Agricultural and Environmental Sciences Faculty Research

Department of Agricultural and Environmental Sciences

Costimulation of soil glycosidase activity and soil respiration by nitrogen addition

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Recommended Citation

Chen, J., Luo, Y., Li, J., Zhou, X., Cao, J., Wang, R.-W., Wang, Y., Shelton, S., Jin, Z., Walker, L.M., Feng, Z., Niu, S., Feng, W., Jian, S. and Zhou, L. (2017), Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. Glob Change Biol, 23: 1328-1337. https://doi.org/10.1111/gcb.13402

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7   Co-stimulation of soil glycosidase activity and soil respiration by nitrogen addition

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9   Running title: glycosidase activity and soil respiration

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Abstract

Unprecedented levels of nitrogen (N) have been deposited in ecosystems over the past

century, which is expected to have cascading effects on microbially mediated soil

respiration (SR). Extracellular enzymes play critical roles on the degradation of soil

organic matter, and measurements of their activities are potentially useful indicators

of SR. The links between soil extracellular enzymatic activities (EEAs) and SR under

N addition, however, have not been established. We therefore conducted a

meta-analysis from 62 publications to synthesize the responses of soil EEAs and SR

to elevated N. Nitrogen addition significantly increased glycosidase activity (GA) by

13.0%, α-1,4-glucosidase (AG) by 19.6%, β-1,4-glucosidase (BG) by 11.1%,

β-1,4-xylosidase (BX) by 21.9% and β-D-cellobiosidase (CBH) by 12.6%. Increases

in GA were more evident for long duration, high rate, organic and mixed N addition

(combination of organic and inorganic N addition), as well as for studies from

farmland. The response ratios (RRs) of GA were positively correlated with the

SR-RRs, even when evaluated individually for AG, BG, BX and CBH. This positive

correlation between GA-RR and SR-RR was maintained for most types of vegetation

and soil as well as for different methods of N addition. Our results provide the first

58   evidence that GA is linked to SR under N addition over a range of ecosystems and

59   highlight the need for further studies on the response of other soil EEAs to various

60   global change factors and their implications for ecosystem functions.

61

62   **Keywords** Nitrogen addition, glycosidase activity, soil respiration, vegetation type,

63   soil type, meta-analysis

64   **Introduction**

65   Atmospheric nitrogen (N) deposition has already increased by three- to five-fold

66   compared to pre-industrial levels, and future global N deposition rates are expected to

67   increase by a factor of 2.5 over the next century (Davidson, 2009, Galloway *et al.*,

68   2008, Vitousek *et al.*, 1997). Additional N has been shown to stimulate plant growth

69   and sequestration of atmospheric CO_2 (LeBauer & Treseder, 2008, Maaroufi *et al.*,

70   2015). However, large uncertainties still remain concerning belowground C cycles,

71   since the dynamics of soil C are often mediated by many complicated processes, such

72   as soil microbial activities and extracellular enzymatic activities (EEAs) (Fog, 1988,

73   Knorr *et al.*, 2005, Lu *et al.*, 2011, Rousk *et al.*, 2016, Zhou *et al.*, 2014). More than

74   half of the C sequestered annually by terrestrial plants is ultimately allocated

75   belowground, and soils contain about four times as much C as vegetation biomass

76   (Chen *et al.*, 2015b, Vitousek *et al.*, 1997). Such large uncertainties concerning the

77   effects of N addition on belowground C cycles would therefore constitute important

78   challenges to quantifying and predicting the dynamics of terrestrial C, especially

79   under the current scenarios of global climate change (Gruber & Galloway, 2008,

80   Thomas *et al.*, 2015, Thornton *et al.*, 2007). To advance our understanding of

81   C-climate feedbacks, more information of N regulation of terrestrial C dynamics is

82   urgently needed.

83   Soil respiration (SR) represents the largest flux of C from soils to the atmosphere

84   (Chen *et al.*, 2016a, Zhou *et al.*, 2007), but the effects of N addition on SR vary

85   greatly for results from field observations (Eberwein *et al.*, 2015, Liu *et al.*, 2015),

86   models (Devaraju *et al.*, 2015, Magnani *et al.*, 2007) and meta-analyses (Janssens *et*

87   *al.*, 2010, Liu & Greaver, 2010, Lu *et al.*, 2011, Zhou *et al.*, 2014). The underlying

88   mechanisms for these divergent responses remain largely unclear. Whatever the exact

89   mechanisms, changes in microbial enzymatic activities are likely the fundamental

90   drivers of the response of SR to N addition since more than half of SR is derived from

91   enzyme-mediated decomposition of litter and soil organic matter (SOM) (Allison *et*

92   *al.*, 2010b, Carreiro *et al.*, 2000, Chen *et al.*, 2016b, Shahzad *et al.*, 2015, Waldrop *et*

93   *al.*, 2004). It is therefore likely that understanding how soil EEAs respond to N

94   addition could provide novel ways to reconcile the divergent responses of SR to N

95   addition (Allison *et al.*, 2008, Stone *et al.*, 2014, Weedon *et al.*, 2011), a concept also

96   suggested by several recent reviews (Fog, 1988, Janssens *et al.*, 2010, Knorr *et al.*,

97   2005). Evidence from recent enzymatic kinetics-based modeling efforts also

98   supported that inclusion of soil EEAs into models have greatly improved the estimates

99   of SR and C-climate feedbacks (Ali *et al.*, 2015, Allison *et al.*, 2010b). Therefore, it is

100   clear that our understanding of the effects of N addition on SR will greatly benefit

101   from the study of soil EEAs.

102   Soil EEAs are commonly regarded as potential indicators of microbial nutrient

103   requirements and depolymerization rates of SOM, yet their activities largely depend

104   upon nutrient availability (Sinsabaugh *et al.*, 2008, Treseder, 2004, Waldrop *et al.*,

105   2004). Nitrogen limitation is widespread in terrestrial ecosystems (LeBauer &

106   Treseder, 2008), soil microorganisms and soil EEAs are therefore highly sensitive to

107   increased levels of N (Allison *et al.*, 2008, Treseder, 2008). The effects of N addition

108   on soil EEAs, however, are highly variable between individual studies (Currey *et al.*,

109   2010, Cusack *et al.*, 2010), which have greatly hindered our understanding of the

110   possible mechanisms driving the response of soil EEAs following N addition. It has

111   been reported that soil EEAs responded positively to N addition in farmland and

112   grassland (Nowinski *et al.*, 2009, Zeglin *et al.*, 2007), but the responses were neutral

113   or negative in temperate and boreal forests (Allison *et al.*, 2010a, Janssens *et al.*,

114   2010). The lack of consistency suggests the importance of evaluating the effects of N

115   addition on EEAs and SR in specific types of ecosystems (Schmidt *et al.*, 2011).

116   Addition of organic or combination of organic and inorganic N (mixed N addition)

117   generally had positive effects on soil EEAs, while NH_4^+ or NO_3^- addition had

118 negative impacts (Du *et al.*, 2014, Li *et al.*, 2014b). It stands to reason that the forms

119 of added N should be reflected in soil N availability or pH, and that each of these

120 effects should influence soil EEAs and SR (Sinsabaugh *et al.*, 2008). Therefore, it is

121 critically necessary to synthesize results from a variety of studies to accurately

122 characterize the principle effects of N addition on soil EEAs and their possible

123 impacts on SR.

124 We conducted a meta-analysis on the responses of soil EEAs and SR to N

125 addition. Our goal was to focus more broadly on the links between soil EEAs and SR

126 rather than evaluating them separately. The objectives of this study were (1) to test

127 whether the response of SR to N addition could be linked to the responses of soil

128 EEAs, (2) and if so, to investigate the responses of these EEAs to the additional N in

129 various types of ecosystems and the different methods of N addition and (3) to further

130 explore the factors affecting the possible links between soil EEAs and SR, such as

131 substrate N availability and pH.

132

133 **Materials and methods**

134 **Sources of data**

135 We searched journal articles published before October 2015 using the Web of Science

136 (http://apps.webofknowledge.com/). The keywords and phrases used for the literature

137 search were (1) "nitrogen deposition" OR "nitrogen addition" OR "nitrogen

138 enrichment" OR "nitrogen fertilizer" OR "nitrogen amendment" OR "nitrogen

139 elevated", AND (2) "soil extracellular enzymes", AND (3) "terrestrial" OR "soil" OR

140 "land".

141 Articles were selected based on the following criteria. (1) Only field experiments

142 were included, and we selected only studies with durations longer than 1 y. Control

143 and N-addition treatments had to be at the same experimental site, i.e. the

144 microclimate and vegetation and soil types had to be similar between treatments. (2)

145 Standard deviations (SDs) and numbers of replicates were reported. If standard errors

146 (SEs) were reported, the following equation was used to estimate SDs:

147     $SD = SE \times \sqrt{n}$ (1),

148 where n is the number of replicates. (3) The methods of N addition (rate, frequency,

149 form and duration) were clearly described. (4) If more than one field manipulation

150 experiment was reported in the same article but for different environmental variables

151 or types of ecosystem (e.g. experiments conducted under various types of vegetation

152 and soil), each experiment was treated as an independent study. (5) If parameters were

153 measured more than once in the same year, we chose only the last set of

154 measurements. (6) If the results were reported for different soil layers, we included

155 only the uppermost soil layer (García-Palacios *et al.*, 2014). We selected a total of 62

156 publications based on these criteria (Supplementary Notes (iii) and Fig. S1).

157

158 **Data acquisition**

159 For each of the selected studies, we recorded the study site, location, vegetation type,

160 soil type (http://www.fao.org/about/en/), N-addition rate (low < 5 g N m^{-2} y^{-1}, medium

161 $5–15$ g N m^{-2} y^{-1} and high > 15 g N m^{-2} y^{-1}), N addition duration (short < 5 y, medium

162 $5–10$ y and long >10 y), N-addition frequency (low < 4 y^{-1}, medium $4–12$ y^{-1} and

163 high > 12 y^{-1}), N-addition form (NH_4^+, NO_3^-, NH_4NO_3, organic N or mixed N) and

164 soil EEAs. Wherever possible, we also recorded SR, soil pH, soil total N, soil

165 dissolved organic N (DON), substrate C: N ratios and aboveground biomass. We

166 defined SR as the amount of soil CO_2 measured by soil chambers in the field studies

167 or during laboratory incubations (Chen *et al.*, 2015a, Treseder, 2008). If the results

168 were presented graphically, we used Engauge Digitizer 4.1

169 (http://digitizer.sourceforge.net) to digitize and extract the data. The authors were

170 contacted when critical information could not be directly acquired from the selected

171 articles or their references.

172

173 **Data analysis**

174 The effects of N addition were evaluated using the response ratio (RR), which was

175 calculated as:

176 $$RR = \ln\left(\frac{\overline{X_N}}{\overline{X_C}}\right) = \ln\left(\overline{X_N}\right) - \ln\left(\overline{X_C}\right) \tag{2},$$

177 where $\overline{X_N}$ and $\overline{X_C}$ are the arithmetic mean concentrations of the soil EEAs in the

178 experimental (N addition) and control treatments, respectively. The statistical

179 distribution of the RRs calculated in this way was found to be nearly normally

180 distributed, and only minor biases were detected (Hedges *et al.*, 1999). The variances

181 (*v*) were calculated by:

182 $$v = \frac{s_N^2}{n_N \overline{X_N^2}} + \frac{s_C^2}{n_C \overline{X_C^2}} \tag{3},$$

183 where n_N and n_C are the replicate numbers of experimental and control treatments,

184 respectively, and S_N and S_C are the SDs for the experimental and control treatments,

185 respectively. The reciprocal of the variance was used as the weight (w) for each RR.

186 The overall mean response ratio (RR_{++}) was calculated from the individual RRs for

187 the experimental and control treatments:

188 $$RR_{++} = \frac{\sum_{i=1}^{m} \sum_{j=1}^{k} w_{ij} RR_{ij}}{\sum_{i=1}^{m} \sum_{j=1}^{k} w_{ij}} \tag{4},$$

189 where *m* is the number of compared groups and *k* is the number of comparisons in

190 the corresponding groups. The SE of RR_{++} was estimated by:

191 $$\text{SE}(RR_{++}) = \sqrt{\frac{1}{\sum_{i=1}^{m} \sum_{j=1}^{k} w_{ij}}} \tag{5}.$$

192 To determine whether N addition had a significant effect, we applied a random model

193 using MetaWin (Sinauer Associates Inc., Sunderland, USA), in which bootstrap 95%

194 confidence intervals (CIs) were calculated for each categorical group. The effect of a

195 treatment was deemed significant if the bootstrap CI did not overlap with zero.

196 Changes induced by N addition were calculated by:

197 $$[\exp(RR_{++}) - 1] \times 100\% \tag{6}.$$

198 Pearson correlation analyses were used to evaluate the relationships between the RRs

199 for the soil EEAs and (1) environmental variables, (2) N-addition methods and (3)

200 substrate N concentrations.

201 The total heterogeneity (Q_T) for each categorical group was divided into

202 heterogeneities among groups (Q_M) and between groups (Q_B). The chi-square

203 distribution for these Q statistics was approximately normal in previous studies (Liu

204 & Greaver, 2009, Treseder, 2008), which allowed us to test our null hypothesis that all

205 RRs were equal within a categorical group. The statistical confidence level was set at

206 $p < 0.05$.

207 The frequency distribution of the individual RRs of each enzyme was tested by:

208 $y = \alpha\exp\left[-\frac{(x-\mu)^2}{2\sigma^2}\right]$ (7),

209 where y is the frequency, x is the RR of each enzyme, α is a coefficient for the

210 expected number of RR values at $x = \mu$, and μ and σ are the mean and variance of

211 the frequency distributions of RR, respectively. The frequency distributions are shown

212 in Fig. S2.

213 Due to the preferential publication of larger over smaller effects, we used a series

214 of statistical measures to evaluate publication bias within each group (Table S1).

215 Kendall's tau rank correlation and Spearman's rank correlation were used to test the

216 rank correlations between replicate numbers of each study and the standardized effect

217 size (Begg, 1994, Rosenberg *et al.*, 2000). A fail-safe number (Rosenthal's method at

218 $\alpha = 0.05$) was next calculated to determine the number of non-significant, unpublished

219 studies that would be needed to change the summary of results from significant to

220 non-significant (LeBauer & Treseder, 2008, Rosenthal, 1979).

221

222 **Results**

223 *Effects of N addition on glycosidase activity and SR*

224 Our preliminary analysis of 12 kinds of soil extracellular enzymes showed that

225 SR-RRs were only positively correlated with RRs for α-1,4-glucosidase (AG),

226 β-1,4-glucosidase (BG), β-1,4-xylosidase (BX) and β-D-cellobiosidase (CBH)

227 activities (Table S2 and Fig. S3), but no correlation was found for the other 8 kinds of

228 EEAs. In addition, there was no between-group heterogeneity among the four kinds of

229 glycosidase and RRs for glycosidase activity (GA-RRs) did not vary with sample size

230   (Figs. 1 and S4). We therefore focused on the responses of GA to N addition and the

231   factors affecting the relationships between the SR-RRs and GA-RRs.

232   Nitrogen addition significantly increased GA by an average of 13.0% (Fig. 1). In

233   detail, N addition profoundly enhanced AG, BG, BX and CBH activities by 19.6, 11.1,

234   21.9 and 12.6%, respectively. GA-RRs were positively correlated with SR-RRs (Fig.

235   1), and this relationship was independent of SR measurement methods (Fig. S5).

236

237   *Methods of N addition*

238   NH_4NO_3, organic and mixed N addition significantly increased GA by 13.2, 18.8 and

239   16.5%, respectively, but the addition of NH_4^+ or NO_3^- had no effects on GA (Fig. 2).

240   Positive correlations between GA-RRs and SR-RRs were consistently identified when

241   N was added as NH_4NO_3, organic or mixed forms (Fig. 3).

242   We found a significant positive linear relationship between GA-RRs and duration

243   of N addition (Fig. S4). Specifically, short, medium and long durations of N addition

244   significantly increased GA by 6.5, 11.2 and 46.6%, respectively (Fig. 2). Similarly,

245   the positive correlations between GA-RRs and SR-RRs were maintained for all

246   durations of N addition (Fig. 3).

247   Low, medium and high rates of N addition increased GA by 8.0, 11.7 and 20.8%,

248   respectively (Fig. 2), and GA-RRs were positively correlated with N-addition rates

249   (Fig. S4). The positive correlations between GA-RRs and SR-RRs held true for

250   medium and high rates but not for a low rate of N addition (Fig. 3).

251   Low, medium and high frequencies of N addition increased GA by 13.2, 15.0 and

252   1.6%, respectively. No between-group heterogeneity was found when studies were

253   grouped by the frequency of N addition, and no clear relationship was detected

254   between GA-RRs and frequency of N addition (Figs. 2 and S4). GA-RRs were

255   positively correlated with SR-RRs for low and medium frequencies of N addition, but

256   this analysis was limited by the paucity of data for treatments with a high frequency

257   of N addition (Fig. 3).

258

259   *Vegetation and soil types*

260 The responses of GA to N addition varied significantly among the types of vegetation

261 and soil (Fig. 4). Nitrogen addition significantly increased GA by 34.7, 6.4, 5.5 and

262 19.9% for farmland, forest, grassland and shrubland, respectively, but N addition had

263 no effect on GA in wetland. GA-RRs were positively correlated with SR-RRs for

264 grassland and farmland but not for forest (Fig. 5). No SR data were available for

265 wetland and shrubland, so we could not evaluate the links between GA and SR for

266 those systems.

267 Nitrogen addition significantly increased GA in Chernozems, Cambisols,

268 Gleysols, Entisols, Histosols, Podzols, Lithosols and Luvisols by 38.4, 13.8, 46.4, 8.6,

269 17.5, 10.2, 15.7 and 27.2%, respectively. In contrast, N addition decreased GA by 5.2%

270 in Ferralsols and had no effects on GA in Solonchaks and Arenosols (Fig. 4).

271 Significant positive relationships between the GA-RRs and SR-RRs were found for

272 Cambisols and Luvisols, but the relationship was negative for Podzols (Fig. 5). The

273 small numbers of studies of Solonchaks, Entisols and Ferralsols, however, limited the

274 statistical power of the analysis.

275

276 **Discussion**

277 This meta-analysis is among the first global syntheses of the effects of N addition on

278 soil EEAs and their impacts on SR. The objectives herein go beyond separate

279 assessments of soil EEAs and SR; rather, we address the broader question of whether

280 the effects of N addition on soil EEAs can help to explain the enhanced SR reported

281 in previous meta-analyses (Fig. S6) (Lu *et al.*, 2011, Zhou *et al.*, 2014). Our results

282 show that SR-RRs are closely correlated with GA-RRs, and this correlation still holds

283 true when evaluated for most types of vegetation and soil, as well as for different

284 methods of N addition. These results suggest that the effects of N addition on four

285 kinds of glycosidase are likely the effective proximate agents of the effects of N

286 addition on SR (Fig. 1). But, what is the basis for linking the four kinds of glycosidase

287 with SR under N addition? Preliminary analysis of 12 kinds of soil EEAs that were

288 frequently investigated in previous studies (Table S2), indicate that only the RRs for

289 AG, BG, BX and CBH are closely correlated with SR-RRs (Fig. S3). These four kinds

290 of glycosidase are all classified as hydrolysis C-targeting enzymes. Our results

291 suggest that shifts in the activities of various kinds of soil extracellular enzymes likely

292 account for the highly divergent responses of SR to N addition.

293

294 *Co-stimulation of GA and SR by N addition*

295 Nitrogen addition significantly increased GA, and the stimulatory effects were

296 positively correlated with SR-RRs (Fig. 1). The added N not only provides the

297 building blocks for enzymatic production because enzymes are fundamentally N-rich

298 molecules, but it also increases microbial C demands due to stoichiometry of

299 microbial nutrients (Allison *et al.*, 2008, Sistla & Schimel, 2013, Weedon *et al.*, 2011).

300 Increases in microbial C demands were expected to be alleviated by promoting the

301 activities of C-degrading enzymes (Buchkowski *et al.*, 2015). This expectation is

302 consistent with the typical economic strategy in which soil microbes would adjust

303 themselves in physiology or community composition in order to produce enzymes

304 needed for acquiring the most limited resources (Sinsabaugh *et al.*, 2008, Stone *et al.*,

305 2012). Despite the fact that various kinds of C-degrading enzymes that can contribute

306 to alleviating microbial C demands, N addition had negative effects on the oxidative

307 lignin-degrading enzymes (Fig. S7). Therefore, C requirements of microbes may be

308 mainly fulfilled by synthesizing glycosidase (Fig. 1), which would accelerate the

309 hydrolysis of cellulolytic compounds.

310

311 *Factors that affect the relationships between GA and SR*

312 Higher substrate N concentrations lead to a more pronounced positive response of GA

313 (Fig. S10), suggesting that high N availability enhances microbial C demands

314 (Nguyen *et al.*, 2016, Treseder, 2008). Longer durations and higher rates of N addition

315 can produce high and stable N concentrations (Bragazza *et al.*, 2012), so the positive

316 responses of GA and the steeper slopes between GA-RRs and SR-RRs under these

317 two conditions were not surprising (Figs. 2 and S4). In farmland, N is often added at

318 high rates through anthropogenic fertilization (Chang *et al.*, 2007, McDaniel *et al.*,

319 2014). Cambisols and Luvisols are among the most productive soils on Earth, and

320 these soils make good agricultural lands given their high soil fertility (i.e. N content).

321 Consistently, we also observed positive responses of GA and positive correlations

322 between GA-RRs and SR-RRs in these systems (Figs. 4 and 5).

323   We found a more pronounced increase for GA in farmland (34.7%) than the other

324 types of vegetation and soil (Fig. 2). In addition to the higher N rates discussed above,

325 another possible explanation might be related to the forms of N addition, because 92%

326 of the added N in farmland was in organic or mixed N forms (Table S3). Organic and

327 mixed N must be transformed or converted by enzymes and so they had positive

328 effects on GA and SR (Figs. 2 and 3) (Ajwa *et al.*, 1999, Hawkins *et al.*, 2000).

329 Considering the current ongoing intensified anthropogenic fertilization with organic

330 and mixed N (McDaniel *et al.*, 2014), our results indicate that there might be a

331 stronger positive response of SR in farmland under future N fertilization.

332   We did not find significant responses of GA for Ferralsols, Solonchaks,

333 Arenosols and wetlands. These types of soils have relatively low pHs due to the high

334 concentrations of iron and aluminum (Chesworth, 2008). Soil nutrient availability and

335 microbial activities may thus be constrained by the low pH or possibly other chemical

336 properties (Evans *et al.*, 2008, Tian & Niu, 2015). This explanation was supported by

337 our regression analysis which indicates that GA-RRs are closely correlated with soil

338 pH (Fig. S11). A comprehensive assessment of the relationships between GA-RRs and

339 SR-RRs under these conditions are currently not feasible due to the small data set, but

340 other studies have shown that N addition had negative or no effects on SR under these

341 conditions (Tao *et al.*, 2013, Wang *et al.*, 2013, Zhou *et al.*, 2014). Our results suggest

342 that soil pH should be considered when implementing GA-RRs as the proximate

343 agents of SR-RRs.

344   GA-RRs were not correlated with SR-RRs for forests, Podzols or low rates of N

345 addition, even though GA did respond positively to N addition in these cases (Figs.

346 2-5). This finding for forests was consistent with two recent meta-analyses indicating

347 that N addition repressed SR (Janssens *et al.*, 2010, Zhou *et al.*, 2014), perhaps

348 because degradation of the low-quality forest litter responded negatively to the

349 additional N (Fog, 1988, Knorr *et al.*, 2005). Podzols are typical in coniferous and

350 temperate forests, and most podzols are not suitable for agriculture due to their low

351 water-retention capacity and low pH (Chesworth, 2008). These two characteristics of

352 Podzols were likely responsible for the negative effects of N addition on SR. The lack

353 of correlation between GA-RRs and SR-RRs with a low rate of N addition may be

354 due in part to undetectable effects caused by the small amount of additional N

355 (Bowden *et al.*, 2004, Knorr *et al.*, 2005). These results would therefore provide

356 valuable information for Earth system models when models are to be applied to

357 various ecosystem types.

358

359 *Uncertainties and implications*

360 The GA-RRs were on average 2.5 times higher than SR-RRs (Fig. 1), suggesting that

361 N-induced changes in SR might not be the direct result of changes in microbial

362 growth. Assuming that enzyme expression is directly related to production rate, these

363 results imply that N addition increases microbial carbon-use efficiency (CUE)

364 (Allison *et al.*, 2010b). Alternatively, microbial growth and death rates could respond

365 differentially to the elevated N (Hagerty *et al.*, 2014, Treseder, 2008). Nonetheless,

366 the responses of microbial CUE and growth and death rates to N addition are

367 currently unclear. Major limitations also may stem from the paucity of data for the

368 various types of vegetation and soil, such as those in arid and semiarid regions and

369 wetlands (Table S3). Future field experiments should be conducted with a wide range

370 of variables to clarify the underlying principles.

371   The highly consistent responses of GA and SR to N addition identified here,

372 support the utility of explicitly incorporating microbial activities and soil EEAs into

373 models for predicting the dynamics of soil C under various global change scenarios

374 (Allison *et al.*, 2010b, Wieder *et al.*, 2013). Actually, several recent modeling efforts

375 have confirmed that incorporation of soil EEAs into enzymatic-kinetics models have

376 substantially improved the projection of both the direction and magnitude of

377 C-climate feedbacks (Ali *et al.*, 2015, Allison *et al.*, 2010b, Li *et al.*, 2014a, Xu *et al.*,

378 2014). Our results also have important implications for other global change factors,

379 for example, climatic warming. Warming has globally increased substrate N

concentrations, aboveground biomass, SR (Bai *et al.*, 2013, Lu *et al.*, 2013), and it is

likely that warming will also lead to increases in GA, as discussed above. This

extrapolation seems reasonable, but we are well aware that ecosystems are often

highly heterogeneous and may respond in unexpected ways to environmental changes.

Our meta-analysis is a first attempt at linking GA to SR under N addition, and future

studies are clearly needed to determine how other soil EEAs and associated ecosystem

functions respond to various global change factors.

Competing financial interests: The authors declare no competing financial interests

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574

575

576   Figure captions:

577

578   Figure 1. The effects of N addition on soil glycosidase activity (a, GA) and the

579   relationships between the response ratio (RR) for soil respiration and the RR for

580   glycosidase activity following N addition (b). Error bars represent bootstrap 95%

581   confidence intervals (CIs). The effect of N addition was considered significant if the

582   CI of the effect size did not overlap with zero. The sample size for each variable is

583   shown next to the CI. Q_B and Q_w are defined in the Materials and methods section.

584   The RRs of the activities of the following enzymes were positively correlated with

585   RR-soil respiration: α -1,4-glucosidase (AG, $Y = 0.763X - 0.037$, $R^2 = 0.423$, $p <$

586   0.001), β-1,4-glucosidase (BG, $Y = 0.323X - 0.063$, $R^2 = 0.255$, $p < 0.001$),

587   β-D-cellobiosidase (CBH, $Y = 0.2X - 0.009$, $R^2 = 0.145$, $p = 0.003$) and

588   β-1,4-xylosidase (BX, $Y = 0.515X - 0.134$, $R^2 = 0.473$, $p < 0.001$).

589

590   Figure 2 The effects of nitrogen addition on soil glycosidase activity for various

591   methods of nitrogen addition. See Figure 1 for detailed information.

592

593   Figure 3 Relationships between the response ratios (RRs) for soil respiration and the

594   RRs for soil glycosidase activity for the methods of nitrogen addition. Relationships

595   between the RRs for glycosidase activity and RR-soil respiration for (a) N-addition

596   rate (medium: $Y = 0.442X - 0.014$, $R^2 = 0.760$, $p < 0.001$; high: $Y = 0.583X + 0.095$,

597     $R^2 = 0.286$, $p = 0.027$), (b) N-addition frequency (low: $Y = 0.418X + 0.053$, $R^2 =$

598   0.548, $p < 0.001$; medium: $Y = 0.480X + 0.083$, $R^2 = 0.430$, $p = 0.001$), (c) N-addition

599   duration (short: $Y = 0.641X + 0.051$, $R^2 = 0.473$, $p < 0.001$; medium: $Y = 0.206X -$

600   0.068, $R^2 = 0.552$, $p < 0.001$; long: $Y = 0.550X - 0.118$, $R^2 = 0.764$, $p < 0.001$) and (d)

601   N-addition form (NH_4NO_3: $Y = 0.253X - 0.135$, $R^2 = 0.185$, $p < 0.001$; organic: $Y =$

602     $0.396X + 0.044$, $R^2 = 0.727$, $p < 0.001$; mixed: $Y = 0.733X + 0.095$, $R^2 = 0.436$, $p =$

603   0.002).

604

605   Figure 4 The effects of nitrogen addition on soil glycosidase activity for various types

606   of vegetation and soil. See Figure 1 for detailed information.

607

608   Figure 5 Relationships between the response ratios (RRs) for soil respiration and the

609   RRs for glycosidase activity for different types of (a) vegetation and (b) soil.

610   Relationships between the RRs of glycosidase activity and RR-soil respiration within

611   vegetation types (grassland: $Y = 0.885X - 0.103$, $R^2 = 0.626$, $p = 0.019$; farmland: $Y =$

612     $0.355X + 0.170$, $R^2 = 0.532$, $p < 0.001$) and soil types (Cambisols: $Y = 0.487X - 0.079$,

613     $R^2 = 0.640$, $p < 0.001$; Luvisols: $Y = 1.340X - 0.158$, $R^2 = 0.638$, $p = 0.031$; Podzols: Y

614     $= -0.295X + 0.013$, $R^2 = 0.420$, $p = 0.004$).

615

616   Supplementary tables and figures:

617

618   Table S1 Results for publication bias.

619

620   Table S2 Description of the 12 kinds of enzymes included in our preliminary analysis.

621

622   Table S3 Distribution of the methods of nitrogen addition for the various types of

623   vegetation and soil.

624

625   Figure S1 Global distribution of the nitrogen-additon experiments selected in this

626   meta-analysis. The map was created with ArcGIS.

627

628   Figure S2 Frequency distributions of the response ratios (RR) of (a) α-1,4-glucosidase

629   (AG), (b) β-1,4-glucosidase (BG), (c) β-D-cellobiosidase (CBH) and (d)

630   β-1,4-xylosidase (BX).

631

632   Figure S3 Relationships between the response ratio (RR) of soil respiration (SR) and

633   the RRs of (a) α-1,4-glucosidase (AG), (b) β-1,4-glucosidase (BG), (c)

634   β-D-cellobiosidase (CBH), (d) β-1,4-xylosidase (BX), (e) phenol oxidase (PO), (f)

635   polyphenol oxidase (PHO), (g) invertase, (h) urease, (i) peroxidase (PER), (j)

636   β-1,4-N-acetylglucosaminidase (NAG), (k) acid (alkaline) phosphatase (AP) and (l)

637   leucine amino peptidase (LAP).

638

639   Figure S4 Relationships between the response ratio (RR) of soil glycosidase activity

640   and (a) N-addition rate, (b) N-addition duration, (c) N-addition frequency and (d)

641   sample size.

642

643   Figure S5 Relationships between the response ratio (RR) of glycosidase activity and

644   the RR of soil respiration (SR) for the different methods of SR measurement.

645

646   Figure S6 The effects of N addition on soil respiration from previous meta-analyses.

647   Error bars represent bootstrap 95% confidence intervals (CIs). The effect of N

648   addition was considered significant if the CI of the effect size did not overlap zero.

649   The sample size for each variable is shown next to the CI. This figure was redrawn

650 from previous meta-analyses published by (a, b and c) Zhou *et al.* 2014, (d) Liu *et al.*

651 2010, (e) Lu *et al.* 2011 and (f) Janssens *et al.* 2010. Ra, autotrophic respiration; Rh,

652 heterotrophic respiration; SR, soil respiration.

653

654 Figure S7 (a) The effects of N addition on the activities of soil oxidative C-acquiring

655 enzymes. Frequency distributions of the response ratios (RR) of (b) oxidative

656 enzymes, (c) phenol oxidase (PO), (d) peroxidase (PER) and (e) polyphenol oxidase

657 (PHO). Error bars represent bootstrap 95% confidence intervals (CIs). The effect of N

658 addition was considered significant if the CI of the effect size did not overlap zero.

659 The sample size for each variable is shown next to the CI. Q_B and Q_w are defined in

660 the Materials and methods section.

661

662 Figure S8 Relationships between the response ratio (RR) of glycosidase activity and

663 the RR of aboveground biomass (AGB).

664

665 Figure S9 Relationships between the possible changes in microbial communities and

666 physiology and the response ratios (RR) of glycosidase activity of (a) microbial

667 abundance, (b) bacterial abundance, (c) fungal abundance, (d) fungi/bacteria, (e)

668 microbial biomass carbon (MBC), (f) microbial biomass nitrogen (MBN) and (g)

669 MBC/MBN. The relationships between the changes in microbial communities and

670 physiology induced by N addition and their links with the corresponding changes in

671 soil respiration were synthesized by Treseder *et al.* (2008).

672

673 Figure S10 Relationships between the response ratio (RR) of glycosidase activity and

674 the (a) RR of soil total nitrogen (STN), (b) RR of dissolved organic nitrogen (DON),

675 (c) RR of the substrate C:N ratio and (d) substrate C:N ratio.

676

677 Figure S11 Relationships between the response ratio (RR) of glycosidase activity and

678 (a) the substrate pH and (b) the RR of the substrate pH.

(a) $Q_B = 6.831$, $Q_W = 1189.381$, $p = 0.328$

GA — 496
AG — 31
BG — 238
CBH — 179
BX — 48

Weighted response ratio

(b) $Y = 0.455\ X - 0.034$
$R^2 = 0.509$
$p < 0.001$

RR-Soil respiration

AG
BG
CBH
BX

RR-glycosidase activities

Figure with x-axis "Weighted response ratio" ranging from -0.1 to 0.5.

Form — $Q_B = 36.467$, $Q_W = 1192.884$, $p = 0.015$
- NO3-: 71
- NH4+: 16
- NH4NO3: 186
- Organic: 111
- Mixed: 110

Duration — $Q_B = 132.282$, $Q_W = 1146.690$, $p = 0.001$
- Short duration: 292
- Medium duration: 109
- Long duration: 87

Frequency — $Q_B = 4.218$, $Q_W = 1151.271$, $p = 0.561$
- Low frequency: 303
- Medium frequency: 138
- High frequency: 3

Rate — $Q_B = 242.600$, $Q_W = 912.986$, $p = 0.001$
- Low rate: 174
- Medium rate: 265
- High rate: 51