

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

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11 **Estimated length of the Manuscript:** 5600 words

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

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

20 **ABSTRACT**

21 Both low air ( $T_{\text{air}}$ ) and root-zone ( $T_{\text{root}}$ ) temperatures can inhibit resource (e.g. carbon and nutrients)  
22 acquisition by leaves and roots through various aspects, such as morphology, biomass allocation and  
23 assimilation/absorption capacity. However, it is still ambiguous whether  $T_{\text{air}}$  and  $T_{\text{root}}$  influence  
24 carbon (C) and nutrient acquisition via the same approach. To this end, in this study, cucumber  
25 (*Cucumis sativus* L.) seedlings were hydroponically grown under treatments arranged in complete  
26 factorial combination of two levels of  $T_{\text{air}}$  (26/18°C and 20/12°C, day/night) and two levels of  $T_{\text{root}}$   
27 (19°C and 13°C, constant). In general, both  $T_{\text{air}}$  and  $T_{\text{root}}$  affected leaf and root sizes mainly by  
28 regulating their morphology rather than biomass investment. Under low  $T_{\text{air}}$  conditions (20/18°C),  
29 elevated  $T_{\text{root}}$  (compare 19°C versus 13°C) did not influence C acquisition, but increased nitrogen (N)  
30 acquisition mainly due to an increase in relative root length, resulting in decreased C : N acquisition  
31 ratio. However, under low  $T_{\text{root}}$  conditions (13°C), elevated  $T_{\text{air}}$  (compare 26/18°C versus 20/12°C)  
32 enhanced both C and N acquisition mainly because of an increase of both C assimilation in leaves  
33 and N absorption by roots, resulting in relatively constant C : N acquisition ratio. In addition, the  $T_{\text{air}}$   
34 and  $T_{\text{root}}$  interaction was mainly observed in relative growth rate and root growth-related variables.  
35 Our results infer that  $T_{\text{air}}$  and  $T_{\text{root}}$  have distinct impacts on resource acquisition and carbon-nitrogen  
36 balance in plants.

37

## 38 INTRODUCTION

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

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

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

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

83 colimitation (Ryser and Eek, 2000; Maire et al., 2013). This mechanism is known as the ‘balanced  
84 growth’, ‘optimal partitioning’ or ‘functional equilibrium’ hypothesis (Brouwer, 1963; Davidson,  
85 1969; Shipley and Meziane, 2002), which can be formalized as follows:

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

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

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

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

89 Carbon-nutrient balance may be achieved through various strategies such as maintaining relatively  
90 smaller root size but higher SAR, or maintaining relatively constant size/activity ratio of leaves to  
91 roots through proportionately increasing NAR and SAR. For instance, in the study of Engels et al.  
92 (1992), nutrient uptake by roots is stimulated by increased temperature of maize shoot base (apical  
93 shoot meristem and zone of leaf extension) via raising shoot growth, even under low  $T_{\text{root}}$  condition.  
94 Getting to know what kind of strategies plants choose can help us better understand the mechanisms  
95 of integrated plant responses to temperature limitation.

96 In this study, we conducted a complete factorial experiment, with two levels of  $T_{\text{air}}$  (high and low)  
97 and two levels of  $T_{\text{root}}$  (high and low), to investigate the independent and interactive effects of  $T_{\text{air}}$   
98 and  $T_{\text{root}}$  on leaf and root growth, and carbon and nutrient assimilation in cucumber (*Cucumis sativus*  
99 L.) seedlings. The objectives were to examine (1) how  $T_{\text{air}}$  and  $T_{\text{root}}$  affect relative contributions of  
100 various plant components to plant growth and resource acquisition, and (2) whether  $T_{\text{air}}$  has different  
101 effects on plant carbon-nutrient balance compared to  $T_{\text{root}}$ . Cucumber was chosen as a model plant  
102 because its growing point locates above the ground surface, which favors separate control of  $T_{\text{air}}$  and  
103  $T_{\text{root}}$ , and because it is a major greenhouse crop that is sensitive to low temperature (Terashima et al.,  
104 1998) and often grows under different  $T_{\text{air}}$  and  $T_{\text{root}}$  conditions due to artificial control (Gosselin and  
105 Trudel, 1985; Teitel et al., 1999; Urrestarazu et al., 2008).

## 106 MATERIAL AND METHODS

### 107 Plant Material and Growth Conditions

108 Cucumber (*Cucumis sativus* L. cv. Zhongnong No.16) seedlings were hydroponically cultured  
109 according to the procedure described by Wang et al. (2016). Briefly, cucumber seeds were  
110 pregerminated at 28°C for 26 h, sown onto hydroponic devices and then cultured at 28°C for 30 h  
111 under darkness. Germinated seedlings were maintained in hydroponic devices and cultured at  
112 26/18°C (day 10h/night 14h) for 10 days, with 60-80% relative humidity (RH) and approximately  
113 100  $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$  during the day. Then, seedlings were transplanted onto brown glass bottles  
114 placed and cultured at 26/18°C (day 10h/night 14h) for another 5 days, with 60-80% RH and 250  
115  $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$  during the day. Full-strength Yamazaki nutrient solution (Yamazaki, 1982) at  
116 pH 6.0 was used for hydroponics throughout this experiment, and was refreshed every 5 days. The  
117 seedlings ready for treatment each had two intact cotyledons, one fully unfolded true leaf and one  
118 new leaf beginning to unfold.

### 119 Temperature Treatments

120 On day 16 after germination, the cucumber seedlings were transferred into temperature controlling  
121 devices as described by Wang et al. (2016), which can respectively set and maintain temperature  
122 regimes around the shoots and roots. There were two regimes of both  $T_{\text{air}}$  and  $T_{\text{root}}$  in this experiment:  
123 26/18°C (day/night, “high”) and 20/12°C (day/night, “low”) for  $T_{\text{air}}$ , and 19±1°C (all-day, “high”)  
124 and 13±1°C (constant, “low”) for  $T_{\text{root}}$ . A 2 × 2 factorial design was employed to create treatments  
125 that included low  $T_{\text{air}}$ /low  $T_{\text{root}}$  (L/L), low  $T_{\text{air}}$ /high  $T_{\text{root}}$  (L/H), high  $T_{\text{air}}$ /low  $T_{\text{root}}$  (H/L) and high  
126  $T_{\text{air}}$ /high  $T_{\text{root}}$  (H/H). To assure the comparability of the morphology and biomass allocation of  
127 seedlings among different treatments, each treatment lasted until the same stage of seedling  
128 development (i.e., for each seedling the second true leaf fully unfolded and the third true leaf was just  
129 about to unfold). The actual treatment periods were 10 days for low  $T_{\text{air}}$  treatments (L/L and L/H) and  
130 5 days for high  $T_{\text{air}}$  treatments (H/L and H/H), respectively. Forty seedlings per treatment were  
131 cultivated.

### 132 **Growth Characteristics**

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

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

145 where  $W_1$  and  $W_2$  are the total plant dry mass before and after treatment, respectively, and  $T_2 - T_1$  is the  
146 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  
147 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  
148 proportions of leaf dry mass and root dry mass of the total plant dry mass, respectively. LAR ( $\text{cm}^2 \cdot \text{g}^{-1}$ )  
149 and RLR ( $\text{m} \cdot \text{g}^{-1}$ ) were calculated as the leaf area and root length per total plant dry mass, respectively.

### 150 **Element Content and Absorption Rates**

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

157 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  $x$   
158 can be N, P, K, Ca, Mg, Fe, Mn, Zn or Cu) and specific absorption rate on a root-length basis ( $\text{SAR}_x$ ,  
159  $\text{mg element} \cdot \text{m}^{-1} \text{ root length} \cdot \text{d}^{-1}$ ) were calculated as mean values over the treatment period according  
160 to Welbank (1962) as follows:

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

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

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

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

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

### 169 Net Assimilation Rate of True Leaves

170 Gas-exchange was measured on both true leaves with the LI-6400xt gas exchange analyzer (Li-Cor  
171 6400xt, Lincoln, NE, USA) (four seedlings per treatment). Determination started from the third hour  
172 of a light period in the last day of treatment. The block temperature was set at the air temperature of  
173 the corresponding treatment, and the PAR and air relative humidity were maintained at  $1200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$   
174 and 60-70%, respectively. Net assimilation rate under  $400 \mu\text{mol CO}_2\cdot\text{mol}^{-1}$  reference  $\text{CO}_2$   
175 concentration ( $A_{400}$ ) was recorded.

### 176 Data Analysis

177 Following the variance partitioning method described by Rees et al. (2010) and Freschet et al.  
178 (2015b), we calculated the relative contributions of variance in LMF and SLA to variance in LAR, of  
179 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  
180 results, the variance partitioning was not performed if less than 15% variation was observed in LAR,  
181 RLR,  $R_C$  or  $R_N$ . Instead of relative contributions, direct effects of  $R_C$  and  $R_N$  on RGR were worked  
182 out using path coefficient analysis (Dewey and Lu, 1959).

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

## 188 RESULTS

### 189 Plant Growth Parameters under Different Temperature Conditions

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



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

### 205 **Effects of $T_{\text{air}}$ and $T_{\text{root}}$ on the Components of Leaf and Root Size**

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

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

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

### 236 **Effects of $T_{\text{air}}$ and $T_{\text{root}}$ on Carbon and Nutrient Acquisition and Allocation**

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

242 different from that of  $NAR_C$ . All elevated-temperature treatments significantly increased the  $A_{400}$  (net  
243 photosynthetic rate) in both true leaves of seedlings (compare H/L, L/H and H/H versus L/L; **Table**  
244 **4**). However, compared to H/H seedlings,  $T_{root}$  limitation did not affect the  $A_{400}$  in any leaf of H/L  
245 seedlings, and  $T_{air}$  limitation only decreased the  $A_{400}$  in the 2nd true leaf of L/H seedlings. No  
246 significant interaction between  $T_{air}$  and  $T_{root}$  was observed in  $R_C$ ,  $R_N$  and  $NAR_C$  (**Figure 4**).

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

251 The value of  $R_C : R_N$  is equal to the ratio of newly gained total carbon to nitrogen per day.  
252 Considering the  $R_C : R_N$  of H/H seedlings as a balanced standard,  $T_{air}$  limitation did not influence the  
253 ratio in L/H seedlings. This was mainly because of the counteracting effect of decreased root  
254 absorption activity (increased  $NAR_C : SAR_N$ ) and increased root size (decreased  $\overline{LAR} : \overline{RLR}$ ) (**Table**  
255 **3**).  $T_{root}$  limitation raised  $R_C : R_N$  in H/L seedlings by increasing both  $NAR_C : SAR_N$  and  $\overline{LAR} : \overline{RLR}$ .  
256  $T_{all}$  limitation led to aggregated nitrogen limitation in L/L seedlings, similar to that in H/L seedlings.  
257 This similarity was due to no significant difference in either  $NAR_C : SAR_N$  or  $\overline{LAR} : \overline{RLR}$  between  
258 L/L and H/L seedlings.

259 The allocation of newly gained carbon and nitrogen to each part of a seedling was not always  
260 proportional to that of biomass, and was distinct among different organs (**Figure 6A and B, Figure**  
261 **2**). Nitrogen allocation was apparently more flexible than carbon allocation. Newly gained nitrogen  
262 was allocated more to new leaf, stem and root than to old leaves. Such trend of heterogeneity was  
263 more apparent at high  $T_{air}$  (H/H and H/L). As a result of aggregated nitrogen limitation, L/L and H/L  
264 seedlings had relatively higher carbon concentration and lower nitrogen concentration, and thus  
265 higher C/N ratios than L/H and H/H seedlings (**Figure 6C, D and E**). However, the more  
266 heterogeneous nitrogen allocation decreased the C/N ratios of the second leaf, stem and root  
267 (compare H/L versus L/L, or compare H/H versus L/H).

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

## 272 **DISCUSSION**

### 273 **Morphology Responds More than Mass Allocation to Temperature Variation in Both Leaf and** 274 **Root**

275 In this study, changes in SLA always accounted for a major part of the temperature-induced variation  
276 in LAR, irrespective of  $T_{all}$ ,  $T_{air}$  or  $T_{root}$  (**Figure 3**). Similar results were reported by Loveys et al.  
277 (2002) and Poorter et al. (2012), where temperature was managed based on  $T_{all}$  or  $T_{air}$ . However, very  
278 few studies to date has attempted to examine the  $T_{root}$ -induced variation in SLA. According to the  
279 results reported by Weih and Karlsson (2001) and Danyagri and Dang (2014), the variation of SLA is  
280 more consistent with that of LAR when compared to LMF, indicating that SLA may contribute more  
281 to LAR.

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

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

## 295 **The Role of Root Activity and Size in Nitrogen Uptake Depends on Temperature Management** 296 **Strategies**

297 When  $T_{\text{air}}$  was elevated alone,  $\text{NAR}_C$  contributed more than LAR to total C assimilation (**Figures 4**  
298 **and 5**). However, the contribution of  $\text{NAR}_C$  was reduced when  $T_{\text{all}}$  was elevated and was nearly  
299 eliminated when  $T_{\text{root}}$  was elevated alone, indicating the inhibiting role of elevated  $T_{\text{root}}$  in  $\text{NAR}_C$ .  
300  $\text{NAR}_C$  is the result of leaf photosynthetic rate minus total plant respiration per unit leaf area. Since  
301 both leaf photosynthetic capacity and LAR increased at elevated  $T_{\text{root}}$  (the higher LAR, the lower leaf  
302 mass per unit area; **Table 4** and **Figure 1**), stimulated respiration rate should be mainly responsible  
303 for the reduction in  $\text{NAR}_C$  at elevated  $T_{\text{root}}$ . Additionally, because the maintenance respiration rate is  
304 generally higher in roots than in leaves (Lambers et al., 1983), and because root respiration increase  
305 with increasing temperature (Atkin et al., 2005), the increased RMF : LMF ratio also contributed to  
306 the reduction of net carbon accumulation at elevated  $T_{\text{root}}$  in this study. For nitrogen absorption,  
307 compared with  $T_{\text{root}}$ ,  $T_{\text{air}}$  had only a slight influence on root size but a predominant effect on  $\text{SAR}_N$   
308 (**Figure 4**). Such distinct impacts resulted in that when  $T_{\text{air}}$  was changed,  $\text{SAR}_N$  contributed more  
309 than RLR to the variation of  $R_N$ , but this trend was reversed when  $T_{\text{root}}$  was changed (**Figure 5** and  
310 **Figure 7**). It is generally comprehensible that  $T_{\text{air}}$  has less influence on root size than  $T_{\text{root}}$ , since the  
311  $T_{\text{root}}$  has more direct effects on root hydraulic status (Wan et al., 1999; Lee et al., 2004; Lee et al.,  
312 2005). Supportive results can be found in the researches of Engels and Marschner (1990) and  
313 Larigauderie et al. (1991), although in these studies data were not presented in the form of RLR or  
314 root area ratio. The influence of  $T_{\text{air}}$  on nutrient uptake is generally considered to be regulated by  
315 sugar signals (Stitt and Krapp, 1999). For instance, exogenous application of sugars can increase  
316 nitrate reductase activity (Reda, 2015). Moreover, a bZIP transcription factor *Arabidopsis*  
317 *ELONGATED HYPOCOTYL5* (HY5) has been reported to mediate sucrose signal and promote root  
318 nitrate uptake by activating *NRT2.1* (Cerezo et al., 2001; Chen et al., 2016). A previous study (Engels  
319 et al. 1992) has reported a promotion effect of elevated shoot base temperature on nutrient  
320 translocation rates per unit root fresh weight, but this effect was not examined at elevated  $T_{\text{root}}$ . Our  
321 observation indicates that the sink-mediated regulation, induced by  $T_{\text{air}}$  management, may overtake  
322 the direct effects of  $T_{\text{root}}$  on length-based  $\text{SAR}_N$ . However, Weih and Karlsson (2001) showed that  
323 raising  $T_{\text{root}}$  was more efficient than raising  $T_{\text{air}}$  in increasing nitrogen uptake rate per unit root dry  
324 weight. Actually, this result doesn't go against our observation, since the trend was also reversed  
325 when transforming length-based  $\text{SAR}_N$  into weight-based  $\text{SAR}_N$  (**Supplementary Table S2**) by  
326 multiplying SRL in our study. Thus, the significant influence of  $T_{\text{root}}$  on SRL may be partly  
327 responsible for counteracting the extent of  $T_{\text{root}}$  effect on length-based  $\text{SAR}_N$ .

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



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

### 334 **Adaptive Phenotypic Plasticity in Response to Altered $T_{\text{air}}$ and $T_{\text{root}}$ in Plants**

335 In a heterogeneous temperature ( $T_{\text{air}}$  vs  $T_{\text{root}}$ ) environment, cucumber seedlings tended to invest less  
336 biomass and generate relatively smaller organ in the cooler zone. Such passive response would  
337 potentially strengthen rather than relieve the limitation of the corresponding resource. This trend  
338 seems to go against the functional equilibrium hypothesis, that a plant would invest more biomass in  
339 the organ responsible for acquiring the most limiting resource (Brouwer, 1963; Freschet et al.,  
340 2015b). Based on previous studies regarding biomass allocation, there are both supportive  
341 (Tachibana, 1982; Clarkson et al., 1986; Delucia et al., 1992; Danyagri and Dang, 2014) and opposed  
342 (Davidson, 1969; Engels and Marschner, 1990; Li et al., 1994; Yan et al., 2012) evidences. It is  
343 possible that various factors (e.g. species, temperature, growth medium and ontogenetic stage) may  
344 also have impacts on the direction of biomass allocation. For the morphological response, low  
345 temperature-induced hydraulic limitation (Murai-Hatano et al., 2008; Wang et al., 2016) and abscisic  
346 acid accumulation (Zhang et al., 2008; Ntatsi et al., 2014), which are generally less combined with  
347 nutrient and/or light limitation, are both responsible for retarding leaf and root growth (Walter et al.,  
348 2009; Pantin et al., 2011). Therefore, in response to temperature variation, changes in the trend of  
349 biomass allocation and relative leaf : root size ratio may be more passive rather than adaptive  
350 compared with those in respond to nutrient and/or light variation.

351 According to the above response and the ‘balanced growth’ hypothesis,  $NAR_C : SAR_N$  should vary  
352 against the trend of  $LAR : RLR$  in order to maintain balanced carbon-nutrient acquisition (i.e.  $R_C :$   
353  $R_N$ ). Actually, in this study,  $NAR_C : SAR_N$  increased no matter which of  $T_{\text{air}}$  and  $T_{\text{root}}$  was cooled. At  
354 low  $T_{\text{air}}$ , the higher  $NAR_C : SAR_N$  counterbalanced the lower  $LAR : RLR$ , resulting in a relatively  
355 constant  $R_C : R_N$ . At low  $T_{\text{root}}$ , however, the higher  $NAR_C : SAR_N$  was accompanied by a higher  $LAR$   
356  $: RLR$ , leading to a large increase in  $R_C : R_N$  (i.e. carbon accumulation or nitrogen limitation). As  
357 discussed above, the counteracting effect of root respiration on total carbon acquisition could be one  
358 of the main reasons for carbon accumulation when  $T_{\text{root}}$  was cooled. In addition, the sugar-induced  
359 increase in nitrogen acquisition could be largely inhibited by limited transporter activity (Reay et al.,  
360 1999) and root size at low  $T_{\text{root}}$ . In response to nitrogen limitation, newly gained nitrogen was more  
361 unevenly distributed between older leaves and other organs to ensure adequate nitrogen concentration  
362 for growth in the latter (L/L vs L/H, H/L vs H/H seedlings, **Figure 6**).

### 363 **The $T_{\text{air}}$ and $T_{\text{root}}$ Interactively Determine Root Size**

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

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

381 In this study, cucumber seedlings with the same number of leaves were compared after different  
382 treatments, and this was originally designed to avoid ontogenetic effects. The period used for new  
383 leaf initiation was changed only by varying  $T_{\text{air}}$ . This is consistent with the report of Savvides et al.  
384 (2016), which showed that the rate of cucumber leaf initiation was completely determined by apical  
385 bud temperature independent of the temperature of other plant organs. Although apical bud  
386 temperature was not monitored in our experiment, it can be regarded as varying along with  $T_{\text{air}}$  rather  
387 than  $T_{\text{root}}$ . Field experiment on tomato also reported that cropping was delayed by low  $T_{\text{air}}$   
388 irrespective of  $T_{\text{root}}$  (Jones et al., 1978). However, the treatment period aiming for a uniform shoot  
389 developmental stage induced ontogenetic drift in roots, as discussed above. Thus, besides plant  
390 growth, the distinct influence of  $T_{\text{air}}$  and  $T_{\text{root}}$  variation on shoot and root development or phenology  
391 should also be taken into consideration when designing temperature control strategy for experiment  
392 or for protected cultivation.

## 393 CONCLUSION

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

## 404 AUTHOR CONTRIBUTIONS

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

## 407 FUNDING

408 This work was supported by the Special Fund for the National Key Research Development Program  
409 of China (2016YED201003) and the earmarked fund for the China Agriculture Research System  
410 (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 ( $T_{\text{air}}$ ) variation (solid lines) and root-zone temperature ( $T_{\text{root}}$ ) variation (dashed lines).  
591 Each variable is expressed on a  $\log_2$ -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  $T_{\text{air}}$  and  $T_{\text{root}}$  is  
594 displayed in each panel. L/L, low  $T_{\text{air}}$ /low  $T_{\text{root}}$ ; L/H, low  $T_{\text{air}}$ /high  $T_{\text{root}}$ ; H/L, high  $T_{\text{air}}$ /low  $T_{\text{root}}$ ; H/H,  
595 high  $T_{\text{air}}$ /high  $T_{\text{root}}$ . 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  $T_{\text{air}}$ /low  
600  $T_{\text{root}}$ ; L/H, low  $T_{\text{air}}$ /high  $T_{\text{root}}$ ; H/L, high  $T_{\text{air}}$ /low  $T_{\text{root}}$ ; H/H, high  $T_{\text{air}}$ /high  $T_{\text{root}}$ .

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 " $T_{\text{all}}$  Variation" refers  
603 to L/L versus H/H; " $T_{\text{root}}$  Variation" refers to the mean value of L/L versus L/H and H/L versus H/H;  
604 " $T_{\text{air}}$  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  $T_{\text{root}}$ ) and L/H versus H/H (optimal  $T_{\text{root}}$ )  
606 respectively, due to the significant difference between the two conditions. LAR, leaf area ratio; RLR,  
607 root length ratio. L/L, low  $T_{\text{air}}$ /low  $T_{\text{root}}$ ; L/H, low  $T_{\text{air}}$ /high  $T_{\text{root}}$ ; H/L, high  $T_{\text{air}}$ /low  $T_{\text{root}}$ ; H/H, high  
608  $T_{\text{air}}$ /high  $T_{\text{root}}$ .

609 **Figure 4.** Responses of leaf and root size and assimilation/absorption rate to air temperature ( $T_{\text{air}}$ )  
610 variation (solid lines) and root-zone temperature ( $T_{\text{root}}$ ) variation (dashed lines). Each variable is  
611 expressed on a  $\log_2$ -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  $T_{\text{air}}$  and  $T_{\text{root}}$  is  
614 displayed in each panel. L/L, low  $T_{\text{air}}$ /low  $T_{\text{root}}$ ; L/H, low  $T_{\text{air}}$ /high  $T_{\text{root}}$ ; H/L, high  $T_{\text{air}}$ /low  $T_{\text{root}}$ ; H/H,  
615 high  $T_{\text{air}}$ /high  $T_{\text{root}}$ . 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 ( $\text{NAR}_C$  or  $\text{SAR}_N$ ) and  
617 size ( $\overline{\text{LAR}}$  or  $\overline{\text{RLR}}$ ) variables to the total variation in  $R_C$  and  $R_N$ . The category " $T_{\text{all}}$  Variation" refers to  
618 L/L versus H/H; " $T_{\text{root}}$  Variation" refers to the mean value of L/L versus L/H and H/L versus H/H;  
619 " $T_{\text{air}}$  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  $T_{\text{root}}$ . L/L, low  $T_{\text{air}}$ /low  $T_{\text{root}}$ ; L/H,  
621 low  $T_{\text{air}}$ /high  $T_{\text{root}}$ ; H/L, high  $T_{\text{air}}$ /low  $T_{\text{root}}$ ; H/H, high  $T_{\text{air}}$ /high  $T_{\text{root}}$ .

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

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

## 639 TABLES

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

Treatment	Total dry mass /mg	RGR /mg·g <sup>-1</sup> ·d <sup>-1</sup>	Leaf dry mass /mg	Root dry mass /mg	Total leaf area /cm <sup>2</sup>	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						
$T_{\text{air}}$	409 ***	577 ***	341 ***	369 ***	129 ***	453 ***
$T_{\text{root}}$	79 ***	191 ***	37 ***	228 ***	1129 ***	2612 ***
$T_{\text{air}} \times T_{\text{root}}$	12 **	9 **	8 **	27 ***	60 ***	254 ***
Treatment	Leaf area /cm <sup>2</sup>			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												
$T_{\text{air}}$	0.566	ns	31	***	113	***	4.4	*	38	***	29	***
$T_{\text{root}}$	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 ( $T_{\text{air}}$ ), root-zone temperature ( $T_{\text{root}}$ ) 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  $T_{\text{air}}$ /low  $T_{\text{root}}$ ; L/H, low  $T_{\text{air}}$ /high  $T_{\text{root}}$ ; H/L, high  $T_{\text{air}}$ /low  $T_{\text{root}}$ ; H/H,  
 652 high  $T_{\text{air}}$ /high  $T_{\text{root}}$ .

Treatment	SLA : SRL /cm <sup>2</sup> ·m <sup>-1</sup>	LMF : RMF	LAR : RLR /cm <sup>2</sup> ·m <sup>-1</sup>
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						
$T_{\text{air}}$	12	**	84	***	245	***
$T_{\text{root}}$	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 ( $T_{\text{air}}$ ), root-zone  
 658 temperature ( $T_{\text{root}}$ ) and  $T_{\text{air}} \times T_{\text{root}}$ . L/L, low  $T_{\text{air}}$ /low  $T_{\text{root}}$ ; L/H, low  $T_{\text{air}}$ /high  $T_{\text{root}}$ ; H/L, high  $T_{\text{air}}$ /low  
 659  $T_{\text{root}}$ ; H/H, high  $T_{\text{air}}$ /high  $T_{\text{root}}$ .

Treatment	$\overline{\text{LAR}} : \overline{\text{RLR}}$ , /cm <sup>2</sup> ·m <sup>-1</sup>	$\text{NAR}_{\text{C}} : \text{SAR}_{\text{N}}$	$\text{R}_{\text{C}} : \text{R}_{\text{N}}$
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						
$T_{\text{air}}$	100	***	14	**	4	ns
$T_{\text{root}}$	439	***	14	***	317	***
$T_{\text{air}} \times T_{\text{root}}$	65	***	23	***	0	ns

660

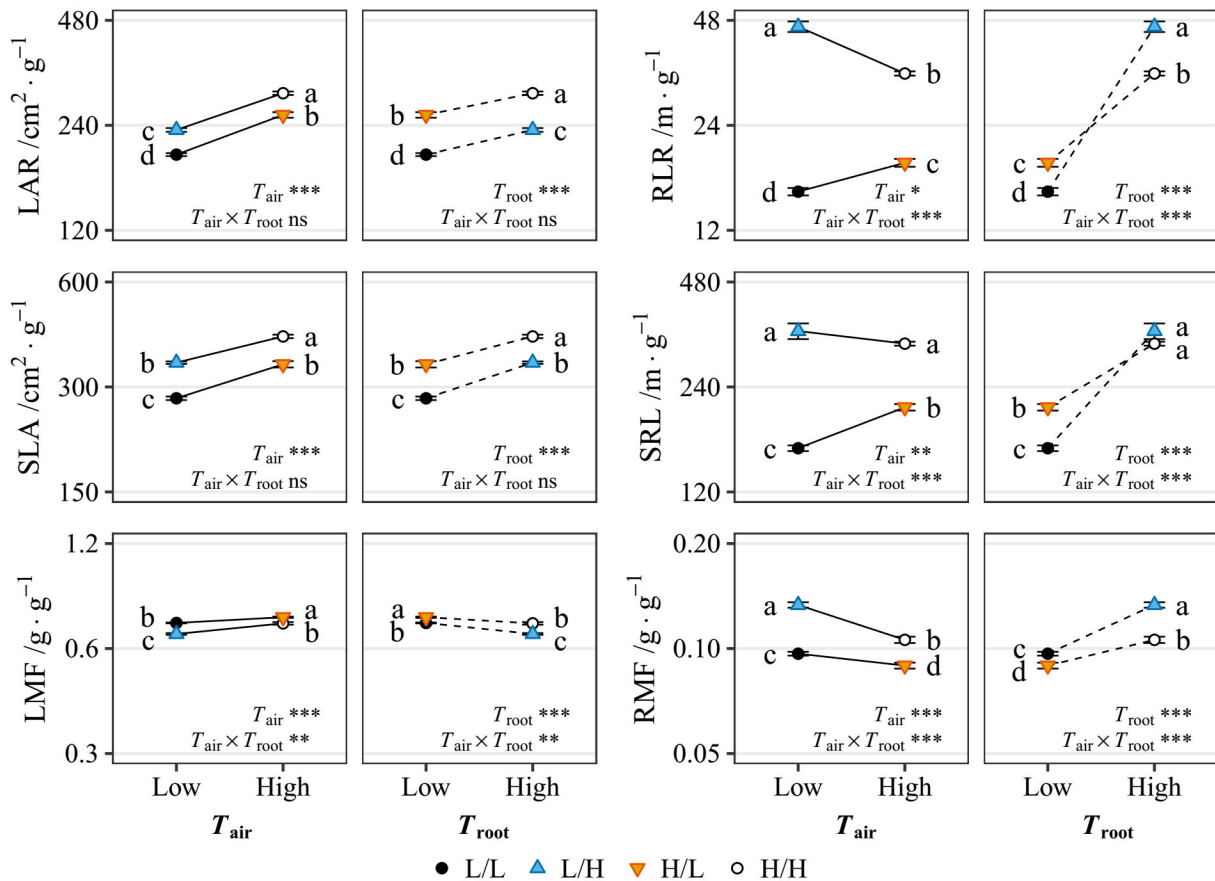
661 **Table 4.** Net photosynthetic rates of cucumber true leaves at 400  $\mu\text{mol CO}_2 \cdot \text{mol}^{-1}$  reference  $\text{CO}_2$   
 662 concentration. Means with different letters are significantly different ( $P < 0.05$ ,  $n = 7$ ) by Tukey HSD.  
 663 Source of variation:  $F$  values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

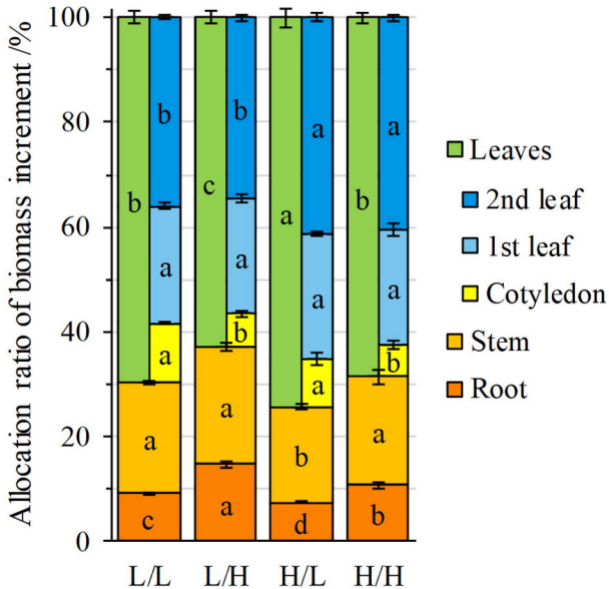
Treatment	$A_{400}$ $\mu\text{mol CO}_2 \cdot \text{m}^{-1} \cdot \text{s}^{-1}$			
	1st leaf		2nd leaf	
L/L	9.6	b	9.3	c
L/H	18.7	a	13.2	b
H/L	20.8	a	16.7	a
H/H	19.0	a	18.3	a

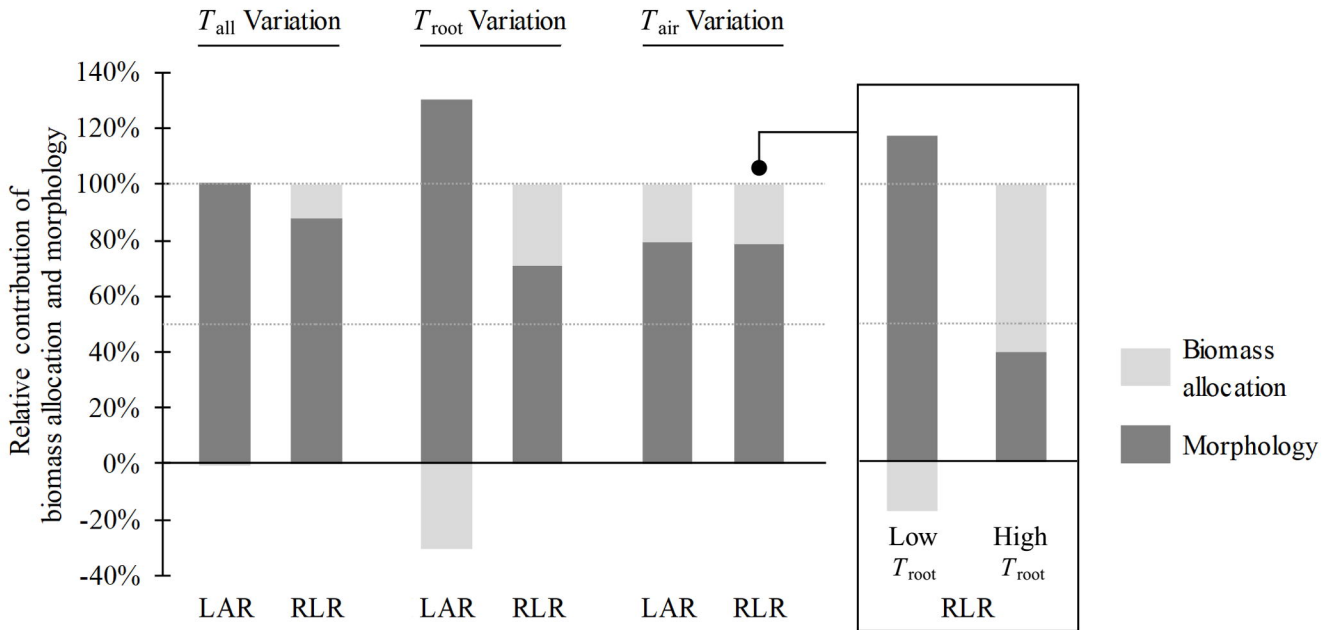
Source of variation					
$T_{\text{air}}$	54	***	207	***	
$T_{\text{root}}$	30	***	40	***	
$T_{\text{air}} \times T_{\text{root}}$	66	***	14	**	

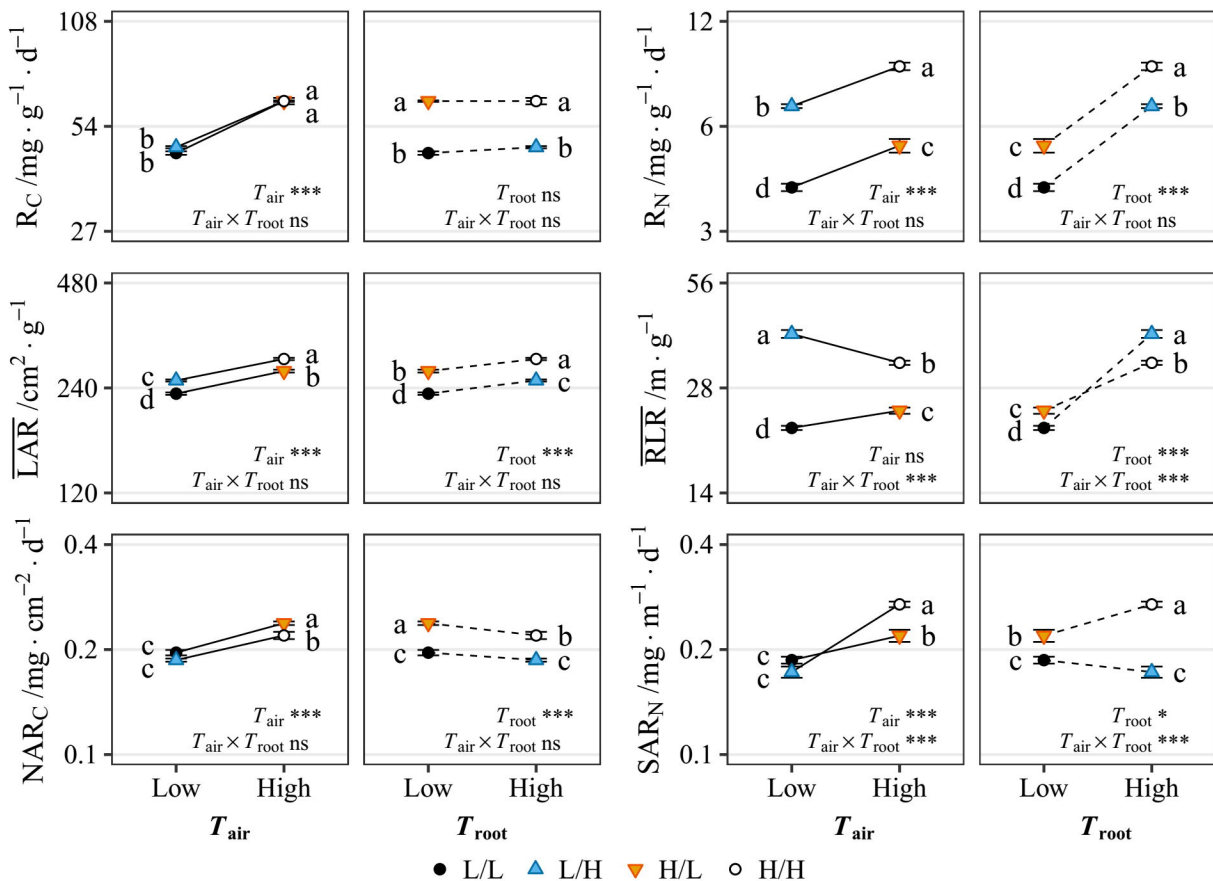
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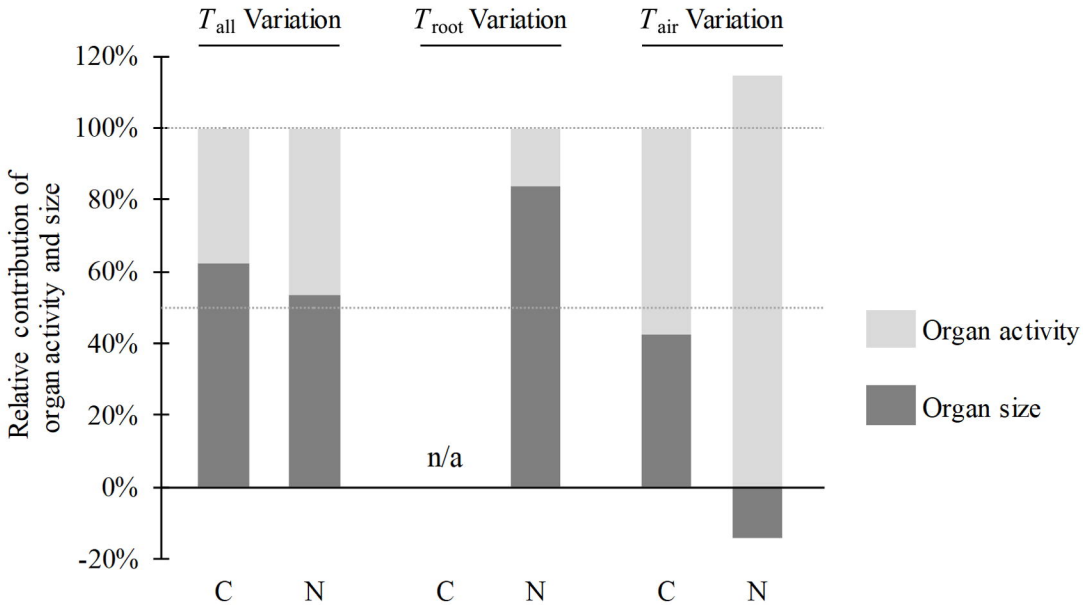


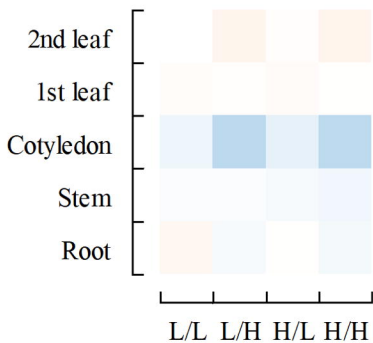
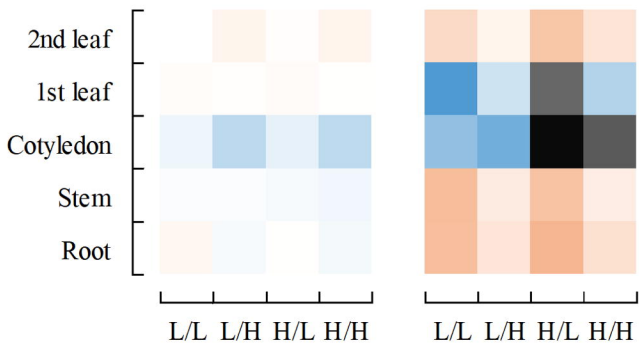
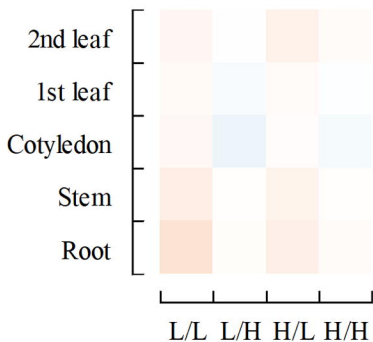
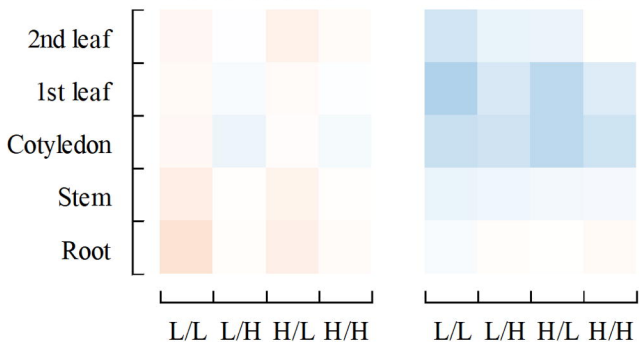










**A**Relative  
C allocation**B**Relative  
N allocation**C**Relative  
C concentration**D**Relative  
N concentration**E**

Relative C/N ratio

