Influence of Seaweed Extracts on the Degradation and Effectiveness of 3,4-Dimethylpyrazole Phosphate

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3,4-Dimethylpyrazole phosphate (DMPP) is acknowledged as one of the most efficient nitrification inhibitors (NIs); however, researchers have suggested that its effectiveness needs to be further improved. The authors combined the biostimulant seaweed extract (SE) with DMPP and conducted an indoor incubation experiment to initially investigate the impact of the influence of SE on the degradation and effectiveness of DMPP. Meanwhile, a method for the extraction and determination of DMPP was developed. DMPP performed better, with a longer effective time in loess than black soil, and SE showed a delayed effect on DMPP degradation of DMPP at the beginning of the incubation and then accelerated the degradation process. This was attributed to the adsorption effect of SE on DMPP, consequently reducing its effectiveness. The SE delayed the hydrolysis of urea in various soils; however, no significant impact on urease activity was observed ($P < 0.05$) ($P < 0.05$). Furthermore, it also increased potential nitrification rate (PNR) from 10 to 21 days and promoting the rapid transformation of NH_4^+-N in black soil. The SE reduced PNR within 21 days and inhibited nitrification in loess. In addition, the SE appeared to mitigate the adverse effects of excessive nitrogen on microorganisms The combination of DMPP and SE was not conducive to the inhibition of soil nitrification, and this formulation in field applications requires further investigation.

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INTRODUCTION

It has been estimated that the world population will reach 10 billion by 2050, and the use of nitrogen (N) fertilizers in agricultural production is essential to meet the food demand of the growing population (Cakmak 2002). Generally, urea is the major form of N applied worldwide (Zanin *et al.* 2016). It is well established that urea applied to the soil is rapidly hydrolyzed by urease and transformed into ammonium nitrogen (NH_4^+-N) and then nitrate nitrogen (NO_3^--N); by such pathways, more than 50% of the N will be lost to the environment and not used by plants (Zhu and Chen 2002; Cruchaga *et al.* 2011). Farmers usually apply excessive N to achieve high crop yields, which results in various environmental risks and a reduction in economic efficiency (Snyder *et al.* 2009; Tian and Niu 2015; Mahmud *et al.* 2021).

Most researchers consider the utilization of nitrification inhibitors (NIs) as an effective strategy to reduce nitrogen losses from fertilizers (Wu *et al.* 2007). Among these

inhibitors, 3,4-dimethylpyrazole phosphate (DMPP) was developed by BASF and is widely recognized as one of the most efficient NIs (Zerulla *et al.* 2001; Hatch *et al.* 2005). Previous studies have suggested that DMPP can affect the metabolic activity and growth of ammonia oxidizing archaea (AOA) and bacteria (AOB) in soil, and that it has a positive environmental effect when combined with urea to reduce the emission of N_2O in soil (Chen *et al.* 2019). In addition, the combination of DMPP with urea has been shown to reduce the risk of eutrophication of water bodies due to its ability to reduce NO_3^--N leaching (Wu *et al.* 2007). However, DMPP appears to have limitations in terms of increasing crop yield. Yin *et al.* (2017) found that although DMPP significantly decreased CH_4 and N_2O emissions, there was no significant difference in crop yields under DMPP treatment, and similar phenomena were confirmed in other studies (Migliorati *et al.* 2014; Li *et al.* 2018; Nauer *et al.* 2018). The application effectiveness of DMPP products still requires improvement; thus, the development of a new generation of DMPP fertilizer is an inevitable development trend.

Biostimulants, as defined by Kauffman *et al.* (2007), with modifications: "are materials, other than fertilizers, that promote plant growth when applied in low quantities," (du Jardin 2015). Recently, the biostimulant industry has been rapidly developing in agriculture globally. Seaweed extract (SE), as one of the mainstream biostimulants, is widely used because it is simple to obtain and inexpensive (Alvarado *et al.* 2008); thus, it occupies more than one-third of the global biostimulant market (Nanda *et al.* 2021). SE is rich in various components that are lacking in terrestrial ecosystems, growth hormones, carbohydrates, betaine, and polysaccharides (Xiao *et al.* 2022). It has been demonstrated that SE can increase the chlorophyll, carotenoid, and protein content of spinach leaves, thereby improving the photosynthetic efficiency and capacity of the plant (Vafa *et al.* 2022). SE can effectively promote plant seedling growth and root development, as well as positively impact fruit quality (Mukherjee and Patel 2020). Furthermore, SE has been shown to enhance crop resistance to environmental stresses and increase yield (Sharma *et al.* 2019; Arioli *et al.* 2021). It has also been demonstrated that SE exerts a positive influence on the soil microbial community by favoring the proliferation of plant growth-promoting bacteria (du Jardin 2015). However, there is currently no research on whether the combination of DMPP and SE could produce positive effects. Additionally, the effectiveness of DMPP is influenced by various environmental factors (Barth *et al.* 2008; Vitale *et al.* 2013; Li *et al.* 2020). Consequently, the objective of this study was to formulate a new fertilizer with DMPP and SE, with the aim of combining the benefits of DMPP with the plant growth-promoting effects of SE. The authors conducted an indoor incubation experiment in two representative Chinese soils (black soil and loess), respectively, to investigate the effect of seaweed extract on the degradation characteristics and effect of DMPP, thus providing a theoretical basis for the future field application and development of new DMPP fertilizer products.

EXPERIMENTAL

Preparation of Soil Sample

The black soil and loess (0 to 20 cm depth) were collected from farmland of Nong' an (44°43'N, 125°18'E), Jilin Province of China and Chang' Wu (34°59'N, 107°48'E), Shanxi Province of China, respectively. Maize (*Zea mays* L.) was the main crop grown in these fields. Surface soil samples were air dried and passed through a 2-mm sieve to remove

visible fine plant roots, stones, and other debris, and then stored at room temperature. Detailed chemical properties of both soils are shown in Table 1.

Table 1. Chemical Properties of Two Different Soils

Soil type	pH	Organic matter (g/kg)	Total N (g/kg)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Total P (g/kg)	Available P (mg/kg)	Total K (g/kg)	Available K (mg/kg)
Black soil	6.23	32.19	1.68	11.15	59.73	0.79	78.88	50.50	322.15
Loess	8.01	31.26	1.14	9.17	10.70	0.67	11.45	40.45	257.37

Experimental Design

Air-dried soil samples (1.5 kg) were placed into a plastic pot (17 cm in diameter and 15 cm in height). Deionized water was added to maintain the soil water holding capacity (WHC) at 16% and keep at a constant temperature (25 ℃) for 2 weeks to recover soil biological activities. Plastic pots containing soil samples were sealed with plastic wrap, and ten holes were made in the wrap using a needle to facilitate gas exchange. The following five treatments were established with three replicates each: CK: No urea, NIs, and SE; U: urea; DMPP: urea+DMPP; SE: urea+SE; DMPP+SE: urea+DMPP+SE. The N was added at a level of 0.8 g N kg^{-1} air-dried soil. The DMPP and SE were added at a level of 2% and 12‰, respectively, on the w/w basis of N. Throughout the 110-day incubation period, the temperature was maintained at 25 ℃, and deionized water was added to maintain the soil WHC at 20%.

Urea was produced by China National Pharmaceutical Group Corporation, containing 46% N. The SE was produced by a company in Qingdao, Shandong Province, China, pH 7.4, containing 40% alginate, 7% polysaccharides laminaran, 30.2% organic matter, and 4.5% moisture. The DMPP was produced by Maya Reagent Biotechnology, with a purity of 97%.

Soil samples were collected from each treatment of three replicates at specific time intervals (1, 3, 5, 7, 10, 14, 21, 28, 35, 50, 65, 80, 95, and 110 days). Soil samples were divided into two parts: one portion was stored at -20 °C to determine the DMPP content, and the other portion was stored at -4 °C to determine the content of urea-N, NH_4^+-N, and NO_3^--N (at the same time intervals as before), as well as urease activity, potential nitrification rate (PNR), nitrate reductase (NR) activity, and microbial biomass carbon (MBC) content (1, 5, 10, 21, 35, 65, and 95 days).

Analytical Methods

Numerous studies have investigated the extraction method of DMPP from soil; however, previous methods mostly use alkaline environment to disproportionate DMPP into DMP for determination (Benckiser *et al.* 2013; Chen *et al.* 2019; Adhikari *et al.* 2021). This approach is comprised of multiple complex steps, which increases the likelihood of human error. As a result, an alternative method for the extraction and determination of DMPP in soil was developed. The method does not require the disproportionation of DMPP into DMP for the determination, and instead directly determines the concentration of DMPP in the extracted sample using simple and easily accessible steps. Moreover, it exhibits excellent reproducibility and recovery rate.

A soil sample weighing 3.5 g was added to a triangular flask. Subsequently, 35 mL of deionized water was added to the flask, which was then placed on a reciprocating shaker set at 160 rpm for a duration of 2 hours. After shaking, the shaken soil suspension was transferred to a 50 mL centrifuge tube and centrifuged at 20,000 g for 20 min. The liquid present in the centrifuge tube was filtered until it achieved clarity, and then it was stored at a temperature of -20 °C for subsequent DMPP analysis. The authors employed a high-performance liquid chromatography (HPLC) instrument (UltiMate 3000, Thermo Fisher Scientific, Waltham, MA, USA) for the determination of DMPP in the extracts, using a 3 μm, 3 × 150 mm Thermo C18 column. The HPLC eluent was pumped at a flow rate of 1 mL min^{-1} at 25 °C. UV adsorption at 224 nm was used for detection. The setup detection conditions were as follows: the mobile phase consisted of 9.75 mM monosodium phosphate in water for Channel "A" and acetonitrile without additives for Channel "B". From 0 to 0.2 min, it was maintained at 2% A and 98% B. From 0.2 to 3 min, the gradient was linearly ramped from 2 to 98% A. From 3 to 5 min, it was maintained at 98% A and 2% B. From 5 min to end (10 min), the gradient was linearly ramped from 98 to 2% A. A calibration curve was prepared at six concentration levels: 0, 0.4, 0.8, 1.2, 1.6, and 2 mg·L^{-1} DMPP. The DMPP eluted at approximately 7 min.

To assess the recovery rate of DMPP, the experiment involved meticulous mixing of DMPP with urea, based on the specified addition ratio. Subsequently, the fertilizer was thoroughly mixed with soil samples (black soil and loess). Then the content of DMPP in the soil was quickly determined. Lastly, the recovery rate was determined, which was 99.7% and 98.6% in black soil and loess, respectively. The content of DMPP is calculated according to the following Eq. 1,

$$DMPP\ (\mathrm{mg \cdot kg^{-1}\ dry\ soil}) = \frac{S \times 35 \times 10^{-3}}{3.5 \times (1-w) \times R \times 10^{3}} \quad (1)$$

where S is the mass concentration (ppm) of DMPP in the extracts calculated from the sample peak areas using the calibration curve; 35 is the volume of extracts in mL; 10^{-3} is the factor for converting mL to L; 3.5 is the fresh soil weight in g; w is the quality water content of soil; R is the recovery rate of DMPP in soil; and 103 is the factor for converting g to mg.

The nitrification inhibition percentage was calculated using the following (Xiao *et al.* 2022) Eq. 2,

$$\text{Nitrification inhibition (\%)} = (a - b) / a \times 100 \quad (2)$$

where a is the NO_3^--N of soil applied urea only (mg·kg^{-1}) and b is the NO_3^--N of soil applied with DMPP and / or SE (mg·kg^{-1}).

The chemical properties of both soils were determined using conventional methods (Xiao *et al.* 2022). Soil urea-N and inorganic N (NH_4^+-N and NO_3^--N) were extracted using 5 g of soil and 50 mL of solution, urea-N was extracted with KCl-PMA (2 mol·L^{-1} KCl and 5 mg·L^{-1} PMA) (Wu *et al.* 2022), and inorganic N was extracted with 2 mol·L^{-1} KCL (Cui *et al.* 2021), and then determined on a continuous flow analyzer (AA III, Norderstedt, Germany).

Determination of urease activity, PNR, and NR activity with reference to the method of Xiao *et al.* (2022), and results were expressed as mg·kg^{-1}·h^{-1}, mg·kg^{-1}·5 h^{-1} and mg·kg^{-1}·24 h^{-1}, respectively. The MBC was extracted with reference to the method of Yang *et al.* (2016), and measured on a total carbon analyzer (TOC Analyzer, Elementar,

Germany). The difference in extractable C contents between the fumigated and non-fumigated samples was divided by 0.45 to calculate MBC.

Statistical Analysis

Mean comparisons were conducted using the Tukey test, and significant differences were determined at $P < 0.05$. All statistical analyses were performed using Microsoft Excel 2010 and SPSS Version 21.0 (SPSS Inc., Chicago, IL, USA). Graphs were prepared with Origin 2021 (Origin Lab Corp., Northampton, MA, USA). Data were presented as the means of the three replicates.

RESULTS

Content of DMPP in Both Soils

The retention time of DMPP exceeded 110 days in both soils, with the black soil exhibiting a higher degradation rate for DMPP (Fig. 1). In black soil, approximately 70% of initially added DMPP degraded within 7 days, after which the degradation rate of DMPP stabilized. DMPP+SE further delayed the degradation of DMPP from 1 to 28 days. After 95 days, the DMPP content reached a stable level for both treatments (Fig. 1a). In loess, the degradation rate of DMPP was more consistent than black soil, with SE delaying the degradation of DMPP from 1 to 3 days, followed by an accelerated degradation (Fig. 1b). In both soils, SE delayed the degradation of DMPP at the early stage of the incubation period, subsequently increasing the degradation rate of DMPP.

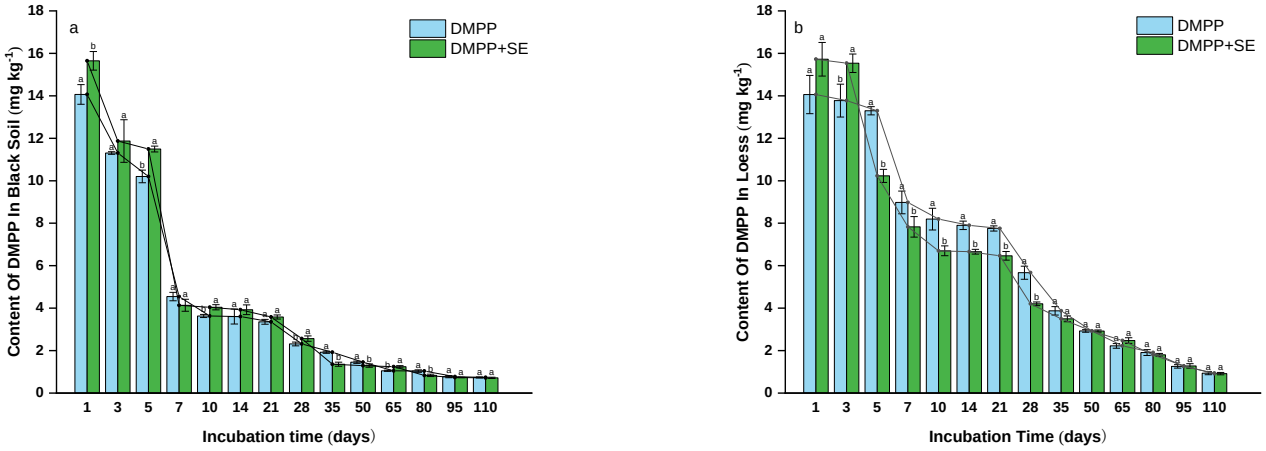

Fig. 1. Changes in DMPP content in black soil (a) and loess (b). Error bars represent standard deviations (n = 3). Different letters indicate significant differences between different treatments at $p < 0.05$ by Tukey test. DMPP: urea +DMPP; DMPP+SE: urea+DMPP+SE

N Transformations of Urea in Both Soils

Urea-N

The hydrolysis rate was faster in black soil compared to loess. In both soils, urea added alone was hydrolyzed within 5 days (Fig. 2). In both soils, DMPP, SE, and their

combination could delay the hydrolysis of urea. However, SE demonstrated better effects in loess than in black soil (Fig. 2) and extended the hydrolysis of urea until 10 days (Fig. 2b). In comparison to DMPP, DMPP+SE proved to be more effective in delaying urea hydrolysis.

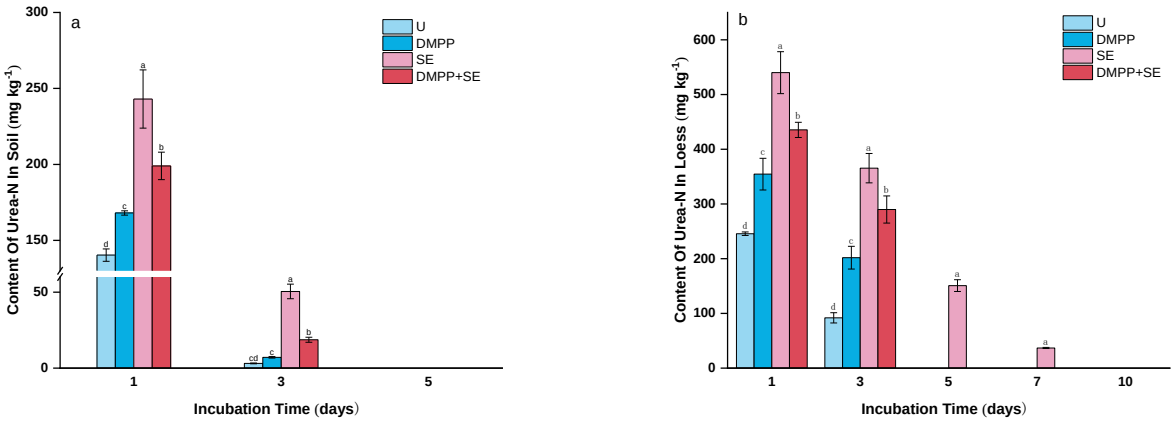

Fig. 2. Changes in Urea-N content in black soil (a) and loess (b). Error bars represent standard deviations (n = 3). Different letters indicate significant differences between different treatments at $p < 0.05$ by Tukey test. U: urea; DMPP: urea+DMPP; SE: urea+SE; DMPP+SE: urea+DMPP+SE

Inorganic N

The transformation rate of NH_4^+-N in black soil was lower than loess, and the NH_4^+-N content of urea alone tended to stabilize after 65 days, while the NH_4^+-N content in loess stabilized after 21 days (Fig. 3). From 10 to 50 days, SE showed a decrease in NH_4^+-N content and an increase in NO_3^-N content compared to U—, which suggests that SE accelerated nitrification in black soil (Fig. 3 a, c). On the other hand, in loess, SE increased NH_4^+-N content from 5 to 28 days compared to U, and it significantly increased NO_3^--N content from 10 to 28 days (Fig. 3 b, d), the phenomenon was different from that in black soil ($P < 0.05$).

The nitrification inhibition rate (NIR) of the soil was calculated before stabilizing the NH_4^+-N content in both soils to facilitate a more accurate comparison of the effect of DMPP (Fig. 4).

The DMPP demonstrated a superior nitrification inhibition effect in loess compared to black soil (Figs. 3 and 4), and it could inhibit soil NH_4^+-N transformation for approximately 50 days when compared to urea alone. In black soil, DMPP significantly increased NH_4^+-N content from 5 to 21 days compared to urea alone. However, the NIR of DMPP added treatments became negative value after 28 days, and the NH_4^+-N content in these treatments was significantly lower than U (Figs. 3 and 4).

These findings suggest that DMPP accelerated NH_4^+-N transformation in black soil after 28 days ($P < 0.05$). In both soils, compared to DMPP, DMPP+SE significantly decreased NH_4^+-N content and NIR from 7 to 21 days in black soils, and decreased NH_4^+-N content and NIR from 10 to 65 days in loess (Figs. 3 and 4) ($P < 0.05$).

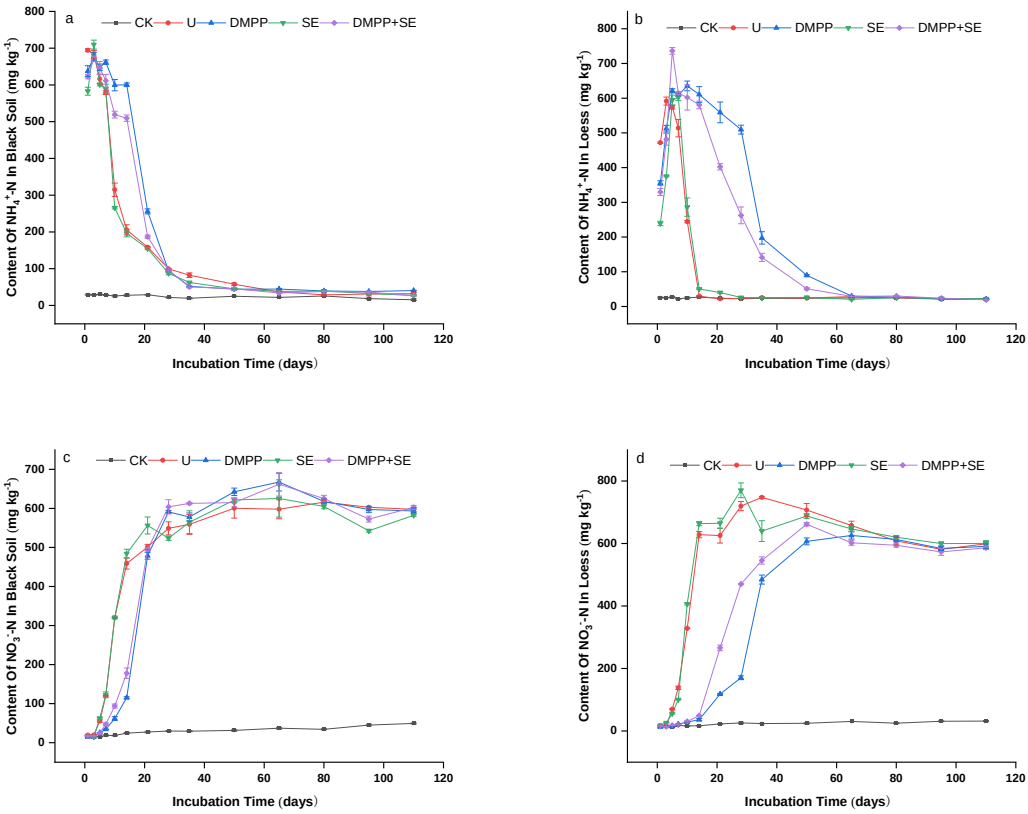

Fig. 3. Changes in NH_4^+-N and NO_3^--N content in black soil (a, c) and loess (b, d). Error bars represent standard deviations (n = 3). CK: no urea, NIs, and SE; U: urea; DMPP: urea+DMPP; SE: urea+SE; DMPP+SE: urea+DMPP+SE

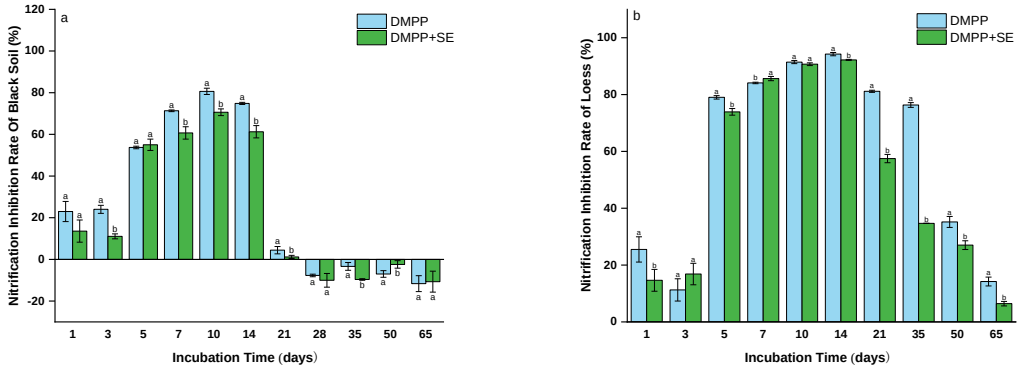

Fig. 4. Changes in nitrification inhibition rate of black soil (a) and loess (b). Error bars represent standard deviations (n = 3). Different letters indicate significant differences between different treatments at $p < 0.05$ by Tukey test. CK: no urea, NIs, and SE; U: urea; DMPP: urea+DMPP; SE: urea+SE; DMPP+SE: urea+DMPP+SE

Urease Activity

In black soil, the addition of urea alone resulted in a significant increase in urease activity on day 5, followed by a decrease and stabilization ($P < 0.05$) (Fig. 5 a). Urease

activity of loess had a faster response to urea compared to black soil, with the addition of urea alone significantly increasing the soil urease activity from 1 to 10 days ($P < 0.05$) (Fig. 5). Compared to urea alone, in both soils, DMPP and SE did not significantly affect urease activity on day 1, indicating that the delayed urea hydrolysis was not caused by inhibiting urease activity ($P < 0.05$). Additionally, compared to DMPP, DMPP+SE did not significantly affect soil urease activity on 1 day in both soils ($P < 0.05$) (Fig. 5). After 10 days, the soil urease activity of each treatment tended to be stabilized.

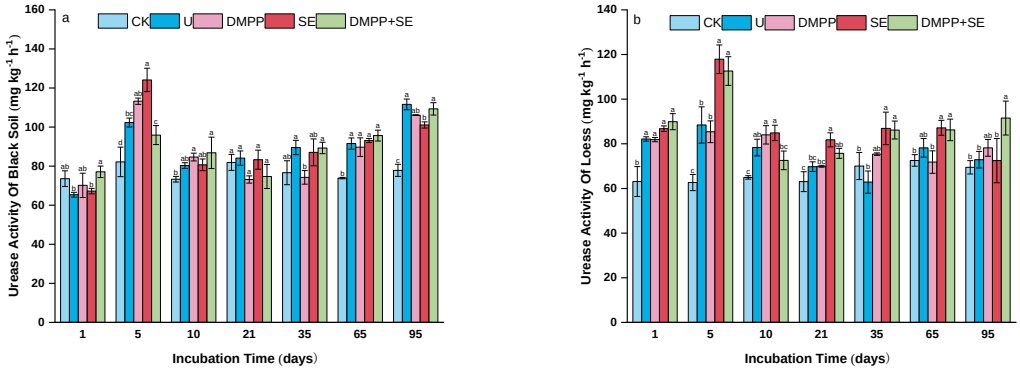

Fig. 5. Changes of urease activity of the black soil (a) and loess (b). Error bars represent standard deviations (n = 3). Different letters indicate significant differences between different treatments at $p < 0.05$ by Tukey test. CK: no urea, NIs, and SE; U: urea; DMPP: urea+DMPP; SE: urea+SE; DMPP+SE: urea+DMPP+SE

Soil Potential Nitrification Rate (PNR)

There was a higher PNR in loess compared to black soil, which was consistent with the changes of soil inorganic N (Fig. 6). In black soil, the application of urea alone significantly increased PNR within 5 days but it subsequently decreased and was significantly lower than CK, while after 21 days the PNR of added N treatments was significantly lower than CK ($P < 0.05$) (Fig. 6 a). In loess, the PNR of added N treatments was significantly lower than CK from 1 to 10 days, and after 21 days, PNR of treatments without DMPP increased rapidly and were significantly higher than CK (Fig. 6 b), which contrasts with the results observed in black soil ($P < 0.05$). Compared to U, SE in black soil increased PNR from 10 to 21 days, in contrast to SE in loess that decreased PNR within 21 days. This was consistent with the changes of inorganic N, further demonstrating that SE showed completely opposite effects on nitrification in both soils. The DMPP exhibited a more effective inhibition of nitrification in loess, which correlates with the findings mentioned above. DMPP could inhibit nitrification for about 35 days (Figs. 3, 4, and 6), while the action time in black soil was only about 10 days. Furthermore, it was found that the added DMPP treatments resulted in a higher PNR compared to U from 10 to 21 days in black soil (Fig. 6 a). This implies that the nitrification inhibition effect of DMPP would subsequently lead to an increase in soil nitrification after its reduction. Compared to DMPP, DMPP+SE significantly increased PNR from 10 to 21 days in black soil and from 21 to 35 days in loess. However, this combination was not conducive to the action effectiveness of DMPP, which aligns with the aforementioned findings ($P < 0.05$) (Fig. 6).

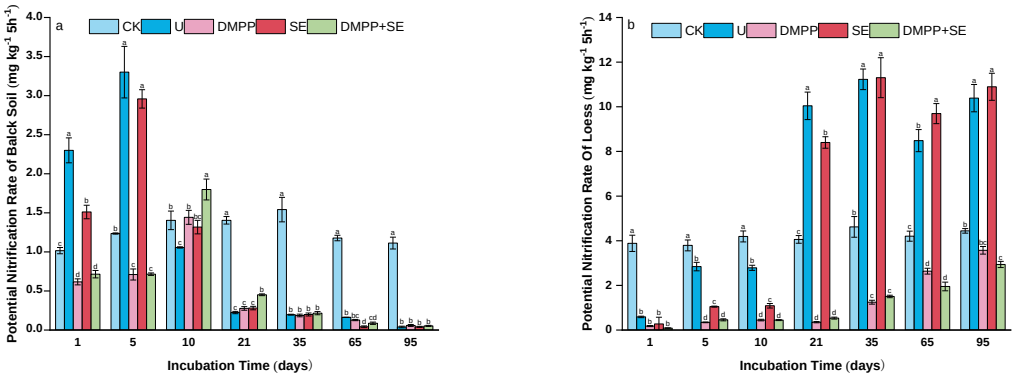

Fig. 6. Changes of PNR of black soil (a) and loess (b). Error bars represent standard deviations (n = 3). Different letters indicate significant differences between different treatments at $p < 0.05$ by Tukey test. CK: no urea, NIs, and SE; U: urea; DMPP: urea+DMPP; SE: urea+SE; DMPP+SE: urea+DMPP+SE

Soil Nitrate Reductase (NR) Activity

In both soils, compared to CK, urea alone significantly increased the soil NR activity during the incubation period ($P < 0.05$) (Fig. 7). The SE showed an inhibition effect on soil denitrification, compared to urea alone. SE reduced soil NR activity from 1 to 21 days in black soil and from 2 to 21 days in loess. The DMPP inhibited NR activity more effectively in loess than black soil. It inhibited NR activity for approximately 35 days in loess and about 10 days in black soil, which was the same time as DMPP reduced PNR. Compared to DMPP, DMPP+SE increased NR activity within 35 days in black soil; however, DMPP+SE did not significantly affect NR activity in loess ($P < 0.05$) (Fig. 7).

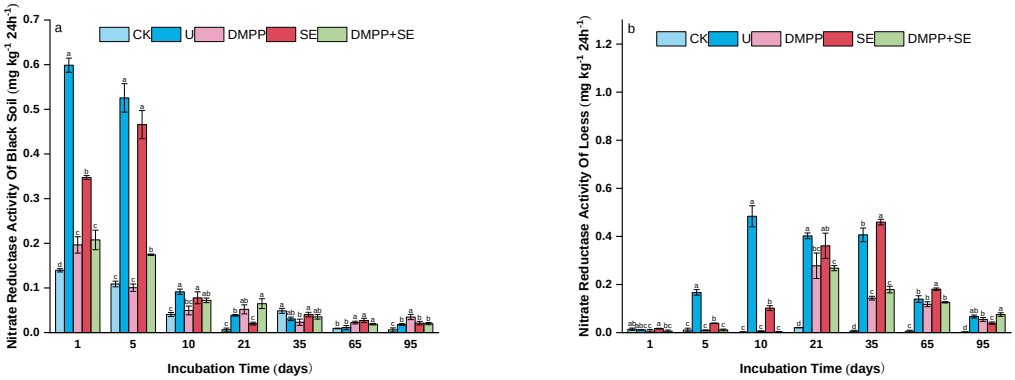

Fig. 7. Changes of nitrate reductase activity of the black soil (a) and loess (b). Error bars represent standard deviations (n = 3). Different letters indicate significant differences between different treatments at $p < 0.05$ by Tukey test. CK: no urea, NIs, and SE; U: urea; DMPP: urea+DMPP; SE: urea+SE; DMPP+SE: urea+DMPP+SE

Soil Microbial Biomass Carbon (MBC) Content

Carbon (C) is a vital component of microorganisms. Therefore, the authors determined the MBC content to show the population of microorganisms in the soil. The effect of urea alone on MBC content differed between the two soils (Fig. 8). In the loess, microorganisms exhibited a quicker response to urea application, resulting in a significant

increase in MBC content from 1 to 21 days compared to CK, followed by a decrease (Fig. 8b). In black soil, urea alone significantly increased MBC content from 5 to 35 days compared to CK and then decreased, and MBC content was significantly lower than CK on the 95th day ($P < 0.05$) (Fig. 8 a). In both soils, compared to urea alone, SE increased the MBC content during the incubation period (Fig. 8). Compared to urea alone, DMPP was beneficial to increase the content of MBC in both soils. There was no significant difference in MBC content between DMPP and DMP+SE before 65 days in black soil ($P < 0.05$), and compared to DMPP, DMPP+SE increased the MBC content from 1 to 5 days, after which both treatments exhibited similar impact in loess soil.

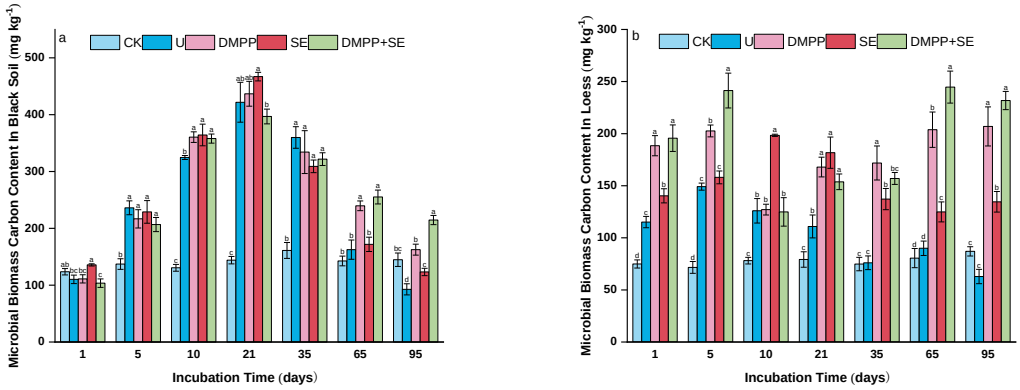

Fig. 8. Changes of microbial biomass carbon in the black soil (a) and loess (b). Error bars represent standard deviations (n = 3). Different letters indicate significant differences between different treatments at $p < 0.05$ by Tukey test. CK: no urea, NIs, and SE; U: urea; DMPP: urea +DMPP; SE: urea+SE; DMPP+SE: urea+DMPP+SE

The Relationship Between Content of DMPP and MBC

To investigate the correlation between the degradation rate of DMPP and the population of microorganisms, a Pearson correlation analysis was conducted, the result demonstrated the content of DMPP was negatively correlated with the content of MBC ($P < 0.01$). However, in the case of loess, there was no significant correlation between the two variables ($P > 0.05$) (Table 2).

Table 2. Correlation Between Content of DMPP and MBC in Both Soils

Variable	Soil Type	MBC
DMPP	Black soil	-0.565**
	Loess	-0.096

MBC: microbial biomass carbon
**Correlation is significant at the 0.01 level

DISCUSSION

Effect of SE on the Degradation Rate of DMPP on Different Soils

Weiske *et al.* (2001) found that DMPP remained detectable in the soil until the end of the growth season of crops in a three-year field experiment. The present study yielded similar results, with approximately 5% and 6% of the originally added DMPP amount still

detectable in black and loess at the end (110 days) of incubation. In black soil, the DMPP content ceased to decrease after 95 days (Fig. 1), a phenomenon also found in the results of Weiske *et al.* (2001). This phenomenon can be attributed to the adsorption of DMPP by soil organic matter and pore space. However, there is currently a lack of research reporting on whether the residues of DMPP contribute to a decline in soil environmental quality. Therefore, future studies should be conducted to monitor and assess these residues through long-term field experiments. This is necessary to determine the potential effects of DMPP residues on the soil environment and decide whether DMPP fertilizer products are suitable for long-term application in agricultural production. Previous research has demonstrated that soil temperature, pH, organic matter content, and microbial activity can influence the degradation characteristics and action effect of DMPP in soil (Xue *et al.* 2012; Guardia *et al.* 2018; Li *et al.* 2022); however, the soil temperature was constant during the incubation period in the current study. Therefore, it was hypothesized that another three factors might affect the degradation of DMPP during the incubation. The rapid hydrolysis of urea could lead to a short-term and rapid increase in pH (Engel *et al.* 2013), while acidic soil environments can accelerate the hydrolysis of urea (Liu *et al.* 2008). Consequently, this pH change was found to be more substantial in black soil. Xue *et al.* (2012) found that changes in soil pH might influence the rate of DMPP by affecting its degradation behavior in soil, and its half-life increased with higher pH, which leads to the degradation of DMPP in black soil at a relatively lower rate within 5 days (Fig. 1 a). Upon application to soil, the phosphate group within the DMPP molecule is broken down by microorganisms (Xiao *et al.* 2022). However, in their study, the authors conducted a Pearson correlation analysis and determined that the degradation of DMPP in loess was not significantly influenced by the population of microorganisms (Table 2), which was attributed to the lower microbial population and activity in loess, as well as the inherent difficulty in biodegrading DMPP itself due to its status as a heterocyclic compound (Chaves *et al.* 2006). Interestingly, the authors noticed that the increase in the populations of loess microorganisms during the incubation period was not accompanied by a faster degradation rate of DMPP, while black soil exhibited a similar phenomenon after 5 days (Figs. 1 and 8). Based on these findings, the authors concluded that soil pH plays a more significant role in influencing the degradation rate of DMPP compared to the population and activity of microorganisms.

In both soils, the application of SE was observed to delay the degradation of DMPP during the early stage of incubation followed by an acceleration of the degradation process until the DMPP content reached a stable state (Fig. 1). These consistent effects on DMPP degradation in different soils suggest that changes in soil pH and microbial population are responsible for the delay in degradation. The SE contains various amino acids and carbohydrates (Hashem *et al.* 2019; Hamouda *et al.* 2022), and these exogenous C can be mineralized by soil microorganisms (Cheng *et al.* 2022), particularly in an N-rich environment (Tang *et al.* 2020). Additionally, soil microorganisms are able to quickly utilize this exogenous free amino acid (Henry and Jefferies 2003). Based on the preceding discussion, the authors propose that the prioritization of organic matter mineralization by microbial communities is responsible for the delayed degradation of DMPP. Blagodatsky *et al.* (2010) support the authors' hypothesis of a sequential decomposition scheme. Because of the low bioavailability of DMPP, the microorganisms preferentially mineralize SE in soil, thus resulting in a lower biodegradation pressure and degradation rate on DMPP. Compared to loess, black soil exhibits a higher concentration of soluble organic carbon (C). DMPP+SE did not significantly affect the MBC content in black soil compared to DMPP (Fig. 8). In contrast, the lower soluble C content in loess made the microorganisms

more sensitive to exogenous amino acids and carbohydrates (Lu and Scheu 2021). Additionally, the MBC content in loess increased significantly at 5 days ($P < 0.05$) (Fig. 8 b), indicating that SE in loess decomposed at a faster rate and DMPP was subjected to stronger biodegradation pressure. This presumably caused the difference in the delay of the degradation process of DMPP by SE in both soils. Regrettably, there are no existing research reports that directly demonstrate the sequential decomposition order of organic matter in soil with different bioavailability; therefore, further investigation is required to explore this topic. Khan *et al.* (2009) demonstrated that alginate, algal polysaccharides, and growth hormones in SE increase the activity of soil microorganisms, which caused an increase in the degradation rate of DMPP after the SE decomposed in both soils.

Effect of SE and DMPP Application on Urea N Transformation, Urease Activity, PNR, NR Activity, and MBC Content

Huang *et al.* (2006) utilized infrared spectroscopy to observe that SE can form α-helical or polymer lattice fertilizer systems with urea molecules through hydrogen bonding. This interaction force delays the hydrolysis of urea, and this result was confirmed in the current study. The hydrolysis of urea was also found to be delayed in various soils (Fig. 2), while the urease activity was not inhibited (Fig. 5). SE also exerted opposite effects on nitrification in black soil and loess, and the authors suggested that the carboxyl groups contained in the SE led to this result (Dang *et al.* 2022). Carboxyl groups have been demonstrated to adsorb ammonium in soil (Mia *et al.* 2017; Zheng *et al.* 2018; Sumaraj *et al.* 2020). Moreover, this adsorption is enhanced with an increase in environmental pH ranging from 3 to 8 (Ding *et al.* 2017). Furthermore, SE have a greater adsorption capacity for ammonium in loess compared to black soil., while SE contains active substances and carbohydrates that increase the MBC content of the soil (Khan *et al.* 2009) (Fig. 8). This represents an increase in the abundance of ammonia oxidizing microorganisms and accelerates soil nitrification. In loess, the positive effect of SE in inhibiting nitrification outweighs the negative effect, whereas the opposite is observed in black soil. Consequently, there is a significant difference in the performance of SE in different soils. Xu *et al.* (2022) found that the addition of SE did not significantly affect the total soil nitrous oxide (N_2O) emissions during the crop growth season. However, the present study revealed that SE exhibited varying degrees of inhibitory effects on soil NR activity denitrification in both soils (Fig. 7). These differences in experimental environments may have contributed to the varying results. Further studies should investigate whether SE has the potential to reduce soil N oxide emissions under different soil types. The DMPP is widely recognized as one of the most efficient NIs (Zerulla *et al.* 2001). However, the authors were surprised to discover that DMPP also delayed the hydrolysis of urea in various soils, although this delaying effect only lasted about 3 days (Fig. 2). In the research of Duncan *et al.* (2017), the NH_4^+-N content of urea added with DMPP was significantly lower than that of urea alone within 7 days of incubation, which indirectly suggests that DMPP indeed delays the hydrolysis of urea. It is known that the surface area of urea particles impacts the rate of hydrolysis (Shah and Wolfe 2003), and in this study, DMPP did not exert a significant effect on urease activity in different soils ($P < 0.05$) (Fig. 5). Therefore, the authors hypothesized that the interaction between DMPP and urea leads to a reduction in the surface area of urea particles that come into contact with the soil, which meant that imply that DMPP occupies the binding site of urease on urea, resulting in a delayed hydrolysis process.

There were notable variations in the effects of DMPP in different soils. However, it was surprising that the content of DMPP was approximately 23% of the original amount added during the rapid transformation of NH_4^+-N in the different soils (21 days in black soil and 35 days in loess) (Figs. 1 and 3). This implies that after roughly 80% degradation of DMPP in the soil, the residual DMPP (approximately 3.5 $mg \cdot kg^{-1}$) does not have an inhibitory impact on soil nitrification. This finding could be beneficial in determining the minimum effective concentration of DMPP in the soil. In loess, soil PNR of applied N treatments without DMPP increased rapidly after 21 days, conversely, in black soil, the PNR of applied nitrogen treatments showed a decreasing trend (Fig. 6). Soil pH controls the abundance of different species of ammonia oxidizing microorganisms. Nitrification is primarily driven by ammonia-oxidizing archaea (AOA) in acidic environments and by ammonia-oxidizing bacteria (AOB) in alkaline environments (Nicol *et al.* 2008; Ying *et al.* 2017). Ying *et al.* (2017) discovered that the accumulation of NO_3^--N in soil had contrasting effects on the abundance of AOA and AOB. As the concentration of NO_3^--N increased, the abundance of AOA decreased, while the abundance of AOB increased. This resulted in opposite changes of PNR in both soils during the incubation experiment. Previous studies have shown that DMPP can reduce the abundance of AOB in soil (Shi *et al.* 2016; Fuertes-Mendizabal *et al.* 2019), but it is difficult to significantly reduce the abundance of AOA (Li *et al.* 2019; Luchibia *et al.* 2020; Kaveney *et al.* 2022). Therefore, it was concluded that the presence of residual DMPP in loess led to a relatively stable soil PNR activity, while the PNR increased slowly as the DMPP content continuously decreased after 35 days (Figs. 1 and 6), which further supports the authors' viewpoint. The added DMPP treatments in black soil showed a dramatic increase in nitrification potential at 10 days (Fig. 1). The effect of DMPP in black soil was diminished and instead accelerated soil nitrification (Fig. 4 a). Based on the experiment results, the authors speculate that there is a threshold value for the effective concentration of DMPP in the black soil. The concentration of DMPP constantly decreased within 10 days (Fig. 1a), yet the PNR and NH_4^+-N content in the black soil remained stable (Figs. 3 a, 6 a), which indicates that the inhibitory effect of DMPP did not decrease with the reduction of DMPP content during this period. Previous studies have demonstrated that DMP is the efficient compound that can inhibit nitrification, not DMPP (Corrochano-Monsalve *et al.* 2021), so it proves challenging to establish a precise threshold for the effective concentration of DMPP solely based on the experimental results of the authors. The mechanism should be investigated in subsequent studies. Based on this hypothesis, once DMPP concentrations fall below the threshold, high concentrations of substrate promoted nitrification (Sanz-Cobena *et al.* 2008).

In both soils, SE had a negative impact on the effect of DMPP, and the authors hypothesized that the adsorption of SE onto DMPP led to the weakened effect of DMPP. Chen *et al.* (2019) suggested that the combination of DMPP with biochar resulted in increased soil N_2O emissions and reduced DMPP effectiveness. This is similar to the current experiment results. Current research attributes the negative effect of biochar on the effect of DMPP to the adsorption of DMPP by biochar (Chen *et al.* 2019; Li *et al.* 2021; Li *et al.* 2022), and Fuertes-Mendizabal *et al.* (2019) concluded that the protonated N present in DMPP molecules could be immobilized or adsorbed by carboxyl groups through electrostatic attraction. SE not only contains a large amount of carboxyl groups (Dang *et al.* 2022), but it is also characterized by its viscosity and adsorption capacity (Geng *et al.* 2017; Spain *et al.* 2022), which the authors thought caused the reduction in the effectiveness of DMPP.

A long-term environment with high nitrogen (N) levels would be toxic to microorganisms (Bunch and Bernot 2012). Moreover, the results of this study suggest that the addition of DMPP and SE may delay the decrease in the population of microorganisms. It was concluded that the preference of microorganisms for NH_4^+-N may have contributed to the increased MBC content of the added DMPP treatments (Murphy *et al.* 2003) (Fig. 8). Current studies reported that SE could change the functional diversity and community structure of soil microorganisms (Chen *et al.* 2020). However, further investigation is needed to determine whether SE can mitigate the negative effects of heavy nitrogen application on microorganisms.

CONCLUSIONS

1. In both soils, seaweed extract (SE) reduced the effectiveness of 3,4-dimethylpyrazole phosphate (DMPP) and increased the potential nitrification rate (PNR) from 10 to 21 days in black soil and 21 to 35 days in loess, which may be caused by the adsorption behavior of SE on DMPP molecules. This effect may be attributed to the adsorption behavior of SE on DMPP molecules, although the exact mechanism requires further investigation. Furthermore, SE was found to delay the hydrolysis of urea-N, but it did not exhibit any inhibitory effect on urease activity.
2. In different soils, SE exhibited contrasting effects on nitrification, SE was found to enhance soil PNR from 10 to 21 days, facilitating the transformation of NH_4^+-N in black soil. On the other hand, SE was observed to decrease soil PNR from 1 to 21 days, impeding nitrification in loess.
3. Additionally, SE was shown to enhance the content of MBC over the course of the incubation period. The DMPP exhibited higher effectiveness in loess. It is worth noting that the use of DMPP resulted in a delay of the urea hydrolysis process in various types of soils, albeit this effect was temporary.
4. Additionally, SE may mitigate the deleterious impact of prolonged exposure to high N levels on microorganisms.

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