Tree stoichiometry and nutrient resorption along a chronosequence of *Metasequoia glyptostroboides* forests in coastal China

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\begin{abstract}
Variations in carbon (C), nitrogen (N) and phosphorus (P) stoichiometry and nutrient resorption during stand development are essential indicators for assessing forest degradation. Yet, their patterns along a chronosequence have been inconsistent. In this study, we analyzed the patterns of tree (leaf, branch, and root) C, N, P stoichiometry and nutrient resorption along a chronosequence of *Metasequoia glyptostroboides* forests (young forests: 7-, 12-year old; middle aged forests: 22-, 28-year old; and mature forests: 33-, 37-year old) in subtropical coastal China. We found that no significant trend was displayed along the chronosequence for both tree N concentrations and N resorption efficiency (NRE). Both tree P concentrations and P resorption efficiency (PRE) were higher, while tree C:P and N:P were lower in young forests than those in middle-aged and mature forests. These results suggested that tree P stoichiometry and PRE were most strongly associated with forest degradation. Leaf P stoichiometry (P concentration, C:P, and N:P) was impacted more by soil stoichiometry, and leaf P concentration and N:P increased exponentially along soil P concentration and N:P, respectively. While branch, root P stoichiometry and PRE were affected more by tree age. Overall, our findings suggested that P deficiency was the leading factors, in terms of nutrients, for the *M. glyptostroboides* forests degradation in coastal China.
\end{abstract}

1. Introduction

Coastal windbreaks play an important role in wind prevention, shore fixation, and crop protection (Wu et al., 2013; Qu et al., 2013). While, coastal windbreaks degrades gradually, for example, mature or over-mature forests account for up to 40% of total coastal windbreaks area in coastal China. *Metasequoia glyptostroboides* is one of the main species of windbreaks in coastal China (Zhang et al., 2015a). Older *M. glyptostroboides* plantations, established since the 1980s, account for up to 50% of the total coastal windbreaks area in subtropical coastal China (Zhang et al., 2015a). Older *M. glyptostroboides* stands (i.e., those planted in the 1980s) have degraded gradually due to combination of biotic (e.g., tree aging, regeneration difficulty) and abiotic (e.g., infertile soil, wind disturbance, and human activity) factors (Wu et al., 2017). To date, the degradation mechanism has not yet been revealed.

Tree nutrient stoichiometry reflects the balance of carbon (C) and nutrient uptake during plant growth, and thus plays a fundamental role in growth and development for forests (Imaran and Gurmani, 2011; Wang et al., 2015; Yan et al., 2017). Nutrient resorption represents an important mechanism with profound influences on many processes, including decomposition, plant competition, and resource use efficiency (Huang et al., 2012; Yan et al., 2015). Foliar nutrient resorption is vital to productivity and elemental cycling in forests, especially in nutrient-poor forest ecosystems (Lü et al., 2012; Cleveland et al., 2013). Therefore, it is important to study the patterns of tree nutrient stoichiometry and resorption along a chronosequence, which may help to reveal nutrients limitations for forest degradation.

Nutrient stoichiometry and nutrient resorption in forests generally vary along a chronosequence, due to huge differences in photosynthetic capability and nutrition requirement at different growth stages (Amazonas et al., 2011; Li et al., 2013; Yan et al., 2017). How tree nutrient stoichiometry and resorption vary along the chronosequence has been the subject of numerous studies, with inconsistent results (Elser et al., 2010; Wang et al., 2014; Zhou et al., 2016). For example, Yang and Luo (2011) found that plant tissue C:N increased significantly with stand development for global secondary forests, while Clinton

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et al. (2002) found that there was no significant variation in a *Fagus* forest. The N resorption efficiency (NRE) and P resorption efficiency (PRE) increased from young to mature forests (Ye et al., 2012; Sun et al., 2016), but NRE and PRE were found to be stable along a chronosequence of *Larix kaempferi* forests (Chang et al., 2017). These inconsistencies may result from element-specific (Wang et al., 2015), or species-specific responses (Gyenge and Fernández, 2014; Wang et al., 2014). Therefore, it is important to investigate stoichiometry and nutrient resorption along chronosequence to reveal the possible degradation mechanism due to nutrient limitation for a given species.

Apart from tree development, soil nutrients may also be responsible for degradation along a forest chronosequence (Amazonas et al., 2011; Sardans et al., 2011; Tully et al., 2013). Soil nutrients might increase (Huang et al., 2013; Fan et al., 2015), decrease (Mao et al., 2010; Wu et al., 2013), or display no change (Smal and Olzewska, 2008) with forest stand development. Furthermore, there were no consistent results on how soil nutrients supply affects tree internal nutrient cycling and demand (Wang et al., 2013; Yuan and Chen, 2015; Liu et al., 2016). Plant growth does not only rely on a single nutrient's availability, but on the balance between multiple nutrients (Marschner, 2012; Zhou et al., 2016). Thus, soil nutrient stoichiometry (i.e., C, N, P concentrations and their ratio) is an important determinant of nutrients limitation in ecosystems (Han et al., 2013; Mo et al., 2015). In coastal China, soil nutrient deficiency and imbalance are serious problems (Zhang et al., 2015b; Luo et al., 2017), and it is important to understand the effects of soil nutrient stoichiometry on tree stoichiometry and nutrient resorption along a chronosequence of *M. glyptostroboides* forests.

In this study, we examined the patterns of tree (leaf, branch and root) C, N, and P stoichiometry and nutrient resorption along a chronosequence of *M. glyptostroboides* forests in coastal China. We also analyzed the influences of tree age and soil stoichiometry on tree C, N, P stoichiometry and nutrient resorption. This study aimed to test the following two hypotheses: (1) tree C, N, P concentrations decrease, and N and P resorption efficiency increase as forest stands developed, and (2) soil stoichiometry, varying with stand age, also significantly impact on tree C, N, and P stoichiometry and nutrient resorption. Answering the hypotheses could reveal which nutrient was most critical in stand degradation. The results should provide novel insights for us to better understanding the degradation of *M. glyptostroboides* forest in view of nutrients limitations, and also provide a theoretical foundation for fertilization practices in coastal windbreaks.

### 2. Materials and methods

#### 2.1. Study site

*M. glyptostroboides* is a rare deciduous conifer of the family Cupressaceae. It is native to western Hubei, northern Hunan and eastern Sichuan provinces in central China (Chu and Cooper, 1950; Fu and Jin, 1992). The climate of the native range of *M. glyptostroboides* is characterized as a temperate mesothermal climate with a winter dry season. Following its discovery as a living species, the species has been planted in numerous locations throughout the world, with a wide range of mean annual temperature (4.5–20.8 °C) and precipitation (497–2859 mm) (Williams, 2005). Since 1980s, the species have been planted along eastern coastline of China for controlling coastal erosion and reducing wind damage. *M. glyptostroboides* becomes the main species of shelterbelts in China's coasts (Wu et al., 2013).

The study was conducted in Huanghai forest farm (121.12'E, 32.46'N), which was located in eastern Jiangsu Province, China (Fig. 1). The climate of the study site is that of monsoon subtropical moist marine climate zone. The mean annual temperature, precipitation and potential evapotranspiration are 14.50 °C, 1055.70 mm and 1006.70 mm, respectively. The study site is alkaline sandy soils, which is more infertile than yellow-earth soils in the natural range of *M. glyptostroboides*. The study site was the pioneer area to plant the *M. glyptostroboides* in coastal China. These old *M. glyptostroboides* forests were degrading. Tree growth rate went down from 0.50 (in 7-year old forest) to 0.08 cm year$^{-1}$ (in 37-year old forest, Appendix S1).

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foreco.2018.08.037.

#### 2.2. Experiment design and sample collection

In late August 2015, *M. glyptostroboides* forests of different ages (young forests: 7- and 12-year old; middle aged forests: 22- and 28-year old; mature forests: 33- and 37-year old) were investigated in Huanghai forest farm. These forests were with similar wind disturbance and less human activity. Three replicates of plot, 20 m × 20 m, were established in each age class.

The diameter at breast height (DBH) was measured for all trees in each plot (Table 1). Based on DBH, three average trees in each plot were selected for collecting leaves, branches and roots. Fully expanded leaves and branches from the upper and outer part of tree crowns were sampled. The fine roots (< 2 mm) of each selected individual were sampled through careful removal of the soil surrounding the roots. Five soil cores (2.5 cm in diameter) per plot were randomly collected from 0 to 20 cm depth following removal of understory plants and surface litter, and thoroughly mixed to homogenize a sample. Leaves, branches, roots and soils were sampled in August 2015. Three litter traps (1.0 m$^2$, made of nylon mesh) per plot were fixed 1.0 m above the ground. Leaf litter was collected in late November 2015.

#### 2.3. Chemical measurements

All plant samples were ground with a mechanical grinder after oven drying for 72 h at 60 °C. The C and N concentration were determined for each sample using an autoanalyzer (Kjeltec 2300 Analyzer Unit, Foss, Sweden). P concentration was determined using the standard ammonium molybdate method (reference code GBW08513; General Administration of Quality Supervision, PRC).

Soil samples were air-dried after being sieved (2-mm mesh). Soil organic carbon (C) was determined with wet oxidation by sulfuric acid and potassium dichromate and back titration with ferrous sulfate. Hydrolysable N concentration (N) was determined by titration with dilute solution of H$_2$SO$_4$ after extraction with a mixture of ferrous
sulfate and sodium hydroxide. Available P concentration (P) was
determined with molybdate blue colorimeter after extraction with 0.5 M
calcium bicarbonate.

2.4. Data analysis

Nutrient resorption efficiency (NuRE) was defined as the propor-
tional withdrawal of a nutrient during senescence and was calculated as follows:

\[
\text{NuRE} = \left(1 - \frac{X_{\text{litter}}}{X_{\text{leaf}}}ight) \times \text{MLCF} \times 100\%
\]

where NuRE is N or P resorption efficiency, \(X_{\text{leaf}}\) and \(X_{\text{litter}}\) represent N or P concentration (mass based) in leaf and litter respectively, and

MLCF is mass loss correction factor with a value of 0.745 for deciduous
coniferous species (Vergutz et al., 2012).

One-way ANOVA was used to test the differences of soil, tree C, N, P stoichiometry and nutrient resorption among age classes. Hierarchical partitioning (HP) analysis was used to examine the effects of tree age and soil stoichiometry on tree stoichiometry and nutrient resorption of forests. Regression analyses were performed to test the relationships between tree stoichiometry, nutrient resorption and soil, leaf stoichiometry. All analyses were performed using R statistical platform 3.3.1 (R Development Core Team).

**3. Results**

**3.1. Variations in soil C, N, P stoichiometry along the chronosequence of M. glyptostroboides forests**

Soil C, N, P stoichiometry differed significantly among M. glyptostroboides forests of different ages (Table 1). As forest stands developed, soil C concentration decreased first and then increased, soil P concentration decreased, and soil C:P and N:P increased. However, no change in soil N concentration was found.

**3.2. Variations in tree C, N, P stoichiometry along the chronosequence of M. glyptostroboides forests**

Tree (leaf, branch, and root) C, N concentrations, and C:N showed significant differences among age classes, but displayed no significant trends along the chronosequence (Fig. 2a, b, d). Tree P concentrations were higher, and tree C:P and N:P were lower in young forest (7-12-year) than those in middle aged (22-, 28-year) and mature forests (33-, 37-year) (Fig. 2c, e, f, scatter plots see Appendix S2). HP analysis revealed that tree P stoichiometry (P concentrations, C:P, and N:P) were significantly impacted by tree age and soil stoichiometry (Table 2). Leaf P stoichiometry was impacted more by soil stoichiometry than tree age, while branch and root P stoichiometry was affected more by tree age than soil stoichiometry (Table 2). Leaf P concentration and N:P increased exponentially with soil P concentration and N:P, respectively.
3.3. Variations in litter C, N, P stoichiometry and nutrient resorption along the chronosequence of *M. glyptostroboides* forests

Litter N concentration was lower, and litter C:N was higher in 22-year-old forest than the other forests (Fig. 2b, d). NRE showed non-significant differences among age classes (Fig. 4).

Litter P concentration was lower, and C:P, N:P, PRE were higher in young forests than mature forests (Figs. 2c, e, f and 3, scatter plots see Appendix S2). PRE was impacted more by tree age (Table 3), and was also positively related to leaf P concentration (Fig. 5b).

4. Discussion

Different tree nutrient elements (e.g., N and P) often display different variations along a forest chronosequence (Zhou et al., 2016; Liang et al., 2017). In this study, both tree and litter N concentration showed non-significant trends along a chronosequence of *M. glyptostroboides* forest, which was contrary to Hypothesis 1, but was consistent with some previous studies (Freschet et al., 2010; Hayes et al., 2014; Chen et al., 2015). NRE also remained constant along forest chronosequence, contrary to Hypothesis 1. This did not support the view that tree growth depended more on N resorption to supply its N need with stand development for *L. principis-rupprechtii* (Sun et al., 2016) and *Pinus massoniana* plantations (Liu et al., 2016). A possible explanation was that soil N supply was sufficient after the decomposition of dead organisms (Yang and Luo, 2011).

In contrast to the N, both tree P concentrations and PRE varied greatly along the chronosequence of *M. glyptostroboides* forest. Tree P concentrations were higher in young forests than other forests, supporting Hypothesis 1. The results could be caused in part by the increase in woody biomass (Yang and Luo, 2011; Wu et al., 2011; Lee...
et al., 2012). The proportion of woody biomass increases substantially during stand development. The increasing proportion of woody biomass could lead to a decrease in tree P concentrations because the increasing rate of tree P concentrations are much lower than that in woody biomass. In other words, tree P concentrations were diluted by increasing biomass (Wu et al., 2011; Lee et al., 2012). Moreover, the pattern of tree P concentrations could be due to shifts in tree physiological function from active to passive (Wang et al., 2015). Leaves are mainly responsible for photosynthesis, transpiration, and gas exchange. Roots are responsible for nutrient absorption and support, and branches are responsible for support and storage (Zhang et al., 2017). Whereas, as M. glyptostroboides aging, capacity of P absorption in roots, P transportation and storage in branches, and P metabolism in leave, gradually reduced, which led to the decline in tree P concentrations. This suggested that tree P concentrations were more indicative for forest degradation than other nutrients.

Then, we also found that leaf P concentration were also impacted by soil P concentration, which suggested that leaf was more sensitive to changes in soil nutrients than other tissues (Fernandez-Moya et al., 2013; Schreeg et al., 2014). These results confirmed that soil P supply was an important factor responsible for the variations of tree P concentrations along a chronosequence (Fan et al., 2015), supporting Hypothesis 2. As Table 1 showed, soil P concentration decreased with forest age, which could be explained by the uptake of P by the rapidly growing trees (Fan et al., 2015). Therefore, soil P deficiency was also a contribution for the M. glyptostroboides forests degradation.

PRE were higher in young forests than other forests, while litter P concentration showed an inverse trend. The patterns contrasted with Hypothesis 1 and general results (Ye et al., 2012; Wang et al., 2014; Chang et al., 2017; Yan et al., 2017), which suggested that P resorption played a less important role in conserving P, as P-limitation increased with M. glyptostroboides ageing in our study. Such a result could be ascribed to vary in storage capacity with tree age (Sardans and Peñuelas, 2015). Long tree life is related to a greater capacity to continuously accumulate wood tissues. The wood can thus become an effective reservoir of resources. The old tree, coupled to a large capacity to store P in wood (Sardans and Peñuelas, 2013), reduced the resorption efficiency of P from leaves. This phenomenon showed that the degradation process of metabolically active organs (leaves) possibly was earlier than that of non-functional organs (stems).

Tree C:P and N:P increased along the chronosequence of M. glyptostroboides. These trends reflected that tree P concentrations were limited for the growth in middle aged and mature M. glyptostroboides forests. This was consistent with Huang et al. (2013), who reported that P limitation increased along three successional forests in southern China. Moreover, these results corresponded with the view that P limitation was more pronounced in forest growth and productivity in many subtropical areas (Houlton et al., 2008; Zhou et al., 2016).

Our results revealed that P deficiency was the leading factors, in terms of nutrients, for the M. glyptostroboides forests degradation in coastal China. P addition in soils should an appropriate way to slow the forests degradation. In addition, it is a feasible method that selecting proper low phosphor tolerance breedings of M. glyptostroboides in establishment of coastal windbreaks in future.

5. Conclusions

Tree N concentrations and NRE showed non-significant trends along the chronosequence of M. glyptostroboides forests. Tree P concentrations and PRE were significantly higher, but tree C:P and N:P were significantly lower in younger forests compared to older forests. These results suggested that tree P stoichiometry and PRE were more responsive indicators for M. glyptostroboides forest degradation. Leaf P stoichiometry was impacted more by soil than tree age, while branch, root P stoichiometry and PRE were more affected by tree age.

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