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Atmospheric phenanthrene pollution modulates carbon allocation in red clover (*Trifolium pratense* L.)

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ABSTRACT

The influence of atmospheric phenanthrene (PHE) exposure (160 μg m^{-3}) during one month on carbon allocation in clover was investigated by integrative (plant growth analysis) and instantaneous $^{13}CO_2$ pulse-labelling approaches. PHE exposure diminished plant growth parameters (relative growth rate and net assimilation rate) and disturbed photosynthesis (carbon assimilation rate and chlorophyll content), leading to a 25% decrease in clover biomass. The root-shoot ratio was significantly enhanced (from 0.32 to 0.44). Photosynthates were identically allocated to leaves while less allocated to stems and roots. PHE exposure had a significant overall effect on the ^{13}C partitioning among clover organs as more carbon was retained in leaves at the expense of roots and stems. The findings indicate that PHE decreases root exudation or transfer to symbionts and in leaves, retains carbon in a non-structural form diverting photosynthates away from growth and respiration (emergence of an additional C loss process).

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent, ubiquitous organic by-products of the incomplete combustion of fossil fuel and biomass. They reach ecosystems through short or long-range atmospheric deposition of particles or gas (Wild and Jones, 1995). Since they can exert toxic mutagenic and carcinogenic properties the contamination of ecosystems by PAHs raises environmental and human health issues (IARC, 1983). The pivotal position of plants in natural and agro ecosystems provides an exposure route for PAHs to the higher trophic levels (Kipopoulou et al., 1999).

Atmospheric PAHs are well studied in field experiments for environmental monitoring (Srogi, 2007). In such a context, PAH effects mainly depend on the exposure concentration, the time and the ability of plants to take up and accumulate such pollutants. Nevertheless, the physiological mechanism accounting for the effects of PAHs on plant growth was sparsely considered mostly because of inhomogeneous environments. Hence, experimental devices that simulate atmospheric PAH exposures were required to clarify such physiological aspects (Zuo et al., 2006). Until now, PAH effects have been mainly studied in the case of root exposure through contaminated soil or water (Aina et al., 2006). In such a context, PAHs induced physiological disturbances, such as the decrease in germination, growth (root length and shoot biomass) and in chlorophyll content (chlorophyll *a* and *b*) (Kummerova et al., 2006, 2008, 2010; Liu et al., 2009).

In the few studies concerning atmospheric exposure to PAHs, plant biomass was usually affected but contradictory results were reported for biomass partitioning, with either leaf or root biomass diminished by PAH exposure. For example, biomass production of *Cucumbis sativus* and *Brassica napus* was altered by foliar application of an aqueous solution of PAHs (Huang et al., 1996). Root biomass of *Lactuca sativa* (lettuce) and *Raphanus sativus* (radish) was affected by 2 weeks of foliar application of anthracene in aerosol form (Wieczorek and Wieczorek, 2007). Physiological disturbances in primary metabolic processes in plants were also reported. For instance, the photosynthetic rate decreased by about 20% in lettuce and radish exposed to anthracene (Wieczorek and Wieczorek, 2007). A decrease in photosynthetic and respiration activities was also observed in *Pinus densiflora* and *Lycopersicon*

Abbreviations: PAHs, polycyclic aromatic hydrocarbons; PHE, phenanthrene; R:S, root–shoot ratio; RGR, relative growth rate; NAR, net assimilation rate; C_{MIC}, microbial biomass C; chl, chlorophyll.

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esculentum when their foliage was fumigated with fluoranthene (Oguntimehin et al., 2008, 2010).

In a previous work (Desalme et al., 2011), a significant decrease in shoot biomass was highlighted in clover exposed to atmospheric phenanthrene, while root biomass remained unchanged. Such a biomass decrease in clover remained unexplained but suggested a change in carbon allocation. Plants do regulate carbon allocation in response to abiotic stress in a way to ensure their survival (Grantz, 2003; Pell et al., 1994; Ruehr et al., 2009). Carbon allocation in plants is assessed by integrative (plant growth analysis) and/or instantaneous ($^{13}C-CO_2$ labelling) approaches. Plant growth analysis is an explanatory and holistic approach to interpret plant form and function across harvest-intervals that relates growth to carbon acquisition and allocation (Hunt et al., 2002). Additional information is given by the $^{13}C-CO_2$ labelling technique which permits to elucidate the fate of recently assimilated carbon within the plant soil system (Olsson and Johnson, 2005). The impact of atmospheric PAHs on carbon allocation in plants has been sparsely investigated solely in plants grown in petroleum-polluted soil. Results demonstrated that carbon allocation was modified in favor of belowground parts in response to root exposure to PAHs (Nie et al., 2010).

The main objective of this work was to elucidate how carbon allocation in plant and soil compartments was affected by atmospheric phenanthrene exposure. Clover was selected as a functionally important model grassland species that is found in both natural and agroecosystems. Phenanthrene (PHE) is a gaseous PAH generally considered as a model of PAH because of its ubiquity and its preponderance in the atmosphere (Gioia et al., 2006). Clover was grown in agricultural soil and exposed to a PHE-enriched atmosphere during one month. We hypothesized that, at the end of the PHE exposure, 1) the relative growth rate (RGR), net assimilation rate (NAR) could diminish and 2) the root–shoot (R:S) allometric coefficient and the carbon allocation (i.e. ^{13}C labelling) to roots could be impaired.

2. Material and methods

2.1. Culture and exposure

Red clover (*Trifolium pratense* L., var. Jonas, Caillard) seeds were disinfected (5 min in ethanol followed by 2 h in sodium hypochlorite solution (4%, v/v)) and germinated in Petri dishes with Vincent medium during 3 days (Purchase et al., 1997). The soil that came from an agricultural field in Maconcourt (Vosges, France) was characterized as an eutric cambisol (WRB). Thirty-eight plastic pots (12 cm high, 8 cm diameter) were filled with 300 g dry weight (DW) of 2 mm-mesh sieved soil. Nine seedlings were transplanted into each pot and cultivated in a thermostatic growth chamber (25 °C, photoperiod 12 h/12 h, 70% constant relative humidity, 180 $\mu mol\, m^{-2}\, s^{-1}$ photosynthetically active radiation (PAR). After one month of culture, 12 pots were harvested as a starting point for growth analysis.

Four pots were placed inside polluted and control chambers ($n = 3$ chambers for both treatments, i.e. 24 pots in total) during one month (20 °C, photoperiod 12 h/12 h, 135 $\mu mol\, m^{-2}\, s^{-1}$ PAR). An additional set of 1 polluted and 1 control chamber was installed to provide unlabelled plant and soil materials. As described in Desalme et al. (2011), the air was enriched with PHE before reaching each polluted chamber by passing through an evaporator filled with PHE pills. PHE concentrations in air were monitored throughout the experimentation in each chamber (polluted and control) with passive samplers made of activated carbon.

2.2. Labelling

After one month of PHE exposure, the ^{13}C-labelling was performed using the exposure chambers with the holes fitted with a rubber septum. The 3 replicate chambers were labelled simultaneously. Evolution of $^{12}CO_2$ and $^{13}CO_2$ concentrations inside each chamber was monitored simultaneously with a $^{12}CO_2/^{13}CO_2$ infrared gas analyzer (S710, SICK/MAIHAC). When the chamber was closed, the ambient CO_2 concentration declined at a rate of 0.825 and 1.05 $\mu mol\, CO_2\, s^{-1}$ in polluted and control system, respectively. When $^{12}CO_2$ concentration reached values around 120 $\mu mol\, mol^{-1}$, pure $^{13}CO_2$ (99.299 atom %; Eurisotop, Cambridge Isotope Laboratory Inc., Andover, MA) was injected at a flow rate set to maintain $^{13}CO_2$ concentration between 600 and 800 $\mu mol\, mol^{-1}$ while $^{12}CO_2$ concentration ranged between 200 and 300 $\mu mol\, mol^{-1}$. After 1.5-h labelling, the chambers were opened and vented for 10 min to remove all $^{13}CO_2$ in excess then the light was turn off. The first sampling was done 15 min after turning off the lights (i.e. 2 h after the beginning of the labelling) (T0).

2.3. Sample collection

Leaves, stems, roots and soil were sampled over a 4-day period: 2 h (T0), 14 h (T1), 38 h (T2) and 86 h (T3) after the beginning of the labelling. Samples of unlabelled pots (^{13}C natural abundance) were collected just before the labelling to avoid any risk of contamination from 6 additional chambers (3 polluted and 3 controls) devoted to provide unlabelled samples. At each sampling time and in each pot ($n = 3$ per treatment), plant shoots were rapidly cut from roots, separated into leaves and stems. The root/soil system was separated into top (0–1 cm depth; 50 g DW) and deep layer (1–15 cm depth; 250 g DW) where the roots were recovered and rinsed with distilled water. All the plant samples were weighted and frozen in liquid nitrogen (−80 °C) before being freeze-dried and ground to powder for further analyzes. Soil samples were all divided into 2 subsamples for PHE quantification (dry soil) and microbial biomass measurements (fresh soil), respectively.

2.4. Total organic content and ^{13}C abundance determination in the different C pools

Plant samples were analyzed for total C and N with a NC elemental analyzer (CE instruments, NA 1500, Italy). Each soil sample was divided into 2 samples: one kept as is (non-fumigated samples) and the other submitted to chloroform fumigation extraction during 24 h to solubilize microflora (fumigated samples). Microbial biomass was extracted from each soil samples (40 g of fresh soil) by with of K_2SO_4 (100 mL of 0.05 M) (Vance et al., 1987). Total organic content in the soil and in K_2SO_4 extracts was determined with a TOC analyzer (TOC-V 5000, Shimadzu). Microbial biomass C (C_{MIC}) was calculated as the difference of C content between the fumigated and the non-fumigated extracts. ^{13}C isotope composition ($\delta^{13}C$) in plant, soil and K_2SO_4 extracts were determined by an isotope ratio mass spectrometer (IRMS Finnigan, Delta S, Germany). By convention, ^{13}C isotope composition was expressed relative to Pee Dee Belemnite standard ($R_{PDB} = 0.011179602$). The following equation was used to calculate the $\delta^{13}C$ of microbial biomass ($\delta^{13}C_{MB}$, ‰)

$$\delta^{13}C_{MB} = \frac{\delta^{13}C_f \times C_f - \delta^{13}C_{nf} \times C_{nf}}{\left(C_f - C_{nf}\right)} \quad (1)$$

where C_f and C_{nf} refer to the amount of C extracted from fumigated and non-fumigated soil, respectively, and $\delta^{13}C_f$ and $\delta^{13}C_{nf}$ to their $\delta^{13}C$ values.

Isotope composition of CO_2 respired by the plant soil system was recorded during 3 consecutive days on the 3 polluted and the 3 control pots collected at T3. Each pot was introduced inside an airtight 2.4 L-chamber. The accumulation of $^{12}CO_2$ and $^{13}CO_2$ in the dark were measured during 30 min with the $^{12}CO_2/^{13}CO_2$ infrared gas analyzer (S710, SICK/MAIHAC, Germany). The [$^{12}CO_2$] and [$^{13}CO_2$] were used to calculate the isotope composition of the air inside the chamber ($\delta^{13}C$).

$$\delta^{13}C = \frac{\frac{^{13}CO_2}{^{12}CO_2}}{R_{PDB}} - 1 \quad (2)$$

$\delta^{13}C$ was plotted against the inverse of total CO_2 concentration (Keeling plots) to estimate the isotope composition of respired CO_2 (Keeling, 1958; Ngao et al., 2005).

Total respiration (in mg C h^{-1}) was calculated from the slope of the linear increase in total CO_2 concentration in the chamber.

The relative abundance of ^{13}C in any compartment including respiration (A_i, expressed in atom %) can be calculated as

$$A_i = \frac{^{13}C_i}{(^{12}C + ^{13}C)_i} = \frac{\left(\frac{\delta^{13}C_i}{1000} + 1\right) \times R_{PDB}}{\left[\left(\frac{\delta^{13}C_i}{1000} + 1\right) \times R_{PDB} + 1\right]} \quad (3)$$

The ^{13}C label recovered in any compartment i (LR_i, mg ^{13}C) was calculated as

$$LR_i = \frac{\left(A_{i,lp} - A_{i,up}\right)}{100} \times Q_i \quad (4)$$

with Q_i the quantity of C in the compartment i (mg C). lp and up denote labelled and unlabelled pots.

For plant materials, Q_i was the product of the carbon content (C_i) and the dry mass (DM_i) of the compartment i. For microbial biomass, Q_i was the product of C_{MIC} and the soil mass per pot.

Cumulative ^{13}C recovered in respiration (LR_r) in the dark was determined assuming an exponential decay of the amount of excess ^{13}C recovered in respiration at a given time ($R_{13Cex,t}$) according to Plain et al. (2009). $R_{13Cex,t}$ (mg $^{13}C\, h^{-1}$) was calculated according to Eqn. (4) using R instead of Q_i. An exponential function was fitted to the data

$$R_{13Cex,t} = R_{13Cex,0} \times \exp(-K \times t)) \quad (5)$$