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1   **Temperature sensitivities of extracellular enzyme Vmax and Km across thermal**
2   **environments**
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4   Running head: Temperature sensitivities of Vmax and Km
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Abstract

The magnitude and direction of carbon cycle feedbacks under climate warming remain uncertain

due to insufficient knowledge about the temperature sensitivities of soil microbial processes.

Enzymatic rates could increase at higher temperatures, but this response could change over time

if soil microbes adapt to warming. We used the Arrhenius relationship, biochemical transition

state theory, and thermal physiology theory to predict the responses of extracellular enzyme

Vmax and Km to temperature. Based on these concepts, we hypothesized that Vmax and Km

would correlate positively with each other and show positive temperature sensitivities. For

enzymes from warmer environments, we expected to find lower Vmax, Km, and Km temperature

sensitivity but higher Vmax temperature sensitivity. We tested these hypotheses with isolates of

the filamentous fungus *Neurospora discreta* collected from around the globe and with

decomposing leaf litter from a warming experiment in Alaskan boreal forest. For *Neurospora*

extracellular enzymes, Vmax Q_{10} ranged from 1.48 to 2.25, and Km Q_{10} ranged from 0.71 to

2.80. In agreement with theory, Vmax and Km were positively correlated for some enzymes, and

Vmax declined under experimental warming in Alaskan litter. However, the temperature

sensitivities of Vmax and Km did not vary as expected with warming. We also found no

relationship between temperature sensitivity of Vmax or Km and mean annual temperature of the

isolation site for *Neurospora* strains. Declining Vmax in the Alaskan warming treatment implies

a short-term negative feedback to climate change, but the *Neurospora* results suggest that

climate-driven changes in plant inputs and soil properties are important controls on enzyme

kinetics in the long term. Our empirical data on enzyme Vmax, Km, and temperature sensitivities

should be useful for parameterizing existing biogeochemical models, but they reveal a need to

develop new theory on thermal adaptation mechanisms.

Introduction

By 2100, human-caused emissions of greenhouse gases are expected to warm the planet by 3-5°C with larger increases over the land surface (IPCC, 2013). This warming will influence biological processes and ecosystems, potentially leading to feedbacks that mitigate or exacerbate greenhouse gas levels and the ultimate magnitude of planetary warming. Given that soils contain around three times as much carbon as the atmosphere (Jobbágy & Jackson, 2000), feedbacks involving soil carbon are particularly important for future climate projections. Yet these feedbacks are poorly resolved and remain difficult to predict (Todd-Brown *et al.*, 2014).

Losses of soil carbon under warming are a major source of uncertainty in climate feedbacks (Carey *et al.*, 2016; Crowther *et al.*, 2016). Although many processes contribute to these losses, microbially-driven decomposition of soil organic matter is expected to increase with warming due to the positive temperature sensitivity of most biochemical reactions (Davidson & Janssens, 2006). In particular, the degradation of complex organic molecules depends on microbial extracellular enzymes with temperature-sensitive kinetic properties (Wallenstein *et al.*, 2011; Steinweg *et al.*, 2012). For example, glycoside hydrolases such as α-glucosidase, β-glucosidase, cellobiohydrolase, and β-xylosidase degrade starch, cellulose, and hemicellulose (Burns *et al.*, 2013). Other enzymes such as *N*-acetyl-glucosaminidase, leucine-aminopeptidase, and phosphatase target organic forms of nitrogen and phosphorus, while oxidative enzymes degrade aromatic polymers (Sinsabaugh, 1994, 2010).

Although enzyme kinetics have long been studied by biochemists in the laboratory (Somero, 1978), the temperature sensitivity of soil enzymes remains unclear. Changes in temperature may affect the kinetic properties of individual enzymes, the expression of isozymes with different kinetic properties by specific organisms, and the relative abundances of microbes

76   expressing different enzymes (Davidson & Janssens, 2006; Bradford, 2013). Any combination of

77   these factors may influence observed kinetic properties and their temperature sensitivities within

78   a given environment and in response to temperature change.

79   There is a rich theoretical literature on the kinetics and thermodynamics of enzyme-

80   catalyzed reactions. Many enzymes are assumed to follow Michaelis-Menten kinetics that

81   describe the reaction velocity (V):

82    $V = V_{max}[S]/(K_m + [S])$ [eq. 1]

83   where V_{max} is the maximum reaction velocity, [S] is substrate concentration, and K_m is the

84   half-saturation constant (i.e. substrate concentration at which V is one-half V_{max}). Note that K_m

85   appears in the denominator of the Michaelis-Menten equation, so increases in K_m lead to lower

86   reaction velocities. For purified enzymes, V_{max} is proportional to $k_{cat}*[E]$ where k_{cat} is the

87   catalytic turnover rate (number of substrate molecules converted to product per enzyme per unit

88   time), and [E] is the enzyme concentration. In enzyme mixtures, such as soils or culture fluid,

89   measured V_{max} and K_m depend on the proportions of individual enzymes with different kinetic

90   properties (Wallenstein *et al.*, 2011).

91   Transition state theory provides a framework for predicting how V_{max} and K_m respond

92   to warming (Fig. 1). According to this theory, enzyme catalysis requires enzyme-substrate

93   binding, formation of an enzyme-substrate activated complex, and product formation and

94   dissociation. Each step involves a change in Gibbs free energy:

95    $\Delta G_{ES} = \Delta H_{ES} - T\Delta S_{ES}$ for substrate binding [eq. 2]

96   and

97    $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$ for formation of the activated complex [eq. 3]

4

98   where ΔH is the change in enthalpy (heat of reaction), ΔS is the change in entropy (a metric of

99   disorder), and T is temperature. In a typical enzymatic reaction (Fig. 1), ΔG_{ES} is negative and

100   $\Delta G^{\ddagger}$ is positive.

101   Vmax and k_{cat} are governed by formation of the activated complex and depend on

102   temperature through the Arrhenius relationship (Davidson & Janssens, 2006):

103   $k_{cat} = A*exp(-Ea/RT)$ [eq. 4]

104   where A is a pre-exponential factor, Ea is activation energy, and R is the ideal gas constant.

105   Arrhenius and transition state theory are related because $Ea = \Delta H^{\ddagger} + RT$, and $\Delta S^{\ddagger}$ influences the

106   pre-exponential factor (see Lonhienne et al. (2000) for details). Reaction rates increase as Ea

107   declines or as temperature increases due to an increasing fraction of reactants with sufficient

108   energy to form an activated complex.

109   With long-term warming, as expected under climate change, Vmax and its temperature

110   sensitivity could shift due to mechanisms of thermal adaptation (Bradford, 2013). Cold-adapted

111   enzymes are thought to be optimized through reductions in $\Delta H^{\ddagger}$ that reduce Ea (Lonhienne *et al.*,

112   2000; Georlette *et al.*, 2004; Siddiqui & Cavicchioli, 2006). However, at higher temperatures,

113   selection to minimize Ea declines because enzymes and substrates have higher kinetic energy. If

114   Ea increases under long-term warming, so should Vmax temperature sensitivity in accordance

115   with the Arrhenius relationship. Still, this mechanism is not widely recognized, as some recent

116   studies have hypothesized the opposite pattern—lower Vmax temperature sensitivity in warmer

117   environments (Wallenstein *et al.*, 2009; Brzostek & Finzi, 2012; Nottingham *et al.*, 2016).

118   For Km, transition state theory dictates that the temperature response depends on the

119   energetics of enzyme-substrate binding. Binding affinity (1/Km) is dependent on the free energy

120   change upon binding, ΔG_{ES} (Tsuruta & Aizono, 2003):

121      $1/Km = \exp(-\Delta G_{ES}/RT)$ [eq. 5]

122   where $\Delta G_{ES} = \Delta H_{ES} - T\Delta S_{ES}$ (eq. 2). Therefore, affinity increases with greater enthalpy release

123   (more negative ΔH_{ES}) and greater entropy upon substrate binding (more positive ΔS_{ES}).

124   Rearranging the equation yields

125      $Km = \exp(\Delta H_{ES}/RT - \Delta S_{ES}/R)$ [eq. 6]

126   A negative ΔH_{ES} in eq. 6 dictates that Km increases with increasing T. In contrast, Km should

127   decrease with warming if ΔH_{ES} is positive because the term $\Delta H_{ES}/RT$ will decline as T increases

128   (Siddiqui & Cavicchioli, 2006). Note that substrate binding is still thermodynamically favorable

129   with a positive ΔH_{ES} if increases in entropy counteract the enthalpy term (Snider *et al.*, 2000;

130   Tsuruta & Aizono, 2003; Siddiqui & Cavicchioli, 2006).

131   Recently, macromolecular rate theory (MMRT) has been proposed to account for

132   empirical evidence that enzyme catalytic rates do not follow the Arrhenius relationship with

133   increasing temperature (Hobbs *et al.*, 2013; Schipper *et al.*, 2014; Alster *et al.*, 2016). MMRT

134   posits that Ea is not constant as assumed under Arrhenius theory but varies with increasing

135   temperature due to changes in enzyme heat capacity. Although MMRT offers promise as a more

136   mechanistic explanation of enzyme temperature sensitivity, the theory has not yet been applied to

137   questions regarding Km or thermal adaptation of Vmax and Km.

138   The goal of this study was to determine the temperature sensitivities of Vmax and Km

139   and whether these sensitivities shift with long-term warming. Temperature sensitivity is defined

140   here as the slope of the relationship between the log(Vmax) or log(Km) value and laboratory

141   incubation temperature. We hypothesized that 1) temperature sensitivity would be positive for

142   enzyme Vmax based on Arrhenius theory and positive for Km due to a broader distribution of

143   enzyme-substrate conformational states at higher temperatures (Hochachka & Somero, 2002;

144   Georlette *et al.*, 2004). We also predicted that Vmax would correlate positively with Km if

145   stronger enzyme-substrate binding increases the activation energy barrier as implied by transition

146   state theory (Fig. 1). Based on biochemical theory, we hypothesized that 2a) the magnitude of

147   Vmax at a common temperature would be lower and the temperature sensitivity of Vmax would

148   be greater for enzymes from warmer environments. For Km, we hypothesized 2b) a lower

149   magnitude and lower temperature sensitivity of Km for enzymes from warmer environments.

150   Warm environments should select for enzymes with greater rigidity to enhance substrate binding,

151   thereby reducing Km at a common temperature and limiting changes in enzyme conformation as

152   temperature increases (Georlette *et al.*, 2004; Dong & Somero, 2009).

153   We tested these hypotheses with individual strains of the filamentous fungus *Neurospora*

154   *discreta* isolated across a gradient of mean annual temperatures and with whole microbial

155   communities growing on leaf litter in a warming manipulation in boreal Alaska. These two

156   systems are complementary because the litter communities produce a more complex mixture of

157   enzymes compared to individual *Neurospora* strains. Also, the warming experiment captures the

158   short-term response of communities to warming whereas the *Neurospora* strains have evolved

159   under the long-term, integrated effects of different climate conditions.

160

161   **Materials and methods**

162   *Neurospora* strains

163   *Neurospora discreta* strains from Côte d'Ivoire, Thailand, Switzerland, and Spain were obtained

164   from the Fungal Genetic Stock Center (Gladieux *et al.*, 2015). Other strains from the United

165   States were from a culture collection maintained in J. Taylor's laboratory (Table 1). *Neurospora*

166   species occur on all continents, show a complex population structure, and include populations

167   that inhabit the soil and reproduce following fire in temperate and boreal forests (Jacobson *et al.*,

168   2004).

169

170   Growth conditions

171   *Neurospora* strains were inoculated from stock cultures onto the center of an agar plate

172   containing Vogel's minimal medium (VMM). Plates were sealed with Parafilm and incubated for

173   5-7 days at 21ºC until fully covered by mycelium. The mycelium was then transferred to a 250

174   ml flask containing 100 ml VMM and incubated at 28ºC with shaking at 150 rpm. After 3 days,

175   the medium was centrifuged to separate the mycelium which was then rinsed and resuspended in

176   VMM, homogenized in a blender (4 x 10 sec pulses), and transferred to a flask with 120 ml fresh

177   VMM. This procedure was necessary to reduce aggregation of mycelium that inhibited the

178   growth of some strains. After 4 more days of growth at 28ºC and shaking at 150 rpm, the

179   mycelium was separated from the medium by centrifugation, and the supernatant was used in

180   enzyme assays.

181

182   Litter warming experiment

183   We analyzed enzyme parameters in litterbags collected from a soil warming experiment in

184   Alaskan boreal forest near Delta Junction, AK, USA (63º55'N, 145º44'W). The warming

185   experiment began in July 2005 with five 2.5 m x 2.5 m unwarmed control plots paired with five

186   2.5 m x 2.5 m warmed plots in a 1 km^2 area (Allison & Treseder, 2008). Warming was

187   accomplished with closed-top greenhouses. The top panel of each greenhouse was removed in

188   September and replaced in each subsequent May to allow snowfall to reach the plots. Rainfall

189   entered the greenhouses through a system of gutters and tubing. On average, the warming

190   treatment increased air temperature by 1.6ºC, increased soil temperature by 0.5ºC (5 cm depth),

191   and reduced soil moisture by 22% (0-5 cm depth).

192   Litterbags containing 2 g senescent black spruce needles were placed in control and

193   warmed plots on May 22, 2013 (Romero-Olivares *et al.*, 2017). Each bag was 10 cm × 10 cm

194   and constructed from a layer of elastic 1 mm nylon mesh and a layer of 1 mm fiberglass window

195   screen. Each plot received 5 sets of 2 bags placed on the forest floor. One set of bags was

196   harvested on July 5, 2013, August 28, 2013, May 29, 2014, September 7, 2014, and July 4, 2015,

197   and the contents of the 2 bags were combined. Subsamples were removed for determination of

198   enzyme activity (~0.6 g fresh weight) and dry weight. The enzyme subsamples were stored at -

199   80ºC until analysis.

200

201   Enzyme assays

202   Potential activities of extracellular enzymes were measured according to established fluorimetric

203   and colorimetric protocols for 96-well microplates (German *et al.*, 2011). For culture assays, 125

204   µl *Neurospora* culture supernatant was combined with 125 µl substrate (Table 2) dissolved in 50

205   mM maleate buffer, pH 6.0. For litter assays, material was homogenized in 50 mM maleate

206   buffer, pH 6.0, using a hand held Bamix Homogenizer (BioSpec Products, Bartlesville, OK,

207   USA) at a ratio of ~2.7 mg litter ml^{-1} buffer. This homogenate (125 µl) was combined with 125

208   µl substrate dissolved in ultrapure water. Substrate solutions were serially diluted by two-fold

209   from the maximum concentrations shown in Table 2 to create a gradient with eight substrate

210   concentrations. The OX assay also received 10 µl 0.3% H_2O_2 in all wells with substrate. All

211   assays included homogenate (or culture) blanks and substrate controls. Fluorimetric assays also

212   included 7-amino-4-methylcoumarin (AMC) and 4-methyumbelliferone (MUB) standards for

213   LAP and the other hydrolytic enzymes, respectively. Fluorescence was measured from 125 µl of

214   25 µM MUB or AMC with 125 µl water or buffer as the standard or with 125 µl homogenate or

215   culture supernatant as a quench control.

216   Assay plates were incubated for 1-5 h (hydrolytic enzymes) or 24 h (oxidases) at 4, 10,

217   16, 22, 28, and 34ºC. Fluorescence was read at 365 nm/450 nm excitation/emission for the

218   hydrolytic enzymes, and absorbance was read at 410 nm for OX and PPO on a BioTek Synergy

219   H4 microplate reader (Winooski, VT, USA). Enzyme activities were expressed as nmol h^{-1} ml^{-1}

220   culture supernatant or µmol h^{-1} g^{-1} dry litter according to German et al. (2011, 2012) using an

221   extinction coefficient of 3.9 $µM^{-1}$ for pyrogallol.

222

223   Statistical analyses

224   Enzyme activity data were checked for outliers, and values below detection limits were

225   converted to 0.0001, thereby removing negative activities from the dataset. Quality-checked

226   activities were fit to the Michaelis-Menten equation using the non-linear least squares (nls)

227   function in *base* R (R Development Core Team, 2011). Vmax and Km parameters were extracted

228   from the model fit, and parameters from poor fits were discarded.

229   Extracted parameters were log-transformed and analyzed with linear regression using

230   incubation temperature as the independent variable. Regression slopes were extracted as a metric

231   of Vmax or Km temperature sensitivity (TS) in terms of change in log(Vmax) or log(Km) per

232   °C. These slopes were converted to Q_{10} values using the relationship $Q_{10} = \exp(10 \times slope)$. For

233   *Neurospora* cultures, we also used this approach to analyze the ratio Vmax/Km for each strain

234   and enzyme. Regression parameters were used to compute enzyme Vmax or Km at 16ºC,

235   hereafter referred to as Vmax or Km for simplicity. We chose 16ºC as the common temperature

236   at which to compare parameters because it falls within the range of our laboratory assays and

237   approximates growing season temperatures for many of our strains.

238   Further analyses were conducted on the enzyme parameters. For the *Neurospora* cultures,

239   we tested for significant Spearman correlations between Vmax and Km using the corr.test

240   function of the *psych* package in R. We used simple linear regression to test for significant

241   relationships between strain isolation site mean annual temperature (MAT) and enzyme kinetic

242   parameters or temperature sensitivities. To account for non-independence among strains isolated

243   from the same site, we also tested for these relationships after including site as a random effect in

244   the regression model. For the litter analyses, we tested for the effects of warming treatment and

245   collection date on log enzyme kinetic parameters and temperature sensitivities using mixed-

246   model analysis of variance (ANOVA) with block as the random factor.

247

248   **Results**

249   *Neurospora* isolate Vmax and Km

250   Under culture conditions, enzyme kinetic parameters varied substantially across enzymes and

251   strains (Tables 3-5, Table S1). On average, Vmax at 16ºC was greatest for *N*-acetyl-

252   glucosaminidase (NAG) at 140.8 nmol h^{-1} ml^{-1} and lowest for the oxidases (<0.16 nmol h^{-1} ml^{-1}).

253   The other Vmax means ranged between 0.37 and 3.25 nmol h^{-1} ml^{-1} (Table 3, Fig. 2a). Average

254   Km values at 16ºC were less than 100 μM for NAG, *α*-glucosidase (AG), and the oxidases but

255   ranged up to 850 μM for the other enzymes (Table 4, Fig. 2b). These patterns for Vmax and Km

256   resulted in a very high mean Vmax/Km of 1.17 for NAG, an intermediate value of 0.032 for AG

257   and values below 0.004 for the other enzymes (Table 5, Fig. 2c). Vmax and Km were

258 significantly positively correlated across strains for β-xylosidase (BX; r = 0.76), total oxidase

259 (OX; r = 0.92), and polyphenol oxidase (PPO; r = 0.94).

260

261 *Neurospora* isolate Vmax and Km temperature sensitivity

262 As with the kinetic parameters themselves, Vmax TS and Km TS varied across strains and

263 enzymes (Tables 3-4, S2). On average, all Vmax TS values were positive with Q_{10} ranging from

264 1.48 (PPO) to 2.25 (NAG). TS values were generally positive except for some strains that

265 showed negative values for cellobiohydrolase (CBH), leucine aminopeptidase (LAP), and OX

266 (Fig. 3a).

267   The results for Km TS were much more variable. Km TS was consistently positive for

268 NAG with a cross-strain average of Q_{10} of 2.80 (Table 4, Fig. 3b). Most, but not all, strains

269 showed positive Km TS for AG, acid phosphatase (AP), and the oxidases with average Q_{10}

270 ranging from 1.17 to 1.48 (Table 4). Km TS for the carbohydrate-degrading enzymes β-

271 glucosidase (BG), BX, and CBH varied substantially across strains including both positive and

272 negative values (Fig. 3b). Only LAP showed a consistently negative Km TS for most strains with

273 an average Q_{10} of 0.71 (Table 4). The TS of Vmax/Km was generally positive for all enzymes

274 except NAG, which was consistently negative, and the oxidases which showed low or variable

275 Vmax/Km TS (Table 5, Fig. 3c).

276

277 Enzyme kinetic response to MAT for *Neurospora* isolates

278 There were some significant positive relationships between enzyme kinetic parameters and MAT

279 of the strain isolation site. Vmax showed a positive relationship with MAT for AP, CBH, and

280 LAP with R^2 values ranging from 0.13 to 0.33 (Fig. 4). Similar patterns were observed for Km,

281   with significant positive relationships for LAP ($R^2 = 0.24$) and NAG ($R^2 = 0.26$). When isolation

282   site was included as a random effect in the regression model, only the CBH Vmax relationship

283   with MAT remained significant ($P = 0.016$). We did not find a significant relationship between

284   MAT and Vmax TS or Km TS for any enzyme.

285

286   Litter Vmax and Km under Alaskan warming treatment

287   Effects of warming on litter enzyme kinetic parameters depended on date and enzyme (Tables 6,

288   S3). The warming effect on Vmax was negative and significant for all enzymes except CBH, and

289   there was also an interaction with date for AP, BG, NAG, OX, and PPO (Fig. 5a). For Km, the

290   warming effect was positive, at least on some dates, for AP, BG, CBH, LAP, and NAG (Fig. 5b).

291   In contrast, there was a significant negative warming effect on Km for the oxidases.

292

293   Litter Vmax and Km temperature sensitivity

294   On average, Vmax TS was positive for all litter enzymes (Fig. 6a, Table S4). Warming treatment

295   significantly increased Vmax TS of AP, BG, BX, and NAG, and there was a significant date by

296   treatment interaction for AG, indicating increased TS under warming on the earlier collection

297   dates (Table 6). In contrast, warming treatment significantly reduced Vmax TS of LAP.

298   Km TS varied by enzyme and in some cases warming treatment (Fig. 6b, Table 6). For

299   AG, Km TS was low in the control plots but increased significantly with warming. In contrast,

300   Km TS of AP was generally positive in control plots but declined significantly with warming on

301   some dates. On average, Km TS for BG was close to zero, but there was a significant treatment

302   by date interaction. BX and CBH values were close to zero and showed no warming effects. Km

303   TS for LAP was consistently negative and significantly reduced by warming treatment, but for

13

304   NAG it was consistently positive with the warming effect dependent on collection date. Km TS

305   for the oxidases was generally positive but declined significantly with warming for OX.

306

307   **Discussion**

308   We found that the Vmax values of extracellular enzymes involved in decomposition increased

309   exponentially with increasing temperature, consistent with Arrhenius theory and a positive

310   feedback to climate warming (Davidson & Janssens, 2006). Still, the strength of this feedback

311   could be reduced if enzymes exhibit thermal adaptation such that Ea rises under warming,

312   thereby reducing absolute Vmax while increasing Vmax TS. Our litter enzyme results provide

313   support for this thermal adaptation mechanism, as most enzymes showed lower Vmax values and

314   some showed higher Vmax TS under warming treatment (Fig. 7). In contrast, we did not find

315   support for thermal adaptation when comparing enzymes from *Neurospora* strains native to

316   different thermal environments. These contrasting results suggest that community-level

317   processes may influence thermal adaptation of Alaskan litter enzymes, whereas *Neurospora* taxa

318   were limited in their thermal response due to phylogenetic constraints or ecological factors, such

319   as changing substrate availability across the MAT gradient.

320

321   Vmax-Km relationship

322   In the *Neurospora* study, significant positive correlations between Vmax and Km for BX, OX,

323   and PPO supported predictions from transition state theory (Fig. 1). A deeper free energy well

324   for enzyme-substrate binding leads to lower Km but also a greater free energy barrier to

325   overcome during activation of the transition state (Lonhienne *et al.*, 2000; Georlette *et al.*, 2004;

14

326   Siddiqui & Cavicchioli, 2006). This greater Ea barrier results in a slower reaction rate (lower

327   Vmax), consistent with the relationships we saw.

328

329   Temperature sensitivity of enzyme kinetics

330   The temperature sensitivity of Vmax, represented as Q_{10} values, varied from 1.48 to 2.25 for

331   *Neurospora* enzymes, similar to other studies (Koch *et al.*, 2007; Hui *et al.*, 2013; Nottingham *et*

332   *al.*, 2016). Whereas our Q_{10} values were based on Arrhenius theory, MMRT has been proposed

333   as a more appropriate framework for analyzing enzyme temperature sensitivity because it does

334   not assume constant Q_{10} as temperature changes (Schipper *et al.*, 2014). However, our study was

335   not ideal for testing MMRT because we used a temperature range below the thermal optima of

336   the enzymes. In addition, thermal adaptation predictions have not yet been developed for

337   MMRT. Nonetheless, we successfully fit the MMRT equation to obtain thermodynamic

338   parameters and heat capacities for many of our *Neurospora* and litter enzymes (Supporting

339   Information). This analysis showed that most enzyme heat capacities in our study were close to

340   zero where Arrhenius theory and MMRT yield similar Q_{10} values. Still, if MMRT is developed

341   further to make predictions about thermal adaptation, our data could be used to test them.

342   Km TS was positive for some enzymes but close to zero or negative for others in both

343   experimental systems. Km TS is measured less frequently than Vmax TS in environmental

344   contexts, but our results are partially consistent with previous studies (German *et al.*, 2012b;

345   Stone *et al.*, 2012). As in these studies, Km TS was generally lower than Vmax TS; however, the

346   magnitude of Km TS for *Neurospora* and Alaskan litter enzymes was lower than in most of the

347   soils measured previously, including those from our Alaskan field site. NAG was an exception to

348   this pattern with higher Km TS among *Neurospora* strains and Alaskan litter compared to soils

349   from Alaska and several other sites (German *et al.*, 2012b; Stone *et al.*, 2012).

350   Km TS may be lower than Vmax TS, or even negative, owing to the thermodynamic

351   processes controlling enzyme-substrate binding (Fig. 1). Our data indicate that enzymes like

352   NAG and PPO, with strong positive Km TS, have negative ΔH_{ES} values. These enzymes also

353   have relatively low Km values, consistent with a dominant role for the enthalpy term in eq. 6.

354   Based on these results, the temperature sensitivities of Vmax and Km may have consequences

355   for nitrogen cycling under warming. For NAG, which is involved in chitin degradation, Km TS

356   was more positive than Vmax TS, such that NAG was the only enzyme with a consistently

357   negative Vmax/Km TS. In contrast, Vmax/Km TS for LAP was very high due to a negative Km

358   TS. Because LAP is involved in protein degradation, these results imply that rising temperatures

359   might favor increased nitrogen cycling from protein sources relative to peptidoglycans,

360   particularly if substrate concentrations for these enzymes are near Km.

361

362   Response to thermal environment

363   Physiological theory predicts that organisms from cold environments should minimize enzyme

364   Ea to maximize catalytic rates; conversely warm-adapted enzymes should have higher Ea

365   (Georlette *et al.*, 2004; Somero, 2004; Siddiqui & Cavicchioli, 2006). Based on the Arrhenius

366   relationship (eq. 4), warm-adapted enzymes should therefore have *higher* temperature

367   sensitivities. Multiple studies suggest that soil enzyme properties respond to thermal

368   environment (Fenner *et al.*, 2005; Stone *et al.*, 2012), but in contrast to physiological theory,

369   some studies have found higher Vmax TS in enzymes from *cooler* environments. For instance, in

370   some tundra and forest soils, Vmax TS increased during cool seasons (Wallenstein *et al.*, 2009;

371   Brzostek & Finzi, 2011), although this pattern reversed for proteolytic activity in a sugar maple

372   forest (Brzostek & Finzi, 2012). Across an elevation gradient in the Andes, Vmax TS was greater

373   at higher, cooler elevations for some extracellular enzymes (Nottingham *et al.*, 2016).

374   Our study lends support for physiological theory under controlled conditions, with

375   enzymes from the warming experiment, but not across the MAT gradient where we observed

376   higher Vmax for some *Neurospora* enzymes from warmer sites (Fig. 4A). We suspect that

377   factors aside from thermal environment can modulate the enzyme parameters of *Neurospora*

378   strains. The ecosystems hosting these strains differed not only in MAT, but also precipitation,

379   vegetation type, biotic community composition, and soil edaphic characteristics. All these factors

380   may select on the complement of extracellular enzyme genes present in fungal genomes as well

381   as the kinetic properties of the expressed enzymes (Riley *et al.*, 2014). As in previous studies

382   (German *et al.*, 2012b), such differences may have obscured the influence of temperature on the

383   biochemical properties of individual enzymes or enzyme classes.

384   In the warming experiment with Alaskan litter, many of these characteristics were better

385   controlled because we used a paired sampling design. This design may have afforded the power

386   to detect changes in enzyme Vmax consistent with physiological theory and driven by

387   community-level processes. A previous study showed that our warming treatment alters fungal

388   community composition and the genetic capacity to degrade lignin-like compounds, which is

389   consistent with changes in soil enzyme functioning (McGuire *et al.*, 2010). It is also possible that

390   greater late-season drying in the warming treatment affected our enzyme Vmax results (Allison

391   & Treseder, 2008), but other studies suggest that drying can increase measured Vmax in contrast

392   to the pattern we observed (Alster *et al.*, 2013).

17

393   Trends in Vmax TS differed across our two study systems. Again, under the better-

394   controlled conditions of the warming experiment, we found some support for the physiological

395   prediction that warmer environments allow for relatively higher Ea and therefore higher

396   temperature sensitivity. Yet in our *Neurospora* study, there were no relationships between Vmax

397   TS and strain origin MAT. These results align with observations from Elias et al. (2014), who

398   found no differences in Q_{10} among enzymes from psychrophiles versus thermophiles. Likewise,

399   no relationship between MAT and Vmax TS was found in a previous cross-latitudinal study with

400   five enzymes (German *et al.*, 2012b). As a whole, these findings challenge classic physiological

401   theory and suggest a need to further develop MMRT (Schipper *et al.*, 2014) or theory on enzyme

402   rigidity (Elias *et al.*, 2014) to address enzyme thermal adaptation at the global scale.

403   Based on biochemical and physiological theory, we predicted that Km and Km TS should

404   be lower in warmer environments. We found little support for this theory—Km and Km TS both

405   varied inconsistently with thermal environment in the *Neurospora* and Alaskan litter studies

406   (Figs. 4B, 5B, 6B). Although Km TS of soil β-glucosidase was previously found to decline with

407   increasing MAT, no relationship was observed for four other enzymes (German *et al.*, 2012b).

408   Taken together, these results suggest that there may be biochemical limits to the thermal

409   adaptation of Km, or that adaptation is difficult to observe in mixtures of enzymes influenced by

410   confounding ecological factors.

411   Predictions of carbon-climate feedbacks require data on enzymatic rate changes under a

412   warmer climate (Wieder *et al.*, 2013). Overall, the results from our Alaskan warming study

413   suggest a negative feedback, as litter communities showed lower enzyme Vmax under warming

414   treatment (Fig. 7). This result is consistent with physiological theory and with reduced soil

415   respiration and increased surface soil carbon pools observed in the warming treatment (Allison &

416 Treseder, 2008; Crowther *et al.*, 2016). Our results also suggest that thermal adaptation

417 responses may be weaker or more difficult to observe across broad climate gradients. Even

418 though the *Neurospora* strains evolved under very different MAT, there was little evidence for

419 thermal adaptation of *Neurospora* enzymes. Temperature may be a weak selective force on

420 enzyme properties compared to other edaphic and ecological factors that vary across broad

421 climate gradients.

422   Regarding temperature sensitivity, our study identifies a need for new theory

423 development. Existing physiological theory could not account for observed variation in Vmax or

424 Km temperature sensitivities across thermal environments, especially in our *Neurospora* study.

425 In general, the response was weaker than expected, suggesting that most enzymes will maintain

426 their temperature sensitivities under a warmer climate. Even if the mechanisms and underlying

427 theory are unclear at this point, this empirical result is important for parameterizing trait-based

428 models that require Vmax, Km, and temperature sensitivity data on microbial enzymes (Allison,

429 2012; Kaiser *et al.*, 2014). For example, our findings suggest that the relative cycling of different

430 nutrient forms, particularly proteins versus peptidoglycans, may change due to differential

431 kinetic responses to temperature across enzyme classes. Altogether, our study provides enzyme

432 data and theoretical insight that should help improve predictions of soil biogeochemical

433 feedbacks under climate change (Wang *et al.*, 2013; Wieder *et al.*, 2013, 2014).

434

435 **Acknowledgments**

440

References

441 Allison SD (2012) A trait-based approach for modelling microbial litter decomposition. *Ecology*
443 *Letters*, **15**, 1058–1070.

444 Allison SD, Treseder KK (2008) Warming and drying suppress microbial activity and carbon
445 cycling in boreal forest soils. *Global Change Biology*, **14**, 2898–2909.

446 Alster CJ, German DP, Lu Y, Allison SD (2013) Microbial enzymatic responses to drought and
447 to nitrogen addition in a southern California grassland. *Soil Biology and Biochemistry*, **64**,
448 68–79.

449 Alster C, Baas P, Wallenstein M, Johnson N, von Fischer J (2016) Temperature Sensitivity as a
450 Microbial Trait Using Parameters from Macromolecular Rate Theory. *Frontiers in*
451 *Microbiology*, **7**, 1821.

452 Bradford MA (2013) Thermal adaptation of decomposer communities in warming soils.
453 *Frontiers in Microbiology*, **4**, 333.

454 Brzostek ER, Finzi AC (2011) Substrate supply, fine roots, and temperature control proteolytic
455 enzyme activity in temperate forest soils. *Ecology*, **92**, 892–902.

456 Brzostek ER, Finzi AC (2012) Seasonal variation in the temperature sensitivity of proteolytic
457 enzyme activity in temperate forest soils. *Journal of Geophysical Research*, **117**, G01018,
458 doi:10.1029/2011JG001688.

459 Burns RG, DeForest JL, Marxsen J et al. (2013) Soil enzymes in a changing environment:
460 Current knowledge and future directions. *Soil Biology and Biochemistry*, **58**, 216–234.

461 Carey JC, Tang J, Templer PH et al. (2016) Temperature response of soil respiration largely
462 unaltered with experimental warming. *Proceedings of the National Academy of Sciences*,
463 **113**, 13797–13802.

464 Crowther TW, Todd-Brown KEO, Rowe CW et al. (2016) Quantifying global soil carbon losses

465   in response to warming. *Nature*, **540**, 104–108.

466 Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and

467   feedbacks to climate change. *Nature*, **440**, 165–173.

468 Dong Y, Somero GN (2009) Temperature adaptation of cytosolic malate dehydrogenases of

469   limpets (genus *Lottia*): differences in stability and function due to minor changes in

470   sequence correlate with biogeographic and vertical distributions. *The Journal of*

471   *Experimental Biology*, **212**, 169–177.

472 Elias M, Wieczorek G, Rosenne S, Tawfik DS (2014) The universality of enzymatic rate-

473   temperature dependency. *Trends in Biochemical Sciences*, **39**, 1–7.

474 Fenner N, Freeman C, Reynolds B (2005) Observations of a seasonally shifting thermal optimum

475   in peatland carbon-cycling processes; implications for the global carbon cycle and soil

476   enzyme methodologies. *Soil Biology & Biochemistry*, **37**, 1821–1841.

477 Georlette D, Blaise V, Collins T et al. (2004) Some like it cold: Biocatalysis at low temperatures.

478   *FEMS Microbiology Reviews*, **28**, 25–42.

479 German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD (2011)

480   Optimization of extracellular enzyme assay methods for ecosystem studies. *Soil Biology &*

481   *Biochemistry*, **43**, 1387–1397.

482 German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD (2012a)

483   Corrigendum to "Optimization of hydrolytic and oxidative enzyme methods for ecosystem

484   studies" [Soil Biol. Biochem. 43 (2011) 1387-1397]. *Soil Biology & Biochemistry*, **44**, 151.

485 German DP, Marcelo KRB, Stone MM, Allison SD (2012b) The Michaelis-Menten kinetics of

486   soil extracellular enzymes in response to temperature: a cross-latitudinal study. *Global*

487   *Change Biology*, **18**, 1468–1479.

488   Gladieux P, Wilson BA, Perraudeau F et al. (2015) Genomic sequencing reveals historical,

489   demographic and selective factors associated with the diversification of the fire-associated

490   fungus *Neurospora discreta*. *Molecular Ecology*, **24**, 5657–5675.

491   Hobbs JK, Jiao W, Easter AD, Parker EJ, Schipper LA, Arcus VL (2013) Change in heat

492   capacity for enzyme catalysis determines temperature dependence of enzyme catalyzed

493   rates. *ACS Chemical Biology*, **8**, 2388–2393.

494   Hochachka PW, Somero GN (2002) *Biochemical Adaptation: Mechanism and Process in*

495   *Physiological Evolution*. Oxford University Press, Oxford.

496   Hui D, Mayes MA, Wang G (2013) Kinetic parameters of phosphatase: A quantitative synthesis.

497   *Soil Biology and Biochemistry*, **65**, 105–113.

498   IPCC (2013) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group*

499   *I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. 1535

500   pp.

501   Jacobson DJ, Powell AJ, Dettman JR et al. (2004) Neurospora in temperate forests of western

502   North America. *Mycologia*, **96**, 66–74.

503   Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation

504   to climate and vegetation. *Ecological Applications*, **10**, 423–436.

505   Kaiser C, Franklin O, Dieckmann U, Richter A (2014) Microbial community dynamics alleviate

506   stoichiometric constraints during litter decay. *Ecology Letters*, **17**, 680–690.

507   Koch O, Tscherko D, Kandeler E (2007) Temperature sensitivity of microbial respiration,

508   nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. *Global*

509   *Biogeochemical Cycles*, **21**, 11.

510 Lonhienne T, Gerday C, Feller G (2000) Psychrophilic enzymes: Revisiting the thermodynamic

511   parameters of activation may explain local flexibility. *Biochimica et Biophysica Acta -*

512   *Protein Structure and Molecular Enzymology*, **1543**, 1–10.

513 McGuire KL, Bent E, Borneman J, Majumder A, Allison SD, Treseder KK (2010) Functional

514   diversity in resource use by fungi. *Ecology*, **91**, 2324–2332.

515 Nottingham AT, Turner BL, Whitaker J et al. (2016) Temperature sensitivity of soil enzymes

516   along an elevation gradient in the Peruvian Andes. *Biogeochemistry*, **127**, 217–230.

517 R Development Core Team (2011) *R: a language and environment for statistical computing*. R

518   Foundation for Statistical Computing, Vienna, Austria.

519 Riley R, Salamov AA, Brown DW et al. (2014) Extensive sampling of basidiomycete genomes

520   demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi.

521   *Proceedings of the National Academy of Sciences*, **111**, 9923–9928.

522 Romero-Olivares AL, Allison SD, Treseder KK (2017) Decomposition of recalcitrant carbon

523   under experimental warming in boreal forest. *PLoS ONE*, **12**, e0179674.

524 Schipper LA, Hobbs JK, Rutledge S, Arcus VL (2014) Thermodynamic theory explains the

525   temperature optima of soil microbial processes and high Q_{10} values at low temperatures.

526   *Global Change Biology*, **20**, 3578–3586.

527 Siddiqui KS, Cavicchioli R (2006) Cold-Adapted Enzymes. *Annual Review of Biochemistry*, **75**,

528   403–433.

529 Sinsabaugh RL (1994) Enzymic analysis of microbial pattern and process. *Biology and Fertility*

530   *of Soils*, **17**, 69–74.

531 Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil*

532   *Biology & Biochemistry*, **42**, 391–404.

533 Snider MJ, Gaunitz S, Ridgway C, Short SA, Wolfenden R (2000) Temperature effects on the

534   catalytic efficiency, rate enhancement, and transition state affinity of cytidine deaminase,

535   and the thermodynamic consequences for catalysis of removing a substrate "anchor."

536   *Biochemistry*, **39**, 9746–9753.

537 Somero GN (1978) Temperature adaptation of enzymes: biological optimization through

538   structure-function compromises. *Annual Review of Ecology and Systematics*, **9**, 1–29.

539 Somero GN (2004) Adaptation of enzymes to temperature: Searching for basic "strategies."

540   *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, **139**,

541   321–333.

542 Steinweg JM, Dukes JS, Wallenstein MD (2012) Modeling the effects of temperature and

543   moisture on soil enzyme activity: Linking laboratory assays to continuous field data. *Soil*

544   *Biology and Biochemistry*, **55**, 85–92.

545 Stone MM, Weiss MS, Goodale CL, Adams MB, Fernandez IJ, German DP, Allison SD (2012)

546   Temperature sensitivity of soil enzyme kinetics under N-fertilization in two temperate

547   forests. *Global Change Biology*, **18**, 1173–1184.

548 Todd-Brown KEO, Randerson JT, Hopkins F et al. (2014) Changes in soil organic carbon

549   storage predicted by Earth system models during the 21st century. *Biogeosciences*, **11**,

550   2341–2356.

551 Tsuruta H, Aizono Y (2003) Catalytic efficiency and some structural properties of cold-active

552   protein-tyrosine-phosphatase. *Journal of Biochemistry*, **133**, 225–230.

553 Wallenstein MD, McMahon SK, Schimel JP (2009) Seasonal variation in enzyme activities and

554   temperature sensitivities in Arctic tundra soils. *Global Change Biology*, **15**, 1631–1639.

555 Wallenstein M, Allison S, Ernakovich J, Steinweg JM, Sinsabaugh R (2011) Controls on the

556   temperature sensitivity of soil enzymes: A key driver of in-situ enzyme activity rates. In:

557   *Soil Enzymology* (ed Shukla GC), pp. 245–258. Springer-Verlag.

558   Wang G, Post W, Mayes M (2013) Development of microbial-enzyme-mediated decomposition

559   model parameters through steady-state and dynamic analyses. *Ecological Applications*, **23**,

560   255–272.

561   Wieder WR, Bonan GB, Allison SD (2013) Global soil carbon projections are improved by

562   modelling microbial processes. *Nature Climate Change*, **3**, 909–912.

563   Wieder WR, Grandy AS, Kallenbach CM, Bonan GB (2014) Integrating microbial physiology

564   and physio-chemical principles in soils with the MIcrobial-MIneral Carbon Stabilization

565   (MIMICS) model. *Biogeosciences*, **11**, 3899–3917.

566

567

568 Table 1. *Neurospora* strain isolation sites and site characteristics. Mean annual temperature
569 (MAT) and mean annual precipitation (MAP) over the period 1981-2010 were obtained from
570 PRISM (http://www.prism.oregonstate.edu/) for sites inside the continental United States. MAT
571 and MAP for other sites were obtained with Climate Reanalyzer (http://cci-reanalyzer.org),
572 Climate Change Institute, University of Maine, USA, using the University of Delaware Air
573 Temperature and Precipitation dataset.

ID	Genotype	Site	Latitude	Longitude	MAT	MAP
FGSC 8565	*Neurospora discreta* from the USA	Wells, Nevada	41.11	-114.96	7.1	259
FGSC 8567	*Neurospora discreta* from the USA	Cobalt, Idaho	45.00	-114.30	4.1	470
FGSC 8572	*Neurospora discreta* from the USA	Perma-2, Montana	47.35	-114.59	7.1	495
FGSC 8991	*Neurospora discreta* from USA W963	Weaverville, California	40.73	-122.94	12.4	950
FGSC 8994	*Neurospora discreta* from USA W1070	Chelan Lake, WA	47.86	-120.12	9.9	276
FGSC 9957	*Neurospora discreta* from Thailand P3004	Pakchong-2, Thailand	14.70	101.42	25.1	1171
FGSC 9967	*Neurospora discreta* from Ivory Coast P3650	Fougbesso, Ivory Coast	7.59	-5.56	26.4	1061
FGSC 9979	*Neurospora discreta* from USA W854	Tok, Alaska	63.33	-142.99	-4.4	256
FGSC 9983	*Neurospora discreta* from USA W745	Pecos, New Mexico	35.57	-105.66	9.4	439
FGSC 9992	*Neurospora discreta* from Switzerland W-1303	Leuk, Switzerland	46.32	7.63	0.4	798
MM10	*Neurospora discreta* from USA	Fairbanks, AK	64.83	-147.72	-1.9	276
MM2	*Neurospora discreta* from USA	Fairbanks, AK	64.83	-147.72	-1.9	276
MM20	*Neurospora discreta* from USA	Fairbanks, AK	64.83	-147.72	-1.9	276
MM23	*Neurospora discreta* from USA	Fairbanks, AK	64.83	-147.72	-1.9	276
MM26	*Neurospora discreta* from USA	Fairbanks, AK	64.83	-147.72	-1.9	276

MM30	*Neurospora discreta* from USA	Fairbanks, AK	64.83	-147.72	-1.9	276
MM31	*Neurospora discreta* from USA	Fairbanks, AK	64.83	-147.72	-1.9	276
MM6	*Neurospora discreta* from USA	Fairbanks, AK	64.83	-147.72	-1.9	276
W1099	*Neurospora discreta* from USA	Morgan Hill, CA	37.11	-121.65	15.7	560
W1101	*Neurospora discreta* from USA	Morgan Hill, CA	37.11	-121.65	15.7	560
W1103	*Neurospora discreta* from USA	Morgan Hill, CA	37.11	-121.65	15.7	560
W1111	*Neurospora discreta* from USA	Morgan Hill, CA	37.11	-121.65	15.7	560
W1289	*Neurospora discreta* from Spain	Macanet de la Selva, Spain	41.78	2.73	13.7	701
W792	*Neurospora discreta* from USA	Bernalillo, NM	35.3	-106.55	13.0	268
W793	*Neurospora discreta* from USA	Bernalillo, NM	35.3	-106.55	13.0	268
W794	*Neurospora discreta* from USA	Bernalillo, NM	35.3	-106.55	13.0	268
W795	*Neurospora discreta* from USA	Bernalillo, NM	35.3	-106.55	13.0	268

574

575

576 Table 2. Enzymes and substrates analyzed in the current study.

Enzyme and abbreviation		Substrate target	Synthetic substrate and maximum concentration (µM)	
α-glucosidase	AG	Starch degradation products	4-MUB-*α*-D-glucopyranoside	1000
Acid phosphatase	AP	Organic phosphorus	4-MUB Phosphate	4000
β-glucosidase	BG	Cellulose degradation products	4-MUB-*β*-D-glucopyranoside	2000
β-xylosidase	BX	Hemicellulose degradation products	4-MUB-*β*-D-xylopyranoside	2000
Cellobiohydrolase	CBH	Cellulose degradation products	4-MUB-*β*-D-cellobioside	1000
Leucine-aminopeptidase	LAP	Polypeptides	L-leucine-7-amido-4-methylcoumarin hydrochloride	1000
N-acetyl-*β*-D-glucosaminidase	NAG	Chitin degradation products	4-MUB-*N*-acetyl-*β*-D-glucosaminide	2000
Total oxidase	OX	Lignin and phenolics	Pyrogallol + H_2O_2	1000
Polyphenol oxidase	PPO	Lignin and phenolics	Pyrogallol	1000

577
578

579 Table 3. *Neurospora* cross-strain average Vmax parameters (computed at 16ºC) and temperature
580 sensitivities (TS). SEM = standard error of the mean.

	Log(Vmax)	SEM	Vmax (nmol h^{-1} ml^{-1})	Vmax TS (°C^{-1})	SEM	Q$_{10}$
AG	0.90	0.26	2.47	0.0576	0.0029	1.78
AP	1.18	0.19	3.25	0.0759	0.0044	2.14
BG	-0.47	0.25	0.62	0.0468	0.0045	1.60
BX	-0.74	0.30	0.48	0.0771	0.0090	2.16
CBH	-0.56	0.27	0.57	0.0621	0.0078	1.86
LAP	-1.00	0.21	0.37	0.0521	0.0122	1.68
NAG	4.95	0.17	140.75	0.0813	0.0044	2.25
OX	-1.81	0.50	0.16	0.0502	0.0138	1.65
PPO	-2.18	0.46	0.11	0.0391	0.0078	1.48

581
582

583 Table 4. *Neurospora* cross-strain average Km parameters (computed at 16ºC) and temperature
584 sensitivities (TS). SEM = standard error of the mean.

	Log(Km)	SEM	Km (μM)	Km TS ($°C^{-1}$)	SEM	Q_{10}
AG	4.35	0.090	77.4	0.0185	0.0023	1.20
AP	6.74	0.140	847.8	0.0156	0.0038	1.17
BG	5.21	0.266	184.0	-0.0076	0.0081	0.93
BX	6.61	0.282	746.0	0.0272	0.0127	1.31
CBH	5.57	0.189	261.4	-0.0061	0.0117	0.94
LAP	5.58	0.188	264.5	-0.0338	0.0068	0.71
NAG	4.79	0.209	119.8	0.1031	0.0050	2.80
OX	4.54	0.408	93.5	0.0274	0.0206	1.31
PPO	4.01	0.354	55.1	0.0393	0.0123	1.48

585
586

587 Table 5. *Neurospora* cross-strain average Vmax/Km parameters (computed at 16ºC) and
588 temperature sensitivities (TS). SEM = standard error of the mean.

	Log(Vmax/Km)	SEM	Vmax/Km	Vmax/Km TS (°C^{-1})	SEM	Q$_{10}$
AG	-3.44	0.30	0.03205	0.0385	0.0029	1.47
AP	-5.54	0.21	0.00392	0.0582	0.0024	1.79
BG	-5.67	0.34	0.00343	0.0531	0.0072	1.70
BX	-7.18	0.18	0.00076	0.0447	0.0061	1.56
CBH	-5.91	0.31	0.00271	0.0743	0.0082	2.10
LAP	-6.58	0.27	0.00139	0.0856	0.0091	2.35
NAG	0.16	0.24	1.17491	-0.0219	0.0017	0.80
OX	-6.32	0.21	0.00179	0.0247	0.0118	1.28
PPO	-5.92	0.14	0.00268	0.0012	0.0062	1.01

589
590

591 Table 6. *p*-values from mixed model analyses of variance on log(Vmax), log(Km), and Vmax
592 and Km temperature sensitivities (TS). Significant values are shown in bold text.

		Vmax	Km	Vmax TS	Km TS
AG	Warming	**<0.001**	0.713	**0.047**	**0.045**
	Date	0.362	0.093	**0.042**	0.146
	Warm×Date	0.590	0.663	**0.002**	0.117
AP	Warming	**<0.001**	**0.019**	**0.012**	**<0.001**
	Date	**0.001**	0.088	0.063	0.230
	Warm×Date	**<0.001**	0.066	0.399	**0.034**
BG	Warming	**<0.001**	**<0.001**	**0.013**	0.141
	Date	**<0.001**	**<0.001**	**0.002**	0.113
	Warm×Date	**<0.001**	**<0.001**	0.226	**0.003**
BX	Warming	**<0.001**	0.646	**<0.001**	0.687
	Date	**0.007**	0.900	0.354	0.362
	Warm×Date	0.509	0.083	0.123	0.103
CBH	Warming	0.061	**<0.001**	0.177	0.290
	Date	**0.003**	**<0.001**	0.349	0.318
	Warm×Date	0.976	0.071	0.443	0.651
LAP	Warming	**<0.001**	**0.004**	**0.006**	**<0.001**
	Date	**0.015**	**<0.001**	0.070	**0.029**
	Warm×Date	0.332	0.385	0.068	0.548
NAG	Warming	**<0.001**	**<0.001**	**<0.001**	0.609
	Date	0.406	**<0.001**	**<0.001**	**0.026**
	Warm×Date	**0.002**	**<0.001**	0.126	**0.006**
OX	Warming	**<0.001**	**<0.001**	0.379	**0.006**
	Date	0.067	**0.005**	**0.004**	**<0.001**
	Warm×Date	**<0.001**	0.079	0.076	0.549
PPO	Warming	**<0.001**	**<0.001**	0.276	0.566
	Date	**0.003**	0.331	0.841	0.313
	Warm×Date	**0.011**	0.112	0.975	0.734

593

594
595

596   Figure captions

597

598   Fig. 1. Conceptual diagram of thermodynamic changes during an enzyme-catalyzed reaction.

599   Enzyme (**E**) binds to substrate (**S**) to form a complex (**ES**) with binding energy ΔG_{ES}. Formation

600   of an activated complex (**ES‡**) requires a change in free energy $\Delta G^{\ddagger}$ prior to product (**P**)

601   formation. More negative values for ΔG_{ES} result in stronger substrate binding (lower Km) but

602   can also increase $\Delta G^{\ddagger}$, thereby reducing Vmax. More negative ΔG_{ES} can occur via greater release

603   of enthalpy (more negative ΔH_{ES}) or greater increase in entropy (more positive ΔS_{ES}) upon

604   substrate binding.

605

606   Fig. 2. Heatmaps of (a) log(Vmax), (b) log(Km), and (c) log(Vmax/Km) for individual strains of

607   *Neurospora* computed at 16°C. Gray boxes are missing data.

608

609   Fig. 3. Heatmaps of temperature sensitivities for (a) Vmax (b) Km, and (c) Vmax/Km for

610   individual strains of *Neurospora*. Red values are positive, blue values are negative, and gray

611   values are missing.

612

613   Fig. 4. (a) log(Vmax) and (b) log(Km) versus strain mean annual temperature (MAT) for each

614   extracellular enzyme. Significant simple linear regressions are shown.

615

616   Fig. 5. (a) log(Vmax) and (b) log(Km) over time for litter extracellular enzymes in the Alaskan

617   boreal soil warming experiment. Points represent means $\pm$ standard error of the mean.

618

619   Fig. 6. Temperature sensitivities of (a) Vmax and (b) Km over time for litter extracellular

620   enzymes in the Alaskan boreal soil warming experiment. Dashed lines represent zero

621   temperature sensitivity. Points represent means ± standard error of the mean.

622

623   Fig. 7. Hypothesized changes in enzyme log(Km), log(Vmax), and activation energy (Ea) under

624   thermal adaptation to cold versus warm environments. Temperature ranges indicate laboratory

625   assay conditions. Empirical agreement with the hypotheses is indicated for the *Neurospora*
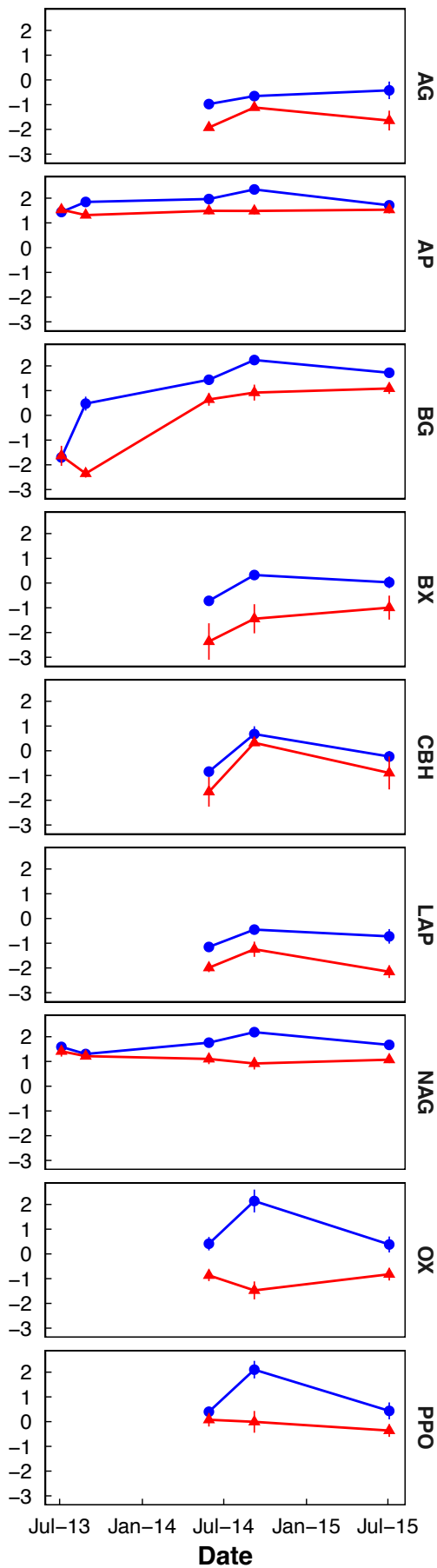
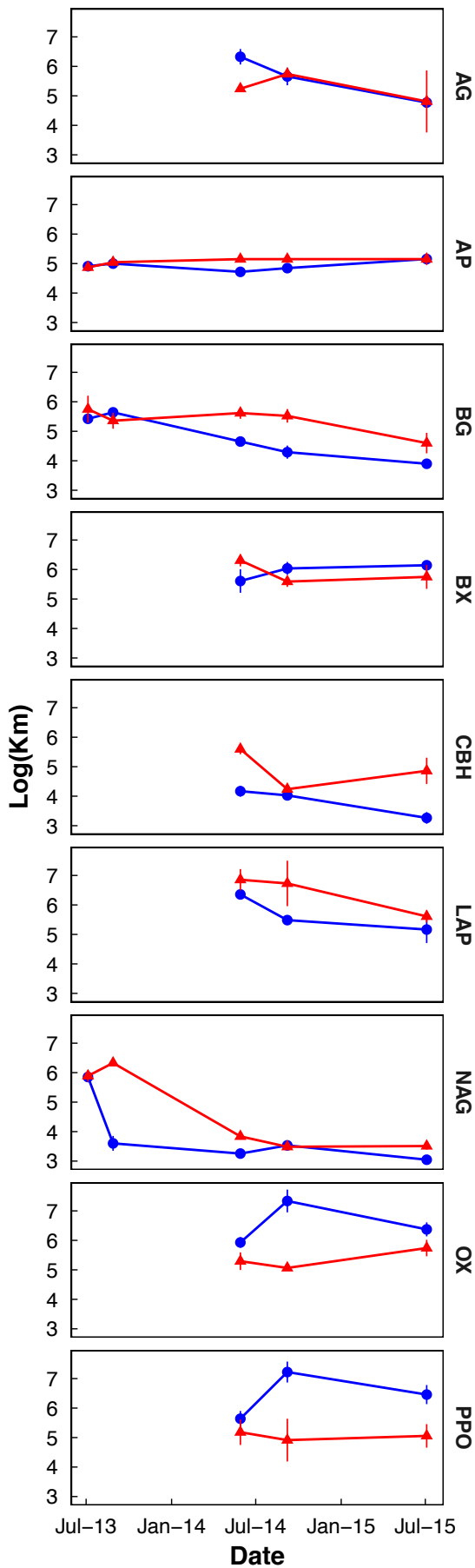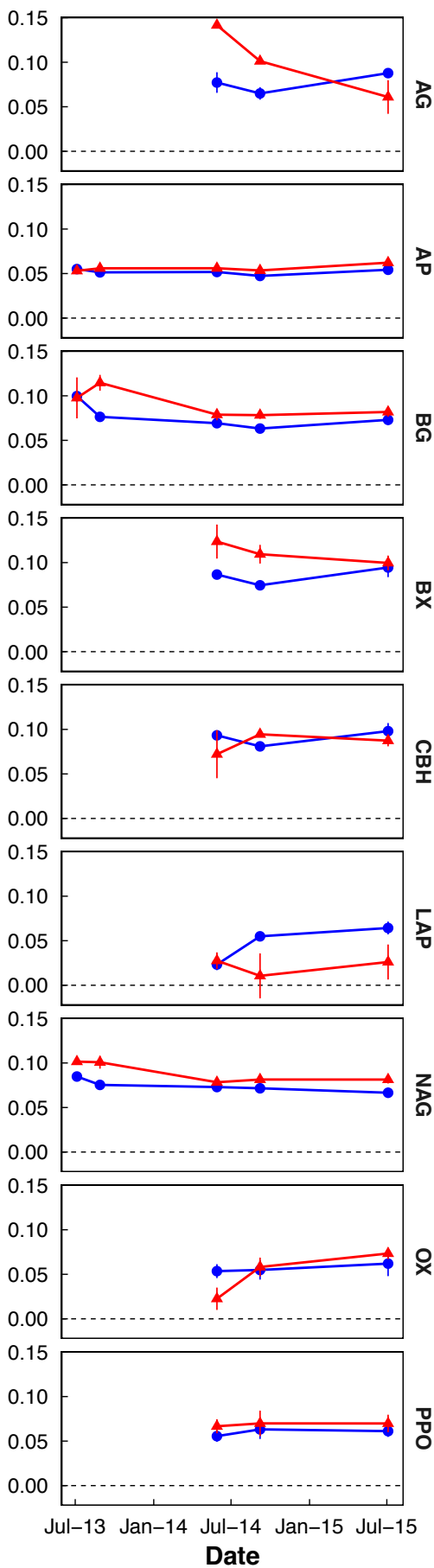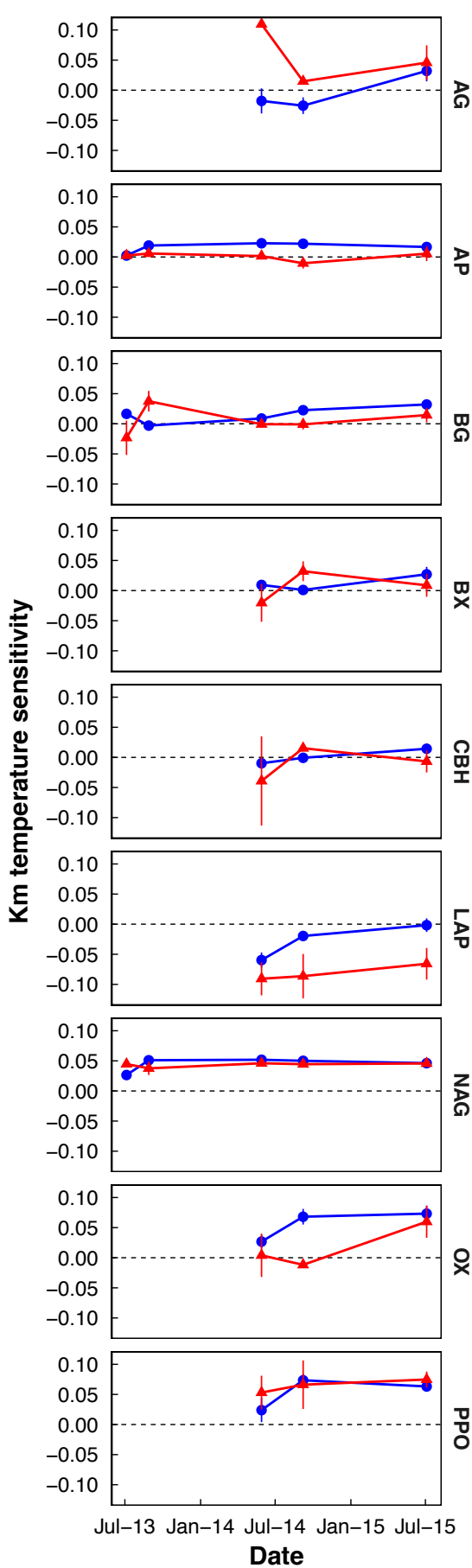626   versus litter studies in the last two columns.

627
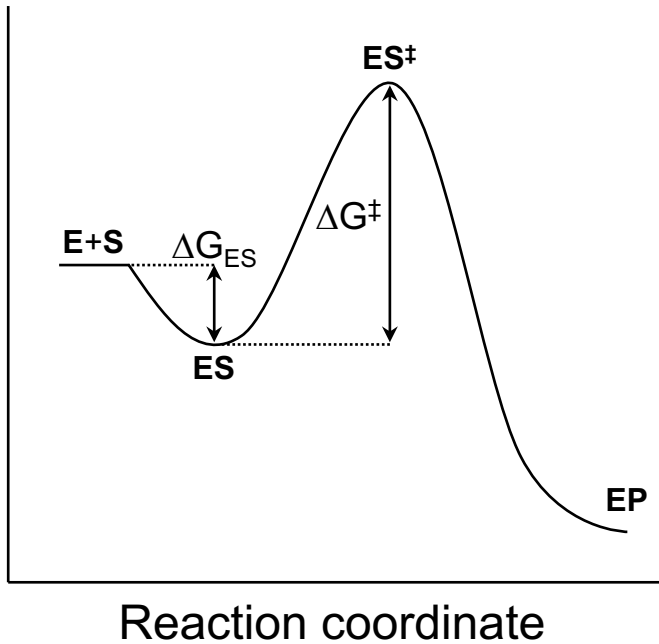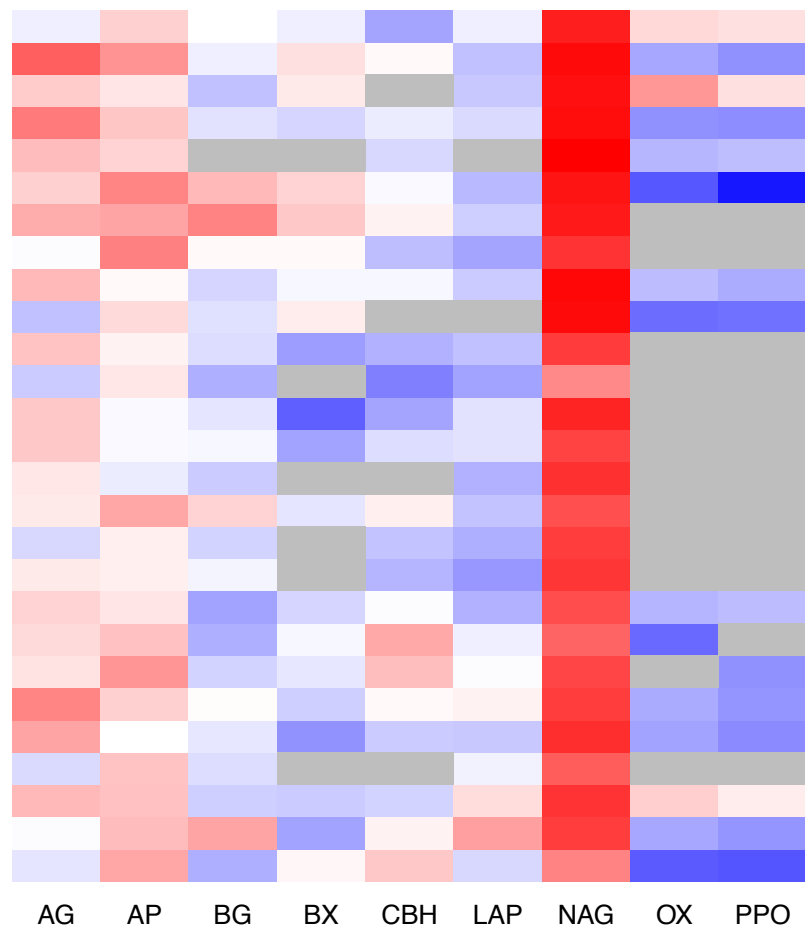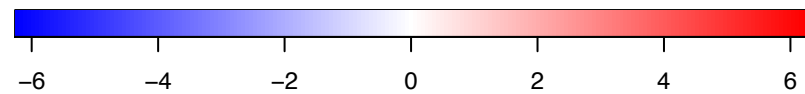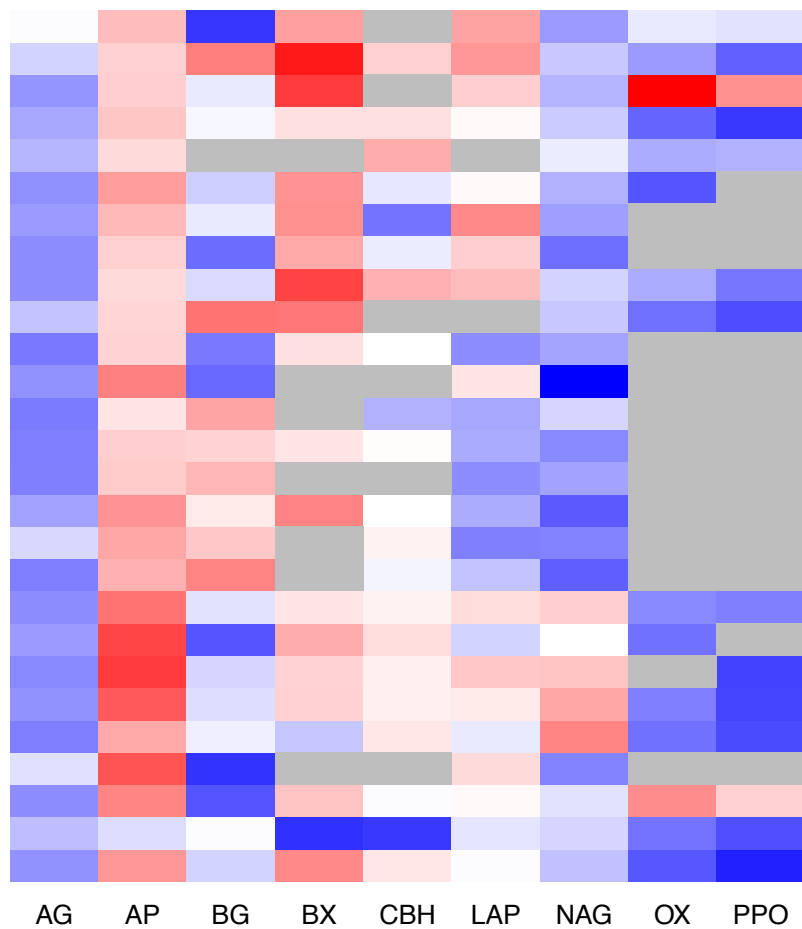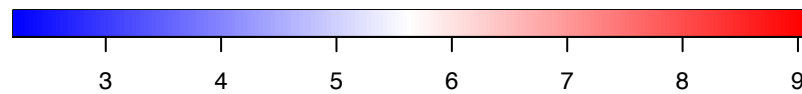
(a) Log(Vmax) **(b) Log(Km)** **(c) Log(Vmax/Km)**
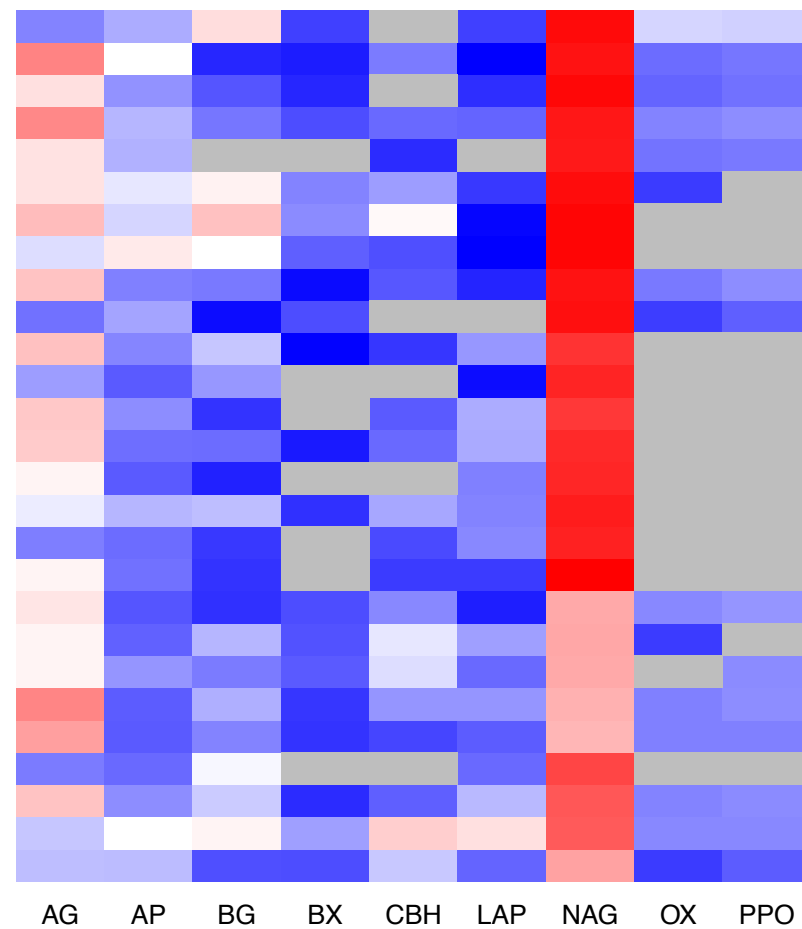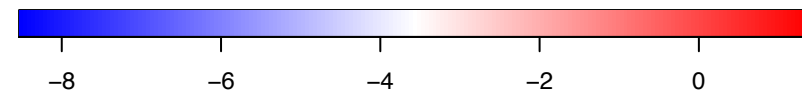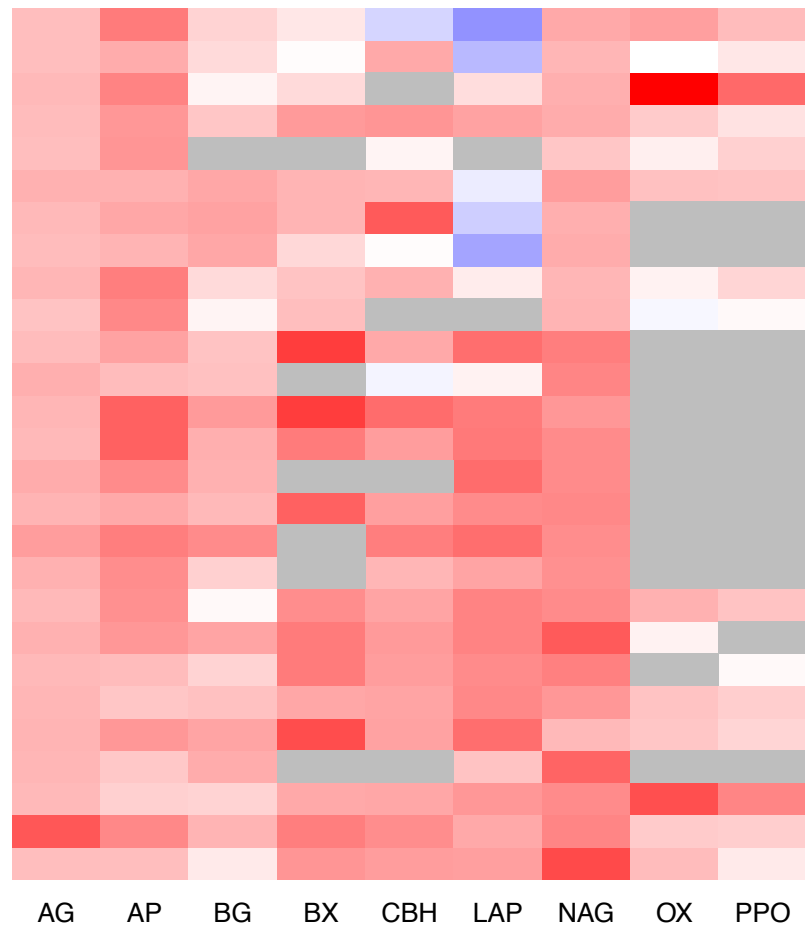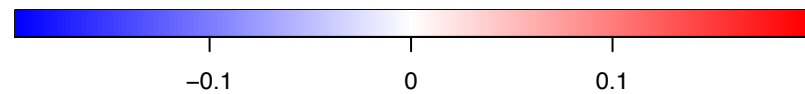
(a) Vmax temperature sensitivity **(b) Km temperature sensitivity** **(c) Vmax/Km temperature sensitivity**
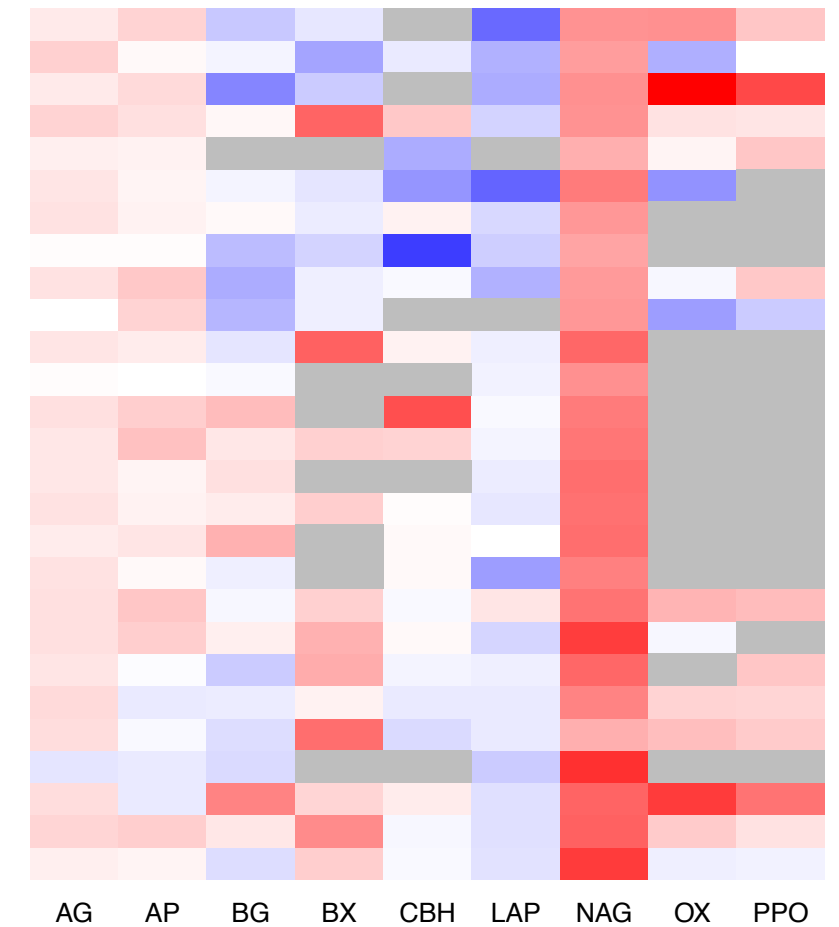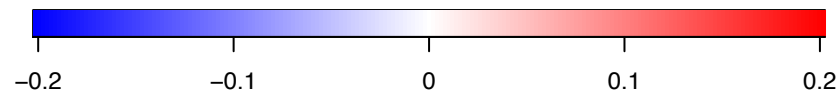
(a) Log(Vmax)

(b) Log(Km)

AG

AP — $R^2 = 0.15$, $p = 0.028$

BG

BX

CBH — $R^2 = 0.33$, $p = 0.002$

LAP — $R^2 = 0.13$, $p = 0.045$; $R^2 = 0.24$, $p = 0.007$

NAG — $R^2 = 0.26$, $p = 0.004$

OX

PPO

MAT (°C)