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RESEARCH REPORT

Effect of divalent manganese (Mn^{2+}) concentration on the growth and nitrate nitrogen content of lettuce during aeroponic intercropping with cherry radish

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Abstract
Manganese (Mn) plays an important role in regulating metabolism, especially nitrogen metabolism, in plants. Considering the desired levels required for plant growth and the most popular concentration in nutrient solutions in soilless cultures, lettuce plants were exposed to 4 μM, 10 μM, or 40 μM Mn^{2+} as $MnSO_4{\cdot}4H_2O$ during aeroponic intercropping with cherry radish plants with a 1:1 quantity ratio of lettuce/cherry radish. The effects of Mn^{2+} on plant growth, nitrate nitrogen (NO_3^--N), and metabolism of lettuce were investigated. The results showed that the fresh weight (FW) and dry weight (DW) of lettuce increased by 20.9% and 24.7%, respectively, at 30 days after transplanting when the Mn^{2+} concentration ranged from 4 (treatment C1) to 40 μM (treatment C3). The NO_3^--N content in the edible parts of lettuce decreased by 34.4% and 44.9% with increasing Mn^{2+} concentrations on the 10th day and the 20th day after transplanting, respectively, but the maximal reduction of the NO_3^--N content was only 9% on the 30th day when the Mn^{2+} concentration ranged from 4 (treatment C1) to 40 μM (treatment C3). Additionally, our results showed that increased but not excess Mn^{2+} could markedly promote nitrate reductase (NR) activity instead of limiting the stomata, which was one reason why the NO_3^--N content in edible parts decreased. During aeroponic intercropping with cherry radish plants, Mn^{2+} thresholds were found that improved organic biomass and nitrogen assimilation in the edible parts of lettuce. The Mn^{2+} thresholds could be similar or different, but both were within the range of 10 (treatment C2) –40 μM (treatment C3).

Keywords Intercropping aeroponics · Lettuce · Manganese ion · Nitrate · Nitrate reductase · Nitrogen metabolism

1 Introduction

Accumulation of nitrate nitrogen (NO_3^--N) often occurs in horticultural leaf vegetables (Colla et al. 2018). NO_3^--N is not a threat to human health, but its reaction products and metabolites, such as nitrite and N-nitroso compounds, may pose potential harm to public health (EFSA 2008). The international Food and Agriculture Organization/World Health Organization (FAO/WHO) and the European commission for food science (SCF) give the acceptable daily intake (ADI) at 3.7 mg/kg BW. Studies have indicated that NO_3^--N accumulation could be affected by cultural practices, including intercropping cultivation. Intercropping is an innovation for soilless culture systems. The fresh weight (FW) of lettuce was increased by 7.9% and the NO_3^--N content was decreased by 16.6% in an intercropping aeroponics system of lettuce (*Lactuca sativa L.*) and cherry radish (*Raphanus sativus L.*) at a ratio of 1:1 (Wang et al. 2017a; Yu et al. 2017).

To date, there have been no reports of specialized nutrient solutions and management modes for intercropping in aeroponics, hydroponics, or other soilless culture systems. However, NO_3^--N metabolism has been shown to be directly or indirectly influenced by macroelements, including nitrogen (N), phosphorus (P), sulfur (S), and chloride (Cl), and microelements, including molybdenum (Mo), iron (Fe), and manganese (Mn) (Colla et al. 2018; Hewitt and Gundry 1970; Borlotti et al. 2012; Yang et al. 2006).

Excess Mn is potentially toxic to plants. However, Mn plays an important role in protein structure and participates

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in metabolic processes, such as chlorophyll biosynthesis, photosynthetic activity, and phosphorylation of enzymes (Santos et al. 2017; Ribera et al. 2013). Divalent manganese (Mn^{2+}) may contribute to the regulation of metabolism in plants via beneficial metal substitutions and interacting with divalent magnesium (Mg^{2+}). In some cases, Mn^{2+} has been substituted for divalent iron (Fe^{2+}) and divalent nickel (Ni^{2+}) in proteins and sustained the normal activity of related enzymes in plants (Imlay 2014; Deshpande et al. 2017). Changes in Mg^{2+} or Mn^{2+} influence the use of nitrate or ammonium as a nitrogen source in plants and mitigate changes in nitrate assimilation, which regulates the carbon-nitrogen balance (Bloom and Kameritsch 2017). Exchange of Mn^{2+} for Mg^{2+} in several chloroplast enzymes also maintains the plant carbon-nitrogen balance (Bloom 2019). Mn deficiency disturbs photosynthesis by damaging chloroplasts and affecting water photolysis in photosystem II (Fernando and Lynch 2015). Dry matter production and net photosynthesis rapidly declines. However, respiration and transpiration are not affected in Mn-deficient plants (Marschner 2013).

In contrast to the wide range of critical toxicity concentrations (Kochian et al. 2004; Inostroza-Blancheteau et al. 2017), the critical deficiency concentration of Mn^{2+} narrowly varies in most plant species between 10 and 20 mg kg^{-1} dry weight (DW) in leaves (Marschner 2013). Based on the Mn tolerance of plants and increasing experience with soilless culture, the desired levels of Mn^{2+} required for plant growth usually range from 1.8 to 40 μM, and 10 μM is the most popular concentration in nutrient solutions in soilless cultures (Smith and Dalton 1999).

The following hypotheses need to be tested in an intercropping aeroponics system: (a) high but not excess Mn^{2+} will improve growth of lettuce; (b) high but not excess Mn^{2+} will decrease the NO_3^--N content in edible parts of lettuce; (c) high NR activity will result in low NO_3^--N content under high but not excess Mn^{2+} treatment; and (d) low but not deficient Mn^{2+} concentration will decrease photosynthesis due to non-stomatal limitation.

Therefore, the aim of this study is to investigate the effects of different Mn^{2+} concentrations on the growth and NO_3^--N content in the edible parts of lettuce in an intercropping aeroponics system. Cherry radish was selected for intercropping with lettuce, but it is considered to be an associated plant instead of an edible vegetable, and the effects on cherry radish were not considered.

The results of this study will aid in producing high-quality leaf vegetables with low nitrate levels in the aerosol cultivation system via a biocontrol method.

2 Materials and methods

2.1 Plant materials and experimental conditions

Cherry radish (*Raphanus sativus L.*) plants were intercropped with Italian lettuce (*Lactuca sativa L.*) plants in a 1:1 ratio to establish an aeroponics system in this study (Fig. 1a) based on previous work (Wang et al. 2017a, b; Yu et al. 2017).

Specific buckets shown in Fig. 1b (12 plants per bucket; $53 \times 37 \times 23$ cm^3, L × W × H) for aeroponic cultivation were used in this experiment. A closed pipeline with atomizing nozzles was placed over the nutrient solution to make nutrient solution circulate in every bucket. There was a half-hour interval between 15-minute sprays by a timer control. In the nutrient solution, macroelements and microelements except for Mn^{2+} were both kept at constant concentrations, and quivalents of the above elements are shown in Table 1. The electrical conductivity (EC) value of nutrient solution was maintained at 2000 ± 200 μS cm^{-1} by adding water, and pH was maintained in a range of 5.8–6.2 by adding diluted hydrochloric acid (0.1M HCl) because that they kept increasing with growth was observed in this experiment.

The experiment was conducted in a glass greenhouse with an automatic environmental control system at the Nanling campus of Jilin University (43°51′05″N, 125°19′51″E). The average temperature was maintained at 22 ± 4 °C in the daytime (8:00 am to 7:59 pm) and 18 ± 4 °C at night (8:00 pm to 7:59 am). The average photosynthetic photon flux density (PPFD) was 180 ± 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the photoperiod (9:00am to 4:00 pm). The Italian lettuce 'annual bolting resistant' and the cherry radish 'Hongding' cultivars used in the experiments were obtained from the Fengke Seed Industry Co. LTD in Jilin province.

Table 1 Equivalent of each element in the nutrient solution

Macroelements[z]	Concentration (mM)	Microelements[y]	Concentration (mM)
$Ca(NO_3)_2 \cdot 4H_2O$	4.00	H_3BO_3	0.0500
KNO_3	8.00	$ZnSO_4 \cdot 7H_2O$	0.0008
$NH_4H_2PO_4$	1.33	$CuSO_4 \cdot 5H_2O$	0.0003
$MgSO_4 \cdot 7H_2O$	2.00	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.0001
		EDTA-2NaFe	0.0100

[z]Concentrations of macroelements according to Japanese garden nutrient solution

[y]Concentrations of microelements according to Hoagland and Anrnon's nutrient solution

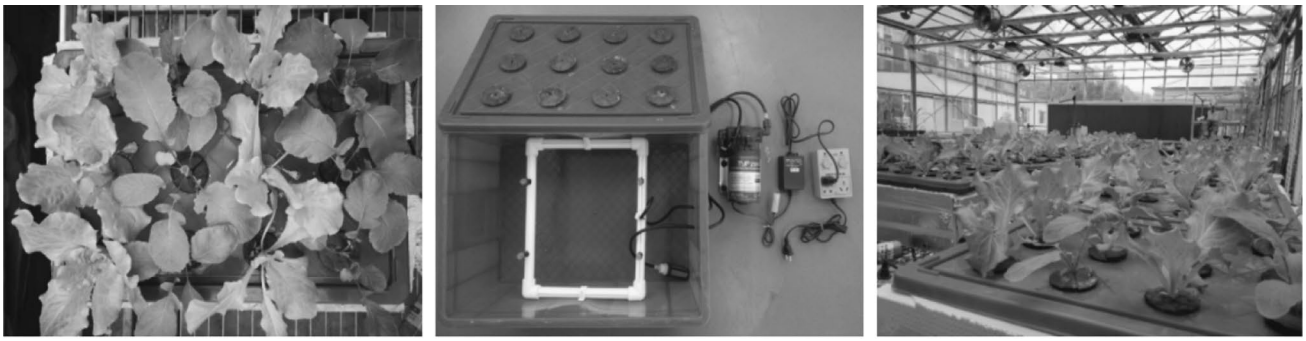

Fig. 1 Intercropping in an aeroponics system. **a** Intercropping of radishes and lettuce in a 1:1 ratio in one bucket. **b** Specific bucket for aeroponics culture. **c**.Transplantation of vegetables into buckets in the summer of 2017 (the spacing between plants was 15 cm)

2.2 Experimental design

The experiment was performed in the summer of 2017 (the 24th of May to the 24th of July) and was designed to discover how Mn^{2+} concentration affected the growth and NO_3^--N content of lettuce during intercropping cultivation. Lettuce and cherry radish plants underwent intercropping cultivation with three Mn^{2+} concentrations, 4 μM (treatment C1), 10 μM (treatment C2), and 40 μM (treatment C3). $MnSO_4{\cdot}4H_2O$ was used to regulate Mn^{2+} concentrations. Each treatment included six buckets (shown in Fig. 1c) to guarantee sufficient replicates for all measurements at each sampling time.

Seeds of lettuce and cherry radish were sown on the 24th of May and the 9th of June, respectively. Seedlings were transplanted together on the 24th of June. After transplanting, the cultivation period of 30 days was divided into three stages (10 days per stage) to guarantee a visible difference as shown in previous studies (Wang et al. 2017a; Ibraimo et al. 2017).

2.3 Sampling and analytical methods

The growth, nitrogen compound content, photosynthesis, and nitrogen reductase (NR) activity in the intercropping cultivation were only measured in lettuce. Sampling was performed on the 4th of July, the 14th of July, and the 24th of July. Six lettuce plants (one plant per bucket) were randomly harvested for each treatment for destructive testing. Additionally, six cherry radishes were also removed simultaneously from the buckets to guarantee the same intercropping ratio (1:1) when sampling lettuce.

Growth parameters Half of the harvested plants were used to measure growth parameters. The edible parts of plants were used to get the FW of edible parts using an electronic scale (ME104E, Mettler Co., Switzerland), and then the DW was obtained by drying the parts at 105 °C for 30 min and then holding at 80 °C in a vacuum oven (DZF-6050, Shanghai, China). Roots of fresh plants were removed to collect root surface area (SA) and root volume (VOL) using a root analysis system (WinRHIZO, Canada). The relative chlorophyll content was determined by a chlorophyll meter (SPAD502, Konica-Minolta, Japan) before harvesting. The second youngest leaf was selected for determination, and leaves were assessed in a position near the major veins.

Nitrogen compound content Dried pieces were used to assay the total nitrogen (TN) and protein nitrogen (PN) after determining DW. The TN was determined according to the micro-Kjeldahl method (Li 2003). Dried material 200 (mg) was placed in a 250-mL digestion tube. Then, a 2.5 g mixture of K_2SO_4 and $CuSO_4{\cdot}5H_2O$ (3:1, W/W), as a catalyst, and 5 mL of concentrated sulfuric acid were added successively. Digestion was carried out at 200 °C for 0.5 h and then at 400 °C for 0.5–1 h until the solution was completely clear. Once digestion was complete, the sample was put into a micro-Kjeldahl Analyzer (JK9830, China) for distillation and then titrated. PN was assayed according to the method of micro-Kjeldahl (Li 2003). Dried material (200 mg) was put into a 10-mL centrifuge tube, and 5 mL of 5% trichloroacetic acid was added. The samples were extracted for 15 min in a water bath at 90 °C, cooled, and then centrifuged at 4000 r min^{-1} for 15 min. The supernatant was discarded, and the precipitate was washed with 5% trichloroacetic acid two or three times. The process of centrifugation and discarding the supernatant was repeated. Then, the precipitate and filter paper were dried at 50 °C in an oven after thoroughly washing the filter paper with distilled water. The nitrogen content of the precipitate was then determined.

NO_3^-*-N content and NR activity analyses* The other half of the harvested plants was used to directly determine the NO_3^--N content and nitrate reductase (NR) activity. The NO_3^--N content of edible parts was determined by the method of Li(2003; Cataldo et al. 1975). Leaves (2.0 g) were ground using a Waring blender (JJ-2B, China) and then suspended in

10 mL of distilled water. The sample was boiled at 100 °C in a water bath for 30 min. The extracts were filtered and then diluted with distilled water to 25 mL after cooling with tap water. Then, 5% salicylic acid-concentrated sulfuric acid (0.4 mL) was added to 0.1 mL of the exact. After 20 min of standing at room temperature in the dark, the mixture was further diluted with 9.5 mL of NaOH (2.0 M) solution and shaken until a yellow color appeared. The absorption at 410 nm was used to calculate the NO_3^--N content according to the standard curve.

NR activity was determined in vitro according to the method of Li (2003), Lei et al. (2018). Fresh leaf tissue (1 g) was cut into pieces, ground, and homogenized with 8 mL of 25 mM phosphate buffer (containing 1 mM EDTA and 10 mM cysteine, pH 8.7). The sample was centrifuged at 10,000 r·min^{-1} for 30 min at 4 °C for crude enzyme extraction and assays. Then, 1.2 mL of 0.1 mM phosphate buffer (pH 7.5), 0.4 mL of 2 mg mL^{-1} NADH, and 0.4 mL of crude enzyme extract were mixed together for an enzyme assay. After incubation for 30 min at 25 °C in water bath, 1 mL of sulfanilamide (1%, W/V) and 1 mL of N-1-naphthylethylenediamine dihydrochloride (0.02%, W/V) were added to stop the reaction. The absorbance was recorded at 540 nm with a spectrometer.

Photosynthesis characteristics Three plants (three replicates per plant) were randomly selected to determine their photosynthesis parameters in each treatment. The net photosynthetic rate (Pn), stomatal conductance (Gs), and intercellular carbon dioxide (Ci) were measured using a portable open gas exchange system (Li-6400XT, LI-COR, USA) with the 6-cm^2 chamber. The measurement was conducted from 9:00 to 12:00 before each sampling, under the ambient CO_2 concentration of 400 μmol mol^{-1}, photosynthetically active radiation (PAR) of 1500 μmol s^{-1}, air-flow rate of 500 μmol s^{-1}, leaf temperature of 25 °C, and relative humidity (RH) of 70% (Kwon et al. 2019). The measurements were initiated until the values of Pn fluctuated less than 0.1 for stabilization. The second youngest leaf was selected for determination, and leaves were assessed in a position near the major veins.

Statistical analysis Data are presented as the mean ± SD of three replicates. Pearson's correlation analysis of various measures was conducted with SPSS 24.0 software. Duncan's test at a value of $p < 0.05$ was performed to compare significant differences among the groups. All the figures were created with Origin 9.0 software (Origin Lab, Northampton, MA, USA).

3 Results

3.1 Effects of Mn^{2+} on lettuce growth

The growth parameters of lettuce under different treatments are indicated in Fig. 2. Increasing the Mn^{2+} concentration from 4 (treatment C1) to 40 μM (treatment C3) showed positive effects on growth. The FW, DW, SA, VOL, and SPAD all increased with increasing Mn^{2+} concentration at different ages as shown in Fig. 2. Compared to treatment C1, the FW, root surface area, and root volume increased by 20.9%, 17.0%, and 22.7%, respectively, for treatment C3 on the 30th day after transplantation. The maximum values of SPAD were detected for treatment C3 on the 20th day.

The responses to different Mn^{2+} concentrations were slightly different from those of the FW. No significant differences in DW were observed between treatment C1 (4 μM) and treatment C2 (10 μM) under any condition, but a remarkable increase in the DW was found under treatment C3 (40 μM) starting on the 20th day. The DW was increased by 27.5% and 24.7% on the 20th day and the 30th day, respectively, when the Mn^{2+} concentration was increased from 4 (treatment C1) to 40 μM (treatment C3).

3.2 Effects of Mn^{2+} on nitrogen compound content in the edible parts of lettuce

Increasing the Mn^{2+} concentration positively affected the total nitrogen (TN) and protein nitrogen (PN) in the edible parts of lettuce starting on the 20th day (Fig. 3). The PN under the high Mn^{2+} concentration (treatment C3, 40 μM) was obviously higher than those of the other two Mn^{2+} concentrations (4 μM and 10 μM) on the 20th day (28.64 mg g^{-1} DW). The PN was 23.32 mg g^{-1} DW under treatment C3 (40 μM) and was remarkably higher than that for treatment C1 (4 μM) on the 30th day ($p < 0.05$). However, no significant difference was found between treatments C2 and C3 as shown in Fig. 3. The NO_3^--N content showed a negative trend with increasing Mn^{2+} concentration in the first two stages (Fig. 3), decreasing by 34.4% and 44.9% with increasing Mn^{2+} concentration on the 10th day and 20th day, respectively. However, the NO_3^--N content was 567.90, 539.45, and 515.97 mg kg^{-1} FW under treatments C1, C2, and C3, respectively, and the content was reduced by up to 9% on the 30th day. No significant differences ($p > 0.05$) between any two groups were found, and the content was obviously higher than that on the 20th day as shown in Fig. 3 ($p < 0.05$, data not shown).

3.3 Nitrate reductase activity analysis

Increasing the Mn^{2+} concentration from 4 (treatment C1) to 40 μM (treatment C3) caused positive effects on NR activity in the edible parts of lettuce over 30 days, which was contrary to the findings for the NO_3^--N content (Fig. 4). The maximum NR activity for all three treatments occurred on the 20th day, with values of 19.47, 21.19, and 22.33 μg g^{-1} h^{-1} FW, respectively. Significant differences in NR activity were found between the low

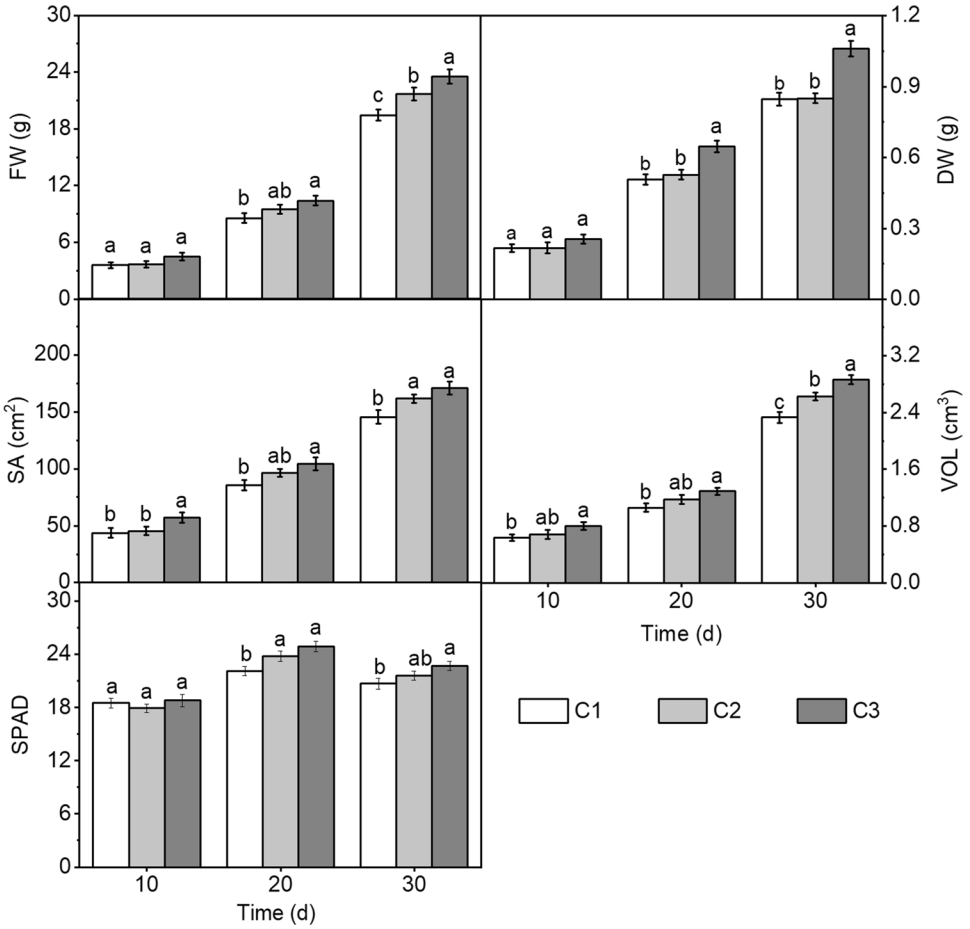

Fig. 2 Effect of manganese ions on lettuce growth. Different letters indicate significant differences ($p < 0.05$) based on Duncan's multiple range test. Values indicate means ± SE

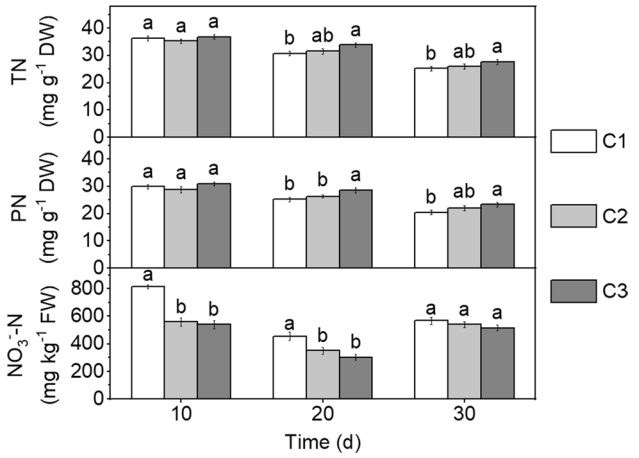

Fig. 3 Effects of manganese on different forms of nitrogen in the above-ground parts of lettuce. Different letters indicate significant differences ($p < 0.05$) based on Duncan's multiple range test. Values represent means ± SE ($n = 3$) for individual plants

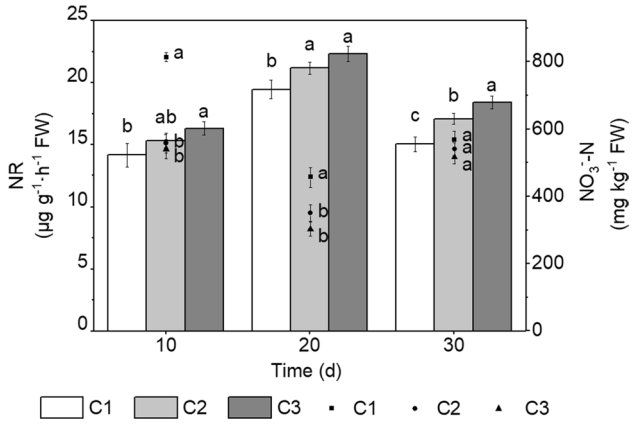

Fig. 4 Effects of manganese on the activities of key enzymes in the above-ground parts of lettuce. Different letters indicate significant differences ($p < 0.05$) based on Duncan's multiple range test. Values represent means ± SE ($n = 3$) for individual plants

Mn^{2+} concentration (treatment C1, 4 μM) and the high Mn^{2+} concentration (treatment C3, 40 μM) on the 10th day and were maintained until harvesting on the 30th day. However, remarkable variation of NR activity between the low Mn^{2+} concentration (treatment C1, 4 μM) and the middle Mn^{2+} concentration (treatment C2, 10 μM) was observed on the 20th day (Fig. 4). On the 30th day,

significant differences of NR activity were found between all pairs of treatments.

3.4 Effects of Mn^{2+} on photosynthesis parameters

The photosynthetic parameters of lettuce plants under different Mn^{2+} concentrations are summarized in Fig. 5. The Pn and Gs were increased in response to increasing Mn^{2+} concentrations. The maximum Pn and Gs values all occurred on the 20th day under the high Mn^{2+} concentration (treatment C3, 40 μM). Significant differences in these two parameters were found between the low concentration (treatment C1, 4 μM) and the high concentration (treatment C3, 40 μM) starting on the 20th day (Fig. 5). The highest Ci values for the three treatments all occurred for the middle Mn^{2+} concentration (treatment C2, 10 μM), and the maximum value was found on the 20th day (Fig. 5).

4 Discussion

In most plants, both growth and biomass decline when Mn^{2+} is deficient or present in excess (Marschner 2013; Singh et al. 2001; Li et al. 2015). This study revealed that the yield and organic matter content were obviously increased under increasing concentrations of Mn^{2+} from 4 to 40 μM in an intercropping aeroponics system, and a similar trend was found for manganese in modulating the responses of the nitrogen supply (Pelaez et al. 2010). This finding proves our hypothesis that a high but not stressful Mn^{2+} concentration will improve the growth of lettuce. An appropriate Mn^{2+} concentration in nutrient solution favored root growth and development (Fig. 2), which improved water and nutrition absorption and chlorophyll biosynthesis in lettuce (Marschner 2013). SA and VOL were significantly positively correlated with FW, and correlation coefficients were 0.991 and 0.997 based on Pearson correlation analysis. The correlation between FW and Pn was not significant ($r = 0.382$). These findings demonstrated that the effect of Mn^{2+} concentration on lettuce yield was mainly through regulating root development instead of organic synthesis. Additionally, the results indicate that the most rapid growth occurred during the period from the 20th day to the 30th day, and the growth rates were all greater than 125% for the three treatments. Because it has been reported that restricted xylem loading leads to a delay in Mn^{2+} transport to the needles of nearly 2 weeks (Dučić et al. 2006), it is reasonable that the most obvious effect of increasing the Mn^{2+} concentration on the FW was found on the 30th day because of a transport lag of water, Mn^{2+}, and other elements. The significant difference in the DW response observed with the high Mn^{2+} concentration (treatment C3, 40 μM) compared to the low and middle concentrations indicates that a threshold of increased Mn^{2+} concentration exists that can improve organic matter accumulation in the edible parts of lettuce, and this threshold should be within the range of 10–40 μM under the same cultivation conditions used this study.

The lowest NO_3^--N content was observed on the 20th day in the three treatments, and a decrease in NO_3^--N content with increasing Mn^{2+} concentration was found before the 30th day (Fig. 3). No effect of Mn^{2+} concentration on NO_3^--N was found in the edible parts upon harvesting on the 30th day. These results are similar to Yang's report about effective control of nitrate content using a foliar spray

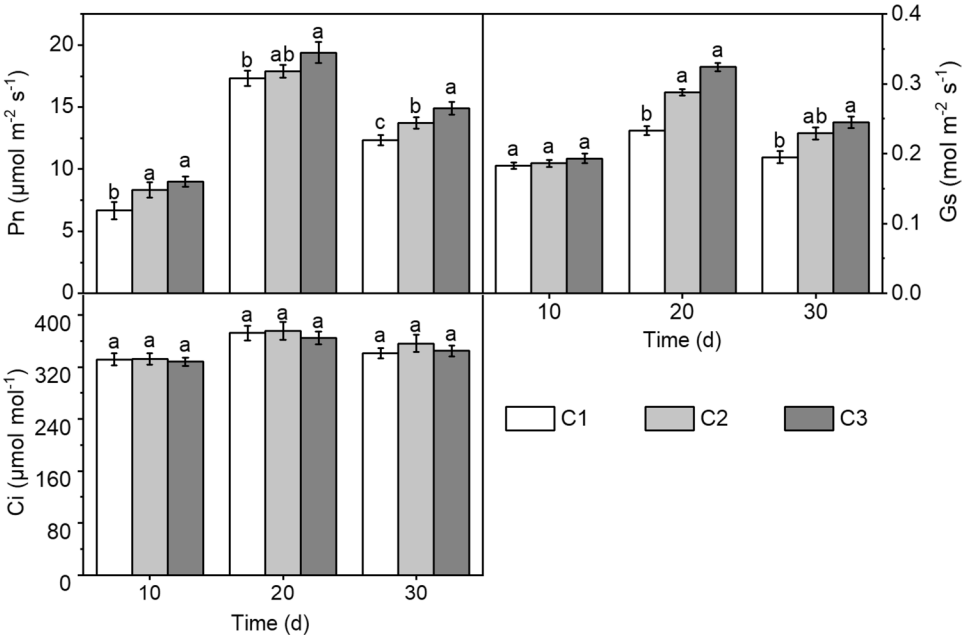

Fig. 5 Effects of manganese on photosynthesis in lettuce. Different letters indicate significant differences ($p < 0.05$) based on Duncan's multiple range test. Values represent means ± SE ($n = 3$) for individual plants

containing manganese on lettuce during the rapid growth stage (Yang et al. 2006). Therefore, our hypothesis that a high but not stressful Mn^{2+} concentration will decrease the NO_3^--N content in edible parts of lettuce is proved for 20-day-old plants, while this effect can not be observed for older plants. Moreover, the findings of this study reveal that it is feasible to harvest lettuce earlier than 30 days to guarantee a low NO_3^--N content. Considering both the yield and NO_3^--N content, a better choice would be to increase Mn^{2+} concentration to 40 μM and still harvest on the 30th day based on Figs. 2 and 3. In particular, the highest yield and a lower NO_3^--N content can be obtained when the Mn^{2+} concentration is increased to 40 μM and lettuce is harvested on the 30th day. However, the combination of 4 μM Mn^{2+} and harvesting on the 30th day is the best when the input and output of the system and the NO_3^--N content of lettuce are considered simultaneously. The above cultivation and harvesting suggestions are valid under the same conditions as this study.

In order to find out the variable closely related to NO_3^--N content of edible parts under different Mn^{2+} concentration, Pearson correlation analysis was also carried out on variables including NO_3^--N content, growth parameters, TN, PN, NR activity, and Pn. The results indicated that NO_3^--N content was controlled mainly through the influence of Mn^{2+} concentration on NR ($r = -0.899$, $p = 0.001$). This finding supports our hypothesis that high NR activity causes low NO_3^--N content under high but not excess Mn^{2+} treatment. However, the mechanism needs to be extensively investigated.

The NR activity increased with increasing Mn^{2+} concentration over the entire growth period. Significant variations of NR activity among different treatments gradually appeared with age (Fig. 4), which again shows that Mn^{2+} transport lags from the roots to the above-ground parts.

From the view of the direct effect, the absolute requirement of Mn^{2+} for many enzymes is related to nitrogen metabolism, such as allantoate amidohydrolase and arginase (Dabir et al. 2005; Werner et al. 2008). And allantoate amidohydrolase was found to be in charge of transporting N in soybean (Marschner 2013). Although an important role of Mn^{2+} in nitrate reductase was presumed because of the higher nitrate concentration in Mn-deficient leaves (Marschner 2013), there is no evidence that Mn plays a direct role in NR (Leidi and Gomes 1985). Wang (2013) suggested that Mn^{2+} acted as a co-factor of nitrate reductase and influenced nitrogen assimilation. This may be related to the fact that Mn^{2+} can readily displace Mg^{2+} to form Mn-ATP instead of Mg-ATP to disturb other reactions, including ammonia assimilation, because the normal functioning of Mg-ATP is energy transmission (Clarkson 1988). The studies found that Mn deficiency did not disrupt protein synthesis so that PN content of plants supplied with deficient Mn^{2+} was similar to or a little higher than that of plants supplied with adequate Mn^{2+} (Marschner 2013; Lerer 1976).

On the 30th day, the NR activity was increased with the Mn^{2+} concentration, but no significant differences in NO_3^--N content were found between any two treatments (Fig. 3). We assumed that NO_3^--N absorption was decreased because vegetables grow slowly. However, NO_3^--N transportation was increased from the roots to the above-ground parts in the later period. Then, the increased NO_3^--N, which was continuously transported to above-ground parts, was reduced and assimilated into organic nitrogen compounds by the increased NR activity as well as the other assimilation enzymes in the edible parts of lettuce. This finding is in accordance with the trends of TN and PN under different Mn^{2+} concentrations (Fig. 3). Although no remarkable differences in TN and PN were observed between treatment C1 (4 μM) and treatment C2 (10 μM), these values were obviously higher under treatment C3 (40 μM) than under the other two treatments. This indicates that a threshold of increasing Mn^{2+} concentration exists that can improve nitrogen assimilation in edible parts of lettuce, and this threshold should be within the range of 10 μM to 40 μM under the same cultivation conditions as this study.

A decreased Mn^{2+} concentration led to a lower Pn in lettuce. Figure 5 shows parallel decreases in the Pn and Ci when the Mn^{2+} concentration was decreased from 10 (treatment C2) to 4 μM (treatment C1). We calculated stomatal limitation values (Ls, Ls = Ca/Ci, where Ca is the CO_2 concentration in the air) and found that the Ls increased (data not shown) with decreasing the Ci and Pn. The relationship of Ci and Ls supports that the decrease in the Pn is somewhat affected by stomatal limitations when the Mn^{2+} concentration is less than 10 μM (Xu 1997; Pan et al. 2018). However, no significant differences in Ci were observed between any two treatments. In other words, the Mn^{2+} concentration had no obvious influence on Ci under the conditions of this study. This indicates that a variable Pn with increasing Mn^{2+} did not mainly depend on Ci. Mn has been reported to play an indispensable role in the oxygen-evolving complex (OEC) of photosystem II by influencing photosynthetic electron transport; thus, photosynthesis always is a target of Mn deficiency (Schmidt et al. 2016). These findings reveal that photosynthesis of lettuce is affected by Mn^{2+} concentrations in the safe and reasonable range mainly due to non-stomatal limitation. Therefore, our hypothesis that low but not deficient Mn^{2+} will decrease photosynthesis due to non-stomatal limitation is proven. However, this is different from Pan's result that the photosynthesis activity of *Xanthium strumarium* was inhibited by high levels of Mn stress (more than 1000 μM) due to the limitation of stomatal conductance (Pan et al. 2018). Therefore, the mechanism by which the Mn^{2+} concentration influences photosynthesis is related to its concentration.

5 Conclusions

Increasing the Mn^{2+} concentration within a safe range can improve the yield, root growth, and photosynthesis and decrease NO_3^--N content in the edible parts of lettuce in an intercropping aeroponics system containing lettuce and radish plants. The fact that increased Mn could increase NR activity is one reason why the NO_3^--N content in the above-ground parts was decreased. However, whether the yield, NO_3^--N content, or both is the focus of attention determines the optimal Mn^{2+} concentration and harvest time for cultivation.

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Author contribution All authors contribute equally in this paper.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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