Transcriptome-wide gene expression plasticity in *Stipa grandis* in response to grazing intensity differences

Running Head: Plastic gene expression with grazing of *S. grandis*

Zhenhua Dang [1], Yuanyuan Jia [1], Yunyun Tian [2], Jiabin Li [1], Yanan Zhang [1], Lei Huang [1], Cunzhu Liang [1], Peter J. Lockhart [3], Cory Matthew [4], Frank Yonghong Li [1]

Zhenhua Dang and Yuanyuan Jia should be considered joint first author.

[1] Ministry of Education Key Laboratory of Ecology and Resource Use of the Mongolian Plateau & Inner Mongolia Key Laboratory of Grassland Ecology, School of Ecology and Environment, Inner Mongolia University, Hohhot 010021, Inner Mongolia, People's Republic of China

[2] Ministry of Education Key Laboratory of Herbage & Endemic Crop Biotechnology, School of Life Sciences, Inner Mongolia University, Hohhot 010021, Inner Mongolia, People's Republic of China

[3] School of Fundamental Sciences, College of Sciences, Massey University, Palmerston North 4442, New Zealand

[4] Institute of Agriculture and Environment, Massey University, Palmerston North 4442, New Zealand

Abstract

Organisms have evolved effective and distinct adaptive strategies to survive. *Stipa grandis* is one of the widespread dominant species on the typical steppe of the Inner Mongolian Plateau,

and is regarded as a suitable species for studying the effects of grazing in this region. Although phenotypic (morphological and physiological) variations in *S. grandis* in response to long-term grazing have been identified, the molecular mechanisms underlying adaptations and plastic responses remain largely unknown. Accordingly, we performed a transcriptomic analysis to investigate changes in gene expression of *S. grandis* under four different grazing intensities. A total of 2,357 differentially expressed genes (DEGs) were identified among the tested grazing intensities, suggesting long-term grazing resulted in gene expression plasticity that affected diverse biological processes and metabolic pathways in *S. grandis*. DEGs were identified that indicated modulation of Calvin–Benson cycle and photorespiration metabolic pathways. The key gene´expression profiles encoding various proteins (e.g., Ribulose-1,5-bisphosphate carboxylase/oxygenase, fructose-1,6-bisphosphate aldolase, glycolate oxidase etc.) involved in these pathways suggest that they may synergistically respond to grazing to increase the resilience and stress tolerance of *S. grandis*. Our findings provide scientific clues for improving grassland use and protection, and identify important questions to address in future transcriptome studies.

KEY WORDS

Comparative transcriptomic analysis, Differentially expressed genes, Gene expression plasticity, Grazing adaptation, Calvin–Benson cycle, Photorespiration, *Stipa grandis*

1 | INTRODUCTION

Grassland covers about 40% of the total land area worldwide (Bai et al., 2014). It plays an

crucial role in ecological security by regulating the climate, conserving water resources, preventing wind and water erosions, and in prevision of forages for pastoral production (Song, Liu, Si, & Yin, 2012; T. & K., 2007; G. Y. Zhou et al., 2017). Grazing is the most common land use in grassland regions. Unfortunately, because of long-term inappropriate use, human activities, and adverse natural factors (e.g., warming, drought, and pest damage), grasslands have been extensively damaged, resulting in serious ecological issues. In particular, 74% of the grassland in northern China has become degraded because of decades of over-grazing and the fact the region is gradually becoming more arid. This has seriously threatened the survival and growth of the grassland vegetation, the local biodiversity, and the livelihoods of local herders.

Grazing affects the availability of resources essential for plant growth, including nutrients, water, and light, while also influencing ground temperatures. The ability of plants to use these resources is altered accordingly. These changes result in the redistribution of materials and energy in plants, thereby affecting herbage growth (Hou & Yang, 2006). Plant phenotypes are influenced by the impact of the environment on the formation of individual traits, including morphological, physiological, or behavioral responses (Chevin, Lande, & Mace, 2010; Schlichting & Smith, 2002). Many previous studies have explored how plant phenotypes are affected by grazing disturbance (Louault, Pillar, Aufrère, Garnier, & Soussana, 2005; Rusch, Skarpe, & Halley, 2009). Under continuous grazing disturbance, herbage usually exhibits dwarfism-related characteristics, including decreased height and short internodes, short and narrow leaves, stiff branches, small clumps and seeds, and shallow root distribution (Cingolani, Posse, & Collantes, 2005; X. L. Li et al., 2014). These traits ultimately decrease

the biomass of an individual plant and the whole community. A comparison by Louault et al. (2005) of the functional traits between plants in a grassland area that has been grazed for 12 years and plants in a grassland area with no grazing indicated that among 22 traits (e.g., leaf economy, root morphology, reproductive characteristics, and phenology), seven traits related to plant height were significantly affected by grazing. In sward-forming grasses responses to grazing have been referred to as tiller size-density compensation, a phenomenon which operates to optimize sward leaf area index through a higher density of smaller shoots in conditions of more intense grazing and a lower density of larger shoots under laxer grazing. Under more extreme grazing intensity, both size and density of shoots are reduced (Matthew et al., 1995). In clump- or tussock-forming grasses, a phenomenon similar to tiller size-density compensation can occur at the tussock level, with grazing altering the size and density of tussocks (da Silva et al., 2015). At the physiological level, grazing has been found to induce a series of changes in mesophyll cells, including changes to levels of expression and activity of photosynthetic enzymes, chlorophyll content, electron transport capacity, and hormone regulation (M. Liu et al., 2019; S. Q. Zhou, Lou, Tzin, & Jander, 2015). More specifically, the physiological processes of the photosynthetic system, carbon assimilation capacity, and water use efficiency are substantially enhanced in grazed plants in response to defoliation disturbance and other stress conditions (Peng et al., 2007; Zhao, Chen, & Lin, 2008). Moreover, several biological events, such as cell division, meristem elongation, leaf growth, and tillering, are promoted. These changes have been linked with recovery from damage incurred by grazing (M. Liu et al., 2019; Siddappaji et al., 2015; Z. H. Zhang et al., 2020) while some physiological responses to grazing may not help plant growth. Increased

photosynthetic activity can result in the over-accumulation of metabolic by-products, such as reactive oxygen species and free radicals, and these can damage membrane lipid structures and disrupt water and ion homeostasis in plant cells (Sunil, Saini, Bapatla, Aswani, & Raghavendra, 2019). However, during the regrowth of herbage after grazing, accumulation of large amounts of osmoprotectants (e.g. proline and betaine) and activation of the reactive oxygen scavenging system has been observed. Both could help to eliminate and/or minimize the toxic effects of the metabolic by-products (Hu et al., 2013; M. Liu et al., 2019; Ma, 2008). Additionally, the feeding behavior of livestock has been found to stimulate plants to produce large quantities of defensive compounds, such as terpenes, flavonoids, and alkaloids, which can decrease the palatability and nutritional quality of herbage to protect plants from further grazing and pest damage (Karinho-Betancourt & Nunez-Farfan, 2015).

The plastic phenotypic response of an organism is mediated through regulation of the transcriptome. In this process, gene expression is an intermediate molecular phenotype that links the genotype to environmental changes, while also linking diverse types of cells, tissues, and organs to express different phenotypes (Hodgins-Davis & Townsend, 2009; Kenkel & Matz, 2016). Gene expression changes in response to changing environmental conditions [i.e., gene expression plasticity (GEP)], are crucial for phenotypic plasticity and adaptive evolution (Lopez-Maury, Marguerat, & Bahler, 2008; Tirosh, Barkai, & Verstrepen, 2009). For example, in *Saccharomyces cerevisiae*, altered levels of gene expression of many genes in the ergosterol biosynthesis pathway have been attributed to an adaptive lineage specific response (Fraser, Moses, & Schadt, 2010). In a recent study of seven *Drosophila* species, 64% of the observed expression divergence was associated with adaptive changes driven by directional

selection, and the adaptive gene expression was enriched in functional classes, including regulation, sensory perception, sexual behavior, and morphology (Nourmohammad et al., 2017). Over shorter time frames, modulation of gene expression has been found to be an effective regulator and indicator of the phenotypic status of organisms exposed to fluctuating environmental conditions. Examples of the latter include the response of *Mytilus* mussels to changing tidal conditions (Lockwood & Somero, 2011), and the plastic response of fish *Fundulus heteroclitus* undergoing thermal acclimation (Dayan, Crawford, & Oleksiak, 2015). *Stipa grandis* (Poaceae, 2n = 44) is a dominant species on the typical steppe of the Inner Mongolian Plateau, thus the responses of this species to over-grazing represent a major part of the ecosystem response to grazing disturbance in this region (Smith & Knapp, 2003). To date, several investigations have been conducted regarding this topic. These pioneering studies examined how grazing influences the succession and construction of the *S. grandis* community (Dong et al., 2019; X. T. Wang et al., 2020) as well as the *S. grandis* biomass, functional traits, physiological ecology characteristics, population genetic diversity, and gene expression profiles (X. B. Li et al., 2018; Ren, Schonbach, Wan, Gierus, & Taube, 2012). Although studies evaluating responses to grazing suggested different populations of *S. grandis* exhibit phenotypic divergence to varying degrees, the transcriptome changes underlying such changes remain largely unknown. To better understand these phenotypic changes in *S. grandis* populations, we sequenced, assembled, and compared the transcriptomes of *S. grandis* under four grazing intensities. We identified differentially expressed genes (DEGs) under different grazing treatments and identified transcriptional regulation of genes closely associated with the Calvin–Benson cycle (CBC) and

photorespiration. We herein elucidate the inferred divergent gene expression response of *S. grandis* under different grazing conditions.

2 | MATERIALS AND METHODS

2.1 | Study site and grazing intensities

The grazing experiment was performed at the eastern edge of Xilinhot, Inner Mongolia, China (44°08′31″ N, 116°18′45″ E, Altitude 1, 129 m), characterized as a semi-arid continental climate with very cold and dry winters but warm and humid summers (Y. F. Bai et al., 2010). The average annual temperature in this region is 0–4°C, and the average precipitation is less than 300 mm. The rainfall is mostly concentrated in the June–September period (plant growth season stage). The soil type in this area is a 'chestnut soil' (i.e. calcic orthic Aridisol according to the US soil taxonomic system) (W. M. Bai et al., 2010), and the vegetation type is a typical steppe dominated by the perennial bunchgrass *Stipa grandis* and the perennial rhizomatous grass *Leymus chinensis*. Other companion species, such as *Cleistogenes squarrosa*, *Agropyron cristatum*, and *Carex korshinskyi*, were also common in the study region. The grazing treatments were initiated in 2013. Specifically, 12 paddocks (120 m × 120 m) were fenced off, and four different grazing intensities were established in a randomized complete block design with three replicates. The four grazing intensities were as follows: no grazing (CK, no sheep), light grazing (G3, 2 sheep·ha^{-1}), moderate grazing (G6, 4 sheep·ha^{-1}), and heavy grazing (G12, 8 sheep·ha^{-1}). For each grazing intensity, 28 Inner Mongolian Ujimqin sheep (3 years old, 60 kg body weight) were allowed to repeatedly graze for 3, 6, and 12 days per month for the G3, G6, and G12 treatments (one after the other in series), respectively, during the

vegetation growing season (i.e., June–September) every year. The sheep were allowed to graze from 7 am to 6 pm every day and were housed at night, and had free access to water and minerals.

2.2 | Sample collection, RNA extraction, and transcriptome sequencing

In order to identify the gene expression profiling related to the compensatory photosynthesis behavior of *S. grandis* in the recovery growth stage after grazing, plant samples were collected from 9:00 am to 11:00 am on a sunny day at the end of July (two weeks after grazing). The steppe grasslands of this area have a net primary productivity in the region of 3 – 5 t dry matter (DM) ha^{-1} y^{-1}, with July being the time of peak herbage accumulation rate in mid growing-season (J. F. Wang et al., 2020; Wu, Lin, Yang, Song, & Bai, 2020). Figure 1 shows the vegetation status of the grazing plots when the samples were collected. Emerging and healthy leaves of three *S. grandis* individuals were collected for each of the four grazing intensities. All samples were immediately frozen in liquid nitrogen and stored at −80°C for the subsequent transcriptomic analysis. Total RNA was extracted from the frozen tissues with the TRIzol reagent (Invitrogen, Life Technologies, USA) according to the manufacturer's instructions. The extracted RNA was treated with deoxyribonuclease I (TaKaRa Bio Inc., Otsu, Shiga, Japan) for 30 min at 37°C to remove residual DNA. The total RNA was quantified, and the quality was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, Calif.), with a minimum RNA integrity number of 8.0. The poly (A) mRNA was isolated with Oligo (dT) Beads and then purified and fragmented for the construction of cDNA libraries. The cDNA library for each sample was constructed and sequenced with the BGISEQ-500 platform by BGI Tech Solution Co., Ltd. (Wuhan, China).

2.3 | Data filtering and *de novo* assembly

The raw sequence reads for all samples were filtered with Trimmomatic (version 0.36) (Bolger, Lohse, & Usadel, 2014) to remove adapter-contaminated reads, low-quality reads (> 20% low-quality nucleotides), and reads with ambiguous nucleotides (> 5% 'N') to obtain clean reads, which were counted with SOAPnuke (version 1.4.0) (Chen et al., 2018). The clean reads of each sample were then *de novo* assembled into a transcriptome using Trinity (version 2.0.6) (Grabherr et al., 2011), with an optimized k-mer length (25-mer). The subsequent clustering and elimination of redundancies were completed with TGICL (version 2.1) (Pertea et al., 2003) to obtain unigenes. The unigenes assembled for all samples were then clustered with TGICL to obtain the non-redundant and unextendable assemblies (i.e., all-unigenes). To assess the completeness of the assembled transcriptomes, a BUSCO analysis was performed based on 303 conserved sequences in the eukaryotic database (Simão, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015) (http://busco.ezlab.org/v2/datasets/eukaryota_odb9.tar.gz).

2.4 | Gene expression quantification and functional annotation

Bowtie2 (Version 2.3.4.1) was used to map the reads of each sample to the merged transcriptome to quantify the expression level for each all-unigene in 12 samples (Langmead & Salzberg, 2012). The number of mapped reads was then estimated using RSEM (version 1.2.8) (B. Li & Dewey, 2011), with the default setting, for each sample. The normalized FPKM (Fragments per kilobase of exon model per million mapped reads) values for each unigene in the 12 libraries were used to represent gene expression levels. To identify genes with reproducible expression levels in three biological replicates, we calculated the coefficient

of variance (CV) for the gene expression of each grazing intensity. For each treatment, only genes with CV ≤ 0.5 were retained for further analyses. The correlations between all pairs of samples were analyzed with the hierarchical clustering of Pearson's correlation coefficients based on the gene expression levels. Symmetrical heat maps were generated with ggplot2 (version 1.0.0) (Gómez-Rubio, 2017) within R version 3.0.2 (R Development Core Team, 2012).

To predict the probable functions of the retained all-unigenes, their sequences were aligned with the sequences in public databases with the BLASTX algorithm, with a significance threshold E-value < 10^{-5}. The following databases were screened: non-redundant (Nr) protein database (http://www.ncbi.nlm.nih.gov), the Swiss-Prot protein database (http://www.expasy.ch/sprot), the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.genome.jp/kegg), and the Cluster of Orthologous Groups (COG) database (http://www.ncbi.nlm.nih.gov/COG). The Blast2GO software (version 2.5.0) (Conesa et al., 2005) and the Gene Ontology (GO) database were used to functionally annotate unigenes and assign them to the main GO functional categories (molecular function, cellular component, and biological process).

2.5 | Identification of differentially expressed genes and analysis of functional enrichment

Unigenes with a mean FPKM value greater than 10.0 in all samples were retained for identifying DEGs. Specifically, the DEGs among the four grazing intensities were identified as described by Wang *et al*. (Wang, Feng, Wang, Wang, & Zhang, 2010). The p-values were adjusted for multiple testing using the Benjamini-Hochberg method to control the false

discovery rate (FDR) (Benjamini & Hochberg, 1995) and with Storey and Tibshirani's statistical methods (Storey & Tibshirani, 2003). A FDR ≤ 0.001 and $|\log_2(\text{fold-change})| \geq 1$ were used as the significance threshold to infer gene expression differences. To evaluate the expression patterns of the identified DEGs for the four grazing intensities, a K-means clustering analysis was performed by using the MeV software (version 4.9) (Howe, Sinha, Schlauch, & Quackenbush, 2011). The DEGs for each cluster were then subjected to GO and KEGG Ontology enrichment analyses by using MapMan (version 3.6.0) (Thimm et al., 2004).

3 | RESULTS

3.1 | Sequencing output and *de novo* assembly

Approximately 10 gigabase clean data at the Q20 level (an error probability of 1%) were obtained for each sample. The clean reads for all samples were *de novo* assembled into 67,705–115,918 unigenes, with a mean length and N50 value of 906–1,373 bp and 1,243–2,078 bp, respectively (Table 1). To obtain the non-redundant and unextendable assemblies, the assembled unigenes of the 12 samples were further clustered into 251,412 all-unigenes, with an average length of 1,854 bp and an average N50 value of 2,536 bp (Table 1). Nearly 90% of the paired-end reads for each sample were mapped back to their own *de novo* assembled transcriptome (Table 1). A BUSCO analysis revealed that of 303 conserved sequences in the eukaryotic database, 24%–35% complete and single-copy, 39%–70% complete and duplicated, and 3%–23% fragmented BUSCOs were identified in the assembled *S. grandis* transcriptomes (Additional file 1).

3.2 | Gene quantification and functional annotation

An analysis of the CV indicated the expression of 33,241 all-unigenes in each grazing treatment was highly reproducible (CV ≤ 0.5) (Additional file 2). Thus, these all-unigenes were retained for further analyses (results of the correlation analysis of the retained genes are presented in Figure S1). The number of these all-unigenes with at least one sequence match based on a BLAST search of public databases was as follows: 31,029 (93.35%) in the Nr database, 30,460 (91.63%) in the NT database, 25,313 (76.15%) in the Swiss-Prot database, 22,071 (66.40%) in the GO database, 26,133 (78.62%) in the KOG database, and 26,583 (79.97%) in the KEGG database (Additional file 3). Overall, the top five species with BLAST hits to annotated unigenes were *Brachypodium distachyon* (39.47%), *Aegilops tauschii* (16.65%), *Oryza sativa* (6.72%), *Hordeum vulgare* (6.69%), and *Oryza brachyantha* (3.47%) (Figure S2). The GO annotation results indicated that in the three major GO categories, biological process, cell component, and molecular function, 'cellular process' (11,620; 35%), 'cell' (13,537; 40.7%), and 'binding' (11,381; 34%) were the dominant categories (Figure S3). Many of the identified transcripts were classified in the 'biological process' and 'cell component' categories, whereas only a few genes belonged to the 'molecular function' category (Figure S3). The KEGG pathway analysis indicated that the most enriched pathways were 'global and overview maps' (5,223; 15.7%), 'translation' (2,678; 8.1%), 'signal transduction' (1,277; 3.8%), and 'transport and catabolism' (1,397; 4.2%) (Figure S4).

3.3 | Identification and clustering of differentially expressed genes

Of the 33,241 all-unigenes, 4,701 had a mean FPKM value greater than 10.0 across all samples (Highlighted in bold in Additional file 2), and were used for identifying DEGs. Pairwise comparisons of the four grazing intensities identified 2,357 DEGs (Additional file

4). More specifically, the number of DEGs in each comparison was as follows: 736 DEGs in CK *vs* G3, 979 in CK *vs* G6, 800 in CK *vs* G12, 307 in G3 *vs* G6, 1,674 in G3 *vs* G12, and 1,176 in G6 *vs* G12 (Highlighted in bold in Additional file 4). The K-means clustering analysis assigned the identified DEGs to 12 transcriptional clusters (Figure 2A, Additional file 5), with more than 200 DEGs in clusters 1, 2, 7, 9, and 12. The KEGG pathway enrichment analysis revealed that many of the DEGs in clusters 1, 3, 4, 5, 6, 10, and 11 were associated with photosynthesis-related metabolic pathways. These pathways included 'photosynthesis', 'carbon fixation in photosynthetic organisms', 'carbon metabolism', 'porphyrin and chlorophyll metabolism', 'photosynthesis-antenna proteins', and 'glyoxylate and dicarboxylate metabolism' (Figure 2B). For the GO enrichment analysis, the relatively predominant GO terms included organelle (chloroplast, ribosome, mitochondrial, etc.) organization, response to stimuli (heat and light), and cellular processes (translation, protein folding, gene silencing, etc.) (Figure 2C).

3.4 | Differentially expressed genes related to the Calvin–Benson cycle

Among the analyzed transcripts, 114 transcripts encoded 11 enzymes involved in the CBC, and 38 were DEGs identified by the paired comparisons of the four grazing intensities (Table 2, Additional file 6). The DEGs were divided into two categories based on their expression profiles (Figure 3, Table 2). One group included the DEGs encoding ribulose-1,5-bisphosphate carboxylase/oxygenases (Rubiscos), 3-phosphoglycerate kinases (PGKases), glyceraldehyde-3-phosphate dehydrogenases (GAPDHases), transketolases (Tkases), and ribose-phosphate isomerases (RPIases), and the gene expression levels varied slightly from CK to G3, but were up-regulated significantly in response to grazing pressure (G6 to G12).

The other group comprised DEGs encoding fructose-1,6-bisphosphate aldolases (ALDases), sedoheptulose-1,7-bisphosphatases (SBPases), phosphoribulokinases (PRKases), and ribulose-phosphate epimerases (RPEases). The expression levels of these transcripts were significantly down-regulated from CK to G3, relatively stable from G3 to G6, and then sharply up-regulated from G6 to G12. Additionally, the expression levels of the unigenes encoding ALDases, PRKases, and RPEases under CK and G12 conditions were almost the same (except for Unigene48588 and CL16574.Contig28). Among these DEGs, those that were highly expressed, with mean FPKM values greater than 100.0 across all samples (Table 2), encoded the following: four Rubiscos, two PGKases, four GAPDHases, five ALDases, one fructose-1,6-bisphosphatase (FBPase), one TKase, one SBPase, one RPEase, one RPIase, one PRKase, and one Rubisco activase (RCA) (Figure 3, Table 2). The expression profiles revealed significant changes for several transcripts in the CBC in response to grazing (Figure 3, Table 2). Among the PGKase-encoding unigenes, the CL3380.Contig6 and CL3380.Contig10 expression levels were up-regulated nearly 2.4-times from G3 (141.49 and 75.71, respectively) to G12 (339.03 and 184.49, respectively). Regarding the GAPDHases, Unigene64285 expression was up-regulated significantly from G3 (141.73) to G12 (356.98). For the RPIases, CL843.Contig1 and CL843. Contig10 were similarly expressed from CK to G3, but exhibited the opposite expression trend from G3 to G12. Among the ALDases, Unigene48588 and Unigene48595 expression levels changed by as much as 9.08-times (1,099.92 *vs* 121.14) and 5.29-times (1,500.74 *vs* 283.65), respectively. The expression of the RCA unigene (Unigene11360) was sharply down-regulated by approximately 27-times following the grazing treatments, reflecting the apparent negative regulation of this unigene

(Figure 3, Table 2).

3.5 | Differentially expressed genes related to photorespiration

A total of 127 all-unigenes and 38 DEGs were identified encoding 12 enzymes related to the photorespiratory pathway (Additional file 7). The expression levels of the DEGs encoding one 2-phosphoglycolate phosphatase (PGLPase) (Unigene4982), five catalases (CATases) (Unigene20659, Unigene17925, Unigene11709, Unigene15777, and CL8442.Contig9), two serine hydroxymethyltransferases (SHMTases) (CL3053.Contig39 and CL3053.Contig41), three glycolate oxidases (GOXases) (CL95.Contig34, Unigene9650, and CL95.Contig47), and two glycine decarboxylases (GDCases) (CL1834.Contig3 and CL1834.Contig22) were down-regulated from CK to G3, after which they were relatively steady before being up-regulated from G6 to G12 (Table 3, Figure 4). The expression levels of the glutamine synthetases (GSases) (CL3536.Contig26), aminomethyltransferases (AMTases), glutamate: glyoxylate aminotransferases (GGTases), and hydroxypyruvate reductases (HPRases) unigenes were down-regulated from CK to G3 and then gradually up-regulated from G3 to G12 (Table 3, Figure 4). The GOXase (Unigene9650), dihydrolipoamide dehydrogenases (DIDases) (CL12727.Contig15), and serine: glyoxylate aminotransferases (SGTases) (CL593.Contig32) expression levels were gradually up-regulated from CK to G6 and then sharply down-regulated from G6 to G12 (Table 3, Figure 4). Regarding the GOXase unigene (CL14974.Contig19) and five CATase unigenes (CL10067.Contig2, CL10067.Contig4, CL8442.Contig1, CL10067.Contig 5, and CL8442.Contig4), their expression levels were up-regulated from CK to G3, after which they changed slightly from G3 to G6 and decreased from G6 to G12 (Table 3, Figure 4). The expression levels of the unigenes encoding

SHMTase (Unigene49346) and SGTase (CL593.Contig33) were generally down-regulated from CK to G12 (Table 3, Figure 4). The DEGs for one PGLPase, two GOXases, one GGTase, two SGTases, and two SHMTases were relatively highly expressed, with mean FPKM values exceeding 100.0 across all samples (Table 3). Additionally, Unigene4982 (PGLPase) expression was up-regulated nearly 3-times from G3 (84.81) to G12 (253.03) (Table 3). Unigene9650 (GOXase) was highly expressed, with a G6 expression level (443.96) that was about 3-times higher than the CK expression level (143.59) (Table 3). The expression of the SHMTase-encoding unigenes (CL3053.Contig39 and CL3053.Contig41) changed by as much as 3.43-times (326.94 *vs* 95.39) and 2.15-times (595.44 *vs* 276.86), respectively (Table 3). Among SGTase unigenes, the CL593.Contig1 and CL593.Contig32 expression level changes were similar from G6 to G12, but the opposite expression trend was detected from CK to G3 (Figure 4, Table 3).

4 | DISCUSSION

4.1 | Gene expression plasticity dataset for *Stipa grandis* under different grazing intensities

Gene expression is substantially affected by fluctuations in environmental conditions (Groen et al., 2020). To explore the changes in gene expression patterns in *S. grandis* in response to various long-term grazing intensities, we collected plant samples directly from the experimental grazing fields and analyzed the effects of the grazing intensities on the *S. grandis* transcriptome. In this study, the following strategies were adopted to minimize the influence of an open environment on gene expression and to obtain an accurate transcriptomic

346 dataset.

347 First, we applied rigorous sampling practices. Grazing reportedly affects photosynthesis-
348 related pathways, which are characterized by obvious dynamic diurnal changes (Yan et al.,
349 2013). Accordingly, we strictly controlled the sampling time, with all samples collected
350 between 9:00 am and 11:00 am. Under light conditions, the genes encoding many key
351 enzymes are abundantly expressed in plants. These enzymes have important roles related to
352 the regulation of photosynthetic activities in diverse plant species (Nikkanen, Toivola, &
353 Rintamaki, 2016; Raines, 2003). For example, Rubisco constitutes 30%–50% of the soluble
354 proteins in C_3 plant leaves (Feller, Anders, & Mae, 2008). Consistent with this expectation,
355 Rubisco unigenes (CL2153.Contig7, CL2153.Contig1, and Unigene53513) were highly
356 expressed in our field samples, with mean FPKM values of 3,543.93 across the grazing
357 intensities (Tables 2).

358 We sequenced with high coverage three biological replicates for each grazing intensity.
359 According to the amount the sequencing outputs, several quality assessment parameters of the
360 assembled transcripts (i.e., number, average length, and N50), the mapping rate of reads of
361 each sample to their assembled transcriptome (Table 1), and the BUSCO analysis (Additional
362 file 1), we can infer that the obtained transcriptional sequences were of high quality, integrity
363 and validity, indicating our sample collection method was suitable for the complex
364 environment in the field.

365 Third, we applied multiple data filtering levels. Although an informative transcriptome dataset
366 was constructed, several factors (e.g., variable splicing, incomplete assembly, tandem
367 variations, and assembly bias) caused the merged transcriptome to contain an unusual number

of transcripts. However, by filtering out the unigenes identified as not reproducibly expressed based on the CV, a rational number (33,241) of unigenes was retained for subsequent analyses. This process not only significantly decreased the sample bias resulting from the assembly method, it also ensured a comprehensive coverage of the *S. grandis* transcriptome. Gene annotations are considered useful for assessing the accuracy of the transcript sequences assembled from short-read data (Hao, Ge, Xiao, Zhang, & Yang, 2011). Among the analyzed transcripts, 93.42% were annotated based on at least one of the screened public databases, and these transcripts were matched with a high probability score with homologs from model species, including *Brachypodium distachyon*, *Aegilops tauschii*, *Oryza sativa*, and *Hordeum vulgare* (Figure S2). This suggests that the *S. grandis* transcriptome in the present study was assembled and annotated correctly and that the unannotated transcripts probably represent genetic information unique to *S. grandis*. To identify the most representative gene expression dataset, an FPKM value greater than 10.0 was used as a threshold to further filter the analyzed unigenes. Accordingly, 4,701 unigenes were retained, with functions suggesting they may have major roles related to basic metabolic activities and grazing responses in *S. grandis* (Highlighted in bold in Additional file 2 and 3). Additionally, among these unigenes, 2,357 were significantly differentially expressed across the four grazing intensities (Additional file 4). Several GO terms (e.g., catalytic activity, metabolic process, and cell part) and KEGG pathways (e.g., ribosome, glyoxylate and dicarboxylate metabolism, carbon metabolism, photosynthesis, and carbon fixation in photosynthetic organisms) were enriched among these unigenes (Figure S3 and S4). This information provides some insight into the cryptic physiological, plastic and adaptive responses of *S. grandis* to varying levels of grazing stress.

Our results strongly suggest that photosynthesis-related pathways in *S. grandis* are affected by competition between species in the ungrazed top level plant community but more significantly so in response to grazing.

4.2 | The expression patterns of genes related to the Calvin-Benson cycle in *Stipa grandis* have changed under different grazing intensities

Photosynthesis is a sensitive indicator of grazing stress in various grassland plants. The CBC is the initial pathway for photosynthetic carbon fixation, and changes to the expression of genes encoding the associated enzymes reportedly influence the growth of higher plants (Raines, 2003; Uematsu, Suzuki, Iwamae, Inui, & Yukawa, 2012). In the current study, grazing-induced transcriptional changes were detected for 114 unigenes encoding 12 enzymes involved in the Calvin–Benson cycle (CBC; Figure 2, Table 2, Additional file 6), suggesting that various grazing conditions altered the expression patterns of these CBC-related unigenes. Expression levels of CBC-related genes were compared in *S. grandis* between grazed plots (G3, G6, G12) and non grazing (CK) conditions where the vegetation represents the top-level community (Bai, Han, Wu, Chen, & Li, 2004; Westoby, Walker, & Noy-Meir, 1989). Important to note is that herbivory is a form of predation in which animals draw off for their own use, energy and nutrients from the plants they graze, and that grasses in general are adapted to herbivory as a feature of their natural environment. Previous studies have shown that with increased grazing intensity, the exposed soil surface area of plots increases, the canopy height of the plant community decreases, and the existing aboveground biomass (including the litter) decreases (Figure 1) (Y. H. Li & Wang, 1999; Liang, Gornish, Mariotte, Chen, & Liang, 2019; Z. L. Liu, Wang, Hao, & Liang, 2002). It follows logically that a

gradient of defoliation intensity such as in this experiment, also introduces a gradient of biomass removal intensity that will have impact on both the energy status and nutrient status of the plant. In the temperate sward forming forage grass *Lolium perenne,* carbohydrate levels fall rapidly after defoliation and recover gradually over approximately two weeks (Fulkerson & Donaghy, 2001) while a phenomenon known as shoot size-density compensation comes into play such that a higher density of smaller shoots will help in the restoration of lost leaf area under more severe defoliation. In very severe defoliation new shoots do not appear, likely because of substrate limitations (Matthew et al., 1995). More complex processes come into play in determining grazing effects on tussock forming grasses (da Silva et al., 2015), of which *S. grandis* is an example (Figure 1); however, it would be expected that more intense defoliation would impose greater substrate limitations in tussock forming grasses like *S. grandis*, as in the sward forming grasses. This principle of progressively reducing plant substrate status with increasing grazing intensity thus provides one framework against which to understand the photosynthesis responses observed in the present experiment. While the CBC performed similarly under non-grazing (CK) and heavy grazing (G12) conditions, the expression levels of CBC genes relevant for *S. grandis* growth and survival varied considerably between CK and G12. Specifically, the G12 treatment induced the up-regulated expression of several unigenes encoding CBC enzymes. We propose that this response may be part of an adaptive strategy and plastic response that enables substrate-depleted or damaged plants to use their limited photosynthetic units to reconstruct organs and to maintain an appropriate balance in the materials and energy metabolism in the above- and below-ground plant parts (Lin, Klinkhamer, & Vrieling, 2018; Z. H. Zhang et al., 2020).

This suggestion is consistent with a grazing optimization hypothesis that states that the unaffected biomass and small stature of plants under grazing stress reflect the promotion of net primary production (McNaughton, 1979). Increased photosynthetic rate is a mechanism that has been suggested to support this hypothesis (Painter & Detling, 1981). Furthermore, studies on the Inner Mongolia steppe have revealed that under frequent grazing stress, *S. grandis* plants exhibit dwarfism and induce efficient compensatory photosynthetic activities that can promote leaf regeneration and resistance to severe grazing (Hou & Yang, 2006; X. B. Li et al., 2018). This regenerative ability is crucial because *S. grandis* survival requires rapid restoration of active photosynthesis and growth (Tito, Castellani, Fáveri, Lopes, & Vasconcelos, 2016). After grazing, the remaining or newly developed organs can undergo physiological changes that enhance photosynthesis, which can further increase the photosynthetic capacity of the grazed plants (Lin et al., 2018; Zhao, Chen, Han, & Lin, 2008). Under G3 and G6 conditions, when plant diversity is relatively high, livestock will selectively graze on the highly palatable vegetation, such as *Leymus chinensis*, *Cleistogenes squarrosa*, and *Chenopodium glaucum*, resulting in minimal damage to *S. grandis* (J. Zhang et al., 2019). Thus, the competition for resources decreases for *S. grandis* compared with the competition under CK conditions. The relatively abundant resources make it easier for *S. grandis* plants to maximize physiological growth under G3 and G6 conditions (Hou & Yang, 2006). Therefore, it is unnecessary to invest as much material and energy in resource competition. In the present study, the expression levels of unigenes encoding Rubisco, SBPase, TKasse, and ALDase were down-regulated significantly under G3 and G6 conditions, which was consistent with the observed changes to photosynthetic-related physiology and phenotypic characteristics of

S. grandis in response to grazing.

However, the above hypothesis of response to substrate depletion and herbivory damage does not explain the elevated levels of expression of CBC-related genes in the CK plot compared with those in the G3 plot. In regions with a high plant density, vegetation with a large above-ground biomass, and rich biodiversity, competition is the main driving force of the community (Dusenge, Duarte, & Way, 2019). To gain a competitive advantage with limited resources in this situation, we propose *S. grandis* will increase its carbon fixation capacity to generate more resources. Thus, key genes encoding CBC enzymes would be expected to be highly expressed to increase photosynthetic activity under conditions of increased competition, if this assumption is correct.

Control over the rate of carbon fixation in the CBC is shared by a few enzymes. Analyses of antisense plants generated direct experimental evidence that expression-level changes to Rubisco, SBPase, ALDase, and TKase genes can influence the carbon flux through the CBC, with consequences for photosynthesis and growth (Raines, 2003). In our study, of the identified unigenes in this cycle, 38 were defined as DEGs, and several Rubisco, SBPase, ALDase, TKase, and GAPDHase unigenes were highly expressed, with mean FPKM values greater than 100.0 (Additional file 6, Table 2). These results suggest these enzymes have significant regulatory functions affecting the CBC during *S. grandis* responses to grazing.

Rubisco catalyzes the carboxylation of the CO_2 acceptor molecule ribulose 1,5-bisphosphate (RuBP) to initiate the CBC (Figure 2) (Yang et al., 2017). This enzyme comprises eight large (rbcL) and eight small (rbcS) subunits (Tabita, Hanson, Satagopan, Witte, & Kreel, 2008), and its catalytic properties are determined by the large subunit encoded by the chloroplast genome

(Andersson, 2008). In the current study, the five Rubisco DEGs were rbcS-encoding unigenes. Previous studies demonstrated that rbcS influences Rubisco catalytic efficiency, CO_2 specificity, activity, quantity, assembly, and stability (Bracher, Starling-Windhof, Hartl, & Hayer-Hartl, 2011; Genkov, Meyer, Griffiths, & Spreitzer, 2010). Moreover, rbcS and rbcL gene expression levels are positively correlated (Suzuki, Nakabayashi, Yoshizawa, Mae, & Makino, 2009) and rbcS may function as a CO_2 storage reservoir (Lun, Hub, Spoel, & Andersson, 2014). Thus, the highly expressed *S. grandis* rbcS unigenes (mean FPKM of 2,156.42) identified in this study may indicate that regulating the Rubisco content is important for regulating the CBC in *S. grandis* as a response to differential grazing stresses.

RCA is an AAA^+ ATPase that uses the energy from ATP hydrolysis to remove inhibitory sugars at the RCA site to generate a catalytically active enzyme with a temperature optimum below 40°C (Barta, Dunkle, Wachter, & Salvucci, 2010; Lu, Nawaz, Wei, Cheng, & Bie, 2020). In the present study, an RCA unigene (Unigene11360) was highly expressed under CK conditions (FPKM of 653.40), but with grazing its expression was significantly down-regulated (Table 2, Figure 2), indicative of its varying roles under the four grazing conditions. Under CK conditions, the abundant RCA can accelerate CO_2 fixation, activate Rubisco, and induce the expression of key genes in the CBC (Lu et al., 2020). However, the microenvironment of plants that survives grazing changes because of a decrease in humidity and increases in the temperature, surface exposure, light radiation, and evaporation, which ultimately lead to unstable and inactive RCA (DeRidder, Shybut, Dyle, Kremling, & Shapiro, 2012; Perdomo, Capo-Bauca, Carmo-Silva, & Galmes, 2017). Additionally, because RCA is a labile protein in *vivo*, the cost of accumulating RCA is quite high (Fukayama et al., 2018).

Therefore, in response to grazing, *S. grandis* does not actively synthesize RCA, and the carbon turnover in the CBC is mediated by other CBC enzymes to maintain photosynthesis and regeneration.

Highly efficient photosynthetic CO_2 fixation depends not only on the carboxylation capacity of Rubisco, but also on the regeneration of RuBP (Raines, 2011). This regeneration is largely regulated by SBPase, TKase, and ALDase (Raines, 2011), which catalyze the irreversible reactions and induce the metabolic branches of the CBC (Raines, 2003). The over-expression of ALDase and SBPase genes individually or together in tobacco and *Arabidopsis thaliana* significantly increases photosynthetic activities as well as the overall biomass and seed yield, especially under elevated CO_2 conditions (Simkin et al., 2017; Uematsu et al., 2012). However, a small decrease in the plastid TKase activity can dramatically inhibit photosynthesis and growth in antisense tobacco and cucumber transformants (Bi, Dong, Wu, Wang, & Ai, 2015; Henkes, Sonnewald, Badur, Flachmann, & Stitt, 2001). In the current study, ALDase, SBPase, and TKase unigenes in the *S. grandis* CBC were highly and differentially expressed (Table 2, Figure 2), suggesting that the transcriptional regulation and/or GEP of these enzymes may have important effects on RuBP regeneration, the photosynthetic capacity, and re-growth during *S. grandis* responses to grazing. Among these enzymes, ALDase and SBPase unigenes were similarly expressed (Figure 2, Table 2), indicating that when photosynthesis was relatively strong under CK and G12 conditions, the branching reaction efficiency of the CBC increased significantly in *S. grandis*. Consequently, ALDase effectively catalyzed the conversion of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP) to fructose-1,6-bisphosphat (FBP) as well as the

conversion of DHAP and erythrose 4-phosphate to sedoheptulose-1,7-bisphosphate (SBP) (Nakahara, Yamamoto, Miyake, & Yokota, 2003) (Figure 2), after which SBPase catalyzed the dephosphorylation of SBP to S7P (sedoheptulose-7-phosphate). These reactions can lead to the formation of a metabolic flux that enhances the carbon partitioning in the cycle and avoids the negative feedback regulation due to metabolic intermediates (e.g., glycolate and glyoxylate) (Laxa & Fromm, 2018; Messant et al., 2018). Additionally, up-regulated ALDase and SBPase gene expression might further activate Rubisco by promoting the regeneration of RuBP in the CBC (Miyagawa, Tamoi, & Shigeoka, 2001; Uematsu et al., 2012), thereby accelerating the carbon turnover to achieve compensatory photosynthesis and to stimulate the restorative growth of *S. grandis* plants . Interestingly, however, the expression of the TKase unigene (CL14956.Ct14) increased as the grazing intensity increased, with expression levels significantly higher than that under CK conditions. This suggests that the enzyme was actively engaged in regenerating RuPB in the grazed *S. grandis* plants and that it can effectively alleviate the limitation of RuBP to maintain photosynthesis under grazing stress. The significant up-regulation of TKase unigene expression might be highly related to the hyper-compensatory photosynthesis of *S. grandis*, especially under the G3 and G6 conditions.

4.3 | Gene expression plasticity affecting the photorespiration is important for *S. grandis* adaptations to grazing

Plant photorespiration involves a complex network of enzymatic reactions and is linked to the CBC to form a photosynthetic photorespiratory supercycle that is responsible for nearly all of the biological CO_2 fixation on Earth (Husic, Husic, Tolbert, & Black, 1987). Photorespiration begins with the oxygenation of RuBP by Rubisco, and the synthesized 2-phosphoglycolate (2-

PG) causes a significant carbon loss and impedes carbon fixation as well as allocation (Flügel et al., 2017). Therefore, the degradation of 2-PG during photorespiration directly affects the overall carbon metabolism in plants (Flügel et al., 2017).

The PGLPase enzyme catalyzes the formation of glycolate from 2-PG, and its activity is essential for plant carbon fixation and distribution. In this study, a PGLPase unigene (Unigene4982) was highly expressed in response to the CK and G12 treatments, but its expression was significantly down-regulated under G3 and G6 conditions (Figure 3, Table 3). A previous study indicated that PGLPase expression and the 2-PG content are inversely related (Flügel et al., 2017). Therefore, the 2-PG levels under the G3 and G6 conditions were higher than those under the CK and G12 conditions, reflecting the differences in carbon fixation and allocation between these treatments. Enzymatic analyses have demonstrated that high 2-PG levels inhibit *A. thaliana* triose-phosphate isomerase (TPI) and SBPase (Flügel et al., 2017), and SBPase further limits the carbon flux of the RuBP regeneration phase in the CBC (Ding, Wang, Zhang, & Ai, 2016). In *S. grandis*, PGLPase unigene expression was consistent with SBPase unigene expression, indicating RuBP regeneration was negatively regulated by 2-PG under G3 and G6 conditions. In contrast, under CK and G12 conditions, the CBC carbon flux leading to RuBP regeneration was probably attributed to the substantial metabolism of 2-PG. Under heavy grazing conditions, abiotic stressors, such as high light intensity, water scarcity, increased temperatures, and elevated O_2 partial pressures, might promote the oxidation of RuBP to form 2-PG (S. Timm & Hagemann, 2020). The relatively high PGLPase unigene expression level (FPKM of 253.03) under this condition suggests that the PGLPase activity was not correlated with the photorespiratory flux (2-PG hydrolysis), and

566 an increase in PGLPase activity may be beneficial for *S. grandis* under G12 conditions.
567 Hence, PGLPase is not a limiting factor for the photorespiratory flux, but it prepares the cycle
568 for a considerable influx of 2-PG due to abiotic stresses (Flügel et al., 2017; S. Timm,
569 Woitschach, Heise, Hagemann, & Bauwe, 2019) and enhances plant stress tolerance.
570 Another photorespiratory enzyme related to abiotic stress tolerance is GOXase, which
571 catalyzes the conversion of glycolate to glyoxylate and produces H_2O_2. As a second
572 messenger, H_2O_2 plays an important role in plant defense reactions (Taler, Galperin,
573 Benjamin, Cohen, & Kenigsbuch, 2004). Because it produces glyoxylate, GOXase represses
574 Rubisco and RCA in maize and rice (Xu et al., 2009; Zelitch, Schultes, Peterson, Brown, &
575 Brutnell, 2009). In *S. grandis*, the GOXase unigene (CL95.Ct34) expression pattern was
576 similar to that of the RCA and Rubisco unigenes, reflecting the likely effect of GOXase on
577 RCA and Rubisco. Nevertheless, under G12 conditions, PGLPase and GOXase unigene
578 expression levels were significantly up-regulated, which would have increased the tolerance
579 of *S. grandis* to stressors due to grazing.
580 In the photorespiratory pathway, GGTase, SGTase, GDCase, and SHMTase form networks
581 regulating glycine and serine (Figure 3). The plant cellular glycine-to-serine ratio is a
582 sensitive indicator of photorespiratory activity (Wingler, Lea, Quick, & Leegood, 2000). The
583 over-expression of GGTase genes can increase glycine and serine levels, whereas up-
584 regulated SGTase gene expression has the opposite effect on serine levels (Modde et al.,
585 2017). These two reactions share common metabolites and exhibit a mutual decreasing trend
586 (Figure 3). In this study, the unigenes encoding these two enzymes had the opposite
587 expression patterns (GGTase: CL3941.Contig15; SGTase: CL593.Contig32), indicative of

their roles in regulating the balance between glycine and serine contents in *S. grandis* under different grazing conditions. The GDCase gene expression level is reportedly a key determinant of photorespiratory flux control (S Timm et al., 2012). Remarkably, increases in GDCase activity facilitate carbon conversion throughout the photorespiratory cycle (Lopez-Calcagno et al., 2019). Simkin et al. (2017) determined that plant growth and photosynthetic activities increase following the combined over-expression of GDC-H, SBPase, or ALDase genes. Therefore, the positive correlation between the photorespiration carbon flux and the CBC is one of the determinants of photosynthetic efficiency and biomass, indicating that adjusting the carbon flux via photorespiration to achieve compensatory photosynthesis is an important strategy adopted by *S. grandis* in response to differing grazing intensities.

5 | CONCLUSION

Comparative transcriptomic analyses revealed that the gene expression of *S. grandis* has plasticity induced by long-term differential grazing intensities, which involves altered regulation of many biological processes and metabolic pathways. The similar expression profiles of genes related to the CBC and photorespiration pathways suggest that these pathways synergistically respond to grazing to promote stress tolerance. Our findings provide novel insights into the grazing responses of *S. grandis* on the gene expressional level, and will facilitate future investigations of the relevant regulatory roles and mechanisms of genes underlying the plastic response of grassland plant species to grazing.

ACKNOWLEDGMENTS

We thank Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac) for editing the English

text of a draft of this manuscript. This work was supported by the National Key Research and Development Program of China Grant 2016YFC0500501, the National Natural Science Foundation of China Grant 31860078 to Dr. Zhenhua Dang, and the Open Project Program of 'Ministry of Education Key Laboratory of Ecology and Resources Use of the Mongolian Plateau, Hohhot, Inner Mongolia, 010021, China.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

FUNDING INFORMATION

This work was supported by the National Key Research and Development Program of China Grant 2016YFC0500501, the National Natural Science Foundation of China Grant 31860078 to Pro. Zhenhua Dang, and the Open Project Program of 'Ministry of Education Key Laboratory of Ecology and Resources Use of the Mongolian Plateau, Hohhot, Inner Mongolia, 010021, China.

ORCID

Zhenhua Dang ID: https://orcid.org/0000-0002-9196-159X

Yuanyuan Jia ID: https://orcid.org/0000-0003-1296-634X

REFERENCES

Andersson, I. (2008). Catalysis and regulation in Rubisco. *Journal of Experimental Botany, 59*(7), 1555-1568. doi:10.1093/jxb/ern091

Bai, W. M., Wan, S. Q., Niu, S. L., Liu, W. X., Chen, Q. S., Wang, Q. B., . . . Li, L. H. (2010). Increased

temperature and precipitation interact to affect root production, mortality, and turnover in a temperate steppe: implications for ecosystem C cycling. *Global Change Biology, 16*(4), 1306-1316. doi:10.1111/j.1365-2486.2009.02019.x

Bai, Y. F., Han, X. G., Wu, J. G., Chen, Z. Z., & Li, L. H. (2004). Ecosystem stability and compensatory effects in the Inner Mongolia grassland. *Nature, 431*(7005), 181-184. doi:10.1038/nature02850

Bai, Y. F., Huang, J. H., Zheng, S. X., Pan, Q. M., Zhang, L. X., Zhou, H. K., . . . Ma, J. (2014). Drivers and regulating mechanisms of grassland and desert ecosystem services. *Chinese Journal of Plant Ecology, 38*(2), 93-102. doi:10.3724/sp.J.1258.2014.00009

Bai, Y. F., Wu, J. G., Clark, C. M., Naeem, S., Pan, Q. M., Huang, J. H., . . . Han, X. G. (2010). Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. *Global Change Biology, 16*(2), 889-889. doi:10.1111/j.1365-2486.2009.02142.x

Barta, C., Dunkle, A. M., Wachter, R. M., & Salvucci, M. E. (2010). Structural changes associated with the acute thermal instability of Rubisco activase. *Archives of Biochemistry and Biophysics, 499*(1-2), 17-25. doi:10.1016/j.abb.2010.04.022

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B: Methodological, 57*(1), 289-300. doi:10.2307/2346101

Bi, H. G., Dong, X. B., Wu, G. X., Wang, M. L., & Ai, X. Z. (2015). Decreased TK activity alters growth, yield and tolerance to low temperature and low light intensity in transgenic cucumber plants. *Plant Cell Reports, 34*(2), 345-354. doi:10.1007/s00299-014-1713-5

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics, 30*(15), 2114-2120. doi:10.1093/bioinformatics/btu170

Bracher, A., Starling-Windhof, A., Hartl, F. U., & Hayer-Hartl, M. (2011). Crystal structure of a chaperone-bound assembly intermediate of form I Rubisco. *Nature Structural and Molecular Biology, 18*(8), 875-880. doi:10.1038/nsmb.2090

Chen, Y. X., Chen, Y. S., Shi, C. M., Huang, Z. B., Zhang, Y., Li, S. K., . . . Chen, Q. (2018). SOAPnuke: A MapReduce Acceleration supported Software for integrated Quality Control and Preprocessing of High-Throughput Sequencing Data. *GigaScience, 7*(1), 1-6. doi:10.1093/gigascience/gix120

Chevin, L. M., Lande, R., & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS biology, 8*(4), e1000357. doi:10.1371/journal.pbio.1000357

Cingolani, A. M., Posse, G., & Collantes, M. B. (2005). Plant functional traits, herbivore selectivity and response to sheep grazing in Patagonian steppe grasslands. *Journal of Applied Ecology, 42*(1), 50-59. doi:10.1111/j.1365-2664.2004.00978.x

Conesa, A., Gotz, S., Garcia-Gomez, J. M., Terol, J., Talon, M., & Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics, 21*(18), 3674-36766. doi:10.1093/bioinformatics/bti610

Dayan, D. I., Crawford, D. L., & Oleksiak, M. F. (2015). Phenotypic plasticity in gene expression contributes to divergence of locally adapted populations of Fundulus heteroclitus. *Molecular Ecology, 24*(13), 3345-3359. doi:10.1111/mec.13188

DeRidder, B. P., Shybut, M. E., Dyle, M. C., Kremling, K. A. G., & Shapiro, M. B. (2012). Changes at the 3'-untranslated region stabilize Rubisco activase transcript levels during heat stress in Arabidopsis. *Planta, 236*(2), 463-476. doi:10.1007/s00425-012-1623-0

Ding, F., Wang, M. L., Zhang, S. X., & Ai, X. Z. (2016). Changes in SBPase activity influence photosynthetic capacity, growth, and tolerance to chilling stress in transgenic tomato plants. *Scientific reports, 6*, 32741. doi:10.1038/srep32741

Dong, K., Hao, G., Yang, N., Zhang, J. L., Ding, X. F., Ren, H. Q., . . . Gao, Y. B. (2019). Community assembly mechanisms and succession processes significantly differ among treatments during the restoration of Stipa grandis - Leymus chinensis communities. *Scientific reports, 9*(1), 16289. doi:10.1038/s41598-019-52734-0

Dusenge, M. E., Duarte, A. G., & Way, D. A. (2019). Plant carbon metabolism and climate change: elevated CO2 and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytologist, 221*(1), 32-49. doi:10.1111/nph.15283

Feller, U., Anders, I., & Mae, T. (2008). Rubiscolytics: fate of Rubisco after its enzymatic function in a cell is terminated. *Journal of Experimental Botany, 59*(7), 1615-1624. doi:10.1093/jxb/erm242

Flügel, F., Timm, S., Arrivault, S., Florian, A., Stitt, M., Fernie, A. R., & Bauwe, H. (2017). The Photorespiratory Metabolite 2-Phosphoglycolate Regulates Photosynthesis and Starch Accumulation in Arabidopsis. *Plant Cell, 29*(10), 2537-2551. doi:10.1105/tpc.17.00256

Fraser, H. B., Moses, A. M., & Schadt, E. E. (2010). Evidence for widespread adaptive evolution of gene expression in budding yeast. *Proceedings of the National Academy of Sciences of the United States of America, 107*(7), 2977-2982. doi:10.1073/pnas.0912245107

Fukayama, H., Mizumoto, A., Ueguchi, C., Katsunuma, J., Morita, R., Sasayama, D., . . . Azuma, T. (2018). Expression level of Rubisco activase negatively correlates with Rubisco content in transgenic rice. *Photosynthesis Research, 137*(3), 465-474. doi:10.1007/s11120-018-0525-9

Fulkerson, W.J., Donaghy, D.J. (2001). Plant-soluble carbohydrate reserves and senescence – key criteria for developing and effective grazing management system for ryegrass-based pastures: a review. A*ustralian Journal of Experimental Agriculture, 41(2)*, 261-275. doi: 10.1071/EA00062

Gómez-Rubio, V. (2017). ggplot2 - Elegant Graphics for Data Analysis (2nd Edition). *Journal of Statistical Software, 77*(Book Review 2), 1-3. doi:10.18637/jss.v077.b02

Genkov, T., Meyer, M., Griffiths, H., & Spreitzer, R. J. (2010). Functional hybrid rubisco enzymes with plant small subunits and algal large subunits: engineered rbcS cDNA for expression in chlamydomonas. *The Journal of biological chemistry, 285*(26), 19833-19841. doi:10.1074/jbc.M110.124230

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., . . . Regev, A. (2011). Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nat Biotechnol, 29*(7), 644-652. doi:10.1038/nbt.1883

Groen, S. C., Calic, I., Joly-Lopez, Z., Platts, A. E., Choi, J. Y., Natividad, M., . . . Purugganan, M. D. (2020). The strength and pattern of natural selection on gene expression in rice. *Nature, 578*(7796), 572-576. doi:10.1038/s41586-020-1997-2

Hu, X., Zhao, M. L., Han, B., Wang, J., Niu, H., Cheng, Y., & Han, X. (2013). The Change of the Amino Acid Content in the Leaves of Agropyron cristatum under the Stress of Grazing. *Journal of Anhui Agricultural Sciences, 41*(30), 12045-12048. doi:10.13989/j.cnki.0517-6611.2013.30.063

Hao, D. C., Ge, G. B., Xiao, P. G., Zhang, Y. Y., & Yang, L. (2011). The first insight into the tissue specific taxus transcriptome via Illumina second generation sequencing. *PLoS One, 6*(6), e21220. doi:10.1371/journal.pone.0021220

Henkes, S., Sonnewald, U., Badur, R., Flachmann, R., & Stitt, M. (2001). A small decrease of plastid transketolase activity in antisense tobacco transformants has dramatic effects on photosynthesis and phenylpropanoid metabolism. *Plant Cell, 13*(3), 535-551. doi:10.2307/3871405

Hodgins-Davis, A., & Townsend, J. P. (2009). Evolving gene expression: from G to E to GxE. *Trends in Ecology & Evolution, 24*(12), 649-658. doi:10.1016/j.tree.2009.06.011

Hou, F. J., & Yang, Z. Y. (2006). Effects of grazing of livestock on grassland. *Acta Ecologica Sinica, 26*(1), 244-264.

Howe, E. A., Sinha, R., Schlauch, D., & Quackenbush, J. (2011). RNA-Seq analysis in MeV. *Bioinformatics, 27*(22), 3209-3210. doi:10.1093/bioinformatics/btr490

Husic, D. W., Husic, H. D., Tolbert, N. E., & Black, C. C. (1987). The oxidative photosynthetic carbon cycle or C2cycle. *Critical Reviews in Plant Sciences, 5*(1), 45-100. doi:10.1080/07352688709382234

Karinho-Betancourt, E., & Nunez-Farfan, J. (2015). Evolution of resistance and tolerance to herbivores: testing the trade-off hypothesis. *Peer J, 3*, e789. doi:10.7717/peerj.789

Kenkel, C. D., & Matz, M. V. (2016). Gene expression plasticity as a mechanism of coral adaptation to a variable environment. *Nature Ecology & Evolution, 1*(1), 14. doi:10.1038/s41559-016-0014

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods, 9*(4), 357-359. doi:10.1038/nmeth.1923

Laxa, M., & Fromm, S. (2018). Co-expression and regulation of photorespiratory genes in Arabidopsis thaliana: A bioinformatic approach. *Current Plant Biology, 14*, 2-18. doi:10.1016/j.cpb.2018.09.001

Li, B., & Dewey, C. N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC bioinformatics, 12*(1), 323. doi:10.1186/1471-2105-12-323

Li, X. B., Huang, Q., Mi, X., Bai, Y. X., Zhang, M., & Li, X. (2018). Grazing every month minimizes size but boosts photosynthesis in Stipa grandis in the steppe of Inner Mongolia, China. *Journal of Arid Land, 10*(4), 601-611. doi:10.1007/s40333-018-0011-4

Li, X. L., Hou, X. Y., Wu, X. H., Sarula., Ji, L., Chen, H. J., . . . Ding, Y. (2014). Plastic responses of stem and leaf functional traits in Leymus chinensis to long-term grazing in a meadow steppe. *Chinese Journal of Plant Ecology, 38*(5), 440-451. doi:10.3724/sp.J.1258.2014.00040

Li, Y. H., & Wang, S. P. (1999). Response of plant and plant community to different stocking rates. *Grassland of China, 3*, 11-19.

Liang, M. W., Gornish, E. S., Mariotte, P., Chen, J. Q., & Liang, C. Z. (2019). Foliar Nutrient Content Mediates Grazing Effects on Species Dominance and Plant Community Biomass. *Rangeland Ecology & Management, 72*(6), 899-906. doi:10.1016/j.rama.2019.08.001

Lin, T. T., Klinkhamer, P. G. L., & Vrieling, K. (2018). Evolutionary changes in growth, regrowth and carbohydrate storage in an invasive plant. *Scientific reports, 8*, 14917. doi:10.1038/s41598-018-33218-z

Liu, M., Gong, J. R., Li, Y., Li, X. B., Yang, B., Zhang, Z. H., . . . Hou, X. Y. (2019). Growth-defense trade-off regulated by hormones in grass plants growing under different grazing intensities. *Physiologia Plantarum, 166*(2), 553-569. doi:10.1111/ppl.12802

Liu, Z. L., Wang, W., Hao, D. Y., & Liang, C. Z. (2002). Probes on the degeneration aand recovery succeession mechanisms of Inner Mongolia steppe. *Journal of Arid Land Resources and Environment, 16*(1), 84-91. doi:10.13448/j.crki.jalre.2002.01.015

Lockwood, B. L., & Somero, G. N. (2011). Transcriptomic responses to salinity stress in invasive and native blue mussels (genus Mytilus). *Molecular Ecology, 20*(3), 517-529. doi:10.1111/j.1365-294X.2010.04973.x

Lopez-Calcagno, P. E., Fisk, S., Brown, K. L., Bull, S. E., South, P. F., & Raines, C. A. (2019). Overexpressing the H-protein of the glycine cleavage system increases biomass yield in glasshouse and field-grown transgenic tobacco plants. *Plant Biotechnology Journal, 17*(1), 141-151. doi:10.1111/pbi.12953

Lopez-Maury, L., Marguerat, S., & Bahler, J. (2008). Tuning gene expression to changing environments: from

rapid responses to evolutionary adaptation. *Nature Reviews Genetics, 9*(8), 583-593. doi:10.1038/nrg2398

Louault, F., Pillar, V. D., Aufrère, J., Garnier, E., & Soussana, J.-F. (2005). Plant traits and functional types in response to reduced disturbance in a semi-natural grassland. *Journal of Vegetation Science, 16*(2), 151-160. doi:10.1111/j.1654-1103.2005.tb02350.x

Lu, J. Y., Nawaz, M. A., Wei, N. N., Cheng, F., & Bie, Z. L. (2020). Suboptimal Temperature Acclimation Enhances Chilling Tolerance by Improving Photosynthetic Adaptability and Osmoregulation Ability in Watermelon. *Horticultural Plant Journal, 6*(1), 49-60. doi:10.1016/j.hpj.2020.01.001

Lun, V. M., Hub, J. S., Spoel, D. V. D., & Andersson, I. (2014). CO2 and O2 distribution in Rubisco suggests the small subunit functions as a CO2 reservoir. *Journal of the American Chemical Society, 136*(8), 3165-3171. doi:10.1021/ja411579b

Ma, H. B. (2008). Influence of different grazing intensities in desert steppe on some physiological indexes of several pastures. *Agricultural Research in the Arid Areas, 26*(2), 79-84. doi:10.1145/1344411.1344416

McNaughton, S. J. (1979). Grazing as an optimization process: grass-ungulate relationships in the Serengeti. *American Naturalist, 113*(5), 691-703. doi:10.1086/283426

Messant, M., Timm, S., Fantuzzi, A., Weckwerth, W., Bauwe, H., Rutherford, A. W., & Krieger-Liszkay, A. (2018). Glycolate Induces Redox Tuning Of Photosystem II in Vivo: Study of a Photorespiration Mutant. *Plant physiology, 177*(3), 1277-1285. doi:10.1104/pp.18.00341

Miyagawa, Y., Tamoi, M., & Shigeoka, S. (2001). Overexpression of a cyanobacterial fructose-1,6-/sedoheptulose-1,7-bisphosphatase in tobacco enhances photosynthesis and growth. *Nature Biotechnology, 19*(10), 965-969. doi:10.1038/nbt1001-965

Modde, K., Timm, S., Florian, A., Michl, K., Fernie, A. R., & Bauwe, H. (2017). High serine:glyoxylate aminotransferase activity lowers leaf daytime serine levels, inducing the phosphoserine pathway in Arabidopsis. *Journal of Experimental Botany, 68*(3), 643-656. doi:10.1093/jxb/erw467

Nakahara, K., Yamamoto, H., Miyake, C., & Yokota, A. (2003). Purification and characterization of class-I and class-II fructose-1,6-bisphosphate aldolasesfrom the cyanobacterium Synechocystis sp. PCC 6803. *Plant & Cell Physiology, 44*(3), 326-333. doi:10.1007/s10126-011-9402-7

Nikkanen, L., Toivola, J., & Rintamaki, E. (2016). Crosstalk between chloroplast thioredoxin systems in regulation of photosynthesis. *Plant, Cell & Environment, 39*(8), 1691-1705. doi:10.1111/pce.12718

Nourmohammad, A., Rambeau, J., Held, T., Kovacova, V., Berg, J., & Lassig, M. (2017). Adaptive Evolution of Gene Expression in Drosophila. *Cell Reports, 20*(6), 1385-1395. doi:10.1016/j.celrep.2017.07.033

Painter, E. L., & Detling, J. K. (1981). Effects of defoliation on net photosynthesis and regrowth of western wheatgrass. *Journal of Range Management, 34*(1), 68-71. doi:10.1175/JCLI4099.1

Peng, Y., Jiang, G. M., Liu, X. H., Niu, S. L., Liu, M. Z., & Biswas, D. K. (2007). Photosynthesis, transpiration and water use efficiency of four plant species with grazing intensities in Hunshandak Sandland, China. *Journal of Arid Environments, 70*(2), 304-315. doi:10.1016/j.jaridenv.2007.01.002

Perdomo, J. A., Capo-Bauca, S., Carmo-Silva, E., & Galmes, J. (2017). Rubisco and Rubisco Activase Play an Important Role in the Biochemical Limitations of Photosynthesis in Rice, Wheat, and Maize under High Temperature and Water Deficit. *Frontiers in plant science, 8*, 490. doi:10.3389/fpls.2017.00490

Pertea, G., Huang, X. Q., Liang, F., Antonescu, V., Sultana, R., Karamycheva, S., . . . Quackenbush, J. (2003). TIGR Gene Indices clustering tools (TGICL): a software system for fast clustering of large EST datasets. *Bioinformatics, 19*(5), 651-652. doi:10.1093/bioinformatics/btg034

Raines, C. A. (2003). The Calvin cycle revisited. *Photosynthesis Research, 75*(1), 1-10. doi:10.1023/A:1022421515027

Raines, C. A. (2011). Increasing photosynthetic carbon assimilation in C3 plants to improve crop yield: current and future strategies. *Plant physiology, 155*(1), 36-42. doi:10.1104/pp.110.168559

Ren, H. Y., Schonbach, P., Wan, H. W., Gierus, M., & Taube, F. (2012). Effects of grazing intensity and environmental factors on species composition and diversity in typical steppe of Inner Mongolia, China. *PLoS One, 7*(12), e52180. doi:10.1371/journal.pone.0052180

Rusch, G. M., Skarpe, C., & Halley, D. J. (2009). Plant traits link hypothesis about resource-use and response to herbivory. *Basic and Applied Ecology, 10*(5), 466-474. doi:10.1016/j.baae.2009.01.004

Schlichting, C. D., & Smith, H. (2002). Phenotypic plasticity: linking molecular mechanisms with evolutionary outcomes. *Evolutionary Ecology, 16*(3), 189-211. doi:10.1023/A:1019624425971

Siddappaji, M. H., Scholes, D. R., Krishnankutty, S. M., Calla, B., Clough, S. J., Zielinski, R. E., & Paige, K. N. (2015). The role of invertases in plant compensatory responses to simulated herbivory. *BMC Plant Biology, 15*(278), 1-13. doi:10.1186/s12870-015-0655-6

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics, 31*(19), 3210-3212. doi:10.1093/bioinformatics/btv351

Simkin, A. J., Lopez-Calcagno, P. E., Davey, P. A., Headland, L. R., Lawson, T., Timm, S., . . . Raines, C. A. (2017). Simultaneous stimulation of sedoheptulose 1,7-bisphosphatase, fructose 1,6-bisphophate aldolase and the photorespiratory glycine decarboxylase-H protein increases CO2 assimilation, vegetative biomass and seed yield in Arabidopsis. *Plant Biotechnology Journal, 15*(7), 805-816. doi:10.1111/pbi.12676

Smith, M. D., & Knapp, A. K. (2003). Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters, 6*(6), 509-517. doi:10.1046/j.1461-0248.2003.00454.x

Song, Z. L., Liu, H. Y., Si, Y., & Yin, Y. (2012). The production of phytoliths in China's grasslands: implications to the biogeochemical sequestration of atmospheric CO2. *Global Change Biology, 18*(12), 3647-3653. doi:10.1111/gcb.12017

Storey, J. D., & Tibshirani, R. (2003). Statistical Methods for Identifying Differentially Expressed Genes in DNA Microarrays. *Methods in Molecular Biology, 224*, 149-157. doi:10.1385/1-59259-364-X:149

Sunil, B., Saini, D., Bapatla, R. B., Aswani, V., & Raghavendra, A. S. (2019). Photorespiration is complemented by cyclic electron flow and the alternative oxidase pathway to optimize photosynthesis and protect against abiotic stress. *Photosynthesis Research, 139*(1-3), 67-79. doi:10.1007/s11120-018-0577-x

Suzuki, Y., Nakabayashi, K., Yoshizawa, R., Mae, T., & Makino, A. (2009). Differences in expression of the RBCS multigene family and rubisco protein content in various rice plant tissues at different growth stages. *Plant & Cell Physiol, 50*(10), 1851-1855. doi:10.1093/pcp/pcp120

T., A., & K., K. (2007). Grassland degradation in China: Methods of monitoring, management and restoration. *Grassland Science, 53*(1), 1-17. doi:10.1111/j.1744-697X.2007.00073.x

Tabita, F. R., Hanson, T. E., Satagopan, S., Witte, B. H., & Kreel, N. E. (2008). Phylogenetic and evolutionary relationships of RubisCO and the RubisCO-like proteins and the functional lessons provided by diverse molecular forms. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 363*, 2629-2640. doi:10.1098/rstb.2008.0023

Taler, D., Galperin, M., Benjamin, I., Cohen, Y., & Kenigsbuch, D. (2004). Plant eR genes that encode photorespiratory enzymes confer resistance against disease. *Plant Cell, 16*(1), 172-184. doi:10.1105/tpc.016352

Thimm, O., Bläsing, O., Gibon, Y., Nagel, A., Meyer, S., Krüger, P., . . . M, S. (2004). MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes.

Plant Journal, 37(6), 914-939. doi:10.1111/j.1365-313X.2004.02016.x
Timm, S., Florian, A., Arrivault, S., Stitt, M., Fernie, A., & Bauwe, H. (2012). Glycine decarboxylase controls photosynthesis and plant growth. *FEBS Letters, 586*(20), 3692-3697. doi:10.1016/j.febslet.2012.08.027
Timm, S., & Hagemann, M. (2020). Photorespiration-how is it regulated and how does it regulate overall plant metabolism? *Journal of Experimental Botany, 71*(14), 3955-3965. doi:10.1093/jxb/eraa183
Timm, S., Woitschach, F., Heise, C., Hagemann, M., & Bauwe, H. (2019). Faster Removal of 2-Phosphoglycolate through Photorespiration Improves Abiotic Stress Tolerance of Arabidopsis. *Plants (Basel), 8*(12), 563. doi:10.3390/plants8120563
Tirosh, I., Barkai, N., & Verstrepen, K. J. (2009). Promoter architecture and the evolvability of gene expression. *Journal of Biology, 8*(11), 95. doi:10.1186/jbiol204
Tito, R., Castellani, T. T., Fáveri, S. B., Lopes, B. C., & Vasconcelos, H. L. (2016). From over to undercompensation: Variable responses to herbivory during ontogeny of a Neotropical monocarpic plant. *Biotropica, 48*(5), 608-617. doi:10.1111/btp.12340
Uematsu, K., Suzuki, N., Iwamae, T., Inui, M., & Yukawa, H. (2012). Increased fructose 1,6-bisphosphate aldolase in plastids enhances growth and photosynthesis of tobacco plants. *Journal of Experimental Botany, 63*(8), 3001-3009. doi:10.1093/jxb/ers004
Wang, J. F., He, L., Lu, S. J., Lu, D., Huang, T., Cao, Q., . . . Liu, B. Y. (2020). Photosynthetic vegetation cover response to precipitation on the Inner Mongolian Steppe. *Acta Ecologica Sinica, 40*(16), 5620-5629. doi:10.5846/stxb201910092097
Wang, L. K., Feng, Z. X., Wang, X., Wang, X. W., & Zhang, X. G. (2010). DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics, 26*(1), 136-138. doi:10.1093/bioinformatics/btp612
Wang, X. T., Chai, J., Jiang, C., Tai, Y., Chi, Y. Y., Zhang, W., . . . Li, S. Y. (2020). Population spatial pattern of Stipa grandis and its response to long-term overgrazing. *Biodiversity Science, 28*(2), 128-134. doi:10.17520/biods.2019276
Westoby, M., Walker, B., & Noy-Meir, I. (1989). Opportunistic Management for Rangelands Not at Equilibrium. *Journal of Range Management, 42*(4), 266-274.
Wingler, A., Lea, P. J., Quick, W. P., & Leegood, R. C. (2000). Photorespiration: metabolic pathways and their role in stress protection. *Philosophical Transactions B: Biological Sciences, 355*(1402), 1517-1529. doi:10.1098/rstb.2000.0712
Wu, N. T., Lin, G. X., Yang, Y., Song, X. Y., & Bai, H. H. (2020). Dynamics monitoring of net primary productivity and its response to climate factors in native grassland in Inn Mongolia using a light-use efficiency model. *Acta Prataculturae Sinica, 29*(11), 1-10. doi:10.11686/cyxb2019543
Xu, H. W., Zhang, J. J., Zeng, J. W., Jiang, L. R., Liu, E., Peng, C. L., . . . Peng, X. X. (2009). Inducible antisense suppression of glycolate oxidase reveals its strong regulation over photosynthesis in rice. *Journal of Experimental Botany, 60*(6), 1799-1809. doi:10.1093/jxb/erp056
Yan, X., Gong, J. R., Zhang, Z. Y., Huang, Y. M., An, R., Qi, Y., & Liu, M. (2013). Responses of photosynthetic characteristics of Stipa baicalensis to grazing disturbance. *Chinese Journal of Plant Ecology, 37*(6), 530-541. doi:10.3724/sp.J.1258.2013.00054
Yang, B., Liu, J., Ma, X. N., Guo, B. B., Liu, B., Wu, T., . . . Chen, F. (2017). Genetic engineering of the Calvin cycle toward enhanced photosynthetic CO2 fixation in microalgae. *Biotechnology for Biofuels, 10*, 229. doi:10.1186/s13068-017-0916-8
Zelitch, I., Schultes, N. P., Peterson, R. B., Brown, P., & Brutnell, T. P. (2009). High glycolate oxidase activity is required for survival of maize in normal air. *Plant physiology, 149*(1), 195-204.

doi:10.1104/pp.108.128439
Zhang, J., Li, Z., Li, J., Liu, J., LV, S., & Han, G. (2019). Comprehensive Evaluation and Correspondence Analysis of Nutritional Value of Common Grass Species in Ewenki Grassland. *Chinese Journal of Grassland, 41*(5), 33-.
Zhang, Z. H., Gong, J. R., Wang, B., Li, X. B., Ding, Y., Yang, B., . . . Zhang, W. (2020). Regrowth strategies of Leymus chinensis in response to different grazing intensities. *Ecological Applications, 30*(5), e02113.
Zhao, W., Chen, S. P., Han, X. G., & Lin, G. H. (2008). Effects of long-term grazing on the morphological and functional traits of Leymus chinensis in the semiarid grassland of Inner Mongolia, China. *Ecological Research, 24*(1), 99-108. doi:10.1007/s11284-008-0486-0
Zhao, W., Chen, S. P., & Lin, G. H. (2008). Compensatory growth responses to clipping defoliation in Leymus chinensis (Poaceae) under nutrient addition and water deficiency conditions. *Plant Ecology, 196*(1), 85-99. doi:10.1007/s11258-007-9336-3
Zhou, G. Y., Zhou, X. H., He, Y. H., Shao, J. J., Hu, Z. H., Liu, R. Q., . . . Hosseinibai, S. (2017). Grazing intensity significantly affects belowground carbon and nitrogen cycling in grassland ecosystems: a meta-analysis. *Global Change Biology, 23*(3), 1167-1179. doi:10.1111/gcb.13431
Zhou, S. Q., Lou, Y. R., Tzin, V., & Jander, G. (2015). Alteration of Plant Primary Metabolism in Response to Insect Herbivory. *Plant physiology, 169*(3), 1488-1498. doi:10.1104/pp.15.01405

DATA ACCESSIBILITY

The raw sequencing data have been deposited in the Sequence Read Archive under accession number PRJNA658710.

AUTHOR CONRIBUTIONS

Project design, Zhenhua Dang; sampling, Zhenhua Dang, Yuanyuan Jia, Lei Huang, Jiabin Li, Yanan Zhang; wet lab work, Yunyun Tian and Yuanyuan Jia; data submission, Yunyun Tian; bioinformatic analyses, data analysis, writing, and review, Zhenhua Dang, Yuanyuan Jia, Yunyun Tian, Peter J. Lockhart, Cory Matthew, and Frank Yonghong Li; Grazing experimental design, Cunzhu Liang. All authors have read and agreed to publish of the manuscript.

TABLES

TABLE 1 Summary of sequencing and assembly results

Sample	RD (Mb)	CR (Mb)	GCC (%)	Q20 (%)	AU (No.)	ML (bp)	N50 (%)	TM (%)
STICK_1	106.66	101.8	47.79	97.73	101,788	1,373	2,078	91.72
STICK_2	105.8	101.64	47.57	97.59	101.392	1,322	1,953	92.22
STICK_3	104.76	100.89	47.81	97.83	89,023	1,363	2,030	93.14
STIG3_1	105.96	102.07	47.62	97.72	108,636	1,300	1,890	92.27
STIG3_2	107.36	102.71	47.92	98.03	113,212	1,327	1,956	90.52
STIG3_3	105.71	101.88	47.95	97.78	115,918	1,373	2,052	91.75
STIG6_1	107.08	102.77	47.42	98.12	67,705	906	1,243	89.19
STIG6_2	107.54	107.54	47.51	97.75	103,648	1,304	1,917	92.73
STIG6_3	105.94	105.94	47.51	97.94	107,998	1,304	1,919	91.35
STIG12_1	105.73	101.37	47.37	97.89	70.978	1,144	1,636	93.49
STIG12_2	106	102.09	47	98.2	88,736	1,239	1,806	92.09
STIG12_3	106.01	102.9	47.07	98.43	90,147	1,138	1,644	92.28
All-Unigene	—	—	47.27	—	251,412	1,854	2,536	—

RD and CR denote(s) raw data and Clean Reads respectively. GCC represents GC content. Q20 means the percentage of bases with a Phred value > 20. AU represents the number of assembled Unigenes. ML indicates mean length of assembled sequences. N50 represents 50% of the assembled basses were incorporated into sequences with length of N50 or longer. TM indicates total mapped reads to an assembled transcriptome.

TABLE 2 DEGs identified in the Calvin Benson Cycle

Gene ID	CK-FPKM	G3-FPKM	G6-FPKM	G12-FPKM	FC-G3/CK	FC-G6/CK	FC-G12/CK	FC-G6/G3	FC-G12/ G3	FC-G12/G6
Rubisco										
CL2153.Ct7	4316.15	3309.66	6219.92	9028.56	-0.38	+0.54	**+1.07**	+0.91	**+1.45**	+0.54
Ug53513	814.46	541.78	933.75	1710.03	-0.59	+0.20	**+1.07**	+0.79	**+1.66**	+0.87
Ug53512	11.87	12.05	17.27	41.67	+0.04	+0.58	**+1.84**	+0.54	**+1.81**	**+1.26**
Ug53514	88.58	97.20	147.86	184.79	+0.14	+0.76	**+1.07**	+0.62	+0.93	+0.32
CL2153.Ct1	2309.53	1729.17	4911.67	6702.50	-0.41	**+1.09**	**+1.54**	**+1.50**	**+1.96**	+0.45
PGKase										
CL3380.Ct10	93.12	75.71	130.53	184.49	-0.29	+0.49	+0.99	+0.79	**+1.29**	+0.50
CL3380.Ct6	191.12	141.49	232.50	339.03	-0.43	+0.29	+0.83	+0.72	**+1.26**	+0.54
GAPDHase										
CL293.Ct12	91.46	69.83	151.08	182.89	-0.39	+0.73	**+1.00**	**+1.12**	**+1.39**	+0.27
CL293.Ct13	145.40	72.75	221.96	134.56	**-1.00**	+0.62	-0.11	**+1.61**	+0.89	-0.72
CL293.Ct30	22.02	34.77	60.47	90.62	0.66	**+1.47**	**+2.05**	+0.80	**+1.38**	+0.58
CL293.Ct33	100.97	55.92	109.13	129.76	-0.85	+0.12	+0.37	+0.97	**+1.22**	+0.24
Ug64285	202.91	141.73	228.68	356.98	-0.51	+0.18	+0.82	+0.69	**+1.33**	+0.64
Ug64290	365.55	216.81	170.85	316.10	-0.75	**-1.09**	-0.20	-0.34	**+0.55**	+0.89
ALDase										
Ug48591	358.17	165.06	117.60	439.33	**-1.12**	**-1.60**	+0.30	-0.48	**+1.41**	**+1.90**
Ug48588	560.38	139.93	121.14	1099.92	**-2.00**	**-2.20**	+0.98	-0.21	**+2.98**	**+3.18**
CL1648.Ct49	185.81	118.93	191.64	243.47	-0.64	+0.05	+0.39	+0.69	**+1.03**	+0.34
CL1717.Ct15	237.81	71.48	72.81	196.39	**-1.73**	**-1.70**	-0.27	+0.03	**+1.46**	**+1.43**
CL1717.Ct8	13.17	3.94	2.73	20.76	**-1.74**	**-2.25**	+0.66	-0.51	**+2.40**	**+2.91**
CL1717.Ct14	154.75	53.44	70.83	97.33	**-1.53**	**-1.11**	-0.66	+0.42	+0.87	+0.45
CL7818.Ct5	59.21	63.48	32.89	29.33	+0.10	-0.84	**-1.00**	-0.94	**-1.11**	-0.16
Ug48595	1500.74	347.73	283.65	1181.09	**-2.11**	**-2.39**	-0.34	-0.28	**+1.77**	**+2.05**
FBPase										
CL9825.Ct2	77.37	54.01	148.83	299.25	-0.51	+0.95	**+1.96**	**+1.47**	**+2.47**	**+1.01**
CL9825.Ct23	65.55	26.24	41.26	67.44	**-1.32**	-0.65	+0.05	+0.66	**+1.37**	**+0.70**
TKase										
CL14956.Ct14	71.74	81.01	148.78	167.68	+0.18	**+1.05**	**+1.23**	+0.87	**+1.05**	+0.18
SBPase										
CL5196.Ct26	7.49	4.00	9.38	23.56	-0.90	+0.33	**+1.66**	**+1.23**	**+2.56**	**+1.33**
CL5196.Ct6	217.25	99.64	154.61	385.86	**-1.12**	-0.48	+0.84	+0.64	**+1.96**	**+1.32**
CL5196.Ct21	27.09	9.47	8.81	24.16	**-1.52**	**-1.61**	-0.16	-0.10	**+1.36**	**+1.46**
CL5196.Ct17	83.88	29.11	48.06	124.86	**-1.52**	-0.80	+0.58	+0.73	**+2.10**	**+1.38**
RPEase										
CL16574.Ct15	124.79	63.59	77.27	147.24	-0.97	-0.68	+0.25	+0.29	**+1.21**	+0.93
Ug66782	25.28	11.86	13.55	22.78	**-1.09**	-0.88	-0.14	+0.20	+0.94	+0.74
CL16574.Ct28	63.42	43.77	69.90	150.81	-0.53	+0.15	**+1.26**	+0.68	**+1.79**	**+1.11**
RPIase										

CL2119.Ct70	11.74	12.85	16.72	23.62	+0.14	+0.52	**+1.02**	+0.38	+0.88	+0.50
CL843.Ct1	136.56	111.92	97.67	53.30	-0.28	-0.46	**-1.35**	-0.18	**-1.07**	-0.89
CL843.Ct10	91.10	74.08	119.66	159.89	-0.29	+0.41	+0.82	+0.70	**+1.12**	+0.42
CL12672.Ct4	10.40	17.30	19.59	9.55	+0.74	+0.92	-0.11	+0.19	-0.85	**-1.04**
CL13185.Ct8	23.02	12.48	10.94	16.15	-0.88	**-1.07**	-0.51	-0.18	+0.37	+0.56
PRKase										
CL2846.Ct13	387.88	149.49	149.53	332.08	**-1.37**	**-1.36**	-0.22	+0.01	**+1.15**	**+1.15**
RCA: Rubisco activase										
Ug11360	653.40	27.81	3.11	40.32	**-4.55**	**-7.71**	**-4.01**	**-3.15**	+0.54	**+3.70**

Bold values indicate DEG identified in pairwise comparisons. DEGs were filtered using a threshold of FDR ≤ 0.001 and absolute value of $\log_2$Ratio ≥ 1. Ug indicates unigene. FC indicates fold change. FPKM indicates FPKM values of unigenes in each grazing treatment. FC equals $\log_2$ value of a paired comparison for two grazing intensities. "+" indicates up-regulated transcription and "-" represents down-regulated transcription.

940 **TABLE 3 DEGs identified in photorespiratory pathway**

Gene ID	CK-FPKM	G3-FPKM	G6-FPKM	G12-FPKM	FC-G3/CK	FC-G6/CK	FC-G12/CK	FC-G6/G3	FC-G12/G3	FC-G12/G6
PGLPase										
Ug4982	212.93	84.81	97.38	253.03	**-1.32**	**-1.12**	+0.26	+0.20	**+1.58**	**+1.38**
GOXase										
CL14974.Ct1										
9	10.71	15.71	11.18	5.78	+0.56	+0.07	-0.88	-0.49	**-1.44**	-0.95
CL95.Ct35	63.01	23.46	21.87	27.53	**-1.42**	**-1.51**	**-1.19**	-0.09	+0.23	+0.32
CL95.Ct34	191.35	76.98	109.89	122.89	**-1.31**	-0.79	-0.63	+0.52	+0.68	+0.15
Un9650	143.59	194.60	443.96	256.77	+0.44	**+1.63**	+0.85	**+1.19**	+0.40	-0.79
CL95.Ct47	155.69	65.21	74.17	89.60	**-1.25**	**-1.06**	-0.79	+0.19	+0.46	+0.27
GGTase										
CL3941.Ct15	190.65	69.86	172.11	191.19	**-1.44**	-0.13	+0.01	**+1.31**	**+1.46**	+0.15
CL3941.Ct28	18.60	7.80	14.30	11.00	**-1.25**	-0.38	-0.76	+0.88	+0.50	-0.38
SHMTase										
CL3053.Ct39	326.94	127.95	95.39	194.27	**-1.35**	**-1.77**	-0.75	-0.41	+0.60	**+1.02**
Ug49346	29.27	30.66	15.07	8.65	+0.07	-0.95	**-1.75**	**-1.02**	**-1.82**	-0.81
CL3053.Ct41	595.44	292.29	276.86	541.90	**-1.02**	**-1.10**	-0.13	-0.07	+0.89	+0.96
Ug49351	17.58	21.40	6.87	8.60	+0.28	**-1.34**	**-1.03**	**-1.63**	**-1.31**	+0.32
SGTase										
CL593.Ct19	59.64	49.37	51.81	29.63	-0.27	-0.20	**-1.01**	+0.07	-0.74	-0.81
CL593.Ct33	103.75	60.52	49.47	35.20	-0.78	**-1.06**	**-1.56**	-0.28	-0.78	-0.50
CL593.Ct32	177.56	218.05	303.41	145.89	+0.30	+0.78	-0.28	+0.48	-0.58	**-1.06**
CL593.Ct1	190.01	155.49	197.01	80.32	-0.29	+0.06	**-1.24**	+0.35	-0.95	**-1.30**
CATase										
Ug20659	665.35	268.29	268.35	437.80	**-1.31**	**-1.30**	-0.60	+0.01	+0.71	+0.70
CL10067.Ct2	14.83	23.59	15.93	7.35	+0.67	+0.11	**-1.01**	-0.56	**-1.68**	**-1.12**
CL10067.Ct4	12.83	17.75	14.33	6.80	+0.47	+0.17	-0.91	-0.30	**-1.38**	**-1.08**
Ug17925	48.22	12.97	16.50	26.53	**-1.89**	**-1.54**	-0.86	+0.35	**+1.03**	+0.68
Ug11709	390.91	102.84	104.05	311.38	**-1.92**	**-1.90**	-0.32	+0.02	**+1.60**	**+1.58**
CL8442.Ct1	12.64	14.57	28.12	8.48	+0.21	**+1.15**	-0.57	+0.95	-0.78	**-1.72**
CL10067.Ct5	11.15	18.52	10.95	5.32	+0.73	-0.02	**-1.06**	-0.75	**-1.79**	**-1.04**
CL8442.Ct4	37.22	59.31	65.18	24.18	+0.67	+0.81	-0.62	+0.14	**-1.29**	**-1.43**
Ug15777	42.24	40.03	70.97	137.85	-0.07	+0.75	**+1.71**	+0.82	**+1.79**	+0.96
CL8442.Ct9	40.03	9.55	8.96	11.92	**-2.07**	**-2.15**	**-1.74**	-0.08	+0.32	+0.41
GSase										
CL3536.Ct26	42.38	35.39	86.49	105.57	-0.26	**+1.03**	**+1.32**	**+1.28**	**+1.58**	+0.29
CL12113.Ct1										
4	61.13	91.60	36.22	25.12	+0.58	-0.74	**-1.28**	**-1.33**	**-1.86**	-0.53
GDCase										
CL1834.Ct22	96.09	36.07	39.11	84.10	**-1.41**	**-1.29**	-0.19	+0.12	**+1.22**	**+1.10**

79
80

CL1834.Ct3	93.14	44.47	45.19	123.96	**-1.06**	**-1.04**	+0.41	+0.03	**+1.48**	**+1.45**
AMTase										
CL14900.Ct8	47.24	47.01	79.11	114.67	0.00	+0.75	**+1.28**	+0.75	**+1.29**	+0.54
DIDase										
CL12727.Ct1										
5	8.66	13.63	17.80	6.41	+0.66	**+1.05**	-0.42	+0.39	**-1.08**	**-1.47**
HPRase										
CL9071.Ct1	34.36	34.24	22.49	46.78	0.00	-0.60	+0.45	-0.60	+0.46	**+1.05**
CL738.Ct1	97.15	79.52	144.23	233.41	-0.28	+0.58	+1.28	+0.86	**+1.56**	+0.70
CL738.Ct17	60.15	25.65	26.99	37.49	-1.23	**-1.14**	-0.68	+0.09	+0.55	+0.47
CL738.Ct27	181.46	78.90	81.30	94.01	-1.20	**-1.14**	-0.94	+0.06	+0.26	+0.20
CL738.Ct39	97.28	52.19	75.97	105.65	-0.89	-0.35	+0.12	+0.55	**+1.02**	+0.47
CL9071.Ct2	11.27	3.46	8.67	20.73	-1.70	-0.37	+0.89	+1.33	**+2.59**	**+1.26**

Bold values indicate DEG identified in pairwise comparisons. DEGs were filtered using a threshold of FDR ≤ 0.001 and absolute value of $\log_2$Ratio ≥ 1. Ug indicates unigene. FC represents fold change. FPKM indicates FPKM values of unigenes in each grazing treatment. FC equals $\log_2$ value of a paired comparison for two grazing intensities. "+" indicates up-regulated transcription and "-" represents down-regulated transcription.

FIGURE LEGENDS

FIGURE 1: Vegetation status of plots of the four grazing intensity treatments. (Photographed 28 July 2018)

FIGURE 2: Expression patterns and functional annotations of the DEGs. (A) Hierarchical clustering of DEGs. The red-to-blue gradient indicates high-to-low expression levels. (B) KEGG pathway enrichment analysis. (C) GO enrichment analysis. In panels B and C, only the top five enriched GO terms and KEGG pathways are shown, respectively; the x-axis indicates the number of the unigenes, and clusters are separated by left-extended short black bars.

FIGURE 3: Expression patterns of DEGs in the CBC. The line and symbol chart next to each enzyme represents the expression profiles of DEGs shown in table 2 and Additional file 6. The grazing gradient is shown on the *x*-axis and the gene expression level (the mean FPKM value of three biological replicates) is shown on the *y*-axis. The carboxylation reaction catalyzed by Rubisco fixes CO_2 into the acceptor molecule RuBP, forming 3-PGA. The reductive phase of the cycle follows with two reactions catalyzed by PGKase and GAPDHase, producing GAP. The GAP enters the regenerative phase catalyzed by ALDase and either FBPase or SBPase, producing F6P (fructose-6-phosphate) and S7P (sedoheptulose-7-phosphate). The F6P and S7P are then used in reactions catalyzed by TKase, RPIase, and RPEase, producing Ru5P (ribulose 5-phosphate). The final step, which is catalyzed by PRKase, converts Ru5P to RuBP. Rubisco is the initiating enzyme for the Calvin–Benson

968 cycle and the photorespiratory cycle, fixing O_2 into the acceptor molecule RuBP to form 2-
969 PG, which is then metabolized via the photorespiratory pathway.
970
971 **FIGURE 4:** Expression patterns of DEGs in the photorespiratory pathway. The line and
972 symbol chart next to each enzyme represents the expression profiles of DEGs shown in table
973 3 and Additional file 7. The grazing gradient is shown on the *x*-axis and the gene expression
974 level (the mean FPKM value of three biological replicates) is shown on the *y*-axis. The
975 photorespiratory cycle is a process in photosynthetic cells involving the chloroplasts,
976 peroxisomes, mitochondria, and the cytosol. In chloroplasts, Rubisco catalyzes the
977 oxygenation of RuBP, which generates one molecule of 3-PGA and one molecule of 2-PG.
978 The 2-PG is first dephosphorylated to glycolate by PGLPase, after which it diffuses into the
979 peroxisome. In the peroxisome, the O_2-dependent glycolate is oxidized to glyoxylate by
980 GOXase to produce H_2O_2, which is quickly detoxified by CATase. Glyoxylate is
981 transaminated to glycine by the parallel action of GGTase or SGTase. Glycine then moves
982 into the mitochondrion, wherein the GDCase multienzyme system and SHMTase convert two
983 molecules of glycine to one molecule of serine, NH_3, and CO_2. After being transported from
984 the mitochondrion to the peroxisome, serine is converted by SGTase to hydroxypyruvate,
985 which is reduced to glycerate by HPRase. The glycerate returns to the chloroplast to be
986 phosphorylated by GLYKase (glycerate 3-kinase), and the resulting 3-PGA is converted to
987 RuBP in the CBC.

SUPPORTING INFORMATION

Additional file 1. Statistics of BUSCO for the transcriptome assembly quality assessment of the 12 *Stipa grandis* samples.

Additional file 2. All coefficient of variance filtered Genes.

Additional file 3. Functional annotation of all analyzed transcripts.

Additional file 4. DEGs identified by pairwise comparisons of *Stipa grandis* under different grazing treatments.

Additional file 5. Transcriptional clustering analysis of DEGs.

Additional file 6. Transcripts Related to the Calvin–Benson cycle

Additional file 7. Transcripts Related to the photorespiratory pathway

Figure S1. Correlation analysis of the CV filtered transcripts

Figure S2. Similarity comparison of *Stipa grandis* Unigenes with other species in Nr database

Figure S3. GO functional annotation of DEGs

Figure S4. KEGG enrichment analysis of DEGs