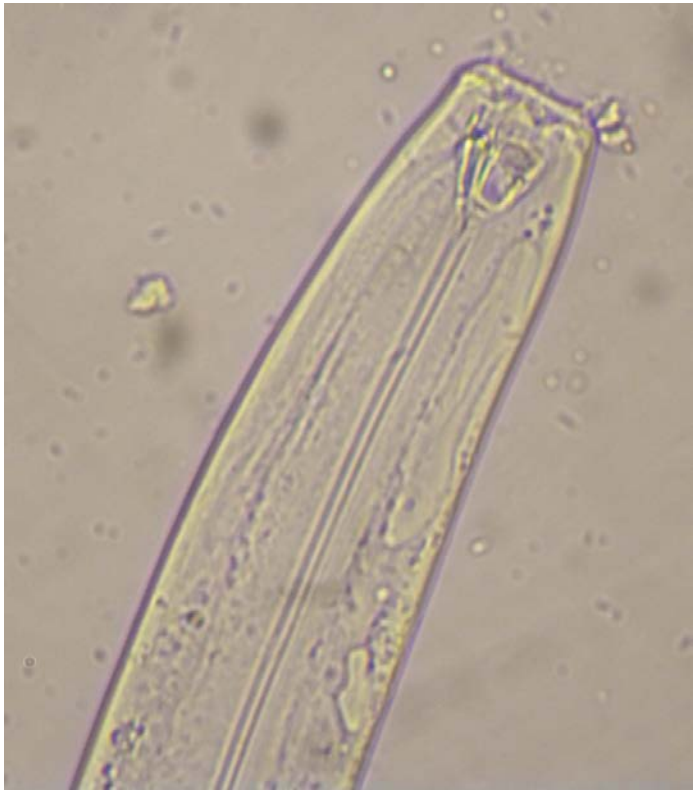
Uncovering nematode assemblages with molecular tools



Project AgriNem

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Mountain Agriculture Research Unit

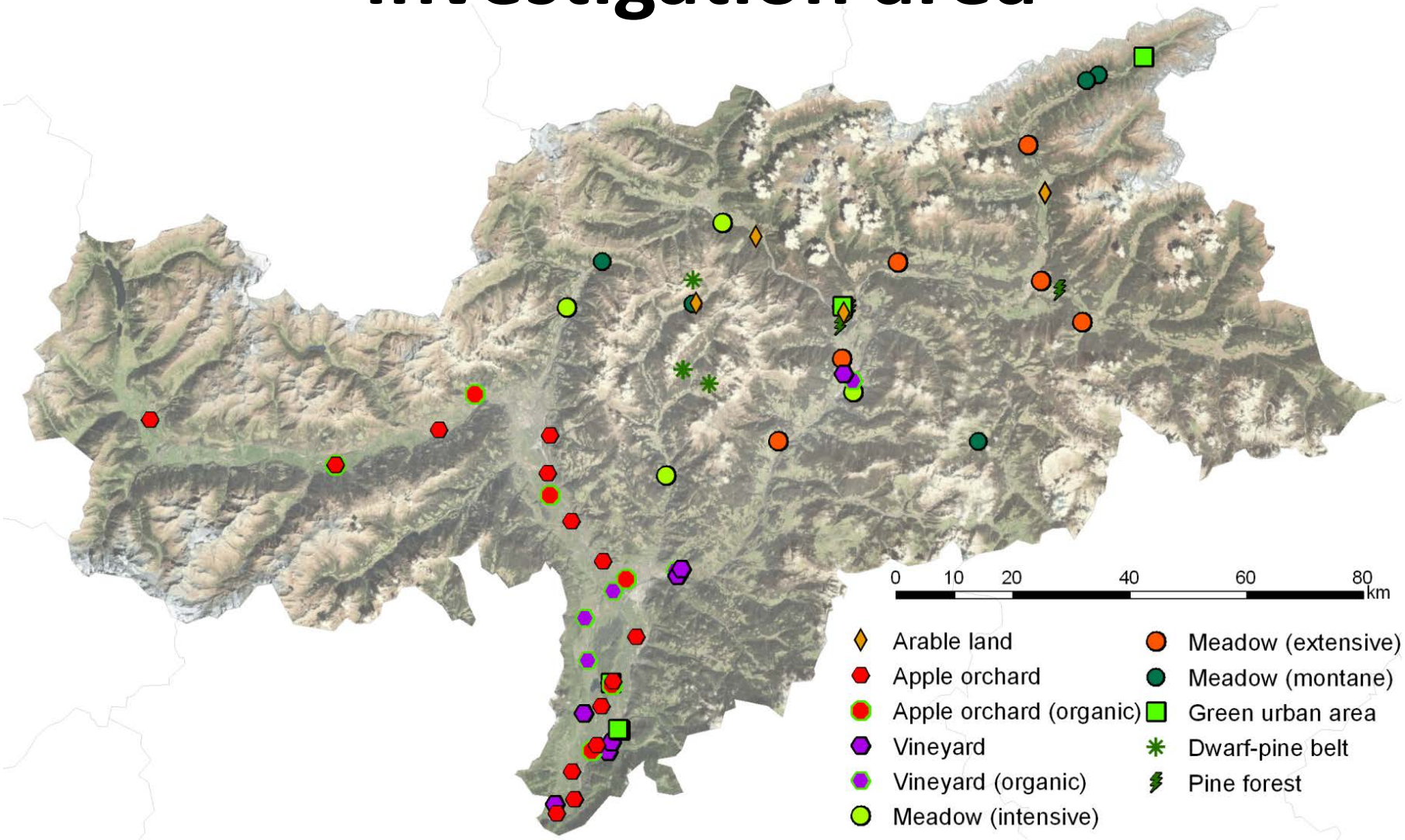
Annual Meeting - 28.02.2014

Aims of the AgriNem project

(i) Survey and assessment of the nematode diversity in soils of agricultural land-uses from South Tyrol

(i) Evaluation of the complementarity of morphological und molecular tools in biodiversity assessment.

Investigation area



Methods

Direct extraction of nematode DNA from soil

Use of commercial soil DNA kits to extract DNA and remove inhibitors with respect to the sample volume

Detection of nematode DNA

Quantitative real-time PCR with specific primers

Soil DNA kit test

Is it possible to extract nematode DNA directly from soil samples?

Are inhibitors successfully removed?

Is it necessary to dilute the DNA-extracts?

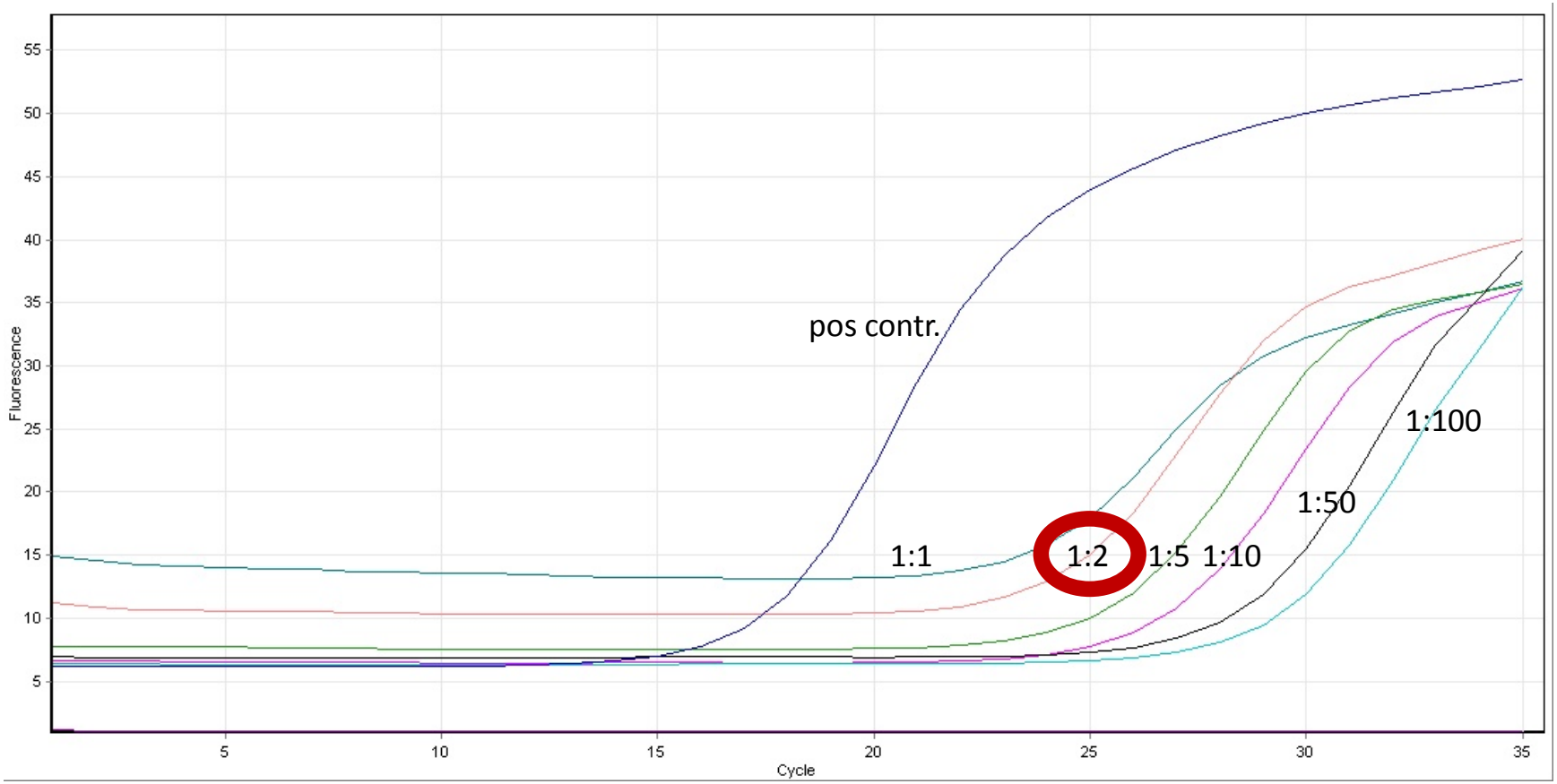
Does the extract represent the nematode community of the sampling site?

Efficiency of soil DNA kits

Comparison of six different extraction kits with a meadow soil sample, two replications and the spiking with *Drosophila nigrosparsa*:

Soil DNA kit	*D. nigrosparsa*	General nematodes
Nucleospin Soil	✓	
Precellys	✓	✓
MoBio Power Lyzer	✓	
MoBioPower Soil	✓	
MoBio Power Max	✓	
E.Z.N.A. Mag Bind Soil	✓	✓

Extract dilution test



Family detection

Nine families were tested:

Family	Trophic guilde
Alaimidae	Bakterial feeding
Aphelenchoididae	Hyphal feeding
Cephalobidae	Bakterial feeding
Dorylaimidae 1	Plant parasite
Dorylaimidae 3	Omnivorous
Filenchus group 1	Hyphal feeding
Helicotylenchus	Plant parasite
Mylonchulidae 1	Predacious
Tylenchus	Algal-Lychen-Moss-feeding

➔ No amplification

Cloning and sequencing

Evidence of at least four different families out of 16 clones of one extract:

N1 = *Plectus minimus*

N8 = *Eucephalobus striatus*

N9 = *Meloidogyne hapla*

N13 = *Acrobeloides* sp. oder *Cephalobus* sp.

BUT also a proturan was found in the sample and the Cephalobidae family primer should have amplified the group N13

First results

Is it possible to extract nematode DNA directly from soil samples? **Yes**

Are inhibitors successful removed? **Yes**

Is it necessary to dilute the DNA-extracts? **1:2**

Does the extract represent the nematode community of the sampling site? **Yes**

Next steps

Selection of the extraction kit

Optimization of quantitative real-time PCR with family primers

Testing of the samples from the main sample set

Thank you for your attention!