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Do plants modulate biomass allocation in response to petroleum pollution?

Ming Nie[1,2], Qiang Yang[1], Li-Fen Jiang[1], Chang-Ming Fang[1], Jia-Kuan Chen[1,2] and Bo Li[1,2,*]

[1]*Key Laboratory for Biodiversity Science and Ecological Engineering, Institute of Biodiversity Science, Fudan University, Shanghai 200433, People's Republic of China*
[2]*Centre for Watershed Ecology, Institute of Life Science and Key Laboratory of Poyang Lake Environment and Resource Utilization, Nanchang University, Nanchang 330031, People's Republic of China*
**Author for correspondence (bool@fudan.edu.cn).*

Biomass allocation is an important plant trait that responds plastically to environmental heterogeneities. However, the effects on this trait of pollutants owing to human activities remain largely unknown. In this study, we investigated the response of biomass allocation of *Phragmites australis* to petroleum pollution by a $^{13}CO_2$ pulse-labelling technique. Our data show that plant biomass significantly decreased under petroleum pollution, but the root–shoot ratio for both plant biomass and ^{13}C increased with increasing petroleum concentration, suggesting that plants could increase biomass allocation to roots in petroleum-polluted soil. Furthermore, assimilated ^{13}C was found to be significantly higher in soil, microbial biomass and soil respiration after soils were polluted by petroleum. These results suggested that the carbon released from roots is rapidly turned over by soil microbes under petroleum pollution. This study found that plants can modulate biomass allocation in response to petroleum pollution.

Keywords: biomass allocation; $^{13}CO_2$ pulse-labelling; petroleum pollution; photosynthesis; *Phragmites australis*

1. INTRODUCTION

Biomass allocation of plants is an important trait that constantly senses environmental changes (Hermans *et al.* 2006; Berendse & Moller 2009). Many studies have suggested that plants can adaptively respond to belowground stresses by altering biomass allocation to the belowground parts, to alleviate the stresses in a manner that optimizes the capture of soil nutrients and maximizes plant growth rate (Bonifas *et al.* 2005; Mahoney & Swanton 2008). However, the impacts of pollutants on plants' biomass allocation remain largely unknown. Understanding biomass allocation of plants in response to environmental pollution will enable us to better understand the mechanisms by which plants respond adaptively to an imbalance of resources and predict what plant features increase their fitness under environmental changes.

Petroleum pollution, as an important global change, has broadly threatened the environments of plants owing to human activities (Kingston 2002; Peterson *et al.* 2003). In China, for example, the area for petroleum exploration accounts for 3 per cent of the total land area and about 60 million tons of petroleum on average enters the soil every year (Liu *et al.* 2007). While several studies have documented that petroleum pollution has negative effects on plant growth, little is known about how biomass allocation of plants responds to petroleum pollution (Dowty *et al.* 2001; Culbertson *et al.* 2008). In this paper, we conducted a controlled study with the common plant *Phragmites australis* of how plants alter biomass allocation in response to varying petroleum concentrations. *Phragmites australis* was selected because of its tolerance to petroleum pollution and important ecosystem functions (Engloner 2009; Nie *et al.* 2009). According to a simple model of flux and fate of carbon from photosynthesis (electronic supplementary material, figure S1) (Kuzyakov *et al.* 1999), the $^{13}C–CO_2$ pulse-labelling technique was used to investigate the allocation of photosynthetically fixed carbon in response to petroleum pollution.

2. MATERIAL AND METHODS

Phragmites australis seeds were collected from the Shengli oilfield in Shandong Province, China (37°33′ N; 118°30′ E). The topsoil (0–20 cm) was sampled from a site with no history of previous petroleum pollution in the National Nature Reserve of the Yellow River Delta near the oilfield (Nie *et al.* 2009). Three treatments of petroleum-polluted soils were designated by mixing the soil with crude oil from the Shengli oilfield, i.e. control (0 mg kg^{-1}), low pollution (6000 mg kg^{-1}), and high pollution (12 000 mg kg^{-1}). After emergence of the first euphylla, one seedling of *P. australis* was transferred to the centre of a pot filled with 228.15 g of prepared soil (dry weight). A total of 27 pots (three treatments × three replicates × two incubation times (24 h or 72 h after the initiation of pulse labelling) + three non-labelled pots (blank) for each treatment) were randomly placed in a growth chamber (14 h daytime period, light intensity 800 μ mol m^{-2} s^{-1}, with daytime and nighttime temperatures of 25°C and 20°C respectively). After two months growth, pots were transferred to the gas-tight system for pulse labelling (figure 1). Owing to the negative impacts of petroleum pollution on plant biomass (table 1), the upper chambers were designed at different heights, and thus created volumes of 875, 625 and 375 cm^3 (the same basal area, 25 cm^2), which were used to label plants growing in control, low-polluted and high-polluted soils, respectively. After CO_2 concentrations fell to about 200 ppm (v/v) in the upper chambers, three pulses of $^{13}CO_2$ at an interval of 2 h were applied from 9.00 h to 15.00 h (figure 1). Each pulse contained 400 ppm of $^{13}CO_2$ (v/v) by mixing 1 ml of lactic acid (1.5 M) and 1 ml of $NaH^{13}CO_3$ solution (different contents according to the upper chambers' volumes; greater than 99 atom%, Cambridge Isotope Laboratories; Butler *et al.* 2004).

Soils and plants were sampled at 24 and 72 h after the initiation of pulse-labelling. C and ^{13}C contents in plant tissues, soils and microbial biomass (MBC) were determined by a mass spectrometer (MAT 253, Finnigan, Germany) coupled with an elemental analyser (FlashEA 1112, Finnigan, Italy; Bardgett *et al.* 2003). CO_2 captured in an NaOH solution was quantified by titration, and then the solutions were freeze-dried for analysing ^{13}C content through evolving CO_2 by adding phosphoric acid in a Gasbench II Device (Finnigan; Landi *et al.* 2003). All ^{13}C data were corrected from average values of blanks. The total ^{13}C recovery expressed as the proportion of total assimilated ^{13}C in total supplied ^{13}C (Fan *et al.* 2008).

The effects of petroleum concentration on plant biomass and total recovery of ^{13}C were examined using one-way ANOVA. The effects of petroleum concentration and incubation time on the ^{13}C proportions in the total assimilated ^{13}C in plant biomass, soil, soil respiration and MBC were examined using two-way ANOVA.

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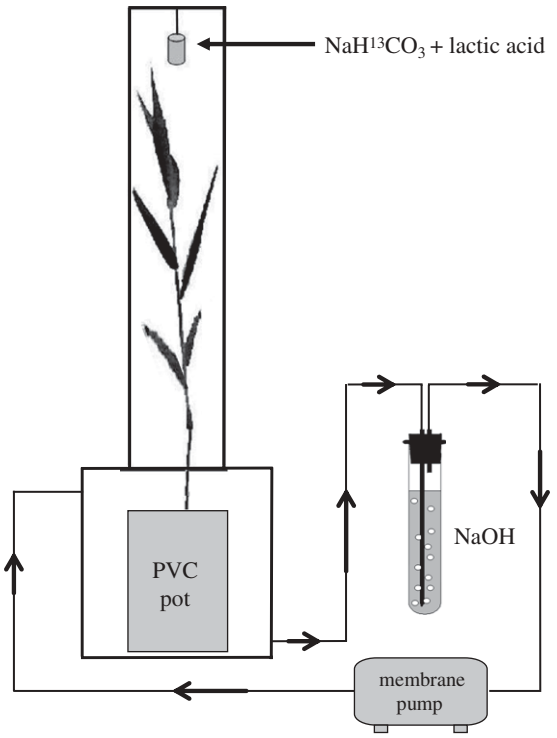

Figure 1. Sketch of experimental set-up used in this study. The gas-tight system consisted of independent upper and bottom chambers made from Plexiglas, and an installation for capturing soil respiration CO_2.

3. RESULTS

(a) *Plant biomass and recovery of ^{13}C*

Plant shoot ($F = 352.59$, $p < 0.001$) and root ($F = 98.31$, $p < 0.001$) biomass significantly decreased as petroleum concentration increased. However, the root–shoot ratio ($F = 11.65$, $p < 0.001$) significantly increased with increasing petroleum concentration (table 1). The total recovery of ^{13}C did not vary significantly among treatments ($F = 0.054$, $p = 0.948$; table 1).

(b) *^{13}C allocation to plant biomass*

The ^{13}C proportion in plant shoots expressed as the percentage of shoot ^{13}C in the total assimilated ^{13}C was significantly affected by petroleum concentration (table 2), and the ^{13}C allocation to plant shoots decreased as petroleum concentration increased at both 24 (71–48%) and 72 h (69–29%) (figure 2*a*). Although incubation time did not significantly affect the ^{13}C proportion in plant shoots (table 2), the decrease in the ^{13}C proportion at 72 h relative to that at 24 h increased as petroleum concentration increased, i.e. control (−2%), low pollution (−4%), high pollution (−19%). Although petroleum concentration and incubation time did not significantly affect the ^{13}C proportion in plant roots (table 2, figure 2*b*), the root–shoot ratio of ^{13}C was significantly influenced by petroleum concentration and its interaction with incubation time (table 2). The root–shoot ratio of ^{13}C increased as petroleum concentration increased at both 24 (0.36–0.64) and 72 h (0.33–1.12) (figure 2*c*).

(c) *^{13}C allocation in soil and respiration*

Petroleum pollution and incubation time had significant effects on the total soil ^{13}C proportion (table 2), and petroleum pollution increased ^{13}C allocation to the soil at both 24 and 72 h (figure 2*d*). The ^{13}C proportion in MBC was quite similar to the total soil ^{13}C proportion in response to petroleum pollution and incubation time (table 2 and figure 2*e*). Petroleum pollution also had significant effects on the ^{13}C proportion in soil ^{13}C–CO_2 respiration (table 2), which sharply increased with increasing petroleum concentration within 24 h (3–17%). At 72 h, a high ^{13}C proportion in soil ^{13}C–CO_2 respiration occurred in high-polluted soil (33%) but not in control (6%) and low-polluted soils (9%) (figure 2*f*).

4. DISCUSSION

Phragmites australis biomass significantly decreased under petroleum pollution, but the root–shoot ratio both in plant biomass and ^{13}C increased with increasing petroleum concentration. Furthermore, assimilated ^{13}C was found to be higher in soil, MBC and soil respiration after soils were polluted by petroleum. These results suggested that plants increased biomass allocation to its belowground parts, and carbon released from roots is rapidly turned over by soil microbes under petroleum pollution.

Being similar to the previous studies (Butler *et al.* 2004; Fan *et al.* 2008), the total ^{13}C recoveries in this study ranged between 50 and 58 per cent (table 1). There was no significant difference in the ^{13}C recovery among treatments from the same initial $^{13}CO_2$ content (table 1); therefore our gas-tight system with different sizes of upper chambers was suitable for studying ^{13}C allocation of plants under petroleum pollution.

In this study, we found that the root–shoot ratio in both biomass and ^{13}C positively responded to petroleum pollution (tables 1, 2 and figure 2*c*), indicating that *P. australis* could allocate more biomass to roots in response to petroleum pollution. High ^{13}C proportions in soil, MBC and respiration were also found in this study (figure 2), indicating that plants released a high proportion of biomass as root exudates to petroleum-polluted soils (Fan *et al.* 2008). Thus petroleum pollution appeared to not only promote the carbon allocation to plant roots but also enhanced the release of carbon from roots. These results provide evidence that plant biomass allocation also has profound effects on soil processes and microbial activities (Hogberg *et al.* 2001; Hogberg & Read 2006).

High ^{13}C proportions in MBC and soil respiration also indicated that rapid turnover of soil resources occurred in petroleum-polluted soils (figure 2*e*,*f*) (Zhou & Crawford 1995; Caravaca & Roldan 2003). Furthermore, petroleum concentration had a greater effect than incubation time on the proportions of ^{13}C in plants, soil, MBC and soil respiration (table 2), suggesting that transfer of the photosynthetically fixed carbon from shoots to the roots and into the soil was mainly petroleum-dependent and fast. These observations are consistent with the previous reports about rapid turnover of photosynthetically fixed

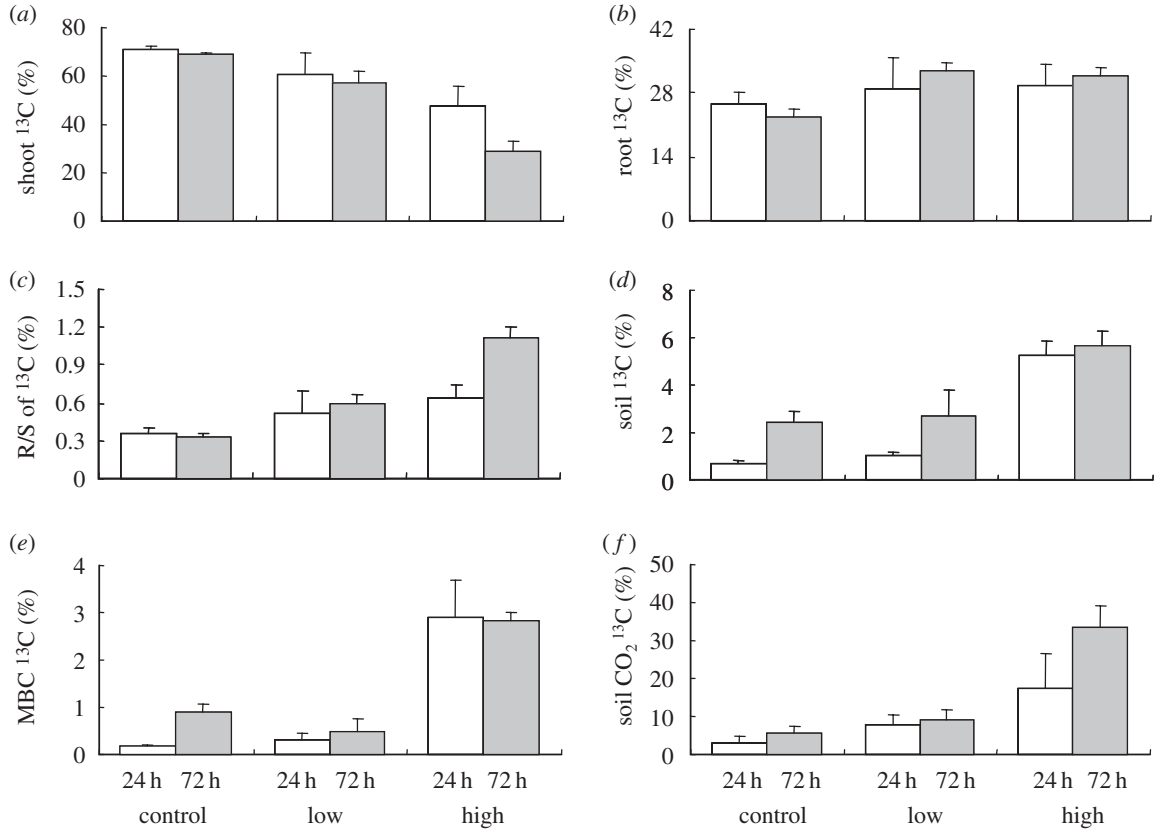

Figure 2. The proportions of total assimilated ^{13}C in (*a*) plant shoots, (*b*) roots, (*c*) the root–shoot (R/S) ratio of assimilated ^{13}C, (*d*) total soil carbon, (*e*) microbial biomass carbon (MBC) and (*f*) soil respiration (CO_2) at different petroleum concentrations after being incubated for 24 and 72 h. Values are means $\pm$ s.e.

Table 1. Plant shoot and root biomass (dry weight), the root–shoot ratios and the ^{13}C recoveries (expressed as the proportion of total supplied ^{13}C) at different petroleum concentrations. (Values are means $\pm$ s.e. The same letters denote non-significant differences between treatments ($p > 0.05$).)

	shoot biomass (g)	root biomass (g)	root–shoot	^{13}C recovery (%)
control	0.194 ± 0.011^{a}	0.071 ± 0.007^{a}	0.364 ± 0.033^{c}	52 ± 7^{a}
low	0.051 ± 0.002^{b}	0.024 ± 0.002^{b}	0.462 ± 0.022^{b}	58 ± 10^{a}
high	0.023 ± 0.002^{c}	0.015 ± 0.001^{c}	0.671 ± 0.071^{a}	50 ± 7^{a}

Table 2. Summary of two-way ANOVAs to test the effects of petroleum pollution and incubation time on the ^{13}C proportions of total assimilated ^{13}C in plant biomass, soil, microbial biomass (MBC) and soil respiration.

variables	source of variation	*F*	*p*
shoot ^{13}C proportion	petroleum	16.593	$p < 0.001$
	time	3.172	0.100
	petroleum × time	1.405	0.283
root ^{13}C proportion	petroleum	2.232	0.150
	time	0.145	0.710
	petroleum × time	0.504	0.616
root/shoot of ^{13}C	petroleum	15.797	$p < 0.001$
	time	4.715	0.051
	petroleum × time	3.974	$p < 0.05$
soil ^{13}C proportion	petroleum	23.933	$p < 0.001$
	time	14.175	$p < 0.01$
	petroleum × time	3.557	0.061
MBC ^{13}C proportion	petroleum	21.260	$p < 0.001$
	time	5.012	$p < 0.05$
	petroleum × time	2.230	0.150
soil CO_2 ^{13}C proportion	petroleum	10.732	$p < 0.01$
	time	2.087	0.174
	petroleum × time	1.936	0.187

carbon (Butler *et al.* 2004; Kastovska & Santruckova 2007).

In conclusion, our study suggests that plants, in response to petroleum pollution, increase biomass allocation to their belowground parts, and carbon released from roots is rapidly turned over by soil microbes. Our observed responses of plants to petroleum pollution are similar to those other belowground stresses (Nguyen 2003; Jones *et al.* 2004; Paterson *et al.* 2009). Therefore, this study suggested that plants can modulate biomass allocation in response to petroleum pollution.

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