g DM mg N^{-1})	(g DM mg P^{-1})
Block	n.s.	n.s.	n.s.	n.s.
Stage	***	***	***	***
O_3	***	***	***	***
Straw	**	***	***	***
O_3 × Straw	*	***	***	***
Stage × O_3	***	***	***	***
Stage × Straw	***	***	***	***
Stage × O_3 × Straw	***	***	***	***
***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., not significant				

C, N, P concentrations and stoichiometric ratios of leaves and shoots

The O_3 stress showed significant effects on the N and P concentrations and C/N and C/P ratios of soybean leaf according to GLM results (Fig. 1B-E). The concentrations of C, N and P of leaf were significantly increased at branching and decreased at podding stage by the O_3 stress (Fig. 1A, C, E). There were significant effects of the growth stage × O_3 stress on the C, N, P concentrations and stoichiometric ratios of soybean leaf. The straw return significantly increased C concentration and ratios of C/N and C/P of soybean leaf under the O_3 stress at podding stage, while the straw return significantly decreased C concentration and ratios of C/N and C/P of soybean leaf under the O_3 stress at branching stage (Fig. 1A, B and D). Generally, the interaction effects of the straw return and the O_3 stress were significant on the C, N, P concentrations and stoichiometric ratios of soybean leaf.

The O_3 stress showed significant effects on C/N, C/P and N/P ratios and insignificant effects on the concentrations of C, N and P of soybean shoot (Fig. 2). The ratios of C/N and C/P of shoot were significantly decreased at the stages of branching and podding by the O_3 stress (Fig. 2B, D). The O_3 stress significantly increased the C/N ratio of shoot at podding stage (Fig. 2B). There were insignificant differences in C/P ratio of shoot between the O_3 stress and non-filtered air treatment (CK) at podding stage (Fig. 2D). There were no significant effects of the O_3 stress × straw return on C concentration and C/P ratio of soybean shoot (Fig. 2A and D). The straw return significantly increased C concentration and C/N ratio of soybean shoot under the O_3 stress at flowering and podding stages, while there were insignificant differences in C concentration and C/N ratio of soybean shoot under the O_3 stress between straw return treatment and no straw return treatment at branching stage (Fig. 2A and B).

C, N, P concentrations and stoichiometric ratios of roots, soils and soil microbial biomass

Generally, the O_3 stress and the straw return × O_3 stress had significant effects on the C concentration and C/N ratio of soybean root according to GLM results (Fig. 3). The C concentration of root was significantly decreased at the stages of branching and podding by the O_3 stress (Fig. 3A). There were insignificant differences in C concentration of root between the O_3 stress and non-filtered air treatment (CK) at flowering stage. The O_3 stress significantly decreased the C/P ratio of root at the stages of branching and flowering and increased the C/P ratio of root at the podding stage (Fig. 3A). There were insignificant effects of the straw return × O_3 stress on the N and P concentrations and C/P and N/P ratios of soybean shoot (Fig. 3B, D, F). The straw return significantly increased P concentration and significantly decreased N/P ratio of soybean root under the O_3 stress at podding stage, while there were insignificant differences in P concentration and N/P ratio of soybean root under the O_3 stress between straw return treatment and no straw return treatment at branching and flowering stages (Fig. 3E and F).

According to GLM results, the O_3 stress and the interaction effects of the O_3 stress and straw return showed significant effects on SOC and P concentration of soybean soil (Fig. 4A, E). The O_3 stress significantly decreased SOC and P concentration of soil at the stages of flowering and podding (Fig. 4A, E). There were insignificant differences in SOC and P concentration of soil between the O_3 stress and non-filtered air treatment (CK) at branching stage. The straw return showed insignificant effects on C/N and N/P ratios (Fig. 4B, F). The straw return significantly increased SOC, N concentration and C/P ratio under the O_3 stress at the branching stage, while there were insignificant differences in SOC, N concentration and C/P ratio under the O_3 stress between straw return treatment and no straw return treatment at podding stage (Fig. 4A, C, D).

Generally, the O_3 stress, the straw return and the interaction effects of O_3 stress and straw return showed significant effects on soil microbial biomass according to GLM results (Fig. 5). The O_3 stress significantly decreased the ratios of MBC/MBP and MBN/MBP at the stages of branching and flowering and increased the ratios of MBC/MBP and MBN/MBP at the podding stage (Fig. 5D, F). The MBC and MBC/MBN ratio were significantly decreased at the branching stage by the O_3 stress (Fig. 5A, B). There were insignificant differences in MBC and MBC/MBN ratio between the O_3 stress and non-filtered air treatment (CK) at podding stage. There were insignificant differences in MBC, MBN, MBP, MBC/MBN ratio, MBC/MBP ratio and MBN/MBP ratio under the O_3 stress between straw return treatment and no straw return treatment at branching stage. Straw return significantly increased MBC and ratios of MBC/MBN, MBC/MBP and MBN/MBP, and decreased MBN and MBP under the O_3 stress at flowering stage.

Relationships Between Plants And Soils

There were significant relationships between the conversion efficiency of N and root N concentration under the O_3 stress, and between the conversion efficiency of P and root P concentration under the combined effects of O_3 stress and straw return (Fig. 6). According to the results of the path analyses, the O_3 stress had significant positive correlation with leaf stoichiometric ratios (Fig. 8A). There were significant positive relationships between the stoichiometric ratios of leaf and shoot, and between the stoichiometric ratios of microbial biomass and root biomass, and between the stoichiometric ratios of root and root biomass. There were significant negative relationships between the soil stoichiometric ratios and root biomass, and between the stoichiometric ratios of microbial biomass and root. Considering the combined effects of the O_3 stress and straw return, the O_3 stress showed significant positive correlation with the C, N, P concentrations of leaf (Fig. 8B). There were significant positive relationships between the C, N, P concentrations of leaf and shoot, and between the C, N, P concentrations of shoot and root. The stoichiometric ratios of microbial biomass showed negative relationships with the C, N, P concentrations of root and root biomass.

Discussion

In the present study, the O_3 stress significantly inhibited root growth during the whole stage of soybean, consistent with our previous study (Mao et al. 2017; 2018). Furthermore, straw return significantly increased root biomass exposed to the O_3 stress at branching and flowering stages. There were insignificant differences in root biomass between straw return treatment and no straw return treatment at podding stage. Indicated that straw return promoted root growth during branching and flowering stage, while straw return inhibited root growth at podding stage of soybean exposed to the O_3 fumigation. Thus, straw return effectively prevented the O_3 damage only at branching and flowering stage.

Previous studies have reported that the concentrations of the plant elements were increased by O_3 (e.g., Fangmeier et al. 2002; Wang et al. 2014; Zhang et al. 2014; Zheng et al. 2013; Shang et al. 2018). For instance, the results of a meta-analysis (143 experiments; O_3 stress (61 ppb and 34 ppb)) found that N concentration of leaves was significantly increased by 5% (Wittig et al. 2009). In the present study, when comparing leaves of the same position, we found that the O_3 stress tended to increase leaf N and P concentrations at branching and flowering stages, and most of the increases in N and P concentrations were statistically significant. Increasing N concentration may be a plant response in order to enhance the defense capability against O_3 stress and can be an adaptive strategy for plants against this pollutant (Cao et al., 2016). Braun et al. (2017) suggested that the nutrient imbalance between N and P may change the physiological responses of trees to elevated O_3. In fact, N and P may change the contents of secondary metabolites related to antioxidant systems against the oxidative stress caused by O_3 (Koricheva 2002; Fares et al. 2008). Differently, the present study showed that the O_3 significantly decreased the N and P concentrations of leaf at podding stage, indicating that the defense capability against O_3 stress at the podding stage was weaker than that at the branching and flowering stages.

Leaves and roots of plants are the main plant organs of carbon assimilation and absorption of nutrient, respectively, and the shoots are important intermediaries for linking leaves to roots (Zhao et al. 2016a; Shang et al. 2018). In the present study, the O_3 significantly increased the N and P concentrations of shoot during the whole growth stage. While, the N and P concentrations of roots responses to the O_3 stress in each growth stages were inconsistent with changes observed in leaves and shoots. Differently, Shang et al. (2018) found that the roots responses to the O_3 stress were consistent with changes observed in leaves. On the other hand, several studies have shown that the effect of elevated O_3 on dry matter and C assimilation of plants is the opposite. O_3 stress tends to decrease plant biomass and increase the nutrient concentration (Wang et al. 2014). In the present study, C concentration of leaves, shoots and roots responses to the O_3 stress in branching stage were inconsistent with changes observed in flowering stage. Concentrations of N and P of leaves and roots responses to the O_3 stress in flowering stage were inconsistent with changes observed in podding stage. It indicated that the significant effects of O_3 stress on C, N, P concentrations of soybean leaves, shoots and roots varied greatly and depended on the growth stage of soybean, consistent with the first hypothesis of present study.

Generally, MBC/MBP and MBN/MBP ratios responses to the O_3 stress in each growth stages were consistent with changes observed in C/P and N/P ratios of roots, while the O_3 stress showed insignificant effects on soil C/N and N/P ratios in the present study. Indicated that the O_3 stress could significantly affect root nutrient indirectly by affecting soil microbial biomass. Furthermore, C/N and C/P ratios of shoots responses to the O_3 stress in each growth stages were consistent with changes observed in leaves. The results of path analyses showed that there were significant correlations between leaf stoichiometry and shoot stoichiometry, and between shoot stoichiometry and root stoichiometry. Thus, the O_3 stress showed significant influence on root biomass indirectly by affecting soil microbial biomass stoichiometry which were directly linked with leaf stoichiometry in the present study (Nikolova et al. 2010; Pregitzer et al. 2008).

Nutrient use efficiency from leaves to root is an important process of nutrients storage or direct nutrients use by plant, and this process is less depended on the available nutrient of soils, and thus the process has important consequences for nutrient cycling in ecosystems (Aerts and Chapin 2000). So far, several studies have evaluated the effects of the O_3 stress on the response of the nutrient use efficiency of plant, however, with inconsistent results. It has been reported that the response of nutrient use efficiency to the O_3 stress was significant decreased (Gyu et al. 2015; Uddling et al. 2005), or was insignificant differences (Baker and Allen 1995; Lindroth et al. 2001), or was significant increased (Temple and Riechers 1995). The inconsistent results are probably because the different nutrient availability of soils, different O_3 stress, or plant species-specific differences (Shang et al. 2018). In the present study, leaf C/N and C/P ratios and conversion efficiency were used as the nutrient use efficiency. Generally, the O_3 stress significantly inhibited the conversion efficiency of N and P in the present study. Although the straw return significantly increased the conversion efficiency of N and P at the branching and flowering stages, the conversion efficiency of N and P was still significant lower in the O_3 stress than that in the non-filtered air treatment during the whole stages of soybean. Thus, the straw return had limited mitigation effect on the damage from the O_3 stress. Meanwhile, the conversion efficiency of P showed significant relationship with root P concentration under the combined effects of O_3 stress and straw return. Indicated that the responses of P concentration of plants (above-ground) and roots (below-ground) in the soybean cropland were strongly linked. And the limited mitigation effect of straw return on the damage from the O_3 stress was might due to the changes of P concentrations between plants and roots.

Generally, the straw return significantly increased the SOC and N concentrations of the soil cropped to soybean, consistent with the previous studies (Zhao et al. 2018; Liu et al. 2014). Meanwhile, under O_3 stress, there were insignificant differences in N and P concentrations of leaves between straw return treatment and no straw return treatment. Indicated that straw return might not alleviate the destructive effect of O_3 stress on leaves. However, under O_3 stress, N and P concentrations and C/P and N/P ratios of soil in straw return treatments were significantly higher than that in no straw return treatments in branching and flowering stages, indicating that straw return might alleviate the destructive effect of O_3 stress on soil. Moreover, the path analyses results suggested that the straw return showed significant effects on C, N, and P concentrations of soils and roots. Thus, the limited mitigation effects of straw return on the damage from the O_3 stress might be due to the changes of C, N, P concentrations of soils and roots.

Meanwhile, the present study also found that the effects of the straw return under the O_3 treatment on the C, N and P concentrations varied greatly and depended on the growth stage of soybean, consistent with the first hypothesis of the present study. For instance, the trends of C concentration and N concentration of soils and roots in the flowering stage were inconsistent with the observed in the podding stage. The trends of MBC, MBN and MBP in the flowering stage were inconsistent with the observed in the podding stage. Furthermore, the path analyses results indicated that the relationship between the responses of the plants and soils to the combined effects of the O_3 stress and the straw return were not mainly driven by the stoichiometric ratios but more generally by the chemical concentration of the plants and soils, contrary to the second hypothesis of the present study. In other words, plant chemical traits might be able to override ecological stoichiometric ratios as a driver of defense against the O_3 stress in the case of straw return.

Conclusions

This study contributes to address the influence of the O_3 stress and/or straw return on the C, N, P concentrations and stoichiometric ratios of plants and soils during a soybean growing season. The O_3 stress significantly inhibited soybean root growth, and the straw return effectively prevented the O_3 damage on soybean root at branching and flowering stages of soybean. Confirming the first hypothesis, the significant effects of the O_3 stress and/or straw return on C, N, P concentrations and stoichiometric ratios of leaf, shoot, root and soils varied greatly and depended on the growth stage of soybean. The root growth of soybean might be indirectly affected by the O_3 stress through the alterations of soil microbial biomass stoichiometry which were directly induced by the leaf stoichiometric ratios. The straw return had limited mitigation effect on the damage from the O_3 stress via the changes of C, N, P concentrations of soils and roots. Furthermore, plant C, N, and P concentrations might be more important than ecological stoichiometric ratios as a driver of defense against the O_3 stress in the case of straw return, contrary to the second hypothesis of present study. Collectively, these findings of present study provide a further understanding and forecasting on nutrient utilization and feedbacks between plants and soils during a growth season of soybean.

Declarations

Acknowledgements

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Figures

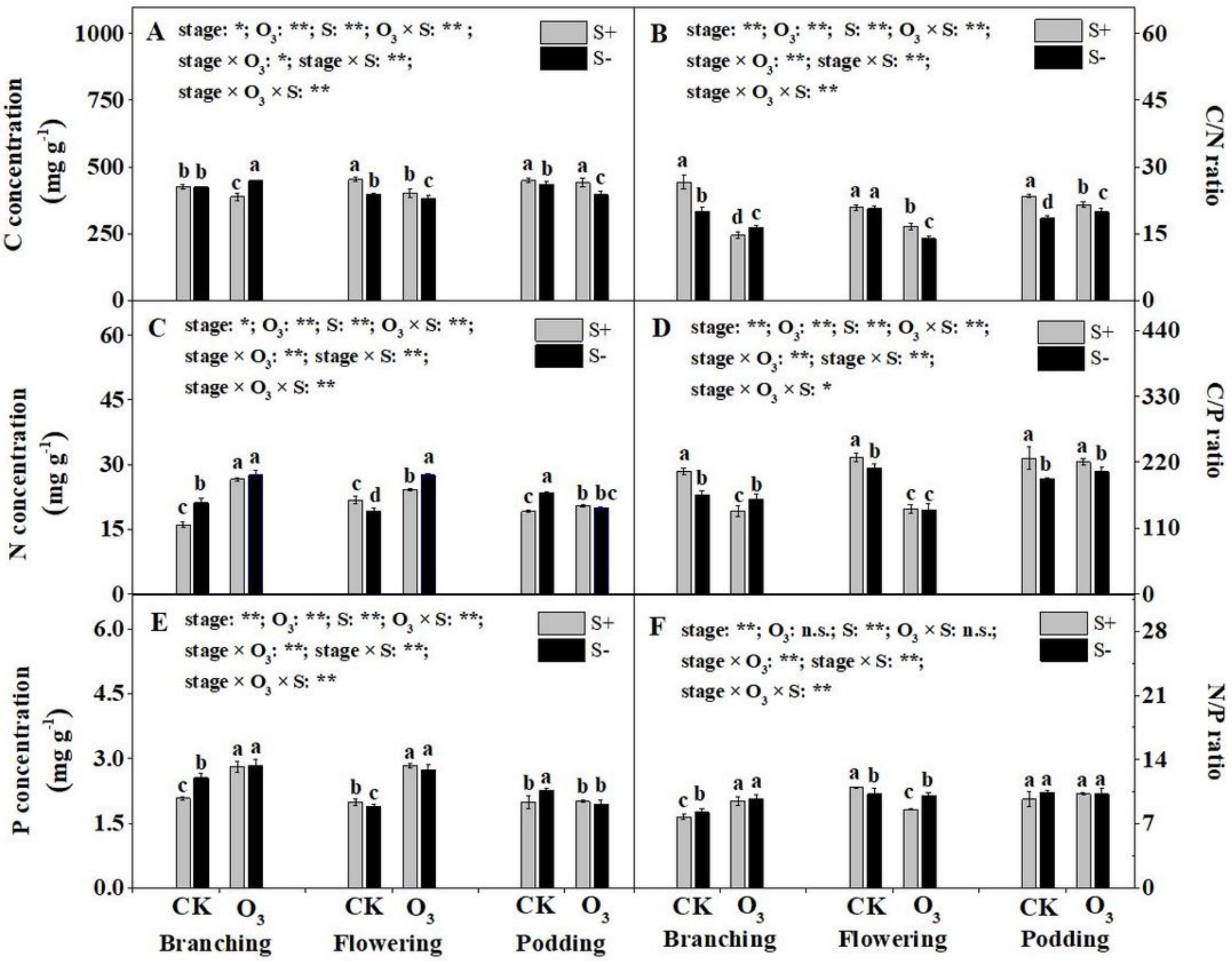

Figure 1

C, N, P concentrations and stoichiometric ratios of soybean leaves in response to non-filtered air treatment (CK), O3 stress, straw return (S+) and no straw return (S-) at growth stages of branching, flowering and podding. Data are shown as mean ± standard error (n=3). For each parameter, results of general linear model (GLM) are reported, with asterisks showing the significant effect of O3 stress (O3) and growth stage (stage) and their interaction: ** P < 0.01, * P < 0.05, n.s. not significant. Different letters above the bars represent significant differences from Tukey's multiple comparisons among the treatments (P<0.05).

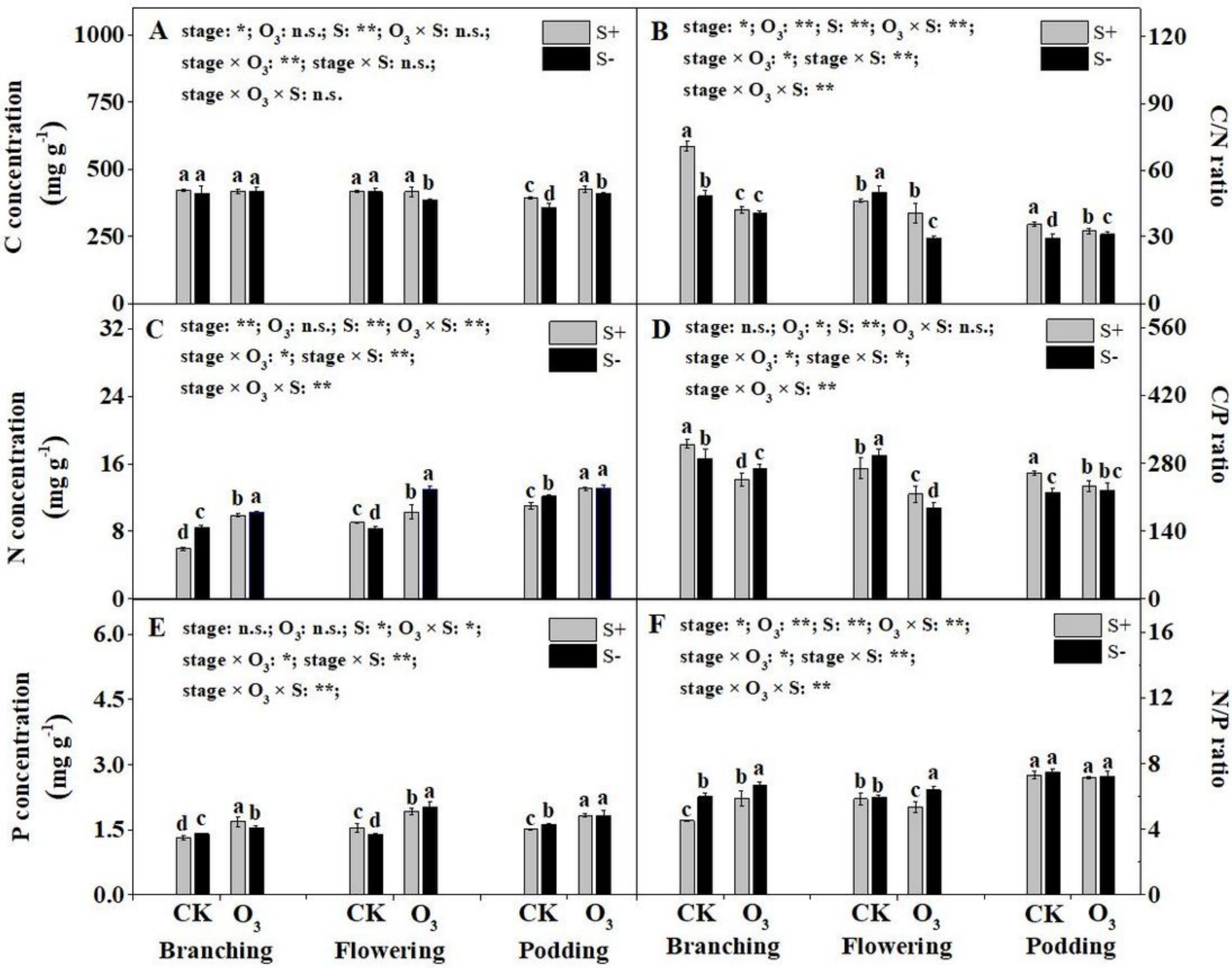

Figure 2

C, N, P concentrations and stoichiometric ratios of soybean shoots in response to non-filtered air treatment (CK), O3 stress, straw return (S+) and no straw return (S-) at growth stages of branching, flowering and podding. Data are shown as mean ± standard error (n=3). For each parameter, results of general linear model (GLM) are reported, with asterisks showing the significant effect of O3 stress (O3) and growth stage (stage) and their interaction: ** P < 0.01, * P < 0.05, n.s. not significant. Different letters above the bars represent significant differences from Tukey's multiple comparisons among the treatments (P<0.05).

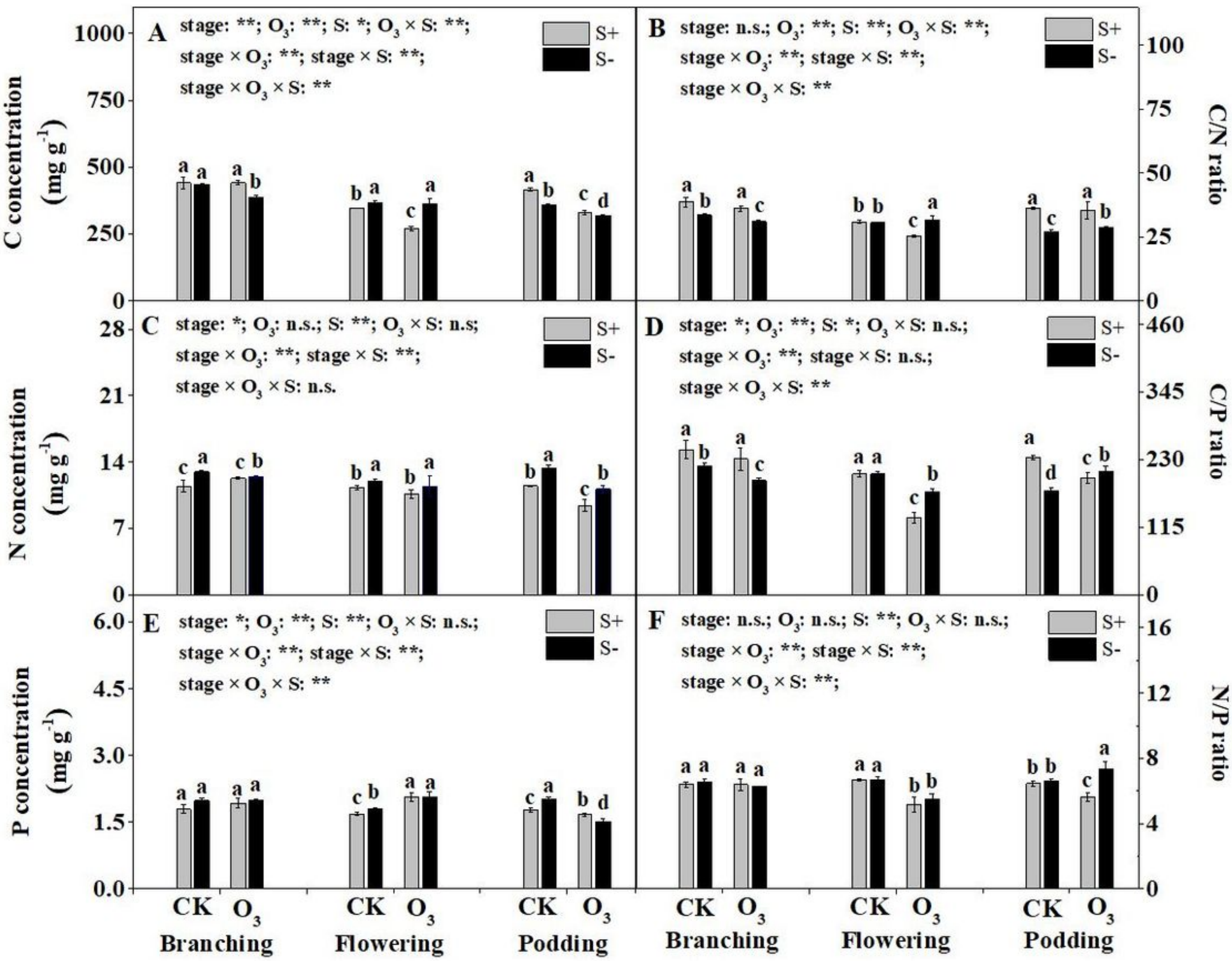

Figure 3

C, N, P concentrations and stoichiometric ratios of soybean roots in response to non-filtered air treatment (CK), O3 stress, straw return (S+) and no straw return (S-) at growth stages of branching, flowering and podding. Data are shown as mean ± standard error (n=3). For each parameter, results of general linear model (GLM) are reported, with asterisks showing the significant effect of O3 stress (O3) and growth stage (stage) and their interaction: ** P < 0.01, * P < 0.05, n.s. not significant. Different letters above the bars represent significant differences from Tukey's multiple comparisons among the treatments (P<0.05).

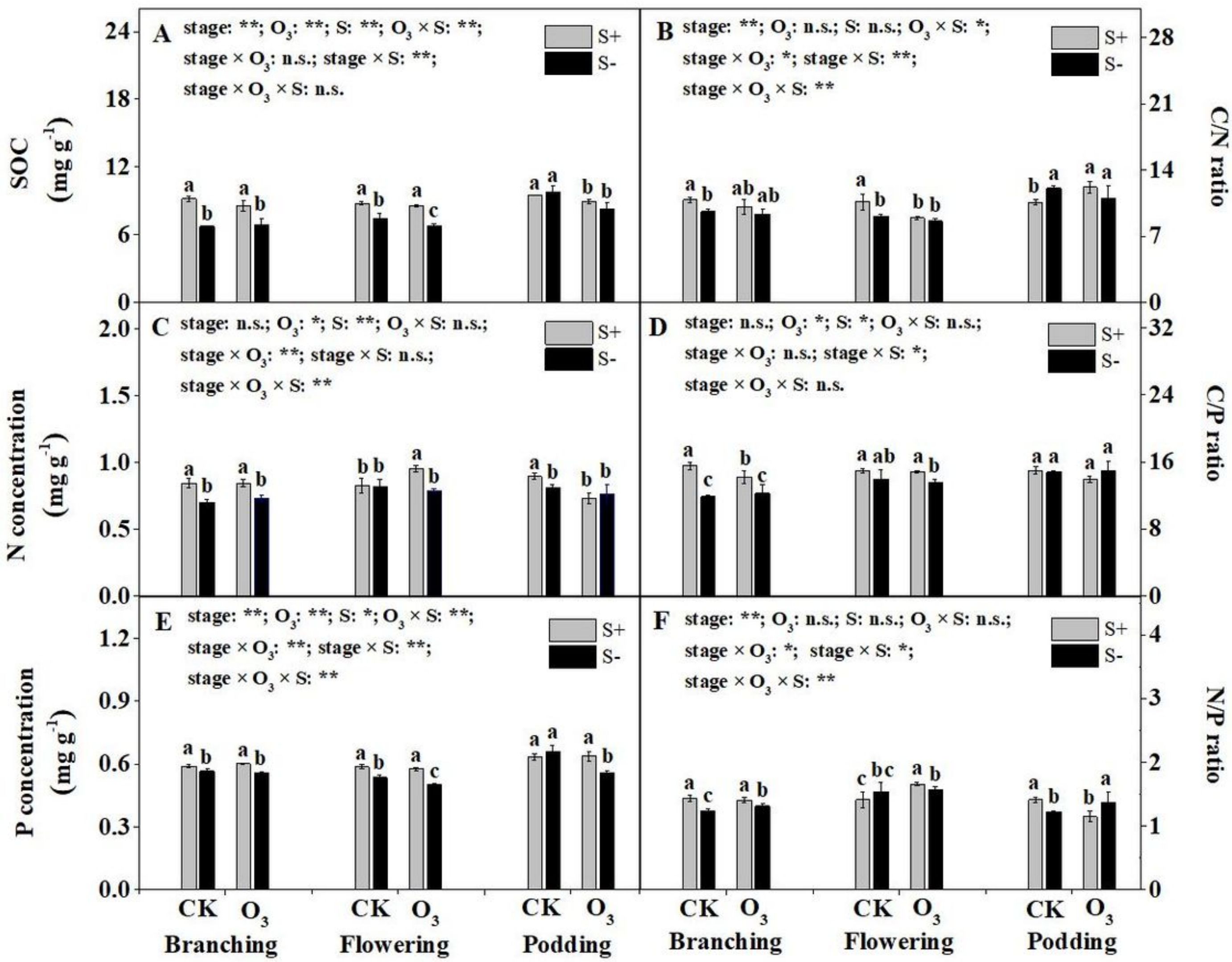

Figure 4

C, N, P concentrations and stoichiometric ratios of soils cropped to soybean in response to non-filtered air treatment (CK), O3 stress, straw return (S+) and no straw return (S-) at growth stages of branching, flowering and podding. Data are shown as mean ± standard error (n=3). For each parameter, results of general linear model (GLM) are reported, with asterisks showing the significant effect of O3 stress (O3) and growth stage (stage) and their interaction: ** P < 0.01, * P < 0.05, n.s. not significant. Different letters above the bars represent significant differences from Tukey’s multiple comparisons among the treatments (P<0.05). SOC, soil organic C.

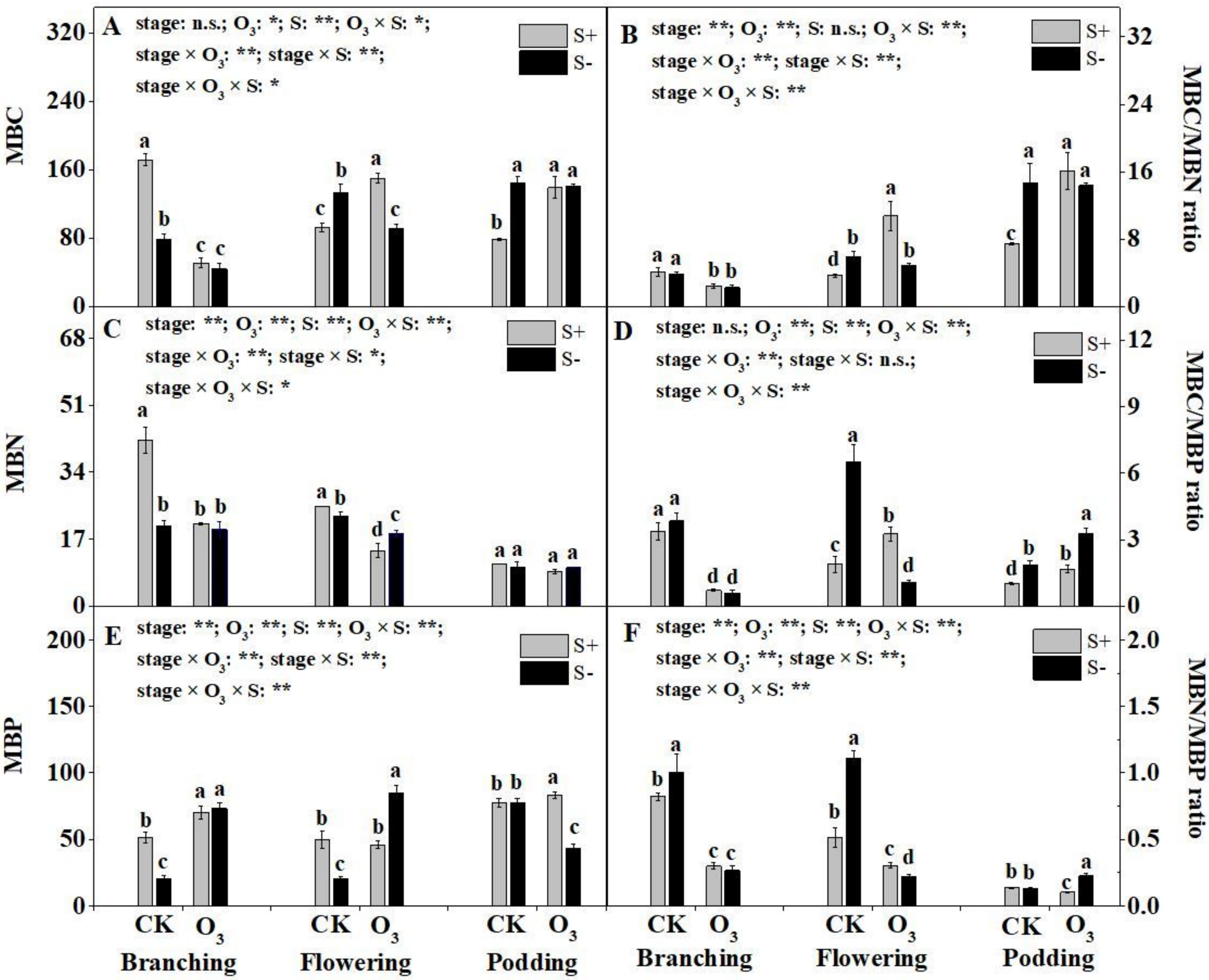

Figure 5

C, N, P concentrations and stoichiometric ratios of soil microbial biomass in response to non-filtered air treatment (CK), O3 stress, straw return (S+) and no straw return (S-) at growth stages of branching, flowering and podding. Data are shown as mean ± standard error (n=3). For each parameter, results of general linear model (GLM) are reported, with asterisks showing the significant effect of O3 stress (O3) and growth stage (stage) and their interaction: ** P < 0.01, * P < 0.05, n.s. not significant. Different letters above the bars represent significant differences from Tukey's multiple comparisons among the treatments (P<0.05). MBC, microbial biomass C; MBN, microbial biomass N; MBP, microbial biomass P.

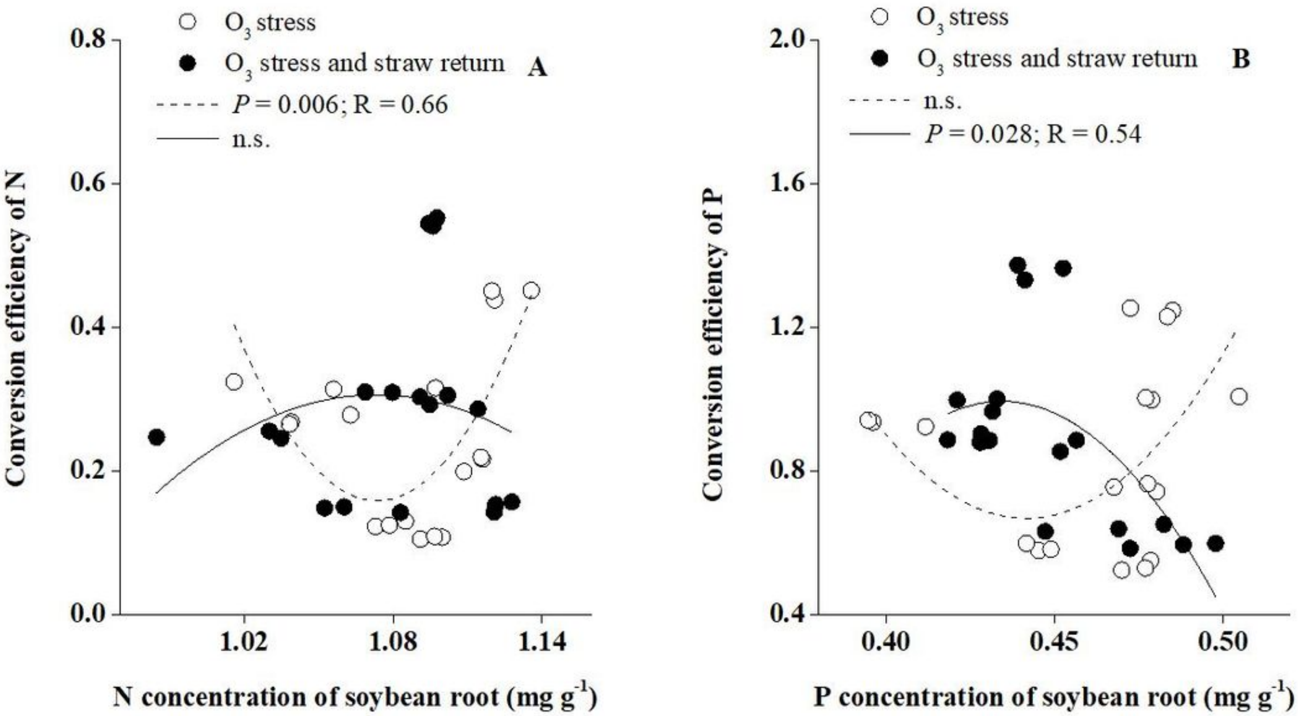

Figure 6

Relationship between conversion efficiency of N and root N concentration (A) and between conversion efficiency of P and root P concentration (B). regression lines in panel: solid black = the combined effects of O3 stress and straw return; dotted black = the O3 stress.

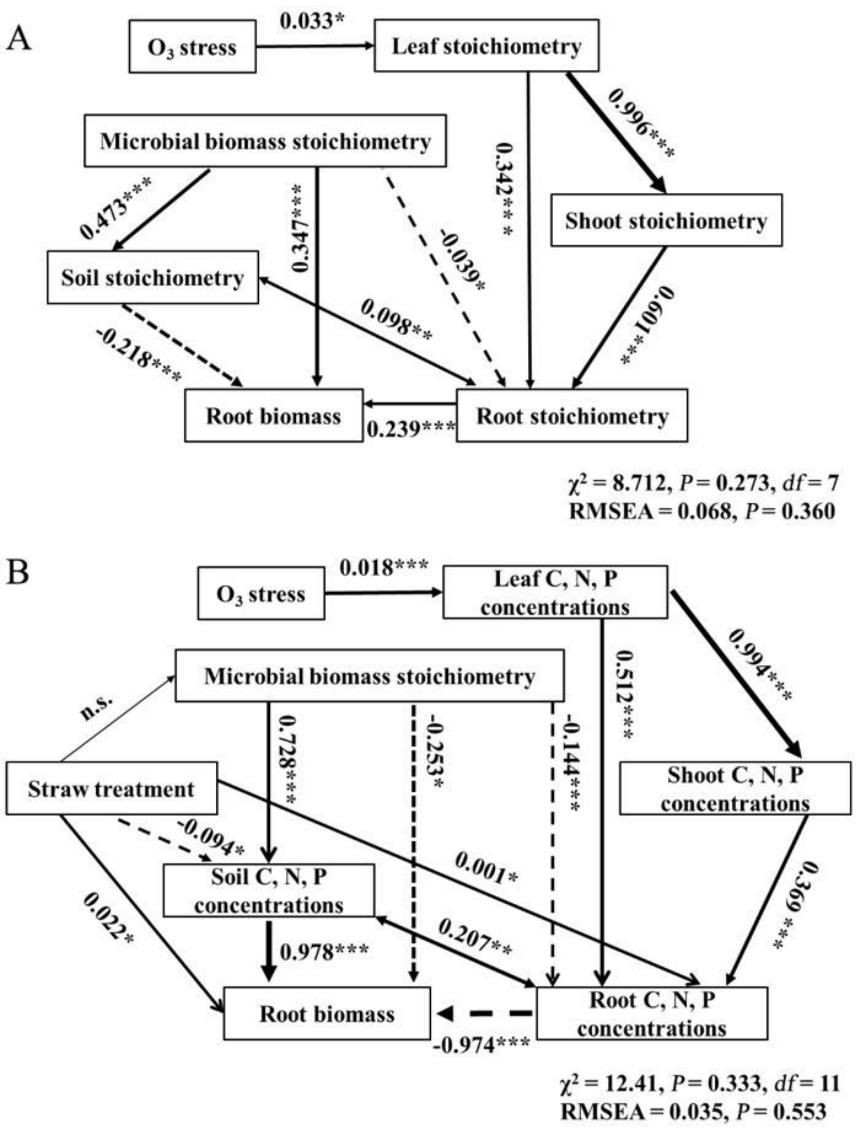

Figure 7

The path analyses of the responses of root biomass to the O3 stress (A) and to the combined effects of O3 stress and straw return (B). Measured variables are represented by boxes. Causal relationships are represented by one-headed arrows. Numbers adjacent to arrows are standardized path coefficients, analogous to relative regression weights, indicating the effect size of the relationship. Continuous and dashed black arrows indicate positive and negative relationship, respectively. * $p < 0.05$, ** $p < 0.01$, n.s. not significant.

Supplementary Files

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