Effects of substrate availability on growth and metabolism in soil microbes: Insights from theoretical modeling of studies of the Warburg effect and substrate-induced respiration

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Abstract

Carbon Use Efficiency (CUE) is a popular concept for measuring the efficiency of biomass production in different biological systems and, is frequently employed to understand effects of microbial processes on soil carbon dynamics. CUE in soil microbes is often measured through respiration-based studies, especially through the addition of a labile carbon substrate such as glucose. Therefore, exploring the response of microbial respiration to availability of labile substrates is crucial to understand microbial CUE in soils. In this work, we build upon a cellular model of the Warburg effect, where cells simultaneously utilize inefficient aerobic glycolysis/fermentation and efficient oxidative phosphorylation pathways for energy synthesis even at high oxygen availability, to predict microbial community response to various levels of substrate availability. We test our predictions systematically using a series of substrate-induced respiration (SIR) experiments to demonstrate prevalence of the Warburg effect in soil microbial communities. We further discuss the relevance of the underlying metabolic processes behind the Warburg effect in interpreting soil microbial CUE.

1. Introduction

Carbon Use Efficiency (CUE) of the soil microbial community, usually defined as carbon biomass produced per substrate carbon utilized, is considered an important variable that influences what proportion of organic carbon is released into the atmosphere as CO₂ and what stays back as soil organic matter in (living or dead) microbial biomass (Billings and Ballantyne, 2013; Bradford, 2013; Hagerty et al., 2014). CUE depends strongly on the nature and quantity of available substrate and the compounds being produced, as well as on the physiology of the cells (e.g., active growth and division, survival when substrate availability is low, dormancy) (Dijkstra et al., 2015). However, CUE is usually considered to be low because of low carbon availability in soil and the supposedly recalcitrant nature of soil organic matter (Anderson and Domsch, 2010; Manzoni et al., 2012; Sinsabaugh et al., 2013; Reischke et al., 2015). Studies usually assume that the limited substrate available is used to satisfy basal metabolic demands for the cells with negligible growth. However, this assumption is not supported by numerous studies that find high values of CUE (Brant et al., 2006; Frey et al., 2013; Hagerty et al., 2014; Steinweg et al., 2008; Ziegler et al., 2005; Geyer et al., 2019), which sometimes approach pure culture values (Dijkstra et al., 2015). Moreover, the assumption that soils are oligotrophic and that microbes are normally in a 'starvation-survival lifestyle' is flawed (Hobbie and Hobbie, 2013). Although most of the soil organic carbon (SOC) is dominated by recalcitrant carbon, the small portion involving labile carbon (or, low molecular weight carbon compounds) has very high turnover (Schneckenberger et al., 2008). Consequently, this labile carbon plays a major part in soil nutrient dynamics, and influences measurements of CUE.

CUE is often measured in soils using respiration studies that quantify the rate of mineralization of a substrate (mostly labile carbon compounds such as glucose) into CO₂ or other gaseous or soluble simple compounds (Manzoni et al., 2012; Sinsabaugh et al., 2016). However, these respiration-based methods assume that CO₂ emission in heterotrophic microbial communities is

a good proxy for measuring microbial metabolic processes and biomass, and further assume that the proxy relationship is linear. Previous studies have reported the presence of non-linear effects of increased labile substrate concentration on CO₂ emissions, especially the reduction of CO₂ emissions at high substrate concentrations (Molenaar et al., 2009). This nonlinearity occurs because the rate of oxidative phosphorylation declines relative to other pathways as substrate concentrations increase. Eventually, at the highest substrate concentrations, aerobic glycolysis becomes the primary energy producing pathway, even in the presence of ample oxygen; this phenomenon is termed the Warburg effect (or overflow metabolism) (Schuster et al., 2015). This effect, initially discovered in cancer cells, has been well documented across a broad range of organisms (summarized in Swain and Fagan, 2018) including yeast (de Deken et al., 1966), bacteria (Basan et al., 2015, Zhuang et al., 2011, Molenaar et al., 2009), and various human cell types including activated lymphocytes, Kupffer cells, fast twitch muscle fibers, and microglial cells (Schuster et al., 2015, Vander Heiden et al., 2009). Here, we will focus on microbial (bacterial) respiration processes in the soil and its impact on CUE.

Although seemingly paradoxical at first, the Warburg effect occurs in bacterial cells due to three major trade-offs that occur between the two dominant energy producing pathways: oxidative phosphorylation (OP) and fermentation/aerobic glycolysis. These tradeoffs involve (1) the rate and yield tradeoff between OP and fermentation; (2) OP being a surface process whereas fermentation is volume-based; and (3) fermentation produces precursors to be used for growth whereas OP cannot do so directly (Molenaar et al., 2009; Zhuang et al., 2011; Schuster et al., 2015). Taking these three trade-offs into account, previous studies developed an array of frameworks including linear maximization models (Swain and Fagan, 2018) and flux balance analysis (Schuster et al., 2015) to explain the occurrence of Warburg effect at a cellular level.

Here, we use the cellular level linear maximization model from our earlier study (Swain and Fagan, 2018) to develop a set of hypotheses detailing the community-level consequences of the Warburg effect. We test these predictions using a series of simple substrate-induced respiration (SIR) experiments, and discuss these results in the context of CUE as a function of substrate availability in the soil and consequences for respiration-based studies.

2. Hypothesis Generation

Increased substrate availability tips trade-offs in favor of fermentative processes in bacterial cells at the expense of OP. In contrast, OP is favored at low substrate concentrations where efficiency of energy production is necessary to meet basal energy requirements (Swain and Fagan, 2018; Kempes et al., 2017). OP yields about 30 moles of ATP per mole of glucose consumed and is much more efficient compared to fermentation, which produces 2-4 moles of ATP per mole of glucose. However, fermentation is about two orders of magnitude faster than OP (Voet, 2004). Therefore, at high labile substrate availability, kinetic factors and availability of enzymatic centers would lead to an increased rate of fermentation. The enzymes that help facilitate OP, cytochrome oxidases, are located exclusively as transmembrane proteins, whereas enzymes that facilitate fermentation occur in the cytoplasmic space (Zhuang et al., 2011). To maintain membrane integrity, only a small proportion of the transmembrane space can be occupied by proteins; this limits the upper rate of OP to be much lower than fermentation. Moreover, at high substrate concentrations, transporters compete for membrane space with OP (Zhuang et al., 2011), thereby reducing OP. In addition, the products of fermentation facilitate biomass production (Vander Heiden et al., 2009) and therefore promote growth. However, the energy produced by fermentation is insufficient to support biosynthesis of all the carbon precursors produced through its enzymes, and energy produced from OP would be utilized in biosynthesis. Therefore, with increasing substrate concentrations, the total energy produced from both OP and fermentation would not suffice to process the fermentation-based

products for biosynthesis. As a result, these excess products are expelled from the cell as overflow products, leading to the term overflow metabolism (or, Warburg effect) (Schuster et al., 2015).

These processes happen at a cellular level, but the surface-area-to-volume-ratio (henceforth termed Z) varies widely among individual cells, and therefore plays a major role in the prevalence of overflow metabolism as well as in relative growth, competitive substrate uptake, and minimum energy requirements (Swain and Fagan, 2018). Higher values of Z enable growth at lower substrate concentrations due to higher rate of substrate uptake at a given concentration. However, cells with lower Z values grow slower than those with larger Z values (Young, 2006; Swain and Fagan, 2018).

Given great diversity and high abundance of bacterial cells in soils, we can assume that Z is normally distributed in a sample of soil. This assumption takes into account both the size and shape of the cell, and controls the extent of overflow metabolism in each individual. We use the framework from a previous linear maximization model (see Swain and Fagan, 2018 for details), and apply it to this distribution of hypothetical cells which differ in Z (but, for simplicity, have the same volume) to calculate the CO_2 mineralization rate from a simple substrate such as glucose. We assume the parameter values associated with various biochemical processes (see Swain and Fagan, 2018 for details) in the model to be normally distributed and randomly assigned.

For 100 starting substrate concentrations, we ran simulations of the model outlined by Swain and Fagan (2019) for 1 million different initializations each, using Latin hypercube sampling (LHS) (via the *lhs* package (Carnell, 2020) in R), to evaluate the effect of substrate availability on carbon usage in bacterial communities. Each simulation started with a set of parameter values for various cellular processes and an initial value of substrate concentration. The

substrate was assumed to be homogeneously distributed and was depleted when consumed. Although the exact values of cumulative mineralization of carbon substrate to CO₂ after a fixed time obtained using the Warburg effect model differed among simulations, they had the same underlying qualitative behavior (see Figure 1A).

We observed that the cumulative CO₂ production in our Warburg Effect model increased smoothly with increasing substrate concentration until a certain concentration (let us call it - C₀) where the maximum CO₂ production occurred and, after which the production declined slowly (Fig 1A). We used a partial rank correlation coefficient (PRCC) via the R package *sensitivity* (Iooss et al., 2020) to identify which parameters affected different parts of the curve (Fig S1). We found that the initial rate of CO₂ production increase depended negatively on the basal energy requirement, glucose transport efficiency, and OP efficiency. The substrate concentration where the maxima occurred depended on the mean value of the starting Z distribution, and OP efficiency. The rate of decrease of the curve beyond the threshold depended on the competition for membrane surface, variance of the starting Z distribution, OP efficiency, and glucose transport efficiency.

This behavior, seen in Fig. 1A, is also possible in two possible alternative scenarios: (1) microbes die due to very high substrate concentrations or, (2) microbes become inactive due for the same reason. Some previous studies have shown that high substrate concentration can result in cell inactivation or even death (Edwards, 1970; Han and Levenspiel, 1988). To simulate this, we built two simple models with similar resource usage patterns and cell distributions (and their properties) as the original Warburg effect model – one for cell death and one for cell inactivation. We assigned a threshold substrate concentration (referred to as the threshold parameter) at which the probability of death or becoming inactive (but alive) was 0.5 for the microbial population and increased linearly from zero substrate and, this slope of increasing

probability is the second parameter that can be controlled in these two alternative models. We also removed the constraints associated with the Warburg effect (e.g., competition for membrane space, and upper limit of OP enzymes on the membrane) in these two models – so that the bacteria could make maximal use of the substrate input, but were subject to death or inactivation due to increased substrate levels.

Figure 1: Theoretical predictions from our model: (1) CO₂ production at different substrate concentrations and the factors that affect them (estimated using PRCC; see Fig. S1-S3) for three models discussed in the text; the values for cumulative CO₂ production scaled with increasing time, as expected. The concentration where maximal CO₂ (C_0) production occurs is represented with a vertical dotted line. (2) Variations in CO₂ production rates among the three models for three concentrations of substrate 0.5x, 1x and 2x (depicted using solid lines)

followed by an abrupt halving of substrate concentration in 1x and 2x samples resulting in 1xA (1x altered) and 2xA (2x altered) samples (CO₂ production rates are depicted in dashed lines).

We outline the parameters that affect the CO₂ production in figure 1A (and provide details in Figures S1-S3). We can differentiate between the scenarios by abruptly changing our substrate regime where the value of CO₂ production is greater than the maximal CO₂ production rate in Figure 1A to one where it is lower. To systematically compare this, we selected a specific concentration (1x) such that it is slightly less than the concentration where maximal CO₂ production occurs. Twice this concentration (2x) is then substantially greater than the concentration leading to maximal CO₂ production, and 0.5x is far below that concentration (see Figure 1A). On abruptly changing the concentration from 1x to 0.5x and 2x to 1x in a given simulation, we see different responses in the three models (the Warburg Model, the Death model, and the Inactivation model; see Figure 1B). For example, because the biomass production rate is higher at higher substrate concentrations in the Warburg model, we observe altered 2x (2xA) and altered 1x (1xA) having higher respiration than that of 1x and 0.5x respectively. In the cell death model, we see higher respiration in altered concentrations, but only for 1xA and not for 2xA. This occurred because the reduction in respiration was actually due to cell death at 2x despite higher biomass production with increased substrate. The inactivation model showed either the same or lower values of respiration at 1xA and 2xA as compared to 0.5x and 1x respectively.

To validate our predictions, we used a system of simple substrate-induced respiration (SIR) experiments (Anderson and Domsch, 1973) to investigate two things. First, we examined the effect of substrate concentration on CO₂ emission (after a fixed time) to look for the humped pattern predicted by the simulations. Second, we inspected the rate of CO₂ production at three

focal concentrations (as described above and in Figure 1B) to understand the humped patterns. Although more advanced kinetic respiration methods (such as Panikov and Sizova, 1996) are available, we used basic SIR as we expected it to be adequate for our purposes and easier to implement without any advanced equipment.

3. Materials and Methods

3.1 Sample collection and treatment

Soil samples used in this study were collected from three different sites each in North Bhubaneswar and adjacent Barang region in the state of Odisha, India. All six sites were non-agricultural and formed a part of a semi-forested landscape. The details about the soils in each plot appear in table 1. Soil cores were taken using a metal boring sampler (4 cm diameter) from five randomly chosen spots at each site. The soil from 1-8 cm below ground level was collected.

Property	Soil A	Soil B	Soil C	Soil D	Soil E	Soil F
Collection Location	20.397° N, 85.825° E	20.399° N, 85.819° E	20.400° N, 85.816° E	20.366° N, 85.824° E	20.367° N, 85.822° E	20.370° N, 85.835° E
Clay %	31	25	43	23	24	50
Sand %	35	33	31	38	47	29
Silt %	34	42	26	39	29	21
pH _{1:5}	5.6	5.6	5.7	5.4	5.2	5.6
Max. Water Holding Capacity (g kg ⁻¹)	390	340	410	380	300	430
Total Organic Carbon (g kg ⁻¹)	35	29	32	28	24	30
Total Nitrogen (g kg ⁻¹)	2.7	2.3	2.5	2.3	1.8	2.9
Total Phosphorus (g kg ⁻¹)	0.5	0.5	0.4	0.4	0.3	0.4

Table 1: Details of the soil at six sampling locations

211

212 The soils were passed through a 2 mm mesh to remove plant matter, roots, nematodes and
213 other undesired material, and to homogenize the distribution of substrate and produce a more
214 dispersed release of carbon dioxide. Soil properties (pH, water holding capacity, texture, total
215 organic Carbon, total Nitrogen and total Phosphorus) were estimated, after open-air drying of
216 the soil for 24 hours at 35° C, and samples were then stored at 5° C in aerated plastic bags. Soil
217 water-holding capacity was measured at matric potential 10 kPa following previous studies
218 (Wilke, 2005). A 1:5 soil/water suspension ratio was used to estimate soil pH after 60 minutes
219 shaking at 22 °C. Total organic Carbon was measured using the Walkley-Black procedure and
220 multiplied with a factor of 1.33 to adjust for the carbon recovery rate (Schumacher, 2002). Total
221 Nitrogen was estimated using the Kjeldahl digestion method (Taylor, 2000), and Total
222 Phosphorus was estimated using the fusion method (Olsen and Sommers, 1982).

223

224 *3.2 Substrate Induced Respiration and test for Warburg effect*

225 All samples were kept at 22° C for 24 hours before assay and were subjected to three sets of
226 trials. For each of the thirty soil samples (five for each of the six sites), the following basic
227 protocol was followed across all trials: 10g of sieved soil was put in a 25 mL dry Erlenmeyer
228 conical flask, with sufficient headspace (15-20 mL) to ensure there was no oxygen limitation.
229 Glucose solution of a desired concentration was added with frequent mixing, keeping total
230 (deionized) water added to the sample at 50 percent of the maximum water holding capacity.
231 The mouth of the flask was corked using a rubber structure with a tube inserted through it and
232 the other end of the tube ended in a corked conical flask containing 30 mL 0.01 M KOH solution.
233 The setup was incubated at 22° C. The KOH solution, after a stipulated time as needed for a
234 specific set of trials, was treated with 0.03 M Barium nitrate solution and titrated against 0.005 M
235 HCl solution to estimate CO₂ emission. Three replicates of every trial were performed for each
236 of the 30 soil samples.

237

238 For the first set of trials, the basic protocol was performed for differing concentrations of glucose
 239 ranging from 0.05 g to 0.80 g per 10 g of soil (with increments of 0.05 g of glucose between
 240 samples). The cumulative total CO₂ emission was measured after 4, 5 and 6 hours (with 3
 241 replicates for each soil for each time point). We also maintained two control groups (with three
 242 replicates each for each of the three end timepoints), one with the addition of water to the
 243 normal soil, and the other to autoclaved soil at 0.5 maximum water holding capacity. Final
 244 estimates of respiration were calculated by subtracting the respiration from the normal soil
 245 without glucose addition from the respective sample. Autoclaved soil samples were maintained
 246 to see if there was any abnormal respiration.

247

248 The second set of trials were exact replicates of the first set in two groups except for the
 249 addition of 1000 ppm chloramphenicol and 2000 ppm cycloheximide combined with 50 mg of
 250 talc into the soil (and mixed well) to function as selective inhibitors for bacterial and fungal
 251 growth, respectively (based on Nakamoto and Wakahara, 2004). Their respective controls were
 252 also amended with chloramphenicol and cycloheximide. Although these treatments are not
 253 completely effective (Nakamoto and Wakahara, 2004) and there might be streptomycin-resistant
 254 bacteria and cycloheximide-resistant fungi in soils, such colonies are usually slow growing and
 255 small (Anderson and Domsch, 1975). Consequently, these experimental treatments would give
 256 us a good estimate of bacterial and fungal biomass separately.

257

258 From the theoretical predictions (Fig 1A), we expected a dip in the production of carbon dioxide
 259 after a certain concentration C_0 of glucose (see Hypothesis Generation section). Using the data
 260 from the first two sets of trials, we estimated this concentration C_0 and further focused only on
 261 the bacterial respiration (i.e., cycloheximide treatment) for two major reasons. The first reason is
 262 that our models are based on bacterial metabolic mechanisms and extrapolating them to all soil

organisms could be inappropriate. Second, anti-bacterial compounds like chloramphenicol and streptomycin can stimulate microbial respiration, especially fungal respiration, which can cancel the decline seen in bacterial respiration (Johnson et al., 1996; Lin and Brooks, 1999). Therefore, looking at the cycloheximide treatment in the third set of trials is the most appropriate, as it involves mostly bacterial respiration.

For doing the same, four specific glucose concentrations were taken for each soil sample, as described in the hypothesis generation section (Fig 1A-B): a base concentration (1x) was chosen such that it was less than C_0 (the concentration of glucose where maximal CO_2 release happens) but twice the concentration (2x) was greater than C_0 .

Processing data from first two sets of trials, we fixed 1x to be 0.30 g glucose/10 g of soil (see Fig 2A-F). For each soil sample, we established SIR experiments for glucose concentrations of 0.15 g (0.5x), 0.30 g (1x) and 0.60 g (2x) per 10 g of soil and performed the titration every 30 minutes starting at 0.5 hr timepoint and ending at 7 hr for three replicates of a given sample. After periods of 4, 5 and 6 hours, 10 g of autoclaved soil (with cycloheximide amendment and water added at 0.5 maximum water holding capacity) was added to one set of three replicates each of 1x and 2x series of samples. CO_2 emission rates were observed at the same 30-minute observation intervals. The addition of autoclaved soil functions as a diluter, thus reducing the substrate concentration to half – therefore making the 2x runs into 1x and 1x into 0.5x. For every soil we had 42 (3 x 14 time-points), 78 (3 x 14 time-points + 3 x 12 amendment samples (6 for 4 hr, 4 for 5 hr and 2 for 6 hr)) and 78 replicates for 0.5x, 1x and 2x respectively. We also maintained 78 replicates of each of the two control groups (i.e. normal soil and autoclaved soil) with cycloheximide amendment. A similar treatment (addition of autoclaved soil) was done to 3 replicates each of the two control groups for comparison after 4, 5 and 6-hour timepoints. To estimate the respiration after addition of autoclaved soil to 1x and 2x concentrations, we

subtracted the respective respiration from autoclaved soil-added normal soil sample control. To estimate the rate, we subtracted the total CO₂ produced at a given time-step from that of the previous time-step.

All comparisons and analyses were performed using R statistical software. For comparing the emissions from the 0.5x, 1x and 2x series of data, we used paired t-tests to identify significant differences.

4. Results

respectively, see Table 1) after 4 hours of incubation at various concentrations of glucose and three different treatments for each soil – unamended soil (total biomass), cycloheximide treatment (primarily bacterial biomass) and chloramphenicol treatment (primarily fungal

biomass). Note that the decreases in cycloheximide treated soils were larger and faster than their corresponding chloramphenicol treated samples.

Cumulative carbon dioxide emissions due to total, fungal, and bacterial soil microbial biomass after 4 hours of incubation are plotted in figure 2. Comparable emissions results after 5 and 6 hours of incubation appear in figures S4 and S5, respectively. As predicted by simulations (Fig. 1A), CO₂ production from the same soil sample decreased beyond a threshold substrate concentration and saturated to a lower value at all the six soil sites and for all three treatments. The threshold concentration varied slightly across the six soil samples, which may be a function of the microbial composition and soil properties. These basic trends in our experimental results match predictions described by our model in Figure 1A, even though the model was specifically made for bacterial metabolism.

Respiration in the cycloheximide-treated soils (which involved predominantly bacterial respiration) underwent a sharper decrease than the respiration in the chloramphenicol-treated soils (which involved predominantly fungal respiration) (Fig. 2A-F). This pattern suggests that the decrease in overall microbial respiration was likely caused primarily by the decrease in predominantly bacterial respiration. The sum of the cycloheximide- and chloramphenicol-treated soil respirations together exceeded the total untreated/unamended microbial respiration (Fig. 2A-F).

The temporal carbon dioxide emissions from the third set of trials where autoclaved soil was added after 4 hours is shown in figure 3A-F. The results of soil addition after five and six hours show similar responses across all soils (Figs. S4, S5). The addition of the autoclaved soil caused the respiration level of the soil to increase for 2x concentration samples (2xA), matching and even exceeding the CO₂ production levels of 1x concentration. The same addition to 1x

concentration (1xA) samples reduced the CO₂ levels to just more than the CO₂ levels produced by the 0.5x concentration; this observation held true for all six sites. Paired t-tests (for all 4, 5 and 6 hr trials) showed that the emissions from the 2xA samples were significantly higher than 1x sample ($p < 0.05$ for soils A, B, D and F for all the three timepoints; $p < 0.05$ for C and E for 5 and 6 hr timepoints; $p < 0.1$ for C and E for 4 hr timepoint) Likewise, emissions from 1xA samples were significantly higher than 0.5x samples ($p < 0.05$ for soils B and F for all the three timepoints, and for soils A, C and E for 5 and 6 hr timepoints; $p < 0.1$ for all other cases).

determined concentrations of glucose: 0.5x (0.15g/10g of soil), 1x (0.3g/10g of soil) and 2x (0.6g/10g soil). Autoclaved soil was added after 4 hours (denoted by black vertical line) in 1x and 2x samples, resulting in 1xA (1x altered) and 2xA (2x altered) samples respectively.

5. Discussion

The first two sets of trials for all accumulation periods (4, 5, and 6 hours of incubation) matched the qualitative shape of the curve predicted by our model (Fig 1A, Fig 2A-F, Fig S4A-F, Fig S5A-F). We observed that the additive total of CO₂ emissions from the cycloheximide- and chloramphenicol-treated soils was greater than the total for the unamended soils (Fig 2A-F, Fig S4A-F, Fig S5A-F). This may be due to the presence of resistant microorganisms - thus contributing to both the measurements. Although our model is specifically made for bacterial metabolism, certain predictions from it might be applicable to fungal processes. However, for the sake of clarity, we focused on cycloheximide-amended soil (primarily bacterial biomass) for our third set of trials.

The third set of trials were conducted to differentiate among mechanisms that could have resulted in the curve observed in Fig 1A and the cumulative CO₂ incubations (Fig 2A-F, Fig S4A-F, Fig S5A-F), allowing us to compare against the predictions for three possible models (the Warburg model, the cell death model, and the cell inactivation model) in Fig 1B. Based on the results (Fig 3A-F, Fig S6A-F, Fig S7A-F) for the third trials and the measured statistical differences between the two pairs (1x, 2xA) and (0.5x, 1xA) – the model that matched the data best was the Warburg model. A few cases were statistically inconclusive (i.e., $p < 0.1$ but not < 0.05), and we could not rule out the cell inactivation model completely.

It is also possible that different sets of microbes were active at different substrate regimes and that might cause small differences in respiration, as seen in previous studies (Derrien et al., 2014). Our model assumption in which the microbial community can be characterized as having a distribution of Z (surface area to volume ratio) values takes care of this scenario. By allowing microbes with different Z, we allow for a wide range of different energy requirements and growth regimes (Swain and Fagan, 2018). Although this normal distribution is a good approximation. Nevertheless, because our aim here was to create a framework for understanding the Warburg

effect as a function of the responses of microbial processes to labile substrate availability, this represents a reasonable starting point. To capture a certain breadth of this complexity, we performed the experiments on six different soils with different physical and chemical properties (Table 1), and we observed similar trends of observations in soils across the six sampled sites. A further step in this direction might be to understand the consequences of the Warburg effect in different soil types and across different physical and chemical gradients.

We are not the first to see these effects in SIR-based studies. Indeed, past research has uncovered results similar to our first set of trials (e.g. Schneckenberger et al., 2008; Tian et al., 2015), where mineralization of glucose into CO₂ follows a humped curve as a function of substrate concentration (Fig 1A). However, the questions in those studies were quite different from ours here, where we were primarily interested in understanding the implications of the Warburg effect (and its associated trade-offs) in the context of soil microbial community ecology. For example, our results can be assimilated in terms of carbon use efficiency (CUE), which is widely used in the soil literature (Lipson, 2015). Fig 1A can be repurposed to think about CUE as a function of substrate concentration by calculating the biomass C produced as a function of the input of cellular substrate C using the Warburg model (see Swain and Fagan, 2018; Fig 4).

Figure 4: Conceptual model of Carbon Use Efficiency (CUE) as a function of substrate concentration

The overall trend of CUE represented here is similar to the model presented in a previous work by Lipson (2015), which was based on a different population-level tradeoff between metabolic rate and growth (Lipson, 2015; Beardmore et al., 2011) (Fig 4). At low substrate concentrations, microbial metabolism is dominated by high Z microbes, which have higher substrate use efficiency but slow growth. As the substrate availability increases, more cells cross the energy requirement barrier to achieve growth with high CUE. This continues until a certain threshold concentration of substrate is reached, at which fermentation-induced biomass production utilizes all precursors based on total energy availability. Beyond this threshold, the total energy produced is not sufficient to use all the precursors formed in glycolytic pathways – leading to overflow products. Even though the maximal OP rate might be reached at a higher substrate value, it is the ratio of fermentation-to-OP that matters. The higher the ratio, the larger the amount of overflow products that is expelled from the cell, leading to low CUE.

402

403 This trend in CUE can lead to different ecological ‘strategies’ among the members of microbial
 404 communities – both spatially and temporally. For example, inefficient fast-growers, efficient
 405 slow-growers and extremely efficient very-slow-growers could all coexist with each group
 406 predominating for particular levels of environmental substrate availability. Many previous studies
 407 also show that high CUE is representative of cooperative populations, and these can be
 408 particularly successful in spatially structured environments with varying resource regimes
 409 (Pfeiffer et al., 2001; Kreft and Bonhoeffer, 2005). However, the tradeoff between rate and yield
 410 can lead to coexistence of different microbial growth strategies even in a well-mixed
 411 environment (Beardmore et al., 2011). Temporal variations in substrate availability can also
 412 dynamically alter the microbial composition such as increased slow growing population at lower
 413 substrate concentrations (which happen with increased time of incubation) (see Derrien et al.,
 414 2014). The value of CUE also hinges on limiting nutrients such as N and P (Sinsabaugh et al.,
 415 2016; Sterner and Elser, 2002). In this work we have not considered the limitation imposed by N
 416 and P availability in the soils above the substrate availability; this topic remains open for further
 417 exploration.

418

419 Deciphering the relationships among the Warburg effect, CUE, and soil microbial respiration can
 420 be crucial for several reasons. For example, numerous methods that are commonly used to
 421 calculate soil microbial biomass and substrate utilization models assume merely a linear
 422 response of respiration to changes in substrate concentration (Schnecker et al., 2019). If this
 423 relationship is commonly nonlinear, as evident in scenarios where the Warburg effect is active,
 424 the applications employing the respiration-substrate concentration relationship could be over- or
 425 under-estimating aspects of microbial dynamics that can have downstream implications for the
 426 environment or the study system. In other words, understanding the nonlinear response of
 427 microbial respiration to changes in substrate concentration in an experimental setting can

improve analyses that use substrate priming techniques for biomass estimation (Dilly and Zyakun, 2008) as well as understanding differential microbial community dynamics (Derrien et al., 2014). In addition, exploring these non-linear relationships can help explain the effects of sudden/periodic substrate/nutrient addition to soil systems such as leaf litter addition during fall season in temperate soils, rewetting of arid soils (allowing for pulsed usage of substrates), or other forms of nutrient periodicity and variability (Carrero-Colon et al., 2006; Lennon and Cottingham, 2008).

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569 **Supplementary Figures**

Figure S1: PRCC coefficients for the Warburg model for three properties of CO₂ production

Figure S2: PRCC coefficients for the Death model for three properties of CO₂ production

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575 Figure S3: PRCC coefficients for the Inactivation model for three properties of CO₂ production

letter, respectively; see Table 1) after 5 hours of incubation at various concentrations of glucose and three different treatments for each soil – unamended soil (total biomass), cycloheximide treatment (primarily bacterial biomass) and chloramphenicol treatment (primarily fungal biomass).

letter, respectively; see Table 1) after 6 hours of incubation at various concentrations of glucose and three different treatments for each soil – unamended soil (total biomass), cycloheximide treatment (primarily bacterial biomass) and chloramphenicol treatment (primarily fungal biomass).

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591 Figure S6: SIR emission rate values for cycloheximide amended samples of soils, with pre-
592 determined concentrations of glucose: 0.5x (0.15g/10g of soil), 1x (0.3g/10g of soil) and 2x
593 (0.6g/10g soil). Autoclaved soil was added after 5 hours (denoted by clack vertical line) in 1x
594 and 2x samples, resulting in 1xA (1x altered) and 2xA (2x altered) samples respectively.

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596

597 Figure S7: SIR emission rate values for cycloheximide amended samples of soils, with pre-
 598 determined concentrations of glucose: 0.5x (0.15g/10g of soil), 1x (0.3g/10g of soil) and 2x
 599 (0.6g/10g soil). Autoclaved soil was added after 4 hours (denoted by clack vertical line) in 1x
 600 and 2x samples, resulting in 1xA (1x altered) and 2xA (2x altered) samples respectively.

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