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

Zhaoan Sun [1], **Shuxia Wu** [2], **Biao Zhu** [3], **Yiwen Zhang** [4], **Roland Bol** [5], **Qing Chen** [1], **Fanqiao Meng** [Corresp. 1]

[1] Beijing Key Laboratory of Farmland Soil Pollution Prevention and Remediation, College of Resources and Environmental Sciences, China Agricultural University, Beijing, China

[2] Institute of Agricultural Resources and Regional Planning, China Academy of Agricultural Sciences, Beijing, China

[3] Institute of Ecology, College of Urban and Environmental Sciences, Key Laboratory for Earth Surface Processes of the Ministry of Education, Peking University, Beijing, China

[4] Dryland-Technology Key Laboratory of Shandong Province, Qingdao Agricultural University, Qingdao, China

[5] Institute of Bio- and Geosciences, Agrosphere Institute (IBG-3), Forschungszentrum Jülich GmbH, Jülich, Germany

Corresponding Author: Fanqiao Meng
Email address: mengfq@cau.edu.cn

There is a lack of information on homogeneity and distribution of ^{13}C and ^{15}N labeling in winter wheat. We conducted a dual labeling experiment to examine the variability of ^{13}C and ^{15}N enrichment in aboveground parts of labeled winter wheat. ^{13}C and ^{15}N labeling were performed on non-N fertilized (−N) and N fertilized (+N, 250 kg N ha^{-1}) winter wheat at elongation and grain filling stages. Aboveground parts of wheat were destructively sampled at 28 days after each labeling. As winter wheat growth progressed, δ^{13}C values of wheat ears significantly increased, whereas those of leaves and stems significantly decreased. 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 (new) leaves were more highly enriched with ^{13}C compared with lower (old) 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 ^{13}C labeling, differences of ^{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, while the materials were more highly ^{13}C-enriched at the elongation stage although the δ^{13}C values were more variable. ^{15}N-enriched straw tissues via urea fertilization were relatively uniformly labeled at the grain filling stage than at the elongation stage.

1 **TITLE PAGE**

2 **Title:**

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

4 L.)

5 **Authors:**

6 Zhaoan Sun[a], Shuxia Wu[b], Biao Zhu[c],Yiwen Zhang[d], Roland Bol[e], Qing Chen[a], Fanqiao Meng[a*]

7 **Affiliations and addresses:**

8 [a]Beijing Key Laboratory of Farmland Soil Pollution Prevention and Remediation, College of Resources and

9 Environmental Sciences, China Agricultural University, Beijing 100193, China

10 [b]Institute of Agricultural Resources and Regional Planning, China Academy of Agricultural Sciences, Beijing

11 100081, China

12 [c]Institute of Ecology, College of Urban and Environmental Sciences, Key Laboratory for Earth Surface

13 Processes of the Ministry of Education, Peking University, Beijing 100871, China

14 [d]Dryland-Technology Key Laboratory of Shandong Province, Qingdao Agricultural University, Qingdao,

15 Shandong 266109, China

16 [e]Institute of Bio- and Geosciences, Agrosphere Institute (IBG-3), Forschungszentrum Jülich GmbH, Jülich

17 52425, Germany

18 *Corresponding author: Dr. Fanqiao Meng, College of Resources and Environmental Sciences, China

19 Agricultural University, Beijing 100193, China

20 Telephone: 0086 10 62731538; Fax: 0086 10 62827958; Email: mengfq@cau.edu.cn

21   **ABSTRACT**

22   There is a lack of information on homogeneity and distribution of ^{13}C and ^{15}N labeling in winter wheat. We

23   conducted a dual labeling experiment to examine the variability of ^{13}C and ^{15}N enrichment in aboveground

24   parts of labeled winter wheat. ^{13}C and ^{15}N labeling were performed on non-N fertilized (−N) and N fertilized

25   (+N, 250 kg N ha^{-1}) winter wheat at elongation and grain filling stages. Aboveground parts of wheat were

26   destructively sampled at 28 days after each labeling. As winter wheat growth progressed, δ^{13}C values of wheat

27   ears significantly increased, whereas those of leaves and stems significantly decreased. At the elongation stage,

28   N addition tended to reduce the aboveground δ^{13}C values through dilution of C uptake. At the two stages,

29   upper (new) leaves were more highly enriched with ^{13}C compared with lower (old) leaves. Variability between

30   individual wheat plants and among pots at the grain filling stage was smaller than that at the elongation stage,

31   especially for the −N treatment. Compared with ^{13}C labeling, differences of ^{15}N excess between aboveground

32   components (leaves and stems) under ^{15}N labeling conditions were much smaller. We conclude that non-N

33   fertilization and labeling at the grain filling stage may produce more uniformly ^{13}C-labeled wheat materials,

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

35   variable. ^{15}N-enriched straw tissues via urea fertilization were relatively uniformly labeled at the grain filling

36   stage than at the elongation stage.

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

INTRODUCTION

The accurate quantification of carbon (C) and nitrogen (N) cycling in the plant–soil system requires the use of special techniques, especially for tracking above- and belowground residue C and N dynamics in soil (*Shan et al., 2012*; *Meng et al., 2017*; *Zheng et al., 2018*; *Xu et al., 2019*). This is because that small changes in soil organic C and N are difficult to detect on the background of large soil C and N pools (*Felini et al., 2007; Liang et al., 2013; Sun al., 2018*; *Pausch and Kuzyakov et al., 2018*). Isotopic labeling of plants using ^{13}C and ^{15}N is a very powerful tool in tracking the fate of C and N derived from different plant parts in a soil-plant system when these ^{13}C and ^{15}N-labeled plant parts decompose simultaneously in situ (*An et al., 2015; Xu et al., 2019*) or laboratory (*Meng et al., 2017; Fang et al., 2018*) incubation. However, for obtaining unbiased conclusions from the fate of labeled straw C and N, quantitatively tracing the distribution of labeled straw C and N in various soil C and N pools require uniform ^{13}C or ^{15}N-labeled plant materials, since the calculations assume all plant parts become evenly labeled (*Thompson, 1996; Girardin et al., 2009; Nguyen Tu et al., 2013; Soong et al., 2014*). It is therefore important to assess the variability and the degree of the ^{13}C or ^{15}N label enrichment among plant parts because different plant parts decompose at different rates, and the homogeneity of label signal in the labeled plant residues may impact the interpretation of quantification of the fractional contribution of soil organic matter and added plant material to plant uptake in subsequent decomposition studies.

Temporal ^{15}N variability among sources of N from soil and in allocation of N to different plant organs will produce labeled plant tissues with quite different ^{15}N contents (*Wagger et al., 1985; Crozier et al., 1993*). Production of more uniformly labeled tissue is a potential advantage of using a single source of N in a controlled growth system—for instance, in soil-free culture supplied with ^{15}N-fertilizer solution. The

61   homogeneous ^{15}N enrichment among different plant parts can be relatively easy to obtain through the growing

62   crops fertilized with ^{15}N tracer (*Liang et al., 2013; Soong et al., 2014*), while the production of uniformly ^{13}C-

63   labeled crop residues through ^{13}CO$_2$ fixation poses a number of challenges (*Thompson, 1996; Girardin et al.,*

64   *2009; Nguyen Tu et al., 2013; Soong et al., 2014*). This difficulty is because rates of C incorporation

65   (photosynthesis) differ widely among plant compartments, which further increases ^{13}C isotope variation of the

66   obtained plant tissues (*Thompson, 1996; Girardin et al., 2009; Nguyen Tu et al., 2013; Soong et al., 2014*).

67   Pulse or continuous labeling of above-ground plant parts has frequently been used to the production of ^{13}C or

68   ^{14}C-labeled plant parts. A continuous supply with either ^{14}C or ^{13}C presents an advantage over pulse labeling

69   for uniformly label all plant parts, but it requires special facilities and was applied from first leaf emergence to

70   harvest time, therefore it is not possible to use this approach under field conditions (*Zhu et al., 2014*). Field ^{13}C

71   (or ^{14}C) and ^{15}N labeling has shown potential in investigating C and N dynamics derived from crop residues

72   under actual field conditions, as affected by chemical composition and quantities of crop residue (*Berg et al.,*

73   *1991; Tahir et al., 2018*). For instance, concentrations of phenolic compounds, which may alter decomposition

74   rates (*Fox et al., 1990; Ranells and Wagger, 1992*), may be sensitive to differences in field, greenhouse, or

75   growth-chamber UV light intensities (*Crozier et al., 1993*). For field isotopic labeling, pulse labeling is better

76   than continuous labeling due to the advantage of being easier to handle and simple instrumentation (*Berg et al.,*

77   *1991; Tahir et al., 2018*). However, most of the labeled crop residues that were used for C and N dynamic

78   studies were labeled with ^{13}C (or ^{14}C) and ^{15}N tracer under controlled conditions.

79   For the production of the labeled crop residues, the field-labeling experiments that were developed in the

80   past were mainly focused on the degree of ^{13}C and ^{15}N enrichment in crop residues with ^{13}C and ^{15}N. However,

81   few researchers evaluated the homogeneity and distribution of ^{13}C and ^{15}N labels within the labeled plant

82   compartments, especially from winter wheat residues, one of the most important staple crops and frequently

83   used plants in labeling studies (*Jia et al., 2011*; *Butterly et al., 2015*; *Chen et al., 2016; Sun et al., 2018*). In

84   annual cereal crops, the plants grow vigorously and the enrichment of labeled C is higher at the early stage

85   than at the mature stage (*Meng et al., 2013; Liu et al., 2015; Sun et al., 2018*). However, the majority of C

86   labeling studies have been conducted at the early growth stages of wheat, e.g., 60 days after emergence by

87   continuous labeling (*Liljeroth et al., 1994; Marx et al., 2007*) and <150 days after emergence by pulse labeling

88   (*Martens et al., 2009; Butterly et al., 2015*), and few studies have compared the enrichment of labeled C for

89   different labeling periods. In addition, heavy use of N fertilizer delayed senescence in wheat, which resulted in

90   much nonstructural carbohydrate left in the straw and affected the photosynthesis (*Yang and Zhang, 2006*),

91   might lead to changes in the enrichment degree of labeled C in straws. To assess uniformity of labeling within

92   plants, the shoot of a single plant was divided into leaves and stem from three sections of equal length using

93   the method described by *Thompson* (*1996*). Thus, we set up a ^{13}C pulse-labeling and ^{15}N fertilization

94   experiment for labeling winter wheat under field pot-grown conditions at different growth stages, and the

95   objectives were: (i) to assess the homogeneity and the degree of ^{13}C and ^{15}N enrichment among different wheat

96   organs and among different positions of identical organs such as stems and leaves; and (ii) investigate the

97   impacts of wheat growth and N fertilization on the isotope variability among aboveground compartments after

98   ^{13}C-labeling.

99

100   **MATERIAL AND METHODS**

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

102   In current study, we used winter wheat (*Triticum aestivum* L. 'Luyuan 502'), one of the main staple crops

103   grown in the world (*Zhao et al., 2016*). To examine the impacts of wheat growth stages and N fertilization on

104   the variation of ^{13}C enrichments of winter wheat, two N levels (0 mg N kg^{-1} soil, -N; and 90 mg N kg^{-1} soil,

105   equivalent to 250 kg N ha^{-1}, +N) were applied, and wheat plants grown on a calcareous soil (fluvo-aquic sandy

106   loam) were $^{13}CO_2$ pulse-labeled at elongation (168 days after sowing; (DAS)) and grain filling (205 DAS)

107   stages (Figure 1a and b). To examine the variation degree of ^{15}N enrichment among aboveground

108   compartments, the ^{15}N-labeled fertilizer was applied in the form of ^{15}N-labeled urea by directly adding N to

109   soil for +N treatment (the atom% ^{15}N was 10.14%; about 10 US dollars per gram of ^{15}N labeled urea), which

110   was provided by Shanghai Research Institute of Chemical Industry. Meanwhile, the other wheat plants were

111   selected as ^{15}N natural abundance controls fertilized with unlabeled urea for +N treatment. Ten pre-germinated

112   (24–48 h) winter wheat seeds were sown directly into pots (30 × 20 cm i.d.) containing 8.7 kg air-dried soil,

113   and the soil was rewetted to 70% of the water holding capacity. One week after germination (October 20,

114   2014), all except the six strongest seedlings were removed (equivalent to a field planting density of 1.5 million

115   plants ha^{-1}). The experiment was conducted under field pot-grown condition. For the N addition treatment

116   (+N), half of the ^{15}N labeled or unlabeled urea was applied basally at sowing (0.84 g ^{15}N labeled or unlabeled

117   urea was applied to each pot) and the other half was used to top-dress plants at the elongation stage (March 15,

118   2015). The wheat plants for the first $^{13}CO_2$ labeling were fertilized in only one basal application and received

119   45 mg N kg^{-1} air-dried soil. The wheat plants for the second $^{13}CO_2$ labeling received a total of 90 mg N kg^{-1}

120   air-dried soil in two applications during the experiment (1.68 g ^{15}N labeled or unlabeled urea was applied to

121   each pot; corresponding to a total fertilization of 250 kg N ha^{-1}), which was split into 2 × 45 mg N kg^{-1} air-

122   dried soil (basal urea N and topdressing urea N). KH_2PO_4 fertilizer was applied basally at a rate of 17 mg P

123 and 18 mg K kg^{-1} air-dried soil (same to local agricultural practice). To produce plant litter similar to field-

124 grown conditions in this study, we therefore applied ^{15}N-urea to soil of field pot-grown winter wheat rather

125 than supplying ^{15}N-fertilizer solution to soil-free culture.

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

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

128 Station of China Agricultural University, Huantai County, Shandong Province (36°57′N, 117°59′E, 18 m

129 elevation). The soil of the experimental field was derived from alluvial sediments of Yellow River and was

130 classified as aquic inceptisol (a calcareous, fluvo-aquic sandy loam; *Shi et al., 2013*). Soil properties were as

131 follows: soil organic C = 8.4 g kg^{-1}, soil inorganic C = 5.2 g kg^{-1}, total N = 0.67 g kg^{-1}, NH$_4$-N = 1.6 mg kg^{-1},

132 NO$_3$-N = 17.2 mg kg^{-1}, pH = 8.2 (soil: water = 1: 2.5), available K = 174 mg kg^{-1}, and Olsen P = 5.2 mg kg^{-1}.

133 After sampling, the soil was air-dried, homogenized, and sieved (5-mm screen) prior to the experiment. Each

134 pot (height = 30 cm; inner diameter = 20 cm) for wheat-growing was filled with 8.7 kg of air-dried soil to a

135 bulk density of 1.38 g cm^{-3}. Wheat mainly concentrated in 0–20 cm soil layer and contributed the proportion

136 to 0 to 100 cm total rooting weight were 57.6%–78.6% in typical farmland of Northern China Plain (*Hou,*

137 *2011*), hence pots with 30 cm soil layer was enough for wheat root development in this study. The pot-planted

138 winter wheat seedlings were transferred to the field of the above-described Agro-Ecosystem Experimental

139 Station. The PVC pots containing wheat plants were placed in cropland holes (30 cm in depth; 24 cm in

140 diameter) to simulate local wheat-growing conditions. The total growth period was 230 days, with six different

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

142 DAS), (iv) anthesis (180–193 DAS), (v) grain filling (194–214 DAS), and (vi) dough ripening (215–230

143 DAS). The precipitation was 147 mm and soil temperature varied from -1.6 to 29.7℃ during the wheat season

144   (*Zhao et al., 2017*). The soil water content of each pot, which was controlled gravimetrically to simulate local

145   wheat production and was adjusted daily to 65% (during the seedling stage), 70% (tillering), 80% (elongation),

146   80% (anthesis) and 70%–75% (grain-filling) of the field water-holding capacity, according to the amount of

147   rainfall and evaporation.

148   The $^{13}CO_2$ labeling was performed at elongation and grain filling stages (i.e., 168 and 205 DAS). On each

149   occasion, four pots were randomly selected for $^{13}CO_2$ labeling under -N treatment, and four pots were also

150   randomly chosen for $^{13}CO_2$ labeling under +N treatment (^{15}N labeling with ^{15}N-urea), with an additional eight

151   pots selected as $^{13}CO_2$ unlabeled controls and maintained separately from the labeled plants. Hence, we used 32

152   pots in this experiment with three replicates for each N treatment, for destructive sampling at 28 days after

153   labeling during each of the growth stages (three from four pots per sampling for each treatment): 8 pots were

154   labeled with dual ^{13}C and ^{15}N labeling in +N treatment; 8 pots were labeled with single ^{13}C labeling for -N

155   treatment; and an additional 16 pots were selected as unlabeled controls (8 pots for –N and 8 pots for +N). We

156   considered each pot as the technical replicate for each treatment (three from four pots per sampling for each

157   treatment), and took each plant within pot as the biological replicate (three plants chosen within the pot). A

158   chamber (0.6 m long × 1.0 m high; Figure 1c) adapted from *Swinnen et al.* (1994) and *Meng et al.* (2013) was

159   used for the $^{13}CO_2$ labeling. On the previous day, the soil surface was covered with a PVC board and sealed

160   with silicon, including around stems. To remove all $^{12}CO_2$ before labeling, the air inside the chamber was

161   continuously circulated for 30 min through a 10 M NaOH solution using a membrane pump. The ^{13}C labeled

162   $Na_2^{13}CO_3$ at 98 atom% ^{13}C (about 100 US dollars per gram of $Na_2^{13}CO_3$) was provided by Shanghai Research

163   Institute of Chemical Industry. A beaker containing $Na_2^{13}CO_3$ (8.0 g of $Na_2^{13}CO_3$ for each labeling occasion)

164   was placed inside the chamber. To determine CO_2 concentrations within the chamber using an infrared gas

165   analyzer (CI301PS; CID Bio-Science, Camas, WA, USA), an unlabeled control treatment with an additional

166   four pots was set up in another chamber under the same conditions except that Na_2CO_3 was used to produce

167   non-labeled CO_2. This arrangement was used because of the different wavelengths for maximum absorption of

168     $^{13}CO_2$ and $^{12}CO_2$ in the infrared detector (*Meng et al., 2013*). If the rate of CO_2 concentration decline slowed

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

170   injected until CO_2 concentrations increased to approximately 360 μL L^{-1}. The same amount of H_2SO_4 solution

171   was also injected into the chamber housing the plants being labeled. A fan was used to homogenize the

172   atmosphere within each chamber. After 7 h of labeling, plants were removed from the chamber to prevent the

173   re-assimilation of shoot-respired $^{13}CO_2$.

174   **Sampling and chemical analyses**

175   ^{13}C-labeled winter wheat and soils were destructively sampled 28 days after each labeling. There was no

176   significant difference between the mean weights of the aboveground and root biomass of the ^{13}C pulse labeled

177   plants and the unlabeled control plants when they were harvested 28 days after labeling during each of the

178   growth stages. This indicated that ^{13}C pulse labeling and did not affect plant growth. To examine the

179   uniformity of labeling within a wheat plant, individual entire wheat plants were collected at final harvest and

180   their shoots (stem and leaves) were divided into three equal-length portions (upper, middle and lower portion;

181   Figure 1d). Separate analyses of ^{13}C and ^{15}N enrichment were undertaken on leaf and stem portions as well as

182   the ears. Three plants per pot were assessed for the variation of ^{13}C and ^{15}N enrichments. Shoots (stems and

183   leaves) and ears were oven-dried at 60 °C to a constant weight. All samples were ground to a fine powder

184   (<500 μm) in a ball mill (Restol MM2000, Retsch, Haan, Germany) prior to analysis.

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

186   (DELTAplus XP, ThermoFinnigan, Bremen, Germany). The abundance of ^{13}C was expressed as parts per

187   thousands (‰) relative to the international standard (Pee Dee Belemnite, PDB; 0‰) expressed as delta units

188   (δ) (*Craig, 1953*). The ^{15}N enrichment of ^{15}N labeled plant parts fertilized with labeled urea was reported as

189   atom% ^{15}N excess calculated by subtracting the ^{15}N natural abundance control samples fertilized with

190   unlabeled urea (*Coplen, 2011*).

191   **Data processing and statistical analysis**

192   To estimate the weight-average ^{13}C and ^{15}N values of the aboveground and three equal-length portions

193   (upper, middle and lower portion) of shoot in the winter wheat, a simple isotopic mass balance mixing

194   equation was used (*Thompson, 1996*):

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

196    $^{15}N_{abveground} = {}^{15}N_{ear} \times F_{ear} + {}^{15}N_{leaves} \times F_{leaves} + {}^{15}N_{stem} \times F_{stem}$ (2)

197    $1 = F_{ear} + F_{leaves} + F_{stem}$ (3)

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

199   stem of winter wheat, respectively; $^{15}N_{abveground}$, $^{15}N_{ear}$, $^{15}N_{leaves}$, and $^{15}N_{stem}$ is ^{15}N excess in atom% for

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

201   aboveground dry weight of winter wheat in the respective ear, leaves and stem fractions.

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

203    $^{15}N_{i\ portion} = {}^{15}N_{i\ leaves} \times F_{i\ leaves} + {}^{15}N_{i\ stem} \times F_{i\ stem}$ (5)

204    $1 = F_{i\ leaves} + F_{i\ stem}$ (6)

205   where $\delta^{13}C_{i\,\text{portion}}$ and $^{15}N_{i\,\text{portion}}$ is C isotope values (δ) and ^{15}N excess in atom% for upper, middle and lower

206   portion of shoot of winter wheat, respectively; and $F_{i\,\text{leaves}}$ and $F_{i\,\text{stem}}$ is the respective leaves and stem fractions

207   in the same portion of shoot.

208   Statistical analysis was carried out with SPSS (version 11.0, 2002, SPSS, Chicago, Illinois, USA). Two-

209   way analysis of variance was performed to analyze the effect of N fertilization and/or growth stage on ^{13}C and

210   ^{15}N distribution in different aboveground wheat components. The LSD test was used to examine differences in

211   ^{13}C and ^{15}N isotope values between N treatments or different aboveground components. A level of 0.05 was

212   chosen to indicate statistical significance.

213   **RESULTS**

214   **Wheat biomass, total C and N**

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

216   whole wheat was at a significant level (Table 1 and Figure 2a), indicating that the effect of N fertilization tend

217   to be more profound as wheat growth progressed. Straw, ear and whole wheat plant biomass were significantly

218   higher in the +N treatment than in the -N at the elongation and grain filling stages (Figure 2a). With plant

219   growth, the C and N content of wheat straw and ear significantly increased (Table 1 and Figure 2b, c), whereas

220   no significant differences in roots were observed from the elongation to grain filling stages (Table 1 and Figure

221   2a). Compared with -N, N fertilization significantly decreased the C content of straw, and significantly

222   increased the N content of wheat straw and ear at the elongation and grain filling stages (Table 1 and Figure

223   2b, c).

224   **$\delta^{13}C$ values of aboveground components of winter wheat**

225   In the ^{13}C labeled wheat plants, δ^{13}C values in leaves were the highest, followed by stems and ears at the

226   elongation stage; however, significantly higher ^{13}C enrichment was observed in the ears than in the other

227   aboveground parts at the grain filling stage (Table 2). As growth progressed, δ^{13}C values of ears significantly

228   increased, by 130% and 170% for −N and +N treatments, respectively; in contrast, δ^{13}C values of leaves and

229   stems significantly decreased, by 82.3% (−N) and 72.2% (+N) in leaves and 72.4% (−N) and 65.5% (+N) in

230   stems. This indicated that growth stage had a significant impact on ^{13}C distribution among ears, leaves, and

231   stems (Table 3). N fertilization decreased δ^{13}C values of aboveground wheat components at the elongation

232   stage and had no significant effects at the grain filling stage; however, the change was only significant in

233   leaves at the elongation stage (36.9%; Tables 2 and 3).

234   Shoots (leaves and stems) of single wheat plants were divided into three equal-length portions (upper,

235   middle, and lower), to analysis the labeled ^{13}C distribution within the shoot. Following pulse labeling at the

236   elongation stage, δ^{13}C values of leaves and stems in different portions was significantly different: the leaves of

237   lower portion was least ^{13}C enriched for both +N and -N treatments, and ^{13}C enrichment was highest in the

238   middle and upper portions under −N and +N treatments, respectively; however, δ^{13}C values of stems were

239   highest in the lower portion, followed by middle and upper portions, both under −N and +N conditions (Figure

240   3). A significant difference was observed at the grain filling stage: for both leaves and stems, δ^{13}C values were

241   significantly highest in the upper portion, with no significant differences between middle and lower portions

242   (Figure 3).

243   When we combined δ^{13}C values from the same portions of leaves and stems, at elongation stage, we

244   observed that there were no significant differences among different portions for +N treatment, and δ^{13}C values

245   of upper and middle portions was significantly higher than that of lower portion for −N treatment; at the grain

246   filling stage, the $\delta^{13}C$ values of upper portion was the highest, followed by that in the middle and lower

247   portions under both –N and +N conditions (Table 4).

248   Labeled plants had coefficients of variation (CVs) for intra-pot (between individual plants) $\delta^{13}C$ of 9.4%–

249   76.4% and 6.3%–31.5% at elongation and grain filling stages, respectively, while inter-pot CVs (among plants

250   across different pots) for $\delta^{13}C$ ranged from 16.5%–59.0% at the elongation stage and 3.8%–70.9% at the grain

251   filling stage (Table 5). Generally, CVs at the grain filling stage were lower than those at the elongation stage,

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

253   **^{15}N enrichment variation in aboveground components of winter wheat**

254   Following ^{15}N-labeling, the order of ^{15}N excess (in atom%) of ears, leaves, and stems were similar at

255   elongation and grain filling stages, with ^{15}N excess highest in ears, followed by stems and leaves (Table 2). In

256   regard to the three shoot portions (without ears), combined ^{15}N excess of leaves and stems were similar among

257   different portions at both elongation and grain filling stages (Table 4). ^{15}N excess of stems and leaves were

258   similar at the elongation stage, but were highest in the upper portion and lowest in the lower portion at the

259   grain filling stage (Figure 4). As growth advanced, i.e., with the increase in biomass, ^{15}N excess were increased

260   by 32.1%–41.8% (Table 2). Stage had a significant impact on ^{15}N excess for all shoot components except

261   lower portions of leaves (Table 3). Labeled wheat exhibited an intra-pot CV of 7.7%–18.1% at both elongation

262   and grain filling stages; the inter-pot CV was 1.7%–23.4% at the elongation stage, with much smaller variation

263   at the grain filling stage (0.1%–6.2%; Table 5). At both stages, the variation in ^{15}N excess was smaller than

264   that of $\delta^{13}C$. For both ^{15}N excess and $\delta^{13}C$ values, intra-pot and inter-pot CVs were comparable, showing

265   similar levels of variation for ^{13}C and ^{15}N distributions.

266   **DISCUSSION**

Variation in ^{13}C enrichment

267

268   Average total $\delta^{13}C$ values of aboveground wheat parts resulting from $^{13}CO_2$ release from $Na_2^{13}CO_3$ (98

269   atom% ^{13}C) in the field labeling chamber ranged from 81‰ to 295‰ (Table 2). Because most studies

270   involving pulse labeling of wheat used ^{14}C rather than ^{13}C, we compared our findings with those of studies

271   using ^{13}C labeling in other plants. By injection of $^{13}CO_2$ gas (90 atom% ^{13}C) in a field labeling chamber, *Tahir*

272   *et al.* (2018) produced a large amount of wheat with a final $\delta^{13}C$ enrichment of 493‰ in leaves and 357‰ in

273   stems, while *Meng et al.* (2013) enriched maize shoots to approximately 125‰–178‰ via $^{13}CO_2$ release from

274    $Ba^{13}CO_3$ (98 atom% ^{13}C) in a laboratory labeling chamber. The direct $^{13}CO_2$ labeling method has also been

275   used for the production of very highly ^{13}C-enriched crop plants such as rice (533‰–927‰) (*Liu et al., 2015*)

276   and clover (>1000‰) (*Thompson, 1996*). The ^{13}C enrichment of plant materials is dependent on both the

277   quantity of ^{13}C per gram dry weight of plant labeled, which is influenced by the quantity of ^{13}C added to the

278   labeling chamber, and net ^{12}C and ^{13}C assimilation rates.

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

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

281   increased, whereas those of leaves and stems significantly decreased (Tables 1, 2). This pattern, i.e., an

282   increase in the carbohydrate sink during plant development, is typical of cereal crops. In a ^{14}C pulse-labeling

283   experiment, *Swinnen et al.* (1994) found that up to 85% of the ^{14}C allocated to aboveground parts was

284   recovered in the ears at the ripening stage. In our study, the proportion in ears relative to all aboveground

285   portions increased from 30% at the elongation stage to 50% at the grain filling stage (Figure 2a). Among

286   aboveground components, $\delta^{13}C$ values were also the highest in ears at grain filling stage (Table 2). At the grain

287   filling stage, the ears were conditioned by the size and availability of C pools. At the grain filling stage, the

288   main C sources for wheat ears are mainly from the remobilization of labeledphotosynthesized C from leaves

289   and stems , and the photosynthesis by the ears itself (*Palta et al., 1997; Aranjuelo et al., 2013; Zhou et al.,*

290   *2016*). Several studies using the natural abundance of ^{13}C and ^{13}C labeling techniques have proved that ear

291   photosynthesis may account for 40% to 80% of the C accumulated in the ears (*Palta et al. 1997; Aranjuelo et*

292   *al. 2011; Sanchez-Bragado et al. 2014*). The fact that the leaves and stems had the lowest labeled C in wheat is

293   also indicative that these organs acted as a C source for grain filling, with ears as sink having more labeled C.

294   This result is in agreement with previous C pulse labeling study for wheat during grain filling (*Aranjuelo et al.,*

295   *2013*). However, at the elongation stage, the larger ^{13}C-enriched values of shoots (leaves and stems) was

296   observed in this study (Table 2). This suggested that shoots acted as major labeled C sinks due to the poor sink

297   strength of ears at the elongation stage, while ears represented the major labeled C sink that competed with the

298   shoots as C sink at the grain filling stage (*Palta et al., 1994; Aranjuelo et al., 2013; Zhou et al., 2016*).

299   At the elongation stage, N addition tended to reduce δ^{13}C values of aboveground components through a

300   dilution effect (Table 2). Compared with the –N treatment, net ^{13}C assimilation in plant biomass increased by

301   7.8% under the +N treatment (76 mg ^{13}C pot^{-1} for –N vs. 82 mg ^{13}C pot^{-1} for +N), while the increase in plant

302   biomass was 35% (Figure 2a). Another likely relevant factor was the reduced carbohydrate accumulation under

303   over-supplied N conditions, i.e., under the +N treatment in the current study (*Palta, 1991; Gooding and*

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

305   components (Table 2). This result was probably due to the presence of more green leaves for the +N treatment

306   at the later stage of maturity (grain filling), which have a stronger photosynthetic capacity and hence can affect

307   the ^{13}C-isotopic signature of aboveground components.

308   At both labeled stages, ^{13}C enrichment was higher in upper (new) leaves than in lower (old) leaves

309   (Figure 3), which may be explained by the more rapid uptake of $^{13}CO_2$ by younger leaves. Leaf age influences

310   photosynthetic capacity, and also the ^{13}C-isotopic signature of plant leaves (*Girardin et al., 2009*). In addition,

311   young expanded leaves provide most labeled C assimilates for stem growth (*Ryle and Powell, 1972;*

312   *Subrahmanyam and Rathore, 2004*). Other factors, such as light intensity (*Ryle and Powell, 1972;*

313   *Subrahmanyam and Rathore, 2004*), air temperature (*Meharg and Killham, 1989*), and relative humidity

314   (*Farquhar et al., 1982*), are also known to influence photosynthetic rates and change the C-isotopic signature.

315   Wheat leaves had higher $\delta^{13}C$ values than stems (Table 2), which is in agreement with previous $^{13}CO_2$ pulse-

316   labeling studies such as that of grain sorghum (*Berg et al., 1991*). Leaf and stem positions (upper, middle, and

317   lower) also influence the $\delta^{13}C$ values of aboveground components (*Thompson, 1996*). In our study, upper

318   leaves tended to be more enriched in ^{13}C compared with upper stems, whereas lower leaves were generally less

319   ^{13}C-enriched than stems at a similar position (Table 2) (*Thompson, 1996*).This result was due to the

320   remobilization of photosynthate and more recently fixed C from leaves to stems during reproductive (grain

321   filling) and vegetative (elongation) stages. The great variation in $\delta^{13}C$ values in leaves and stems indicated that

322   a single $^{13}CO_2$ pulse-labeling did not produce uniformly ^{13}C-labeled plant material. Repeat-pulse labeling has

323   been suggested to overcome the technical constraints of continuous labeling and the high variation issues of

324   single-pulse labeling approache, and may obtain adequate homogeneous ^{13}C-labeled plant material (*Roper et*

325   *al., 2013; Tahir et al., 2018*).

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

327   individual plants within a pot and among the three replicate pots (Table 5). Approximately 75% of leaf and

328   stem components had a CV for intra-pot $\delta^{13}C$ higher than 30% at the elongation stage, while 67% had a CV

329   less than 20% at the grain filling stage. This result demonstrates that the variability between individual wheat

330   plants at the grain filling stage was smaller than that at the elongation stage, especially for the −N treatment.

331   The ^{13}C-enrichment variation in different portions of shoot at the elongation stage are much higher than that of

332   the grain filling stage, which may explain that young leaves are the most frequently labeled wheat organs

333   (*Girardin et al., 2009; Nguyen Tu et al., 2012*). The stronger labeling of starch is related to its rapid synthesis

334   during leaves photosynthesis compared with that of stems during vegetative growth stages, while the

335   redistribution of labeled C from source organs (leaves and stems) toward sink organs (ears) of C assimilated

336   during the grain filling period (*Aranjuelo et al., 2013; Zhou et al., 2016*) may lead to a less extent of variations

337   of ^{13}C-enrichment among plant organs. Such different variations between +N and -N were because C and N

338   remobilization starts earlier and stronger when plants are under low N fertilization compared to high N

339   fertilization condition (*Aranjuelo et al., 2013*). Approximately 83% of leaf and stem components had an inter-

340   pot δ^{13}C CV higher than 20% at the elongation stage, while 67% had an intra-pot δ^{13}C CV lower than 20% at

341   the grain filling stage (Table 5). This result might indicate that the photosynthesized C in leaves and stem has

342   many destinations at the elongation stage, whereas the reallocation of labeled C from leaves and stem mainly

343   goes to the ears at the grain filling stage (*Palta et al., 1994; Aranjuelo et al., 2013; Zhou et al., 2016*). We

344   therefore suggest that labeling of winter wheat at the grain filling stage without N fertilization may produce

345   more uniformly ^{13}C-labeled materials, while labeling of winter wheat at the elongation stage without N

346   fertilization may produce more highly ^{13}C-enriched plant materials albeit it can be more variable.

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

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

349   labeling conditions were much smaller (Table 2). Variation among individual plants (intra-pot) and among

350   different pots (inter-pot) was also much lower (Table 5). No significant differences in ^{15}N excess were

351   observed among wheat aboveground components; the greatest difference, between ears and leaves, was only

352   11% at the grain filling stage (Table 2). This result suggests that the ^{15}N-enriched urea application used in this

353   study can produce relatively uniformly labeled straw tissues at the late (grain filling) stage in wheat. It is

354   because N is a very mobile nutrient in the plant, because there are other nutrients (i.e., C) that do not move so

355   easily inside the plant. This outcome is due to the fact that N is utilized as a nutrient by plants, whereas C is a

356   basic element of plant photosynthesis. In contrast to plant ^{13}C labeling, which requires complex environmental

357   conditions, such as temperature, humidity, and ^{13}CO$_2$ concentration, to be maintained throughout the labeling

358   period (*Soong et al., 2014*), ^{15}N labeling is relatively easy to achieve through ^{15}N fertilization. Ideally, studies

359   of straw tissue decomposition rate should be conducted, both to select uniformly enriched materials (*Crozier et*

360   *al., 1993; Soong et al., 2014*) and to choose straw tissue similar to that of actual field-grown plant tissues

361   (*Crozier et al., 1993*). The chemical composition of straw tissue at late maturity differs substantially from that

362   at an early stage of maturity. For instance, most plant N at later stages of maturity is associated with higher

363   proteins, nucleic acids, and amino sugars (*Wagger et al., 1985*). Some components may exist in a form more

364   resistant to microbial decomposition, such as N associated with cell wall material (*Wagger et al., 1985*) .

365   As wheat growth progressed, i.e., with an increase in biomass, ^{15}N excess increased by 30.4%–41.1%

366   (Table 2), indicating that plant N demand was satisfied by available soil N derived from ^{15}N fertilizer between

367   elongation and grain filling stages. N demand per plant is largely met through N uptake by roots pre-anthesis,

368   with ear N content also supplemented by the amount of N absorbed by root systems between anthesis and ear

369   maturity *(Kichey et al., 2007)*.

370   The ^{15}N enrichment of shoots and ears were different at different wheat stages. At the elongation stages,

371   there was no significant differences of ^{15}N enrichment among leaves, stems and ears. This result may be

372   explained by the fact that the redistribution of labeled N from source organs to ears was low level and the

373   heading ears N was mainly taken up from the soil during vegetative growth stages (*Aranjuelo et al., 2013*).

374   However, at the grain filling stage, wheat ears labeled with ^{15}N tended to accumulate a significantly higher ^{15}N

375   compared with other aboveground components (Table 2). Other researchers have described similar phenomena

376   following ^{15}N labeling, such as in wheat (*Ladd et al., 1981; Palta et al., 1994*) and in winter wheat and grain

377   sorghum (*Wagger et al., 1985*). The ears was a critical pool of N sink that influenced the N accumulation in the

378   ears during the grain filling, while the shoots and roots serve to feed the ears (*Palta et al., 1994; Aranjuelo et*

379   *al., 2013; Zhou et al., 2016; Sun et al., 2018; Zhou et al., 2018*). In the same experiment, we found that 65% of

380   total N of grains was remobilized from the N accumulation in the shoots and roots during vegetative growth

381   stage, while the remaining 35% was derived from the N taken up from the soil during grain filling stage (*Sun*

382   *et al., 2018*). Although ears exhibited a significantly higher ^{15}N enrichment compared with leaves and stems in

383   winter wheat at the grain filling, the differences of ^{15}N enrichment between leaves and stems were only slight

384   (Table 2), thereby suggesting relatively uniform labeling in the plants because N accumulation and

385   redistribution in grain occurs many days after N accumulation in leaves and stems (*Jha and Ladd, 1985;*

386   *Wagger et al., 1985*).

387   **CONCLUDING REMARKS**

388   Weight-average ^{13}C values of aboveground wheat components resulting from ^{13}CO$_2$ release from

389   Na$_2$^{13}CO$_3$ (98 atom% ^{13}C) in the field labeling chamber ranged from 81‰ to 295‰. ^{13}C labeling of winter

390   wheat at the grain filling stage without N fertilization may produce more uniformly ^{13}C-labeled materials,

391   while the materials were more highly ^{13}C-enriched at the elongation stage although the ^{13}C values of the wheat

392   materials were more variable. The photosynthesized C in leaves and stems has many destinations at the

393   elongation stage, whereas at the grain filling stage, the reallocation mainly goes to the ears. The presence of

394   more green leaves for the +N treatment than for -N at the grain filling stage had a stronger photosynthesis

395   capacity and led to a higher variation of ^{13}C-isotopic signature of aboveground components. The pulse labeling

396   did not produce uniformly ^{13}C-labeled plant materials. Different from ^{13}C labeling, which asks for the control

397   of various environmental conditions throughout the labeling period, such as temperature, humidity and ^{13}CO$_2$

398   concentration, a uniform ^{15}N labeling is relatively easier to achieve through ^{15}N fertilization; differences in

399   isotope excess among aboveground wheat components were much smaller under ^{15}N labeling than under ^{13}C

400   labeling condition. The application of ^{15}N-enriched urea as carried out in this study may produce more

401   uniformly labeled wheat straw at the grain filling stage than at the elongation stage.

402   **Acknowledgement**

403   This study was financially supported by the National Key Research and Development Program of China

404   (grant no. 2016YFD0800100) and NSFC program (grant no. 31370527). We thank Zhao Zichao for his

405   assistance with isotope analysis. We also thank the anonymous reviewers for their valuable comments.

406

407   **References**

408   **An T, Schaeffer S, Zhuang J, Radosevich M, Li S, Li H, Pei J, Wang J. 2015.** Dynamics and distribution of

409   ^{13}C-labeled straw carbon by microorganisms as affected by soil fertility levels in the Black Soil region of

410   Northeast China. *Biology and Fertility of Soils* **51**: 605-613

411   **Aranjuelo I, Cabrera-Bosquet L, Araus JL, Nogués S. 2013.** Carbon and nitrogen partitioning during the

412   post-anthesis period is conditioned by N fertilisation and sink strength in three cereals. *Plant Biology* **15**: 135-

413   143

414   **Aranjuelo I, Cabrera-Bosquet L, Morcuende R, Avice JC, Nogues S, Araus JL, Martínez-Carrasco R,**

415   **Pérez P. 2011.** Does ear c sink strength contribute to overcoming photosynthetic acclimation of wheat

416   plants exposed to elevated CO_2?. *Journal of Experimental Botany* **62**: 3957-3969

417   **Berg JD, Hendrix PF, Cheng WX, Dillard AL. 1991.** A a labeling chamber for ^{13}C enrichment of plant-

418   tissue for decomposition studies. *Agriculture, Ecosystems and Environment* **34**: 421-425

419   **Bird JA, Kessel CV, Horwath WR. 2003.** Stabilization of C-carbon and immobilization of N-nitrogen from

420   rice straw in humic fractions. *Soil Science Society of America Journal* **67**: 806-816

421   **Butterly CR, Armstrong R, Chen D, Tang C. 2015.**Carbon and nitrogen partitioning of wheat and field pea

422   grown with two nitrogen levels under elevated CO_2. *Plant and Soil* **391**: 367-382

423   **Chen Z, Wang H, Liu X, Liu Y, Gao S, Zhou J. 2016.** The effect of N fertilizer placement on the fate of

424   urea-^{15}N and yield of winter wheat in Southeast China. *PLoS One* **11**: e0153701

425   **Coplen, TB. 2011.** Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio

426   measurement results. *Rapid Communications in Mass Spectrometry* **25**: 2538-2560

427   **Craig H. 1953.** The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta* **3**: 53-92

428   **Crozier CR, King LD, Volk RJ. 1993**. Variation in ^{15}N enrichment of crimson clover labeled under field

429   conditions. *Plant and Soil* **152:** 255-260

430   **Fang Y, Nazaries L, Singh BK, Singh BP. 2018.** Microbial mechanisms of carbon priming effects revealed

431   during the interaction of crop residue and nutrient inputs in contrasting soils. *Global Change Biology* **24**:

432   2775-2790

433   **Farquhar GD, Oleary MH, Berry JA. 1982**. On the relationship between carbon isotope discrimination and

434   the inter-cellular carbon-dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9**: 121-137

435   **Fox RH, Myers RJK, Vallis I. 1990.** The nitrogen mineralization rate of legume residues in soil as influenced

436   by their polyphenol, lignin, and nitrogen contents. *Plant and Soil* **129**: 251-259

437   **Gavrichkova O, Kuzyakov Y. 2010.** Respiration costs associated with nitrate reduction as estimated by $^{14}CO_2$

438   pulse labeling of corn at various growth stages. *Plant and Soil* **329**: 433-445

439   **Girardin C, Rasse DP, Biron P, Ghashghaie J, Chenu C. 2009.** A method for ^{13}C-labeling of metabolic

440   carbohydrates within French bean leaves (*Phaseolus vulgaris* L.) for decomposition studies in soils. *Rapid*

441   *Communications in Mass Spectrometry* **23**: 1792-1800

442   **Gooding MJ, Davies WP. 1992**. Foliar urea fertilization of cereals - a review. *Fertilizer Research* **32:** 209-

443   222

444   **Hou HT. 2011**. Effects of fertilization application rate on crop cultivation and soil organic carbon. Doctoral

445   dissertation, Nanjing Agricultural University, Nanjing,China

446   **Jha B, Ladd JN. 1985.** Symbiotically-fixed and soil-derived nitrogen in legumes grown in pots in soils with

447   different amounts of available nitrate. *Soil Biology and Biochemistry* **17**: 47-55

448   **Jia S, Wang X, Yang Y, Dai K, Meng C, Zhao Q, Zhang X, Zhang D, Feng Z, Sun Y, Wu X, Cai D,**

449   **Grant C. 2011.** Fate of labeled urea-^{15}N as basal and topdressing applications in an irrigated wheat-maize

450   rotation system in North China Plain: I winter wheat. *Nutrient Cycling in Agroecosystems* **90**: 331-346

451   **Kichey T, Hirel B, Heumez E, Dubois F, Le GJ. 2007**. In winter wheat (*Triticum aestivum* L.), post-anthesis

452   nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological

453   markers. *Field Crops Research* **102**: 22-32

454   **Ladd J N, Oades JM, Amato M. 1981.** Distribution and recovery of nitrogen from legume residues

455   decomposing in soils sown to wheat in the field. *Soil Biology and Biochemistry* **13**: 251-256

456   **Liang B, Yang X, Murphy DV, He X, Zhou J. 2013**. Fate of ^{15}N-labeled fertilizer in soils under dryland

457   agriculture after 19 years of different fertilizations. *Biology and Fertility of Soils* **49**: 977-986

458   **Liljeroth E, Kuikman P, Van Veen JA. 1994.** Carbon translocation to the rhizosphere of maize and wheat

459   and influence on the turnover of native soil organic matter at different soil nitrogen levels. *Plant and Soil*

460   **161**: 233-240

461   **Liu P, Jiang C, Li Z. 2015**. Quantitative research on effects of nitrogen application rate on distribution of

462   photosynthetic carbon in rice-soil system using ^{13}C pulse labeling technique. *Acta Pedologica Sinica* **52**:

463   567-575

464   **Marc M, Buegger F, Gattinger A, Marschner B, Zsolnay Á, Munch JC. 2007.** Determination of the fate of

465   [13]C labelled maize and wheat rhizodeposit-C in two agricultural soils in a greenhouse experiment under [13]C-

466   CO_2-enriched atmosphere. *Soil Biology and Biochemistry* **39**: 3043-3055

467   **Martens R, Heiduk K, Pacholski A, Weigel HJ. 2009.** Repeated [14]CO_2 pulse-labelling reveals an additional

468   net gain of soil carbon during growth of spring wheat under free air carbon dioxide enrichment (FACE). *Soil*

469   *Biology and Biochemistry* **41**: 2422-2429

470   **Meharg AA, Killham K. 1989**. Distribution of assimilated carbon within the plant and rhizosphere of *Lolium*

471   *perenne*: Influence of temperature. *Soil Biology and Biochemistry* **21**: 487-489

472   **Meng F, Dungait JAJ, Xu X, Bol R, Zhang X, Wu W. 2016.** Coupled incorporation of maize (*Zea mays* L.)

473   straw with nitrogen fertilizer increased soil organic carbon in Fluvic Cambisol. *Geoderma* **304**: 19-27

474   **Meng F, Dungait JAJ, Zhang X, He M, Guo Y, Wu W. 2013**. Investigation of photosynthate-C allocation

475   27 days after ^{13}C-pulse labeling of *Zea mays* L. at different growth stages. *Plant and Soil* **373**: 755-764

476   **Nguyen Tu TT, Biron P, Maseyk K, Richard P, Zeller B, Quénéa K, Alexis M, Bardoux G, Vaury V,**

477   **Girardin C, Pouteau V, Billiou D, Bariac T. 2013.** Variability of ^{13}C-labeling in plant leaves. *Rapid*

478   *Communications in Mass Spectrometry* **27**: 1961-1968

479   **Palta JA. 1991**. Assessing the use of ^{15}N-^{13}C-urea for studying the long term storage and remobilization of

480   carbon and nitrogen in wheat. p-441-452. *In* The stable isotopes in plant nutrition, soil fertility and

481   environment studies. International Atomic EnergyAgency, Vienna

482   **Palta JA, Kobata T, Turner NC, Fillery IR. 1994.** Remobilization of carbon and nitrogen in   wheat as

483   influenced by postanthesis water deficits. *Crop Science* **34**: 118-124

484   **Pausch J, Kuzyakov Y. 2018.** Carbon input by roots into the soil: Quantification of rhizodeposition from root

485   to ecosystem scale. *Global Change Biology* **24**: 1-12

486   **Pollierer, MM, Langel R, Körner C, Maraun M, Scheu S. 2007**. The underestimated importance of

487   belowground carbon input for forest soil animal food webs. *Ecology Letters* **10**: 729-736

488   **Ranells NN, Wagger MG. 1992.** Nitrogen release from crimson clover in relation to plant growth stage and

489   composition. *Agronomy Journal* **84**: 424-430

490   **Roper MM, Fillery IRP, Jongepier R, Sanford P, Macdonald LM, Sanderman J, Baldock JA. 2013.**

491   Allocation into soil organic matter fractions of ^{14}C captured via photosynthesis by two perennial grass

492   pastures. *Soil Research* **51**: 748-759

493   **Ryle GJA, Powell CE. 1972.** The export and distribution of ^{14}C-labelled assimilates from each leaf on the

494   shoot of *Lolium temulentum* during reproductive and vegetative growth. *Annals of Botany* **36**: 363-375

495   **Sanchez-Bragado R, Molero G, Reynolds MP, Araus JL. 2014.** Relative contribution of shoot and ear

496   photosynthesis to grain filling in wheat under good agronomical conditions assessed by differential organ

497   δ^{13}C. *Journal of Experimental Botany* **65**: 5401-5413

498   **Shan HX, Lu CA, Zhang JT, Wang JZ, Xu MG. 2012.** Effect of maize straw applied with N fertilizer on

499   nitrogen adsorption of winter wheat under different soil fertility. *Plant Nutrition and Fertilizer Science* **18**:

500   35-41

501   **Shi YF, Wu WL, Meng FQ, Zhang ZH, Zheng L, Wang DP. 2013**. Integrated management practices

502   significantly affect N_2O emissions and wheat–maize production at field scale in the North China Plain.

503   *Nutrient Cycling in Agroecosystems* **95**: 203-218

504   **Soong JL, Reuss D, Pinney C, Boyack T, Haddix ML, Stewart CE, Cotrufo MF. 2014**. Design and

505   operation of a continuous ^{13}C and ^{15}N labeling chamber for uniform or differential, metabolic and structural,

506   plant isotope labeling. *Jove-Journal of Visualized Experiments* **83**: e51117

507   **Studer MS, Siegwolf RTW, Abiven S. 2014.** Carbon transfer, partitioning and residence time in the plant-soil

508   system: a comparison of two ^{13}C-CO_2 labelling techniques. *In* EGU General Assembly Conference **16**:

509   1637-1648

510   **Subrahmanyam D, Rathore VS. 2004.** $^{14}CO_2$ assimilation and ^{14}C-photosynthate translocation in different

511   leaves of sunflower. *Photosynthetica* **42**: 313-316

512   **Sun Z, Chen Q, Han X, Bol R, Qu B, Meng F. 2018.** Allocation of photosynthesized carbon in an intensively

513   farmed winter wheat–soil system as revealed by $^{14}CO_2$ pulse labelling. *Scientific Reports* **8**: 3160

514   **Swinnen J, Van Veen JA, Merckx R. 1994.** ^{14}C pulse-labelling of field-grown spring wheat: An evaluation

515   of its use in rhizosphere carbon budget estimations. *Soil Biology and Biochemistry* **26**: 161-170

516   **Tahir MM, Recous S, Aita C, Pfeifer IC, Chaves B, Giacomini Sandro José. 2018.** Field ^{13}C pulse labeling

517   of pea, wheat, and vetch plants for subsequent root and shoot decomposition studies. *Revista Brasileira de*

518   *Ciência do Solo* **42**: e0160449

519   **Thompson RB. 1996**. Pulse-labelling a cover crop with ^{13}C to follow its decomposition in soil under field

520   conditions. *Plant and Soil* **180**: 49-55

521   **Wagger MG, Kissel DE, Smith SJ. 1985.** Uniformity of nitrogen-15 enrichment in different plant parts and

522   subsequent decomposition monitoring of labeled crop residues. *Soil Science Society of America Journal* **49**:

523   1205-1208

524   **Wang YP, Law RM, Pak B. 2009.** A global model of carbon, N and phosphorus cycles for the terrestrial

525   biosphere. *Biogeosciences* **7**: 9891-9944

526   **Xu X, An T, Zhang J, Sun Z, Schaeffer S, Wang J. 2019.** Transformation and stabilization of straw residue

527   carbon in soil affected by soil types, maize straw addition and fertilized levels of soil. *Geoderma* **337**: 622-

528   629

529   **Yang J, Zhang J. 2006.** Grain filling of cereals under soil drying. *New Phytologist* **169**: 223-236

530   **Yu Z, Li Y, Jin J, Liu X, Wang G. 2017.** Carbon flow in the plant-soil-microbe continuum at different

531   growth stages of maize grown in a Mollisol. *Archives of Agronomy and Soil Science* **63**: 362-374.

532   **Zhao Y, Zhang W, Wang X, Fu X, Jia D, Gao Z, Shi Z, He M. 2016**. Analysis of winterness-springness

eager

533   type and vernalization genes in the main wheat cultivars from the North of Yellow and Huai valley of China.

534   *Journal of Triticeae Crop* **36**: 1440-1448

535   **Zhao Z, Wu D, Bol R, Shi Y, Guo Y, Meng F, Wu W. 2017.** Nitrification inhibitor's effect on mitigating N_2O

536   emissions was weakened by urease inhibitor in calcareous soils. *Atmospheric Environment* **166:** 142-150

537   **Zhou B, Serret MD, Elazab A, Pie JB, Araus JL, Aranjuelo I, Sanz-Saez A. 2016.** Wheat ear carbon

538   assimilation and nitrogen remobilization contribute significantly to grain yield. *Journal of Integrative Plant*

539   *Biology* **58**: 914–926

540   **Zhou B, Serret MD, Pie JB, Shah SS, Li Z. 2018.** Relative contribution of nitrogen absorption,

541   remobilization, and partitioning to the ear during grain filling in chinese winter wheat. *Frontiers in Plant*

542   *Science* **9**: 1351

543   **Zhu B, Cheng W. 2011.** Rhizosphere priming effect increases the temperature sensitivity of soil organic

544   matter decomposition. *Global Change Biology* **17**: 2172-2183

545

546

547

548

549

550

551

552

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

554   **(grain filling stage) (b). Field labeling chamber for winter wheat (c). Schematic diagram of the three**

555   **sampled portions of wheat shoots (d).** –N = no N addition; +N = N addition.

556

557   **Figure 2 Biomass (a), C content (b), and N content (c) of different wheat components.** Error bars indicate

558   standard errors ($n = 4$). –N = no N addition; +N = N addition.

559

560

561   **Figure 3 δ^{13}C (‰) values of different wheat components.** Different lowercase letters denote significant

562   differences (LSD, $p < 0.05$) among wheat components at the same stage. Error bars indicate standard errors (n

563    $= 9$). –N = no N addition; +N = N addition.

564

565   **Figure 4 ^{15}N excess in atom% of different wheat components with ^{15}N labeling.** Different lowercase letters

566   denote significant differences among wheat components at the same stage (LSD, $p < 0.05$). Error bars indicate

567   standard errors ($n = 9$).

568

569

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 wheat shoots (d).

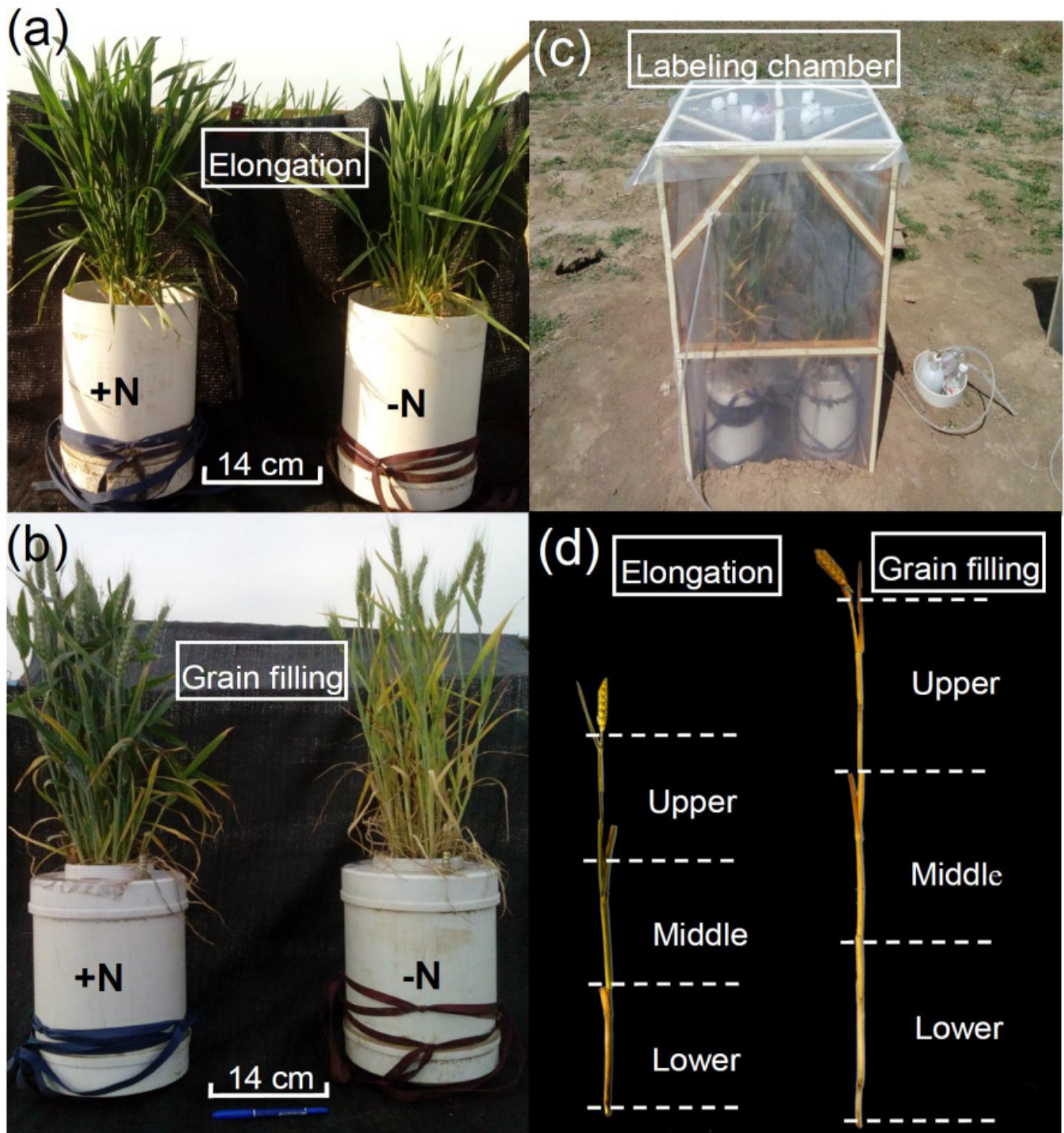



Figure 2

Biomass (a), C content (b), and N content (c) of different wheat components.

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

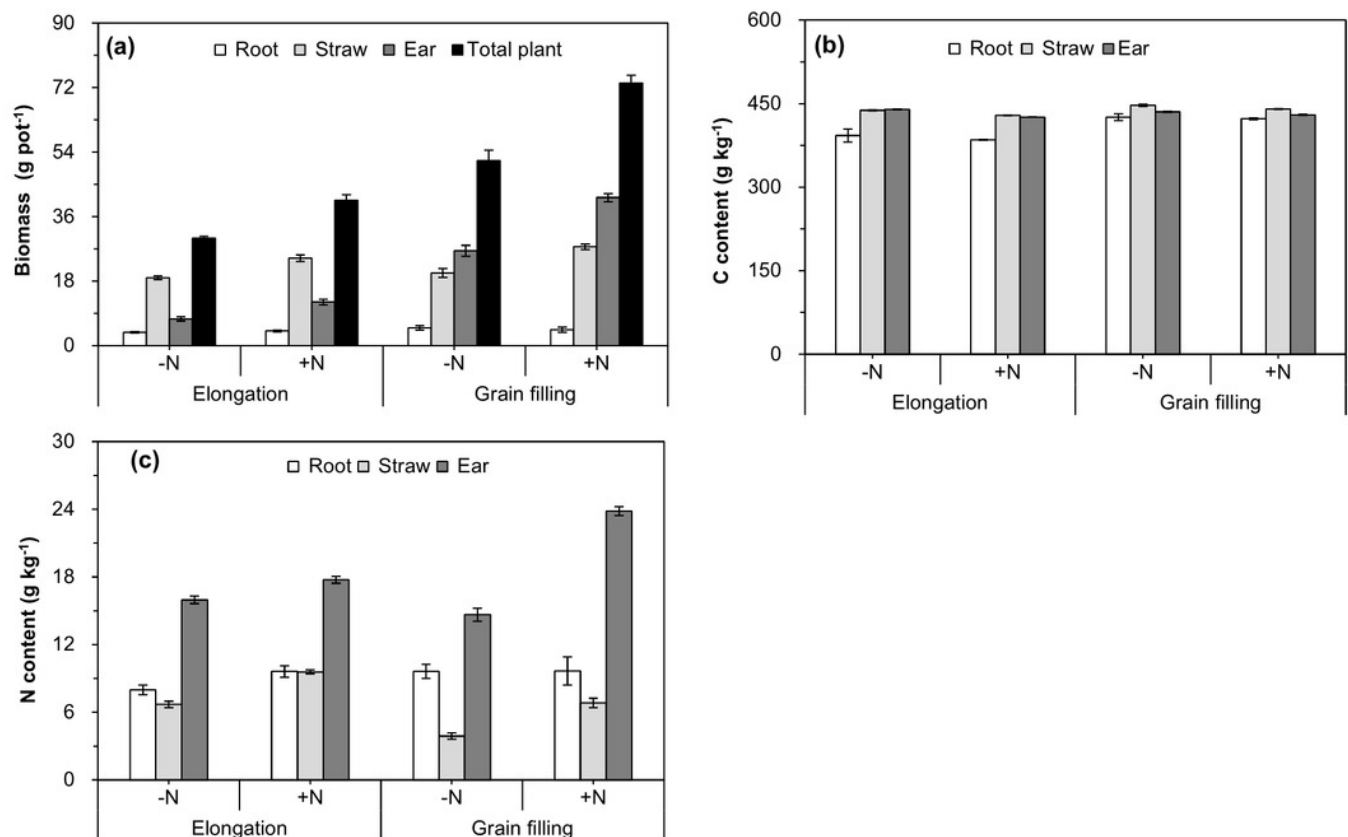



Figure 3

δ^{13}C (‰) values of different wheat components.

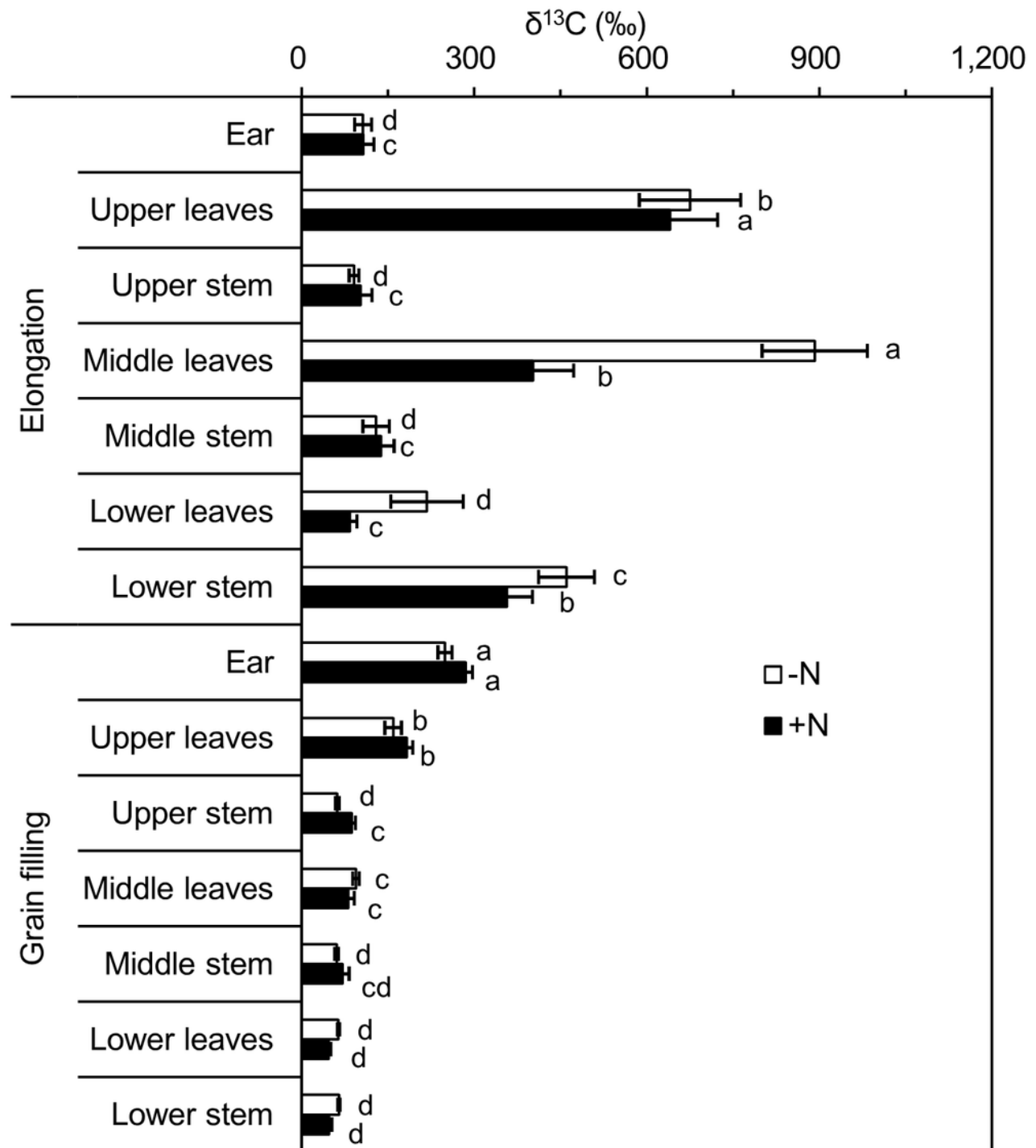
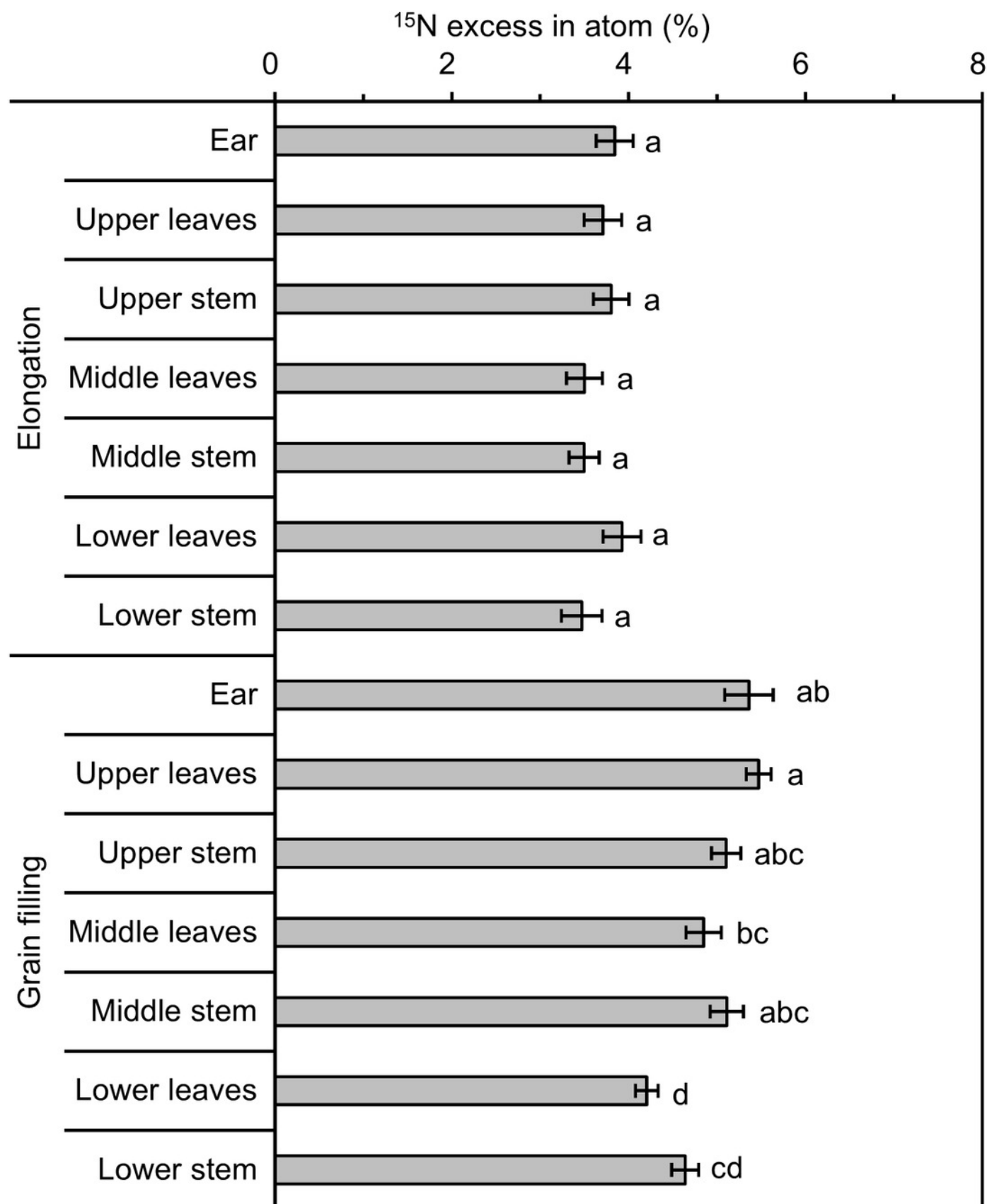
Different lowercase letters denote significant differences (LSD, $p < 0.05$) among wheat components at the same stage. Error bars indicate standard errors ($n = 9$). –N = no N addition; +N = N addition.

δ¹³C (‰)

Figure 4

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

Different lowercase letters denote significant differences among wheat components at the same stage (LSD, $p < 0.05$). Error bars indicate standard errors ($n = 9$).

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

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

2 **C content, and N content of different 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% (mean ± SE, $n = 9$) of different wheat components.

Different lowercase letters in the same row denote significant differences (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 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% (mean ± SE, *n* = 9) of different wheat components.** Different

2 lowercase letters in the same row denote significant differences (LSD, $p < 0.05$) in δ^{13}C or ^{15}N excess in atom%

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

4 δ^{13}C values between N treatments at the same stage or significant differences (*t*-test, $p < 0.05$) in ^{15}N excess in

5 atom% between different stages under the same N treatment (^{15}N labeling). †−N = no N addition; ††+N = N

6 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 filling	-N	249.1±12.1a	105.7±4.6b	62.4±1.0c	81.2±3.1
		+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 of two-way analysis of variance for the effects of stage (S) and nitrogen (N) on δ^{13}C value and δ^{15}N excess in atom% in different wheat components.

1 **Table 3 *P*-values of two-way analysis of variance for the effects of stage (S) and nitrogen (N) on $\delta^{13}C$**

2 **value and $\delta^{15}N$ excess in atom% in different 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 (mean ± SE, n = 9) of upper, middle, and lower portions of shoot in wheat.

Different lowercase 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 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 fertilization; $^{††}+N$ = N fertilization.

1   **Table 4 Isotope values (mean ± SE, *n* = 9) of upper, middle, and lower portions of shoot in wheat.**

2   Different lowercase letters in the same row denote significant differences in $\delta^{13}C$ or ^{15}N excess in atom% (LSD,

3    $p < 0.05$) between the three portions. Asterisks (*) in the same column denote either significant differences (*t*-

4   test, $p < 0.05$) in $\delta^{13}C$ between N treatments at the same stage or significant differences (*t*-test, $p < 0.05$) in ^{15}N

5   excess in atom% between different stages under the same N treatment (^{15}N labeling). [†] −N = no N fertilization;

6   [††] +N = N fertilization.

			Upper portion	Middle portion	Lower portion
$\delta^{13}C$ (‰)	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 $\delta^{13}C$ value and ^{15}N excess in atom% of different wheat components.

[†] $-N$ = no N addition; [††] $+N$ = N addition.

1   **Table 5 Intra-pot and inter-pot variation in δ¹³C value and ¹⁵N excess in atom% of different wheat components.** † −N = no N addition; †† +N = N

2   addition.

			CV (%) of intra-pot							CV (%) of inter-pot						
				Leave			Stem				Leave			Stem		
			Ear	Upper	Middle	Lower	Upper	Middle	Lower	Ear	Upper	Middle	Lower	Upper	Middle	Lower
$\delta^{13}C$ (‰)	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 in atom%	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
	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