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THE EFFECT OF EXOGENOUS NITRIC OXIDE ON BENZO[A]PYRENE STRESS IN RYEGRASS (*Lolium perenne* L.)

The pot experiment investigated the role of exogenous nitric oxide (NO) in mitigating benzo[a]pyrene (B[a]P) stress and regulating mineral element dynamics in ryegrass. Exposure to 30 μmol/dm^3 B[a]P significantly suppressed seed germination, root vitality, nitrate reductase (NR) activity, and dry weight, while altering mineral distribution, copper (Cu) and manganese (Mn) accumulated in roots, whereas nitrogen (N), phosphorus (P), lead (Pb), and cadmium (Cd) were translocated to leaves. Seed germination, plant height, root vitality, and NR activity initially increased at 100–300 μmol/dm^3 sodium nitroprusside (SNP, NO donor) but declined at 400 μmol/dm^3. SNP treatments enhanced root retention of N, P, Mn, Cu, and Pb, reducing toxic metal mobility. PCA, cluster, and correlation analyses identified 200-300 μmol/dm^3 SNP as optimal for alleviating B[a]P stress, with N and P dynamics most strongly correlated with growth recovery. Exogenous NO counteracts B[a]P-induced phytotoxicity by regulating root-to-leaf translocation of N and P to sustain metabolic activity, restricting Pb/Cd mobility and redistributing essential minerals (Cu, Mn) to minimize aerial tissue exposure, optimizing SNP concentration to enhance stress tolerance without overburdening detoxification pathways. This study underscores NO's dual role as a nutrient coordinator and detoxification agent, offering strategies to enhance plant resilience in B[a]P-contaminated environments.

1. INTRODUCTION

Soil has been seriously polluted by polycyclic aromatic hydrocarbons (PAHs) in agricultural production in developing countries. More than 90% of PAHs enter organisms via soil accumulation and migration, which is highly dangerous for human survival and the environment [1, 2]. B[a]P, as a representative of PAHs, is widely found in the

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atmosphere and soil. It has a high level of toxicity and chemical stability, inducing carcinogenesis by altering the cell cycle [3]. Studies have found that B[a]P reduced the stem length, root length, and fresh weight of wheat (*Triticum aestivum* L.) seedlings [4]. Low concentration of B[a]P alleviated lead stress of ryegrass, enhanced root vitality, and promoted adventitious root differentiation of plants [5, 6]. However, the effect of B[a]P on the content of mineral elements in plants is still unclear.

NO has been recognized as an important signaling factor in plants, which participates in plant growth and physiological processes such as seed germination, growth, antioxidant capacity, osmotic regulation, photosynthesis, signal transduction, gene expression, etc. [7–9]. It can relieve abiotic stresses such as heavy metal stress, low temperature stress, salt stress, and drought stress on plants [10–12]. Studies have shown that low concentration of NO donor sodium nitroprusside (SNP) promoted the seed germination of tomato (*Lycopersicon esculentum* Mill.) under chromium (Cr) stress and enhanced root vitality of tomato under cadmium (Cd) stress [7, 13]. Nitrate reductase (NR) activity in rice (*Oryza sativa* L.) can be decreased, and the content of mineral elements increased through adding SNP [14]. However, little information is available on the effect of NO on the absorption and transport mechanism of mineral elements in plants under B[a]P stress.

Ryegrass is often used as one of the appropriate plants for phytoremediation owing to its strong tolerance to heavy metals and PAHs [7, 8]. Ryegrass can grow in soils with very high concentrations of B[a]P, extracting it through their roots. Bai et al. [15] found that under lead stress, the contents of copper (Cu), potassium (K), and zinc (Zn) in the aboveground parts of ryegrass decreased. Current research has predominantly centered on examining the impacts of heavy metal pollution on photosynthesis, antioxidant systems, and growth parameters in ryegrass [16]. However, little attention is paid to the changes of mineral elements in ryegrass under PAH stress and the effects of NO on the transport and absorption of mineral elements. Heavy metals in ryegrass under PAH stress have not been fully investigated simultaneously. The objective of this study was to examine the effects of exogenous NO on the absorption, migration, and accumulation of mineral elements, heavy metal elements of ryegrass, and its antagonistic mechanism under B[a]P stress, providing a scientific basis for the application of NO in B[a]P contaminated soil.

2. METHODS AND MATERIALS

Materials. Ryegrass (*Lolium perenne* L.) seeds were purchased from Liaoning Fuyou Seed Industry Co., Ltd., China. SNP (> 97% purity), B[a]P (> 98% purity) were purchased from Sigma Company, USA. All other reagents (analytical purity) were purchased from Sinopharm Chemical Reagents Shenyang Co., Ltd., China.

Seed germination. An experiment on the seed germination of ryegrass under different concentrations of B[a]P (0, 10, 20, 30, 40 μmol/dm^3) was conducted to select the optimal B[a]P stress concentration. Evenly sized plump ryegrass seeds were selected and sterilized for 4–5 min in 5 vol. % sodium hypochlorite solution. The seeds were rinsed thoroughly with deionized water, and then dried and soaked in warm water at 30 °C for 30 min. 50 seeds and 100 cm^3 of B[a]P solutions of different concentrations were placed on four Petri dishes (14 cm in diameter). Another 50 seeds were placed onto clean Petri dishes, a mixture of 100 cm^3 30 μmol/dm^3 B[a]P and different concentrations of SNP (100, 200, 300, and 400 μmol/dm^3) were added. Each treatment had six replicates, and seeds were germinated in a constant temperature incubator at 28 °C.

Experiment of seeding culture. Soil preparation. The tested soil was collected from the topsoil in the Liaoning University Ecological Park (0–20 cm in depth) in Shenyang, Liaoning, China. The basic physical and chemical properties of soil are given in Table 1. The soil samples were air-dried. After removing rocks and debris, the plant roots were ground, sieved through a 10-mesh sieve, and homogenized. No PAHs were detected in the collected soil. Ammonium nitrate 35% N, super phosphate 50% P_2O_5, and potassium chloride 55% K_2O were added as a base fertilizer and fully stirred to prepare the sample. Samples were added to deionized water to adjust soil moisture to 60% and put into pots after 7 days.

Table 1

Basic physical and chemical properties of the soil

Soil parameter	Value
pH	6.02
Organic matter, g/kg	19.8
Cation-exchange capacity, cmol/kg	22.7
Total nitrogen (N), %	0.151
Total phosphorus (P), mg/kg	561
Total potassium (K), %	1.68
Copper (Cu), mg/kg	40.35
Zinc (Zn), mg/kg	24.56
Manganese (Mn), mg/kg	322.78
Lead (Pb), mg/kg	0.47
Cadmium (Cd), mg/kg	2.74
B[a]P, mg/kg	ND

SNP experiment. Ryegrass seedlings were placed in the pots after germination, and 50 seedlings were left in each pot when the ryegrass seedlings grew to 20 days. When seedlings grew to 58 days, 50 cm^3 30 μmol/dm^3 B[a]P as the stress concentration and 50 cm^3 different concentrations of SNP (100, 200, 300, and 400 μmol/dm^3) were mixed to spray leaves of ryegrass in each group, and each group had 6 replicates. 100 cm^3 mixtures

were evenly sprayed onto the ryegrass leaves to keep them moist and free of water droplets. Plant height and root length of ryegrass were measured in each treatment group 14 days after spraying. After the aboveground and underground parts of ryegrass were harvested, each part was rinsed with deionized water to determine fresh weight. After drying, the dry weight was determined, and the samples were stored in the refrigerator at –80 °C. The root vitality, NR activity, and element content of ryegrass were determined once a day.

Germination indices. The germination of seeds was observed every day during the germination period. With the first root visible on seed skin as a sign of protuberance, the number of protuberant seeds was observed 7 days later, and the seeds with consistent growth status were observed 14 days later (the bud length was more than 2 mm as a sign of germination). The seed germination and seedling growth of ryegrass on the 7th and 14th day were recorded [17]:

$$\text{Germination rate} = \left(\sum \frac{n}{N} \right) \times 100\%$$

$$\text{Germination capacity} = \frac{C_t}{N} \times 100\%$$

$$\text{Germination index} = \sum \frac{G_t}{D_t}$$

where n is the cumulative number of germinated seeds in t days, C_t is the number of germinated seeds in t days, N is the total seed number for the testing, G_t is the number of germinated seeds on day t, D_t is the number of days since sowing at time t.

Determination of biomass. 20 seedlings were selected from each treatment group, and their plant height and root length were measured with Vernier calipers. The experiment was repeated 3 times in each treatment group. The roots and leaves of the seedlings were separated, and their fresh weight was determined separately, and then the seedlings were placed in an oven at 65 °C and dried for 45–48 h to constant weight. Then the dry weight was recorded [18].

Root vitality. The root vitality of ryegrass was determined by the 2,3,5-triphenyltetrazolium chloride (TTC) reduction method [19]. Ryegrass root tips were weighed and fully immersed in an equal mixture of 0.4% TTC solution and phosphoric acid buffer. After dark insulation at 37 °C for 1–3 h, H_2SO_4 was added to stop the reaction. The roots were taken out, and 0.01 g of $Na_2S_2O_4$ powder was added before shaking well to extract

triphenylmethane. The standard curve was analyzed using a spectrophotometer at a wavelength of 485 nm. The root vitality, mg/(g·h), was evaluated by the following equation:

$$\text{Root vitality} = \frac{\text{Weight of the reduced product of TCC}}{\text{Root weight} \times \text{time}}$$

Determination of nitrate reductase (NR) activity. Ten seedling leaves in each treatment were removed and rinsed with deionized water, then dried with gauze and filter paper. The leaves were finely chopped, homogenized, and divided into three labeled portions. These portions were then added to either a 0.1 mol/dm^3 phosphate buffer solution (pH 7.5) or a 0.2 mol/dm^{30} potassium nitrate reagent. The leaf samples and reaction solution were combined in a syringe. The system was repeatedly evacuated to create a vacuum, after which the leaves were incubated in the dark at 30 °C for 30 min. Subsequently, the samples were treated with trichloroacetic acid to quench the enzymatic reaction. Following centrifugation (3000*g*, 10 min), 2 cm^3 of the supernatant was collected to quantify nitrite $\left(NO_2^-\right)$ concentration. Enzyme activity was calculated as the rate of nitrite$^-$ production, expressed in μg/(g·FW·h) [19].

Determination of mineral and heavy metal elements content. The samples of roots and leaves were rinsed with distilled water. 3 g of fresh samples were dehydrated at 80–90 °C for 30 min, dried in an oven at 60 °C to constant weight. 0.1 g of dry samples were put into the test tube before adding 5 cm^3 concentrated H_2SO_4. The test tube was kept at a constant volume after repeated heating, taking out, and cooling. The contents of N and P were determined by UV-Vis spectrophotometry (Shimadzu-1601PC) [9]. 1 g of ryegrass roots and leaves were added to 10 cm^3 of concentrated nitric acid in 150 cm^3 triangular flasks. It was covered with the curved neck funnel and soaked overnight. The triangular flask had been heated on an electric heating plate until white smoke came out. 2 cm^3 $HClO_4$ was added to dissolve them until the solution was clear and colorless. A small amount of distilled water was added to a 50 cm^3 volumetric flask for constant volume. The contents of K, Cu, Zn, Mn, Pb, Cd were determined with a TAS-990 atomic absorption spectrophotometer [9].

Calculation of the transfer coefficient. The transfer coefficient (*TF*) refers to the ratio of leaf element content to root element content:

$$TF = \frac{\text{Element concentration in the leave}}{\text{Element concentration in the root}}$$

Quality control (QC). QC measures were implemented to evaluate potential contamination and ensure data reliability. QC samples, prepared from certified standard solutions of K, Cu, Zn, Mn, Pb, and Cd, were analyzed alongside experimental samples

to validate metal recovery rates. Mean recoveries were within acceptable ranges: 97% (K), 96% (Cu), 95% (Zn), 91% (Mn), 98% (Pb), and 101% (Cd). The variation coefficients were below 5%. The limit of detection for K, Cu, Zn, Mn, Pb and Cd were 0.25, 0.19, 0.43, 0.2, 0.13, and 0.4 mg/kg, respectively.

Statistical analysis. Origin 2018 was used for mapping, and Microsoft Excel 2010 and SPSS 22.0 software were used for data statistics, principal analysis, cluster analysis and correlation coefficient. Two-way analysis of variance (ANOVA) was used for comparison between different groups, and LSD was used for difference significance analysis ($p < 0.05$). Growth parameters, physiological traits, and aboveground/belowground elemental content of ryegrass were incorporated as variables in a hierarchical cluster analysis using the squared Euclidean distance method.

3. RESULTS

3.1. EFFECTS OF EXOGENOUS NO ON THE GROWTH OF RYEGRASS UNDER B[a]P STRESS

After 14 days of cultivation, the inhibition of 30 μmol/dm^3 B[a]P stress on seed germination rate, capacity, and index was lower than for 40 μmol/dm^3 but higher than for 20 μmol/dm^3 B[a]P. The optimal stress concentration 30 μmol/dm^3 B[a]P was selected to use in the experiment.

Figure 1a illustrates that 30 μmol/dm^3 B[a]P stress significantly reduced the ryegrass germination rate, capacity, and index by 88.21, 79.43, and 84.12% respectively ($p < 0.05$), compared to the control. These results indicated that B[a]P stress strongly inhibited ryegrass seedling germination. However, treatment with 200 μmol/dm^3 SNP markedly alleviated this stress: the germination rate (at 7 and 14 days) and germination index increased by 11.5-, 5.5-, and 7.5-fold, respectively ($p < 0.05$), relative to B[a]P alone. While 400 μmol/dm^3 SNP exhibited a weaker effect than the control, it still outperformed the 30 μmol/dm^3 B[a]P treatment. Collectively, these findings demonstrate that exogenous NO supplementation can mitigate B[a]P-induced inhibition of ryegrass germination.

Compared to the control, Fig. 1b demonstrates that root length under 30 μmol/dm^3 B[a]P stress increased by 21.05% ($p < 0.05$). Plant heights of ryegrass with 100, 200, 300, and 400 μmol/dm^3 SNP treatment increased by 41.51, 19.35, 32.26, and 38.71% ($p < 0.05$ for all), respectively. However, compared to 30 μmol/dm^3 B[a]P, 100, 300, and 400 μmol/dm^3 SNP significantly inhibited root length, with inhibition rates of 42.11, 13.33, and 26.67%, respectively ($p < 0.05$). These results indicate that 30 μmol/dm^3 B[a]P stress did not significantly affect plant height but markedly enhanced root elongation. In contrast, exogenous NO strongly promoted the ryegrass plant height, with the 100 μmol/dm^3 SNP exhibiting the greatest stimulatory effect.

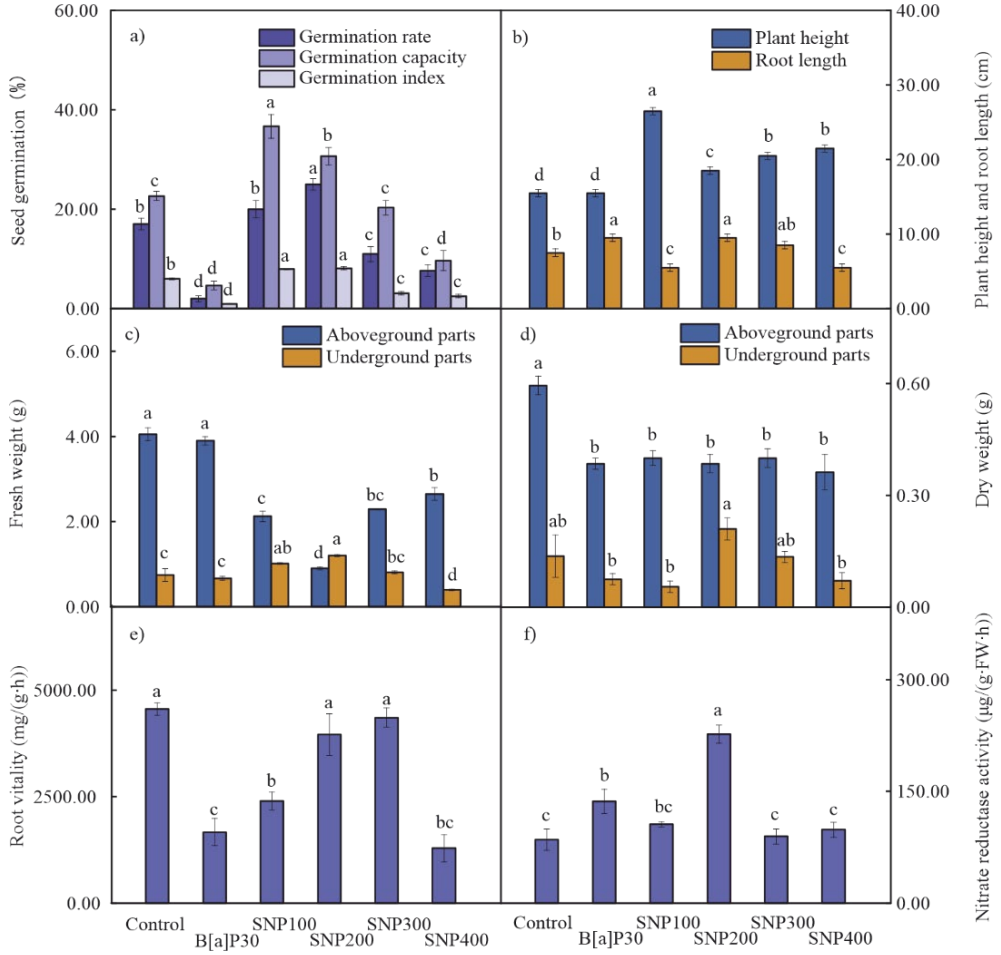

Fig. 1. Effects of exogenous NO on the seed germination, biomass, root vitality, and nitrate reductase activity of ryegrass under B[a]P stress. The data in the figure are mean ± SD. Small letters a–d indicate the statistically significant difference between treatment groups at $p < 0.05$. Detailed description of figures a)–f) in the text

Figure 1c demonstrates that exogenous NO supplementation significantly reduced the aboveground fresh weight of ryegrass compared to the control. Specifically, treatments with 100, 200, 300, and 400 μmol/dm^3 SNP decreased aboveground fresh weight by 47.53, 77.65, 43.46, and 34.57%, respectively ($p < 0.05$), with the most pronounced reduction (77.65%) occurring at 200 μmol/dm^3 SNP.

In contrast, the underground fresh weight of ryegrass exhibited a biphasic response to SNP: compared to 30 μmol/dm^3 B[a]P alone, it increased by 34.31%, 44.40%, and 17.28% at 100, 200, and 300 μmol/dm^3 SNP, respectively, but decreased by 40.30% at 400 μmol/dm^3 SNP ($p < 0.05$ for all). Notably, 30 μmol/dm^3 B[a]P stress alone had no

significant effect on overall fresh weight. These results indicate that while exogenous NO strongly suppresses aboveground biomass accumulation in ryegrass, its impact on underground biomass depends on concentration, with moderate SNP levels enhancing root growth but higher doses causing inhibition.

Figure 1d demonstrates that 30 μmol/dm^3 B[a]P stress significantly reduced the dry weight of ryegrass, decreasing aboveground and underground biomass by 35.32 and 45.33%, respectively ($p < 0.05$). Following exogenous NO treatment, no significant recovery in dry weight was observed compared to the B[a]P-stressed group ($p > 0.05$), suggesting that B[a]P-induced reductions in ryegrass biomass are largely irreversible.

The underground dry weight exhibited a biphasic response to increasing SNP concentrations: it initially increased by 34.76% at 200 μmol/dm^3 SNP compared to the control, but decreased sharply by 47.81% at 400 μmol/dm^3 SNP ($p < 0.05$). 100 μmol/dm^3 SNP showed no significant difference from the B[a]P-stressed group ($p > 0.05$). These results indicate that while exogenous NO partially restored root biomass at 200 μmol/dm^3 SNP, higher doses exacerbated suppression. Overall, B[a]P stress severely impaired ryegrass dry matter accumulation, and exogenous NO modulated underground biomass in a concentration-dependent manner, with 200 μmol/dm^3 SNP showing the most pronounced restorative effect on root growth.

Under 30 μmol/dm^3 B[a]P stress, ryegrass root vitality decreased by 63.45% ($p < 0.05$) (Fig. 1e), while NR activity increased by 37.56% compared to the control ($p < 0.05$), (Fig. 1f). The addition of SNP elicited a biphasic response in root vitality: the values initially increased at lower SNP concentrations but decreased sharply at higher doses. 100 and 400 μmol/dm^3 SNP reduced root vitality by 47.37 and 71.64%, respectively, relative to the control ($p < 0.05$). NR activity peaked at 200 μmol/dm^3 SNP, showing a 1.66-fold increase over the control and a 0.66-fold increase over the B[a]P-stressed group ($p < 0.05$), respectively. These results indicate that B[a]P stress suppresses root vitality but moderately enhances NR activity. Exogenous NO partially counteracted these effects, with 200 μmol/dm^3 SNP optimally boosting NR activity and mitigating B[a]P-induced root damage. Thus, NO supplementation may enhance stress resilience in ryegrass by modulating enzymatic and physiological responses.

3.2. EFFECTS OF EXOGENOUS NO ON CONTENTS OF MINERAL ELEMENTS AND HEAVY METAL ELEMENTS IN RYEGRASS UNDER B[a]P STRESS

As shown in Figs. 2a–2c, exposure to 30 μmol/dm^3 B[a]P stress significantly changed nutrient dynamics in ryegrass compared to the control. Leaf N content increased by 10.84% ($p < 0.05$). P content increased by 48.49% in leaves and 72.55% in roots ($p < 0.05$). Leaf K content was significantly reduced ($p < 0.05$). These results demonstrate that B[a]P stress enhances N and P accumulation in ryegrass but suppresses K uptake. When SNP was applied alongside B[a]P stress, nutrient partitioning shifted concentration-dependently.

At 100, 200, and 400 μmol/dm^3 SNP, leaf N decreased sharply by 96.82, 75.36, and 96.30, respectively ($p < 0.05$), while root N increased by 70.33%, 45.90%, and 57.53%, respectively ($p < 0.05$), indicating N translocation from leaves to roots. 300 μmol/dm^3 SNP uniquely enhanced N accumulation in both leaves and roots ($p < 0.05$). Root P content under SNP and B[a]P exceeded levels observed under B[a]P alone or SNP alone. Leaf P exhibited a V-shaped trend, declining initially but peaking at 400 μmol/dm^3 SNP. At 300 μmol/dm^3 SNP, leaf K content surpassed control levels ($p < 0.05$), counteracting B[a]P-induced suppression.

As shown in Figs. 2d–2f, exposure to 30 μmol/dm^3 B[a]P stress significantly suppressed Cu and Mn uptake in ryegrass compared to the control. Leaf and root Cu content decreased by 98.40% and 95.58%, respectively ($p < 0.05$). Leaf Mn content declined by 50.69% ($p < 0.05$). The results indicated that B[a]P stress inhibited Cu and Mn accumulation in ryegrass. The addition of 400 μmol/dm^3 SNP counteracted B[a]P-induced suppression. Leaf and root Cu content surged by 97.40 and 98.00%, respectively ($p < 0.05$). Both leaf and root Mn content increased progressively with SNP concentration, peaking at 78.74% in the leaves and 74.95% in the roots under 400 μmol/dm^3 SNP ($p < 0.05$). Neither B[a]P stress nor SNP treatments significantly changed Zn content ($p > 0.05$).

As shown in Figs. 2g, 2h, exposure to 30 μmol/dm^3 B[a]P stress elicited contrasting effects on heavy metal dynamics in ryegrass compared to the control. Leaf Pb content showed a sharp increase by 55.98% ($p < 0.05$), while root Pb decreased by 51.61% ($p < 0.05$), indicating enhanced Pb translocation from roots to leaves. Leaf Cd declined by 47.04% ($p < 0.05$), and root Cd was substantially reduced by 75.53% ($p < 0.05$), demonstrating B[a]P's capacity to suppress Cd uptake. Leaf Pb exhibited a dose-dependent decline with increasing SNP concentrations. Root Pb displayed a non-linear response, rising initially under 100–300 μmol/dm^3 SNP treatment before dropping, reaching its lowest level at 300 μmol/dm^3 SNP. Both leaf and root Cd content were significantly reduced across all SNP treatments compared to the control ($p < 0.05$). Exogenous NO under B[a]P stress mitigates Pb and Cd accumulation.

The variability in elemental content within ryegrass was governed by differences in translocation efficiency across plant tissues. Under 30 μmol/dm^3 B[a]P stress, the *TF*s for Cu and Mn were 0.91 and 0.53, respectively, representing increases of 64.5% (Cu) and 61.6% (Mn) compared to the control (CK) (Table 2). In contrast, *TF*s for N, P, K, Zn, Pb, and Cd all exceeded 1.0. These findings suggest that ryegrass roots preferentially retained Cu and Mn under B[a]P stress, while actively translocating N, P, K, Zn, Pb, and Cd to the leaves.

Following SNP application, *TF*s for N, P, and Cu in ryegrass roots and leaves exhibited distinct trends. *TF*s ranged from 0.03–2.46 (N), 0.34–0.66 (P), and 0.70–1.04 (Cu), all lower than control values. These elements displayed a biphasic response that *TF*s initially rose but declined at higher SNP concentrations.

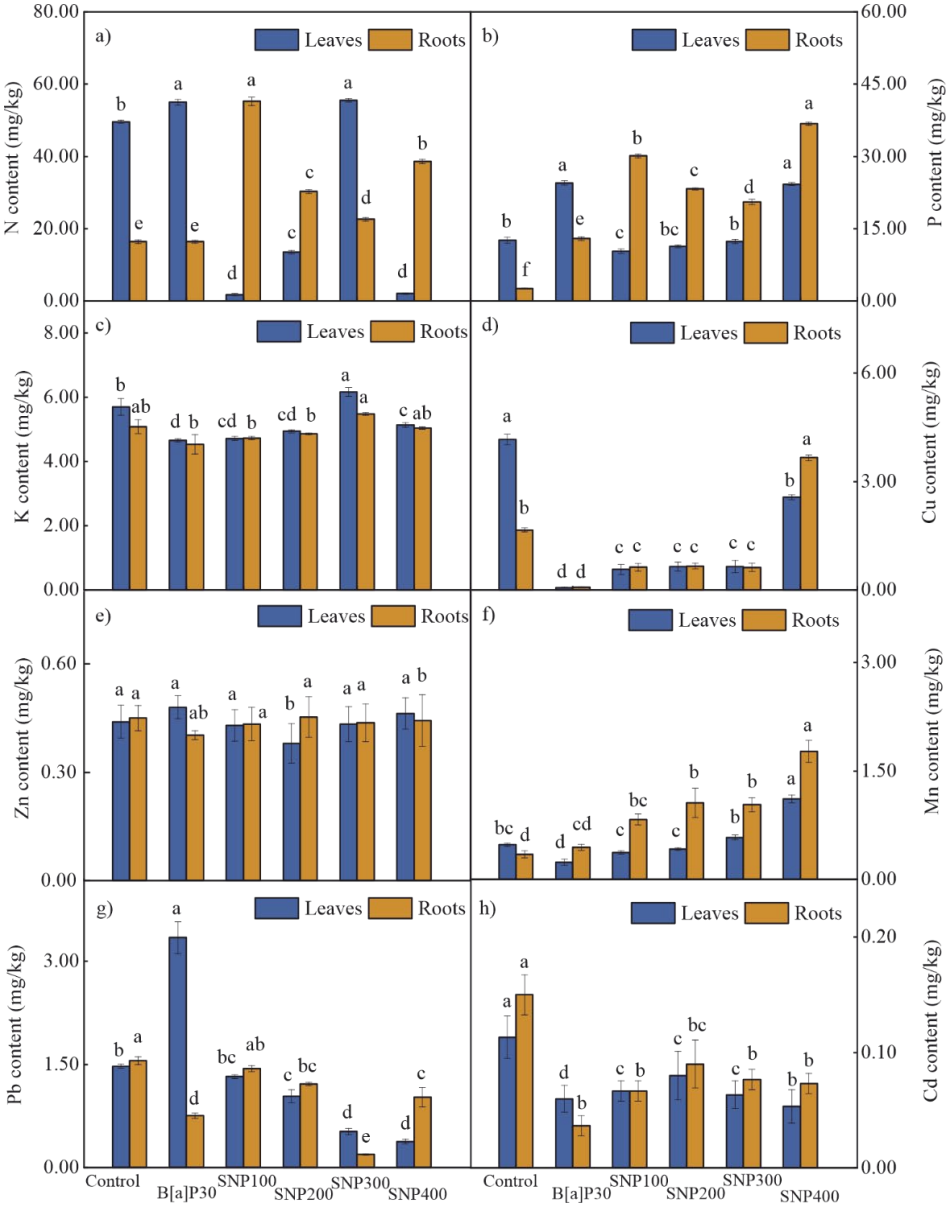

Fig. 2. Effects of exogenous NO on N, P, K, Cu, Zn, Mn, Pb, and Cd contents of ryegrass under B[a]P stress. The data in the figure are mean ± SD. Small letters a–d indicate the statistically significant difference between treatment groups at $p < 0.05$. Detailed description of figures a)–h) in the text

300 μmol/dm^3 SNP significantly enhanced *TF*s for N, P, and Cu compared to other concentrations. *TF*s ranged from 0.40 to 0.63 (Mn), with transport capacity suppressed at

100–200 μmol/dm^3 SNP but restored at 300–400 μmol/dm^3 SNP. At 400 μmol/dm^3 SNP, Zn shifted from root retention (*TF* < 1) to active translocation to leaves (*TF* > 1). *TF*s ranged from 0.37 to 2.77 (Pb), decreasing at 100–200 μmol/dm^3 SNP. 300 μmol/dm^3 SNP markedly increased Pb translocation from leaves to roots, but this effect diminished at 400 μmol/dm^3 SNP. *TF*s of Cd (0.73–1.00) revealed a progressive weakening of leaf-to-root transport with increasing SNP concentrations. No significant *TF* of K changes occurred at any SNP concentration. 300 μmol/dm^3 SNP optimally enhanced translocation of N, Cu, Mn, and Pb. 400 μmol/dm^3 SNP uniquely altered Zn and Mn dynamics, promoting Zn leaf accumulation and restoring Mn transport.

Table 2

TF of each element in ryegrass

Treatment	N	P	K	Cu	Zn	Mn	Pb	Cd
Control	3.02±0.13b	4.94±0.23a	1.12±0.08a	2.56±0.02a	0.98±0.04b	1.38±0.15a	0.95±0.02c	0.80±0.06a
B[a]P30	3.36±0.13a	1.89±0.09b	1.03±0.06a	0.91±0.04b	1.19±0.10a	0.53±0.14b	4.44±0.09a	1.64±0.15a
SNP100	0.03±0.00e	0.34±0.02c	1.00±0.05a	0.90±0.39b	0.99±0.00b	0.45±0.03b	0.92±0.00c	1.00±0.09a
SNP200	0.45±0.00d	0.49±0.00c	1.02±0.00a	0.97±0.19b	0.84±0.22b	0.40±0.09b	0.85±0.09c	0.89±0.03a
SNP300	2.46±0.08c	0.60±0.00c	1.12±0.01a	1.04±0.11b	0.99±0.02b	0.56±0.02b	2.77±0.31b	0.83±0.24a
SNP400	0.05±0.00e	0.66±0.01c	1.02±0.00a	0.70±0.00b	1.05±0.30a	0.63±0.08b	0.37±0.02d	0.73±0.05a

Control (CK): no SNP and B[a]P added.

3.3. THE CORRELATION COEFFICIENT CLUSTER, AND PRINCIPAL COMPONENT ANALYSIS OF EACH INDEX OF RYEGRASS

Correlation analysis (Table 3) was performed to assess relationships between elemental content and physiological indices in ryegrass. A strong positive correlation with plant height ($r = 0.939$, $p < 0.05$), underscoring N's critical role in ryegrass growth. Leaf Zn significantly negatively correlated with NR activity ($r = –0.894$) and underground fresh weight ($r = –0.881$, $p < 0.05$). Root Zn exhibited a strong negative correlation with plant height ($r = –0.955$) but a positive correlation with underground dry weight ($r = 0.955$, $p < 0.05$). Leaf Mn negatively correlated with germination potential ($r = –0.904$, $p < 0.05$). Leaf Cd positively correlated with aboveground dry weight ($r = 0.938$, $p < 0.05$), suggesting preferential Cd accumulation in leaves.

PCA was performed to investigate the interplay between elemental distribution, biomass parameters, and exogenous NO supplementation in ryegrass under B[a]P stress. The first two principal components, PC1 and PC2, collectively explained 70.6% of the total variance (PC1: 39.2%, PC2: 31.4%) (Fig. 3). PC1 was strongly associated with plant height and mineral elements linked to growth and nutrient allocation, including root N, leaf/root P, root K, root Cu, leaf Zn, and leaf Mn. PC2 reflected correlations between developmental metrics (seed germination, NR activity) and biomass parameters (underground fresh weight, root length) alongside elements such as root N, root P,

root Mn, and leaf/root Pb. PC3 (18.5% variance) captured interconnected responses in root-system indices following exogenous NO application, suggesting NO-mediated root metabolic adjustments. Exogenous NO induced systematic reallocation of elements to physiological indices critical for stress adaptation. Comprehensive PCA scores (Table 4) ranked treatments as follows: Control > 200 μmol/dm^3 SNP > 300 μmol/dm^3 SNP, indicating that 200–300 μmol/dm^3 SNP optimally mitigated B[a]P stress.

Table 3

Correlation coefficients

	NR activity	Root vitality	Germination			Plant height	Root length	Fresh weight		Dry weight	
			Rate	Capacity	Index			Ground	Underground	Ground	Underground
N leave	–0.370	0.833	–0.179	–0.169	–0.283	–0.653	0.503	0.463	–0.187	0.269	0.522
N root	0.069	–0.759	0.128	0.391	0.297	0.939*	–0.610	–0.353	0.290	–0.258	–0.805
P leave	–0.255	–0.700	–0.741	–0.863	–0.721	0.066	–0.475	0.151	–0.791	–0.361	–0.297
P root	0.153	–0.855	–0.257	–0.131	–0.201	0.774	–0.388	–0.562	0.011	–0.759	–0.792
K leave	–0.496	0.612	–0.493	–0.452	–0.599	–0.540	0.392	0.442	–0.416	0.001	0.313
K root	–0.441	0.428	–0.628	–0.576	–0.750	–0.398	0.384	0.254	–0.449	–0.296	0.135
Cu leave	–0.444	0.076	–0.293	–0.490	–0.250	–0.574	–0.317	0.834	–0.706	0.679	0.445
Cu root	–0.319	–0.651	–0.670	–0.821	–0.621	–0.033	–0.568	0.346	–0.842	–0.094	–0.177
Zn leave	–0.894*	–0.483	–0.842	–0.638	–0.694	0.214	–0.773	0.731	–0.881*	0.084	–0.464
Zn root	0.411	0.593	0.253	–0.212	0.072	–0.955*	0.629	0.061	–0.048	0.320	0.955*
Mn leave	–0.304	–0.653	–0.818	–0.904*	–0.813	0.071	–0.414	0.134	–0.806	–0.453	–0.332
Mn root	0.123	–0.740	–0.518	–0.570	–0.548	0.375	–0.207	–0.447	–0.320	–0.823	–0.518
Pb leave	0.100	0.399	0.728	0.724	0.814	–0.154	–0.038	0.291	0.444	0.871	0.396
Pb root	0.177	–0.147	0.541	0.402	0.664	–0.030	–0.398	0.247	0.149	0.748	0.232
Cd leave	–0.054	0.632	0.418	0.241	0.421	–0.670	0.148	0.577	0.023	0.938*	0.762
Cd root	–0.191	0.620	0.182	–0.021	0.165	–0.779	0.140	0.693	–0.215	0.873	0.781

*Significantly correlated at the 0.05 level ($p < 0.05$).

Cluster analysis was performed to evaluate the impact of exogenous NO on ryegrass physiological and biochemical indices. Hierarchical clustering (Euclidean distance cut-off: 2.25) categorized the measured parameters into three distinct clusters (Fig. 4). Cluster 1: NR activity, germination rate, germination index, germination potential, underground fresh weight. Exogenous NO primarily influences seed germination efficiency, NR activity, and initial biomass accumulation. Cluster 2: root activity, root length, underground dry weight, root Zn, leaf/root N, leaf/root K, leaf/root Cu, leaf/root Cd, leaf/root Pb. Exogenous NO regulates root system vitality and modulates metal/nutrient partitioning (Zn, Cu, Cd, Pb) between roots and leaves. Cluster 3: Plant height, root N, root P, root Mn, leaf P, leaf Mn, root Cu, leaf Zn. NO-driven changes in plant height correlate with nutrient redistribution (N, P, Mn, Cu, Zn), suggesting coordinated growth-elemental homeostasis.

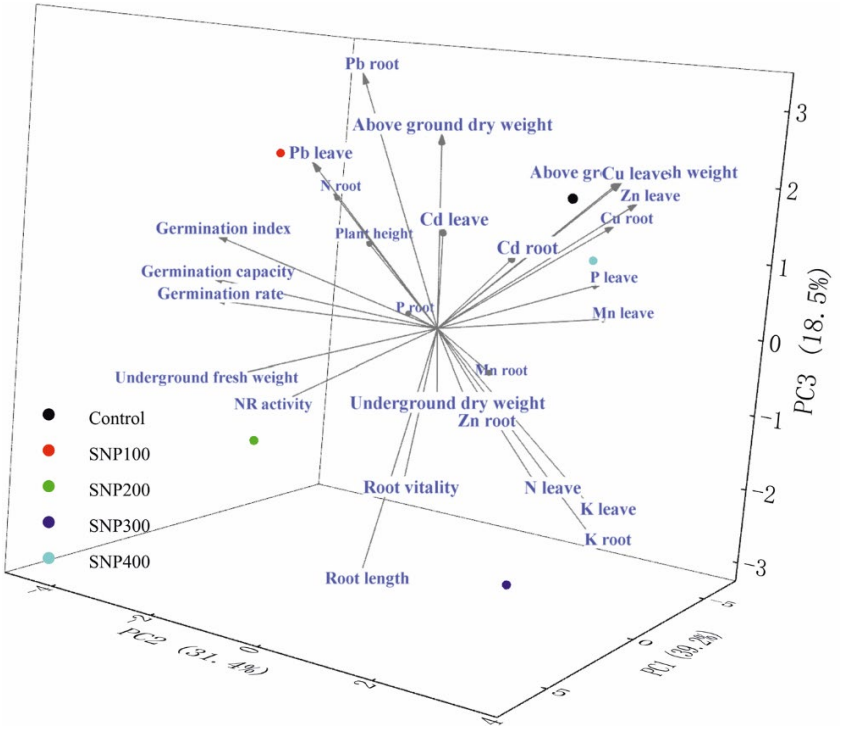

Fig. 3. Principal component analysis of each index of ryegrass

Table 4

Comprehensive weight and ranking of different treatment groups

Treatment group	Control	SNP100	SNP200	SNP300	SNP400
Comprehensive weight	280.91	–84.97	–16.35	–29.99	–149.60
Rank	1	4	2	3	5

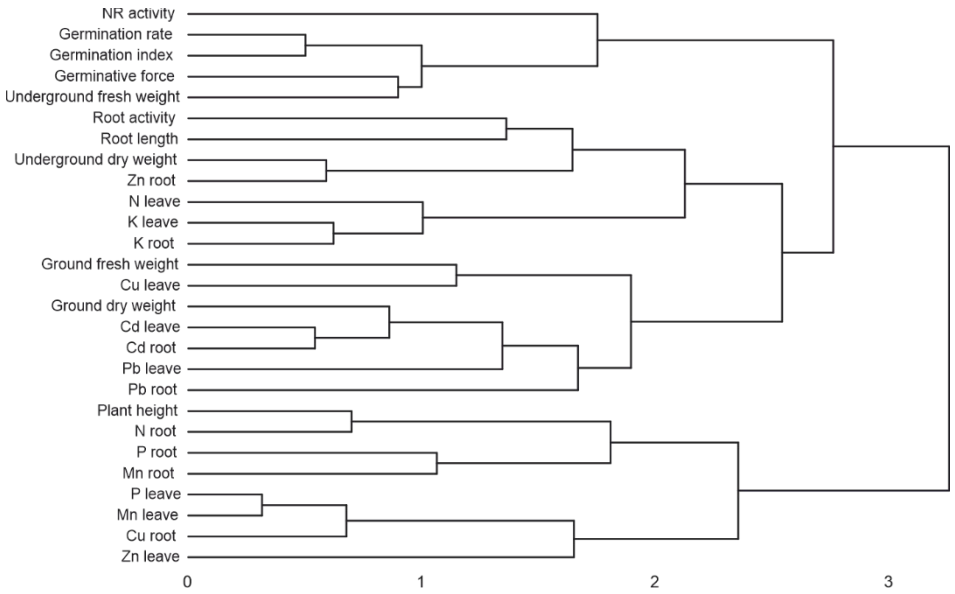

Fig. 4. Cluster tree pattern of growth parameter and mineral element in ryegrass

4. DISCUSSION

B[a]P, a representative PAH, poses significant risks to human health and disrupts plant growth. In this study, exposure to 30 μmol/dm^3 B[a]P stress suppressed ryegrass germination metrics (germination rate, potential, and index), reduced dry weight, and impaired root vitality, while paradoxically enhancing NR activity and root elongation (Fig. 1). These findings contrast with those of Li et al. [16], who reported that low-concentration B[a]P (0–30 mg/kg) progressively increased ryegrass biomass and enzymatic activity. This discrepancy may stem from differences in stress exposure duration, plant developmental stages, or specific physiological endpoints measured.

NO, a key signaling molecule in plants, plays a pivotal role in enhancing stress tolerance and promoting growth under adverse conditions [20]. Exogenous NO supplementation demonstrated significant potential to optimize plant development and resilience [14, 21]. In this study, low SNP concentrations (100–200 μmol/dm^3) improved ryegrass germination metrics (rate, potential, and index, Fig. 1), aligning with findings by Khan et al. [7], where 100 μmol/dm^3 SNP alleviated Cr stress in tomato seed germination. Notably, ryegrass root biomass (fresh and dry weight) exhibited a biphasic trend, initially increasing but declining at higher SNP concentrations (Fig. 1). Furthermore, SNP treatments enhanced plant height, root vitality, and NR activity, underscoring NO's capacity to mitigate B[a]P-induced growth suppression by counteracting phytotoxicity.

PCA, cluster analysis, and correlation analysis were employed to unravel interactions between exogenous NO, mineral elements, and physiological indices in ryegrass

(Table 3, Figs. 3, 4). Exogenous NO exerted pronounced effects on plant height, with N, P, K, Cu, Zn, and Mn identified as key contributors to growth modulation. Cluster analysis changes in plant height correlated with dynamic shifts in N, P, Mn, Cu, and Zn allocation between roots and leaves, highlighting NO's role in balancing nutrient partitioning. Exogenous NO elevated N and P content while enhancing their root-to-leaf translocation. Correlation analysis linked these elements to seed germination, plant height, and NR activity, suggesting NO stimulates growth organs to counteract B[a]P stress. Exogenous NO reduced root Pb/Cd content and restricted their leaf-to-root transport, potentially through internal detoxification mechanisms that shield photosynthetic tissues from metal toxicity [15]. NO enhanced N translocation via *TF* analysis and suppressed Pb/Cd mobility imply NO prioritizes essential nutrient distribution while immobilizing non-essential metals.

5. CONCLUSIONS

Exposure to 30 μmol/dm^3 B[a]P stress suppressed ryegrass seed germination, dry weight, and root vitality, while enhancing NR activity and altering elemental distribution. Cu and Mn accumulated preferentially in roots. N, P, Pb, and Cd were translocated to leaves. Exogenous NO significantly promoted vertical growth. Seed germination, root vitality, and NR activity initially increased at 100–300 μmol/dm^3 SNP but decreased at 400 μmol/dm^3 SNP. N, P, Mn, Cu, and Pb were increasingly accumulated in roots at higher SNP concentrations. PCA, cluster, and correlation analyses identified 200–300 μmol/dm^3 SNP as most effective in alleviating B[a]P stress. Changes in N and P content exhibited the strongest correlations with seed germination, plant height, and NR activity, underscoring their pivotal role in stress adaptation. In conclusion, B[a]P stress disrupts ryegrass growth and mineral homeostasis. Exogenous NO counteracts these effects by enhancing absorption and root-to-leaf translocation of N and P, restricting Pb/Cd mobility while redistributing essential elements (Cu, Mn), and optimizing elemental partitioning to sustain growth under stress. This study highlights NO's potential as a phytoprotectant, leveraging nutrient and metal contents to mitigate B[a]P toxicity in plants.

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