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Research article

Metabolic response and correlations between ions and metabolites in *Phragmites communis* under long-term salinity toxicity

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ABSTRACT

Phragmites communis has a long history in Songnen grassland of China and has a series of biological, ecological as well as genetic characteristics contributing to its adaptation to the specific local climatic and edaphic conditions. The aim of the present study was to investigate the ions balance and their relationship to metabolites in *P. communis* under three salinity stress conditions. Results showed that the contents of Na^+, Cl^-, and SO_4^{2-} significantly increased in *P. communis* leaves, while K^+, Mg^{2+}, and Mn^{2+} decreased. Moreover, Na^+ and Cl^- had significant negative correlations with metabolites involved in the tricarboxylic acid cycle (*TCA* cycle), and significant positive correlations with glycolysis. The metabolite results showed that high contents of sugars and proline played important roles in developing salinity tolerance, indicating that glycolysis and proline biosynthesis were enhanced; however, this consumes large amounts of energy and likely caused the *TCA* cycle to be inhibited. The results suggested that *P. communis* might enhance its salinity tolerance mainly through increased glycolysis and energy consumption. In addition, restricting Na^+ accumulation and increasing of Cl^-, and rearrangement of metabolite production in *P. communis* tissues are possible causes of salinity tolerance. Therefore, salinity caused systems alterations in widespread metabolic networks involving *TCA* cycle, glycolysis and proline biosynthesis. These findings provided new insights for the *P. communis* metabolic adaptation to salinity and demonstrated the ions balance and metabolites in *P. communis* are possibly attributable to development of salinity tolerance.

1. Introduction

The Songnen Plain, part of the Northeast plain, is located in the center of northeastern China. This area covers approximately $22.35 \times 10^4\ km^2$, and was formed by the flushing effect of the Songhua and Nen Rivers (Zheng et al., 2015). The Songnen Plain is an important national grassland, its grain production accounts for 12% of China's total, and it plays a key role in the ecosystem of north China (Shi and Guo, 2006). However, the land surface properties of the Songnen Plain have been changed remarkably by human reclamation activities, overgrazing, and global climate change, sand, desertification, and salinity now affect 11.2%, 11.05%, and 22.64% of the plain, respectively (Ye et al., 2009). Soil salinization poses a significant threat to agriculture. Understanding the metabolic characteristics of dominant

Abbreviations: 3-PGA, 3-phosphoglycerate; α-KG, α-ketoglutaric acid; Aco, aconitic acid; Ala, Alanine; Asco, ascorbic acid; Asn, asparagine; Asp, aspartic acid; Chlor, chlorogenic acid; Cit, citric acid; EC, electrical conductivity; ETA, Ethanolamine; Feru, ferulic acid; Fru, fructose; Fum, Fumaric acid; F-6-P, fructose-6-phosphate; Ile, isoleucine; GABA, γ-aminobutyric acid; Galac, galactose; Glc, glucose; Gln, Glutamine; Gly, glycine; Glya, glyceric acid; Glyc, glycerol; Glu, glutamate; G-6-P, glucose-6-phosphate; KEGG, kyoto encyclopedia of genes and genomes; Lac, lactose; Leu, Leucine; LS, low salinity soil; Man, mannose; Mal, Malic acid; Malt, maltose; Met, methionine; mIno, myo-Inositol; MS, moderate salinity soil; Nle, norleucine; OAA, Oxalic acid; Pala, palmitic acid; PCA, Principal component analysis; PC1, the first principal component; PC2, the second principal component; Phe, phenylalanine; PEP, Phenylpyruvate; pH, hydrogen ion concentration; PLS-DA, partial least squares discrimination analysis; Pro, proline; Pyr, Pyruvate; Put, putrescine; Quin, quinic acid; Rib, ribose; Ribi, ribitol; Ser, serine; Shik, shikimic acid; Sor, sorbitol; SS, severe salinity soil; SSA, succinate semialdehyde; Suc, sucrose; Succ, Succinic acid; Tag, tagatose; TCA cycle, tricarboxylic acid cycle; Thr, threonine; Thre, threonic acid; Tre, trehalose; TSC, total salinity concentration; Tyr, tyrosine; Val, valine; VIP, variable importance into projection; Xyl, xylose

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grassland populations on salinity land can reveal the ecophysiological adaptive mechanisms of the plant community and provide scientific evidence for the restoration and reconstruction of degraded grassland.

Phragmites communis is widely distributed as the dominant population in the Songnen Plain, and can form a single-species community on the microtopography of low-lying areas. It has high tolerance to salinity soils, and can live with an extreme saline-alkaline locations (Li et al., 2009; Qiu, 2014). *Phragmites communis* is recognized as a major gramineous forage because of its high productivity and high protein, mineral, and carbohydrate contents (Hocking, 1989; Van et al., 2003). It also has important ecological and economic value, and is the most fundamental vegetation of the Songnen Plain, constructs the community synusia, and limits the balance of ecosystems; as a result, the specific local climatic and edaphic conditions should be accommodated (Li et al., 2009; Qiu, 2014; Liu et al., 2016). Therefore, *P. communis* plays an important multifold role in nature conservation, environmental protection, and grassland rehabilitation (Du et al., 2006; Hansen et al., 2007; Takahashi et al., 2009). *Phragmites communis* responds to salinity stress through a set of combating mechanisms, including expression of genes for Na^+/H^+ antiporter proteins that participate in excluding toxic ions, synthesizing proline and soluble sugars, and inducing enzymatic and non-enzymatic antioxidants for scavenging of reactive oxygen species (ROS) – all of which are considered crucial biochemical strategies (Takahashi et al., 2009; Gorai et al., 2010; Ding et al., 2015). Our previous studies indicated that the different physiological adaptive mechanisms in old and young leaves of *P. communis* under salinity stress, however, there has been no work, for the time being, published on comprehensive investigations on the whole-plant metabolomic responses of *P. communis* to different salinity concentrations (Guo et al., 2018). Plant responses to adverse natural salinity conditions should be analyzed to identify the metabolites associated with a specific response to high ion concentrations and high pH. These metabolites should also be determined to elucidate the mechanisms of plant tolerance and adaptation to salinity stress (Ruan and Silva, 2011; Barding et al., 2013).

In this study, *P. communis* growth, nutrient elements, metabolic profiles, and the relationships among these factors were investigated for their effects on population development in different salinity soils. The purpose of this study was to determine the primary effects of salinity stress on the growth of *P. communis*, to evaluate the correlations between ions and metabolites in *P. communis*. Our results should help in understanding the physiological adaptive mechanisms and reveal *P. communis* population development in a degenerated meadow. This study provides a new strategy to contribute to research on global climate change and environmental conservation and restoration. This study also yielded a scientific basis for the restoration and reconstruction of arid, acidic, and saline areas of grassland experiencing desertification and degeneration worldwide.

2. Materials and methods

2.1. Study site and plot selection

This research was conducted in ChangLing County, JiLin Province, China (44°30′–44°N, 123°31′–123°56′E). This area is characterized by a typical mesothermal monsoon climate, with mean annual rainfall of 400–500 mm and annual evaporation capacity 2–3 times than the rainfall. The mean annual temperature and accumulated temperatures are 4.6–6.4 °C and 2545–3374 °C, respectively (Zhang et al., 2014). Soils of the Songnen Plain are of various types, including black soil, chernozem, meadow soil, swamp soil, and saline soil, with salinized meadow soil the main type (Zhang et al., 2013). NaCl, Na_2SO_4, $NaHCO_3$, and Na_2CO_3 are the main salt components of salinity soil in Songnen grassland (Ge and Li, 1990; Shi and Wang, 2005). In this research, three large sites were selected for sampling according to the different degrees of retrogressive succession (File 1). In the first site, single dominant *P. communis* communities were found in low salinity soil (LS) (File 1A). In the second site, *P. communis* coexisted with *Leymus chinensis* communities in moderate salinity soil (MS) (File 1B). In the third site, *P. communis* coexisted with *Suaeda salsa* communities in severe salinity soil (SS) (File 1C). Each area consisted of five 3 × 3 m plots, and the distance between plots was < 100 cm – consequently, they shared similar climatic conditions and soil types. Each plot represented one replicate in all experiments, and the duration of the experiments is 2 years (2015 and 2016). In experiment plots, the mean annual temperature is 7.05 ± 0.73 °C and 6.42 ± 0.76 °C in 2015 and 2016, and the annual precipitation is 395.6 mm and 450.2 mm.

Table 1
The properties and ion contents in soil of *P. communis* habitats in Songnen grass land. The total salinity concentration (TSC), the electrical conductivity (EC). Values are means of five replicates. Means followed by different letters in the same graph are significantly different at $P < 0.05$ according to Duncan's method.

Soil properties and ion contents	The three concentrations for the salinity stresses in soil on the Songnen grassland		
	LS	MS	SS
K^+ (μmol/g)	0.37 ± 0.08^c	1.99 ± 0.28^b	2.87 ± 0.36^a
Na^+ (μmol/g)	6.70 ± 0.64^c	23.03 ± 2.71^b	40.30 ± 8.51^a
Ca^{2+} (μmol/g)	1.38 ± 0.12^a	0.88 ± 0.07^b	0.80 ± 0.05^b
Mg^{2+} (μmol/g)	0.38 ± 0.06^c	1.18 ± 0.42^b	5.68 ± 0.71^a
CO_3^{2-} (μmol/g)	2.86 ± 0.43^c	9.86 ± 1.46^b	18.12 ± 3.11^a
HCO_3^- (μmol/g)	10.20 ± 0.56^b	20.04 ± 2.27^a	25.75 ± 4.97^a
Cl^- (μmol/g)	1.87 ± 0.14^c	7.16 ± 0.86^b	15.25 ± 3.41^a
SO_4^{2-} (μmol/g)	0.36 ± 0.04^c	2.08 ± 0.33^b	4.06 ± 0.77^a
TSC (μmol/g)	24.13 ± 1.67^c	66.21 ± 7.28^b	112.83 ± 19.49^a
pH	8.85 ± 0.51^c	9.64 ± 0.63^b	10.13 ± 0.76^a
EC (μs/cm)	135.77 ± 4.36^c	439.33 ± 10.43^b	690.67 ± 23.02^a

2.2. Measurement of properties of soil

Soil samples were collected in each plot at depths of 0–10, 10–20, 20–30, and 30–40 cm in 2015 before this study. Afterward, the obtained samples were mixed together to obtain a uniform sample and passed through a 0.25-mm soil sieve after being naturally dried. The electrical conductivity (EC) and potential of hydrogen (pH) of salinity soil were measured using a conductivity meter (DDG-2080-S, Anhui, China) and a pH meter (PSH-3C, Jiangsu, China), respectively. The Na^+, K^+, Ca^{2+}, and Mg^{2+} contents were determined using an inductively coupled plasma-optical emission spectroscopy (ICP-OES) spectrometer (iCAP 6000). The Cl^- and SO_4^{2-} contents were determined through ion exchange chromatography (DX300 ion chromatographic system). The CO_3^{2-} and HCO_3^- contents were determined using a dual indicator (phenolphthalein and bromophenol)-neutralization titrimetric method with H_2SO_4 as a standard acid. The contents of the eight ions were summed to give the total salinity concentration (TSC).

2.3. Samplings collected and measurement of growth characteristics

In the middle of the years in which the experiments were conducted, the length of shoot was measured before harvesting, and then the shoot and root were separated. Meanwhile, the first fully expanded leaves of reed were separated and frozen immediately in liquid nitrogen which were used for physiological index measurements. Dry weight of shoot was determined after incubating in an 80 °C oven for 15 min and in a vacuum dryer at 40 °C until the weight was constant.

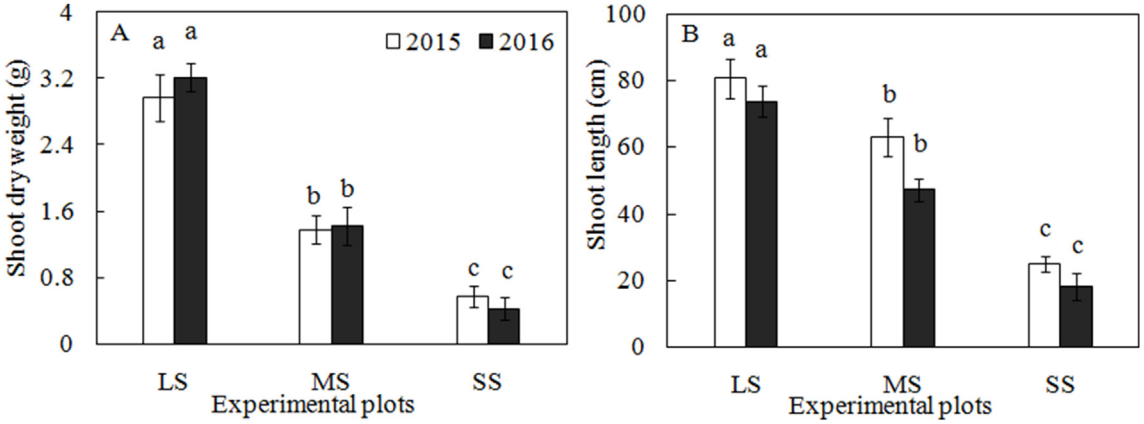

Fig. 1. Changes in the shoot dry weight (A) and length (B) of *P. communis* under different salinity concentration soil on the Songnen grassland. Values are means of five replicates. Means followed by different letters in the same graph are significantly different at $P < 0.05$ according to Duncan's method. Single dominant *P. communis* communities were found in low salinity soil (LS), *P. communis* coexisted with *Leymus chinensis* communities in moderate salinity soil (MS), *P. communis* coexisted with *Suaeda salsa* communities in severe salinity soil (SS).

2.4. Measurement of ions

The samples were digested with HNO_3, and the cation contents (Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}) were assayed using an ICP-OES spectrometer (iCAP 6000 series, Thermo Fisher Scientific Inc.). And samples used treated with 20 cm^3 of deionized water at 100 °C for 20 min, and the obtained extracts used to measure the contents of free inorganic anions, including NO_3^-, Cl^-, SO_4^{2-}, and $H_2PO_4^-$, through ion exchange chromatography (DX300 ion chromatographic system; AS4A-SC ion-exchange column, CD M-II electrical conductivity (EC) detector; Dionex, Sunnyvale, CA, USA).

2.5. Measurement of metabolic profiles

Metabolites were extracted from *P. communis* leaves, as described by Lisec with slight modification (Lisec et al., 2006). Approximately 100 ± 10 mg of fresh leaves were pooled, homogenized, transferred into 2-cm^3 centrifuge tubes with 60 mm^3 of distilled water containing 0.012 mg of ribitol, and vortexed for 10 s. The samples were extracted by using a thermomixer (Eppendorf, Hamburg, Germany) at 70 °C and 950 rpm for 15 min and subsequently centrifuged at 11,000 × *g* at 4 °C for 10 min. After extraction was completed, the supernatant was transferred into 2-cm^3 glass tubes and dried at 30 °C for 2 h in a vacuum concentrator. Afterward, 80 mm^3 of methoxamine pyridine solution was added to the tubes and the mixture vortexed for 30 s. The samples were further derivatized with bis(trimethylsilyl) trifluoroacetamide containing 1% trimethylchlorosilane (100 mm^3) at 70 °C for 1 h, and after the reactions were complete their metabolites were determined with an Agilent 7890 gas chromatograph system (CA, USA) with DB-5MS coated capillary column (J&W Scientific, Folsom, CA, USA). The analyte (1 mm^3) was injected in a splitless mode. The front inlet purge flow was 3 $cm^3\,min^{-1}$ of helium. The temperature of the measured process was followed by an initial temperature at 90 °C for 0.25 min, 10 °C/min increase rate to 180 °C, 5 °C/min increase rate to 240 °C, and 5 °C/min increase rate to 285 °C, maintained at 285 °C for 8 min, and gradually decreased. Mass spectrometry was conducted via a full-scan method with a range of 20–600 (*m/z*) at a rate of 100 spectra s^{-1} after a solvent delay of 492 s. The R software platform (http://cran.r-project.org/) provides information regarding raw signal extraction, data baseline filtering peak identification, and integration. TagFinder software was used to correct the analysis of mass debris, peak alignment, and deconvolution (Luedemann et al., 2008). The integral areas of all samples were normalized, the normalized data analyzed using the SIMCA-P 14.0 software package (Umetrics, Umea, Sweden; http://www.umetrics.com/simca), and partial least squares discrimination analysis (PLS-DA) model utilized using the first principal component of variable importance in the projection (VIP) values combined with similarity values of > 700. Duncan's method at $P < 0.05$ was used to determine differentially expressed metabolites. Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/) and MetaboAnalyst websites (www.metaboanalyst.ca/) were used to search metabolites in commercial databases.

2.6. Statistical analysis

All measurements were based on five replicates. The statistical analyses of the physiological indexes and evaluation of statistical significance and correlations were conducted using SPSS v. 16. Metabolic data were acquired, preprocessed using the manufacturer's ChromaTOF software (versions 2.12, 2.22 and 3.34; LECO, St. Joseph, MI, USA), and further identified by FiehnLib (commercial EI-MS library) (Kind et al., 2009). According to the previous research methods, we performed data preprocessing and replaced it with one-half of the minimum value (Zhang et al., 2016). The data were filtered using the interquartile range, and the total mass of the signal integration area was normalized for each sample. The normalized data were analyzed by SIMCA-P 13 software package (Umetrics), thereby resulting in VIP values obtained through partial least squares discriminant. The obtained data were evaluated using principal component analysis (PCA) and PLS-DA. Additionally, different metabolites were compared using Duncan's method ($P < 0.05$) and VIP (VIP > 1), combined with similarity values of > 700. Afterward, the metabolic pathway was constructed according to KEGG (http://www.genome.jp/kegg/) and MetaboAnalyst websites (www.metaboanalyst.ca/) and compared with samplings in LS (Xia et al., 2012). The correlations between the main nutrition elements and the main metabolites were analyzed by the spearman correlation analysis, with the detailed method according to Chong et al. (2018) and Xia and Wishart (2011).

3. Results

3.1. Soil properties and ion contents

With a serious degree of salinity and alkalinity, Na^+, K^+, Mg^{2+}, CO_3^{2-}, HCO_3^-, Cl^-, SO_4^{2-}, TSC, pH, and EC contents increased, but Ca^{2+} decreased, as compared with LS (Table 1, $P < 0.05$). The changes for the SS site was greater than for MS. Results showed that TSC, Na^+, CO_3^{2-}, HCO_3^-, pH, and EC were important factors indicating the degree of soil salinization (Table 1, $P < 0.05$). Furthermore, Na^+ was the dominant toxic ion in salinity soil (Table 1, $P < 0.05$).

Table 2
Changes in the nutrients elements (μmol/g DW) in the leaves of *P. communis* under different salinity concentration soil in 2015 and 2016. Values are means of five replicates. Means followed by different letters in the same graph are significantly different at $P < 0.05$ according to Duncan's method. Single dominant *P. communis* communities were found in low salinity soil (LS), *P. communis* coexisted with *Leymus chinensis* communities in moderate salinity soil (MS), *P. communis* coexisted with *Suaeda salsa* communities in severe salinity soil (SS).

Years	Study Plots	Metal elements and anion (μmol/g DW)											
		K^+	Na^+	Ca^{2+}	Mg^{2+}	Fe^{2+}	Cu^{2+}	Zn^{2+}	Mn^{2+}	Cl^-	NO_3^-	$H_2PO_4^-$	SO_4^{2-}
2015	LS	305.02 ± 13.82^a	13.10 ± 3.10^c	64.52 ± 4.53^a	30.16 ± 2.03^a	2.89 ± 0.22^a	2.25 ± 0.14^a	0.37 ± 0.07^a	1.24 ± 0.06^a	135.05 ± 0.25^c	10.39 ± 0.16^a	15.87 ± 0.03^a	5.26 ± 0.04^b
	MS	255.40 ± 16.44^b	78.64 ± 5.64^b	54.45 ± 3.23^b	39.22 ± 2.89^a	3.27 ± 0.16^a	2.80 ± 0.16^a	0.44 ± 0.05^a	0.56 ± 0.04^b	195.71 ± 0.22^b	10.97 ± 0.12^a	11.70 ± 0.03^a	21.84 ± 0.03^a
	SS	188.58 ± 8.86^c	200.64 ± 7.05^a	42.76 ± 2.45^b	20.36 ± 1.88^b	3.00 ± 0.12^a	2.43 ± 0.15^a	0.32 ± 0.06^a	0.62 ± 0.06^b	237.25 ± 0.31^a	10.55 ± 0.13^a	14.83 ± 0.02^a	28.44 ± 0.03^a
2016	LS	287.98 ± 12.46^a	20.76 ± 3.48^c	69.07 ± 4.77^a	43.52 ± 4.65^a	2.39 ± 0.25^a	2.32 ± 0.16^a	0.36 ± 0.03^a	0.49 ± 0.04^a	141.05 ± 10.37^c	10.91 ± 0.89^a	17.08 ± 2.55^a	6.99 ± 1.25^b
	MS	218.02 ± 11.86^b	84.86 ± 3.96^b	60.61 ± 2.25^b	36.05 ± 6.01^b	3.03 ± 0.22^a	2.80 ± 0.12^a	0.33 ± 0.03^a	0.32 ± 0.03^b	228.87 ± 11.81^b	10.48 ± 0.91^a	14.99 ± 1.42^a	25.81 ± 1.66^a
	SS	194.29 ± 13.23^c	138.13 ± 4.91^a	50.12 ± 3.33^b	31.74 ± 5.03^b	2.54 ± 0.31^a	2.20 ± 0.13^a	0.24 ± 0.02^a	0.33 ± 0.03^b	266.19 ± 11.23^a	10.11 ± 0.75^a	18.13 ± 1.47^a	30.66 ± 1.58^a

3.2. Growth characteristics and ion balance in P. communis response to different level of salinity stress

Both the shoot dry weight and the length of shoot decreased under increasing salinity concentration, but the effects of stress was much more pronounced in severe salinity soil (Fig. 1, $P < 0.05$). The changes of ions in two years were similar (Table 2, $P < 0.05$). With a serious degree of salinity, the K^+ contents consistently decreased; in contrast, Na^+ contents increased significantly (Table 2, $P < 0.05$). The Ca^{2+}, Mg^{2+}, and Mn^{2+} contents decreased under salinity stress, but Fe^{2+}, Cu^{2+}, and Zn^{2+} contents showed no significant change (Table 2, $P < 0.05$). The Cl^- and SO_4^{2-} contents increased with salinity stress, especially for Cl^-; however, NO_3^- and $H_2PO_4^-$ contents showed no significant change among sites, as compared with LS (Table 2, $P < 0.05$).

3.3. Metabolic changes in P. communis response to different level of salinity stress

Metabolomic analyses identified 98 metabolites in the leaves of *P. communis* growing in different retrogressive degrees on the Songnen grassland (File 2). All samples were distributed within the 95% confidence interval of Hotelling's T2 ellipse. The score plots of PCA results showed approximately 80.5% and 90.4% variability in the three groups of samples in 2015 and 2016, respectively (Fig. 2A_1 and A_2). In 2015, the contribution of metabolites in leaves for PC1 and PC2 were dominated by sugars/polyols (e.g., mannose, fructose, tagatose, erythrose, and ETA) and amino acids (e.g., proline, γ-aminobutyric acid or GABA, and alanine). Sugars/polyols, amino acids, and organic acids (e.g., succinic, gallic, ascorbic, and vanillylmandelic acids) were major contributors to PC1 and PC2 in 2016 (Fig. 2B_1 and B_2). The PCA analysis showed clear separations between samples for the different salinity soils (Fig. 2).

3.4. Metabolic profiles in P.communis response to different level of salinity stress

The PCA results showed the response of metabolites in *P. communis* leaves to the different degrees of salinity stress (Fig. 2). A total of 31 and 54 metabolites significantly changed in MS and SS in 2015, respectively, and correspondingly 29 and 50 metabolites showed significant change in 2016 (File 2, $P < 0.05$). The progressive metabolite alterations caused by salinity stress showed the duration dependence of *P. communis*, which grew in MS and SS. In MS there was no significant effect on the TCA cycle; however, this was remarkably inhibited in SS, as shown by low oxaloacetic acid, citric acid, and GABA contents in both 2015 and 2016 (Fig. 3, $P < 0.05$). Furthermore, as compared with LS, in the glycolysis pathway, several metabolites, including glucose, fructose-6-P, glucose-6-P, PEP, and pyruvate, were enhanced in *P. communis* leaves, but increased considerably for SS compared with MS (Fig. 3, $P < 0.05$). These results indicated that sugar synthesis in the glycolysis pathway was enhanced with SS and increased accumulation of several kinds of sugars and polyols in leaves, including sucrose, glucose, mannose, maltose, xylitol, and sorbitol (File 2, $P < 0.05$). Proline, a compatible solute well-known to play an important role in osmotic adjustment, increased by 4.642- and 4.271-fold in *P. communis* in 2015 and 2016 for SS, respectively (File 2 and Fig. 3, $P < 0.05$). Amino acid synthesis was inhibited under salinity stress, especially in SS. Consequently, most amino acid contents decreased significantly, including glutamic acid, alanine, phenylalanine, and tyrosine (File 2 and Fig. 3, $P < 0.05$). With increased salinity stress, the levels of shikimic, quinic, and chlorogenic acids, which act in the shikimic pathway, were enhanced substantially, as compared with *P. communis* under LS (File 2 and Fig. 3, $P < 0.05$). In addition, urea contents significantly increased under salinity stress, whereas SSA and putrescine significantly decreased, as compared with *P. communis* under LS (Fig. 3,

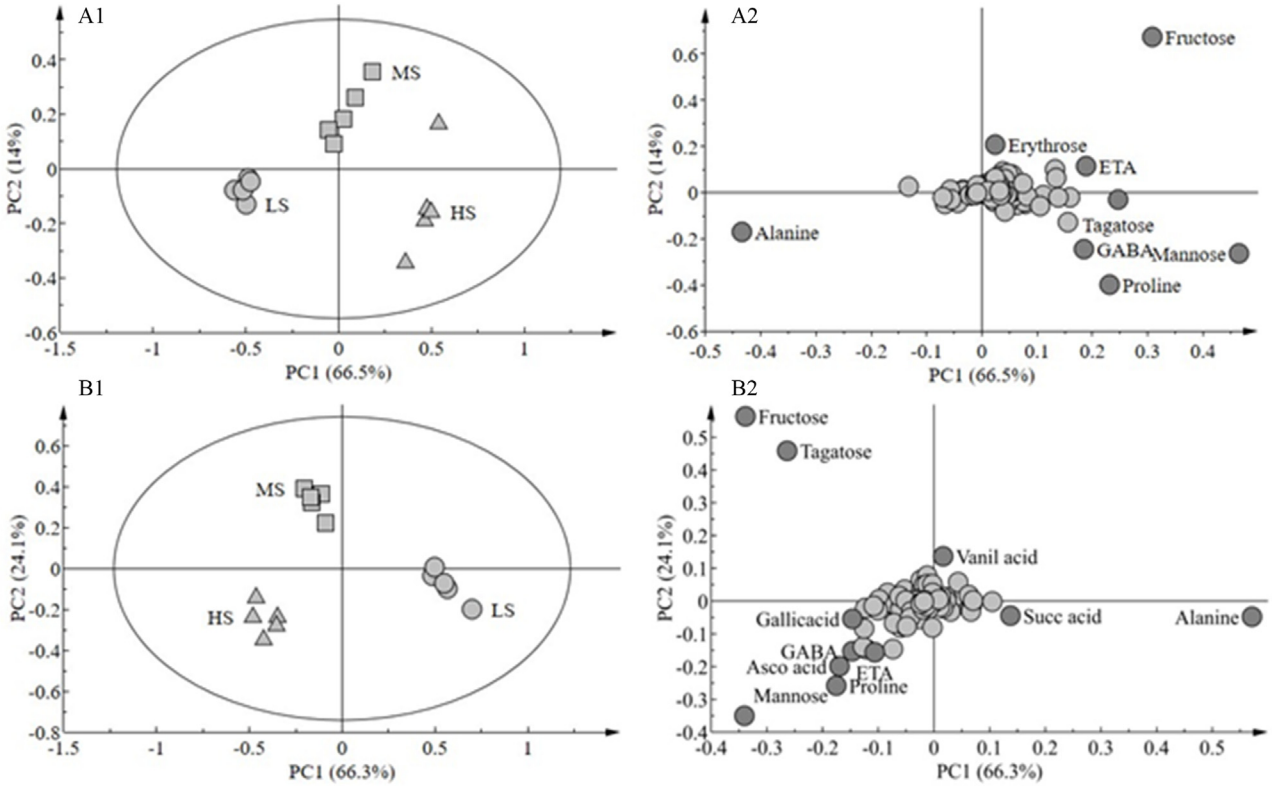

Fig. 2. Score and Loading Scatter Plots of metabolic profiles in leaves of *P. communis* along different soil salinity concentration on the Songnen grassland by Principal component analysis (PCA) method (five biological replicates). (A1) Score scatter plots in 2015; (A2) Score scatter plots in 2016; (B1) Loading Scatter Plots PCA in 2015; (B2) Loading Scatter Plots PCA in 2016. ETA: Ethanolamine; GABA:γ-aminobutyric acid; PC1, the first principal component; PC2, the second principal component.

$P < 0.05$).

4. Discussion

4.1. Growth characteristics and ion balance in P. communis response to different level of salinity stress

Our results showed that *P. communis* displays different levels of tolerance towards salinity stress. Salinity stress inhibits growth and the reduction was more severe in a high-salinity environment. Ion transport in plants is closely related to salinity tolerance; therefore, studies on ion transport should reveal the mechanisms of salinity tolerance and corresponding regulation (Munns and Tester, 2008; Bui, 2013; Latef and Tran, 2016). Our results revealed uptake competition between K^+ and Na^+ in response to salinity stress. The K^+ content decreased, whereas that of Na^+ increased significantly – effects that are more pronounced in soil salinization (Dodd et al., 2010; Wang et al., 2012a). The results also showed that Na^+ contents of *P. communis* increased sharply in severely salinized soil, indicating the Na^+ accumulation impact on ionic balance, and further impact on the physiology of leaves activity. In addition, our results showed that Ca^{2+} and Mg^{2+} accumulations were inhibited significantly with increased stress intensity, consistent with findings of several previous investigations, implying salinity stress had a negative impact on the mechanisms that regulate Ca^{2+} and Mg^{2+} (Lissner et al., 1999; Lee et al., 2004). This suggested that high Na^+ contents reduce the ability of plants to regulate the uptake of cations.

With critical roles in photosynthesis, turgor regulation, and cytoplasmic enzyme regulation, Cl^- is considered an essential micronutrient (White and Broadley, 2001). Several previous studies reported that large amounts of Cl^- inhibited NO_3^- and $H_2PO_4^-$ absorption or accumulation in plants under salinity stress. However, in the present study, Cl^- had no significant effect on NO_3^- and $H_2PO_4^-$ contents in *P. communis* leaves, implying that if Cl^- content increased sharply, it could balance the massive influx of Na^+ and so maintain ionic balance and pH homeostasis (White and Broadley, 2001; Hartzell and Jordan, 2012; Zribi et al., 2012). Although sulfur is an essential nutrient for plants, excessive S content can adversely affect plant growth via H_2S gas produced by reduction of SO_4^{2-}. The increased SO_4^{2-} in *P. communis* leaves under salinity stress might be related to increased Na^+ assimilation.

4.2. Metabolomics in P.communis response to different level of salinity stress

When grown in saline soil, toxic ions and osmotic stress-induced generation of ROS cause protein degradation and intracellular hyperammonia, which further affects the whole metabolic network of the plant (Parida and Das, 2005; Zushi and Matsuzoe, 2015; Mohanty et al., 2016). GC-MS results showed that *P. communis* metabolome was dominated by 98 metabolites, including organic acids, sugars and polyols, and amino acids, which participate in the TCA cycle, glycolysis, membrane phospholipid metabolism pathway, shikimic pathway, GABA shunt, urea cycle, methionine cycle, and GS/GOGAT cycle (File 2 and Fig. 3, $P < 0.05$). This result revealed more details on adverse metabolomic changes caused by salinity stress compared with reports for plant cells and seedlings under saline conditions. Such results could also reveal the metabolic adaptation mechanisms of plants to salinity stress.

An evident difference in the metabolic pathway existed for LS compared with MS and SS. The TCA cycle was inhibited, but glycolysis was enhanced in *P. communis* leaves under salinity stress, suggesting that high levels of sugars were important for leaves to tolerate salinity stress and that energy metabolism was inhibited (Sancheza et al., 2008; Wu et al., 2013a). The common sugars in plant cells are obtained from

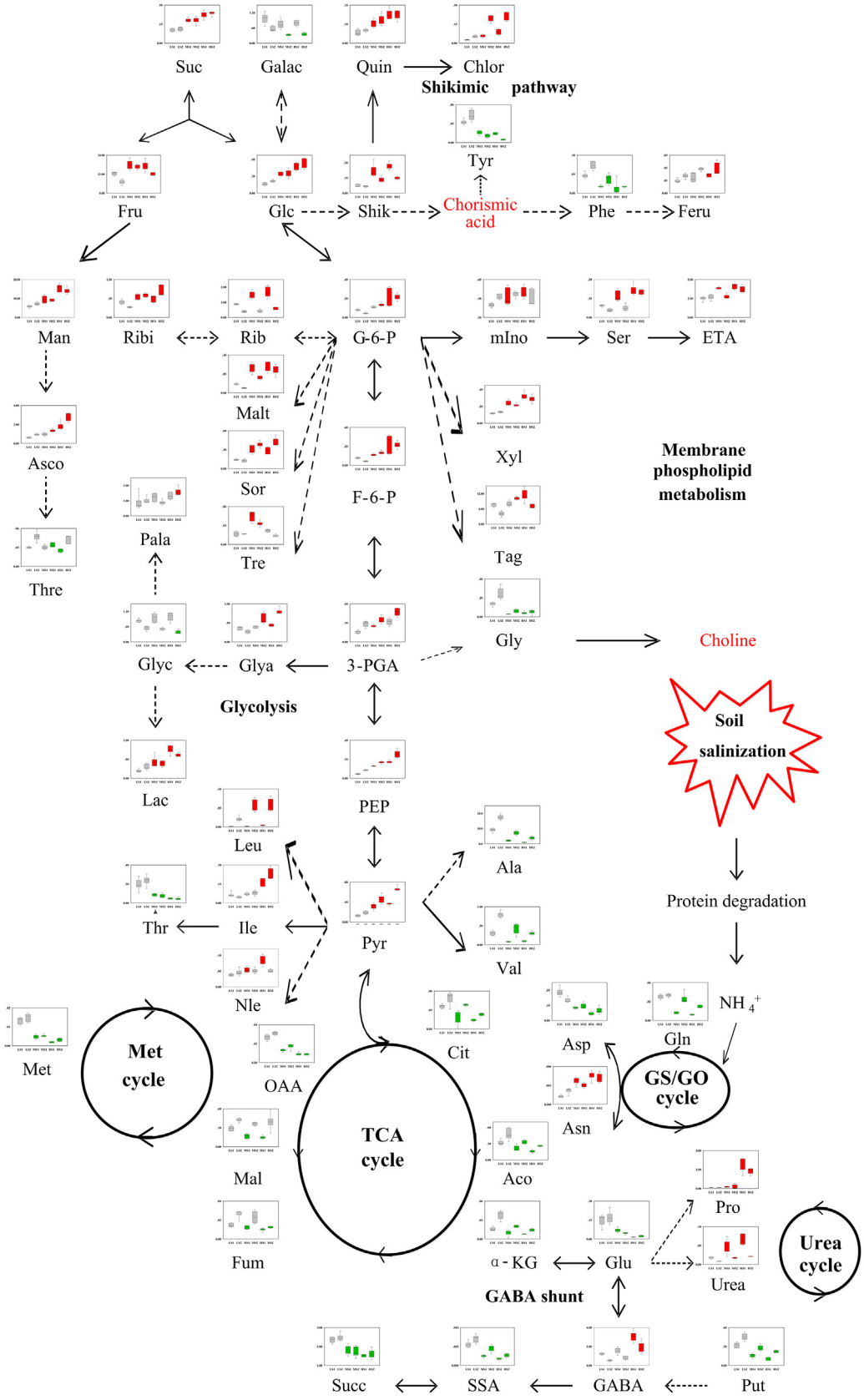

Fig. 3. Changes in the metabolism pathways in the leaves of *P. communis* under different soil salinity concentration on the Songnen grassland in 2015 and 2016. As compared with salinity soil (LS), red boxes denote significantly enhanced metabolites, whereas those in green boxes were significantly reduced ($p < 0.05$). Single dominant *P. communis* communities were found in low salinity soil (LS), *P. communis* co-existed with *Leymus chinensis* communities in moderate salinity soil (MS), *P. communis* coexisted with *Suaeda salsa* communities in severe salinity soil (SS). Number 1 behind LS, MS and SS indicate experiment in 2015, number 2 indicate experiment in 2016. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

polysaccharide degradation, gluconeogenesis, and photosynthesis (Zhang et al., 2011). Our results showed that gluconeogenesis and the degradation of polysaccharides as a carbon source might be enhanced to maintain the osmotic balance in *P. communis* under salinity stress.

Proline has important functions in cell membranes and proteins and, as a ROS scavenger, protects plants under drought and saline stress (Delauney and Verma, 1993; Hare and Cress, 1997). In the present study, proline was the most significantly changed metabolite with

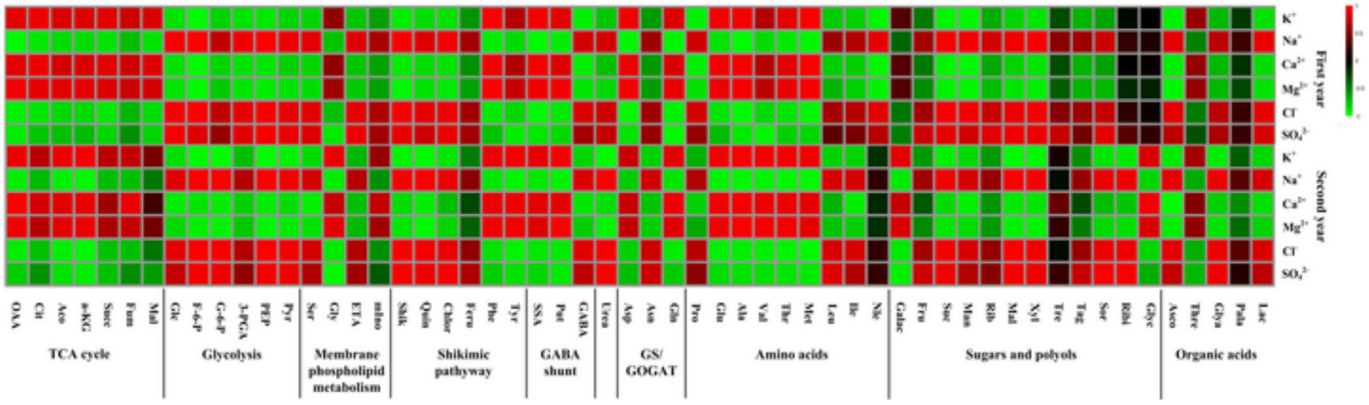

Fig. 4. The correlations analysis between the main nutrition elements and the main metabolites associated with metabolites pathway in *P. communis* leaves along different soil salinity concentration on the Songnen grassland in 2015 and 2016. The colors indicates a positive correlation (red) or negative correlation (green) according to the color-legend beside the figure. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

salinity stress, especially in SS. A similar dramatic increase in proline levels in *P. communis* leaves has been previously reported (Gorai et al., 2007; Pagter et al., 2009). The significantly reduced glutamate levels indicated that proline and urea syntheses were enhanced, especially under high salinity stress. Additionally, declines in aspartate and glutamine contents and increases in asparagine implied that the GS/GOGAT cycle of transamination-related metabolites was converted into proline biosynthesis.

Of the metabolites of the GABA shunt pathway, SSA and Put were decreased, and GABA increased. This result indicated that these metabolites may act in protecting plants from salinity stress (Bouche and Fromm, 2004; Fait et al., 2008). Shikimic acid is the precursor to aromatic amino acids, and the shikimic pathway belongs to the basic cellulose synthesis metabolism of higher plants. In the present study, shikimic acid was enhanced in leaves, indicating that it might be part of a mechanism for *P. communis* to tolerate salinity stress (Cardoso et al., 2014). Organic acid synthesis (including ascorbic, glyceric, and lactic acids) was significantly enhanced in leaves (Fig. 3), which favored effective ROS-scavenging metabolites and so increases salinity tolerance (Chen et al., 2007; Wu et al., 2013a). The increased urea contents could help reduce the negative effects of salt stress on *P. communis* physiology, which could produce NH_3 and CO_2 to enhance N metabolism and photosynthesis processes.

The NH_4^+ absorbed by roots or produced by NO_3^- reduction is assimilated to glutamine by glutamine synthetase or incorporated into glutamate dehydrogenase (Galvan and Fernandez, 2001). Crawford and Glass (1998) and Wang et al. (2012b) demonstrated that the NRT and AMT protein families control the uptake of NO_3^- and NH_4^+ through the transmembrane proton gradient, respectively. Our results revealed that salinity stress caused a large Na^+ influx and left NO_3^- contents unchanged. Therefore, *P. communis* could maintain the NRT protein activity and the H^+/NO_3^- symport mechanism process to keep the intracellular ionic balance and reduce ion injury in leaves. The N and total amino acid contents of leaves decreased with increased salinity stress and thus accelerated remobilization. Salinity stress enhanced the glycine conversion into serine ratio showed improvement, and branched chain amino acid contents increased consistently (Novitskaya et al., 2002; Florian et al., 2013). This phenomenon was probably due to the increase in glycine decarboxylase and threonine deaminase complex activities that are related to increased salinity stress. This result suggested that serine and branched chain amino acid accumulation may be a passive adaptive response to the shortage of negative charge (Robards and Lucas, 1988).

4.3. The co-relationship between ions and metabolites in P. communis

The ions balance will affect plant metabolism, with a subsequent impact on metabolic synthesis and pathway (Amtmann and Armengaud, 2009; Wu et al., 2013b). In our study, the results of the metabolic profiles indicated that with increasing salinity, sugars increased including sucrose, glucose, mannose and maltose in leaves, but some amino acids were reduced when plants were exposed to high salinity. Therefore, the metabolites which are involved in glycolysis were increased, however, metabolites associated with the TCA cycle and amino acid syntheses were reduced. Fig. 4 showed that there were strong correlations between ions and metabolites in *P. communis* leaves. We found that Na^+, Cl^-, and SO_4^{2-} contents also showed remarkable positive correlations with glycolysis and proline syntheses but significant negative correlations with the *TCA* cycle and some amino acid syntheses (Fig. 4, $P < 0.05$). Those ions as poisonous ions cause ion imbalance and oxidative stress in plants, especially Na^+, it is associated with Na transportation through ion transporters (Munns and Tester, 2008). These results indicated that sugars play pivotal roles in osmoregulation and chelating abilities to bind Na^+, Cl^-, and SO_4^{2-} to starch granules (Boriboonkaset et al., 2012; Kumar and Khare, 2016). Moreover, we also found that K^+ accumulation to maintain a lower Na/K ratio in tissue, and there was a significant positive correlation between K^+ and TCA cycle, while significant negative correlations with glycolysis (Fig. 4, $P < 0.05$). In addition, Ca^{2+} as a signal in salinity stress and Mg^{2+} participates in many metabolic activities, we found that both Ca^{2+} and Mg^{2+} share the same changing trend with K^+ (Fig. 4, $P < 0.05$). In conclusion, it may be assumed that ions play important roles in metabolic synthesis and physiological processes, and *P. communis* develops its adaptation to salinity conditions through ionic balance and the interaction of ions with metabolic profiles in leaves.

5. Conclusion

Better understanding of effects of salinity stress on ions balance and metabolites in *P. communis* is essential to reveal the responses of mechanisms to natural soil salinization. When grown in salinity soil, Na^+ greatly accumulated in leaves with no significant change in NO_3^-, demonstrating that salinity stress have broken ions balance, and inhibited the synthesis of amino acids. However, salinity stress also induced metabolic changes in glycolysis and led to accumulation of sugars; probably as a reaction to attenuate stress. In addition, soil salinization also caused alterations in widespread metabolic networks including the membrane phospholipid metabolism pathway, shikimic pathway, GABA shunt, urea cycle, methionine cycle, and proline biosynthesis. In addition, rearrangement of ions and metabolites in *P.*

communis leaves under salinity stress and restricted accumulation of Na or extension of Cl^- could be attributed to salt tolerance. To tolerate soil salinization, *P. communis* has a special mechanism of self-regulating physiology and metabolism; however, this consumes a lot of energy, limiting normal plant growth. This is also important in affecting establishment of *P. communis* populations. These findings provided new insights into *P. communis* metabolic adaptation to soil salinization.

Competing financial interests

The authors declare no competing financial interests.

Contributions

RG designed the study. DJ, JZ, XZ, and FG performed the experiments. DJ, JZ, XZ, FG and HL analyzed the data. RG and DJ wrote the manuscript. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.04.027.

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