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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

Soil microbial communities with greater investment in resource acquisition have lower growth yield

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Abstract

Resource acquisition and growth yield are fundamental microbial traits that affect biogeochemical processes and have consequences for ecosystem functioning. However, there is a lack of empirical observations linking these traits. Using a landscape-scale survey of temperate near-neutral pH soils, we show tradeoffs in key community-level parameters linked to these traits. Increased investment into extracellular enzymes estimated using specific potential enzyme activity was associated with reduced growth yield obtained using carbon use efficiency measures from stable isotope tracing. Reduction in growth yield was linked more to carbon than nitrogen acquisition highlighting smaller stoichiometric than energetic constraints on community metabolism in examined soils.

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

Microorganisms are known to affect biogeochemical cycling of elements with consequences for ecosystem functioning. Of particular interest is how microbial metabolic strategies affect the fate of plant carbon (C) entering soils (Schimel and Schaeffer 2012, Gleixner 2013). Soil microorganisms partition detrital carbon into biomass production and respiration, and this partitioning is key in determining the amount of carbon stored in soil (Schimel 2013, Liang et al. 2017). Microbial growth yield often measured in C units as carbon use efficiency (CUE), is defined as the amount of new growth production per unit of resource consumed (Manzoni et al. 2012, Roller and Schmidt 2015, Geyer et al. 2016). It determines the fraction of carbon that is allocated to biosynthetic processes (excluding that excreted as metabolites and enzymes) versus the fraction that is respired for cellular energy requirements. Thus, growth yield integrates microbial physiology and is a measure of the energetic and material costs for survival and growth. Resource limitation can reduce growth yield by increasing the investment into metabolic machinery to degrade and take up complex substrates (Frank 2010, Allison 2014, Lipson 2015). This investment to acquire energy- and nutrient-rich molecules comes in the form of extracellular enzymes that depolymerise complex macromolecules to be then taken up and assimilated. Extracellular enzyme activity is widely believed to reflect cellular metabolism specifically regulated by resource availability in the environment (Sinsabaugh et al. 2010). Although there is some theoretical support to verify tradeoffs in growth versus resource acquisition, empirical validation of these tradeoffs in soil microbial communities is lacking (Middelboe and Sndergaard 1993). Nutrient limitation, particularly nitrogen (N), can also affect growth yield as cells need to maintain the elemental stoichiometry of their biomass (Manzoni et al. 2012, Sinsabaugh et al. 2013, Geyer et al. 2016). Under such conditions where carbon availability exceeds growth requirements, microbes may take up substrates in excess to meet nutrient requirements, leading to overflow 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

neutral pH soils (38 sites). We excluded those from acidic (pH < 6.2) wet soils that exhibited very slow growth rates and low CUE (Figure 1a, Supplementary information figure S1) resulting from alternate physiological constraints (Malik et al. 2018b). From each of the geographically distributed sites, 3 dispersed soil cores (5 cm diameter, 15 cm deep) were sampled. After all visible roots were removed, aliquots of the homogenized soil were used for the following functional analyses. For microbial respiration measurements, a soil aliquot (1 g) from each replicate was placed in a 10 mL glass vial, 100 µL of ^{13}C-labeled plant leaf litter DOC solution (0.13 mgC) was added and incubated overnight (for ~ 16 h) in the dark at room temperature (21°C). The filter-sterilised DOC solution was prepared from ^{13}C-labeled powdered plant leaf litter that was produced by growing a temperate herb in a $^{13}CO_2$ atmosphere (Malik et al. 2015). Respired $^{13}CO_2$ collected in the headspace of incubation vials was measured using a gas chromatography isotope ratio mass spectrometer (GC-IRMS, Delta+ XL, Thermo Fisher Scientific, Germany) coupled to a PAL-autosampler (CTC Analytics) with general purpose (GP) interface (Thermo Fisher Scientific, Germany). Soil microbial total DNA was used as a proxy for biomass; DNA extraction was carried out on a soil aliquot of 0.25 g from each replicate using PowerSoil-htp 96-well soil DNA isolation kit following manufacturer instructions (MO BIO Laboratories, UK). Another set of identical DNA extraction was performed following addition of 25 µL of the DO^{13}C solution and overnight (16 h) incubation in dark. Both extracts with and without the tracer were analysed in the size exclusion chromatography (SEC) mode on a liquid chromatography isotope ratio mass spectrometer LC-IRMS (HPLC system coupled to a Delta+ XP IRMS through an LC IsoLink interface; Thermo Fisher Scientific, Germany, Malik et al., 2015). This allowed us to obtain DNA-C content and the proportion of DO^{13}C in microbial DNA. Microbial CUE was 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 during respiration. More analytical details are given elsewhere (Malik et al. 2015, 2018b).

Potential activity of the extracellular enzymes was estimated with the common assay protocol using fluorigenic substrates (Puissant et al. 2015). ß-1,4-glucosidase, acetyl esterase, leucine aminopeptidase and N-acetyl glucosaminidase activity were assayed at saturated substrate concentration (300 μM). Briefly, we homogenized 1.5 g soil in 20 ml of deionized water. The resultant slurry was used to perform enzyme activity assays using methylumbelliferyl (MUF) and 7-amino-4-methylcoumarin (AMC) conjugated substrates. The reaction was performed for 3 hours at 28°C, with one fluorometric measure every 30 minutes (BioSpa 8 Automated Incubator). Fluorescence intensity was measured using a Cytation 5 spectrophotometer linked to the automated incubator. Biomass specific enzyme activities were calculated using DNA-C measures as biomass proxy. Visualisations and regression analyses were performed with R software 2.14.0 (R Development Core Team 2013) using ggplot2 and lme4 packages, respectively (Bates et al. 2015).

We linked biomass specific potential activities of a set of extracellular enzymes to community metabolism in order to assess microbial resource demand and its impact on microbial growth yield. We observed a negative linear-log relationship between community CUE and C acquisition activity (R^2=0.22, p<0.001, figure 1b). BG catalyses a terminal reaction in the hydrolysis of glucose from cellobiose (Sinsabaugh et al. 2010) and AE is involved in non-specific deacetylation including that of xylans (Zhang et al. 2011). Both cellulose and hemicellulose, targets of the two enzymes, do not contain N and hence can be used as a proxy for C acquisition. Whereas, LAP catalyses the hydrolysis of proteins and NAG is involved in the hydrolysis of chitin and peptidoglycan, these target compounds are principle sources of N for microorganisms. LAP and NAG have thus been widely used as a proxy for N acquisition (Burns and Dick 2002, Sinsabaugh et al. 2010). A similar negative

linear-log relationship was observed between community CUE and N acquiring enzyme activity (R^2=0.04, p=0.028, figure 1c), and although statistically significant this relationship was weaker relative to that between CUE and C acquiring enzyme activity. These relationships were assessed using linear mixed models to account for within site variation (three replicates per site) across the geographically distributed soils, while assuming community CUE to be a dependent variable for statistical purposes. 30-40% of the variation in CUE was explained by site which was added as a random factor in the mixed effect model (Table 1). The distribution of these traits across the landscape was also related to the soil organic carbon (SOC) concentration gradient (overlaid in figure 1a-d). We have previously observed, in the same set of soils, that decreasing community CUE and biomass is related to decreasing SOC concentration (R^2=0.34, p<0.0001; Malik et al. 2018b). Here we show that decreasing SOC was also linked to increasing biomass specific C enzyme activity (R^2=0.3, p<0.0001), and to a very small extent to increasing N enzyme activity (R^2=0.06, p=0.02). C enzyme activity and to a smaller degree N enzyme activity was positively correlated to biomass specific respiration and community aggregated growth rate (table S1). These patterns suggest that in soils with lower SOC (usually intensive grasslands and arable croplands), resource limitation drives microbial communities to invest heavily into resource acquisition traits that trades off against growth yield. On the other hand, communities grow efficiently in more resource-rich soils with higher SOM and more readily available precursor molecules (usually “pristine” or less intensive grasslands) as they possess substrate uptake mechanisms like ABC transporters and have lower biomass specific activity of extracellular enzymes (Malik et al. 2018b, Zhalnina et al. 2018). Lower maintenance requirement of these communities is corroborated by observations of lower biomass specific respiration in such soils. It is also interesting to note that certain communities exhibited lower enzyme activity 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,

likely stress tolerance (Schimel et al. 2007, Malik et al. 2018b, Wood et al. 2018). In support of this interpretation, we previously found lower growth yield in acidic soils (Figure 1a; Malik et al. 2018b) highlighting much higher maintenance costs of acid stress tolerance in such soils. Thus, we demonstrate strong support for the growth-maintenance tradeoff hypothesis and show trait tradeoffs have consequences for soil carbon dynamics. In line with the empirical trends, we propose a microbial Y-A-S (high yield-resource acquisition-stress tolerance) life history framework (Malik et al. 2018a), which suggests that tradeoffs in resource allocation among traits linked to high yield, resource acquisition and stress tolerance prevent microbes from excelling at multiple strategies such that different strategies are favoured under different environmental conditions. However, more work is required in estimating trait values for stress tolerance strategies and how they trade off with microbial growth yield. We also show, in the temperate soils under study, that stoichiometric imbalances have smaller impacts on microbial community growth yield in comparison to energetic requirements. This finding suggests that C flow in cellular systems is a fundamental constraint on microbial growth efficiency that affects the fate of plant and soil organic carbon.

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Author contributions

AAM, JP and RIG designed research; AAM and TG performed the stable isotope analyses; JP and TG performed the enzyme assays; AAM and JP performed statistical analyses; AAM and SDA developed the conceptual framework, AAM drafted the manuscript and all authors were involved in critical revision and approval of the final version.

Figure legends

Figure 1: a) Regression trends of microbial CUE with soil pH across the landscape scale gradient of soils. Data from all 56 sites with three replicates at each site are presented here. The threshold was determined at pH 6.2 below which microbial CUE was very low, hence excluded from this study. **b-c)** Regression trends of community-aggregated growth yield or carbon use efficiency-CUE (unitless) with biomass specific C and N acquiring enzyme activity expressed as nmol min^{-1} μg-DNA-C^{-1} (DNA as a biomass proxy) from 38 sites with pH > 6.2. **d)** Relationship between growth yield and enzymatic C:N ratio. Overlaid in the scatterplots is the variation in soil C concentration. The x-axes in b-d are on a $\log_2$ scale as a means to transform a skewed variable into a more approximate normal distribution.

Figure 2: Conceptual framework assigning dominant life history strategies to microbial communities superimposed on the observed trait distribution patterns.

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Table 1

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

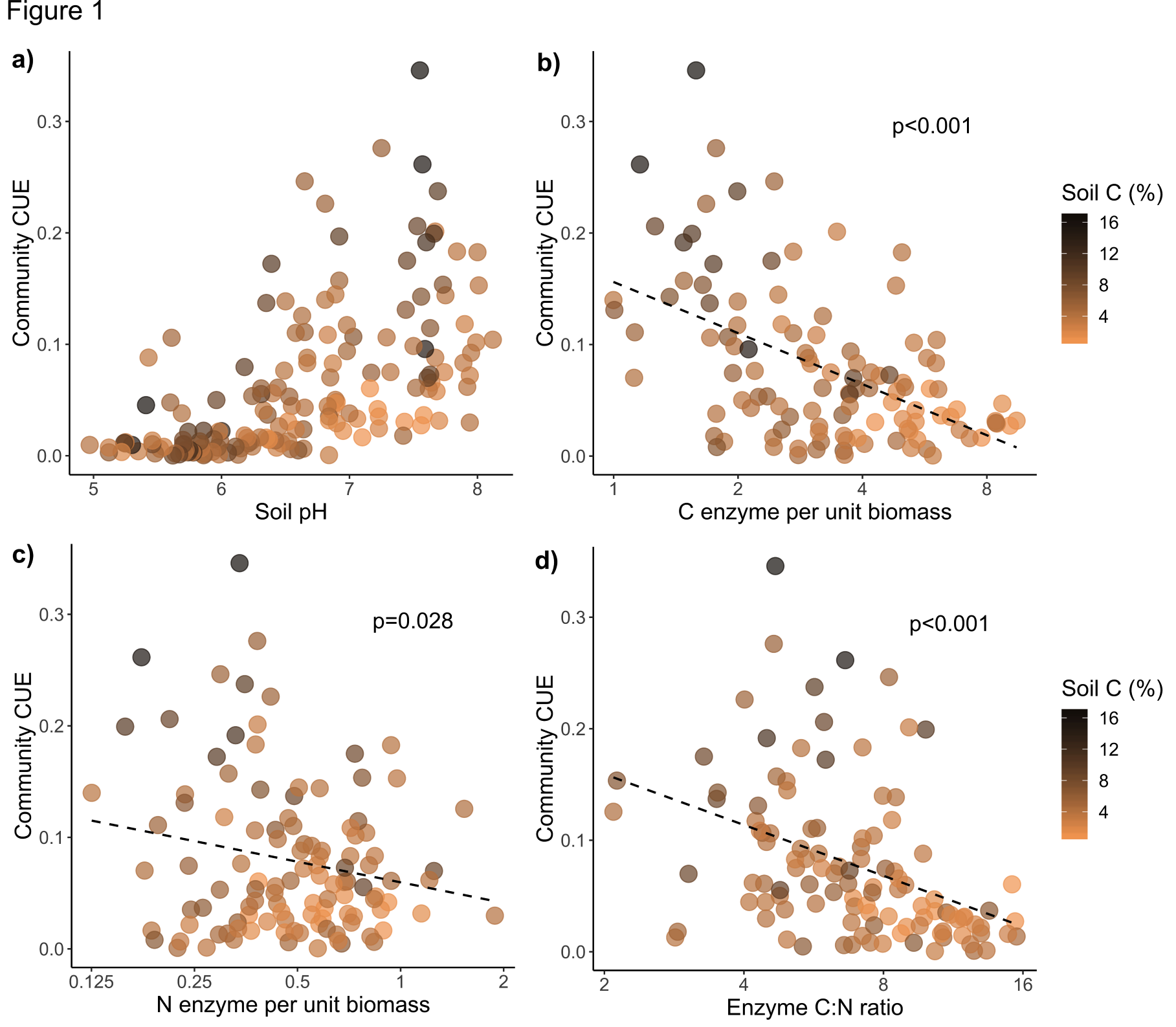
a)
b)
c)
d)
Community CUE
Soil pH
C enzyme per unit biomass
N enzyme per unit biomass
Enzyme C:N ratio
p<0.001
p=0.028
p<0.001
Soil C (%)
16
12
8
4

Figure 2

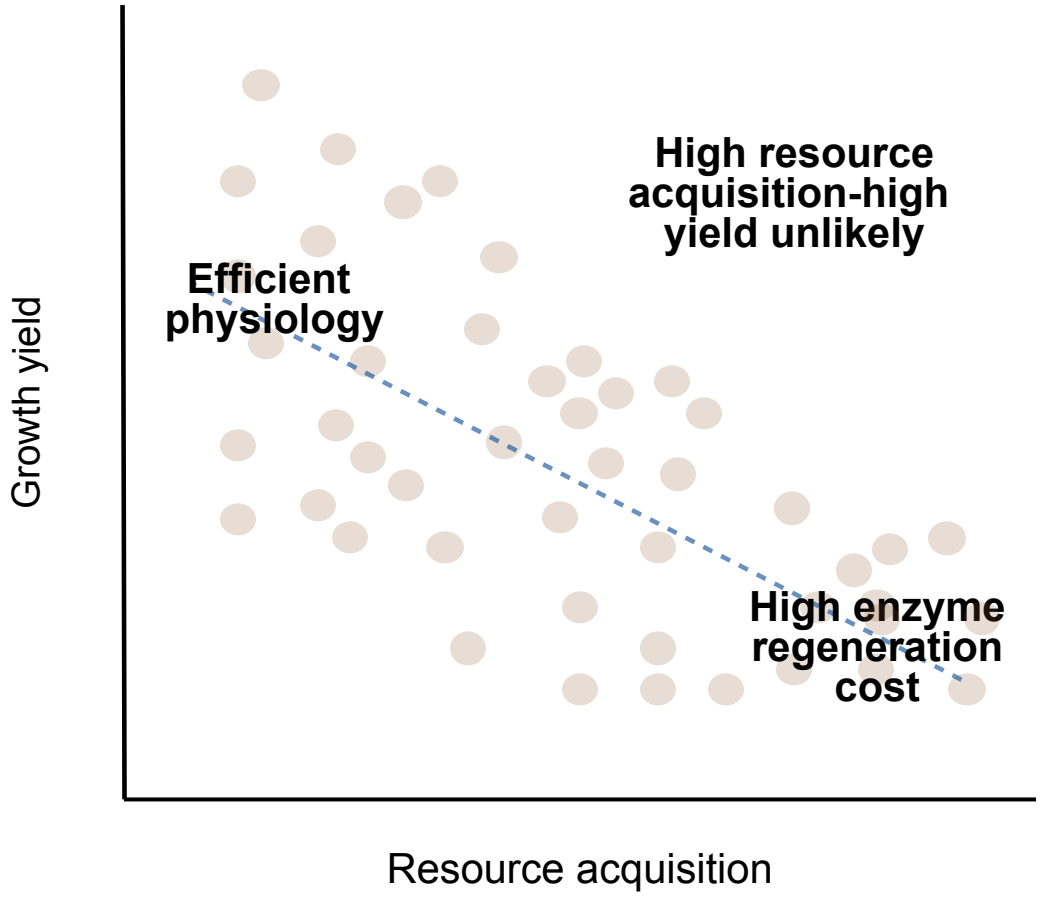

Figure 2