Homeostatic responses and growth of *Leymus chinensis* under incrementally increasing saline-alkali stress

Shujie Li Equal first author, 1, 2 , **Yujin Huang** Equal first author, 1, 3 , **Yuefen Li** Corresp. 1, 2

1 College of Earth Sciences, Jilin University, Changchun 130061, China

2 Key laboratory of Mineral Resources Evaluation in Northeast Asia, Ministry of Land and Resources, Changchun 130061, China

3 Institute of Geographic Sciences and Natural Resources Research, CAS, Beijing 100101, China

Corresponding Author: Yuefen Li
Email address: yfli@jlu.edu.cn

Despite considerable tolerance to salt and alkali stress, *Leymus chinensis* populations on the southwestern Songnen Plain in northern China are threatened by increasing soil salinity and alkalinity. To explore the species' responses to saline-alkali stress, we grew it in soils with varying concentrations of nitrogen (N) and phosphorus (P) while applying varying levels of saline-alkali stress (increasing in 14-, 17- or 23-day intervals). We measured the plants' contents of N and P, and the N:P ratio, and calculated their homeostasis indices (H_N, H_P and $H_{N:P}$) under each nutrient and saline-alkali stress treatment. We also calculated the specific growth rate of aboveground parts of *L. chinensis* under each treatment, and investigated its relationship with the N:P ratio. The N content was found to be more sensitive to saline-alkali stress than the P content. The N and P contents were highest and the N:P ratio was stable at pH 8.4. At both pH 8.1 and 8.4, $H_{N:P}>H_N>H_P$, but the indices and their relations differed at other pH values. In conclusion, when *L. chinensis* plants lack N, applying N fertilizer will be extremely efficient, but excessive N concentrations in the environment will inhibit their growth. Conversely, applying excess P-fertilizer will not inhibit growth. The optimal concentrations of N and P appeared to be 16 and 1.2 mmol/L, respectively. When the *L. chinensis* plants were N- and P-limited, the specific growth rate correlated positively with N:P, when limited by N it correlated positively with the environmental N concentration, and when limited by P it was weakly positively correlated with the environmental P concentration. Exposure to saline-alkali stress for the shortest (14 d) incremental interval had weaker effects on the plants.

INTRODUCTION

Ecological stoichiometry (ES) is the study of the balances of elements and energy in ecosystems, which have profound effects on living organisms, their interactions, and associated ecological processes (Cambardella and Elliott, 1993; Elser *et al.*, 2000; Güsewell, 2004; Bradshaw *et al.*, 2012; Wang *et al.*, 2018). ES theory focuses primarily on elements required by all living organisms, so it can be readily generalized across taxa and systems (Sanders and Taylor, 2018). A key concept is homeostasis: a system's capacity to maintain constant conditions internally when external conditions vary, a fundamental property of organisms (Kooijman, 1995; Cooper, 2008; Halvorson *et al.*, 2019). Characterizing both the degrees and consequences of homeostasis is important for understanding responses to environmental changes (Meunier *et al.*, 2014; Halvorson *et al.*, 2019). In ES-based analyses, the homeostasis concept is used to infer organisms' responses to changes in ratios of elemental resources, and predict their feedback effects on resources' availability through changes in the acquisition, storage, and release of nutrients, particularly limiting nutrients such as nitrogen (N) and phosphorus (P) (Halvorson *et al.*, 2019). A general assumption is that stoichiometric homeostasis is stronger in heterotrophs than autotrophs (Sterner and Elser, 2002), so environmental stoichiometry is believed to be reflected more closely by plant and algal stoichiometry than by animals' stoichiometry. However, (Yu *et al.*, 2011) found that homeostatic regulation of N and P varies widely in vascular plants, so the patterns and processes involved are complex and far from fully understood.

Another core concept of ES is the growth rate hypothesis (GRH), which postulates that organisms' growth rates correlate positively with their phosphorus (P) contents because P is essential for the ribosomal RNA production needed to sustain growth (Moody *et al.*, 2017) and for many other processes including photosynthesis, respiration, enzyme production, and

24   generation of ATP (Li *et al.*, 2019). Thus, organisms with high growth rates have high nutrient

25   demands and (*inter alia*) low tissue C:nutrient ratios, low N:P ratios, and potentially competitive

26   advantages in high-P environments, but disadvantages in low-P environments. Hence, plants'

27   abilities to compete for nutrients depend on both their tissue nutrient contents and life history

28   traits (Mulder and Elser, 2009; González *et al.*, 2010; Sanders and Taylor, 2018).

29   ES has been widely applied in various areas of plant science, including in studies on the

30   growth of individual plants, population dynamics, limiting elements, succession, and stability of

31   vegetative communities (Güsewell, 2004). Soil is a key component of terrestrial ecosystems

32   because it supports numerous ecological processes (Normand *et al.*, 2017; Wang *et al.*, 2018),

33   plays crucial roles in plant growth, and directly affects plant communities' composition, stability,

34   and succession (Wardle *et al.*, 2004). N and P are key elements with profound effects on plants

35   growth because they play major roles in microbial dynamics, litter decomposition, food webs,

36   and the accumulation and cycling of nutrients in soil (Elser et al., 2003; Griffiths et al., 2012).

37   Moreover, dynamic changes in concentrations and distributions of soil nutrients are highly

38   interactive and have tightly integrated effects on ecological processes (Wang *et al.*, 2018).

39   Changes in plants' N:P ratios (*inter alia*) may thus reflect adaptations to environmental

40   conditions (Tang *et al.*, 2018), including (of particular interest here) salinity and alkalinity.

41   According to incomplete UNESCO and FAO statistics, the area of saline-alkali land is

42   growing globally (Kerr, 2017) and amounts to 9.5438×10^8 hm^2, including 0.9913×10^8 hm^2 in

43   China (Li *et al.*, 2017a). This is a severe problem because salinization impairs seed germination,

44   root extension, and plant development, resulting in land degradation, reductions in cultivated

45   land quality, and limitation of agricultural development (Wu *et al.*, 2016). Most saline-alkali soil

46   in northeast China is on the Songnen Plain, and its area is increasing through grassland

47   salinization (Li, 2000). The dominant species in saline-alkali grassland on the Songnen Plain is

48   *Leymus chinensis (L. chinensis)*, which can provide high yields of high quality gramineous

49   forage if the salinity and alkalinity are not too severe. It can play an important role in restoring

50   saline-alkali land (Liu *et al.*, 2014), but there is a need to reduce the salinity and alkalinity of

51   affected grassland and improve the species' yield and stress tolerance. It is therefore important to

52   study the ecological stoichiometric homeostasis of N and P, associated ecological processes, and

53   the effects of saline-alkali stress on the growth rate and survival strategy of *L. chinensis*.

54   Previous research on saline-alkali tolerance in *L. chinensis* has mainly focused on

55   physiological processes (such as the osmoregulatory roles of ions, organic acids, soluble sugars

56   and other substances) and morphological traits (such as tiller buds, root distribution, and

57   rhizomes' internode length and branching angles) (Zheng *et al.*, 2017). C, N, and P dynamics in

58   soil-*L. chinensis* systems have also been addressed (Li *et al.*, 2016; Li *et al.*, 2017b; Li *et al.*,

59   2018). However, soil salinization is a long-term dynamic process, and there is relatively little

60   information on relations between the effects of saline-alkaline stress (and of variation in its

61   duration) and ecological stoichiometric characteristics. Here, we demonstrate that the ecological

62   stoichiometric characteristics of *L. chinensis* are influenced by the duration of saline-alkali stress

63   and the available concentrations of both N and P.

64   ## MATERIALS AND METHODS

65   **The experimental site**

66   We performed experiments in a polytunnel on the campus of the College of Earth Sciences, Jilin

67   University, Changchun, Jilin Province, northeast China. This is in the north temperate

68   continental climatic zone, which has four distinct seasons. The mean annual temperature and

69   precipitation are 4.8°C and 567 mm, respectively, and mean winter and summer temperatures are

70   -14 and 24 °C, respectively (Zhang *et al.*, 2017).

71   **Experimental design**

72   **Experimental preparation**

73   Experiments were performed from August 7 to November 6, 2017. To minimize interference

74   from impurities in the soil, the substrate used was fine nutrient-poor sand, sieved with 10 and 75

75   mesh sieves and thoroughly washed with distilled water to minimize its nutrient content. To

76   avoid effects of different soil loadings on growth of experimental plants, the sand was air-dried

77   and equal portions were placed in plastic flowerpots (diameter 30 cm, height 23 cm). The

78   portions were roughly equivalent to two-thirds of the pots' volume. Small holes in the bottom of

79   each flowerpot were sealed with waterproof tape. *L. chinensis* seeds were immersed in water for

80   48 hours and air-dried in the dark to avoid dormancy and increase the germination rate, then

81   about 100 seeds were sown evenly in each flowerpot. About seventy percent of the seeds

82   survived, and all pots were similar with respect to the number, size, and vitality of surviving

83   seedlings.

84   **N and P addition**

85   After the seeds germinated, a 200 ml portion of Hoagland's nutrient solution (obtained from

86   South China Agricultural University) with adjusted N and P concentrations was added to each

87   pot once every two days. The elemental composition of Hoagland's solution is described in

88   detail elsewhere (Li et al., 2018); briefly, the version used here contained $Ca(NO_3)_2$ (945 mg/L),

89   KNO_3 (607 mg/L), $NH_4H_2PO_4$ (115 mg/L), $MgSO_4.7H_2O$ (493 mg/L) and various microelements.

90   In five sets of nine pots, the N concentration was adjusted using ammonium nitrate to establish

91   five N concentration treatments (2, 4, 8, 16, and 24 mmol/L) in which the concentration of P was

92   fixed at 1 mmol/L. In another five sets of nine pots, the P concentration was adjusted using

93   potassium dihydrogen phosphate to establish five P concentration treatments (0.3, 0.6, 1.2, 2.4,

94   and 4.8 mmol/L) in which the concentration of N was fixed at 14 mmol/L. The experiment thus

95   involved 10 nutrient treatments and a total of 90 pots (10 treatments×3 replicates×3 salinity

96   stress intervals). The N and P concentration gradients were set on the basis of previously

97   reported results (Yu et al., 2011).

98   **Saline-alkali stress**

99   On 7 Aug 2017, after the *L. chinensis* plants had grown to adulthood, plants in triplicate pots

100   were subjected to the saline-alkali stress treatments shown in Table 1, by adding $NaHCO_3$ to the

101   previously described treatment solution to simulate various degrees of land salinization

102   encountered in the western regions of Jilin Province. To avoid excessively stressing the plants,

103   the pH was increased at intervals of 14, 17, or 23 days. For the 14-day interval treatment, the pH

104   was raised from 7.5 to 8.1, and then to 8.4, 8.7, and finally 9.3. For the 17-day interval treatment,

105   the pH was raised from 7.5 to 8.1 and then to 8.4 and finally 8.7. For the 23-day interval

106   treatment, the pH was raised from 7.5 to 8.1 and finally to 8.4. Aboveground parts of *L.*

107   *chinensis* plants subjected to these treatments were harvested according to the schedule shown in

108   the table.

109   **Sample collection and testing methods**

110   Aboveground parts of *L. chinensis* plants were cut according to the harvesting schedule shown in

111   Table 1. The samples were rinsed with distilled water, dried with absorbent paper, deactivated

112   for 15 min in a dry air oven (105 °C) and dried for 12 h at 65 °C to eliminate water completely.

113   After cooling the samples to room temperature, they were weighed, crushed, screened with a 100

114   mesh sieve, and finally quartered. The resulting sub-samples were then packed in sealed bags for

115   analysis.

116   Total N and total P in the *L. chinensis* samples were respectively determined by Chinese

117   standard methods LY/T 1269-1999 (using a SAN++ Continuous Flow Analyzer; Skalar,

118   Netherlands) and LY/T 1270-1999 (using an ICPS-7500 inductively coupled plasma atomic

119   emission spectrometer; Shimadzu, Kyoto, Japan).

120   **Data analysis**

121   The acquired data were statistically analyzed using Excel 2010 and SPSS 24 (SPSS Inc., USA).

122   Homeostasis indices were calculated using the model $y = cx^{\frac{1}{H}}$. Here, x is the measured content of

123   an element in the soil, y is the measured content of the same element in *L. chinensis*, c is a

124   constant coefficient, and H is the homeostasis index. The results were visualized using Sigmaplot

125   12.5 (Systat Software, Inc.). We calculated growth rates (day^{-1}) based on recorded changes in dry

126   mass and instantaneous growth rates using the equation $u = \ln(M_t / M_0)/t$, where u is the growth

127   rate (day^{-1}), M_t and M_0, are the final and initial dry mass, respectively, and t is experiment

128   duration in days. Details of several statistical analyses are included in Appendix 1 and Appendix

129   2.

130   ## RESULTS

131   **Effects of incremental increases in saline-alkali stress on N, P contents and N:P ratios**

132   The 14-day stress increment yielded the highest N and P contents in the plants when the N

133   concentration in the substrate was low (2-8 mmol/L), but not when the N concentration in the

134   substrate was higher (16 or 24 mmol/L). The variation in the N and P contents, and the N:P ratio,

135   was similar under all three stress intervals: the N content and N:P ratio were highest with 17-day

136   intervals, while the P content was highest with 14-day intervals (Fig. 1).

137   **Homeostasis characteristics of *L. chinensis***

138   The homeostasis index of N (H_N) in *L. chinensis* ranged from 2.35 to 7.25, and first rose then fell

139   as salinity-alkalinity increased, independently of interval length (Fig. 2). Under the 14-, 17- and

140   23-day intervals, H_N ranges were 3.10-7.25, 2.35-5.36 and 3.19-3.87, respectively. It was

141   consistently highest at pH 8.4, and higher at pH 8.1 and 8.7 than at pH 7.5 and 9.3 (Table 2).

142   Under the 14-day stress intervals, the changes in H_N were moderate, and H_N was high at all pH

143   values. The index was lower at both pH 8.1 and 8.7 under the 17- and 23-day intervals.

144   Under our treatments, H_P of *L. chinensis* ranged from 2.60 to 5.33. Under all three saline-

145   alkali stress intervals, it first rose and then declined (Fig. 2). Under the 14-, 17-, and 23-day

146   saline-alkali stress intervals, the ranges of H_P were 2.84-5.33, 2.65-4.90 and 2.60-4.10,

147   respectively, and the highest values of H_P occurred at pH 8.1, 8.7, and 8.1, respectively (Table 2).

148   At pH 8.1, H_P was significantly higher under 14-day intervals than under longer intervals. At pH

149   8.4, it was highest under 17-day intervals, and at pH 8.7 it declined in the order 17-day > 14-day >

150   23-day intervals.

151   Plants have strong self-regulating mechanisms, and generally keep their N:P ratios within a

152   narrow range by adjusting their N and/or P contents in response to environmental changes. For

153   example, the $H_{N:P}$ of *L. chinensis* varied from 3.06 to 9.23 in plants exposed to different P

154   concentrations, while its ranges under the 14-, 17- and 23-day stress interval regimes were 3.15-

155   9.23, 3.11-6.83 and 3.06-6.44, respectively, and its peak values occurred at pH 8.4, 8.4, and 8.1,

156   respectively (Table 2). *L. chinensis* plants are thus able to effectively regulate their *N:P* ratio in

157   the pH range 8.1 to 8.7, but less so at pH 7.5 and 9.3.

158   **The specific growth rate of *L. chinensis***

159   The specific growth rate of the plants rapidly increased then decreased as the N concentration in

160   the substrate increased, peaking at 16 mmol/L (Fig. 3). This indicates that when *L. chinensis*

161   plants lack N, initial N addition will be extremely efficient, but saturating or excessive amounts

162   will not promote (and may even hinder) further growth. Conversely, the plants' specific growth

163   rate first increased, then decreased, and then increased again with increases in environmental P

164   concentration. Specifically, it was lowest at a P concentration of 2.4 mmol/L, and higher at both

165   1.2 and 4.8 mmol/L. Thus, high addition of P is beneficial for plant growth but may lead to

166   wastage of resources. Therefore, the optimum P concentration under our experimental conditions

167   was 1.2 mmol/L.

168   In summary, the threshold concentrations of N and P in the substrate solution at which the

169   biomass of *L. chinensis* began to decrease (or stopped increasing) were 16 and 1.2 mmol/L,

170   respectively. Moreover, plants exposed to 1.2-4.8 mmol/L P were mainly restricted by N, while

171   those exposed to 16 and 24 mmol/L N were mainly restricted by P, and those exposed to lower

172   concentrations of P and N were restricted by P and N, respectively (Fig. 4).

173   **DISCUSSION**

174   **N and P contents and the N:P ratio in *L. chinensis***

175   N and P are key nutrients that play major roles in myriad processes in plants and have important

176   effects on one-another's uptake and metabolism (Han *et al.*, 2009). N:P ratios in plants fluctuate

177   within relatively narrow ranges, and variations in their N and P contents are restricted by

178   homeostatic mechanisms. Although P and N contents can influence each other, N affects P

179   contents more strongly than vice versa, putatively due to the overall higher abundance of N in

180   plants (Yu, 2005). (Tao *et al.*, 2017) found that increasing deposition of N in soils could facilitate

181   P absorption and accumulation in *Suaeda heteroptera*, indicating that N supply limited the plants'

182   growth. Li *et al.* (2012) detected a significant positive correlation between N and P contents in *S.*

183   *heteroptera* plants in a coastal saline environment and observed relatively consistent variations in

184   their N and P demands. We found that *L. chinensis* plants absorbed limited amounts of P from

185   soils with a low N content, and changes in the soil's N contents between 2 and 8 mmol/L had

186   minor effects on the P content of their aboveground parts. However, their P contents were

187   substantially increased by higher N concentrations in the soil. Similarly, we found that N

188   contents of aboveground parts of *L. chinensis* are influenced by the environmental P

189   concentration: they changed greatly when the soil's P content was low but remained very similar

190   at high P concentrations. In summary, P content is the main constraint on plant growth when the

191   soil's P content is low, but N content becomes the main constraint of plant growth when the P

192   content is high.

193   Our experiment examined saline-alkaline environments with different pH values. The N and

194   P contents in aboveground parts of *L. chinensis* were both high at pH 8.4, indicating that the

195   plants maintained strong control over their N:P ratios at this pH and confirming the previously

196   reported finding that this pH promotes growth of *L. chinensis* (Li *et al.*, 2018). Yan et al. (2006)

197   found that *L. chinensis* can resist mild pH stress but it is inhibited by strong saline-alkali stress,

198   which (*inter alia*) reduces its tillering rate, average number of roots, relative water content,

199   relative growth rate, and contents of both chlorophyll and N (Yan *et al.*, 2006). At high pH (8.7

200   and 9.3) we found that its P content was relatively high when there were high soil concentrations

201   of either N (16 or 24 mmol/L) or P (2.4 or 4.8 mmol/L). However, plants need more P to cope

202   with pH stress. Cheng et al. (2017) found that plants' nutrient adsorption is related to both their

203   taxa and pH, together with indications that luxury uptake of N and P under moderate pH stress

204   enables some plants (e.g. *Rhizoma acori graminei* and *Lythrum salicaria*) to resist strong pH

205   stress (Cheng *et al.*, 2017). The N and P contents in aboveground parts of *L. chinensis* we

206   observed at various pH values indicate that N contents in plants are more sensitive to saline

207   conditions than their P contents.

208 **Homeostasis characteristics of N, P, and N:P in *L. chinensis***

209 Homeostasis theory postulates that plants can adjust their growth rate, and rates of both resource

210 allocation and utilization in mechanisms that maintain their internal homeostasis (Mendez and

211 Karlsson, 2005). Homeostasis is stronger in animals than in plants, and in higher plants than

212 lower plants (Zeng *et al.*, 2016), suggesting that homeostatic mechanisms have become more

213 powerful over the course of evolutionary history (Nie *et al.*, 2016). We found that the

214 homeostasis indices for N content, P content and the N:P ratio in *L. chinensis* were within

215 reported ranges for algae, fungi (Sterner and Elser, 2002), and animals (Yu, 2005; Karimi and

216 Folt, 2006). On the basis of experiments on Inner Mongolian grassland and sand culture, Yu *et al.*

217 (2009) concluded that aboveground parts of *L. chinensis* have slightly higher H_N, H_P and $H_{N:P}$

218 values (5.88-8.80, 3.37-6.67 and 4.49-9.46, respectively) than those observed in our study,

219 possibly due to differences in climatic conditions.

220   We also found that all three homeostasis indexes first increased then decreased with

221 increases in pH. At pH 8.1 and 8.4, they were all high, showing that *L. chinensis* can maintain

222 stable levels of nutrients in weakly alkaline environments. However, the indices were lower

223 under more strongly alkaline conditions (pH>8.7), indicating that such conditions have toxic

224 effects. This is consistent with previous observations that saline and alkaline stress has both

225 physiological and biochemical effects on roots and leaves of *L. chinensis* (Liu *et al.*, 2014).

226   Another finding is that $H_{N:P}>H_N>H_P$, indicating that the stronger homeostasis of N content

227 than of P content in plants is mainly due to their significantly higher N content. Accordingly,

228 homeostasis in zooplankton is reportedly highest for macronutrients, followed by micronutrients,

229 and lowest for non-essential elements (Karimi and Folt, 2006). Since high N contents in plant

230 tissues promote high P contents and plants' N:P ratios vary less than their N and P contents, the

231   N:P ratio may be subject to stronger homeostasis than either N or P individually (Sterner and

232   Elser, 2002). Plants maintain N:P stability by their adjusting resource allocation and utilization

233   of resources. The degree of stability may reflect their environmental adaptability, so the N:P ratio

234   appears to be more important than N and P contents in this respect.

235   We also found that the plants' homeostasis is related to the duration of the incremental

236   increases in salinity-alkalinity stress. With 14-day intervals (rapid changes in alkalinity), the

237   positive effects of a weakly alkaline environment (pH 7.5-8.4) on leaves of *L. chinensis* are

238   stronger than those observed with longer intervals: the homeostasis indices of the aboveground

239   parts are relatively high, and further increases in alkalinity (to pH 8.7 or 9.3) induce relatively

240   little damage to leaves. $H_{N:P}$ and H_P are strongly affected by changes in the duration of the

241   incremental increases. For example, the variation in $H_{N:P}$ of aboveground parts is higher with 23-

242   day intervals than with either 14- or 17-day intervals (rising to a peak and then quickly falling

243   with increases in pH, as shown in Fig. 2).

244   **Specific growth rate**

245   Growth rates are crucial indicators of organisms' adaptation to (and thus ability to survive and

246   reproduce within) an environment. Clearly, variations in growth capacity can be linked to

247   variations in elemental demand (Moody *et al.*, 2017). Our results confirm previous findings (and

248   intuitive expectations) that adding N can significantly enhance growth of N-deficient plants.

249   However, they also show that adding N can inhibit plants' growth if the environmental content of

250   N exceeds the plants' requirements. We found that the optimal N and P contents in the soil under

251   our experimental conditions were 16 and 1.2 mmol/L, respectively. Our results suggest that

252   adding excess P will not have inhibitory effects, and may even further stimulate growth slightly,

253   but would be a waste of resources.

254   Plants can absorb elements in soils selectively. However, the C:N:P ratios in plants vary

255   more widely than in animals and microorganisms. Moreover, theoretical analyses based on

256   experiments with algae and birch seedlings indicate that the N:P ratio has a relatively complex

257   relationship with growth rates: they are positively correlated at low growth rates, but negatively

258   correlated once the growth rate exceeds a certain threshold (Agren, 2004; Agren, 2008) that

259   depends on both the taxon in question and the abundance of other nutrients. Other studies

260   (Cernusak *et al.*, 2010) indicated that the N:P ratios of 13 categories of tropical trees and lianas

261   are positively related to growth rates when the growth rate is low, but negatively related at high

262   growth rates. Additionally, Reef et al. found an interspecific (but not intraspecific) relationship

263   between N:P ratios and growth rates of two species of *Rhizophoraceae* (Reef *et al.*, 2010). Yu

264   found that growth rates of belowground parts of plants were positively correlated with N:P under

265   N constraints, but negatively correlated with N:P under P constraints (Yu, 2009). In our

266   experiment, growth of *L. chinensis* was restricted by both N and P. The growth rate of

267   aboveground parts was positively related to N:P under N constraint, but the positive correlation

268   was weakened under P constraint. This is consistent with the conclusions of Yu *et al.* with

269   respect to the effects of N constraint, but not those of P constraint. We speculate that this was

270   due to the plant's generally low growth rate in the experiment, and also possibly to effects of

271   salinity-alkalinity on the relationships between plant growth and other environmental variables.

272   Although effects of plants' nutrient storage on their growth rates have been discussed (Sterner

273   and Elser, 2002; Agren, 2008; Yu, 2009), the relationship between their growth rates and C:N:P

274   ratios clearly requires further study.

275   According to the growth rate hypothesis, high amounts of ribosomal RNA (rRNA), and thus

276   P, are needed to synthesize the large quantities of proteins required to sustain high growth rates

277   (Sterner and Elser, 2002). Therefore, organisms with high growth rates have relatively high P

278   contents and low N:P ratios. This hypothesis is supported by both theoretical considerations and

279   empirical observations of zooplankton, arthropods, and bacteria (Elser *et al.*, 2003; Watts *et al.*,

280   2006; Hessen *et al.*, 2007). However, the relationships may be more complex in higher plants.

281   Accordingly, we observed a nonlinear relationship between the specific growth rate and N:P

282   ratio of *L. chinensis*, rather than the negative correlation predicted by the hypothesis. This may

283   have been because we monitored adult *L. chinensis* plants rather than juveniles, and/or because

284   the specific growth rate of the studied plants was strongly influenced by the variation of several

285   environmental factors (N content, P content, and pH) and thus does not reflect their intrinsic

286   potential growth rates.

287   ## CONCLUSION

288   The findings presented here demonstrate that the N content of *L. chinensis* is more sensitive to

289   the environmental pH than its P content. At substrate pH values of 8.4 and 8.7, the N:P ratio of *L.*

290   *chinensis* was stable and effectively regulated, indicating that the species possesses good

291   environmental adaptability in this pH range. In particular, at a substrate pH of 8.4, the H_P, H_N,

292   and $H_{N:P}$ indices of *L. chinensis* were all high, indicating that the plants were well able to control

293   their contents of N and P as well as the N:P ratio. Weak alkalinity (pH 7.5-8.4) is beneficial for

294   growth and N accumulation in *L. chinensis*, but more strongly alkaline conditions (pH 8.7 or 9.3)

295   inhibit its growth. Rapid changes in salinity-alkalinity (at intervals of 14 or 17 days) weakened

296   both the positive effects of the weakly alkaline conditions (pH 7.5-8.4) and the negative effects

297   of more strongly alkaline conditions (pH 8.7 or 9.3) on *L. chinensis*. At pH values above 8.7, the

298   interval between stress increments clearly affected the plants' contents of N and P as well as the

299   N:P ratio.

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304 **REFERENCES**

305 Agren, G.I., 2004. The C : N : P stoichiometry of autotrophs - theory and observations. Ecology Letters 7, 185-
306 191.
307 Agren, G.I., 2008. Stoichiometry and nutrition of plant growth in natural communities. Annual Review of
308 Ecology Evolution and Systematics, pp. 153-170.
309 Bradshaw, C., Kautsky, U., Kumblad, L., 2012. Ecological stoichiometry and multi-element transfer in a
310 coastal ecosystem. Ecosystems 15, 591-603.
311 Cambardella, C.A., Elliott, E.T., 1993. Carbon and nitrogen distribution in aggregates from cultivated and
312 native grassland soils. Soil Science Society of America Journal 57, 1071-1076.
313 Cernusak, L.A., Winter, K., Turner, B.L., 2010. Leaf nitrogen to phosphorus ratios of tropical trees:
314 experimental assessment of physiological and environmental controls. New Phytologist 185, 770-779.
315 Cheng, X., Liang, Y., Zhu, H., Chen, X., Yan, B., Zhou, Q., Yu, X., 2017. A hydroponic experiment on
316 response of six plant species to salt stress and their denitrification potentiality. Wetland Science 15, 635-640.
317 Cooper, S.J., 2008. From Claude Bernard to Walter Cannon. Emergence of the concept of homeostasis.
318 Appetite 51, 419-427.
319 Elser, J.J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., Watts, T., Hobbie, S., Fagan, W.,
320 Schade, J., Hood, J., Sterner, R.W., 2003. Growth rate-stoichiometry couplings in diverse biota. Ecology Letters 6,
321 936-943.
322 Elser, J.J., Sterner, R.W., Gorokhova, E., Fagan, W.F., Markow, T.A., Cotner, J.B., Harrison, J.F., Hobbie, S.E.,
323 Odell, G.M., Weider, L.W., 2000. Biological stoichiometry from genes to ecosystems. Ecology Letters 3, 540-550.
324 Güsewell, S., 2004. N : P ratios in terrestrial plants: variation and functional significance. New Phytologist 164,
325 243-266.
326 González, A.L., Kominoski, J.S., Danger, M., Ishida, S., Iwai, N., Rubach, A., 2010. Can ecological
327 stoichiometry help explain patterns of biological invasions? Oikos 119, 779-790.
328 Halvorson, H.M., Fuller, C.L., Entrekin, S.A., Scott, J.T., Evans-White, M.A., 2019. Interspecific homeostatic
329 regulation and growth across aquatic invertebrate detritivores: a test of ecological stoichiometry theory. Oecologia
330 190, 229-242.
331 Han, W., Wu, Y., Tang, L., Chen, Y., Li , L., He, J., Fang, J., 2009. Leaf carbon, nitrogen and phosphorus
332 stoichiometry across plant species in Beijing and its periphery. Acta Scientiarum Naturalium Universitatis
333 Pekinensis 45, 855-860.
334 Hessen, D.O., Jensen, T.C., Kyle, M., Elser, J.J., 2007. RNA responses to N- and P-limitation; reciprocal
335 regulation of stoichiometry and growth rate in *Brachionus*. Functional Ecology 21, 956-962.
336 Karimi, R., Folt, C.L., 2006. Beyond macronutrients: element variability and multielement stoichiometry in

337   freshwater invertebrates. Ecology Letters 9, 1273-1283.

338   Kerr, J.G., 2017. Multiple land use activities drive riverine salinization in a large, semi-arid river basin in

339   western Canada. Limnology and Oceanography 62, 1331-1345.

340   Kooijman, S., 1995. The stoichiometry of animal energetics. Journal of Theoretical Biology 177, 139-149.

341   Li, B., Liu, G., Yang, J., Wang, X., 2017a. Effects of agronomic measures on soil nutrients in light-moderate

342   salinization blocked farmland. Chinese Journal of Soil Science 48, 190-194.

343   Li, J., Hou, X., Ma, W., Li, X., Ding, Y., Wu, Z., Guo, F., Li, Y., Zhao, J., 2019. Effect of interspecies

344   relationships on nitrogen and phosphorus uptake and utilization efficiency of *Leymus chinensis* and *Artemisia*

345   *frigida* under different phosphorus environment. Chinese Journal of Grassland 41, 17-24.

346   Li, X., 2000. The alkili-saline land and agricultural sustainable development of the western Songnen plain in

347   China. Scientia Geographica Sinica 20 51-55.

348   Li, Y., Li, Q., Guo, D., Liang, S., Wang, Y., 2016. Ecological stoichiometry homeostasis of *Leymus chinensis*

349   in degraded grassland in western Jilin Province, NE China. Ecological Engineering 90, 387-391.

350   Li, Y., Li, Q., Liu, H., Li, S., Wang, Y., Liu, Y., 2018. Ecological stoichiometry-based study of the influence of

351   soil saline-alkali stress on nutrient homeostasis in *L. chinensis*. Ecotoxicology and Environmental Safety 165, 243-

352   249.

353   Li, Y., Liang, S., Zhao, Y., Li, W., Wang, Y., 2017b. Machine learning for the prediction of *L. chinensis* carbon,

354   nitrogen and phosphorus contents and understanding of mechanisms underlying grassland degradation. Journal of

355   Environmental Management 192, 116-123.

356   Liu, B., Kang, C., Wang, X., Bao, G., 2014. Physiological and biochemical response characteristics of *Leymus*

357   *chinensis* to saline-alkali stress. Transactions of the Chinese Society of Agricultural Engineering 30, 166-173.

358   Mendez, M., Karlsson, P.S., 2005. Nutrient stoichiometry in *Pinguicula vulgaris*: Nutrient availability, plant

359   size, and reproductive status. Ecology 86, 982-991.

360   Meunier, C.L., Malzahn, A.M., Boersma, M., 2014. A new approach to homeostatic regulation: towards a

361   unified view of physiological and ecological concepts. PloS one 9, e107737.

362   Moody, E.K., Rugenski, A.T., Sabo, J.L., Turner, B.L., Elser, J.J., 2017. Does the Growth Rate Hypothesis

363   apply across temperatures? Variation in the growth rate and body phosphorus of neotropical benthic Grazers. Front.

364   Environ. Sci. 5, 11.

365   Mulder, C., Elser, J.J., 2009. Soil acidity, ecological stoichiometry and allometric scaling in grassland food

366   webs. Global Change Biology 15, 2730-2738.

367   Nie, L., Wu, Q., Yao, B., Fu, S., Hu, Q., 2016. Leaf litter and soil carbon, nitrogen, and phosphorus

368   stoichiometry of dominant plant species in the Poyang Lake wetland. Acta Ecologica Sinica 36, 1898-1906.

369   Normand, A.E., Smith, A.N., Clark, M.W., Long, J.R., Reddy, K.R., 2017. Chemical composition of soil

370   organic matter in a subarctic peatland: Influence of shifting vegetation communities. Soil Science Society of

371   America Journal 81, 41-49.

372   Reef, R., Ball, M.C., Feller, I.C., Lovelock, C.E., 2010. Relationships among RNA : DNA ratio, growth and

373   elemental stoichiometry in mangrove trees. Functional Ecology 24, 1064-1072.

374   Sanders, A.J., Taylor, B.W., 2018. Using ecological stoichiometry to understand and predict infectious diseases.

375   Oikos 127, 1399-1409.

376   Sterner, R.W., Elser, J.J., 2002. Ecological stoichiometry: the biology of elements from molecules to the

377   biosphere. Princeton University Press.

378   Tang, M., Xiao, M., Yuan, H., Wang, G., Liu, S., Zhu, Z., GE, T., Wu, J., 2018. Effect of CO_2 doubling and
379   different plant growth stages on rice carbon, nitrogen, and phosphorus and their stoichiometric ratios. Environmental
380   Science 39, 5708-5716.
381   Tao, W., Wu, J., Liu, C., Fang, L., Liu, Y., Yuan, J., Li, J., 2017. Response of stoichiometric homeostasis and
382   allometric scaling in halophyte *Suaeda heteroptera* Kitag. to simulated nitrogen and phosphorus deposition. Journal
383   of Hydroecology 38, 18-26.
384   Wang, L., Wang, P., Sheng, M., Tian, J., 2018. Ecological stoichiometry and environmental influencing factors
385   of soil nutrients in the karst rocky desertification ecosystem, southwest China. Global Ecology and Conservation 16,
386   e00449.
387   Wardle, D.A., Walker, L.R., Bardgett, R.D., 2004. Ecosystem properties and forest decline in contrasting long-
388   term chronosequences. Science 305, 509-513.
389   Watts, T., Woods, H.A., Hargand, S., Elser, J.J., Markow, T.A., 2006. Biological stoichiometry of growth in
390   *Drosophila melanogaster*. Journal of Insect Physiology 52, 187-193.
391   Wu, C., Huang, C., Liu, G.H., Liu, Q., 2016. Spatial prediction of soil salinity in the Yellow River Delta based
392   on geographically weighted regression. Resources Science 38, 704-713.
393   Yan, H., Zhao, W., Yin, S., Shi, D., Zhou, D., 2006. Different physiological responses of
394   *Aneurolepidiumchinense* to NaCl and Na_2CO_3. Acta Prataculturae Sinica 15, 49-55.
395   Yu, Q., 2005. The study on the homeostasis and growth rate of a higher plant and their mechanism Master's
396   thesis, Gansu Agricultural University, Lanzhou.
397   Yu, Q., 2009. Ecological stoichiometric study on vascular plants in the Inner Mongolia steppe. Doctoral
398   dissertation, Institute of Botany, the Chinese Academy of Sciences.
399   Yu, Q., Elser, J.J., He, N., Wu, H., Chen, Q., Zhang, G., Han, X., 2011. Stoichiometric homeostasis of vascular
400   plants in the Inner Mongolia grassland. Oecologia 166, 1-10.
401   Zeng, Q., Li, X., Dong, Y., An, S., Darboux, F., 2016. Soil and plant components ecological stoichiometry in
402   four steppe communities in the Loess Plateau of China. Catena 147, 481-488.
403   Zhang, D., Wang, W., Zheng, H., Ren, Z., Zhai, C., Tang, Z., Shen, G., He, X., 2017. Effects of urbanization
404   intensity on forest structural-taxonomic attributes, landscape patterns and their associations in Changchun, Northeast
405   China: Implications for urban green infrastructure planning. Ecological Indicators 80, 286-296.
406   Zheng, C., Wang, Y., Sun, H., Wang, X., Gao, Y., 2017. Effects of clipping on nitrogen allocation strategy and
407   compensatory growth of *Leymus chinensis* under saline-alkali conditions. Chinese Journal of Applied Ecology 28,
408   2222-2230.

409

Saline-alkali stress treatments, including durations of exposure to each pH, dates of exposure, and harvest dates.

Table 1. Saline-alkali stress treatments, including durations of exposure to each pH, dates of exposure, and harvest dates.

Notes: There were 10 nutrient treatments and a total of 90 pots were used in the experiment. All treatments were last harvested on 2017/10/13.

N-P level	Duration (days)	pH	Dates of exposure	Harvest date
		7.5	2017/8/07-2017/8/17	No harvest
3×N1-P		8.1	2017/8/18-2017/8/31	2017/8/18
3×N2-P	Incremental interval 14 days	8.4	2017/9/1-2017/9/14	2017/9/1
3×N3-P		8.7	2017/9/15-2017/9/28	2017/9/15
3×N4-P		9.3	2017/9/29-2017/10/12	2017/9/29
3×N5-P		7.5	2017/8/07-2017/8/22	No harvest
3×N-P1	Incremental interval	8.1	2017/8/23-2017/9/8	2017/8/23
3×N-P2	17 days	8.4	2017/9/9-2017/9/25	2017/9/9
3×N-P3		8.7	2017/9/26-2017/10/12	2017/9/26
3×N-P4		7.5	2017/8/07-2017/8/27	No harvest
3×N-P5	Incremental interval	8.1	2017/8/28-2017/9/19	2017/8/28
	23 days	8.4	2017/9/20-2017/10/12	2017/9/20

Homeostasis indices (H) of aboveground parts of *L. chinensis*, and their linear regression correlation coefficients (R^2), under the indicated saline-alkali stress treatments.

Table 2. Homeostasis indices (H) of aboveground parts of *L. chinensis*, and their linear regression correlation coefficients (R^2), under the indicated saline-alkali stress treatments.

Notes: "—" indicates that no valid data were obtained, and "*" indicates an outlier.

pH	Incremental interval 14 days		Incremental interval 17 days		Incremental interval 23 days		Average H_N
	H_N	R²	H_N	R²	H_N	R²	
7.5	3.23	0.93	3.20	0.92	3.81	0.93	3.41
8.1	5.53*	0.22*	3.70	0.98	3.87	0.95	4.37
8.4	7.25	0.95	5.36	0.83	15.58*	0.34*	6.31
8.7	5.57	0.64	2.35	0.96	3.19	0.93	3.70
9.3	3.10	0.89	—	—	—	—	3.10
	H_P	R²	H_P	R²	H_P	R²	Average H_P
7.5	2.97	0.99	3.19	0.99	3.60	0.92	3.25
8.1	5.33	0.80	3.92	0.96	4.10	0.79	4.45
8.4	3.84	0.70	4.35	0.84	3.65	0.85	3.95
8.7	3.97	0.96	4.90	0.95	2.60	0.94	3.82
9.3	2.84	0.99	2.64	0.90	—	—	2.74
	$H_{N:P}$	R²	$H_{N:P}$	R²	$H_{N:P}$	R²	Average $H_{N:P}$
7.5	3.15	0.96	5.47	0.82	3.06	0.95	3.89
8.1	4.84	0.82	3.68	0.93	6.44	0.79	4.98
8.4	9.22*	0.31*	6.83	0.86	4.76	0.89	6.94
8.7	5.27	0.84	5.89	0.91	3.94	0.84	5.03
9.3	3.46	0.86	3.11	0.96	—	—	3.29

Figure 1

Figure 1 Relationships between the N and P contents, and N:P ratios of *L. chinensis* plants and the intervals between saline-alkali stress increments. Solid circles, hollow circles and triangles refer to intervals of 14, 17, and 23

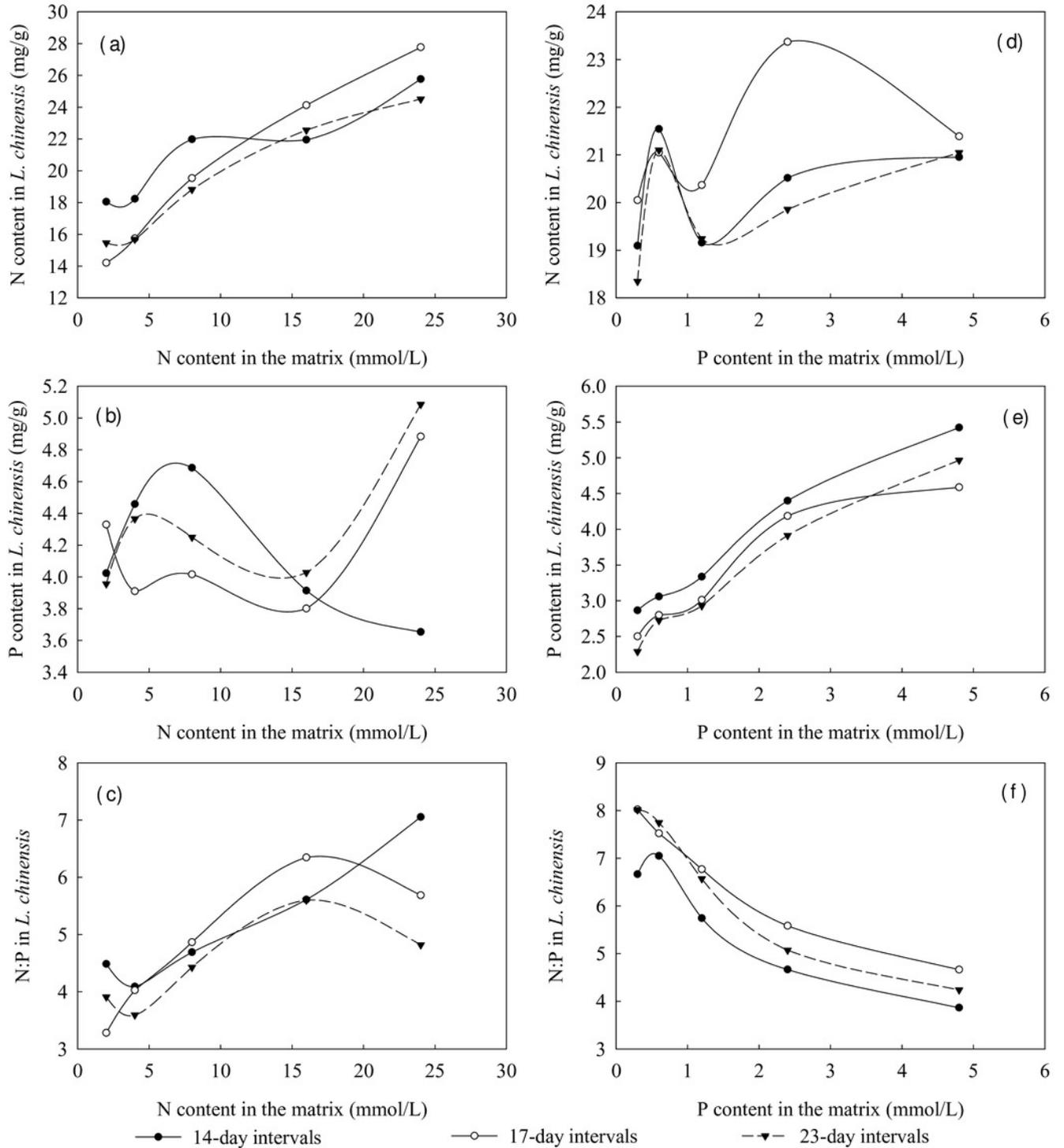
Figure 1 Relationships between the N and P contents, and N:P ratios of *L. chinensis* plants and the intervals between saline-alkali stress increments. Solid circles, hollow circles and triangles refer to intervals of 14, 17, and 23 days, respectively.

- ● — 14-day intervals   —○— 17-day intervals   --▼-- 23-day intervals

Figure 2

Homeostasis indices of *L. chinensis* at the indicated pH values and saline -alkali stress incremental intervals.

Figure 2 Homeostasis indices of *L. chinensis* at the indicated pH values and saline -alkali stress incremental intervals.

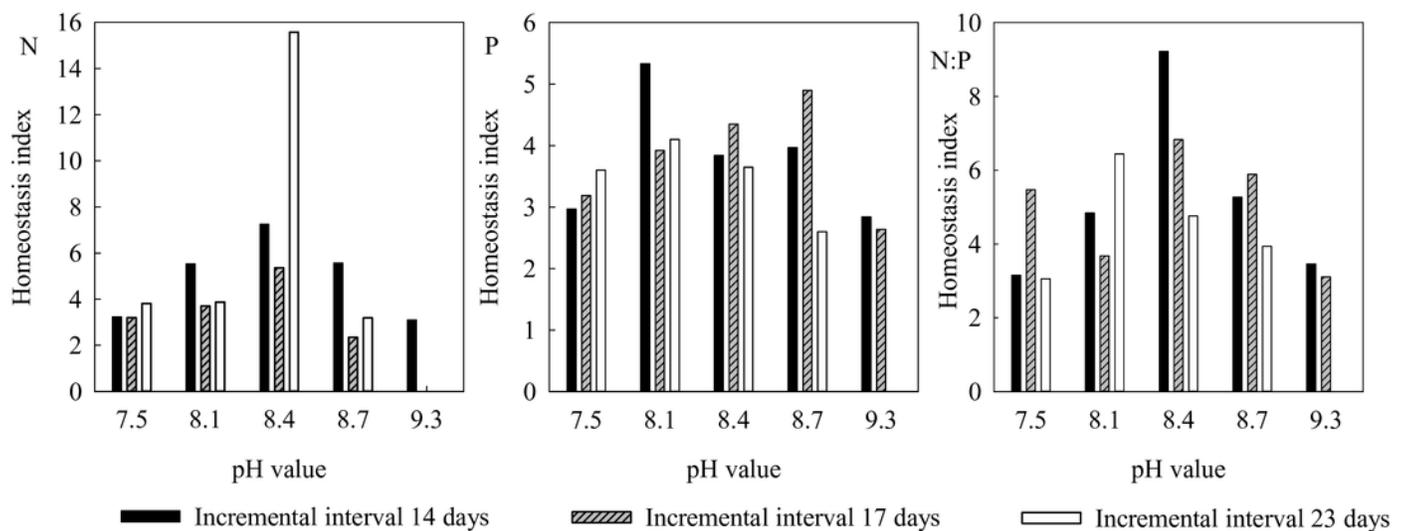



Figure 3

Specific growth rates of *L. chinensis* at indicated environmental N and P concentrations, and pH.

Figure 3 Specific growth rates of *L. chinensis* at indicated environmental N and P concentrations, and pH.

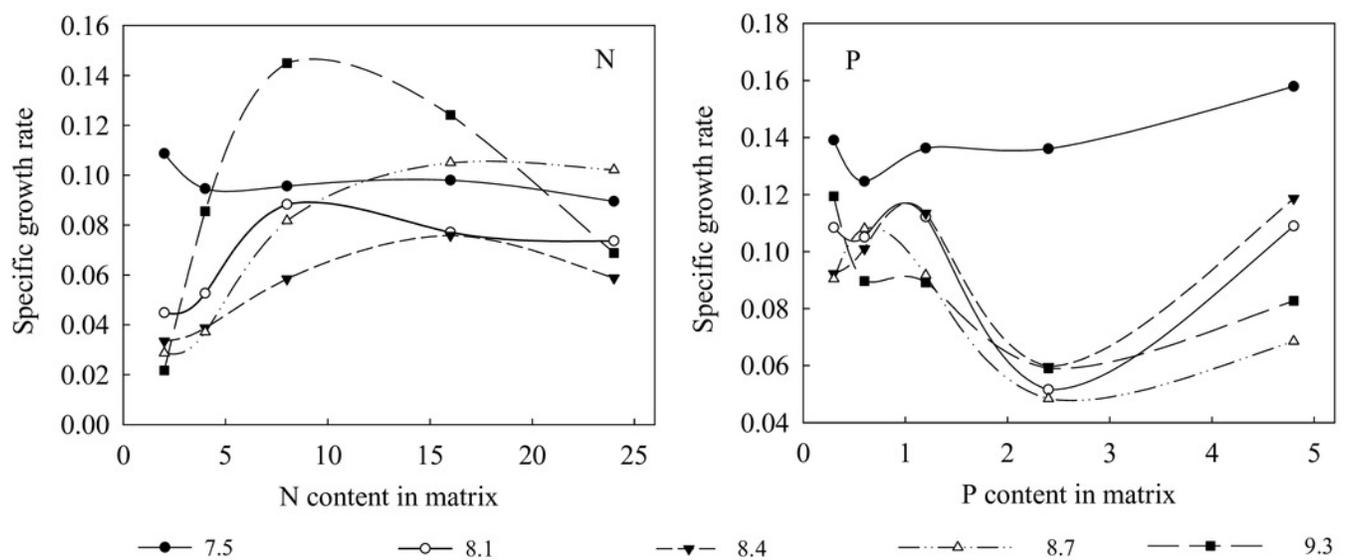



Figure 4

Relationship between specific growth rate and the N:P ratio in the aboveground parts of *L. chinensis*.

Figure 4 Relationship between specific growth rate and the N:P ratio in the aboveground parts of *L. chinensis*.