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Nitrification inhibitor 3,4-dimethylpyrazole phosphate alleviates the dissolution of soil inorganic carbon caused by nitrogen fertilization

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ABSTRACT

In carbonate soils, soil inorganic carbon (SIC) dissolves and releases CO_2 due to exogenous application of nitrogen fertilizer, which makes the soil an important carbon source. Application of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) is likely to be an effective measure to alleviate SIC dissolution. A 5-year field experiment was conducted to observe SIC dynamics under three treatments: No N fertilizer (CK), N fertilizer (NF), and N fertilizer combined with DMPP (N + NI). Soil samples were collected from 0 to 20, 20–40, 40–60, 60–80, and 80–100 cm in depth, and soil organic carbon (SOC), SIC, total carbon (TC), total nitrogen (TN), and carbon isotope composition ($\delta^{13}C$) of the soil were analyzed. The proportion of pedogenic carbonate (PIC) in SIC was estimated using an isotopic mass balance equation. Results showed that: (1) compared with the application of N only, DMPP had no significant effect on SOC content and $\delta^{13}C_{SOC}$ in the 0–100 cm soil profile; (2) the application of N fertilizer significantly reduced the SIC content by 10.0 $g\cdot kg^{-1}$ (58.3 %) in the 0–80 cm soil profile. In the 0–20, 20–40, and 60–80 cm soil layers, the SIC content was reduced by 2.9, 1.9, and 3.8 $g\cdot kg^{-1}$, respectively. However, the SIC content significantly increased by 3.2 $g\cdot kg^{-1}$ (58.9 %) in the 80–100 cm soil layer. DMPP application significantly increased the SIC content in the 0–20 and 20–40 cm soil layers by 1.3 $g\cdot kg^{-1}$ (50.9 %) and 1.2 $g\cdot kg^{-1}$ (48.2 %), respectively; (3) DMPP combined with N significantly alleviated PIC dissolution in the 0–40 cm soil layers and increased PIC stock in 0–100 cm soil by 6.2 $Mg\cdot ha^{-1}$ (64.6 %). Applying DMPP to farmland soil containing carbonate significantly alleviated the dissolution of N fertilizer on PIC, which may decrease CO_2 emissions in the field.

1. Introduction

The soil carbon (C) pool contains a soil organic carbon (SOC) reserve of approximately 1,500–2,400 Pg (Batjes, 1996; Stockmann et al., 2013) and a soil inorganic carbon (SIC) reserve of approximately 700–1,750 Pg (Lal, 2007), which is the largest C pool in the terrestrial biosphere. Small changes in the soil C pool can affect the atmosphere and global climate (Luo et al., 2010). Therefore, many researchers have been exploring effective strategies to sequester soil C, such as the “4 per 1000” initiative (Minasny et al., 2017; Soussana et al., 2019). The SOC content is highly important for plant growth (Lal, 2007; Gelaw et al., 2014; Costantini et al., 2020). Sequestering SOC has received more attention than SIC in the last few decades. However, more and more researchers have shown that SIC dissolves and releases CO_2, making it a carbon source that should be taken seriously (Zamanian et al., 2018; Zhao et al., 2022b).

The application of chemical fertilizers has caused soil acidification, which may be the main cause of a substantial amount of SIC dissolution and CO_2 emission from carbonate-containing soils (Chevallier et al., 2016; Zamanian et al., 2018). China has considered to be a country with high N fertilizer application rates (Potter et al., 2010), which has led to a substantial amount of SIC dissolution. Raza et al. (2020) showed that SIC from a 0–40 cm depth of Chinese cropland soil dissolved at a rate of 5 ×

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10^6 Mg y^{-1} during 2001–2020. At a global scale, the CO_2 emitted from carbonate containing soils due to nitrogen (N) fertilization is approximately 7.5 × 10^{12} g C y^{-1} (Zamanian and Kuzyakov, 2018). While N fertilizers are being applied, the acidified SIC / soil CO_2 cannot reach equilibrium. Complete SIC dissolution and CO_2 release will take centuries or millennia (Zamanian et al., 2018). The H^+ released into carbonate-containing soil during the nitrification process after N fertilizer application may be one of the main factors leading to SIC dissolution (Zhao et al., 2022b; Yu et al., 2018), but there is a lack of long-term field tests to confirm this. Therefore, it is necessary to explore the impact mechanism of N fertilizer application on SIC with long-term experiment and find an effective strategy to inhibit or alleviate SIC evolved-CO_2 emitted into the atmosphere.

Two types of SIC are lithogenic carbonate (LIC) which originates from parent material carbonate, such as limestone (Zamanian et al., 2016), and pedogenic carbonate (PIC) which originates from the reprecipitation of Ca^{2+} (and/or Mg^{2+}) and CO_3^{2-} (and/or HCO_3^-) (Bughio et al., 2017). A proportion of the CO_3^{2-} (and/or HCO_3^-) may be derived from CO_2 emitted by SOC decomposition and SIC dissolution in the soil water (Zhao et al., 2022a). Ca^{2+} and Mg^{2+} in agricultural soil are predominantly derived from irrigation, fertilizer, straw, and the dissolution of the original SIC (Zhao et al., 2022b). The carbonate formed from that which has been dissolved and reprecipitated does not increase soil C stocks (Bughio et al., 2017). Net SIC loss is the difference between SIC dissolution and newly formed PIC. The depth of PIC formation and accumulation was predominantly in the 0–100 cm soil layer (Lal, 2007; Zamanian and Kuzyakov, 2018). Therefore, evaluation of PIC and LIC in the 0–100 cm soil layer is important for understanding the dynamic changes in SIC. To explore the effects of N fertilizer on SIC and find an effective strategy to inhibit or alleviate CO_2, it is necessary to clarify the effects of N fertilizer on PIC and LIC. Changes in PIC and LIC stocks in different soil layers after N fertilizer application have rarely been reported.

Nitrification inhibitors (NI) are a class of compounds that can decrease the bacterial oxidation of NH_4^+ to NO_2^- by inhibiting the activity of *Nitrosomonas* sp. (Wu et al., 2018; Zerulla et al., 2001). This means that the application of NI can reduce the release of H^+ after N fertilizer application, which is likely to slow the SIC dissolution, but it has not been confirmed. Over the last few years, the research on NI has mainly focused on reducing N losses and improving fertilizer use efficiency (Menéndez et al., 2012; Schwenke et al., 2018). One of the most widely used NI is 3, 4-dimethylpyrazole phosphate (DMPP) (Barrena et al., 2017; Wu et al., 2018). DMPP shows no toxic effect in crops (Zerulla et al., 2001), and it requires significantly lower application rates (0.5–1.5 kg ha^{-1}) compared to other NI (Friedl et al., 2017). The DMPP can also effectively reduce N_2O emissions from the field by up to 50 % (Schwenke et al., 2018). Small yield and quality benefits have been observed by applying DMPP (Linzmeier et al., 2001; Pasda et al., 2001; Duncan et al., 2017). However, whether DMPP reduces soil CO_2 emissions in the field is controversial. Menéndez et al., (2012); Menéndez et al. (2009) reported that CO_2 emissions were unaffected by DMPP, while others reported significant reductions in CO_2 emissions (Weiske et al., 2001; Li et al., 2017). There has been no published mechanism for how DMPP would decrease CO_2 emissions. It is likely that any discrepancies in research results may be due to the different soil types. For carbonate-containing soils, DMPP may inhibit CO_2 emissions because it can inhibit the release of hydrogen ions and alleviate SIC dissolution, but this requires long-term field experiments to confirm.

To determine whether the application of DMPP can be an effective means to alleviate SIC dissolution and reduce CO_2 emissions. We examined the changes in SOC, SIC, total carbon (TC), and total nitrogen (TN) stocks in the 0–100 cm layers after the DMPP had been applied continuously in the field for 5 years. We measured and analyzed the isotope δ^{13}C, and then estimated the PIC content using an isotopic mass balance equation. We hypothesized that after 5 years of continuous application of DMPP: i) SOC stocks would increase, ii) DMPP would effectively alleviate SIC dissolution and increase its stocks, and iii) the relief of DMPP on LIC and PIC dissolution would be the same.

2. Materials and methods

2.1. Study site

This long-term experiment was initiated at the Huantai Agricultural Experiment Station, China Agricultural University, Shandong Province (36°58′ N, 117°59′ E), which is a typical intensively farmed region in northern China. The climate at the site is temperate monsoon, with a mean annual air temperature of 13.4 °C. The mean annual precipitation is 543 mm, which predominantly occurs from June to September, and the mean annual sunshine duration is 2,833 h. A winter wheat–summer maize cropping system has been implemented since the 1980 s. Wheat and maize are usually harvested in June and October each year. The soil at the site is classified as an aquic inceptisol (calcareous, clay loam). The soil pH, bulk density (BD), SOC content, sand (0.02–2 mm), silt (0.002–0.02 mm), and clay (<0.002 mm) content of the top layer (0–20 cm) were 7.80 ($CaCl_2$ method), 1.55 g·cm^{-3}, 11.6 g·kg^{-1}, 30 %, 32 %, and 38 %, respectively.

2.2. Experimental design

The long-term experiment was initiated in June 2012. The experiment comprised three treatments with three replicates: (1) CK (no N fertilizer input). (2) NF (urea input), 300 kg N ha^{-1} (50 % as basal fertilization and 50 % as topdressing fertilization), which were applied in the wheat and maize seasons. (3) N + NI (urea combined with DMPP), DMPP was 1 % of the amount of urea N applied at the same time, that is, 3 kg ha^{-1} was applied every season. Nine planting plots were randomly established, each encompassing an area of 60 m^2 (8 × 7.5 m).

All the plots were fertilized with phosphorus (P) (140 kg P_2O_5 ha^{-1}) and potassium (K) (60 kg K_2O ha^{-1}) for the winter wheat seasons and P (100 kg P_2O_5 ha^{-1}) and K (60 kg K_2O ha^{-1}) for the summer maize seasons. The previous season's wheat straw was incorporated into the soil the approximate C:N was 98:1 and was applied at a rate of 0.86 kg m^{-2} (Xu et al., 2021). Straw from the previous maize season with an approximate C:N of 76:1 was thoroughly incorporated into the soil via plowing at 0.96 kg m^{-2}, which was consistent with local practice. Flood irrigation was practiced and carried out immediately after fertilization two to four times in the wheat season and one to three times in the maize season. The irrigation amount was 75 mm each time.

2.3. Soil sampling and analysis

After the winter wheat harvest in July 1, 2017, composite soil samples were collected using a soil auger of 3.0 cm in diameter at five depths of 0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm. Three cores of soil from each replicate were mixed into the composite samples. The soil samples were air-dried, crushed, and then passed through a 2.0 mm sieve. The BD was determined during sampling using the ring-cutter method. The soil pH was measured using a pH meter ($CaCl_2$ method). Subsamples were ground and passed through a 0.15 mm sieve before analysis of the soil TC content, TN content, SOC content, SIC content, and isotopic analyses.

The TC and TN contents were measured using a CN analyzer (Flash EA 2000, Thermo Electron Corporation, Italy) at the Chinese Academy of Agricultural Sciences. The SIC content was measured by the pressure–calcimeter method (Sherrod et al., 2002). The SOC content was calculated by the difference between TC content and SIC content. Samples were analyzed for δ^{13}C using a DELTA plus isotope ratio mass spectrometer at the Chinese Academy of Agricultural Sciences (Bughio et al., 2015; Zhao et al., 2022b). All the δ^{13}C values reported relative to VPDB, the international stable carbon isotope scale.

2.4. Calculation of SOC, SIC, TC, PIC, and LIC stocks

The SOC and SIC reserves for each soil depth were calculated by adjusting the soil mass for bulk densities, using Eq. (1):

$$C_{stock} = BD \times C_{conc} \times D \tag{1}$$

where D is the thickness of the soil depth, C_{conc} is the SOC or SIC content, and BD is the soil bulk density. The TC stock is the sum of the SOC and SIC stocks.

The proportion of PIC in SIC was estimated using the isotopic mass balance Eq. (2) (Salomons and Mook, 1976; Bughio et al., 2015):

$$PIC\% = \frac{\left({}^{13}C_{SIC} - {}^{13}C_{par}\right)}{\left({}^{13}C_{PIC} - {}^{13}C_{par}\right)} \times 100 \tag{2}$$

where $^{13}C_{Par}$ is the $\delta^{13}C$ value of the parent materials (−2.94 ‰), $^{13}C_{PIC}$ is the $\delta^{13}C$ value of PIC, $^{13}C_{SIC}$ is the $\delta^{13}C$ value of SIC in the soil, which was calculated using Eq. (3)

$$^{13}C_{SIC} = \frac{{}^{13}C_{TC}C_{TC} - {}^{13}C_{SOC}C_{SOC}}{C_{TC} - C_{SOC}} \tag{3}$$

where $^{13}C_{SOC}$ is the $\delta^{13}C$ value of SOC and $^{13}C_{TC}$ is the $\delta^{13}C$ value of TC. C_{TC} is the TC content. C_{SOC} is the SOC content.

The $\delta^{13}C$ values for PIC were estimated from the $\delta^{13}C$ values of the soil organic matter (SOM) using Eq. (4) and Eq. (5) (Nordt et al., 1998; Bughio et al., 2017):

$$^{13}C_{PIC} = {}^{13}C_{SOM} + \Delta_{CO2\ diffusion} + \Delta_{CO2-CaCO3} \tag{4}$$

$$^{13}C_{PIC} = (1 - \alpha - \beta)\,{}^{13}C_{SOM} + \alpha^{13}C_{C3} + \beta^{13}C_{C4} \tag{5}$$

where $^{13}C_{SOM}$ is the $\delta^{13}C$ value of the SOM and $\Delta_{CO2\ diffusion}$ is the $\delta^{13}C$ difference between $^{12}CO_2$ and $^{13}CO_2$, with a value of 4.4 ‰ (PDB) (Bughio et al., 2017; Cerling, 1984; Cerling et al., 1991), or 2.45 ‰ on the VPDB scale. $\Delta_{CO2-CaCO3}$ is the $\delta^{13}C$ difference between C in $CaCO_3$ and CO_2 during equilibrium reactions, and the estimated value was 10.27 ‰ (PDB) (Cerling, 1999), or 8.3 ‰ on the VPDB scale. $^{13}C_{C3}$ is the $\delta^{13}C$ value of wheat (−25.9 ‰(VPDB)) and $^{13}C_{C4}$ is the $\delta^{13}C$ value of maize (−15.92 ‰(VPDB)). Following Cerling (1999) and Bughio et al. (2017), we set α and ß at 25 % and 25 % for 0–20 cm soil layer, 20 % and 20 % for 20–40 cm soil layer, 15 % and 15 % for 40–60 cm soil layer, 10 % and 10 % for 60–80 cm soil layer, and 5 % and 5 % for the 80–100 cm soil layer, respectively.

2.5. Statistical analysis

Data were analyzed using SAS statistical software (SAS Institute Inc., 2000). Data were tested for normality using the Shapiro–Wilk test, followed by one-way analysis of variance (ANOVA), which was used to evaluate the differences among treatments. Differences between means greater than the least significant difference (LSD) were considered statistically significant ($P < 0.05$). Data are reported as mean ± standard deviation.

3. Results

3.1. Soil pH and soil BD

Compared with the CK treatment, the NF and N + NI treatments had reduced soil pH in the 0–100 cm soil profile (Table 1). The NF and N + NI treatments showed significant differences in the surface soil layer (0–20 cm; $P < 0.05$). DMPP played a role in slowing down the acidification of the surface soil caused by N application ($P < 0.05$). While the soil BD decreased with increasing soil depth in the 0–100 cm soil profile, BD in each soil layer did not change significantly after 5 years of N application and DMPP application (Table 1).

Table 1
The soil pH and soil bulk density in the soil profile under different treatments.

Layer (cm)	pH			Bulk density (g cm^{-3})		
	CK	NF	N + NI	CK	NF	N + NI
0–20	7.8 ± 0.2a	6.3 ± 0.1c	6.6 ± 0.1b	1.52 ± 0.03a	1.46 ± 0.02a	1.48 ± 0.03a
20–40	7.8 ± 0.2a	6.2 ± 0.3b	6.6 ± 0.2b	1.49 ± 0.04a	1.42 ± 0.03a	1.45 ± 0.05a
40–60	7.9 ± 0.1a	6.3 ± 0.2b	6.5 ± 0.2b	1.46 ± 0.04a	1.35 ± 0.06a	1.36 ± 0.08a
60–80	7.9 ± 0.1a	6.5 ± 0.3b	6.5 ± 0.3b	1.42 ± 0.05a	1.32 ± 0.07a	1.35 ± 0.04a
80–100	7.8 ± 0.3a	6.9 ± 0.2b	6.9 ± 0.2b	1.46 ± 0.07a	1.36 ± 0.04a	1.36 ± 0.02a

Data are presented as mean ± SD (n = 3). Different lowercase letters indicate significant differences among the three treatments in the same soil layer ($P < 0.05$). CK, nitrogen-free; NF, nitrogen application; N + NI, combined application of nitrogen fertilizer and DMPP.

3.2. SOC and SIC content in the 0–100 cm soil profile

The SOC content in the CK, NF, and N + NI treatments decreased with increasing soil depth in the 0–100 cm soil profile (Fig. 1A). At 0–20 cm, 20–40 cm, and 40–60 cm, the SOC content in the NF and N + NI treatments was higher than that in the CK treatment ($P < 0.05$). However, there was no significant difference between the NF and N + NI treatments. There was also no significant difference in the SOC content among the three treatments in the two soil layers (60–80 cm and 80–100 cm). Compared with the CK treatment, N application showed increased SOC content in the 0–60 cm soil layer ($P < 0.05$), and compared with NF, the addition of DMPP had no significant effect on the SOC content in the 0–100 cm soil layer.

In the 0–80 cm soil profile, the SIC content was the highest in the CK treatment, followed by the N + NI treatment. The SIC content was the lowest in the NF treatment, that is, CK > N + NI > NF (Fig. 1B). However, in the 80–100 cm soil layer, the SIC content order of the three treatments was reversed, with NF > N + NI > CK. Compared with the CK treatment, NF treatment reduced the SIC content by 10.0 g·kg^{-1} (58.3 %) in the 0–80 cm soil profile. In the 0–20 cm, 20–40 cm, and 60–80 cm soil layers the SIC content was reduced by 2.9, 1.9, and 3.8 g·kg^{-1}, respectively. However, the SIC content increased by 3.2 g·kg^{-1} (58.9 %) in the 80–100 cm soil layer (Fig. 1B). Compared with the NF treatment, the DMPP application increased the SIC content in the 0–20 cm and 20–40 cm soil layers by 1.3 g·kg^{-1} (50.9 %) and 1.2 g·kg^{-1} (48.2 %), respectively ($P < 0.05$). Meanwhile, the DMPP did not cause significant differences in the SIC content in the 20–40 cm, 40–60 cm, and 80–100 cm soil layers. The application of N promoted SIC dissolution in the 0–80 cm soil profile and accumulation in the 80–100 cm soil layer. Meanwhile, DMPP application slowed SIC dissolution in the 0–80 cm soil layer (Fig. 1B).

3.3. TC and TN content in the 0–100 cm soil profile

For the three treatments, the TC content decreased with increasing soil depth in the 0–60 cm soil layer, whereas in the 60–100 cm soil layer, TC increased with increasing depth (Fig. 2A). Compared with the CK treatment, the NF treatment showed an increase in the TC content by 1.8 g·kg^{-1} (12.2 %) and 3.1 g·kg^{-1} (36.2 %) in the 0–20 cm and 80–100 cm soil layers respectively ($P < 0.05$). However, the NF treatment had a reduced TC content of 3.1 g·kg^{-1} (35.7 %) in the 60–80 cm soil layer (Fig. 2A). For the 0–100 cm soil layer, N application increased the TC content. Compared with the NF treatment, DMPP application increased the TC content by 1.1 g·kg^{-1} (11.2 %) in the 20–40 cm soil layer ($P < 0.05$). There was no significant difference between the two treatments in the other soil layers (Fig. 2A).

In the 0–20 cm and 20–40 cm soil layers, compared with CK, the NF

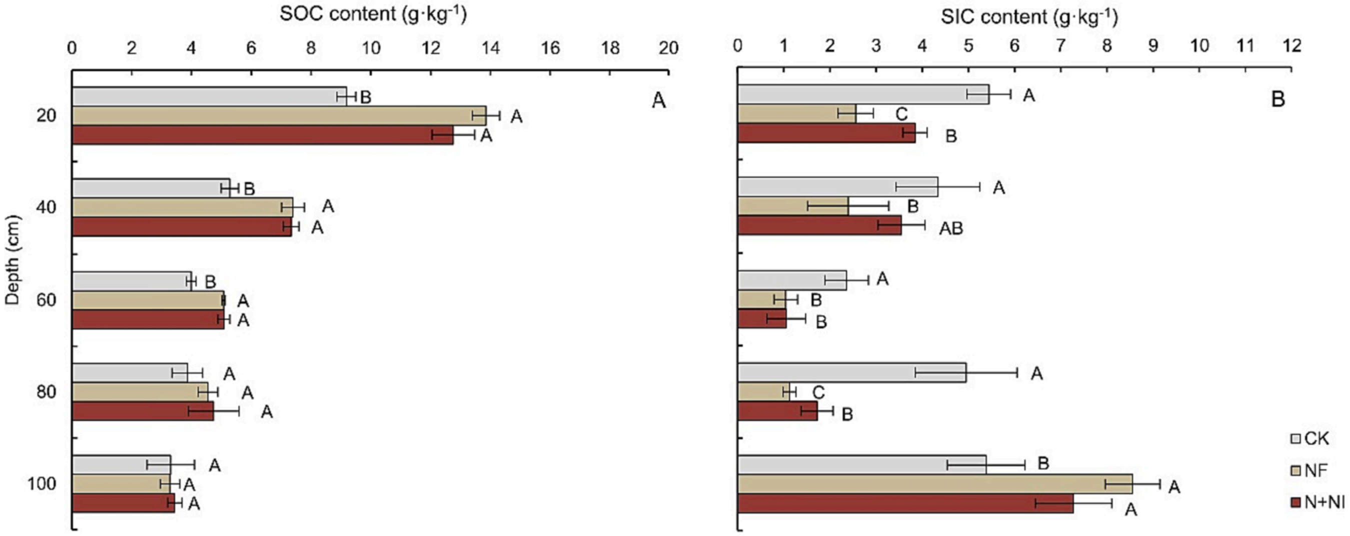

Fig. 1. SOC and SIC content in 0–100 cm soil profile. Bars show standard deviation (n = 3). Different upper-case indicate significant differences among the three treatments in the same soil layer ($P < 0.05$). CK, nitrogen-free; NF, nitrogen application; N + NI, combined application of nitrogen fertilizer and DMPP.

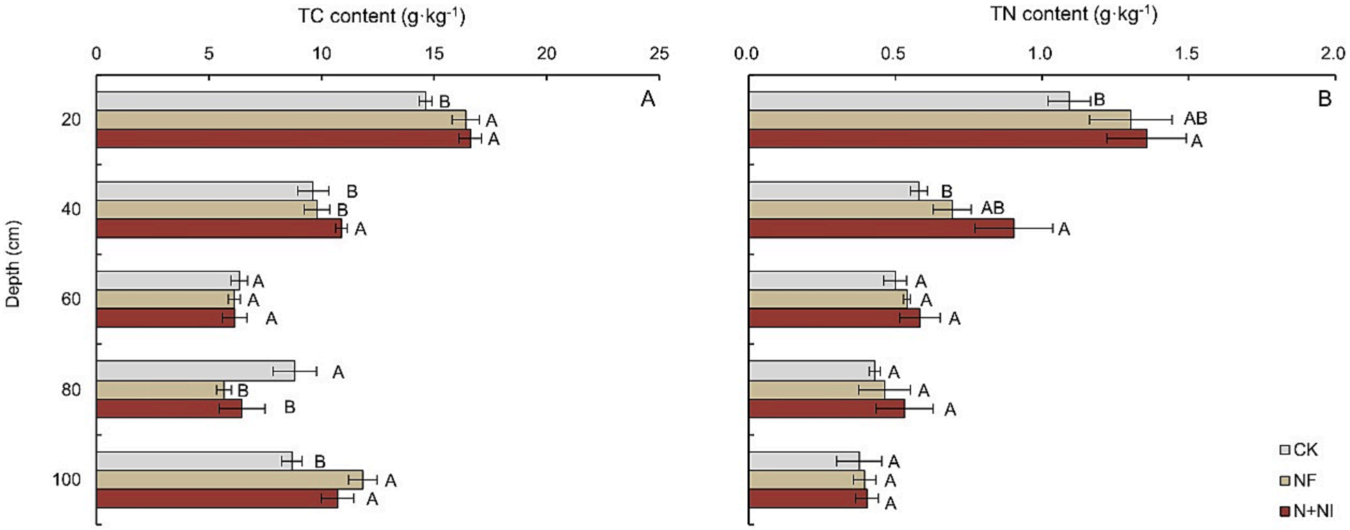

Fig. 2. TC and TN content in 0–100 cm soil profile Bars show standard deviation (n = 3). Different upper-case indicate significant differences among the three treatments in the same soil layer ($P < 0.05$). CK, nitrogen-free; NF, nitrogen application; N + NI, combined application of nitrogen fertilizer and DMPP.

treatment did not show significant changes in the TN content. The TN content in the N + NI treatment was higher than that of CK ($P < 0.05$), while there was no significant difference between the N + NI and NF treatments, that is, N + NI > NF > CK (Fig. 2B). There was no significant difference in the TN content among the three treatments in the 40–100 cm soil layer (Fig. 2B).

3.4. *δ^{13}C values of SOC and TC in the 0–100 cm soil profile*

In the three treatments, $\delta^{13}C_{SOC}$ gradually increased with increasing soil depth in the 0–100 cm soil profile. There was no significant difference between the three treatments in the same soil layer (Fig. 3A). In the CK, NF, and N + NI treatments, the $\delta^{13}C_{TC}$ values differed at different soil depths (Fig. 3B). There was no significant difference in the $\delta^{13}C_{TC}$ among the three treatments in the 0–20 cm and 20–40 cm soil layers. The $\delta^{13}C_{TC}$ in the CK treatment was greater than that of the NF and N + NI treatments ($P < 0.05$). Meanwhile, there was no significant difference between the NF and N + NI treatments in the 40–60 cm and 60–80 cm soil layers. The $\delta^{13}C_{TC}$ in the NF treatment was greater than that in the CK treatment in the 80–100 cm soil layer ($P < 0.05$). The order of $\delta^{13}C_{TC}$ in the three treatments was NF > N + NI > CK.

3.5. *SOC, SIC, and TC stocks*

The distribution of SOC stocks in each soil layer was similar to the SOC content. The SOC stocks of the NF and N + NI treatments were higher than those of CK in 0–20, 20–40, and 40–60 cm soil layers ($P < 0.05$). There was no significant difference between NF and N + NI (Table 2). There was also no significant difference among the three treatments in the 60–80 cm and 80–100 cm soil layers. In the 0–100 cm soil profile, the SOC stock in all three treatments gradually decreased with increasing soil depth. Compared with the CK treatment, the SOC stock in the NF and N + NI treatments increased by 20.3 Mg·ha^{-1} (26.7 %) and 19.1 Mg·ha^{-1} (25.1 %), respectively (Table 2).

The SIC stock distribution pattern was opposite to those of SOC (Table 2). In the 0–20, 20–40, 40–60, and 60–80 cm soil layers, the SIC stock of the CK treatment was higher than that of the NF and N + NI treatments ($P < 0.05$). In the 0–20 cm and 20–40 cm soil layers, the SIC

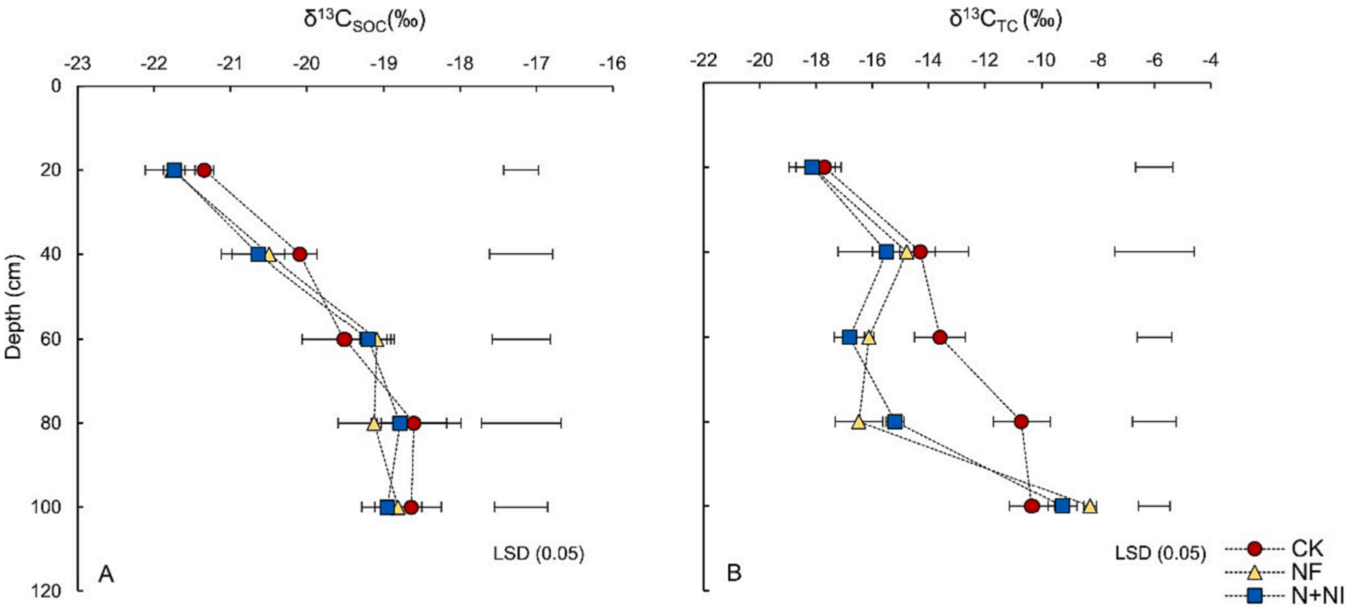

Fig. 3. $\delta^{13}C_{SOC}$ and $\delta^{13}C_{TC}$ in 0–100 cm soil profile under different treatments. Bars show standard deviation (n = 3). Error bars shows LSD ($P < 0.05$) for comparison among different treatments at the same layer. CK, nitrogen-free; NF, nitrogen application; N + NI, combined application of nitrogen fertilizer and DMPP.

Table 2
The soil organic carbon (SOC), soil inorganic carbon (SIC) and total carbon (TC) stocks (Mg·ha^{-1}).

Layer	SOC			SIC			TC		
(cm)	CK	NF	N + NI	CK	NF	N + NI	CK	NF	N + NI
0–20	28.0 ± 1.2b	40.4 ± 1.2a	37.7 ± 1.8a	16.6 ± 1.5a	7.5 ± 1.2c	11.4 ± 1.0b	44.6 ± 1.5b	47.9 ± 2.0a	49.0 ± 1.5a
20–40	15.8 ± 1.2b	21.0 ± 0.7a	21.3 ± 1.4a	12.9 ± 2.3a	6.8 ± 2.6b	10.3 ± 1.2ab	28.6 ± 1.3b	27.9 ± 2.0b	31.6 ± 0.5a
40–60	11.6 ± 0.6b	13.7 ± 0.6a	13.9 ± 1.1a	6.9 ± 1.5a	2.8 ± 0.8b	2.9 ± 1.2b	18.5 ± 1.5a	16.6 ± 1.4a	16.8 ± 2.1a
60–80	11.0 ± 1.1a	12.0 ± 0.3a	12.7 ± 2.0a	14.2 ± 3.7a	3.0 ± 0.4b	4.6 ± 0.8b	25.1 ± 3.4a	15.0 ± 0.4b	17.4 ± 2.2b
80–100	9.6 ± 1.9a	9.0 ± 0.8a	9.4 ± 0.4a	15.8 ± 3.1b	23.5 ± 2.2a	19.9 ± 2.4ab	25.3 ± 1.8b	32.4 ± 2.3a	29.2 ± 2.1ab

Data are presented as mean ± SD (n = 3). Different lowercase letters indicate significant differences among the three treatments in the same soil layer ($P < 0.05$). CK, nitrogen-free; NF, nitrogen application; N + NI, combined application of nitrogen fertilizer and DMPP.

stock of the N + NI treatment was higher than that of the NF treatment ($P < 0.05$). In the 80–100 cm soil layer, the SIC stock of the NF treatment was higher than that of the other two treatments in the order of NF > N + NI > CK ($P < 0.05$). In the 0–100 cm soil layer, compared with the CK treatment, the SIC stock in the NF and N + NI treatments were reduced by 22.7 Mg·ha^{-1} (34.2 %) and 17.3 Mg·ha^{-1} (26.0 %), respectively (Table 2).

The TC in each soil layer did not show any pronounced regularity in differences with soil depth (Table 2). In the 0–20 cm and 20–40 cm soil layers, the N + NI treatment had the largest TC stock. There was no significant difference among the three treatments in the 40–60 cm soil layer; in the 60–80 cm soil layer, the TC stock of the CK treatment was larger than that of the other two treatments ($P < 0.05$). Meanwhile, in the 80–100 cm soil layer, the NF treatment had the largest TC stock. In the 0–100 cm soil layer, the TC stocks of the CK, NF, and N + NI treatments were 142.2, 139.8, and 144.0 Mg·ha^{-1} (Table 2), respectively, with no significant difference.

3.6. *PIC and LIC stocks*

Using stable isotope $\delta^{13}C$ to estimate the PIC and LIC stocks in the 0–100 cm soil layer in the three treatments (Table 3), the results showed that in the 0–20 cm and 20–40 cm soil layers, the order of PIC stock was CK > N + NI > NF. In the 0–20 cm soil layer, the PIC stock of the NF treatment approached 0. In the 40–60 cm soil layer, the PIC stocks of the NF treatment were close to those of the CK treatment. In the 60–80 cm soil layer, there was no significant difference among the three treatments. However, in the 80–100 cm soil layer, the PIC stock of the NF treatment was the highest, followed by N + NI, and then CK. In the 0–100 cm soil layer, the PIC stocks of the CK treatment were higher than those of the other two treatments ($P < 0.05$), and the PIC stocks of the NF treatment were the lowest. Compared with the CK treatment, PIC stocks of the NF and N + NI treatments were reduced by 17.5 Mg·ha^{-1} (64.4 %)

Table 3
Pedogenic carbonates (PIC) and lithogenic carbonates (LIC) stocks (Mg·ha^{-1}).

Layer	PIC			LIC		
(cm)	CK	NF	N + NI	CK	NF	N + NI
0–20	12.7 ± 1.1a	0.0 ± 0.0c	4.7 ± 1.3b	3.9 ± 0.5b	7.6 ± 1.2a	6.6 ± 1.8a
20–40	5.3 ± 0.8a	1.0 ± 0.4c	4.1 ± 0.5b	7.6 ± 1.6a	5.9 ± 2.2a	6.2 ± 0.7a
40–60	1.4 ± 0.7a	0.9 ± 0.2ab	0.5 ± 0.2b	5.5 ± 2.1a	1.9 ± 0.6b	2.4 ± 1.0b
60–80	4.4 ± 3.0a	1.9 ± 0.3a	1.6 ± 0.5a	9.8 ± 2.1a	1.0 ± 0.2b	3.1 ± 0.5b
80–100	3.3 ± 1.2b	6.0 ± 1.2a	5.0 ± 1.5ab	12.4 ± 2.6b	17.5 ± 2.5a	14.9 ± 0.9ab
0–100	27.2 ± 4.6a	9.7 ± 1.2c	15.9 ± 1.3b	39.1 ± 5.4a	33.9 ± 1.8a	33.1 ± 1.3a

Data are presented as mean ± SD (n = 3). Different lowercase letters indicate significant differences among the three treatments in the same soil layer ($P < 0.05$). CK, nitrogen-free; NF, nitrogen application; N + NI, combined application of nitrogen fertilizer and DMPP.

and 11.2 Mg·ha^{-1} (41.4 %), respectively. Compared with the NF treatment, N + NI increased the PIC stock by 6.2 Mg·ha^{-1} (64.6 %) in the 0–100 cm soil layer ($P < 0.05$). This indicates that N fertilizer application reduced the PIC stocks in the 0–100 cm soil layer, while the application of DMPP reduced the impact of N fertilizer on PIC stocks.

There were no significant differences in the LIC stocks between the NF and N + NI treatments in the five soil layers (Table 3). However, there were significant differences between the CK and the other two treatments ($P < 0.05$). In the 0–20 cm and 80–100 cm soil layers, the LIC stocks of the CK treatment were lower than those of NF and N + NI. Meanwhile, in the 40–60 cm and 60–80 cm soil layers, the LIC stocks of the CK treatment were higher than those of the other two treatments ($P < 0.05$). In the 0–100 cm soil layer, there were no significant differences among the LIC stocks under the three treatments.

4. Discussion

4.1. Effects of DMPP on soil organic carbon

Compared with the NF treatment, the application of DMPP did not have a significant impact on SOC stock in the 0–100 cm soil profile, which is in contrast with our hypothesis. Some researchers have suggested that DMPP application reduces the mineralization of SOC by soil microorganisms and reduces soil CO_2 emissions (Weiske et al., 2001). DMPP inhibits nitrification and inhibits the growth and activity of *Nitrosomonas* sp. in the soil (Shi et al., 2017; Wu et al., 2018), whereas overall soil microbial activities do not respond to the application of DMPP (Kong et al., 2017). Observing the overall effect of DMPP on SOC is unlikely, because nitrifying bacteria occupy only a small proportion of soil microorganisms (Duncan et al., 2017). Although our team has shown that DMPP has increased the maize yield in this field (Han, 2018), it is likely that more exogenous carbon comes from plants, which may increase the SOC stock. Our research suggested that DMPP has a low overall impact on SOC increase despite its known effects on microbial nitrifying activity and crop yield. There have been no significant changes in SOC stock after a 5-year test, and a longer study duration may be required to further verify this.

4.2. Effects of DMPP on soil inorganic carbon

Ca^{2+} and/or Mg^{2+} are required for PIC formation, which in our research were predominantly supplied by the decomposition of straw amendments, irrigation, and LIC dissolution. PIC was predominantly distributed in the 0–40 cm soil layer in the CK treatment, which is consistent with many previous studies (Zamanian et al., 2016; Zamanian and Kuzyakov, 2018). Our research has shown that N fertilizer application mainly caused PIC dissolution rather than LIC dissolution in 0–100 cm soil, which led to a decrease in SIC stock. DMPP application significantly increased the PIC stock in the 0–100 cm soil layer, particularly in the 0–40 cm soil layer. This may be because the effect of DMPP on PIC is to reduce the H^+ produced during the reaction by inhibiting nitrification, thereby alleviating PIC dissolution caused by the application of N fertilizer (Zamanian and Kuzyakov, 2018). NH_4^+ was predominantly concentrated in the upper soil layer because of the adsorption by soil colloids (Tamir et al., 2013; Xu, 2018). Therefore, DMPP inhibition of nitrification is mainly concentrated in the 0–40 cm soil layer.

The C in the dissolved PIC may change into two forms: (1) CO_2, which is emitted into the atmosphere, or (2) CO_3^{2-} and HCO_3^-, which are leached into the deeper soil layers to re-form PIC or exported as soluble carbonate in the surface water (Zhao et al., 2022b). We found that the PIC stock of N fertilizer application was significantly higher than that of CK in the 80–100 cm soil layer. This may be because Ca^{2+} and Mg^{2+} result in PIC dissolution of the surface layer and gradually accumulate at depth with irrigation, before promoting a balanced response and further PIC formation. However, the amount of PIC increasing in the 80–100 cm layer was significantly lower than the PIC dissolved in the 0–80 cm soil layer. Although the PIC is potentially formed in the deeper soil layer, the PIC formed in the soil layer below 100 cm is lower in farmlands in semi-arid areas (Zamanian et al., 2018; Zamanian and Kuzyakov, 2018). Therefore, in our research, a substantial proportion of the PIC in the surface soil decomposed into CO_2 and was released into the atmosphere, and application of DMPP may alleviate this process.

The change in LIC in the soil may be related to the Ca^{2+} and/or Mg^{2+} balance in the soil environment (Sanderman, 2012; Bughio et al., 2017). The LIC stock in CK was significantly lower than that in NF and N + NI in the 0–20 cm soil layer, which is consistent with some studies (Bughio et al., 2015; Bughio et al., 2017). This may be because in the CK treatment, PIC is generated in the surface layer, and the formation of PIC requires the consumption of Ca^{2+} and Mg^{2+} in the soil. This affects the Ca^{2+} and Mg^{2+} balance in the soil (Zamanian et al., 2016), thereby promoting LIC dissolution. In contrast, PIC was dissolved in the soil surface layer in the NF and N + NI treatments, releasing $Ca^{2+}$and Mg^{2+} into the soil. This increased the $Ca^{2+}$and Mg^{2} concentrations in the soil, thereby suppressing LIC dissolution. In the 40–80 cm soil layer, the LIC stock was significantly reduced in the NF and N + NI treatments, perhaps because this was an intermediate soil layer. External $Ca^{2+}$and Mg^{2+} ions which were mainly from irrigation and straw decomposition could not act directly on this soil layer, and these ions may be soluble in lower soil layers. When PIC is formed, $Ca^{2+}$and Mg^{2+} may be derived from LIC dissolution. Compared with a single N fertilizer, DMPP application did not significantly impact the LIC stock in each soil layer. This may be because LIC is more stable than PIC. The hydrogen ions released by nitrification first dissolved the PIC to reduce its stock, which provided additional buffer time for LIC dissolution.

Therefore, the impact of DMPP on SIC is predominately reflected in the effects of PIC. Applying DMPP to farmland soil containing carbonate significantly alleviated the dissolution of PIC by N fertilizer, which may reduce CO_2 emissions in the field. If the DMPP can be applied over a wide range, it may be an effective way to reduce CO_2 emissions, especially in China. Although the average N fertilizer input in Chinese cropland is at least 2–3 times that of other countries (Raza et al., 2020; Zhao et al., 2022b), the average N-use efficiency is 25 % (Zhang et al., 2015). As one of the NI, DMPP also plays an important role in improving the N efficiency and reducing the N loss. Therefore, the role of DMPP in protecting farmland SIC stocks and soil health should always be considered.

5. Conclusion

By comparing the NF treatment for 5 consecutive years, we found that DMPP had no significant effect on SOC content and $\delta^{13}C_{SOC}$ in the 0–100 cm soil profile. The application of N fertilizer significantly reduced the SIC content in the 0–80 cm soil profile, but significantly accumulated SIC in the 80–100 cm soil layer. DMPP combined with N significantly alleviated PIC dissolution in the 0–20 cm and 20–40 cm soil layers and increased PIC stocks in 0–100 cm soil by 6.2 Mg·ha^{-1} (64.6 %). However, there was no significant effect on LIC. Applying DMPP to farmland soil containing carbonate significantly alleviated the dissolution of PIC by N fertilizer, which may reduce CO_2 emissions in the field.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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