Data Repository Item 2009144

Methods

Organisms and experimental set-up.

Seeds of Scots pine (*Pinus sylvestris*) were surface sterilized in 30% H_2O_2 for 25 min, washed with sterile distilled water and transferred aseptically to water agar plates (7.5 g L^{-1}). Meanwhile, *Paxillus involutus* (Mich. Ex Pers.; Coker & Cough) was grown in a sterile square petri dishes (10×10×2 cm) on cellophane covered 1/10 strength, modified Melin-Norkrans (MMN) nutrient agar (Table DR2). After four week of growth, Scots pine seedlings were transferred aseptically to the petri dishes, with the shoot protruding through a slot along the top side of the dish. The hole was covered with autoclaved lanolin and the root system compartments were sealed with two layers of parafilm to prevent contamination by other microorganisms and covered in aluminium foil to protect the roots and fungus from light. After 10 weeks the roots of the tree seedlings were well-colonized by their fungal symbiont and ready for transfer to the experimental microcosms. These comprised another sterile square Petri dish, containing 80 mL nutrient agar, based on Rorison's nutrient solution (Table DR2). A sheet of acid washed and autoclaved cellophane was laid over the agar, and the system was incubated for at least one week at 25º C to check for microbial contamination at this stage. In systems confirmed to be sterile, an additional 20 mL of nutrient agar was poured on top of the cellophane and before it set 50 g of HCl-washed, rinsed and autoclaved, perlite (inert amorphous volcanic glass) of 2.0- 2.4 mm grain size were sprinkled over the agar to form a monolayer.

A well-colonized tree seedling was transferred aseptically into the sterile experimental microcosm. The roots were spread evenly over the perlite layer, with the shoot protruding outside the dish through a hole cut in the topside and the microcosms were sealed with sterile lanolin. Freshly cleaved biotite flakes (~ 0.5×1 cm) were autoclaved for 20 min at 121ºC and laid flat on the perlite grains. The microcosm system was incubated for 19 weeks in a climate controlled room at 15ºC day and 10ºC night temperature, with an 18 hours photoperiod (550 μmol m^{-2} s^{-1} irradiance) with the shoots exposed to 80% humidity. The biotite was from Moen, Norway (X01481), purchased from Krantz Company, Bonn (Germany). The bulk chemical composition of the biotite was determined by Electron Microprobe Analysis (EPMA - Cameca SX50 equipped with three wavelength detectors and an Oxford Instruments INCA 250 EDX system) operated at 15 kV and 15 nA (Table DR1).

Preparation of FIB lamella

Preparation of cross-sections was performed in the Institute for Materials Research (University of Leeds), using a dual beam FEI Nova 200 NanoLab system equipped with a Field Emission Gun Scanning Electron Microscope, (FEG-SEM) for high resolution imaging and a Gallium source Focused Ion Beam (FIB) for milling of the sections. A biotite flake colonized by mycorrhizal mycelium connected to the plant, but not experiencing any contact with any roots was retrieved from an experimental microcosm on the day of the cross section preparation by FIB. Samples were first observed using an Environmental Scanning Electron Microscope (ESEM) (Philips XL30 in “wet” mode operating at 30kV, 4°C and 4-5 Torr chamber pressure of H_2O) in order to determine the

hydrated state of the fungi-biotite interface. Subsequently the biotite flake was sputter coated with a 3 nm platinum-palladium layer and imaged using the FEG-SEM capability of the dual beam FIB system. No difference in the aspect ratio, shape or width of the hyphae were visible between ESEM ("wet" mode) and FEG-SEM (under high vacuum) observations suggesting that dehydration under high vacuum conditions did not cause a shrinkage of the hypha on the mineral surface (Figure DR2).

Avoiding cracks and crystals boundaries visible at the surface of the biotite, specific areas of interest were selected for FIB sectioning (Figure 1B in main text). To protect the upper side of the lamella from Gallium-ion damage, prior to sectioning an additional Pt-layer (about 0.5 μm in thickness) was deposited onto the milling region using a Pt-organic gas injector (Gaseous Injection System, GIS) at a typical ion beam current of 30nA (Figure DR1a). Trenches were first milled on either side of the Pt strap with a high ion beam current (20 nA) to reach approximately 5 μm in depth (Figure DR1b). After tilting the sample stage, the lamella was cut free along the bottom and side edges of the thin section, although it remained attached to the biotite flake at the top of each side. Using a chamber mounted Kleindieck micromanipulator; a tungsten microprobe was brought into contact with the FIB cross-section and subsequently welded onto the lamella by a thin Pt strap (Figure DR1c). The top sides of the cross-sectional lamella were then cut and *in-situ* lift-out of the cross-section was performed. The average dimensions of the cross sections were 12 μm in length and 4 μm in depth. After translating the sample stage, the FIB lamellas were welded onto the Omniprobe Transmission Electron Microscopy (TEM) half-grid by two Pt straps on each side (Fig. DR1d). The Pt straps attaching the lamella to the tungsten microprobe were subsequently cut (Fig. DR1e). For

the final thinning and low angle polishing of the lamella, the ion beam current was reduced stepwise (30 nA to 10 nA to 50 pA and finally 30 pA) resulting in an ultra-fine region of around 80-90 nm thickness centered at the biotite-fungi interface allowing for observation using transmission electron microscopy (Figure DR1f). All FIB processing was carried out at 30 kV and FEG-SEM imaging at 5 kV.

HR-TEM observations and analysis

FIB cross-sections were imaged using a Philips CM 200 Field Emission Gun (FEG) transmission or scanning transmission electron microscope (TEM/ STEM) operated at 200 kV and equipped with an ultra thin window Energy Dispersive X-ray detector (EDX, Oxford Instruments) and a GIF200 (Gatan) imaging filter. Samples, consisting of two cross sections per half grid, were placed into a double tilt TEM sample holder allowing for the optimal alignment of the fungi-biotite interface with respect to the electron beam. Selected area electron diffraction (SAED) patterns were obtained with the smallest selected-area aperture available (180 nm in diameter). EDX linescans and maps were performed in STEM mode using a spot size of 5 nm, which represented the best compromise between spatial resolution and signal. The EDX line profiles were recorded along a 300 nm (4 nm step size) perpendicular line traversing the fungi-biotite interface and with the FIB lamella oriented such that the interface was as close as possible to being "edge-on" (i.e., with the incident beam perpendicular to the section) in order to minimize geometric aberrations leading to overestimation of the interface thickness. For the elemental line profiles, the intensities of the Kα X-ray lines of Si and K were monitored as a function of probe position. Total typical acquisition times over the whole profile

length were around 10 minutes. During a preliminary study (data not shown), the effect of beam damage on the FIB lamella chemical composition was shown to be negligible for the first 30 minutes of continuous exposure using the beam settings of the STEM-EDX linescans described above.

XANES measurements

Scanning transmission X-ray microscopy (STXM) was used to perform high spatial and energy resolution X-ray absorption near edge structure (XANES) spectroscopy at the Fe $L_{2,3}$-edges (in the energy range 700 to 730 eV) on FIB lamella 2 and the control lamella. The XANES measurements were performed on the PolLux beamline of the Swiss Light Source (SLS) at Villigen, Switzerland. The SLS synchrotron storage ring was operated at 2.4 GeV and 400 mA current in a top-up mode during data collection. STXM is a transmission microscopy technique using a monochromated X-ray beam produced by synchrotron radiation. The energy of the X-ray beam on the PolLux STXM can be varied by less than 0.1 eV increments over a wide energy range (200–1200 eV). The beam is focused on the sample using a condenser zone plate and a 2-D image is collected by scanning the sample stage at a fixed photon energy with a spot size of 30-40 nm. The image contrast results from differential absorption of X-rays, which depends on the chemical composition of the sample.

Scans were recorded by scanning linearly over selected areas of the sample which were orientated perpendicular to X-ray beam direction at energy increments of 0.1 eV over the energy range of interest (700 to 730 eV). The stack image scan procedure consists of measuring the XANES spectrum for Fe on each pixel (one pixel can be as small as

30 nm). Counting times are of the order of few milliseconds or less per pixel. Normalization and background correction of the Fe $L_{2,3}$-edge NEXAFS spectra were performed by dividing each spectrum by a second spectrum from a region without sample material. AXis2000 software (ver2.1n)[1] was used to align image stacks and extract XANES spectra from image stack measurements.

[1]Hitchcock, A.P., 2000. AXis2000 software (ver2.1n) http://unicorn.mcmaster.ca/aXis2000.html

Figure DR1: Succesive steps in the preparation of the FIB lamella: (**A**) deposition of a platinum protective strap at the surface of the biotite colonized by fungi; (**B**) ion milling of the trenches on both sides of the lamella; (**C**) welding of the micromanipulator onto the lamella;(**D**) mounting of the FIB lamella on the dedicated TEM half-grid; (**E**) view of the FIB lamella before thinning (dashed lines represent the regions to be thinned) and (**F**) FIB lamella after final thinning of the central region.

Figure DR2: Comparison of the same hypha imaged in "wet mode" by ESEM (100% relative humidity) and in FEG-SEM mode (high vaccum) indicating that the aspect ratio or width of the attached hypha was not affected by sample handling in vacuum.

Figure DR3: STEM EDX Si and K elemental line profiles across the interface of the section with the longest exposure (lamella 3). Note that the interface is marked as 0 nm. Elemental line profiles exhibited an increase from the background level in the hypha to a maximum in the biotite with an interfacial boundary width of ~ 25 ± 4 nm for the Si profile and 50 ± 4 nm for the K profile, indicating that the topmost part of the biotite in contact with the fungi was depleted in K.

Table DR1: Biotite chemical composition in oxide weight percent, O by stoichiometry (average of 25 analysis with standard deviation in brackets) determined by EPMA.

Oxide	**Wt %**
SiO_2	35.12 (0.22)
TiO_2	3.32 (0.30)
Al_2O_3	16.23 (0.22
Cr_2O_3	0.03 (0.04)
FeO	24.73 (0.42)
MnO	0.83 (0.08)
MgO	6.56 (0.18)
CaO	0.02 (0.01)
Na_2O	0.12 (0.03)
K_2O	9.23 (0.21)
H_2O	3.32 (0.07)
F	1.04 (0.13)
Cl	0.11 (0.02)
Total	100.68 (0.40)

Table DR2: Chemical composition of inoculation medium (cultivation stage) and in the agar perlite microcosms.

	Inoculation **Melinin-Norkrans**	**Microcosm** **Rorison's agar**
	Concentration in mmol L^{-1}	
$CaNO_3$	-	2.0
$MgSO_4$	6.0×10^{-2}	1.0
K_2HPO_4	$3.7\ 10^{-1}$	1.2
$(NH_4)_2SO_4$	-	8.0×10^{-2}
FeEDTA	-	2.0
$MnSO_4$	-	9.0×10^{-3}
H_3BO_4	-	5.0×10^{-2}
$(NH_4)_6Mo_7O_{24}$	-	1.5×10^{-4}
$ZnSO_4$	-	1.5×10^{-3}
$CuSO_4$	-	1.5×10^{-3}
$(NH_4)_2HPO_4$	1.9×10^{-1}	-
$CaCl_2$	4.3×10^{-2}	-
$FeCl_3$	2.2×10^{-2}	-
	Concentration in g L^{-1}	
Malt extract	10.0	-
Glucose	1.0	-
Agar	15.0	10.0

Figure DR1 (Data Repository)

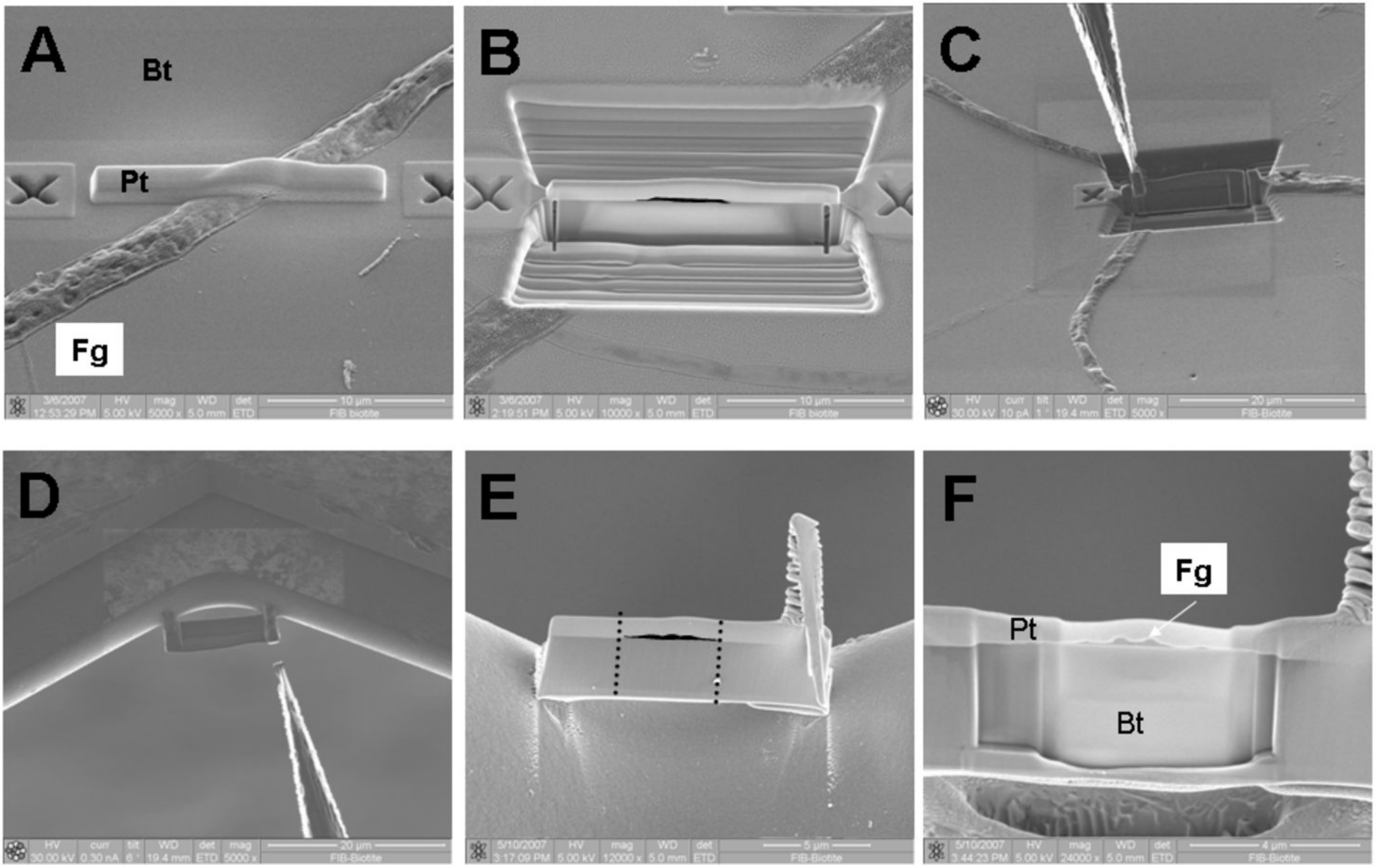

Figure DR2 (Data Repository)

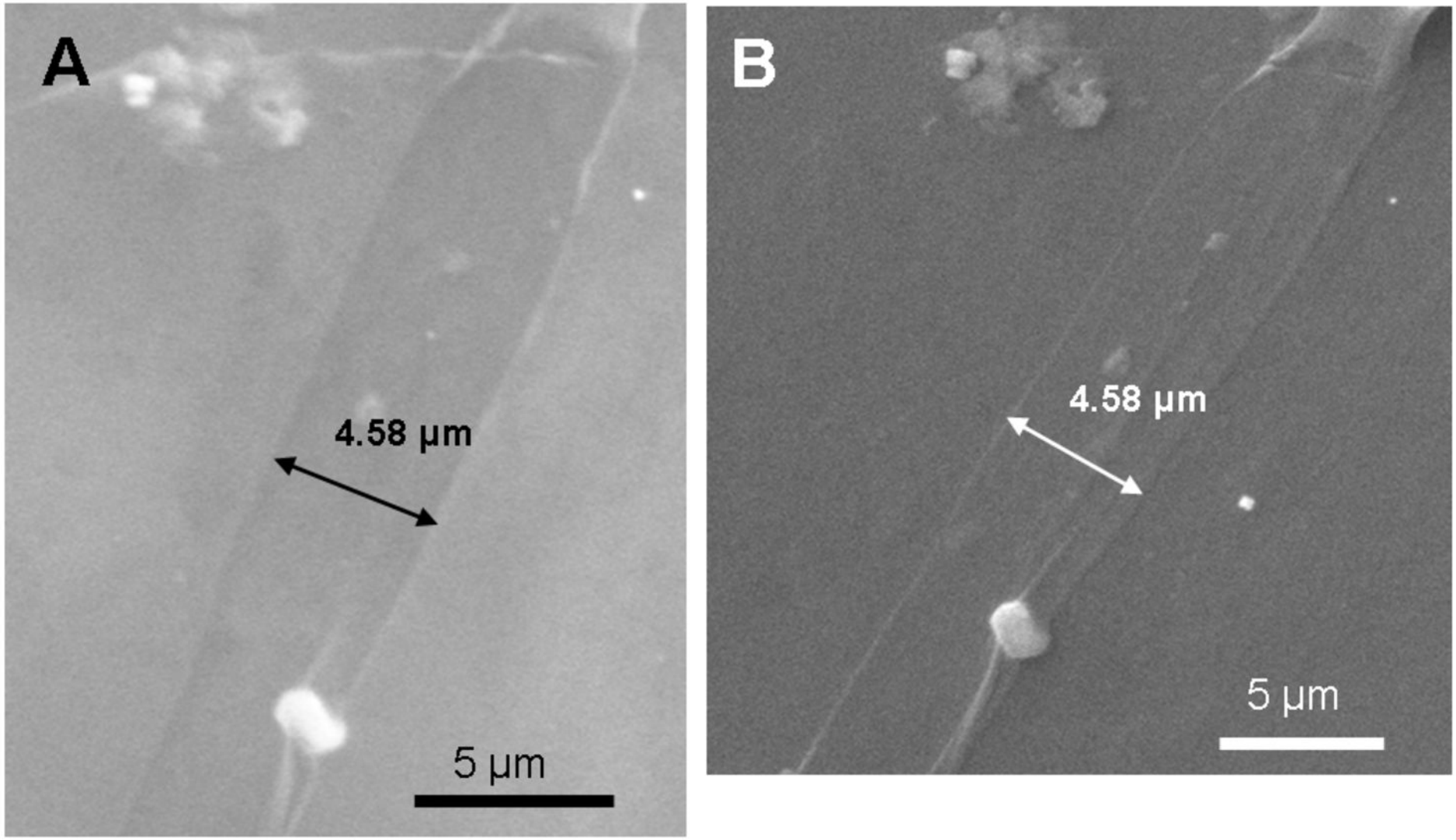

Figure DR3 (Data Repository)