Exploring the activity of microorganisms in the forest soil by metatranscriptomics

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Ecology of forest topsoil

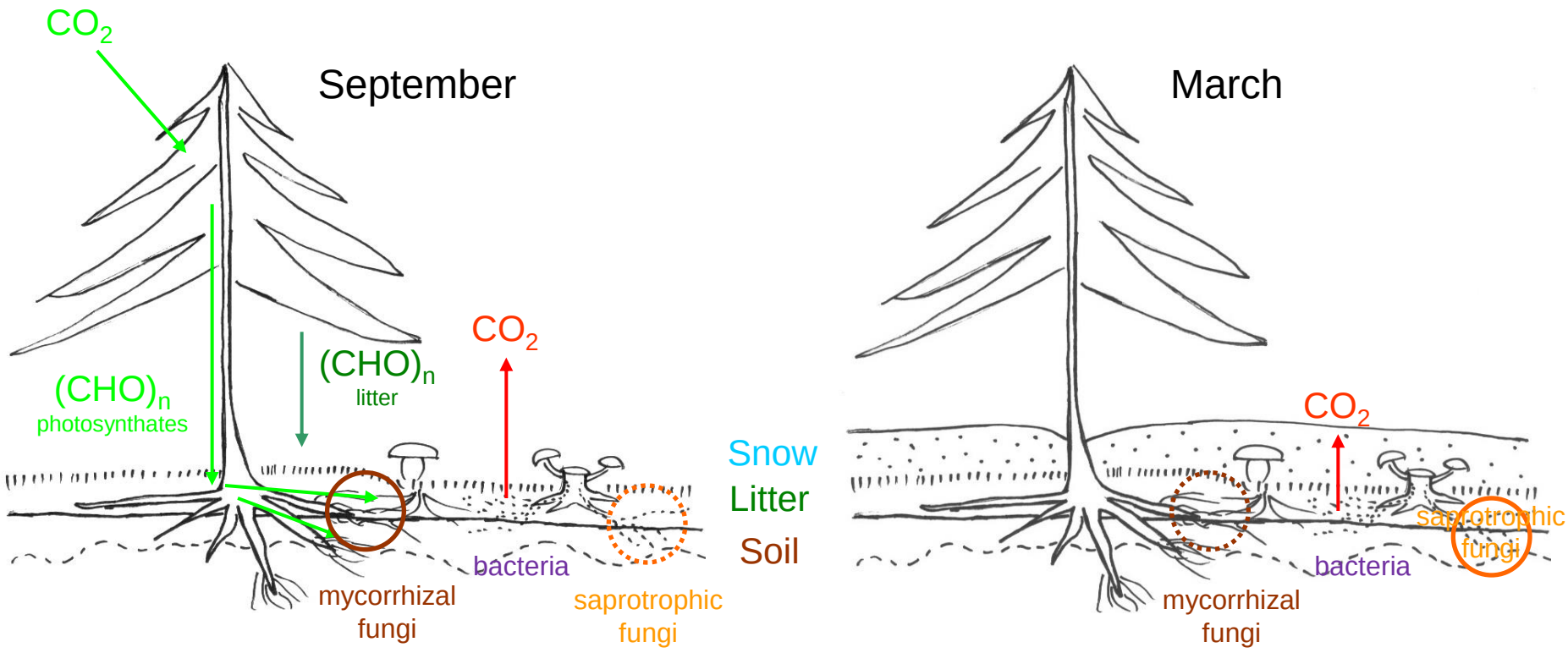
Nutrients

- leaf and root litter	- major source of organic matter in soil - cellulose, lignin, hemicellulose, pectin, proteins - numerous enzymes required for degradation
- root exudates	- nutrient source in mineral and organic soil - more readily available C

Microorganisms

- saprotrophic fungi	- obtain carbon by organic matter decomposition - production of extracellular degradative enzymes
- mycorrhizal fungi	- obtain carbohydrates from symbiotic plants - provide soil nutrients to plants - saprotrophic abilities vary
- bacteria	- preferential utilization of easily available compounds - degradation of biopolymers by some taxa

Microbial activity in litter and soil in contrasting seasons



- High share of fungi in the ecosystem is reflected by high fungal contribution to transcription and protein production, especially in litter
- Fungal activity is important for decomposition of complex organic matter
- Seasonal differences in rhizodeposition affect the nutrient availability in soil
- Activity of root-associated microbes, such as mycorrhizal fungi, decreases in winter

Sampling

September - soil temperature 15°C

March - soil temperature 2°C

Metatranscriptomics – opportunities

- - can indicate real activity in the studied ecosystem; fast response to disturbance / experimental treatment
- little danger of „ancient“ RNA from dead cells – such RNA decomposes rapidly
- for gene-coding sequences, functional and taxonomic assignment is more simple than for DNA (no introns, no noncoding DNA)
- relative rate of soil processes can be assessed by comparison of transcription in individual samples
- while metagenomics tell which genes **may be involved**, metatranscriptomics tell which genes actually **are involved** (expressed)

Metatranscriptomics – limitations and challenges

- expression is highly regulated and corresponds to „actual“ conditions, not „usual“ conditions of the site
- the amount of RNA extractable from soils usually makes amplification necessary which brings some bias
- extracted RNA contains much rRNA that may be difficult to remove
- there is little (if any useful) information on mRNA stability in time and translation rate and thus the amount of protein molecules synthesized per mRNA molecule in its lifetime
- metagenomics can theoretically deliver long contigs - chromosome fragments with multiple genes that co-occur in one genome; this is impossible for metatranscriptomics
- Soil environment is highly complex: one gram of soil typically contains >10000 of bacterial species and >500 fungal species

Analysis of microbial activity in summer and winter

Sampling

6 sites x 2 horizons (litter, soil) x 2 seasons (September, March) = 24 samples

Community analysis

Amplicon sequencing of DNA and RNA-deriver ITS2 sequences (MiSeq)

Metatranscriptomics: Shotgun sequencing of rRNA-depleted RNA

Isolation of total RNA
Deletion of bacterial rRNA and eukaryotic rRNA
(communities analysed by 16S and ITS sequening of DNA and RNA)

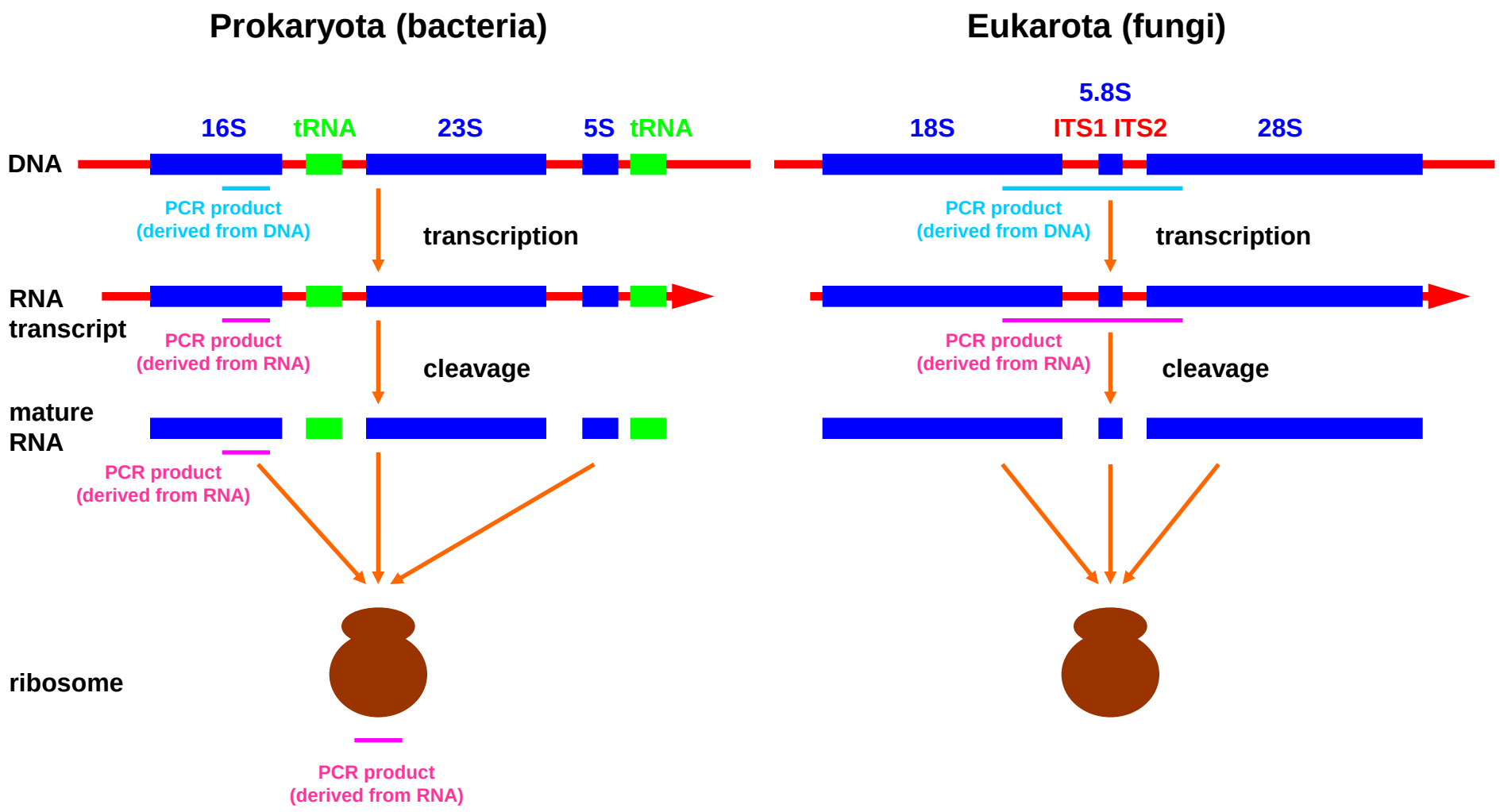
Sequencing on Illumina HiSeq – 2 lanes, 2x150 b *673 000 000 sequences*
Assembly of reads from all samples together *4 500 000 contigs >200 bases*
Annotation using MG-RAST and GenBank

44% reads mapped to contigs, 21% to identified contigs (taxon, function)

Metadata: Microbial biomass, enzyme activity, chemistry

Metaproteomics: Identification of fungal / bacterial proteins

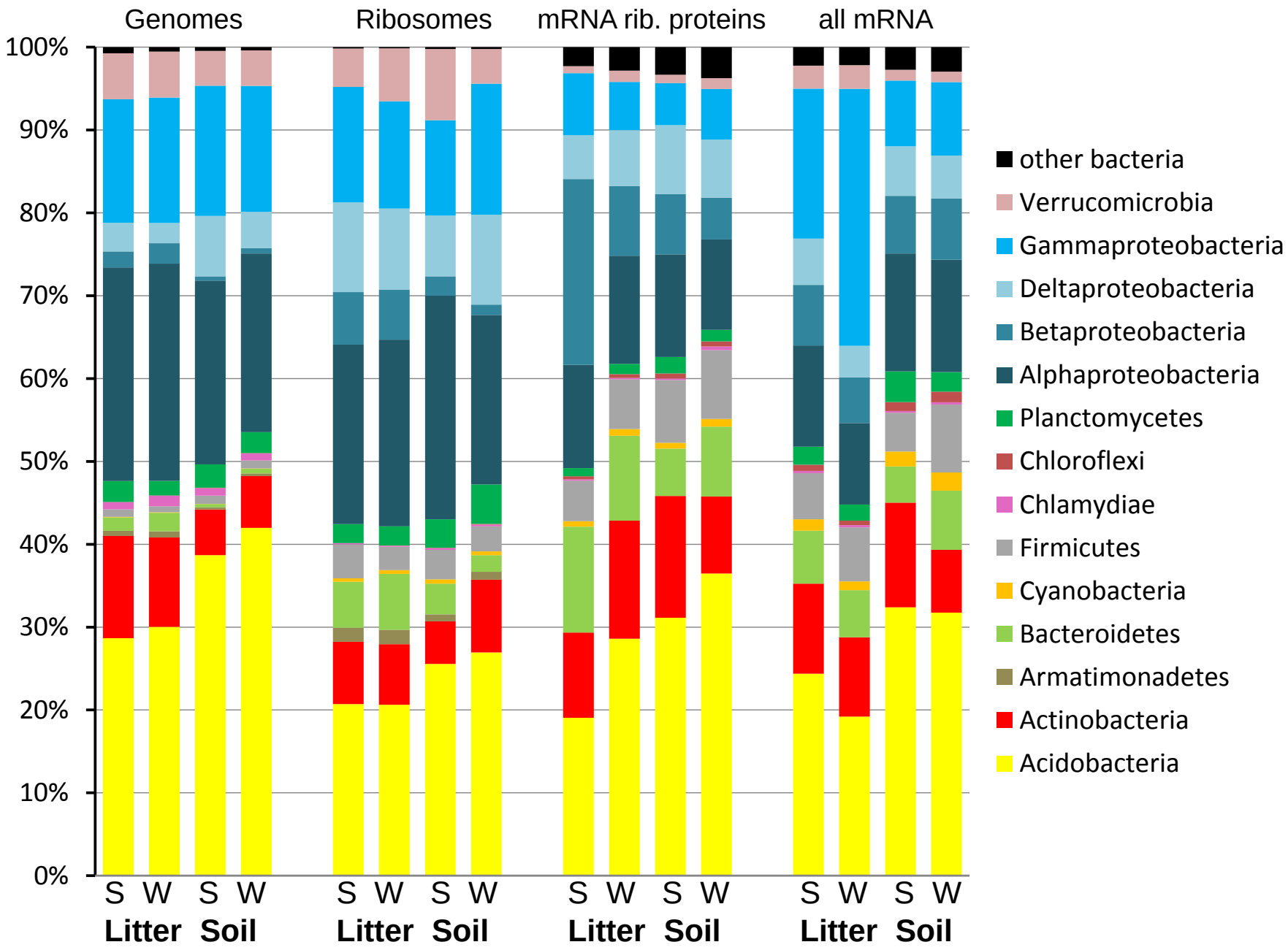
Identification of active microbes by 16S / ITS sequencing from RNA



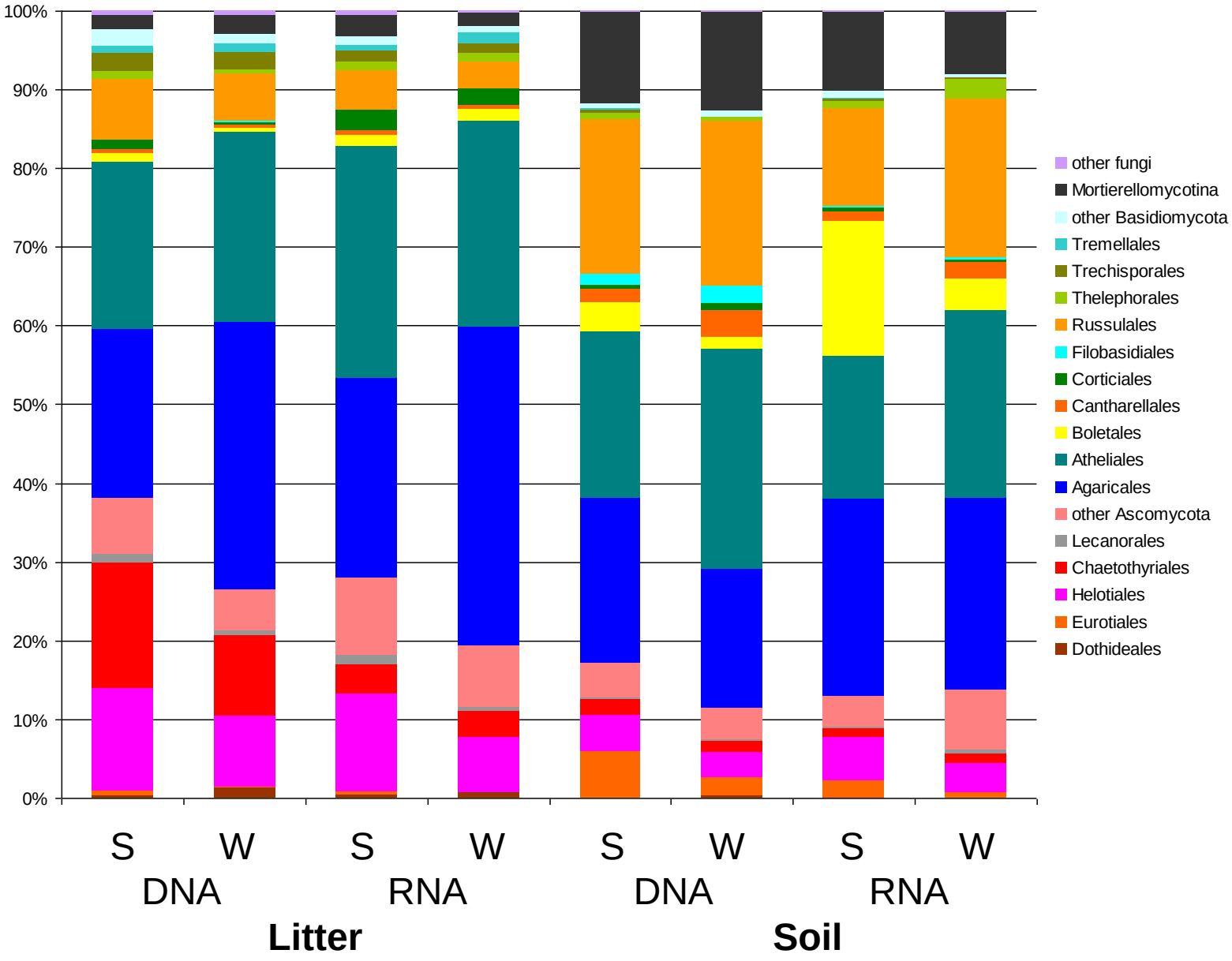
RNA amplicons:
microorganisms possessing ribosomes

RNA amplicons:
microorganisms producing ribosomes

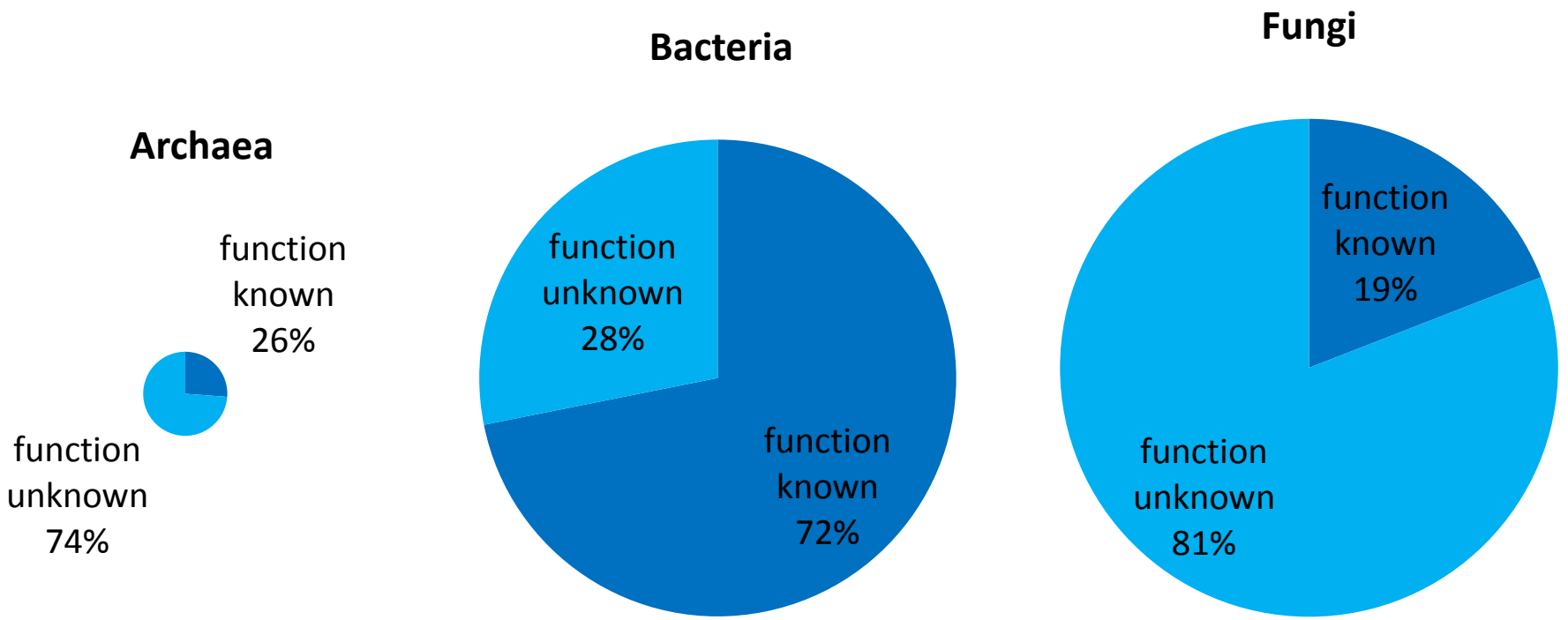
Community composition and activity of bacteria



Composition of total (DNA) and active (RNA) communities of fungi



Exploring microbial activity: assignment of mRNA taxonomy and function

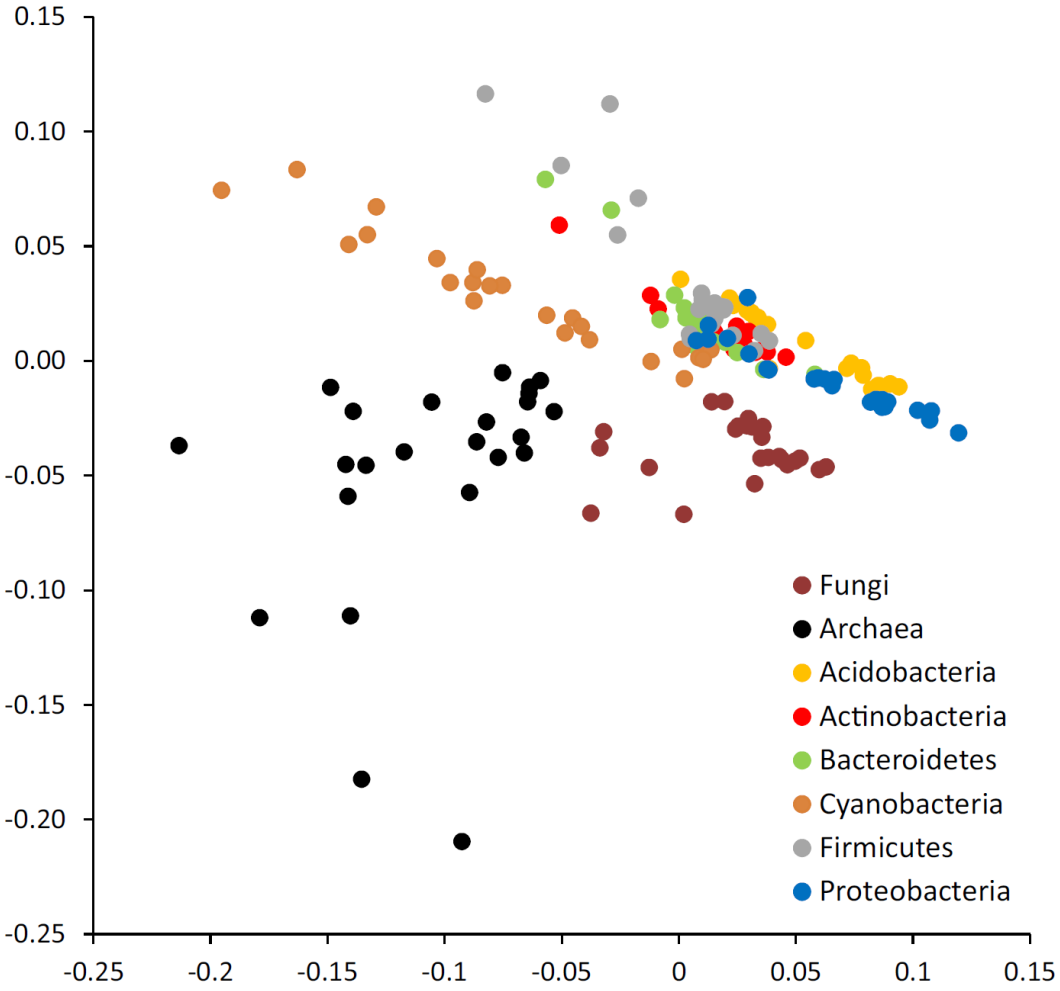


Functional annotation of predicted genes works well for bacteria but far less well for fungi and archaea. There, many hits are to „hypothetical proteins“.

The situation is even worse for nonmicrobial sequences (protozoa, invertebrates…)

Size of charts corresponds to numbers of transcripts.

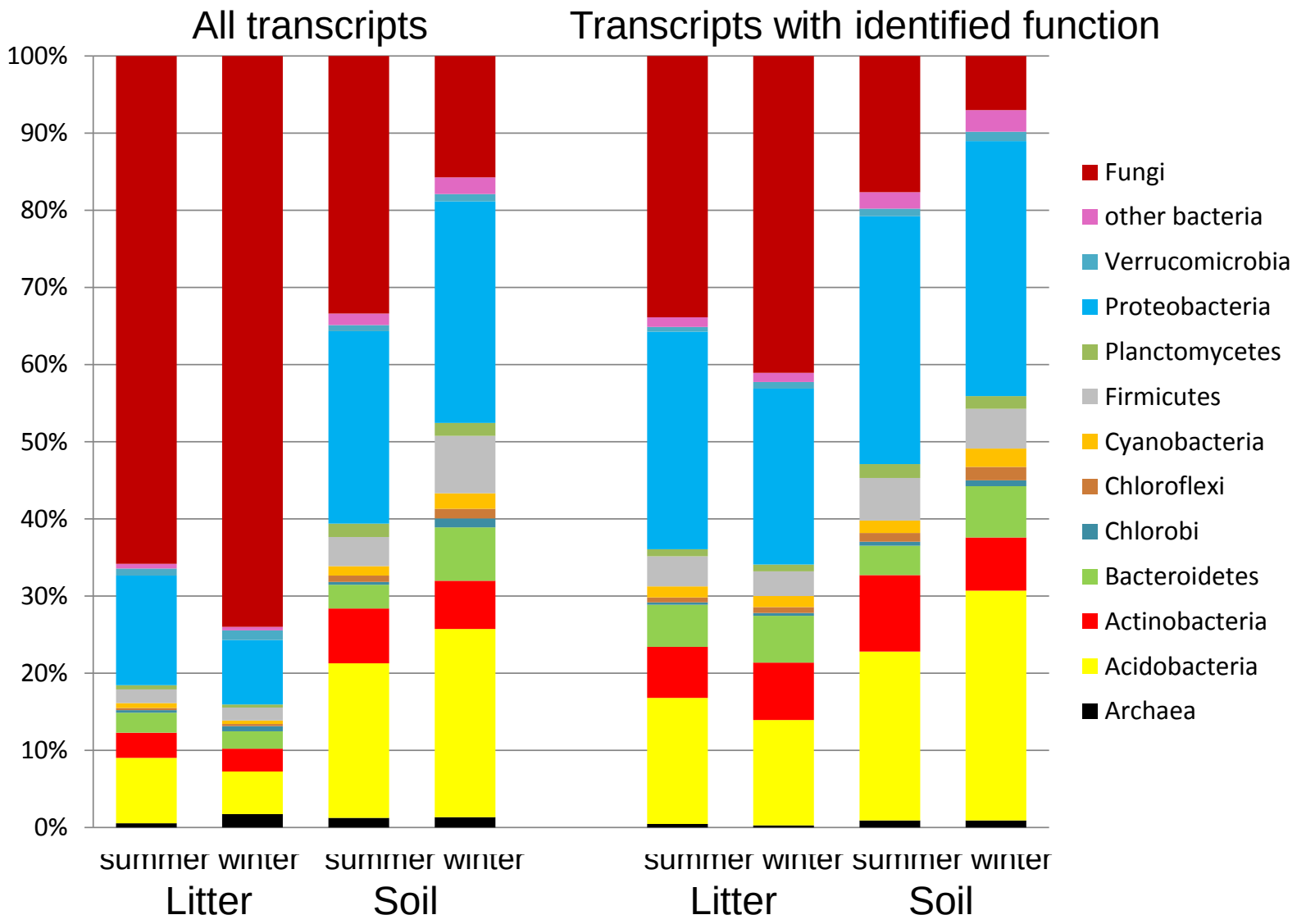
Exploring microbial activity: combining taxonomy and function



Bacterial (but not fungal) reads can be reliably identified on the level of phyla.

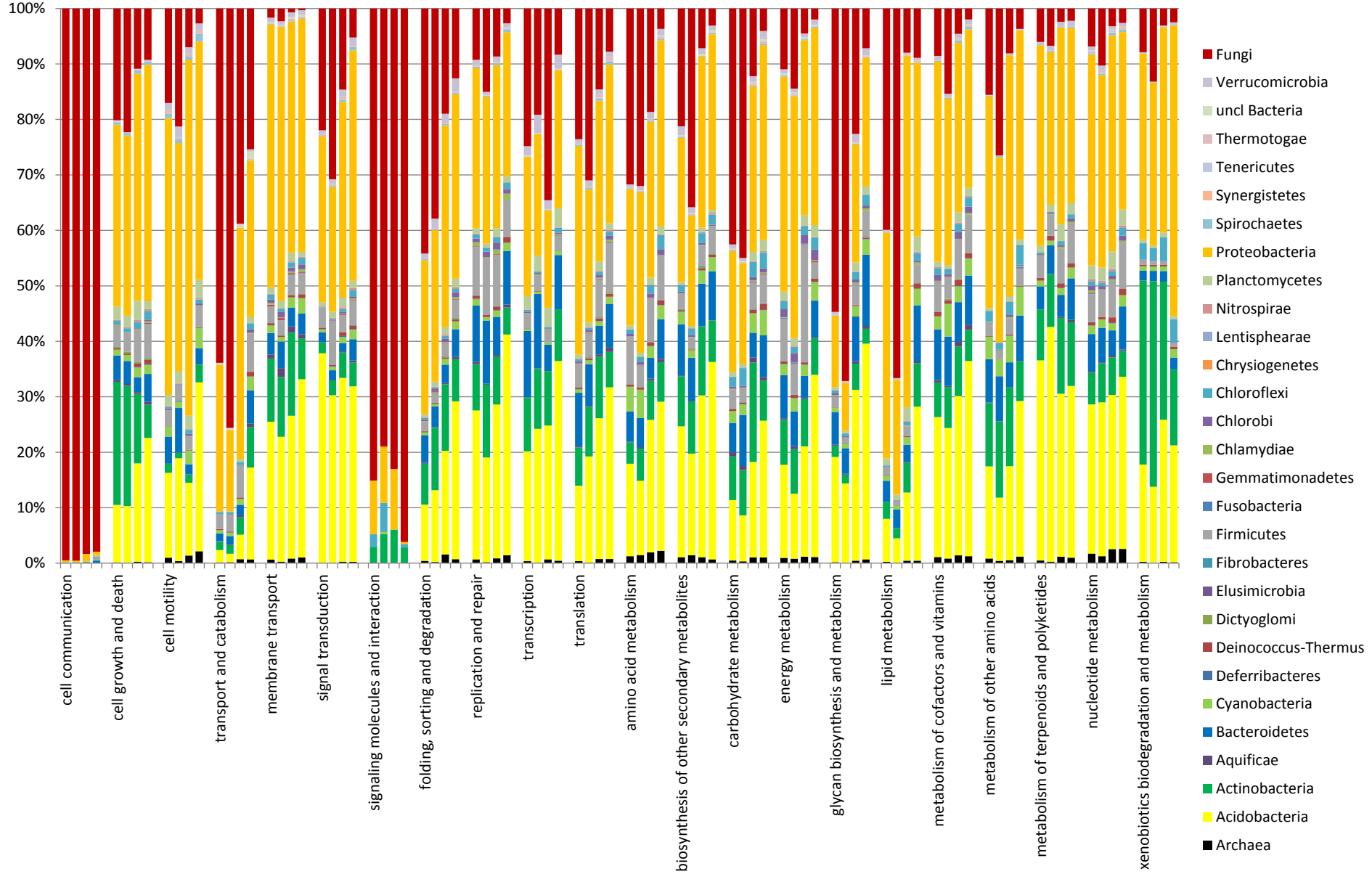
NMDS shows that profiles of functions of various microbial taxa differ

Seasonal contribution of microbial taxa to mRNA production



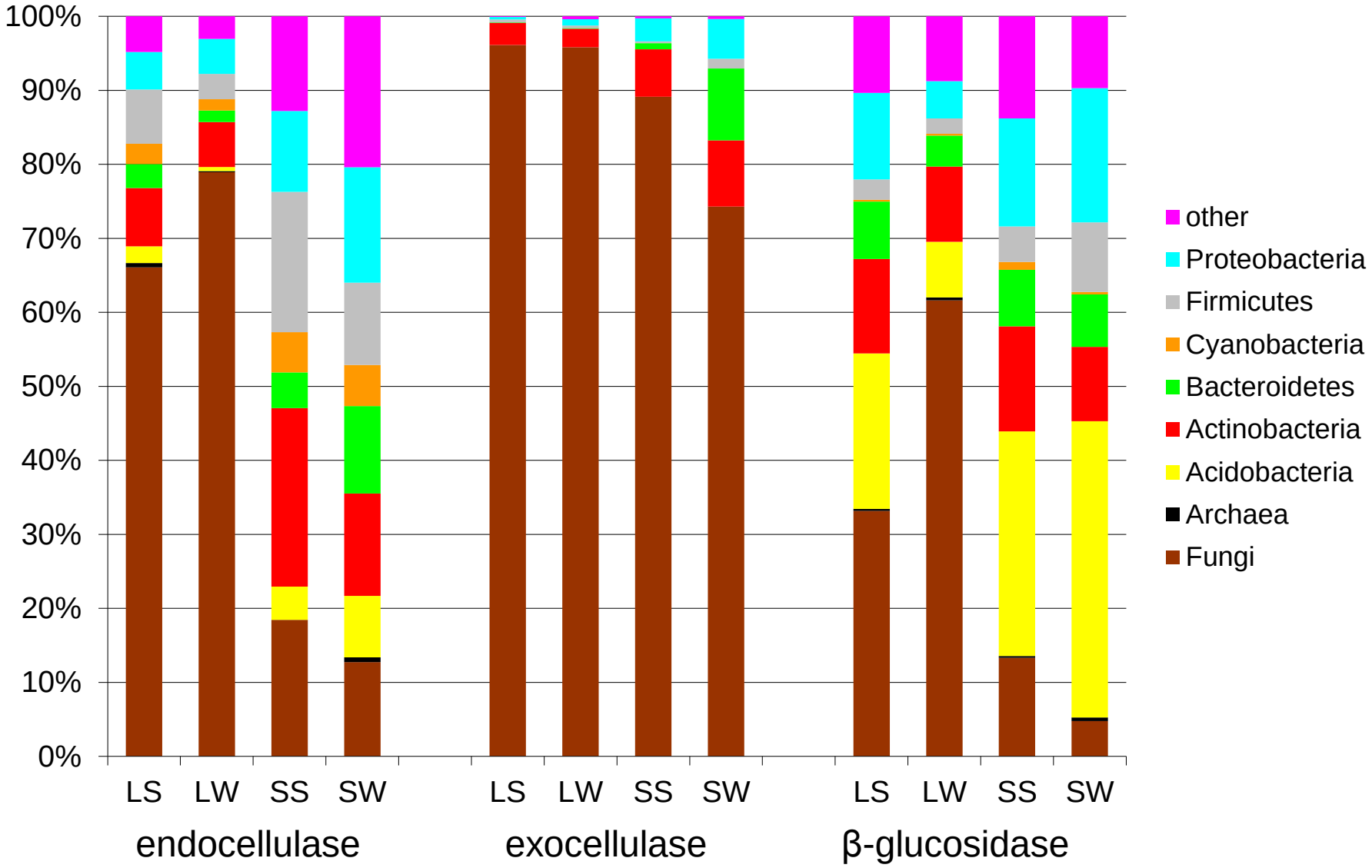
The share of fungal transcripts is higher in litter than in soil.
In soil, fungal share of fungal transcripts dramatically decreases in winter.

Involvement of microbial taxa in soil processes



Contributions to activity in litter/summer, litter/winter, soil/summer and soil/winter

Involvement of taxa in the decomposition of cellulose



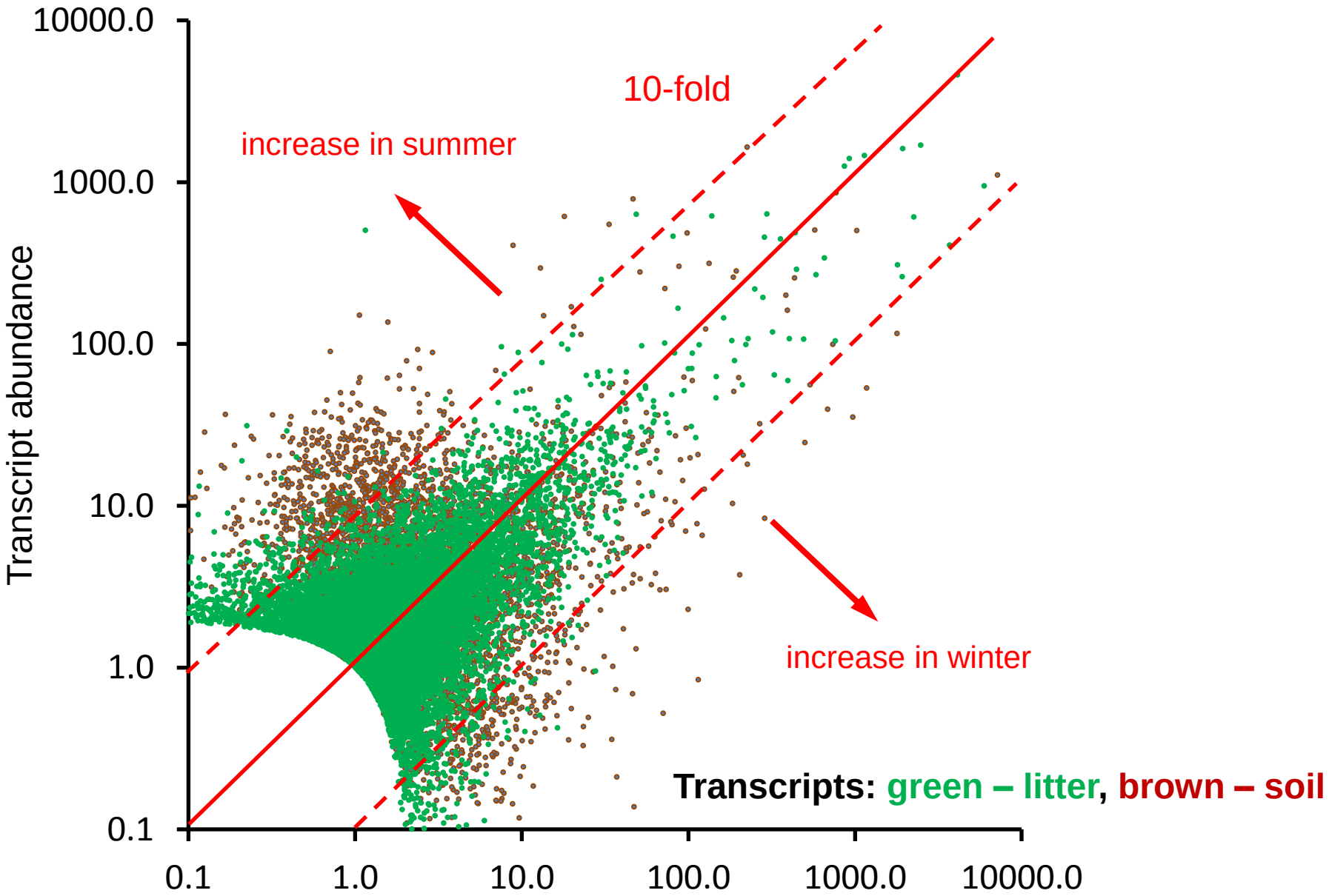
Fungi are dominant producers of cellulolytic enzymes in litter and important (but not dominant) producers in soil.

Functional biodiversity: high redundance of functions (starch and sucrose metabolism as an example)

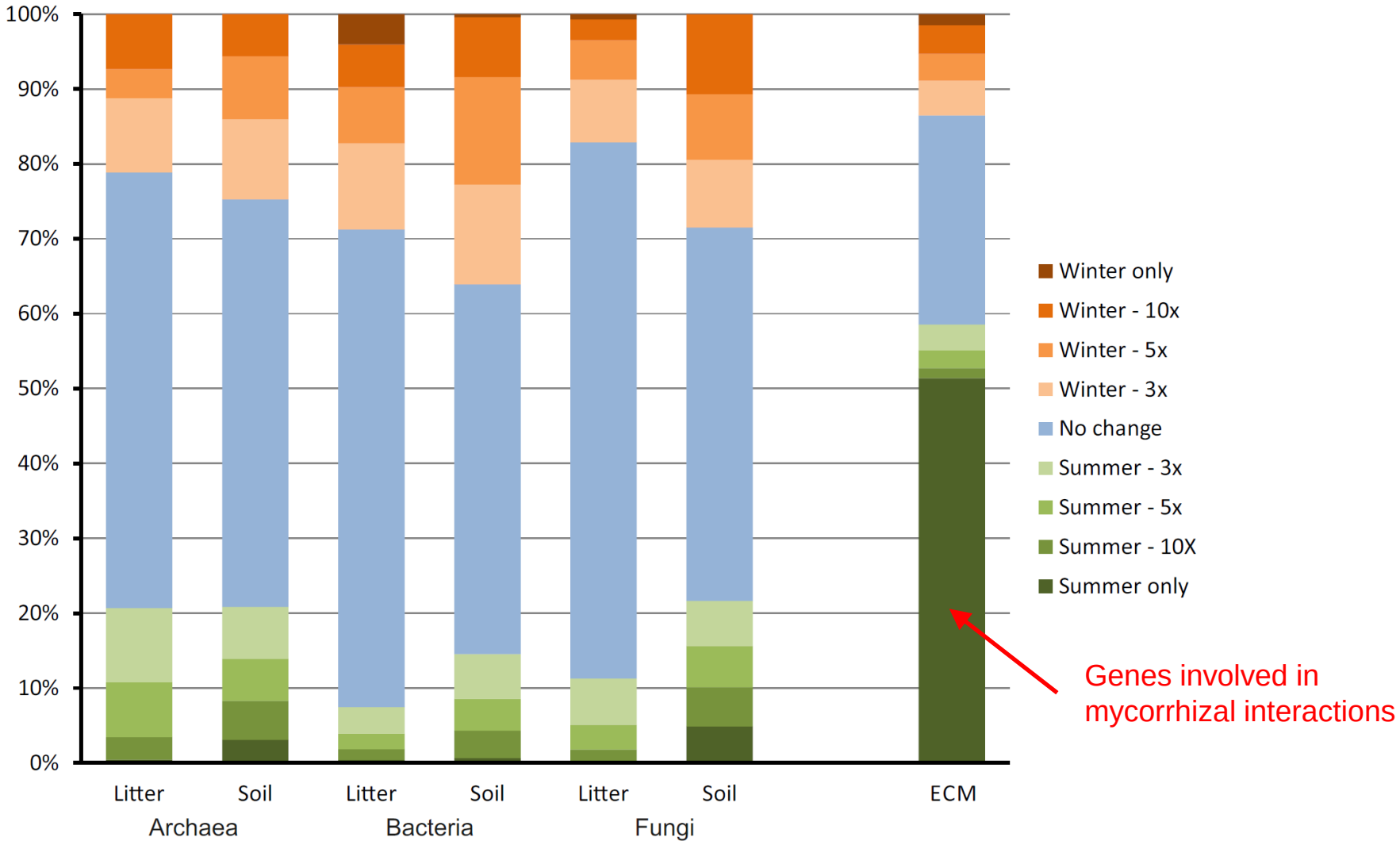
		fungi	bacteria
AGL	glycogen debranching enzyme	117	0
bcsA	cellulose synthase (UDP-forming)	0	145
E2.4.1.1	starch phosphorylase	191	1023
E2.4.1.20	cellobiose phosphorylase	0	11
E2.4.1.21	starch synthase	0	284
E2.4.1.34	1,3-beta-glucan synthase	463	0
E3.2.1.4	endoglucanase	136	204
glgB	1,4-alpha-glucan branching enzyme	132	666
glgC	glucose-1-phosphate adenylyltransferase	0	355
malQ	4-alpha-glucanotransferase	0	396
otsA	trehalose 6-phosphate synthase	213	511
rfbF	glucose-1-phosphate cytidylyltransferase	0	317
treS	maltose alpha-D-glucosyltransferase/ alpha-amylase	0	664

Numbers indicate transcript counts for each function.
(one species may produce one or more transcripts)

Seasonal changes in fungal expression are more intensive in soil



Seasonal changes in expression



29-51% of dominant transcripts show seasonal changes in relative abundance

Transcription of fungal genes involved in mycorrhizal interactions with plant roots is highly increased in summer

Metatranscriptomics – what we learned

- higher percentage of reads receive taxonomy annotation than functional annotation (due to proteins of hypothetical function known from sequenced genomes)

- functional annotation is more reliable than taxonomic annotation

- current resources are much more approppriate for annotation of bacterial genes (thousands of genomes included in annotation pipelines) than fungal ones (<200 published genomes, <50 included in annotation databases)

- many microbial transcripts/functions represent basic metabolism which can be of limited value for the exploration of environmental processes

Soil metatranscriptomics is currently technically feasible and can deliver interesting data

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