An investigation on possible effect of leaching fractions physiological responses of hot pepper plants to irrigation water salinity

Rangjian Qiu¹, Chunwei Liu¹, Fusheng Li², Zhenchang Wang³, Zaiqiang Yang¹ and Ningbo Cui⁴*

Abstract

Background: The modification effect of leaching fraction (LF) on the physiological responses of plants to irrigation water salinity (ECiw) remains unknown. Here, leaf gas exchange, photosynthetic light–response and CO₂–response curves, and total carbon (C) and nitrogen (N) accumulation in hot pepper leaves were investigated under three ECiw levels (0.9, 4.7 and 7.0 dS m⁻¹) and two LFs treatments (0.17 and 0.29).

Results: Leaf stomatal conductance was more sensitive to ECiw than the net photosynthesis rate, leading to higher intrinsic water use efficiency (WUE) in higher ECiw, whereas the LF did not affect the intrinsic WUE. Carbon isotope discrimination was inhibited by ECiw, but was not affected by LF. ECiw reduced the carboxylation efficiency, photosynthetic capacity, photorespiration rate, apparent quantum yield of CO₂ and irradiance–saturated rate of gross photosynthesis; however, LF did not influence any of these responses. Total C and N accumulation in plants leaves was markedly increased with either decreasing ECiw or increasing LF.

Conclusions: The present study shows that higher ECiw depressed leaf gas exchange, photosynthesis capacity and total C and N accumulation in leaves, but enhanced intrinsic WUE. Somewhat surprisingly, higher LF did not affect the intrinsic WUE but enhanced the total C and N accumulation in leaves.

Keywords: Photosynthetic light–response curve, CO₂–response curve, δ¹⁵N, Δ¹³C, Photosynthetic capacity

Background

In many countries, the shortage of fresh water is a principal factor restricting the development of irrigated agriculture. The use of saline water is a possible alternative to meet the increased water demands for irrigation [1]. A prototypical case is the cultivation of pepper (Capsicum annuum L.), which is now one of the most widely grown crops in the world. In 2016, global pepper production (fresh and dry) from some 4 million ha was estimated at some 39 million tonnes, increasing by some 30% in the last decade [2]. Increasing demand for pepper is perhaps not surprising for high nutritional value of pepper. However, the total water requirement for pepper cultivation is by no means small ranging from 500 to 900 mm and up to 1250 mm in some areas [3]. In arid and semi-arid regions where much of the pepper cultivation occurs, fresh water resources are scarce necessitating the use of recycled (and often saline) water. In some areas, up to 1200–1400 mm of saline water with salinity levels ranging from 2.2 to 3.7 dS m⁻¹ have been successfully used to meet pepper water requirements [4]. Unsurprisingly, as with many other crops, irrigation with saline water can result in the accumulation of salt in the root zones, leading to the reduction in pepper growth and yield [5, 6]. Such reduction is the consequence of several physiological responses including lower CO₂ uptake, intercellular CO₂ concentration, and availability of intercellular CO₂ for carboxylation by decreasing stomatal conductance (gs), as well as the reduction in photosynthesis capacity, photosynthesis rate (Pn), and depression in both the photochemical and Calvin cycle reactions [7, 8]. To maintain the minimum salinity in the root zones and enhance crop growth, a considerable amount of water is needed to drain salinity when the field is irrigated with saline water [9]. Leaching fraction (LF) is the volume of drainage water passing through the

* Correspondence: cuiningbo@scu.edu.cn

¹State Key Laboratory of Hydraulics and Mountain River Engineering &
College of Water Resource and Hydropower, Sichuan University, Chengdu
610065, China

Full list of author information is available at the end of the article

© The Author(s). 2019 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
root-zones divided by the volume of irrigation water. Crop yield with saline water irrigation depends on plant evapotranspiration as well as soil salinity leaching [10]. Previous studies have focused on the effects of LF on root growth [11], root-zone salinity, evapotranspiration and yield [10, 12-14]. However, little information is available on the physiological response of hot pepper leaves to LF.

Intrinsic water use efficiency (WUE), defined as the ratio of $P_n$ to $g_s$ at leaf level, can explain instantaneous responses to environmental factors [15]. Intrinsic WUE can be enhanced either by lowering $g_s$ or by maintaining or enhancing the $P_n$ [16, 17]. As salinity stress simultaneously decreases $g_s$ and $P_n$, the intrinsic WUE varies under different salinity levels. Assessing the Brazilian pepper tree (Schinus terebinthifolius Raddi), Ewe and Sternberg (2005) [18] reported that the intrinsic WUE did not statistically differ among their salinity treatments, ranging from 0 to 21.4 dS m$^{-1}$. Likewise, Yarami and Sepaskhah (2015) [19] noted that the intrinsic WUE of saffron (Crocus sativus) was not affected when irrigation water salinity (EC$\text{iw}$) was lower than 3.0 dS m$^{-1}$. However, for some crop species, including water melon (Citrullus lanatus) [20], henna (Lawsonia inermis) [21] and plantain (Plantago coronopus) [22], high salinity improved the intrinsic WUE as the sensitivity of $g_s$ to salinity increased relative to $P_n$. Further investigation is therefore necessary to assess whether EC$\text{iw}$ and LF can affect intrinsic WUE for hot pepper.

Stable carbon isotope composition ($\delta^{13}$C), which is frequently expressed as carbon isotope discrimination ($\Delta^{13}$C), has been correlated with gas exchange responses in the plant growth cycle. $\delta^{13}$C in plants therefore provides a time-integrated measurement of intrinsic WUE to environmental stress, such as water and salinity stresses [16, 23]. Consequently, the variation of $\Delta^{13}$C has been suggested as an indicator of intrinsic WUE since there is a negative relationship between leaf $\Delta^{13}$C and intrinsic WUE [15, 24].

Crop nitrogen (N) is important for plant growth. The natural variation of the N isotope composition ($\delta^{15}$N) in plants under salinity stress is useful as it is related to N metabolism [23]. Isotope fractionation may occur during the N enzymatic assimilation of nitrate, recycling, translocation, exudation, or volatilization [25, 26]. Salinity-induced impacts on metabolism may cause a substantial change in the isotopic content of metabolites. For instance, increased salinity results in a significant reduction of $\delta^{15}$N in wheat shoots, which may result from reduction in the loss of ammonia and nitrous oxide [27]. Many studies have also shown that $\delta^{15}$N in plants can be used as an indicator to assess the mineralization rate of soil organic N [28]. Higher $\delta^{15}$N in plants indicates more N is absorbed from soil organic N pools than from inorganic mineral N. In addition, the uptake and assimilation of ammonium, plant growth and root length density or surface area may also affect plant N accumulation. Previous studies showed that increasing salinity leads to a reduction in the N content and total N accumulation [23, 27, 29, 30]. However, the modification effect of LF on the uptake of hot pepper N uptake to EC$\text{iw}$ remains unclear. In addition, the salinity-induced reduction in hot pepper N may affect C retention in the plant.

Therefore, the objectives of this study are (1) to analyze the response of photosynthetic capacity, intrinsic WUE and total C and N accumulation of hot pepper leaves exposed to different EC$\text{iw}$ treatments, and (2) to assess the modification effect of LF on leaf gas exchange, intrinsic WUE, and total C and N accumulation to EC$\text{iw}$.

### Results

#### Gas exchange, intrinsic WUE, photosynthetic light-response and CO$_2$-response curves

Higher EC$\text{iw}$ induced the lower $P_n$ and $g_s$. Compared to the EC$\text{iw}$ of 0.9 dS m$^{-1}$, the treatment with EC$\text{iw}$ of 7.0 dS m$^{-1}$ decreased $P_n$ by 37.7 and 60.5%, respectively, showing that $P_n$ declined slower than $g_s$, which led to a higher intrinsic WUE (i.e. $P_n / g_s$) with higher EC$\text{iw}$ (Table 1). Interestingly, high LF did not affect $P_n$ and $g_s$ significantly. As a consequence, the intrinsic WUE of the South American pepper tree, Schinus terebinthifolius Raddi, was improved by LF when EC$\text{iw}$ was lower than 3.0 dS m$^{-1}$, but not when EC$\text{iw}$ was 7.0 dS m$^{-1}$, showing that LF is a suitable method for increasing the intrinsic WUE of the plant.

| Factors | $P_n$ | $g_s$ | $C_i / C_a$ | Intrinsic WUE |
|---------|------|------|------------|--------------|
| EC$\text{iw}$ | | | | |
| 0.9 | 21.2a | 0.81a | 0.80a | 32.6c |
| 4.7 | 16.0b | 0.41b | 0.74b | 48.8b |
| 7.0 | 13.2c | 0.32c | 0.70c | 58.4a |
| LF | | | | |
| 0.17 | 16.9 | 0.51 | 0.75 | 45.5 |
| 0.29 | 17.5 | 0.56 | 0.75 | 45.4 |
| ANOVA | | | | |
| LF | NS | NS | NS | NS |
| EC$\text{iw}$ | *** | *** | *** | NS |
| LF × EC$\text{iw}$ | * | NS | * | * |

* and *** represent significant differences between means at 0.05 and 0.001 level of probability, respectively; NS, no significant. Different letters within a column indicate significant difference at $P<0.05$ by Duncan’s multiple range tests.
WUE had no statistical difference between the two LFs treatments (Table 1). There were significant relationships (i.e., a typical logarithmic correlation) between $P_n$ and $g_s$ under different EC $iw$ levels and LF treatments (Fig. 1a, b), showing that partial stomatal closure would result in an increase in intrinsic WUE [31]. A clear logarithmic decrease of intrinsic WUE with increasing $g_s$ was also found based on the pooled data from all treatments (Fig. 1c). Collectively, based on these results, it is suggested that EC $iw$ reduced $g_s$ more than $P_n$, resulting in an increase in intrinsic WUE; in contrast LF had no marked effect on $g_s$ and $P_n$, leading to an identical intrinsic WUE. ANCOVA analyses also show that the EC $iw \times g_s$ or LF $\times g_s$ interactions were not significant, indicating that the slopes of the regression lines between $P_n$ and $g_s$ under different levels of EC $iw$ and LFs were not significantly different. These results also further suggest that at a certain $g_s$, the differences in $P_n$ among the EC $iw$ or LF were consistent (Fig. 1).

The effects of EC $iw$ and LF on gas exchange were further investigated by measuring the photosynthetic light–response ($P_n$–PPFD) and CO$_2$–response ($P_n$–Ci) curves. Figure 2 shows the $P_n$–PPFD and $P_n$–Ci curves of hot pepper leaves under varying EC $iw$ and LF treatments. The photosynthetic characteristics including $\alpha$, $P_n$ max, $\kappa$ and $R_d$ derived from $P_n$–PPFD curve and $e$, $P_n$ sat, and $R_p$ derived from $P_n$–Ci curve are shown in the Table 2. There were no significant interactions between EC $iw$ and LF in terms of the parameters derived from the $P_n$–PPFD and $P_n$–Ci curves. $\kappa$ was also not influenced by EC $iw$ and LF, indicating $P_n$ increased identically to $P_n$ max as increasing PPFD. The identical $R_d$ under various levels of EC $iw$ and LFs indicate steady early symptom of carbon metabolism [32]. However, salinity–induced reductions in $P_n$ max, $\alpha$ and $P_n$ sat were observed in this study (Table 2).

In agreement with the prior analysis for $P_n$, $g_s$ and intrinsic WUE in this study, the improvement of carboxylation capacity, electron transport, $P_n$ max and $P_n$ sat in the higher LF were not observed on the $P_n$–PPFD and $P_n$–Ci curves (Fig. 2, Table 2), indicating that the higher LF treatment did not enhance $g_s$, which ultimately affected photosynthesis capacity and intrinsic WUE.

$\delta^{13}$C, $\delta^{15}$N and total C and N accumulation in leaves

Although no significant interaction between EC $iw$ and LF was found for the $\Delta^{13}$C of leaves, $\Delta^{13}$C decreased by 2.4 and 6.1% in the EC $iw$ treatments of 4.7 and 7.0 dS m$^{-1}$, respectively, when compared to the EC $iw$ of 0.9 dS m$^{-1}$ (Table 3). This suggests that higher EC $iw$ had greater stomatal closure. A significantly negative linear relationship between the $\Delta^{13}$C and electrical conductivity of soil saturated paste extract measured at the end of the experiment was observed regardless of the LF treatments (Fig. 3), indicating that soil salinity restricted CO$_2$ diffusion in $P_n$ [33]. A previous study has shown that salinity–induced reductions in $\Delta^{13}$C accompany decreases in $C_i / C_a$ [34]. In this study, the decline in $\Delta^{13}$C as EC $iw$ increased from 0.9 to 7.0 dS m$^{-1}$ corresponded to a reduction of $C_i / C_a$ from 0.8 to 0.7 (Table 1). In addition, a significant positive relationship between the $\Delta^{13}$C and $C_i / C_a$ between the LF treatments was also found ($R^2 = 0.92, n = 6, P < 0.01$). Partial stomatal closure or higher photosynthetic capacity or a combination of both could lead to a decrease in $C_i / C_a$.
In this study, a significantly positive relationship between $C_i/C_a$ and $g_s$ represents partial stomatal closure caused by salinity as a result of lower $C_i/C_a$ levels (Fig. 4, Table 1). Previous studies have shown that salinity markedly reduced the $\delta^{15}N$ in leaves of broccoli and barley plants [36, 37]. However, the $\delta^{15}N$ in leaves of hot pepper plants was not affected by EC$_{iw}$ (Table 3), indicating that the similar soil organic N mineralization and therefore the identical soil N bioavailability under different levels of EC$_{iw}$ [16]. However, total C and N accumulation in leaves decreased with increasing EC$_{iw}$ (Table 3).

It should be noteworthy that LF did not affect $\Delta^{13}C$ with values ranging from 22.87‰ to 23.09‰. Additionally, in accordance with similar $\Delta^{13}C$ values in two LF treatments, the $C_i/C_a$ was also identical for two LFs, which may attribute to similar stomatal opening and photosynthetic capacity as discussed earlier (Tables 1 and 2). Furthermore, LF also did not influence the $\delta^{15}N$ in leaves of hot pepper plants. However, higher LF enhanced total C and N accumulation in leaves (Table 3).

**Discussion**

Pepper is considered moderately sensitive to salinity (generally no yield loss when EC$_{iw}$ was lower than 1.5–2.0 dS m$^{-1}$ [14, 38]). Hence higher EC$_{iw}$ in this study markedly inhibited the $P_n$ and $g_s$, leading to a higher intrinsic WUE. In addition, a significant linear positive correlation between intrinsic WUE and EC$_{iw}$ was observed within the range of EC$_{iw}$ levels considered here regardless of LF treatments ($R^2 = 0.993$, $n = 6$, $P < 0.001$). However, additional data on more severe EC$_{iw}$ levels are necessary to assess the aforementioned correlation. For instance, when

---

**Fig. 2** Photosynthetic light–response (a) and CO$_2$–response curves (b) in the leaves of hot pepper plants under different levels of irrigation water salinity (EC$_{iw}$) and leaching fractions (LF) (the measurements were made at a CO$_2$ concentration of 400 μmol mol$^{-1}$ and at a PPFD of 1200 μmol mol$^{-1}$, respectively for light–response curves and CO$_2$–response curves). The three regression curves are made for the leaves of EC$_{iw}$ of 0.9, 4.7 and 7 dS m$^{-1}$, respectively, across the two LFs.

| Factors | $\alpha$ | $P_{n \text{ max}}$ | $\kappa$ | $R_i$ | $\epsilon$ | $P_{n \text{ sat}}$ | $R_{p}$ |
|---------|---------|----------------|--------|------|--------|----------------|------|
| EC$_{iw}$ |         |                 |        |      |        |                 |      |
| 0.9     | 0.052a  | 19.9a           | 0.53   | 2.07 | 0.224a | 61.0a          | 12.7a|
| 4.7     | 0.047a  | 15.7b           | 0.78   | 1.78 | 0.188a | 55.7a          | 11.2a|
| 7.0     | 0.048b  | 8.6c            | 0.83   | 1.43 | 0.109b | 42.8b          | 7.5b |
| LF      |         |                 |        |      |        |                 |      |
| 0.17    | 0.045   | 16.4            | 0.64   | 1.68 | 0.179  | 52.1           | 10.5 |
| 0.29    | 0.042   | 14.2            | 0.75   | 1.96 | 0.168  | 54.2           | 10.4 |
| ANOVA   |         |                 |        |      |        |                 |      |
| LF      | NS      | NS              | NS     | NS   | NS     | NS              | NS   |
| EC$_{iw}$ | * ***  | NS              | NS     | NS   | NS     | NS              | NS   |
| LF × EC$_{iw}$ | NS | NS              | NS     | NS   | NS     | NS              | NS   |

* * * and ** represent significant differences between means at 0.05, 0.01 and 0.001 level of probability, respectively; NS, no significant. Different letters within a column indicate significant difference at $P < 0.05$.
recalculating data in Table 4 from Chartzoulakis and Klapaki (2000) [6], only a small increase in intrinsic WUE was found when ECw higher than 12.6 dS m\(^{-1}\), showing that intrinsic WUE did not appreciably increase for the aforementioned correlation.

Salinity–induced reductions in \(P_{\text{nm}ax}\) and \(\alpha\) from \(P_n–PPFD\) curves were observed in this study, revealing a comparatively lower capacity of the biochemical reactions responsible for \(CO_2\) fixation and lower photochemical efficiency of photosystem in hot pepper leaves in higher ECw [39]. Similarly, \(P_{n-\text{sax}}\) derived from \(P_n–C_i\) curves also restricted in the ECw of 7.0 dS m\(^{-1}\) treatment as shown by the decline in the initial slope and the level of the upper plateau in the \(P_n–C_i\) curve (Fig. 2b) [40]. Brugnoli and Lauteri (1991) [41] observed similar results in bean and cotton plants, with the effect more marked in bean plants. A decline in carboxylation efficiency (\(\varepsilon\)) was a major component among those inhibiting \(P_n\) by mesophyll limitations in higher salinity (e.g. ECw of 7.0 dS m\(^{-1}\) in this study); this was likely produced by a reduction in enzyme activities in the carbon reduction cycle [42]. In addition, owing to the decreases in the \(CO_2/O_2\) ratio in the mesophyll, an increase in salinity may increase the rate of photorespiration (\(R_p\)) in \(C_3\) plants [8, 43]. However, analysis of the \(P_n–C_i\) curves of hot pepper leaves in this study suggested that \(R_p\) decreased significantly when ECw was higher than 4.7 dS m\(^{-1}\).
In this study, lower leaf dry biomass and C content (Table 2). Similar findings have also been reported in P. Similar factors indicates significant difference at *.* and *** represent significant differences between means at 0.05, 0.01 and 0.001 level of probability, respectively; NS, no significant. Different letters within a column indicate significant difference at P < 0.05 by Duncan’s multiple range tests.

Table 4 Dry biomasses of leaves and roots (g plant−1) and Na+ content (mg g−1 DW) in hot pepper leaves measured at the end of the experiment subjected to varying levels of irrigation water salinity (ECiw, dS m−1) and two leaching fractions (LF). Mean values were calculated from four replications.

| Factors | Dry biomass of leaves | Dry biomass of roots | Na+ content          |
|---------|-----------------------|----------------------|----------------------|
| ECiw    |                       |                      |                      |
| 0.9     | 14.3a                 | 5.5a                 | 2.73c                |
| 4.7     | 9.8b                  | 3.4b                 | 8.53b                |
| 7.0     | 7.0c                  | 2.5c                 | 12.60a               |
| LF      |                       |                      |                      |
| 0.17    | 9.6b                  | 3.6                  | 8.51a                |
| 0.29    | 11.1a                 | 4.1                  | 7.39b                |
| ANOVA   |                       |                      |                      |
| LF      | *                     | NS                   | *                    |
| ECiw    | ***                   | ***                  | ***                  |
| LF × ECiw | NS               | **                   | NS                   |

*, ** and *** represent significant differences between means at 0.05, 0.01 and 0.001 level of probability, respectively; NS, no significant. Different letters within a column indicate significant difference at P < 0.05 by Duncan’s multiple range tests.

(Table 2). Similar findings have also been reported in mallow [44] and mangrove [45] leaves based on the measurements of gas exchange. The enhanced PEPCase may account for the reduction in Rp [45], however further research is needed to explore the physiological mechanisms of reduced Rp within hot pepper leaves under high salinity levels.

It is well established that Δ13C analysis in leaf samples is one of the most versatile methodologies in assessing the environmental effects on the efficiency of photosynthesis in plants [32]. For instance, variation of Δ13C was found when plants were subjected to water and salinity stresses [33, 46], which was confirmed by salinity stress in this study. Variation in Δ13C relies not only on changes within C i / C a, but also the variation in intrinsic WUE [26]. This is confirmed by the negative correlation between the intrinsic WUE and Δ13C regardless of LF treatments in this study (R² = 0.92, n = 6, P < 0.01).

LF did not affect the gas exchange, photosynthesis capacity and hence intrinsic WUE, which further confirmed by the identical value of Δ13C. The possible reason is that no creditable soil salinity may leach from root zone in high LF in this study, as indicated by that the electrical conductivities of soil saturated paste extract measured at the end of the experiment were no more than 2.5 dS m−1 between two LFs, especially for lower salinity levels [47].

Higher ECiw induced lower total C accumulation in leaves (Table 3). A lower leaf biomass or a decreased C content in the biomass could retain less C in plant [48]. In this study, lower leaf dry biomass and C content might account for lower total C accumulation in leaves in the higher ECiw treatments (Tables 3 and 4). It is noteworthy that the reduction in leaf dry biomass in higher ECiw levels could result from lower Pn sat and limited root water uptake ability (Table 2). Root water uptake is mainly depended on soil’s matric and osmotic potentials [49, 50]. The salinity reduces the osmotic potential [51], causing the plant to spend more energy in taking up water from the soil solution, leading to a reduction in root water uptake [52, 53]. Salinity-induced reduction of root growth and excessive Na+ absorption also limited the root water uptake rate (Table 4).

As expected, high LF enhanced total C accumulation in leaves because of high leaf dry biomass and C content (Table 4), where the enhanced leaf dry biomass in high LF may result from the reduction in Na+ uptake and increased osmotic potential (Table 4). However, the reasons for the reduction in C content in higher ECiw and lower LF treatments remain unclear. Wang et al. (2010) [48] suggested that the C content in the plant is affected by the ability of C utilization in the plant. Plant N nutrition is one of the essential factors regulating C metabolism in plants because N is an important element for enzymes concerning metabolism, carbohydrate transport, and utilization in plants [54].

Based on literature surveys, at least four factors may determine plant N uptake from the soil. Firstly, the decreased leaf N accumulation in higher ECiw or lower LF could be attributed to a decrease in plant available N in the soil [28]. If this was the case, the δ15N in the high ECiw or low LF treatment should be low because the source of N taken up by plants could be reflected by variations in δ15N [55]. However, neither the ECiw nor LF affects δ15N in this study (Table 3). Alternatively, the reduced leaf N accumulation may result from the inhibited uptake and assimilation of ammonium as a result of competitive inhibition of Na+ [30]. We observed that the Na+ content in roots was greater in the higher ECiw and lower LF treatments (Table 4), which might imply that the uptake and assimilation of ammonium was restricted by higher Na+ in the higher ECiw and lower LF, and reduced leaf total N accumulation. Thirdly, the reduction in N accumulation in the higher ECiw treatment may result from the decrease in the root surface area for N uptake [28]. Even though the root length density or surface area was not investigated in this study, the root dry biomass declined with increasing ECiw or was not affected by LF (Table 4). This might indicate the lower root density in higher ECiw and similar root density between the two LF treatments. This implies that the lower root length density and root surface area in the higher ECiw might account for the reduction in leaf N accumulation. Lastly, plant N uptake is also affected by plant growth, as shown by significant positive linear
correlation between total N content and dry biomass of leaves, regardless of the LFs in this study ($R^2 = 0.98$, $n = 6$, $P < 0.001$), indicating leaf total N accumulation was in accordance with the dry biomass accumulation of leaves.

**Conclusions**

In summary, our results indicated that higher salinity impacted $g_s$ more than $P_n$, which resulted in higher intrinsic WUE. High salinity also inhibited photosynthesis capacity and retained less C and N in leaves. The novelty of this study is that we found higher LF did not improve leaf gas exchange, photosynthesis capacity and intrinsic WUE. However, higher LF did enhanced C and N accumulation in leaves of hot pepper plants.

**Methods**

**Experimental design**

The experiment was conducted under a rain shelter from April 28 to July 22, 2015 at the Agro-Meteorology Research Station located in Nanjing, Jiangsu, Eastern China (32.2°N, 118.7°E, altitude 14.4 m). Plastic pots (top diameter 27 cm, bottom diameter 26 cm, and height 22 cm) with holes in the bottom were used. Each pot was filled with 11 kg of air-dried soil (sandy loam, with sand = 75.7%, silt = 20.4% and clay = 3.9%) sieved with a 5-mm sieve. The bulk density of soil was 1.47 g cm$^{-3}$, field water capacity was 0.27 cm$^3$ cm$^{-3}$ and wilting point was 0.04 cm$^3$ cm$^{-3}$. The electrical conductivity of soil (paste) was 0.59 dS m$^{-1}$, and the pH was 7.4.

One hot pepper plant, *Capsicum annuum* L., Bocuiwang cultivar, purchased from Jingshiyuan Co. Ltd., China, was transplanted into each pot on April 28, 2015. All the pots were saturated with tap water before the transplanting. Five days after the transplanting, each plant was irrigated using tap water with an irrigation amount of 0.9 L pot$^{-1}$ (all pots observed drainage). Five days after this irrigation event, three different saline water treatments were initiated for two LF treatments. The three EC$_{iw}$ levels assessed were 0.9, 4.7 and 7.0 dS m$^{-1}$ and the two LFs treatments were 0.17 and 0.29 according to the method proposed by Letey et al. (2011) [1].

Leaf gas exchange, $\delta^{13}$C and $\delta^{15}$N of hot pepper leaves and Na$^+$ content in roots

Leaf gas exchange parameters, including $P_n$, $g_s$, $P_{n}$, and $g_{s}$, were measured at 9:00–11:00 am on three sunny days (i.e. 23, 39, and 76 days after transplanting) using a portable photosynthesis system with a red–blue light source (LI

| EC$_{iw}$ (dS m$^{-1}$) | SAR (mmol L$^{-1}$) | Cation (mmol L$^{-1}$) | Anion (mmol L$^{-1}$) |
|------------------------|---------------------|------------------------|------------------------|
|                        | Na$^+$              | Ca$^{2+}$              | K$^+$                  | Mg$^{2+}$              | NH$_4^+$              | Cl$^-$              | NO$_3^-$              | SO$_4^{2-}$             | H$_2$PO$_4^-$ |
| 0.9                    | 0.0                 | 0                      | 4                      | 225                    | 1                      | 0.5                  | 0                      | 6.5                    | 1                      | 0.25                  |
| 4.7                    | 5.4                 | 17                     | 21                     | 225                    | 1                      | 0.5                  | 34                     | 6.5                    | 1                      | 0.25                  |
| 7.0                    | 7.3                 | 29                     | 33                     | 225                    | 1                      | 0.5                  | 58                     | 6.5                    | 1                      | 0.25                  |

The electrical conductivity of soil (paste) was 0.59 dS m$^{-1}$, and the pH was 7.4.

At each irrigation event, the plants were irrigated with 120 and 140% of ET for each EC$_{iw}$ treatment, which lead to an LF of 0.17 and 0.29 according to the method proposed by Letey et al. (2011) [1]:

$$\frac{AW}{ET} = \frac{1}{1-LF}$$

Therefore a different amount of water based on actual ET for each pot was applied to maintain the target LF. At the end of the experiment, the average actual LF based on the amount of seasonal drainage water and applied water was 0.17 and 0.27, respectively [47], showing that the amount of applied irrigation water is reasonable.

The drainage water of individual pots was collected with a glass bottle positioned beneath each pot, and the amount was collected after each irrigation event. Just before each irrigation event, each pot was weighed with an electronic scale of 20 kg with an accuracy of 0.1 g, afterwards the evapotranspiration and irrigation amounts were calculated. During the experimental period, the plants were irrigated every two to five days and a total of 24 irrigations were applied.

Leaf gas exchange, $\delta^{13}$C and $\delta^{15}$N of hot pepper leaves and Na$^+$ content in roots

Leaf gas exchange parameters, including $P_n$, $g_s$, $P_{n}$, and $g_{s}$, were measured at 9:00–11:00 am on three sunny days (i.e. 23, 39, and 76 days after transplanting) using a portable photosynthesis system with a red–blue light source (LI
6400, LI–COR, Lincoln, NE, USA). Three to six fully grown leaves per treatment were measured with a fixed PPFD level of 1200 μmol m⁻² s⁻¹. The intercellular to ambient CO₂ concentration ratio (Cᵢ / Cₐ) were also obtained from the gas exchange measurements. As noted earlier, intrinsic WUE is defined as the ratio of Pₙ to gs.

The plants were harvested on July 22, 2015. The biomass of the leaves were dried in an oven at 70 °C for 72 h to obtain constant weight. Dry leaf samples were ground and used for δ¹³C and δ¹⁵N measurements. The values of δ¹³C and δ¹⁵N as well as the total C and N content in the leaves were measured using a MAT253 Stable Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, USA). The δ¹³C in leaf dry biomass can be calculated as:

\[
\delta^{13}C = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

where R_{sample} and R_{standard} are the ¹³C/¹²C ratio of the sample and PDB (Pee Dee Belemnite) standard, respectively.

The δ¹⁵N in the leaf biomass is calculated as:

\[
\delta^{15}N = \left( \frac{R_s}{R_b} - 1 \right) \times 1000
\]

where Rₛ and Rₜ are the N¹⁵ (N¹⁴ + N¹⁵) ratios of the leaf sample to standard, respectively. Δ¹³C in leaf dry biomass can be calculated as:

\[
\Delta^{13}C = \frac{\delta_a - \delta_p}{1 + \delta_p}
\]

where δ_a and δ_p are the carbon isotope composition of source air and plant material, respectively. The δ_a was taken as ~8% [34].

The roots of each plant were washed with fresh water, and dried in an oven at 70 °C to obtain constant weight. The dried roots were then ground into a powder, broken down with concentrated HNO₃ that was warmed with a heating block, and finally dissolved in 5% (v/v) high-purity HNO₃. The sodium ion (Na⁺) content in the dry roots was determined using an Inductively Coupled Plasma–Optical Emission Spectrometry (ICP–OES, Perkin Elmer Optima 8000). The electrical conductivity of soil saturated paste extract was determined at the end of the experiment by a dual channel pH/mV/Ion/Conductivity benchtop meter (MP522, Shanghai San–Xin Instrumentation Inc., China).

The Pₙ–PPFD and Pₙ–Cᵢ curves

The Pₙ–PPFD and Pₙ–Cᵢ curves for different levels of ECᵢw and LFs were determined using a LI–6400 photosynthesis system (LI–COR, Lincoln, NE, USA). The Pₙ–PPFD curves were measured at a fixed CO₂ concentration of 400 μmol mol⁻¹ on 2–4 plants per treatment. Measurements were made at PPFD levels of 2000, 1500, 1000, 700, 400, 200, 100, 50, 20 and 0 μmol m⁻² s⁻¹. The non-rectangular hyperbola model was used to simulate Pₙ–PPFD curve [58]:

\[
P_n = \frac{\alpha Q + P_n \text{ max} - \sqrt{(\alpha Q + P_n \text{ max})^2 - 4\alpha QP_n \text{ max}}}{2\kappa} - R_d
\]

where Pₙ is the rate of net photosynthesis (μmol CO₂ m⁻² s⁻¹); Q is the PPFD (μmol m⁻² s⁻¹); Pₙ max is the irradiance–saturated rate of gross photosynthesis (μmol CO₂ m⁻² s⁻¹); R_d is the dark respiration rate (μmol CO₂ m⁻² s⁻¹) at Q = 0; α is the maximum apparent quantum yield of CO₂ (mol CO₂ mol⁻¹ photons); and κ is a dimensionless convexity term [0, 1].

Measurements of Pₙ–Cᵢ curves were made at CO₂ levels of 400, 250, 150, 100, 50, 500, 700, 1000 and 1500 μmol mol⁻¹ at a fixed PPFD of 1200 μmol m⁻² s⁻¹. The Pₙ were plotted against the respective Cᵢ. A non-rectangular hyperbola curve was used to simulate Pₙ–Cᵢ curve [59, 60]:

\[
P_n = \frac{\epsilon P_n \text{ sat} C_i}{\epsilon C_i + P_n \text{ sat} - R_p}
\]

where ε is carboxylation efficiency (mol m⁻² s⁻¹); Pₙ sat is the photosynthetic capacity (μmol CO₂ m⁻² s⁻¹); and Rₚ is the rate of photorespiration (μmol CO₂ m⁻² s⁻¹).

Statistical analysis

Two-way analysis of variation using the general linear model-univariate procedure was performed to assess the effects of the ECᵢw and LF on gas exchange parameters, intrinsic WUE, Δ¹³C, δ¹⁵N, C content and total C and N accumulation, dry biomass of leaves and roots, Na⁺ content, the parameters obtained from the Pₙ–PPFD and Pₙ–Cᵢ curves. All analyses were conducted in the SPSS software package (Version 21.0, IBM Corp., Armonk, NY). Correlations between the measured parameters were determined with regression analyses. The slopes of the relationships between Pₙ and gₛ under different ECᵢw levels and LFs were tested by a standard analysis of covariance (ANCOVA). Pₙ was analyzed through a General Linear Model (GLM) of the natural logarithm of gₛ. The ECᵢw (or LF) and the interaction with the linear predictor were included to test for differences in slope. If there was no significant interaction between ECᵢw (or LF) and linear predictor, the slopes were assumed to be the same.

Abbreviations

C: Carbon; Cᵢ / Cₐ: Intercellular to ambient CO₂ concentration ratio; ECᵢw: Irrigation water salinity; gₛ: Stomatal conductance; intrinsic WUE: Intrinsic water use efficiency; LF: Leaching fraction; N: Nitrogen;
Na⁺: Sodium ion; \( P_{\text{max}} \): Irradiance--saturated rate of gross photosynthesis; \( P_{\text{sat}} \): Photonsynthetic capacity; \( R_{D} \): Net photosynthesis rate; \( R_{o} \): Dark respiration rate; \( R_{PH} \): Rate of photorespiration; \( \alpha \): Maximum apparent quantum yield of \( \text{CO}_2 \); \( \delta^{13}\text{C} \): 13C isotope composition; \( \Delta^{13}\text{C} \): Carbon isotope discrimination; \( \delta^{15}\text{N} \): 15N isotope composition; \( \epsilon \): Carboxylation efficiency; \( \kappa \): A dimensionless convexity term

Acknowledgements
We are grateful to Jinqin Xu, Jun Chen, Shanshan Cheng, Xu Liu and Hongzhou Chen for the assistance of the experiment, and to three anonymous reviewers for valuable comments.

Authors’ contributions
R. Q. conducted most of the experiment, analyzed the data and wrote the manuscript. C. L., Z. W., Z. Y. and N. C. analyzed the data and improved the manuscript. F. L. provided important advice and improved the standard of the manuscript. All authors discussed the results and approved the final version of the manuscript.

Funding
We are grateful for the research grants from the National Natural Science Foundation of China (51509130, 41475107, 51779161), the Natural Science Foundation of Jiangsu Year Plan (2014BAD10B07) and the Priority Academic Program Development of Jiangsu – Foundation of China (51509130, 41475107, 51779161), the Natural Science We are grateful for the research grants from the National Natural Science and the manuscript.

Availability of data and materials
The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. The matlab program fitting \( P_{NPFD} \) and \( P_{NN} \) curves using aforementioned methods in this study were shared freely in https://github.com/shuhlbithe/lab-repositories.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Collaborative Innovation Center on Forecast and Evaluation of Meteorological Disasters, Jiangsu Key Laboratory of Agricultural Meteorology, Nanjing University of Information Science and Technology, Nanjing 210044, China. 2College of Agriculture, Guangxi University, Nanning 530005, Guangxi, China. 3College of Water Conservancy and Hydropower Engineering, Hohai University, Nanjing 210098, China. 4State Key Laboratory of Hydraulics and Mountain River Engineering & College of Water Resource and Hydropower, Sichuan University, Chengdu 610065, China.

Received: 9 October 2018  Accepted: 27 June 2019
Published online: 08 July 2019

References
1. Letey J, Hoffman GJ, Hopmans JW, Grattan SR, Suarez D, Conwin DL, Oster JD, Wu L, Amrhein C. Evaluation of soil salinity leaching requirement guidelines. Agr Water Manage. 2011;98:502–6.
2. FAO-STATISTICS. Food and Agricultural Organization (online report): FAOSTAT Section, http://www.fao.org/faostat/en/data/QC/visualize, accessed: 2018-05-09, 2018.
3. FAO. Food and Agricultural Organization (online report): Land and Water Section, http://www.fao.org/land-water/databases-and-software/crop-information/pepper, accessed: 2018-05-09, 2018.
4. Ben-Gal A, Ityel E, Dudley L, Cohen S, Yermiyahu U, Presnov E, Zigmond L, Shani U. Effect of irrigation water salinity on transpiration and on leaching requirements: a case study for bell peppers. Agr Water Manage. 2008;95:587–97.
5. Qiu RL, Jing YS, Liu CW, Yang ZQ, Wang ZC. Response of hot pepper yield, fruit quality, and fruit ion content to irrigation water salinity and leaching fractions. HortScience. 2017;52:979–85.
6. Chartoulakis K, Kapaki G. Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Sci Hortic-Amsterdam. 2000;86:247–60.
7. Neto MCL, Lobo AK, Martins MO, Fontenele AV, Silveira JAG. Dissipation of excess photosynthetic energy contributes to salinity tolerance: a comparative study of salt-tolerant Ricinus communis and salt-sensitive Jatropha curcas. J Plant Physiol. 2014;171:29–30.
8. Hossain MS, Dietz K. Tuning of redox regulatory mechanisms, reactive oxygen species and redox homeostasis under salinity stress. Front Plant Sci. 2016;7:548.
9. Aktas H, Abak K, Cakmak I. Genotypic variation in the response of pepper to salinity. Sci Hortic-Amsterdam. 2006;109:260–6.
10. Ben-Gal A, Beiersdorff I, Yermiyahu U, Soda N, Presnov E, Zoupon I, Crisostomo RR, Dag A. Response of young bearing olive trees to irrigation-induced salinity. Irrig Sci. 2017;35:90–109.
11. Soda N, Epaphridi AE, Pag A, Beiersdorff I, Presnov E, Yermiyahu U, Ben-Gal A. Root growth dynamics of olive (Olea europaea L.) affected by irrigation induced salinity. Plant Soil. 2017;414:305–18.
12. Heakal MS, Modahlsh AS, Mathiady AS, Metwally AI. Combined effects of leaching fraction, salinity, and potassium content of waters on growth and water-use efficiency of wheat and barley. Plant Soil. 1990;125:177–84.
13. Heidarpour M, Mostafaezadeh Fard B, Arzani A, Aghakhani A, Feizi M. Effects of irrigation water salinity and leaching fraction on yield and evapotranspiration in spring wheat. Commun Soil Sci Plan. 2009;40:2521–35.
14. Semz G, Suarez DL, Unlikar A, Yurtsen E. Interactive effects of salinity and N on pepper (Capsicum annuum L) yield, water use efficiency and root zone and drainage salinity. J Plant Nutr. 2014;37:595–610.
15. Cil N, Du T, Kang S, Li F, Hu X, Wang M, Li Z. Relationship between stable carbon isotope discrimination and water use efficiency under regulated deficit irrigation of pear-jujube tree. Agr Water Manage. 2009;96:1615–22.
16. Wang YS, Janz B, Engedal T, de Neergaard A. Effect of irrigation regimes and nitrogen rates on water use efficiency and nitrogen uptake in maize. Agr Water Manage. 2017;179:271–6.
17. Gilbert ME, Zwienericka MA, Holbrook NM. Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. J Exp Bot. 2011;62:2875–87.
18. Ewe SM, Sternberg LDSL. Growth and gas exchange responses of Brazilian pepper (Schinus terebinthifolius) and native South America species to salinity. Trees. 2005;19:119–28.
19. Yaram N, Separkhah AR. Physiological growth and gas exchange response of saffron (Crocus sativus L.) to irrigation water salinity, manure application and planting method. Agr Water Manage. 2015;154:443–51.
20. Colla G, Roupahel Y, Cardarelli M, Rea E. Effect of salinity on yield, fruit quality, leaf gas exchange, and mineral composition of grafted watermelon plants. Hortscience. 2006;41:622–7.
21. Fernández-García N, Olmos E, Bardisi E, García-De La Garma J, López-Berenguer C, Rubio-Aneas JS. Intrinsic water use efficiency controls the adaptation to high salinity in a semi-arid adapted plant, henna (Lavsonia inermis L.). J Plant Physiol. 2014;171:64–75.
22. Koyro H. Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte Plantago coronopus (L.) Environ Exp Bot. 2006;56:136–46.
23. Youssi S, Serret MD, Márquez AI, Valtos J, Araus JL. Combined use of \( \delta^{13}\text{C} \), \( \delta^{16}\text{O} \) and \( \delta^{15}\text{N} \) tracks nitrogen metabolism and genotypic adaptation of durum wheat to salinity and water deficit. New Phytol. 2012;194:320–44.
24. Wei ZH, Du TS, Zhang J, Xu SJ, Cambre PJ, Davies WJ. Carbon isotope discrimination shows a higher water use efficiency under alternate partial root-zone irrigation of field-grown tomato. Agr Water Manage. 2016;165:33–43.
25. Tcharkhov G. \( \delta^{11}\text{N} \) NaCl isotope composition in C3 leaves: are enzymatic isotope effects informative for predicting the \( \delta^{15}\text{N} \)-abundance in key metabolites? Funct Plant Biol. 2011;38:1–12.
26. Youssi S, Serret MD, Araus JL. Comparative response of 6\(^{13}\text{C} \), 6\(^{16}\text{O} \) and \( \delta^{15}\text{N} \) in durum wheat exposed to salinity at the vegetative and reproductive stages. Plant Cell Environ. 2013;36:1214–27.
27. Yourell S, Serret MD, Voltas J, Araus JL. Effect of salinity and water stress during the reproductive stage on growth, ion concentrations, Δ13C, and δ15N of durum wheat and related amphloids. J Exp Bot. 2010;61:3529–42.
28. Wang ZC, Liu FL, Kang SZ, Jensen CR. Alternate partial root-zone drying irrigation improves nitrogen nutrition in maize (Zea mays L.) leaves. Environ Exp Bot. 2012;75:36–40.
29. Shaheen R, Hood Nowotny RC. Carbon isotope discrimination: potential for screening salinity tolerance in rice at the seedling stage using hydroponics. Plant Breed. 2005;124:220–4.
30. Jampeetong A, Bri x H. Effects of NaCl salinity on growth, morphology, photosynthesis and proline accumulation of Salvinia natans. Aquat Bot. 2009;91:181–6.
31. Liu F, Shahnazari A, Andersen MN, Jacobsen S, Jensen CR. Physiological responses of potato (Solanum tuberosum L) to partial root-zone drying: ABA signalling, leaf gas exchange, and water use efficiency. J Exp Bot. 2006;57:3727–35.
32. Wang ZC, Kang SZ, Jensen CR, Liu FL. Alternate partial root-zone irrigation reduces bundle-sheath cell leakage to CO2 and enhances photosynthetic capacity in maize leaves. J Exp Bot. 2011;63:1145–53.
33. Choi W, Ro H, Chang SX. Carbon isotope composition of Phragmites australis in a constructed saline wetland. Aquat Bot. 2005;82:27–38.
34. Farquhar GD, Ehleringer JR, Hubick KT. Carbon isotope discrimination and photosynthesis. Annu Rev Plant Biol. 1989;40:503–30.
35. Condon AG, Richards RA, Rebetzke GJ, Farquhar GD. Improving intrinsic photosynthetic capacity in three mangrove species, Aegiceras corniculatum, to long term salinity and humidity conditions. Plant Physiol. 1984;74:1–6.
36. Amor FMD, Cuadra-Crespo P. Alleviation of salinity stress in broccoli using foliar urea or methyl-jasmonate: analysis of growth, gas exchange, and isotope composition. Plant Growth Regul. 2011;63:55–62.
37. Pérez-López U, Robredo A, Miranda-Apodaca J, Lacuesta M, Muñoz-Rueda A, Mena-Petite A. Carbon dioxide enrichment moderates salinity-induced effects on nitrogen acquisition and assimilation and their impact on growth in barley plants. Environ Exp Bot. 2013;97:148–58.
38. Maas EV, Hoffman GJ. Crop salt tolerance-current assessment. J Irrig Drain Div. 1977;103:115–34.
39. Suárez N, Medina E. Influence of salinity on Na+ and K+ accumulation, and photosynthesis of Phragmites australis L. in cultures of different NaCl concentration. J Experimental Botany. 2006;57:3727–35.
40. Farquhar GD, Ehleringer JR, Hubick KT. Carbon isotope discrimination and photosynthesis. Annu Rev Plant Biol. 1989;40:503–30.
41. Condon AG, Richards RA, Rebetzke GJ, Farquhar GD. Improving intrinsic water-use efficiency and crop yield. Crop Sci. 2002;42:121–32.
42. Brugnoli E, Lauteri M. Effects of salinity on stomatal conductance, photosynthetic capacity, and carbon isotope discrimination of salt-tolerant mangrove species, Avicennia marina, to long term salinity and humidity conditions. Plant Physiol. 1984;74:1–6.
43. Amor FMD, Cuadra-Crespo P. Alleviation of salinity stress in broccoli using foliar urea or methyl-jasmonate: analysis of growth, gas exchange, and isotope composition. Plant Growth Regul. 2011;63:55–62.
44. Everard JD, Gucci R, Kann SC, Flore JA, Loescher WH. Gas exchange and compartment-specific differences in the regulation of cysteine synthesis. J Exp Bot. 2000;51:231–42.
45. Wei L. Carbon stable isotope composition in three mangrove species under salinity. Xiamen: Xiamen University; 2007.
46. Centritto M, Lauteri M, Monteverdi MC, Serraj R. Leaf gas exchange, carbon isotope discrimination, and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. J Exp Bot. 2006;57:3727–40.
47. Qiu RJ, Liu CW, Wang ZC, Yang ZQ, Ling JY. Effects of irrigation water salinity on evapotranspiration modified by leaching fractions in hot pepper plants. Sci Rep. 2011;7:7231.
48. Wang YS, Liu FL, Andersen MN, Jensen CR. Carbon retention in the soil-plant system under different irrigation regimes. Agr Water Manage. 2010;98:419–24.
49. Bhatn a P, Lazarovitch N. Evapotranspiration, crop coefficient and growth of two young pomegranate (Punica granatum L.) varieties under salt stress. Agr Water Manage. 2010;97:715–22.
50. Hopmans JW, Bristow KL. Current capabilities and future needs of root water and nutrient uptake modeling. Adv Agron. 2002;77:103–83.
51. Corwin DL, Rhoades JD, Simúněk J. Leaching requirement for soil salinity control: steady-state versus transient models. Agr Water Manage. 2009;50:165–80.