Responses of soil C, N, and P stoichiometric ratios to N and S additions in a subtropical evergreen broad-leaved forest

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Abstract

Acid deposition from the emission of nitrogen (N) and sulfur (S) has become an important factor affecting the soil nutrient balance and biogeochemical cycling in terrestrial ecosystems. The average levels of N and S deposition in the rainy area of southwestern China from 2008 to 2010 were 9.5 g N m^{-2} y^{-1} and 19.3 g S m^{-2} y^{-1}, respectively. External additions of N and S fertilizers combined with high levels of acid deposition may affect the soil ecological stoichiometry in the region's widely distributed subtropical evergreen broad-leaved forest. Therefore, we investigated the responses of the soil stoichiometric ratios and enzyme activities to added N (+N), added S (+S), added N and S (+NS), and a control (Ctr) in the 0-20 cm layer in an evergreen broad-leaved forest in the rainy area of southwestern China from April 2013 to April 2015. The results showed that the soil total N (TN) concentration and N/P ratio were higher and the soil organic C (SOC) concentration and C/N ratio were lower in the fertilization treatments than the Ctr, although N and S additions did not significantly alter the soil total P (TP) concentration. The +N, +S, and +NS treatments increased the soil acid phosphatase activity and reduced the soil invertase, cellulase, catalase, and polyphenol oxidase activities. The +N and +NS treatments increased the soil urease activity and reduced soil peroxidase activity. The +S treatment reduced the soil urease activity and did not alter soil peroxidase activity. N and S additions had synergistic decreasing effects on the SOC concentration, C/N ratio, and soil cellulose and catalase activities. Moreover, structural equation models identified that N and S additions regulated the SOC, TN, and TP concentrations via shifting the activities of soil enzymes and the pathways differed between N addition and S addition. In conclusion, N and S additions decreased the SOC concentration, C/N ratio, and most soil C-cycle enzyme activities and increased the TN concentration, N/P ratio, and soil acid phosphatase activity. All these results indicated that external N

40   and S additions combined with acid deposition increased soil N concentrations and exacerbated soil

41   C and P limitations in this forest.

42   **Keywords:** Acid deposition; Soil organic carbon; Nutrient limitation; Nitrogen-phosphorus imbalance;

43   Subtropical forest; Soil enzyme activities.

1. Introduction

45   Atmospheric acid deposition is an important component of global climate change (Bouwman et

46   al., 2002; Oulehle et al., 2013), and nitrogen (N) and sulfur (S) are the main elemental components of

47   atmospheric acid deposition (Anatolaki and Tsitouridou, 2007). The global atmospheric N and S

48   deposition in 2010 was estimated at approximately 123 Tg N y^{-1} and 84 Tg S y^{-1}, respectively

49   (Galloway et al., 2008; Tan et al., 2018). Although the regional scales of atmospheric acid deposition

50   exhibit varying trends, acid deposition is still increasing in China (Gao et al., 2018; Tan et al., 2018).

51   As one of the regions with the highest level of acid deposition in the world, China accounts for

52   approximately 20% of the global atmospheric N deposition and approximately 17% of global

53   atmospheric S deposition (Du et al., 2015; Tan et al., 2018). Especially in some subtropical forests in

54   southern China, the acid deposition level is two to three times that of the national average (Du et al.,

55   2015). Evidence suggests that inputs of N and S have affected soil nutrient imbalance (Gao et al., 2018;

56   Mori et al., 2019) and altered the biogeochemical cycles of terrestrial ecosystems (Güsewell and

57   Gessner, 2009; Lal, 2004; Sardans and Peñuelas, 2015). Therefore, external N and S fertilizers may

58   interact with high levels of atmospheric acid deposition to seriously threaten the soil nutrient balance

59   and alter biogeochemical cycles in these subtropical forests.

60   Soil stoichiometric ratios have been widely used to study the responses of soil ecosystem nutrient

61   balances and soil carbon (C), N, and phosphorus (P) biogeochemical cycles to the inputs of external

nutrient elements (Sardans and Peñuelas, 2012; van Dijk et al., 2012). External N addition generally

increases the availability of soil N (Magill et al., 2004; Michopoulos et al., 2004). However, the effects

of N addition on soil C and P concentrations and C/N, C/P, and N/P ratios vary with the level of

atmospheric deposition (Cleveland and Townsend, 2006), duration of fertilizer application (de Vries

et al., 2014), and type of ecosystem (Keeler et al., 2009). Thus, different results have been reported

for the effects of N addition on soil C and P concentrations, including increases (Cleveland and

Townsend, 2006; Marklein and Houlton, 2012), decreases (de Vries et al., 2014; Huang et al., 2016),

and no effects (Chen et al., 2018a; Hu et al., 2013). The effects of S addition on soil nutrient

concentrations and stoichiometric ratios are more uncertain than those of N addition, although S

addition can also affect nutrient dynamics (Fenn et al., 1999; Gao et al., 2018; Matejko et al., 2009).

Moreover, the inputs of external N can interact with S to alter the nutrient cycles of terrestrial

ecosystems (Keeler et al., 2009; Saiya-Cork et al., 2002), thereby increasing the uncertainty of the

responses of soil nutrient concentrations and stoichiometric ratios to N and S additions.

Soil enzymes are key in the control of biogeochemical cycles and soil nutrient concentrations

(Aragón et al., 2014). The activities of soil enzymes are very sensitive to the inputs of external nutrients

(Allison and Vitousek, 2005; Hu et al., 2013). Thus, the addition of N and S may alter the activities of

soil enzymes to regulate soil nutrient concentrations and stoichiometric ratios. For example, a meta-

analysis reported that N addition increased soil C concentrations in terrestrial ecosystems via shifting

the activities of soil enzymes (Chen et al., 2018b). However, the responses of soil nutrient

concentrations and stoichiometric ratios to N and S additions and how these responses are regulated

by soil enzymes in subtropical forests under high levels of acid deposition are poorly understood.

Therefore, investigating how N and S additions alter soil nutrient concentrations and stoichiometric

84   ratios via shifting soil enzyme activities will improve our understanding of the mechanism underlying

85   the effect of adding N and S on the concentrations and ratios.

86   The rainy area of southwestern China has received considerable acid deposition and represents

87   one of the areas of China with the greatest levels of deposition (Tu et al., 2013). Subtropical evergreen

88   broad-leaved forests are the most widely distributed and representative type of forest in this region.

89   They provide excellent experimental sites for examining the responses of soil stoichiometric ratios

90   and soil enzyme activities to N and S additions in subtropical forests under high levels of acid

91   deposition. In this study, we conducted a field experiment in a subtropical evergreen broad-leaved

92   forest in the rainy area of southwestern China for two years. We hypothesized that (i) N and S additions

93   would affect the concentrations and stoichiometric ratios of soil nutrients via shifting the activities of

94   soil enzymes in this forest. N and S additions can have different effects on the activities of soil enzymes

95   (Hu et al., 2013; Lv et al., 2014), and the mechanisms underlying the effects of N and S additions on

96   soil nutrient concentrations and stoichiometric ratios may be different. Therefore, we also

97   hypothesized that (ii) the responses of soil nutrient concentrations and stoichiometric ratios to N

98   addition and S addition would differ. In addition, due to the strong coupling between N and S cycles

99   (Gao et al., 2018), we hypothesized that (iii) N and S additions would have interactive effects on the

100   nutrient concentrations and stoichiometric ratios in the study subtropical forest.

101   **2. Materials and methods**

102   *2.1. Study site*

103   The rainy area of southwestern China is an ecotone between the Sichuan Plateau and the Sichuan

104   Basin that spans 70 km from east to west and 450 km from north to south, and the total area is

105   approximately 25,000 km^2 (Zhou et al., 2018). The Sichuan Plateau and Basin differ greatly in

106 elevation. Warm and humid air from the Sichuan Basin and the city of Chongqing (east of the basin)

107 usually condenses into rain in this rainy area as the elevation increases, thereby providing abundant

108 rainfall (Tu et al., 2013; Xu et al., 2013). Rapid agricultural intensification, industrial and urban

109 development, and increased fuel use for transportation and energy production in the Sichuan Basin

110 and the city of Chongqing have resulted in dramatic increases in N and S pollution since the 1980s

111 (Xu et al., 2013). N and S pollutants are transported to this rainy area and deposited on the ground

112 with rain due to the special climate and topography of the area (Tu et al., 2013; Xu et al., 2013). From

113 2008 to 2010, the annual average wet N and S depositions were 9.5 g N m^{-2} y^{-1} and 19.3 g S m^{-2} y^{-1},

114 respectively (Lin and Hu, 2011; Tu et al., 2013). The pH of the precipitation ranged from 3.9 to 5.0,

115 and the annual average was 4.4 (Lin and Hu, 2011).

116 Ya'an, Sichuan Province is located in the center of this rainy area. Thus, we selected a subtropical

117 evergreen broad-leaved forest on Jinfengsi Mountain (30°02′N, 103°03′E; 800 m a.s.l.) in Ya'an in

118 October 2012 to study the effects of N and S additions on the concentrations and stoichiometric ratios

119 of soil nutrients. The study site has a subtropical humid monsoon climate. The annual average

120 temperature is 16.1 °C, and the annual average rainfall is 1772 mm. Approximately 70% of the rain

121 falls from May to October, and < 20 mm falls in December and January. Both the temperature and

122 rainfall are lowest in January and highest in August. The stand age at the study site is approximately

123 40 years. The dominant tree species are *Lindera megaphylla*, *Quercus serrata*, *Choerospondias*

124 *axillaris*, and *Eurya japonica*. The main species in the undergrowth is *Dicranopteris linearis*. The soil

125 is classified as a Lithic Dystrudept (according to USDA Soil Taxonomy) derived from purple

126 sandstone and shale. The average thickness of the surface organic layer is approximately 2 cm. The

127 average soil depth to bedrock is approximately 80 cm. The surface soil horizon (0-20 cm) organic C

128   was 16.2±0.7 (mean ± standard deviation) g kg^{-1}, total N (TN) was 1.60±0.06 g kg^{-1}, and pH was

129   4.50±0.09 (H$_2$O extraction) before the experimental treatments began. The annual temperature and

130   water content of the surface soil horizon (0-20 cm) were 11.9±0.4 °C and 27.9±1.1% from April 2013

131   to April 2015, respectively (Fig. S1).

132   *2.2. Experimental design*

133   Considering that the levels of atmospheric N and S depositions in this rainy area are very high

134   (Tu et al., 2013; Xu et al., 2013) and expected to continue increasing until at least 2030 (Du et al.,

135   2015) and drawing on the experience of N addition in this region (Peng et al., 2020; Tu et al., 2013;

136   Zhou et al., 2018), 15 g N m^{-2} y^{-1} and 30 g S m^{-2} y^{-1} additions were tested in this study. The treatments

137   consisted of a control (Ctr, 0 g N m^{-2} y^{-1} and 0 g S m^{-2} y^{-1}), added N (+N, 15 g N m^{-2} y^{-1}), added S (+S,

138   30 g S m^{-2} y^{-1}), and added N and S (+NS, 15 g N m^{-2} y^{-1} and 30 g S m^{-2} y^{-1}). Urea and sodium sulfate

139   were used as the N and S fertilizers, respectively.

140   Twelve plots (5 × 5 m) were established in the study forest in March 2013. The distance between

141   each plot was > 5 m. To reduce errors caused by slope, topographic position, and nutrient heterogeneity,

142   the twelve plots were divided into three blocks perpendicular to the maximum slope. The four

143   treatments were randomly distributed to plots inside each block, and each treatment was repeated three

144   times. The N and S fertilizers were divided into 48 equal parts and applied every half month from

145   April 2013 to April 2015. The fertilizers were dissolved in 2 L of water for each application and then

146   sprayed evenly on the soil surface. The Ctr plots were sprayed with 2 L of water without fertilizer to

147   avoid experimental error. Based on the rainfall and N and S concentrations in the precipitation, we

148   estimated that approximately 7.75 g N m^{-2} y^{-1} and 15.8 g S m^{-2} y^{-1} were deposited onto the soil surface

149   from natural rain during the study period.

150   *2.3. Soil sampling and analysis*

151   Soil samples were collected eight times: July and October 2013; January, April, July, and October

152   2014; and January and April 2015. Moss and leaf litter were removed from the soil surface, and five

153   soil samples (0-20 cm) were collected in each plot using a soil auger (diameter of 5 cm). The five

154   samples from each plot were then fully mixed and transported to the laboratory. In the laboratory, the

155   roots and gravel in the samples were removed by tweezers. The soil sample was homogenized and

156   then divided into two subsamples. One subsample was sieved (< 2 mm) and stored at 4 °C for

157   measuring the activities of soil enzymes within one week. The other subsample was air-dried and

158   sieved (< 2 mm) for measuring the concentrations of soil organic C (SOC), TN, total P (TP), and pH.

159   The SOC concentration was determined using dichromate oxidation-ferrous sulfate titration

160   (Schinner et al., 1996), the TN concentration was determined by Kjeldahl digestion (Allen et al., 1974),

161   the TP concentration was determined by molybdenum-antimony colorimetry (Allen et al., 1974), and

162   the soil pH was determined by a glass electrode in aqueous extracts (Allen et al., 1974). Soil enzyme

163   activities were determined by spectrophotometrically. The activity of invertase (E.C. 3.2.1.26) was

164   determined using sucrose as the substrate (Ohshima et al., 2007), the activity of urease (E.C. 3.5.1.5)

165   was determined using urea as the substrate (Kandeler and Gerber, 1988), the activity of acid

166   phosphatase (E.C. 3.1.3.2) was determined using para-nitrophenyl phosphate as the orthophosphate

167   monoester analog substrate (Schinner et al., 1996; Tabatabai, 1982), the activity of cellulase (E.C.

168   3.2.1.4) was determined using carboxymethylcellulose sodium as the substrate (Ghose, 1987), the

169   activity of catalase (E.C. 1.11.1.6) was determined using hydrogen peroxide as the substrate (Tabatabai,

170   1982), and the activities of peroxidase (E.C. 1.11.1.7) and polyphenol oxidase (E.C. 1.10.3.2) were

171   determined using L-3,4-dihydroxyphenylalanine as the substrate (Keeler et al., 2009). The detailed

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172 measurements of soil enzyme activities are shown in Table S1. All measurements were repeated three

173 times.

174 *2.4. Data analysis*

175 The soil stoichiometric ratios in this text are mass-based, e.g., C/N is the ratio of SOC to TN

176 concentrations (g kg^{-1}), C/P is the ratio of SOC to TP concentrations, and N/P is the ratio of TN to TP

177 concentrations.

178 The variables were statistically analyzed using SPSS 20.0 for Windows (SPSS Inc., Chicago,

179 USA). All variables were first tested for a normal distribution and homogeneity of variance using the

180 one-sample Kolmogorov-Smirnov test and Levene's test, respectively. Variables with non-normal or

181 unequal variances, i.e., the C/N ratio and soil cellulose, peroxidase, and polyphenol oxidase activities,

182 were transformed using the Box-Cox method. A two-way ANOVA was performed to examine the

183 effects of N and S additions on the SOC, TN, and TP concentrations; soil C/N, C/P, and N/P ratios;

184 and soil enzyme activities at each sampling time. To understand how N and S additions affected the

185 soil nutrient concentrations, stoichiometric ratios, pH value, and enzyme activities during the whole

186 fertilization process, linear mixed effects models employing the restricted maximum likelihood

187 estimation method were used to examine the effects of N addition, S addition, and their interactions

188 on the mean SOC, TN, and TP concentrations; soil C/N, C/P, and N/P ratios; soil pH; and soil enzyme

189 activities during two years of fertilization. We included N addition, S addition, and sampling time as

190 fixed factors and blocks and plots nested in blocks as random factors. The Bonferroni adjustment

191 method was applied to the confidence intervals and significance values to account for multiple

192 comparisons. The pairwise relationships among the SOC, TN, and TP concentrations and the soil

193 stoichiometric ratios in the different treatments and control were examined by using distinct regression

194   approaches, such as curve estimation with the unary linear regression model, logarithmic regression

195   model, quadratic regression model, and exponential regression model. Then, the optimal model was

196   chosen in each case. To understand how N and S additions directly and indirectly affect soil nutrient

197   concentrations, we also used the structural equation model (SEM) in AMOS 22.0 (IBM SPSS, Chicago,

198   USA) to examine the effects of the N and S additions on the soil nutrient concentrations and to compare

199   the relative contributions of the soil enzyme activities to the SOC, TN, and TP concentrations.

200   Coefficients were estimated using the maximum likelihood method, and confidence intervals were set

201   at 95%. Differences were deemed significant at $P < 0.05$ for all analyses.

202   To understand the overall differences in the soil nutrient concentrations, stoichiometric ratios,

203   and enzyme activities among different treatments, we performed multivariate statistical analyses using

204   a general discriminant analysis (GDA) to determine the overall differences in SOC, TN and TP

205   concentrations; C/N, C/P, and N/P ratios; and soil enzyme activities among the Ctr and different

206   fertilization treatments. We also assessed the component of the variance caused by the sampling time

207   as an independent categorical variable. This analysis used the squared Mahalanobis distance statistic

208   that depends on the Euclidean distance in the model between two sets of samples: as the sets became

209   closer and less different and the squared Mahalanobis distance decreased; and as the sets became more

210   distant and different and the squared Mahalanobis distance increased (De Maesschalck et al., 2000).

211   GDA is thus an appropriate tool for identifying the variables most responsible for the differences

212   among groups while controlling for the component of the variance caused by other categorical

213   variables, e.g., sampling time. The GDA was performed using Statistica 8.0 (StatSoft, Inc., Tulsa,

214   USA).

215   **3. Results**

216 *3.1. Effects of N and S additions on soil nutrient concentrations and soil pH*

217   The SOC concentration was lower in the +N, +S, and +NS than the Ctr treatment after fifteen

218 months ($P < 0.05$; Fig. S2a), and the TN concentration was higher in the fertilization treatments than

219 the Ctr after eighteen months ($P < 0.05$; Fig. S2b). In contrast, the TP concentration in all treatments

220 ranged from 0.4 to 0.6 g kg^{-1} (Fig. S2c). The +N, +S, and +NS decreased the mean SOC concentration

221 by 14.0%, 10.5%, and 19.7%, respectively ($P < 0.05$; Fig. 1a), increased the mean TN concentration

222 by 15.4%, 15.3%, and 34.3%, respectively ($P < 0.05$; Fig. 1b), but did not affect the mean TP

223 concentration ($P > 0.05$; Fig. 1c). The mean TN concentration was higher in the +NS treatment than

224 the +N and +S treatments ($P < 0.05$). N and S additions interactively decreased the SOC concentrations

225 ($P < 0.05$; Table 1). The soil pH in the +N, +S, and +NS were lower than that in the Ctr by 3.07%,

226 1.98%, and 4.58%, respectively ($P < 0.05$; Fig. S3).

227 *3.2. Effects of N and S additions on soil stoichiometric ratios*

228   The C/N ratio was lower in the +N, +S, and +NS than the Ctr after fifteen months ($P < 0.05$; Fig.

229 S4a), and the N/P ratio was higher in the fertilization treatments than the Ctr after twelve months ($P <$

230 0.05; Fig. S4c). The +N, +S, and +NS reduced the mean C/N ratio by 27.1%, 22.7%, and 39.8%,

231 respectively ($P < 0.05$; Fig. 1d), and increased the mean N/P ratio by 17.7%, 22.4%, and 41.2%,

232 respectively ($P < 0.05$; Fig. 1f). The C/N ratio was lower and the N/P ratio was higher in the +NS than

233 the +N and +S ($P < 0.05$). Moreover, the mean C/P ratio was reduced in the +N and +NS ($P < 0.01$;

234 Fig. 1e) but was not altered by the +S ($P > 0.05$). The N and S additions interactively decreased the

235 soil C/N ratios ($P < 0.01$; Table 1).

236 *3.3. Effects of N and S additions on soil enzyme activities*

237   The activities of the soil enzymes were seasonally dynamic, and the responses of the activities

238 varied among treatments (Fig. S5a-g). The +N, +S, and +NS treatments reduced the mean activities of

239 soil invertase, cellulose, catalase, and polyphenol oxidase ($P < 0.05$; Fig. 2a, d, e, and g) and increased

240 the mean activity of acid phosphatase ($P < 0.05$; Fig. 2c). The +N and +NS increased the mean activity

241 of soil urease by 10.6% and 8.9%, respectively ($P < 0.05$; Fig. 2b), and reduced the mean activity of

242 soil peroxidase by 12.3% and 14.8%, respectively ($P < 0.05$; Fig. 2f). The +S decreased the mean

243 activity of soil urease by 9.8% ($P < 0.05$; Fig. 2b) but did not alter the mean activity of soil peroxidase

244 ($P > 0.05$; Fig. 2f). N and S additions interactively decreased the activities of cellulase and catalase (P

245 < 0.05; Table 2).

246 *3.4. Relationships among the SOC, TN, and TP concentrations and soil stoichiometric ratios*

247 The SOC and TN concentrations, SOC concentration and N/P ratio were negatively and linearly

248 correlated in the samples from the +N, +S, and +NS treatments ($P < 0.05$; Fig. 3a and b) but

249 quadratically correlated in the Ctr ($P < 0.05$). The TN and C/P were negatively and linearly correlated

250 in the +N, +S, and +NS ($P < 0.05$; Fig. 3d) but were not significantly correlated in the Ctr ($P > 0.05$).

251 The SOC and TP concentrations were not significantly correlated in the Ctr, +N, or +S ($P > 0.05$; Fig.

252 3c) but were positively correlated in the +NS ($P < 0.05$).

253 *3.5. Contributions of the enzyme activities to the SOC, TN, and TP concentrations*

254 Although N and S additions directly increased the SOC and TN concentrations, their indirect

255 effects on nutrient concentrations through soil enzyme activities were the dominant effects (Table 3).

256 N and S additions both decreased SOC concentration, with N addition decreasing the activities of

257 invertase, cellulase, and peroxidase and increasing the activities of urease and acid phosphatase (Fig.

258 4a) and S addition decreasing the activities of invertase, urease, and peroxidase and increasing the acid

259 phosphatase activity (Fig. 4b). N and S additions mainly increased the acid phosphatase activity and

260 decreased the peroxidase activity to increase the TN concentration (Fig. 4c and d). N addition also

261 increased the soil TN concentration by improving the soil urease activity, but this result cannot be

262 taken into account because the added N was in the form of urea. N and S additions did not directly

263 affect the soil TP concentration but indirectly affected the TP concentration by altering the soil enzyme

264 activities (Fig. 4e and f, Table 3). The standardized total effects of N and S additions on the soil TP

265 concentrations were 0.0022 and -0.157, respectively ($P < 0.05$; Table 3).

266 *3.6. General discriminant analysis*

267   The 2D layout represented by the two first roots (explaining 80.6% of the total variance) was

268 clearly consistent with the observations from the univariate and SEM analyses (Fig. 5). The main

269 variables loading on Root 1 were soil TN concentration, N/P ratio, and acid phosphatase activity, which

270 were higher towards the S addition treatments, and soil invertase activity and C/N ratio, which were

271 lower towards the S addition treatments. The main variables loading on Root 2 were soil TN

272 concentration, urease activity, and N/P ratio, which were higher towards the N addition treatments,

273 and SOC concentration, C/N and C/P ratios, and peroxidase and cellulase activities, which were lower

274 towards the N addition treatments. N and S additions had synergistic increasing effects on the soil TN

275 concentration, N/P ratio, and acid phosphatase activity but synergistic decreasing effects on the SOC

276 concentration, C/N and C/P ratios, and invertase, catalase, and peroxidase activities. The main effects

277 of the variables in the GDA showed that soil TN concentration, soil C/N ratio, and all of the soil

278 enzyme activities were the main variables responsible for the overall differences among soils receiving

279 distinct treatments (Table S2 and S3). This information reinforces the results observed in the univariant

280 analyses.

281 **4. Discussion**

4.1. Effects of N and S additions on SOC concentration

283   The responses of SOC concentration to N addition vary with the fertilization level and duration

284   of application (Cleveland and Townsend, 2006; Lu et al., 2013). Short-term (< 3 years) high levels of

285   N input (> 10 g N m^{-2} y^{-1}) generally decrease SOC concentration (Cleveland and Townsend, 2006; de

286   Vries et al., 2014). Similarly, N addition in our study (15 g N m^{-2} y^{-1}) also decreased the SOC

287   concentration in the 0-20 cm soil layer during the two years of fertilization (Fig. 1a). Two potential

288   mechanisms may account for the effect of N addition on SOC concentration. First, the associated

289   decrease in SOC concentration may be related to the reduction of most soil C-cycle enzyme activities

290   (Fig. 4a), e.g., soil invertase, cellulase, and peroxidase activities, which strongly suggests a decrease

291   in soil biological activity (Allison and Vitousek, 2005; Aragón et al., 2014). This decrease in biological

292   activity may reduce the potential plant litter decomposition rate and transformation rate into SOC,

293   which results in lower soil C inputs. A meta-analysis also showed that N addition reduced most soil

294   C-cycle enzyme activities when the level of fertilization reached 15 g N m^{-2} y^{-1} (Jian et al., 2016),

295   which supported the results in our study. Second, the relatively high levels of both N addition and

296   atmospheric N deposition can exacerbate soil C leaching, which may account for the decrease of SOC

297   concentration. For example, Scott et al. (2015) reported that N addition (10 g N m^{-2} y^{-1}) increased the

298   amount of soil dissolved organic C leaching by 70%.

299   The S addition in our study reduced SOC concentration of the 0-20 cm soil layer (Fig. 1a). In

300   general, lower concentrations of soil fulvic acid, total carbohydrates, and total organic C were found

301   in the soil samples with lower pH (Hay et al., 1985). We found that the addition of S lowered the soil

302   pH (Fig. S3), which may have caused a decrease in SOC concentration. Moreover, S interacted with

303   N to reduce SOC concentration (Table 1). On the one hand, the decrease of soil pH value was stronger

304   under the +NS treatment than the +N or +S treatments (Fig. S3). Thus, the +NS treatment may have a

305   stronger inhibitory effect on the biogeochemical cycle of soil C than +N or +S treatments (Keeler et

306   al., 2009; Zhang et al., 2018). On the other hand, the N and S additions in our study synergistically

307   decreased the activities of cellulase and catalase (Table 2), which may have synergistically reduced

308   the return of plant organic C back to soil (Allison and Vitousek, 2005), resulting in a synergistic

309   decreasing effect on SOC concentration (Fig. 5).

310   Our previous study (Tie et al., 2018) reported that N and S additions inhibited the decomposition

311   of leaf litter in the same forest and that organic matter accumulated in the layers of leaf litter. The

312   organic C in the leaf litter could not return to the soil with short-term fertilization, which supported

313   our inferences above. Therefore, short-term N and S additions may have interacted the high levels of

314   atmospheric acid deposition to inhibit the C cycle, stimulate C sequestration in the litter layer, and

315   decrease the C concentration in the 0-20 cm soil layer of this study forest. These findings are in line

316   with those of other studies (Magill and Aber, 1998; Xu et al., 2017). However, Hu et al. (2013) reported

317   that N (3.0 g N m^{-2} y^{-1}) and S (3.0 g S m^{-2} y^{-1}) additions did not change the SOC concentration in a

318   boreal forest, where the levels of atmospheric N and S deposition were 0.08 and 0.11 g m^{-2} y^{-1},

319   respectively. The effects of external N and S inputs on soil C concentration vary with their levels

320   (Cleveland and Townsend, 2006), and the levels of atmospheric deposition and fertilization differed

321   greatly between our site and the site studied by Hu et al. (2013), which may account for the different

322   results between the two studies. In addition, the decrease in SOC with time under N and S additions

323   (Fig. 1a) supports the idea that their additions may have cumulative effects on SOC concentration (de

324   Vries et al., 2014; Mori et al., 2019); thus, medium- and long-term N and S additions are likely to

325   accelerate the reduction of SOC concentration in our study forest.

326   *4.2. Effects of N and S additions on soil TN concentration*

327   The addition of N generally directly increases external N and thus increases the soil TN

328   concentration (Magill et al., 2004; Michopoulos et al., 2004). The direct effects of N addition on soil

329   TN concentration in our study were consistent with previous studies (Magill et al., 2004; Michopoulos

330   et al., 2004). Moreover, the addition of N can indirectly alter the concentration of soil N via shifting

331   soil enzyme activities (Allison, 2005; Keeler et al., 2009). SEM analyses showed that N addition led

332   to increased soil TN concentration, at least partially, by indirectly increasing the soil acid phosphatase

333   activity and decreasing the soil peroxidase activity (Fig. 4c). Peroxidases can oxidize phenols, amines,

334   and hydrocarbons (Keeler et al., 2009). N addition in our study decreased peroxidase activity (Fig. 2f),

335   which could inhibit the decomposition of nitrogenous phenols, amines, and hydrocarbons and may

336   lead to the accumulation of N. Organic P and organic N are mainly present in carbonaceous organic

337   matter (Prescott, 2010). Soil acid phosphatase can hydrolyze P groups from various organic-P

338   substrates in organic matter (Saiya-Cork et al., 2002) and may indirectly promote the hydrolysis of

339   organic N (Jian et al., 2016). The increase of soil acid phosphatase activity under N addition in our

340   study therefore may have promoted the hydrolysis of organic N. Moreover, N addition increased the

341   negative correlations between SOC and TN, SOC and N/P, and TN and C/P (Fig. 3a, b, and d), which

342   may also be related to the increased soil acid phosphatase activity and decreased soil peroxidase

343   activity.

344   The addition of sodium sulfate can increase the leaching of soil exchangeable cations (e.g., K^+,

345   Mg^{2+}, and Ca^{2+}) because Na^+ can displace the rest of soil cations from the exchange complex, which

346   usually alters microbial and enzyme activities to influence N cycling in forest ecosystems (Gao et al.,

347   2018; Mori et al., 2019). In our study, the increased TN concentration was stronger under the +NS

16

348   treatment than the +N or +S treatments (Fig. 1b). S addition thus assisted the N fertilizer in increasing

349   the soil TN concentrations at the 0-20 cm soil layer, which may be related to S addition reducing the

350   soil N output (e.g., N_2O emission). For example, Gao et al. (2014) reported that Na_2SO_4 addition

351   decreased N_2O emissions by 79% from the soil in the Tibetan Plateau, China. Moreover, ammonium

352   sulfate could be formed in the soil when Na_2SO_4 was added in combination with urea, which is

353   important because ammonium sulfate releases ammonium more slowly than urea and leads to a greater

354   reduction in the potential volatilization and leaching of ammonium compared with urea (Chien et al.,

355   2011). The effects of N and S additions on TN concentration in this forest may thus be more

356   complicated than previously thought.

357   Most of the external N inputs generally leach and erode into rivers. For example, Fang et al.

358   (2009) reported that 25-66% of the input of external N leached and eroded into rivers in three seasonal

359   monsoon subtropical forests (evergreen broad-leaved forest, needle-leaved forest, and needle- and

360   broad-leaved mixed forest) with the same annual average rainfall of 1927 mm (approximately 75% of

361   rain falls from March to August). Our study site was also in a seasonal monsoon subtropical area, with

362   an annual average rainfall of 1772 mm (approximately 70% of rain falls from May to October).

363   Although S addition assisted the N fertilizer in increasing the soil TN concentration, some external N

364   may still have leached and eroded. Previous studies found that the Yangtze River has undergone

365   eutrophication mainly due to increases in the levels of nitrogenous compounds in the water (Chi et al.,

366   2017). Our study site is in the upper reaches of the Yangtze River; therefore, the eutrophication of the

367   Yangtze River may become more serious with high levels of urea and sodium sulfate addition. More

368   studies, however, are needed.

369   *4.3. Effects of N and S additions on soil TP concentration*

370   P is an essential element in all life and responsible for a considerable proportion of the growth

371   and development of vegetation (Paytan and McLaughlin, 2007; Turner, 2008). However, the effects of

372   external N and S inputs on the soil P cycle are still unclear. The N and S additions in our study did not

373   significantly alter the soil TP concentration (Fig. 1c) but rather increased the soil acid phosphatase

374   activity at the 0-20 cm soil layer (Fig. 2c), strongly suggesting that the demand for P in the 0-20 cm

375   soil ecosystem was increased (Peñuelas et al., 2013). Our previous study (Tie et al., 2018) also reported

376   that N and S additions slowed the release of P during litter decomposition, which may have reduced P

377   return to soil and strongly suggests a soil P limitation at the 0-20 cm soil layer.

378   The soil P cycle is a slow and complex biogeochemical process (Paytan and McLaughlin, 2007;

379   Peñuelas et al., 2013), so the TP concentration at 0-20 cm did not respond strongly to the short-term

380   additions of N and S. However, after 21 months of fertilization, N and S additions slightly decreased

381   the TP concentration (Fig. S2c), suggesting that long-term N and S additions potentially increase the

382   P demand and medium- and long-term additions may decrease the TP concentration in this forest.

383   *4.4. Effects of N and S additions on the soil C/N, C/P, and N/P ratios*

384   Soil stoichiometric ratios are at the core of C, N, and P biogeochemistry (Sardans and Peñuelas,

385   2015), which regulates the above- and belowground nutrient cycles of forest ecosystems (Güsewell

386   and Gessner, 2009; Peng et al., 2019). Soil stoichiometric ratios are inherently stable until external

387   nutrients are added (Huang et al., 2018). In our study, N and S additions decreased the soil C/N ratio

388   and increased the soil N/P ratio (Fig. 1d and f), which is consistent with the findings reported in other

389   studies (Huang et al., 2018; van Dijk et al., 2012). Changes in soil stoichiometric ratios can alter

390   nutrient cycles in plant-soil systems (Peng et al., 2019; Sardans and Peñuelas, 2015), which may affect

391   tree health and forest services (Aherne and Posch, 2013; Yuan and Chen, 2015).

392   Soil C and P limitations are generally caused by decreases in the soil C/N ratio and increases in

393   the soil N/P ratio, respectively (Peng et al., 2019; Zechmeister-Boltenstern et al., 2015). The lower soil

394   C/N ratio and higher soil N/P ratio in the soil samples from the N and S addition treatments in our

395   study indicated that the N and S additions may have aggravated the soil C and P limitations at the 0-

396   20 cm soil layer of the forest (Cleveland and Townsend, 2006). Moreover, microorganisms and plants

397   generally secrete more phosphatase in the case of P limitation (Zechmeister-Boltenstern et al., 2015).

398   The N and S additions in our study synergistically increased the acid phosphatase activity (Fig. 2c),

399   which reinforced the overall results strongly suggesting a clear exacerbation of P as the main limiting

400   factor. Atmospheric acid deposition in the rainy area of southwestern China is projected to remain high

401   until at least 2030 (Du et al., 2015). Therefore, the addition of N and S fertilizers combined with high

402   levels of atmospheric acid deposition may continue increasing the soil N concentrations and

403   exacerbating the soil C and P limitations at the 0-20 cm soil layer of these subtropical forests in the

404   rainy area of southwestern China in the future.

405   **5. Conclusions**

406   N and S additions decreased the SOC concentration, soil C/N ratio, and most soil C-cycle enzyme

407   activities and increased the soil TN concentration, soil N/P ratio, and soil acid phosphatase activity at

408   the 0-20 cm soil layer of the forest. These results indicated that N and S additions combined with acid

409   deposition increased soil N concentrations and exacerbated soil C and P limitations in this forest. N

410   and S additions regulated SOC, TN, and TP concentrations via shifting the activities of soil enzymes.

411   The responses of the soil C, N, and P concentrations and stoichiometric ratios to the N and S additions

412   differed because the effects of their additions on the activities of soil urease, cellulase, catalase, and

413   peroxidase differed. Moreover, the N and S additions synergistically decreased the SOC concentration,

414　C/N ratio, and soil cellulose and catalase activities, which suggested that N and S additions may have

415　synergistically exacerbated soil C limitation at the 0-20 cm soil layer. The responses of soil nutrient

416　concentrations and stoichiometric ratios to N and S additions, however, are complex. Therefore, the

417　long-term effects of N and S additions on soil C, N, and P stoichiometric ratios in this forest should

418　be investigated.

Competing interests

The authors declare that they have no competing financial interests.

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610

Tables

612 **Table 1** *F*- and *P*-values of linear mixed effects models on the effects of nitrogen (N) addition, sulfur

613 (S) addition, sampling time (month) and their interactions on the concentrations of SOC, TN, and TP

614 and the ratios of C/N, C/P, and N/P. d.f., degrees of freedom; SOC, soil organic carbon; TN, total

615 nitrogen; TP, total phosphorus; C/N, ratio of SOC to TN concentrations; C/P, ratio of SOC to TP

616 concentrations; and N/P, ratio of TN to TP concentrations. n = 576.

	d.f.	*F*-value	*P*-value		d.f.	*F*-value	*P*-value
SOC (g kg⁻¹)				*C/N ratio*			
N	1	66.7	<0.001	N	1	564	<0.001
S	1	17.6	<0.010	S	1	363	<0.001
Time	7	2265	<0.001	Time	7	2170	<0.001
N × S	1	5.70	<0.050	N × S	1	28.9	<0.001
N × Time	7	118	<0.001	N × Time	7	124	<0.001
S × Time	7	26.3	<0.001	S × Time	7	194	<0.001
N × S × Time	7	85.4	<0.001	N × S × Time	7	159	<0.001
TN (g kg⁻¹)				*C/P ratio*			
N	1	32.1	<0.001	N	1	40.7	<0.001
S	1	30.4	<0.001	S	1	1.65	0.223
Time	7	682	<0.001	Time	7	439	<0.001
N × S	1	0.352	0.564	N × S	1	0.600	0.454
N × Time	7	118	<0.001	N × Time	7	30.8	<0.001
S × Time	7	124	<0.001	S × Time	7	13.1	<0.001
N × S × Time	7	94.7	<0.001	N × S × Time	7	30.4	<0.001
TP (g kg⁻¹)				*N/P ratio*			
N	1	0.001	0.975	N	1	57.1	<0.001
S	1	1.16	0.303	S	1	91.1	<0.001
Time	7	80.6	<0.001	Time	7	219	<0.001
N × S	1	0.307	0.590	N × S	1	0.009	0.926
N × Time	7	11.0	<0.001	N × Time	7	18.3	<0.001
S × Time	7	16.8	<0.001	S × Time	7	39.5	<0.001
N × S × Time	7	8.49	<0.001	N × S × Time	7	12.1	<0.001

617

618   **Table 2** *F*- and *P*-values of linear mixed effects models on the effects of nitrogen (N) addition, sulfur

619   (S) addition, sampling time (month) and their interactions on the activities of soil invertase, urease,

620   acid phosphatase, catalase, cellulase, peroxidase, and polyphenol oxidase. d.f., degrees of freedom. n

621   = 672.

	d.f.	*F*-value	*P*-value		d.f.	*F*-value	*P*-value
Invertase (µmol g^{-1} h^{-1})				*Catalase (µmol g^{-1} h^{-1})*			
N	1	90.7	<0.001	N	1	18.4	<0.010
S	1	379	<0.001	S	1	1.12	0.310
Time	7	119	<0.001	Time	7	458	<0.001
N × S	1	0.610	0.501	N × S	1	5.95	<0.050
N × Time	7	8.80	<0.001	N × Time	7	23.4	<0.001
S × Time	7	15.9	<0.001	S × Time	7	64.4	<0.001
N × S × Time	7	4.07	<0.010	N × S × Time	7	17.5	<0.001
Urease (µmol g^{-1} h^{-1})				*Peroxidase (µmol g^{-1} h^{-1})*			
N	1	41.8	<0.001	N	1	12.2	<0.010
S	1	6.42	<0.050	S	1	4.38	0.058
Time	7	553	<0.001	Time	7	652	<0.001
N × S	1	3.21	0.098	N × S	1	1.30	0.277
N × Time	7	80.8	<0.001	N × Time	7	99.1	<0.001
S × Time	7	8.98	<0.001	S × Time	7	101	<0.001
N × S × Time	7	12.7	<0.001	N × S × Time	7	34.1	<0.001
Acid phosphatase (µmol g^{-1} h^{-1})				*Polyphenol oxidase (µmol g^{-1} h^{-1})*			
N	1	4.51	<0.050	N	1	15.6	<0.010
S	1	5.09	<0.050	S	1	23.4	<0.001
Time	7	122	<0.001	Time	7	7277	<0.001
N × S	1	0.963	0.346	N × S	1	0.205	0.659
N × Time	7	14.6	<0.001	N × Time	7	20.3	<0.001
S × Time	7	12.6	<0.001	S × Time	7	33.2	<0.001
N × S × Time	7	9.24	<0.001	N × S × Time	7	80.0	<0.001
Cellulase (µmol g^{-1} h^{-1})							
N	1	37.1	<0.001				
S	1	0.014	0.907				
Time	7	81.9	<0.001				
N × S	1	16.3	<0.010				
N × Time	7	23.6	<0.001				
S × Time	7	27.1	<0.001				
N × S × Time	7	42.3	<0.001				

622

623 **Table 3** Standardized total effects, direct effects, and indirect effects of nitrogen (N) and sulfur (S)

624 additions on soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) in the structural

625 equation models. **: highly significant ($P < 0.01$); *: significant ($P < 0.05$). n = 1088.

Relationships	Direct effects	Indirect effects	Total effects
N addition on SOC	0.214*	-0.582**	-0.368**
S addition on SOC	0.157*	-0.346**	-0.189**
N addition on TN	0.147*	0.317**	0.464**
S addition on TN	0.210**	0.241**	0.451**
N addition on TP	No significant effects	0.0022*	0.0022*
S addition on TP	No significant effects	-0.157*	-0.157*

626

627

628 **Figure captions**

629

630 **Fig. 1.** Effects of nitrogen and sulfur additions on the mean concentrations of SOC (a), TN (b), and TP

631 (c), and mean ratios of C/N (d), C/P (e), and N/P (f) during two years of fertilization based on linear

632 mixed effects models (mean ± standard deviations). Different lowercase letters denote significant

633 differences (multiple comparisons with Bonferroni adjustment, $P < 0.05$) among different treatments

634 during two years of fertilization. SOC: soil organic carbon; TN: soil total nitrogen; TP: soil total

635 phosphorus; C/N: ratio of SOC to TN concentrations; C/P: ratio of SOC to TP concentrations; N/P:

636 ratio of TN to TP concentrations; Ctr: no added nitrogen or sulfur; +N: added nitrogen; +S: added

637 sulfur; and +NS: added nitrogen and sulfur. n = 576.

638

639 **Fig. 2.** Effects of nitrogen and sulfur additions on the mean activities of soil invertase (a), urease (b),

640 acid phosphatase (c), cellulase (d), catalase (e), peroxidase (f), and polyphenol oxidase (g) during two

641 years of fertilization based on linear mixed effects models (mean ± standard deviations). Different

642 lowercase letters denote significant differences (multiple comparisons with Bonferroni adjustment, P

643 < 0.05) among different treatments during two years of fertilization. Ctr: no added nitrogen or sulfur;

644 +N: added nitrogen; +S: added sulfur; and +NS: added nitrogen and sulfur. n = 672.

645

646 **Fig. 3.** Relationships between SOC and TN (a), SOC and N/P (b), SOC and TP (c), TN and C/P (d),

647 TP and TN (e), and TP and C/N (f) in the treatments during two years of fertilization. **: highly

648 significant ($P < 0.01$) correlation; *: significant ($P < 0.05$) correlation. The solid regression lines

649 denote significant ($P < 0.05$) or highly significant ($P < 0.01$) correlations. SOC: soil organic carbon;

650 TN: soil total nitrogen; TP: soil total phosphorus; C/N: ratio of SOC to TN concentrations; C/P: ratio

651 of SOC to TP concentrations; N/P: ratio of TN to TP concentrations; Ctr: no added nitrogen or sulfur;

652 +N: added nitrogen; +S: added sulfur; and +NS: added nitrogen and sulfur. n = 576.

653

654 **Fig. 4.** Structural equation models of the effects of nitrogen (N) and sulfur (S) additions on the soil

655 enzyme activities and the relative contributions of the activities to the SOC (a, b), TN (c, d), and TP

656 (e, f) concentrations during two years of fertilization. Solid black and dashed blue arrows denote

657 significant (*P* < 0.05) positive and negative effects, respectively. SOC, soil organic carbon; TN, soil

658 total nitrogen; TP, soil total phosphorus; INV, invertase; URE, urease; AP, acid phosphatase; CA,

659 catalase; CEL, cellulase; and POD, peroxidase. **, highly significant (*P* < 0.01); and *, significant (*P*

660 < 0.05). n = 1088.

661

662 **Fig. 5.** Two-dimensional figure formed by the two first roots of the general discriminant analysis

663 (GDA) showing the area formed by the mean ± confidence interval (95%) of the scores of the control

664 and three treatment cases. The loads in these two roots of the independent continuous variables SOC,

665 TN and TP concentrations; C/N, C/P and N/P ratios; and soil enzyme activities are also represented.

666 SOC: soil organic carbon; TN: soil total nitrogen; TP: soil total phosphorus; C/N: ratio of SOC to TN

667 concentrations; C/P: ratio of SOC to TP concentrations; N/P: ratio of TN to TP concentrations; Ctr: no

668 added nitrogen or sulfur; +N: added nitrogen; +S: added sulfur; and +NS: added nitrogen and sulfur.
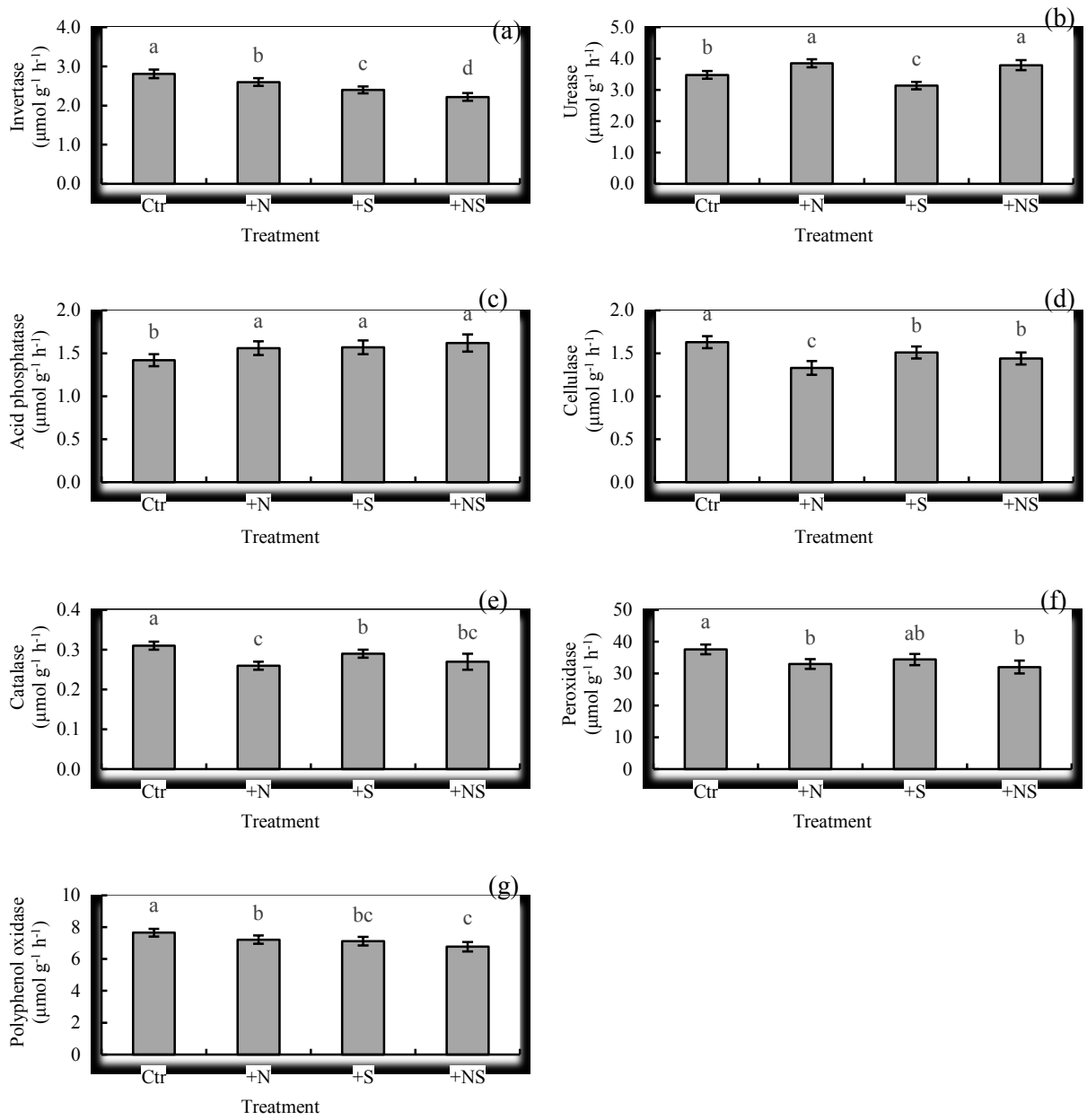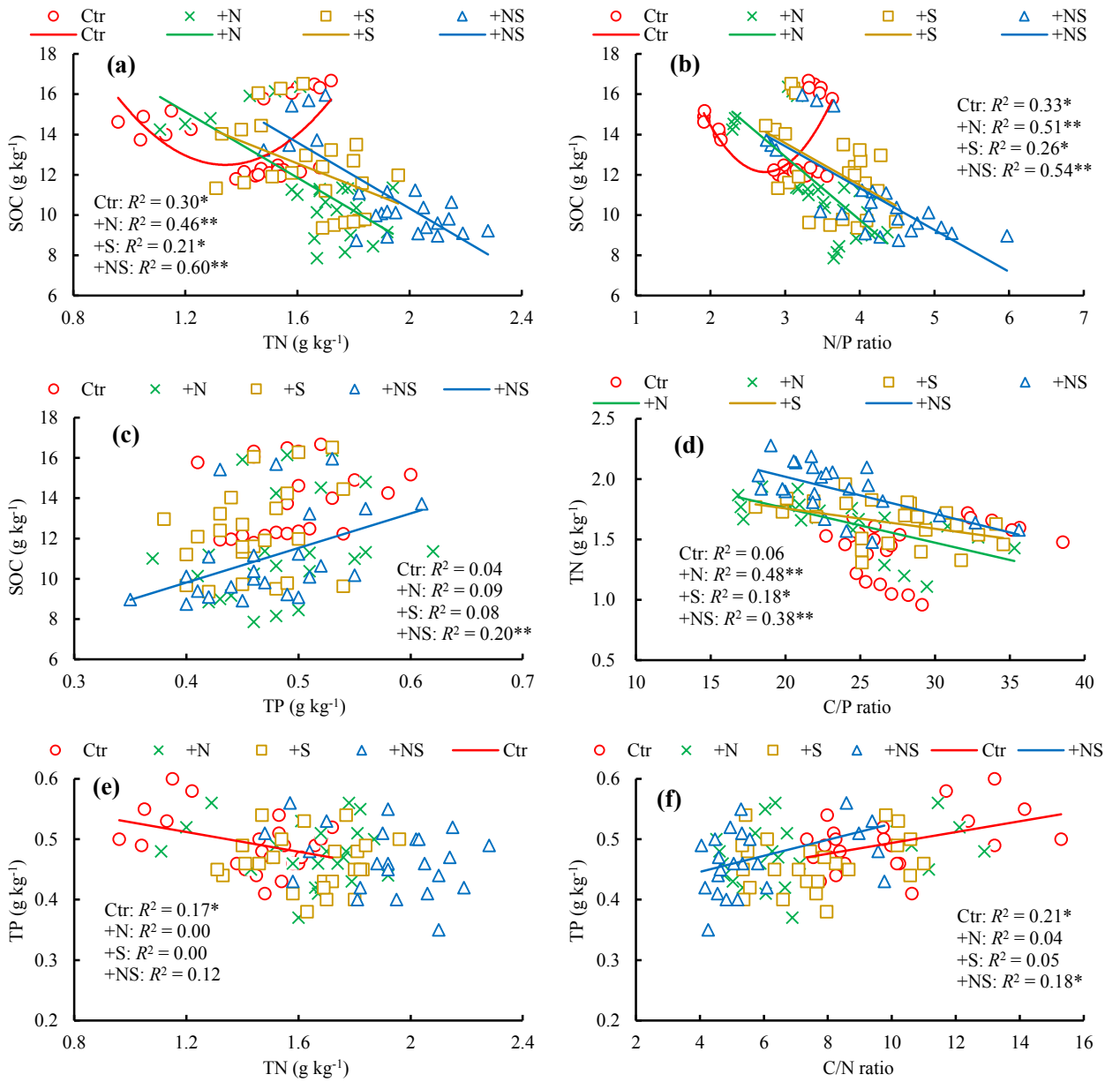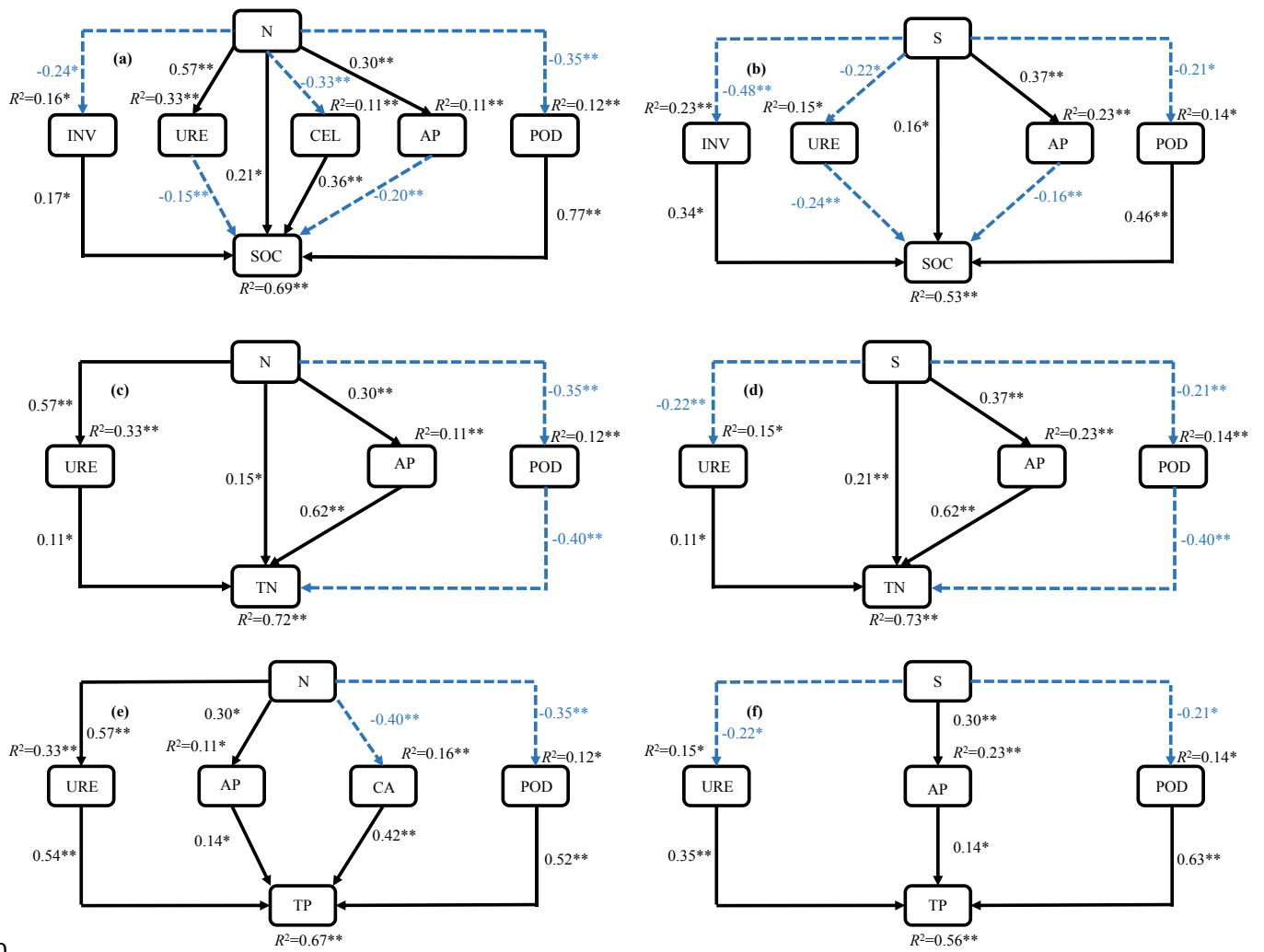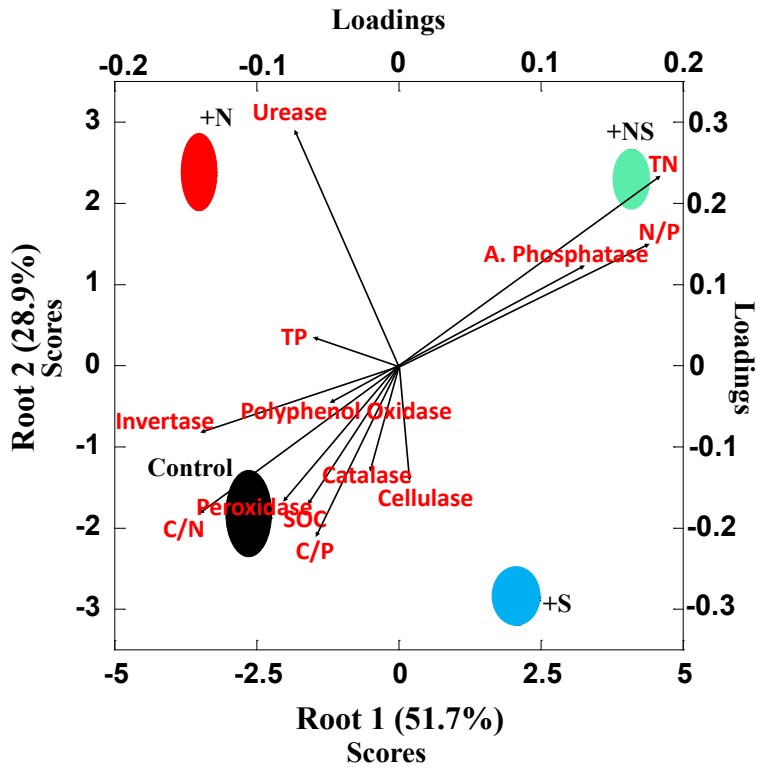
669

Figures

Fig. 1

674   **Fig. 2**

Fig. 3

Fig. 4

681 **Fig. 5**

682
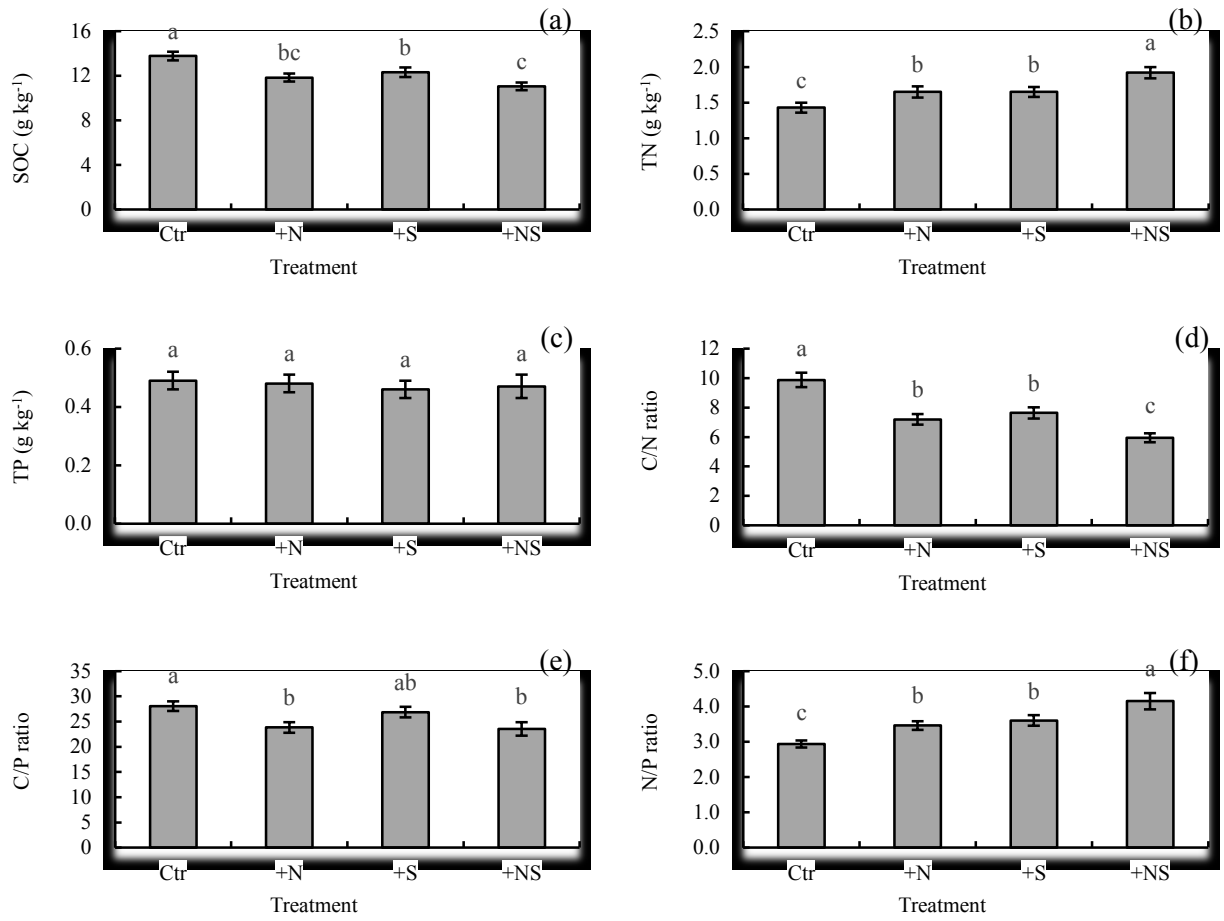
Supplementary tables and figures

Table S1 Enzymes used in this study and their measurements

Enzyme	Preparation of enzyme solution	Incubation condition	Measurement of activity
Invertase	5 mL phosphate buffer (pH 5.5)	37 °C, 24 h	Production rate of glucose from sucrose
Urease	10 mL citrate buffer (pH 6.7)	37 °C, 24 h	Production rate of ammonia from urea
Acid phosphatase	20 mL acetate buffer (pH 5.0)	37 °C, 2 h	Production rate of phenol from para-nitrophenyl phosphate
Cellulase	10 mL acetate buffer (pH 5.5)	37 °C, 72 h	Production rate of glucose from carboxymethylcellulose sodium
Catalase	40 mL distilled water	25 °C, 0.5 h	Degradation rate of hydrogen peroxide
Peroxidase	4 mL citrate-phosphate buffer (pH 4.5)	30 °C, 2 h	Production rate of phenanthraquinone from catechol in the presence of H_2O_2
Polyphenol oxidase	4 mL citrate-phosphate buffer (pH 4.5)	30 °C, 2 h	Production rate of purple gallopoietin from pyrogallic acid without H_2O_2

686 **Table S2** Squared Mahalanobis distances (*SMDs*) and *F*- and *P*-values in the general discriminant

687 analysis (GDA) among the treatments and control, with SOC, TN and TP concentrations; C/N, C/P

688 and N/P ratios; and soil enzyme activities as the continuous independent variables and time of

689 sampling (month) as the categorical independent controlling variable. SOC: soil organic carbon; TN:

690 soil total nitrogen; TP: soil total phosphorus; C/N: ratio of SOC to TN concentrations; C/P: ratio of

691 SOC to TP concentrations; N/P: ratio of TN to TP concentrations; Ctr: no added nitrogen or sulfur;

692 +N: added nitrogen; +S: added sulfur; and +NS: added nitrogen and sulfur.

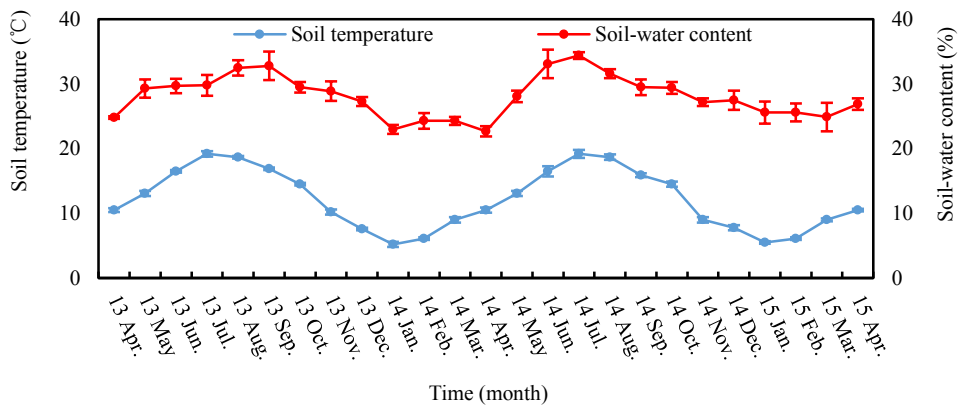| Treatment	+N			+S			+NS		
	SMD	*F-value*	*P*-value	*SMD*	*F-value*	*P*-value	*SMD*	*F-value*	*P*-value
Ctr	36.6	17.4	<0.001	44.4	21.2	<0.001	64.1	30.5	<0.001
+N				58.8	28.0	<0.001	66.3	31.6	<0.001
+S							41.6	19.8	<0.001

693

694 **Table S3** Main effects of the variables in the general discriminant analysis (GDA). Statistics (Wilks'

695 λ and *F*- and *P*-values) of the discriminant functional analysis among the treatments and control,

696 with SOC, TN and TP concentrations; C/N, C/P, and N/P ratios; and soil enzyme activities as the

697 independent continuous variables and time of sampling as the independent controlling categorical

698 variable. The significant effects of a variable in the model are highlighted in bold type ($P < 0.05$).

699 SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; C/N: ratio of SOC to TN

700 concentrations; C/P: ratio of SOC to TP concentrations; N/P: ratio of TN to TP concentrations.

| Variables	Wilks' Lambda	*F-value*	*P*-value
SOC (g kg^{-1})	0.962	0.972	0.410
TN (g kg^{-1})	**0.792**	**6.37**	**<0.001**
TP (g kg^{-1})	0.990	0.240	0.868
C/N	**0.702**	**10.3**	**<0.001**
C/P	0.962	0.964	0.410
N/P	0.994	0.149	0.930
Invertase (µmol g^{-1} h^{-1})	**0.749**	**8.16**	**<0.001**
Urease (µmol g^{-1} h^{-1})	**0.276**	**63.8**	**<0.001**
Cellulase (µmol g^{-1} h^{-1})	**0.792**	**6.38**	**<0.001**
Acid phosphatase (µmol g^{-1} h^{-1})	**0.409**	**35.2**	**<0.001**
Catalase (µmol g^{-1} h^{-1})	**0.604**	**15.9**	**<0.001**
Peroxidase (µmol g^{-1} h-^{1})	**0.831**	**4.94**	**<0.010**
Polyphenol oxidase (µmol g^{-1} h^{-1})	**0.351**	**45.1**	**<0.001**
Time	**0.060**	**16.7**	**<0.001**

701

702   **Fig. S1** Dynamics of soil-water content and soil temperature in the 0-20 cm soil layer from April

703   2013 to April 2015 (mean ± standard deviation).
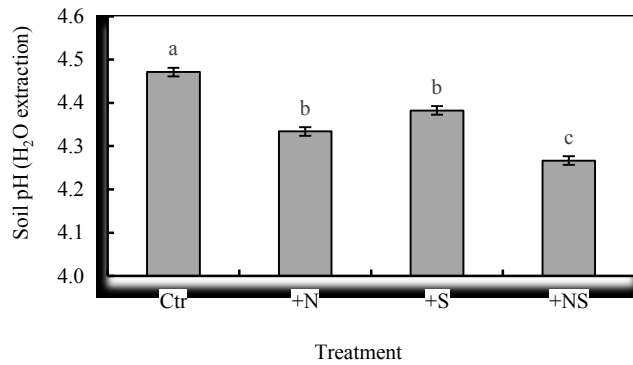

704

705   **Fig. S2** Changes in the SOC (a), TN (b), and TP (c) concentrations in the treatments at each sampling

706   time based on a two-way ANOVA (mean ± standard deviations). Different lowercase letters denote

707   significant differences (multiple comparisons with Bonferroni adjustment, $P < 0.05$) among different

708   treatments at each sampling time. SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total

709   phosphorus; Ctr, no added nitrogen or sulfur; +N, added nitrogen; +S, added sulfur; and +NS, added

710   nitrogen and sulfur. n = 288.
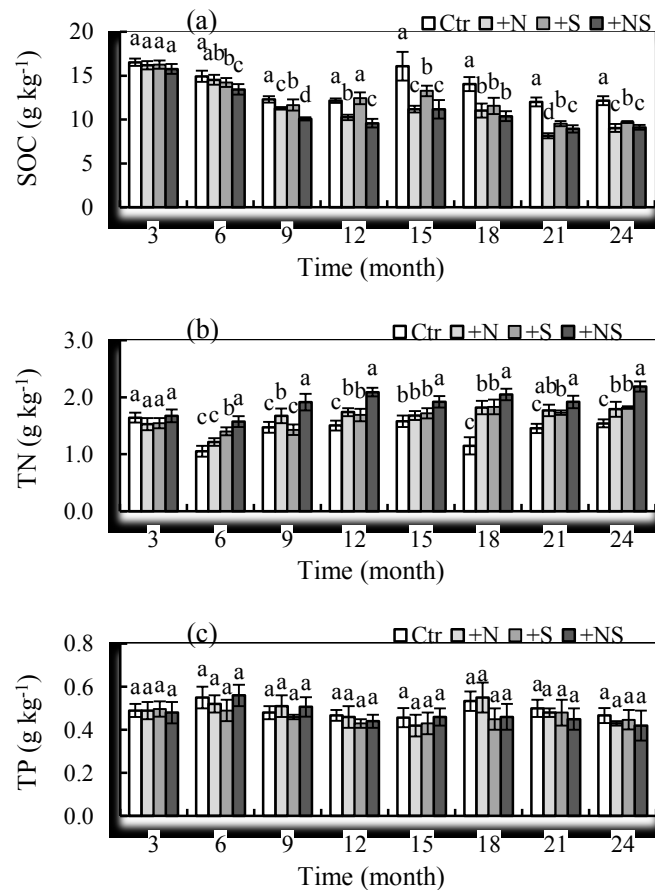

711

712

713   **Fig. S3** Effects of nitrogen and sulfur additions on the mean soil pH value during two years of

714   fertilization based on linear mixed effects models (mean ± standard deviation). Different lowercase

715   letters denote significant differences (multiple comparisons with Bonferroni adjustment, $P < 0.05$)

716   among different treatments during two years of fertilization. Ctr: no added nitrogen or sulfur; +N:

717   added nitrogen; +S: added sulfur; and +NS: added nitrogen and sulfur. n = 96.
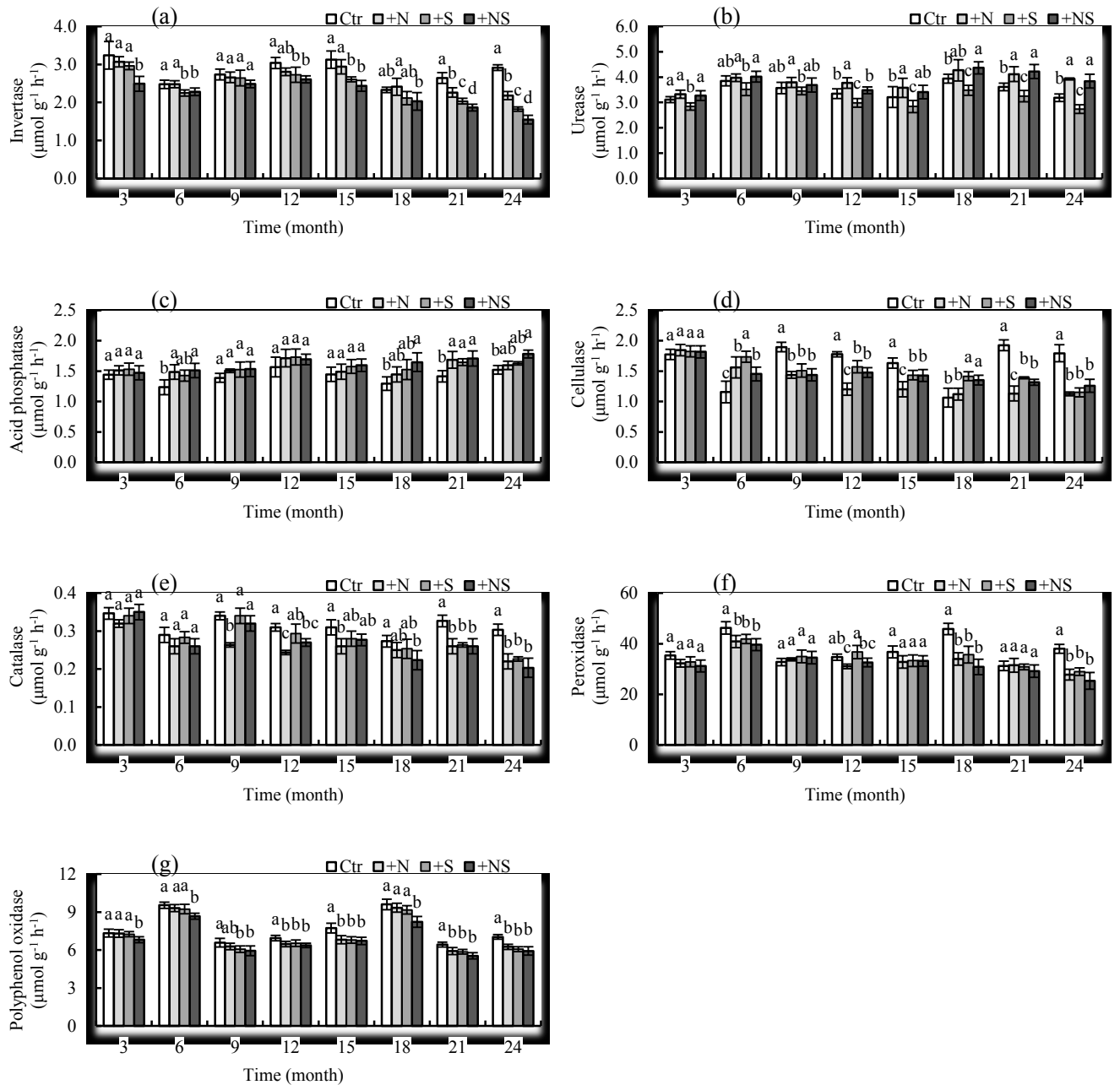

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719

720   **Fig. S4** Changes in the C/N (a), C/P (b), and N/P (c) ratios in the treatments at each sampling time

721   based on a two-way ANOVA (mean ± standard deviations). Different lowercase letters denote

722   significant differences (multiple comparisons with Bonferroni adjustment, $P < 0.05$) among different

723   treatments at each sampling time. C/N, ratio of soil organic carbon to total nitrogen concentrations;

724   C/P, ratio of soil organic carbon to total phosphorus concentrations; N/P, ratio of soil total nitrogen to

725   total phosphorus concentrations; Ctr, no added nitrogen or sulfur; +N, added nitrogen; +S, added sulfur;

726   and +NS, added nitrogen and sulfur. n = 288.
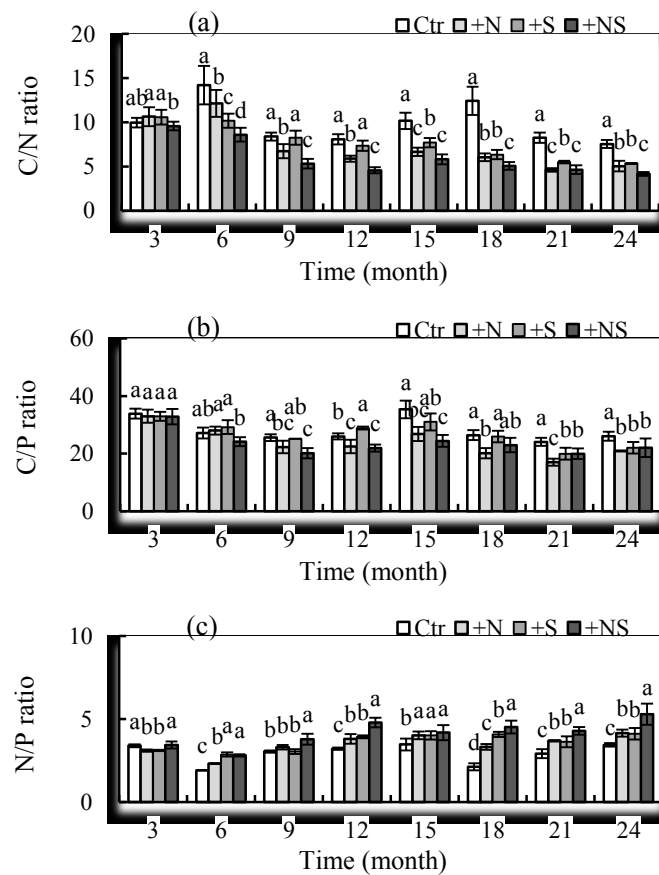
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728

729   **Fig. S5** Changes in the activities of soil invertase (a), urease (b), acid phosphatase (c), cellulase (d),

730   catalase (e), peroxidase (f), and polyphenol oxidase (g) in the treatments at each sampling time based

731   on a two-way ANOVA (mean ± standard deviations). Different lowercase letters denote significant

732   differences (multiple comparisons with Bonferroni adjustment, $P < 0.05$) among different treatments

733   at each sampling time. Ctr, no added nitrogen or sulfur, +N, added nitrogen; +S, added sulfur; and

734   +NS, added nitrogen and sulfur. n = 672.

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736

737