1 Meshes in mesocosms control solute and biota exchange in soils: a step towards disentangling
2 (a)biotic impacts on the fate of thawing permafrost

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Abstract

Environmental changes feedback to climate through their impact on soil functions such as carbon (C) and nutrient sequestration. Abiotic conditions and the interactions between above- and belowground biota drive soil responses to environmental change but these (a)biotic interactions are challenging to study. Nonetheless, better understanding of these interactions would improve predictions of future soil functioning and the soil-climate feedback and, in this context, permafrost soils are of particular interest due to their vast soil C-stores. We need new tools to isolate abiotic (microclimate, chemistry) and biotic (roots, fauna, microorganisms) components and to identify their respective roles in soil processes. We developed a new experimental setup, in which we mimic thermokarst (permafrost thaw-induced soil subsidence) by fitting thawed permafrost and vegetated active layer sods side by side into mesocosms deployed in a subarctic tundra over two growing seasons. In each mesocosm, the two sods were separated from each other by barriers with different mesh sizes to allow varying degrees of physical connection and, consequently, (a)biotic exchange between active layer and permafrost. We demonstrate that our mesh-approach succeeded in controlling 1) lateral exchange of solutes between the two soil types, 2) colonization of permafrost by microbes but not by soil fauna, and 3) ingrowth of roots into permafrost. In particular, experimental thermokarst induced a ~ 60% decline in permafrost nitrogen (N) content, a shift in soil bacteria and a rapid buildup of root biomass (+ 33.2 g roots m⁻² soil). This indicates that cascading plant-soil-microbe linkages are at the heart of biogeochemical cycling in thermokarst events. We propose that this novel setup can be used to explore the effects of (a)biotic ecosystem components on focal biogeochemical processes in permafrost soils and beyond.

Keywords: faunal inoculation, field incubation, lateral soil connection, nitrogen, root, bacterial community

1 Introduction

The growing recognition of the importance of soils for biodiversity, climate regulation and carbon (C) sequestration (Baveye et al., 2016) has highlighted the need to understand how environmental changes affect soils. Arctic soils may form a particularly important case, since they store vast amounts of C (e.g. in permafrost) that, if released to the atmosphere due to shifting environmental conditions, could significantly enhance climate warming (Schuur et al., 2015). Importantly, this soil C-climate feedback is a key uncertainty in future climate projections (Todd-Brown et al., 2014) and to increase their accuracy we must unravel how changes in the abiotic soil environment and the interactions between above- and belowground biota will affect soil C and nitrogen (N) cycling (Bardgett et al., 2013; De Vries and Shade, 2013). Field experiments, in which soils are warmed with, for example, greenhouses or by manipulating snow depth (e.g. Dorrepaal et al., 2009; Weedon et al., 2012; Väisänen et al., 2019), realistically assess ecosystem-level responses to environmental changes, but provide often only a limited mechanistic understanding, resulting in varying context-dependent responses (Wardle et al. 2004). Laboratory incubations on the other hand, allow for explicit linking of the activity of particular soil organisms to processes (e.g. Briones et al., 2014), but at the same time greatly oversimplify the soil system and the environment. Filling this gap between controlled yet over-simplified laboratory incubations and realistic but less-controlled field studies could advance our understanding of biotic interactions in soil and ecosystem process responses to environmental changes.

Field-deployed mesh bags filled with plant litter are widely used to assess the effects of specific biotic interactions on the initial stages of organic-matter decomposition (Eno, 1960). Varying mesh sizes of litterbags can control the access of differently sized groups of decomposers, such as macro- or micro-invertebrates and fungi, to litter and thus control their contribution to organic matter decomposition (Cornelissen et al., 2007; Makkonen et al., 2012). With a similar

rationale, plant belowground production and the roles of roots and root-associated mycorrhizae on soil biogeochemistry are studied with soil bags that control root in-growth (Steingrobe et al., 2001; Neumann and Matzner, 2013). These mesh approaches can provide control over the biotic components in order to assess their roles in ecosystem processes under realistic conditions (Johnson et al., 2001, 2005; Heinemeyer et al., 2006, 2007; Morrison et al., 2017). Such mesocosm-mesh approaches could be used to study and unveil ecological interactions among soil chemistry, decomposer organisms and roots in the thawing permafrost soil.

In thawing permafrost, soil temperature and aeration are important controls of decomposition (Schädel et al. 2016) but other soil abiotic and biotic drivers could play additional significant roles. For example, permafrost contains large pools of bioavailable N (Keuper et al., 2012; Salmon et al., 2018) that upon thawing could remain in the permafrost or, alternatively, be lost via leaching and/or gaseous emission (Abbott and Jones, 2015). Nitrogen forms an essential component of extracellular enzymes that microorganisms synthesize to catalyze the decomposition of organic polymers (Schimel and Weintraub, 2003) and drives microbial C use-efficiency – *i.e.* the ability of microorganisms to incorporate C into their biomass instead of respiration (Manzoni et al., 2012). Declines in permafrost N upon thaw could therefore contribute to the mineralization and stability of permafrost C.

Permafrost decomposer biota is low in biomass and diversity (Mackelprang et al., 2017). Thus, the gradual invasion of microorganisms and soil invertebrates from seasonally thawing active layer soil to thawing permafrost could alter the decomposition of permafrost-C via increased decomposer biomass or by alleviating functional limitations of the permafrost decomposer community (Monteux et al., 2018). How rapidly and to what extent such colonization occurs could control C cycling in thawing permafrost and interact with the possible changes in permafrost N content.

Plant roots growing into thawing permafrost can take up permafrost bioavailable N inducing increased plant biomass (Keuper et al., 2017; Blume-Werry et al., 2019), and potentially simultaneously increasing availability of root exudates while decreasing N availability for permafrost microbes. Increased plant productivity might result in higher permafrost-C decomposition rates through rhizosphere priming effects (Wild et al., 2016). Roots may simultaneously contribute to soil C pools in permafrost affected soils via input of root litter (Blume-Werry et al., 2019).

To improve our mechanistic understanding and facilitate future research on the impact of environmental changes on soil processes in permafrost ecosystems we designed mesocosms fitted with different pore-size meshes. With this novel setup, we aimed to specifically manipulate the lateral exchange of solutes (0.5 μm mesh), soil microbes and invertebrate fauna (20 μm mesh, fauna inoculation) and roots (2 mm mesh) via the soil from the seasonally thawing active layer peat into the thawed permafrost (and implicitly vice versa) while exposing the permafrost to ambient field conditions. This lateral connection simulates permafrost thaw-induced soil subsidence, thermokarst (Olefeldt et al, 2016). We hypothesize that: 1) Solute exchange through a 0.5 μm mesh between active layer and permafrost soil alters permafrost chemistry relative to permafrost incubated in the field without any connection to active layer peat (i.e. hermetic barrier). 2) Microbial colonization through a 20 μm mesh barrier increases microbial biomass and alters bacterial community composition in permafrost soil relative to a 0.5 μm mesh barrier, through which (most) bacteria cannot actively pass. 3) Soil fauna inoculation into fauna-free permafrost soil (i.e. 20 μm mesh barrier) increases soil fauna abundance and alters fauna community relative to no inoculation. 4) Root ingrowth through a 2 mm mesh between active layer and permafrost soil allows roots and fauna to actively forage into permafrost soil relative to the case of passive diffusion and microbial-driven change of nutrient availability in thawed permafrost (i.e. 20 μm

mesh). Following assessment of the proposed technique, we briefly discuss the ecological implications of our findings for permafrost degradation and suggest additional research questions that could be addressed using this setup.

2 Material and methods

2.1 Experimental setup and the expected effects of different-sized meshes

The experimental setup was based on a mesocosm design, a custom-made chamber ($l=30$ cm, $w=20$ cm, $h=40$ cm) made from 8 mm thick clear, acrylic plastic with 5 mm drainage holes (covered with a 0.5 μ m and a 2 mm mesh) near the bottom of the chamber to enable water drainage (Fig. 1a). The mesocosm was split into two compartments of unequal sizes, $2/3$ (i.e. $20 \times 20 \times 40$ cm) and $1/3$ (i.e. $10 \times 20 \times 40$ cm), by an acrylic frame (2 mm thick, Fig. 1a). This frame was either completely solid or used to mount mesh barriers with one of three different mesh sizes (Nylon, PlastOK Ltd., UK) that formed our treatments of incremental physical connectivity between the two compartments (Fig. 1a, b).

Each mesh size was intended to allow for an exchange of specific abiotic and biotic ecosystem components. The treatments were: a solid/hermetic barrier, a 0.5 μ m mesh barrier, a 20 μ m mesh barrier, a 20 μ m mesh barrier accompanied by microarthropod inoculation, and 2 mm mesh barrier. The hermetic barrier does not allow any transfer into/from permafrost to active layer through the soil, but allows exposure to weather and airborne deposition. Such a scenario, i.e. permafrost thawing in isolation from active layer, can occur in thermokarst events, when thawed permafrost locates at a further distance from active layer, such as in thaw slumps (Abbott and Jones, 2015). The 0.5 μ m mesh barrier additionally allows transfer of soil solutes, while excluding the lateral movement of the majority of soil microorganisms (not microprokaryotes, Courties et al., 1994). The transfer of solutes was expected to contribute to changes in soil chemistry with

permafrost thaw/thermokarst. The 20 μm mesh barrier allows movement of microorganisms smaller than 20 μm , including most bacteria and fungi (Mikusinska et al., 2013), which was expected to contribute to changes in soil microbial biomass and microbial community. As fauna and fine roots may have similar dimensions, the 20 μm mesh barrier was complemented by inoculation of microarthropods (springtails and mites). Soil microarthropods were collected on moist gypsum by live-extraction using the Tullgren-method (Van Straalen and Rijninks, 1982) from peat cores (diam. 12 cm, 8 cm depth) taken in mid-July 2015 from the same subarctic palsabog (Storflaket) where the mesocosm-soils were collected and the mesocosms were deployed (see 2.2 and 2.3). Microarthropods were transferred to the permafrost every 2–3 days (until no more animals came out of the peat core after approximately 2 weeks) by flushing the gypsum with 10 ml of demineralized water. The same amount of demineralized water without soil fauna inoculation was added to the other treatments. During flushing, additional elements, such as calcium, may have leached from the gypsum but soil pH was not higher in the inoculated treatment (5.50 ± 0.02 ; mean $\pm$ standard error, $n=6$) in comparison to the treatment with 20 μm mesh barrier (5.68 ± 0.09 ; mean $\pm$ standard error, $n=6$) in September 2015. As the surface area of the peat core (113 cm^2) equaled that of the permafrost compartment of the mesocosm (100 cm^2), microarthropods were assumed to be added in field densities (Krab et al., 2014). In addition to the transfers in soil solutes and microorganisms, the inoculated treatment was expected to contribute to changes in microarthropod biomass and community in the permafrost. The 2 mm mesh allows the growth of roots with a diameter smaller than 2 mm (often considered as fine roots) and movement of all soil fauna, such as enchytraeid worms (Cui et al., 2016) into the permafrost soil. Consequently, and in addition to all other treatment effects, the treatment with 2 mm mesh was expected to contribute to new root growth into permafrost and to induce increased fauna biomass and shift in faunal community.

2.2 Study area

The study area was in the Torneträsk region of subarctic northern Sweden, which has discontinuous and sporadic permafrost classified as Cryosols (FAO, 2001). The mean annual precipitation is 342 mm yr⁻¹ and mean annual air temperature is 0.3 °C (meteorological data 1991–2015, measured at 321 m a.s.l. at the Abisko Scientific Research Station: www.polar.se/abisko). Because the permafrost temperature in the area is very close to zero (Åkerman and Johansson, 2008; Johansson et al., 2011), and the average air temperature is above freezing, the study area is highly susceptible to permafrost thawing and subsequent permafrost collapse (Olefeldt et al., 2016). We chose an ombrotrophic palsa bog as the site for the experiment (Storflaket, 68°20.836' N, 18°58.398' E), where the mean active layer thickness in 2015 was 69.3 ± 3.7 cm (mean ± SE, n = 6, Monteux et al., 2018) and the water table was 30.2 ± 2.8 cm below the surface in October 2013 (Blume-Werry et al., 2019). The active layer soil is organic and classified as Histosols (FAO, 2001). The vegetation consists of low (<20 cm) evergreen and deciduous dwarf shrubs (*Empetrum nigrum* ssp. *hermaphroditum* L., *Andromeda polifolia* L., *Betula nana* L., *Vaccinium uliginosum* L.), forbs (*Rubus chamaemorus* L.) and sedges (*Eriophorum vaginatum* L.) above peat mosses (*Sphagnum fuscum* (Schimp.) H. Klingg; Johansson et al., 2013).

2.3 Permafrost and active layer and the mesocosm installation

Permafrost cores were taken with a diamond concrete drill bit (diam. 10.2 cm) mounted on an ice auger motor, from 70 cm below the surface to a maximum depth of 250 cm, at the experimental site in Storflaket (11 May – 15 June 2015). A thin outer layer of the permafrost cores was removed by scraping and the cores were rinsed with sterile water to minimize contamination by microbes from the above active layer soil, and stored at -18 °C. Before the experiment was set up, permafrost was slowly thawed at 2°C, homogenized and analyzed for selected properties before the start of

the mesocosm experiment (Table 1). Vegetated active layer sods with comparable vegetation cover (excluding *B. nana* due to the large size of local individuals) were excavated from the experimental site in Storflaket in late June 2015. Sods were cut to fit exactly into the larger mesocosm compartment and to 20 cm depth, which covers the main rooting zone at 5–15 cm below the surface (Iversen et al., 2015). The selected properties of active layer peat were analyzed from additional samples collected in Storflaket during the growing season 2015 (Table 1). We used the thawed permafrost and excavated active layer sods to fill the mesocosms (11–12 July 2015). We filled the larger compartment with a vegetated active layer peat sod up to 20 cm height and the smaller compartment with thawed permafrost (2351 ± 24 g; mean $\pm$ SE, $n = 45$) up to 20 cm height. Due to the practical challenges of obtaining large volumes of permafrost, the rear half of the smaller compartment (5×20 cm up to 20 cm height) was fitted with a Styrofoam block in a sealed plastic bag to fill up excess volume with inert material (Fig. 1c).

At the experimental site, we established nine spatial blocks over an area of 1 000 m². Each block was 10–15 m² and adjacent blocks were 2–10 m apart. Each block consisted of five mesocosms, representing the five different treatments, thus resulting in 45 mesocosms in total. Within each block, the mesocosms were fitted randomly into rectangular pits, excavated *c.* 20 cm deep on hummocks in a west-east direction, and adjusted so that the soil surfaces inside each compartment and outside the mesocosms were level. The treatment in which 20 μ m mesh was accompanied by inoculation of the permafrost with microarthropods (see 2.1) was started 8 days after setting up the field experiment. During winter (7 October 2015 – 15 April 2016), the mesocosms were covered with insulating gardening fleece, chicken wire and tarpaulin to protect the experiment from frost and animals, because of low snow cover in the area.

2.4 Soil pore water

At the onset of the experiment, rhizon samplers (Rhizosphere Research Products, The Netherlands) were installed (10 cm depth) for pore water sampling in the permafrost compartments in six blocks. Pore water was sampled four times during the frost-free period in 2016 into evacuated opaque syringes over a period of 24–48 hours, filtered (0.45µm, sterile nylon, Sarstedt, Germany) and frozen (-18°C). Unfrozen samples were analyzed for pH during the last sampling in 2016 (MP-220, Mettler-Toledo). Samples from all sampling campaigns were analyzed for total dissolved nitrogen (TN; TOC-VCPH equipped with Total N measuring unit TNM-1, Shimadzu) and for NH₄-N and the sum of NO₂-N and NO₃-N (FIA star 5000 Analyzer, FOSS). The results for each sampling time are reported in the supplements (Supplementary table S1) and were used to calculate the average pore water N over the frost-free period.

2.5 Mesocosm harvesting and measurements

In 2016, mesocosms were destructively harvested in mid-July (three blocks, 15 mesocosms, data not shown) and in mid-September (six blocks, 30 mesocosms). During both campaigns, harvesting and subsequent analyses followed the same protocols. Mesocosms were transported to the laboratory where harvesting was done in separate spaces for the active layer and permafrost to limit cross-contamination. The permafrost compartment was always processed first and the intact permafrost slab was carefully detached from the mesocosm and placed on an aseptic tray so that the mesh barrier side of the slab faced upwards. After this, the permafrost was divided into subsections that were subsequently assigned for different analyses.

For bacterial community analyses, soil (*c.* 2 g field moist weight) was collected into sterile 2 ml tubes from a location 5 cm below the top and at the side that had faced towards the mesh barrier, using ethanol-cleaned tweezers. The samples were immediately snap-frozen in liquid nitrogen, and stored at -80°C for two months until nucleic acid extraction followed by DNA- and

RNA-based profiling to obtain profiles of the active and total bacterial community (Ulrich et al., 2008; Baldrian et al., 2012; Weedon et al., 2017). RNA and DNA were co-extracted from soil (c. 1 g field moist weight) by the phenol:chloroform:isoamyl alcohol (Sigma Aldrich) method using the RNeasy PowerSoil RNA extraction kit (Qiagen) along with RNeasy PowerSoil DNA elution kit (Qiagen), following the manufacturer's instructions. First-strand complementary DNA was synthesized from the RNA extracts treated with DNase I, then the V3-V4 region of the 16S rRNA or rRNA gene was amplified (341F; 805R) and sequenced on an Illumina MiSeq using V3 chemistry (2x300bp). *De novo* 97% OTUs (Operational Taxonomic Units) were clustered and classified through a custom VSEARCH-QIIME 1.9.1 pipeline (Greengenes 13.8 taxonomy; Caporaso et al., 2010; McDonald et al., 2012; Rognes et al., 2016), after which OTU tables were created from averaging 100 rarefactions at 6000 reads per sample, and UniFrac dissimilarity matrixes were computed (Lozupone et al., 2011). More details are provided in Supplementary information 1, raw sequence data is deposited at EBI (project number PRJEB31437) and the bioinformatics script is available as a Jupyter notebook (https://bitbucket.org/smouteux/mesocosms_methods).

For measuring microbial biomass and abiotic soil properties, a column of soil ($l=5$ cm, $w=5$ cm) from the top of the permafrost (10 cm deep) was sampled, and the overlying thin crust layer (0.5 cm) was cut off. The samples were stored in plastic bags at 2°C and processed within 7–10 days after sampling. The permafrost columns were manually homogenized and all *Eriophorum* roots were collected (see further below). Then, a sub-sample (2 g field moist weight) was analyzed for soil moisture (+105°C, 16h) and loss on ignition (475°C, 4h). Across all treatments, the gravimetric moisture content in the permafrost soil was 57.4–77.1 % and organic matter content was 19.6–44.6 %. Sub-samples (7.5 g field moist weight) were used for water-based extraction in 50 mL MilliQ (2 h, 240 rpm), the extract filtered (0.45µm, sterile nylon Sarstedt) and analyzed for

dissolved organic C (DOC) after acidification (TOC-VCPH equipped with Total N measuring unit TNM-1, Shimadzu). Another set of sub-samples (7.5 g field moist weight) was extracted and analyzed for DOC after chloroform fumigation (20–22 h, Brookes et al., 1985). Microbial biomass C was calculated as ‘DOC_{fumigated}’ – ‘DOC_{soil}’, and the difference divided by 0.49 to correct for extraction efficiency (k_{EC} , Joergensen, 1996).

For measuring soil fauna, two different-sized soil columns were cut from the permafrost slab, one for microarthropods (identified to broad functional groups, Collembola, Oribatid mites, ‘predatory mites’, and ‘nymphs/other mites’) and another one for enchytraeids. A column of soil ($l=5$ cm, $w=3.5$) from the top of the permafrost (8 cm deep) was used for extracting microarthropods into 70% ethanol (Tullgren-extraction, Van Straalen and Rijninks, 1982) and another soil column ($l=5$ cm, $w=5$ cm) again from the top (8 cm deep) was used to extract enchytraeids into water (a modified O’Connor extraction, O’Connor, 1955). Fauna were counted under a dissecting microscope and the densities (individuals m^{-2}) of microarthropod groups were used to determine relative abundances (% of total microarthropod density). No enchytraeids were found from the permafrost soil columns. After extractions, soil columns were homogenized and roots of *Eriophorum* were collected (see further below).

All treatments were searched for plant roots even though roots were expected to occur only in the 2 mm mesh treatment. *Eriophorum vaginatum* roots were collected throughout the entire permafrost sample (*i.e.* in all subsamples) and pooled together. In addition, a 20 cm deep column of soil ($l=5$ cm, $w=2$ cm) was sampled from each treatment and searched for the very fine “glass” roots for at least 30 min up to a maximum of 2 hours per sample. *Eriophorum* roots and glass roots were dried (60°C, 72 h and 24 h, respectively) and the dry weights standardized per area of soil (Suppl. table S2). The total root biomass for each replicate in the 20 μm and 2 mm mesh treatments was calculated by summing up the biomass of glass and *Eriophorum* roots.

2.6 Environmental data

To monitor microclimate inside and outside the mesocosms without disturbing the ecosystem processes in the experimental mesocosms, we established an additional mesocosm in exactly the same way as the treatment with 2 mm mesh in six of the experimental blocks. We recorded soil temperature and volumetric water content (5 cm depth, 5TM sensor, Decagon, USA) in active layer compartment in the mesocosms, and outside each of these mesocosms hourly on Decagon EM50 loggers over the duration of the experiment. We recorded air temperature hourly close to the ground (5 cm height, TinyTags, Intab, Sweden, insulated by custom-made plastic radiation shields) in the active layer compartment of the mesocosms and outside the mesocosms in one central location at the study site over the summer seasons of the experiment (July 21 – September 18, 2015 and June 1 – September 18, 2016). Due to pauses in the functioning of loggers and/or sensors, replication for different parameters for different seasons varied from three to six (Table 3). We did not analyze the effects of mesocosm for the permafrost compartment (2 mm mesh) due to the lack of a natural nearby thermokarst site with disturbance of similar age as in our experiment.

2.7. Statistics

We used one-sample t-tests to analyze the effects of different-sized meshes in changing the permafrost parameters – soil N, pH, microbial biomass, and density of microarthropods and roots. To test our first hypothesis (connection via 0.5 μ m mesh changes permafrost chemistry relative to a situation with no connection to active layer), we calculated the mesh effect (0.5 μ m) for soil chemistry as '(N or pH in the 0.5 μ m mesh treatment) – (N or pH in the hermetic treatment)' for each block. Then, we tested whether this effect was smaller than zero, as permafrost has higher N and pH than active layer (Table 1) and these differences were expected to decrease due to the mesh.

To test our second hypothesis (connection via 20 μm mesh increases microbial biomass relative to a situation when only solutes can move), we calculated the mesh effect (20 μm) for microbial biomass as ‘(microbial biomass C in the 20 μm mesh treatment) – (microbial biomass C in the 0.5 μm mesh treatment)’. Then, we tested whether this effect was greater than zero, as active layer has higher microbial biomass (Table 1) that was expected to spread into permafrost. To test our third hypothesis (faunal inoculation increases microarthropod density relative to a situation when only microbes and solutes can move), the effects of faunal inoculation on soil fauna were calculated as ‘(microarthropod density in the inoculated 20 μm mesh treatment) – (microarthropod density in the 20 μm treatment)’. Then, we tested whether this effect was greater than zero, as active layer soil fauna was expected to spread into permafrost. To test our fourth hypothesis (microarthropods and roots will colonize permafrost through 2 mm mesh even though soil solutes diffusion and changes in microorganisms have altered permafrost chemistry), the effect of full connection (2 mm mesh) on microarthropods and roots was calculated as ‘(microarthropod density / total roots in the 2 mm mesh treatment) – (microarthropod density / total roots in the 20 μm mesh treatment)’. Then, we tested whether this effect was greater than zero, since active layer soil fauna and plant roots were expected to spread into permafrost.

The effects of possible lateral movement of microorganisms on DNA- and RNA-based bacterial community composition in the permafrost compartment (0.5 μm vs 20 μm) were visualized using non-metric multidimensional scaling on weighted UniFrac dissimilarity (Fig. 3b, Suppl. information 2). The overall effects were tested with the *anova.manyglm* function (in the R package mvabund, Wang et al., 2012) using treatment, nucleic acid, their interaction and block as fixed factors, and 999 PIT-trap resampling permutations. The differences between the effects of microarthropod inoculum and the unhindered lateral movement (20 μm mesh vs 20 μm mesh with

inoculation and 2 mm mesh) were tested with *anova.manyglm* function using treatment and block as fixed factors, and 999 PIT-trap resampling permutations.

We analyzed the effects of ‘mesocosm’ (active layer compartment, 2 mm mesh) vs surrounding peatland on air temperature during the growing seasons 2015 (July 21 – September 18) and 2016 (June 1 – September 18) and for soil temperature and moisture year-round. We calculated the effect size of the mesocosm-effect as ‘(temperature or moisture inside mesocosm) - (ambient temperature or moisture)’ for each block. We then analyzed the deviation from zero of the effect-size of the mesocosms separately for the two summers and for winter (September 19, 2015 – May 31, 2016), using one-sample t-tests.

All data were visually checked for normality by quantile – quantile plots and by plotting residuals against fitted values. All statistical analyses were conducted using the statistical software R (R Development Core Team, version 3.4.4).

3 Results

3.1 Effects of meshes in allowing (a)biotic changes in permafrost

In general, inorganic forms of N – ammonium, nitrite + nitrate – constituted approximately 80% of permafrost total dissolved N in the treatment with hermetic barrier, with no connection to the active layer compartment (Fig. 2a). In comparison, the permafrost in the treatment with 0.5 μ m mesh had a 63% lower ammonium concentration, but not significantly lower total dissolved N or nitrite+nitrate concentrations (Table 2, Fig. 2a–c). Regardless of the treatment (hermetic, 0.5 μ m mesh), total dissolved N and ammonium decreased over time and were 60% and 70% lower, respectively, at the last sampling campaign than during the first sampling campaign (Table S1). In addition, soil pH was 0.842 pH units lower in the treatment with 0.5 μ m mesh than in the treatment with hermetic barrier (Table 2, Fig. 2d).

Microbial biomass C was lower in the treatment with 20 μm mesh relative to the treatment with 0.5 μm mesh but this effect was not statistically significant (Table 2, Fig. 3a). Similarly, there was no significant main effect of the treatment on bacterial community composition (Table S3). However, the DNA- and RNA-based communities differed from each other ($p=0.001$, along axis MDS1, Fig. 3b), and there was a significant ‘treatment $\times$ nucleic acid’ interaction ($p=0.012$), reflecting a more marked separation in the DNA-based communities between the 0.5 μm mesh and 20 μm mesh treatments than in the RNA-based communities (Fig. 3b). In addition, the DNA- and RNA-based communities were affected differently by the 20 μm mesh, resulting in converse effects along MDS2 axis (Fig. 3b). This converse effect of the 20 μm mesh is reflected, for instance, in the decrease in the relative abundance of Alpha-proteobacteria and increase in the relative abundance of Bacteroidetes in the DNA-based communities whereas the opposite happened in the RNA-based communities (Fig. S1).

The density of microarthropods was also lower in the inoculated treatment and in the treatment with 2 mm mesh relative to the treatment with 20 μm mesh, but these differences were not significant (Table 2, Fig. 3c). The composition of microarthropod communities did not significantly differ between these treatments, although predatory mites were found only in the treatment with 2 mm mesh (Fig. 3d, Table S4).

Eriophorum roots and glass roots were found in all six replicates in the treatment with 2 mm mesh, the biomass being 2.1–25.8 g m^{-2} soil and 4.1–41.8 g m^{-2} soil, respectively (Table S2). Very low amounts of *Eriophorum* roots were found in two replicates in the 20 μm mesh treatment with faunal inoculum, and low amounts of glass roots in one replicate in the treatment with 0.5 μm mesh and two replicates in the treatment with 20 μm mesh (Table S2). The total abundance of roots in the treatment with 2 mm mesh, including *Eriophorum* and glass roots, was on average $33.2 \pm 8.0 \text{ g m}^{-2}$ soil (mean $\pm$ SE, Table S2) and significantly higher than in the treatment with 20 μm

382 mesh, in which the abundance of (glass) roots was on average $0.18 \pm 0.12 \text{ g m}^{-2}$ soil (mean $\pm$ SE,
383 Tables 2 and S2).

384

385 3.2 Active layer microclimate in mesocosms compared to the ambient peatland

386 The mean air temperature at canopy height (5 cm) inside the mesocosm active layer compartment
387 did not differ from ambient values at the experimental site (Table 3). Similarly, the mean soil
388 temperature and volumetric water content did not differ between the active layer compartment and
389 the ambient values (Table 3).

4 Discussion

4.1. Do meshes in mesocosms effectively control both abiotic and biotic soil components?

Our mesh-based field mesocosm setup proved successful in controlling lateral exchange of soil solutes between thawed permafrost and active layer soils. This exchange of solutes was confirmed by the lower pH and ammonium concentration in permafrost soil with lateral connection to active layer peat via 0.5 μm mesh, than in permafrost soil without connection. We suspect that these changes were primarily caused by diffusion along concentration gradients, as active layer peat contains considerably less N and is more acidic than permafrost soil (Table 1, Keuper et al., 2012). In addition, total dissolved N and ammonium in the permafrost decreased consistently over time in both treatments (Supplementary table S1). In tundra soil N availability generally declines over summer due to microbial and plant N uptake (Schimel et al., 2004), whereas in thermokarst sites soluble N may also disappear via percolation into deeper soil horizons and/or release in gaseous forms (Abbott and Jones, 2015; Voigt et al., 2017). Thus, physical connectivity between soils, even to the limited extent that only solutes can transfer, leads to changes in permafrost chemistry that significantly differ from those induced by isolated thawing and exposure to ambient surface conditions. This finding may have implications for the ecological interactions taking place in thawing permafrost soil, since lowered N could alter microbial C metabolism (Manzoni et al., 2012), but also via altered substrate availability and conditions (pH) could more directly affect nitrification and denitrification (Palmer et al., 2010; Voigt et al., 2017; Siljanen et al., 2019). Moreover, the observed changes in soil chemistry due to 0.5 μm mesh may have more general implications for the execution and interpretation of future mesh studies. This is because movement of soil water and solutes is likely to always occur and contribute to e.g. N or C loss, especially so when greater mesh sizes are used to identify the roles of soil biota on decomposition (Eno, 1960; Cornelissen et al., 2007; Makkonen et al., 2012). Therefore, we suggest that mesh studies should

also include smaller mesh sizes to control for the role of soil solutes exchange, when aiming to determine the role of biotic drivers.

Increased lateral connectivity between permafrost and active layer peat via a 20 μm mesh was expected to result in altered bacterial community composition. The permafrost bacterial DNA- and RNA-based communities indeed shifted in comparison to the lower connectivity (0.5 μm mesh), although in different directions. This result is difficult to interpret, but most likely reflects the fact that the different nucleic acids capture different aspects of the bacterial community (Emerson et al. 2017). RNA-based community measures will be dominated by the portion of the community that is actively growing (but see Blazewicz et al., 2013), whereas DNA gives an overall picture of the total community, including dormant members that will contribute less to the RNA pool. For this reason, the observed interaction between mesh size and nucleic acid may reflect different degrees of succession, which depends on the rates of migration and colonization that are expected to vary due to our mesh treatments. In that interpretation, the higher relative abundance of certain phyla (e.g. Bacteroidetes) in the RNA-based community of 0.5 μm compared to 20 μm mesh treatment could reflect an increased growth rate earlier in the succession. In the 20 μm mesh treatment, this increased growth rate happened earlier and has resulted in higher relative abundance in the DNA-based community, while at this point members of other phyla (e.g. α -proteobacteria) are becoming more active, thus explaining the apparent contradiction between the changes visible at DNA and RNA levels (Fig. S1). This interpretation is supported by Hultman et al. (2015), who found distinct bacterial communities along a permafrost thaw gradient. Despite the shifting bacterial assemblages, microbial biomass did not increase, indicating that permafrost (a)biotic conditions may not support the overall buildup of microbial biomass. Although we focused on bacteria, changes may have also occurred in the fungal assemblages, as those are particularly common in northern systems (Lamit et al., 2017). We conclude that connection via 20 μm mesh

induces shifts in soil bacterial assemblages that differ from those induced by changing soil chemistry alone (0.5 μm mesh) and thus provides insight in the drivers affecting the temporal dynamics of soil microbial community changes.

Both the faunal inoculum treatment (applied in the permafrost soil one year before the sampling), and the full lateral connection (2 mm mesh) treatment, were expected to result in increased microarthropod density and shift soil fauna community in the permafrost soil, relative to permafrost soil separated by a 20 μm mesh barrier (through which microarthropods cannot pass) from active layer soil. Inoculation did not, however, increase microarthropod densities, even though the inoculum was estimated to contain microarthropods in active layer field densities (25 000 *Collembola* m^{-2} ; Krab et al., 2014). Similarly, full lateral connection (2 mm mesh) overwhelmingly failed to allow establishment of microarthropods. Correspondingly, the more connected treatments did also not significantly shift the microarthropod community composition in the permafrost. Interestingly, microarthropods were found in the permafrost connected to the active layer via 20 μm mesh – the mesh size expected to prevent microarthropods from colonizing permafrost – suggesting that these animals entered permafrost by aboveground migration (cf. Dunger et al., 2002). Overall, across all three treatments, densities of microarthropods in the permafrost soil were much lower (3000–5000 individuals m^{-2}) in comparison to typical active layer densities (Krab et al., 2014). Permafrost soil may thus provide an only sub-optimal habitat for active layer-borne fauna due to low availability of suitable forage (litter, microbial biomass; Ponge et al., 2000) and microhabitats. We conclude that faunal inoculation, at least with a long lag time between inoculation and sampling, may not be an efficient way to study the impacts of fauna on (recently) thawed permafrost soil processes. Yet, since fauna seem to be unable to establish in high densities in permafrost, their impacts on biogeochemical processes are probably not very strong.

Connection via 2 mm mesh allowed ingrowth of roots into thawed permafrost soil and in root densities (Table S2) comparable to active layers soil beneath some tundra plants (e.g. *Carex* sp. 1–16 g m⁻²; Iversen et al., 2015). In comparison to this, roots occurred in amounts that were 1–2 orders of magnitude lower in the less connected treatment, and these few roots could be explained by recent seed dispersal and seedling establishment (*personal observation*). Thus, our set-up demonstrated that roots will actively colonize thawed permafrost even when permafrost chemistry has changed due to diffusion and that lateral connection to neighboring vegetation speeds up the colonization of permafrost soil by plant roots dramatically. Permafrost thaw and deepening of the active layer have been associated with increasing root biomass and deeper root systems leading to enhanced plant N uptake from the thaw front and increased input of root litter (Blume-Werry et al., 2019; Keuper et al., 2017). Since the topmost and deeper soil horizons differ in their thermal conditions and oxygen availability, the consequences of lateral superficial expansion of roots on permafrost C could differ from the case of thaw front deepening. Beyond the potential role of rhizosphere priming effects (Wild et al., 2016) and the effects of roots (*Eriophorum*) on the balance between methane and CO₂ release from different soil horizons (Ström et al., 2012), the decomposition of root litter and thus root litter contribution to the build-up of soil C pools can also vary among shallow and deeper soil horizons.

All levels of lateral connection manipulated here, excluding the microarthropod inoculation, led to a significant change in both abiotic conditions and biotic components of the permafrost soil relative to the more restricted level of connectivity. Thus, with this experimental setup, the effects of exchange of a(biotic) components on soil processes can be studied individually and, further, the apparent biotic effects can be corrected by taking into account the preceding (a)biotic effects. The microclimate in the mesocosms' active layer compartment was similar to the surrounding peatland. Thus, our mesocosms provide realistic microclimatic settings to investigate

the role of different ecological components and how they interact with soil processes in thawing permafrost.

4.2 Conclusions

Permafrost responses to climate change pose great uncertainty for forecasting our future climate, since our understanding about the ecological mechanisms that drive permafrost biogeochemical cycles is not complete (Schaefer et al., 2014; Todd-Brown et al., 2014; Koven et al., 2015; Turetsky et al., 2019). Our experimental setup helps untangle the roles of different ecological interactions in thermokarst events. We demonstrate that diffusion of soil solutes, shifts in bacterial communities and ingrowth of roots could all, individually, be potential factors controlling the fate of permafrost C and N soon after soil subsidence, but that soil fauna will, most likely, not play an important part. Even though we introduced our setup in the scenario of permafrost thaw-induced lateral soil mixing, a similar setup could be used for the scenario of thaw front deepening, by replacing the lateral design with a vertical one. In addition, this setup is not only relevant to thawing permafrost systems but could be applied to other soil mixing events, such as landslides, cryoturbation and even to test for the ecological impacts of agricultural practices (soil tillage, mulching, application of organic fertilizer). To conclude, we believe that our setup has great potential to unravel the individual roles of the abiotic soil environment, and above- and belowground biota, as well as to quantify the impact of interactions between above- and belowground biota on soil functioning across various terrestrial ecosystems.

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Literature

Abbott, B. W., Jones, J. B., 2015. Permafrost collapse alters soil carbon stocks, respiration, CH₄, and N₂O in upland tundra. *Glob. Change Biol.* 21, 4570–4587.

Baldrian, P., Kolařík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., Žifčáková, L., Šnajdr, J., Rídl, J., Vlček, Č., Voříšková, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J.* 6, 248–258.

Bardgett, R. D., Manning, P., Morriën, E., De Vries, F. T., 2013. Hierarchical responses of plant-soil interactions to climate change: consequences for the global carbon cycle. *J. Ecol.* 101, 334–343.

Baveye, P. C., Berthelin, J., Munch, J.-C., 2016. Too much or not enough: Reflection on two contrasting perspectives on soil biodiversity. *Soil Biol. Biochem.* 103, 320–326.

Blazewicz, S. J., Barnard, R. L., Daly, R. A., Firestone, M. K., 2013. Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. *ISME J.* 7, 2061–2068.

Blume-Werry, G., Milbau, A., Teuber, L. M., Johansson, M., Dorrepaal, E., 2019. Dwelling in the deep – strongly increased root growth and rooting depth enhance plant interactions with thawing permafrost soil. *New Phytol.* 223, 1328–1339.

530 Briones, M. J. I., McNamara, N. P., Poskitt, J., Crow, S. E., Ostle, N. J., 2014. Interactive biotic
531 and abiotic regulators of soil carbon cycling: evidence from controlled climate experiments on
532 peatland and boreal soils. *Glob. Chan. Biol.* 20, 2971–2982.

533 Brookes, P. C., Kragt, J. F., Powlson, D. S., Jenkinson, D. S., 1985. Chloroform fumigation and
534 the release of soil nitrogen: the effects on fumigation time and temperature. *Soil Biol. Biochem.*
535 17, 831–835.

536 Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E. K., Fierer,
537 N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig,
538 J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky,
539 J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R.,
540 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7,
541 335–336.

542 Cornelissen, J. H. C., van Bodegom, P. M., Aerts, R., Callaghan, T. V., van Logtestijn, R. S. P.,
543 Alatalo, J., Chapin, S., Gerdol, R., Gudmundsson, J., Gwynn-Jones, D., Hartley, A. E., Hik, D. S.,
544 Hofgaard, A. Jónsdóttir, I. S., Karlsson, S., Klein, J. A., Laundre, J., Magnusson, B., Michelsen,
545 A., Molau, U., Onipchenko, V. G., Quested, H. M., Sandvik, S. M., Schmidt, I. K., Shaver, G. R.,
546 Solheim, B., Soudzilovskaia, N. A., Stenström, A., Tolvanen, A., Totland, Ø., Wada, N. Welker,
547 J. M., Zhao, X., M.O.L. Team, 2007. Global negative vegetation feedback to climate warming
548 responses of leaf litter decomposition rates in cold biomes. *Ecol. Lett.* 10, 619–627.

549 Courties, C., Vaquer, A., Troussellier, M., Lautier, J., Chrétiennot-Dinet, M. J., Neveux, J.,
550 Machado, C., Claustre, H., 1994. Smallest eukaryotic organism. *Nature* 370, 255–255.

551 Cui, Y., Wang, S., Yan, S., 2016. Influence of soil faunal properties and understory fine root on
552 soil organic carbon in a “mesh bag” approach. *Eur. J. Soil Biol.* 76, 19–25.

553 De Vries, F. T., Shade, A., 2013. Controls on soil microbial community stability under climate
554 change. *Front. Microbiol.* 4: article number 265.

555 Dorrepaal, E., Toet, S., van Logtestijn, R. S. P., Swart, E., van de Weg, M. J., Callaghan, T. V.,
556 Aerts, R., 2009. Carbon respiration from subsurface peat accelerated by climate warming in the
557 subarctic. *Nature* 460, 616–U79.

558 Dunger, W., Schulz, H. J., Zimdars, B. 2002. Colonization behaviour of Collembola under different
559 conditions of dispersal. *Pedobiologia* 46, 316–327.

560 Emerson, J. B., Adams, R. I., Román, C. M. B., Brooks, B., Coil, D. A., Dahlhausen, K., Ganz, H.
561 H., Hartmann, E. M., Hsu, T., Justice, N. B., Paulino-Lima, I. G., Luongo, J. C., Lymperopoulou,
562 D. S., Gomez-Silvan, C., Rothschild-Mancinelli, B., Balk, M., Huttenhower, C., Nocker, A.,
563 Vaishampayan, P., Rothschild, L. J., 2017. Schrödinger’s microbes: Tools for distinguishing the
564 living from the dead in microbial ecosystems. *Microbiome* 5, 86.

565 Eno, C. F., 1960. Nitrate production in the field by incubating the soil in polyethylene bags. *Soil*
566 *Sci. Soc. Am. J.* 24, 277 – 279.

567 Food and Agriculture Organization (FAO), 2001. World Reference Base (WRB) for Soil Resources
568 2001, Lecture Notes on the Major Soils of the World. FAO, Rome World Soil Resource Reports.

569 Heinemeyer, A., Ineson, P., Ostle, N., Fitter, A. H., 2006. Respiration of the external mycelium in
570 the arbuscular mycorrhizal symbiosis shows strong dependence on recent photosynthates and
571 acclimation to temperature. *New Phytol.* 171, 159–170.

572 Heinemeyer, A., Hartley, I. P., Evans, S. P., Carreira de la Fuente, J. A., Ineson, P., 2007. Forest
 573 soil CO₂ flux: uncovering the contribution and environmental responses of ectomycorrhizas. *Glob.*
 574 *Change Biol.* 13, 1786–1797.

575 Hultman, J., Waldrop, M. P., Mackelprang, R., David, M. M., McFarland, J., Blazewicz, S. J.,
 576 Harden, J., Turetsky, M. R., McGuire, A. D., Shah, M. B., VerBerkmoes, N. C., Lee, L. H.,
 577 Mavrommatis, K., Jansson, J. K., 2015. Multi-omics of permafrost, active layer and thermokarst
 578 bog soil microbiomes. *Nature* 521, 208–212.

579 Iversen, C. M., Sloan, V. L., Sullivan, P. F., Euskirchen, E. S., McGuire, A. D., Norby, R. J.,
 580 Walker, A. P., Warren, J. M., Wullschleger, S. D., 2015. The unseen iceberg: plant roots in arctic
 581 tundra. *New Phytol.* 205, 34–58.

582 Joergensen, R. G., 1996. The fumigation-extraction method to estimate soil microbial biomass:
 583 calibration of the kEC value. *Soil Biol. Biochem.* 28, 25–31.

584 Johansson, M., Åkerman, J., Keuper, F., Christensen, T. R., Lantuit, H., Callaghan, T. V., 2011.
 585 Past and present permafrost temperatures in the Abisko area: redrilling of boreholes. *Ambio* 40,
 586 558–565.

587 Johansson, M., Callaghan, T. V., Bosiö, J., Åkerman, H. J., Jackowicz-Korczynski, M.,
 588 Christensen, T. R., 2013. Rapid responses of permafrost and vegetation to experimentally increased
 589 snow cover in sub-arctic Sweden. *Environ. Res. Lett.* 8, 035025.

590 Johnson, D., Leake, J. R., Read, D. J., 2001. Novel in-growth core system enables functional
 591 studies of grassland mycorrhizal mycelial networks. *New Phytol.* 152, 555–562.

592 Johnson, D., Krsek, M., Wellington, E. M. H., Stott, A. W., Cole, L., Bardgett, R. D., Read, D. J.,
 593 Leake, J. R., 2005. Soil invertebrates disrupt carbon flow through fungal networks. *Science*. 309,
 594 1047.

595 Keuper, F., van Bodegom, P. M., Dorrepaal, E., Weedon, J. T., van Hal, J., van Logtestijn, R. S.
 596 P., Aerts, R., 2012. A frozen feast: thawing permafrost increases plant-available nitrogen in
 597 subarctic peatlands. *Glob. Change Biol.* 18, 1998–2007.

598 Keuper, F., Dorrepaal, E., van Bodegom, P. M., van Logtestijn, R., Venhuizen, G., van Hal, J.,
 599 Aerts, R., 2017. Experimentally increased nutrient availability at the permafrost thaw front
 600 selectively enhances biomass production of deep-rooting subarctic peatland species. *Glob. Change*
 601 *Biol.* 23, 4257–4266.

602 Koven, C. D., Lawrence, D. M., Riley, W. J., 2015. Permafrost carbon–climate feedback is
 603 sensitive to deep soil carbon decomposability but not deep soil nitrogen dynamics. *P. Natl. Acad.*
 604 *Sci.* 112, 3752–3757.

605 Krab, E. J., Aerts, R., Berg, M. P., van Hal, J., Keuper, F., 2014. Northern peatland Collembola
 606 communities unaffected by three summers of simulated extreme precipitation. *Appl. Soil Ecol.* 79,
 607 70–76.

608 Lamit, L. J., Romanowicz, K. J., Potvin, L. R., Rivers, A. R., Singh, K., Lennon, J. T., Tringe, S.
 609 G., Kane, E. S., Lilleskov, E. A., 2017. Patterns and drivers of fungal community depth
 610 stratification in *Sphagnum* peat. *FEMS Microbiol. Ecol.* 93(7) fix082

611 Lozupone, C., Lladser, M. E., Knights, D., Stombaugh, J., Knight, R., 2011. UniFrac: an effective
 612 distance metric for microbial community comparison. *ISME J.* 5, 169–172.

613 Mackelprang, R., Burkert, A., Haw, M., Mahendrarajah, T., Conaway, C. H., Douglas, T. A.,
614 Waldrop, M. P., 2017. Microbial survival strategies in ancient permafrost: insights from
615 metagenomics. *ISME J.* 11, 2305–2318.

616 Makkonen, M., Berg, M. P., Handa, I. T., Hättenschwiler, S., van Ruijven, J., van Bodegom, P. M.,
617 Aerts, R., 2012. Highly consistent effects of plant litter identity and functional traits on
618 decomposition across a latitudinal gradient. *Ecol. Lett.* 15, 1033–1041.

619 Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G. I., 2012. Environmental and
620 stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytol.* 196, 79–91.

621 McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., Andersen,
622 G. L., Knight, R., Hugenholtz, P., 2012. An improved Greengenes taxonomy with explicit ranks
623 for ecological and evolutionary analyses of bacteria and archaea. *ISME J.* 6, 610–618.

624 Mikusinska, A., Persson, T., Taylor, A. F. S., Ekblad, A., 2013. Response of ectomycorrhizal
625 extrametrical mycelium production and isotopic composition to in-growth bag size and soil fauna.
626 *Soil Biol. Biochem.* 66, 154–162.

627 Monteux, S., Weedon, J. T., Blume-Werry, G., Gavazov, K., Jassey, V. E. J., Johansson, M.,
628 Keuper, F., Olid, C., Dorrepaal, E., 2018. Long-term in situ permafrost thaw effects on bacterial
629 communities and potential aerobic respiration. *ISME J.* 12, 2129–2141.

630 Morrison, E., Lagos, L., Al-Agely, A. Johnson, W., Jorquera, M. A., Ogram, A., 2017. Mycorrhizal
631 inoculation increases genes associated with nitrification and improved nutrient retention in soil.
632 *Biol. Fertil. Soils* 53, 275–279.

633 Neumann, J., Matzner, E., 2013. Biomass of extramatrical ectomycorrhizal mycelium and fine
634 roots in a young Norway spruce stand — a study using ingrowth bags with different substrates.
635 Plant Soil 371, 435–446.

636 O'Connor, F., 1955. Extraction of Enchytraeid worms from a coniferous forest soil. Nature
637 175(4462), 815–816.

638 Olefeldt, D., Goswami, S., Grosse, G., Hayes, D., Hugelius, G., Kuhry, P., McGuire, A. D.,
639 Romanovsky, V. E., Sannel, A. B. K., Schuur, E. A. G., Turetsky, M. R., 2016. Circumpolar
640 distribution and carbon storage of thermokarst landscapes. Nat. Commun. 7, 13043.

641 Palmer, K., Drake, H. L., Horn, M. A., 2010. Association of novel and highly diverse acid-tolerant
642 denitrifiers with N₂O fluxes of an acidic fen. Appl. Environ. Microb. 76, 1125–1134.

643 Ponge, J.F., 2000. Vertical distribution of Collembola (Hexapoda) and their food resources in
644 organic horizons of beech forests. Biol. Fert. Soils 32, 508e522.

645 Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source
646 tool for metagenomics. PeerJ. 4, e2584.

647 Salmon, V. G., Schädel, C., Bracho, R., Pegoraro, E., Celis, G., Mauritz, M., Mac, M. C., Schuur,
648 E. A. G., 2018. Adding depth to our understanding of nitrogen dynamics in permafrost soils. J.
649 Geophys. Res. Biogeosci. 123, 2497–2512.

650 Schaefer, K., Lantuit, H., Romanovsky, V. E., Schuur, E. A. G., Witt, R., 2014. The impact of the
651 permafrost carbon feedback on global climate. Environ. Res. Lett. 9, 085003.

652 Schimel, J. P., Weintraub, M. N., 2003. The implication of exoenzyme activity on microbial carbon
653 and nitrogen limitation in soil: a theoretical model. Soil Biol. Biochem. 35, 549–563.

654 Schimel, J. P., Bilbrough, C., Welker, J. M., 2004. Increased snow depth affects microbial activity
 655 and nitrogen mineralization in two Arctic tundra communities. *Soil Biol. Biochem.* 36, 217–227.

656 Schuur, E. A. G., McGuire, A. D., Schädel, C., Grosse, G., Harden, J. W., Hayes, D. J., Hugelius,
 657 G., Koven, C. D., Kuhry, P., Lawrence, D. M., Natali, S. M., Olefeldt, D., Romanovsky, V. E.,
 658 Schaefer, K., Turetsky, M. R., Treat, C. C., Vonk, J. E., 2015. Climate change and the permafrost
 659 carbon feedback. *Nature* 520, 171–179.

660 Schädel, C., Bader, M. K.-F., Schuur, E. A. G., Biasi, C., Bracho, R., Capek, P., De Baets, S.,
 661 Diáková, K., Ernakovich, J., Estop-Aragones, C., Graham, D. E., Hartley, I. P., Iversen, C. M.,
 662 Kane, E., Knoblauch, C., Lupascu, M., Martikainen, P. J., Natali, S. M., Norby, R. J., O'Donnell,
 663 J. A., Roy Chowdhury, T., Šantrůvková, H., Shaver, G., Sloan, V. L., Treat, C. C., Turetsky, M.
 664 R., Waldrop, M. P., Wickland, K. P., 2016. Potential carbon emissions dominated by carbon
 665 dioxide from thawed permafrost soil. *Nat. Clim. Change* 6, 950–954.

666 Siljanen, H. M. P., Alves, R. J. E., Ronkainen, J. G., Lamprecht, R. E., Bhattarai, H. R., Bagnoud,
 667 A., Marushchak, M. E., Martikainen, P. J., Schleper, C., Biasi, C., 2019. Archaeal nitrification is a
 668 key driver of high nitrous oxide emissions from arctic peatlands. *Glob. Change Biol.* 137, 107539.

669 Steingrobe, B., Schmid, H., Claassen, N., 2001. The use of the ingrowth core method for measuring
 670 root production of arable crops – influence of soil and root disturbance during installation of the
 671 bags on root ingrowth into the cores. *Eur. J. Agron.* 15(2), 143–151.

672 Ström, L., Tagesson, T., Mastepanov, M., Christensen, T. R., 2012. Presence of *Eriophorum*
 673 *scheuchzeri* enhances substrate availability and methane emission in an Arctic wetland. *Soil Biol.*
 674 *Biochem.* 45, 61–70.

675 Todd-Brown, K. E. O., Randerson, J. T., Hopkins, F., Arora, V., Hajima, T., Jones, C.,
 676 Shevliakova, E., Tjiputra, J., Volodin, E., Wu, T., Zhang, Q., Allison, S. D., 2014. Changes in soil
 677 organic carbon storage predicted by Earth system models during the 21st century. *Biogeosciences*
 678 11, 2341–2356.

679 Turetsky, M. R., Abbott, B. W., Jones, M. C., Walter Anthony, K., Olefeldt, D., Schuur, E. A. G.,
 680 Koven, C., McGuire, A. D., Grosse, G., Kuhry, P., Hugelius, G., Lawrence, D. M., Gibson, C.,
 681 Sannel, A. B. K., 2019. Permafrost collapse is accelerating carbon release. *Nature* 569, 32–34.

682 Urich, T., Lanzén, A., Qi, J., Huson, D. H., Schleper, C., Schuster, S. C., 2008. Simultaneous
 683 assessment of soil microbial community structure and function through analysis of the meta-
 684 transcriptome. *PLOS ONE* 3, e2527.

685 Van Straalen, N., Rijninks, P., 1982. The efficiency of Tullgren apparatus with respect to
 686 interpreting seasonal changes in age structure of soil arthropod populations. *Pedobiologia* 24, 197–
 687 209.

688 Voigt, C., Marushchak, M. E., Lamprecht, R. E., Jackowicz-Korczyński, M., Lindgren, A.,
 689 Mastepanov, M., Granlund, L., Christensen, T. R., Tahvanainen, T., Martikainen, P. J., Biasi, C.,
 690 2017. Increased nitrous oxide emissions from Arctic peatlands after permafrost thaw. *PNAS*
 691 114(24), 6238–6243.

692 Väisänen, M., Gavazov, K., Krab, E. J., Dorrepaal, E., 2019. The legacy effects of winter climate
 693 on microbial functioning after snowmelt in a subarctic tundra. *Microb. Ecol.* 77, 186–190.

694 Wang, Y., Naumann, U., Wright, S., Warton, D. E., 2012. *mvabund: Statistical Methods for*
 695 *Analysing Multivariate Abundance Data*.

696 Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H., Wall, D. H.,
697 2004. Ecological linkages between aboveground and belowground biota. *Science* 304, 1629–1633.

698 Weedon, J. T., Kowalchuk, G. A., Aerts, R., van Hal, J., van Logtestijn, R., Taş, N., Röling, W. F.
699 M., van Bodegom, P. M., 2012. Summer warming accelerates sub-arctic peatland nitrogen cycling
700 without changing enzyme pools or microbial community structure. *Glob. Change Biol.* 18, 138–
701 150.

702 Weedon, J. T., Kowalchuk, G. A., Aerts, R., Freriks, S., Röling, W. F. M., van Bodegom, M. P.,
703 2017. Compositional stability of the bacterial community in a climate-sensitive sub-arctic peatland.
704 *Front. Microbiol.* 8, 317.

705 Wild, B., Gentsch, N., Čapek, P., Diáková, K., Eloy Alves, R. J., Bárta, J., Gittel, A., Hugelius, G.,
706 Knoltsch, A., Kuhry, P., Lashchinskiy, N., Mikutta, R., Palmtag, J., Schleper, C., Schneckner, J.,
707 Shibistova, O., Takriti, M., Torsvik, V. L., Urich, T., Watzka, M., Šantrůčková, H., Guggenberger,
708 G., Richter, A., 2016. Plant-derived compounds stimulate the decomposition of organic matter in
709 arctic permafrost soils. *Sci. Rep.* 6, 25607.

710 Åkerman, H. J., Johansson, M., 2008. Thawing permafrost and thicker active layers in sub-arctic
711 Sweden. *Permafrost Periglac.* 19, 279–292.

Figure legends

Figure 1. Experimental setup. (a) Dimensions of the mesocosms, the smaller permafrost compartment and the larger active layer compartment, separated by a frame to which the meshes are mounted. The mesocosm has eight drainage holes located at the lower half and covered with 0.5 μm and 2 mm mesh (inside and outside). (b) Two mesocosms made of 8 mm thick acrylic plastic. At the left hand side, the frame that separates the two compartments is solid, the hermetic treatment, and at the right hand side the frame is covered by a 2 mm mesh, the 2 mm mesh treatment. (c) A mesocosm, in which the active layer compartment is filled with peat sod having *Andromeda polifolia* and *Eriophorum vaginatum* growing over *Sphagnum* mosses. The right (rear) half of the permafrost compartment is filled with inert material (Styrofoam), and the left half (next to the mesh barrier) is filled with recently thawed and homogenized permafrost soil.

Figure 2. Permafrost soil pore-water chemistry measured in the treatment with no lateral connection to the active layer compartment, hermetic treatment, and in the treatment with a connection through a 0.5 μm mesh to the active layer compartment. (a) Total dissolved N (TN), (b) ammonium, (c) nitrite + nitrate, and (d) soil pH during growing season 2016. Values represent mean $\pm$ standard error (n=6). The asterisks (*) denote significance at $p < 0.05$.

Figure 3. Soil microbes and microarthropods in permafrost with different mesh connections to the active layer in September 2016. (a) Microbial biomass C (mean $\pm$ standard error) in the treatment with a connection to active layer peat through a 0.5 μm mesh (n=6), which allows lateral movement of soil solutes through the soil, but not microbes; and in the treatment with a connection through a 20 μm mesh (n=6), which allows lateral movement of microbes through the soil. (b) Bacterial community composition (NMDS, mean $\pm$ standard error) based on DNA (n=6) and RNA

736 amplicons (n=5) in the 0.5 μ m and 20 μ m mesh treatments. (c) Total density of microarthropods
737 (mean $\pm$ standard error) in the 20 μ m mesh treatment, which allows no lateral movement of soil
738 fauna through the soil; in the treatment, which has been inoculated with microarthropods (n=6);
739 and in the 2 mm mesh treatment (n=6), which has full lateral connection through the soil. (d) The
740 relative abundances of broad groups of microarthropods – nymphs/other mites, predatory mites,
741 Collembola and Oribatid mites (mean – standard error) – in the 20 μ m, inoculated and 2 mm
742 treatments.

Figure 1.

745

746 Figure 2.

a) Microbial biomass carbon

b) Bacterial community

c) Microarthropod density

d) Microarthropod community

747

748 Figure 3.

Table 1. Properties of the thawed permafrost soil and the ambient active layer peat at the experimental site, Storflaket, in subarctic Sweden (68°20.836' N, 18°58.398' E). The properties of permafrost were analysed using six subsamples of the recently thawed and homogenized permafrost at the onset of the experiment in July 2015. The properties of the active layer surface peat were analysed using six samples collected from the experimental site during summer 2015. The pH of both permafrost and active layer was analysed using soil pore water that was collected from the mesocosms with no physical connection between active layer and permafrost (n=6) in September 2015. Values show mean (standard error). For more details about the methods, see 2.4 and 2.5.

Parameter	Permafrost	Active layer
Gravimetric moisture (%)	76.4 (0.83)	86.3 (0.10)
Organic matter (% of dry soil)	33.5 (1.35)	96.0 (0.03)
Total dissolved organic C (mg g ⁻¹ SOM)	0.78 (0.12)	0.76 (0.06)
Total dissolved N (mg g ⁻¹ SOM)	0.06 (0.01)	0.02 (0.001)
Microbial biomass C (mg g ⁻¹ SOM)	2.03 (0.23)	5.19 (0.75)
pH	5.82 (0.07)	3.68 (0.05)

Table 2. The effects of different levels of physical connectivity between permafrost and active layer peat on permafrost chemistry, microbial biomass and the density of microarthropods and roots. The effect of soil solubles diffusion through 0.5 μm mesh on soil N and pH was tested by comparing the treatments with hermetic barrier and 0.5 μm mesh. The effect of connection via 20 μm mesh on microbial biomass was tested by comparing the treatments with 0.5 μm and 20 μm meshes. The effect of inoculation on microarthropod density was tested by comparing the treatments with 20 μm mesh and faunal inoculum in combination with 20 μm mesh, while the effect of full lateral connection via 2 mm mesh on microarthropods and roots was tested by comparing the treatments with 20 μm and 2 mm meshes. The mesh effects were tested with one-sample t-test, and effects with $p < 0.05$ are in bold.

Parameter	Treatment pair	t-value	p-value
Total dissolved N		-1.23	0.135
Ammonium	Hermetic vs 0.5 μm	-3.16	0.012
Nitrite + nitrate		1.20	0.859
pH		-2.30	0.041
Microbial biomass C	0.5 μm vs 20 μm	-1.45	0.897
Microarthropod density	20 μm vs inoculum	-0.89	0.794
	20 μm vs 2 mm	-1.93	0.944
Total root abundance	20 μm vs 2 mm	4.15	0.004

Table 3. Environmental data. Average seasonal soil temperature and volumetric moisture content (5 cm depth) and air temperature close to the ground (5 cm height) in summer 2015 (July 21–September 18) and in summer 2016 (June 1–September 18), and soil temperature and moisture during winter (September 19, 2015–May 31, 2016). Soil parameters were measured in the active layer compartment and at the close vicinity of these mesocosms in the surrounding ambient peatland, whereas air temperature was also measured in the active layer compartment and at one central location of the experimental site. The values represent mean (standard error) and the number of replicates. There were no significant differences (assessed with one-sample t-test) in the environmental conditions between mesocosm compartment and the ambient peatland.

	location	air temperature (°C)	soil temperature (°C)	volumetric soil moisture (m ³ /m ³)
Summer 2015	mesocosm	11.4 (0.28), n=4	10.1 (0.61), n=6	0.241 (0.024), n=6
	ambient	11.3 (n.a.), n=1	9.4 (0.20), n=6	0.256 (0.015), n=6
Winter 2015/2016	mesocosm		-0.4 (0.33), n=3	0.183 (0.023), n=4
	ambient		-0.6 (0.32), n=3	0.175 (0.015), n=4
Summer 2016	mesocosm	10.1 (0.18), n=4	8.3 (0.76), n=4	0.310 (0.029), n=4
	ambient	10.1 (n.a.), n=1	8.2 (0.59), n=4	0.269 (0.010), n=4

Supplementary table S1. Soil pore water N for four sampling times (indicated as weeks) during the frost-free period in 2016. The pore water N in nitrite+nitrate, ammonium, and total dissolved N (unit is mg L⁻¹), were measured from permafrost soil with hermetic barrier, i.e. no physical connection to active layer peat (n=6), and in permafrost soil that was laterally connected to active layer peat via 0.5 µm mesh, allowing exchange of solutes (n=6). The values represent mean (standard error).

treatment	week	NO ₂ +NO ₃ -N	NH ₄ -N	TN
hermetic	25	5.76 (2.20)	9.13 (1.45)	24.20 (3.38)
	28	14.85 (6.15)	8.54 (2.45)	22.72 (3.79)
	32	7.11 (3.48)	4.61 (1.37)	12.69 (0.83)
	37	2.78 (0.93)	3.48 (1.22)	11.14 (1.10)
0.5 µm	25	8.27 (2.04)	4.59 (1.33)	24.22 (3.72)
	28	17.28 (5.98)	3.06 (1.27)	19.83 (4.65)
	32	10.33 (3.57)	1.09 (0.59)	9.39 (1.99)
	37	2.37 (0.89)	0.50 (0.20)	7.61 (3.44)

Supplementary information 1

We amplified the V3 and V4 region of the 16S rRNA gene from the DNA extracts and of the first-strand complementary DNA synthesized from the RNA extracts. The RNA extracts were treated with DNase I (Invitrogen) to remove residual DNA. A random subset of DNase-treated samples were tested for absence of DNA by PCR (primers 341F 5' CCT ACG GGN GGC WGC AG 3' and 805R 5' GAC TAC HVG GGT ATC TAA TCC 3', sequences do not include Illumina adapters) and agarose gel electrophoresis. PCR conditions were as follows: hot-start at 72°C, initial denaturation 30s at 98°C; 25 cycles of denaturation at 98°C for 10s, annealing at 55°C for 30s, elongation at 72°C for 30s; and a final elongation step at 72°C for 5min. First-strand cDNA was synthesized (RevertAid First Strand cDNA synthesis kit, Thermo Scientific) with random hexamer primers and the undiluted product was used for PCR. To reduce PCR inhibitors concentration and improve amplification, the DNA extracts were diluted up to 1:50 with ultra-pure water prior to PCR. Both DNA and cDNA were amplified

by PCR (primers and conditions as above), using either 2 μ L of the cDNA synthesis product or 2 μ L of the diluted DNA extract as template in a 25 μ L reaction volume with 12.5 μ L Phusion high-fidelity polymerase mastermix (Thermo Scientific), 1.25 μ L of each primer at 10 μ M and 8 μ L PCR-grade water.

PCR products were normalized using SequalPrep Plate Normalization Kit (Thermo Scientific) with 15 μ L PCR product and 15 μ L elution volume. After this, a second PCR step was used to add sequencing indexes to the samples (conditions as above, with 9 cycles instead of 25). Indexing-PCR products were normalized with SequalPrep Plate Normalization Kit as above. After this, 2 μ L of each sample was pooled together and quantified by qPCR using a KAPA SYBR-FAST qPCR Kit (Sigma Aldrich). The library was sequenced on an Illumina MiSeq system (V3 chemistry, paired-end 2 x 300 cycles) loaded with 8 pM of denatured library at 15% PhiX target concentration (the Molecular Biology department of Umeå University, Sweden). Reads assigned to each sample were demultiplexed using the MiSeq Reporter GenerateFASTQ workflow with default parameters.

Raw forward and reverse reads were merged with VSEARCH 2.7.1 (Rognes et al., 2016) allowing 15 mismatches over the overlapping region. VSEARCH *fastq_filter* command was used to trim primers from the reads and apply quality filtering (discarding reads with more than one expected error). Sequences were then dereplicated and clustered in 97% similarity operational taxonomic units (OTUs) using VSEARCH *cluster_size* algorithm. The OTUs were checked for putative chimeras using the UCHIME (Edgar et al., 2011) algorithm in VSEARCH against the GOLD database (Haas et al., 2011). Quality-filtered reads were mapped to OTUs using the *usearch_global* algorithm, and the resulting OTU table was imported in QIIME 1.9.1 (Caporaso et al., 2010a) for further processing. Representative sequences for each OTU were aligned using pyNAST (Caporaso et al., 2010b), gaps in the alignment were filtered out, and the taxonomy for each OTU was assigned using the RDP naïve Bayesian classifier (Wang et al., 2007) against the Greengenes 13.8 reference set (McDonald et al., 2012). OTUs that were not found in at least two samples were removed along with two samples from which no cDNA could be amplified (i.e. no RNA data for one replicate in the treatment with 20 μ m mesh and for one replicate in the treatment with 0.5 μ m mesh). A normalized abundance table was created by averaging the output of 100 rarefactions at 6000 sequences per sample depth, from which pairwise weighted UniFrac dissimilarity matrixes were computed (Lozupone et al., 2011). For the full description about bioinformatics, see

https://bitbucket.org/smouteux/mesocosms_methods.

References

- Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010a. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 335–336.
- Caporaso, J. G., Bittinger, K., Bushman, F. D., DeSantis, T. Z., Andersen, G. L., Knight, R., 2010b. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* 26, 266–267.
- Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27, 2194–2200.
- Haas, B. J., Gevers, D., Earl, A. M., Feldgarden, M., Ward, D. V., Giannoukos, G., Ciulla, D., Tabbaa, D., Highlander, S. K., Sodergren, E., Methé, B., DeSantis, T. Z., Consortium, T. H. M., Petrosino, J. F., Knight, R., Birren, B. W., 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res.* 21, 494–504.
- Lozupone, C., Lladser, M. E., Knights, D., Stombaugh, J., Knight, R., 2011. UniFrac: an effective distance metric for microbial community comparison. *ISME J.* 5, 169–172.
- McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., Andersen, G. L., Knight, R., Hugenholtz, P., 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J.* 6, 610–618.
- Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4, e2584.

Wang, Q., Garrity, G. M., Tiedje, J. M., Cole, J. R., 2007. Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Appl. Environ. Microb.* 73, 5261–5267.

Supplementary table S2 The abundances of *Eriophorum vaginatum* roots and glass roots found from the permafrost compartment of the mesocosms in each spatial block in September 2016. Roots of *Eriophorum* were found from the treatments with faunal inoculum and 2 mm mesh and glass roots were found from the treatments with 0.5 μm , 20 μm and 2 mm mesh. The values represent g dry root m^{-2} of soil and the mean values and their standard error for each treatment are reported in bold.

Spatial block	<i>Eriophorum</i> roots		Glass roots		
	Inoculum	2 mm	0.5 μm	20 μm	2 mm
1	0.07	16.81	0	0.44	41.85
2	0	13.97	0	0	16.30
3	0.18	7.24	0	0	4.07
4	0	25.79	0	0	10.11
6	0	2.12	0	0	9.46
8	0	16.63	0.285	0.63	34.73
Mean	0.042	13.76	0.048	0.18	19.42
Standard error	0.029	3.368	0.047	0.115	6.241

Supplementary information 2

To compare bacterial communities in permafrost soil without and with facilitating lateral dispersal from active layer soil through a mesh, non-metric multidimensional scaling (NMDS) was computed from the weighted UniFrac distance matrix (Figure 3b). We tested the differences due to treatment (0.5 μm mesh, 20 μm mesh) and nucleic acid (DNA, RNA) by fitting the normalized abundances of the OTUs, to a *manyglm* model (*mvabund* package; Warton et al., 2012; Wang et al., 2012). Validity of the *manyglm* assumptions were visually checked, and the overall effects were tested with the *anova, manyglm* function using treatment, nucleic acid and their interaction and spatial block as fixed factors, and 999 PIT-trap resampling permutations (Supplementary table 3).

These analyses were carried out using R v3.4.4 (R Core Team, 2018) with the packages *mvabund* and *vegan* (Wang et al., 2012; Oksanen et al., 2015).

Supplementary table S3 Analysis of Deviance Table for the manyglm model of the response of bacterial OTU counts to treatment (treatment pair 0.5 µm vs 20 µm mesh, n=6) and nucleic acid (DNA vs RNA) and spatial block. P-values were calculated using 999 resampling iterations (PIT-trap resampling). Bold numbers indicate * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

	Res. Df	Df. diff	Dev	Pr (>Dev)
(Intercept)	21			
Treatment	20	1	8324	0.396
Nucleic acid	18	2	20758	0.001 ***
Block	17	1	10935	0.007 **
Treatment × Nucleic acid	17	2	4831	0.012 *

References

- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., 2015. *vegan*: Community Ecology Package.
- Wang, Y., Naumann, U., Wright, S., Warton, D. E., 2012. *mvabund*: Statistical Methods for Analysing Multivariate Abundance Data.
- Warton, D. I., Wright, S. T., Wang, Y., 2012. Distance-based multivariate analyses confound location and dispersion effects. *Methods Ecol. Evol.* 3, 89–101.

Supplementary figure S1 The relative proportions of different bacterial phyla in the permafrost based on DNA and RNA (average values, n=6 for DNA and n=5 for RNA). The phyla were analysed in two different treatments that were accomplished using 0.5 µm mesh, which allows lateral transfer of soil solutes but excludes the lateral movement of most microorganisms, and 20 µm mesh, which allows lateral movement of microbes.

Supplementary table S4 Analysis of Deviance Table for the manyglm model of the response of the permafrost microarthropod community (identified to broad functional groups – Collembola, Oribatid mites, nymphs/other mites, predatory mites) to different levels of physical connectivity between permafrost and active layer peat and to spatial block. The treatments were connection through the 20 μ m mesh (allows no lateral movement of soil fauna, n=6), the inoculum treatment (20 μ m mesh accompanied by microarthropod inoculum, n=6), and unhindered lateral connection through the 2 mm mesh (n=6). P-values were calculated using 999 resampling iterations (PIT-trap resampling).

Treatment		Res. Df	Df. diff	Dev	Pr (>Dev)
20 μ m vs inoculum	(Intercept)	11			
	Treatment	9	2	2.738	0.281
	Block	9	1	2.182	0.440
20 μ m vs 2 mm	(Intercept)	11			
	Treatment	9	2	2.437	0.543
	Block	9	1	1.136	0.764