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Enhanced summer warming reduces fungal decomposer diversity and litter mass loss more strongly in dry than in wet tundra

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Abstract

Many Arctic regions are currently experiencing substantial summer and winter climate changes. Litter decomposition is a fundamental component of ecosystem carbon and nutrient cycles, with fungi being among the primary decomposers. To assess the impacts of seasonal climatic changes on litter fungal communities and their functioning, *Betula glandulosa* leaf litter was surface-incubated in two adjacent low Arctic sites with contrasting soil moisture regimes: dry shrub heath and wet sedge tundra at Disko Island, Greenland. At both sites, we investigated the impacts of factorial combinations of enhanced summer warming (using open-top chambers; OTCs) and deepened snow (using snow fences) on surface litter mass loss, chemistry and fungal decomposer communities after approximately 1 year. Enhanced summer warming significantly restricted litter mass loss by 32% in the dry and 17% in the wet site. Litter moisture content was significantly reduced by summer warming in the dry, but not in the wet site. Likewise, fungal total abundance and diversity were reduced by OTC warming at the dry site, while comparatively modest warming effects were observed in the wet site. These results suggest that increased evapotranspiration in the OTC plots lowered litter moisture content to the point where fungal decomposition activities became inhibited. In contrast, snow addition enhanced fungal abundance in both sites but did not significantly affect litter mass loss rates. Across sites, control plots only shared 15% of their fungal phylotypes, suggesting strong local controls on fungal decomposer community composition. Nevertheless, fungal community functioning (litter decomposition) was negatively affected by warming in both sites. We conclude that although buried soil organic matter decomposition is widely expected to increase with future summer warming, surface litter decay and nutrient turnover rates in both xeric and relatively moist tundra are likely to be significantly restricted by the evaporative drying associated with warmer air temperatures.

Keywords: Arctic, Ascomycota, Basidiomycota, *Betula glandulosa*, climate warming, deepened snow, fungi, litter decomposition, litter moisture

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Introduction

In tundra ecosystems, microbial decomposition of plant litter remobilizes nutrients for plant growth and thereby exerts strong controls on the balance between net plant carbon (C) uptake through photosynthesis and ecosystem C release through respiration. Climate-driven changes in plant litter decomposition rates may therefore have profound implications for tundra ecosystem functioning, possibly shifting the C balance from a sink to a net source of C (Davidson & Janssens, 2006; McGuire *et al.*, 2009). However, despite its importance to the global climate, Earth System Models are currently hampered by limited knowledge on how climate change will affect litter decomposition rates in cold tundra regions across the Arctic (Wieder *et al.*, 2013).

Litter decomposition rates are determined by microclimate (in particular the temperature and moisture regimes), the physicochemical properties of the litter substrate, and the decomposer and detritivore communities (e.g., Aerts, 2006; Adair *et al.*, 2008; Wickings *et al.*, 2012). Warmer conditions generally promote litter

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decay (Hobbie, 1996; Cornelissen *et al*., 2007), and decomposition rates are accordingly slower than net primary production in the cold Arctic and boreal biomes, leading to substantial accumulation of soil organic matter (Hugelius *et al*., 2014). However, the rate of climate warming in the Arctic is currently at least twofold faster than the global mean warming rate (e.g., Serreze & Francis, 2006; Hartmann *et al*., 2013). Consequently, it is of concern whether warming-induced changes in decomposition rates can shift tundra ecosystems from net sinks to sources of C (McGuire *et al*., 2009; Hayes *et al*., 2011).

Furthermore, climate models project that by the end of this century, winter warming will be up to four times greater than summer warming in arctic regions (Bintanja & van der Linden, 2013). Winter warming is projected to be accompanied by a substantial (>50%) increase in snowfall in many circumpolar regions (Collins *et al*., 2013). Snow insulates the ground from freezing winter air temperatures and, consequently, increasing snow accumulation up to depths of 1 m leads to a relatively warm and less fluctuating winter ground surface temperature regime (Brooks *et al*., 2011). When soils are below 0 °C, some liquid water persist as tiny water films but this unfrozen water availability is reduced drastically as temperatures decline further (Elberling & Brandt, 2003). When soil water freezes, it hinders diffusion of enzymes and substrates and thereby limits microbial activity (Mikan *et al*., 2002; Öquist *et al*., 2009). Warmer winter microclimates under deepened snow may therefore promote microbial respiration and nutrient mobilization rates (Schimel *et al*., 2004; Nobrega & Grogan, 2007; Semenchuk *et al*., 2015).

Enhanced summer warming may also stimulate litter decomposition activities (Hobbie, 1996), but only as long as the associated increases in evapotranspiration do not lead to moisture limitation of microbial activity, as previous studies observed a decrease in litter mass loss rates following experimental warming (Robinson *et al*., 1995; Sjogersten & Wookey, 2004; Hicks Pries *et al*., 2013; Blok *et al*., 2016). Similar to liquid water limitation during freezing, summer drought lowers soil water availability and microbial activity by restricting diffusion of solutes (Schimel *et al*., 2007). Thus, in a changing climate with both warmer summer air temperatures and greater snowfall, water may become increasingly important in regulating the effects of temperature on microbial communities and C-cycling rates (Davidson *et al*., 1998; Illeris *et al*., 2004; Geml *et al*., 2015; Semenova *et al*., 2015). Despite the importance of litter decomposition as a critical ecosystem process, only few Arctic studies so far have investigated the combined effects of both summer and winter climate manipulations on ecosystem C-cycling processes (Jones *et al*., 1998; Welker *et al*., 2000; Natali *et al*., 2011; Leffler *et al*., 2016), with even fewer focusing on foliar litter responses (Aerts *et al*., 2012; Blok *et al*., 2016).

Both fungi and bacteria are important for litter decomposition, but fungi have greater enzymatic capabilities to decompose complex organic compounds such as lignin (Osono, 2007). For example, fungi are the primary producers of the hydrolytic exoenzymes in leaf litter during decomposition, whereas bacterial hydrolases are negligible (Schneider *et al*., 2012). Thus, free-living saprotrophic fungi are critically important colonizers and decomposers of leaf litter. This is also true for Arctic soils, as soil fungal species richness does not decline with increasing latitude (Tedersoo *et al*., 2014; Timling *et al*., 2014) and fungi dominate mesic Arctic soil microbial communities year-round (Buckeridge *et al*., 2013). Although little is known about the effects of climate change on surface litter fungi, experimental warming has been found to markedly alter tundra soil fungal communities (Clemmensen *et al*., 2006; Deslippe *et al*., 2011, 2012; Geml *et al*., 2015; Morgado *et al*., 2015; Semenova *et al*., 2015). Even though surface litter fungal communities are generally distinct from soil communities (Clemmensen *et al*., 2013), this suggests that climate warming could lead to similar compositional shifts in Arctic litter fungi, potentially affecting future litter C and nutrient cycling as well. However, to the best of our knowledge, no studies have so far linked the climate sensitivity of litter decomposition to climate-induced changes in litter fungal communities in Arctic tundra – and only very few *in situ* experimental studies on the effects of climate change on litter fungal communities have been performed in other biomes, such as boreal forests (McGuire *et al*., 2010). This knowledge gap complicates our understanding of how the primary litter decomposers will respond to a changing environment and ultimately our ability to determine ecosystem carbon and nutrient cycling rates in the future.

Here, we investigated the effects of season-specific climate change manipulations on litter decomposition rates and the associated fungal communities. The tundra is a mosaic of distinct vegetation types that are likely to respond differently to changes in climate. Therefore, we performed a litter decomposition experiment using litterbags placed on the ground surface in two adjacent, widespread low Arctic ecosystem types with contrasting moisture regimes: dry-mesic heath and wet sedge tundra. At both sites, seasonal warming was simulated using a full-factorial experiment with open-top chambers (OTCs) that warmed the litter surface layer during the growing season and snow fences that promoted deeper snow accumulation. We expected

litter layer and soil fungal communities to differ between sites (Morgado *et al*., 2015), and that therefore the litterbag-colonizing fungal communities, colonizing fresh litter from the already present litter layer, would be site dependent. Consequently, we hypothesized that: (H1) Enhanced summer warming would stimulate litter mass loss more in the wet site than in the dry site due to greater warming-induced evaporative declines in water availability at the latter site; (H2) deepened snow would promote litter mass loss equally in both sites; and (H3) experimental treatment effects on litter mass loss would be mirrored by corresponding changes in the fungal communities.

Materials and methods

Site description and experimental setup

We conducted our study in the Blæsedalen Valley on the southernmost tip of Disko Island, West Greenland (69°16′N, 53°27′W), from July 2013 to August 2014. The research site is located in the discontinuous permafrost zone and has a typical low Arctic climate with mean annual air temperatures of −3 °C, and monthly mean temperatures ranging from −14 °C in March to +8 °C in July (1992–2012 averages), recorded by the meteorological station at Arctic Station, approximately 2 km from our study site (Hollesen *et al*., 2015).

In July 2012, we established an experimental site in a dry-mesic shrub heath (from here on referred to as 'dry' site) with vegetation consisting mainly of deciduous (*Betula nana* and *Salix glauca*) and evergreen (*Vaccinium vitis-idaea*, *Empetrum nigrum* and *Cassiope tetragona*) low shrubs (height <10 cm), with a mixture of lichens covering the ground (Blok *et al*., 2016). In July 2013, a similar, second, experimental site was established in a wet sedge area approximately 200 m away from the dry site. The vegetation in the wet site is generally taller (height <25 cm) than in the dry site and is dominated by sedges (*Carex rariflora*, *C. aquatilis* and *Eriophorum angustifolium), Equisetum arvense,* and the low shrub *Salix arctophila*, with a mixture of mosses (*Tomentypnum nitens* and *Aulacomnium turgidum*) covering the ground surface. Both shrub heath (Circumpolar Arctic Vegetation Map (CAVM) subunits P1, P2 and P3) and wet sedge (CAVM subunits W1, W2 and W3) ecosystems occur abundantly across the low Arctic (CAVM subzones D and E), where they cover about 25% and 9% of the nonglaciated land area, respectively (Walker *et al*., 2005). Generally, soil thaw depth varies between the dry and wet sites, with the permafrost table being located 2–3 m belowground in the dry site and 0.5 m in the wet site.

In each of the two sites, we established a climate manipulation experiment combining two treatments in a fully factorial block design. The 'deepened snow' treatment was achieved through passive snow accumulation by six snow fences (14.7 m wide, 1.5 m tall), reducing wind speeds on their lee side, thus creating ~140- to 150-cm-deep snowdrifts approximately 2–3 times deeper than ambient (control) snow accumulation. The 'summer warming' treatment used polycarbonate OTCs (in place year-round; Marion *et al*., 1997), measuring 150 cm diameter at the base and 35 cm tall, to warm air and soil during the snow-free season. The two main treatments were combined in a full-factorial design and laid out in six blocks for each site. The original setup also included a shrub removal treatment that was laid out in a factorial arrangement with the other two above-mentioned main treatments. However, an earlier litter decomposition study performed in the same experimental plots showed that the shrub removal treatment had no effect on litter decomposition rates (Blok *et al*., 2016). Therefore, we added the shrub treatment plots into the summer warming and deepened snow treatments (doubling *n* from six to twelve per treatment), resulting in 96 plots in total (48 plots per site).

Although the dry and wet sites were established during different years (summer 2012 and summer 2013, respectively), our litterbag incubations were initiated at the same time. Furthermore, at the start of our study, plant community composition remained unchanged by the experimental treatments (A. Michelsen, unpublished data). We therefore assume that we can reasonably test for site-specific treatment effects on litterbag properties, as well as make inter-site effect size comparisons.

Temperature and moisture

We measured near-surface air (1–2 cm above ground) and soil temperatures at 5 cm depth using TinyTag thermistors (Gemini Data Loggers, Chichester, UK), logging mean hourly temperatures. Similarly, volumetric soil moisture integrated over 0–5 cm depth was measured using Decagon EC-5 water content sensors (Decagon Devices, Pullman, WA, USA) and logged every 10 min. Regardless of soil type, these sensors have an accuracy of 0.03 m^3 H_2O m^{-3} soil between 0 and 50 °C. We used the default sensor calibration optimized for soils with volumetric soil water ranging between 0 and ~55%. Volumetric soil moisture in the wet site may occasionally have exceeded the top end of this range, and, therefore, values in this site could be slightly underestimated. All temperature and moisture data were recorded in half of all plots in both dry and wet sites (n = 24 per site). Winter snow depth was estimated from daily photographs by automated cameras (n = 3 blocks $site^{-1}$) aimed at graduated meter sticks within the blocks at both sites.

Litter bag preparation, incubation, and processing

Freshly senesced *Betula glandulosa* (American Dwarf Birch) leaves were collected near Daring Lake, Northwest Territories, Low Arctic Canada in early September 2012. Leaf litter was collected from shrubs spanning a 4 km^2 lowland valley, representing broad-scale variation in birch shrub litter chemistry at the site. We carefully collected leaves by gently shaking shrub branches into large plastic bags, thereby only collecting leaves that were fully ready to senesce. The leaf litter contained 51.1 ± 0.8% C, 0.5 ± 0.01% nitrogen (N) and 0.1 ± 0.01% phosphorus (P; dry weight concentrations; n = 6; ± 1 SE). All collected litter was air-dried and well mixed before sealing

into 192 litterbags (0.5 g litter per bag; 0.68 mm mesh size), as previously described in detail by Blok *et al.* (2016).

We placed two litterbags in each of the 96 experimental plots across the dry and wet sites between July 16, 2013, and July 18, 2013. All litterbags were incubated on top of the moss/lichen surface and held in place by noncorroding steel nails. Our measurements therefore reflect the integrated effects of microbial decomposition, photodegradation and leaching by freeze/thaw and dry/wetting episodes, thus mimicking the natural conditions during leaf litter decomposition. We harvested all litterbags on 2 August, 2014 (after 380–382 days of incubation). Subsequently, soil or foreign litter material was carefully hand-picked or shaken off. Litter material from one set of litterbags was then weighed, dried at room temperature for a minimum of 96 h and reweighed for determination of moisture content and mass loss. The dried litter material was subsequently homogenized using a fine ball mill (Mini-Mill Pulverisette 23, Fritsch, Idar-Oberstein, Germany). The second set of litterbags was frozen at −18 °C until extracted for genomic DNA.

Litter carbon, nitrogen and phosphorus contents

Subsamples (40 mg) of one set of litterbags were analyzed for total C and N content using an Elementar VARIO Micro Cube elemental analyzer (Elementar Analysensysteme, Hanau, Germany). Total P content was determined photometrically after acid digestion (Kedrowski, 1983) on a FIAstar™ 5000 Flow Injection Analyzer (FOSS, Hillerød, Denmark).

Initial litter mass remaining was calculated using the equation $M_r = \frac{M_t}{M_0}$, where M_r is the fraction of total litter mass remaining at time t (after 380–382 days of incubation), M_t is total litter mass (g) remaining at time t, and M_0 is initial total litter mass (g). Similarly, initial C, N and P remaining were calculated using the equation $\text{CNP}_r = \frac{M_t \times \text{CNP}_t}{M_0 \times \text{CNP}_0}$, where CNP_r is the fraction of litter C, N or P remaining at time t; M_t and M_o are as described above; CNP_t is the concentration of litter C, N or P remaining at time t; and CNP_0 is the initial litter concentration of C, N or P. We report C and P as % loss ($(1 - \text{CP}_r) \times 100\%$), whereas N is reported as % immobilization, that is, N import into litter, ($(\text{N}_r - 1) \times 100\%$).

Fungal DNA extraction, quantitative PCR and sequencing of the fungal community

DNA was extracted from ~0.10 g fresh litter subsamples and from original nonincubated leaf litter using a PowerLyzer® PowerSoil® DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) following their standard protocol. The extracted DNA was stored at −18 °C and used for both quantitative PCR (qPCR) and PCR amplicon sequencing.

The operational taxonomic unit (OTU) abundance of fungal ITS2 copies in the litter samples was quantified using 20 μL SYBR green reactions on Bio-Rad CFX96™ (Bio-Rad, Hercules, CA, USA). All qPCRs were run in technical triplicates and contained 10 μL SyGreen Mix Lo-ROX (PCR Biosystems Ltd, London, UK), 1 μL forward primer gITS7 (Ihrmark *et al.*, 2012), 0.6 μL reverse primer ITS4 (White *et al.*, 1990), 2 μL BSA, 2 μL template DNA and 4.4 μL ddH$_2$O. The qPCR conditions were 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s, 56 °C for 20 s, 72 °C for 45 s, an extension step of 72 °C for 6 min and with a melt curve analysis as the final step. The standard curve was generated using a plasmid containing the ITS2 region amplified from *Aureobasidium pullulans* (de Bary) G. Arnaud. The qPCR results were adjusted for any amplified plant DNA after obtaining sequencing results of the ITS2 region.

The primers gITS7 and ITS4 were also used to PCR amplify the fungal ITS2 for sequencing. PCR amplifications were performed as a two-step process: In the first step (PCR-I), we added illustra™ puReTaq Ready-To-Go PCR Beads (GE Healthcare, Little Chalfont, UK), the primers and 1 μL of 10 × diluted template DNA. The PCR-I conditions were 94 °C for 2 min; 30 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s, followed by 72 °C for 5 min. The gel check on 1% agarose gels after PCR-I showed no DNA bands from four nonincubated litter samples, suggesting negligible fungal presence before incubation, and they were therefore not analyzed further. In the second step (PCR-II), we added 2 μL of the PCR-I product to a reaction mixture including 0.15 μL DNA polymerase (AccuPrime™ Taq DNA Polymerase High Fidelity, Invitrogen™, Thermo Fisher Scientific, Waltman, MA, USA), 2 μL 10 × AccuPrime™ PCR buffer II, 1 μL (770 nM) of each primer (tagged forward and reverse primer) and 13.85 μL ddH$_2$O to a 20 μL reaction volume. The conditions for PCR-II were 94 °C for 2 min, 14 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s, followed by 72 °C for 5 min. Amplicon concentrations were then quantified for all samples using the PicoGreen® dsDNA Assay Kit (Invitrogen™, Thermo Fisher Scientific) and measured in a 96-well microplate by a Modulus™ Microplate Fluorometer (Turner Biosystems, Sunnyvale, CA, USA). All samples were pooled and paired-end sequenced on a MiSeq™ (Illumina, San Diego, CA, USA).

Processing of fungal ITS2 sequence data

The fungal DNA sequences were processed using QIIME 1.7 (Caporaso *et al.*, 2010). The sequences were trimmed of primer sequences and barcodes and paired sequences assembled using a custom script. Sequences that could not be assembled as well as chimeras and sequences with a quality score less than 20 were removed. The remaining sequences (9.8×10^6 reads with an average read length of 435 bp) were clustered into OTUs, using USEARCH v.5.2.236 (Edgar, 2010) at 97% similarity level in QIIME. OTU clusters containing <100 reads were removed. The remaining OTUs were classified using Ribosomal Database Project classifier (Wang *et al.*, 2007) against the UNITE database v.7.0 (Abarenkov *et al.*, 2010). The samples were rarefied to 5000 reads per sample before the between-sample community analysis. The final dataset consisted of 4.8×10^5 reads.

Statistical analyses

All data distributions were visually inspected and natural log-transformed where needed to achieve variance homogeneity.

We used the PROC MIXED procedure in SAS Enterprise Guide 6.1 (SAS Institute Inc., Cary, NC, USA) to test the effects of the deepened snow and summer warming treatments, as well as their interaction (all assigned as fixed effects), on litter and fungal variables. The original setup also included a factorial shrub removal treatment (Blok *et al*., 2016) but because of our focus on seasonal climate change effects, we do not discuss the (nonsignificant) shrub removal treatment effects. Nevertheless, we kept the shrub removal treatment as explanatory factor in the statistical models as a conservative measure to limit denominator degrees of freedom to the original three-way factorial design (Blok *et al*., 2016). We set block and half-block (deepened snow treatment nested within snow fence block) as random factors to take into account any potential spatial effects at the block and half-block scale, respectively. All models were step-wise reduced, eliminating three-way and two-way interactions when these were nonsignificant, and run separately for each site. Fixed effects were assessed using the restricted maximum likelihood method, and degrees of freedom were estimated by the Kenward–Roger method.

Based on our daily snow monitoring data, we divided the seasonal environmental data into three main periods: autumn (from time of litterbag incubation to start of snow accumulation), winter (continuous snow cover) and summer (from spring snow melt until fall litterbag harvest) – with two shorter transitional periods in between (snow buildup and snow melt, respectively). See Table S1 for detailed information on period dates. Snow buildup took slightly longer at the wet site compared to the dry site, but complete snow melt in the deepened snow treatment plots was consistently delayed by 10 days compared to ambient plots in both sites. For each period, we separately analyzed treatment effects on environmental parameters (using period means) as described above for analyses of treatment effects on litter and fungal parameters.

When we observed significant treatment effects on a variable across sites or between treatments within sites, we calculated treatment effect sizes (standardized mean difference, Cohen's d; McGrath & Meyer, 2006) to facilitate comparisons. We report mean treatment effect sizes (d) and 95% confidence intervals (CI) where effect sizes of 0.2–0.49, 0.5–0.79 and ≥0.8 were considered small, medium and large, respectively (Cohen, 1992). Positive values of d indicate that the treatment had a positive effect on the parameter, while negative values indicate a negative treatment effect.

We analyzed site and treatment effects on fungal community composition by nonmetric multidimensional scaling on a matrix of treatment plots by OTUs using PRIMER version 7 (Clarke & Gorley, 2015). The dataset was subjected to 500 iterations per run using the Bray–Curtis similarity index. We tested for site and treatment effects by permutation-based analysis of similarity (ANOSIM) and subsequently determined which fungal OTU contributed most to community structure by similarity percentage analysis. Finally, principal component analyses were performed for each site – and overlain with biplots of the ten OTUs with the largest impact on fungal community composition in ordination space.

Results

Effects of the experimental treatments on seasonal microclimate

The summer warming treatment increased mean autumn and summer near-surface temperatures by ~1–2 °C in both the dry ($F_{1,10}$ = 64.4, and $F_{1,25}$ = 42.9, respectively; both P < 0.0001) and wet site ($F_{1,17}$ = 17.2; P < 0.0001; and $F_{1,17.2}$ = 27.8; P < 0.0001, respectively; Fig. 1 and Table S1). The associated effect sizes were similar across sites and seasons (Cohen's d ± 95% CI in autumn and summer in the dry site: 1.7 ± 0.3; and 1.6 ± 0.3, respectively; and in autumn and summer in the wet site: 1.8 ± 0.3; and 1.3 ± 0.2, respectively). Additionally, the summer warming treatment slightly enhanced winter near-surface temperatures in the dry site ($F_{1,22}$ = 5.1, P = 0.03), but this effect was less than ~0.5 °C and primarily occurred in the summer warming + deepened snow plots (Table S1), presumably due to some snow trapping by the OTC frames during early winter (Aerts *et al*., 2012). Summer warming also tended to reduce soil moisture by 22% relative to non-warmed plots in the dry site during summer $F_{1,11}$ = 4.0, P = 0.07; Fig. S1 and Table S2), but not in the wet site. Similarly, the deepened snow treatment enhanced winter surface temperatures by ~2.5 °C in the dry site ($F_{1,3}$ = 54.9, P < 0.01; Fig. 1 and Table S1), but not in the wet site. There were no significant effects of deepened snow on soil moisture in either site (Fig. S1 and Table S2). Seasonal soil temperatures at 5 cm depth for each treatment combination are shown in Table S3.

Climate manipulation effects on litter mass loss and litter biogeochemistry

Litter moisture content in the unmanipulated control plots was about three times greater and more heterogeneous among plots in the wet site than in the dry site (Table 1). Similarly, litter mass and C loss as well as N immobilization rates were all greater in the wet site (by up to a factor of 2.5), while P loss rates were similar across sites. Litter mass loss showed a significant, albeit weak, positive correlation with litter moisture content in the dry site (Adj. r^2 = 0.09, P < 0.05, n = 48 litterbags), but not in the wet site.

Summer warming reduced litter moisture content significantly by 24% (relative %-change) in the dry site ($F_{1,39}$ = 9.0; P < 0.01; Table 1), but not in the wet site.

The summer warming treatment reduced litter mass loss relative to non-warmed plots by 32% ($F_{1,34}$ = 58.8, P < 0.0001) and C loss by 40% ($F_{1,39}$ = 63.5, P < 0.0001) in the dry site and by 17% and 21%, respectively, in the

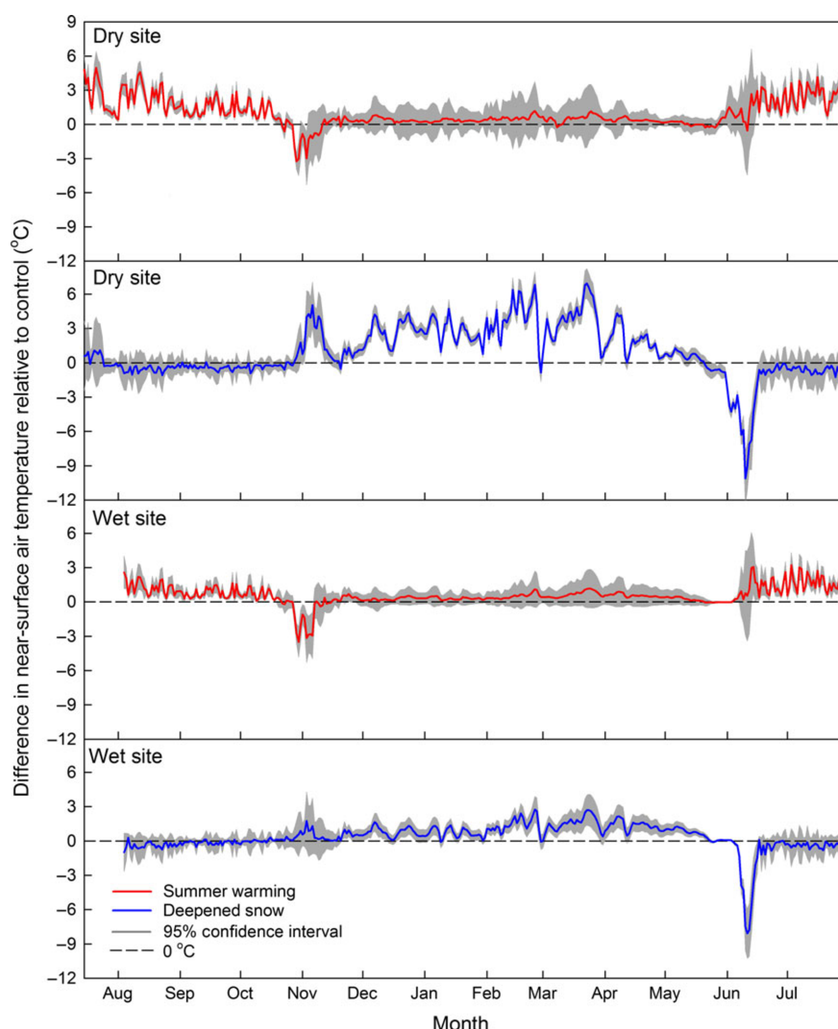

Fig. 1 Effects of experimental summer warming (by open-top chambers; red lines) and deepened snow (using snow fences; blue lines) on diel ground surface temperature (plotted as differences relative to their corresponding controls) from July 15, 2013, to August 2, 2014, in the dry and wet tundra sites at Disko Island, W. Greenland, respectively (n = 6–16 probes per treatment per site). Positive values (i.e., above the zero line) indicate warmer surface temperatures in the treatment plots, and the gray fills indicate 95% confidence intervals. Note that temperature data in the wet site were not available prior to August 5, 2013.

wet site ($F_{1,39} = 13.6$, $P < 0.001$; and $F_{1,44} = 9.7$, $P < 0.02$, respectively; Table 1 and Fig. 2). Accordingly, the associated summer warming effect sizes were markedly larger in the dry relative to the wet site (mass and C loss d; dry site, both: -2.3 ± 0.1; wet site: -0.9 ± 0.1 and -1.3 ± 0.1, respectively). In the dry site, the summer warming treatment had no effect on litter N content but significantly reduced %P loss by 31% ($F_{1,36} = 31.5$; $P < 0.0001$; Table 1). In the wet site, summer warming reduced litter N immobilization by 26% ($F_{1,34} = 4.2$; $P = 0.047$) but had no significant effect on litter P content.

There was a strong tendency for deepened snow to increase litter mass loss in the dry site by 12% ($F_{1,5} = 5.8$; $P = 0.06$; Cohen's $d = 0.4 \pm 0.1$), but it had no impact on mass loss in the wet site (Fig. 2, Table 1). Similarly, deepened snow increased litter N immobilization rates by a factor of 2.6 in the dry site ($F_{1,44} = 12.5$; $P = 0.001$), but had no effect in the wet site. The deepened snow treatment did not affect %C or %P loss in either site (Table 1).

Climate manipulation effects on litter-associated fungal communities

In accordance with greater decomposition rates in the unmanipulated control plots at the wet site compared to the dry site, the abundance of fungal ITS copies in the incubated litter was higher in the wet site (Fig. 3). Furthermore, and consistent with the above, there was

Table 1 Effects of experimental summer warming and deepened snow on litter chemistry and fungal community structure in *Betula glandulosa* leaves following one full year of *in situ* litterbag incubation in the dry (top) and wet (bottom) sites. The factorial treatments are control, summer warming (by open-top chambers), deepened snow (using snow fences), and warming + snow is the combination thereof (n = 12 per treatment per site)

	Control	Warming	Snow	Warming + snow	Treatment effects
Dry site					
Litter carbon loss (%)	17.9 ± 0.6	10.1 ± 1.5	18.4 ± 0.9	11.8 ± 0.7	Warming***
Litter nitrogen immobilization (%)	11.9 ± 4.3	7.0 ± 3.3	25.1 ± 4.4	23.2 ± 4.5	Snow***
Litter phosphorus loss (%)	63.8 ± 0.8	42.3 ± 6.1	62.0 ± 1.0	44.1 ± 4.8	Warming***
Litter moisture (%)	4.2 ± 0.4	3.7 ± 0.4	4.5 ± 0.3	3.2 ± 0.4	Warming**
Fungal Shannon–Weaver diversity	2.5 ± 0.1	2.1 ± 0.1	2.5 ± 0.1	1.5 ± 0.1	Warming***; Snow*; W × S**
Wet site					
Litter carbon loss (%)	22.3 ± 1.0	17.5 ± 2.8	20.6 ± 0.8	16.4 ± 1.2	Warming*
Litter nitrogen immobilization (%)	30.1 ± 3.6	21.2 ± 5.3	33.8 ± 3.8	25.9 ± 5.5	Warming*
Litter phosphorus loss (%)	58.3 ± 2.3	50.9 ± 5.1	51.0 ± 5.4	51.8 ± 2.7	
Litter moisture (%)	13.2 ± 5.2	6.8 ± 0.8	6.6 ± 1.4	7.0 ± 0.6	
Fungal Shannon–Weaver diversity	2.9 ± 0.2	2.5 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	

All values are means (±1 SE), and significant main factors (warming and snow) and interactions (W × S) are shown as *$P \leq 0.05$; **$P \leq 0.01$; and ***$P \leq 0.001$.

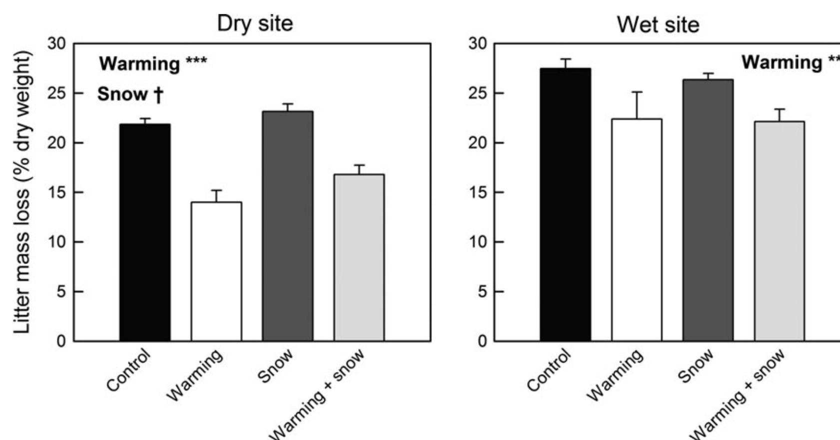

Fig. 2 Effects of experimental summer warming and deepened snow on total mass loss in *Betula glandulosa* leaves following one full year of *in situ* litterbag incubation in the dry (left) and wet (right) tundra sites at Disko Island, W. Greenland. The factorial treatments are control, summer warming (by open-top chambers), deepened snow (using snow fences), and warming + snow is the combination thereof (n = 12 per treatment per site). All values are means (±1 SE) and significant main factor treatment effects (warming and snow) are shown inside panels as $^{\dagger}P \leq 0.1$; **$P \leq 0.01$; and ***$P \leq 0.001$.

a corresponding drop in fungal abundance and/or richness in the summer warming plots. Litter mass and C loss showed weak-to-moderate positive correlations with fungal richness and diversity in both sites (Adj. r^2 = 0.08–0.26 across sites and correlations), as well as with fungal abundance in the dry site (Adj. r^2 = 0.15–0.18; Table S4). In addition, litter N immobilization showed a weak, albeit significant, correlation with fungal abundance in the dry site (Adj. r^2 = 0.08), while litter P loss also correlated positively with fungal richness and diversity in the dry but not the wet site (Adj. r^2 = 0.09–0.12; Table S4).

Deepened snow almost tripled fungal ITS copy numbers in the dry site ($F_{1,36}$ = 14.2; P < 0.0001; d = 0.5 ± 0.2; Fig. 3), but this was primarily driven by a large positive interaction between the warming and deepened snow treatment, enhancing fungal abundance more so than expected from each single main treatment effect ($F_{1,36}$ = 7.5; P < 0.01; d = 0.9 ± 0.2). Although the summer warming treatment reduced fungal abundance in the dry site by one-third and therefore had the largest effect size (d = −1.5 ± 0.2), this effect remained a tendency ($F_{1,36}$ = 3.6; P = 0.07) due to the strong positive effect of the interaction

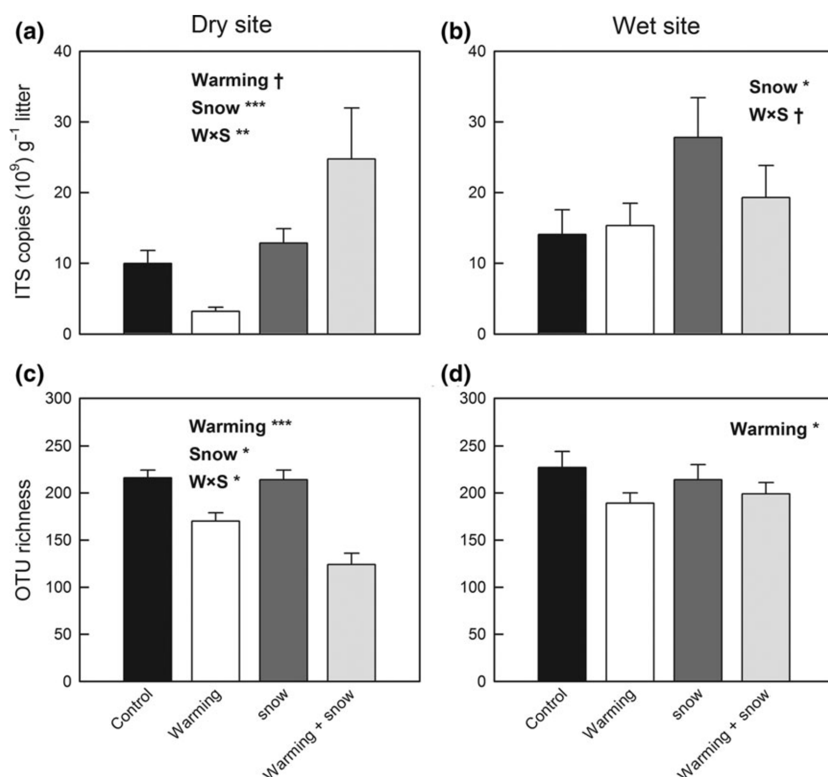

Fig. 3 Effects of experimental summer warming and deepened snow on (a) + (b) fungal abundance (total number of fungal ITS gene copies) and (c) + (d) fungal operational taxonomic unit richness in litterbags containing *Betula glandulosa* leaves following one full year of *in situ* incubation in the dry (left) and wet (right) tundra sites at Disko Island, W. Greenland. The factorial treatments are control, summer warming (by open-top chambers), deepened snow (using snow fences), and warming + snow is the combination thereof (n = 12 per treatment per site). All values are means (±1 SE) and significant main factor treatment effects (warming and snow) and interactions (W × S) are shown inside panels as $^{\dagger}P \leq 0.1$; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; and $^{***}P \leq 0.001$.

between the summer warming and deepened snow treatments.

In the wet site, deepened snow increased fungal ITS copy numbers by 60% ($F_{1,35} = 5.1$; $P < 0.05$; Fig. 3b) and similar to the dry site, we detected a deepened snow and warming interaction effect as fungal abundance was lower than expected from main treatments alone ($F_{1,31} = 3.6$; $P = 0.07$).

In both sites, the fungal communities were generally dominated by Ascomycetes followed by Basidiomycetes (Fig. 4 and Table S5). However, Basidiomycetes constituted a larger fraction of the fungal community in the unmanipulated control plots in the wet site compared to control plots in the dry site.

Furthermore, the fungal community in the dry site was dominated by fewer, but more abundant OTUs than in the wet site (Table S5), leading to high dissimilarity in community structure between the sites ($r = 0.603$; $P < 0.0001$; Fig. 5a), with only ~15% of the fungal OTUs present in both sites (Fig. S2).

In the dry site, the summer warming ($F_{1,36} = 46.4$; $P < 0.0001$) and deepened snow ($F_{1,36} = 7.8$; $P < 0.01$) treatments reduced fungal diversity by 28% and 13%, respectively, and richness by 32% and 10%, respectively ($F_{1,36} = 54.0$; $P < 0.0001$; and $F_{1,36} = 6.7$; $P = 0.01$, respectively; Fig. 3 and Table 1). However, the deepened snow effects were almost entirely driven by a 40–43% decline in both diversity and richness (relative to control plots) in the combined warming and deepened snow plots with no corresponding change in the plots with deepened snow alone, leading to significant warming and deepened snow interaction effects ($F_{1,36} = 7.5$; $P < 0.01$; and $F_{1,36} = 4.9$; $P = 0.03$, respectively). Consequently, main treatment effect sizes (Cohen's $d \pm 95\%$ CI) regarding fungal diversity were -1.2 ± 0.2 (summer warming), 0 ± 0.2 (deepened snow) and -3.0 ± 0.4 (warming + snow combination). Similarly, the main treatment effect sizes for OTU richness were -1.6 ± 0.3 (summer warming), -0.1 ± 0.2 (deepened snow) and -2.7 ± 0.4 (warming + snow combination).

In the wet site, there were no treatment effects on fungal diversity, whereas summer warming reduced fungal richness but to a lesser extent (~12%) compared

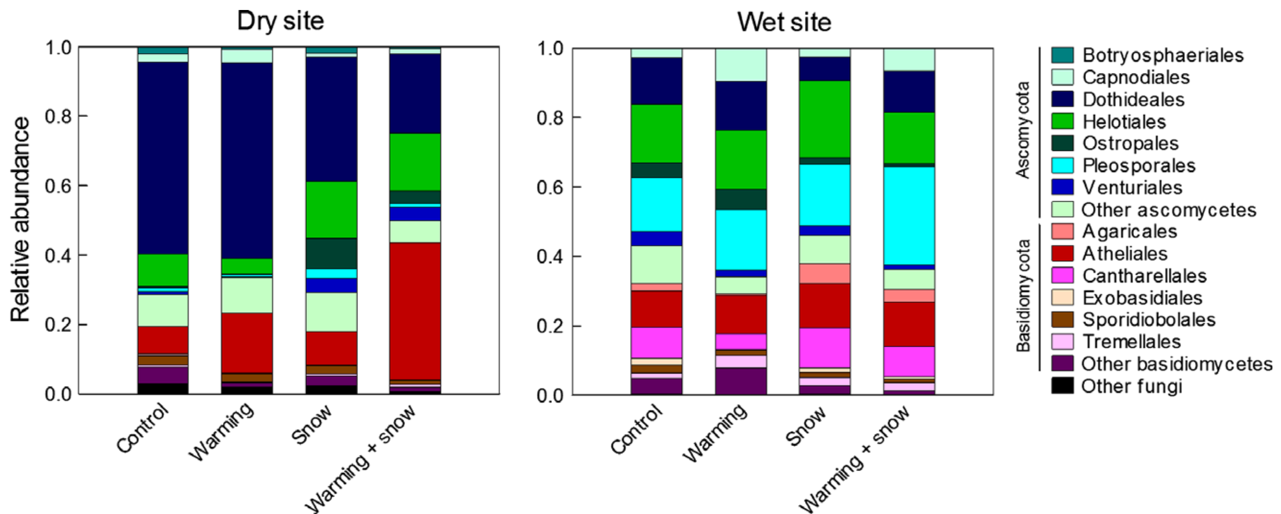

Fig. 4 Effects of experimental summer warming and deepened snow on relative abundance (i.e., operational taxonomic unit (OTU) frequencies) of phylogenetically assigned fungal groups (orders) following 1 year of litterbag incubation in the dry (left) and wet (right) tundra sites at Disko Island, W. Greenland. The factorial treatments are control, summer warming (by open-top chambers), deepened snow (using snow fences), and warming + snow is the combination thereof (n = 12 per treatment per site). The blue-green color scheme represents fungal orders belonging to the phylum Ascomycota, and the red-purple-brown color scheme represents orders belonging to the phylum Basidiomycota. All other fungal OTUs are grouped as other fungi (black).

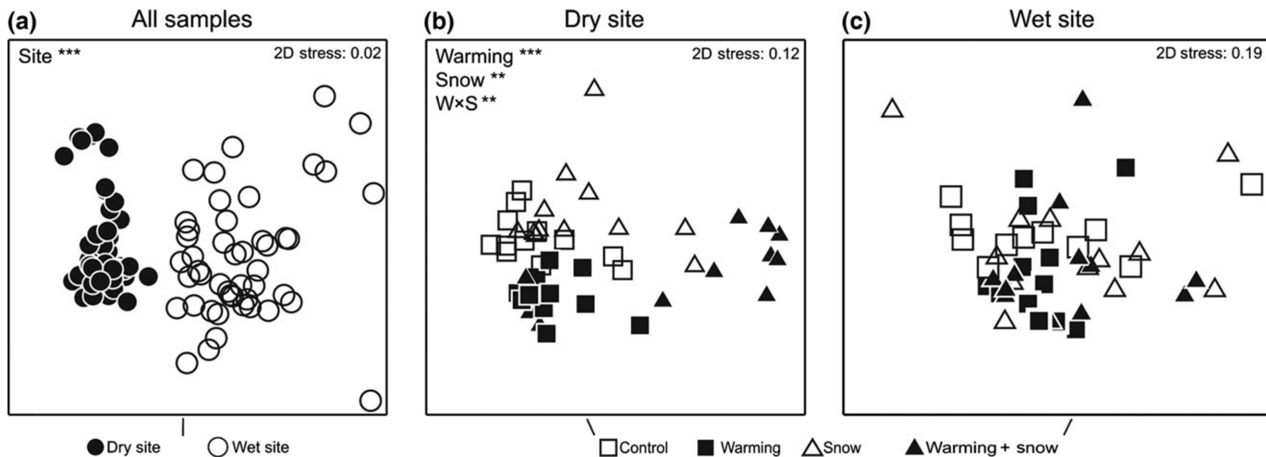

Fig. 5 Nonmetric multidimensional scaling analyses of the fungal communities extracted from *Betula glandulosa* senesced leaves following one full year of *in situ* litterbag incubation on the ground surface. All summer warming and deepened snow treatment data were combined for an overall site comparison (a) and were also analyzed separately for the dry (b) and wet (c) sites, respectively. The factorial treatments are control, summer warming (by open-top chambers), deepened snow (using snow fences), and warming + snow is the combination thereof (n = 12 per treatment per site). Significant main factor treatment effects (warming and snow) and interactions (W×S) are shown inside ordinations as $^{**}P \leq 0.01$; and $^{***}P \leq 0.001$.

to the dry site ($F_{1,31}$ = 5.1; P = 0.03; d = −0.2 ± 0.1; Fig. 3).

The ANOSIM confirmed that summer warming significantly altered fungal community composition in the dry site but not in the wet site (r = 0.541; P < 0.001; Fig. 5b and c). Deepened snow also changed fungal community composition (r = 0.232; P < 0.002) in the dry site, although this effect largely was driven by the interaction between the warming and deepened snow treatment (r = 0.414; P < 0.001). When performing a pairwise comparison between controls and deepened snow plots alone, we found no difference in community structure between these two treatments, although deepened snow decreased the abundance of Capnodiales (P < 0.05; Fig. 4). The warming-induced shifts in dry site fungal community composition were primarily driven by an increased relative abundance of Basidiomycota OTUs from the order Atheliales ($F_{1,36}$ = 5.2;

$P < 0.05$; Figs 4 and S3) – as shown by the OTU *Fibulorhizoctonia* sp. being the main driver of the distinction between warmed and non-warmed plots ($P < 0.05$; Fig. S3). Furthermore, warming increased the relative abundance of Capnodiales ($P < 0.05$), whereas the Dothideales ($P < 0.01$), Helotiales ($P < 0.05$), Ostropales ($P < 0.05$), Venturiales ($P < 0.05$) and Sporidiobolales ($P < 0.05$) all decreased in abundance (Fig. 4). There were no treatment effects on relative abundance of fungal orders (Fig. 4) or fungal community composition (Fig. 5c) in the wet site.

Discussion

Here, we demonstrate that – at least during the initial decomposition stages of *B. glandulosa* leaf litter – season-specific climate change effects on litter mass loss are mirrored by changes in fungal abundance, richness and diversity across distinct tundra ecosystems. The effects of our experimental climate manipulations on near-surface microclimate – including the litter layer – were clearly constrained by site-specific moisture regimes, suggesting that ecosystem hydrology will be an important determinant of the impacts of a future warmer and potentially drier climate on surface litter decomposition (Figs 1 and S1; Tables S1–S3). The two sites only shared 15% of the total fungal OTUs found in all litterbags, indicating relatively strong species sorting by the different local edaphic properties (e.g., Martiny *et al.*, 2006; Fig. S2). Nevertheless, despite the relatively large site differences in litter decomposer fungal community structure, the functional responses to environmental perturbations were broadly similar. However, litter fungal abundance and richness, as well as litter decay rates, were greatest in the wet site, suggesting that the relatively moist conditions there stimulated fungal litter decomposer communities more so than in the dry site (Figs 2–4).

Effects of experimental warming on litter decay and fungal communities

Warming generally increases microbial enzyme activity, but warming-induced drying of a substrate below a certain moisture level reduces diffusion rates and therefore ultimately slows biogeochemical processes (Schimel *et al.*, 2007). Accordingly, litter decay rates were not just lower in the unmanipulated control plots in the dry site relative to the wet site, they were also more strongly reduced by the warming treatment in the dry site (greater effect sizes; Fig. 2). This was likely due to the inherent differences in moisture regimes, with summer warming reducing litter moisture content to a greater extent in the dry site. Previous studies have also reported reductions in litter mass loss rates following experimental warming in predominantly mesic and xeric tundra sites (Robinson *et al.*, 1995, 1997; Sjogersten & Wookey, 2004; Blok *et al.*, 2016). However, to the best of our knowledge, this study is the first to report warming-induced declines in litter decay rates in distinct dry *and* wet ecosystems, partially refuting H1.

Phosphorus is well known to leach readily from surface litter (Chapin & Moilanen, 1991; Schreeg *et al.*, 2013), and litter P loss was significantly reduced following summer warming in the dry site (i.e., enhanced litter P retention). Greater P availability generally correlates with fungal abundance (Schneider *et al.*, 2012), and while we did not observe this relationship in our data, fungal diversity positively correlated with litter P loss in the dry site (Table S4). Furthermore, across both sites, fungal richness and diversity positively correlated with litter mass and C loss, suggesting that microbial diversity promotes decomposer community function (i.e., litter decay; Bell *et al.*, 2005). Accordingly, summer warming reduced dry site fungal OTU richness and diversity by one-third, while abundance was reduced by two-thirds (control plots compared to the summer warming treatment alone; Fig. 3), resulting in an overall changed fungal community (Fig. 5). In particular, summer warming increased the relative abundance of the dominant basidiomycete *Fibulorhizoctonia* sp. (order: Atheliales; Figs 4, 5, and S3). Such hyphal-forming fungi are generally more tolerant to drying than the black yeast *Hormonema carpetanum* (which were highly dominant in the control plots) because they can translocate water and other limiting resources from other locations (Schimel *et al.*, 2007).

We did not detect a significant decrease in litter moisture following warming in the wet site (Table S2). Accordingly, OTU richness was the only fungal community parameter that declined in the wet site – and overall, community structure remained similar across treatments (Figs 3, 5 and S3). Litters incubated in the summer warming plots in the wet site contained five times more fungal copy numbers compared to the dry site (single warming treatments; Fig. 3). Nevertheless, litter decay and N immobilization rates were reduced by the warming treatment in the wet site – albeit with a lower effect size for litter decay than in the dry site. Microbes adapted to mesic and moist microclimates are generally more strongly affected by reductions in moisture availability than microbes from xeric environments due to differences in specialized physiologies and stress tolerance (Manzoni *et al.*, 2011). Together, this suggests that the fungal community in the wet site most likely experienced some moisture limitation, possibly, as litter

moisture was lowered by the warming treatment during earlier parts of the growing season not covered by our single sampling in early August (e.g., during July that typically is the warmest summer month). If so, this would mean that the fungal community had not yet recovered in August.

Effects of experimentally deepened snow on litter decay and fungal communities

We observed a tendency for enhanced litter mass loss (by 12%, $P = 0.06$) in the deepened snow treatment in the dry but not in the wet site, partially refuting H2. In both sites, the effect of deepened snow was minor in comparison with the summer warming treatment. A few other longer-running (3–5 years) Arctic snow manipulation studies have previously reported no significant effects of deepened snow on mass loss of similar quality *Betula* sp. leaf litter as in our study (Aerts *et al.*, 2012; DeMarco *et al.*, 2014). However, in the study by DeMarco *et al.* (2014), litterbags were incubated beneath the moss layer (as it is common for litter to accumulate within the moss layer in moss-rich tussock tundra), and their results therefore do not directly reflect the ground surface litter layer microenvironment, whereas the bog study site used by Aerts *et al.* (2012) may more closely resemble the moisture regime at our wet site. Direct comparisons across studies are therefore not straightforward. However, Blok *et al.* (2016) detected substantially enhanced mass loss (47%) of surface-incubated *Salix glauca* leaf litter using the same experimental plots as our study. We attribute this relatively large difference in effect sizes between studies to the unusually early snow melt in ambient snow plots during the previous study by Blok *et al.* (2016), resulting in an ~8 week delay in snow melt in the deepened snow plots (compared to a 10 day snow melt delay in our study). Consequently, the deepened snow plots stayed warmer relative to ambient conditions during the winter–spring transition with frequent subzero air temperatures.

Litter N immobilization was enhanced by a factor of 2.6, and fungal copy numbers were three times greater in the deepened snow treatment plots in the dry site. This strongly suggests that the deepened snow treatment enhanced microbial growth rates in winter, due to relatively warm temperatures and therefore likely greater unfrozen water availability (Tilston *et al.*, 2010; Aanderud *et al.*, 2013), although litter mass loss rates did not increase significantly. Large microbial community shifts occur between winter and summer in tundra soils (Björk *et al.*, 2008; Buckeridge *et al.*, 2013), and the deepened snow treatment would therefore likely only affect the fungal OTUs that dominate in winter. Accordingly, plots receiving a combination of summer warming and deepened snow treatments had ~eightfold greater fungal abundance than summer warming plots without deepened snow, suggesting that deepened snow promoted fungal growth primarily during winter and not due to potential legacy effects during summer. This could be due to large parts of the winter fungal community downregulating their activity (i.e., entering an inactive resting state) in the growing season (McMahon *et al.*, 2011). Consequently, the legacy effect of a more abundant winter-adapted fungal community on litter decomposition rates could remain a relatively small effect every year – depending on the severity of the local winter microclimate. Nevertheless, as the circumpolar winter climate is projected to warm four times more than in summer during the 21st century (Bintanja & van der Linden, 2013) and involve increased snowfall (Collins *et al.*, 2013), enhanced fungal activity under deeper snow could become exceedingly important regarding annual litter decay rates (Blok *et al.*, 2016).

Will litter decay rates in tundra ecosystems with contrasting moisture regimes respond similarly to changes in climate?

The high heat capacity of water generally kept autumn and summer surface soils cooler in the wet site relative to the dry site (control plots). In contrast, winter temperatures were less cold in the wet site due to the high energy demand associated with the phase shift between liquid water and ice (Brooks *et al.*, 2011). Furthermore, the greater soil water content in combination with water movement through the plots in the wet site also constrained the microclimatic warming effects of both experimental climate treatments compared to the dry site. Accordingly, litter decay and fungal communities were more strongly affected by the warming and deepened snow treatments in the dry site relative to the wet site (greater effect sizes). Nevertheless, we observed broadly similar treatment responses across sites. For example, the summer warming treatment reducing litter decomposition rates at both sites, and the deepened snow treatment having either no (wet site) or only very minor impacts (dry site) (Fig. 6), and changes in litter decay rates were generally mirrored by changes in the fungal community, largely supporting H3. However, only relatively few fungal taxa showed significant changes in their relative abundance, and we were not able to assign these taxa with specific roles relevant to leaf litter degradation. Nevertheless, fungal taxa generally differ in their breakdown of different carbon

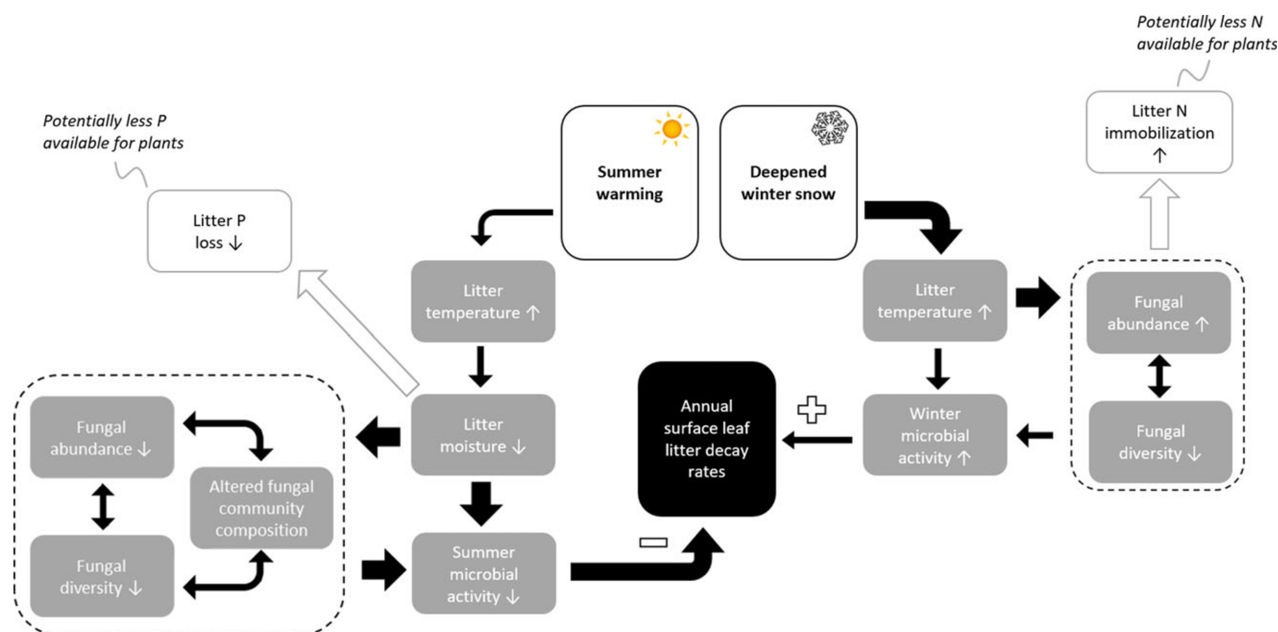

Fig. 6 Conceptual diagram showing predicted feedbacks associated with future summer warming (left) and deepened winter snow (right) on surface leaf litter decay rates. The feedbacks included here are solely based on the significant treatment effects from the present study, and other feedbacks (e.g., litter moisture likely increases with warmer temperatures in winter, and changes in litter quantities and quality) may also be important. Arrow sizes indicate the relative magnitude of the effect between linked factors. The impacts of both summer warming and deepened snow are constrained by initial ecosystem moisture regime, where drier moisture regimes lead to greater effects on litter decay rates and wet moisture regimes may be considered relatively more resilient to climate change. Dashed boxes contain factors belonging to the fungal community that cannot be teased apart based on the present study, and open arrows indicate effects on litter nitrogen and phosphorus import (i.e., fungal immobilization) and loss (i.e., leaching). See text for discussion.

substrates found in litter (Hanson *et al.*, 2008; McGuire *et al.*, 2010) and the observed decline in litter mass loss rates may (in addition to effects of warming-induced surface drying) be related to a reduced ability of the fungal communities to degrade specific carbon sources when a number of taxa is lost. Thus, the observed reductions in fungal diversity and richness following our warming treatment appear to have had substantial consequences for litter degradation and overall ecosystem functioning.

Geml *et al.* (2015) and Semenova *et al.* (2015) investigated the long-term effects of 18 years of experimental summer warming on Alaskan soil fungi in dry and wet tundra ecosystems. Seemingly contrasting with our findings on litter-associated fungi, they reported larger treatment effects on soil fungi in the wet site compared to the dry. However, indirect interactions between plants and soil fungi (e.g., changes in the vegetation, which are not apparent in our study site, leading to altered quantity and quality of leaf litter inputs, and root exudates) are likely driving a large part of these site-specific differences (Waldrop *et al.*, 2006; Sterkenburg *et al.*, 2015). In addition, soil moisture could have been constraining both the plant and fungal responses in their dry site following warming (Allison & Treseder, 2008; Nobrega & Grogan, 2008). Our surface litter study reflects the direct initial response in the initial steps of the ecosystem C-cycle to a changing abiotic surface microclimate (warming-induced drying). This important ecosystem carbon and nutrient cycling process ultimately transforms leaf litter into soil organic matter. By contrast, the soil fungal communities investigated by Geml *et al.* (2015) and Semenova *et al.* (2015) are primarily responsible for soil organic matter decomposition (i.e., the steps in the C-cycle following breakdown of surface litter) and may be more protected from desiccation than fungi in the litter layer.

The size and activity of litter microbial communities can be strongly affected by stress history. For example, Schimel *et al.* (1999) found that dry surface litter decayed slower upon rewetting than litter that was kept continuously moist. This suggests that the inhibitory effects of summer warming, presumably in response to drought, may persist during subsequent stages of litter decay and therefore have important implications for plant growth. In general, plant growth

in Arctic (and most other) terrestrial ecosystems is strongly nutrient limited (Harpole *et al.*, 2011) and a decrease in mobilization of N and other nutrients from degrading litter, for example, may limit the ongoing 'greening' shrub expansion across the Arctic (Elmendorf *et al.*, 2012). In addition, a shift in mineralization from summer to winter and shoulder seasons will not only decrease nutrient release during the plant-growing season but also potentially facilitate leaching loss (especially during snow melt).

Our study is novel because, to the best of our knowledge, it is the first to couple the response of *in situ* surface litter fungi and their associated litter decay activities to combinations of projected changes in summer and winter climates in Arctic tundra. We conclude that moisture regime is likely to govern effects of climate warming on the initial stages of surface litter decay, similar to observed climate sensitivity of tundra shrub growth (Myers-Smith *et al.*, 2015). Surface litter fungal decomposer activities in both dry-mesic and wet tundra ecosystems, representative of about one-third of the low Arctic land surface area (Walker *et al.*, 2005), were susceptible to our summer warming (and associated surface drying) treatment, with this effect being more pronounced in the dry relative to the wet site. Consequently, in a warmer future with similar chemical quality and quantities of foliar litter inputs, reductions in litter fungal diversity and abundance may lead to *slower* litter C and nutrient turnover rates that are likely to restrain vegetation responses to climate change. In addition, decreased surface litter turnover rates are likely to slow soil organic matter formation from aboveground foliar sources as microbially processed foliar C inputs to the soil matrix would be reduced (Cotrufo *et al.*, 2015; Soong *et al.*, 2015). Accordingly, near-surface litter accumulation could provide a negative feedback to the climate warming associated with CO_2 release from enhanced SOM decomposition. Such thicker litter layers may also have wider implications for the soil ecosystem – not least with respect to thermal and moisture regimes in deeper soil layers.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Main treatment effects of summer warmed (red lines) and non-warmed plots (black lines) on diel soil volumetric moisture content.
Figure S2. Venn diagram illustrating the relative proportion of shared and unique fungal OTUs between unmanipulated control plots in the dry and wet sites.
Figure S3. Principal component analyses (PCAs) of the fungal communities extracted from litterbags.
Table S1. Seasonal ground surface temperatures (°C) in all factorial treatments.
Table S2. Seasonal volumetric soil moisture (integrated across 0–5 cm depth; %) in all factorial treatments.
Table S3. Seasonal 5 cm soil temperatures (°C) in all factorial treatments.
Table S4. Pearson correlation coefficients for litter fungal and chemical properties.
Table S5. Relative abundance of the 20 most abundant fungal OTUs.