INFLUENCE OF DIFFERENTIAL NITROGEN FORMS ON SEEDLING MORPHOLOGY OF FOUR WIDELY GROWN SPECIES IN SOUTHERN CHINA

GILANI, M. M.[1,2,3] – TIGABU, M.[1] – FAROOQ, T. H.[4] – RASHID, M. H. U.[5] – YASIN, G.[2] – ZUBAIR, M.[2] – AZHAR, M. F.[2] – CAI, L. P.[1,3] – AKRAM, M. A.[6]– LIU, B.[7*] – MA, X. Q.[1,3*]

[1]*Forestry College, Fujian Agriculture and Forestry University, Fuzhou, 350002 Fujian Province, PR China*

[2]*Department of Forestry and Range Management, Bahauddin Zakariya University, Multan 66000, Pakistan*

[3]*Cross-Strait Collaborative Innovation Center of Soil and Water Conservation, Fuzhou, 350002 Fujian Province, PR China*

[4]*Bangor College China, a joint unit of Bangor University and Central South University of Forestry and Technology, Changsha 410004, China*

[5]*Department of Forestry and Range Management, University of Agriculture, Faisalabad 38000, Pakistan*

[6]*State Key Laboratory of Grassland Agro-ecosystems, College of Ecology, Lanzhou University, Lanzhou 730000, China*

[7]*School of Life Sciences, Qufu Normal University, Qufu 273165, Shandong Province, China (phone: +86-137-9940-8187)*

[*]*Corresponding author*
e-mail: lxymxq@126.com; lboshandong@126.com

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Abstract. The rise in environmental nitrogen (N) is a worldwide concern, particularly in southern China, which contains highly N-contaminated areas. The ecological outcome of the increase in N should be paid additional consideration. Thus, this study was conducted to assess the impact of N on seed germination and seedling emergence of four timber species in subtropical southern China. We conducted a growth chamber experiment with three nitrogen fertilizers i.e., Ammonium sulfate $(NH_4)_2SO_4$, Ammonium Nitrate (NH_4NO_3), and Potassium Nitrate (KNO_3) with three concentrations (0.1%, 0.5% & 1%) along with control treatment (CK). We hypothesized that the impact of N on seed germination and seedling emergence would differ among species and N levels. Results showed that higher N concentration affected *Cunninghamia lanceolata, Fokienia hodginisi* and *Pinus massoniana* seedlings compared with *Phoebe zhennan*. Overall, fresh and dry weight as well as the length of broad-leaved species was better than coniferous and pine species. It was observed that 1% KNO_3 solution enhanced the germination capacity, germination time, radicle, and hypocotyl elongation along with fresh and dry weights as compared to $(NH_4)_2SO_4$ and NH_4NO_3. The outcome showed that deposition of 1% KNO_3 is promising to increase the germination of seed and seedlings emergence.

Keywords: *Nitrogen, growth dynamics, germination capacity, germination time, subtropical species*

Introduction

The rise in environmental nitrogen (N) is a worldwide concern, particularly in southern China, which contains highly N-contaminated areas (Baijuan et al., 2022). The atmospheric N deposition level in China is far more than North America and Europe. To circumvent the increased greenhouse gas emission, China gradually increased its area under forest plantations (Gilani et al., 2021) and southern China has the highest percentage (54.3%) of forest cover (Farooq et al., 2019). N is a vital nutrient for plant growth. In numerous species of plants, N encourages germination of seeds at low and high levels and serves as a signal and a nutrient as well (Duermeyer et al., 2018). For a broad range of plant species, N is a germination stimulator (Pill et al., 1991) and the response varies with variation in environment conditions, such as light, moisture, seed storage time and temperature (Shim et al., 2008).

Germination of seed is a vital process in the life of a plant, its dynamics can offer valuable evidence about the beginning, consistency, and ending of germination. For instance, two seed lots can have a similar sprouting percentage but vary in consistency and speed (Joosen et al., 2010). Moreover, Basra et al. (2005) found that N could result in good germination and emergence in crops like maize, wheat, rice and canola. Furthermore, Farooq et al. (2006) stated that N application resulted in an increase in the germination percentage, germination rate, consistency in the germination speed, and development in plant growth and speed of the flowering stage. Various N fertilizers like $(NH_4)_2SO_4$, NH_4NO_3 and KNO_3 are used to stimulate germination of seed and breaking of seed dormancy.

The influence of elevated N content on germination of seeds relies on the range of N concentrations and species. Numerous findings have assessed the impact of N (NH_4^+ and NO_3) on germination of seeds and consequent decrease in dry grasslands along with decline in species richness (Tipping et al., 2013). KNO_3 is a vastly utilized chemical for stimulating germination and its concentration of 0.1 to 0.2% is very common in regular germination analysis and it is suggested by the Association of Official Seed Analysis (AOSA) and the International Seed Testing Association (ISTA) for germination trials of numerous species (Copeland and McDonald, 2012). Nitrate like KNO_3 visibly stimulates the germination of dormant seeds (Alboresi et al., 2005). In some studies, the encouraging influence of N in breaking dormancy has been noticed in soils (Franco-Vizcaíno and Sosa-Ramirez, 1997) and on filter paper moistened with nitrate solutions (Pérez-Fernández et al., 2006). Though increasing levels of N have been well studied in the natural atmosphere (Suding et al., 2005) but not much study has been done on how the rise in N accessibility to forests associated with enhanced N may affect species carrying large seeds. *Cunninghamia lanceolata* is an economically valuable tree known for its high yield and wood quality. *Pinus massoniana* is a woody and evergreen coniferous species in the sub-tropical area famous for paper manufacturing. *Fokienia hodginisi* is well-known as vulnerable worldwide through its distribution range and vital in addressing seed pretreatment, storage conditions, germination ecology, maintenance, and sustainable harvest of this forest. *Phoebe zhennan* is valued for its high timber quality, fragrance, attractive surface color, and medicinal values. Its seed vigor is beneficial for enhancing the process of storing and technology of seedlings cultivation. The present trial is a rare one to report these species' question regarding seed germination and various seed pretreatment. To best of our knowledge, germination of weedy or crop species has frequently been studied, and little attention has been paid to seed growth dynamics of timber species in southern China. This is particularly important as the atmospheric N deposition level is very high in southern China. Previous studies have shown that N treatment exerts a stimulatory, inhibitory, or no influence on the germination of seeds (Haden et al., 2011; Varma et al., 2016).The objectives of this research was to (1) observe the effect of N deposition on the germination of seeds and seedling emergence depending upon the N sources and the concentration of N; and (2) whether the effects differ between species (broadleaved versus coniferous species).

Materials and Methods

In the current trial, seeds were collected from Forest Farm in Fuzhou city. Fujian province has a subtropical climate with high temperatures in summer. Average annual precipitation is 1400 to 2000 mm. Seeds were collected from ten individual species during seed maturity time and kept at 4 °C for limited time before trial.

Treatments

We prepared 0.1%, 0.5%, and 1% solutions of $(NH_4)_2SO_4$, NH_4NO_3 and KNO_3 by mixing them in 1000 ml water. Seeds were treated with these concentrations (0.1% 0.5% and 1%) and N sources ($(NH_4)_2SO_4$, NH_4NO_3 and KNO_3) for 24 hours at room temperature and distilled water as control.

Treatments	**Concentrations**
$(NH_4)_2SO_4$	(0.1% , 0.5% , 1%)
NH_4NO_3	(0.1%, 0.5%, 1%)
KNO_3	(0.1%, 0.5%, 1%)

After treatment with different N treatments, the germination trail was carried out in Fujian Agriculture and Forestry University laboratory. Seeds were disinfected by treating them with concentrated $KMNO_4$ for 20 minutes before sowing and then washed with distilled water for five minutes. Petri dishes of 9 cm in diameter were used for sowing seeds. To each Petri dish was added 2 ml of N solutions or distilled water when the filter papers were about to dry out. For each treatment, 50 seeds were used in each dish with six replicates. Growth chamber temperature was 25 ± 1 °C in day and 12/12 hours a day/night light of 20 µE (Fluorescent lamp F 40 M/33 RS white light) for 30 or 50 days. Seeds that germinated were counted. Fresh and dry weights of hypocotyl and radicle, along with their lengths were determined.

Statistical analysis

For each treatment, germination capacity (GC) and the mean germination time (MGT) were calculated by following formula 1 and 2, respectively:

$$GC\ (\%) = (\sum ni / N) \times 100 \qquad \text{(Eq.1)}$$

$$MGT\ (days) = \sum ti \times ni / \sum ni \qquad \text{(Eq.2)}$$

where ni is number of germinated seeds per day, N is the total number of seeds sown, and ti is the amount of days for germination to occur beginning from sowing date (Bewley et al., 2012; Gilani et al., 2021). The germination capacity data set was arcsine converted prior to data analyses to meet the normality assumption for ANOVA (Zar, 1996). A general linear model, Univariate Analysis, was conducted to examine significant differences in germination time, germination capacity and seedling emergence attributes among species and treatments (3 N sources X 3 concentration levels). Means that showed significant changes were compared by Tukey's Honestly Significant test at the 5% level of significance for each species separately. Data analyses were performed using Statistics 8.1 while all figures were drawn by GraphPad Prism 8 (GraphPad Software, Inc., CA) statistical packages.

Results

Effect of simulated N deposition on germination in the climatic chamber

Highly significant differences in germination capacity were detected among simulated nitrogen deposition treatments, species, and interaction (*Table 1*).

Table 1. *Results of General Linear Model – Univariate Analysis for examining differences in germination capacity (GC), mean germination time (MGT), radicle length (RL), hypocotyl length (HL), fresh weigh of the radicle (RFW) and hypocotyl (HFW), and dry weight of radicle (RDW) and hypocotyl (HDR) among species and different nitrogen treatments (3 N sources X 3 concnetration levels and the control, giving a total of 10 treatments)*

	Treatments			**Species**			**Interaction**		
Attributes	**d.f**	**F**	**P**	**d.f**	**F**	**P**	**d.f**	**F**	**P**
GC	9	9262.00	< 0.001	3	7022.81	<0.001	27	5391.33	< 0.001
MGT	9	272.71	<0.001	3	272.81	< 0.001	27	246.89	< 0.001
RL	9	472.23	< 0.001	3	35.87	< 0.001	27	35.27	< 0.001
HL	9	352.78	< 0.001	3	14.16	< 0.001	27	25.28	< 0.001
RFW	9	241.41	< 0.001	3	6.91	<0.001	27	12.44	< 0.001
HFW	9	15.03	< 0.001	3	0.69	<0.001	27	0.49	<0.001
RDW	9	23.87	< 0.001	3	85.77	< 0.001	27	82.64	< 0.001
HDR	9	273.16	< 0.001	3	56.29	<0.001	27	79.89	<0.001

N treatment with 1% concentration positively affected the germination of *C. lanceolata, P. zhennan* and *F. hodginisi* seeds, respectively (*Fig. 1a,c,d*) while germination of *P. massoniana* seeds was enhanced by treatments involving 0.5% N solution (*Fig. 1b*). Apart from 1% KNO_3 treatment, the germination of *F. hodginisi* seeds was not influenced by other N treatments compared to the control (*Fig. 1d*). Generally, germination of *F. hodginisi* seeds was lower than the other species.

The mean germination time also varied significantly among species, N treatments and their interactions (*Table 1*). For *C. lanceolata* seeds, 1% treatment of $(NH4)_2SO_4$ and NH_4NO_3 prolonged the germination time (*Fig. 1e*); 0.1% N treatment significantly prolong the germination time of *P. massoniana* seeds (*Fig. 1f*); and 1% N treatment prolonged the germination time of *P. zhennan* (*Fig. 1g*) and *F. hodginisi* (*Fig. 1h*) seeds. Among treatments, 0.5% N treatments resulted in quicker germination of *C. lanceolata, P. massoniana* and *F. hodginisi* seeds. Generally, seeds of *P. massoniana* germinated faster and that of *F. hodginisi* germinated slower than the other species.

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Effect of N treatments on seedling emergence

Both hypocotyl and radicle lengths significantly varied among species, N treatments and their interaction (*Table 1*). Hypocotyl length of *C. lanceolata* seedlings was shorter than the other species. With regard to the effects of N treatments on hypocotyl length, 1% N treatments elongated the hypocotyl of *C. lanceolata* seedlings (*Fig. 2a*). While all concentrations of ammonium sulfate resulted in elongation of hypocotyl of *P. massoniana* seedlings, 0.1% ammonium nitrate and potassium nitrate treatments resulted in elongation of *P. massoniana* hypocotyl (*Fig. 2b*). Elongation of hypocotyl of *P. zhennan* (*Fig. 2c*) and *F. hodginisi* (*Fig. 2d*) seedlings were high in response to 1% N treatments.

Radicle length was generally shorter for *F. hodginisi* than the rest of the species. N treatment with 1% concentration resulted in increased radicle length of *C. lanceolata* seedlings (*Fig. 2e*) while 0.1% N treatment increased radicle length of *P. massoniana* seedlings (*Fig. 2f*) compared to the control and other N treatments. For *P. zhennan* seedlings, 1% ammonium sulfate and ammonium nitrate treatments increased radicle length compared to 0.1% and the control while potassium nitrate treatments did not bring significant effect on radicle length (*Fig. 2g*). Radicle length of *F. hodginisi* seedlings was higher in response to 1% N treatments than others and the control (*Fig. 2h*).

Effect of N treatments on seedling fresh and dry weight

Hypocotyl and radicle fresh and dry weights varied significantly among N treatments, species, and their interaction (*Table 1*). Hypocotyl fresh weight of *P. zhennan* seedlings was higher and that of *F. hodginisi* seedlings was lower than the other two coniferous species. While 1% N treatments resulted in significantly higher hypocotyl fresh weight of *C. lanceolata* (*Fig. 3a*), *P. zhennan* (*Fig. 3c*) and *F. hodginisi* (*Fig. 3d*) seedlings, 0.1% N treatments resulted in higher hypocotyl fresh weight of *P. massoniana* seedlings (*Fig. 3b*). Similarly, radicle fresh weight of *C. lanceolata* (*Fig. 3e*), *P. zhennan* (*Fig. 3g*) and *F. hodginisi* (*Fig. 3h*) seedlings treated with 1% N solutions was higher than the control and other concentrations of N treatments while 0.1% N treatments resulted in higher radicle fresh weight of *P. massoniana* seedlings (*Fig. 3f*). Generally, radicle fresh weight of *P. zhennan* seedlings was the highest whereas the lowest radicle fresh weight was observed in *P. massoniana* seedlings compared to other species.

With regard to hypocotyl dry weight, it was noted that 1% N treatments resulted in increased hypocotyl dry weight of *C. lanceolata* (*Fig. 4a*), *P. zhennan* (*Fig. 4c*) and *F. hodginisi* (*Fig. 4d*) while 0.1% and 1% N treatments increased hypocotyl dry weight of *P. massoniana* seedlings (*Fig. 4b*) compared to the control and other treatments. Across all treatments, hypocotyl dry weight was the highest for *P. zhennan*, followed by *F. hodginisi* compared with the coniferous species. Similarly, radicle dry weight showed similar pattern as hypocotyl dry weight; being higher in 1% N treatments in *C. lanceolata* (*Fig. 4e*), *P. zhennan* (*Fig. 4g*) and *F. hodginisi* (*Fig. 4h*) and 0.1% N treatments in *P. massoniana* (*Fig. 4f*).

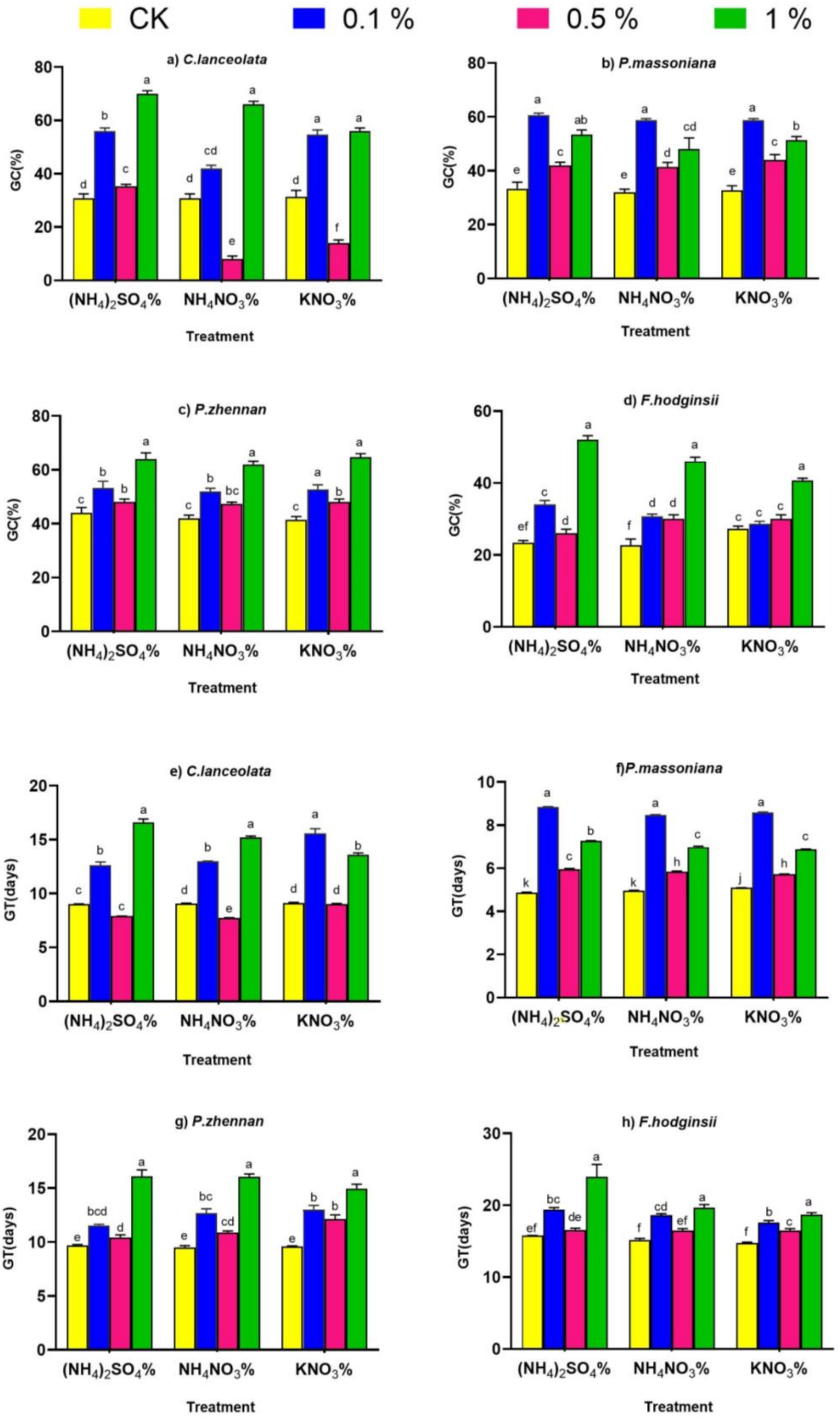

***Figure 1.** Germination capacity (GC; a-d) and mean germination time (MGT; e-h) of seeds of four tree species in response to different nitrogen treatments and the control (Mean ± SE). For each species, bars with different letters are significantly different among N treatments at 5% probability level*

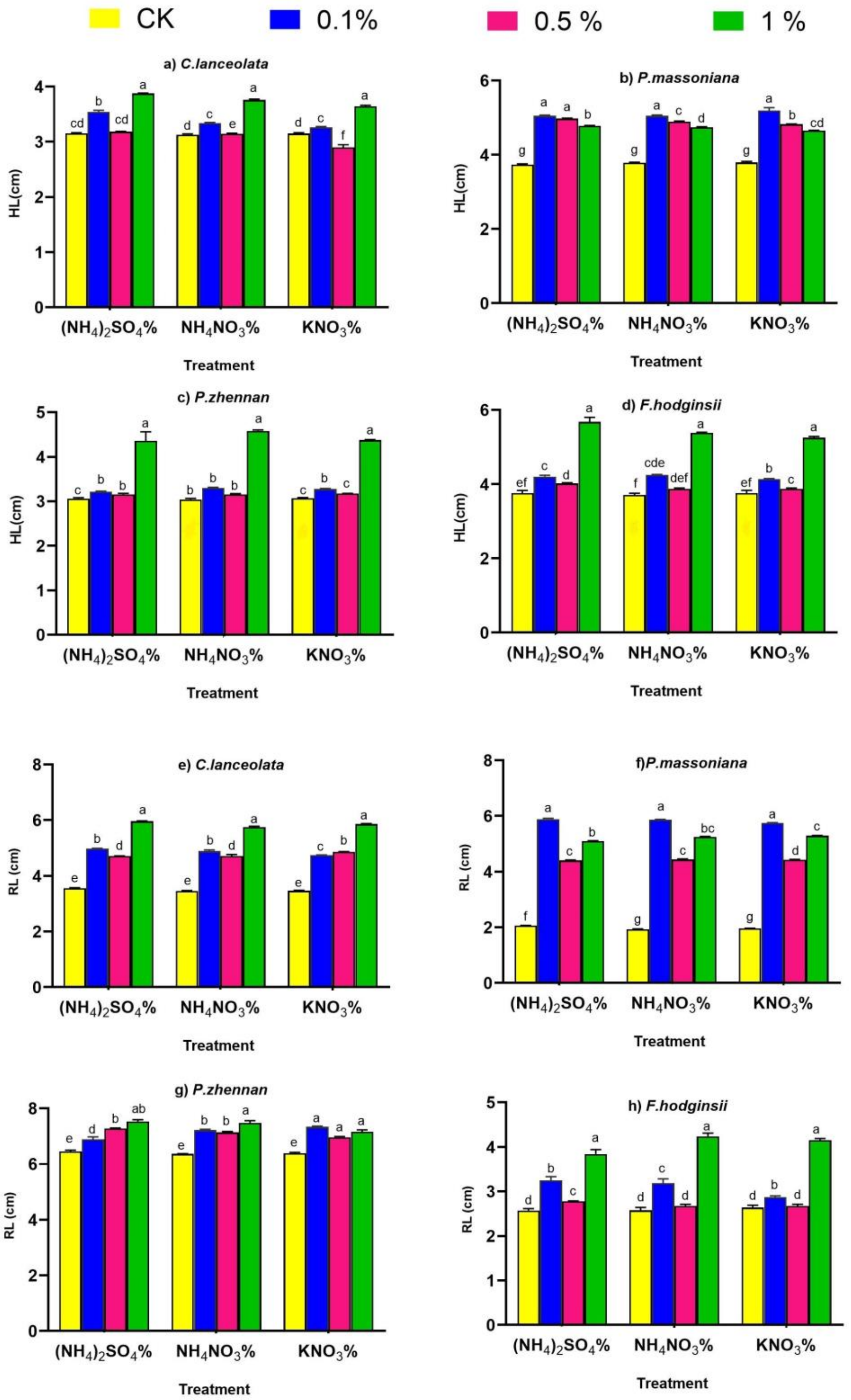

Figure 2. Hypocotyl (HL; a-d) and radicle (RL; e-h) length of seedlings of four tree species in response to different nitrogen treatments and the control (Mean ± SE). For each species, bars with the different letter are significantly different among N treatments at 5% probability level

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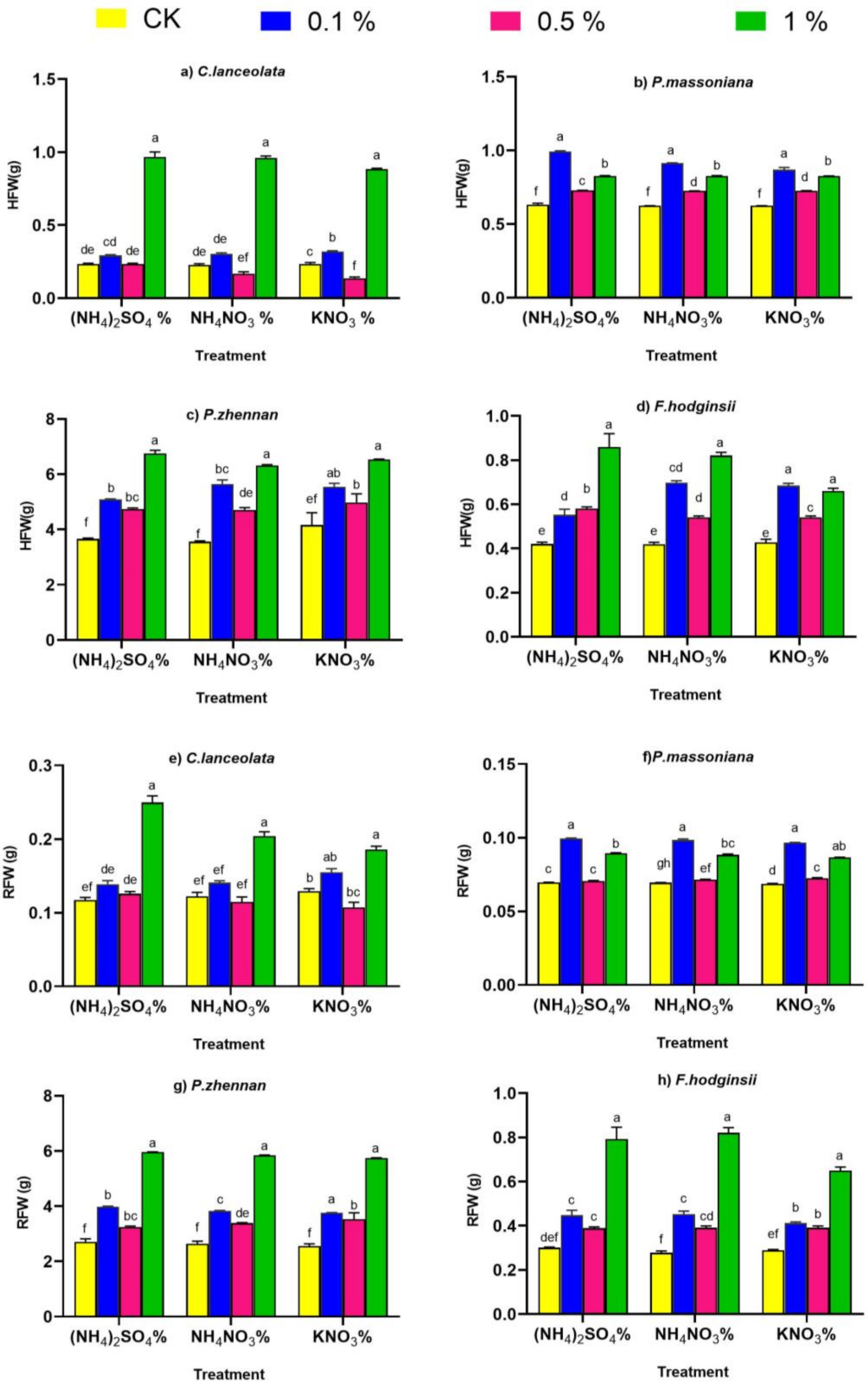

Figure 3. Radicle fresh weight (RFW) and hypocotyl fresh weight (HFW) of seedlings of four tree species in response to different nitrogen treatments and the control (Mean ± SE). For each species, bars with the different letter are significantly different among N treatments at 5% probability level

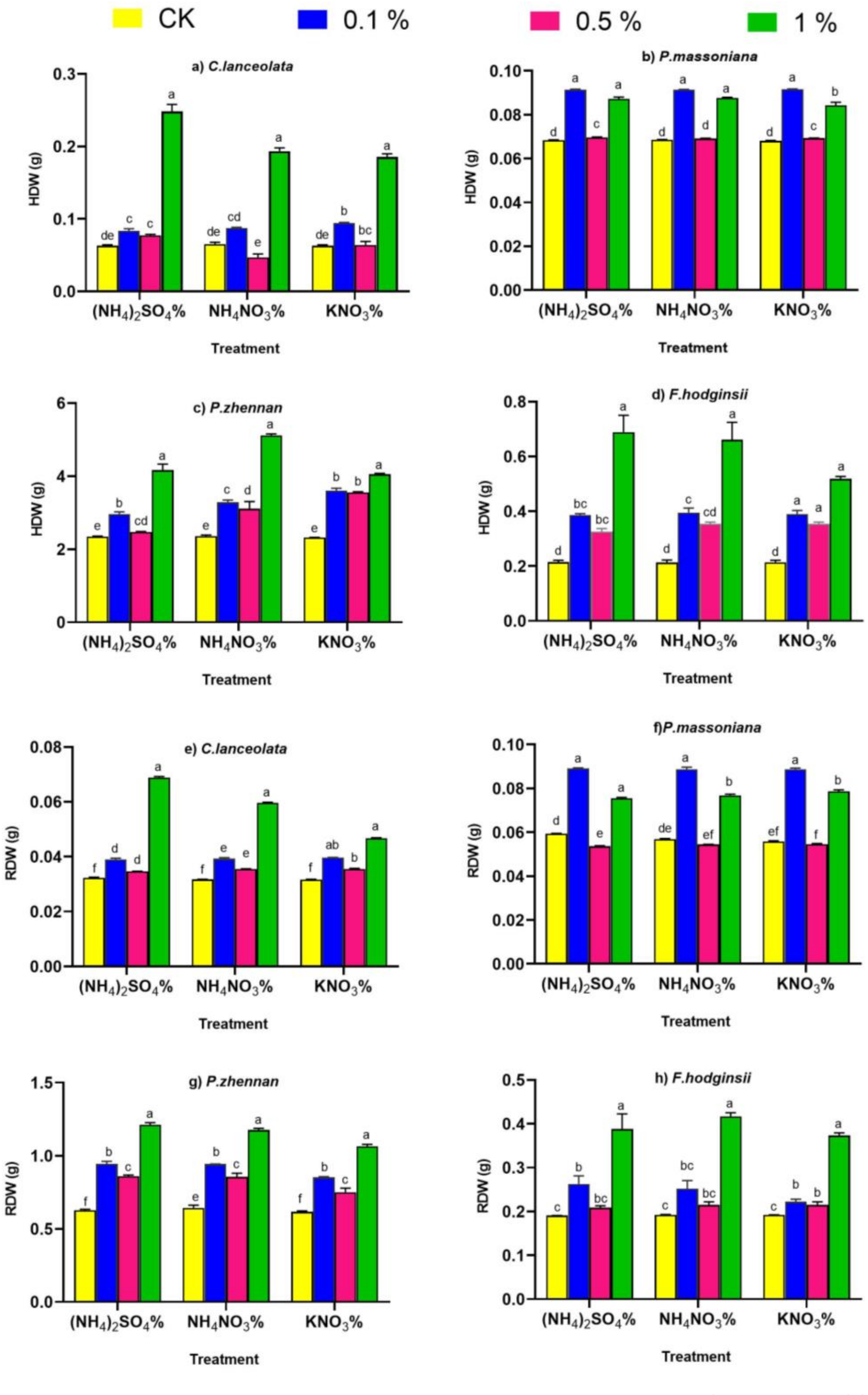

Figure 4. *Radicle dry weight (RDW) and hypocotyl dry weight (HDW) of seedlings of four tree species in response to different nitrogen treatments and the control (Mean ± SE). For each species, bars with the different letter are significantly different among N treatments at 5% probability level*

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Discussion

Species showed that the seeds could germinate in a wide range of N concentration. We found that the seed germination capacity and germination time increased as the amount of N increased. Level of initial dormancy of seeds is influenced by environment and genotype of plant (Penfield, 2017). Previous studies discussed that pre-sowing treatment on seed could improve the growth rate, development process, and seed germination (Wala et al., 2021) although dormancy of seed varies with the degree of seed drying and phase of seed maturity (Gilani et al., 2019).

Earlier it was indicated that N exerts a positive, harmful, or no influence on the germination of seeds (Haden et al., 2011; Varma et al., 2016). In the current experiment, germination of seeds of four sub-tropical forest species had different responses to N. These outcomes showed that the influence of N on germination of seeds might vary according to species. This variation in germination might be due to the seed coat of these species. Hernández et al. (2021) demonstrated that N could improve the performance of poor-quality seeds. N accessibility generally functions as a signal to encourage species germination and their life span (Luna and Moreno., 2009). This appeared to be the case for all species in our study. The encouraging impact of N on seed coating deterioration would not appear. The general impact of N on germination of a particular species depends upon the major aspects affecting germination. Our findings are consistent with the earlier experiments that showed the impact of $(NH_4)_2SO_4$ on germination and seedling growth were positive. (Li et al., 2005) and Agrawal and Dadlani (1987) support the fact that 1% $(NH_4)_2SO_4$ gave the best percentage germination for *Sabal palmetto* seeds.

Several studies have demonstrated a positive effect of low concentrations of NH_4NO_3 on seed germination (Hilhorst and Karssen, 1989), such as germination of *G. tricornotum* and *A. sagitata* was reduced by elevated concentrations (Chauhan et al., 2006). On the contrary, extremely low N concentrations can also adversely affect the species; for example, *P. albida*, whose germination is hindered by concentrations of 2 mg NH_4NO_3 (Ponert et al., 2013). Jain and Foy (1992) stated that germination of *O. aegyptiaca* decreased from 28% to 18% after treatment with 5 mM or 10 mM NH_4NO_3. Even at small level, N can prevent the germination of seeds of few species (Boudell and Stromberg, 2015). Our results also showed diverse response at low concentration of 0.1%. Not much study has been done related to these N applications on the seeds of South China tree species. The outcomes exhibited that the germination for the three N sources were significantly different, with 1% showing improved germination than 0.1% and 0.5%. Physiological dormancy of seed can be improved by chemical stimulants and several N compounds (Farhadi et al., 2013). For testing of germination in many species, KNO_3 is recommended because it stimulate the germination of seeds (Brasil. Ministério da Agricultura, 2009).

For the treatment with KNO_3, significant differences were recorded as 1% concentration gave better results as compared to other treatments. Numerous researchers have stated that KNO_3 increased the germination of several plants seed (Cirak et al., 2004; Olmez et al., 2004).The usage of KNO_3 has been a vital treatment in seed-testing laboratories for several years and successful in dormancy breaking of various species (Baskin and Baskin, 2001).

Abnavi and Ghobadi (2012) stated a related tendency of seed treatment with 1% KNO_3 having an encouraging response and give rise to good germination. Seeds treated with 1% $(NH_4)_2SO_4$ and KNO_3 enhanced germination in sorghum and tomato (Shehzad

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et al., 2012), Additionally, Mohammadi (2009) stated that treating soybean with 1% KNO_3 for 24 h increased the germination of seeds as compared to non-treated seeds in laboratory and field experiments. The germination time of all species was influenced by N treatments. Germination speed based on mean germination time was also improved by 1% KNO_3. The effect of N varied among species, with seeds of *C. lanceolata, P. massoniana, F. hodginisi,* and *P. zhennan* showing stimulating results with the treatment of 1% N.

N affected the pine species, *P. massoniana,* compared to a broadleaved species, *P. zhennan,* at seedling emergence, which is consistent with earlier studies that N hinders the growth of *P. massoniana* (Zhang et al., 2010). Nevertheless, N did not have an effect on the development of *P. zhennan,* in all parameters compared to other species. Mo et al. (2008) stated that N has an encouraging impact on the development of *S. Superba,* which is also a broadleaved species. The variation in four species to N supplementation could be linked to their N demand.

It demonstrates that even if the critical load of N is elevated there is still a little influence on plant cover functional features (Bobbink et al., 2003). This is observed in our study where hypocotyl and radicle length increased by N. Rawat et al. (2008) also described that quick germination in wild pomegranate seeds may result in longest radicle, which improves the establishment of seedlings. Dewir et al. (2011) also studied that maximum seedling length was of gladiolus seeds treated with 1% KNO_3, and the smallest seedlings were of control gladiolus seeds. Hypocotyl and radicle fresh and dry weight reduced in *C. lanceolata, P. massoniana* and *F. hodginisi* among all treatments. As stated in the previous studies N sources, particularly the use of NH_4NO_3, lowered the weight of Orobanche seedlings (Van Hezewijk and Verkleij, 1996).

This study only sets up three N addition levels. The higher sulfate/nitrate ratio in this study have probably exacerbated the adverse effects of N deposition on coniferous seedlings. Thus, further research may be needed to understand if different sulfate to nitrate ratios of simulated N deposition has an impact on the seeds and seedlings establishment. Overall coniferous and pine species' germination was affected by N as compared to broadleaved species, *P. zhennan*. Hence, we suggest that subsequent trials must concentrate on the influences of extended N on the plant with various N concentrations.

Conclusions

This experiment demonstrated the beneficial effects of KNO_3 application on growth compared to $(NH_4)_2SO_4$ and NH_4NO_3. The use of KNO_3 to treat seeds enhanced and drastically increased the germination in laboratory conditions. Untreated seeds (control treatment) showed slower germination time, hypocotyl, radicle length, and weight. Coniferous and pine species germination was affected by N as compared to broadleaved species. The sulfate/nitrate ratio defines the degree to which N impacts the growth. The increased sulfate and nitrate proportion in this experiment perhaps aggravated the adverse impacts of N on coniferous and pine seedling. As a whole, the findings suggest that it is favorable to use a procedure containing 1% KNO_3 in the initial stage as seed pre-sowing treatment to enhance the germination of seeds and subsequent seedling emergence.

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