Elevated atmospheric CO2 concentration triggers redistribution of nitrogen to promote tillering in rice

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Abstract

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Running title: **N redistribution promotes tillering under eCO_2**

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Abstract

Elevated atmospheric CO_2 concentration (eCO_2) reduces nitrogen (N) content in rice plants and stimulates tillering. However, these are contradictory to the general consensus that reduced N would constrain rice tillering. To resolve this, we detected N distribution in organs and transcriptomic changes of different organs after subjected to eCO_2 in combination with different N application rates. Our results indicated that eCO_2 promoted rice tillers more under higher N supply conditions, and confirmed that N availability constrained tillering in the early growth stage. Despite N content declined in the leaf and sheath of rice exposed to eCO_2, the new-born tillers had a stable or higher N content compared to those under ambient CO_2. Apparently the redistribution of N within the plant*per se* was a critical adaptation strategy to eCO_2condition. Transcriptomic analysis revealed that eCO_2introduced less extensive alteration of gene expression than N application. Most importantly, the expression levels of multiple N-related transporters and receptors were differentially regulated, suggesting that multiple genes were involved in sensing the N signal and transporting N metabolites in adapting to eCO_2. The redistribution of N in different organs could be a universal adaptation strategy of terrestrial plants to eCO_2. Keywords: atmospheric CO_2, rice, tiller, nitrogen, distribution, gene expression.

1. Introduction

The accelerating pace of rising atmospheric carbon dioxide (CO_2) concentration has been a serious global climatic issue for a couple of decades. Despite the Kyoto Protocol being adopted in 1997 and entered into force in 2005, followed by Paris Agreement in November 2016, the atmospheric CO_2 concentration ($[CO_2]$) shows no sign of slowing down in its increasing rate. The current historical record of atmospheric $[CO_2]$ reached a climax of 417.07 ppm in May 2020 (ESRL, Mauna Loa, Hawaii, USA). In terms of crop production, the elevated atmospheric CO_2 concentration (eCO_2) and the consequential temperature hike will bring in more extreme weathers (such as drought and flood, extreme heatwave and freezing temperature, hurricane and hails), frequent crop diseases and pests occurrences, and recurrent adverse crop growth conditions. All these will reduce crop productivity, quality, and stability, especially for wheat and maize (Easterling et al, 2005; Rosenzweig et al, 2014; Li et al, 2015; Wang et al, 2019a). It seems that rice and soybean are the mere major crops that may marginally benefit from the eCO_2, especially when considering the collateral temperature and ozone hikes (Long et al, 2006; Ainsworth, 2008; Kimball, 2016; Usui et al, 2016; Zhao et al, 2017). To mitigate the threat and secure the crop production, it is critically important to breed crop varieties adaptable for the future $[CO_2]$ and temperature conditions, and to integrate a novel cultivation management system to take the advantage of the fertilization effect of this irreversible eCO_2 change (Long et al, 2004). However, genes responsive for eCO_2 adaptation are largely unknown (Morita et al, 2015; Nakano et al, 2017; Hasegawa et al, 2019), thus, we need to clarify targets for breeding purpose. Top priority should be given to those yield-limiting agronomic characters that showing beneficially responsive to eCO2.

Rice (*Oryza sativa* L.) is a staple cereal crop for more than half of the world population, especially in the densely populated Asian regions. Despite the divergent responses of the productivity to eCO_2 by different crops, it is generally concluded that rice may marginally benefit from the CO_2 fertilization in temperate and tropical regimes (Ruiz-Vera et al, 2013; Rosenzweig et al, 2014; Kimball et al, 2016). Most studies conclude that the eCO_2 increases grain yield of rice (Hasegawa et al, 2017). For example, multiple-year free-air CO_2enrichment (FACE) field experiments conducted at different locations with various *indica* ,

japonica and hybrid rice varieties concluded that, eCO_2 at 550 ppm can enhance rice grain yield up to 5˜35% compared to the ambient [CO_2] (Kim et al, 2003; Kimball et al, 2016). Among the four major rice yield components, namely, panicles per area, spikelets per panicle, seed setting rate (%), and grain weight, eCO_2 consistently increases panicles per area, while the other three yield components show both negative and positive responses (Kim et al, 2003a; Huang et al, 2004; Lai et al, 2014; Hasegawa et al, 2019). The panicle per area is a fundamental factor that being determined at the earlier stage of rice growth. It impacts the other three yield components at later stage of growth and arbitrates final grain yield. Therefore, endeavor to achieve a stable panicle number is always a top priority in rice production management.

In addition to the main stem, rice plant produces tillers (branches) that may eventually develop into panicle florescence to generate grain yield (Wang and Li, 2011). The tiller number of a rice plant is not only a basis for panicle number, but also an indicator of plant growth status. Rice plants with more tillers at the early stages usually indicate they are in a healthy developmental path toward higher yield. Tiller number also shows a significant accrual response to eCO_2 (Kim et al, 2003b; Huang et al. 2004). Since the discovery of MOC1 in tillering regulation (Li et al, 2003), recent advances in molecular genetics have clarified that more than 60 genes involving in tiller regulation in rice plants (Wang et al, 2011; Wang et al, 2018a). However, most of the knowledge is derived from mutant or gene manipulation experiments, limited info is available on how they coordinate in a regular variety (Zhang et al, 2019).

Moreover, nitrogen (N) is a major macronutrient that constrains tiller growth. Reports have clarified that eCO_2 alters the element stoichiometry in plants, especially a reduction of N content was consistently observed from grasses to crops and trees (Luo et al, 2006; Norby et al, 2010; Deng et al, 2015). Insufficient N availability is a constraint on the growth of perennial grass species in response to eCO_2 (Reich et al, 2006; Mueller et al, 2013). Multiple reviews summarize that quite a range of crops and model plants displaying certain pattern of interaction between N requirement and eCO_2, though interpretation differs but N constraint is a consistency (Stitt and Krapp, 1999; Wang et al, 2010; Bloom et al, 2015; Rubio-Asensio and Bloom 2017; Andrew et al, 2019). eCO_2 reduces the N content in rice plant as well (Makino et al, 1997; Kim et al, 2001; Lieffering et al, 2004; Zhang et al, 2013; Wang et al, 2019b). However, low N content in rice plants is supposed to inhibit tiller occurrence (Jiang et al, 1997). Despite the expected inhibition effect on tillering by reduced N content in rice plants under the eCO_2, multiple reports have confirmed that tillers are promoted by eCO_2 (Jitla et al, 1997; Huang et al, 2004; Yang et al, 2007; Jiang et al, 2020). However, the underlying physiological and molecular mechanisms remain unclear.

We hypothesized that N distribution changes in favor of tillering under eCO2 condition. To test this, we investigated the interactive effects of CO_2 and N rate on rice tillering in growth chambers. Our objectives were 1) to analyze the interaction effect of eCO_2 and N application rate on rice tillering at early stage; 2) to decipher the change in the N distribution among different organs; and 3) to investigate the molecular change in response to the eCO_2. Clarifying the molecular adaptation mechanism of rice to the eCO_2 would directly enable breeders to target certain genes in order to breed varieties that can better benefit from the CO_2 fertilization effect. The mechanism may also help interpreting the adaptation of other terrestrial plants to the eCO_2 and develop new approaches to mitigate the eCO_2.

2. Materials and Methods

2.1 Rice material and culture conditions

The experiment was conducted in two growth chambers (CMP6050, Conviron, Winnipeg, Manitoba, Canada) at the Yangzhou University in Yangzhou, Jiangsu Province, China. Japanese rice variety Nipponbare (*Oryza sativa* , ssp. *japonica*) was used in the experiments. Nipponbare is widely used in rice production, breeding, and molecular genetics studies. It is the first rice variety being deeply genome sequenced as a monocots model plant (Sasaki et al, 2005; Matsumoto et al, 2016).

Rice seeds were acquired from Yangzhou Agricultural Research Center, and surface sterilized with disinfectant prochloraz (Jinbao, Yangzhou Suling Agricultural Chemicals, Jiangdu, China) following the supplier recommendation. Seeds were immersed in tap water for 48 hrs before subjected to germination promotion at 32°C with a humidity of 85% in an incubator for 15 hrs, and at 28°C till shoot and root appearance to 0.5˜1 cm. The germinated seeds were screened for uniformity and planted to soil in plastic trays at a spacing of 3.5 cm x 3.2 cm, with each tray containing 96 plants. The plastic tray contained premixed soil, 12 cm in depth (Zhang et al, 2019), prepared in a similar way as in field nursery for seedling.

During the growth period, soil was checked three times a day to keep its moisture at saturated point but without persistent water layer. The temperature of the growth chamber was preset to 30/22°C (actual records at ± 0.67°C range) in the 12/12hrs day/night regime, respectively. The illumination was programmed to 800 μmol photons $m^{-2}s^{-1}$ (actual records at ± 58 μmol photons m^{-2} s^{-1}) during the day and 0 at night. The relative humidity was set at 70% (actual records range 65-80%).

2.2 Experimental design

This experiment used a split-plot experimental design with four replications. The main treatment factor was [CO_2] and subplot treatment factor was N application rate. There were two [CO_2] levels: ambient CO_2 (400 μmol mol^{-1}) and eCO_2 (600 μmol mol^{-1}). Since we have two independent growth chambers, we set one chamber for ambient CO_2 and the other for eCO_2 treatments, and repeated the whole experiment four times. Each time was considered one replication. The eCO_2 concentration was achieved by pumping in industrial grade pure CO_2 to a partial pressure of 600 μmol mol^{-1} (actual records range 595-640 μmol mol^{-1}), and the ambient condition had a CO_2 partial pressure range of 405 to 430 μmol mol^{-1} during the growth period. As we focused on the adaptation of rice plants to eCO_2 at the early seedling stage, we initiated eCO_2 treatment from seeding. N application rates included four levels: 0 (N0), 5 (N5), 10 (N10) and 15 (N15) kg N 666.7 m^{-2}, which was comparable to the general range of N fertilization rate in the region at this growth phase. Half of the N fertilizer (in the form of urea, 46% N) was premixed into the soil three days prior to seeding, and the remaining portion was top-dressed in a water-solution form at true leaf-age 1, 2, 3 and 4, respectively, each at a 12.5% of the total N application rate. Together, there were eight treatments, combined from two levels of [CO_2] and four levels of N application rate, and each treatment had two plastic trays comprising 192 plants.

2.3 Measurements of total N and carbohydrates content

Leaf-age, plant height and tiller numbers were measured at true leaf-age four and six, respectively. Tiller occurrence percentage was calculated as total tiller number divided by main stem number, reflecting plant consistency at 4^{th} true leaf emerging stage as each plant usually has only one tiller. Plants were harvested at true leaf-age six for biomass measurement (at 25-27 days post-seeding). The plants were separated into leaf, sheath, and new-born tillers (with less than 3 true leaves) for N content analysis using Kjeldhal method with an automatic Kjeltec 8400 Analyzer Unit (Foss Analytical AB, Höganäs, Sweden) following the manufacturer's recommendations. For carbohydrates measurement, plants were further separated into new-born tillers, leaves (leaf positions 1-5), or sheaths (upper and lower parts). Carbohydrates were measured by anthrone reagent (Zhang et al, 2019).

2.3 Samples for RNA sequencing and bioinformatics analysis

Samples for RNA extraction and sequencing were collected at true leaf-age four: the third leaf and tissues near shoot apical meristem (SAM, after removal of outside 2 layers of sheath keeping about ±3 mm of the growth point) were collected swiftly on ice and snap frozen in liquid N_2, then stored at -80°C till use. Tillers

are supposed to start to appear from the leaf axillary zone at this stage, therefore, the SAM were collected for the RNA sequencing to reflect tiller-related genes profile. RNA extraction, pre-treatment, sequencing library generation and bioinformatics algorithm all followed protocols of a previous report (Zhang et al, 2019). We chose four treatments from two [CO_2] combined with two N application rates (N0 and N10) for RNA sequencing analysis. Three independent biological replication samples for each treatment was sequenced, and the acquired RNA sequencing data were no less than 6 Gb bases per sample, which were qualified for gene expression analysis (Table S1).

The filtered data were used in the mapping and expression analysis following previous protocol (Trapnell et al, 2012). The FPKM (Fragments Per Kilo base transcript per Million mapped reads) value was used to indicate the expression level of a gene, which was calculated by this equation: total exon reads / (mapped reads (millions) * exon length (kilo base)). Differentially expressed gene (DEG) was defined as genes showing the ratio of average FPKM between two groups surpassed two-fold ([?]2, or [?]0.5) and the adjusted false discovery rate (adj FDR) being$p$ [?]0.05.

2.4 Statistical analysis

The generalized linear model (GLM) multivariate procedure from SPSS software (Ver22, IBM, Armonk, NY, USA) was used for the ANOVA to test the effects among the treatments. The CO_2 level, N supply rate and tissue type were defined as independent factors, and their full interactions were analyzed using the GLM procedure. Significance level was set at (p [?]0.05) for post-hoc test. Data presented in figures and tables were the average +- standard deviation.

3. Results

3.1 Interactive effects of $eCO_2$and N application rate on tillering

Compared with the ambient [CO_2], eCO_2 significantly increased the number of tillers (Fig. 1A). In respect to N application, the number of tillers increased with N application rate, with more enhancement at leaf-age six than at leaf-age four. Under the higher N application, eCO_2 stimulated more tillers (Fig 1A). Tiller occurrence percentage and uniformity were also enhanced by eCO_2 at leaf-age four (Fig. 1B), especially under eCO_2 and higher N application. Apparently, the tiller enhancement effect by eCO_2depended on N application rate, indicating that the N availability constrained tillering, especially under eCO_2.

Similarly, eCO_2 increased biomass more at the higher than lower N application rate (Fig. 1C). Though N application promoted biomass accumulation in both the ambient and eCO_2conditions, the biomass accumulation reached a plateau at the N10 treatment under the ambient CO_2 condition. At the N15 treatment, biomass still showed a significant increment under the eCO_2 condition (Fig. 1C). It can be concluded that eCO_2 promoted biomass accumulation, especially when combined with higher N application rate.

3.2 Relative distribution change of N to new-born tillers under eCO_2

Higher N application rate increased N content in both leaf and sheath, regardless of CO_2 condition (Fig. 2 A and B). However, eCO_2 reduced the N content consistently under all N application rates, compared to the ambient CO_2treatments. It is worthy to mention that under the ambient CO_2 condition, N content reached a plateau at the N10 treatment, whereas under the eCO_2 it still increased at the N15, regardless of tissue

type (leaf or sheath). This further showed that N application rate was more a constraint to rice growth at the eCO_2 than at the ambient CO_2 condition.

Contrary to the reductions of N contents in the leaf and sheath of rice plant, the new-born tillers under the eCO_2 tended to have higher N content than under the ambient CO_2 (Fig. 2C). Further comparison of their relative changes clearly demonstrated, contrasting to the dramatic decrements of N content in both leaf and sheath, the new-born tillers under the eCO_2 showed a slight increment (Fig. 2D), suggesting distribution favored the new-born tillers. A plausible explanation was that a higher proportion of N distributed to the new-born tillers under eCO_2 than under ambient CO_2 condition.

3.3 Carbohydrate accumulation promoted by the eCO_2

Under the ambient CO_2 condition, soluble sugar content reduced significantly when N application rate increased from N0 to N5 and N10. Further enrichment to N15 significantly increased the soluble sugar content. However, the starch content was relatively stable (Fig. 3A, B, C and D). Likewise, under the eCO_2, enrichment of N from N0 to N5 reduced soluble sugar, but further N enrichment to N10 or N15 increased soluble sugar content. In contrast, the starch content under the eCO_2 was consistently high under all N application rates (Fig. 3E, F, G and H). The new-born tillers under the eCO_2 had higher contents of both soluble sugar and starch than the ambient CO_2, implying that relative higher distribution of N content in them was not a result of carbohydrate drainage. Overall, under the eCO_2condition, all tissues contained higher carbohydrate content than the ambientCO_2, regardless of N application rate.

3.3 Limited transcriptomic change induced by eCO_2 than by N application rate

To understand the underlying transcriptional changes in response to the eCO_2, we employed RNA sequencing approach to compare the gene expression profiles of tissues near the SAM (where the tiller was about to appear) and the third true leaf at leaf-age four. The total number of DEG introduced by tissue type (leaf and SAM), N application rate (N0 and N10), and CO_2 treatment (ambient CO_2 and eCO_2) were strikingly different (Fig. 4A). Tissue had the highest effect, followed by N application rate, and eCO_2. The N application had more influences on leaf than SAM, similar to the impact of CO_2 (Fig. 4A).

Using the Venn diagram, we found that the universal DEG (commonly altered genes) introduced by tissue was the most (11221, Fig. 4B), followed by N application rate (197, Fig. 4C), and lastly by CO_2 effect (5, Fig. 4D). These numbers indicated that the global gene expression profile was overwhelmingly defined by the tissue type, only being altered at limited range by N application rate, and at a very narrow scope by $[CO_2]$.

3.4 Tiller genes displayed a more wide-scale change by eCO_2 than by N application rate

To elaborate their responses to eCO_2 and N application rate, we extracted the transcription data of 65 tiller-related genes from the transcriptome. Among the 51 genes that showed decent expression (FPKM>0.1) in leaf and/or SAM at 4^{th} true leaf emerging stage, there were 50 genes varied between tissues (leaf and SAM), 29 genes being altered by $[CO_2]$, and 24 genes being significantly affected by N application (Table 1). This further corroborated that tissue type (organ) predominantly defines the gene expression profile. However, as there were more tiller genes being responsive to $[CO_2]$ than N application, the $[CO_2]$ was more influential in regulating the tiller-related genes than the N application. Of the 29 $[CO_2]$ responsive tiller-genes and

24 N application responsive tiller-genes, there were 16 genes being commonly responsive to both factors. Eight out of the 22 (36.4%) tiller genes were not responsive to [CO_2] but to N application, whereas 13 out of 27 (48.1%) tiller genes were not responsive to N but to CO_2. This further supported that the [CO_2] was more effective in specifically altering tiller-related genes than the N application, contrary to their differential influences to the global transcriptomic profile.

The relative FPKM changes of these tiller genes introduced by N application (N10/N0), and by [CO_2] (eCO_2/Ambient) in different tissues were shown in Table 2. N application caused more dramatic change in the leaf than in the SAM: 14 (7 up, 7 down) and 12 (11 up, 1 down) tiller genes surpassed two-fold threshold to be DEG under the ambient and the eCO_2 conditions in the leaf, respectively. In the SAM, these numbers were three (all down) and 0 for the ambient and eCO_2, respectively. Direct comparison of the [CO_2] effect showed that 16 (8 up and 8 down) and seven (down) tiller-genes surpassed two-fold in leaf at N0 and N10, respectively; while these values were seven (6 up and 1 down) and two (up) in SAM. These indicated that either most of them were not participating in the tillering response to eCO_2, or the scale of change did not need to surpass the two-fold to affect tillering. This also implies that other genes might be involved in tillering promotion in response to eCO_2.

3.5 N transporters and glutamate receptors were differentially altered in the leaf and SAM

Since the N distribution showed dramatic change, we specifically screened for the N metabolism-related gene expression in the transcriptome profile. Of the 210 N-related genes, 164 had expression (FPKM>0.1). Among them, 158 genes were significantly impacted by the tissue type, 109 genes were affected by N application rate, and 94 were altered by [CO_2] (Table S2). Apparently, eCO_2 exerted a less influential effect to this specific category of genes than the N application. Further ascribing the putative functions of those being significantly affected by [CO_2] had revealed that 20 of the 94 (21.3%) were glutamate receptors and nitrate or ammonium transporters. In contrast, only seven of 70 (10%) CO_2 non-responsive genes belonged to these groups. It seems that more N metabolite receptors and transporters were specifically altered by eCO_2, suggesting that N redistribution/reallocation may become more active under the eCO2. Among them, nine genes displayed contrasting induction and suppression changes between the leaf and SAM (Fig. 5). They were putative major facilitator family transporter (NRT2.3a/b, LOC_Os01g50820, Fig. 5A), ammonium transporters (LOC_Os01g61510, LOC_Os02g34580, and LOC_Os03g62200, Fig. 5B-D), high affinity nitrate transporter (LOC_Os04g40410, Fig. 5E) and peptide transporter PTR2 (NRT1.1a/b, LOC_Os10g40600, Fig. 5F). The others were all putative glutamate receptors (LOC_Os06g08910, LOC_Os09g26144, and LOC_Os09g26160, Fig. 5G-H). These genes were likely the critical contributor to the change of N distribution among organs in the eCO_2 adaptation process in rice plant.

4. Discussion

Significant effects of [CO_2] and N application rate and their interaction on tillering were founded in this study (Fig. 1). Gradual N enrichment promoted tillering under both ambient CO_2 and eCO_2 confirmed that N availability was a constraint to the rice tillering process. However, N promotion effect on tillering diminished at the N10 under the ambient CO_2, but still showed an enhancement effect at the N15 under the eCO_2. These differential responses suggested that N limitation was aggravated under the eCO_2. This was further corroborated by the declining N content in leaf and sheath and the increment of carbohydrates under the eCO_2. It implies that more N input is needed to take advantage of the CO_2 fertilization effect in future eCO_2condition in order to achieve more tillers. The new-born tillers actually had comparable or higher N contents under the eCO_2 condition than under the ambient CO_2 (Fig. 2), whereas leaf and sheath showed a significant decline of N content. The more dramatic decline of N content in the sheath than in the

leaf was consistent with previous findings (Jilta et al, 1997; Huang et al, 2004). Therefore, there could be a redistribution or reallocation of N favoring towards the SAM (new-born tillers include novel SAM) under the eCO_2. As the N constraint is persistent in an unmanaged niche (Reich et al, 2006; Mueller et al, 2013), the plants likely adapt to distribute more of their acquired N to cell proliferating active zones rather than maintaining the original distribution pattern. This implies that future production requires more N input to take advantage of eCO_2, detail may vary in different species and varieties.

To reveal the molecular mechanism underlying this change, we conducted RNA-sequencing for a complete profile of transcriptomic alteration between the SAM and leaf tissues under four combinations of $[CO_2]$ and N application (Fig. 4). Though tissue differences predominantly defined the transcription profile globally, N application generally showed a more influential effect than the $[CO_2]$. This is reasonable as N has been proved to be a major limiting factor in plant growth regulation (Xuan et al, 2017; Zhang et al, 2019). For a specific group of the N metabolism related genes, they displayed a consistency with the general transcriptomic differences, i.e., N application overrode the $[CO_2]$ influences as a more significant effector. However, when tiller-related genes were specifically picked out, they were affected more by the $[CO_2]$ than by the N application. We speculate that the redistribution of N favoring the SAM under the eCO_2strengthened the impact on tiller-related gene regulations.

Further check on the putative functions of those N metabolism related genes altered by eCO_2 revealed that a major portion of N receptors and transporters was inversely regulated in leaf and SAM. Among them, the LOC_Os10g40600 (Fig. 5F) encoded OsNRT1.1a/b, which have been proved to function in nitrate sensing, transport and remobilization (Hu et al, 2015; Fan et al, 2016; Wang et al, 2018b). In addition, there might be other genes that regulating the N signaling and transportation (Feng et al, 2011; Wang et al, 2019c) in response to eCO_2. As evidenced here, gene *OsNRT2.3a/b*(LOC_Os01g50820, Fig. 5A) had a similar expression level (FPKM) with*OsNRT1.1a/b* , and two glutamate receptors (LOC_Os06g08910 and LOC_Os09g26160, Fig. 5G and I, respectively) also showed decent expression levels in both leaf and SAM, which were differently regulated in the tissues by $[CO_2]$ (Fig. 5). This suggests that they were coordinately regulated in the procedure of eCO_2 adaptation. There is an integrative co-expression network that determines the N effect and NUE (Zhang et al, 2019.). Engineering one target gene could bring in certain effect on crop yield improvement or NUE (Zeng et al, 2017; Xu et al, 2019). However, stacking (pyramiding) more targets together, especially targets in certain important network pathways may eventually create substantial yield breakthrough. Our results here provided a list of potential targets for such an approach.

Knowledge of the underlying mechanism of eCO_2 adaptation in crops can provide us useful information for breeders in genetic engineering (Schimel, 2006; Ainsworth and Ort, 2010; Fletcher, 2018). Our preliminary results showed that the N sensor and transporter genes play important roles in the response to eCO_2. We believe that our results can be applied to certain other plants, as under the eCO_2 conditions, the element stoichiometry reveals a generally consistent reduction of N content in most terrestrial plants, but their growth and biomass accumulation are accelerated to a variating degree (Reich et al, 2006; Norby et al, 2010; Deng et al, 2015). The plausible reason probably lies in that plants redistribute N to the division active zones such as SAM and new-branches in a different way. This might be a universal adaptation strategy in most terrestrial plants. Consequently, genetic engineering on these targets that facilitate the N redistribution may improve the CO_2harness and yield in those plants.

In summary, eCO_2 increased carbohydrates in all organs of rice plant and lowered N content in leaves and sheath, as expected. However, the new-born tillers maintained N content comparable to or higher than those under control CO_2 condition. The redistribution among the organs was likely accomplished by coordinated regulations of N metabolism receptor and transporter expression in leaf and SAM. The preliminary understanding of the mechanism provides a group of putative genes for further functional investigation and breeding targets.

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Conflict of Interest Statement

All authors have no conflict of interest to declare.

Author Contributions

JZ, YY, JH and YW: Conceptualization; JZ, YG, JW, ZW, ML, CC, YZ, XZ and GD: Investigation, Data curation and Analysis, Visualization and Validation; JZ and YY: Original draft; YY, DH and ZY: Review and Editing.

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Table 1

Table 1. The significance level of tiller-related gene affected by [CO2], N rate and organ.

Gene name
RFL
OsRPK1
DLT
BES1/BZR1/OsBZR1
D11
CGMC_GSK.4
D2
TAL
LAX1
BRD1
OsSPL14/IPA1/WFP
qPN1
OsNAC2
GID2/14-3-3
slr1
sdg/GID1

Table 1. The significance level of tiller-related gene affected by [CO2], N rate and organ.

MOC1
OsWOX4
Lazy1 (La1)
D14/D88/HTD2
D3/OsFBL27 /AtMAX2/AtOER9
LIC
OsEXP4
BRI1/D61
D10
OsPIN1
OsBIN2
APC/C(TE)
CRCT
D17/HTD1
BAK1
OsCDC27
RCN1/OsABCG5
OsHAP2E
IBH1
SD1
CGMC_GSK.8
LAX2
OsMADS57
LRK1
ILI1
GDH7
OsPIN2
BKI1
OsTB1/fc1
GA2ox1
OsAPC10
D63
DEP1
OsFEN-1
OsH1

Note: Probability level equal or less than 0.05 are displayed in red. [CO2]*N rate, [CO2]*Organ, N rate*Organ, and [CO2]*

Table 2

Table 2. The relative FPKM change of tiller-related genes in response to [CO2] and N rate.	**Table 2. Th**
Gene name	LOC_ID#
RFL	Os04g51000
OsRPK1	Os05g40770
DLT	Os06g03710
BES1/BZR1/OsBZR1	Os07g39220

Table 2. The relative FPKM change of tiller-related genes in response to [CO2] and N rate.	Table 2. Th
D11	Os04g39430
CGMC_GSK.4	Os02g14130
D2	Os01g10040
TAL	Os01g70170
LAX1	Os01g61480
BRD1	Os03g40540
OsSPL14/IPA1/WFP	Os08g39890
qPN1	Os01g70550
OsNAC2	Os08g06140
GID2/14-3-3	Os02g36974
slr1	Os03g49990
sdg/GID1	Os05g33730
MOC1	Os06g40780
OsWOX4	Os04g55590
Lazy1 (La1)	Os11g29840
D14/D88/HTD2	Os03g10620
D3/OsFBL27 /AtMAX2/AtOER9	Os06g06050
LIC	Os06g49080
OsEXP4	Os05g39990
BRI1/D61	Os01g52050
D10	Os01g54270
OsPIN1	Os02g07630
OsBIN2	Os01g10840
APC/C(TE)	Os03g03150
CRCT	Os05g51690
D17/HTD1	Os04g46470
BAK1	Os08g07760
OsCDC27	Os06g41750
RCN1/OsABCG5	Os03g17350
OsHAP2E	Os03g29760
IBH1/OsIBH1	Os04g56500
SD1	Os01g66100
CGMC_GSK.8	Os06g35530
LAX2	Os04g32510
OsMADS57	Os02g49480
LRK1	Os02g05980
ILI1	Os04g54900
GDH7	Os07g15770
OsPIN2	Os06g44970
BKI1	Os09g28550
OsTB1/fc1	Os03g49880
GA2ox1	Os05g06670
OsAPC10	Os05g50360
D63	Os08g01110
DEP1	Os09g26999
OsFEN-1	Os05g46270
OsH1	Os03g51690
Note: Red and blue text denote up or down-regulated fold change surpass two-fold to be DEG, respectively.	Note: Red ar

Figure legends

Figure 1 . Tiller number at leaf-age 4 and 6, tiller occurrence percentage, and biomass accumulation in response to N application rate and CO_2 concentration.

Error bars are standard deviations; Bars with different letters mean they are significantly different at p[?]0.05.

Figure 2 . The responses of N content in leaf, sheath, and new-born tillers to N application rate and CO_2conditions (A, B and C), and their relative change under eCO_2 to ambient CO_2 condition.

Error bars are standard deviations; Bars with different letters mean they are significantly different at p[?]0.05

Figure 3 . Carbohydrates change in response to N application rate and CO_2 condition.

L1-L5 represent leaf positions 1-5, respectively; SU and SD represent up and down part of sheath, respectively; tiller were new-born ones at leaf-age 6; Error bars are standard deviations; Bars with different letters mean they are significantly different at p[?]0.05.

Figure 4. The number of differentially expressed genes (DEG) and Venn diagrams of different comparisons.

A and C represent ambient and enriched CO_2, respectively; L3 and M stand for leaf number 3 and SAM respectively; 0 and 10 represent N application rate N0 and N10, respectively.

Figure 5 . The gene expression (FPKM) in leaf and SAM in response to N application and CO_2 condition.

L for leaf, M for SAM, Ambient and eCO_2 stand for ambient and enriched CO_2 treatments, respectively. Error bars are standard deviations; Bars with different letters mean they are significantly different at p[?]0.05.

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