Lincoln University
Te Whare Wānaka o Aoraki

Bio-Protection
Bioprotection science for New Zealand

SCION
Next generation biomaterials

Ectomycorrhizal communities associated with a Pinus radiata *plantation in the North Island, New Zealand*

PhD Results Seminar
24 September 2008

Katrin Walbert
Scion,
Lincoln University

Project funding and supervisors

- Start in June 2004
- Joint project between Lincoln University and Scion (Former Forest Research Institute)
- Foundation for Research & Technology, contract C04X0302
- Lincoln University Supervisors
  - Dr. Eirian Jones, Dr. Hayley Ridgway
- Scion Supervisors
  - Dr. Tod Ramsfield, Margaret Dick

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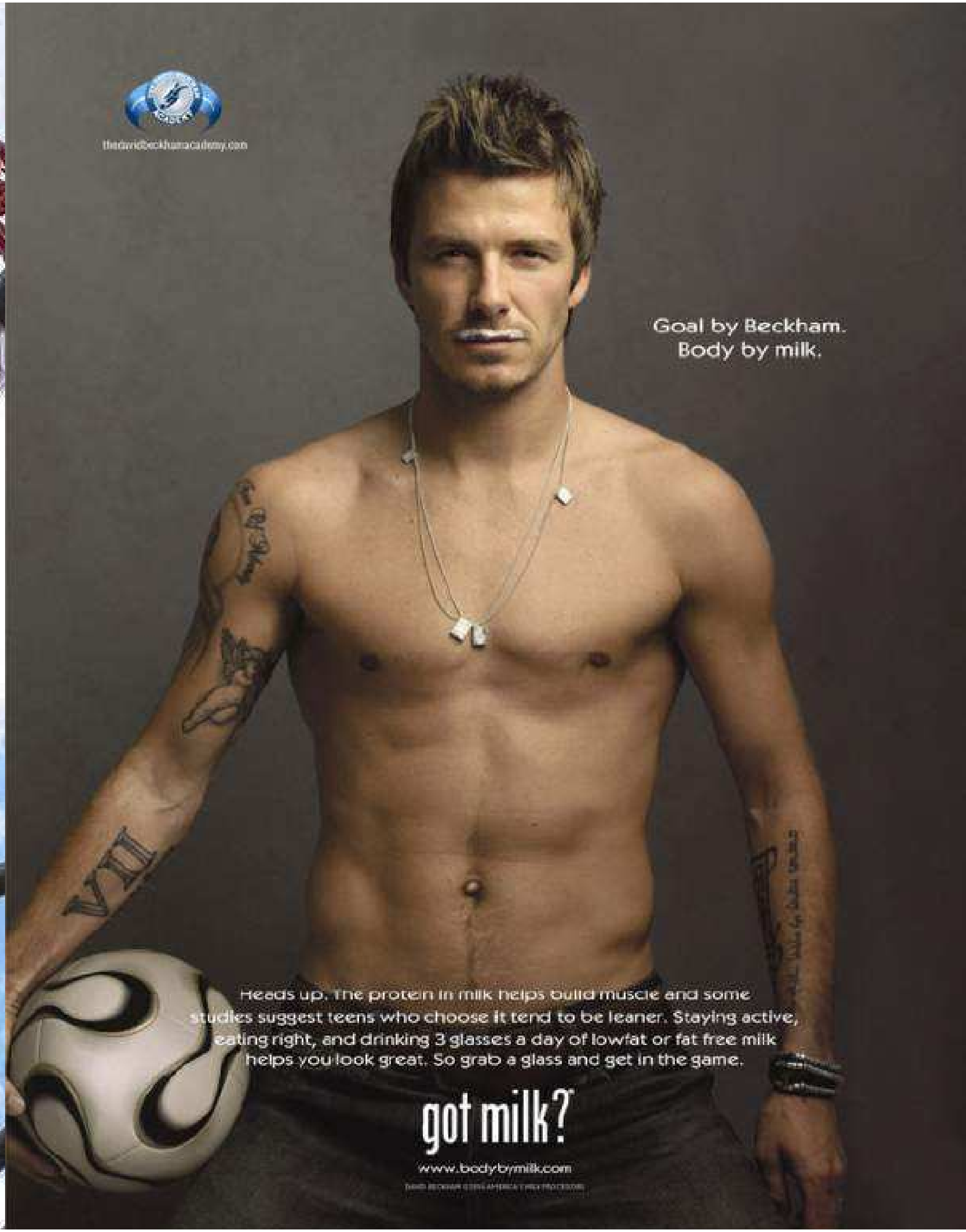
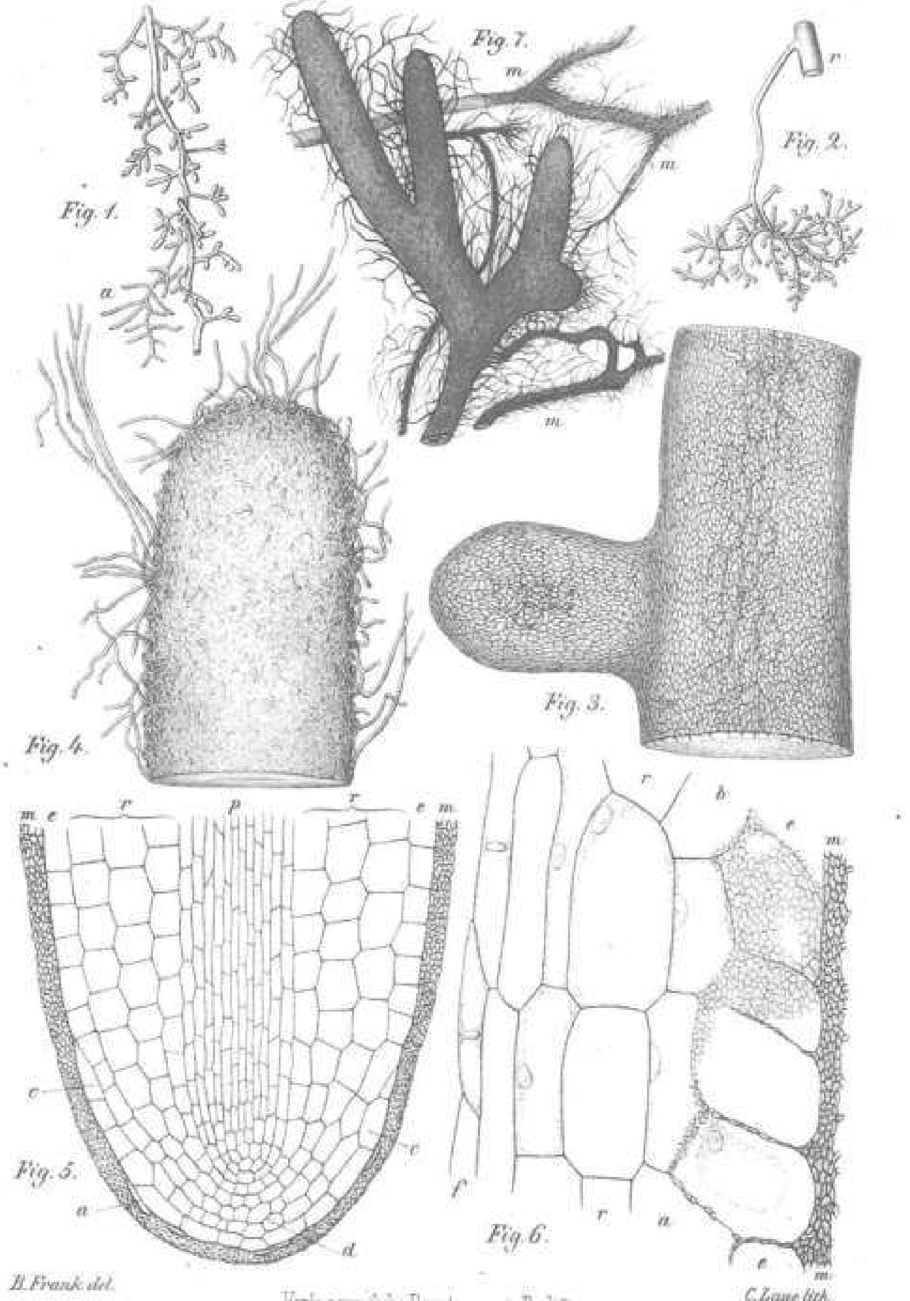

Goal by Beckham.
Body by milk.
Heads up. The protein in milk helps build muscle and some studies suggest teens who choose it tend to be leaner. Staying active, eating right, and drinking 3 glasses a day of lowfat or fat free milk helps you look great. So grab a glass and get in the game.
got milk?
www.bodybymilk.com

Berichte d. Deutschen Bot. Gesellsch. Bd. III. Taf. X.



B. Frank del. Verlag von Gebr. Borntraeger in Berlin. C. Laue lith.

Mycorrhiza

- **"Mycorrhiza":** greek for 'root-fungus', new organ
- **Symbiotic association** between a plant (photobiont) and a fungus (mycobiont)
- **Bi-directional movement of nutrients**:
  - Mycobiont: carbohydrates, vitamins, spore germination is stimulated
  - Plant: phosphorus, nitrogen and other minerals, water uptake, protection from root pathogens

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The symbiosis

Fungi are achlorophyllous

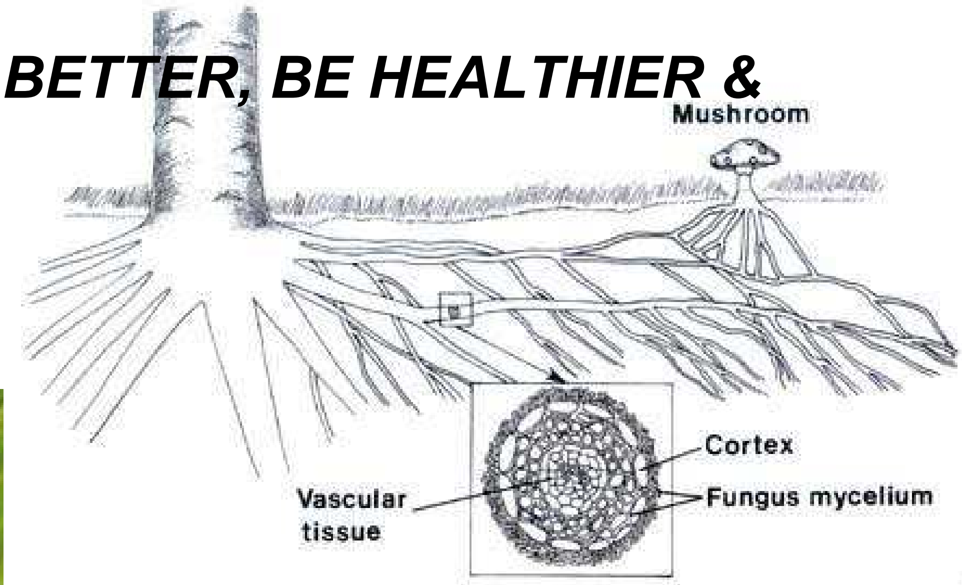
➲ ***FUNGI ARE RELIANT ON PLANT PARTNER***

Benefits for plant multifaceted

- Nutrient & water uptake, pathogen protection, surface area increase, avoidance of nutrient depletion zone, fungal enzymes, CMN…

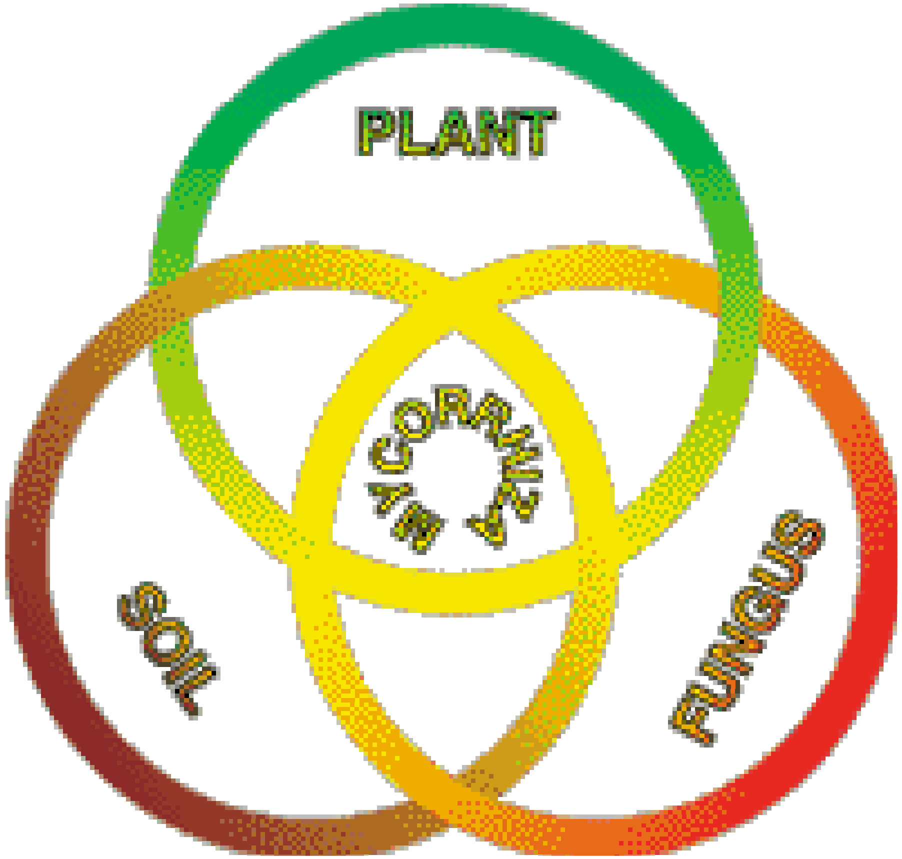
➲ ***MOST HOST SPECIES ARE RELIANT ON FUNGAL PARTNER***

➲ ***PLANT HOST WILL GROW BETTER, BE HEALTHIER & MORE RESISTANT***



Effects of the soil environment on mycorrhiza

- pH
- Fertility
- Moisture
- Temperature
- Other soil biota



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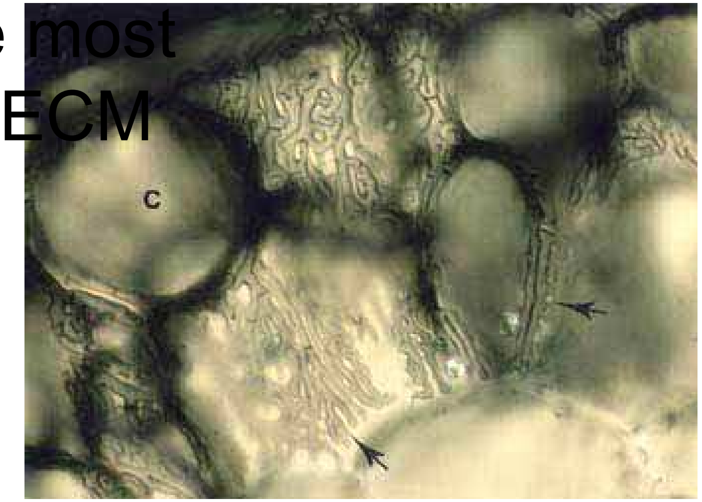
Ectomycorrhiza ECM

- Most diverse type, ~ 6000 fungal species
- Only 3% of phanerogams are ECM
- ***BUT*** importance globally because most coniferous/plantation species are ECM
- Diagnostic features:
  - ***VISIBLE***
  - Mantle
  - Hartig net - intracellular



Mycorrhiza and Forestry

Mycorrhiza essential on plantation species

- Growth in the nursery – limits fertiliser substitution
- Facilitation of establishment
  - E.g. Douglas fir problems in New Zealand
- Pathogen protection – esp. nursery environment
- Nutrient deficiencies without mycorrhiza

The project

Original research aim: to observe serial changes in ECM on *P. radiata* in a plantation, characterisation of indicator species of successional stages

3 Chapters:

- ECM species diversity in a *P. radiata* plantation in NZ
- ECM communities associated with different age classes of *P. radiata*
- Changes in ECM diversity from the nursery to outplanting

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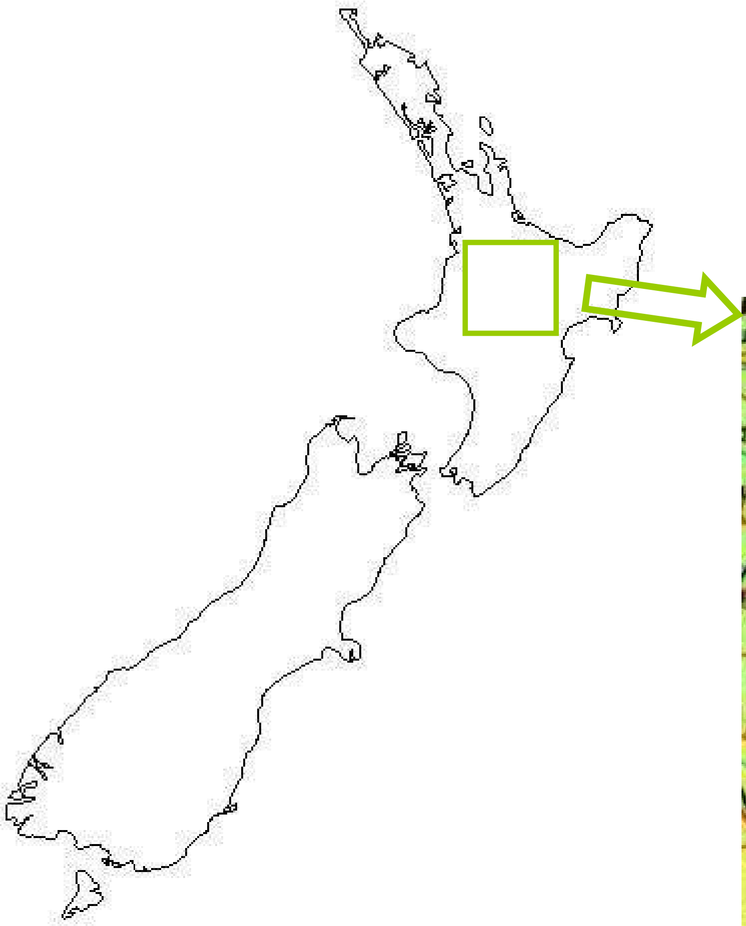
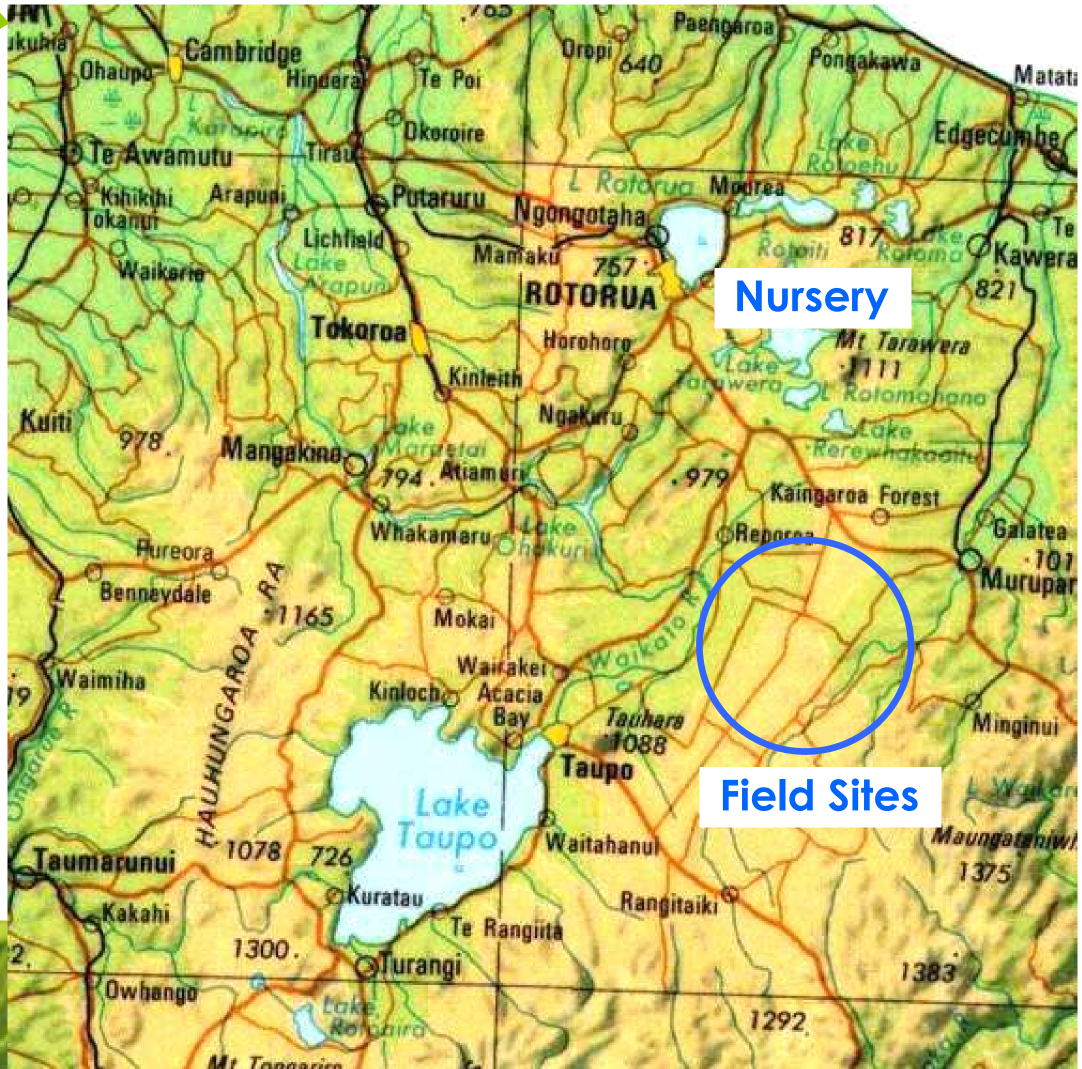
Study sites





Material & Methods

- Nursery and 2, 8, 15, 26 yr old plantation sites plus 1yr old site in 2006 belowground survey
- Sporocarp surveys for aboveground ECM
  - Autumn 2005, 2006
  - Species ID with morphological and molecular methods
- Soil core surveys for belowground ECM
  - Autumn 2005, December 2005, Autumn 2006
  - Species ID with morphotyping and molecular methods

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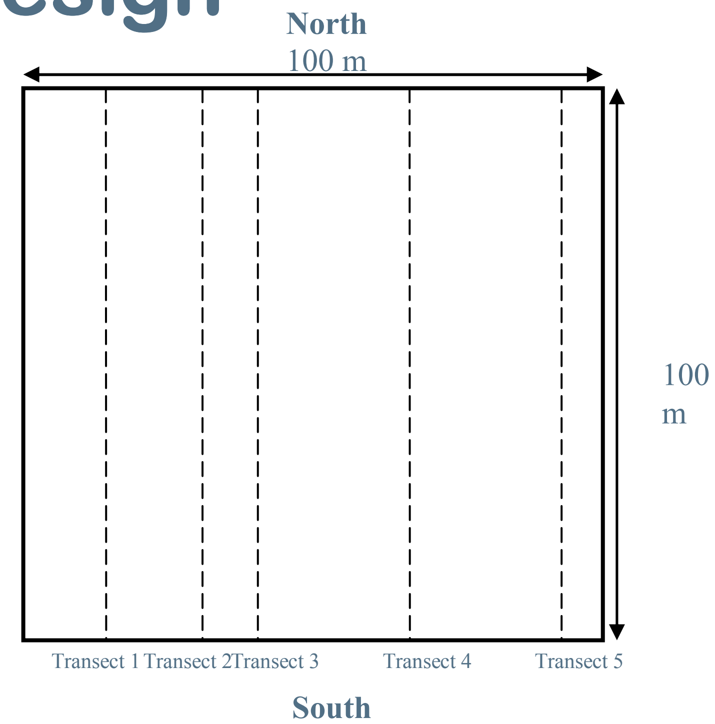
Sampling design



Soil core assessment	K06-N	K05-N	K05F-1	K04-2	K98-8	K91-15	K80-26
SCA 1 June 2005	-	20	-	20	20	20	20
SCA 2 December 2005	-	-	-	10	10	10	10
SCA 3 May/July 2006	8	-	8	8	8	8	8

Molecular identification - PCR

- DNA extraction and PCR unsatisfactory with recommended methods
- REDExtract-N-Amp™ Plant PCR Kit (Sigma)
- ITS1F/ITS4
- Nested PCR with ITS1F/ITS4B followed by ITS1F/ITS4 in case of double-banding

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Molecular identification – RFLP, Cloning and sequencing

- RFLP
  - digest with *Alu*I, *Hinf*I and *Mbo*I
  - Analysis with GERM freeware (Good-Enough RFLP Matcher, Dickie et al. 2003)
- Cloning
  - Representative of each ECM type
  - pGEM-T® Easy Vector System (Promega)
- Sequencing
  - GenBank www.ncbi.nlm.nih.gov
  - UNITE www.unite.zbi.ee

    Nordic-Baltic database focused on ECM

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Statistical analysis

Alpha Diversity – diversity of species present within a community

- Species richness, Jackknife estimate
- Margalef's Index
- Absolute and relative abundance
- Diversity indices
  - Shannon index
  - Simpson index
  - Species Evenness
- PRIMER, MINITAB, GenStat

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Statistical analysis cont'

Beta Diversity – variation in diversity from one community to the next

- Sorensen's Index
- One way ANOVA of Shannon & Simpson index
- Rank-abundance curves
- Community analysis
  - Hierarchical clustering
  - Multidimensional scaling (MDS)
  - Similarity analysis

- PRIMER, MINITAB, GenStat, PAST

ECM diversity – Chapter 3

Aims

- Assess within site (α-) ECM diversity
  - Richness, frequency, diversity indices, evenness
- Compare diversity of above- and belowground ECM communities
- Increase national and international knowledge on ECM diversity in exotic monoculture
- Contribute to international sequence database

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Sporocarp Results

- 18 ECM taxa found
- ID with morphological characteristics
- Molecular analysis
  - RFLP for reference
  - Direct sequencing

ECM species clarification

"*Tricholoma pessundatum*"

"*Hebeloma crustuliniforme*"

"*Laccaria laccata*"

→ ***Tricholoma*** **sp.**

→ ***Hebeloma*** **sp.**

→ ***Laccaria proxima***

ECM species clarification

Inocybe sindonia

I. lacera

***Inocybe* sp.**

Rhizopogon rubescens, R. pseudoroseolus, R. luteolus

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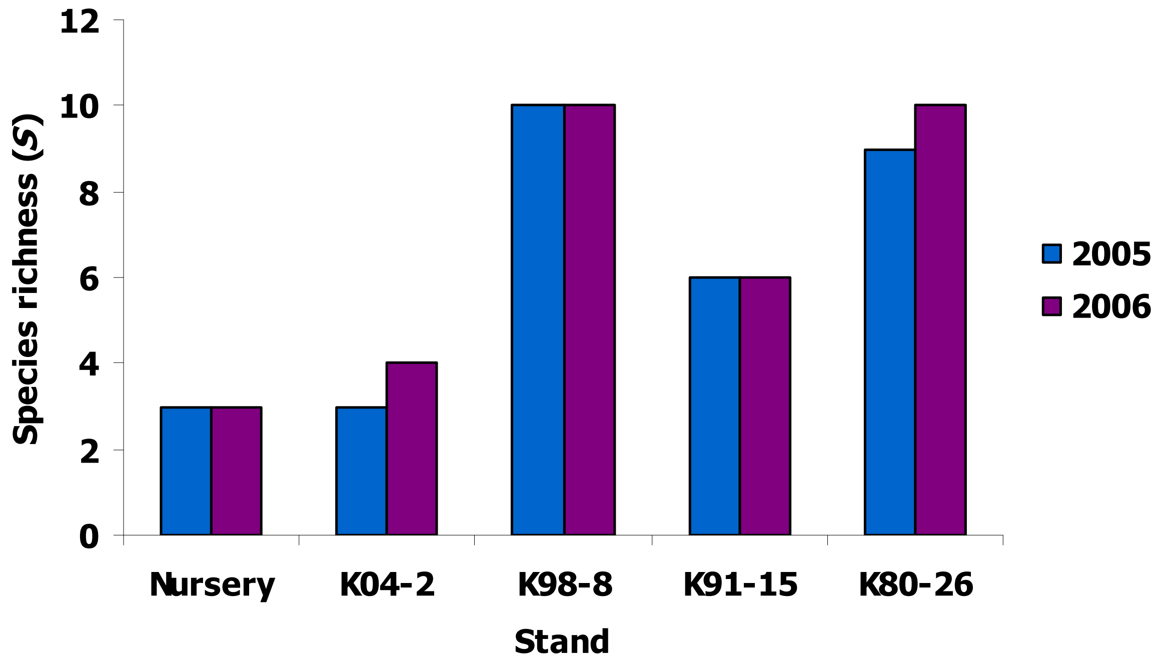
Aboveground species richness



	Observed richness	Jackknife estimate (± SE)
Nursery	3	3.00 (± 0.00)
K04-2	4	4.80 (± 0.80)
K98-8	10	10.80 (± 0.80)
K91-15	6	7.00 (± 0.00)
K80-26	10	12.40 (± 1.60)

ECM ID Results

- 19 morphotypes
- Morphotype grouping followed by RFLP pattern analysis
- RFLP comparison to known sporocarps
- Direct sequencing & cloning
- 8 ECM species – ID to species level through RFLP matching or sequencing
- 5 ECM types to genus level
- 6 ECM types remained unknown

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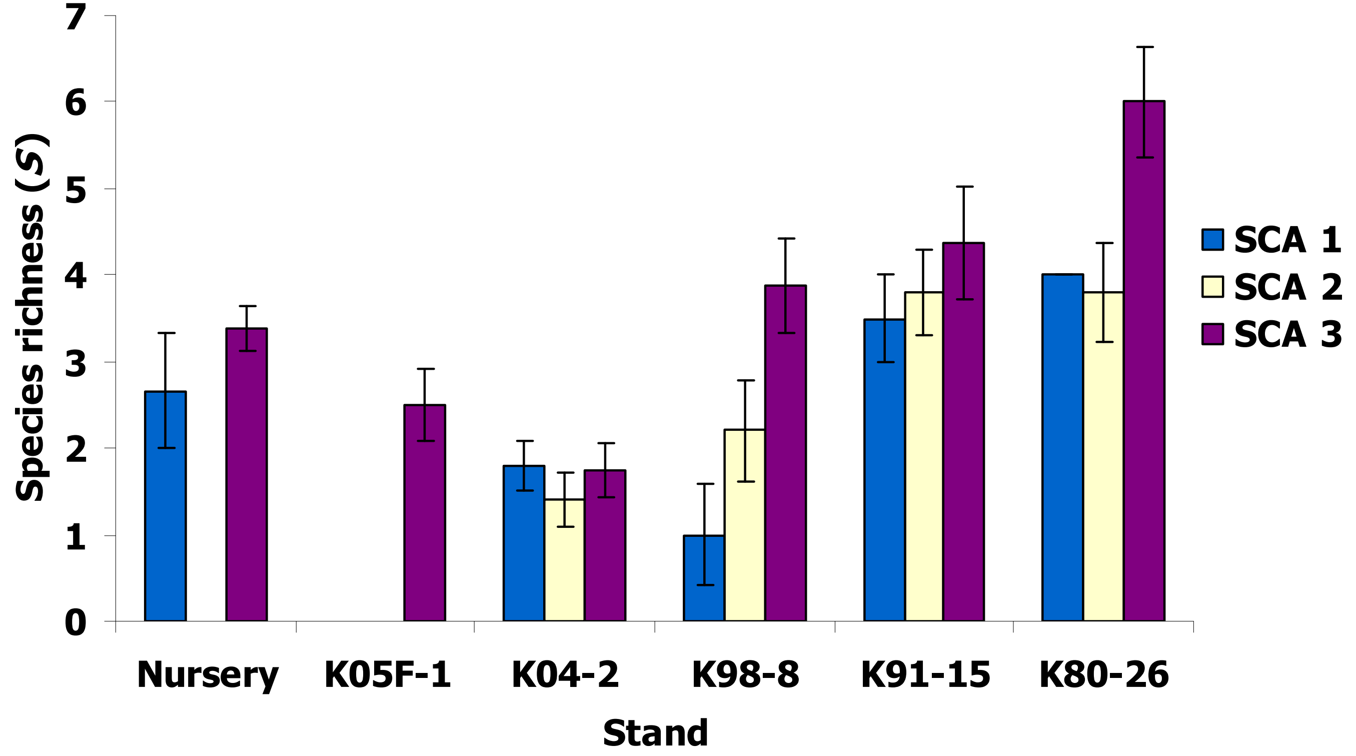
Belowground species richness



	SCA 1			SCA2			SCA 3		
	Observed richness	Jackknife estimate (± SE)		Observed richness	Jackknife estimate (± SE)		Observed richness	Jackknife estimate (± SE)	
Nursery	5	7.00	(± 1.16)	-	-	-	5	5.00	(± 0.00)
K05F-1	-	-	-	-	-	-	5	5.88	(± 0.88)
K04-2	3	3.00	(± 0.00)	4	6.75	(± 1.15)	3	3.00	(± 0.00)
K98-8	4	6.00	(± 0.00)	6	8.40	(± 1.60)	10	10.88	(± 0.88)
K91-15	5	11.00	(± 2.12)	8	9.60	(± 0.98)	10	10.88	(± 0.88)
K80-26	7	10.00	(± 0.00)	8	8.00	(± 0.00)	13	14.75	(± 1.15)

New species/association to New Zealand

Inocybe sindonia

Lactarius rufus

Rhizopogon pseudoroseolus

Rhizopogon luteorubescens

Wilcoxina mikolae

Tomentella sp. – not recorded as ECM, only saprotroph

Pseudotomentella sp.

Pseudotomentella tristis

Diversity - discussion

Low ECM species richness/diversity

- Introduced host
- Young history
- Monoculture
- Short rotation (harvest age < 30, maturing forest)
- Disturbance – clearcutting
- Study duration and assessment frequency (aboveground)

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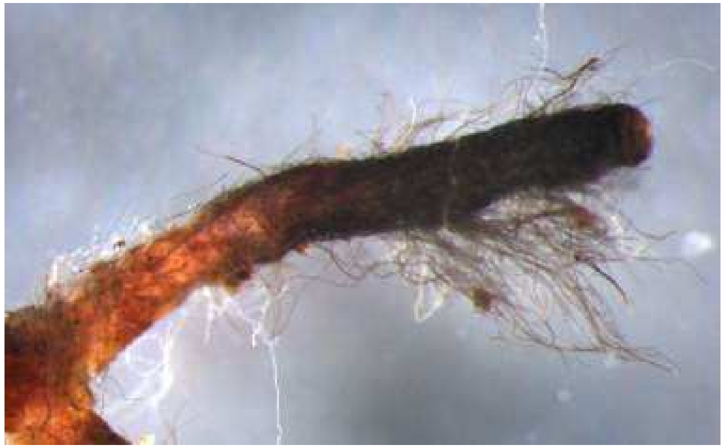
Diversity - discussion

Only exotic ECM associated with *P. radiata*

- *Cenococcum geophilum* potential native



- What is hiding behind the unknown ECM types?

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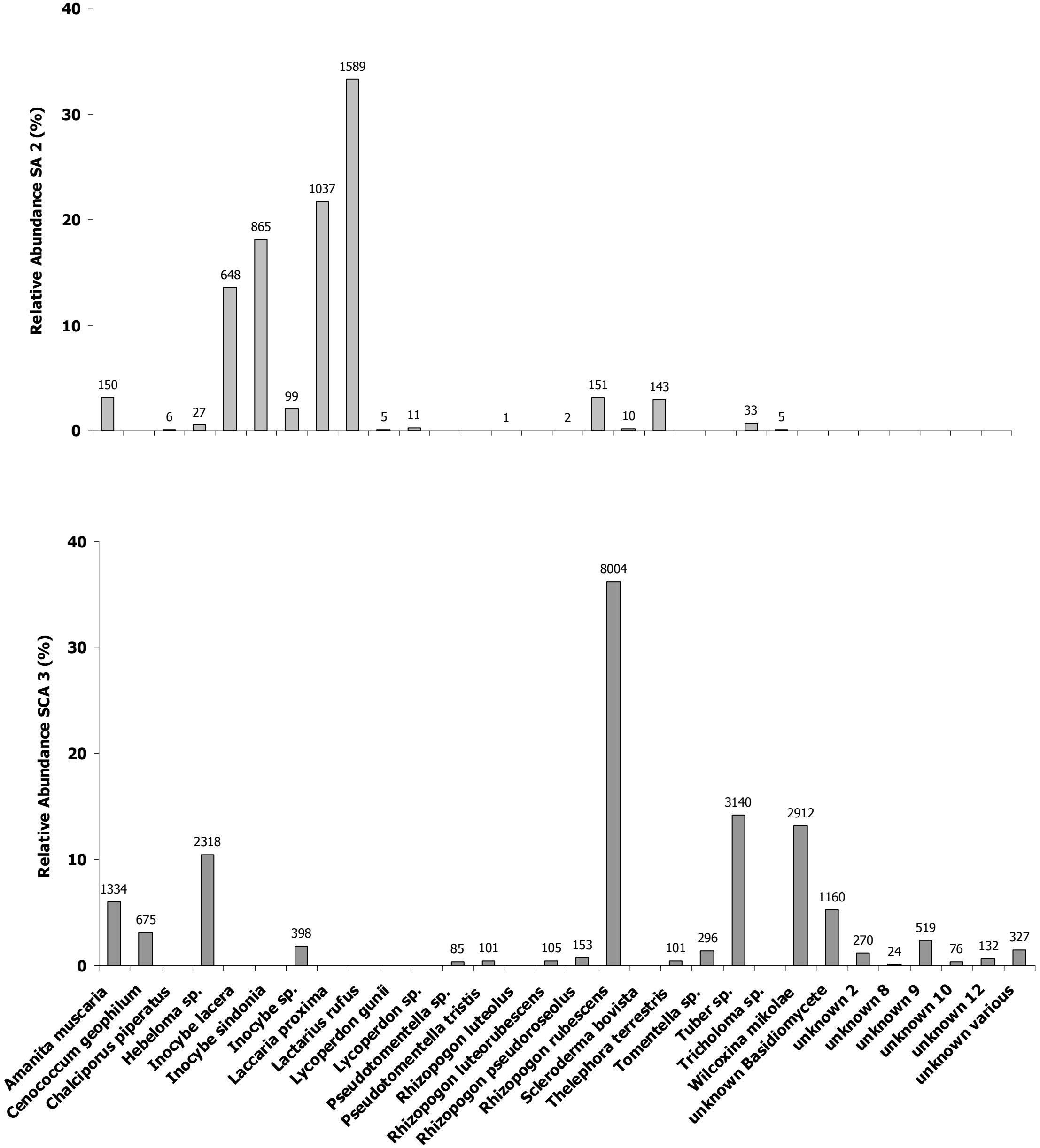
ECM diversity – above vs. below

Above

Below



- Little correlation
- 7 ECM species in common
- dominant species aboveground not observed below

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Above – vs. belowground abundance

- Only nursery sharing same species
- Reason for lack of correlation?
  - Additional food sources – saprophytes
  - Lack of fruiting body – *C. geophilum*
  - Inconspicuous fruiting body – hypogeous and resupinate fruiting structures
  - Sampling frequency esp. aboveground
  - Sampling strategy esp. belowground
  - Thinning – different effect on above- and belowground communities

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ECM communities of different age classes – Chapter 4

- **Aim**: to assess and discuss effect of host age on the between-site diversity (β-diversity)
- Succession: non-seasonal, directional and continuous pattern of colonisation, and extinction on a site by species populations (Begon *et al*. 1998)
- Mycorrhizal succession concept – originated by Mason *et al*. 1982, 1983 based on mycorrhizal fruit body production observed

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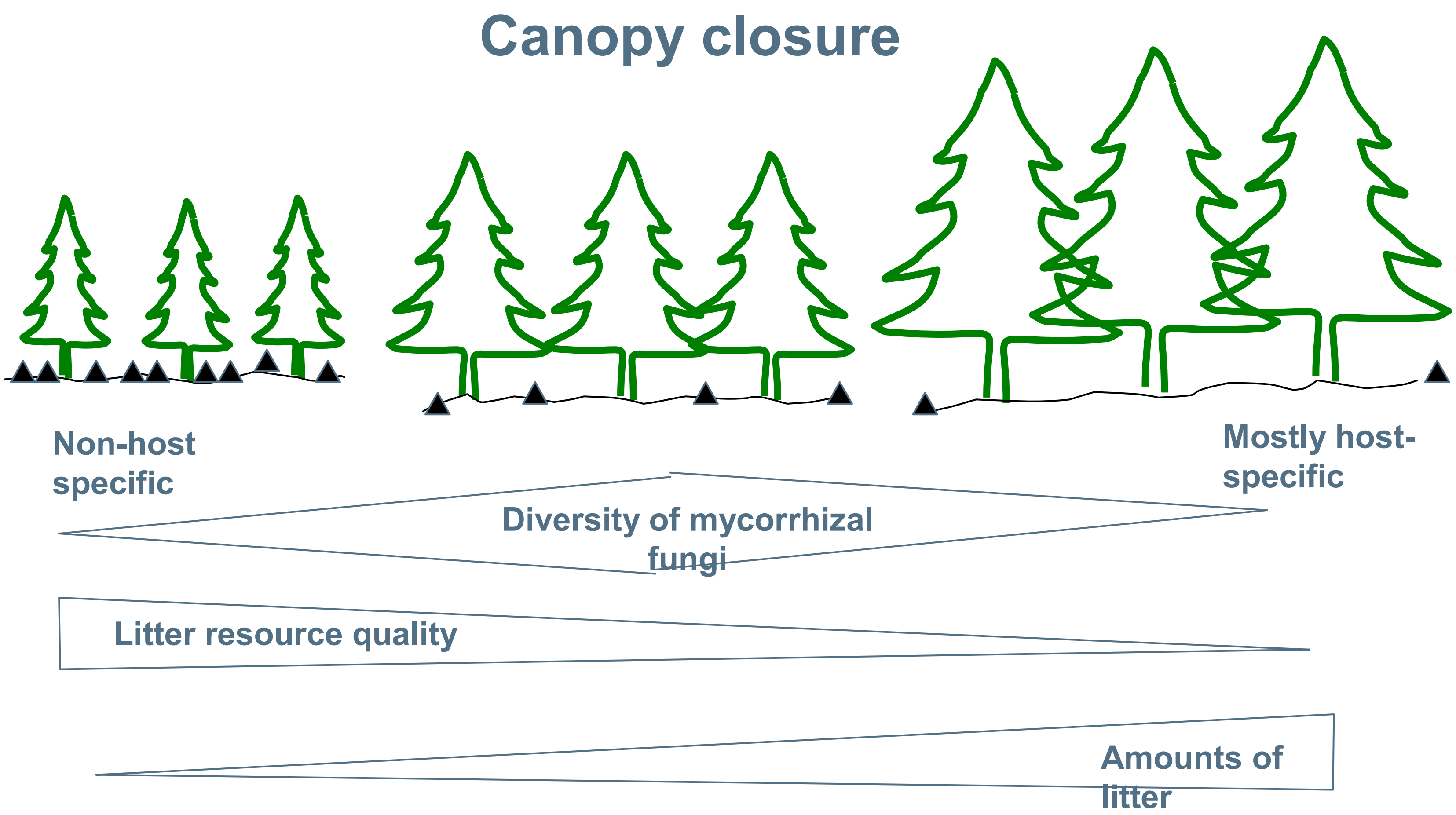

- Little correlation above- belowground → have only fruiting patterns been observed?
- Recent studies did not support theory by Mason *et al.*

Chapter 4 - Study layout

- Based on sporocarp and soil core assessment autumn 2006 – most robust dataset
- Stand ages investigated
  - Nursery
  - 1 yr
  - 2 yrs
  - 8 yrs
  - 15 yrs
  - 26 yrs

Chapter 4 - main results

Change in species richness & diversity over time

- α-diversity: above richness increased over time, below it decreased nursery → 2yr, then increased again
- above diversity increased till 15 yrs then declined, below it decreased nursery → 2yr, then increased thereafter
- Species richness higher above than below
- β-diversity: above 8, 15 and 26 yr stands similar, below 2 different ECM communities: nursery, 1, 2yrs vs. 8, 15, 26 yrs

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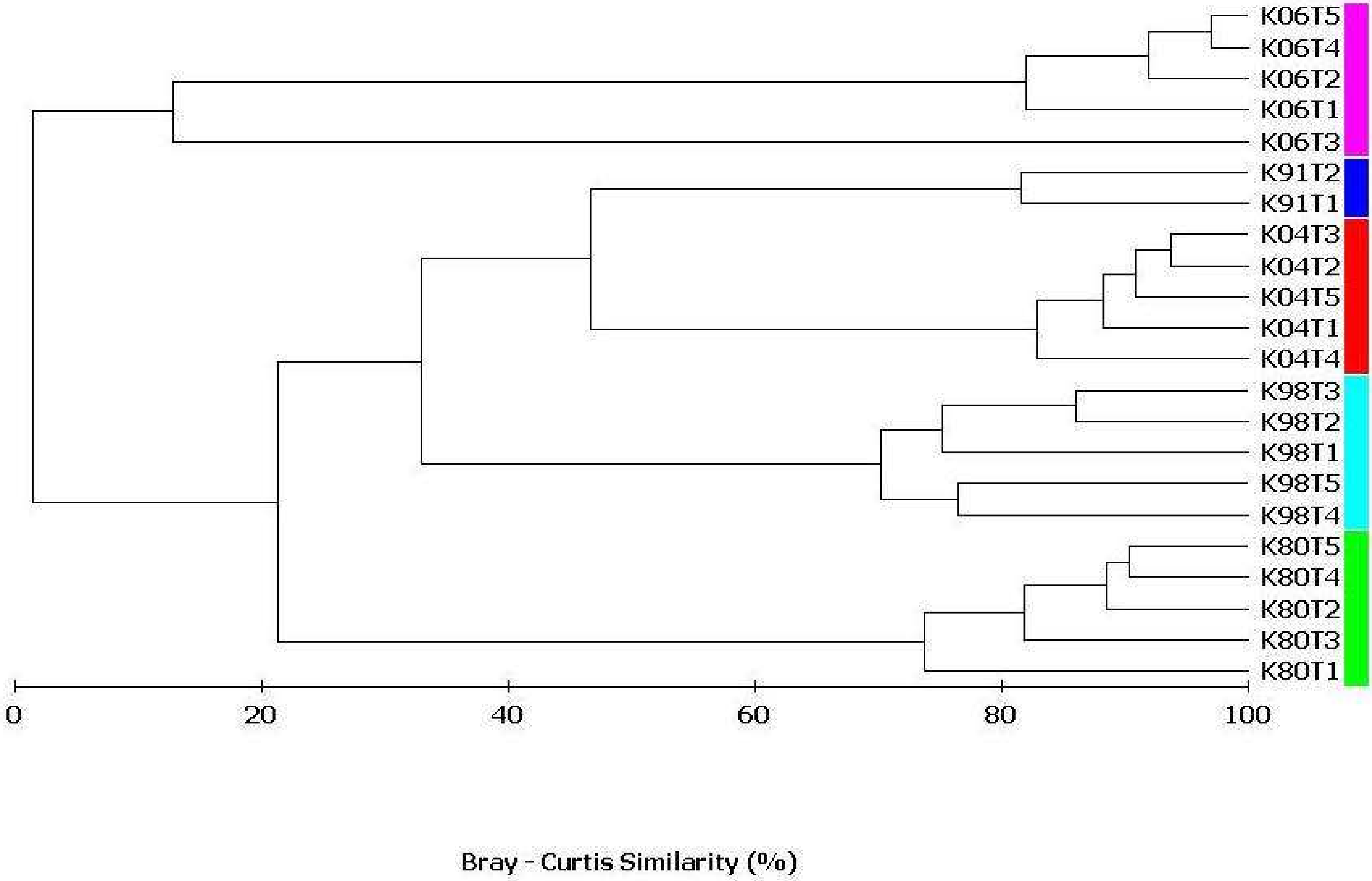
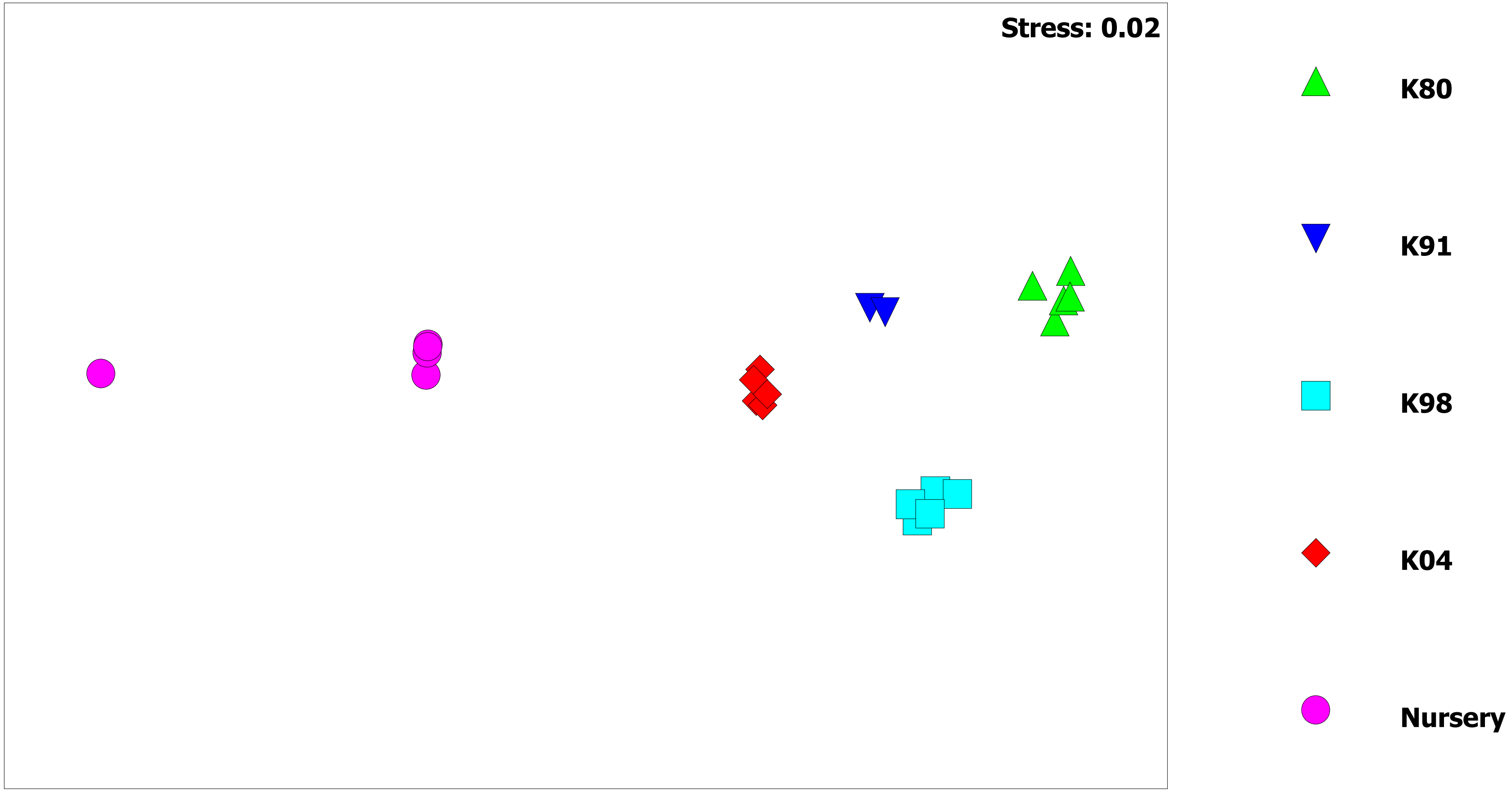
Community analysis – hierarchical clustering - aboveground

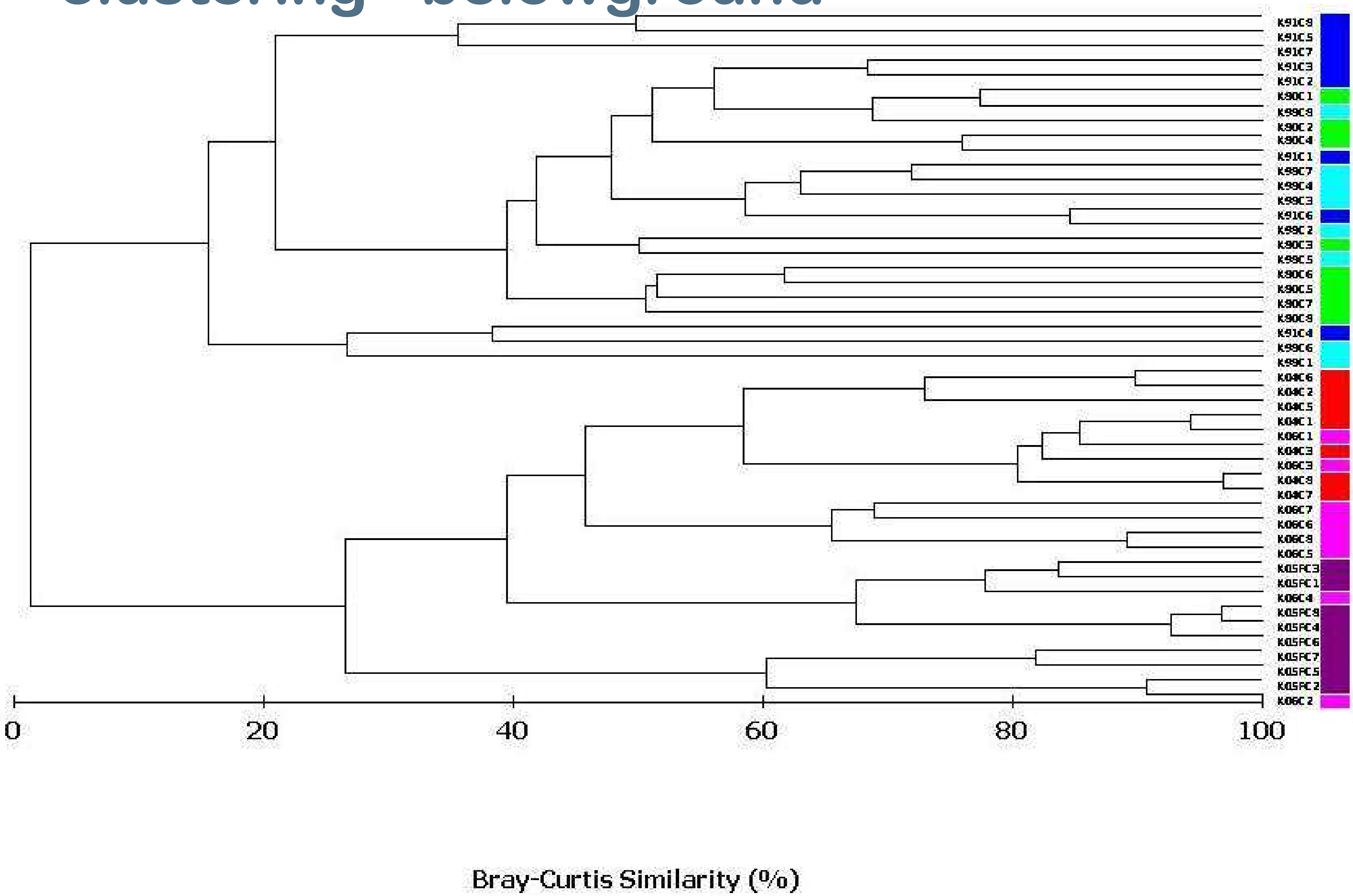


MDS ordination - aboveground

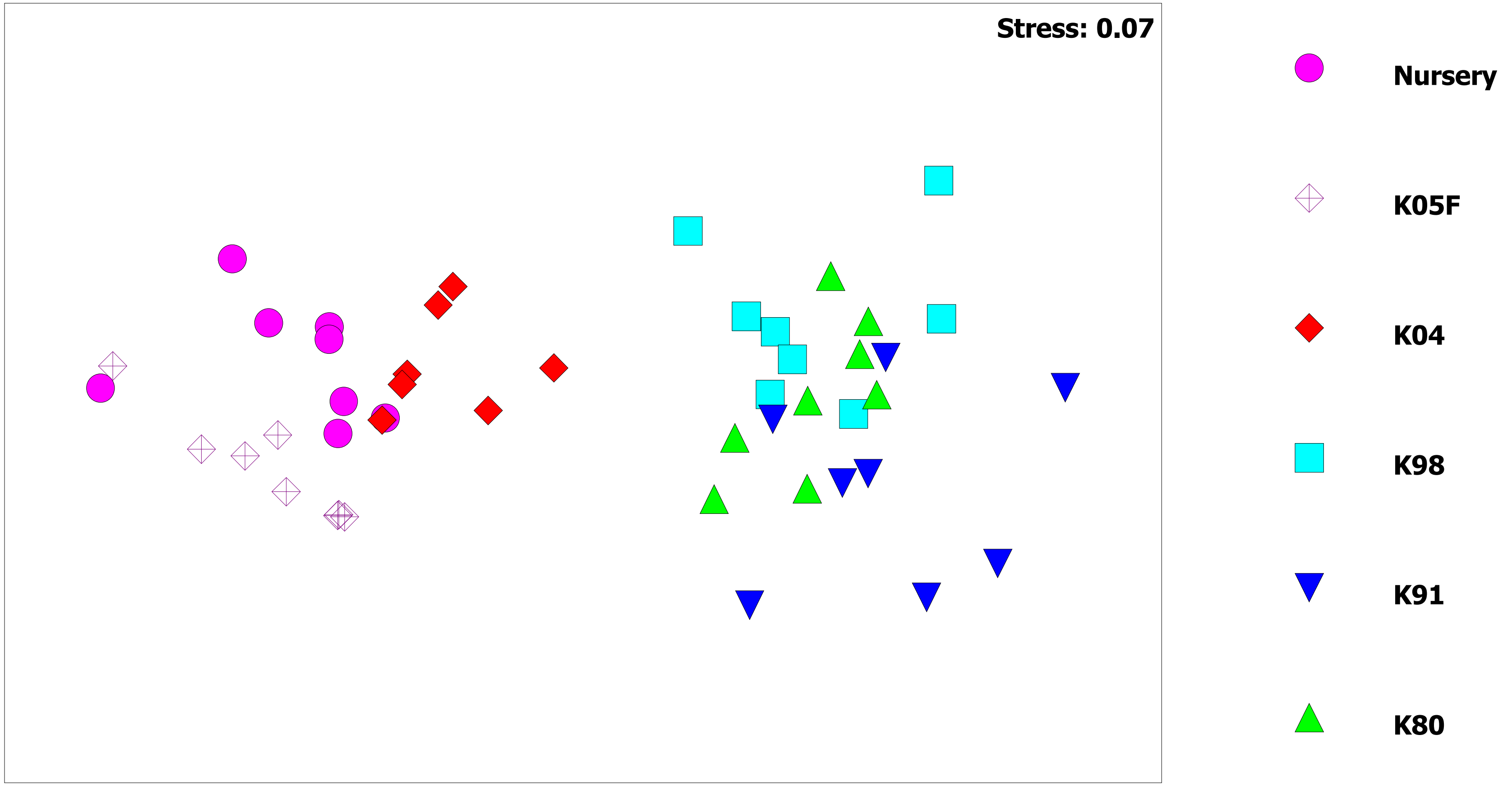


Clear indicator species for each age group found

Community analysis – hierarchical clustering - belowground



MDS ordination - belowground



Indicator species for each, nursery and forest group, age class independent

Richness and diversity

- Hypothesis 1: species richness and diversity increases with host age confirmed
- But: richness/diversity peak not reached

→This study limited by harvesting age of *P. radiata* – full succession range could not be investigated

ECM species communities

- Hypothesis 2: ECM communities change over time in relation to host age
    - Confirmed aboveground
    - Rejected belowground
- ECM change aboveground – function of edaphic conditions influencing fruiting patterns
- ECM change belowground – not a function of host age but edaphic conditions, root density and turnover, competitive pressure

Changes in ECM diversity from nursery to outplanting – Chapter 5

- Aim: to investigate the fate of nursery ECM in the initial years after being planted out on clearcut site
- Do nursery ECM survive, and if, how long?
- When do new, forest ECM species enter the system?
- When does the community fully change?

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Chapter 5 - Study layout

- Assessed nursery ECM in Te Ngae Nursery in May 2006
- Seedlings planted on clearcut site in June 06, followed up for one year, 8 assessments
- Sited planted in 1998 and 2004 assessed – representing the 2 – 7 yrs after outplanting

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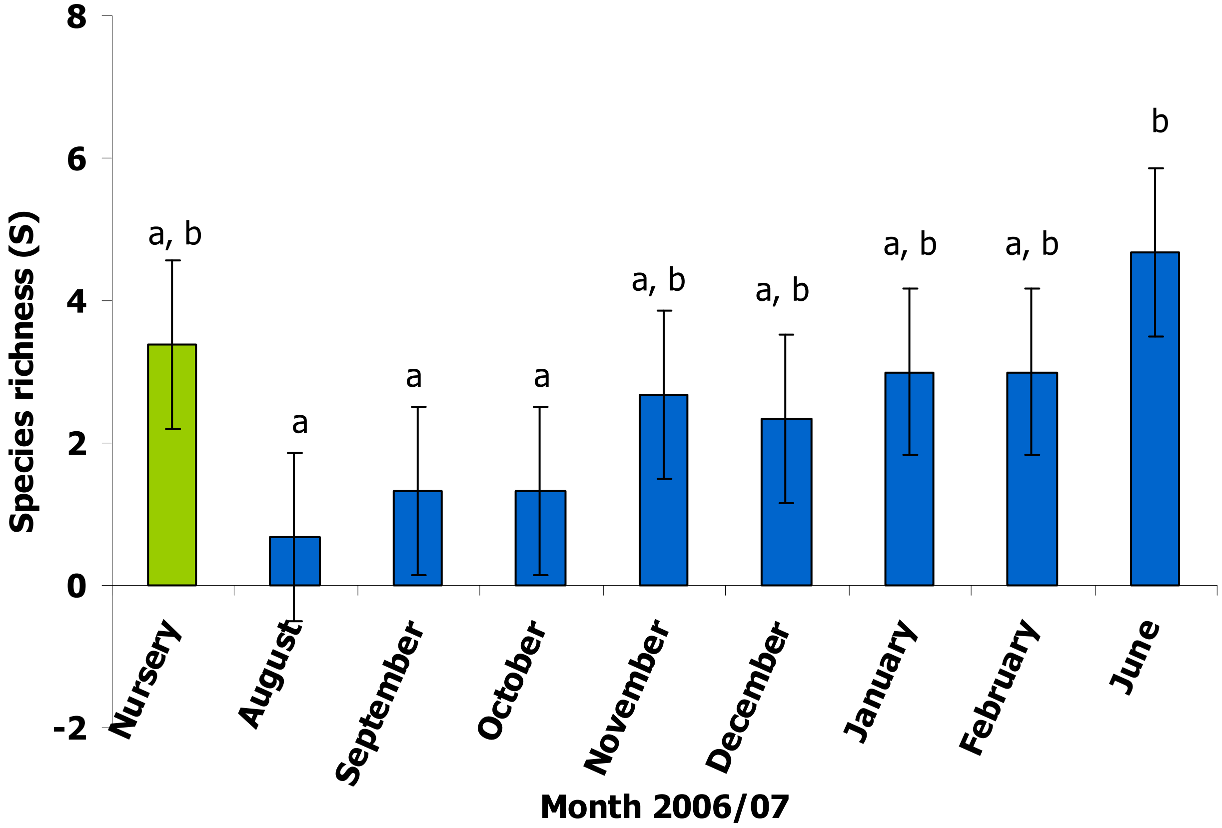
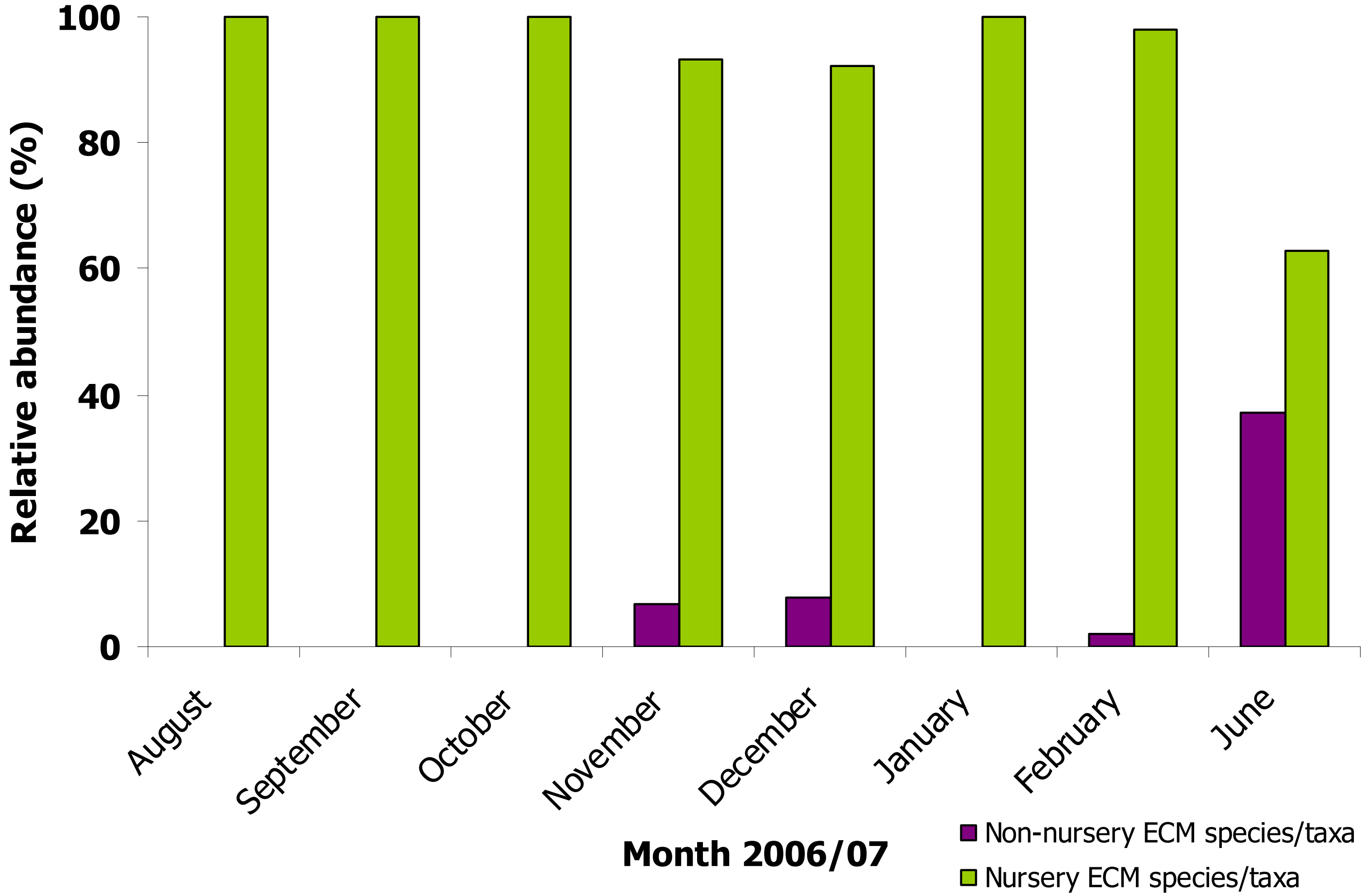
ECM species richness after outplanting



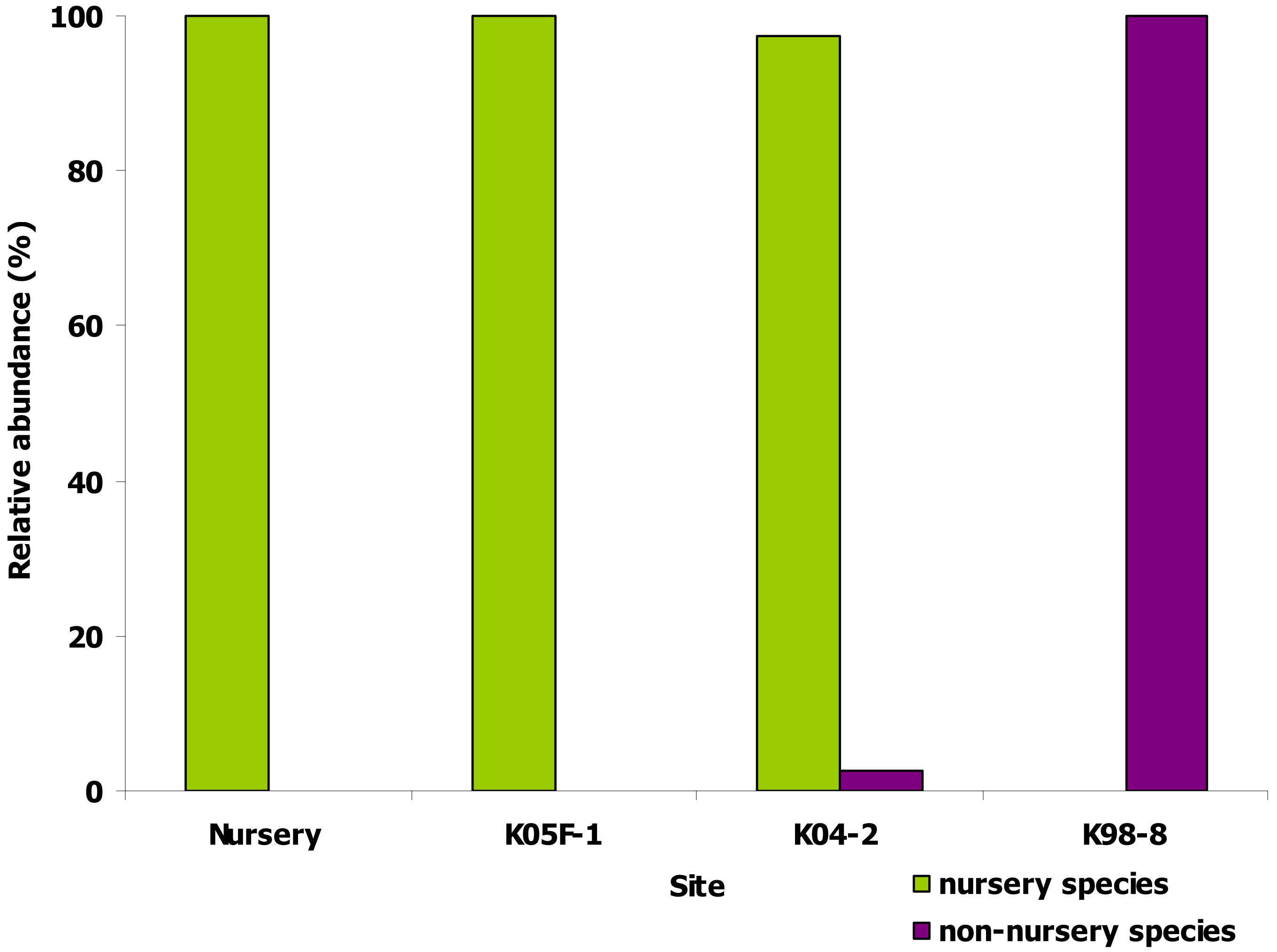
ECM richness declines but recovers within one year to a higher level than previously

Dominance of nursery ECM in 1st year



- All nursery ECM present in 1st year, dominant
- First non-nursery ECM 6mths after outplanting

Change from nursery to forest ECM



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- Kaingaroa Timberlands, Nigel Heron
- Alison Lister, James Ross
- www.dict.leo.org

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"excuse me

but I just have to
explode

explode this body
off me

wake-up tomorrow
brand new

a little tired

but brand new"

björk - pluto