Elemental composition of ectomycorrhizal rhizomorphs grown in contact with different minerals in forest soil

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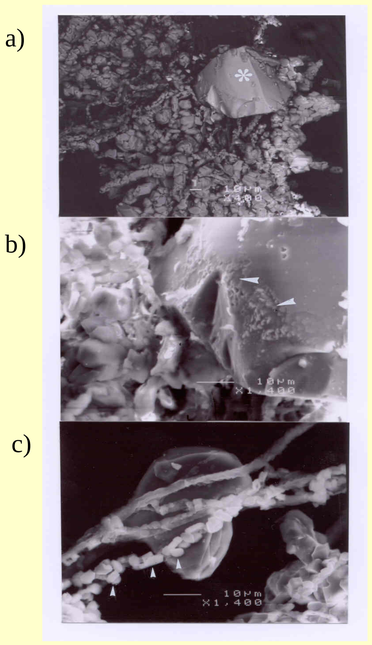
SUMMARY

• Fungal rhizomorphs connected to apatite were sampled both from a laboratory experimental system (*Rhizopogon sp*. and *Pinus Muricata*) and from mesh bags buried in forest soil in the field. The elemental composition of the samples was analysed with particle induced-X-ray emission (PIXE).

• Rhizomorphs connected to apatite contained larger amounts of Ca (mean ranges between 12-38 µg Ca g^{-1}, Table 1 and 2) than similar rhizomorphs connected to acid washed sand (range 0.3-3.5 µg Ca g^{-1}). Ca originating from apatite was deposited as calcium oxalate crystals on the surface of the rhizomorphs (Fig 2).

• EM mycelium produced in mesh bags had a capacity to mobilise 0.6-1.7 mg P kg^{-1} yr^{-1} from apatite-amended sand. A high concentration of K (up to10 mg K g^{-1}) in some rhizomorphs (*e.g. Suillus granulatus*) suggests that these fungi are good accumulators of K and may have a significant role in transporting K to trees.



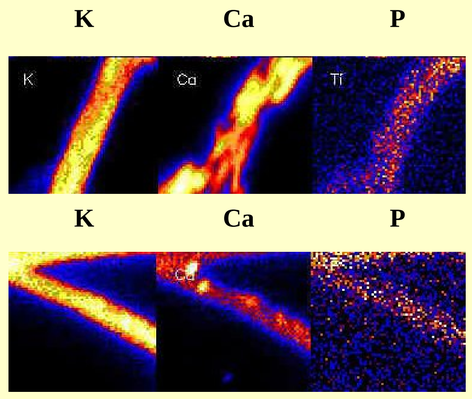


Figure 1. K, Ca and P distribution In *Rhizopogon* rhizomorphs connetcted to a) apatite or b) acid washed sand

Figure 2. A rhizomorph of *Rhizopogon* sp. with an embedded apatite particle (a, *). The apatite particle shows signs of erosion (b, arrowheads) and the hyphae are covered by calcium oxalate crystals (c, arrowheads)

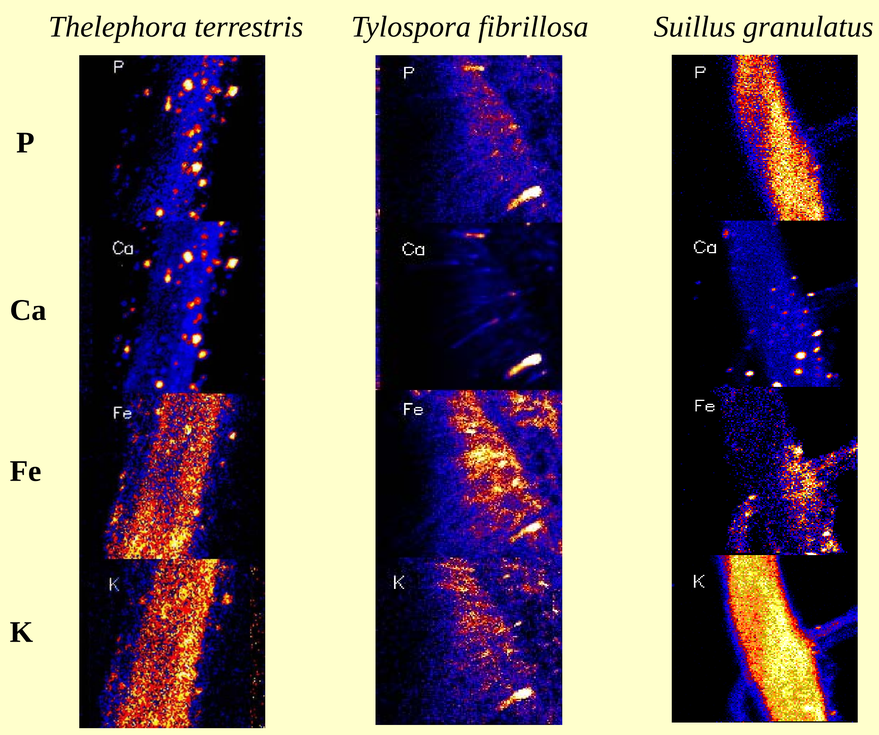


Figure 4. Elemental maps (Ca, P, Fe and K) of three different species of EM rhizomorphs collected from apatite amended mesh bags buried in forest soil. *Thelephora terrestris* and *Tylospora fibrillosa* had numerous particles embedded in the rhizomorphs that contained Ca and P (probably apatite particles). *Suillus granulatus* on the other hand had embedded particles which contained Ca alone (probably calcium oxalate crystals). *S. granulatus* contained large amounts of K suggesting that this fungus may be important in transporting K to trees. The other two species contained large amounts of Fe. The fungi were identified with PCR/RFLP analysis.

Location	N	P (mg g^{-1})	Ca (mg g^{-1})	K (mg g^{-1})
Outside sand	4	1.3±0.5	1.3±0.5 b	10.4±2.3
Outside apatite	4	1.0±0.1	**19±7.6 a**	5.5±1.4
Inside apatite	4	0.6±0.1	**28±12 a**	5.0±1.5
ANOVA (p-value)		0.13	0.02	0.2

Table 1 Elemental content (mean±SE) of rhizomorphs of *Rhizopogon* (isolate 2272, M. Biderthanto, Berkeley) grown in contact with acid washed sand or in contact with apatite. Samples of rhizomorphs were taken outside or inside a container with or without apatite. One-way ANOVA was performed on log-transformed values and LSD was used to separate the means.

Methods
PIXE analysis was performed with the Lund Nuclear Microprobe (Pallon et al. 1999). Protons at an energy of a few MeV are focused with magnetic lenses to a micrometer sized beam, which is scanned across the sample. At each focus spot an elemental analysis may be performed and from the collected data elemental maps of sample composition produced. PIXE is analogous to EDX analysis, but its sensitivity is much higher and easily reaches ppm-level. Scanning Transmission Ion Microscopy (STIM) was performed at the same time as PIXE analysis, to determine the areal mass density of the sample. STIM is based on the detection of proton energy loss when passing through the sample.

SEM analysis
Rhizomorphs were carbon coated and micrographs taken with a JEOL JSM 6400 electron microscope equipped with a back-scattered electron detector and a LINK EDS analysis unit.

Molecular analysis
To estimate the identity of the fungal rhizomorphs collected from mesh bags (Wallander et al. 2001), DNA was extracted and amplified by the polymerase chain reaction (PCR) using the ITS1 and ITS4 primers. Restriction fragment length polymorphism (RFLP) analysis of the PCR products was conducted and ITS–RLFP patterns were compared with those in a reference library of identified EM fruit bodies.

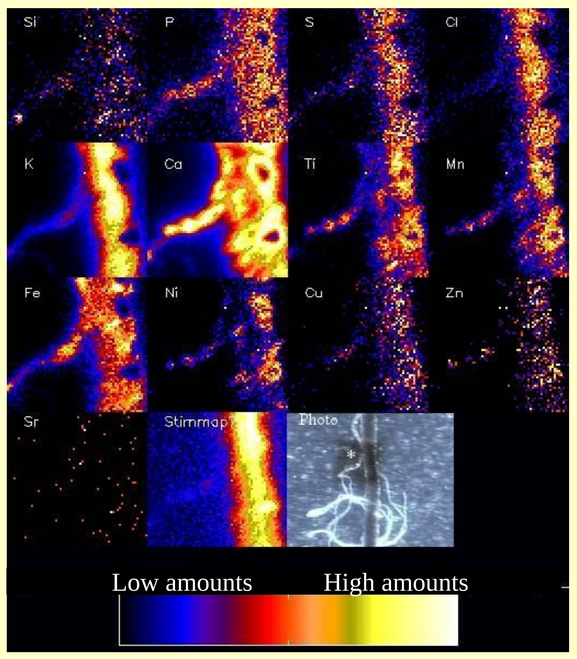


Figure 3 Elemental maps (Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn, Sr) and estimates of the mass (Stimmap7) of a brown rhizomorph collected from an apatite amended mesh bag in a Norway spruce forest. A photograph (photo) of the sample is shown at the bottom. Note the high concentration of Ca in the thinner part of the sample (60 mg Ca g^{-1}, to the left) compared to the thicker part of the rhizomorph (20 mg Ca g^{-1}, to the right). The part of the sample that has been scanned with PIXE is shown as a dark square in the photo image (*).

Background
Many fungi are known to interact with minerals in the soil. The main mechanism by which ectomycorrhizal fungi release nutrients from minerals is thought to be by the production of organic acids, especially oxalic acid which can form complexes with metals from the minerals. In the present study fungal rhizomorphs connected to different minerals were analysed with SEM (scanning electron microscopy) and PIXE (particle induced X-ray emission). With PIXE technique it is possible to quantify the amount and distribution of elements in small samples such as fungal rhizomorphs.

Location	Rhizomorph	Mineral	N	P (mg g^{-1})	Ca (mg g^{-1})	K (mg g^{-1})
Ignaberga	White	Sand	2	0.8±0.3 b	0.6±0.04 b	1.1±0.1 bc
Ignaberga	White	Apatite	2	2.3±0.9 a	**38±17 a**	0.4±0.1 c
Ignaberga	Brown	Sand	3	0.4±0.2 bc	0.9±0.3 b	2.1±0.2 b
Ignaberga	Brown	Apatite	3	0.9±0.3 ab	**13±7 a**	3.1±1.3 b
Ignaberga	Brown	Biotite	3	0.4±0.2 bc	0.5±0.1 b	1.5±0.05 b
Skogaby	White	Sand	2	0.24±0.03c	0.3±0.06 b	3.8±1 b
Skogaby	White	Apatite	2	1.1±0.2 ab	0.7±0.4 b	**10±1 a**
Dynaboda	Brown	Apatite	3	1.2±0.2 ab	15.2±2.8 a	2.3±0.4 b
ANOVA (p-value)				**0.007**	**0.0001**	**0.009**

Table 2 Elemental content (mean±SE) of rhizomorphs collected from mesh bags buried in forest soil in three different Norway spruce forests in southern Sweden. The mesh bags were filled with acid washed sand or sand amended with apatite or biotite One-way ANOVA was performed on log-transformed values and LSD was used to separate the means.

References
Pallon J, Elfman M, Kristiansson P, Malmqvist K, Granéli E, Sellborn A, Karlsson C. 1999. elemental analysis of single phytoplankton cells using the Lund nuclear microprobe. Nuclear Instruments and Methods B158: 312-316

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