

Supplement S1: simulations for sensitivity:

Each simulation was set up under a series of biologically-plausible scenarios. Fungal:bacterial ratio and total community size are presented in terms of DNA, as this is the unit of growth measurement for the ^{18}O method. Definitions for parameters are found in Table 1 of the main text, and for variables defined in the equations below in Table S1 below. Values without subscripts denote true MBC, DNA, and MBC:DNA ratios, while values with subscripts denote observed values were DNA (d), MBC (c), or both (dc) to be extracted inefficiently. In our current manuscript, we only present results for the scenario where both DNA and MBC are extracted inefficiently ("dc" scenario).

A community of size $totalDNA$ was generated as a function of the fungal fraction of the total DNA pool ($FBratio$), where 30 was used as an arbitrary multiplier to determine the amount of DNA, and 3 as an arbitrary additive factor to ensure that bacteria-only ($FBratio$ of zero) still had DNA.

$$totalDNA = 30 * FBratio + 3 \quad (1)$$

The corresponding amount of microbial biomass carbon (MBC) is calculated as the sum of the biomass carbon of bacteria and fungi, which are the products of their DNA and MBC:DNA ratios.

$$MBC = FBratio * totalDNA * MBCDNAratF + (1 - FBratio) * totalDNA * MBCDNAratB \quad (2)$$

The MBC:DNA ratio of the starting community is therefore:

$$MBCDNA = \frac{MBC}{totalDNA} \quad (3)$$

The observed total DNA observed assuming inefficient DNA extraction ($totalDNA_d$) is then:

$$totalDNA_d = FBratio * totalDNA * DNAexteffF + (1 - FBratio) * totalDNA * DNAexteffB \quad (4)$$

The corresponding MBC:DNA ratio assuming inefficient DNA extraction ($MBCDNA_d$) is:

$$MBCDNA_d = \frac{MBC}{totalDNA_d} \quad (5)$$

MBC_c represents the total amount of microbial biomass observed after accounting for inefficient chloroform fumigation extraction:

$$MBC_c = FBratio * totalDNA * MBCDNAratF * MBCexteffF + (1 - FBratio) * totalDNA * MBCDNAratB * MBCexteffB \quad (6)$$

And the corresponding MBC:DNA ratio is:

$$MBCDNA_c = \frac{MBC_c}{totalDNA} \quad (7)$$

If inefficiencies in both MBC and DNA extraction must be accounted for, then the apparent MBC:DNA ratio ($MBCDNA_{dc}$) is:

$$MBCDNA_{dc} = \frac{MBC_c}{totalDNA_d} \quad (8)$$

Steps 4-6 therefore show the MBC:DNA ratios a researcher converting the new DNA produced to MBC would use if they were unaware of extraction biases and did not account for differences in bacterial and fungal growth rates (below).

If not all the microbes are actively growing during the incubation, we multiply the maximum growth rate ($GRfun$ or $GRbact$) by the fraction of the community growing during an incubation ($activeFractionF$ or $activeFractionB$). This gives the effective growth rate, that we can use in place of $GRfun$ and $GRbact$ in the equations below.

Parameter	Description	units
totalDNA	The true total mass of the DNA pool in the soil	ug g ⁻¹ soil
FBratio	Fungal fraction of total DNA pool	dimensionless
MBC	The true total mass of the microbial biomass carbon pool in the soil	ug g ⁻¹ soil
MBCc	The apparent total mass of the microbial biomass carbon pool in the soil, given inefficient microbial biomass carbon extraction	ug g ⁻¹ soil
totalDNA _d	The apparent total size of the true DNA pool in the soil, given a DNA extraction inefficiency	ug g ⁻¹ soil
MBCDNA	True microbial biomass carbon to DNA mass ratio of starting community; Used to convert the DNA growth increment into microbial biomass carbon growth.	dimensionless
MBCDNA _c	Apparent microbial biomass carbon to DNA mass ratio of starting community, given that microbial biomass carbon is not completely extracted; Used to convert the DNA growth increment into microbial biomass carbon growth.	dimensionless
MBCDNA _d	Apparent microbial biomass carbon to DNA mass ratio of starting community, given that DNA is not completely extracted; Used to convert the DNA growth increment into microbial biomass carbon growth.	dimensionless
MBCDNA _{dc}	Apparent microbial biomass carbon to DNA mass ratio of starting community, given that both DNA and microbial biomass carbon are not completely extracted; Used to convert the DNA growth increment into microbial biomass carbon growth.	dimensionless
GR _{mean}	Growth rate for simulations when bacteria and fungi are assumed to grow at the same community-level mean	day ⁻¹
TrueMBC _{same}	The true new microbial biomass carbon produced during the CUE incubation, assuming bacteria and fungi grow at the same rate (GR _{mean})	ug C day ⁻¹
MBC _{samed}	The apparent new microbial biomass carbon produced during the CUE incubation, given that not all the DNA is extracted from the growing community and assuming that bacteria and fungi grow at the same rate (GR _{mean})	ug C day ⁻¹
MBC _{samec}	The apparent new microbial biomass carbon produced during the CUE incubation, given that not all the microbial biomass carbon is extracted from the growing community and assuming that bacteria and fungi grow at the same rate (GR _{mean})	ug C day ⁻¹
MBC _{samedc}	The apparent new microbial biomass carbon produced during the CUE incubation, given that not all the DNA and microbial biomass carbon are extracted from the growing community and assuming that bacteria and fungi grow at the same rate (GR _{mean})	ug C day ⁻¹
H ₂ O _{cont}	The fraction of new oxygen in DNA which comes from extracellular water (ie the 18O-water added) rather than other sources	dimensionless
TrueMBC _{diff}	The true new microbial biomass carbon produced during the CUE incubation assuming bacteria and fungi grow at different rates	ug C day ⁻¹
MBC _{diffd}	The apparent new microbial biomass carbon produced during the CUE incubation assuming bacteria and fungi grow at different rates, given that not all the DNA is extracted from the growing community	ug C day ⁻¹
MBC _{diffc}	The true new microbial biomass carbon produced during the CUE incubation assuming bacteria and fungi grow at different rates, given that not all the microbial biomass carbon is extracted from the growing community	ug C day ⁻¹
MBC _{diffdc}	The true new microbial biomass carbon produced during the CUE incubation assuming bacteria and fungi grow at different rates, given that not all the DNA and microbial biomass carbon are extracted from the microbial community	ug C day ⁻¹

Table 1: Variables defined in the microbial biomass carbon calculations

We assume growth during the incubation is representative of overall community growth. In other words, the community is assumed to be in a steady state and the rate of turnover of a given taxon matches its growth. In turn, the turnover of DNA in the environment is proportionate to its abundance [1]. If bacteria and fungi grow at the same rate, then the community-level growth rate (GR_{mean}) can be set to vary as a function of the community composition:

$$GR_{mean} = F_{Bratio} * GR_{fun} + GR_{bact} * (1 - F_{Bratio}) \quad (9)$$

The corresponding true increase in MBC for bacteria and fungi when they are assumed to grow at the same rate ($TrueMBC_{same}$) is:

$$TrueMBC_{same} = GR_{mean} * totalDNA * F_{Bratio} * MBC_{DNA_{atF}} + GR_{mean} * totalDNA * (1 - F_{Bratio}) * MBC_{DNA_{atB}} \quad (10)$$

We account for a bias towards using extracellular rather than intracellular water using $H2O_{cont}$, which is the fraction of DNA oxygen derived from extracellular water.

If just DNA is extracted inefficiently then the corresponding apparent new MBC produced (MBC_{same_d}) is:

$$MBC_{same_d} = H2O_{cont} * (GR_{mean} * totalDNA * F_{Bratio} * DNA_{exteffF} * MBC_{DNA_d} + GR_{mean} * totalDNA * (1 - F_{Bratio}) * DNA_{exteffB} * MBC_{DNA_d}) \quad (11)$$

If just MBC is extracted inefficiently, then the corresponding apparent new MBC produced (MBC_{same_c}) is:

$$MBC_{same_c} = H2O_{cont} * (GR_{mean} * totalDNA * F_{Bratio} * MBC_{DNA_c} + GR_{mean} * totalDNA * (1 - F_{Bratio}) * MBC_{DNA_c}) \quad (12)$$

If both MBC and DNA are extracted inefficiently, then the apparent new MBC produced ($MBC_{same_{dc}}$) is:

$$MBC_{same_{dc}} = H2O_{cont} * (GR_{mean} * totalDNA * F_{Bratio} * DNA_{exteffF} * MBC_{DNA_{dc}} + GR_{mean} * totalDNA * (1 - F_{Bratio}) * DNA_{exteffB} * MBC_{DNA_{dc}}) \quad (13)$$

When bacterial growth rate (GR_{bact}) and fungal growth rate (GR_{fun}) differ, the true MBC produced ($TrueMBC_{diff}$) is:

$$TrueMBC_{diff} = GR_{fun} * totalDNA * F_{Bratio} * MBC_{DNA_{atF}} + GR_{bact} * totalDNA * (1 - F_{Bratio}) * MBC_{DNA_{atB}} \quad (14)$$

And the values for the true MBC produced under the various extraction bias scenarios are:

$$MBC_{diff_d} = H2O_{cont} * (GR_{fun} * totalDNA * F_{Bratio} * DNA_{exteffF} * MBC_{DNA_d} + GR_{bact} * totalDNA * (1 - F_{Bratio}) * DNA_{exteffB} * MBC_{DNA_d}) \quad (15)$$

$$MBC_{diff_c} = H2O_{cont} * (GR_{fun} * totalDNA * F_{Bratio} * MBC_{DNA_c} + GR_{bact} * totalDNA * (1 - F_{Bratio}) * MBC_{DNA_c}) \quad (16)$$

$$MBC_{diff_{dc}} = H2O_{cont} * (GR_{fun} * totalDNA * F_{Bratio} * DNA_{exteffF} * MBC_{DNA_{dc}} + GR_{bact} * totalDNA * (1 - F_{Bratio}) * DNA_{exteffB} * MBC_{DNA_{dc}}) \quad (17)$$

Ref: [1] Lennon, J. T., M. E. Muscarella, S. A. Placella, and B. K. Lehmkuhl. "How, when, and where relic DNA affects microbial diversity." *MBio* 9, no. 3 (2018).