Article (refereed) - postprint

Malik, Ashish A.; Puissant, Jeremy; Goodall, Tim; Allison, Steven D.;
Griffiths, Robert I. 2019. **Soil microbial communities with greater
investment in resource acquisition have lower growth yield**.

Contact CEH NORA team at
noraceh@ceh.ac.uk

1 **Soil microbial communities with greater investment in resource acquisition**

2 **have lower growth yield**

3

4 **Ashish A. Malik[1,2], Jeremy Puissant[1], Tim Goodall[1], Steven D. Allison[2,3] and Robert I.**

5 **Griffiths[1]**

6 [1]Centre for Ecology and Hydrology, Wallingford, UK

7 [2]Department of Ecology and Evolutionary Biology, University of California, Irvine, USA

8 [3]Department of Earth System Science, University of California, Irvine, USA

9

10 **Abstract**

11 Resource acquisition and growth yield are fundamental microbial traits that affect

12 biogeochemical processes and have consequences for ecosystem functioning. However, there

13 is a lack of empirical observations linking these traits. Using a landscape-scale survey of

14 temperate near-neutral pH soils, we show tradeoffs in key community-level parameters

15 linked to these traits. Increased investment into extracellular enzymes estimated using

16 specific potential enzyme activity was associated with reduced growth yield obtained using

17 carbon use efficiency measures from stable isotope tracing. Reduction in growth yield was

18 linked more to carbon than nitrogen acquisition highlighting smaller stoichiometric than

19 energetic constraints on community metabolism in examined soils.

20

21 **Keywords:** carbon; microbial communities; enzymes; carbon use efficiency; nitrogen; traits

22 Microorganisms are known to affect biogeochemical cycling of elements with consequences

23 for ecosystem functioning. Of particular interest is how microbial metabolic strategies affect

24 the fate of plant carbon (C) entering soils (Schimel and Schaeffer 2012, Gleixner 2013). Soil

25 microorganisms partition detrital carbon into biomass production and respiration, and this

26 partitioning is key in determining the amount of carbon stored in soil (Schimel 2013, Liang et

27 al. 2017). Microbial growth yield often measured in C units as carbon use efficiency (CUE),

28 is defined as the amount of new growth production per unit of resource consumed (Manzoni

29 et al. 2012, Roller and Schmidt 2015, Geyer et al. 2016). It determines the fraction of carbon

30 that is allocated to biosynthetic processes (excluding that excreted as metabolites and

31 enzymes) versus the fraction that is respired for cellular energy requirements. Thus, growth

32 yield integrates microbial physiology and is a measure of the energetic and material costs for

33 survival and growth. Resource limitation can reduce growth yield by increasing the

34 investment into metabolic machinery to degrade and take up complex substrates (Frank 2010,

35 Allison 2014, Lipson 2015). This investment to acquire energy- and nutrient-rich molecules

36 comes in the form of extracellular enzymes that depolymerise complex macromolecules to be

37 then taken up and assimilated. Extracellular enzyme activity is widely believed to reflect

38 cellular metabolism specifically regulated by resource availability in the environment

39 (Sinsabaugh et al. 2010). Although there is some theoretical support to verify tradeoffs in

40 growth versus resource acquisition, empirical validation of these tradeoffs in soil microbial

41 communities is lacking (Middelboe and Sndergaard 1993). Nutrient limitation, particularly

42 nitrogen (N), can also affect growth yield as cells need to maintain the elemental

43 stoichiometry of their biomass (Manzoni et al. 2012, Sinsabaugh et al. 2013, Geyer et al.

44 2016). Under such conditions where carbon availability exceeds growth requirements,

45 microbes may take up substrates in excess to meet nutrient requirements, leading to overflow

46 respiration. Thus, it is also crucial to resolve the energetic and stoichiometric constraints on

47   microbial growth yield in soil environments (Sinsabaugh et al. 2010).

48

49   We hypothesised that due to resource constraints, community-level tradeoffs exist between

50   growth yield and resource acquisition, and that nutrient limitation affects community

51   metabolism and reduces growth yield. To test this hypothesis, we assessed the empirical

52   relationships between key physiological traits of soil microbial communities sampled at a

53   landscape scale. Soil samples were collected in triplicate from 56 sites across Britain with

54   land uses ranging from more pristine species-rich grasslands to intensive grasslands and

55   arable croplands, which together represent a set of distributed samples encompassing a

56   gradient of soil organic carbon concentrations (Malik et al. 2018b). Resource acquisition

57   traits were quantified by assessing the biomass specific potential activities of the extracellular

58   enzymes ß-1,4-glucosidase (BG), acetyl esterase (AE), leucine aminopeptidase (LAP) and ß-

59   1,4-N-acetylglucosaminidase (NAG); BG and AE were used as a proxy for C acquisition and

60   LAP and NAG were used as a proxy for N acquisition. Microbial growth yield was estimated

61   as community CUE by tracing ^{13}C-labelled, plant-derived substrates into total microbial

62   DNA and respired CO_2. Using DNA-C concentration as a proxy for microbial community

63   biomass could lead to its underestimation, however, measuring ^{13}C incorporation into

64   microbial DNA to measure growth is ideal as new DNA formation reflects microbial growth.

65   However, DNA accounts for a smaller proportion of the total cellular biomass and therefore

66   absolute value of microbial CUE measured here could be underestimated in comparison to

67   approaches that employ other biomarkers.

68

69   Following from our recent study comprehensively examining microbial community

70   physiology where we observed soil pH driven shifts in microbial CUE and its links to soil C

71   accumulation (Malik et al. 2018b), here we focus on the physiology of communities in near

72 neutral pH soils (38 sites). We excluded those from acidic (pH < 6.2) wet soils that exhibited

73 very slow growth rates and low CUE (Figure 1a, Supplementary information figure S1)

74 resulting from alternate physiological constraints (Malik et al. 2018b). From each of the

75 geographically distributed sites, 3 dispersed soil cores (5 cm diameter, 15 cm deep) were

76 sampled. After all visible roots were removed, aliquots of the homogenized soil were used for

77 the following functional analyses. For microbial respiration measurements, a soil aliquot (1

78 g) from each replicate was placed in a 10 mL glass vial, 100 µL of ^{13}C-labeled plant leaf litter

79 DOC solution (0.13 mgC) was added and incubated overnight (for ~ 16 h) in the dark at room

80 temperature (21°C). The filter-sterilised DOC solution was prepared from ^{13}C-labeled

81 powdered plant leaf litter that was produced by growing a temperate herb in a ^{13}CO$_2$

82 atmosphere (Malik et al. 2015). Respired ^{13}CO$_2$ collected in the headspace of incubation vials

83 was measured using a gas chromatography isotope ratio mass spectrometer (GC-IRMS,

84 Delta+ XL, Thermo Fisher Scientific, Germany) coupled to a PAL-autosampler (CTC

85 Analytics) with general purpose (GP) interface (Thermo Fisher Scientific, Germany). Soil

86 microbial total DNA was used as a proxy for biomass; DNA extraction was carried out on a

87 soil aliquot of 0.25 g from each replicate using PowerSoil-htp 96-well soil DNA isolation kit

88 following manufacturer instructions (MO BIO Laboratories, UK). Another set of identical

89 DNA extraction was performed following addition of 25 µL of the DO^{13}C solution and

90 overnight (16 h) incubation in dark. Both extracts with and without the tracer were analysed

91 in the size exclusion chromatography (SEC) mode on a liquid chromatography isotope ratio

92 mass spectrometer LC-IRMS (HPLC system coupled to a Delta+ XP IRMS through an LC

93 IsoLink interface; Thermo Fisher Scientific, Germany, Malik et al., 2015). This allowed us to

94 obtain DNA-C content and the proportion of DO^{13}C in microbial DNA. Microbial CUE was

95 estimated as DNA-^{13}C/(DNA-^{13}C+$\sum$CO$_2$-^{13}C), where $\sum$CO$_2$-^{13}C is the cumulative DO^{13}C lost

96 during respiration. More analytical details are given elsewhere (Malik et al. 2015, 2018b).

97

98 Potential activity of the extracellular enzymes was estimated with the common assay protocol

99 using fluorigenic substrates (Puissant et al. 2015). ß-1,4-glucosidase, acetyl esterase, leucine

100 aminopeptidase and N-acetyl glucosaminidase activity were assayed at saturated substrate

101 concentration (300 μM). Briefly, we homogenized 1.5 g soil in 20 ml of deionized water. The

102 resultant slurry was used to perform enzyme activity assays using methylumbelliferyl (MUF)

103 and 7-amino-4-methylcoumarin (AMC) conjugated substrates. The reaction was performed

104 for 3 hours at 28°C, with one fluorometric measure every 30 minutes (BioSpa 8 Automated

105 Incubator). Fluorescence intensity was measured using a Cytation 5 spectrophotometer linked

106 to the automated incubator. Biomass specific enzyme activities were calculated using DNA-C

107 measures as biomass proxy. Visualisations and regression analyses were performed with R

108 software 2.14.0 (R Development Core Team 2013) using ggplot2 and lme4 packages,

109 respectively (Bates et al. 2015).

110

111 We linked biomass specific potential activities of a set of extracellular enzymes to

112 community metabolism in order to assess microbial resource demand and its impact on

113 microbial growth yield. We observed a negative linear-log relationship between community

114 CUE and C acquisition activity (R^2=0.22, p<0.001, figure 1b). BG catalyses a terminal

115 reaction in the hydrolysis of glucose from cellobiose (Sinsabaugh et al. 2010) and AE is

116 involved in non-specific deacetylation including that of xylans (Zhang et al. 2011). Both

117 cellulose and hemicellulose, targets of the two enzymes, do not contain N and hence can be

118 used as a proxy for C acquisition. Whereas, LAP catalyses the hydrolysis of proteins and

119 NAG is involved in the hydrolysis of chitin and peptidoglycan, these target compounds are

120 principle sources of N for microorganisms. LAP and NAG have thus been widely used as a

121 proxy for N acquisition (Burns and Dick 2002, Sinsabaugh et al. 2010). A similar negative

122 linear-log relationship was observed between community CUE and N acquiring enzyme

123 activity (R^2=0.04, p=0.028, figure 1c), and although statistically significant this relationship

124 was weaker relative to that between CUE and C acquiring enzyme activity. These

125 relationships were assessed using linear mixed models to account for within site variation

126 (three replicates per site) across the geographically distributed soils, while assuming

127 community CUE to be a dependent variable for statistical purposes. 30-40% of the variation

128 in CUE was explained by site which was added as a random factor in the mixed effect model

129 (Table 1). The distribution of these traits across the landscape was also related to the soil

130 organic carbon (SOC) concentration gradient (overlaid in figure 1a-d). We have previously

131 observed, in the same set of soils, that decreasing community CUE and biomass is related to

132 decreasing SOC concentration (R^2=0.34, p<0.0001; Malik et al. 2018b). Here we show that

133 decreasing SOC was also linked to increasing biomass specific C enzyme activity (R^2=0.3,

134 p<0.0001), and to a very small extent to increasing N enzyme activity (R^2=0.06, p=0.02). C

135 enzyme activity and to a smaller degree N enzyme activity was positively correlated to

136 biomass specific respiration and community aggregated growth rate (table S1). These

137 patterns suggest that in soils with lower SOC (usually intensive grasslands and arable

138 croplands), resource limitation drives microbial communities to invest heavily into resource

139 acquisition traits that trades off against growth yield. On the other hand, communities grow

140 efficiently in more resource-rich soils with higher SOM and more readily available precursor

141 molecules (usually "pristine" or less intensive grasslands) as they possess substrate uptake

142 mechanisms like ABC transporters and have lower biomass specific activity of extracellular

143 enzymes (Malik et al. 2018b, Zhalnina et al. 2018). Lower maintenance requirement of these

144 communities is corroborated by observations of lower biomass specific respiration in such

145 soils. It is also interesting to note that certain communities exhibited lower enzyme activity

146 per unit biomass and lower growth yield thus weakening the regression trends (Figure 1b-c).

147 Moreover, although the enzymatic C:N ratio increases with decreasing CUE as we

148 hypothesised (R^2=0.17, p<0.001, figure 1d, table 1), there was little evidence to suggest

149 stoichiometric constraints on microbial growth and metabolism. The stronger association of

150 C- relative to N-acquiring enzyme activity with CUE suggests that community-level

151 energetic constraints are greater than stoichiometric constraints (Sinsabaugh et al. 2010,

152 Mooshammer et al. 2014). Still, this result could also reflect the resource and nutrient status

153 of the temperate soils under investigation, which appeared to be C- and not N-limited. We

154 also observed that enzymatic C:N ratio and soil C:N ratio did not covary (Figure S2)

155 indicating that soil C:N ratio is not a good indicator of available resources (Mooshammer et

156 al. 2014).

157

158 Based on empirical relationships, we provide evidence for a clear tradeoff between

159 community-level growth yield and resource acquisition potential in near neutral pH soils.

160 Although the statistical power of these relationships is not strong (given the geographically

161 distibuted nature of this survey and the high amount of variation explained by site), the

162 patterns in trait distribution demonstrate distinct life history strategies. The observed

163 tradeoffs are in line with those assumed or predicted by theoretical models (Allison 2014,

164 Manzoni et al. 2014). On the basis of the trade-off patterns observed in this study, we applied

165 a three-way microbial trait framework similar to Grime's competitor-stress tolerator-ruderal

166 (C-S-R) framework for plants (Grime 1977). Growth yield suffered in communities investing

167 in maintenance requirements like resource acquisition through regeneration of extracellular

168 enzymes (Figure 2, lower right). This tradeoff is reiterated by the absence of scenarios of

169 communities excelling in both traits (Figure 2, upper right). However, a large amount of

170 variation in community growth yield was explained by site thus it is plausible that either or

171 both of these traits trade-off with some other unmeasured trait linked to the soil environment,

172 likely stress tolerance (Schimel et al. 2007, Malik et al. 2018b, Wood et al. 2018). In support

173 of this interpretation, we previously found lower growth yield in acidic soils (Figure 1a;

174 Malik et al. 2018b) highlighting much higher maintenance costs of acid stress tolerance in

175 such soils. Thus, we demonstrate strong support for the growth-maintenance tradeoff

176 hypothesis and show trait tradeoffs have consequences for soil carbon dynamics. In line with

177 the empirical trends, we propose a microbial Y-A-S (high yield-resource acquisition-stress

178 tolerance) life history framework (Malik et al. 2018a), which suggests that tradeoffs in

179 resource allocation among traits linked to high yield, resource acquisition and stress tolerance

180 prevent microbes from excelling at multiple strategies such that different strategies are

181 favoured under different environmental conditions. However, more work is required in

182 estimating trait values for stress tolerance strategies and how they trade off with microbial

183 growth yield. We also show, in the carbon-limited microbial communities under study, that

184 stoichiometric imbalances have smaller impacts on microbial community growth yield in

185 comparison to energetic requirements. This finding suggests that C flow in cellular systems is

186 a fundamental constraint on microbial growth efficiency that affects the fate of plant and soil

187 organic carbon.

188

195

196 **Author contributions**

197 AAM, JP and RIG designed research; AAM and TG performed the stable isotope analyses;

198 JP and TG performed the enzyme assays; AAM and JP performed statistical analyses; AAM

199 and SDA developed the conceptual framework, AAM drafted the manuscript and all authors

200 were involved in critical revision and approval of the final version.

201

202 **Figure legends**

203 **Figure 1: a)** Regression trends of microbial CUE with soil pH across the landscape scale

204 gradient of soils. Data from all 56 sites with three replicates at each site are presented here.

205 The threshold was determined at pH 6.2 below which microbial CUE was very low, hence

206 excluded from this study. **b-c)** Regression trends of community-aggregated growth yield or

207 carbon use efficiency-CUE (unitless) with biomass specific C and N acquiring enzyme

208 activity expressed as nmol min^{-1} µg-DNA-C^{-1} (DNA as a biomass proxy) from 38 sites with

209 pH > 6.2. **d)** Relationship between growth yield and enzymatic C:N ratio. Overlaid in the

210 scatterplots is the variation in soil C concentration. The x-axes in b-d are on a log$_2$ scale as a

211 means to transform a skewed variable into a more approximate normal distribution.

212 **Figure 2**: Conceptual framework assigning dominant life history strategies to microbial

213 communities superimposed on the observed trait distribution patterns.

214

215 **References**

216 Allison, S. D. 2014. Modeling adaptation of carbon use efficiency in microbial communities.

217 Frontiers in Microbiology 5:1–9.

218 Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models

219 Using lme4. Journal of Statistical Software; Vol 1, Issue 1 (2015) .

220 Burns, R. G., and R. P. Dick. 2002. Enzymes in the environment: activity, ecology, and

221 applications. Page (R. G. Burns and R. P. Dick, Eds.). Marcel Dekker, Inc., New York.

222 Frank, S. A. 2010. The trade-off between rate and yield in the design of microbial

223   metabolism. Journal of Evolutionary Biology 23:609–613.

224 Geyer, K. M., E. Kyker-Snowman, A. S. Grandy, and S. D. Frey. 2016. Microbial carbon use

225   efficiency: accounting for population, community, and ecosystem-scale controls over the

226   fate of metabolized organic matter. Biogeochemistry 127:173–188.

227 Gleixner, G. 2013. Soil organic matter dynamics: a biological perspective derived from the

228   use of compound-specific isotopes studies. Ecological Research 28:683–695.

229 Grime, J. P. 1977. Evidence for the Existence of Three Primary Strategies in Plants and Its

230   Relevance to Ecological and Evolutionary Theory. The American Naturalist 111:1169–

231   1194.

232 Liang, C., J. P. Schimel, and J. D. Jastrow. 2017. The importance of anabolism in microbial

233   control over soil carbon storage. Nature Microbiology 2:17105.

234 Lipson, D. A. 2015. The complex relationship between microbial growth rate and yield and

235   its implications for ecosystem processes. Frontiers in Microbiology 6:1–5.

236 Malik, A. A., H. Dannert, R. I. Griffiths, B. C. Thomson, and G. Gleixner. 2015. Rhizosphere

237   bacterial carbon turnover is higher in nucleic acids than membrane lipids: implications

238   for understanding soil carbon cycling. Frontiers in microbiology 6:268.

239 Malik, A. A., J. B. H. Martiny, E. L. Brodie, A. C. Martiny, K. K. Treseder, and S. D.

240   Allison. 2018a. Defining trait-based microbial strategies with consequences for soil

241   carbon cycling under climate change. bioRxiv.

242 Malik, A. A., J. Puissant, K. M. Buckeridge, T. Goodall, N. Jehmlich, S. Chowdhury, S.

243   Gweon, J. Peyton, K. E. Mason, M. van Agtmaal, A. Blaud, I. M. Clark, J. Whitaker, R.

244   F. Pywell, N. Ostle, G. Gleixner, and R. I. Griffiths. 2018b. Land use driven change in

245   soil pH affects microbial carbon cycling processes. Nature Communications 9:3591.

246 Manzoni, S., S. M. Schaeffer, G. Katul, A. Porporato, and J. P. Schimel. 2014. A theoretical

247   analysis of microbial eco-physiological and diffusion limitations to carbon cycling in

248   drying soils. Soil Biology and Biochemistry 73:69–83.

249   Manzoni, S., P. Taylor, A. Richter, A. Porporato, and G. I. Ågren. 2012. Environmental and

250   stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist

251   196:79–91.

252   Middelboe, M., and M. Sndergaard. 1993. Bacterioplankton Growth Yield: Seasonal

253   Variations and Coupling to Substrate. Applied and Environmental Microbiology

254   59:3916–3921.

255   Mooshammer, M., W. Wanek, S. Zechmeister-Boltenstern, and A. Richter. 2014.

256   Stoichiometric imbalances between terrestrial decomposer communities and their

257   resources: mechanisms and implications of microbial adaptations to their resources.

258   Frontiers in Microbiology 5:1–10.

259   Puissant, J., L. Cécillon, R. T. E. Mills, B. J. M. Robroek, K. Gavazov, S. De Danieli, T.

260   Spiegelberger, A. Buttler, and J.-J. Brun. 2015. Seasonal influence of climate

261   manipulation on microbial community structure and function in mountain soils. Soil

262   Biology and Biochemistry 80:296–305.

263   R Development Core Team. 2013. R: A language and environment for statistical computing.

264   Roller, B. R., and T. M. Schmidt. 2015. The physiology and ecological implications of

265   efficient growth. The ISME journal 9:1481–1487.

266   Schimel, J. 2013. Soil carbon: Microbes and global carbon. Nature Climate Change 3:867–

267   868.

268   Schimel, J., T. C. Balser, and M. Wallenstein. 2007. Microbial stress-response physiology

269   and its implications for ecosystem function. Ecology 88:1386–1394.

270   Schimel, J. P., and S. M. Schaeffer. 2012. Microbial control over carbon cycling in soil.

271   Frontiers in Microbiology 3:1–11.

272 Sinsabaugh, R. L., B. H. Hill, and J. J. F. Shah. 2010. Ecoenzymatic stoichiometry of

273   microbial organic nutrient acquisition in soil and sediment. Nature 468:122.

274 Sinsabaugh, R. L., S. Manzoni, D. L. Moorhead, and A. Richter. 2013. Carbon use efficiency

275   of microbial communities: stoichiometry, methodology and modelling. Ecology Letters

276   16:930–939.

277 Wood, J. L., C. Tang, and A. E. Franks. 2018. Competitive traits are more important than

278   stress-tolerance traits in a cadmium-contaminated rhizosphere: A role for trait theory in

279   microbial ecology. Frontiers in Microbiology 9:1–12.

280 Zhalnina, K., K. B. Louie, Z. Hao, N. Mansoori, U. Nunes da Rocha, S. Shi, H. Cho, U.

281   Karaoz, D. Loqué, B. P. Bowen, M. K. Firestone, T. R. Northen, and E. L. Brodie. 2018.

282   Dynamic root exudate chemistry and microbial substrate preferences drive patterns in

283   rhizosphere microbial community assembly. Nature Microbiology. 3:470–480.

284 Zhang, J., M. Siika-aho, M. Tenkanen, and L. Viikari. 2011. The role of acetyl xylan esterase

285   in the solubilization of xylan and enzymatic hydrolysis of wheat straw and giant reed.

286   Biotechnology for Biofuels 4:60.

287

Table 1: Results of linear mixed effect models used to assess community CUE-enzyme activity relationship by analyzing the predictive power of enzyme measures to explain the variance in community CUE. Enzyme variables were used as fixed factors and site was used as a random factor in the mixed model. ICC (intraclass correlation coefficient) accounts for the variance explained by site. Marginal R^2 describes the proportion of variance explained by the fixed factor alone, whereas, conditional R^2 describes the proportion of variance explained by both the fixed and random factors. Number of observations: 114, number of sites: 38.

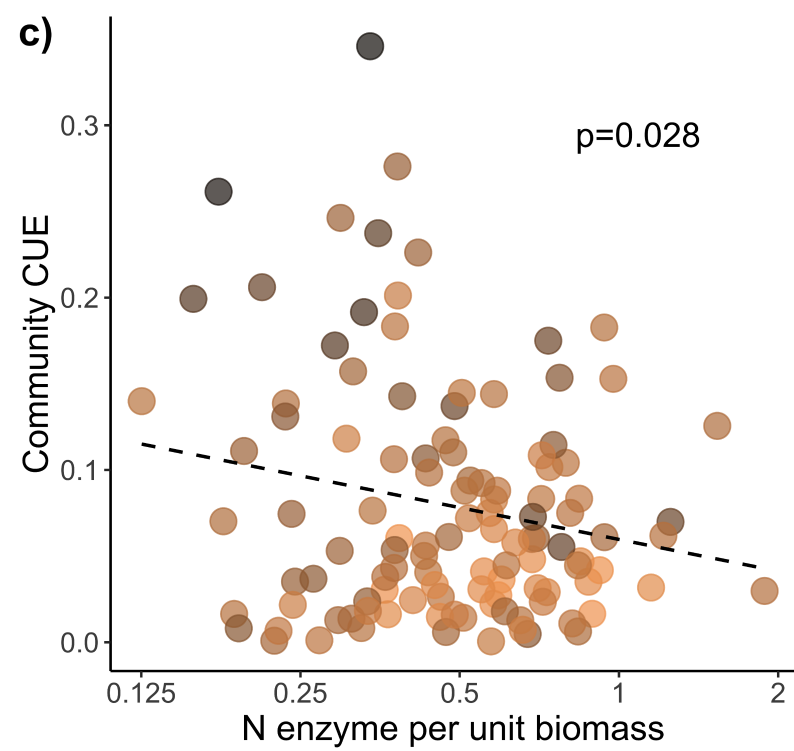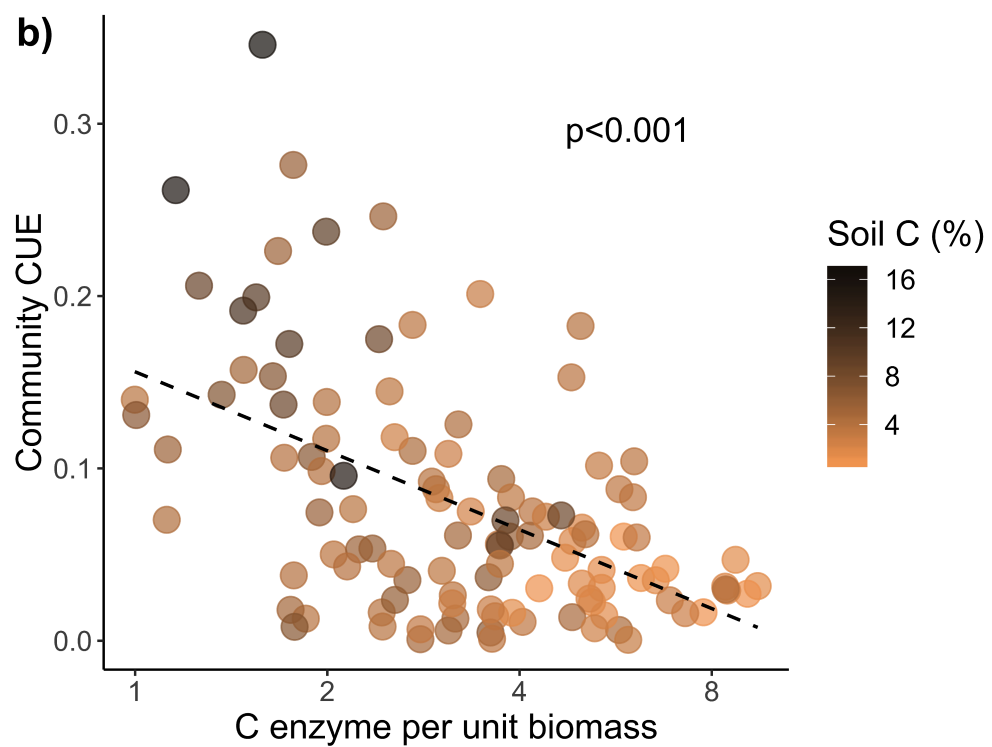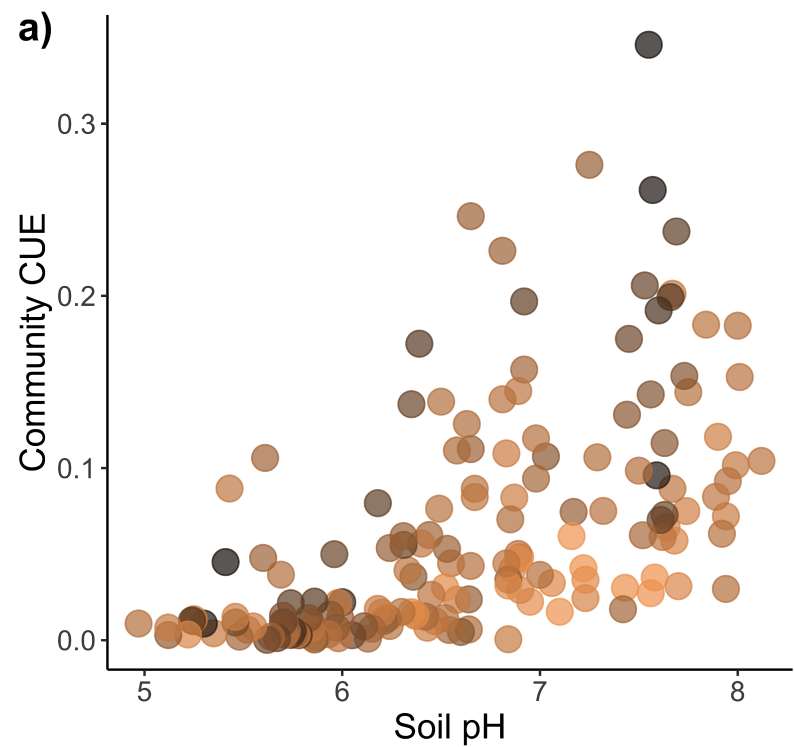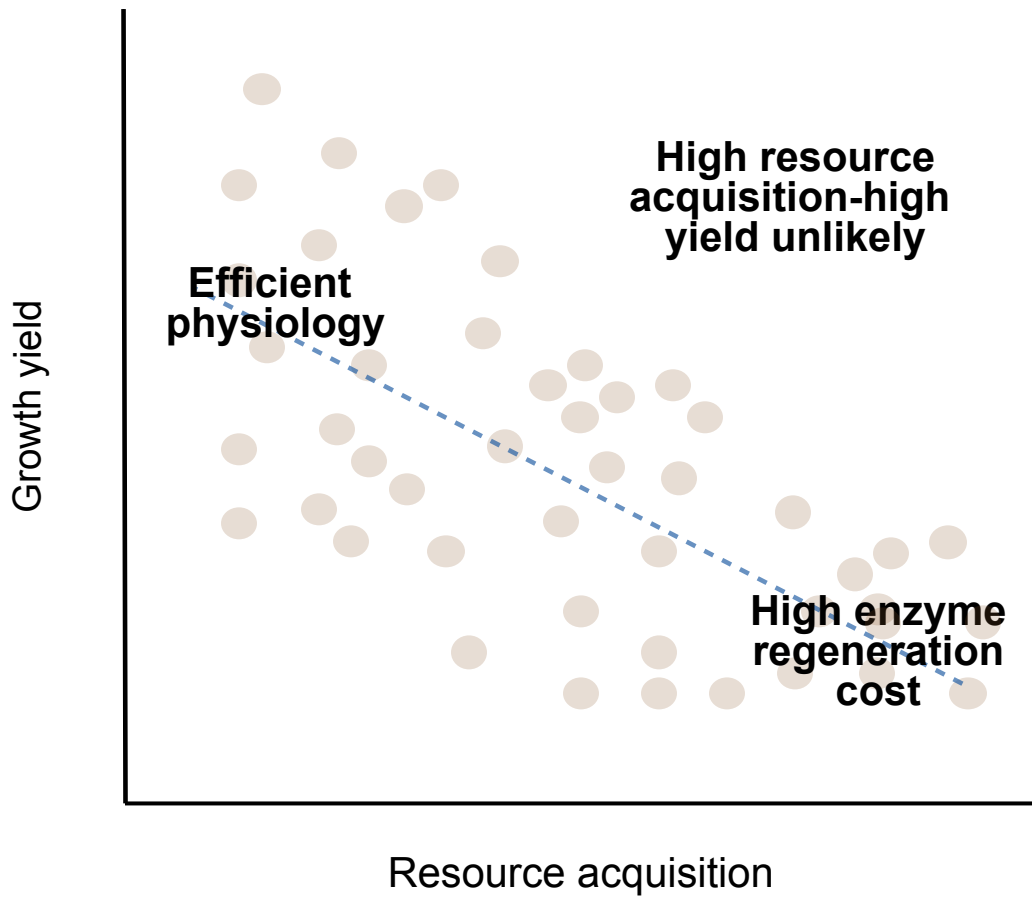| Predictor variable	C enzyme	N enzyme	Enzyme C:N
Intercept	0.14	0.11	0.15
Confidence interval	0.11 - 0.17	0.08 - 0.14	0.12 - 0.18
p	<0.001	0.028	<0.001
ICC_{site}	0.30	0.40	0.34
Marginal R^2	0.22	0.04	0.17
Conditional R^2	0.45	0.43	0.45

Figure 1
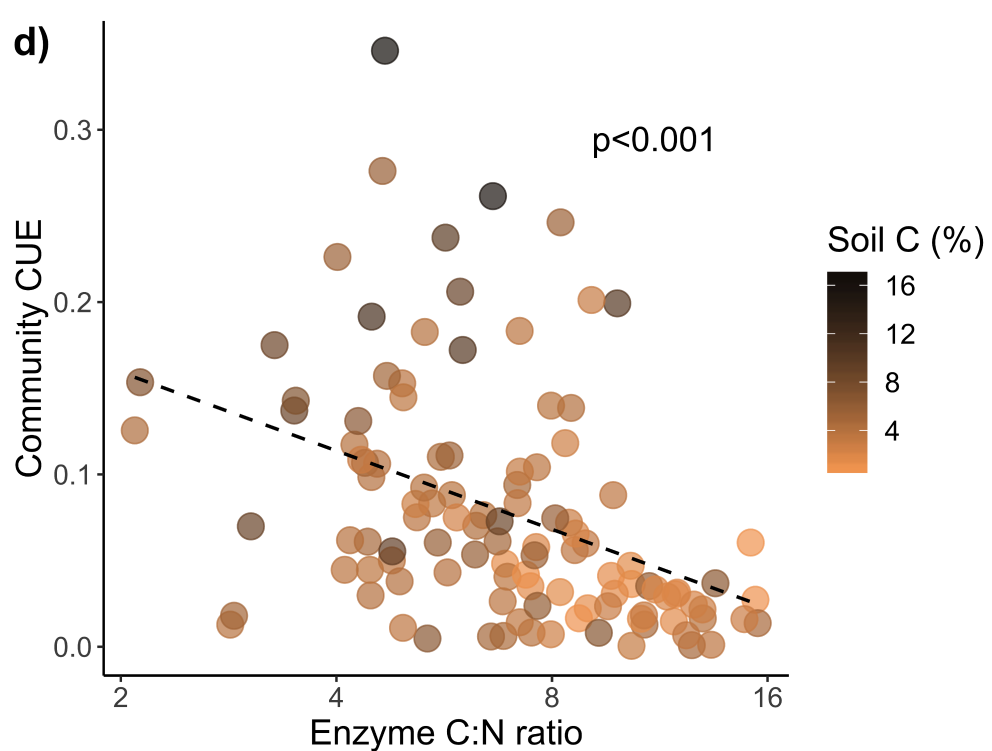
Figure 2

Highlights

- Evidence for tradeoff in microbial resource acquisition and growth yield traits
- Growth yield patterns linked more to carbon than nitrogen enzyme activity
- Smaller stoichiometric than energetic constraints on community metabolism
- Community-aggregated trait tradeoffs have consequences for soil carbon cycling