Nitrogen addition enhances seed yield by improving soil enzyme activity and nutrients

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Nitrogen (N) addition is a simple and effective field management strategy for enhancing plant productivity, but the regulatory mechanisms of nitrogen concentrations on soil enzyme activity, nutrient and seed yield of *Festuca kirilowii* seed field remain unclear. Therefore, the impact of N fertilizer application on soil enzyme activities, soil nutrients, and seed yield of *F. kirilowii* Steud cv. Huanhu, the only domesticated variety in the Festuca genus of Poaceae, was investigated based on two years field experiments in the Qinghai-Tibet Plateau (QTP). Results showed that N input significantly effected soil nutrients (potential of hydrogen, total nitrogen, organic matter, and total phosphorus). Additionally, the soil enzyme activity (urease, catalase, sucrase, and nitrate reductase) were significantly increased under different concentrations N addition, which drove the changes in the contents of soil nutrients. Further, N addition also improved the seed yield and yield components (number of tillers and number of fertile tillers). These results indicate that the application of different concentrations of N fertilizers can stimulate soil enzyme activity, thereby accelerating nutrient conversion and thus increasing seed yield. The comprehensive evaluation of membership function showed that the optimal N fertilizer treatment was N4 (75 $kg \cdot hm^{-2}$) both for 2022 and 2023, which provided a certain practical suggestion for the improvement of seed production of *F. kirilowii* in the QTP.

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Abstract: Nitrogen (N) addition is a simple and effective field management strategy for enhancing plant productivity, but the regulatory mechanisms of nitrogen concentrations on soil enzyme activity, nutrient and seed yield of *Festuca kirilowii* seed field remain unclear. Therefore, the impact of N fertilizer application on soil enzyme activities, soil nutrients, and seed yield of *F. kirilowii* Steud cv. Huanhu, the only domesticated variety in the Festuca genus of Poaceae, was investigated based on two years field experiments in the Qinghai-Tibet Plateau (QTP). Results showed that N input significantly effected soil nutrients (potential of hydrogen, total nitrogen, organic matter, and total phosphorus). Additionally, the soil enzyme activity (urease, catalase, sucrase, and nitrate reductase) were significantly increased under different concentrations N addition, which drove the changes in the contents of soil nutrients. Further, N addition also improved the seed yield and yield components (number of tillers and number of fertile tillers). These results indicate that the application of different concentrations of N fertilizers can stimulate soil enzyme activity, thereby accelerating nutrient conversion and thus increasing seed yield. The comprehensive evaluation of membership function showed that the optimal N fertilizer treatment was N4 (75 kg·hm^{-2}) both for 2022 and 2023, which provided a certain practical suggestion for the improvement of seed production of *F. kirilowii* in the QTP.

Key words: *Festuca kirilowii* Steud cv. Huanhu, Nitrogen addition, soil enzyme activity, nutrient content, seed yield, Qinghai-Tibet Plateau

1. Introduction：

F. kirilowii is one of the few grass species that can produce seed on a large scale in the Qinghai-Tibet Plateau (QTP), which plays a key role in ecological recovery and alleviating the pressure of animal husbandry. Nevertheless, due to the low productivity of native seed fields, low efficiency of eco-restoration, and the high urgency of seed utilization for ecological restoration, there is a demand for more sufficient seeds of *F. kirilowii*. Nitrogen (N) has received extensive attention from a large number of scholars as one of the major limiting factors in the growth and development of most plants(Amanullah et al. 2016; Wang et al. 2018). For perennial forage grass, nitrogen fertilization increases seed yield at different plant ages(Hua et al. 2001; Li et al. 2012; Pei-Sheng et al. 2001). Accordingly, N application is widely used in seed production and is one of the key factors impacting seed quality and yield. Inadequate N addition results in reduced seed yield and reduced proceeds for cultivators. Nevertheless, excessive N addition does not produce a substantial improve in seed yield due to the principle of diminishing returns and results in increased costs(Cassman et al. 2003; Reussi Calvo et al. 2013). In addition, the optimum amount of N to be applied varies according to species, soil fertility, planting density, and climatic conditions(Chen et al. 2023; Peng et al. 2010; Yang et al. 2019). Previous studies on *Elymus nutans* with amounts of

N addition demonstrated that the optimal N addition rate (250 kg·hm^{-2}) could seed yield(Guo-Ping et al. 2010). Yuan et al. (2022)'s study that examined the impact of amounts of N addition (0–240 kg·hm^{-2}) on *Kengyilia melanthera* found that the seed yield and yield components (number of tillers, number of fertile tillers) reached the peak value under 180 kg·hm^{-2}. Therefore, it is especially critical to study the optimal nitrogen application concentration for seed yield of *F. kirilowii*.

Soil is a complex microenvironment and its nutrients are regulated by a variety of factors(Wang et al. 2023b; Zhao et al. 2022). Examples include soil enzymes, soil physical and chemical properties and soil microorganisms. N fertilizer affects soil nutrient balance through direct action on the soil (Chen et al. 2020; Liao et al. 2021). For instance, N addition can affect soil organic matter (SOM) storage in grassland ecosystems. N application can change soil pH, affect soil microbial activity, and major soil biochemical reactions (Gong et al. 2015; Song et al. 2014). Soil enzymes are derived from the cellular secretions of animals, plants and microorganisms in the soil and the breakdown products of their residues(Davies et al. 2022). These enzymes accumulate in the soil as free enzymes or are adsorbed on the surface of soil particles and can reflect soil carbon and nitrogen transformations(Rao et al. 2014). Enzymes commonly used to reflect soil carbon and nitrogen transformations can be divided into two categories: oxidoreductases and hydrolases. Soil oxidoreductases mainly include catalase (CAT), nitrate reductase (NR) and hydroxylamine reductase (HR), etc., which are directly involved in the denitrification process of soil nitrogen. Soil hydrolases (such as urease (UE), sucrase (SC)) hydrolyze proteins and other macromolecules into simple small molecules that are easily absorbed and utilized by plants. Soil enzyme activities affected by N are particularly sensitive to the amount of N applied. N application may have an indirect effect on soil enzyme activities by changing soil physicochemical properties(Nannipieri et al. 2012). In previous studies, the activity of soil enzymes responded differently to N addition(Song et al. 2009). N addition significantly increased the activity of UE because N fertilizer increases microbial demand for C, N, and P, which in turn improves the activities of soil enzymes associated with the acquisition of these three elements(Saiya-Cork et al. 2002; Stone et al. 2012). However, additional studies have shown a negative effect of N addition on UE activity(Andersson et al. 2004). N effectiveness was relatively high during the early part of the growing season, and when N was applied during this phase, the N concentration was so high, resulting in inhibition of UE activity(Weintraub & Schimel 2005). These different observations on the effect of N on soil enzyme activity were attributed to differences in nutrient effectiveness, litter and soil C : N ratios and microbial biomass(Treseder et al. 2001). At the same time, increasing N effectiveness affects soil enzyme function and activity to some extent, promoting SOM decomposition and stimulating plant growth by alleviating N limitation(Cusack & Daniela 2013).

Despite the fact that a large number of studies have been conducted to elucidate the effects of N addition on forage grass, knowledge on the effects of soil enzyme activities and soil nutrients on forage seed yield under N addition is still limited. Especially the internal mechanisms between them, and so further studies are required. In order to understand the effect of N application on soil enzyme activities, soil nutrients, and seed yield, we conducted an experiment over a two years period with N addition in forage seed field in the QTP.

2. Materials and methods

2.1. Experimental sites

The experiment was carried out for two consecutive years, between 2021 and 2023. The experimental site is located in the national perennial forage germplasm resource Nursery of Xihai Town, Haibei Prefecture, Qinghai Province, China (36°59′36″N, 100°52′85″E, 3,156 m a.s.l.). The climate is cold, there is no absolute frost-free period, the annual average temperature is 0.5℃, the annual precipitation is 369.1mm, and mostly concentrated in July to September. The annual evaporation is 1400 mm, the annual sunshine duration is 2980 h, and the average frost-free period is about 93d. The soil of experimental land was chestnut soil, and chemical properties were provided in Table S1..

2.2. Experimental management

Seeds of *F. kirilowii* (variety named *Festuca kirilowii Steud* cv. Huanhu) was supplied by Grassland Institute of Qinghai Academy of Animal Science and Veterinary Science. Its germination rate and 1,000-seed weight were 81% and 0.622 g, respectively.

2.3. Experimental design

The N fertilizer treatments (urea fertilizer, 46% N) were conducted to the experimental plots during the regreening stage, in mid June of 2022 and 2023. This trial is adopted as was a randomized complete block design for seven sample plots with different amounts of N addition (N1 – 0 kg·hm^{-2}, N2 – 25 kg·hm^{-2}, N3 – 50 kg·hm^{-2}, N4 – 75 kg·hm^{-2}, N5 – 100 kg·hm^{-2}, N6 – 125 kg·hm^{-2}, N7 – 150 kg·hm^{-2}). Each N addition treatment had three replications' plots and a total of 21 plots. Sowing was performed by the drill method in early July, 2021 and 2022. The seeding capacity was 22.5kg ·hm^{-2}, the row spacing was 0.5m. The experimental plot was a 20 m^2 (5 m × 4 m) plot and seeds were not sown within 50 cm of the plot boundary to minimize marginal effects.

2.4. Yield components and seed yield

The number of tillers (NTs) and number of fertile tillers (NFTs) were measured to assess yield. At full-blooming stage, NTs and NFTs were measured in a randomly selected row from each sample plot and three replicates were taken from each sample plot.

Approximately 25 days after the peak of flowering, all the seeds in each plot were harvested except for the side rows. These seeds were threshed, cleaned, and dried naturally to convert to seed yield per hectare.

2.5. Soil Enzyme Activities

Soil enzyme (urease (UE), catalase (CAT), sucrase (SC), and nitrate reductase (NR)) activities were determined by using an enzyme activity detection kit (Suzhou Mengxi Biomedical Technology Co., Ltd.). The substrates for enzymes were as follows: UE, CAT, SC, and NR were assayed with the urea(Zibilsk et al. 1994), H_2O_2(Saiya-Cork et al. 2002), sucrose(Yang et al. 2023), and KNO_3(Nardi et al. 2005), respectively. Refer to the kit program for detailed assay procedures for these soil enzyme activities.

2.6. Soil nutrients

Soil pH was determined using the digital pH meter (Seven2Go, Mettler-Toledo Instruments

(Shanghai) Co., Ltd, Shanghai, China) in a 1:5 soil /water suspension after shaking for 5 min. The determination of soil physicochemical properties refers to "Soil Sampling and Methods of Analysis"(Crepin & Johnson 1993). Soil organic matter (OC) was determined by using the SOM $K_2Cr_2O_7$ redox titration method. Total nitrogen (TN) in the soil was determined by Kjeldahl digestion. The total phosphorus (TP) content was measured by the antimony-molybdenum colorimetry after perchloric acid digestion.

2.7. Statistics analysis

The effect of N addition on soil properties, enzyme activities, seed yield and yield components were investigated by one-way analysis of variance (one-way ANOVA) using IBM Statistical Package, SPSS version 27.0 (IBM, Armonk, NY, USA). The two-way analysis of ANOVA (two-way ANOVA) with a general linear model at $P < 0.05$ was applied to determine the combined influence of N fertilizer and trial years on seed yield and yield components. The two-way ANOVA was also applied to determine the combined influence of N fertilizer and soil layers on soil properties and enzyme activities. Correlation and path analysis were conducted using SPSS version 25.0 to identify the effects of soil factors and yield components on seed yield. Origin 2021 (OriginLab, Massachusetts) and R 4.3.1 (R Development Core Team, 2023) software were used to draw graphs.

The membership function method was used to explore the response of yield components, seed yield, soil nutrients and soil enzyme activities to N fertilizer, and to determine the optimal amount of N to apply for *Festuca kirilowii Steud* cv. Huanhu. The calculation formula for the forward membership function was $y=(x_a - x_{min})/(x_{max} - x_{min})$, and the negative membership function was $y=1-(x_a - x_{min})/(x_{max} - x_{min})$, where the x_a is the value of certain index, and the x_{max} and x_{min} represent the maximum and minimum values of an indicator under different N application treatments..

3. Result

3.1. Soil nutrients

The ANOVA of soil nutrients under different treatments is shown in Table 1. N addition and soil layer had significant effects on PH, OC, TN, and TP (P<0.05). However, these soil nutrients were non-significant under the interaction of N addition and soil layer (P>0.05).

The specific results of changes in soil nutrients under different treatments are shown in Figure 1. The negative effect of N addition on soil PH was decreased with the nitrogen concentrations. Nevertheless, SOM, TN and TP all showed an increasing and then decreasing trend with the increase of N application. Additionally, SOM, TN and TP were maximum content in N3, N4 and N4 treatments, respectively, which significantly higher than the CK. Soil PH and SOM did not N1 differ significantly between the two soil layers under the same N application treatment. For the TN, the soil layer of 0~20cm is significantly higher than that of 20~40cm. Under N4-N7 treatment, the TP content in the 0~20cm soil layer was significantly higher than that in the 20~40cm layer.

3.2. soil enzyme activities

The results of ANOVA for each enzyme under each treatment are shown in Table 2. N addition and soil layer had significant effects on four soil enzyme activities (P<0.05). However, the soil enzyme activities were non-significant under the interaction of N addition and soil layer (*P*>0.05).

Soil enzyme activities under different N application treatments are shown in Figure 2. Nitrogen application significantly increased soil UE activity (*P*<0.05). With the increase of nitrogen application, the soil UE activity of different soil layers showed the trend of increasing and then decreasing, and all of them reached the maximum urease activity under N4 treatment. Under N4 treatment, soil urease activity increased by 356.10% and 449.49% in the 0~20 cm and 20~40 cm soil layers, respectively, compared to CK (N1). Meanwhile, compared with 0~20cm soil layer, soil UE activity in 20~40cm soil layer was lower under all nitrogen application treatments. The soil CAT and SC activities reached their maximum activity under N4 and N3 treatments, respectively. There is no significant difference in SC activity among different soil layers under nitrogen application treatment. Interestingly, CAT activity was significantly higher in the 0~20 cm soil layer than in the 20~40 cm under N1-N4 treatments. For the NR, the positive effect of N addition on its activity was increased with the nitrogen concentrations. The differences in NR activity between the two soil layers under the treatments were not significant except for N7.

3.3. yield components and seed yield

The ANOVA of yield components and seed yield under different treatments is shown in Table 3. N addition had significant effects on NTs, NFTs, and SY (*P*<0.01). And no significant difference was observed in all metrics measured in 2022 and 2023.

Compared to N1, the NTs and NFTs in both trial years were significantly increased when N2–N6 of N fertilizer was applied (*P*<0.05, Figure 3). And they reached peak value under N3 or N4. With increasing rates of N fertilizer application rate, the seed yield showed a tendency of first increasing and then decreasing (Figure 4), and reached their peak values under N4 treatment in both 2022 and 2023. What is noteworthy is that excessive rates of N fertilizer application (N6 or N7) had a non-significant improvement on seed yield (*P*>0.05). This was accompanied by a reduction in two seed yield component indicators (Figure 3). During two-year independent field trials, we found up and down fluctuations in seed yield in 2022 and 2023 under the same N application treatment, but the difference was no significant (*P*>0.05).

3.4. Correlation and pathway analysis

The correlation between seed yield and yield components, soil enzyme activity, and soil physicochemical properties in 2022 and 2023 is shown in Table 4 and Figure 5. The result showed a significant correlation between yield components (NTs and NFTs) and seed yield (*P*<0.01). Seed yield in 2022 was significantly and positively correlated with UE, CAT, SC, TP, and TN in 0~20 cm soil layer (*P*<0.05), and positively correlated with NR, PH, and SOM. Seed yield in 2023 was significantly and positively correlated with UE, CAT, SC, and TN in 0~20 cm soil layer (*P*<0.05), and positively correlated with NR, PH, SOM, and TP. In a soil layer of 20~40cm, Seed yield in 2022 was significantly and positively correlated with UE, CAT, SC, and TN (*P*<0.05). Seed yield in 2023 was significantly and positively correlated with UE, CAT, SC, TN, and TP (*P*<0.05).

Next, we performed a path analysis of the metrics (NTs, NFTs, UE, CAT, SC, TN, and TP) that were significantly associated with seed yield. In this case, seed yield and yield components were averaged over 2022 and 2023, and soil enzyme activities and soil nutrients were averaged over two soil layers. The path analysis explained 84% of the variation for SY (Figure 6). SY was directly affected by soil enzyme activities (UE, CAT, and SC), soil nutrients (TP and TN), and yield components (NTs and NFTs). Soil enzyme activities (UE, CAT, and SC) and soil nutrients (TP and TN) also indirectly affected SY by positively influencing NTs and NFTs. In addition, the N addition significantly stimulated the soil enzyme activities by increasing the soil nutrients content, thereby enhancing the seed yield of *F. kirilowii*. The standardized effect sizes of the driving factors further clarify the results of the path analysis (Figure 7). The result suggested that NFTs, UE and TN play important roles in seed yield formation in *F. kirilowii*.

3.5. Comprehensive analysis of membership function

The changes in the seed trait indices of *F. kirilowii* represented a certain degree of correlation and difference based on the N fertilizer, and a single indicator cannot fully show the influence of treatment on seed production. Therefore, it was necessary to carry out a comprehensive evaluation of multiple indices to determine the best combination. Based on the values of each indicators measured under different treatments, including indices of four soil enzyme activities, four soil nutrients and three yield components, to calculated the membership function values (Table 5). Higher membership function values represented more distinct improvement of *F. kirilowii* seed yield. The final ranking was: N4>N3>N5>N2>N6>N7>N1. The results showed that the N4 treatment was the most suitable for *F. kirilowii* seed production.

4. Discussion

The specific processes and mechanisms of N fertilizer transformation in the plant-soil system are regulated by a variety of factors. Soil enzymes, as important biological activators of energy flow and material cycling in the subsurface, are involved in the transformation of soil nutrients. Changes in soil enzyme activities can well reflect changes in soil physicochemical properties and soil nutrients, thus regulating plant growth. Therefore, it is crucial to explore the mechanism between nitrogen-regulated soil enzymes and soil nutrients, and their regulatory effects on plant seed yield formation.

4.1. Effect of N addition on soil nutrients

The state of soil nutrient balance fundamentally determines the direction of soil fertility evolution, and is closely linked to the benefits of fertilizer application and ecological and environmental safety(Bindraban et al. 1998). For quite some time in the past, the focus of fertilizer application has been on increasing yields, to a certain extent ignoring the issues of soil fertility balance and ecological and environmental safety(Saha et al. 2007). Soil acidification has become a global environmental problem as global N deposition continues to increase. It has been shown that long-term N deposition can reduce soil quality by causing soil acidification(Gong et al. 2015). Our results showed that N addition decreased soil pH due to the increase of NH^{+4} and NO^{-3} content in the soil by N input, and the replacement of NH^{+4} with base cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) on the surface of the soil colloid made it easier to leach out the base cations from the soil, and when the NH^{+4} was absorbed by the plants, it released H^{+} into the soil solution, thus causing soil acidification(Alatalo et al. 2017). In this study, N application contributed to soil nutrient (SOM, TN, and TP) content, especially to TP content. This may be due to the coordinating effect between nitrogen and phosphorus, which has the effect of promoting phosphorus with nitrogen. SOM was maximum under N3 treatment and significantly higher than control (N1), and the difference in SOM between the two soil layers was not significant. The TN and TP contents reached their maximum values under N4 treatment. It is shown that the appropriate amount of N application can improve the soil nutrient content(Gong et al. 2015; Presley et al. 2012), mainly due to the appropriate amount of N fertilizer can strengthen the microbial metabolism in the soil, and the increase of metabolic products secreted is conducive to promoting the mineralization of humus and difficult to decompose organic matter in the soil, which improves the soil nutrient content, whereas the excessive application of N, and the stacking effect of N fertilizers will lead to excessive N in the soil, breaking the nutrient balance of the soil ecosystem and destroying the soil biological traits, which will have an effect the ability of the soil to be used sustainably(Jing et al. 2007; Paul & Juma 1981). In addition, the TN and TP content in the 0~20cm soil layer is significantly higher than that in the 20~40cm soil layer. This is due to the lower rainfall in the QTP, which reduces the amount of nutrients that are leached into the deeper soil layers(Chu et al. 2021).

4.2. Effect of N addition on soil enzyme activities

Soil enzymes are an important indicator of soil fertility, and soil enzyme activity is closely related to soil nutrients, reflecting the strength of soil nutrient transformation and characterizing soil fertility(Yang et al. 2012). UE is a hydrolase that has a crucial effect on the soil N cycle. Specifically speaking, it converts small amounts of nitrogenous organic substrates into inorganic nitrogenous compounds (e.g., ammonia) to provide available nitrogen for normal plant growth and development(Iovieno et al. 2009). Furthermore, SC, as another important hydrolyzing enzyme in the soil, is able to degrade and convert sucrose in the soil into glucose and fructose, providing carbon and energy supply for microbial activities(Weige et al. 2016). Several studies have shown that N addition significantly increased soil UE and SC activities(Gong et al. 2015; Weng et al. 2013). This coincides with our findings that N application significantly increased UE and SC activities in both surface (0~20cm) and deep soils (20~40cm). The difference was that UE and SC activities were highest under N4 and N3 treatments, respectively. The enhancement of UE and SC activities under N application may be attributed to the stimulation of microbial activity by the increase in soil available N and the improvement of soil physicochemical properties making the soil environment more suitable for microbial growth and proliferation(Iovieno et al. 2009; Nayak et al. 2007). CAT and NR, as important oxidoreductases in soil, play important roles in energy transfer and material metabolism of organisms in soil. Moreover, they also indirectly affect plant uptake and utilization of N(Pu et al. 2019). NR catalyze NO^{3-} and $NO^{2-}$and transform them into NO^{2-} and NO, respectively(Braker & Conrad 2011). Nitrification, denitrification and mineralization induced by these enzymes are important biochemical processes in soil N metabolism that affect N use and loss. Therefore, various factors affecting soil N metabolism are also recognized as major determinants of the activities of these enzymes, especially N addition(Liu 2018). Previous studies have shown that exogenous N directly affects soil properties and microbial composition, thereby indirectly increasing soil NR and CAT activities(Wang et al. 2023a; Zhao et al. 2022). In our experiment, CAT activity showed an increasing and then decreasing trend with increasing N application, and CAT activity was higher than control under all treatments. In contrast, NR activity was positively correlated with N application. This was in line with the results of Weng et al. (2013). Furthermore, it was also found in our study that N application did not significantly affect NR activity in the 0~20 cm and 20~40 cm soil layers. And the activities of each enzyme decreased with the deepening of the soil layer. This may be due to the fact that soil microorganisms are mainly active in the tillage layer where the root system is well developed.

4.3 Effect of N addition on seed yield and yield components

N is an essential nutrient for plant growth and development, and proper fertilization management has a positive impact on crop growth, yield formation and ecological and environmental protection(Duan et al. 2011; Feng 2018). For perennial herbage, suitable N application can not only improve the aboveground biomass yield and grassland stand vegetation coverage, meet the needs of grassland animal husbandry and ecological restoration, but also effectively increase the seed yield and extend the use life of forage seed field(Gislum & Boelt 2009; Zhao et al. 2023). Nevertheless, seed yield always depends on the multiplicative effect of the yield components (NTs, NFTs, and TSW)(Dewitt et al. 2021). Numerous studies have shown that appropriate nitrogen additions significantly increase seed yield components. Researchers have shown in studies ~~of~~ *Elymus sibiricus*(Wang et al. 2018), *Elymus nutans*(Guo-Ping et al. 2010)and *Poa pratensis*(Bai et al. 2010) that N fertilization improves seed yield by increasing NTs and (or) NFTs. Investigations on seed yield of tall fescue showed that N fertilizer application was positively related to NFTs within a range(Hui et al. 2003). Additionally, similar findings have been found with respect to the seed yield of *Lolium perenne*, N addition increased NTs, NFTs and TSW(Hides et al. 2010). In this study, N application significantly increased NTs, NFTs and seed yield. NTs ($r = 0.75$, $P < 0.01$) and NFTs ($r = 0.91$, $P < 0.01$) were dramatically positively correlated with seed yield by N fertilizer in 2023. Path analysis showed that NTs and NFTs significantly contributed to seed yield. These results demonstrated that N addition was one of the most effective approaches to improving *F. kirilowii* seed yield by enhancing NTs and NFTs.

4.4. Correlation and path analysis

The results showed that there were strong interactions between seed yield and soil enzyme activities, while UE, CAT, and SC were the three vital acting enzymes. The N addition significantly stimulated the UE, CAT, and SC activities by increasing the TN and TP content, thereby enhancing the NTs, NFTs, and seed yield. The three most effective indicators for *F. kirilowii* seed yield were NFTs, UE and TN. The total effects of NFTs, UE and TN on seed yield were 0.493, 0.658 and 0.548, respectively.

5. Conclusion

In this study, we found that N addition significantly affected soil nutrient content, soil enzyme activity, yield components and seed yield. Among them, TN, TP, UE, CAT, NFTs and SY reached their maximum values under N4 treatment. Soil enzyme activities (UE, CAT and NR) were significantly higher than control under each N application treatment. The results showed that N application significantly stimulated soil enzyme activity by increasing soil N and P contents. The increased enzyme activities and soil nutrient availability jointly promoted yield components and seed yields of *F. kirilowii*.

Availability of data

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Author contributions

Wenbo Mi: Writing original draft, Writing review & editing, Visualization, Methodology. Wenhui Liu: Funding acquisition, Writing review & editing. Feng Luo and Kaiqiang Liu: performing the experiments, analyzing the data. Yan Qin, Yongchao Zhang and Wen Li: analyzing the data, preparing figures and/or tables. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figure 1

Figure 1 Effects of nitrogen fertilizer and soil layers treatments on soil nutrients of *F. kirilowii*.

Different lower-case letters above the column indicate significant differences under different nitrogen fertilizer treatments ($P < 0.05$). Different capital letters above the column indicate significant differences under different soil layer treatments ($P < 0.05$). Vertical bar represents the standard error of mean.

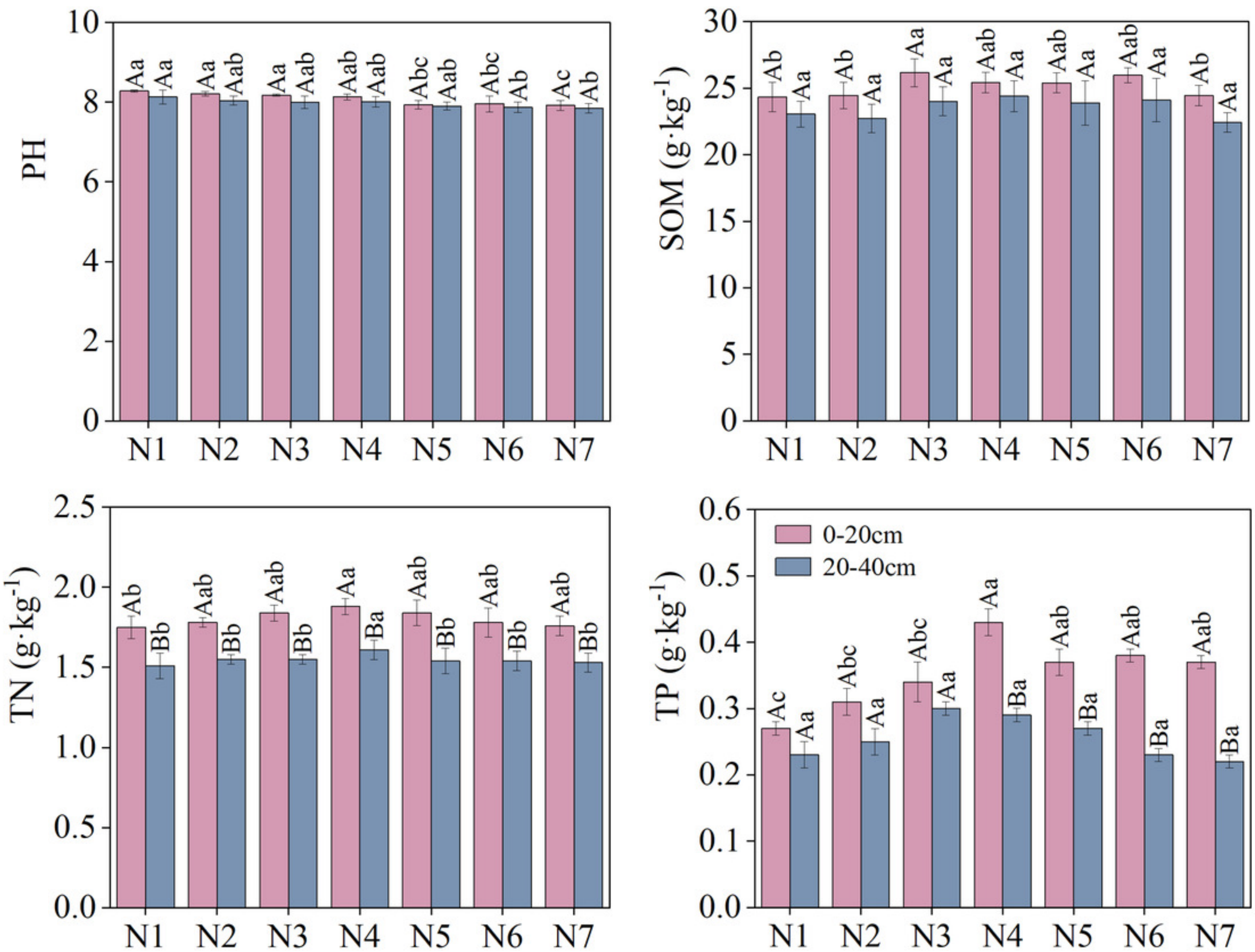

Figure 2

Figure 2 Effects of nitrogen fertilizer and soil layers treatments on soil enzymes activity of *F. kirilowii*.

Different lower-case letters above the column indicate significant differences under different nitrogen fertilizer treatments ($P < 0.05$). Different capital letters above the column indicate significant differences under different soil layer treatments ($P < 0.05$). Vertical bar represents the standard error of mean.

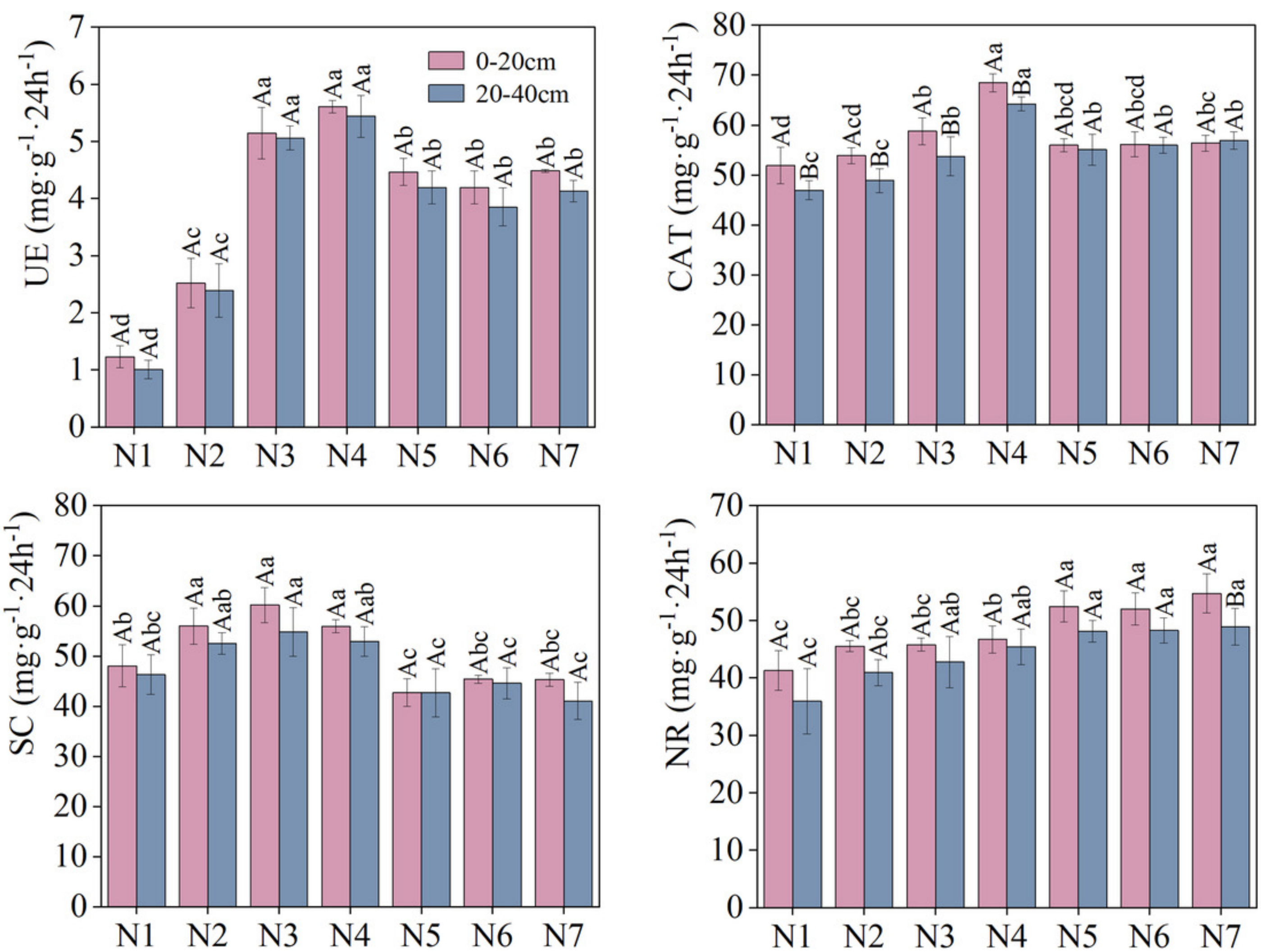

Figure 3

Figure 3 The yield components of *F. kirilowii* as influenced by seven different rates of N fertilizer application during the trial years 2022-2023.

Different lower-case letters above the column indicate significant differences under different nitrogen fertilizer treatments ($P < 0.05$). Vertical bar represents the standard error of mean.

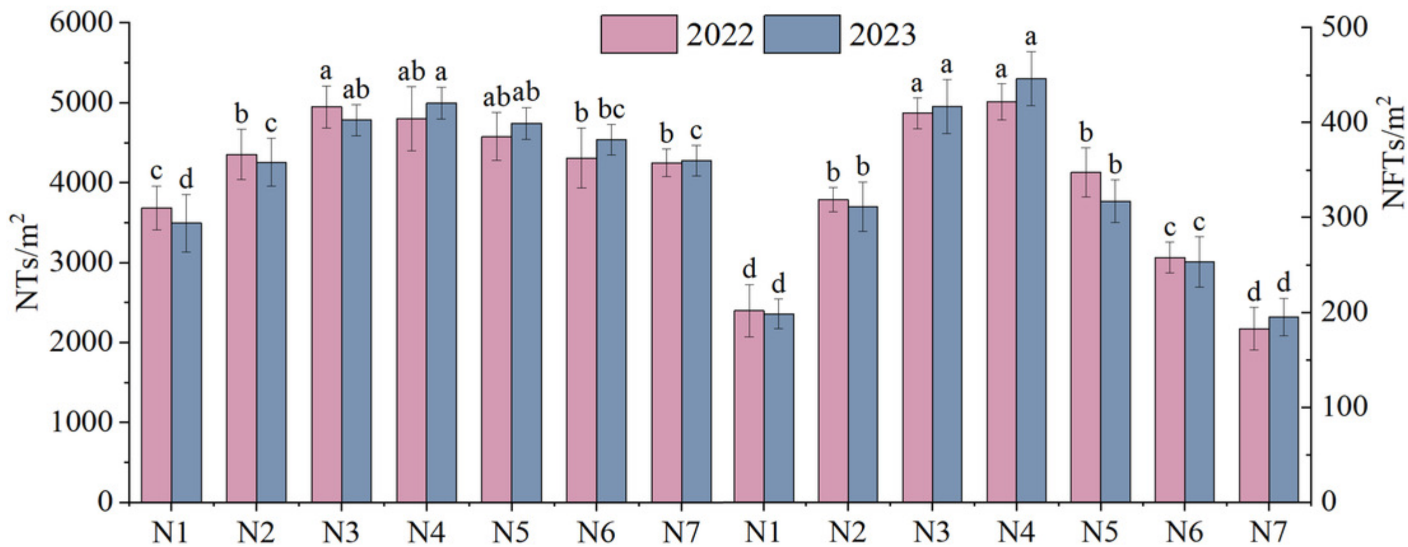

Figure 4

Figure 4 The seed yield (mean ± standard error, n = 4) of *F. kirilowii* as influenced by N fertilizer during the trial years of 2022-2023.

Different lower-case letters above the column indicate significant differences under different nitrogen fertilizer treatments ($P < 0.05$). Vertical bar represents the standard error of mean.

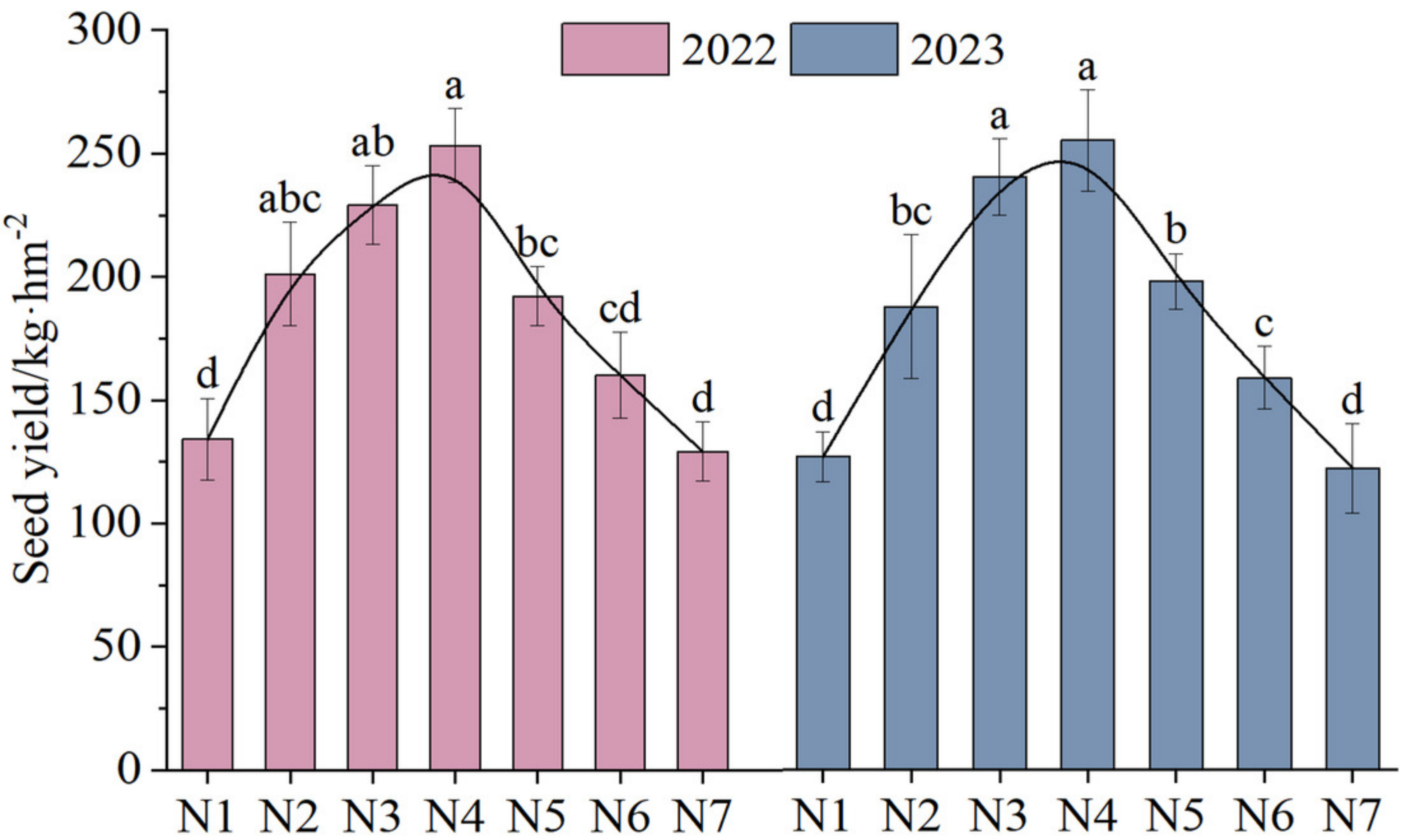

Figure 5

Figure 5 Pearson correlation between seed yield and soil enzyme activities, soil nutrients. (a) 0~20cm soil layer; (b) 20~40cm soil layer.

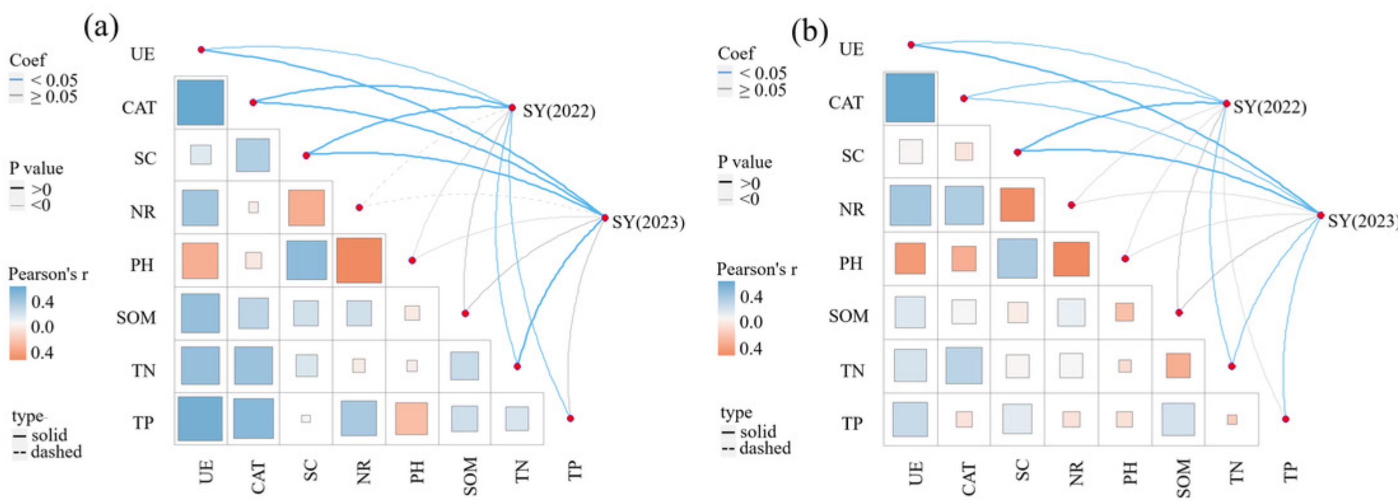

Figure 6

Figure 6 Effects of soil enzyme activities (UE, CAT, and SC), soil physicochemical properties (TP and TN), and yield components (NTs and NFTs) on seed yield.

The lines in black mean positive relationship, while those in red color mean negative relationship. The full and dashed lines represent direct and indirect effects, respectively. The width of lines is proportional to the strength of path coefficients. *, **, and *** represent significant differences at the 0.05, 0.01, and 0.001 level, respectively.

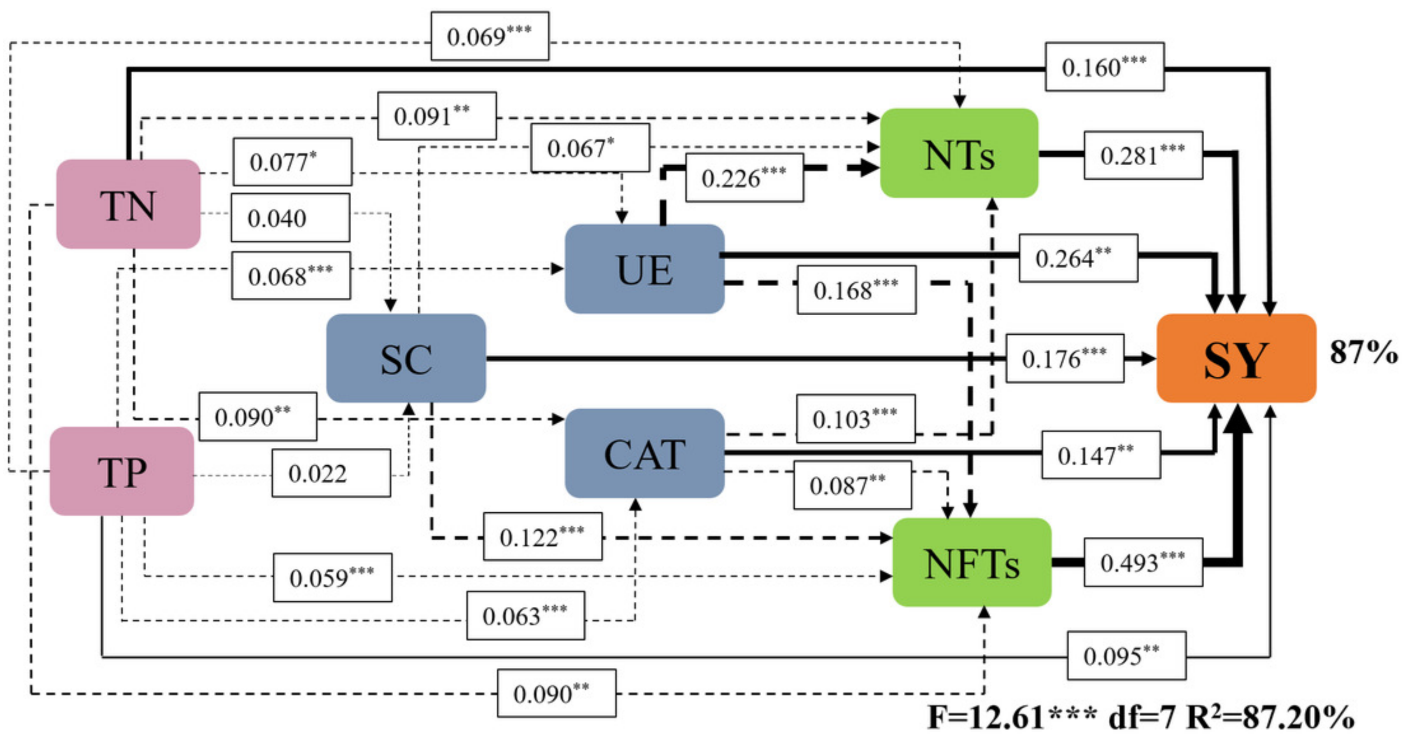

Figure 7

Figure 7 Standardized effects of indicators on seed yield

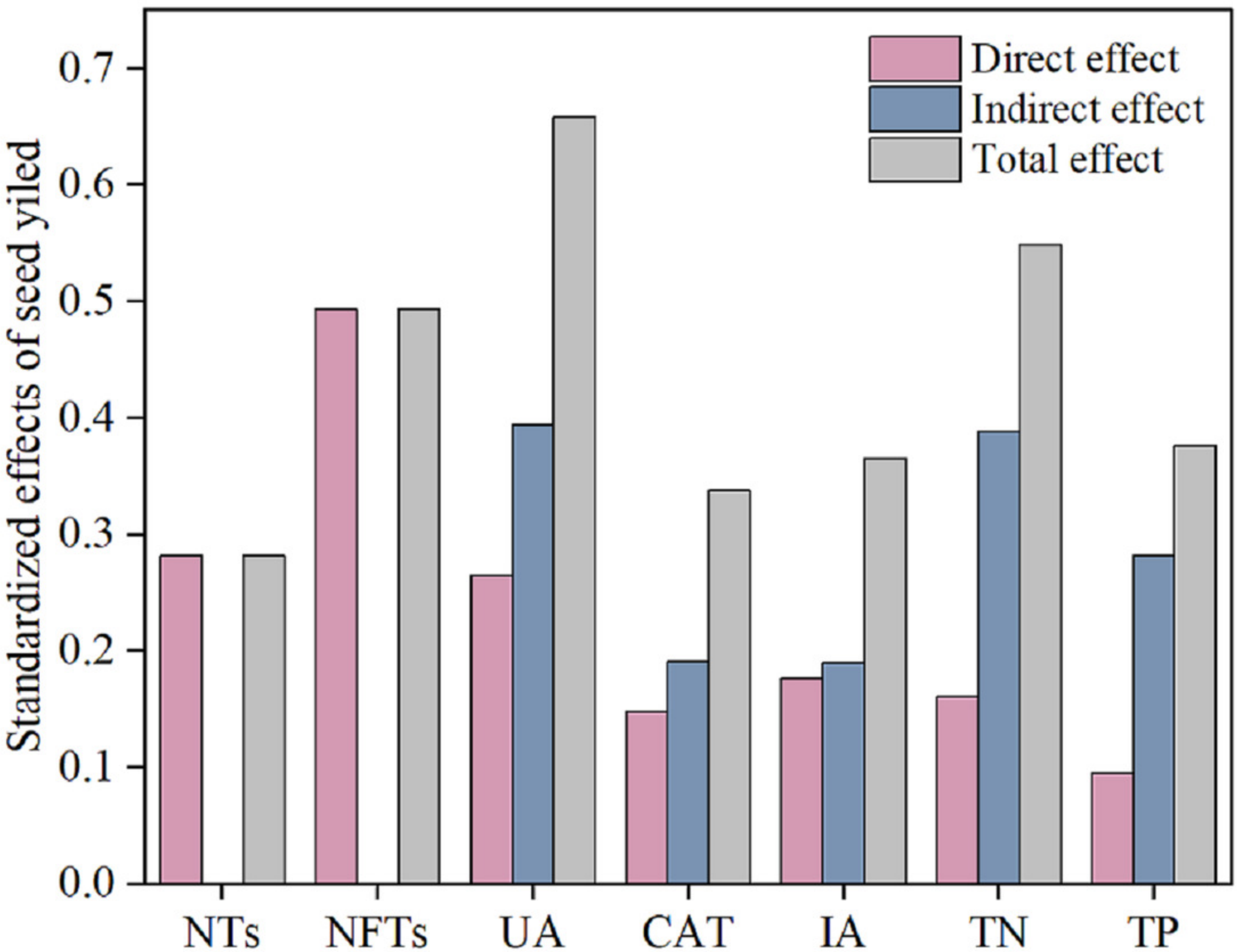

Table 1(on next page)

Table 1 Analysis of variance of the effects of nitrogen fertilizer and soil layers on *F. kirilowii* soil nutrients.

Note: PH, potential of hydrogen; SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus.

Index	Source of variance	Df	F-ratio	P-value
	Soil (S)	1	10.517	0.003
PH	N addition (N)	6	6.500	0.000
	Interaction S×N	6	0.291	0.936
	Soil (S)	1	25.378	0.000
SOM/$g \cdot kg^{-1}$	N addition (N)	6	2.867	0.026
	Interaction S×N	6	0.211	0.970
	Soil (S)	1	189.370	0.000
TN/$g \cdot kg^{-1}$	N addition (N)	6	2.366	0.045
	Interaction S×N	6	0.350	0.904
	Soil (S)	1	42.752	0.000
TP/$g \cdot kg^{-1}$	N addition (N)	6	3.334	0.013
	Interaction S×N	6	1.540	0.202

Table 2 (on next page)

Table 2 Analysis of variance of the effects of nitrogen fertilizer and soil layers on *F. kirilowii* soil enzyme activities.

Note: UE, urease; CAT, catalase; SC, sucrase; NR, nitrate reductase.

Index	Source of variance	Df	F-ratio	P-value
	Soil (S)	1	5.997	0.021
UE/mg·g^{-1}·24h^{-1}	N addition (N)	6	161.212	0.000
	Interaction S×N	6	0.184	0.979
	Soil (S)	1	14.912	0.001
CAT/ mg·g^{-1}·24h^{-1}	N addition (N)	6	30.764	0.000
	Interaction S×N	6	1.682	0.162
	Soil (S)	1	6.855	0.014
SC/ mg·g^{-1}·24h^{-1}	N addition (N)	6	20.199	0.000
	Interaction S×N	6	0.499	0.803
	Soil (S)	1	17.972	0.000
NR/ mg·g^{-1}·24h^{-1}	N addition (N)	6	14.173	0.000
	Interaction S×N	6	0.370	0.892

Table 3 (on next page)

Table 3 Analysis of variance of the influence of N fertilizer on *F. kirilowii* yield components and seed yield.

Note: NTs, number of tillers; NFTs , number of fertile tillers; SY, seed yield.

Index	Source of variance	Df	F-ratio	P-value
	Year (Y)	1	0.075	0.787
NTs/m^2	N addition (N)	6	15.928	0.000
	Interaction Y×N	6	0.610	0.720
	Year (Y)	1	0.000	0.984
NFTs/m^2	N addition (N)	6	109.572	0.000
	Interaction Y×N	6	0.892	0.514
	Year (Y)	1	0.020	0.887
SY/kg·hm^{-2}	N addition (N)	6	23.327	0.000
	Interaction Y×N	6	0.176	0.981

Table 4(on next page)

Table 4 Correlation between seed yield and yield components during trial year 2022 and 2023.

Note: * and ** represent significant differences at the 0.05 and 0.01 level, respectively.

Year	2022		2023	
Source	NTs	NFTs	NTs	NFTs
Seed yield	0.57**	0.84**	0.75**	0.91**

Table 5(on next page)

Table 5 Membership function analysis based on multiple indicators.

Indexes	N1	N2	N3	N4	N5	N6	N7
SY	0.038	0.535	0.848	1.000	0.540	0.262	0.000
NTs	0.000	0.547	0.975	1.000	0.818	0.638	0.513
NFTs	0.045	0.514	0.916	1.000	0.584	0.271	0.000
UE	0.000	0.305	0.904	1.000	0.729	0.659	0.724
CAT	0.000	0.115	0.404	1.000	0.360	0.392	0.426
SC	0.302	0.781	1.000	0.793	0.000	0.154	0.032
NR	1.000	0.651	0.572	0.437	0.117	0.128	0.000
PH	0.000	0.259	0.363	0.420	0.896	0.907	1.000
SOM	0.158	0.098	1.000	0.894	0.735	0.973	0.000
TN	0.000	0.324	0.618	1.000	0.559	0.279	0.132
TP	0.000	0.294	0.676	1.000	0.662	0.529	0.412
Rank	7	4	2	1	3	5	6