Distinct impacts of air and root-zone temperatures on leaf and root features of cucumber seedlings: resource acquisition capacity, organ size and carbon-nitrogen balance

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Short Title: Air and root-zone temperatures influence carbon-nitrogen balance

Estimated length of the Manuscript: 5600 words

Figures 1-7, Tables 1-4, Supplementary Figures S1, Supplementary Table S1-S3

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Keywords: root-zone temperature, air temperature, carbon-nitrogen balance, biomass allocation, cucumber seedling, plant phenotypic plasticity, plant growth analysis.

ABSTRACT

Both low air (T_{air}) and root-zone (T_{root}) temperatures can inhibit resource (e.g. carbon and nutrients) acquisition by leaves and roots through various aspects, such as morphology, biomass allocation and assimilation/absorption capacity. However, it is still ambiguous whether T_{air} and T_{root} influence carbon (C) and nutrient acquisition via the same approach. To this end, in this study, cucumber (*Cucumis sativus* L.) seedlings were hydroponically grown under treatments arranged in complete factorial combination of two levels of T_{air} (26/18°C and 20/12°C, day/night) and two levels of T_{root} (19°C and 13°C, constant). In general, both T_{air} and T_{root} affected leaf and root sizes mainly by regulating their morphology rather than biomass investment. Under low T_{air} conditions (20/18°C), elevated T_{root} (compare 19°C versus 13°C) did not influence C acquisition, but increased nitrogen (N) acquisition mainly due to an increase in relative root length, resulting in decreased C : N acquisition ratio. However, under low T_{root} conditions (13°C), elevated T_{air} (compare 26/18°C versus 20/12°C) enhanced both C and N acquisition mainly because of an increase of both C assimilation in leaves and N absorption by roots, resulting in relatively constant C : N acquisition ratio. In addition, the T_{air} and T_{root} interaction was mainly observed in relative growth rate and root growth-related variables. Our results infer that T_{air} and T_{root} have distinct impacts on resource acquisition and carbon-nitrogen balance in plants.

INTRODUCTION

Low temperature stress is a commonly encountered problem for plants in most temperate or high-altitude regions during cool-season cultivation. Low temperature may inhibit plant growth in a complex manner. Primarily, it limits the size of leaves and roots per unit plant biomass (leaf area ratio, LAR, and root length ratio, RLR) as an integrated result of altered biomass fraction (leaf mass fraction, LMF, and root mass fraction, RMF) and morphological characteristics (leaf area per unit leaf mass, i.e. specific leaf area, SLA, and root length per unit root mass, i.e. specific root length, SRL) (Tachibana, 1982; Weih and Karlsson, 2001). In addition, it decreases the capacity of resource acquisition per unit size of leaves and roots (Clarkson et al., 1986; Delucia et al., 1992). These two aspects together inhibit the access to resources, and thus retard the relative growth rate (RGR) of plants (Loveys et al., 2002).

Knowledge about the relative contributions of various plant components to RGR may help us better predict plant responses to environmental variation and then pursue the right temperature control strategy. Previous researches (Loveys et al., 2002; Poorter et al., 2012) suggest that, for the above-ground part of plants, SLA usually plays a more flexible and important role than LMF in determining LAR, while net assimilation rate (NAR, increase in plant mass per unit leaf area and time) is more important than SLA in determining RGR at cool temperatures. However, such an analysis has not yet been carried out for the below-ground part of plants. Therefore, it is still unclear what the relative contributions are of SRL and RMF to the root length, and whether root absorption activity and root length contribute differently to nutrient acquisition, when plants face low temperature stress. A recent report by Freschet et al. (2015b) suggests that the size ratio of roots to leaves increases as nutrient limitation aggravates, and that RMF contributes more to RLR variation than SRL. It seems that RMF is more important than SRL in determining the root length at cool temperatures, because nutrient limitation can also be aggravated by reducing root-zone temperature. However, despite increased size ratio of roots to leaves, the relative size of roots is generally decreased at cool temperatures (Larigauderie et al., 1991), inferring that RMF and SRL may contribute in different ways to RLR.

Although many studies take temperature as a homogeneous whole, the spatial heterogeneity of temperature (e.g. air and root-zone temperatures) extensively exists either in natural environments (Deanedrummond and Glass, 1983; Walter et al., 2009) or under cultivation conditions (Gosselin and Trudel, 1985; Teitel et al., 1999; Kawasaki et al., 2014). It is well-known that air temperature (T_{air}) is crucial for plant growth. Over the past decades, an increasing number of studies have shed light on the important role played by root-zone temperature (T_{root}) in plant growth (Tachibana, 1987; Ahn et al., 1999; Murai-Hatano et al., 2008; Nagel et al., 2009; Poiré et al., 2010). However, very few studies have attempted to compare the difference between T_{air} and T_{root} by independently and separately changing each type of temperature in one experiment. Therefore, it is still unclear how T_{air} and T_{root} separately affect the relative contributions of various plant components to RGR. Weih and Karlsson (2001) have pointed out that T_{air} and T_{root} have interactive effects on RGR, N productivity (the rate at which dry matter is produced per unit of N in plant biomass per unit of time) and leaf-N content. It means that plant response to root-zone cooling at optimal T_{air} can not be simply predicted as a reverse of response to root-zone warming at low T_{air} . Thus, it is needed to apply a complete factorial design to distinguish the different roles of T_{air} and T_{root} in plant growth.

Low temperature can limit resource (e.g. C and nutrients) acquisition by plants. Either T_{air} or T_{root} limitation alone may lead to unequal accessibilities of above- and below-ground parts to resources. Nevertheless, to maximize growth with minimum resource costs, plants generally tend to balance above- and below-ground resource acquisition capacities to achieve the status of C-nutrient

colimitation (Ryser and Eek, 2000; Maire et al., 2013). This mechanism is known as the ‘balanced growth’, ‘optimal partitioning’ or ‘functional equilibrium’ hypothesis (Brouwer, 1963; Davidson, 1969; Shipley and Meziane, 2002), which can be formalized as follows:

$$\text{NAR} \times \text{LMF} \times \text{SLA} \propto \text{SAR} \times \text{RMF} \times \text{SRL}$$

where NAR is plant C net assimilation rate (per unit time and leaf area), and SAR is plant specific nutrient absorption rate (per unit time and root length) (Freschet et al., 2015a). This equation can be further transformed into:

$$\frac{\text{NAR}}{\text{SAR}} \propto \frac{\text{RMF}}{\text{LMF}} \times \frac{\text{SRL}}{\text{SLA}}$$

$$\frac{\text{NAR}}{\text{SAR}} \propto \frac{\text{RLR}}{\text{LAR}}$$

Carbon-nutrient balance may be achieved through various strategies such as maintaining relatively smaller root size but higher SAR, or maintaining relatively constant size/activity ratio of leaves to roots through proportionately increasing NAR and SAR. For instance, in the study of Engels et al. (1992), nutrient uptake by roots is stimulated by increased temperature of maize shoot base (apical shoot meristem and zone of leaf extension) via raising shoot growth, even under low T_{root} condition. Getting to know what kind of strategies plants choose can help us better understand the mechanisms of integrated plant responses to temperature limitation.

In this study, we conducted a complete factorial experiment, with two levels of T_{air} (high and low) and two levels of T_{root} (high and low), to investigate the independent and interactive effects of T_{air} and T_{root} on leaf and root growth, and carbon and nutrient assimilation in cucumber (*Cucumis sativus* L.) seedlings. The objectives were to examine (1) how T_{air} and T_{root} affect relative contributions of various plant components to plant growth and resource acquisition, and (2) whether T_{air} has different effects on plant carbon-nutrient balance compared to T_{root} . Cucumber was chosen as a model plant because its growing point locates above the ground surface, which favors separate control of T_{air} and T_{root} , and because it is a major greenhouse crop that is sensitive to low temperature (Terashima et al., 1998) and often grows under different T_{air} and T_{root} conditions due to artificial control (Gosselin and Trudel, 1985; Teitel et al., 1999; Urrestarazu et al., 2008).

MATERIAL AND METHODS

Plant Material and Growth Conditions

Cucumber (*Cucumis sativus* L. cv. Zhongnong No.16) seedlings were hydroponically cultured according to the procedure described by Wang et al. (2016). Briefly, cucumber seeds were pregerminated at 28°C for 26 h, sown onto hydroponic devices and then cultured at 28°C for 30 h under darkness. Germinated seedlings were maintained in hydroponic devices and cultured at 26/18°C (day 10h/night 14h) for 10 days, with 60-80% relative humidity (RH) and approximately 100 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$ during the day. Then, seedlings were transplanted onto brown glass bottles placed and cultured at 26/18°C (day 10h/night 14h) for another 5 days, with 60-80% RH and 250 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$ during the day. Full-strength Yamazaki nutrient solution (Yamazaki, 1982) at pH 6.0 was used for hydroponics throughout this experiment, and was refreshed every 5 days. The seedlings ready for treatment each had two intact cotyledons, one fully unfolded true leaf and one new leaf beginning to unfold.

Temperature Treatments

On day 16 after germination, the cucumber seedlings were transferred into temperature controlling devices as described by Wang et al. (2016), which can respectively set and maintain temperature regimes around the shoots and roots. There were two regimes of both T_{air} and T_{root} in this experiment: 26/18°C (day/night, “high”) and 20/12°C (day/night, “low”) for T_{air} , and 19±1°C (all-day, “high”) and 13±1°C (constant, “low”) for T_{root} . A 2 × 2 factorial design was employed to create treatments that included low T_{air} /low T_{root} (L/L), low T_{air} /high T_{root} (L/H), high T_{air} /low T_{root} (H/L) and high T_{air} /high T_{root} (H/H). To assure the comparability of the morphology and biomass allocation of seedlings among different treatments, each treatment lasted until the same stage of seedling development (i.e., for each seedling the second true leaf fully unfolded and the third true leaf was just about to unfold). The actual treatment periods were 10 days for low T_{air} treatments (L/L and L/H) and 5 days for high T_{air} treatments (H/L and H/H), respectively. Forty seedlings per treatment were cultivated.

Growth Characteristics

At both the beginning and ending of the treatments, seedlings were harvested to determine growth characteristics (seven replicates, three seedlings each replicate). Fresh leaves and roots were scanned (EPL/HN EXPREL/LION 4990, Japan), and the scanned images were used to quantify leaf area and total root length with WinRHIZO software (LC4800-II LA2400; Sainte-Foy, Canada). Additionally, the path length of each first-order lateral root (LR) on the basal half of main root, and the number of second-order LRs on each first-order LR were quantified with ImageJ software (V1.50b; Abràmoff et al., 2004). The definition of first- and second-order LR was the same as described by Kellermeier et al. (2014). After scanning, fresh plant tissues were separately (root, stem, cotyledon, the first true leaf and the second true leaf) oven-dried at 105°C for 15 min and at 85°C for 48 h, and weighted for the dry mass. Total plant dry mass was calculated as the sum of all plant tissues. For calculating RGR, plants before and after treatment were paired based on the order of total plant dry weight, and then RGR was calculated for paired plants as described by Hunt (1978):

$$\text{RGR} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

where W_1 and W_2 are the total plant dry mass before and after treatment, respectively, and $T_2 - T_1$ is the treatment period. SLA ($\text{cm}^2 \cdot \text{g}^{-1}$) and SRL ($\text{m} \cdot \text{g}^{-1}$) were calculated as the leaf area per leaf dry mass and the root length per root dry mass, respectively. LMF ($\text{g} \cdot \text{g}^{-1}$) and RMF ($\text{g} \cdot \text{g}^{-1}$) were estimated as proportions of leaf dry mass and root dry mass of the total plant dry mass, respectively. LAR ($\text{cm}^2 \cdot \text{g}^{-1}$) and RLR ($\text{m} \cdot \text{g}^{-1}$) were calculated as the leaf area and root length per total plant dry mass, respectively.

Element Content and Absorption Rates

After being weighted, the dry tissues of seedlings were ground into fine powder with a mortar and pestle for analysis of element contents. The contents of C and N in seedling tissues were determined by combusting the powder at 900°C within an elemental analyzer (vario PYRO cube, Germany). The contents of P, K, Ca, Mg, Fe, Mn, Zn, and Cu were determined by digesting the powder with nitric acid in a microwave digestion system (MARS 240/50, CEM, USA) and then analyzing with an inductively coupled plasma atomic emission spectrometer (ICP-AES, ICP6300, Britain).

For nutrient elements, the whole plant absorption rate (R_x , $\text{mg element} \cdot \text{g}^{-1} \text{ plant biomass} \cdot \text{d}^{-1}$, where x can be N, P, K, Ca, Mg, Fe, Mn, Zn or Cu) and specific absorption rate on a root-length basis (SAR_x , $\text{mg element} \cdot \text{m}^{-1} \text{ root length} \cdot \text{d}^{-1}$) were calculated as mean values over the treatment period according to Welbank (1962) as follows:

$$R_x = \frac{M_2 - M_1}{T_2 - T_1} \cdot \frac{\ln W_2 - \ln W_1}{W_2 - W_1}$$

where M_1 and M_2 are the total content of element before and after treatments, respectively, and R_{L1} and R_{L2} are the total root length before and after treatments, respectively. The estimation of mean RLR ($\overline{RLR}$) over the treatment period was calculated as dividing R_x by SAR_x .

To compare with N and other elements, the influx of C was also estimated in similar methods, the whole plant net assimilation rates of carbon (R_C , $\text{mg C} \cdot \text{g}^{-1} \text{ plant biomass} \cdot \text{d}^{-1}$) and unit leaf rate of carbon (NAR_C , $\text{mg C} \cdot \text{cm}^{-2} \text{ leaf area} \cdot \text{d}^{-1}$) were calculated as follows:

$$R_C = \frac{M_2 - M_1}{T_2 - T_1} \cdot \frac{\ln W_2 - \ln W_1}{W_2 - W_1}$$

where L_{A1} and L_{A2} are the total leaf area before and after treatments, respectively. Similarly, the estimation of mean LAR ($\overline{LAR}$) over the treatment period was calculated as dividing R_C by NAR_C .

Net Assimilation Rate of True Leaves

Gas-exchange was measured on both true leaves with the LI-6400xt gas exchange analyzer (Li-Cor 6400xt, Lincoln, NE, USA) (four seedlings per treatment). Determination started from the third hour of a light period in the last day of treatment. The block temperature was set at the air temperature of the corresponding treatment, and the PAR and air relative humidity were maintained at $1200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and 60-70%, respectively. Net assimilation rate under $400 \mu\text{mol CO}_2 \cdot \text{mol}^{-1}$ reference CO_2 concentration (A_{400}) was recorded.

Data Analysis

Following the variance partitioning method described by Rees et al. (2010) and Freschet et al. (2015b), we calculated the relative contributions of variance in LMF and SLA to variance in LAR, of RMF and SRL to RLR, of NAR_C and $\overline{LAR}$ to R_C , and of SAR_N and $\overline{RLR}$ to R_N . To avoid meaningless results, the variance partitioning was not performed if less than 15% variation was observed in LAR, RLR, R_C or R_N . Instead of relative contributions, direct effects of R_C and R_N on RGR were worked out using path coefficient analysis (Dewey and Lu, 1959).

The data and the graphs were processed using Microsoft Excel 2016 and Microsoft R Open 3.4.1. For multiple comparisons, data were $\log_2$ transformed and then subjected to one-way analysis of variance (ANOVA). When ANOVA indicated significant differences ($P < 0.05$), means were compared using Tukey HSD tests (software: IBM SPL/L Statistics 20, IBM Corporation, USA). Two-way ANOVA was performed to compare sources of variation, including T_{air} , T_{root} , and the $T_{\text{air}} \times T_{\text{root}}$ interaction.

RESULTS

Plant Growth Parameters under Different Temperature Conditions

Seedlings spent less time growing one new leaf under high T_{air} conditions (H/L and H/H) than under low T_{air} conditions (L/L and L/H). Thus, although elevated T_{air} decreased the total dry mass of seedlings at the end of the experiment, it significantly accelerated their RGR (H/L vs L/L, H/H vs L/H; **Table 1**). Elevated T_{root} increased total dry mass and RGR at both levels of T_{air} (L/H vs L/L, H/H vs H/L). Significant interactive effects of T_{air} and T_{root} were observed on both total dry mass and RGR. Elevated T_{air} significantly decreased leaf area at low T_{root} (H/L vs L/L) and total root length at both levels of T_{root} (H/L vs L/L, H/H vs L/H). By contrast, elevated T_{root} significantly increased total leaf area and total root length at both levels of T_{air} (L/H vs L/L, H/H vs H/L). **Table 1** and

Supplementary Figure S1 also display more details about how temperature treatments affected the size of leaves and roots. Compared with older tissues, the 2nd true leaf and the 2nd order LR, which newly developed during treatment, had higher size variation between treatments. Apparently, low T_{air} combined with low T_{root} resulted in a generally small and slowly-developed L/L seedling. On this basis, elevated T_{air} led to a fast-developed but still small seedling (H/L vs L/L), elevated T_{root} led to a large but still slowly-developed seedling (L/H vs L/L), and co-elevated T_{air} and T_{root} led to a large and fast-developed seedling (H/H vs L/L).

Effects of T_{air} and T_{root} on the Components of Leaf and Root Size

Elevated T_{air} significantly raised LAR at each T_{root} (**Figure 1**) and RLR at low T_{root} (H/L vs L/L), but decreased RLR at high T_{root} (H/H vs L/H). Elevated T_{root} raised both LAR and RLR at each T_{air} , and the promoting effect on RLR was obviously stronger at low T_{air} than at high T_{air} . Responses of SLA and SRL to temperature variation were similar to those of LAR and RLR, except for that SRL was not significantly affected by elevated T_{air} at high T_{root} (H/H vs L/H). As to biomass allocation, elevated T_{air} increased LMF and decreased RMF (H/L vs L/L), while elevated T_{root} showed reverse trends (L/H vs L/L), leading to unchanged LMF and increased RMF at co-elevated T_{air} and T_{root} (H/H vs L/L). For different leaves of a seedling, their biomass fractions may respond differently to temperature changes, depending on the leaf order (**Figure 2**). Elevated T_{air} significantly increased the allocation ratio of biomass increment of the 2nd true leaf, while elevated T_{root} significantly decreased the allocation ratio of biomass increment of cotyledon. For the 1st true leaf, temperature variation had no obvious influence on biomass allocation.

Changes in morphological characteristics (SLA and SRL) generally weighted more than biomass allocation (LMF and RMF) on determining responses of LAR and RLR to temperature variation (**Figure 3**). In the above ground parts, changes in SLA always contributed the major part of the variation in LAR no matter how temperature was changed. The relative contribution of SLA even exceeded 1 since LMF contributed negatively to the total variation in LAR. In the below ground parts, changes in SRL contributed more than RMF to the variation in RLR when T_{all} ($T_{\text{air}} + T_{\text{root}}$) or T_{root} was altered. Specially, only when T_{air} changed at high T_{root} (L/H vs H/H), the relative contribution of RMF became predominant.

The ratio of total leaf area to total root length (equivalent to LAR : RLR) varied a lot among different temperature treatments (**Table 2**). Considering the LAR : RLR of H/H seedlings as a balanced standard, T_{air} limitation led to a structure with relatively smaller leaves but larger roots in L/H seedlings, while T_{root} limitation did the opposite thing on H/L seedlings. Instead of proportionately inhibiting both leaf and root sizes, T_{all} limitation led to a high LAR : RLR in L/L seedlings, which was similar to that in H/L seedlings, indicating that root length was more sensitive than leaf area to low temperature. As the components of LAR : RLR, leaf-root morphology (SLA : SRL) and leaf-root biomass allocation (LMF : RMF) varied in a similar way to their product, except for that LMF : RMF was significantly lower in L/L seedlings than in L/H seedlings. This trend was counteracted by SLA : SRL, resulting in no difference in LAR : RLR.

Effects of T_{air} and T_{root} on Carbon and Nutrient Acquisition and Allocation

At each T_{root} , elevated T_{air} significantly raised both R_C and R_N , while elevated T_{root} only raised R_N (**Figure 4**). As to specific resource acquiring rates, elevated T_{air} raised both NAR_C and SAR_N , and the promoting effect on SAR_N was stronger at high T_{root} than at low T_{root} . Elevated T_{root} had no significant influence on both NAR_C and SAR_N at low T_{air} , and had a negative effect on NAR_C but a positive effect on SAR_N at high T_{air} . The response of photosynthetic capacity to temperature variation was

different from that of NAR_C . All elevated-temperature treatments significantly increased the A_{400} (net photosynthetic rate) in both true leaves of seedlings (compare H/L, L/H and H/H versus L/L; **Table 4**). However, compared to H/H seedlings, T_{root} limitation did not affect the A_{400} in any leaf of H/L seedlings, and T_{air} limitation only decreased the A_{400} in the 2nd true leaf of L/H seedlings. No significant interaction between T_{air} and T_{root} was observed in R_C , R_N and NAR_C (**Figure 4**).

When T_{all} was changed, NAR_C contributed 38% of the variation in R_C , and SAR_N and $\overline{RLR}$ contributed almost equally to the variation in R_N (**Figure 5**). When T_{root} was changed, $\overline{RLR}$ contributed a major part of the variation in R_N . When T_{air} was changed, NAR_C contributed 57% of the variation in R_C , and SAR_N contributed a major part of the variation in R_N .

The value of $R_C : R_N$ is equal to the ratio of newly gained total carbon to nitrogen per day. Considering the $R_C : R_N$ of H/H seedlings as a balanced standard, T_{air} limitation did not influence the ratio in L/H seedlings. This was mainly because of the counteracting effect of decreased root absorption activity (increased $NAR_C : SAR_N$) and increased root size (decreased $\overline{LAR} : \overline{RLR}$) (**Table 3**). T_{root} limitation raised $R_C : R_N$ in H/L seedlings by increasing both $NAR_C : SAR_N$ and $\overline{LAR} : \overline{RLR}$. T_{all} limitation led to aggregated nitrogen limitation in L/L seedlings, similar to that in H/L seedlings. This similarity was due to no significant difference in either $NAR_C : SAR_N$ or $\overline{LAR} : \overline{RLR}$ between L/L and H/L seedlings.

The allocation of newly gained carbon and nitrogen to each part of a seedling was not always proportional to that of biomass, and was distinct among different organs (**Figure 6A and B, Figure 2**). Nitrogen allocation was apparently more flexible than carbon allocation. Newly gained nitrogen was allocated more to new leaf, stem and root than to old leaves. Such trend of heterogeneity was more apparent at high T_{air} (H/H and H/L). As a result of aggregated nitrogen limitation, L/L and H/L seedlings had relatively higher carbon concentration and lower nitrogen concentration, and thus higher C/N ratios than L/H and H/H seedlings (**Figure 6C, D and E**). However, the more heterogeneous nitrogen allocation decreased the C/N ratios of the second leaf, stem and root (compare H/L versus L/L, or compare H/H versus L/H).

The final networks under various conditions were illustrated in **Figure 7**. As the components of RGR, R_C and R_N had similar direct effects on determining RGR when T_{all} was changed (**Figure 7A**). R_C had a higher direct effect when T_{air} was changed (**Figure 7C, E**), and R_N had a higher direct effect when T_{root} was changed (**Figure 7B, D**).

DISCUSSION

Morphology Responds More than Mass Allocation to Temperature Variation in Both Leaf and Root

In this study, changes in SLA always accounted for a major part of the temperature-induced variation in LAR, irrespective of T_{all} , T_{air} or T_{root} (**Figure 3**). Similar results were reported by Loveys et al. (2002) and Poorter et al. (2012), where temperature was managed based on T_{all} or T_{air} . However, very few studies to date has attempted to examine the T_{root} -induced variation in SLA. According to the results reported by Weih and Karlsson (2001) and Danyagri and Dang (2014), the variation of SLA is more consistent with that of LAR when compared to LMF, indicating that SLA may contribute more to LAR.

For the below-ground parts, SRL contributed more than RMF to the temperature-induced variation in RLR, irrespective of T_{all} , T_{air} or T_{root} (**Figure 3**). Similar trends for T_{root} could be obtained based on

the data reported by Tachibana (1982) and Engels et al. (1992). However, this trend is opposite to root response to nutrient regulation reported by Freschet et al. (2015b), that is, RMF contributed more than SRL to the nutrient-induced variation in RLR. One possible reason for the reverse trends is that temperature variation also has significant effects on the hydraulic status of roots (Lee et al., 2004; Lee et al., 2005), which plays an important role in determining root morphology (Wan et al., 1999). Another reason is probably due to the difference in culture medium. It seems that, probably because hydroponics favors root elongation better than soils or sands due to lower mechanical impedance (Bengough and Mullins, 1990), the contribution of SRL to RLR was predominant under our hydroponic conditions. However, Engels et al. (1992) found that SRL predominated in RLR under both hydroponic and soil culture conditions, indicating the crucial role played by SRL in determining RLR.

The Role of Root Activity and Size in Nitrogen Uptake Depends on Temperature Management Strategies

When T_{air} was elevated alone, NAR_C contributed more than LAR to total C assimilation (**Figures 4 and 5**). However, the contribution of NAR_C was reduced when T_{all} was elevated and was nearly eliminated when T_{root} was elevated alone, indicating the inhibiting role of elevated T_{root} in NAR_C . NAR_C is the result of leaf photosynthetic rate minus total plant respiration per unit leaf area. Since both leaf photosynthetic capacity and LAR increased at elevated T_{root} (the higher LAR, the lower leaf mass per unit area; **Table 4** and **Figure 1**), stimulated respiration rate should be mainly responsible for the reduction in NAR_C at elevated T_{root} . Additionally, because the maintenance respiration rate is generally higher in roots than in leaves (Lambers et al., 1983), and because root respiration increase with increasing temperature (Atkin et al., 2005), the increased RMF : LMF ratio also contributed to the reduction of net carbon accumulation at elevated T_{root} in this study. For nitrogen absorption, compared with T_{root} , T_{air} had only a slight influence on root size but a predominant effect on SAR_N (**Figure 4**). Such distinct impacts resulted in that when T_{air} was changed, SAR_N contributed more than RLR to the variation of R_N , but this trend was reversed when T_{root} was changed (**Figure 5** and **Figure 7**). It is generally comprehensible that T_{air} has less influence on root size than T_{root} , since the T_{root} has more direct effects on root hydraulic status (Wan et al., 1999; Lee et al., 2004; Lee et al., 2005). Supportive results can be found in the researches of Engels and Marschner (1990) and Larigauderie et al. (1991), although in these studies data were not presented in the form of RLR or root area ratio. The influence of T_{air} on nutrient uptake is generally considered to be regulated by sugar signals (Stitt and Krapp, 1999). For instance, exogenous application of sugars can increase nitrate reductase activity (Reda, 2015). Moreover, a bZIP transcription factor *Arabidopsis* ELONGATED HYPOCOTYL5 (HY5) has been reported to mediate sucrose signal and promote root nitrate uptake by activating *NRT2.1* (Cerezo et al., 2001; Chen et al., 2016). A previous study (Engels et al. 1992) has reported a promotion effect of elevated shoot base temperature on nutrient translocation rates per unit root fresh weight, but this effect was not examined at elevated T_{root} . Our observation indicates that the sink-mediated regulation, induced by T_{air} management, may overtake the direct effects of T_{root} on length-based SAR_N . However, Weih and Karlsson (2001) showed that raising T_{root} was more efficient than raising T_{air} in increasing nitrogen uptake rate per unit root dry weight. Actually, this result doesn't go against our observation, since the trend was also reversed when transforming length-based SAR_N into weight-based SAR_N (**Supplementary Table S2**) by multiplying SRL in our study. Thus, the significant influence of T_{root} on SRL may be partly responsible for counteracting the extent of T_{root} effect on length-based SAR_N .

R_C and R_N are additive in affecting RGR, and both are predominant. When T_{air} was elevated, R_C and R_N were proportionally promoted. However, R_C accounted for more variation in RGR than R_N . This

is because the concentration of carbon is higher than that of nitrogen in plant tissue. In contrast to the situation at T_{air} , when T_{root} was elevated, R_C was not affected, and R_N (together with other elements absorbed by roots) became the main reason for variation in RGR (**Figure 4 and Figure 7**). The trends mentioned above were not applicable to the situation at elevated T_{all} .

Adaptive Phenotypic Plasticity in Response to Altered T_{air} and T_{root} in Plants

In a heterogeneous temperature (T_{air} vs T_{root}) environment, cucumber seedlings tended to invest less biomass and generate relatively smaller organ in the cooler zone. Such passive response would potentially strengthen rather than relieve the limitation of the corresponding resource. This trend seems to go against the functional equilibrium hypothesis, that a plant would invest more biomass in the organ responsible for acquiring the most limiting resource (Brouwer, 1963; Freschet et al., 2015b). Based on previous studies regarding biomass allocation, there are both supportive (Tachibana, 1982; Clarkson et al., 1986; Delucia et al., 1992; Danyagri and Dang, 2014) and opposed (Davidson, 1969; Engels and Marschner, 1990; Li et al., 1994; Yan et al., 2012) evidences. It is possible that various factors (e.g. species, temperature, growth medium and ontogenetic stage) may also have impacts on the direction of biomass allocation. For the morphological response, low temperature-induced hydraulic limitation (Murai-Hatano et al., 2008; Wang et al., 2016) and abscisic acid accumulation (Zhang et al., 2008; Ntatsi et al., 2014), which are generally less combined with nutrient and/or light limitation, are both responsible for retarding leaf and root growth (Walter et al., 2009; Pantin et al., 2011). Therefore, in response to temperature variation, changes in the trend of biomass allocation and relative leaf : root size ratio may be more passive rather than adaptive compared with those in respond to nutrient and/or light variation.

According to the above response and the ‘balanced growth’ hypothesis, $\text{NAR}_C : \text{SAR}_N$ should vary against the trend of $\text{LAR} : \text{RLR}$ in order to maintain balanced carbon-nutrient acquisition (i.e. $R_C : R_N$). Actually, in this study, $\text{NAR}_C : \text{SAR}_N$ increased no matter which of T_{air} and T_{root} was cooled. At low T_{air} , the higher $\text{NAR}_C : \text{SAR}_N$ counterbalanced the lower $\text{LAR} : \text{RLR}$, resulting in a relatively constant $R_C : R_N$. At low T_{root} , however, the higher $\text{NAR}_C : \text{SAR}_N$ was accompanied by a higher $\text{LAR} : \text{RLR}$, leading to a large increase in $R_C : R_N$ (i.e. carbon accumulation or nitrogen limitation). As discussed above, the counteracting effect of root respiration on total carbon acquisition could be one of the main reasons for carbon accumulation when T_{root} was cooled. In addition, the sugar-induced increase in nitrogen acquisition could be largely inhibited by limited transporter activity (Reay et al., 1999) and root size at low T_{root} . In response to nitrogen limitation, newly gained nitrogen was more unevenly distributed between older leaves and other organs to ensure adequate nitrogen concentration for growth in the latter (L/L vs L/H, H/L vs H/H seedlings, **Figure 6**).

The T_{air} and T_{root} Interactively Determine Root Size

In this study, the T_{air} and T_{root} interactively affected RGR, and all root length- and root biomass-related parameters. The interaction effects on RGR were also reported in Larigauderie et al. (1991) and Weih and Karlsson (2001), which showed that increasing T_{air} or T_{root} alone had a greater promotion on RGR than increasing T_{all} . Interestingly, interaction effects were not observed in R_C and R_N , neither in LAR and NAR_C , all of which are components of RGR. Thus the only possible interpretation is that elevated T_{root} had a weaker effect on uptake of other elements except for nitrogen at high T_{air} than at low T_{air} . In addition, elevated T_{root} had a weaker effect on LR length, particularly the length of the second order LRs, at high T_{air} than at low T_{air} (**Table 1 and Supplementary Figure S1**), since the treatment period for seedlings was shorter at high T_{air} (thus less accumulated T_{root}) (KASPAR and BLAND, 1992). This infer that the T_{air} and T_{root} interaction effects on root length and root biomass might be further reduced by the initiation and development of

lateral roots. Generally, the second order LR_s were much thinner than main roots and the first order LR_s (about 0.2mm vs 0.5~2mm), indicating that elevated T_{root} led to a much higher SRL at low T_{air} than at high T_{air} . This interaction effect can interpret some exceptions in the observed trends, e.g., contributed less than RMF to RLR when T_{air} was changed at high T_{root} (**Figure 3**), and that T_{air} variation had less influence on LAR : RLR, SLA : SRL and LMF : RMF at low T_{root} than at high T_{root} (**Table 2**).

In this study, cucumber seedlings with the same number of leaves were compared after different treatments, and this was originally designed to avoid ontogenetic effects. The period used for new leaf initiation was changed only by varying T_{air} . This is consistent with the report of Savvides et al. (2016), which showed that the rate of cucumber leaf initiation was completely determined by apical bud temperature independent of the temperature of other plant organs. Although apical bud temperature was not monitored in our experiment, it can be regarded as varying along with T_{air} rather than T_{root} . Field experiment on tomato also reported that cropping was delayed by low T_{air} irrespective of T_{root} (Jones et al., 1978). However, the treatment period aiming for a uniform shoot developmental stage induced ontogenetic drift in roots, as discussed above. Thus, besides plant growth, the distinct influence of T_{air} and T_{root} variation on shoot and root development or phenology should also be taken into consideration when designing temperature control strategy for experiment or for protected cultivation.

CONCLUSION

Our results revealed the distinct effects of T_{air} and T_{root} on cucumber seedling growth. The primary influence of cooling T_{root} on seedling growth was decrease in SRL, which was the main contributor to decrease in RLR. Lower RLR contributed the major part of decrease in total nitrogen acquisition, which finally retarded RGR in seedlings at lower T_{root} . Variation in T_{root} didn't affect net carbon fixation, although cooling T_{root} also decreased LAR mainly via reducing SLA. The major effect of decreasing T_{air} on seedling growth was decrease in the capacities of carbon assimilation in leaves and nitrogen absorption by roots, which contributed more than LAR and RLR to the reduction in total resource acquisition. The ratio of carbon : nitrogen acquisition was maintained at a relatively constant level when T_{air} was changed, but was increased by decreasing T_{root} . The interactive effect of T_{air} and T_{root} was mainly observed on RGR and root growth related variables.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: XW, YT and LG. Performed the experiments: XW. Analysed the data: XW and YT. Wrote the paper: XW and YT.

FUNDING

This work was supported by the Special Fund for the National Key Research Development Program of China (2016YED201003) and the earmarked fund for the China Agriculture Research System (CARS-25-C-12).

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587

588 FIGURE CAPTIONS

589 **Figure 1.** Responses of leaf and root relative size, morphology and biomass allocation to air
590 temperature (T_{air}) variation (solid lines) and root-zone temperature (T_{root}) variation (dashed lines).
591 Each variable is expressed on a log₂-scale. Data points and error bars are means $\pm$ standard error (n =
592 7). Different letters besides each point denote significance at $P < 0.05$ by Tukey's HSD-test. The
593 significance (*, $P < 0.05$; *** $P < 0.001$; ns, not significant) of interactions between T_{air} and T_{root} is
594 displayed in each panel. L/L, low T_{air} /low T_{root} ; L/H, low T_{air} /high T_{root} ; H/L, high T_{air} /low T_{root} ; H/H,
595 high T_{air} /high T_{root} . The data (in number) of this figure are exhibited in **Supplementary Table S1**.

596 **Figure 2.** Allocation ratios of biomass increment in different organs during treatment. Boxes and
597 error bars are means $\pm$ standard error (n = 7). Different letters besides each point denote significance
598 at $P < 0.05$ by Tukey's HSD-test. LAR, leaf area ratio; RLR, root length ratio; SLA, specific leaf
599 area; SRL, specific root length; LMF, leaf mass fraction; RMF, root mass fraction. L/L, low T_{air} /low
600 T_{root} ; L/H, low T_{air} /high T_{root} ; H/L, high T_{air} /low T_{root} ; H/H, high T_{air} /high T_{root} .

601 **Figure 3.** Relative contribution of leaf and root biomass allocation (LMF or RMF) and morphology
602 (SLA or SRL) variables to the total variation in LAR and RLR. The category " T_{all} Variation" refers
603 to L/L versus H/H; " T_{root} Variation" refers to the mean value of L/L versus L/H and H/L versus H/H;
604 " T_{air} Variation" refers to the mean value of L/L versus H/L and L/H versus H/H. Specially, the
605 inserted viewport displays L/L versus H/L (sub-optimal T_{root}) and L/H versus H/H (optimal T_{root})
606 respectively, due to the significant difference between the two conditions. LAR, leaf area ratio; RLR,
607 root length ratio. L/L, low T_{air} /low T_{root} ; L/H, low T_{air} /high T_{root} ; H/L, high T_{air} /low T_{root} ; H/H, high
608 T_{air} /high T_{root} .

609 **Figure 4.** Responses of leaf and root size and assimilation/absorption rate to air temperature (T_{air})
610 variation (solid lines) and root-zone temperature (T_{root}) variation (dashed lines). Each variable is
611 expressed on a log₂-scale. Data points and error bars are means $\pm$ standard error (n = 7). Different
612 letters besides each point denote significance at $P < 0.05$ by Tukey's HSD-test. The significance (*,
613 $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant) of interactions between T_{air} and T_{root} is
614 displayed in each panel. L/L, low T_{air} /low T_{root} ; L/H, low T_{air} /high T_{root} ; H/L, high T_{air} /low T_{root} ; H/H,
615 high T_{air} /high T_{root} . The data (in number) of this figure are exhibited in **Supplementary Table S2**.

616 **Figure 5.** Relative contribution of leaf and root assimilation/absorption activity (NAR_C or SAR_N) and
617 size ($\overline{\text{LAR}}$ or $\overline{\text{RLR}}$) variables to the total variation in R_C and R_N . The category " T_{all} Variation" refers to
618 L/L versus H/H; " T_{root} Variation" refers to the mean value of L/L versus L/H and H/L versus H/H;
619 " T_{air} Variation" refers to the mean value of L/L versus H/L and L/H versus H/H. n/a, not applicable,
620 as no more than 15% variation in R_C was observed when changing T_{root} . L/L, low T_{air} /low T_{root} ; L/H,
621 low T_{air} /high T_{root} ; H/L, high T_{air} /low T_{root} ; H/H, high T_{air} /high T_{root} .

Figure 6. Relative allocation of carbon (A) and nitrogen (B), relative concentration of carbon (C) and nitrogen (D), and relative C/N ratio (E) in each part of the treated seedlings. (A)(B) relative allocation was calculated through dividing the ratio of carbon or nitrogen allocated to each part by the ratio of biomass allocated to correspond part. (C)(D) (E) relative values was calculated through dividing the value in each part by those in the seedlings before treatment. Specially, values in the second true leaves were divided by those in the first true leaves of un-treated seedlings. Negative value of relative allocation indicates net efflux rather than influx of element in the correspond part of plant during treatment. L/L, low T_{air} /low T_{root} ; L/H, low T_{air} /high T_{root} ; H/L, high T_{air} /low T_{root} ; H/H, high T_{air} /high T_{root} . The data (in number) of this figure and biomass allocation are exhibited in **Supplementary Table S3**.

Figure 7. Networks of relative contribution among leaf and root morphology, biomass allocation, size and capacity variables, and direct path coefficients of carbon and nitrogen acquisition rate to relative growth rate under different conditions of temperature variation. (A) L/L vs H/H seedlings, overall warmed or cooled; (B) L/L vs L/H seedlings, only root-zone was warmed; (C) L/L vs H/L seedlings, only air was warmed; (D) H/H vs H/L seedlings, only root-zone was cooled; (E) H/H vs L/H seedlings, only air was cooled. L/L, low T_{air} /low T_{root} ; L/H, low T_{air} /high T_{root} ; H/L, high T_{air} /low T_{root} ; H/H, high T_{air} /high T_{root} .

TABLES

Table 1. Plant growth parameters of seedlings before and after different temperature treatments. Means with different letters are significantly different ($P < 0.05$, $n = 6-7$) by Tukey HSD. Source of variation: F values and significance (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant) of air temperature (T_{air}), root-zone temperature (T_{root}) and $T_{\text{air}} \times T_{\text{root}}$. L/L, low T_{air} /low T_{root} ; L/H, low T_{air} /high T_{root} ; H/L, high T_{air} /low T_{root} ; H/H, high T_{air} /high T_{root} .

Treatment	Total dry mass /mg		RGR /mg·g ⁻¹ ·d ⁻¹		Leaf dry mass /mg		Root dry mass /mg		Total leaf area /cm ²		Total root length /m	
Before treatment	183		—		135		18.9		51.9		5.89	
L/L	481	b	96	d	342	b	46.5	b	95.1	c	7.44	c
L/H	599	a	118	c	396	a	80.1	a	139.3	a	27.48	a
H/L	358	d	134	b	265	c	32.0	d	92.0	c	6.71	d
H/H	394	c	153	a	279	c	41.7	c	116.8	b	13.31	b
Source of variation												
T_{air}	409	***	577	***	341	***	369	***	129	***	453	***
T_{root}	79	***	191	***	37	***	228	***	1129	***	2612	***
$T_{\text{air}} \times T_{\text{root}}$	12	**	9	**	8	**	27	***	60	***	254	***
Treatment	Leaf area /cm ²						Root length /cm					
	Cotyledon		1st true leaf		2nd true leaf		Main root		1st class LR		2nd class LR	
Before treatment	18.2		33.7		—		29.7		363		197	
L/L	20.2	a	42.7	c	32.2	c	29.9	b	391	c	323	c
L/H	19.7	a	54.8	a	64.9	a	40.7	a	954	a	1754	a
H/L	19.5	a	39.4	d	33.1	c	29.4	b	367	c	275	c
H/H	19.7	a	50.8	b	46.2	b	34.5	ab	567	b	731	b

Source of variation												
T_{air}	0.566	ns	31	***	113	***	4.4	*	38	***	29	***
T_{root}	0.203	ns	330	***	1236	***	27.5	***	187	***	193	***
$T_{\text{air}} \times T_{\text{root}}$	0.848	ns	0	ns	154	***	3.0	ns	21	***	13	**

645

646 **Table 2.** Ratios of leaf : root morphological, biomass fraction and size parameters of seedlings before
647 and after different temperature treatments. Means with different letters are significantly different ($P <$
648 0.05, $n = 7$) by Tukey HSD. Source of variation: F values and significance (** $P < 0.01$; *** $P <$
649 0.001) of air temperature (T_{air}), root-zone temperature (T_{root}) and $T_{\text{air}} \times T_{\text{root}}$. SLA, specific leaf area;
650 SRL, specific root length; LMF, leaf mass fraction; RMF, root mass fraction; LAR, leaf area ratio;
651 RLR, root length ratio. L/L, low T_{air} /low T_{root} ; L/H, low T_{air} /high T_{root} ; H/L, high T_{air} /low T_{root} ; H/H,
652 high T_{air} /high T_{root} .

Treatment	SLA : SRL /cm ² ·m ⁻¹		LMF : RMF		LAR : RLR /cm ² ·m ⁻¹	
Before treatment	1.24		7.12		8.82	
L/L	1.74	a	7.36	b	12.80	a
L/H	1.03	c	4.97	d	5.08	c
H/L	1.66	a	8.28	a	13.75	a
H/H	1.31	b	6.71	c	8.79	b
Source of variation						
T_{air}	12	**	84	***	245	***
T_{root}	179	***	175	***	1201	***
$T_{\text{air}} \times T_{\text{root}}$	26	***	16	***	146	***

653

654 **Table 3.** Ratios of leaf : root average size and resource acquiring capacity, and relative carbon :
655 nitrogen accumulation rate of seedlings during different temperature treatments. Means with different
656 letters are significantly different ($P < 0.05$, $n = 7$) by Tukey HSD. Source of variation: F values and
657 significance (** $P < 0.01$; *** $P < 0.001$; ns, not significant) of air temperature (T_{air}), root-zone
658 temperature (T_{root}) and $T_{\text{air}} \times T_{\text{root}}$. L/L, low T_{air} /low T_{root} ; L/H, low T_{air} /high T_{root} ; H/L, high T_{air} /low
659 T_{root} ; H/H, high T_{air} /high T_{root} .

Treatment	$\overline{\text{LAR}} : \overline{\text{RLR}}$, /cm ² ·m ⁻¹		$\text{NAR}_{\text{C}} : \text{SAR}_{\text{N}}$		$\text{R}_{\text{C}} : \text{R}_{\text{N}}$	
L/L	10.76	a	1.05	a	11.33	a
L/H	6.32	c	1.09	a	6.87	b
H/L	11.15	a	1.10	a	12.23	a
H/H	8.80	b	0.82	b	7.17	b

Source of variation						
T_{air}	100	***	14	**	4	ns
T_{root}	439	***	14	***	317	***
$T_{\text{air}} \times T_{\text{root}}$	65	***	23	***	0	ns

660

661 **Table 4.** Net photosynthetic rates of cucumber true leaves at 400 $\mu\text{mol CO}_2 \cdot \text{mol}^{-1}$ reference CO_2
662 concentration. Means with different letters are significantly different ($P < 0.05$, $n = 7$) by Tukey HSD.
663 Source of variation: F values and significance (** $P < 0.01$; *** $P < 0.001$) of air temperature (T_{air}),
664 root-zone temperature (T_{root}) and $T_{\text{air}} \times T_{\text{root}}$. L/L, low T_{air} /low T_{root} ; L/H, low T_{air} /high T_{root} ; H/L, high
665 T_{air} /low T_{root} ; H/H, high T_{air} /high T_{root} .

Treatment	A_{400} $\mu\text{mol CO}_2 \cdot \text{m}^{-1} \cdot \text{s}^{-1}$			
	1st leaf		2nd leaf	
L/L	9.6	b	9.3	c
L/H	18.7	a	13.2	b
H/L	20.8	a	16.7	a
H/H	19.0	a	18.3	a
Source of variation				
T_{air}	54	***	207	***
T_{root}	30	***	40	***
$T_{\text{air}} \times T_{\text{root}}$	66	***	14	**

666

ARelative
C allocation **B**Relative
N allocation **C**Relative
C concentration **D**Relative
N concentration **E**

Relative C/N ratio