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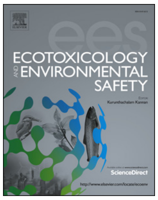

Effects of sulfuric, nitric, and mixed acid rain on Chinese fir sapling growth in Southern China

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ABSTRACT

The influence of acid rain on plant growth includes direct effects on foliage as well as indirect soil-mediated effects that cause a reduction in root growth. In addition, the concentration of NO_3^- in acid rain increases along with the rapid growth of nitrogen deposition. In this study, we investigated the impact of simulated acid rain with different SO_4^{2-}/NO_3^- (S/N) ratios, which were 1:0, 5:1, 1:1, 1:5 and 0:1, on Chinese fir sapling growth from March 2015 to April 2016. Results showed that Chinese fir sapling height growth rate (HGR) and basal diameter growth rate (DGR) decreased as acid rain pH decreased, and also decreased as the percentage of NO_3^- increased in acid rain. Acid rain pH significantly decreased the Chlorophyll a (Chla) and Chlorophyll b (Chlb) content, and Chla and Chlb contents with acid rain S/N 1:5 were significantly lower than those with S/N 1:0 at pH 2.5. The chlorophyll fluorescence parameters, maximal efficiency of Photosystem II photochemistry (Fv/Fm) and non-photochemical quenching coefficient (NPQ), with most acid rain treatments were significantly lower than those with CK treatments. Root activities first increased and then decreased as acid rain pH decreased, when acid rain S/N ratios were 1:1, 1:5 and 0:1. Redundancy discriminant analysis (RDA) showed that the Chinese fir DGR and HGR had positive correlations with Chla, Chlb, Fv/Fm ratio, root activity, catalase and superoxide dismutase activities in roots under the stress of acid rain with different pH and S/N ratios. The structural equation modelling (SEM) results showed that acid rain NO_3^- concentration and pH had stronger direct effects on Chinese fir sapling HGR and DGR, and the direct effects of acid rain NO_3^- concentration and pH on HGR were lower than those on DGR. Our results suggest that the ratio of SO_4^{2-} to NO_3^- in acid rain is an important factor which could affect the sustainable development of monoculture Chinese fir plantations in southern China.

1. Introduction

With the rapid growth of the population and economy, China has established the world's largest area of tree plantations during the past few decades (Tang et al., 2016). Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook), an economically valuable conifer with good wood quality, high yield, and multiple uses, is a mainly indigenous tree species that occupies approximately 25% of plantations in subtropical areas of southern China (Duan et al., 2016; Li et al., 2017; Ma et al., 2017). However, owing to the increasing seriousness of acid rain in southern China, the total Chinese fir plantation areas damaged by acid rain in seven provinces of South China were estimated to be 4.913×10^5 ha (Fan and Wang, 2000; Blanco et al., 2012).

The southern region of China, where precipitation in the late 1980s was found to have average pH's between 3.5 and 4.8, has become the third region in the world seriously affected by acid rain (Fan and Wang, 2000; Singh and Agrawal, 2008; Sun et al., 2016). SO_4^{2-} was the dominant anion in precipitation as the majority of energy is generated from coal combustion (Liang et al., 2016; Zhang et al., 2017). According to the data derived from the State Environmental Protection Administration of China, total SO_2 emissions in China has been increasing compared to the mid-1990s (Tu et al., 2005). The effects of acid rain on plants can be determined by the altering of the biochemical and physiological processes, such as chlorosis and necrosis, nutrient loss from leaves, variation of several enzyme activities (Yu et al., 2002; Singh and Agrawal, 2008; Du et al., 2017). Cape (1993) reported that

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both conifers and broadleaved tree saplings showed subtle changes in the structural characteristics of leaf surfaces after exposure to acid rain at pH 3.5. Liao and Chen (1992) found that acid rain with pH 2.0 can significantly inhibit the fine root growth of Chinese fir. Ramlall et al. (2015) reported that acid rain with pH 3.0 caused leaf tip necrosis, abnormal bilobed leaf tips, leaf necrotic spots and chlorosis, and reduced leaf chlorophyll concentration and root biomass.

Since the late 1990s, China has been implementing flue gas desulfurization and phasing out small inefficient units in the power sector (Chan and Yao, 2008), so sulfate ion (SO_4^{2-}) in acid rain has decreased significantly (Lv et al., 2014). However, the amount of motor vehicle traffic has increased rapidly in China, and NO_x is emitted into atmosphere through tailpipes (Liu et al., 2018). Therefore, acid rain pollution is gradually changing from sulfuric acid dominated rain to nitric acid dominated rain (Niu et al., 2014). Combining the acidification effects of sulfate ion and nitrate ion, the benefits of SO_2 reduction would almost be negated by increased N emissions (Zhao et al., 2009). Prior studies found that the inhibitory effects of nitric acid rain on litter decomposition, soil microbial biomass, and most enzyme activities were more significant than those of sulfuric acid rain in subtropical forests of China (Lv et al., 2014; Liu et al., 2017). Liu et al. (2018) reported that fine-root element contents and antioxidant enzyme activities were significantly affected by the acid rain SO_4^{2-}/NO_3^- ratio. However, there is still a lack of information on the effects of acid rain with different SO_4^{2-}/NO_3^- ratios on Chinese fir growth. This fundamental knowledge would be useful for making informed management decisions to promote sustainable development of monoculture Chinese fir plantations in southern China.

To explore the effects of acid rain with different ratios of SO_4^{2-} to NO_3^- on Chinese fir sapling growth, we established a series of pot experiments. Our primary objective was to discuss the impacts of increasing acid rain NO_3^- concentration and decreasing acid rain pH relative to control in terms of their effects on Chinese fir chlorophyll content, chlorophyll fluorescence parameters, root activity and antioxidant enzymes activities, which are sensitive indicators of forest productivity. Based on the previous studies and reports, we hypothesized that acid rain would depress the Chinese fir sapling growth, and the inhibitory effects would increase with acid rain NO_3^- concentration increases.

2. Materials and methods

2.1. Plant material and treatments

The study was conducted in the intelligent greenhouse of Xiashu Ecological Station of Nanjing Forestry University (31°7′ N, 119°12′ E), Jiangsu Province, China, from March 2015 to April 2016. One-year old Chinese fir saplings of uniform height were selected as our research object. The average height and ground diameter were 27.15 ± 1.33 cm and 5.03 ± 0.28 cm, respectively. The saplings were transplanted in plastic flowerpots (25 cm height × 20 cm diameter) with yellow brown clay soil collected from plantations nearby. The soil pH was 6.31 ± 0.01. These saplings had two months for recover after they were transplanted. During recover period, we watered them with distilled water.

After two months, eighty saplings with healthy growth and uniform height were selected for the simulated acid rain treatments. Five stock solutions of acid rain were prepared by mixing 0.5 mol L^{-1} H_2SO_4 and 0.5 mol L^{-1} HNO_3 at molar ratios of 1:0, 5:1, 1:1, 1:5 and 0:1. The experiment consisted of 16 treatments: CK (distilled water, pH = 7.0), SAR treatments with 1:0 for SO_4^{2-}/NO_3^- (S1 pH = 4.5, S2 pH = 3.5, S3 pH = 2.5), SAR treatments with 5:1 for SO_4^{2-}/NO_3^- (S4 pH = 4.5, S5 pH = 3.5, S6 pH = 2.5), SAR treatments with 1:1 for SO_4^{2-}/NO_3^- (S7 pH = 4.5, S8 pH = 3.5, S9 pH = 2.5), SAR treatments with 1:5 for SO_4^{2-}/NO_3^- (S10 pH = 4.5, S11 pH = 3.5, S12 pH = 2.5), SAR treatments with 0:1 for SO_4^{2-}/NO_3^- (S13 pH = 4.5, S14 pH = 3.5, S15 pH = 2.5). The total amount of simulated acid rain was 670.38 mm based the annual average precipitation (1117.29 mm) and acid rain frequency (60%) (Liu et al., 2017). The monthly volume of simulated acid rain applied to every flowerpot was 1754.16 ml, which was calculated by the monthly amount of acid rain (55.865 mm) and the area of flowerpot (314 cm^2). Each sapling was sprayed four times a month from May 2015 to April 2016, and each time with 438.54 ml of solution.

2.2. Growth measurement

Sapling height was measured using a tape rule from the base of the stem to the terminal bud. Stem basal diameter was measured by a Vernier caliper at the base of stem (Guo et al., 2016). Sapling height and stem basal diameter measurements were taken in April 30, 2015 and April 30, 2016, respectively. The relative growth rate of height (HGR) and basal diameter (DGR) were calculated using the following equations (Mofunanya and Soonen, 2017):

$$HGR = (H_2 - H_1)/H_1 \tag{1}$$

$$DGR = (D_2 - D_1)/D_1 \tag{2}$$

where H_1 is initial sapling height (cm), H_2 is final sapling height (cm), D_1 is initial basal diameter (mm), D_2 is final basal diameter (mm).

2.3. Chlorophyll content

The chlorophyll content was measured according to the procedure described by Gassama et al. (2015). The amount of 0.1 g of leaf was homogenized in a 10 ml mixture with acetone and ethyl alcohol (1:1, v/v) for 10 h in a darkroom, and then was centrifuged at 2500 rpm for 20 min and supernatant was extracted. About 2.5 ml of samples were pipetted into microfuge and the chlorophyll content was measured by using scanning spectrophotometer UV–VIS. The samples were read at wavelength of 663 and 645 nm.

2.4. Chlorophyll fluorescence

Chlorophyll fluorescence (Fs) measurements were performed according to the method of Osório et al. (2013) and Ying et al. (2014) using a chlorophyll fluorescence imager (CF Imager, Technologica, UK). Prior to the measurement of Fo and Fm (minimum and maximum fluorescence), the leaves were dark-adapted for 30 min and then the light-adapted parameters of Fs, Fo′ and Fm′ were determined after applying actinic light [500 μmol m^{-2} s^{-1}] to the leaves for light adaptation. The maximal efficiency of Photosystem II (PSII) photochemistry (Fv/Fm = (Fm-Fo)/Fm), actual PSII efficiency (Fv′/Fm′), the effective efficiency of PSII photochemistry (ΦPSII), photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (NPQ) were calculated using equations according to Wang et al. (2017).

2.5. Root activity

Root activity was measured using the TTC (triphenyl tetrazolium chloride) method (Zhang et al., 2015) and expressed as the deoxidization ability (μg g^{-1} h^{-1}). Dehydrogenase was expressed as the deoxidized TTC quantity, which was an index of root activity. Ten milliliter solutions of equal quantities of TTC (0.4%) and phosphate buffer were added to root samples (0.5 g) and kept in the dark at 37 °C for 2 h. The reaction was stopped with 1 mol L^{-1} H_2SO_4. The roots were ground and transferred into a tube with ethyl acetate to a total volume of 10 ml. The solution was measured at the absorbance of 485 nm using a scanning spectrophotometer UV–VIS.