SUPPLEMENTARY INFORMATION:

Microbial storage and its implications for soil ecology

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# Supplementary Information 1: Overview of microbial storage compounds

## 1 Triacylglycerides

Triacylglycerides (TAG) are ubiquitous across eukaryotes and bacteria. Genomic analysis has not found TAG biosynthetic ability in archaea [1], corroborating the absence of observed TAG accumulation [2]. Microbial TAG has received particular research interest due to potential industrial applications, especially for biofuel production [3]. Many bacteria and fungi can amass TAG stores accounting for substantial proportions of their total biomass, sometimes exceeding 60% of cell dry weight (CDW) [3, 4]. TAG in soil has attracted attention due to the value of the constituent fatty acids as taxonomic and trophic markers (neutral lipid fatty acids – NLFA [5]), but their storage role has been largely overlooked in soil ecosystems.

### *1.1 Chemistry*

TAGs consist of three fatty acids linked, through ester bonds, to a common glycerol molecule. While the triacylglyceride construct is shared by all producing organisms (including mammalian fat), the chain length, branching, and saturation of the constituent fatty acids varies, hence their value as biomarkers [5]. Triacylglycerides are hydrophobic lipids with highly reduced carbon. As a result they represent osmotically neutral storage with very high energy density [6].

### *1.2 Biosynthesis and degradation*

Biosynthesis of triacylgylcerides begins by linking two fatty acids (in the form of fatty acyl-CoA) to a glycerol backbone to form a diacylglyceride, by pathways shared with phospholipid biosynthesis [7]. The key enzyme for TAG synthesis is the acyl-coenzyme A:diacylglycerol acyltransferase that catalyses the transfer of a third fatty acid moiety to the remaining hydroxyl group on the glycerol backbone [8]. In prokaryotes this function is fulfilled by a distinct and promiscuous wax ester synthase/acyl-CoA:DAG acyltransferase enzyme that can accept either diacylglycerol to produce TAG or fatty alcohol to yield wax esters [7, 9]. *De novo* synthesis of the fatty acid is not a prerequisite: incorporation of exogenous or recycled fatty acids into triacylglycerides, with or without modification, has been demonstrated [3, 7].

Triacylglycerides are degraded by lipases to release the fatty acids by hydrolysis. The fatty acids are then catabolized to acetyl-CoA via the beta-oxidation pathway with concomitant generation of reducing equivalents (NADH, $FADH_2$), which in aerobic organisms can drive ATP synthesis by oxidative phosphorylation. TAGs therefore have the potential disadvantage that they can only yield energy under aerobic conditions [6]. The majority of characterized microbial lipases to date are from *Bacillus* spp., but lipases from many other bacteria have been purified including *Burkholderia, Acinetobacter,* and *Enterococcus* [10].

## 2 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA) are a family of bacterial and archaeal storage lipids that have been intensively researched as biodegradable, renewable alternatives to petrochemical plastic [11], and for their importance in wastewater treatment processes [12]. PHA storage is not known among eukaryotes [13]. Intracellular storage of PHA occurs in observable lipid inclusion bodies which can constitute large proportions of biomass, even exceeding 80% of CDW under optimized conditions [11, 14].

### *2.1 Chemistry*

PHAs are straight-chain polyesters, often consisting mainly or exclusively of 3-hydroxybutyrate monomers. However, diverse copolymers are known with different carbon chains or hydroxyl group positions in the

monomer, for example 3-hydroxyvalerate, 4-hydroxybutyrate and 3-hydroxydecanoate [11]. Typically, the polyester chain will comprise hundreds to thousands of monomeric units [15]. Ester bonds are apolar and so, though less reduced than triacylglycerides, PHAs are nevertheless highly hydrophobic [16].

### *2.2 Biosynthesis and degradation*

De novo synthesis of polyhydroxybutyrate begins with the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA, which is then reduced by acetoacetyl-CoA reductase to yield 3-hydroxybutyryl-CoA for polymerization. However, biosynthesis of different hydroxyacyl-CoA monomers can proceed by various anabolic or catabolic pathways [17], including via metabolic links with fatty acid pathways [18]. In the final step of PHA biosynthesis, PHA synthase accepts a hydroxyacyl-CoA precursor and uses this to form a new ester bond, extending the polymer chain from the hydroxyl end.

PHA is degraded through hydrolysis by PHA depolymerase, to release soluble hydroxyacid monomers. In addition to intracellular catabolism by PHA producers, many bacteria that do not themselves accumulate PHA nevertheless secrete extracellular PHA depolymerases to scavenge from necromass in their vicinity [19].

## 3 Glycogen

Glycogen storage occurs in animals, fungi and bacteria [20]. Genomic analysis indicates that the biosynthetic pathways are also present in archaea [1]. Glycogen storage does not occur in plants, although starch is very similar in structure. Inclusions in bacteria and fungi are frequently observed microscopically, while chemical analyses have reported accumulations of up to 9% of CDW [21].

### *3.1 Chemistry*

Glycogen is a polymer of $\alpha$1→4 linked glucose, with $\alpha$1→6 branches, reaching total molecular masses of $10^7$–$10^8$ Da [20]. As a polysaccharide, it is hydrophilic and relatively highly oxidized, implying lower energy density than the more reduced lipids just described. The high degree of polymerization enables storage of glucose without greatly increasing osmotic pressure [20, 22].

### *3.2 Biosynthesis and degradation*

Glycogen is synthesized from ADP-glucose (in bacteria) or UDP-glucose (in eukaryotes) by glycogen synthase, which catalyzes the formation of a new $\alpha$1→4 bond on the non-reducing end of an existing chain [23, 24]. Branching enzyme subsequently relocates the tail of the growing 1→4 chain to the C6 position of an internal glucose residue, forming a new $\alpha$1→6 branch-point. Glycogen is catabolized by the combined action of debranching enzyme and glycogen phosphorylases, to release glucose-1-phosphate [23, 25].

## 4 Trehalose

Trehalose biosynthesis is widespread, occurring in bacteria, archaea, fungi, plants and invertebrates. Storage of C and energy was originally believed to be its principle purpose, but numerous other roles have since been ascribed to the compound, most importantly in osmotic regulation and protection against other stresses. Unlike other storage compounds discussed here, there are relatively few reports of trehalose accumulation to large proportions of biomass, possibly due to the high osmotic pressures that would be generated. However, *Saccharomyces cerevisiae* has been reported to accumulate up to 16% of CDW as trehalose under some conditions [26].

### *4.1 Chemistry*

Trehalose is a soluble, non-reducing sugar consisting of two glucose molecules linked through an α,α-1,1-glycosidic bond [27]. It is hydrophilic, has low molecular mass (342 Da) and is osmotically active.

### *4.2 Biosynthesis and degradation*

The major pathway for trehalose synthesis converts UDP-glucose and glucose-6-phosphate to trehalose-6-phosphate and UDP, catalysed by trehalose-6-phosphate synthase [27, 28]. This is subsequently

dephosphorylated to trehalose by trehalose-6-phosphate phosphatase. Alternative biosynthetic pathways have also been described that convert other glucose polymers, such as maltose or glycogen, to trehalose [28]. In total, five different enzymatic pathways for trehalose synthesis have been described and have been recently reviewed [29]. Trehalose catabolism proceeds by hydrolysis to release the constituent glucose molecules, catalyzed by trehalases [27].

# 5 Wax esters

Wax esters are lipids present as intracellular inclusion bodies in various prokaryotes [7]. They are also widespread among eukaryotes, for example in the hydrophobic cuticles on external plant surfaces [30], but eukaryotic storage functions are uncommon [31–33].

## *5.1 Chemistry*

A wax ester is the condensation product of a fatty acid and a fatty alcohol, each typically 16 or 18 carbon atoms in length but, as for TAGs, with considerable variation in length, saturation and structure of the carbon chain. They share with TAGs the properties of high energy density, hydrophobicity and osmotic neutrality [7].

## *5.2 Biosynthesis and degradation*

Wax ester synthesis by bacteria is catalysed by wax ester synthase/acyl-CoA:DAG acyltransferase, the enzyme also responsible for the final step of bacterial TAG synthesis [34]. In this case a new ester bond is formed from a fatty acyl-CoA and a fatty alcohol, the latter derived from the two-step reduction of another fatty acyl-CoA [7].

Like TAGs, the highly reduced carbon of wax esters can only yield energy through aerobic catabolism. Broad specificity cutinases hydrolyse a variety of wax esters including cutin, which is a major component of plant leaf cuticles. Cutinases have been primarily characterized in plant pathogenic fungi due to their role in pathogenicity [35], but they are also found in some bacteria [36].

# 6 Polyphosphate

Polyphosphate is ubiquitous in all branches of life. It is a truly ancient biomolecule, possibly pre-dating life itself, and plays multiple physiological roles [37, 38]. These include phosphorus and energy storage, but also cellular pH buffering, heavy metal chelation, and involvement in cellular regulation, amongst others [37]. Kornberg et al. argue, however, that the rapid turnover of ATP in *Escherichia coli* implies that even large polyphosphate stores could not support the cellular energy budget for substantial periods of time, and they therefore question its significance for energy storage [39]. Its role in phosphorus storage is more clear-cut [37].

## *6.1 Chemistry*

Polyphosphate is a polymer of inorganic phosphate, consisting of chains of tens to hundreds of phosphate monomers linked by phosphoanhydride bonds, the same phosphate-phosphate binding chemistry as the cellular energy carrier ATP [38]. Hydrolysis of these ‘high-energy’ bonds involves a similar free energy change to ATP hydrolysis, and the polymer can act as a direct ATP substitute in some biochemical reactions or be used for ATP synthesis [37]. When aggregated into storage structures with multivalent cations such as calcium, it has little effect on cellular osmotic pressure [37–39].

## *6.2 Biosynthesis and degradation*

The primary enzymes involved in the biosynthesis of polyphosphates in bacteria are polyphosphate kinases, PPK1 and PPK2. PPK1 reversibly and selectively transfers the gamma-phosphate from ATP to polyphosphate [40], whereas PPK2 can act on either ATP or GTP [41]. In lower eukaryotes including amoebae, the biosynthetic genes were postulated to have been acquired via horizontal gene transfer from prokaryotes [42]. Enzymes responsible for the synthesis of inorganic polyphosphate have not yet been characterized in higher eukaryotes such as mammals [42].

PPK2 is also capable of hydrolysing polyphosphate to convert GDP into GTP [41]. PPK2 is thus bifunctional and involved in both biosynthesis and degradation. Other enzymes involved in polyphosphate hydrolysis include endo- and exopolyphosphatases. As their names suggest, the endopolyphosphatases cleave internal phosphoanhydride bonds whereas exopolyphosphatases work backwards from the terminal phosphate residue [43, 44]. In addition to releasing stored phosphorus and energy, these degradative enzymes are also postulated to regulate levels of polyphosphates in the cytosol since high levels of cytosolic polyphosphate are toxic, at least in yeast [45]. For more detailed coverage of polyphosphate biochemistry, we refer readers to comprehensive reviews [37, 38].

## 7 Cyanophycin

Cyanophycin, sometimes termed CGP (cyanophycin granule peptide), is a nitrogen storage polypeptide with a C:N ratio of 1.7. It occurs in most cyanobacteria and some heterotrophic bacteria [46, 47]. Cyanobacteria are known to accumulate cyanophycin up to 18% of CDM [47], while over 40% of CDM has been reported for *Acinetobacter calcoaceticus* [48]. Genomic analysis suggests a potentially much wider distribution among bacteria, but not in archaea or eukaryotes [46].

### *7.1 Chemistry*

Cyanophycin consists of L-arginyl-poly(L-aspartate), i.e., a polypeptide backbone of aspartic acid, with additional amide bonding through the side-chains to the α-amino group of arginine. The polymer reaches molecular masses of 25-100 kDa [49]. Arginine is present on virtually all aspartate side-chains, so that the molar ratio of the amino acid residues is approximately one [50].

### *7.2 Biosynthesis and degradation*

Biosynthesis from arginine and aspartate is catalysed by cyanophycin synthetase, without the involvement of mRNA templates or ribosomes [50]. Degradation proceeds by hydrolysis from the C-terminus, catalysed by cyanophycinase, which releases aspartate-arginine dimers for subsequent degradation [46].

# Supplementary 1 References


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# Supplementary Information 2A: Figure 1 with genera

Figure 1 in the text was generated from a comprehensive literature survey of taxa shown to accumulate storage compounds. We identified taxa with evidence for storage compound accumulation at the genus level as follows: (i) storage traits have been phenotypically demonstrated for at least one member of the genus as either (a) the build-up of at least 5% of cell dry weight as a known storage compound, or (b) build-up of storage compounds to a sufficient degree for observation by light microscopy; and (ii) the genus has at least one member that occurs in soil. Literature describing storage traits was assembled by searching Web of Science using combinations of keywords “polyhydroxybutyr*”; “polyhydroxyalkano*”; “triacylglyceride”; “glycogen”; “polyphosphate”; “trehalose”; “cyanophycin”; “wax ester”; “PHA”; “PHB”; “NLFA”; “microb*”; “bact*”; “fung*”; “stor*”; “accumul*”; “reserve”, supplemented by literature citing or cited by relevant studies from this search. These studies were surveyed to obtain a shortlist of 89 bacterial and 40 fungal genera that fulfilled criterion (i), based on 126 peer-reviewed journal articles. For each of these genera, a second Web of Science search was then performed using the genus and “soil” to find evidence for its occurrence in soil. References are provided in Supplementary 2B.

To visualize the identified genera in the context of the overall microbial tree of life, we included a selection of representative bacterial and fungal taxa established in phylogenetic studies by Choi et al. [1] and Jun et al. [2]. A cladogram constructed using the NCBI taxonomy database [3] was used to display the relative relationships of the identified genera alongside the storage compounds known to be associated with each taxon at the genus level (Figure S2.1). A total of 488 representative bacterial and fungal genera corresponding to 26 phyla are shown, with 106 genera (10 phyla) that satisfy both criteria (i) and (ii).


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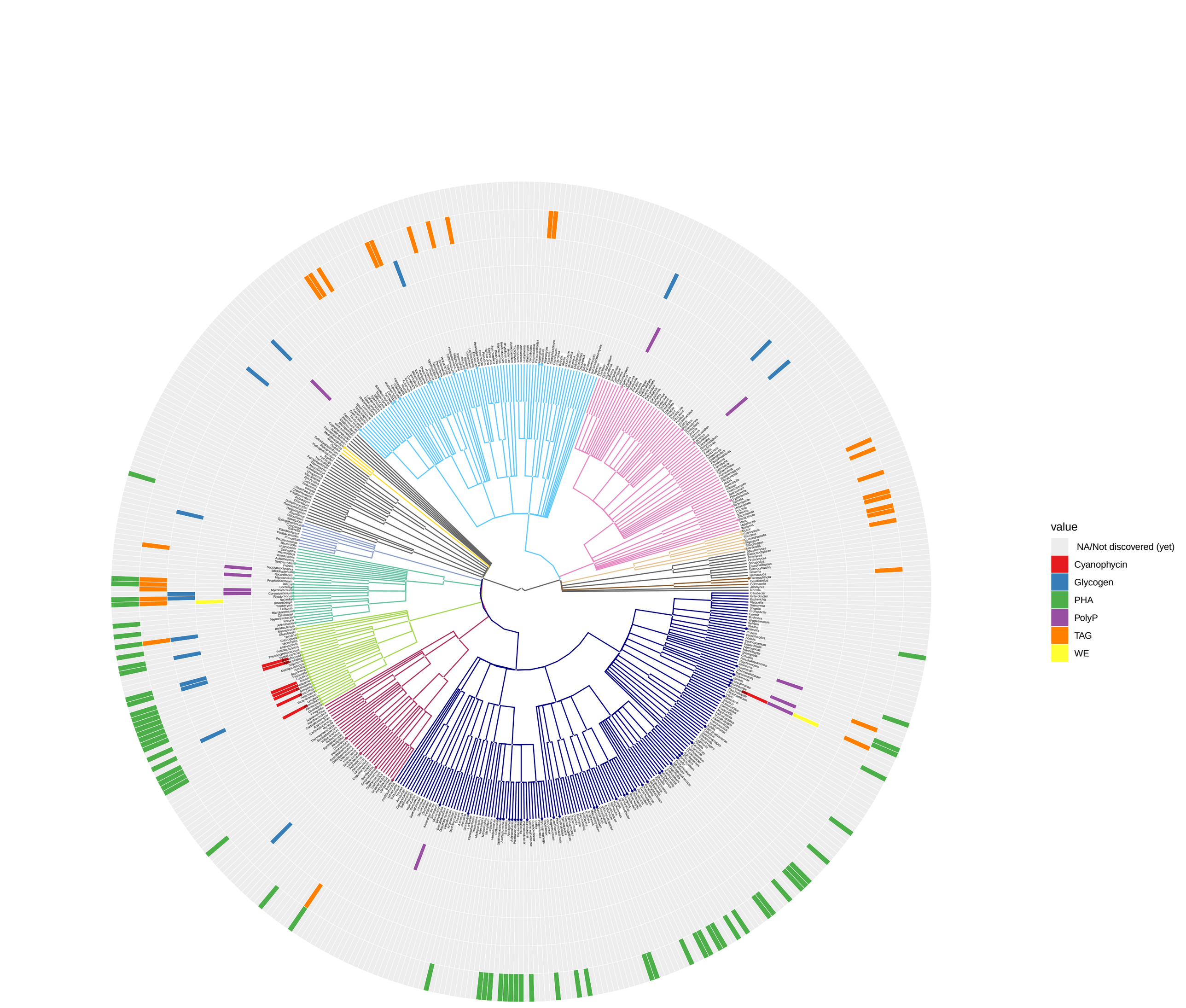
## Figure S2.1

(zoom in to view genus names)


value
NA/Not discovered (yet)
Cyanophycin
Glycogen
PHA
PolyP
TAG
WE

# Supplementary Information 2B: Sources for Figure 1

**Table S2.1: Literature sources for Figure 1 - Fungi**

| Genus | Storage | Soil occurrence |
|---|---|---|
| Agaricus | [1] | [2] |
| Alternaria | [3] | [4] |
| Aspergillus | [5] | [6] |
| Candida | [7] | [8] |
| Chaetomium | [9] | [10] |
| Colletotrichum | [3] | [11] |
| Cryptococcus | [9] | [12] |
| Cunninghamella | [13, 14] | [15] |
| Entomophthora | [16] | [17] |
| Epicoccum | [9] | [18] |
| Gigaspora | [19] | [20] |
| Hebeloma | [21] | [21] |
| Humicola | [9] | [22] |
| Lactarius | [23] | [23] |
| Lipomyces | [24] | [25] |
| Mortierella | [14] | [26] |
| Mucor | [27] | [28] |
| Neurospora | [29] | [30] |
| Penicillium | [31] | [32] |
| Pisolithus | [33] | [34] |
| Pythium | [16] | [35] |
| Rhodosporidium | [36] | [37] |
| Rhodotorula | [9] | [38] |
| Saccharomyces | [39, 40] | [41] |
| Thamnidium | [9] | [42] |
| Trichosporon | [9] | [43] |
| Yarrowia | [44] | [45] |

**Table S2.2: Literature sources for Figure 1 - Bacteria**

| Genus | Storage | Soil occurrence |
|---|---|---|
| Achromobacter | [46] | [47] |
| Acinetobacter | [48–52] | [53] |
| Aeromonas | [54] | [55] |
| Alcaligenes | [56] | [57] |
| Alcanivorax | [58] | [59, 60] |
| Anabaena | [61, 62] | [63] |
| Aneurinibacillus | [64] | [65] |
| Aphanocapsa | [66] | [63] |
| Arthrobacter | [9, 67–69] | [70] |
| Arthrospira | [71, 72] | [63] |
| Aulosira | [73] | [74] |
| Azohydromonas | [75] | [76] |
| Azospirillum | [77] | [78] |
| Azotobacter | [79] | [80] |

| | | |
|---|---|---|
| Bacillus | [81, 82] | [82] |
| Bifidobacterium | [83] | [84] |
| Burkholderia | [85] | [86] |
| Calothrix | [62] | [87] |
| Caulobacter | [88] | [89] |
| Chelatococcus | [90] | [91] |
| Chlorogloea | [62] | [92] |
| Clostridium | [93] | [94] |
| Collimonas | [68] | [95] |
| Corynebacterium | [96, 97] | [98] |
| Cupriavidus | [99] | [82, 100] |
| Cyanothece | [101] | [102] |
| Dietzia | [103] | [104] |
| Ensifer | [68] | [68] |
| Erythrobacter | [105] | [106] |
| Fischerella | [62] | [107] |
| Gordonia | [103, 108] | [109] |
| Halomonas | [110, 111] | [112] |
| Hydrogenophaga | [113] | [114] |
| Janthinobacterium | [115] | [68] |
| Jeongeupia | [116] | [116] |
| Lactobacillus | [117] | [118] |
| Lyngbya | [62] | [119] |
| Mastigocladopsis | [62] | [120] |
| Methylacidiphilum | [121] | [122] |
| Methylobacterium | [123] | [123] |
| Methylocystis | [124] | [125] |
| Methylosinus | [124] | [126] |
| Microbacterium | [68] | [68] |
| Microcoleus | [127, 128] | [63, 129, 130] |
| Microlunatus | [131] | [132] |
| Mycobacterium | [133, 134] | [135] |
| Myxococcus | [136] | [137] |
| Myxosarcina | [62] | [119] |
| Nocardia | [48, 103, 138] | [139] |
| Nostoc | [61, 140] | [63] |
| Oscillatoria | [62] | [63] |
| Paenarthrobacter | [68] | [68] |
| Paraburkholderia | [141] | [142] |
| Paracoccus | [143] | [143] |
| Phormidium | [62, 128] | [144] |
| Plectonema | [62] | [119] |
| Prevotella | [145] | [146] |
| Pseudomonas | [48, 147] | [57] |
| Rahnella | [68] | [148] |
| Ralstonia | [149] | [150] |
| Rhizobium | [151] | [152] |
| Rhodobacter | [153] | [154] |
| Rhodococcus | [103, 155–157] | [82] |
| Rhodopseudomonas | [158] | [154] |

| | | |
|---|---|---|
| Rhodospirillum | [159] | [160] |
| Rhodovulum | [161] | [162] |
| Ruegeria | [163] | [164] |
| Salinivibrio | [165] | [166] |
| Scytonema | [62] | [167] |
| Sinorhizobium | [151] | [168] |
| Sphingobacterium | [169] | [47] |
| Spirulina | [170, 171] | [63] |
| Stenotrophomonas | [46] | [46] |
| Stigonema | [62] | [172] |
| Streptomyces | [173] | [174] |
| Symploca | [62] | [167] |
| Synechococcus | [175, 176] | [177] |
| Synechocystis | [178–180] | [181] |
| Yangia | [182] | [182] |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# Supplementary Information 3: Model description

## 1. Theory

A conceptual model of soil C and N compartments and flows was constructed to assess the effects of internal microbial storage on the short-term dynamics of microbial biomass and substrates. The model is based on Schimel and Weintraub [1], with the addition of a C storage compartment. To illustrate the role of storage in microbial dynamics, we focused on short-term responses to a pulse addition of labile organic matter, neglecting extracellular enzyme synthesis and depolymerization of native soil organic matter. The model includes a bio-available substrate compartment (containing C and N) and a microbial biomass compartment encompassing both 'active' biomass (C and N) and storage compounds (C only). A model schematic is shown in Figure 2 in the main text and symbols are defined in Table S3.1.

### *1.1. Mass balance equations*

Denoting the substrate compartment with the subscript *S*, active biomass with *B*, and storage with *ST*, the mass balance equations for C and N in these compartments can be written as:

$$\frac{dC_S}{dt} = T\left(1 + \frac{C_{ST}}{C_B}\right) - U_S, \tag{1}$$

$$\frac{dN_S}{dt} = \frac{T}{(C{:}N)_B} - \frac{U_S}{(C{:}N)_S}, \tag{2}$$

$$\frac{dC_B}{dt} = U_S - S + U_{ST} - R_G - R_O - R_{ST} - T, \tag{3}$$

$$\frac{dN_B}{dt} = \frac{U_S}{(C{:}N)_S} - \frac{T}{(C{:}N)_B} - M_{net}, \tag{4}$$

$$\frac{dC_{ST}}{dt} = S - U_{ST} - \frac{C_{ST}}{C_B}T, \tag{5}$$

where $T$ is the rate of microbial turnover (recycled as substrate); $U_S$ and $U_{ST}$ are the rates of substrate uptake and storage remobilization, respectively; $S$ is the rate of synthesis of storage compounds; $R_G$, $R_O$, and $R_{ST}$ are the respiration rates associated with growth, overflow, and storage remobilization, respectively; $M_{net}$ is the net N mineralization rate. In Eq. (1), the term $1 + \frac{C_{ST}}{C_B}$ accounts for the fact that storage compounds are recycled at the same rate as microbial turnover.

Inorganic N sources are not modelled explicitly, but a maximum net N immobilization rate ($I_N$) is imposed to account for limited availability of inorganic N. This maximum rate is attained when the N demand for growth is high and the organic substrate is N-poor. The C and N flow rates in the mass balance equations (1)-(5) are described in Section S1.2, and their stoichiometric relations in Section S1.3. Specific choices for the definitions of the storage synthesis and remobilization rates are presented in Section 1.4.

**Table S3.1:** List of symbols and their units (see also Figure S3.1 and main text Figure 2).

| Symbol | Definition | Units |
|---|---|---|
| State variables | | |
| $C_B$ | Microbial biomass C (Eq. (3)) | mg C g soil$^{-1}$ |
| $C_S$ | Substrate C (Eq. (1)) | mg C g soil$^{-1}$ |
| $C_{ST}$ | Storage C (Eq. (5)) | mg C g soil$^{-1}$ |
| $(C:N)_S$ | Substrate C:N ratio (both $C_S$ and $N_S$ are state variables) | mg C mg N$^{-1}$ |
| $N_B$ | Microbial biomass N (Eq. (4)) | mg N g soil$^{-1}$ |
| $N_S$ | Substrate N (Eq. (2)) | mg N g soil$^{-1}$ |
| C and N flow rates (calculated as a function of the state variables) | | |
| $M_{net}$ | Net N mineralization rate (Eq. (6)) | mg N g soil$^{-1}$ day$^{-1}$ |
| $R$ | Total microbial respiration rate | mg C g soil$^{-1}$ day$^{-1}$ |
| $R_G$ | Respiration rate for growth on substrate C | mg C g soil$^{-1}$ day$^{-1}$ |
| $R_{ST}$ | Respiration rate for growth on storage C | mg C g soil$^{-1}$ day$^{-1}$ |
| $R_O$ | Rate of overflow respiration (Eq. (8)) | mg C g soil$^{-1}$ day$^{-1}$ |
| $S$ | Rate of C storage synthesis | mg C g soil$^{-1}$ day$^{-1}$ |
| $T$ | Microbial turnover rate | mg C g soil$^{-1}$ day$^{-1}$ |
| $U_S$ | Substrate C uptake rate | mg C g soil$^{-1}$ day$^{-1}$ |
| $U_{ST}$ | Storage C remobilization rate | mg C g soil$^{-1}$ day$^{-1}$ |
| Model parameters (time invariant) | | |
| $(C:N)_B$ | C:N ratio of active (non-storage) microbial biomass | mg C mg N$^{-1}$ |
| $e$ | C-use efficiency of the active biomass | - |
| $I_N$ | Maximum rate of inorganic N immobilization | mg N g soil$^{-1}$ day$^{-1}$ |
| $k_S$ | Substrate C uptake rate constant | day$^{-1}$ |
| $k_{ST}$ | Storage C remobilization rate constant | day$^{-1}$ |
| $m$ | Microbial turnover rate constant | day$^{-1}$ |
| $\sigma$ | Fraction of growth allocated to reserve storage | - |

## *1.2. Definition of C and N flow rates*

For illustration, we consider the simple case of a single substrate addition at the beginning of the simulations. An initial concentration of substrate is defined, and its change through time is followed as it is consumed by the microbes in the absence of additional inputs. Assuming that the added substrate is immediately available and does not require enzymatic breakdown, it is reasonable to assume that microbial uptake follows first-order kinetics $U_S = k_S C_S$, with a relatively high value of the kinetic constant $k_S$. Using nonlinear kinetics involving microbial biomass or an explicit enzyme compartment would not change the results of these short-term simulations, which are dominated by the initial substrate availability. Similarly, first-order kinetics are also assumed for the mortality rates (i.e., microbial turnover is assumed to be independent of microbial nutritional status). The rates of growth respiration associated with C from the substrate ($C_S$) and storage ($C_{ST}$) are modelled as a fraction 1-$e$ of the respective rates of uptake and use, where $e$ is the C-use efficiency [2–4]. Of the total C uptake rate $U_S$, only the fraction remaining available for growth after C allocation to storage incurs growth

respiration costs; i.e., $R_G = (1-e)(U_S - S)$. Maintenance respiration is neglected as it is expected to be small compared to growth respiration under conditions of substrate-induced growth.

Organic N uptake and microbial N turnover rates are calculated as C flow rates divided by the C:N ratios of the donor compartments. The remaining C and N flow rates ($R_O$ and $M_{net}$) are defined to fulfil specific stoichiometric constraints, as explained in the following section.

### *1.3. Stoichiometric constraints*

We impose the constraint that the active microbial biomass is homeostatic; i.e., its C:N ratio is constant through time, or $dC_B/dt = (C:N)_B\, dN_B/dt$. Note that most models assume that the total microbial biomass C:N ratio is fixed [3], while here we allow for storage C to fluctuate while keeping only the active biomass C:N fixed. This constraint is imposed in two ways depending on whether microbes are C limited or N limited (Figure S3.1). C limitation occurs as long as the microbial N requirements are met by organic or inorganic N—in that case, overflow respiration is zero [1]. In contrast, N limitation occurs when N immobilization is limited by available inorganic N; i.e., N immobilization rate equals a maximum value of $I_N$ and $R_O$ removes the extra C that cannot be converted into biomass. While $R_O$ is interpreted as a respiration term, it could also be regarded as an overflow excretion process that leads to accumulation of extracellular C that is not readily utilized. For the purpose of the present numerical experiments, we consider this extracellular C as 'lost', but for long-term simulations it could become a precursor of stabilized soil organic C.

Under C limitation (when $R_O$=0), the constraint of homeostatic microbial biomass leads to the definition of the net N mineralization rate,

$$M_{net} = \frac{U_S}{(C:N)_S} - \frac{U_S - S + U_{ST} - R_G - R_{ST}}{(C:N)_B} = \frac{U_S}{(C:N)_S} - e\,\frac{U_S + U_{ST} - S}{(C:N)_B}. \quad (6)$$

This equation essentially expresses N mineralization as the difference between supply rate of organic N (i.e., $U_S/(C:N)_S$) and demand by microbes, calculated as the growth rate under C-limited conditions divided by the microbial C:N ratio (i.e., $e(U_S + U_{ST} - S)/(C:N)_B$). When the N demand is higher than the supply, net N immobilization ensues. Without storage ($U_{ST}$=0, $S$=0), Eq. (6) simplifies to

$$M_{net} = \frac{U_S}{(C:N)_S} - \frac{eU_S}{(C:N)_B}, \quad (7)$$

which coincides with the common definition of net N mineralization [3, 5, 6].

Under N limitation, the microbial N demand reaches the maximum rate of inorganic N supply, so that $M_{net}$=-$I_N$. This limits the capacity to grow at a fixed C:N, and requires disposing of extra C from the substrate via overflow respiration [1]. Imposing again the constraint of fixed C:N, but now setting $M_{net}$=-$I_N$, the rate of overflow respiration is found as,

$$R_O = U_S - S + U_{ST} - R_G - R_{ST} - (C:N)_B\left[\frac{U_S}{(C:N)_S} + I_N\right] = e(U_S + U_{ST} - S) - (C:N)_B\left[\frac{U_S}{(C:N)_S} + I_N\right]. \quad (8)$$

Again, without storage dynamics, a previously derived, simpler expression for $R_O$ is recovered [1, 3],

$$R_O = eU_S - (C{:}N)_B \left[\frac{U_S}{(C{:}N)_S} + I_N\right]. \quad (9)$$

Together, Eq. (6) and (8) ensure that the active biomass maintains its C:N ratio at a fixed value, while C accumulates and is depleted in the storage compartment. This means that the overall C:N of total biomass (active microbial biomass plus storage) does vary through time. Equations (6) and (8) are general and hold for any choice of storage synthesis and remobilization kinetics. In Section 1.4, different alternative storage strategies are presented, which result in specific expressions for the rates of net N mineralization and overflow respiration.

## *1.4. Dynamics of microbial internal storage*

Two modes of internal storage synthesis and remobilization are considered, in addition to a baseline scenario in which storage is not used (Figure S3.1): i) reserve storage, in which storage synthesis equals a fixed fraction of substrate uptake, and storage remobilization depends only on the amount of C in storage, and ii) surplus accumulation, in which storage synthesis increases when microbes are N-limited and storage remobilization is activated when they are C-limited.

### *1.4.1. Reserve storage*

This storage mode has been implemented in models of waste water treatment systems. The rate of storage synthesis is modelled as a fraction $\sigma$ of the substrate C uptake rate, $S = \sigma U_S$ [2, 4]. The rate of storage remobilization is assumed to follow first-order kinetics $U_{ST} = k_{ST} C_{ST}$, thus neglecting the inhibition effect that high substrate concentration might have on storage remobilization [2, 4]—a reasonable assumption in soils where substrate concentrations are typically low. With this storage mode, allocation to storage is independent of N availability, so that under N-limitation, C overflow becomes necessary to maintain a stable active biomass C:N ratio.

### *1.4.2. Surplus accumulation*

In this storage mode, C storage can be increased under N limitation and decreased under C limitation to compensate stoichiometric imbalances (Figure S3.1). For simplicity, we assume that under N limitation, all excess C is converted to storage and that no storage is used for growth. In contrast, under C limitation, all C required to convert excess N into biomass is drawn from the storage compartment, whereas no C is allocated to new storage. This storage mode represents an idealized case, as microbes likely cannot attain such a degree of complete flexibility in C storage synthesis and remobilization. However, it serves as a counterpoint to the reserve storage mode, where storage dynamics are independent of N availability.

Following these assumptions, the rate of storage synthesis $S$=0 under C limited conditions, whereas $S$ is defined to maintain fixed active biomass C:N ratio without C overflow under N limited conditions (i.e., $R_O$=0 in Eq. (8)),

$$S = -\left[\frac{1}{e}\frac{(C{:}N)_B}{(C{:}N)_S} - 1\right] U_S - \frac{(C{:}N)_B}{e} I_N. \quad (10)$$

Note that the storage remobilization rate $U_{ST}$ (and associated respiration $R_{ST}$) does not appear in Eq. (10) because we assume that under N limitation, storage C is not used for growth—it would cause a further increase in N demand at a time when N is already scarce.

In contrast, under C limited conditions, the rate of storage C remobilization $U_{ST}$ is defined to maintain a fixed microbial C:N ratio without N losses (i.e., $M_{net}$=0 in Eq. (6)),

$$U_{ST} = \left[\frac{1}{e}\frac{(C{:}N)_B}{(C{:}N)_S} - 1\right] U_S. \quad (11)$$

Note that the storage synthesis rate *S* does not appear in Eq. (11) because we assume that under C limitation, storage C is not synthesized—it would cause a further increase in C demand at a time when C is already limited. Moreover, the rate of storage C remobilization is capped by a maximum rate defined as in the reserve storage mode (i.e., $U_{ST} = k_{ST} C_{ST}$), to avoid remobilization of more C than is actually contained in the storage compartment. Mathematically, this constraint is defined by imposing that $U_{ST}$ is the minimum between the value calculated from Eq. (11) and $k_{ST} C_{ST}$.

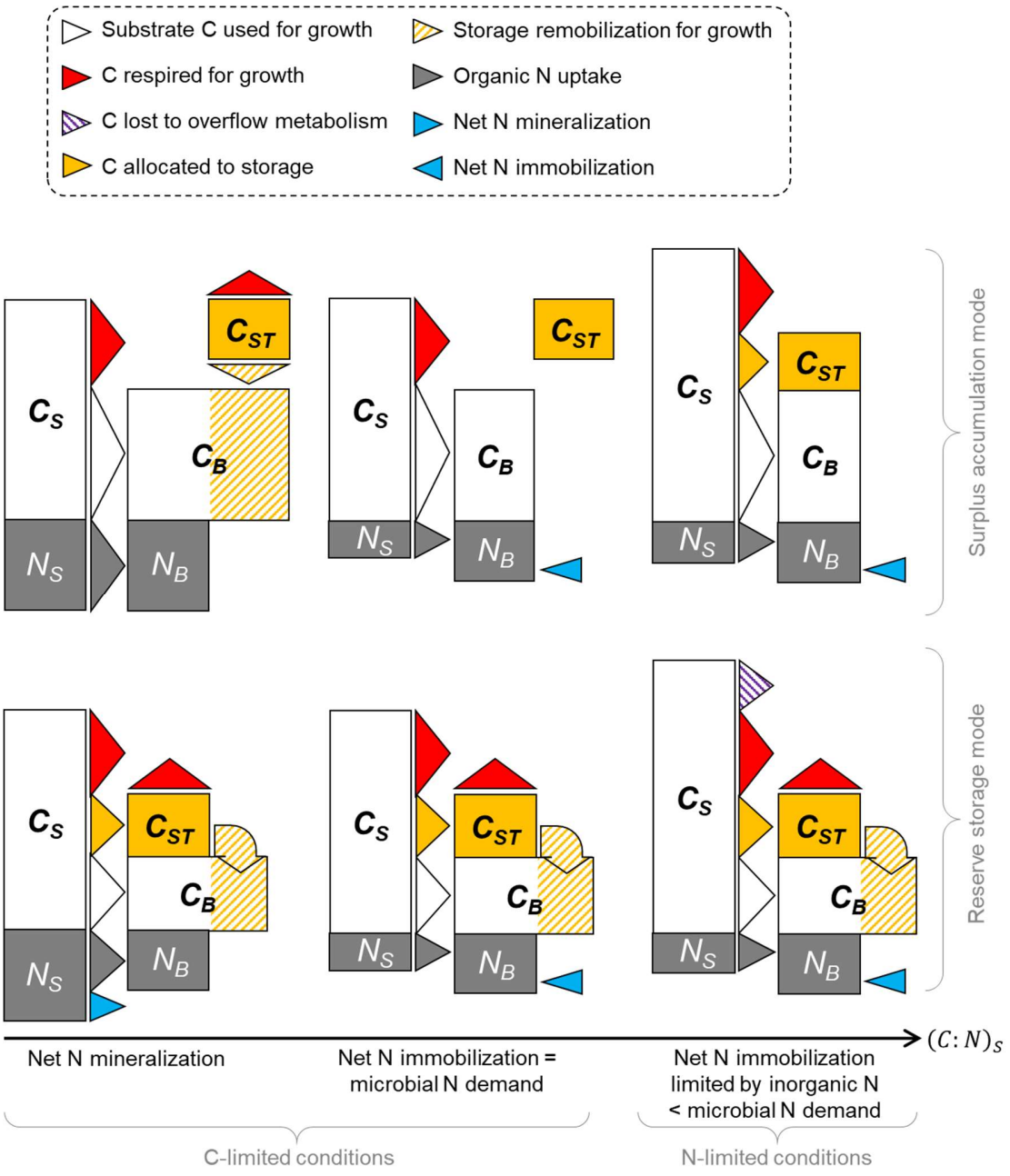


**Figure S3.1:** Schematic of the changes in C and N flows as the substrate C:N ratio is increased (left to right), for two different C storage synthesis and remobilization modes: i) reserve storage, in which storage synthesis is a fixed fraction of microbial growth, independent of N limitation (which results in C overflow under N limitation; bottom row) or ii) surplus accumulation, in which storage synthesis is regulated to compensate stoichiometric imbalances (which results in no C overflow; top row). Color-coded arrows represent C and N flows; rectangles represent substrate and microbial compartments. Note that with the surplus accumulation mode, C storage is only remobilized when N is in excess (i.e., C is limiting) and only synthesized when C is in excess (N is limiting).

## 2. Model parameterization

The goal of the analyses presented here is to explore the effect of contrasting storage modes on substrate use and microbial growth. As such, we do not perform a formal model calibration, but estimate parameter values and initial conditions based on available literature data. The model has only eight microbial parameters, in addition to substrate input rate and C:N ratio, which are varied to simulate different experimental conditions (Table S3.1). To define a set of reasonable microbial parameter values, we consider the dynamics of microbial biomass in the experiment by Chen et al. [7], where microbial biomass increased from approximately 1 to 2 mg C g soil$^{-1}$ in a few days after addition of an amount of labile C double the value of microbial C (in the soil labelled there as 'P-rich'). The microbial turnover rate (=$m$) was ≈0.04 day$^{-1}$ and $(C:N)_B$=8.9 before the amendment. Since we consider additions of labile substrates, we can assume a relatively high C-use efficiency $e$=0.5. Based on previous model results [4], it is reasonable to assume that growth on substrate and storage C occurs with equal efficiency and, for the reserve storage mode, with an equal rate constant (i.e., $k_S$=$k_{ST}$). Due to the speed of microbial uptake of labile substrates (time scales of hours), $k_S$ was set equal to 1 day$^{-1}$. The fraction of substrate C allocated to storage in the reserve storage mode is arbitrarily set to 0.2—somewhat lower than values previously reported [8], but likely more representative of soil conditions. The last parameter to be estimated is $I_N$, which represents the availability of inorganic N in the soil. We chose a value $I_N$=0.01 mg N g soil$^{-1}$ day$^{-1}$, which corresponds to a sufficient inorganic N supply to convert approximately 1 mg C g soil$^{-1}$ of substrate into biomass in 5 days.

We performed two types of numerical experiments: i) simulation of microbial and storage C concentrations and element flows through time following an initial addition of a fixed amount of substrate with $(C:N)_I$=50 (Figure S3.2 and main text Figure 3A); ii) simulations covering a gradient of initial substrate C:N, for a fixed amount of added substrate C, with microbial biomass and cumulative net N mineralization rates evaluated at the end of each simulation (10 days after substrate addition) to summarize the effect of the three storage modes on microbial C-use efficiency and inorganic N availability (main text Figure 3B and C).

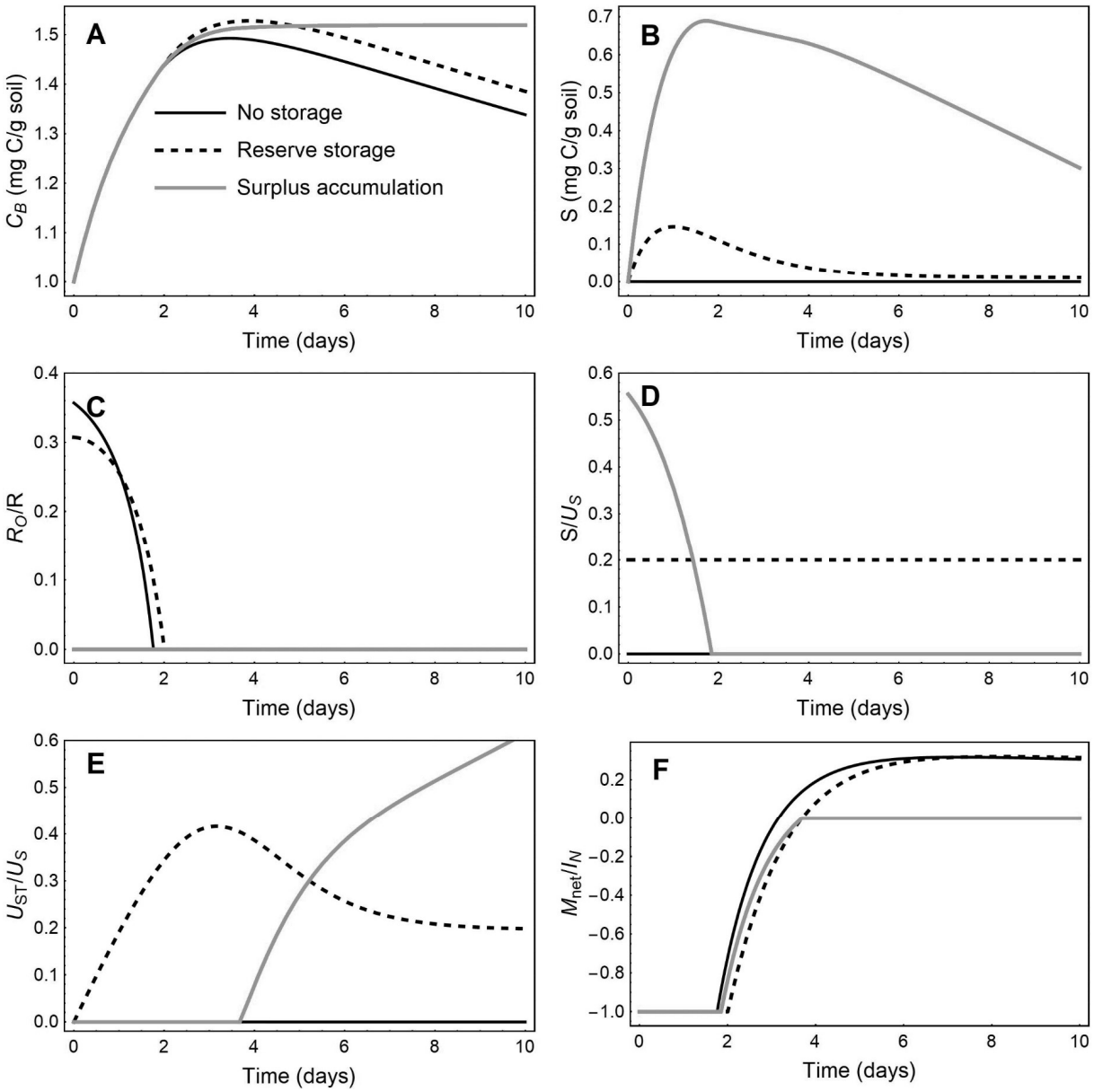


**Figure S3.2:** Temporal dynamics of C and N concentrations and flow rates after substrate addition for two storage modes (compared to a baseline case of no storage use): A) microbial biomass C ($C_B$): B) internal storage C ($S$); C) fraction of total respiration due to overflow ($R_O/R$); D) rate of storage synthesis, $S$, normalized by the rate of substrate uptake, $U_S$; E) rate of storage remobilization, $U_{ST}$, normalized by $U_S$; F) net N mineralization rate, $M_{net}$, normalized by the maximum rate of N immobilization, $I_N$. Initial conditions: $C_B(t=0)$=1 mg C g soil$^{-1}$, $S(t=0)$=0 mg C g soil$^{-1}$, $C_S(t=0)$=2 mg C g soil$^{-1}$; $N_S(t=0)$=0.04 mg N g soil$^{-1}$ (i.e., $(C:N)_I$=50).

## 3. References


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