SUPPLEMENTARY FIGURES



Supplementary Figure 1. Schematic illustration of the microbial N balance. Arrows represent N fluxes (U_N , microbial organic N uptake; U_{inorg} ; microbial inorganic N uptake; G_N , microbial net growth; M_N , release of excess N; E_N , excretion of metabolites including extracellular enzymes; *BD*, microbial mortality).



Supplementary Figure 2. Relationship between resource C:N ($R_{C:N}$) and microbial NUE in decomposing plant litter. Different symbols indicate four beech litter types with different C:N ratios. The solid line is a fitted simple linear regression model (*NUE*=0.601+0.0056× $R_{C:N}$; R²=0.297, $F_{1,36}$ =15.21; P<0.001; n=38).

SUPPLEMENTARY NOTE 1

Integration of NUE in the microbial N balance

Net changes in microbial N (B_N) result from the difference between inward and outward flows from the microbial N pool (see Supplementary Fig. 1 for schematic illustration) and can be described as

$$\frac{\mathrm{d}B_{\mathrm{N}}(t)}{\mathrm{d}t} = U_{\mathrm{N}} + U_{\mathrm{inorg}} - M_{\mathrm{N}} - E_{\mathrm{N}} - BD = G_{\mathrm{N}} - BD, \qquad (1)$$

where U_N is microbial organic N uptake, U_{inorg} is microbial inorganic N uptake, M_N is the release of inorganic N (gross N mineralization), E_N represents the excretion of metabolites (particularly extracellular enzymes), *BD* is microbial mortality and G_N is microbial net growth (adapted from the mass balance of microbial biomass C in¹). Organic and inorganic N taken up by microbes is used for microbial growth (sequestration in biomass) and a small fraction is devoted to extracellular enzyme production. Organic N in excess to microbial requirement can be mineralized (excreted) providing inorganic N (NH₄⁺) for uptake by other organisms. In the shortterm, the difference between N uptake (U_N+U_{inorg}) and N mineralization plus excretion of metabolites and extracellular enzymes (M_N+E_N) represents the fraction of N incorporated in biomass (G_N). Microbial N investment into extracellular enzymes is expected to be less than 5% (e.g., bacteria were shown to use 1–5% of N assimilated for extracellular enzyme production²). Given that E_N is <5% of U_N+U_{inorg} , the term $U_N+U_{inorg}-M_N$ can be replaced by G_N , which represents net microbial growth.

Microbial NUE can then be defined as the ratio of N used for growth over N uptake. Based on this definition and on the mass balance of Supplementary equation 1, NUE is given as

$$NUE = \frac{G_{\rm N}}{U_{\rm N} + U_{\rm inorg}} = \frac{U_{\rm N} + U_{\rm inorg} - E_{\rm N} - M_{\rm N}}{U_{\rm N} + U_{\rm inorg}},\tag{2}$$

where G_N is consistent with G_N of Supplementary equation 1. By solving Supplementary equation 2 for G_N , we obtain

$$G_{\rm N} = NUE(U_{\rm N} + U_{\rm inorg}). \tag{3}$$

Combining Supplementary equations 1 and 3, the mass balance of microbial N becomes,

$$\frac{\mathrm{d}B_{\mathrm{N}}(t)}{\mathrm{d}t} = NUE(U_{\mathrm{N}} + U_{\mathrm{inorg}}) - BD,\tag{4}$$

where N loss pathways are now embedded in the parameter NUE.

Supplementary References

- 1. Manzoni, S., Taylor, P., Richter, A., Porporato, A. & Ågren, G. I. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytol.* **196**, 79-91 (2012).
- 2. Frankena, J., Vanverseveld, H. W. & Stouthamer, A. H. Substrate and energy costs of the production of exocellular enzymes by Bacillus-licheniformis. *Biotechnol. Bioeng.* **32**, 803-812 (1988).