



## The Effect of Simulated Nitrogen Deposition on the Emission of Carbonyl Compounds from *Ormosia pinnata* and *Cinnamomum burmannii*

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### Abstract

Elevated anthropogenic nitrogen (N) deposition has become a limiting factor for plants instead of a nutrient for its dramatic rising at globally scale, which greatly affects the process of carbon (C) cycle from individual plant to ecosystem, and even global levels. It could affect biogenic carbonyl emissions as an important component of carbon pool from plants, but this aspect has not yet been investigated. In this study, we performed a simulated N deposition experiment ( $100 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$ ) on two native tree saplings planted in a nursery in South China. Results showed that three main biogenic carbonyls, i.e. formaldehyde, acetaldehyde, and acetone from tree leaves all had distinct seasonal variations, with much higher emission rates in the wet season than in the dry season. Elevated N addition significantly depressed the emission of biogenic carbonyls in the dry season, but not in the wet season. No big differences were observed in carbonyls emissions from the two tree species responding to N deposition both in the wet season and in the dry season. Thus, we concluded that N deposition might drive plants to change carbon allocation and reduce carbon loss when as a limiting factor in the dry season. This finding is of significance for the theory of carbon allocation and plant adaptability strategy under elevated N deposition.

### Keywords

Biogenic carbonyls emissions; Nitrogen deposition; Dry season; Wet season; Carbon allocation

### Introduction

Carbonyl compounds are unique volatile organic compounds (VOCs) having very strong reactive capacity and short life span ranging from several hours to several days. Plant emission is one of the important carbonyl sources, accounting for 24% of the total volatile organic compounds released by plants [1,2]. The investment of carbon into biogenic VOCs can lead to substantial carbon loss in plant leaves [3-6] and considerable loss of photochemical energy

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[7]. More importantly, biogenic VOCs often plays key ecological functions, e.g. resisting to stress, communicating with other plants as signal and serving for plant survival, etc. [8,9]. Therefore, biogenic carbonyl compounds are very significant for carbon cycles of forest ecosystems for their formation and emission from plants besides their key roles in atmospheric chemical processes, e.g., contributing to the organic components of aerosols, ozone [10], peroxyacyl nitrates [11,12], and haze weather in cities [13].

Biogenic carbonyls are derived from photosynthetic products of plants, so their emissions are remarkably controlled by the factors controlling plant growth including light, temperature, humidity [14-17], water stress [12,18,19], and plant nutrients.

N as an essential nutrient for plant growth, is becoming a limit factor for the dramatic rising of N deposition at the globally scale due to human activities [20]. At present, there are 11% of the world's natural vegetation receiving N deposition higher than the "critical load" threshold of  $10 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$  [21]. N deposition brings significant negative effects on plant growth and the structure and functions of terrestrial forest ecosystems [22-24], also including carbon allocation and plant survival [25,26].

N deposition affects the plant C pool and ecosystem C cycles [27] based on the changes in net photosynthetic rate, net primary productivity, litterfall, soil  $\text{CO}_2$  emission, etc. responding to N deposition [27-29], never in biogenic carbonyls emissions from plant leaves.

N deposition is assumed to affect biogenic carbonyls release for biogenic carbonyls as a part of C from plant and an important component of C cycles. The responses of biogenic carbonyls emissions to elevated N deposition depend on whether plants favor elevated N deposition which could drive plants to change its carbon allocation for better adaptability and even survival, according to the researches that elevated N further worsened water stress [30] in the dry season, which forced plant to reallocate carbon between VOCs and nonstructural carbon (NSC) [26] for better surviving the stressed environment [25]. Therefore, it is of significance to study the effects of nitrogen deposition on biogenic carbonyls emissions for better understanding of the adaptability mechanism of carbon allocation in plants and C cycle of forest ecosystems.

Here, we studied the effects of N deposition on carbonyls emissions from intact leaves of native tree species in south China. A hypothesized was proposed that N deposition could have no significant effects in biogenic carbonyls emissions and no change in carbon allocation to form carbonyls when as a nutrient while have negative effects in the emissions by depressing carbon allocation to form carbonyls when as a stress.

### Materials and Methods

#### Field site

The experiments were performed at the Dinghushan Biosphere Reserve (DHSBR) located in the middle of Guangdong Province in southern China ( $112^{\circ}10'E$ ,  $23^{\circ}10'N$ ). The reserve covers an area of 1,155 ha and has a typical monsoon and humid tropical climate. The average annual relative humidity is 80%, and the mean annual temperature is

21.0°C. The average temperatures of the coldest (January) and hottest (July) months are 12.6°C and 28.0°C, respectively. The mean annual rainfall of 1,927 mm has a distinct seasonal pattern, with 75% rainfall from March to August and only 6% from December to February. The N deposition was 40.23 kg N·ha<sup>-1</sup>·year<sup>-1</sup> in 2011 [31]. The soil is lateritic red earth formed from sandstone.

## Experimental treatments

Two N addition treatments were performed: control (without N addition) and N addition (100 kg N·ha<sup>-1</sup>·y<sup>-1</sup>; N100). N deposition at 100 kg N·ha<sup>-1</sup>·y<sup>-1</sup> was reported to significantly impact plant growth [32] and soil N<sub>2</sub>O emission [33] in this region. Therefore, this level of N deposition (100 kg N·ha<sup>-1</sup>·y<sup>-1</sup>) is considered as a threat to the health and function of forests ecosystems in tropical regions and was used in this study. Three 3.5 m×1 m plots were established, and each plot was separated by a sun board of 50-cm height. Two tree species, i.e., *Ormosia pinnata* and *Cinnamomum burmannii* were chosen to study. *O. pinnata* and *C. burmannii* are native even broadleaved tree species and widely planted in south China for their strong adaptability to environments. Five one-year-old seedlings of each tree species were transplanted at each plot of the study site. NH<sub>4</sub>NO<sub>3</sub> solution was applied in this study. During each application, fertilizer was weighed, mixed with 2 L of water, and applied monthly onto the soils of each plot by using a backpack sprayer at the end of each month from May 2011 to September 2014. Two passes were developed across each plot to ensure an even distribution of fertilizer. The control plots received 2 L of deionized water without N addition. Throughout the experiment, weeds were removed regularly. There were no significant differences in the physical and chemical properties of soils used to grow these saplings at the beginning of the experiment.

**Sample collection and analysis:** Biogenic carbonyl emission samples were collected during the wet season (July-August 2013) and the dry season (January 2014). For each sapling, samples were collected from a single branch. A dynamic enclosure system was constructed using a 40 L (48.2 cm×54.5 cm) Tedlar bag modified to slide over target branches and that could be sealed around the branch with a transparent tape [34,35]. After careful installation to minimize any disturbance, the purged branch enclosure was allowed to equilibrate for 30 min before sampling. Carbonyls were sampled for 150 min (dry season) or 180 min (wet season) from 8:00-18:00 by using 2, 4-dinitrophenylhydrazine (DNPH)-coated silica gel-cartridges (Waters, USA) with flow rates of 0.8-1.1 L·min<sup>-1</sup>. In all, 52 samples were collected by drawing the air through the cartridge by using a sampling pump (Thomas, USA). After sampling, each cartridge was wrapped in aluminum foil, resealed in aluminum foil, transported to the laboratory, and stored in the refrigerator before analysis. Temperature (T), photosynthetic active radiation (PAR), and relative humidity (RH) were monitored by the neighboring weather station within 20 meters.

The sampled cartridges were gradually eluted with 2 mL acetonitrile (ACN) into a 2-mL volumetric flask and stored under refrigerated conditions until analysis. The samples were analyzed using high-performance liquid chromatography (HPLC; Waters 2695) coupled to a UV detector (Waters 2996) and operated at 360 nm. A 10-μL aliquot was injected into the HPLC system through an auto sampler. The analytical conditions were as follows: Agilent SB-C18 reverse column (250 mm×4.6 mm×5μm); gradient mobile phase: 60-70% ACN of water solution for 20 min, 70-100% ACN for 3 min, 100% ACN for 4 min, 100-60% ACN for 1 min, and then 60% ACN for 5 min; mobile-phase flow rate: 1 mL·min<sup>-1</sup>.

Biogenic carbonyl flux rates were calculated using the following equation:

$$F_{\text{carbonyls}} = C_{\text{carbonyls}} \times Q/W \quad (1)$$

Where  $F_{\text{carbonyls}}$  is the flux rate in ng·g<sup>-1</sup>·h<sup>-1</sup>,  $C_{\text{carbonyls}}$  is the measured chamber carbonyl concentration in μg·L<sup>-1</sup>, Q is the flow rate through the chamber to the cartridge in L·h<sup>-1</sup>, and W is the dry weight of the leaves in the chamber in grams.

**Statistical analysis:** Paired-samples *t*-test was performed in order to analyze the differences of carbonyls emissions from plants between the control and N100 treatment at the nursery. Pearson correlation was used to determine the association between carbonyl emission rates and environmental factors. All analyses were conducted using SPSS 13.0 for Windows. Statistically significant differences were set at *P* values of <0.05 unless otherwise stated. Mean values are expressed as ± 1 standard error of the mean.

## Results

Meteorological conditions varied during the measurements between the dry and wet seasons (Table 1). In the wet season, temperature was higher than 30°C, RH was higher than 60%, and PAR was in the range of 850–1,000 μmol·m<sup>-2</sup>·s<sup>-1</sup>; however, in the dry season, their values reduced remarkably. RH had a significantly (*P*<0.01) negative correlation with temperature and PAR in the wet and dry seasons, respectively.

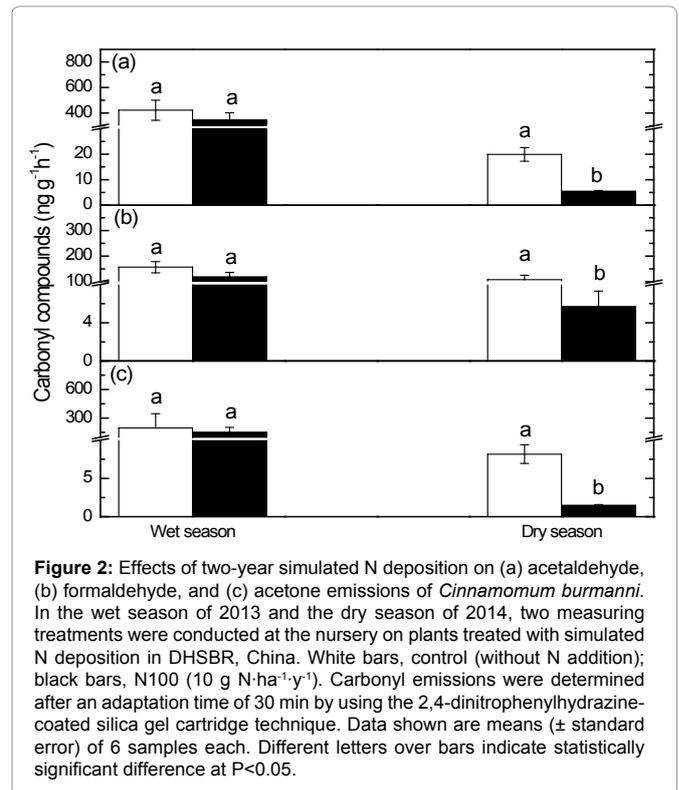
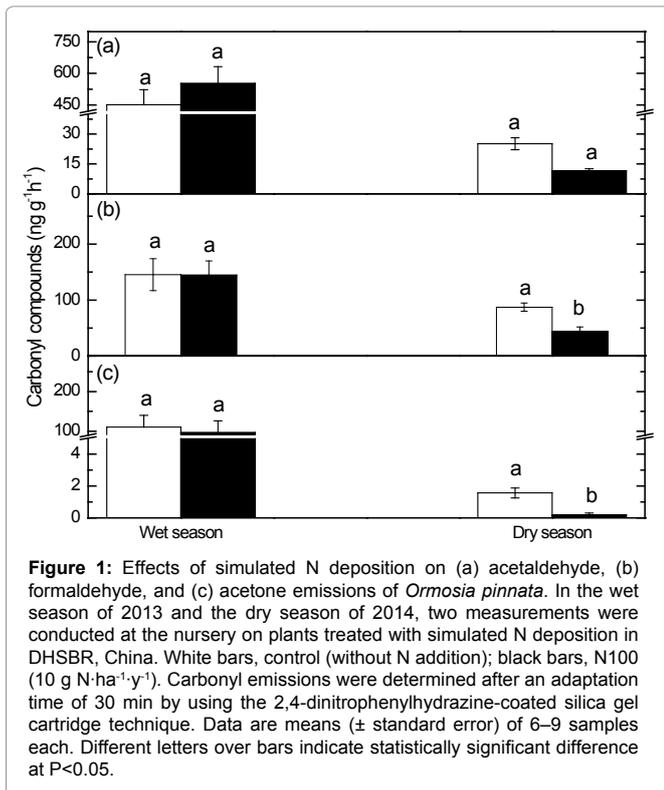
The main carbonyl species emitted in considerable amounts by the leaves of *O. pinnata* and *C. burmannii* were acetaldehyde, formaldehyde, and acetone, with emission rates ranging from approximately 3.4 to 738 ng·g<sup>-1</sup>·h<sup>-1</sup> in the wet season and 0 to 175 ng·g<sup>-1</sup>·h<sup>-1</sup> in the dry season (Figures 1 and 2). In three cases (*O. pinnata*, dry season, 2014, N100 treatment), acetone was deposited, whereas, at all other instances, carbonyls were emitted by the plants. For *O. pinnata*, acetaldehyde was the most abundant carbonyl species in the wet season, but formaldehyde was emitted in higher amounts in the dry season. Acetone emissions in *O. pinnata* were always lower (not exceeding 6%) than those of acetaldehyde and formaldehyde, especially in the dry season. Acetaldehyde, formaldehyde, and acetone emission rates in the control *O. pinnata* plants showed seasonal changes (Figure 1a, b, c), which were higher (450.7, 145.4, and 110.3 ng·g<sup>-1</sup>·h<sup>-1</sup>) in the wet season and lower (25.1, 87.3, and 1.6 ng·g<sup>-1</sup>·h<sup>-1</sup>) in the dry season. N100 treatment further strengthened this trend, and the emission rates remained considerably less in the dry season at approximately 11.6, 44.0, and 0.2 ng·g<sup>-1</sup>·h<sup>-1</sup> for acetaldehyde, formaldehyde, and acetone, respectively, accounting for 12–51% of the levels of the controls in the dry season. However, N100 treatment had no effect on the emissions (*P*>0.05) in the wet season (Figure 1). Similarly, in *C. burmannii* leaves, emissions of carbonyls were higher in the wet season than in the dry season, i.e., formaldehyde emission rates in the wet season were 31% higher and acetaldehyde and acetone emission rates were 95% higher than those in the control in the dry season. Further, N100 treatment did not considerably alter the seasonal emission pattern and also showed significant negative effects (*P*<0.01) only in the dry season (Figure 2). No significant difference was found in carbonyls emissions between the two tree species when responding to N deposition.

Correlation analyses showed that temperature, RH, and PAR had positive effects on the emissions of carbonyls both in *C. burmannii* and *O. pinnata*. In *C. burmannii*, temperature was a more important factor than RH and PAR; there were significant correlations between

**Table 1:** Means ( $\pm$  standard deviation) of environmental factors.

Parameter	<i>Cinnamomum burmannii</i> .		<i>Ormosia pinnata</i>	
	Dry season	Wet season	Dry season	Wet season
T ( $^{\circ}$ C)	17 $\pm$ 4	32 $\pm$ 2	20 $\pm$ 3	33 $\pm$ 2
RH (%)	45 $\pm$ 12	63 $\pm$ 7	52 $\pm$ 10	62 $\pm$ 9
PAR ( $\mu$ mol $\cdot$ m $^{-2}$ $\cdot$ s $^{-1}$ )	634 $\pm$ 428	983 $\pm$ 513	463 $\pm$ 313	866 $\pm$ 476

T: Temperature; RH: Relative humidity; PAR: Photosynthetic active radiation



temperature and formaldehyde and acetaldehyde emissions (P<0.01), but only marginal significant correlation between temperature and acetone emission (P=0.067). However, in *O. pinnata*, temperature, PAR, and RH had significant correlations with the emissions of formaldehyde, acetaldehyde, and acetone (P<0.05) except for a marginal significant correlation between RH and formaldehyde emissions (Table 2). Therefore, temperature was the key influencing factor for both *C. burmannii* and *O. pinnata*, whereas RH and PAR affected carbonyl emissions depending on tree species.

## Discussion

In the present field study, carbonyl emissions from the leaves of native tree saplings in South China in response to elevated N deposition were first investigated. The main carbonyls released into the atmosphere were acetaldehyde, formaldehyde, and acetone, as reported previously [12,34]. Other carbonyls (C $_4$ -C $_{10}$  aldehydes) were not emitted at significant amounts by the sampled trees, especially in the dry season. Acetaldehyde dominated the carbonyl flux with the highest emission rates of 451  $\pm$  72 ng $\cdot$ g $^{-1}$  $\cdot$ h $^{-1}$  in *O. pinnata* and 422  $\pm$  78 ng $\cdot$ g $^{-1}$  $\cdot$ h $^{-1}$  in *C. burmannii* in the wet season. The emission rates of formaldehyde and acetone were slightly lower than those reported in *Populus fremontii* [34,36] and *Populus tremuloides* [36], but were comparable with those reported in *Quercus ilex* [37], *Quercus gambelii* [36] and *Cinnamomum camphora* [38].

Carbonyls were emitted at significantly (P<0.05) higher rates during the wet season than during the dry season (Figures 1,2), suggested that plants allocate more carbon to carbonyls formation in the wet season compared to the dry season. In wet season, plants can use more N as nutrient to their growth under the condition of precipitation associated with the high heat compared to the dry season. Moreover, herbivory (referring to leaf damage by insects, mammals, and pathogens) positively correlating with rapid growth of plants [39] happens more frequently in wet season than in dry season. Coley and Borone [40] reported the highest and lowest herbivory rates in the wet and dry seasons, respectively. So plants can obtain carbon enough and allocate the assimilated carbon to biogenic carbonyls [41-43] in wet season. Higher emissions of biogenic VOCs in wet season are also known to play important roles in overcoming these biotic stresses [4,44-46] and favor plant survival [46].

Decrease in carbonyl emission in the dry season suggested that plants lose less carbon via VOCs emissions and sequester more carbon in this season, which is in agreement with the findings in tropical forests that carbon can be gained in dry season, but not in wet season [43,47]. Considerably low emissions of carbonyls in the dry season also implied that no more carbon is required for plant defense in this season, and more assimilated carbon is allocated to maintain the growth and metabolism of plants. Therein the decrease

**Table 2:** Pearson correlation coefficients between environmental factors and carbonyl emission rates in plant leaves.

	<i>Cinnamomum burmannii</i>			<i>Ormosia pinnata</i>		
	FA	AA	AC	FA	AA	AC
T	0.778**	0.920**	0.389	0.548**	0.798**	0.478*
RH	0.138	0.163	0.475*	0.316	0.642*	0.495*
PAR	0.338	0.600**	0.310	0.482**	0.532**	0.430*

T: Temperature; RH: Relative humidity; PAR: Photosynthetic active radiation; FA: Formaldehyde emission rates; AA: Acetaldehyde emission rates; AC: Acetone emission rates. \* and \*\* represent statistical correlations at P<0.05 and P<0.01, respectively.

in carbonyl emissions during the dry season suggested that the variation in carbon allocation of plants responding to environmental changes is an important mechanism for their defense and survival.

N100 treatment had significant negative effects on carbonyl emissions in the dry season (Figures 1,2), suggesting that N deposition enlarges the seasonal gap of the carbonyls emissions between the wet season and the dry season. This discrepancy was attributed to different responses of plant growth and carbon allocation to N addition between in wet season and in dry season. Plants exhibit good growth and do not change the pattern of their carbon allocation in the wet season when facing increased N deposition, because it is eligible for plants to uptake N under the condition of plenty moisture and light. However, in the dry season, plants cannot uptake the same quantity of N because of water scarcity, and even worse, N deposition becomes a stress for plant growth and forces plants to allocate more carbon to maintain growth and reduce carbon loss via biogenic VOCs emissions. The results further supported the speculation that N deposition depresses biogenic carbonyls emissions as a stress, e.g., in the dry season, and does not affect the emissions as a nutrient, e.g. in the wet season. A similar finding was reported in *Quercus pubescens* seedlings, which showed declined biogenic VOCs emission, but increased non-structural carbon (e.g., soluble sugar) storage under the severe drought condition [26]. Thus, the changes of biogenic VOCs emission indicated the direct response of plants to stress (e.g. N deposition), and suggested carbon allocation becomes a strategy of plants to adapt to the adverse environment. Moreover, the negative effects of N100 on biogenic carbonyls emissions in the dry season supported our previous conclusions that N deposition increases above-ground plant C pool [27] and carbon sequestration of tropical forests [48,49].

Temperature was an important factor that influenced carbonyls emissions from trees (Table 2); this was consistent with the findings of other studies [12,18,19,50]. The positive effect of temperature suggested that it might markedly stimulate carbonyls emissions during global warming, which could further influence atmospheric photochemical reaction and reduce carbon storage in plants. The impacts of RH and PAR on carbonyl emissions (Table 2) were similar to those reported by Cojocariu et al. In this study, the effects of the two factors differed between the two tree species. This could be because the leaves of *C. burmannii* had thin leathery blades, and those of *O. pinnata* had leathery blades; therefore, the leaves of the former species were more sensitive to the changes in RH and PAR.

## Conclusions

Biogenic carbonyls emissions show marked seasonal changes with higher emissions in the wet season than in the dry season; moreover, the seasonal disparity can be strengthened by N deposition at the level of 100 kg N·ha<sup>-1</sup>·y<sup>-1</sup> by depressing their emissions in the dry season. The results suggested that N deposition could induce plants to reallocate carbon and reduce carbon loss via biogenic carbonyls

emissions during the dry season when as a limiting factor. Our findings are of particular significance for better understanding plant adaptation strategies as a form of carbon balance under elevated N deposition.

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