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Responses of *Taxus chinensis* and *Phoebe chekiangensis* seedlings to controlled-release fertilizer in various formulations and application rates

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Decline of species population, low natural regeneration, and heavy competition on field sites require the planting of large seedling stocks to restore *Taxus chinensis* and *Phoebe chekiangensis* in tropical China. In this study, we examined the effects of different formulations and application rates of controlled-release fertilizers (CRF) of nitrogen (N) and phosphorus (P) on nursery seedling growth and nutritional attributes. The objective was to determine optimum formulation (N:P_2O_5 ratio) and application rate to increase nutrient reserves of the seedlings before transplanting to the field. Four formulations (17-9-13 to 19-6-14 N-P_2O_5-K_2O ratios) and four application rates (1.5 kg m^{-3} to 4.5 kg m^{-3}) were used in a double-factors factorial design with 3 replications. The results showed that CRF formulation can affect nutritional attributes, while application rate modified seedling growth and nutritional attributes. The optimum seedling response occurred with the 17-6-16 formulation at the rate of 3.5kg m^{-3}. These findings will guide nursery practice in the production of high-quality seedlings for optimum survival and growth in the field.

Keywords: *Taxus chinensis*, *Phoebe chekiangensis*, Controlled-release Fertilizer, Formulation and Application Rate, Nutrient Utilization Efficiency

Introduction

Taxus chinensis [Pilg.] Rehd. (common name: Chinese yew) and *Phoebe chekiangensis* Shang (common name: Zhejiang Phoebe) are two slow-growing tree species native to tropical China (Deng et al. 2008, Thomas et al. 2013, Zang et al. 2017). The main usage of these species is to harvest timber for manufacture of high end furniture, especially with Zhejiang Phoebe (Wang et al. 2013, Zang et al. 2017). Since the 1990s, natural Chinese yew seeds have been targeted to extract toxoids for the treatment of cancer (Chen et al. 1999). The pharmaceutical use of Chinese yew has heavily impacted the natural populations with a sharp decrease of natural reserves (Thomas et al. 2013), which stimulated the commercial development of this species. Under natural conditions, regeneration of these two species is very difficult (Zang et al. 2017), possibly due to constraints from intense seed dormancy, limited seed dispersal, susceptibility to seed rot, possible animal grazing, and the seed requirements for high moisture and low light during germination, seedling establishment, and early growth (Deng et al. 2008, Zang et al. 2017). Global climate change may also disfavor the natural regeneration of these two species by higher temperatures and increasing drought (Yu et al. 2014, He et al. 2017, Jia & Liu 2017). As a result, both species have been classified as "endangered" in the Red List Category by the International Union for Conservation of Nature (IUCN – World Conservation Monitoring Centre 1998, Thomas et al. 2013). Seedling planting is therefore required for sustaining natural populations and restoring forests for timber harvest (Deng et al. 2008, Zang et al. 2017), especially with the use of competitive seedlings (Wang et al. 2013, Xiao et al. 2015).

Container stock seedlings are superior to bare-root seedlings in terms of survival and growth (Yuan et al. 2012, Wang et al. 2013). The use of polymer-coated controlled-release fertilizer (CRF) at time of planting can promote seedling survival (Irino et al. 2005, Oskarsson et al. 2006, Fu et al. 2017) and early growth (Huat et al. 2002, Hawkins et al. 2005, Irino et al. 2005, Jacobs et al. 2005, Oskarsson et al. 2006, Ruthrof et al. 2010, Burney & Jacobs 2011, Sloan & Jacobs 2013, Fu et al. 2017). It is especially useful to employ CRF to seedlings in the face of harsh site conditions, such as intensive vegetation competition, poor soil nutrient availability, and heavy canopy shade (Devine et al. 2007, Burney & Jacobs 2011, Sloan & Jacobs 2012, Earnshaw et al. 2016). These types of sites are especially hard to reforest with endangered tree species because these species typically grow slower than competitors and are more sensitive to field conditions (Wang et al. 2013).

CRF can also be used to enhance nursery seedling quality through nutrition-loading

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before transplanting (Irino et al. 2004, Zamunér Filho et al. 2012, Klooster et al. 2012, An et al. 2018). In southern nurseries of China, one of the widely-used CRFs by nursery managers is Apex™ (J.R. Simplot Company, USA) which contains nitrogen (N), phosphorus (P, P_2O_5), and potassium (K, K_2O) in a ratio of 18-8-8 and is typically applied at 2.5 kg m^{-3}. This CRF application rate is often supplemented with the application of urea and ammonium phosphate to promote seedling growth. The current formulation and application rate with Apex for container seedlings is largely based on empirical observations, thus significant knowledge gaps exist in terms of the scientific employment of CRF with respect to inherent nutrient uptake and utilization by target seedlings.

There is supporting evidence that seedlings with enhanced nutrient reserves are expected to exhibit increased survival and growth after field transplanting (Reddell et al. 1999, Duan et al. 2013, Wei et al. 2013, Fu et al. 2017, Li et al. 2017), through improved nutrient uptake and enhanced photosynthesis (Irino et al. 2004, Morikawa et al. 2006, Klooster et al. 2012). Among all the elements required by tree seedlings, N and P are the most limiting and therefore generally contained in CRF application (Oskarsson et al. 2006, Ruthrof et al. 2010, Klooster et al. 2012, Sloan & Jacobs 2013, Earnshaw et al. 2016, Zhu et al. 2016, Fu et al. 2017). Both CRF formulation (namely, $N:P_2O_5$ ratio) and application rate influence seedling performances (Oliet et al. 2004, Xiao et al. 2015, Li et al. 2017), but the suggested empirical formulation for optimum growth from previous reports is highly variable (Oliet et al. 2004, Zhao et al. 2010, Chu et al. 2012). In fact, little information is available regarding the optimum combination of CRF formulation and application rate.

In this study, two-year-old containerized seedlings of Chinese yew and Zhejiang Phoebe were treated for one growing season with different formulations and application rates of N and P using CRF. The objective was to determine the combination of CRF formulation and application rate for optimum seedling growth and nutritional attributes, including nutrient recovery and utilization. It was hypothesized that in relation to the standard application of Apex (i) formulations with higher $N:P_2O_5$ ratios would improve seedling growth and nutritional attributes; and (ii) greater application rates would improve seedling growth and nutritional attributes (Wang et al. 2013).

Materials and methods

Study site

The study was conducted in the experimental forest nursery (27° 38′ N, 119° 01′ E) at Qingyuan, Zhejiang, China. The study site belongs to the sub-tropical monsoon region, with elevation of 510 m a.s.l., mean annual temperature of 17.6 °C, mean annual precipitation of 1721.3 mm, and frost-free growing season of 245 days. The experiment was set up in an open steel greenhouse equipped with an automated irrigation system. The greenhouse was 2.2 m from ground to roof and had an average light transmittance around 50%.

Study material

In late April of 2013, Chinese yew and Zhejiang Phoebe seedlings (40 days old after germination) were transplanted into non-woven fabric containers of 5 cm diameter × 10 cm height that were filled with a growing substrate (peat and bran in 7:3 in volume) mixed with 2.5 kg Apex™ (J.R. Simplot Company, USA) per cubic meter. The substrate for CRF treatments was a mixture of peat, chaff, and mineral soil (40%:30%:30% in volume). The mixture had total N of 14.2 g kg^{-1}, total P of 0.7 g kg^{-1}, total potassium (K) of 2.7 g kg^{-1}, bulk density of 0.3 g cm^{-3}, and pH of 6.0. Using an automated irrigation system, seeded substrates were watered twice a day (10-15 minutes in the morning and evening) except for rainy days. In early December, the seedlings reached an average height of 43 cm and root-collar diameter (RCD) of 4.34 mm for Chinese yew, and an average height of 31 cm and RCD of 4.58 mm for Zhejiang Phoebe, based on random samples of 20 seedlings per species. The measurements of the 20 random samples indicated the total seedling N and P of 5.14 mg and 1.87 mg for Chinese yew and of 9.94 mg and 4.50 mg for Zhejiang Phoebe, respectively.

CRF treatment

On April 21, 2014, the two-year-old seedlings were transplanted into larger non-woven fabric pots (18 cm diameter × 20 cm height). CRF granules (Jinzhengda Co. Ltd., Shandong, China), which should provide a continuous supply of nutrients for 6-7 months at 20°C, were incorporated into the potting substrates. The CRF treatments followed a two-way factorial design with four levels of formulation and four levels of application rate. The four formulations (F) differed in concentrations of $N:P_2O_5:K_2O$ (Tab. 1) with four $N:P_2O_5$ratios ranging from 17:9 (F1, reference level) to 19:6 (F4, emulating summer application of additional urea and ammonium phosphate at nursery). The four application rates (A) ranged from 1.5 kg m^{-3} (A1) to 4.5 kg m^{-3} (A4), with the 2.5 kg m^{-3} (A2) being considered a standard rate in southeast China nurseries. The lowest application rate (A1) was designed as a baseline to obtain comparable seedling performance at reduced CRF, while the highest rate (A4) was to evaluate possible gain from enhanced fertilization. These treatments were replicated three times with thirty seedlings assigned to each combination of formulations and application rates. In total, 1440 seedlings (4 formulations × 4 rates × 3 replications × 30 seedlings) were tested for each species. Fans and curtains were used to increase air movement and reduce sunlight when temperatures were above 30 °C during the experiment.

Tab. 1 - Formulation (F) and application rate (A) of controlled-release fertilizers for Chinese yew and *Phoebe chekiangensis* seedlings.

Formulation treatment	$N-P_2O_5-K_2O$ ratio	Application treatment	Application rate (kg m^{-3})	N rate (g $plant^{-1}$)	P rate (g $plant^{-1}$)
F1	17-9-13	A1	1.5	1.30	0.69
		A2	2.5	2.16	1.14
		A3	3.5	3.03	1.60
		A4	4.5	3.89	2.06
F2	16-7-16	A1	1.5	1.30	0.69
		A2	2.5	2.16	1.14
		A3	3.5	3.03	1.60
		A4	4.5	3.89	2.06
F3	17-6-16	A1	1.5	1.30	0.69
		A2	2.5	2.16	1.14
		A3	3.5	3.03	1.60
		A4	4.5	3.89	2.06
F4	19-6-14	A1	1.5	1.30	0.69
		A2	2.5	2.16	1.14
		A3	3.5	3.03	1.60
		A4	4.5	3.89	2.06

Seedling harvest, measurement and statistical analysis

Seedlings were destructively sampled in early December when current growth was complete. Ten randomly-selected seedlings from each treatment combination were measured for height (cm) and RCD (mm) and destructively sampled. For biomass determination, each of the sampled seedlings were washed, separated into leaves, stems and roots, and oven-dried for three days at 68 °C. The dried samples were then ground and sieved through 1.5 mm-mesh, and digested with H_2SO_4-H_2O_2 (Xiao et al. 2015). N and P concentrations were determined using the Kjeldahl method (Xiao et al. 2015) and ICP-OES (Vista-Mpx, Varian®, USA), respectively (Wei et al. 2013).

The Dickson's seedling quality index (*DQI*) (Dickson et al. 1960) was chosen to provide an integrated seedling quality assessment (Li et al. 2017 – eqn.1):

$$DQI=\frac{BAP}{(Height/RCD)+(SB/RB)} \quad (1)$$

where *BAP* is the total seedling biomass (g), *SB* is the shoot biomass (g), and *RB* is the root biomass (g).

Nutrient utilization index (*UI*) was used to evaluate photosynthetic production per unit leaf nutrient mass (Hawkins 2007 – eqn. 2):

$$UI=\frac{BAP}{\%C_{leaf}} \quad (2)$$

where *UI* is N/P utilization index (*NUI*/*PUI*), and $\%C_{leaf}$ is percent leaf nutrient concentration.

Nutrient (N or P) recovery ($C_{recovery}$) was calculated using the following equation (eqn. 3):

$$C_{recovery}=\frac{C_{uptake}-C_{initial}}{C_{fertilizer}}\cdot 100 \quad (3)$$

where C_{uptake} is the seedling nutrient total (mg) at end of the treatments, $C_{initial}$ is the seedling nutrient total prior to CRF treatment (mg), and $C_{fertilizer}$ is the total nutrient supply from CRF.

The utilization efficiency of nutrient uptake by mass input to root (*EuU*) was calculated for N (*ENuU*) and P (*EPuU* – eqn. 4):

$$EuU=\frac{C_{uptake}}{RB} \quad (4)$$

Data analyses followed a two-way factorial design with four levels of CRF formulation and four application rates using the GLM procedure available in SAS (SAS Institute Inc., NC, USA). Measured response variables included height, RCD, total biomass, biomass allocations (component biomass to seedling biomass ratio), R/S ratio (root to stem biomass ratio), *DQI* (Dickson seedling quality index), N and P concentrations, and N and P uptake (seedling total) and allocations (ratios by biomass components). Means were compared with the *post-hoc* Tukey test when treatment effects were significant. When interactive effects of formulation by application rate were significant, data were re-analyzed with one-way ANOVA of sixteen treatments (four formulations by four application rates). Pearson's correlation was calculated among response variables of seedling growth, biomass, nutrient uptake and utilizations.

Results

Growth and biomass

The CRF formulation only affected the stem biomass allocation of Chinese yew seedlings (Tab. 2), with values significantly lower in the F4 (mean ± SE: 38.88 ± 3.18 %) than in the F1 (42.20 ± 2.10 %), F2 (41.14 ± 2.63 %) or F3 (42.07 ± 3.46 %) treatment. Comparatively, the impact of application rate was greater, affecting most of the growth and biomass variables of the two species (Tab. 2). The highest growth and biomass were observed in the A3 treatment, which included height, RCD, seedling biomass and *DQI* of Chinese yew seedlings, and height and seedling biomass of Zhejiang Phoebe seedlings (Tab. 3). The CRF rate also affected stem and root biomass allocations and the root to shoot biomass ratio (R/S) of Zhejiang Phoebe seedlings, with the mean value significantly greater in the A1 treatment for root biomass allocation and R/S, and in the A4 treatment for stem biomass allocation (Tab. 3).

Nutritional attributes

In both species, N and P concentrations and uptakes generally peaked at F3 (Tab. 2, Fig. 1). The effect of application rate varied with species, nutrient type, and biomass component (Fig. 1), as well as with N:P_2O_5 ratio (significant formulation by application rate interactions – Tab. 2). In comparison, the relationship of nutrient uptake with application rate was more consistent with the highest N and P uptake observed at the A3 treatment in both species.

More N and P were allocated to leaf and root than to stem (Fig. 2) in both species. Most allocation variables significantly changed with CRF formulation and application rate (Tab. 2), but the general trends differed by species, biomass components and nutrient types (Fig. 2). For example, root N and P allocations increased, while stem P allocation decreased with the increase of N:P_2O_5 ratio from F1 to F4 in Chinese yew seedlings (Fig. 2a, Fig. 2c). The same formulation change, however, reduced leaf N allocation and increased stem P allocation in Zhejiang Phoebe seedlings (Fig. 2b, Fig. 2d). Peak stem N allocation in Chinese yew and leaf N allocation in Zhejiang Phoebe occurred at F2. These trends, however, varied with application rate (significant treatment interactions – Tab. 2).

Nutrient recovery and utilization

Nutrient recovery decreased with the increase of application rate (A1 to A4, *P*< 0.01 in both species), with N recovery from 23.33% to 10.11% and P recovery 5.0% to 1.77% in Chinese yew seedlings and N recovery

Tab. 2 - *P* values from ANOVA on the effects of different formulations (F) and application rates (A) of controlled-release fertilizers on the growth and nutrient attributes of *Taxus chinensis* and *Phoebe chekiangensis* seedlings. (RCD): root collar diameter; (Leaf biomass allocation): leaf biomass to seedling biomass ratio; (R/S): root to shoot biomass ratio; (*DQI*): Dickson's seedling quality index; (Leaf N allocation): leaf total N to seedling total N ratio.

Variables	*Taxus chinensis*			*Phoebe chekiangensis*		
	F	A	F × A	F	A	F × A
Height	0.098	<0.001	0.844	0.101	<0.001	0.829
RCD	0.077	<0.001	0.165	0.589	0.088	0.292
Seedling biomass	0.200	<0.001	0.607	0.297	<0.001	0.219
Leaf biomass allocation %	0.884	0.966	0.243	0.128	0.064	0.550
Stem biomass allocation %	0.030	0.274	0.578	0.736	0.001	0.068
Root biomass allocation %	0.199	0.649	0.703	0.211	<0.001	0.094
R/S	0.141	0.274	0.897	0.169	<0.001	0.140
DQI	0.484	<0.001	0.914	0.808	0.141	0.481
Leaf N concentration	0.002	<0.001	<0.001	<0.001	<0.001	0.011
Stem N concentration	<0.001	<0.001	<0.001	<0.001	<0.001	0.002
Root N concentration	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Leaf P concentration	<0.001	<0.001	<0.001	<0.001	0.026	0.024
Stem P concentration	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Root P concentration	<0.001	0.001	<0.001	<0.001	<0.001	0.001
Seedling total N uptake	0.359	<0.001	0.047	<0.001	<0.001	0.423
Leaf N allocation %	<0.001	0.158	0.192	0.008	0.033	0.002
Stem N allocation %	<0.001	0.002	<0.001	0.302	<0.001	0.026
Root N allocation %	0.007	0.083	0.625	0.034	0.040	0.005
Seedling total P uptake	0.049	<0.001	0.223	0.001	0.001	0.194
Leaf P allocation %	0.118	0.127	0.009	<0.001	<0.001	0.018
Stem P allocation %	<0.001	<0.001	<0.001	<0.001	0.177	0.038
Root P allocation %	<0.001	0.741	0.069	0.868	<0.001	0.034

Tab. 3 - Mean comparisons of *Taxus chinensis* and *Phoebe chekiangensis* seedling growth and biomass by different application rates of controlled-release fertilizer treatments. (RCD): root collar diameter; (Leaf biomass allocation %): leaf biomass to seedling biomass ratio; (*DQI*): Dickson's seedling quality index. Different letters indicate significant difference (p<0.05) after Tukey test.

Growth variables	Application treatment	n	*Taxus chinensis*			*Phoebe chekiangensis*		
			Mean	SE	diff	Mean	SE	diff
Seedling height (cm)	A1	12	87.81	4.11	bc	77.81	3.01	c
	A2	12	89.84	4.04	bc	82.06	7.23	bc
	A3	12	104.08	4.88	a	92.12	4.24	a
	A4	12	92.6	4.43	b	87.33	3.72	ab
RCD (mm)	A1	12	8.38	0.38	b	11.1	0.54	a
	A2	12	8.81	0.47	b	11.71	0.68	a
	A3	12	9.59	0.61	a	11.78	0.65	a
	A4	12	8.84	0.42	b	11.67	0.65	a
Seedling biomass (g)	A1	12	29.7	2.84	c	51.5	8.81	c
	A2	12	35.3	4.28	b	60.9	7.24	b
	A3	12	43.9	6.16	a	67.7	7.53	a
	A4	12	33.5	4.13	bc	62.7	6.28	ab
Leaf biomass allocation (%)	A1	12	28.1	2.35	a	30.6	2.25	b
	A2	12	28.5	2.66	a	32.4	1.85	a
	A3	12	28.2	2.44	a	33.0	2.01	a
	A4	12	28.4	1.53	a	32.0	2.11	ab
Stem biomass allocation (%)	A1	12	41.8	2.32	a	36.8	2.48	c
	A2	12	41.6	1.58	a	38.8	2.13	b
	A3	12	39.9	4.05	a	38.6	2.42	bc
	A4	12	41.0	3.51	a	40.9	2.31	a
Root biomass allocation (%)	A1	12	30.1	4.03	a	32.5	3.11	a
	A2	12	30.0	3.06	a	28.8	2.38	b
	A3	12	31.9	4.99	a	28.3	1.58	b
	A4	12	30.5	4.47	a	27.1	2.46	b
R/S	A1	12	0.44	0.08	a	0.48	0.07	a
	A2	12	0.43	0.06	a	0.41	0.05	b
	A3	12	0.48	0.11	a	0.40	0.03	b
	A4	12	0.45	0.09	a	0.37	0.05	b
DQI	A1	12	0.36	0.04	b	0.72	0.13	a
	A2	12	0.41	0.05	b	0.86	0.19	a
	A3	12	0.52	0.08	a	0.85	0.10	a
	A4	12	0.4	0.05	b	0.8	0.10	a

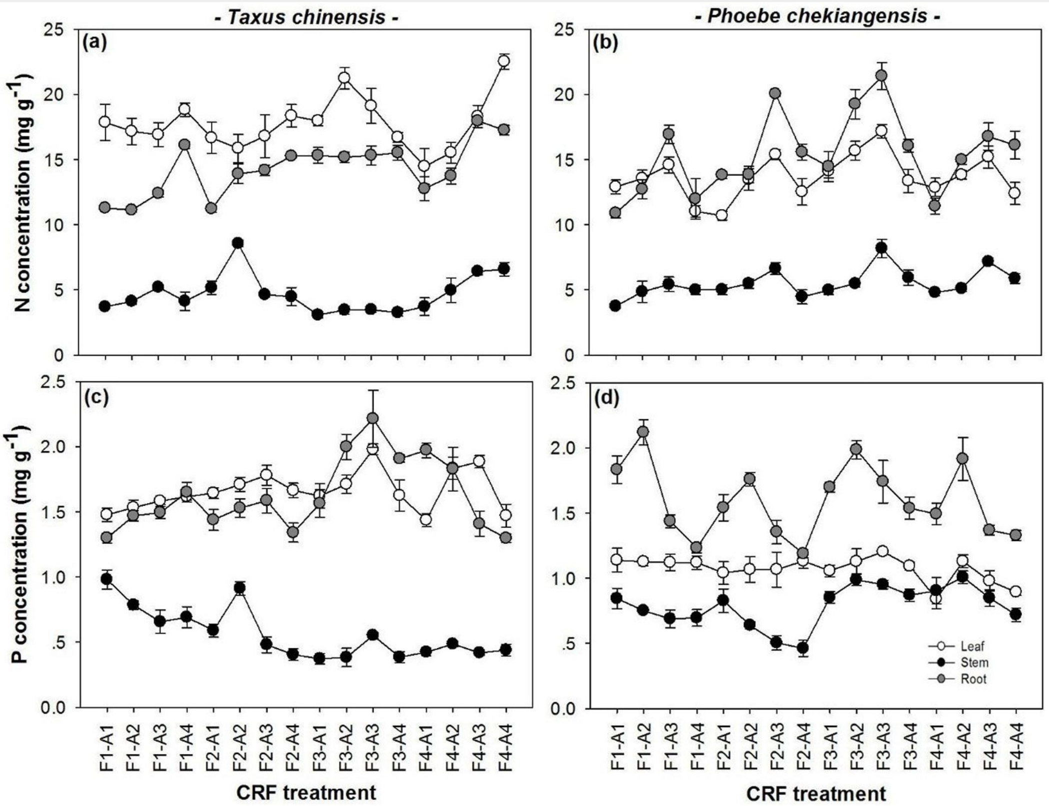

Fig. 1 - N and P concentrations in leaves, stems and roots of *Taxus chinensis* and *Phoebe chekiangensis* seedlings in response to the interactive effects of controlled-release fertilizer formulation (F1-F4) and application rate (A1-A4).

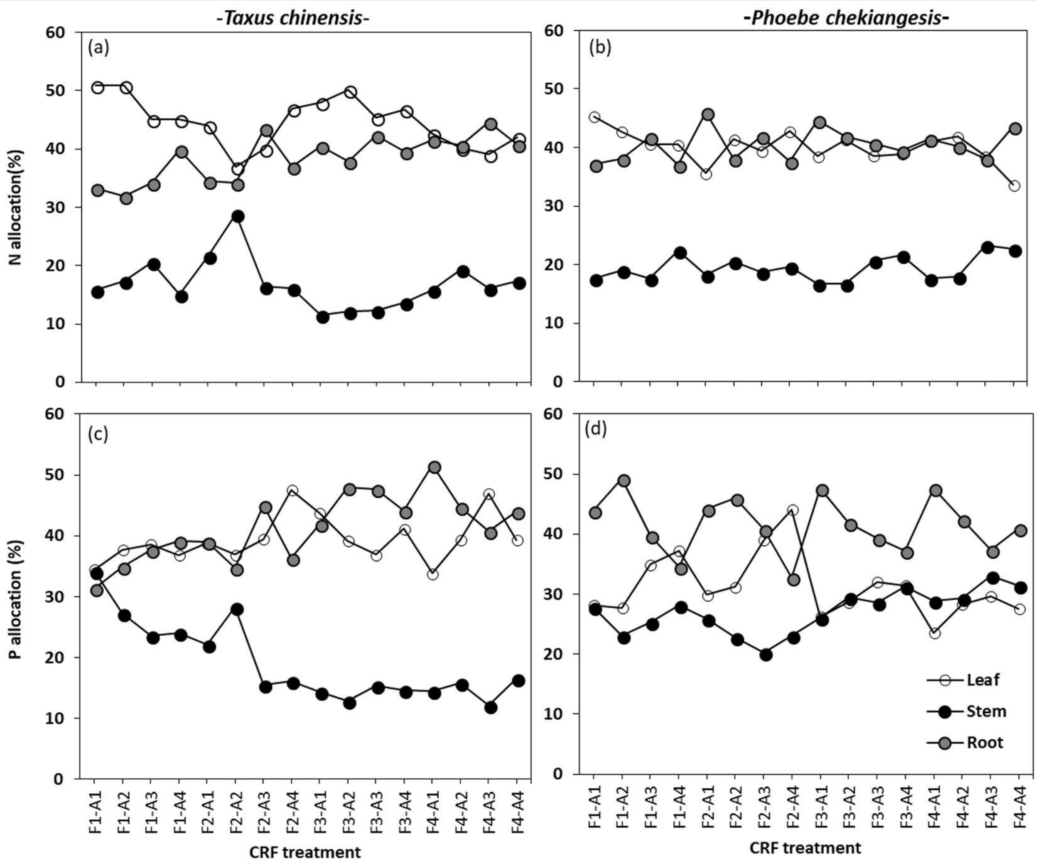

Fig. 2 - N and P allocation (%) to leaves, stems and roots of *Taxus chinensis* and *Phoebe chekiangensis* seedlings in response to the interactive effects of controlled-release fertilizer formulation (F1-F4) and application rate (A1-A4).

from 38.63% to 16.12% and P recovery 8.17% to 2.95% in Zhejiang Phoebe seedlings, but peaked at formulation 3 (F3) in both species (*P*<0.05 only in Zhejiang Phoebe). Interactive effect of formulation by application rate was significant only in Chinese yew where the decreasing pattern of N recovery with application rate was slightly different in F1 and F4.

Nutrient utilization index (*UI*) was generally highest in the A3 treatment for Chinese yew (*NUI*: 6.95 ± 0.86, *P*<0.01; *PUI*: 68.45 ± 9.04, *P*=0.003) and the A4 treatments for Zhejiang Phoebe (*NUI*: 16.08 ± 2.12, *P*=0.001; *PUI*: 187.09 ± 22.28, *P*<0.01). The *NUI* patterns with application rate also varied with formulation in both species (significant interactions).

In Chinese yew, the utilization efficiency of nutrient uptake (*EuU*) was only affected by application rate for N (*P*=0.032) and formulation for P (*P*=0.028), with the highest value observed at A4 (41.13) and F3 (4.27), respectively. In Zhejiang Phoebe, the highest *ENuU* and *EPuU* were recorded at F3 (43.02 and 4.23, respectively; *P*<0.01) and A3 orA2 (46.28 and 4.36, respectively; *P*<0.01) with some variations (significant interactions).

Association among performance variables

In Chinese yew seedlings, height, RCD and biomass were positively correlated with N uptake, P uptake, *NUI* and *PUI*, but not with leaf N concentration (Fig. 3a). Comparatively, leaf P concentration was correlated, not only with height and biomass, but also with *DQI*. Leaf N concentration was positively associated with *ENuU* and *EPuU*, both of which were negatively correlated with P recovery.

In Zhejiang Phoebe seedlings, correlation was positive between height and leaf N + P concentrations, between N uptake and height + RCD + biomass, between R/S and N and P recoveries (Fig. 3b), between P uptake and RCD + biomass, between N + P uptakes and *DQI*, between biomass and *NUI* + *PUI*, and between *EnuU*+ *EPuU* and N + P uptakes, but negative in the relationship of

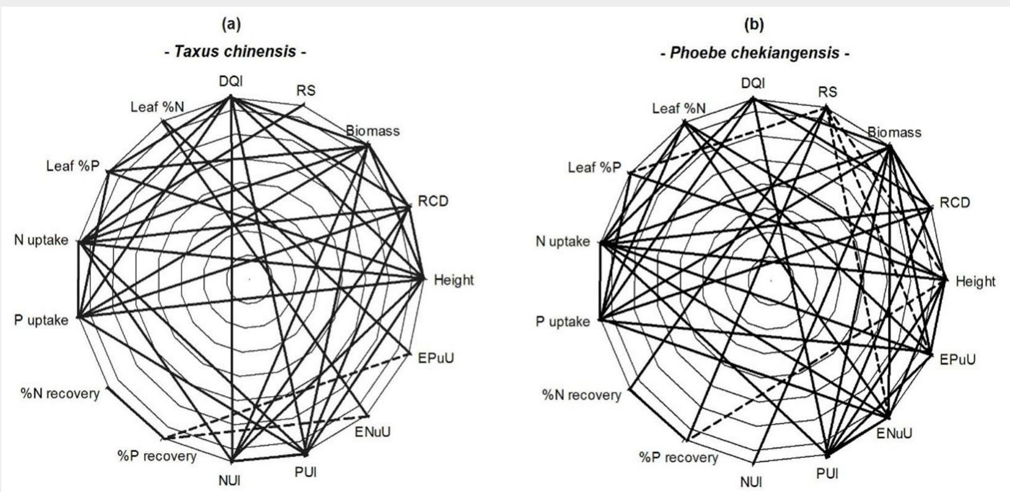

Fig. 3 - Pearson's correlations among seedling growth and nutritional attributes of height, root-collar diameter (RCD), biomass, root to shoot biomass ratio (RS), Dickson seedling quality index (*DQI*), leaf N concentration (leaf %N), leaf P concentration (leaf %P), total N uptake, total P uptake, N recovery, P recovery, N and P utilization index (*NUI* and *PUI*), and utilization efficiency of N and P uptake (*ENuU* and *EPuU*). Solid lines indicate positive correlations and dashed lines indicate negative correlations.

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R/S with height, leaf P concentration, *ENuU* and *EPuU*.

Discussion

Our first hypothesis was only partially confirmed by the findings of this study. The change of CRF formulation did not affect seedling size, biomass, and biomass allocation, possibly due to the similar influences of N and P on the growth of Chinese yew and Zhejiang Phoebe seedlings. Similar responses were found by Walker & Huntt (2000) in *Pinus jeffreyi* seedlings and Oliet et al. (2004) on the root growth of *Pinus halepensis* seedlings. Our results, however, are different from those by Oliet et al. (2004) who found that high N ratio promoted shoot growth and therefore reduced R/S ratio in *Pinus halepensis* seedlings in nursery. Jacobs et al. (2005) and Earnshaw et al. (2016) also indicated that high N ratio in CRF can enhance biomass accumulation of transplanted seedlings in field conditions. Therefore, despite the significant effect of N input on tree seedling growth, a higher proportion of N component in CRF cannot be responsible for the higher quality for tree seedlings of all species.

Although seedling quality index by growth and biomass did not substantially change with formulation, most seedling nutrient variables changed significantly in response to the different formulation levels. Leaf and root nutrient concentrations respond to the change of CRF formulation and contribute heavily to the variation in shoot and root growth (Walker & Huntt 2000, Oliet et al. 2004). Hawkins (2007) discerned that seedlings with high nutrient utilization index tend to produce more biomass. Similarly, in this study, most of growth variables and DQI were positively related with N and P uptakes and *PUI* (Fig. 3). Our results support the connections between seedling growth and DQI (Li et al. 2017).

As expected, seedling height, RCD, biomass, *DQI*, and nutrient uptake all increased with application rate from A1 to A3, consistent with findings by many others (Irino et al. 2004, Klooster et al. 2012, Zamunér Filho et al. 2012, Santelices et al. 2013, Fu et al. 2017). The decrease of growth parameters from A3 to A4 may result from toxic uptake (Oliet et al. 2004). The decrease of nutrient recovery with application rate, as reported by Oliet et al. (2004), indicates a greater leachate with increasing nutrient supply (Oliet et al. 2004). With an increase of application rate, only Zhejiang Phoebe demonstrated a common response of plants to resource change where there is a reduction in the biomass allocation to roots (Wang et al. 2013, Crick & Grime 1987, Hermans et al. 2006), possibly due to greater seedling biomass, than Chinese yew, and therefore pot restriction on root development.

The stronger response of seedling growth to application rate than to the change of $N:P_2O_5$ ratio suggest that N and P elements are equally important to the growth of tree seedlings (Huat et al. 2002, Klooster et al. 2012, Wang et al. 2013, Li et al. 2017), despite some possible influences of formulation on seedling nutrient uptake (Walker & Huntt 2000, Oliet et al. 2004). The equal significance of N and P to Chinese yew and Zhejiang Phoebe seedlings is also revealed from strong correlations of N and P concentrations and uptakes with growth and utilization efficiency of nutrient uptake (*EuU*). In both species, nutrient utilization efficiency was determined primarily by biomass production (Hawkins 2007), as demonstrated from similar treatment response patterns with biomass and strong correlations of utilization index with growth, biomass, and *DQI*, but not with leaf N or P concentrations.

Greater nutrient allocations indicate that leaves and roots are the main nutrient storage of the plants and contribute significantly to new shoot and root growth (Walker & Huntt 2000, Oliet et al. 2004), as also shown by the positive growth correlation with leaf N and P concentrations (Oliet et al. 2004) and uptakes (Fig. 3). Likely, the allocation to leaves would have increased in an earlier season of the year as winter hardening (December) would promote nutrient allocation to stem for winter storage and cold resistance (Zhu et al. 2016, Li et al. 2017).

Substrate property is critical for production of high-quality container seedlings through modified nutrient uptake and utilization. Our study showed a higher nutrient utilization (*NUI*>4.0 and *PUI*>50) for both *Taxus chinensis* and *Phoebe chekiangensis* relative to the values reported previously, *e.g.*, 1.3-1.8 (Hawkins 2007), 1.5-2.1 (Li et al. 2017), and 1.72-4.74 (Li et al. 2018) for *NUI*, and *PUI* < 40 (Li et al. 2017, 2018). These differences likely result from the differences in potting mixtures that are generally mixed by peat and perlite in previous studies. In our study, the mixing chaff and soil with peat has apparently increased total N, compared to the potting mixtures used by others, *e.g.*, *Pinus contorta* (1.2 g kg^{-1} – Amponsah et al. 2004) and *Larix olgensis* (5.7 g kg^{-1} – Wei et al. 2014). The high N and P would favor root development and hence help nutrient uptake, utilization, and recovery (10-38% and 3-9%, respectively) of the two subtropical seedlings, compared to the nutrient recovery values reported by others, *e.g.*, 17-28% in *Pinus contorta* (Amponsah et al. 2004) and 30-56% in *Larix olgensis* (Wei et al. 2014). Therefore, the substrate used in this study is appropriate and unlikely has undesired impacts on seedlings.

Conclusions

The increase of $N:P_2O_5$ratio in CRF formulation did not affect seedlings growth, suggesting that N and P are equally important to Chinese yew and Zhejiang Phoebe seedlings. The optimum nutritional attributes generally occurred at formulation F3 (17-6-16 for $N-P_2O_5-K_2O$), and optimum growth at application rate A3 (3.5 kg m^{-3}). The higher $N:P_2O_5$ ratio (F4, 19-6-14 for $N-P_2O_5-K_2O$) reduced nutrient recovery, utilization index, and utilization efficiency of uptake, possibly due to P limitation, while higher application rate (A4, 4.5 kg m^{-3}) may have resulted in toxic effects on growth. Different interactive effects of formulation by application rate on nutritional attributes indicate a lack of consistent optimum treatment combination for different nutritional attributes. These findings need to be confirmed through connection of seedling growth and nutrient parameters with field performance.

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