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The tolerance of growth and clonal propagation of *Phragmites australis* (common reeds) subjected to lead contamination under elevated CO_2 condition

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ABSTRACT

Phragmites australis is a rhizomatous perennial plant with extensive distribution and tolerance. To explore plant growth and clonal propagative tolerance to lead contamination under elevated CO_2, they were exposed to combinations of five Pb levels (0, 300, 500, 1500, 3000 mg kg^{-1}) and two CO_2 concentrations (380 ± 20 and 760 ± 20 μ mol mol^{-1}) in phytotron. Biomass, photosynthetic parameters and rhizome growth were significantly inhibited, while number of axillary shoot buds and daughter apical rhizome shoots were increased by Pb additions. ~80% of daughter shoots was from daughter axillary shoots, representing a phalanx growth pattern. Under elevated CO_2, photosynthetic parameters (excluding stomatal conductance and transpiration rate), growth of clonal modules were increased, facilitating plant biomass accumulation, phalanx growth and spreading strategy. The results suggest that elevated CO_2 might improve growth and clonal propagative resistance to Pb contamination through increasing photosynthetic, phalanx growth and population expansion of *Phrgagmites australis*.

Keywords: *Phragmites australis*; Elevated CO_2; Pb contamination; Biomass; Photosynthesis; Clonal propagation

Introduction

Due to deforestation and sustained use of fossil fuels, the concentration of atmospheric CO_2 has increased from pre-industrial levels of 280 ppm to approximately 380 ppm, and is predicted to be possibly doubled by the end of the 21st century.[1,2] Elevated CO_2 generally causes reduction in stomatal conductance (g_s) and transpiration rate (E), but increases water use efficiency (WUE) and net photosynthetic rate (P_n).[3,4,5] However, it has been ambiguous about the results regarding effects of elevated CO_2 on plant biomass allocation. Numerous researches have demonstrated that elevated CO_2 leads to increased photosynthetic production allocation to roots by increase in branched root systems, which may stimulate water and nutrient absorption by plant.[6,7,8] Inversely, some scholars have suggested that elevated CO_2 promotes biomass accumulation in stems and leaves instead of root systems.[9]

In recent decades, heavy metals contaminations have been a serious problem around the world. The levels of heavy metal contaminations in soils range from trace to as high as 100,000 mg kg^{-1}.[10] Among the heavy metal-contaminated soils, lead (Pb) is one of the most toxic ones and its phytotoxicity may cause a wide range of adverse effects on the plant growth and physiology. Photosynthesis is considered as one of the most sensitive metabolic processes to Pb toxicity. Substantial literatures have shown that the reasons for inhibitory effects of Pb on photosynthesis include stamotal closure, damaged chloroplast ultrastructural organization, restrained synthesis of chlorophyll, obstructed electron transport, and inhibited activities of Calvin cycle enzymes.[11,12]

Such changes of key processes may eventually lead to an inhibition in plant growth and biomass production.[13] It is noteworthy that, under global elevated CO_2 scenario, the effects of heavy metals on the plant physiology, growth and development may alter. Elevated CO_2 has been shown to alleviate the adverse damage induced by metals through increasing antioxidant enzyme activity and photosynthesis, which increases biomass accumulation.[14,15,16] Biomass accumulation exhibits plant growth, but biomass allocation is an important strategy that is used to maintain and extend plant population and to fight against stress or bad environment.[17,18,19] Therefore, it is necessary to focus on biomass allocation of plants subjected to heavy metals under atmosphere elevated CO_2 scenario, and on what the causes of that biomass changes could be.

The above-ground shoots origin from below-ground bud bank for perennial plants that have predominantly clonal reproduction. The best strategy to resist various disturbances is that they can produce more ramets (daughter shoots) through clonal propagation or below-ground bud bank to increase plant productivity.[20,21,22] Zhang et al. (2015)[23] regarded that the *Phragmites australis* in well-watered environment had stronger clonal propagative ability, exhibiting in more number of buds and daughter shoots. Several scholars have studied effects of elevated CO_2 on the plant clonal growth, and they found that elevated CO_2 improved vegetative propagative ability through enhancing rhizome elongation and growth of tiller ramets.[24,25] However, lack research attention to data that have focused on the combined impacts of elevated CO_2 and heavy metals on below-ground buds or clonal propagation of perennial plants.

In addition, the heavy metal accumulation in plant organs might be altered by atmosphere elevated CO_2. Several literatures have demonstrated that elevated CO_2 has stimulatory effects on heavy metal accumulation.[26,27] Some recent researches however, have documented that elevated CO_2 has no effect or reduce heavy metal uptake by plants.[14,26] It is so far not agreement on the effects of elevated CO_2 on heavy metal accumulation of plants.

Phragmites australis is a typical rhizomatous perennial plant with high biomass production and phytoremediation ability of heavy metal.[28,29] Its population expansion mainly depends on clonal propagation (e.g. vegetation tillering and rhizome spread), because seeding establishment occurs rarely in the field.[30] It remains unclear and has not been reported so far that how P. *australis* grown in Pb contaminated soil will respond to elevated CO_2 in term of biomass allocation, photosynthesis and clonal reproduction. In addition, for soils with pH > 6.5, the toxicity threshold of soil Pb is 500 mg kg^{-1}, according to the environmental quality standard for soil, GB15618-1995, issued by the State Environmental Protection Administration of China. Typically, each plant species' tolerance of Pb can be best assessed with toxicity assays across a range of concentrations.[31] Thus, we designed pot experiments to imitate Pb contamination with a gradient of five Pb concentrations (0, 500, 1000, 1500 and 3000 mg kg^{-1}), and two CO_2 concentrations (380 ± 20 and 760 ± 20 μ mol mol^{-1}) in artificial climate chambers. The objective of present study is to investigate the tolerance of growth and clonal propagation of P. *australis* exposed to Pb contamination under elevated CO_2, measuring the biomass, photosynthesis and clonal propagation parameters as well as

Pb accumulation in organs. We hypothesized that (i) elevated CO_2 might promote photosynthesis and change biomass allocation on organs of P. *australis* subjected to Pb contamination; (ii) to adapt to Pb contaminated environment, some clonal reproduction strategy might be adopted by P. *australis* under elevated CO_2 condition (iii) elevated CO_2 might change the Pb translocation or allocation to organs.

Materials and methods

Preparation of Pb contaminated soils

Soil for use in the pot experiment was collected from the surface layer (0-20 cm) in grassland near the Northeast Normal University field experiment station, located in Changling County, Jilin Province, China (123° 44′E, 44° 44′N, 167 m a.s.l.). Its total nitrogen (N) was 6.9 %, organic carbon was 0.4 %, pH was 8.6, electric conductivity was 91μs cm^{-1}, field capacity was 200 g kg^{-1}, and Pb concentration was 5.9 mg kg^{-1}. The fresh, collected soil was mixed homogeneously and allowed to air dry. It was then passed through a 1-mm sieve, after which it was divided into 3-kg subsamples. Specified concentrations of Pb $(NO_3)_2$ solution (520 ml) were added and thoroughly mixed into the soil subsamples to obtain five levels of Pb contamination: 0, 300, 500, 1500, 3000 mg Pb kg^{-1}. The relative water content of the spiked soils reached precisely 70 % of field capacity. The contaminated soil subsamples were transferred into plastic pots (16 cm diameter × 14 cm height). All the soil-filled pots were placed in a room with ventilation in darkness for 45 days (from June 1 to July 15, 2013). At the time, a small amount of spiked soils was sampled randomly and analyzed for Pb concentrations. The concentrations of Pb in the artificially contaminated soils was 5.9

± 0.2, 304 ± 4.38, 508 ± 7.89, 1513 ± 37.28, 3020 ± 120.41 mg kg^{-1}, respectively, which was very close to the five targeted levels of added Pb.

Plant cultures and treatments

Seeds of *P. australis* were collected from mature wild plants in a wetland, where is located west of Changchun city, Jilin province, China (125° 1′ E, 43° 56′ N, 188 m a.s.l.). Seeds were sown in plastic tanks (80 × 50 × 30 cm) that contained 20 cm of humus soil, which was watered daily to keep it moist. The tanks were housed in a greenhouse at 14-24 °C, on the campus of Northeast Normal University, Jilin City, China (43° 51'N, 125° 19'E, 236 m a.s.l.). After 50 days had elapsed since sowing (from May 26 to July 15, 2013), uniform plants ~8 cm high, with 3 or 4 leaves, were transplanted into the pots, and then placed in the artificial climate chamber (LT/ACR-2002 Phytotron System, E-Sheng Tech., Beijing, China). Knowing that some weak seedlings were unlikely to survive being transplanted, we initially placed twenty seedlings into each pot, and allowed them to grow for 5 days under weak light. Then, the weakest plants were removed to keep 10 strong seedlings in each pot.

These pots with 10 strong seedlings were placed randomly and equally in two phytotrons. There were five levels of Pb treatments for a total of 30 pots (6 replicated pots per treatment) per phytotron. One phytotron was maintained ambient CO_2 at 380 ± 20 μ mol mol^{-1}. The other phytotron was assigned to a doubled level at 760 ± 20 μ mol mol^{-1}. The CO_2 was supplied from a steel can and delivered through 0.64 cm tubing, and the concentrations were monitored every 5 s and adjusted every 10 s for the whole day. The light and temperature regimes in the phytotrons were set according

155 to the diurnal/nocturnal periods and temperature changes of June to September in
156 Northeast China.[32] The high-stress sodium lamps (Philips) with photosynthetically
157 active radiation provided light at a rate of 500 m mol m^{-2} s^{-1} from 5:30-19:30 for 14 h
158 per day, and they were shut in other time. The relative humidity was maintained at
159 40-60 % in the phytotrons. The temperature regimes were 22 °C from 5:30-8:30, 25 °
160 C from 8:30-11:30, 28 ° C from 11:30-14:30, 25 ° C from 14:30-17:30, 22 ° C
161 17:30-19:30 and 18 ° C from 19:30-5:30. Air temperature in each chamber was
162 monitored and adjusted every 10 s for 24 h a day, and maintained within ±1 °C of set
163 points. The pots were watered to 3.42 ~ 3.48 kg using an electronic scale (KaiFeng
164 group co. Ltd, ACS-30, China) at 5:00 pm each day, to maintain the soil water content
165 at 70-80 % of field capacity. In order to ensure that each plant experienced similar
166 light conditions, the pot positions were randomly changed every 2 d in the same
167 phytotron during the treatment period. Furthermore, since there were no chamber
168 replicates in this study, we switched the pots between the two chambers every 2 weeks,
169 changing the environmental settings so that all pots were undergo as similarly as
170 possible during the experiment. The total time of CO_2 enrichment was 60 days (from
171 July 20 to September 19, 2013).

172 **Determination of photosynthetic parameters**

173 Before sampling, photosynthetic parameters were measured with an LI-6400 gas
174 exchange system (LI-6400XT, Li-Cor, Inc., Lincoln, NE, USA) on the youngest
175 available fully expanded leaves (3 leaves per pot, 6 pots per treatment). The net
176 photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), stomatal conductance

(g_s) and transpiration rate (E) were determined under ambient CO_2 (380 μ mol mol^{-1}) and elevated CO_2 (760 μ mol mol^{-1}) under a light intensities of 500 μ mol m^{-2} s^{-1} to equal the light of the phytotron. Leaves were allowed to acclimate for a few minutes until the P_n stabilized and the coefficient of variation was below 0.5. The water use efficiency (*WUE*) was calculated from the ratios of P_n to E.

Classification of below-ground buds and above-ground daughter shoots

In this study, plants developed from seeds were defined as "parent shoots", and those that resprouted from buds of rhizomes or basal nodes of parent shoots were considered as "daughter shoots".

As proposed by Zhang et al. (2009),[33] the three categories of below-ground buds were (i) axillary shoot buds, (ii) axillary rhizome buds, and (iii) apical rhizome buds; The three categories of daughter shoots were (i) daughter axillary shoots, (ii) daughter axillary rhizome shoots, and (iii) daughter apical rhizome shoots. The axillary shoot bud at a basal node of a shoot can grow upwards to form a daughter axillary shoot, or horizontally into a rhizome. The axillary rhizome buds attached to rhizome nodes grow upwards to form daughter axillary rhizome shoots, or horizontally into rhizomes. The apical rhizome buds originating from the end of a rhizome grow upwards to form a daughter apical rhizome shoot, or continue to horizontally extend the rhizome.

Measurement of clonal parameters and biomasses

Each plant per pot was separated from the contaminated soil along with its root systems. We then immediately counted the number of each type of bud > 1mm in length, the number of each type of daughter shoot and the number of rhizomes, and

199 measured the total length of rhizomes. The rhizome length and number of buds,
200 daughter shoots and rhizomes were expressed per parent shoot. Finally, each plant
201 was washed gently with tap water, three times with deionized water, and then divided
202 into leaves, stems and root systems (including root and rhizome) before being
203 oven-dried at 75 ^{o}C to a constant weight. The dry weights were measured, and the
204 dried tissues then were ground into fine powder by ball mill (Retsch, MM400,
205 Germany) for Pb concentration measurement.

206 **Measurement of Pb concentration**

207 The homogeneous soil samples and plant powder (0.15 g) were digested in a
208 microwave oven (ANALYX, CEM Mars5, USA) with solution of 5:1 HNO_3:HF and
209 HNO_3:$HCLO_4$ (v/v) for 120 minutes respectively until clear and transparent liquid.
210 Then, they were continued to digest in electronic heating digestion apparatus
211 (LabTech, DigiBlock ED36, USA) until approximate 0.5 cm height of solutions under
212 high temperature 180 ^{o}C. The cooled solutions were added up to 50 ml of total
213 volume respectively. Concentrations of Pb were determined using a Graphite Furnace
214 Atomic Absorption Spectrometer (Varian, SpectrAA Z220, USA). The detection limits
215 for Pb are from 10 - 100 ppm, and some extracts were further diluted for
216 determination of Pb concentrations within the detection limits.

217 **Statistical analysis**

218 We used two-ways ANOVA to evaluate the response of plants to Pb, CO_2
219 concentrations and their interaction. Data were analyzed as a split-plot design with
220 CO_2 treatments being the main plot and Pb concentrations being the subplot. As the

two-ways ANOVA showed significant effects of Pb and CO_2 treatments, a separate ANOVA was used to evaluate the effects of Pb under the same CO_2 level, with Pb as a fixed factor. An independent sample T-test was used to evaluate the effect of CO_2 on these parameters under the same Pb level, using CO_2 levels as a grouping variable.

Before the ANOVA was conducted, data were checked for their normality and homogeneity of variance, and were squared root-transformed as necessary to meet those assumptions. Multiple comparisons of means were performed using LSD tests at α=0.05 when ANOVA results were significant. All statistical analyses were performed with the SPSS v.13.0 statistical package (SPSS Inc., Chicago, USA), and figures were plotted with SIGMAPLOT 11.0 (Systat Software, Inc., San Jose, CA, USA).

Results

Biomasses in organs

Leaf, below-ground and total biomasses were affected significantly by CO_2 and Pb concentrations, and their interaction affected total biomass significantly (Table 1). Increasing soil Pb concentrations caused a continuous reduction in the stem, leaf and below-ground biomasses and total biomass (Fig. 1). Elevated CO_2 increased biomasses of different organs resulting in an increase in total biomass compared to ambient CO_2 control (Fig. 1).

Photosynthetic parameters

CO_2 and Pb concentrations had significant effects on photosynthetic parameters (P_n, g_s, C_i, E) and WUE, and Pb × CO_2 affected C_i significantly (Table 1). At each CO_2 level, P_n, g_s, C_i, E, WUE were significantly reduced by Pb treatments (Fig. 2). When

compared with the ambient CO_2 control, elevated CO_2 increased P_n, C_i and *WUE* significantly, but decreased g_s, and *E* significantly at the same Pb level. The increased or reduced percentages tended to drop with increasing Pb concentrations in soils (Fig. 2).

Clonal parameters

CO_2 and Pb concentrations showed remarkable effects on number of axillary shoot buds, axillary rhizome buds and total number of buds as well as number and length of rhizomes (Table 1). We observed different responses of various types of buds and daughter shoots to Pb additions. Number of axillary shoot buds was increased firstly, then decreased along with increasing soil Pb contamination, and was still greater at highest Pb level than control. The similar changes occurred in number of daughter axillary shoots, causing that its proportions (in total number of daughter shoots) were increased by Pb additions. Increasing soil Pb concentrations caused a continuous increase in daughter apical rhizome shoots, but decreased the number of axillary and apical rhizome buds and total number of buds significantly (Table 2). There was no daughter axillary rhizome shoots that originated from axillary rhizome buds, but daughter axillary shoots accounted for ~80% of total number of daughter shoots at each Pb level. Elevated CO_2 triggered an increase in number of each type of buds and daughter shoots, especially for axillary shoot buds of plants grown in higher Pb concentrations in soils and for axillary rhizome buds of plants grown in lower Pb treatments, resulting in a significant increase in total number of buds (Table 2).

At each CO_2 level, the number and length of rhizomes were significantly decreased

with increasing soil Pb concentrations. At lower levels of Pb contaminations, elevated CO_2 increased number of rhizomes significantly (Fig. 3*a*). Rhizome length was increased significantly by elevated CO_2 at each Pb (< 3000 mg kg^{-1}) level, and the increases were promoted with increasing Pb concentration (Fig. 3*b*).

Pb concentrations in organs

Under either ambient or elevated CO_2, Pb concentrations in stems, leaves and root systems were significantly increased due to Pb additions in soils, particularly at Pb levels of 1500 and 3000 mg kg^{-1}. The Pb concentration in root systems was greater than that in stems and leaves at the same level of Pb treatments (Fig. 4). Elevated CO_2 triggered a vast amount of Pb accumulation in root systems, but its transportation to above-ground organs was inhibited (Fig. 4).

Discussion

A large body of literatures has shown that elevated CO_2 increases C_i, *WUE* and P_n significantly,[3,4,5] while the presence of Pb has the reverse effects.[11,12] These were similar to our results (Fig. 2*a*, *c*, *e*). The increasing P_n means that elevated CO_2 enhances the capacity of carbon assimilation,[34] which might cause a significant increasing biomass accumulation of leaves where are main photosynthetic organ at lower levels of Pb concentrations (Fig. 1*b*). Simultaneously, the increasing P_n due to elevated CO_2 also promoted photosynthetic production allocation to below-ground parts (Fig. 1*c*), which might enhance resources uptake and storage in plants. It is because that plants mainly obtain water and nutrient through roots, and rhizomes are considered to be main storage organs.[5,24,35] We also found that the increase in

photosynthesis due to relative higher C_i could offset the decreasing carbon assimilation caused by reduced g_s (Fig. 2*b*,*d*).[36] In addition, the decreased percentages of g_s were smaller at higher Pb levels than at lower Pb levels under elevated CO_2 condition (Fig. 2*b*). This might be because both elevated CO_2 and Pb concentrations caused a reduction in g_s to limit E, and elevated CO_2 had a minimum effect on g_s at higher Pb levels in order to maintain photosynthesis. We can conclude that elevated CO_2 could improve plant growth resistance to Pb contaminated environments through biomass allocation to photosynthetic and below-ground resource absorbing organs (i.e. leaves and root systems) due to increasing photosynthesis.

For perennial plants, below-ground bud bank may be a main source for vegetative propagation maintaining population maintenance, because germination and population establishment from seeds happen rarely.[20,37,38] Meanwhile, the ability of bud emergence is also a main factor that might influence above-ground population density and productivity.[39] The research of Zhang et al. (2015)[23] has indicated that higher clonal propagative ability plays a critical role in maintaining population stability and expansion of P. *australis* exposed to Pb stress under well-watered condition. Only a few of scholars has reported effects of elevated CO_2 on plant clonal growth. They regarded that elevated CO_2 might improve clonal propagative ability through increasing rhizome length and daughter shoot growth.[24,25] Clonal modules (e.g. below-ground bud bank) and clonal propagation of perennial plants subjected to heavy metal stress might be affected partly by global elevated CO_2. However, there is no report regarding the combined effects of elevated CO_2 and heavy metal

309 contamination on below-ground bud bank or clonal reproduction of perennial plants.
310 Previous and our present researches observed that daughter axillary shoots were the
311 main source of above-ground population density.[33] We also observed that the
312 proportions of daughter axillary shoots (in total number of daughter shoots) were
313 increased by Pb additions at the same CO_2 level (Table 2), which exhibited a phalanx
314 growth pattern.[40] The daughter axillary shoots (tiller-based ramets) contribute more to
315 population maintenance for rhizomatous clonal plants.[41,42] According to the
316 cost-benefit hypothesis,[43] as axillary shoot buds are attached to basal nodes of stem,
317 their emergence into daughter shoots may incur lower cost.[44] In contrast, not only do
318 plants supply energy to rhizome elongation and development of rhizome buds from
319 the deep soil layer, but also their high rate of respiration should deplete much of their
320 available energy under anoxic conditions, giving rise to higher cost when rhizome
321 buds emerge from the soil surface.[44,45] Therefore, a propagative strategy developed by
322 *P. australis*, which has dominated clonal propagation, was that they produced more
323 axillary shoot buds and daughter axillary shoots (i.e. phalanx growth form) which
324 incurs lowest coast, in order to maintain population stability under Pb contamination
325 condition. Meanwhile, the increased number of axillary shoot buds due to Pb
326 contamination would contribute more to phalanx growth form during the fowling year
327 under elevated CO_2 condition, because each bud can potentially emerge into daughter
328 shoots.[46] We also found that the number of daughter apical rhizome shoots tended to
329 increase with increasing soil Pb contamination, particularly at highest Pb level in both
330 ambient and elevate CO_2 environment (Table 2). Meanwhile, the rhizome length was

significantly increased by elevated CO_2 at each Pb level (Fig. 3*b*). The Rhizome-based ramets (e.g. daughter apical rhizome shoots) contribute more to plant population expansion.[41,42] The spreading rhizome might provide more space and resources that could be mobilized for bud emergence and regrowth of daughter shoots.[47] So, the expanding strategy was also adopted by *P. australis* to adapt to Pb contamination, especially under elevated CO_2 condition. In addition, we also discovered that there was no daughter axillary rhizome shoots that originated from axillary rhizome buds, and similar results were also obtained in previous researches.[23,48] We can conclude that axillary rhizome buds mainly grew horizontally into rhizomes in order to continue their expansion, rather than grew upwards to form daughter shoots. This may be related to biological characteristics of *P. australis*; the growth space must first be expanded, before the number of daughter rhizome shoots can increase.[49] In short, P. *australis* had higher tolerance of clonal propagataion to cope with Pb contamination by the phalanx growth and spreading strategy, particularly in elevated CO_2 environment.

Pb accumulation in plants was enhanced with increasing soil Pb concentrations, and the majority of absorbed Pb was retained in below-ground part of *P. australis* (Fig. 4). These results were in line with findings of previous researches.[28,50,51,52] Although a large amount of Pb was accumulated in below-ground organs of *P. australis*, below-ground biomass accumulations were still increased by elevated CO_2 at each Pb level (Fig. 3*c*, 4*c*). This indicated that below-ground organs had stronger tolerance under elevated CO_2 condition. In addition, elevated CO_2 inhibited Pb translocation to

above-ground stems and leaves (Fig. 4*a*,*b*). The above-ground shoots are considered as the important parts where plants conduct photosynthesis and other metabolisms. Therefore, the Pb allocation form might protect photosynthetic tissues and promote photosynthesis, improving biomass accumulation under elevated CO_2 condition. The Pb allocation strategy can be considered as an adaptive mechanism of plants responded to Pb contamination under elevated CO_2.

Previous work has shown that increasing heavy metal uptake in roots under elevated CO_2 condition is correlated to high metals bioavailability. The high bioavailability is attributed to the decreasing soil pH caused by greater root exudation of carbonic acid, and to the increasing dissolved organic carbon (*DOC*) concentration released from plant roots due to elevated CO_2;[16,27,53] More amounts of heavy metals were released from sediments and soil both through *DOC*-metal complexation reaction and through reducing absorption of heavy metals into soil organic matter as well as clay mineral particles due to reduced pH.[54,55] These two processes account for increasing metals bioavailability leading to more heavy metals uptake by root systerm under elevated CO_2 condition. Furthermore, Guo et al. (2011)[26] regarded that elevated CO_2 increased Cd uptake by rice and wheat, but reduced Cu accumulation. Conversely, several scholars suggested that Cd concentrations in *Lolium mutiforum* and *Lolium perenne* were reduced due to elevated CO_2.[14] Li et al. (2010)[56] discovered that various Cd accumulation patterns occurred in different varieties of rice, and they ascribed this variation to difference in exudation rates and spectrum of organic acids released by plants. We concluded that the inconsistency in heavy metal absorption or

accumulation under elevated CO_2 condition might be closely related to various factors, including plant species and growth media, types of metals and their concentrations in soils, as well as the composition and quantity of plant exudates, etc.

In short, a propagative strategy adopted by *P. australis* to resist high Pb contaminations was that they could develop phalanx growth pattern through increasing formation and output of axillary shoot buds which incurs lowest coast to maintain population stability. Elevated CO_2 might enhance clonal propagation and space expansion of P. *australis* population in Pb contaminated environment through increasing phalanx growth and spreading strategy. Meanwhile, the inhibiting Pb translocation to photosynthetic organs could be beneficial to photosynthesis, promoting biomass allocation to leaves and root system under elevated CO_2 condition.

Conclusions

The phalanx growth form would help *P. australis* resist to Pb contamination. Elevated CO_2 tended to increase photosynthesis, leading to increasing biomass of leaves and root systems. Elevated CO_2 also stimulated increase in number of various types of buds and daughter shoots, enhancing clonal propagative ability of *P. australis* grown in Pb contaminated soils. Clearly, elevated CO_2 could ameliorate Pb toxicity and improve resistance capacity of *P. australis* to Pb contamination. This study would help us better understanding how rhizomatous perennial plants respond to heavy metal contaminations and climate changes. To the best of our knowledge, our experiment represents the first study of the combined effects of elevated CO_2 and Pb stress on clonal propagation of *P. australis*.

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485 **Table 1** Analysis of variance to assess the impacts of Pb, CO_2 and their interaction on biomasses,
486 photosynthetic parameters, no. of buds and daughter shoots and rhizome growth of *Phragmites*
487 *australis*.

	CO_2		Pb		$CO_2 \times$ Pb	
	F-value	P	F-value	P	F-value	P
Biomass						
Stem	1.66	0.21^{ns}	2.69	$\mathbf{0.05}^{*}$	0.13	0.97^{ns}
Leaf	11.03	$\mathbf{0.00}^{***}$	6.37	$\mathbf{0.00}^{***}$	2.73	0.06^{ns}
Below-ground	3.73	$\mathbf{0.02}^{*}$	9.74	$\mathbf{0.00}^{***}$	0.74	0.57^{ns}
Total	18.30	$\mathbf{0.00}^{***}$	19.83	$\mathbf{0.00}^{***}$	3.14	$\mathbf{0.04}^{*}$
Photosynthetic parameters						
P_n	108.60	$\mathbf{0.00}^{***}$	87.79	$\mathbf{0.00}^{***}$	1.98	0.14^{ns}
g_s	10.39	$\mathbf{0.00}^{***}$	2.44	$\mathbf{0.04}^{*}$	0.17	0.95^{ns}
C_i	249.20	$\mathbf{0.00}^{***}$	39.66	$\mathbf{0.00}^{***}$	6.54	$\mathbf{0.00}^{***}$
E	158.81	$\mathbf{0.00}^{***}$	3.79	$\mathbf{0.02}^{*}$	0.10	0.98^{ns}
WUE	139.20	$\mathbf{0.00}^{***}$	5.19	$\mathbf{0.00}^{***}$	0.70	0.60^{ns}
No. of buds and daughter shoots						
Axillary shoot buds	24.76	$\mathbf{0.00}^{***}$	8.80	$\mathbf{0.00}^{***}$	1.64	0.20^{ns}
Axillary rhizome buds	9.02	$\mathbf{0.01}^{*}$	8.85	$\mathbf{0.00}^{***}$	1.65	0.20^{ns}
Apical rhizome buds	3.30	0.08^{ns}	27.51	$\mathbf{0.00}^{***}$	0.18	0.95^{ns}
Total	19.53	$\mathbf{0.00}^{***}$	25.94	$\mathbf{0.00}^{***}$	1.90	0.15^{ns}
Daughter axillary shoots	**0.04**	0.84^{ns}	1.26	0.32^{ns}	0.50	0.74^{ns}
Daughter apical rhizome shoots	1.33	0.26^{ns}	5.90	$\mathbf{0.00}^{***}$	0.26	0.90^{ns}
Total	0.49	0.49^{ns}	1.62	0.21^{ns}	0.34	0.85^{ns}
Rhizome						
No. of rhizomes	4.75	$\mathbf{0.04}^{*}$	15.29	$\mathbf{0.00}^{***}$	0.36	0.83^{ns}
Rhizome length	17.42	$\mathbf{0.00}^{***}$	34.44	$\mathbf{0.00}^{***}$	0.77	0.56^{ns}

488 Note: * = significance at $P \leq 0.05$; ** = significance at $P \leq 0.01$; ***= significance at $P \leq 0.001$; ^{ns} = no significance.

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Table 2 Effects of elevated CO_2 and Pb concentration on the no. of buds and daughter shoots of *Phragmites australis*.

	Pb concentration（mg kg^{-1}）				
No. of buds and daughter shoots ($no.plant^{-1}$)	Control	300	500	1500	3000
Ambient CO_2					
Axillary shoot buds	0.29±0.06b	0.36±0.07ab	0.43±0.03ab	0.51±0.07a	0.30±0.06b
Axillary rhizome buds	2.97±0.19a	2.57±0.32a	1.73±0.20b	1.42±0.19b	1.33±0.29b
Apical rhizome buds	1.87±0.24a	1.57±0.04ab	1.20±0.20b	0.72±0.14bc	0.53±0.07c
Total	5.12±0.28a	4.49±0.28a	3.37±0.13b	2.66±0.06bc	2.07±0.18c
Daughter axillary shoots	0.80±0.15a	1.17±0.18a	1.32±0.12a	1.19±0.15a	1.04±0.10a
Daughter apical rhizome shoots	0.23±0.04b	0.40±0.06ab	0.50±0.12ab	0.50±0.06ab	0.63±0.07a
Total	1.39±0.20b	1.70±0.00ab	1.80±0.12a	1.69±0.12ab	1.43±0.09ab
Elevated CO_2					
Axillary shoot buds	0.38±0.05b	0.43±0.12b	0.73±0.09a*	0.87±0.09a*	0.57±0.09b*
Axillary rhizome buds	3.60±0.55a*	3.41±0.38a*	3.20±0.55a*	1.97±0.52ab	1.40±0.29b
Apical rhizome buds	2.20±0.20a	1.81±0.23ab	1.32±0.06b	0.84±0.15bc	0.67±0.15c
Total	6.18±0.43a*	5.65±0.38a*	5.25±0.55a*	3.67±0.72b	2.30±0.31b
Daughter axillary shoots	1.10±0.22a	1.30±0.06a	1.30±0.21a	1.25±0.28a	1.17±0.20a
Daughter apical rhizome shoots	0.34±0.08b	0.51±0.11ab	0.52±0.06ab	0.54±0.06ab	0.67±0.09a
Total	1.39±0.17a	1.68±0.27a	1.82±0.06a	1.76±0.26a	1.77±0.20a

Different letters indicate significant differences ($P \leq 0.05$) between different Pb levels (within CO_2 one level), and an asterisk indicates significant difference ($P \leq 0.05$) between elevated CO_2 and ambient CO_2 control (within one Pb level).

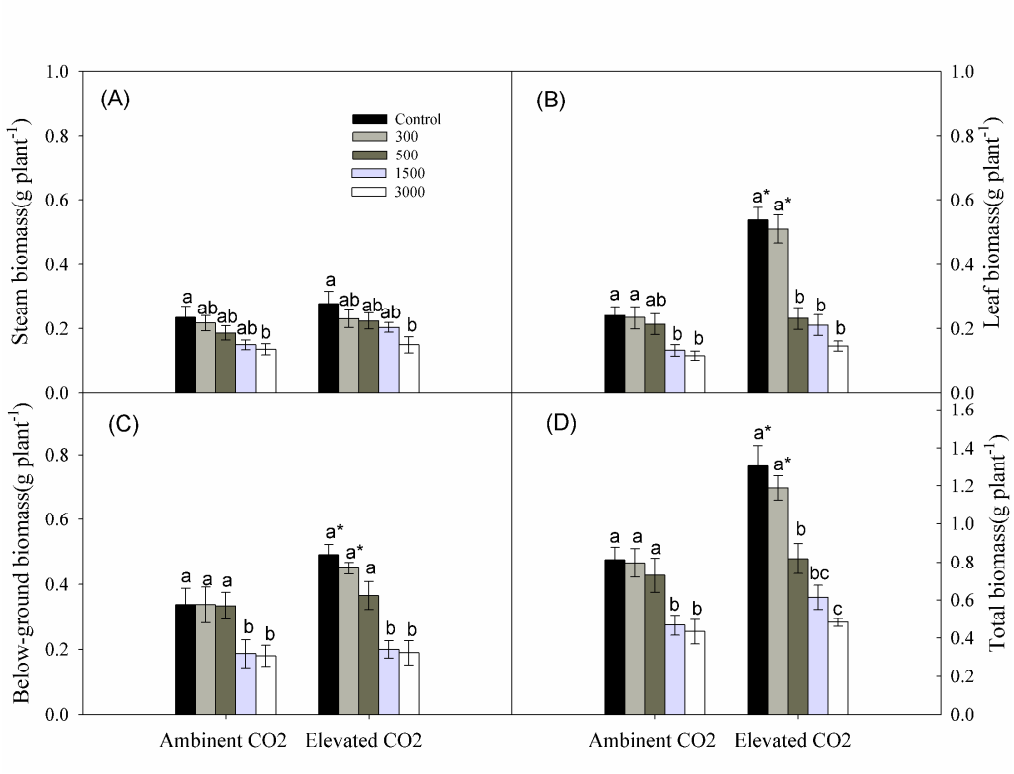


Fig. 1 Effects of elevated CO_2 and Pb contamination on biomass in organs of *Phragmites australis*. (A) indicates stem biomass; (B) indicates leaf biomass; (C) indicates below-ground biomass; (D) indicates total biomass. Different letters indicate significant differences ($P \leq 0.05$) between different Pb levels (within CO_2 one level), and an asterisk indicates significant difference ($P \leq 0.05$) between elevated CO_2 and ambient CO_2 control (within one Pb level).

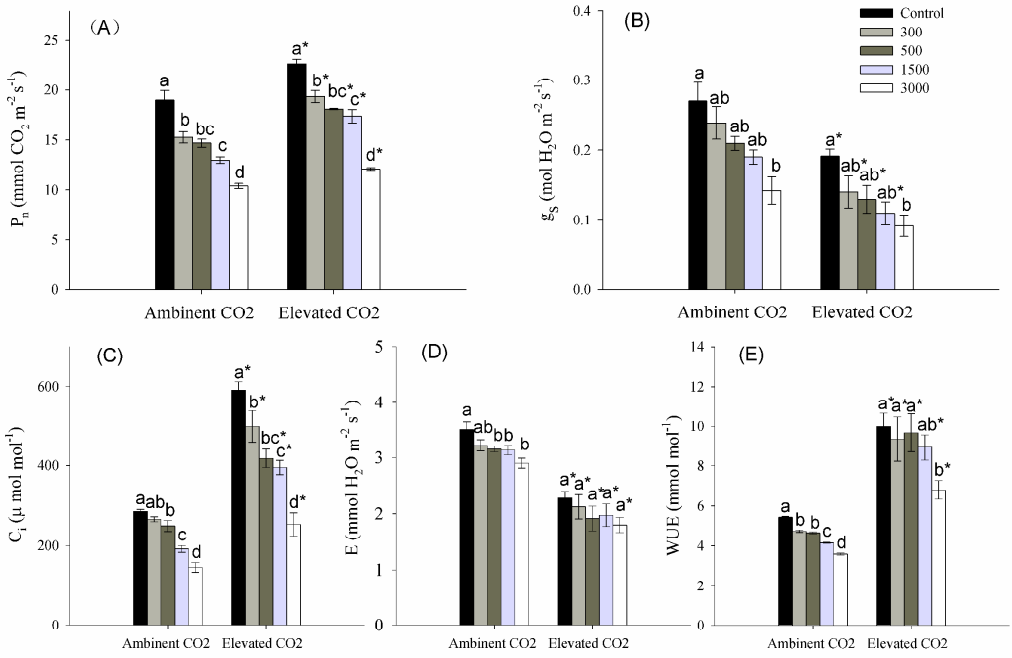


Fig. 2 Effects of elevated CO_2 and Pb contamination on photosynthetic parameters. (A) indicates net photosynthetic rate, P_n; (B) indicates stomatal conductance, g_s; (C) indicates intercellular CO_2 concentration, C_i; (D) indicates transpiration rate, E; (E) indicates water use efficency, WUE. Different letters indicate significant differences ($P \leq 0.05$) between different Pb levels (within CO_2 one level), and an asterisk indicates significant difference ($P \leq 0.05$) between elevated CO_2 and ambient CO_2 control (within one Pb level).

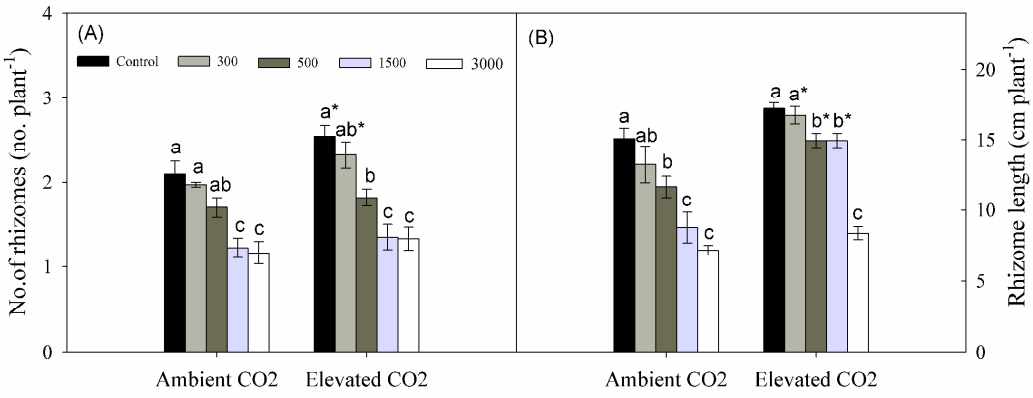
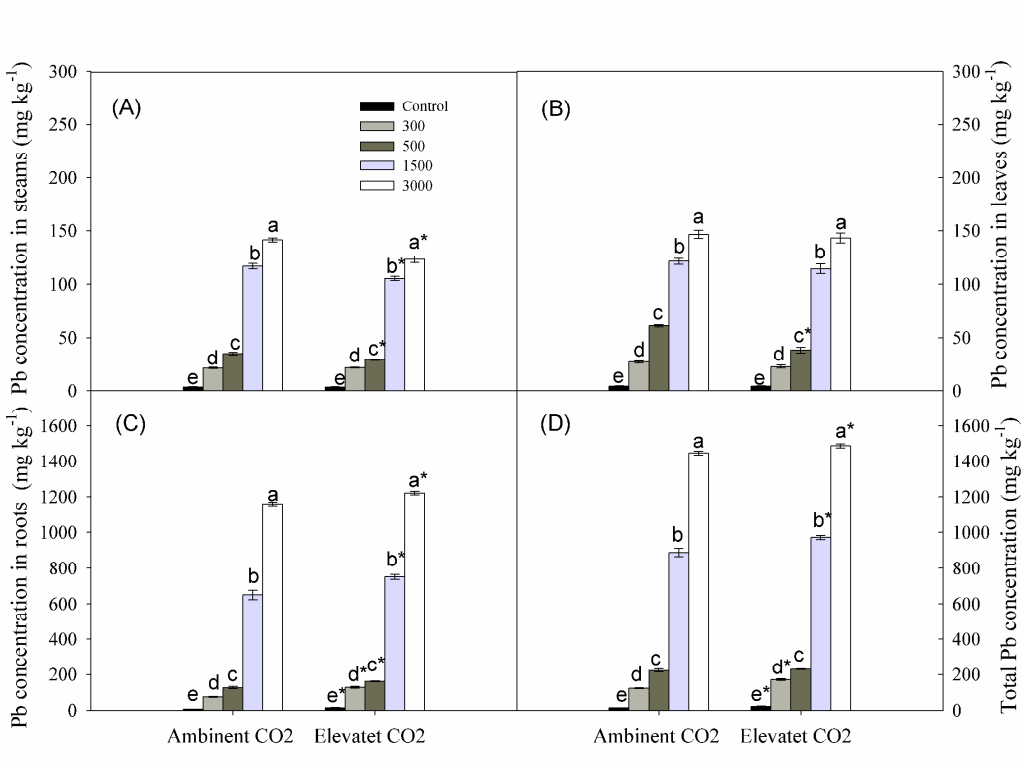


Fig. 3 Effects of elevated CO_2 and Pb contamination on rhizome growth of *Phragmites australis*. (A) indicates the number of rhizomes; (B) indicates the rhizome length. Different letters indicate significant differences ($P \leq 0.05$) between different Pb levels (within CO_2 one level), and an asterisk indicates significant difference ($P \leq 0.05$) between elevated CO_2 and ambient CO_2 control (within one Pb level).



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563 **Fig. 4** Pb concentrations in organs of *Phragmites australis* grown in Pb contaminated soils under

564 two CO_2 levels. (A) indicates Pb concentration in stems; (B) indicates Pb concentration in leaves;

565 (C) indicates Pb concentration in roots; (D) indicates total Pb concentration. Different letters

566 indicate significant differences ($P \leq 0.05$) between different Pb levels (within CO_2 one level), and an

567 asterisk indicates significant difference ($P \leq 0.05$) between elevated CO_2 and ambient CO_2 control

568 (within one Pb level).

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