Variation of ^{13}C and ^{15}N enrichments in different plant components of labeled winter wheat (*Triticum aestivum* L.)

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Information on the homogeneity and distribution of ^{13}carbon (^{13}C) and nitrogen (^{15}N) labeling in winter wheat (*Triticum aestivum* L.) is limited. We conducted a dual labeling experiment to evaluate the variability of ^{13}C and ^{15}N enrichment in aboveground parts of labeled winter wheat plants. Labeling with ^{13}C and ^{15}N was performed on non-nitrogen fertilized (−N) and nitrogen fertilized (+N, 250 kg N ha^{-1}) plants at the elongation and grain filling stages. Aboveground parts of wheat were destructively sampled at 28 days after labeling. As winter wheat growth progressed, δ^{13}C values of wheat ears increased significantly, whereas those of leaves and stems decreased significantly. At the elongation stage, N addition tended to reduce the aboveground δ^{13}C values through dilution of C uptake. At the two stages, upper (newly developed) leaves were more highly enriched with ^{13}C compared with that of lower (aged) leaves. Variability between individual wheat plants and among pots at the grain filling stage was smaller than that at the elongation stage, especially for the −N treatment. Compared with those of ^{13}C labeling, differences in ^{15}N excess between aboveground components (leaves and stems) under ^{15}N labeling conditions were much smaller. We conclude that non-N fertilization and labeling at the grain filling stage may produce more uniformly ^{13}C-labeled wheat materials, whereas the materials were more highly ^{13}C-enriched at the elongation stage, although the δ^{13}C values were more variable. The ^{15}N-enriched straw tissues via urea fertilization were more uniformly labeled at the grain filling stage compared with that at the elongation stage.

1   **TITLE PAGE**

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3   Variation of ^{13}C and ^{15}N enrichments in different plant components of labeled winter wheat

4   (*Triticum aestivum* L.)

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ABSTRACT

Information on the homogeneity and distribution of ^{13}carbon (^{13}C) and nitrogen (^{15}N) labeling in

winter wheat (*Triticum aestivum* L.) is limited. We conducted a dual labeling experiment to

evaluate the variability of ^{13}C and ^{15}N enrichment in aboveground parts of labeled winter wheat

plants. Labeling with ^{13}C and ^{15}N was performed on non-nitrogen fertilized (−N) and nitrogen

fertilized (+N, 250 kg N ha^{-1}) plants at the elongation and grain filling stages. Aboveground

parts of wheat were destructively sampled at 28 days after labeling. As winter wheat growth

progressed, δ^{13}C values of wheat ears increased significantly, whereas those of leaves and stems

decreased significantly. At the elongation stage, N addition tended to reduce the aboveground

δ^{13}C values through dilution of C uptake. At the two stages, upper (newly developed) leaves

were more highly enriched with ^{13}C compared with that of lower (aged) leaves. Variability

between individual wheat plants and among pots at the grain filling stage was smaller than that at

the elongation stage, especially for the −N treatment. Compared with those of ^{13}C labeling,

differences in ^{15}N excess between aboveground components (leaves and stems) under ^{15}N

labeling conditions were much smaller. We conclude that non-N fertilization and labeling at the

grain filling stage may produce more uniformly ^{13}C-labeled wheat materials, whereas the

materials were more highly ^{13}C-enriched at the elongation stage, although the δ^{13}C values were

more variable. The ^{15}N-enriched straw tissues via urea fertilization were more uniformly labeled

at the grain filling stage compared with that at the elongation stage.

KEY WORDS: ^{13}CO$_2$ pulse labeling, ^{15}N labeling, homogeneity, winter wheat.

INTRODUCTION

44   Precise quantification of carbon (C) and nitrogen (N) cycling in plant–soil systems requires

45   the use of specialized techniques, particularly for tracking above- and belowground residue C

46   and N dynamics in soil (*Shan et al., 2012*; *Meng et al., 2017; Zheng et al., 2018; Xu et al., 2019*).

47   This reflects the difficulty of detecting small changes in soil organic C and N against the

48   background of substantial soil C and N pools (*Liang et al., 2013; Sun al., 2018*; *Pausch &*

49   *Kuzyakov et al., 2018*). Isotopic labeling of plants using ^{13}C and ^{15}N is a powerful tool to track

50   the fate of C and N derived from different plant organs in a soil–plant system when the ^{13}C- and

51   ^{15}N-labeled tissues decompose simultaneously during in situ (*An et al., 2015; Xu et al., 2019*) or

52   laboratory (*Meng et al., 2017; Fang et al., 2018*) incubation. However, to draw unbiased

53   conclusions from the fate of isotope-labeled straw C and N, quantitatively tracing the distribution

54   of labeled straw C and N in various soil C and N pools require uniform ^{13}C- or ^{15}N-labeled plant

55   materials, because the calculations assume that all plant parts are evenly labeled (*Thompson,*

56   *1996; Girardin et al., 2009; Nguyen Tu et al., 2013; Soong et al., 2014*). Therefore, assessment

57   of the variability and the degree of ^{13}C or ^{15}N label enrichment among plant organs and tissues is

58   important because the rate of decomposition differs among plant tissues. In addition, the

59   homogeneity of the signal in labeled plant residues may affect quantification of the proportional

60   contribution of soil organic matter and supplementary plant material to plant nutrient uptake in

61   decomposition studies.

62   Temporal variability in ^{15}N among N sources in the soil and in allocation of N to different

63   plant organs will result in labeled plant tissues that differ markedly in ^{15}N contents (*Wagger,*

64   *Kissel & Smith, 1985; Crozier, King & Volk, 1993*). Uniform labeling of tissues is a potential

65   advantage of using a single source of N in a controlled growth system, such as a soil-free culture

66   system supplied with ^{15}N-fertilizer solution. Homogeneous ^{15}N enrichment among plant organs

67   can be relatively easy to achieve by fertilizing the plants with a ^{15}N tracer (*Liang et al., 2013;*

68   *Soong et al., 2014*), whereas attaining uniformly ^{13}C-labeled crop residues by means of ^{13}CO$_2$

69   fixation poses numerous challenges (*Thompson, 1996; Girardin et al., 2009; Nguyen Tu et al.,*

70   *2013; Soong et al., 2014*). The latter impediment is because rates of C incorporation

71   (photosynthesis) differ widely among plant organs and thus enhances variation in ^{13}C isotope

72   incorporation in the plant tissues (*Thompson, 1996; Girardin et al., 2009; Nguyen Tu et al.,*

73   *2013; Soong et al., 2014*). Pulse or continuous labeling of aboveground plant parts has frequently

74   been used to generate ^{13}C- or ^{14}C-labeled plant materials (*Zhu & Cheng, 2011*; *An et al., 2015;*

75   *Meng et al., 2017*). Continuous supply of either ^{14}C or ^{13}C is advantageous over pulse labeling

76   for uniform labeling of all plant organs, but the procedure requires specialized facilities and is

77   applied from emergence of the first leaf until harvest, therefore it is impossible to apply this

78   approach under field conditions (*Zhu & Cheng, 2011*). Labeling with ^{13}C (or ^{14}C) and ^{15}N under

79   field conditions shows potential for analysis of C and N dynamics derived from crop residues,

80   which are affected by the chemical composition and quantities of the crop residue (*Berg et al.,*

81   *1991; Tahir et al., 2018*). Concentrations of phenolic compounds, for example, may alter

82   decomposition rates (*Fox, Myers & Vallis, 1990; Ranells & Wagger, 1992*), may be sensitive to

83   differences in field, greenhouse, or growth-chamber ultraviolet light intensities (*Crozier, King &*

84   *Volk, 1993*). Pulse labeling is advantageous over continuous labeling for field isotopic labeling

85   because pulse labeling is easier to execute and requires simple instrumentation (*Berg et al.,*

86   *1991; Tahir et al., 2018*). The majority of labeled crop residues used in previous studies of C and

87   N dynamics were labeled with ^{13}C (or ^{14}C) and ^{15}N tracers under controlled conditions.

88   To produce labeled crop residues, previous field labeling experiments mainly focused on

89   the degree of ^{13}C and ^{15}N enrichment in crop residues (*Berg et al., 1991; Crozier, King & Volk,*

90   *1993; Tahir et al., 2018*). However, few researchers evaluated the homogeneity and distribution

91   of ^{13}C and ^{15}N labels within the labeled plant tissues, especially in residues of winter wheat,

92   which is among the most important staple crops worldwide and is frequently used in labeling

93   studies (*Jia et al., 2011*; *Butterly et al., 2015*; *Chen et al., 2016; Sun et al., 2018*). In annual

94   cereal crops, the plants grow vigorously and enrichment of labeled C is higher during the

95   juvenile stage than at maturity (*Meng et al., 2013; Liu, Jiang & Li , 2015; Sun et al., 2018*). The

96   majority of C labeling studies have been conducted in the early developmental stages of wheat,

97   e.g., continuous labeling 60 days after emergence (*Liljeroth, Kuikman & Van Veen, 1994; Marx*

98   *et al., 2007*) and pulse labeling <150 days after emergence (*Martens et al., 2009; Butterly et al.,*

99   *2015*). Few studies have compared the enrichment of labeled C for multiple labeling periods. In

100 addition, heavy application of N fertilizer delays senescence in wheat, which results in a

101 substantial quantity of non-structural carbohydrates remaining in the straw and affects

102 photosynthesis (*Yang & Zhang, 2006*), may lead to changes in the degree of labeled C

103 enrichment in straw.

104   To evaluate the uniformity of labeling within plants, in the present study the shoot of an

105 individual plant of winter wheat was divided into leaves and the stem from three sections of

106   equal length using the method described by *Thompson* (*1996*). We conducted a ^{13}C pulse-

107   labeling and ^{15}N fertilization experiment for labeling pot-grown winter wheat under field

108   conditions at different growth stages. The objectives were (i) to assess the homogeneity and

109   degree of ^{13}C and ^{15}N enrichment among different organs and among identical organs (stems and

110   leaves) in different portions of the shoot (upper, middle, and lower); and (ii) to investigate the

111   impacts of wheat growth and N fertilization on isotope variability among aboveground

112   compartments after ^{13}C labeling.

113   **MATERIALS AND METHODS**

114   **Treatments and ^{15}N labeling procedure**

115   In the current study, we used winter wheat (*Triticum aestivum* L. 'Luyuan 502'), a staple

116   crop grown worldwide (*Zhao et al., 2016*). To examine the impacts of wheat growth stage and N

117   fertilization on variation in ^{13}C enrichment of winter wheat, two N treatments (0 mg N kg^{-1} soil,

118   -N; and 90 mg N kg^{-1} soil, equivalent to 250 kg N ha^{-1}, +N) were applied, and wheat plants

119   grown in a calcareous soil (fluvo-aquic sandy loam) were pulse-labeled with ^{13}CO$_2$ at the

120   elongation (168 days after sowing; DAS) and grain filling (205 DAS) stages (Figure 1a and b).

121   To examine the degree of variation in ^{15}N enrichment among aboveground compartments, ^{15}N-

122   labeled fertilizer (^{15}N-labeled urea; Shanghai Research Institute of Chemical Industry, Shanghai,

123   China) was applied by direct addition to the soil for the +N treatment (^{15}N 10.14 atom %; about

124   US$10 per gram of ^{15}N labeled urea). Additional wheat plants were selected as ^{15}N natural

125   abundance controls and were fertilized with unlabeled urea as for +N treatment. Ten pre-

126   germinated (24–48 h) winter wheat seeds were sown directly into PVC pots (30 cm × 20 cm i.d.)

127   containing 8.7 kg air-dried soil, and then the soil was moistened to 70% water-holding capacity.

128   One week after germination (20 October, 2014), all except the six strongest seedlings were

129   removed from each pot (equivalent to a field planting density of 1.5 million plants ha^{-1}). The

130   experiment was conducted under field conditions. For the +N treatment, half of the ^{15}N labeled

131   or unlabeled urea was applied as a basal dressing at sowing (0.84 g ^{15}N labeled or unlabeled urea

132   was applied to each pot) and the remaining half was used to topdress plants at the elongation

133   stage (15 March, 2015). The wheat plants for the first $^{13}CO_2$ labeling were fertilized as a single

134   basal application and received 45 mg N kg^{-1} air-dried soil. The wheat plants for the second

135   $^{13}CO_2$ labeling received a total of 90 mg N kg^{-1} air-dried soil in two applications during the

136   experiment (1.68 g ^{15}N labeled or unlabeled urea was applied to each pot, which corresponded to

137   a total fertilization of 250 kg N ha^{-1}), which was split into 2 × 45 mg N kg^{-1} air-dried soil

138   applications (basal urea N and topdressing urea N). Monopotassium phosphate (KH_2PO_4)

139   fertilizer was applied as a basal dressing at the rate 17 mg P and 18 mg K kg^{-1} air-dried soil,

140   which conformed with the local agricultural practice. To simulate plant litter similar to that

141   occurring under the field-grown conditions in this study, we applied ^{15}N-urea to the soil of pot-

142   grown winter wheat in the field rather than supplying ^{15}N-fertilizer solution to a soil-free culture

143   system.

144   **Growth conditions and ^{13}C pulse-labeling procedure**

145   Soil was collected from the plough layer (0–20 cm) of farmland near the Agro-Ecosystem

146   Experimental Station of China Agricultural University, Huantai County, Shandong Province

147   (36°57′N, 117°59′E, 18 m elevation). The soil of the experimental field was derived from

148   Yellow River alluvial sediments and was classified as an Aquic Inceptisol (a calcareous, fluvo-

149   aquic sandy loam; *Shi et al., 2013*). Soil properties were as follows: soil organic C = 8.4 g kg^{-1},

150   soil inorganic C = 5.2 g kg^{-1}, total N = 0.67 g kg^{-1}, NH_4-N = 1.6 mg kg^{-1}, NO_3-N = 17.2 mg

151   kg^{-1}, pH = 8.2 (soil:water = 1:2.5), available K = 174 mg kg^{-1}, and Olsen P = 5.2 mg kg^{-1}. After

152   sampling, the soil was air-dried, homogenized, and sieved using a 5 mm screen prior to the

153   experiment. Each pot (height = 30 cm; inner diameter = 20 cm) was filled with 8.7 kg of air-

154   dried soil to a bulk density of 1.38 g cm^{-3}. Wheat roots were mainly concentrated in the 0–20 cm

155   soil layer and contributed 57.6%–78.6% of the 0–100 cm total root weight in typical farmland on

156   the North China Plain (*Hou, 2011*). Hence, pots with 30 cm soil depth were considered sufficient

157   for normal wheat root development in this study. The pot-grown winter wheat seedlings were

158   transferred to an experimental field at the Agro-Ecosystem Experimental Station. The pots

159   containing wheat plants were placed in cropland holes (30 cm depth; 24 cm diameter) to simulate

160   local wheat-growing conditions. The total growth period was 230 days, with six developmental

161   stages recognized: (i) seeding (0–17 DAS), (ii) tillering (18–150 DAS), (iii) elongation (151–179

162   DAS), (iv) anthesis (180–193 DAS), (v) grain filling (194–214 DAS), and (vi) dough ripening

163   (215–230 DAS). Precipitation was 147 mm and the soil temperature ranged from −1.6 to 29.7 °C

164   during the wheat growing season (*Zhao et al., 2017*). The soil water content of each pot, which

165   was controlled gravimetrically to simulate local wheat production, was adjusted daily to 65%

166   (during the seedling stage), 70% (tillering), 80% (elongation), 80% (anthesis), and 70%–75%

167   (grain-filling) of the field water-holding capacity in accordance with the amount of rainfall and

168   evaporation.

169   Labeling with $^{13}CO_2$ was performed at the elongation and grain filling stages (i.e., 168 and

170   205 DAS). On each occasion, four pots were randomly selected for $^{13}CO_2$ labeling from each of

171   the −N and +N treatments, and an additional eight pots were selected as $^{13}CO_2$ unlabeled controls

172   and were maintained separately from the labeled plants. Hence, we used 32 pots in the

173   experiment, with three replicates for each N treatment, for destructive sampling at 28 days after

174   labeling during each of the developmental stages (three from four pots per sampling for each

175   treatment): eight pots were dual labeled with ^{13}C and ^{15}N in the +N treatment; eight pots were

176   labeled with ^{13}C for the −N treatment; and an additional 16 pots were selected as unlabeled

177   controls (eight pots for −N and eight pots for +N). We considered each pot to be a technical

178   replicate for each treatment (three from four pots per sampling for each treatment), and took each

179   plant within a pot to be a biological replicate (three plants chosen within the pot). A chamber

180   (0.6 m long × 1.0 m high; Figure 1c), adapted from *Swinnen, Van Veen & Merckx* (1994) and

181   *Meng et al*. (2013), was used for $^{13}CO_2$ labeling. On the previous day, the soil surface was

182   covered with a PVC board and sealed with silicon, including around the stems. To remove all

183      $^{12}CO_2$ before labeling, the air inside the chamber was continuously circulated for 30 min through

184   10 M NaOH solution using a membrane pump. The ^{13}C-labeled $Na_2^{13}CO_3$ at 98 atom % ^{13}C

185   (about US$100 per gram of $Na_2^{13}CO_3$) was provided by the Shanghai Research Institute of

186   Chemical Industry. A beaker containing $Na_2^{13}CO_3$ (8.0 g $Na_2^{13}CO_3$ for each labeling event) was

187   placed inside the chamber. To determine the CO_2 concentration within the chamber using an

188   infrared gas analyzer (CI301PS; CID Bio-Science, Camas, WA, USA), an unlabeled control

189   treatment with an additional four pots was set up in a separate chamber under identical

190 conditions except that Na_2CO_3 was used to produce non-labeled CO_2. This arrangement was used

191 because of the different wavelengths for maximum absorption of $^{13}CO_2$ and $^{12}CO_2$ in the infrared

192 detector (*Meng et al., 2013*). If the rate of decline in CO_2 concentration slowed considerably

193 (below 200 μL L^{-1}) in the chamber containing the unlabeled control treatment, 1 M H_2SO_4 was

194 injected until the CO_2 concentration increased to approximately 360 μL L^{-1}. The same volume of

195 H_2SO_4 solution was also injected into the chamber housing the plants undergoing labeling. A fan

196 was used to homogenize the atmosphere within each chamber. After 7 h of labeling, plants were

197 removed from the chamber to prevent re-assimilation of shoot-respired $^{13}CO_2$.

198 **Sampling and chemical analyses**

199 The ^{13}C-labeled winter wheat plants and soils were destructively sampled 28 days after each

200 labeling. No significant difference between the mean aboveground biomass and root biomass of

201 the ^{13}C pulse-labeled plants and the unlabeled control plants was observed when they were

202 harvested 28 days after labeling during each of the developmental stages. This observation

203 indicated that ^{13}C pulse labeling did not affect plant growth. To examine the uniformity of

204 labeling within a wheat plant, individual wheat plants were collected at the final harvest and the

205 shoots (stem and leaves) were divided into three equal-length portions (upper, middle, and lower

206 portions; Figure 1d). Separate analyses of ^{13}C and ^{15}N enrichment were undertaken on the leaf

207 and stem portions as well as the ears. Three plants per pot were assessed for variation in ^{13}C and

208 ^{15}N enrichment. Shoots (stems and leaves) and ears were oven-dried at 60 °C to a constant

209 weight. All samples were ground to a fine powder (<500 μm) in a ball mill (Restol MM2000,

210 Retsch, Haan, Germany) prior to analysis.

211   The ^{13}C and ^{15}N values of wheat plant materials were determined using isotope ratio mass

212   spectrometry (DELTAplus XP, ThermoFinnigan, Bremen, Germany). The abundance of ^{13}C was

213   expressed as parts per thousands (‰) relative to the international standard (Pee Dee Belemnite;

214   0‰) expressed as delta units (δ) (*Craig, 1953*). The ^{15}N enrichment of ^{15}N-labeled plant parts

215   fertilized with labeled urea was reported as atom % ^{15}N excess calculated by subtracting the

216   values for the ^{15}N natural abundance control samples fertilized with unlabeled urea (*Coplen,*

217   *2011*).

218   **Data processing and statistical analysis**

219   To estimate the weight-average ^{13}C and ^{15}N values of the aboveground parts and three equal-

220   length portions (upper, middle, and lower portion) of the shoot of winter wheat plants, a simple

221   isotopic mass balance mixing equation was used (*Thompson, 1996*):

222    $$\delta^{13}C_{abveground} = \delta^{13}C_{ear} \times F_{ear} + \delta^{13}C_{leaves} \times F_{leaves} + \delta^{13}C_{stem} \times F_{stem} \qquad (1)$$

223    $$^{15}N_{abveground} = {}^{15}N_{ear} \times F_{ear} + {}^{15}N_{leaves} \times F_{leaves} + {}^{15}N_{stem} \times F_{stem} \qquad (2)$$

224    $$1 = F_{ear} + F_{leaves} + F_{stem} \qquad (3)$$

225   where $\delta^{13}C_{abveground}$, $\delta^{13}C_{ear}$, $\delta^{13}C_{leaves}$, and $\delta^{13}C_{stem}$ is the C isotope value (δ) for aboveground

226   parts, ear, leaves, and stem of winter wheat, respectively; $^{15}N_{abveground}$, $^{15}N_{ear}$, $^{15}N_{leaves}$, and

227    $^{15}N_{stem}$ is the ^{15}N excess (in atom %) for aboveground parts, ear, leaves, and stem of winter

228   wheat, respectively; and F_{ear}, F_{leaves}, and F_{stem} is the total aboveground dry weight of winter

229   wheat plants in the ear, leaves and stem fractions, respectively.

230    $$\delta^{13}C_{i\ portion} = \delta^{13}C_{i\ leaves} \times F_{i\ leaves} + \delta^{13}C_{i\ stem} \times F_{i\ stem} \qquad (4)$$

231 $$^{15}N_{i\ portion} = \,^{15}N_{i\ leaves} \times F_{i\ leaves} + \,^{15}N_{i\ stem} \times F_{i\ stem}$$ (5)

232 $$1 = F_{i\ leaves} + F_{i\ stem}$$ (6)

233 where $\delta^{13}C_{i\ portion}$ and $^{15}N_{i\ portion}$ are the C isotope value (δ) and ^{15}N excess (in atom %) for the

234 upper, middle, and lower portion, respectively, of the shoot of winter wheat plants; and $F_{i\ leaves}$

235 and $F_{i\ stem}$ are the respective leaves and stem fractions in the same portion of the shoot.

236   Statistical analysis was carried out with SPSS (version 11.0, 2002; SPSS, Chicago, IL,

237 USA). Two-way analysis of variance was performed to analyze the effect of N fertilization

238 and/or developmental stage on ^{13}C and ^{15}N distribution in different aboveground components of

239 the winter wheat plants. The least significant difference (LSD) test was used to examine

240 differences in ^{13}C and ^{15}N isotope values between the N treatments or different aboveground

241 components. The significance level of 0.05 was chosen to indicate statistical significance.

242 **RESULTS**

243 **Winter wheat biomass and total C and N contents**

244   The interactive effect of plant age and N fertilizer treatment on increasing the biomass of

245 the ear and whole plant was significant (Table 1 and Figure 2a), which indicated that the effect of

246 N fertilization tended to be stronger as wheat growth progressed. Straw, ear, and whole-plant

247 biomass were significantly higher in the +N treatment than in the −N at the elongation and grain

248 filling stages (Figure 2a). With progression of growth, the C and N contents of wheat straw and

249 the ear significantly increased (Table 1 and Figure 2b, c), whereas no significant differences in

250 the roots were observed from the elongation to the grain filling stages (Table 1 and Figure 2a).

251 Compared with those of the −N treatment, N fertilization significantly decreased the C content of

252 straw, and significantly increased the N content of wheat straw and the ear at the elongation and

253 grain filling stages (Table 1 and Figure 2b, c).

$\delta^{13}C$ values of aboveground components

255 In the ^{13}C-labeled wheat plants, the highest $\delta^{13}C$ values were observed in leaves, followed

256 by stems and ears at the elongation stage. However, significantly higher ^{13}C enrichment was

257 observed in the ears compared with the other aboveground parts at the grain filling stage (Table

258 2). As growth progressed, the $\delta^{13}C$ values of ears significantly increased by 130% and 170% for

259 the −N and +N treatments, respectively. In contrast, $\delta^{13}C$ values of leaves and stems significantly

260 decreased by 82.3% (−N) and 72.2% (+N) in leaves and by 72.4% (−N) and 65.5% (+N) in

261 stems. These results indicated that growth stage had a significant impact on ^{13}C distribution

262 among ears, leaves, and stems (Table 3). Nitrogen fertilization decreased $\delta^{13}C$ values of

263 aboveground components at the elongation stage and had no significant effect at the grain filling

264 stage; however, the change was only significant in leaves at the elongation stage (36.9%; Tables

265 2 and 3).

266 Shoots (leaves and stems) of single plants were divided into three equal-length portions

267 (upper, middle, and lower), to analyze the ^{13}C distribution within the shoot. Following pulse

268 labeling at the elongation stage, $\delta^{13}C$ values of leaves and stems in the different portions showed

269 significant differences. The leaves of the lower portion was the least ^{13}C enriched for the +N and

270 −N treatments, and ^{13}C enrichment was highest in the middle and upper portions under the −N

271 and +N treatments, respectively. However, $\delta^{13}C$ values of stems were highest in the lower

272 portion, followed by the middle and upper portions, for the −N and +N treatments (Figure 3). A

273 significant difference was observed at the grain filling stage: for both leaves and stems, $\delta^{13}C$

274 values were significantly higher in the upper portion, with no significant differences between the

275 middle and lower portions observed (Figure 3).

276   When we combined $\delta^{13}C$ values from the same portions of leaves and stems at the

277 elongation stage, no significant differences were observed among the different portions for the

278 +N treatment, and $\delta^{13}C$ values of the upper and middle portions were significantly higher than

279 that of the lower portion for the −N treatment. At the grain filling stage, the $\delta^{13}C$ values of the

280 upper portion was highest, followed by those of the middle and lower portions under the −N and

281 +N treatments (Table 4).

282   Labeled plants showed coefficients of variation (CVs) for intra-pot (between individual

283 plants) $\delta^{13}C$ of 9.4%–76.4% and 6.3%–31.5% at the elongation and grain filling stages,

284 respectively. Inter-pot CVs (among plants across different pots) for $\delta^{13}C$ ranged from 16.5%–

285 59.0% at the elongation stage and 3.8%–70.9% at the grain filling stage (Table 5). Generally,

286 CVs at the grain filling stage were lower than those at the elongation stage, and N addition

287 tended to increase $\delta^{13}C$ variation, particularly at the grain filling stage.

288 **^{15}N enrichment variation in aboveground components of winter wheat**

289   Following ^{15}N-labeling, the rank order of ^{15}N excess (in atom %) of ears, leaves, and stems

290 was similar at the elongation and grain filling stages, with ^{15}N excess highest in ears, followed by

291 stems and leaves (Table 2). With regard to the three shoot portions (without ears), combined ^{15}N

292 excess of leaves and stems were similar among different portions at the elongation and grain

293   filling stages (Table 4). The ^{15}N excess of stems and leaves was similar at the elongation stage,

294   but was highest in the upper portion and lowest in the lower portion at the grain filling stage

295   (Figure 4). As growth progressed, i.e., with increase in biomass, ^{15}N excess increased by 32.1%–

296   41.8% (Table 2). Developmental stage had a significant impact on ^{15}N excess for all shoot

297   components except leaves in the lower portion (Table 3). Labeled wheat exhibited an intra-pot

298   CV of 7.7%–18.1% at the elongation and grain filling stages; the inter-pot CV was 1.7%–23.4%

299   at the elongation stage, with considerably less variation at the grain filling stage (0.1%–6.2%;

300   Table 5). At both stages, the variation in ^{15}N excess was less than that of δ^{13}C. For both ^{15}N

301   excess and δ^{13}C values, intra-pot and inter-pot CVs were comparable, which implied that the

302   degree of variation in the ^{13}C and ^{15}N distributions was similar.

303   **DISCUSSION**

304   **Variation in ^{13}C enrichment**

305   Average total δ^{13}C values of aboveground parts of winter wheat plants, resulting from

306   ^{13}CO$_2$ release from Na$_2^{13}$CO$_3$ (98 atom % ^{13}C) in the field labeling chamber, ranged from 81‰

307   to 295‰ (Table 2). Given that the majority of previous studies that involved pulse labeling of

308   wheat used ^{14}C rather than ^{13}C, we compared our findings with studies that used ^{13}C labeling in

309   other plant species. By injection of ^{13}CO$_2$ gas (90 atom % ^{13}C) in a field-labeling chamber, *Tahir*

310   *et al*. (2018) achieved a high degree of δ^{13}C enrichment in wheat with overall δ^{13}C enrichment of

311   493‰ in leaves and 357‰ in stems. *Meng et al*. (2013) enriched maize shoots to approximately

312   125‰–178‰ via ^{13}CO$_2$ release from Ba^{13}CO$_3$ (98 atom % ^{13}C) in a laboratory labeling chamber.

313   The direct ^{13}CO$_2$ labeling method has also been used to generate highly ^{13}C-enriched crop plants,

314   such as rice (533‰–927‰) (*Liu, Jiang & Li, 2015*) and clover (>1000‰) (*Thompson, 1996*).

315   The ^{13}C enrichment of plant materials is dependent on the net ^{12}C and ^{13}C assimilation rates as

316   well as the quantity of ^{13}C per gram dry weight of plant labeled, which is influenced by the

317   quantity of ^{13}C added to the labeling chamber.

318   The development of the winter wheat ear during the entire growth season was reflected in

319   the increasing allocation of ^{13}C to that reproductive organ. As growth advanced, $\delta^{13}C$ values of

320   ears significantly increased, whereas those of leaves and stems significantly decreased (Tables 1,

321   2). This pattern, i.e., an increase in the carbohydrate sink strength during plant development, is

322   typical of cereal crops. In a ^{14}C pulse-labeling experiment, *Swinnen, Van Veen & Merckx* (1994)

323   observed that as much as 85% of the ^{14}C allocated to aboveground parts of spring wheat plants

324   was recovered in the ears at the ripening stage. In the present study, the proportion of ^{14}C in ears

325   relative to all aboveground portions increased from 30% at the elongation stage to 50% at the

326   grain filling stage (Figure 2a). Among aboveground components, $\delta^{13}C$ values were also highest

327   in ears at the grain filling stage (Table 2). At the grain filling stage, the ears were conditioned by

328   the size and availability of the C pools. At the grain filling stage, the predominant C sources for

329   wheat ears are primarily from remobilization of labeled photosynthesized C from leaves and

330   stems, and from photosynthesis by the ear itself (*Palta et al., 1994; Aranjuelo et al., 2013; Zhou*

331   *et al., 2016*). Previous studies that used the natural abundance of ^{13}C and ^{13}C-labeling techniques

332   revealed that photosynthesis in the ear may account for 40%–80% of the C accumulated in the

333   ears (*Palta et al. 1994; Aranjuelo et al. 2011; Sanchez-Bragado et al. 2014*). Given that the

334   leaves and stems contained the lowest quantities of labeled C in wheat, these organs were

335   indicated to act as a C source for grain filling, with ears as the sink containing higher amounts of

336   labeled C. This result is in agreement with a previous C pulse-labeling study of wheat during

337   grain filling (*Aranjuelo et al., 2013*). However, at the elongation stage, higher ^{13}C-enriched

338   values in the shoots (leaves and stems) were observed in the present study (Table 2). These

339   results suggested that shoots acted as major labeled-C sinks owing to the poor sink strength of

340   ears at the elongation stage, whereas ears represented an important labeled-C sink that competed

341   with the shoots as the C sink at the grain filling stage (*Palta et al., 1994; Aranjuelo et al., 2013;*

342   *Zhou et al., 2016*).

343   At the elongation stage, N addition tended to reduce δ^{13}C values of aboveground

344   components through a dilution effect (Table 2). Compared with those of the −N treatment, net

345   ^{13}C assimilation in plant biomass increased by 7.8% under the +N treatment (76 mg ^{13}C pot^{-1} for

346   −N vs 82 mg ^{13}C pot^{-1} for +N), while the +N treatment showed a 35% increase in plant biomass

347   (Figure 2a). An additional likely relevant factor was reduced carbohydrate accumulation under

348   over-supplied N conditions, i.e., under the +N treatment in the current study (*Palta et al., 1991;*

349   *Gooding & Davies, 1992*). At the grain filling stage, N addition tended to increase δ^{13}C values of

350   aboveground components (Table 2). This result probably reflects the presence of a higher

351   number of green leaves in the +N treatment at an advanced stage of maturity (grain filling),

352   which leads to stronger photosynthetic capacity and hence can affect the ^{13}C-isotopic signature

353   of aboveground components.

354   At both labeled stages, ^{13}C enrichment was higher in upper (newly developed) leaves than

355   in lower (aged) leaves (Figure 3), which may be explained by the more rapid uptake of ^{13}CO$_2$ by

356   younger leaves. Leaf age influences photosynthetic capacity and the ^{13}C-isotopic signature

357   (*Girardin et al., 2009*). In addition, young expanded leaves provide the majority of labeled C

358   assimilates for stem growth (*Ryle & Powell, 1972; Subrahmanyam & Rathore, 2004*). Additional

359   factors, such as light intensity (*Ryle & Powell, 1972; Subrahmanyam & Rathore, 2004*), air

360   temperature (*Meharg & Killham, 1989*), and relative humidity (*Farquhar, Oleary & Berry,*

361   *1982*), also influence the photosynthetic rate and alter the C-isotopic signature. Wheat leaves

362   showed higher δ^{13}C values than those of stems (Table 2), which is consistent with previous

363   ^{13}CO$_2$ pulse-labeling studies, such as a study of grain sorghum (*Berg et al., 1991*). Leaf and stem

364   positions (upper, middle, and lower) also influence the δ^{13}C values of aboveground components

365   (*Thompson, 1996*). In the present study, the upper leaves tended to be more enriched in ^{13}C

366   compared with that of the upper stems, whereas the lower leaves were generally less ^{13}C-

367   enriched than stems at the same position (Table 2) (*Thompson, 1996*). This result was due to the

368   remobilization of photosynthate and translocation of higher amounts of recently fixed C from

369   leaves to stems during reproductive (grain filling) and vegetative (elongation) developmental

370   stages. The strong variation in δ^{13}C values in leaves and stems indicated that a single ^{13}CO$_2$-

371   labeling pulse did not produce uniformly ^{13}C-labeled plant material. Repeat-pulse labeling has

372   been suggested to overcome the technical constraints of continuous labeling and the high

373   variation of the single-pulse labeling approach, and may generate adequately homogeneous ^{13}C-

374   labeled plant material (*Roper et al., 2013; Tahir et al., 2018*).

375   With respect to isotope signals, labeled plants also exhibited different patterns of variation

376   among individual plants within a pot and among the three replicate pots (Table 5).

377 Approximately 75% of the leaf and stem components had a CV for intra-pot $\delta^{13}C$ higher than

378 30% at the elongation stage, whereas 67% had a CV less than 20% at the grain filling stage. This

379 result demonstrates that the variability between individual wheat plants at the grain filling stage

380 was smaller than that at the elongation stage, especially for the −N treatment. The variation in

381 ^{13}C-enrichment in different portions of the shoot at the elongation stage were markedly higher

382 than those at the grain filling stage, which may reflect that young leaves are the most frequently

383 labeled organs in wheat (*Girardin et al., 2009; Nguyen Tu et al., 2013*). The stronger labeling of

384 starch is associated with its rapid synthesis during photosynthesis in leaves compared with that of

385 stems during vegetative growth stages. The redistribution of labeled C from source organs

386 (leaves and stems) to sink organs (ears), consisting of C assimilated during the grain filling

387 period (*Aranjuelo et al., 2013; Zhou et al., 2016*), may lead to a decreased extent of variation in

388 ^{13}C enrichment among plant organs. The difference in variation patterns between the +N and −N

389 treatments is likely because C and N remobilization starts earlier and is stronger when plants are

390 grown under low N fertilization compared with the high N fertilization condition (*Aranjuelo et*

391 *al., 2013*). Approximately 83% of leaf and stem components had an inter-pot $\delta^{13}C$ CV higher

392 than 20% at the elongation stage, whereas 67% had an intra-pot $\delta^{13}C$ CV lower than 20% at the

393 grain filling stage (Table 5). This result might indicate that the photosynthesized C in the leaves

394 and stem is translocated to many destinations at the elongation stage, whereas the reallocation of

395 labeled C from the leaves and stem is predominantly directly to the ears at the grain filling stage

396 (*Palta et al., 1994; Aranjuelo et al., 2013; Zhou et al., 2016*). We therefore suggest that labeling

397 of winter wheat at the grain filling stage without N fertilization may produce more uniformly

398   ^{13}C-labeled materials, whereas labeling of winter wheat at the elongation stage without N

399   fertilization may produce more highly ^{13}C-enriched plant materials, albeit with greater

400   variability.

401   **Variation in ^{15}N enrichment**

402   Compared with that under ^{13}C labeling, differences in ^{15}N excess between aboveground

403   components under ^{15}N labeling conditions were much smaller (Table 2). Variation among

404   individual plants (intra-pot) and among different pots (inter-pot) was also much lower (Table 5).

405   No significant differences in ^{15}N excess were observed among wheat aboveground components;

406   the greatest difference, between ears and leaves, was only 11% at the grain filling stage (Table

407   2). This result suggests that the ^{15}N-enriched urea application used in this study can produce

408   relatively uniformly labeled straw tissues at an advanced (grain filling) developmental stage in

409   wheat. Nitrogen is a highly mobile nutrient in the plant, whereas certain other nutrients (i.e., C)

410   are not translocated so readily within the plant. This outcome reflects that N is utilized as a

411   nutrient by plants, whereas C is a basic element of plant photosynthesis. In contrast to plant ^{13}C

412   labeling, which requires complex environmental conditions, such as temperature, humidity, and

413     $^{13}CO_2$ concentration, to be maintained throughout the labeling period (*Soong et al., 2014*), ^{15}N

414   labeling is relatively easy to achieve by means of ^{15}N fertilization. Ideally, studies of the

415   decomposition rate of straw tissue should be conducted, both to select uniformly enriched

416   materials (*Crozier, King & Volk, 1993; Soong et al., 2014*) and to choose straw tissue similar to

417   that of field-grown plant tissues (*Crozier, King & Volk, 1993*). The chemical composition of

418   straw tissue at late maturity differs substantially from that at an early stage of maturity (*Niu et*

419   *al., 2014*). For instance, the majority of plant N at advanced stages of maturity is associated with

420   proteins, nucleic acids, and amino sugars (*Wagger, Kissel & Smith, 1985; Girardin et al., 2009*).

421   Some nitrogenous components may exist in a form more resistant to microbial decomposition,

422   such as N associated with cell wall material (*Wagger, Kissel & Smith, 1985; Girardin et al.,*

423   *2009*).

424   As wheat growth progressed, i.e., with increase in biomass, ^{15}N excess increased by 30.4%–

425   41.1% (Table 2), which indicated that plant N demand was satisfied by available soil N derived

426   from ^{15}N fertilizer between the elongation and grain filling stages. Nitrogen demand per plant is

427   predominantly satisfied through N uptake by roots prior to anthesis, and ear N content is

428   augmented by the amount of N absorbed by the root system between anthesis and ear maturity

429   (*Kichey et al., 2007*).

430   The ^{15}N enrichment of shoots and ears differed among the developmental stages in winter

431   wheat. At the elongation stage, no significant differences in ^{15}N enrichment among the leaves,

432   stems, and ears were observed. This result reflects that the redistribution of labeled N from

433   source organs to ears was low and N in the heading ears was mainly absorbed from the soil

434   during the vegetative growth stages (*Aranjuelo et al., 2013*). However, at the grain filling stage,

435   wheat ears labeled with ^{15}N tended to accumulate significantly higher quantities of ^{15}N compared

436   with other aboveground components (Table 2). Previous researchers have described similar

437   phenomena following ^{15}N labeling, such as in wheat (*Ladd, Oades & Amato, 1981; Palta et al.,*

438   *1994*) and in winter wheat and grain sorghum (*Wagger, Kissel & Smith, 1985*). The ears are a

439   strong N sink that affect N accumulation in the ears during grain filling, whereas the shoots and

440 roots serve to supply nutrients to the ears (*Palta et al., 1994; Aranjuelo et al., 2013; Zhou et al.,*

441 *2016; Sun et al., 2018; Zhou et al., 2018*). In the present experiment, 65% of the total N in grains

442 was remobilized from the N accumulated in the shoots and roots during vegetative growth

443 stages, whereas the remaining 35% was derived from N absorbed from the soil during the grain

444 filling stage (*Sun et al., 2018*). Although ears exhibited significantly higher ^{15}N enrichment

445 compared with that of leaves and stems in winter wheat at the grain filling stage, the differences

446 in ^{15}N enrichment between leaves and stems were only slight (Table 2), which is suggestive of

447 relatively uniform labeling in the plants because N accumulation and redistribution to the grain

448 occurs many days after N accumulation in the leaves and stems (*Jha & Ladd, 1985; Wagger,*

449 *Kissel & Smith, 1985*).

450 **CONCLUDING REMARKS**

451 Weight-average ^{13}C values for aboveground wheat components, resulting from $^{13}CO_2$

452 release from $Na_2{}^{13}CO_3$ (98 atom % ^{13}C) in a field labeling chamber, ranged from 81‰ to 295‰.

453 ^{13}C labeling of winter wheat at the grain filling stage without N fertilization may produce more

454 uniformly ^{13}C-labeled materials, whereas the materials were more highly ^{13}C-enriched at the

455 elongation stage, although the ^{13}C values of the wheat materials showed greater variability. The

456 photosynthesized C in leaves and stems is transported to many destinations at the elongation

457 stage, whereas at the grain filling stage reallocated C is predominantly translocated to the ears.

458 The presence of a higher number of green leaves under the +N treatment than for the −N

459 treatment at the grain filling stage reflected a stronger photosynthesis capacity and led to greater

460 variation in the ^{13}C-isotopic signature of aboveground components. Pulse labeling did not

461 generate uniformly ^{13}C-labeled plant materials. In contrast to ^{13}C labeling, which requires the

462 control of a variety of environmental conditions throughout the labeling period, such as

463 temperature, humidity, and $^{13}CO_2$ concentration, uniform ^{15}N labeling is relatively easier to

464 achieve through ^{15}N fertilization; differences in isotope excess among aboveground wheat

465 components were much smaller under ^{15}N labeling than under the ^{13}C labeling condition.

466 Application of ^{15}N-enriched urea in this study may produce more uniformly labeled wheat straw

467 at the grain filling stage than at the elongation stage.

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640

641   **Figure 1 Winter wheat growth 168 days after sowing (elongation stage) (a) and 205 days**

642   **after sowing (grain filling stage) (b). Field labeling chamber for winter wheat (c).**

643   **Schematic diagram of the three sampled portions of the shoots (d).** −N = no nitrogen

644   addition; +N = nitrogen addition.

645

646   **Figure 2 Biomass (a), carbon content (b), and nitrogen content (c) of different winter wheat**

647   **components.** Error bars indicate the standard error ($n = 4$). −N = no nitrogen addition; +N =

648   nitrogen addition.

649

650

651   **Figure 3 δ^{13}C (‰) values of different winter wheat components.** Different lower-case letters

652   denote a significant difference (LSD, $p < 0.05$) among components at the same developmental

653   stage. Error bars indicate the standard error ($n = 9$). −N = no nitrogen addition; +N = nitrogen

654   addition.

655

656   **Figure 4 ^{15}N excess (in atom %) of different winter wheat components with ^{15}N labeling.**

657   Different lower-case letters denote a significant difference among components at the same

658   developmental stage (LSD, $p < 0.05$). Error bars indicate the standard error ($n = 9$).

659

660

Figure 1

Winter wheat growth 168 days after sowing (elongation stage) (a) and 205 days after sowing (grain filling stage) (b). Field labeling chamber for winter wheat (c). Schematic diagram of the three sampled portions of the shoots (d).

−N = no nitrogen addition; +N = nitrogen addition.

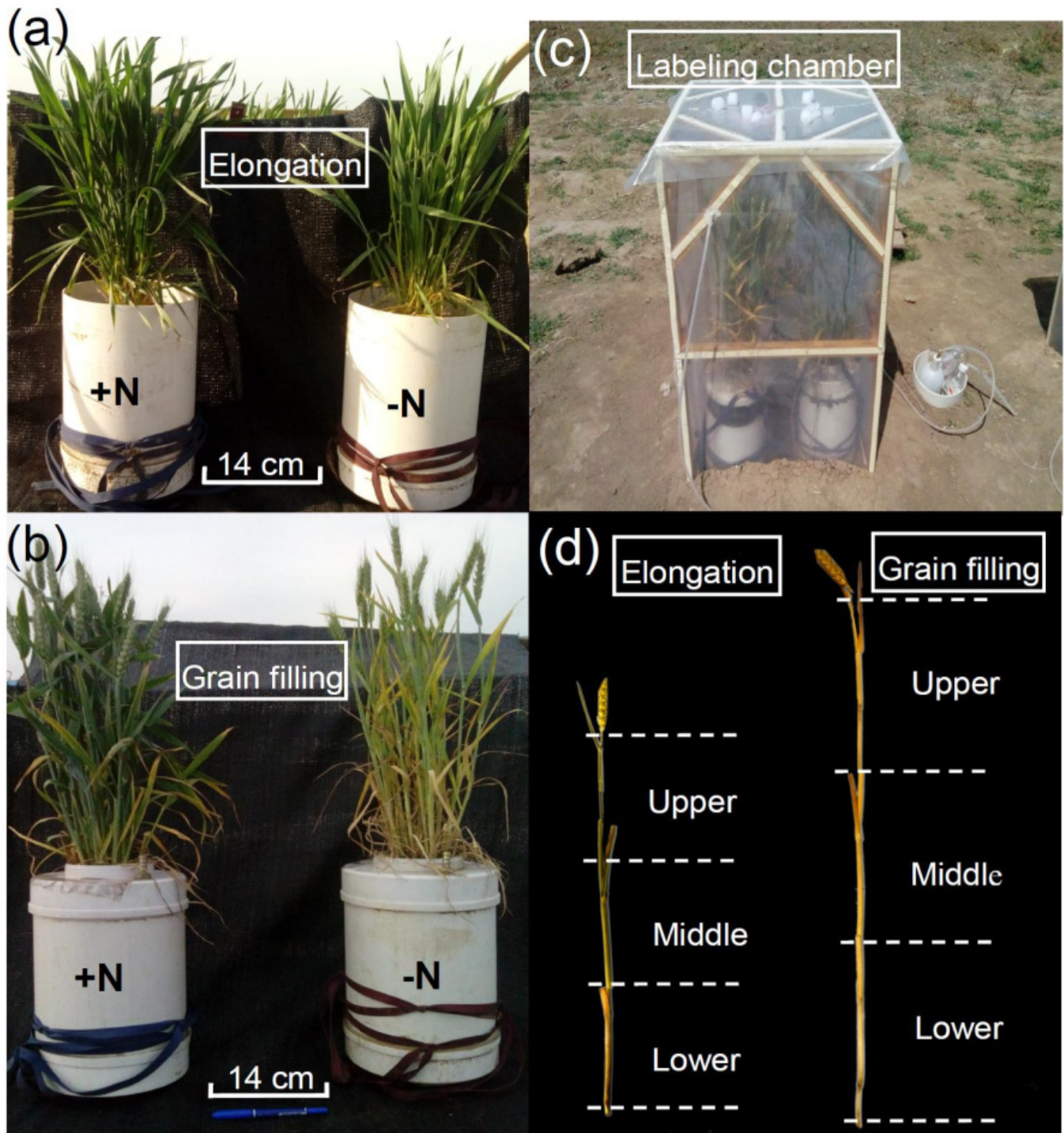



Figure 2

Biomass (a), carbon content (b), and nitrogen content (c) of different winter wheat components.

Error bars indicate standard errors (n = 4). –N = no N addition; +N = N addition.

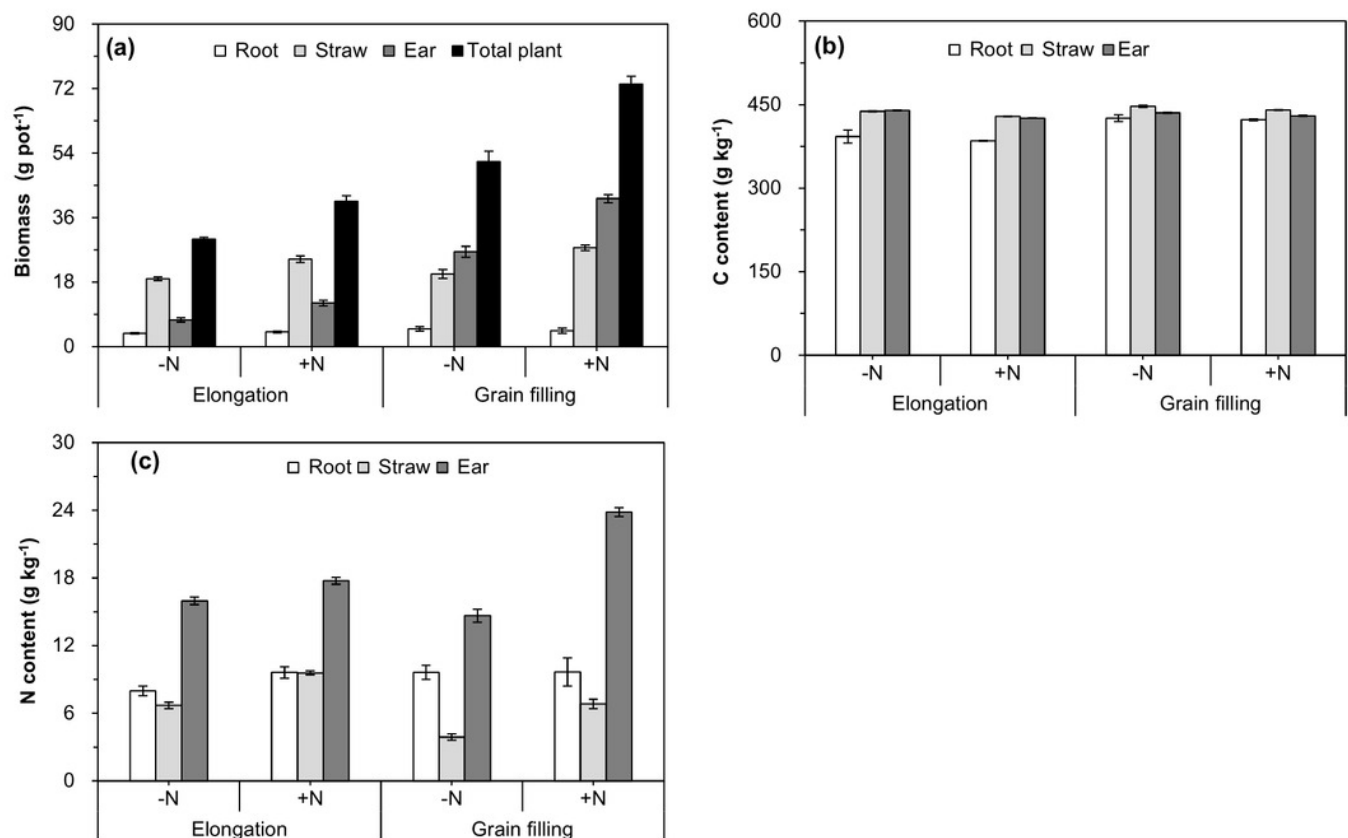



Figure 3

$\delta^{13}C$ (‰) values of different winter wheat components.

Different lower-case letters denote a significant difference (LSD, $p < 0.05$) among components at the same developmental stage. Error bars indicate the standard error ($n = 9$). −N = no nitrogen addition; +N = nitrogen addition.

δ¹³C (‰)

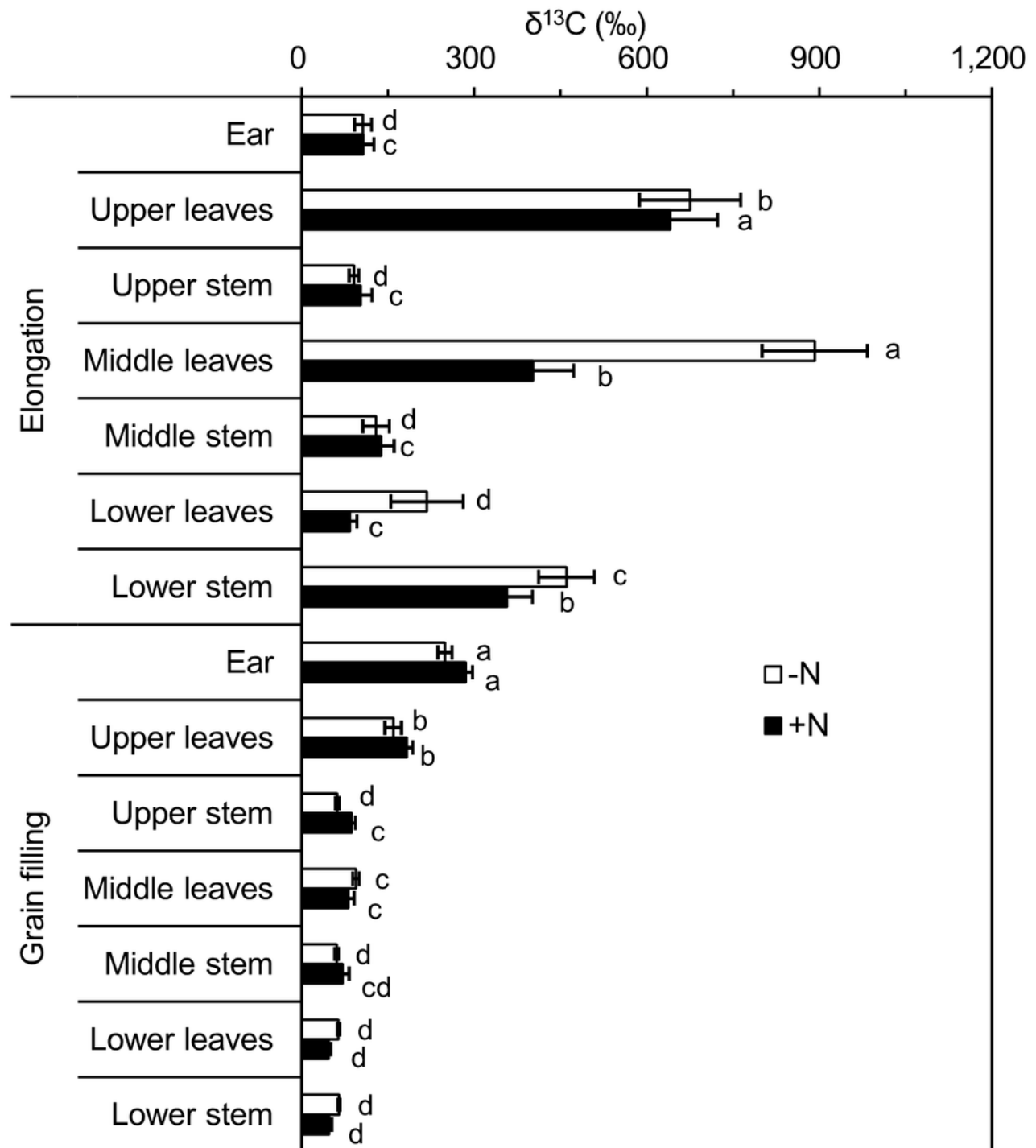
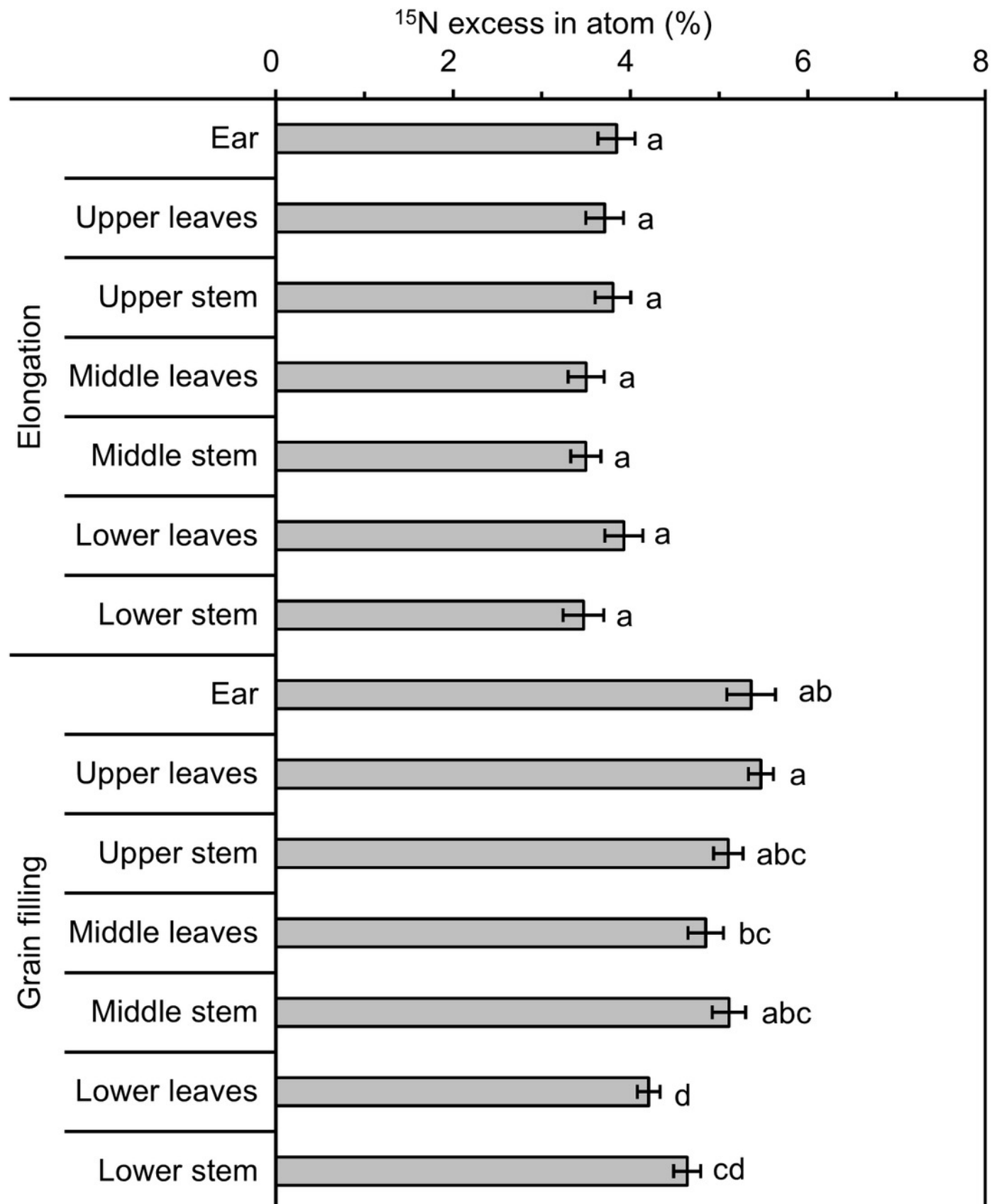



Figure 4

^{15}N excess (in atom %) of different winter wheat components with ^{15}N labeling.

Different lower-case letters denote a significant difference among components at the same developmental stage (LSD, $p < 0.05$). Error bars indicate the standard error ($n = 9$).

P-values for two-way analysis of variance of the effects of stage (S) and nitrogen (N) on biomass, carbon content, and N content of different winter wheat components.

1   **Table 1 *P*-values for two-way analysis of variance of the effects of stage (S) and nitrogen (N)**

2   **on biomass, carbon content, and N content of different winter wheat components.**

Treatment	Biomass				C content			N content		
	Root	Straw	Ear	Total plant	Root	Straw	Ear	Root	Straw	Ear
S	0.886	<0.001	<0.001	<0.001	0.598	0.003	<0.001	0.282	<0.001	<0.001
N	0.136	0.027	<0.001	<0.001	0.118	<0.001	0.947	0.282	<0.001	<0.001
S×N	0.393	0.331	<0.001	0.016	0.528	0.456	0.046	0.310	0.935	<0.001

$\delta^{13}C$ value and ^{15}N excess (in atom %) of different winter wheat components.

Values are the mean ± standard error (n = 9). Different lower-case letters in the same row denote a significant difference (LSD, $p < 0.05$) in $\delta^{13}C$ or ^{15}N excess (in atom %) between components. Asterisks (*) in the same column denote either significant differences (t-test, $p < 0.05$) in $\delta^{13}C$ values between nitrogen (N) treatments at the same stage or significant differences (t-test, $p < 0.05$) in ^{15}N excess (in atom %) between different stages under the same N treatment (^{15}N labeling). [†] −N = no N addition; [††] +N = N addition.

1 **Table 2 δ^{13}C value and ^{15}N excess (in atom %) of different winter wheat components.**

2 Values are the mean ± standard error ($n = 9$). Different lower-case letters in the same row denote

3 a significant difference (LSD, $p < 0.05$) in δ^{13}C or ^{15}N excess (in atom %) between components.

4 Asterisks (*) in the same column denote either significant differences (t-test, $p < 0.05$) in δ^{13}C

5 values between nitrogen (N) treatments at the same stage or significant differences (t-test, $p <$

6 0.05) in ^{15}N excess (in atom %) between different stages under the same N treatment (^{15}N

7 labeling). [†] −N = no N addition; [††] +N = N addition.

			Ear	Leaves	Stem	Aboveground
δ^{13}C (‰)	Elongation	-N[†]	106.7±14.8c	594.9±29.9a*	226.9±15.4b	295.0±15.3*
		+N[††]	107.2±18.5c	375.4±25.0a	198.8±20.1b	212.0±16.8
	Grain	-N	249.1±12.1a	105.7±4.6b	62.4±1.0c	81.2±3.1
	filling	+N	285.3±11.4a	103.7±6.2b	68.5±4.1c	85.9±5.8
^{15}N excess (in atom %)	Elongation		3.8±0.2a	3.71±0.2 a	3.6 ±0.2a	3.6±0.1
	Grain filling		5.4±0.3a*	4.8±0.1b*	4.9±0.1b*	4.9±0.1*

P-values for two-way analysis of variance of the effects of stage (S) and nitrogen (N) on $\delta^{13}C$ value and $\delta^{15}N$ excess (in atom %) in different winter wheat components.

1 **Table 3 *P*-values for two-way analysis of variance of the effects of stage (S) and nitrogen (N)**

2 **on $\delta^{13}C$ value and $\delta^{15}N$ excess (in atom %) in different winter wheat components.**

		Ear	Leaves				Stem			
			Upper	Middle	Lower	Whole	Upper	Middle	Lower	Whole
$\delta^{13}C$	N	0.214	0.929	<0.001	0.025	<0.001	0.116	0.595	0.076	0.398
	S	<0.001	<0.001	<0.001	0.006	<0.001	0.058	<0.001	<0.001	<0.001
	N×S	0.225	0.635	0.000	0.077	<0.001	0.545	0.952	0.199	0.192
^{15}N excess (in atom %)	S	0.000	<0.001	<0.001	0.278	<0.001	<0.001	<0.001	0.001	<0.001

Isotope values of upper, middle, and lower portions of shoots in winter wheat.

Values are the mean ± standard error (n = 9). Different lower-case letters in the same row denote significant differences in $\delta^{13}C$ or ^{15}N excess (in atom %; LSD, $p < 0.05$) between the three portions. Asterisks (*) in the same column denote either significant differences (t-test, $p < 0.05$) in $\delta^{13}C$ between nitrogen (N) treatments at the same stage or significant differences (t-test, $p < 0.05$) in ^{15}N excess (in atom %) between different stages under the same N treatment (^{15}N labeling). †−N = no N addition; ††+N = N addition.

1 **Table 4 Isotope values of upper, middle, and lower portions of shoots in winter wheat.**

2 Values are the mean ± standard error ($n = 9$). Different lower-case letters in the same row denote

3 significant differences in δ^{13}C or ^{15}N excess (in atom %; LSD, $p < 0.05$) between the three

4 portions. Asterisks (*) in the same column denote either significant differences (t-test, $p < 0.05$)

5 in δ^{13}C between nitrogen (N) treatments at the same stage or significant differences (t-test, $p <$

6 0.05) in ^{15}N excess (in atom %) between different stages under the same N treatment (^{15}N

7 labeling). † −N = no N addition; †† +N = N addition.

			Upper portion	Middle portion	Lower portion
δ^{13}C (‰)	Elongation	-N†	383.0±44.6ab	510.6±48.2a*	339.1±37.9b*
		+N††	371.2±48.6a	270.0±38.4ab	220.1±18.9a
	Grain filling	-N	110.4±7.5a	77.4±3.5b	64.3±1.5b
		+N	134.9±6.9a	76.1±9.8b	47.3±3.2c
^{15}N excess	Elongation		3.76 ±0.20a	3.50±0.18 a	3.70±0.20a
(in atom %)	Grain filling		5.29±0.15 a*	4.98±0.19a*	4.42±0.11a*

Intra-pot and inter-pot variation in δ^{13}C value and ^{15}N excess (in atom %) of different winter wheat components.

†−N = no N addition; ††+N = N addition.

1 **Table 5 Intra-pot and inter-pot variation in δ^{13}C value and ^{15}N excess (in atom %) of different winter wheat components.** [†] $-$N

2 = no N addition; [††] +N = N addition.

| | | | CV (%) of intra-pot | | | | | | | CV (%) of inter-pot | | | | | | |
| | | | Ear | Leave | | | Stem | | | Ear | Leave | | | Stem | | |
				Upper	Middle	Lower	Upper	Middle	Lower		Upper	Middle	Lower	Upper	Middle	Lower
δ^{13}C (‰)	Elongation	-N[†]	25.6	28.4	31.0	76.4	9.4	42.8	29.1	41.5	26.0	19.2	59.0	30.9	49.8	22.8
		+N[††]	21.2	31.6	42.6	46.5	36.1	43.7	35.8	47.1	34.3	26.5	24.0	54.1	32.2	16.5
	Grain filling	-N	11.9	25.7	13.0	7.3	11.6	16.2	6.3	9.7	10.8	15.1	6.1	9.7	5.5	8.0
		+N	13.2	16.6	24.3	15.8	19.5	31.5	21.1	60.2	52.5	70.9	39.0	18.3	50.0	3.8
^{15}N excess	Elongation		15.2	14.5	11.1	14.3	13.8	8.6	16.7	1.7	17.1	23.0	18.9	14.9	20.3	23.4
(in atom %)	Grain filling		18.1	7.7	13.6	9.4	10.2	12.3	11.0	1.3	1.5	2.8	0.1	6.2	0.8	2.3