Continuous-cover forestry maintains soil fungal communities in Norway spruce dominated boreal forest

Sanghyun Kim, E. Petter Axelsson

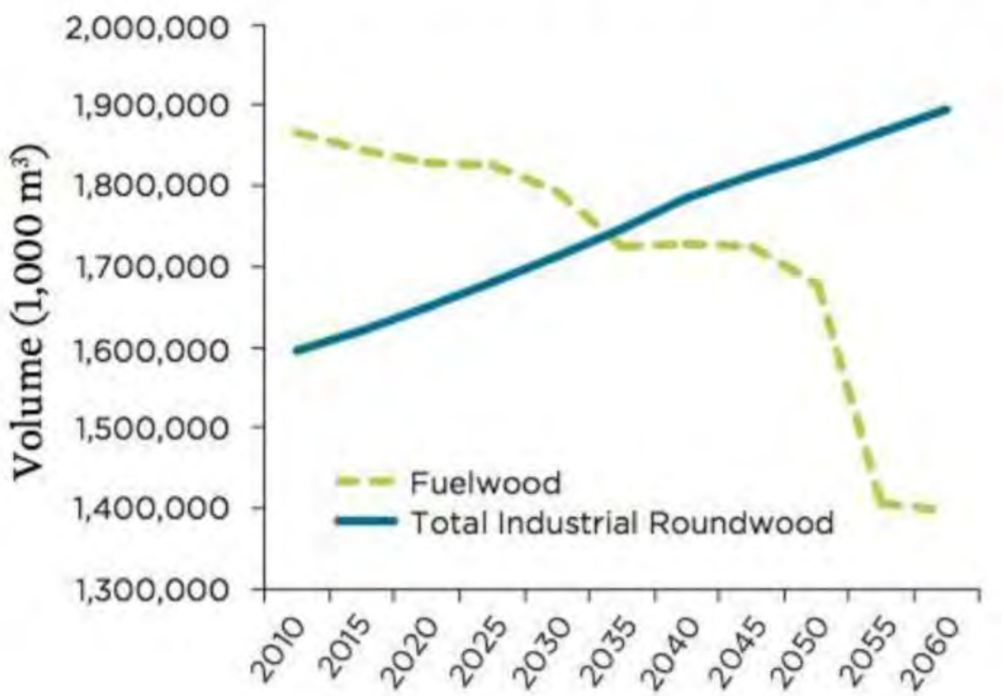
Miguel M. Girona, John K. Senior

Decreasing Forests, Increasing Demands

Proportion and distribution of global forest area by climatic domain, 2020

Source: Adapted from United Nations World map, 2020.

Figure 1.16 Projected global consumption of wood, 2010–2060



Source: Pipa Elias and Doug Boucher, Planting for the Future: How Demand for Wood Products Could Be Friendly to Tropical Forests *(Union of Concern Scientists, 2014).*

The world's forest area has declined by 4.7M ha (> Denmark) a year since 2010 (FAO, 2020)

Size of Denmark

Why Boreal forests are important?

Imported by Amber S. Stephens

- ❑ Boreal forest?

-Coniferous forests (pines, spruces, larches)
-High-latitude environment
-Freezing temperatures: 6-8months

- ❑ 11.5% of total land area.
- ❑ 1/3 global forest area
- ❑ 1/3 Carbon Stock
- ❑ Contains the most freshwater
- ❑ High ECM fungal diversity

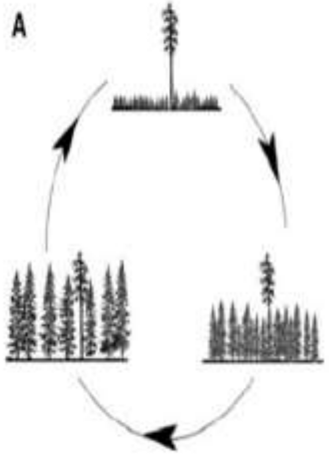
Nilsson et al. 2019
Pew environment group
Dunn et al., 2007

- ❑ 1/3 timber, 1/4 paper in global trade
- ❑ Intensively managed
- ❑ N limited

Gauthier et al. 2015

Even-aged Forestry

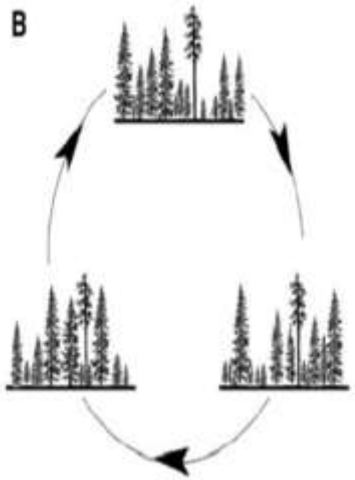
=Clear-fell forestry (CF)
Harvests all trees in a given area (50~60 years)



- ❑ Timber Production
- ❑ Artificial replantation

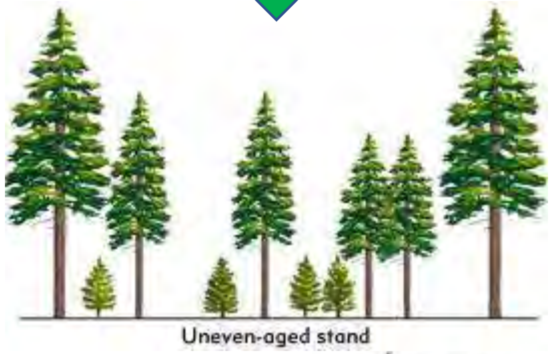
- ❑ Biodiversity, especially EMF
- ❑ Ecosystem function & services
- ❑ Less resilient
- ❑ Carbon storage

Uneven-aged Forestry



=Continuous-cover forestry (CCF)

Harvests 30-40%, every 15-20 years



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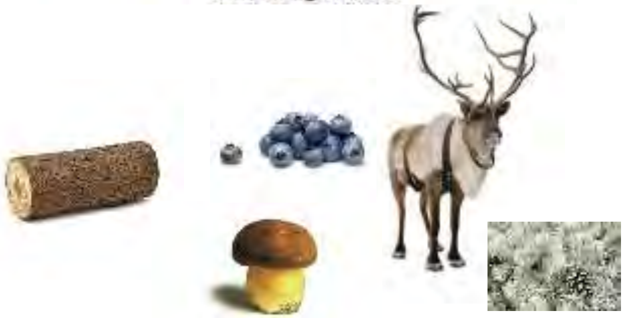
- ❑ Cost-efficient
- ❑ Diversity of plant & invertebrate
- ❑ Bilberry, mushroom production
- ❑ Resistance against wind



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- ❑ Timber production
- ❑ Harder to manage

<u>We don’t know about fungi!</u>

Fungi

R.Henrik Nilsson et al. 2019

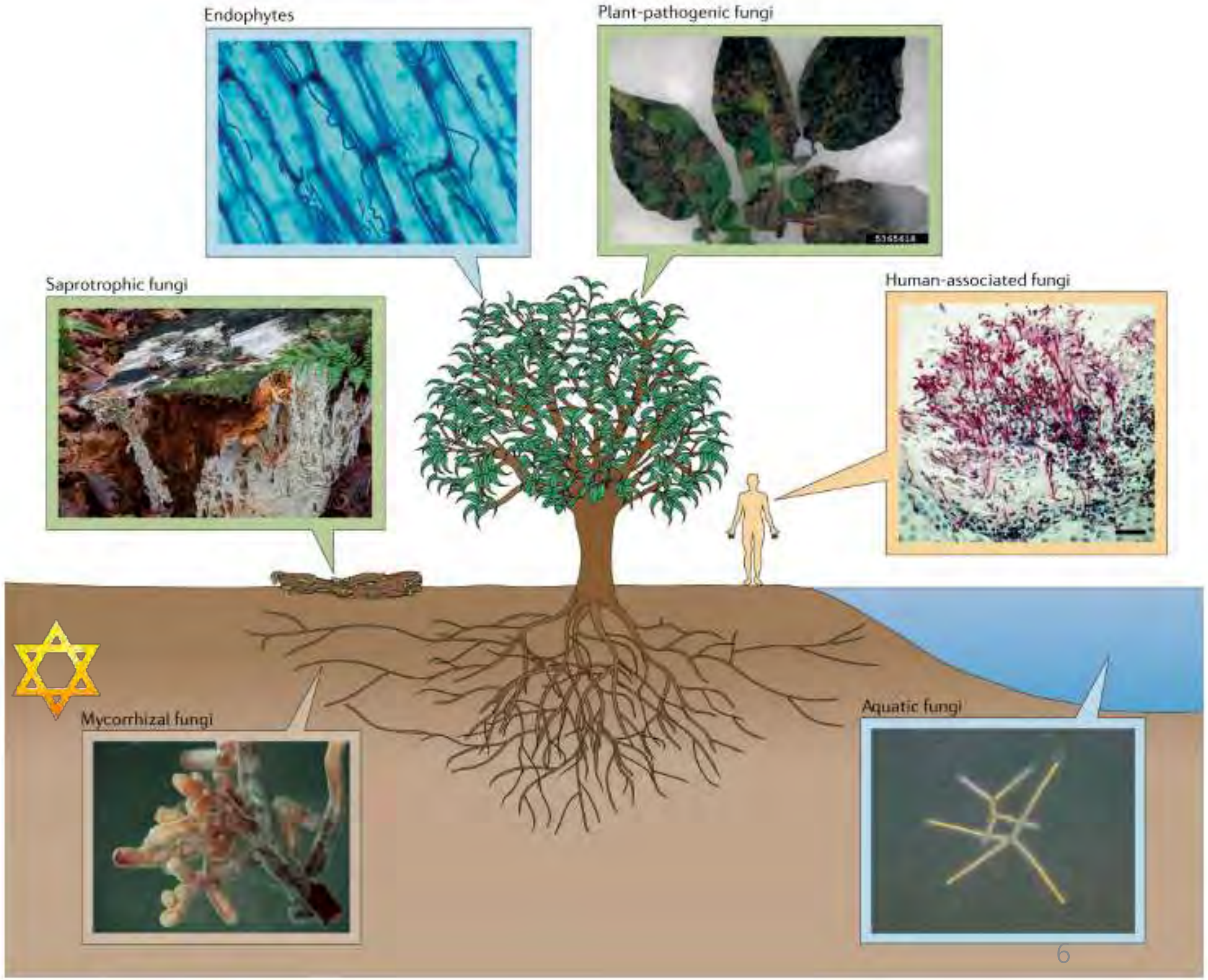
- **Saprotrophic fungi**
mediate the decomposition of organic matter

- **Mycorrhizal fungi**
associate with roots

- **Plant-pathogenic fungi**
decompose living leaves

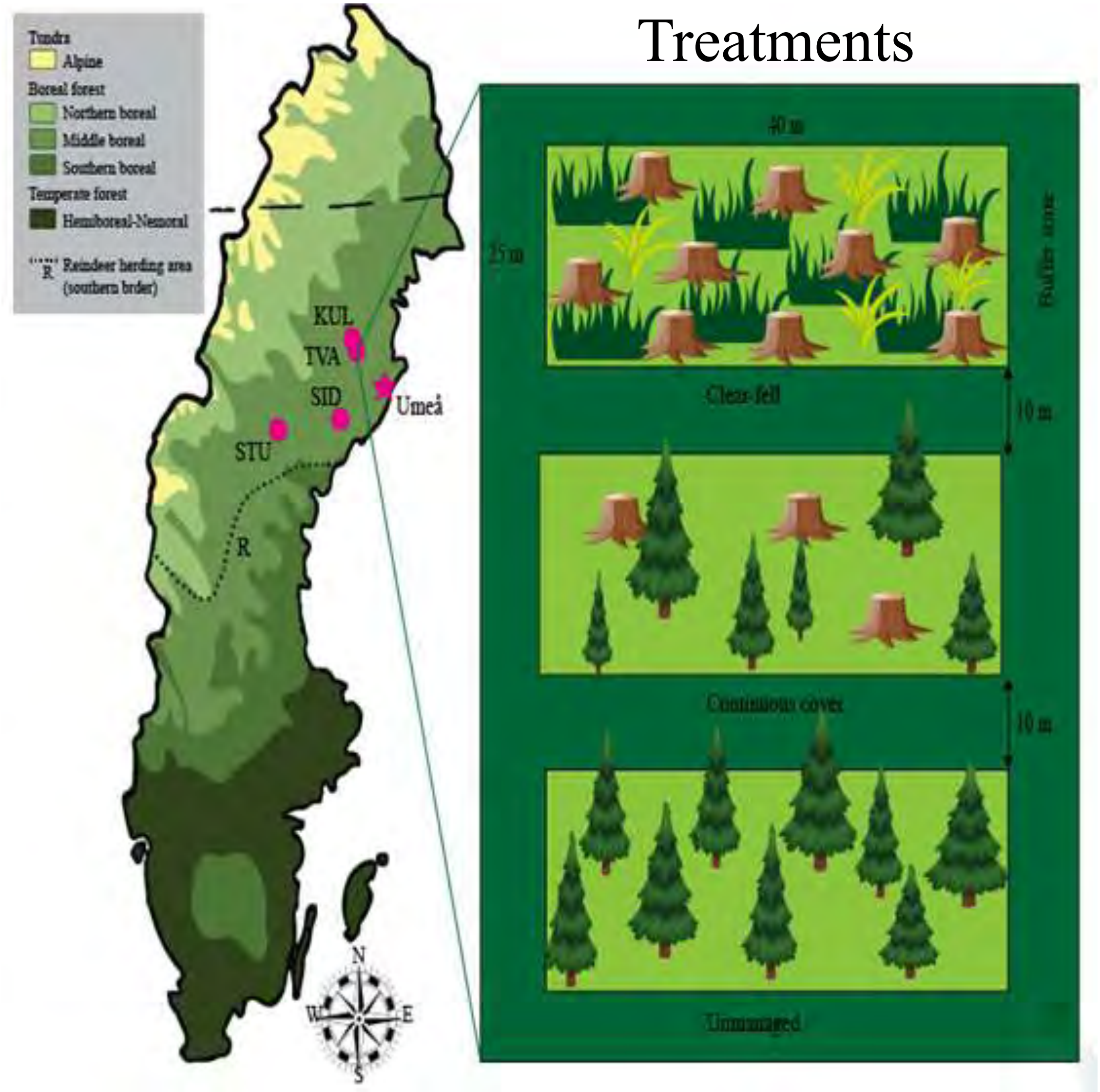
- **Endophytes**
live inside plants and rarely visible to the naked eye
Poorly understood nutritional strategies and taxonomic affiliations.

<u>Mycorrhizal fungi play a key role to provide nutrients to plants in boreal forest</u>



Study Sites

Treatments



- ❑ 4 sites established in 2012
- ❑ 3 treatments(Clear-fell, Continuous-cover, Unmanaged)
- ❑ Similar stands

Stand structure
Species composition
Age of forest
Norway spruce dominated
Field specificity

-Clear-fell condition: 100% cutting, replanted species, site preparation, stump retention

-Harvesting year:
2014-2015 for CF (100%) and CCF (30%) / UF (never logged).

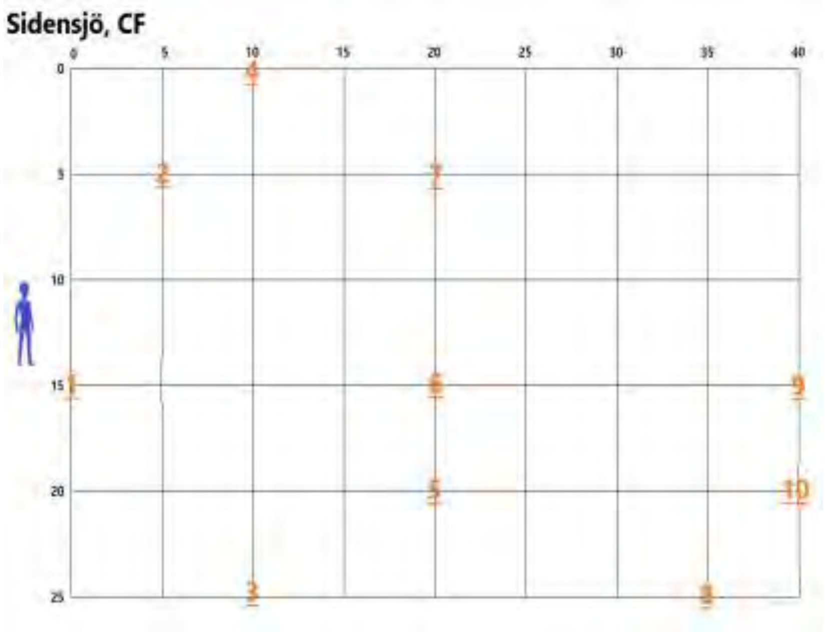
Illustrated by Phong Trans


Kulbäcksliden
CF VS CCF
Tvärålund
Sidensjö
Stugun

Soil Sampling

4 sites * 3 treatments * 10 replicates = 120 soil samples

Soils for DNA Sequencing
Soils for Soil Chemical Analyses (pH, C, N, C/N)



Sampling: Soil Chemicals

pH meter

the 5 ml of 0.01M CaCl2
solution + 10s vortex

**Furnace for soil organic m
atter**

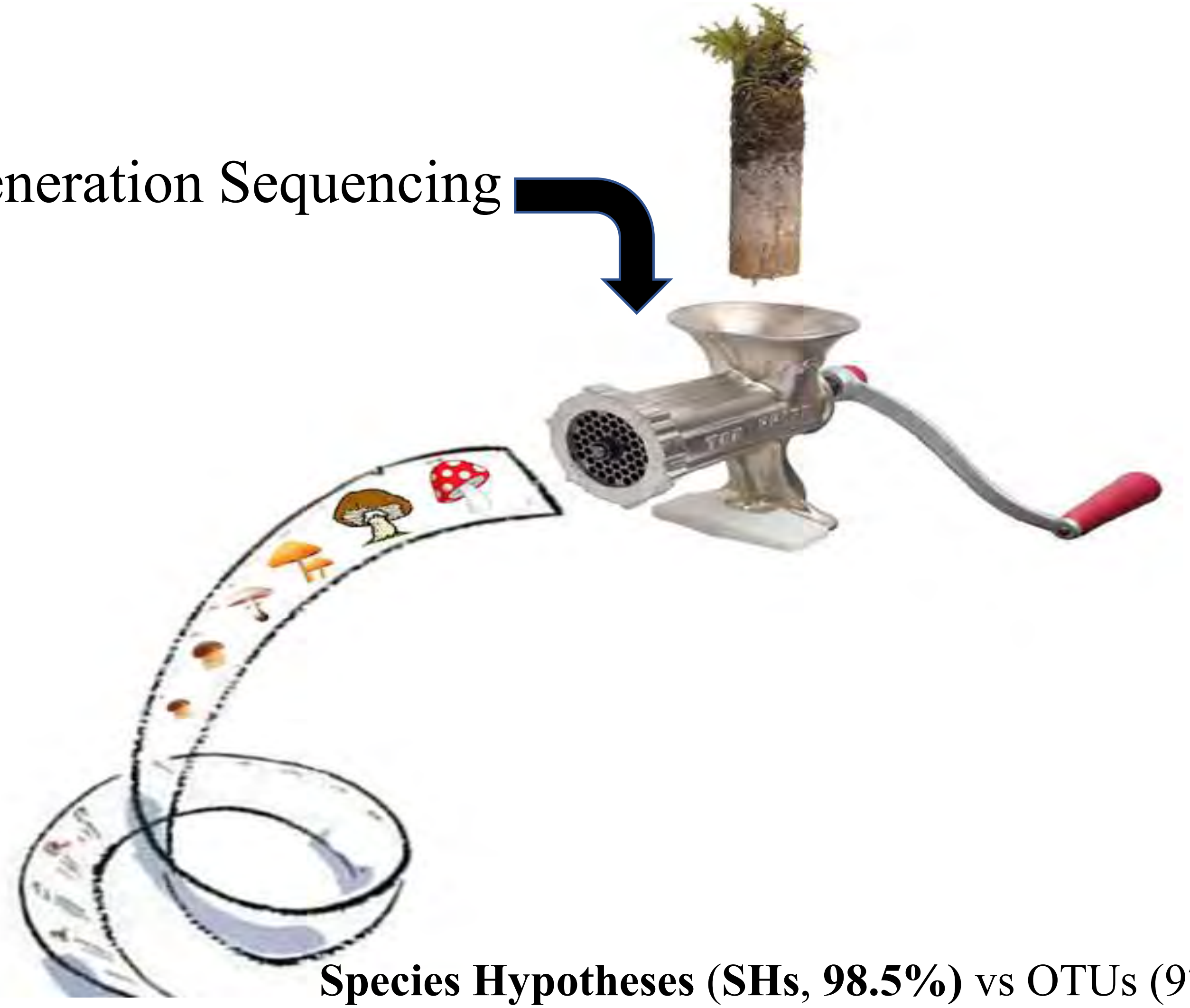
~550C for 6h at 550C for 6h
~70C storing

**Elemental Analyzer - Isotope Ratio Mass Spectrom
eter (EA-IRMS) for soil C, N and C/N**

Pre-treatment – freeze-dried & homogenization

Sampling: Soil fungi

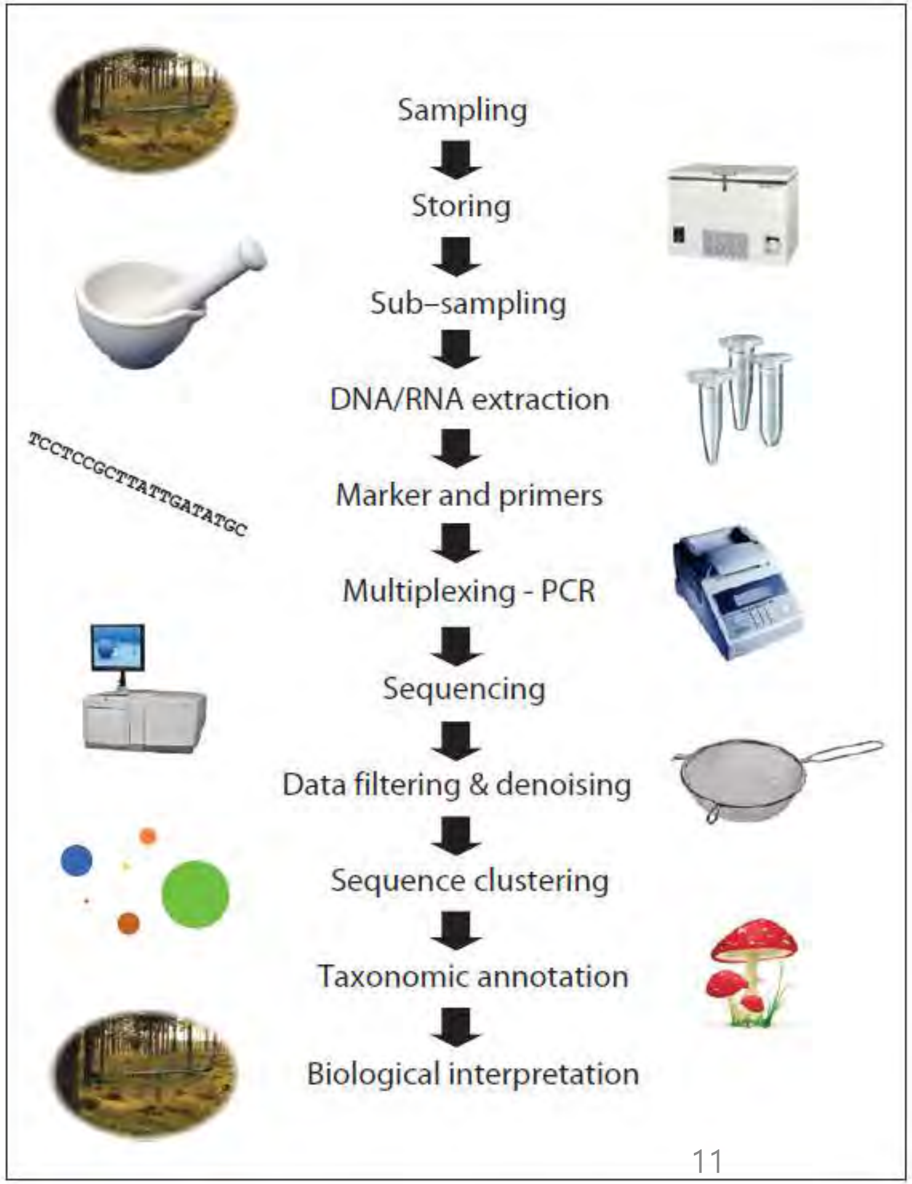
Next Generation Sequencing



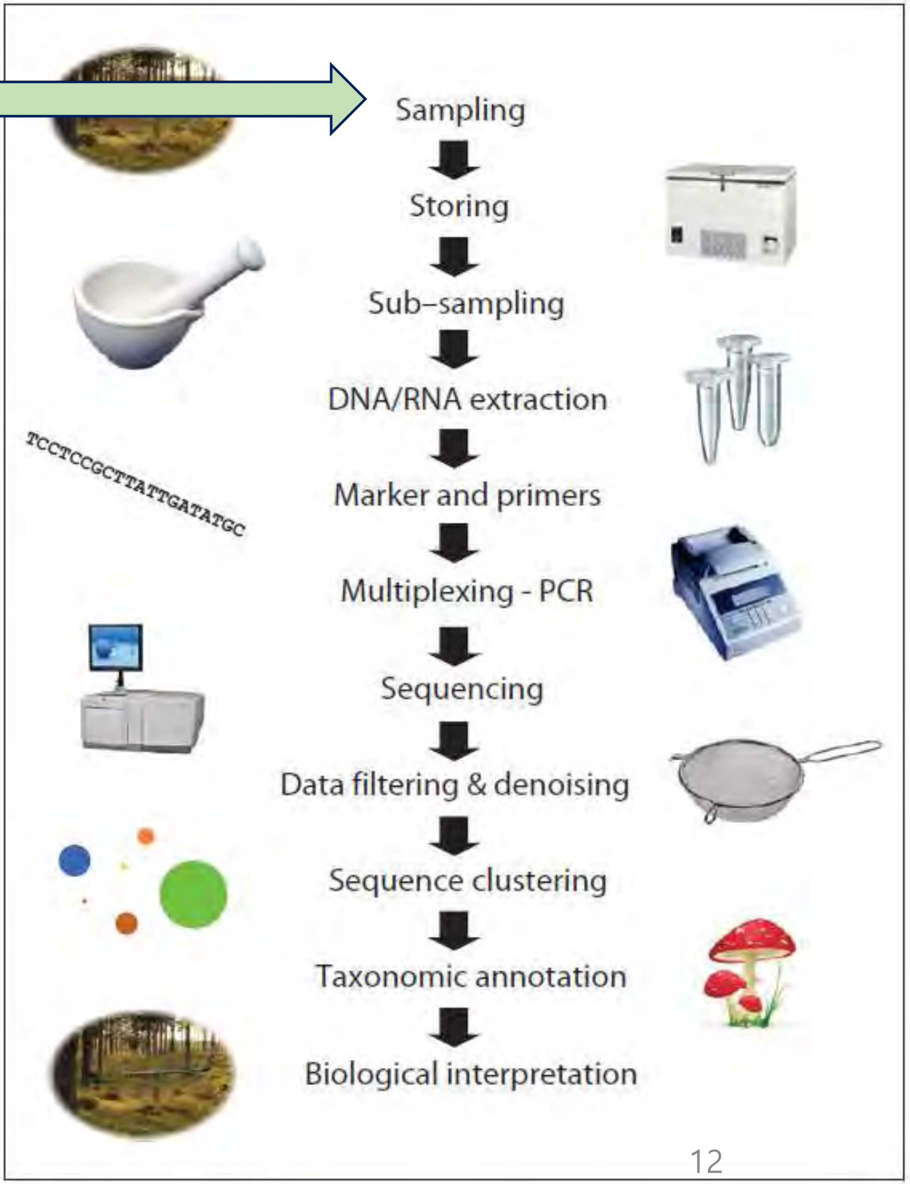
Imported by Björn Lindahl

Species Hypotheses (SHs, 98.5%) vs OTUs (97%) : any species-level group of individuals that share a given set of observed characters

Imported by Björn Lindahl



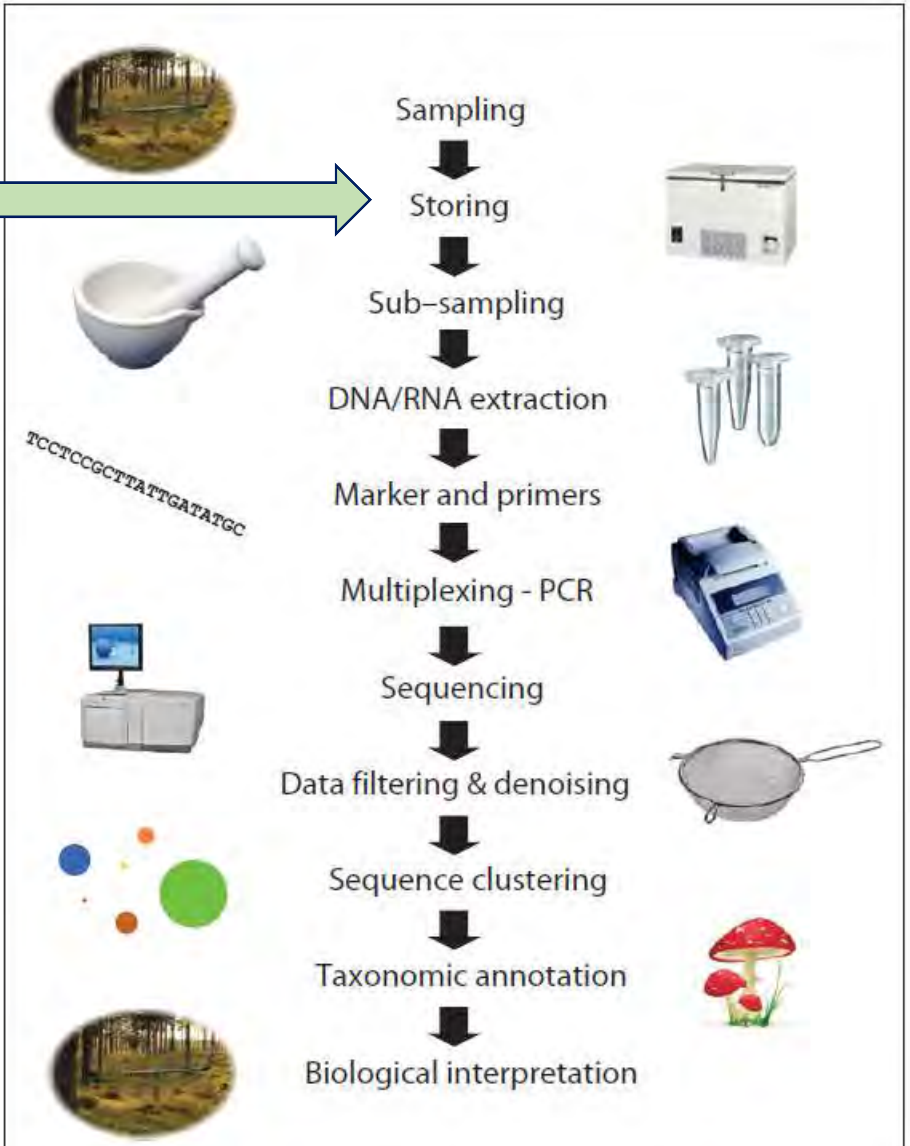
Sampling: Soil fungi



Imported by Björn Lindahl

Sampling: Soil fungi

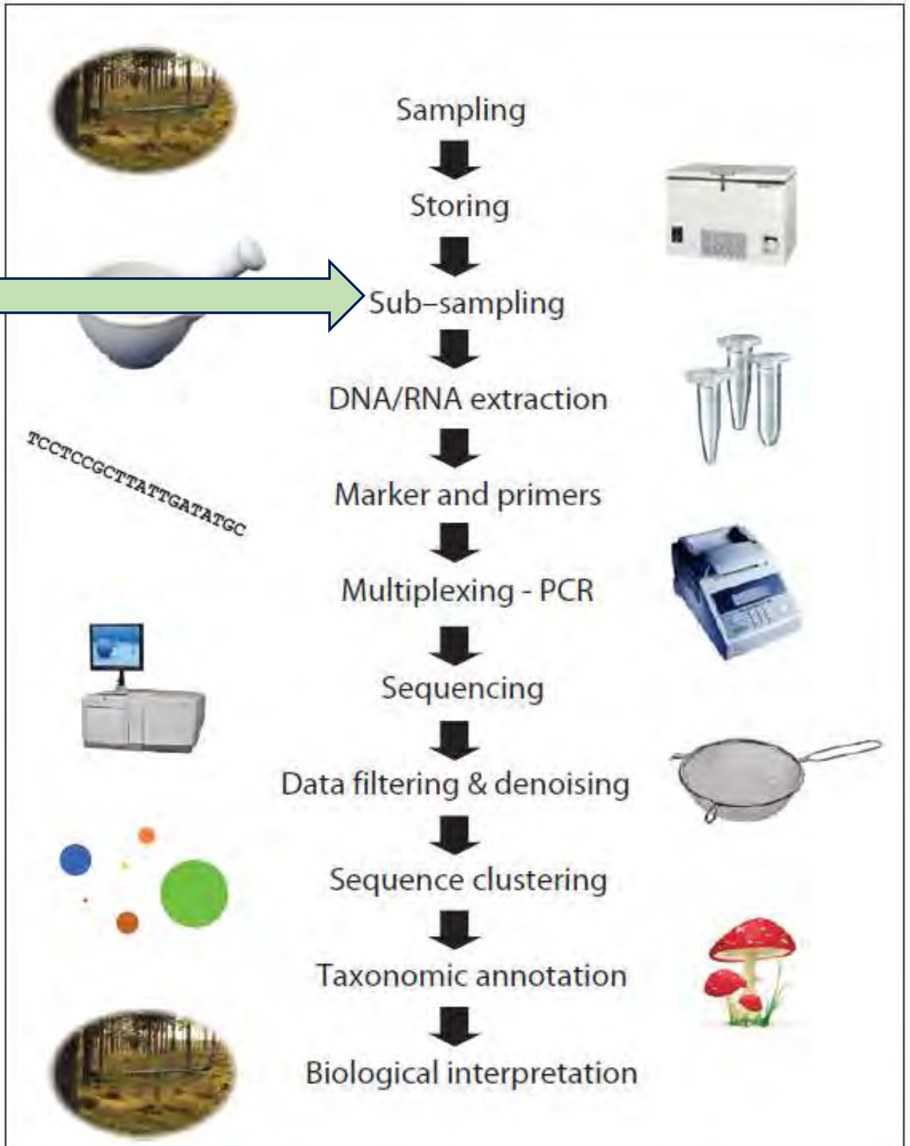
Imported by Thermo Fisher Scientific



Sampling: Soil fungi

Freeze-drier

Roller mill



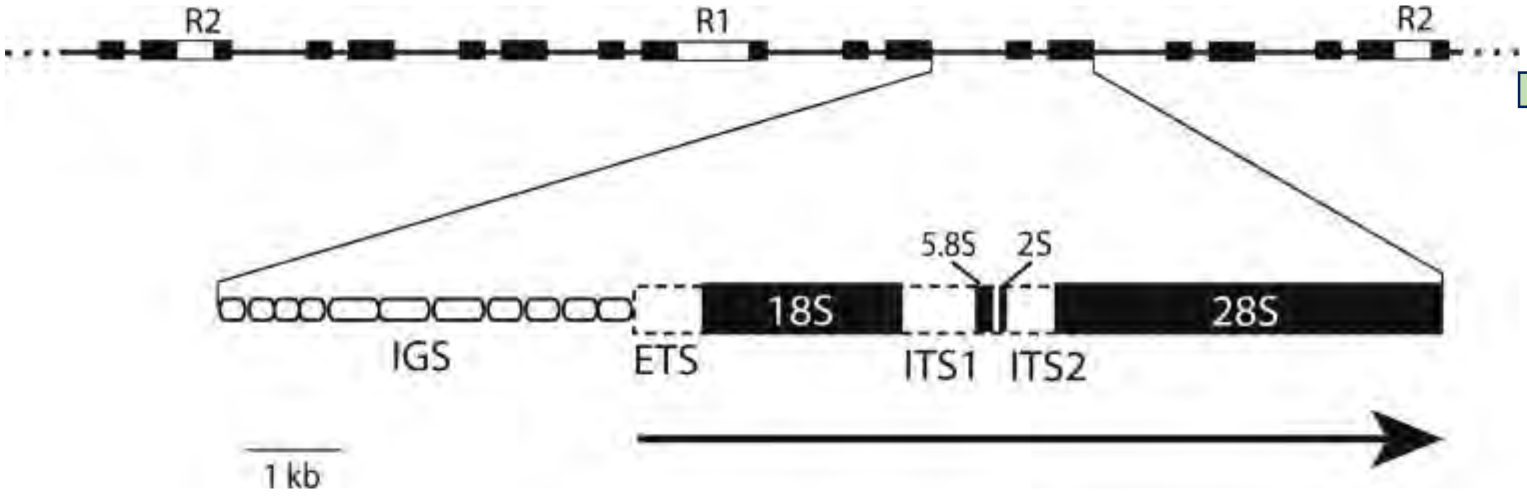
Sampling: Soil fungi

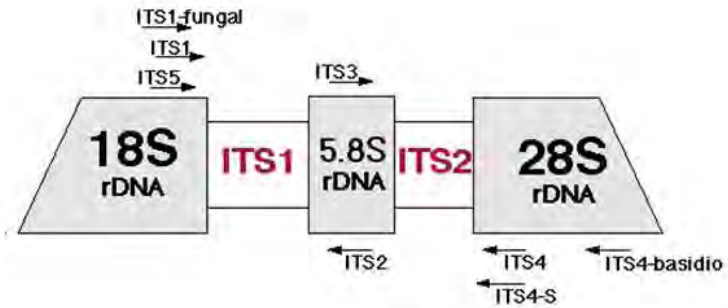
Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*

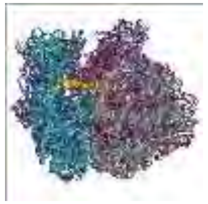
Conrad L. Schoch[a,1], Keith A. Seifert[b,1], Sabine Huhndorf[c], Vincent Robert[d], John L. Spouge[a], C. André Levesque[b], Wen Chen[b], and Fungal Barcoding Consortium[a,2]

[a]National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20892; [b]Biodiversity (Mycology and Microbiology), Agriculture and Agri-Food Canada, Ottawa, ON, Canada K1A 0C6; [c]Department of Botany, The Field Museum, Chicago, IL 60605; and [d]Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre (CBS-KNAW), 3508 AD, Utrecht, The Netherlands

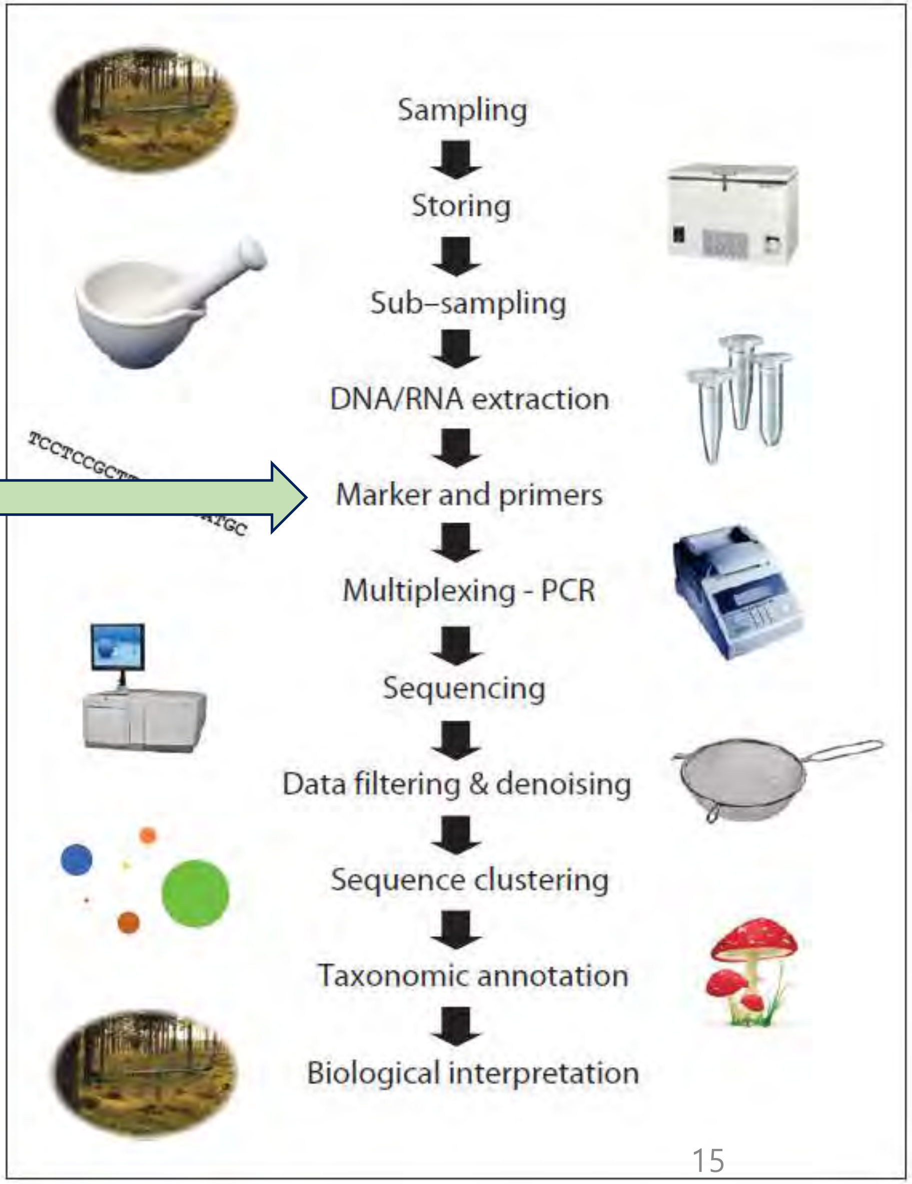
Edited* by Daniel H. Janzen, University of Pennsylvania, Philadelphia, PA, and approved February 24, 2012 (received for review October 18, 2011)







-Routinely amplified
-Easy to detect
-A high degree of variation



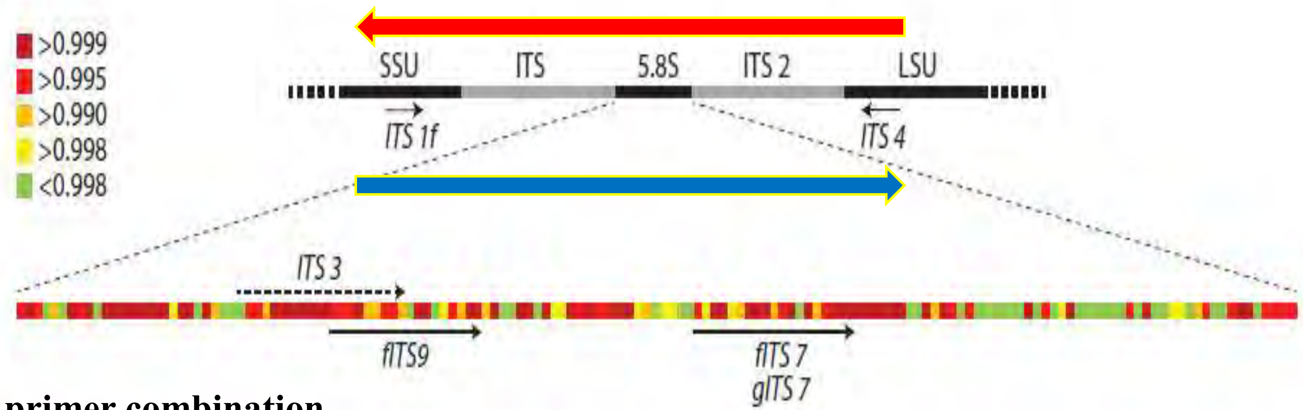
Sampling: Soil fungi


RESEARCH ARTICLE

New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities

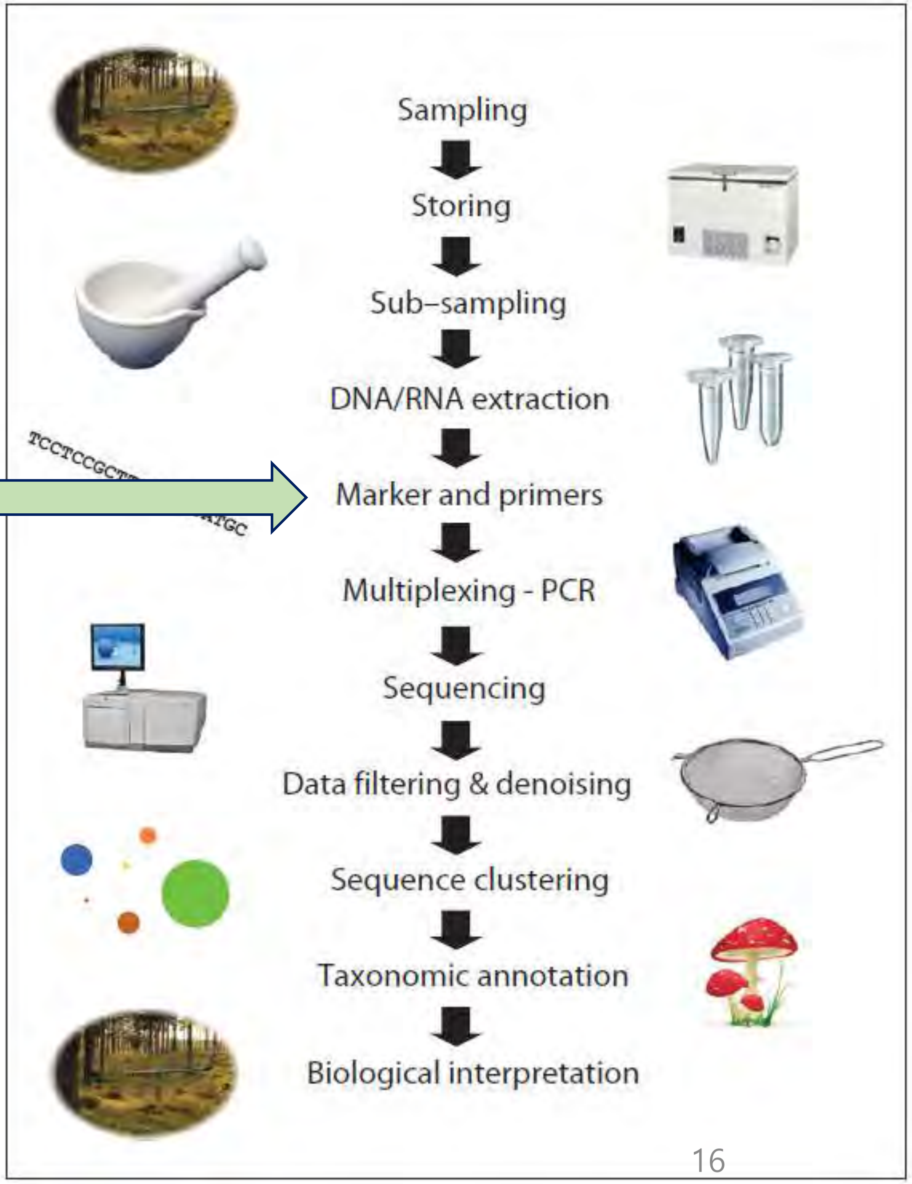
Katarina Ihrmark, Inga T.M. Bödeker, Karelyn Cruz-Martinez, Hanna Friberg, Ariana Kubartova, Jessica Schenck, Ylva Strid, Jan Stenlid, Mikael Brandström-Durling, Karina E. Clemmensen & Björn D. Lindahl

Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden



ITS1f/ITS4 primer combination
+more genetic info
-strong bias against species with longer amplicons

New primer combination (gITS7 + ITS4)
+shorter amplicons and better preserve the quantitative composition of the template
+yielded more diverse amplicon communities
+higher PCR efficiencies
-less genetic info



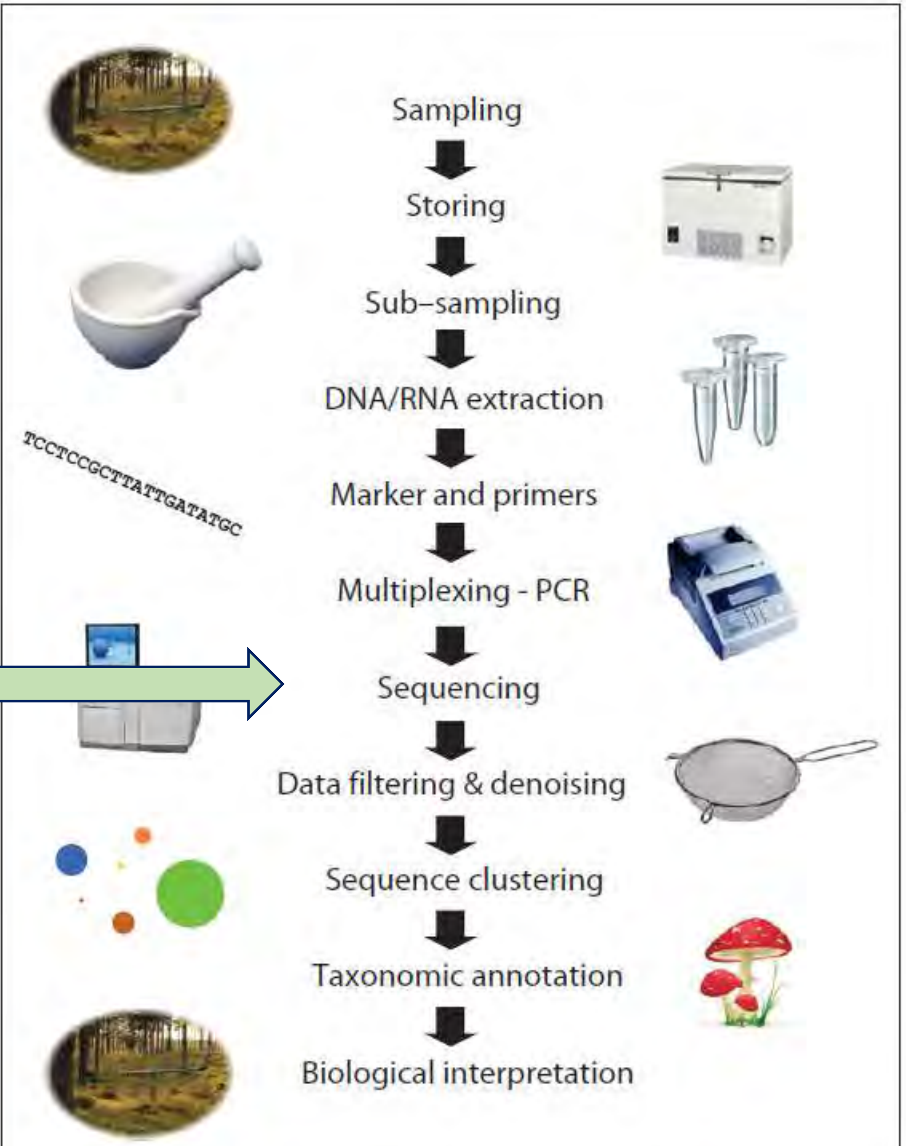
Sampling: Soil fungi

Illumina
(MiSeq)

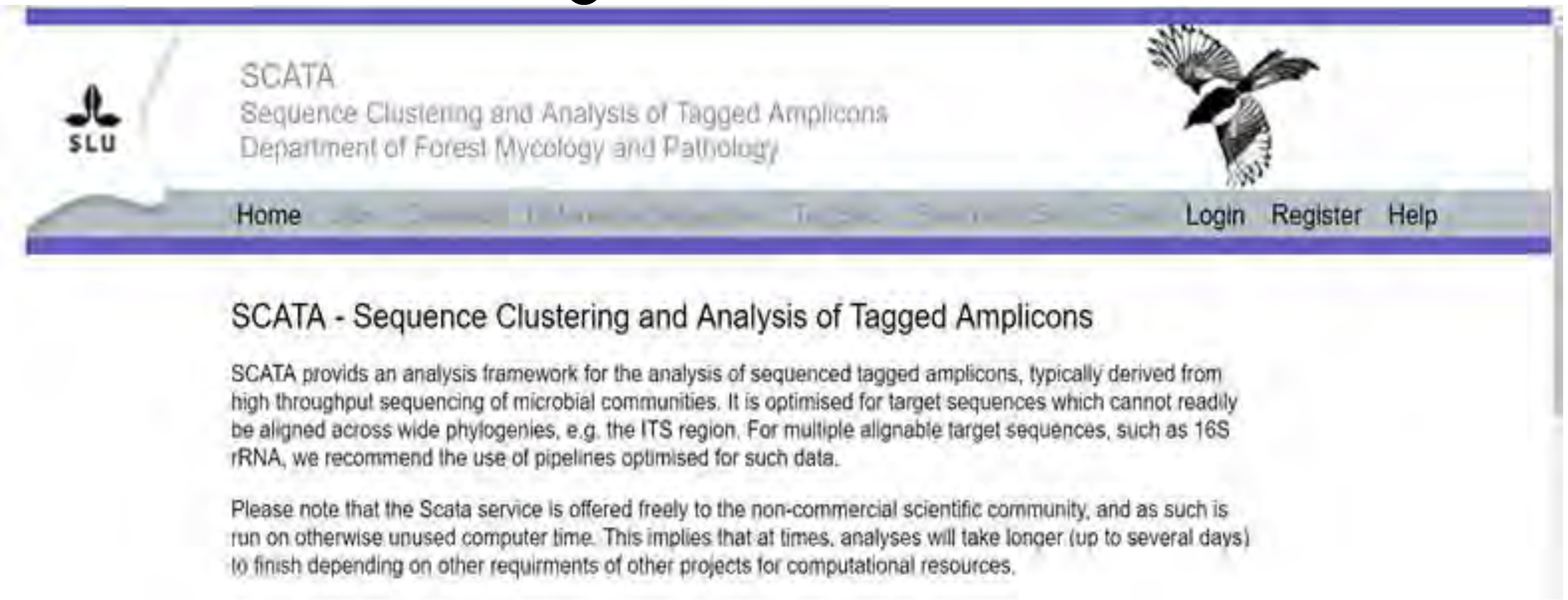
- 25 million sequencing reads
- 2 × 300 bp read lengths

Pacific Biosciences SMRT sequencing
(SEQUEL)

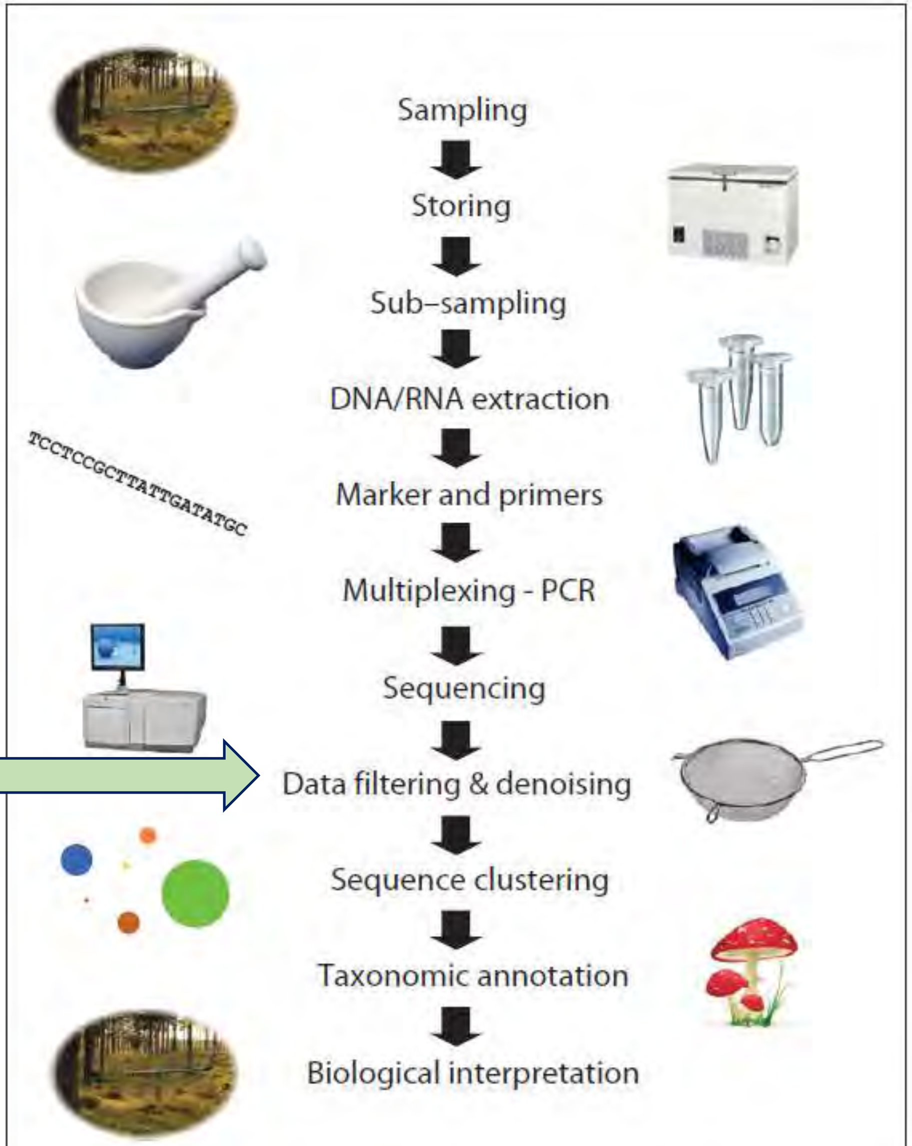
-500 000 sequencing reads
-20 000 bp read lengths



Sampling: Soil fungi



- Quality control
- Remove data with mean quality score below 20 or bases of equality lower than 3
- Screen gITS7 primer and identity tags
- Comparing sequences for similarity from USEARCH (match length – min. 85%)



Sampling: Fruiting body

D5 (CF, SID)

T17 (CCF, TVA)

S22 (UF, STU)

Sampling: Fruiting body

-Mushroom / Moss Identification
>Visual & Microscopic Inspection
>15 Mushroom illustrated books
>5 Fungal DB
>Herbarium of UmU

-Dry mushrooms to measure biomass
(50C, 72h)

= (6 events * ID 4h) + Web-ID + Drying + Cleaning + Measuring + Typing…

Statistics

H1. Soil fungal communities in CCF may be more similar to UF than CF

H2. Variation in soil fungal communities is related to soil chemical properties

Software / Analysis	Univariate	Multivariate	Visualization
R studio	- Mixed effects model - Tukey's HSD pair-wise - Correlation		-Anova table -Scatter plots
PRIMER		-PERMANAOVA -SIMPER -Diversity indices -DistLM	-SIMPER table -Canonical analyses of principal coordinates -DistLM table

Results of Soil fungi

Sequencing output

-549,011 reads -> 355,867 reads
(65% after QC)

-1,016 reads per sample (SD: 142)

-<u>**2157** Species Hypothese</u>

- 5 most common species (9% of total SHs)
- ***Penicillium austroafricanum***
- *Hyaloscyphaceae* (Family)
- ***Oidiodendron pilicola***
- *Luellia* (Genus)
- ***Solicoccozyma terricola***

Results of Fruiting body

- 1825 mushrooms (Identified 93.8%, 325g)
- 120 total species (excluding unidentified fungi)
- 43 Mycorrhizal fungi (145g)
- 3 most common species

Microphale perforans

UF CCF

Mycena epipterygia

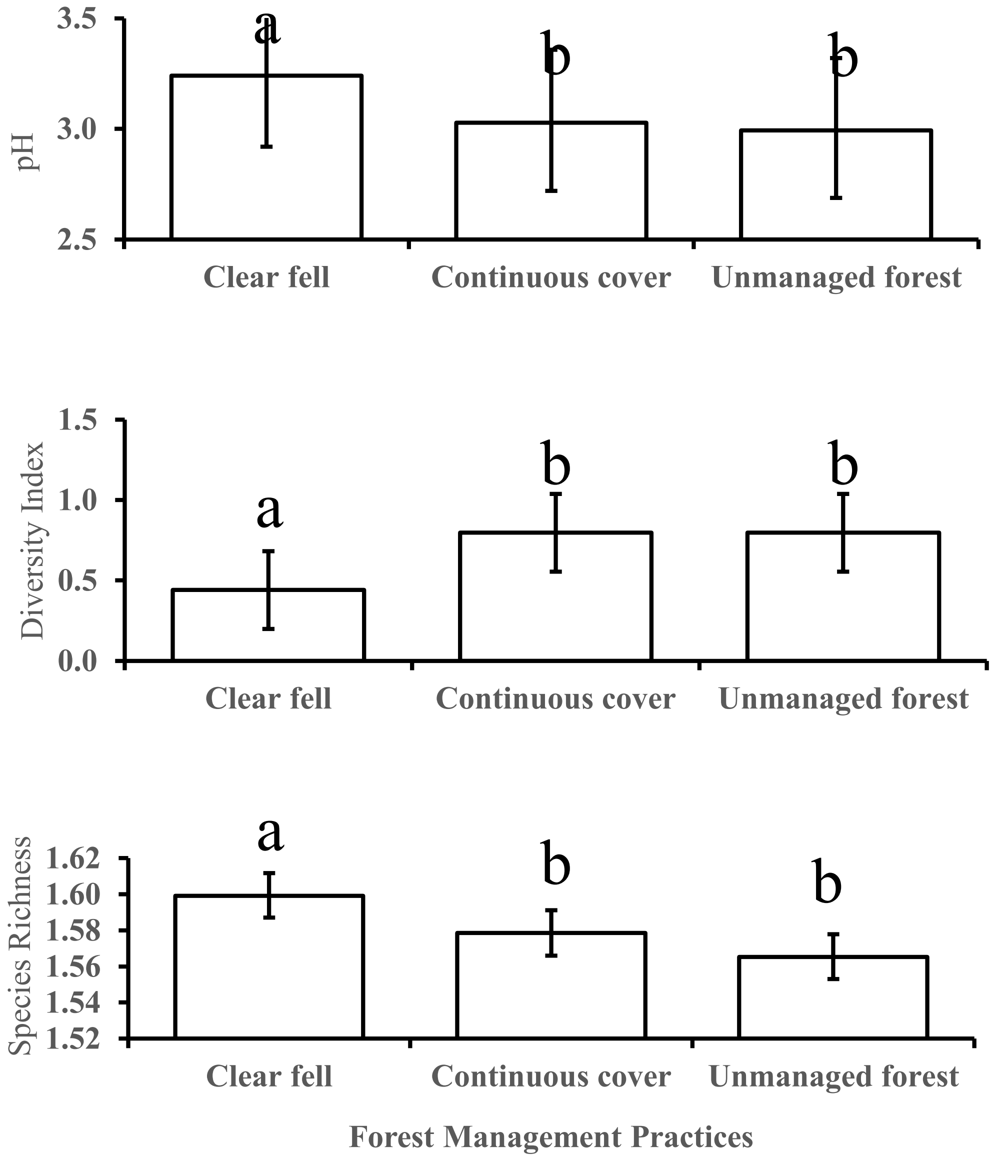
UF CCF

Cortinarius sp.

CF

Mixed Effects Models

1) Impacts of forest management

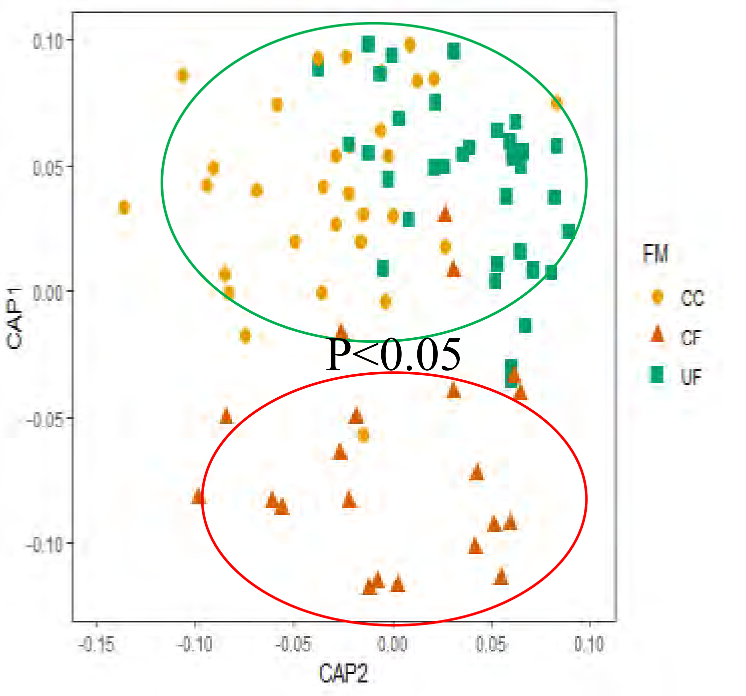


Dataset	Variable	$F_{(df)}$	P-value	Clear fell	Continuous cover	Unmanaged
Soil chemistry	pH***	$12.9_{2,114}$	<0.001	3.2^{a}	3.0^{b}	3.0^{b}
	Organic matter (% soil dry weight)	$2.2_{2,112}$	0.1119	14.9	13.5	17.5
	Carbon* (% soil dry weight)	$2.1_{2,112}$	0.0131	8.3^{a}	7.9^{a}	10.0^{a}
	Nitrogen (% soil dry weight)	$2.9_{2,112}$	0.0617	0.3	0.2	0.3
	Soil C to N ratio*** (%)	$8.4_{2,112}$	<0.001	29.7^{b}	34.1^{a}	32.3^{a}
Fruiting bodies	Dry weight (g)	$3.1_{2,58}$	0.0513	1.0	0.3	0.6
	Abundance**	$5.0_{2,58}$	0.0097	1.9^{b}	2.0^{ab}	2.7^{a}
	Shannon's Diversity Index***	$10.8_{2,113}$	<0.001	0.4^{b}	0.8^{a}	0.8^{a}
	Species richness***	$12.0_{2,113}$	<0.001	2.0^{b}	3.2^{a}	3.3^{a}
Soil fungi	Shannon's	$7.8_{2,115}$	<0.001	5.0^{a}	4.9^{b}	4.8^{b}
	Species richness***	$8.2_{2,115}$	<0.001	137.7^{a}	130.5^{b}	126.6^{b}

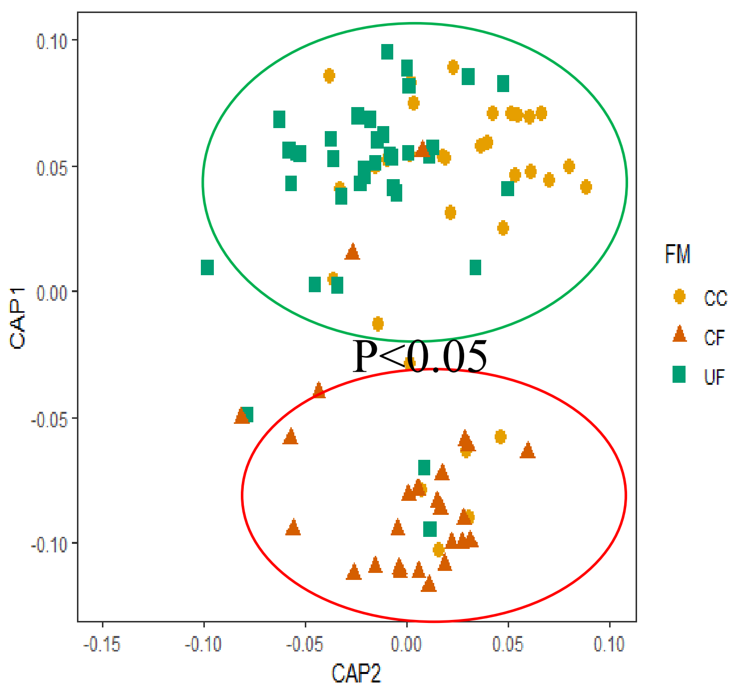
PERMANOVA Models

- Continuous cover
- Clear-fell
- Unmanaged

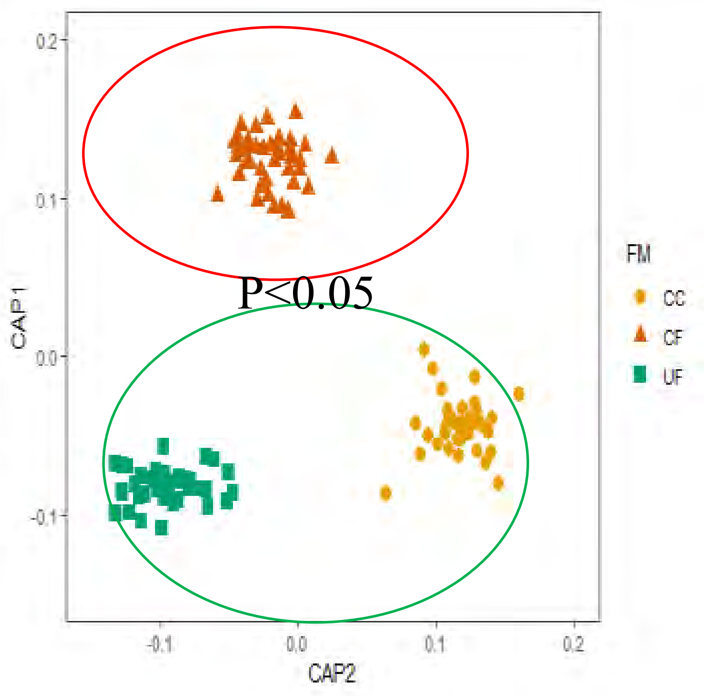
2) The main effect and pair-wise PERMANOVA models – > Canonical Analysis of Principal Coordinates



Fruiting Body Dry Weight



Fruiting Body Abundance



Soil Fungi

Similarity Percentage (SIMPER) Analysis

Species	Group CCF Av.Abund	Group CF Av.Abund	Av.Diss	Diss/SD	Contrib %	Cum.%
Microphale perforance	35.51	1.41	17.75	1.05	18.04	18.04
Cortinarius sp.	1.69	7.06	4.16	0.37	4.23	22.27
Mycena sp.	3.61	3.40	3.34	0.35	3.39	25.66
Lacrarius fulvissimus	0.00	5.97	2.98	0.28	3.03	28.69
Marasmius alliaceus	5.62	0.00	2.81	0.33	2.86	31.54
Mycena galopus	4.98	0.74	2.77	0.37	2.82	34.36
Hygrophoropsis aurantiaca	0.00	5.28	2.64	0.37	2.68	37.04
Collybia peronata	0.95	4.24	2.53	0.28	2.57	39.62
Mycena fagetorum	2.93	1.63	2.23	0.24	2.26	41.88
Mycena epipterygia	3.33	1.28	2.22	0.35	2.26	44.14
Thelephora terrestris	0.00	4.41	2.21	0.28	2.24	46.38
Trichaptum abietinum	1.95	2.10	1.97	0.23	2.00	48.39
Gloeophyllum sepiarium	0.00	3.92	1.96	0.27	1.99	50.38

Fruiting body Abundance

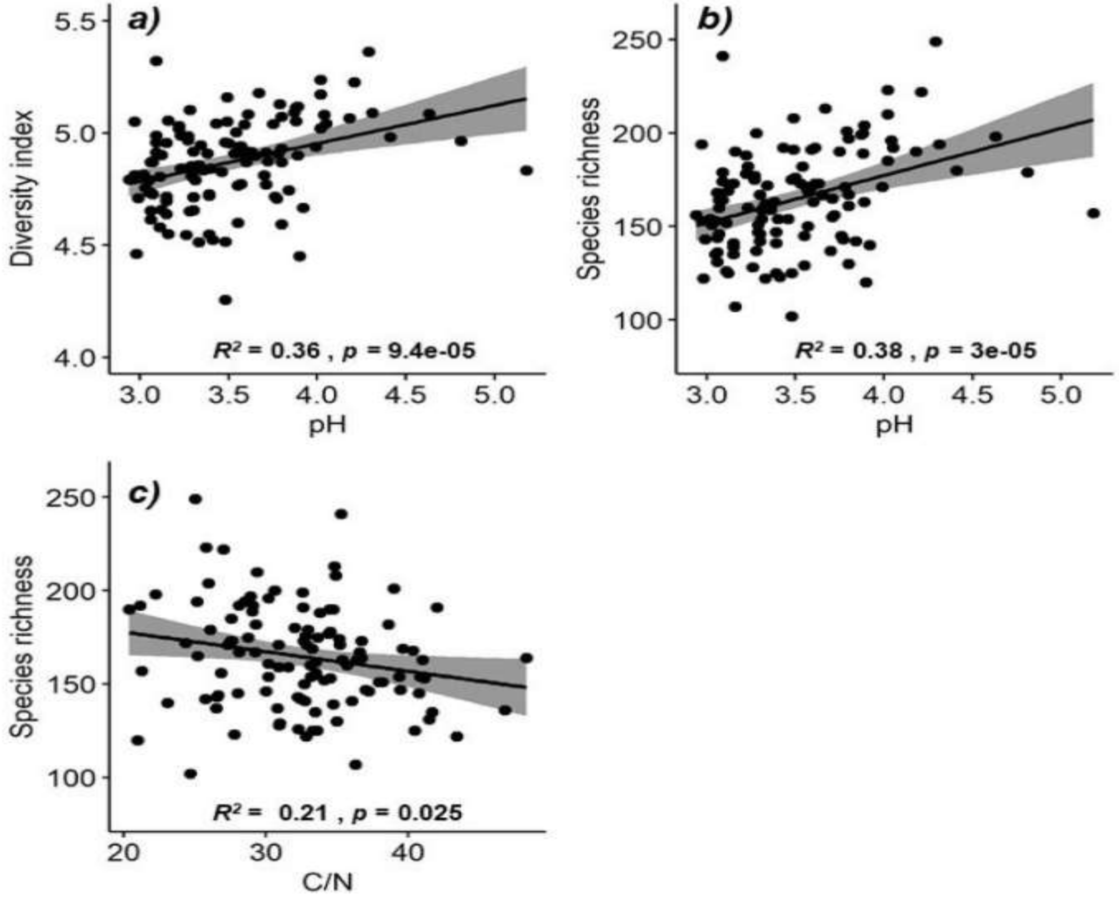
Species	Rank	Group CC Av.Abund	Group CF Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Hyaloscyphaceae	Family	0,87	1,03	0,53	1,14	0,72	0,72
Thelephora terrestris	Species	0,1	1,05	0,53	0,81	0,71	1,43
Luellia	Genus	0,61	0,92	0,5	1,07	0,67	2,1
Venturiaceae	Family	0,87	0,74	0,49	1,12	0,65	2,75
Archaeorhizomyces	Class	0,27	0,82	0,48	0,51	0,64	3,39
Piloderma sphaerosporum	Species	0,98	0,22	0,46	1,05	0,62	4,01
Archaeorhizomyces	Class	0,92	0,41	0,45	1,04	0,61	4,62
Unknown Fungi (34)	Kingdom	0,87	0,3	0,43	0,86	0,58	5,2
Archaeorhizomyces	Class	1,1	0,49	0,43	1,02	0,57	6,34
Penicillium simile	Species	0,47	1,2	0,4	1,59	0,54	7,43
Piloderma	Genus	0,8	0,06	0,4	0,93	0,53	7,96
Solicoccozyma terricola	Species	0,95	1,21	0,39	1,28	0,53	8,49
Umbelopsis	Genus	0,8	0,86	0,39	1,3	0,52	9,02
Penicillium_austroafricanum	Species	1,1	1,45	0,39	1,28	0,52	9,54
Hyaloscyphaceae	Family	1,21	0,7	0,38	1,22	0,52	10,05
Geomyces asperulatus	Species	1,17	1,02	0,38	1,19	0,51	10,56
Cryptococcus neoformans	Species	0,54	0,52	0,37	1	0,49	11,05
Oidiodendron pilicola	Species	1,14	1,04	0,36	1,2	0,48	11,54
Meliniomyces variabilis	Species	0,98	0,71	0,35	1,4	0,47	12,01
Ascomycota	Phylum	0,81	0,77	0,35	1,26	0,47	12,48
Apiotrichum sporotrichoides	Species	0,68	0,92	0,34	0,93	0,46	12,94
Cortinarius stillatitius	Species	0,64	0,04	0,33	0,41	0,44	13,38
Leucosporidiales	Order	0,53	0,71	0,32	1,23	0,43	13,81
Hyaloscyphaceae	Family	0,53	0,39	0,32	0,81	0,42	14,66
Mortierella macrocystis	Species	1,14	0,9	0,28	1,39	0,38	15,04

Soil Fungi

Correlation Analysis

3) Variation of soil fungal communities by soil chemical properties

Adjusted P-value=0.001. *Italic*: significant (P<0.05) before Bonferroni correction.
ns(non-significant)> 0.003, *<0.003



Correlation with
soil chemical properties

Soil fungal diversity
<u>Strong relationship with soil pH.</u>
<u>Close to significant: C to N ratio</u>

Fruiting body diversity
None.

Distance-based Linear Models

3) Variation of soil macro-micro fungal communities by soil chemical properties

Dataset	Variable	Pseudo-F	P-Value	Prop.
Fruiting body abundance	pH**	2.0	**0.002**	3.0%
	OM*	1.5	**0.033**	2.2%
	C	1.4	0.11	2.0%
	N	1.3	0.142	1.9%
	C/N**	1.9	**0.009**	2.9%
Fruiting body dry weight	pH*	1.4	**0.036**	2.1%
	OM	1.1	0.273	1.6%
	C	1.0	0.4	1.6%
	N	1.1	0.312	1.6%
	C/N*	1.4	**0.027**	2.2%
Soil fungi	pH***	7.3	**0.001**	6.1%
	OM***	2.0	**0.001**	1.8%
	N**	1.8	**0.002**	1.5%
	C***	2.1	**0.001**	1.8%
	C/N***	5.3	**0.001**	4.5%

P-value: ns > 0.05, *<0.05, **<0.01, ***<0.001.

> Fungal communities – Soil chemistry

Fruiting body – pH& C/N +(OM)

Soil fungi– All soil variable

Main Findings

⇨ **Continuous-cover forestry maintains similar soil chemical properties and fungal communities to Unmanaged forest.**

⇨ Forest Management Practices significantly altered soil chemical properties, fruiting body and soil f ungal diversity.

⇨ CCF retained similar soil pH and C/N to UF than CF.

<u>Why CF is different?</u>

1. Moisture and sun influx
2. Environmental disturbances (soil erosion, ECM fungi)

Dataset	Variable	Clear fell	Continuous cover	Unmanaged	$F_{(df)}$	P-value
Soil Chemistry	pH***	3.24[a]	3.03[b]	2.99[b]	$12.9_{2,114}$	**<0.001**
	OM	14.9	13.5	17.5	$2.2_{2,112}$	0.1119
	Carbon*	8.27[a]	7.93[a]	10.02[a]	$2.1_{2,112}$	**0.0131**
	Nitrogen	0.28	0.23	0.31	$2.9_{2,112}$	0.0617
	C/N***	29.69[b]	34.11[a]	32.29[a]	$8.4_{2,112}$	**<0.001**

Unexpected Findings

➢ **Fruiting body diversity**

Clear-fell

Continuous-cover

Unmanaged

➢ **Soil fungal diversity**

Unmanaged

Continuous-cover

Clear-fell

Why?!

-Logging residues and stumps

-Soil scarification

-Dormant & external seeds

-Replanted species

Key Species

➢ **Continuous-cover forestry maintains similar soil fungal communities to Unmanaged forest**

Top 3 fruiting bodies: *Micromphale perforans, Hygrophoropsis aurantiaca and Thelephora terrestris*

Decompose coniferous needle

Appears near fallen trees and tree stumps

Abundant in regenerated stands

Top 3 soil fungi: *Piloderma sphaerosporum, Thelephora terrestris and Luellia sp*

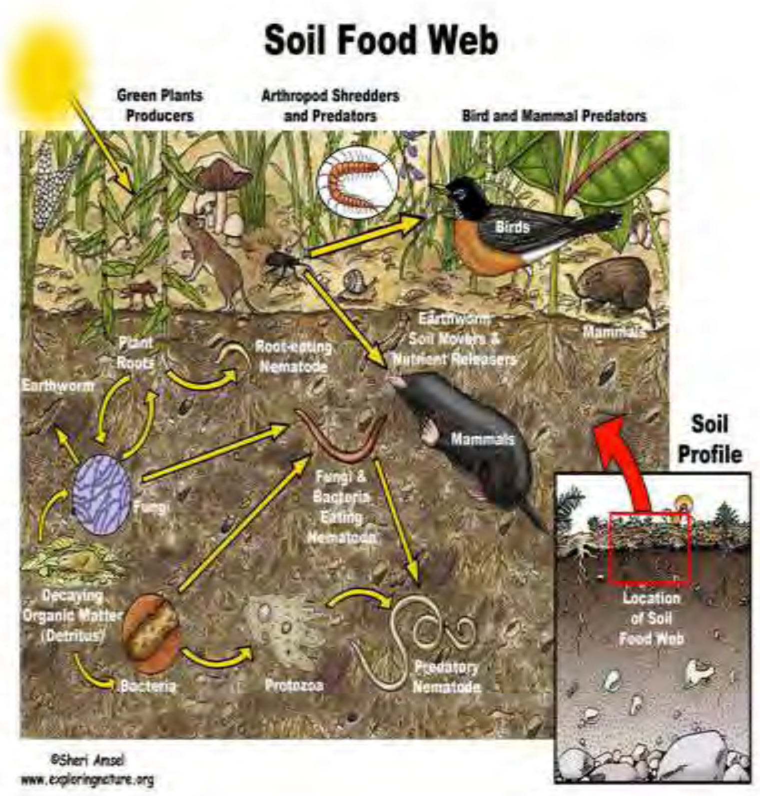
Dominant in late decaying stage

Abundant in regenerated stands

Associated with Orthili secunda

Relationships

- Variation in soil fungal communities is related to soil chemical properties



-Fruiting body --- Soil pH, C/N (4.3~5.9%)+ abiotic factors

-Soil fungi ---- Soil pH, C/N, C, N (15.7%) + abiotic factors

-Correlation with Fruiting body: mushroom is a fraction of fungal communities

-Correlation with Soil fungi: soil pH --- diversity * soil C/N --- diversity ***

Take Home Message

- **Mimicking natural disturbance during harvest may reduce impacts on forest ecosystems.**
- **Clear-felling had pronounced effect on biological and chemical properties of soils.**
- **Partial harvest of 30% maintained similar fungal communities to unmanaged forests.**
- **Variation in soil fungal communities correlated to soil chemical properties.**
- **Continuous-cover forestry is a promising option for maintaining soil ecosystems.**

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Continuous-cover forestry maintains soil fungal communities in Norway spruce dominated boreal forests

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