RESEARCH ARTICLE

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Effect of permafrost thaw on plant and soil fungal community in a boreal forest: Does fungal community change mediate plant productivity response?

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Abstract

1. Permafrost thaw is leading to rapid shifts in boreal ecosystem function. Permafrost thaw affects soil carbon turnover through changes in soil hydrology; however, the biotic mechanisms regulating plant community response remain elusive.

2. Here, we measured the response of fungal community composition and soil nutrient content in an intact permafrost plateau forest soil and an adjacent thermokarst bog and evaluated their potential to mediate shifts in plant composition.

3. We used barcoded amplicon targeting ITS2 and 28S rRNA genes to determine fungal community composition. Next, we used the soils from the permafrost plateau and the thermokarst bog as soil inoculum in a greenhouse experiment to measure whether shifts in fungal community and soil water level regulate plant productivity and composition.

4. Overall, we found that fungal community composition differed significantly between the thawed and intact permafrost sites, but soil nutrient content did not. Relative abundance of mycorrhizal fungal taxa decreased while relative abundance of putative fungal pathogens increased with permafrost thaw. In the greenhouse, we found that ecto- and arbuscular-associated host plants had higher productivity in permafrost-intact soils relative to thawed soils. However, productivity of non-mycorrhizal tussock grass was more affected by soil water levels than soil communities.

5. *Synthesis.* Our results suggest that fungal communities are crucial in mediating plant community response to permafrost thaws inducing hydrology changes.

KEYWORDS
boreal peatland, ecological omics, fungal pathogens, global change ecology, mycorrhizal fungi, permafrost thaw, plant–soil interactions

1 | INTRODUCTION

Climate warming is inducing rapid large-scale shifts in boreal plant communities due to permafrost thaw (Johnstone et al., 2010; Schuur, Crummer, Vogel, & Mack, 2007; Wolken et al., 2011). Thaw changes soil hydrology, with the impacts on carbon dynamics and plant community composition depending on the geomorphology and hydrological changes. In uplands, thaw can shift surface water flow to ground water flow (O'Donnell et al., 2012; Jorgenson et al., 2013), thereby lowering the water-table, drying the soil and accelerating mineralization of soil carbon. Vegetation shifts from black spruce and ericaceous shrubs to more deciduous and herbaceous communities (Chapin, Oswood, Cleve, Viereck, & Verbyla, 2006). In lowlands, thaw can result in the subsidence of the ground, known as thermokarst, affecting both lateral and subsurface water flow, with water post thaw often being confined and creating wetlands such as fens and bogs (Jones et al., 2011; Jorgenson et al., 2013). With rising water-table, plant communities shift from black spruce to sedge-dominated species communities (Camill, Lynch, Clark, Adams, & Jordan, 2001; Jorgenson & Osterkamp, 2005; Jorgenson, Racine, Walters, & Osterkamp, 2001). Permafrost thaw-mediated changes in plant communities and nutrient mineralization are critical ecosystem feedbacks (Chapin et al., 2006), but the biotic mechanisms through which permafrost thaw alters plant communities remain unexplored.

Changes in soil microbial function could mediate effects of permafrost thaw on plant communities. Thaw impacts soil microbial community structure (Blazewicz, Petersen, Waldrop, & Firestone, 2012; Deng et al., 2015; Hultman et al., 2015; Tas et al., 2014), decomposition (Hicks Pries, Schuur, Vogel, & Natali, 2013) and activity (Coolen & Orsi, 2015; Mackelprang et al., 2011; McCalley et al., 2014; Neumann, Blazewicz, Conaway, Turetsky, & Waldrop, 2016). Mackelprang et al. (2011) found that permafrost thaw leads to rapid shifts in many microbial, phylogenetic and functional gene abundances and pathways. Permafrost thaw shifts the expression profile from genes important in stress response, maintenance processes and survival to a profile enriched in expression of genes involved in the decomposition of soil organic matter (Coolen & Orsi, 2015). These changes in microbial communities are thought to influence the release of CO_2 and CH_4 as a result of changes in soil respiration, fermentation, methanogenesis and methane oxidation (McCalley et al., 2014; Schuur et al., 2009). Limited studies have quantified effects of permafrost thaw on fungal communities, with one using 1-year experimental warming of permafrost soils and finding no changes in fungal community composition (Penton et al., 2013).

There is mounting evidence indicating that soil microbial communities, in particular fungal communities, can be important drivers of plant community composition in other environments. Studies have identified that soil microbes play important roles in plant adaptation (Johnson, Wilson, Bowker, Wilson, & Miller, 2010; Schultz et al., 2001), coexistence (Bever, Mangan, & Alexander, 2015), relative abundance (Klironomos, 2002; Mangan et al., 2010), succession (Bauer, Mack, & Bever, 2015; Hewitt et al., 2015; 2016) and plant species invasions (Pringle et al., 2009; Vogelsang & Bever, 2009). Given this growing realization that soil microbiomes can structure plant communities, it is reasonable to expect that changes in fungal composition could mediate plant community response to permafrost thaw and could have key impacts on climate change feedbacks (Averill, Turner, & Finzi, 2014; Hicks Pries et al., 2013).

Mycorrhizal fungi are a potentially keystone component that could mediate plant–microbe feedbacks because they facilitate resource acquisition by plants (Aerts, 2002). While most plant species associate with arbuscular mycorrhizal (AM) fungi, most boreal tree species, including spruce, associate with ectomycorrhizal fungi, and boreal shrubs such as blueberries and cranberries form ericoid mycorrhizal associations. Plants such as sedges are non-mycorrhizal (Miller, Smith, Jastrow, & Bever, 1999) or can associate with dark septate endophytes (DSE; Mandyam & Jumpponen, 2005, Weishampel & Bedford, 2006). Since the common plant species within boreal regions interact with different functional fungal groups, changes in fungal community composition may lead to positive feedbacks that could accelerate plant community shifts (Bever et al., 2010). Resource specialization could also drive changes in relative abundance of these plant–fungal associations, given that AM fungi are thought to be more effective at phosphorus uptake, while ecto- and ericoid mycorrhizal fungi are more effective at nitrogen uptake (Phillips, Brzostek, & Midgley, 2013; Read, Leake, & Perez-Moreno, 2004). Moreover, dominant mycorrhizal associations have been suggested as predictors of net carbon dynamics of forest systems (Cheeke et al., 2017; Phillips et al., 2013; Talbot et al., 2008) and could mediate climate feedbacks in the boreal system.

In this study, we tested the potential for soil community changes to mediate plant community response to permafrost thaw. Specifically, we hypothesized that mycorrhizal fungi would be less dominant at the thermokarst bog as mycorrhizal fungi are not well adapted to water-saturated and anoxic soils. We hypothesized that permafrost thaw would alter the fungal community composition to such an extent that plant growth would be affected. We tested these hypotheses by integrating environmental sequencing of fungal communities with growth assays of four boreal plant species representing the different mycorrhizal associations. The growth assays tested response to inoculum source and their interaction with hydrological changes.

2 | MATERIALS AND METHODS

2.1 | Study site and sampling

The field sites are part of the Alaska Peatland Experiment (APEX) within the Bonanza Creek LTER (64.71°N, 148.28°W) in interior Alaska. This area is a forested peatland where permafrost thaw increased the soil water-table (Finger et al., 2016). These sites are part of ongoing studies evaluating long-term effects of permafrost thaw on plant communities, trace gas fluxes, microbial communities and biogeochemical processes (Blazewicz et al., 2012; Euskirchen, Edgar, Turetsky, Waldrop, & Harden, 2014; Finger et al., 2016; Hultman et al., 2015; Klapstein et al., 2014; Mackelprang et al., 2011; Neumann et al., 2016). In June 2012, we sampled the rooting zone of a permafrost plateau and a thermokarst bog. Within each site, we took 16 samples, with each core (diameter: 5 cm; length 30 cm) being divided
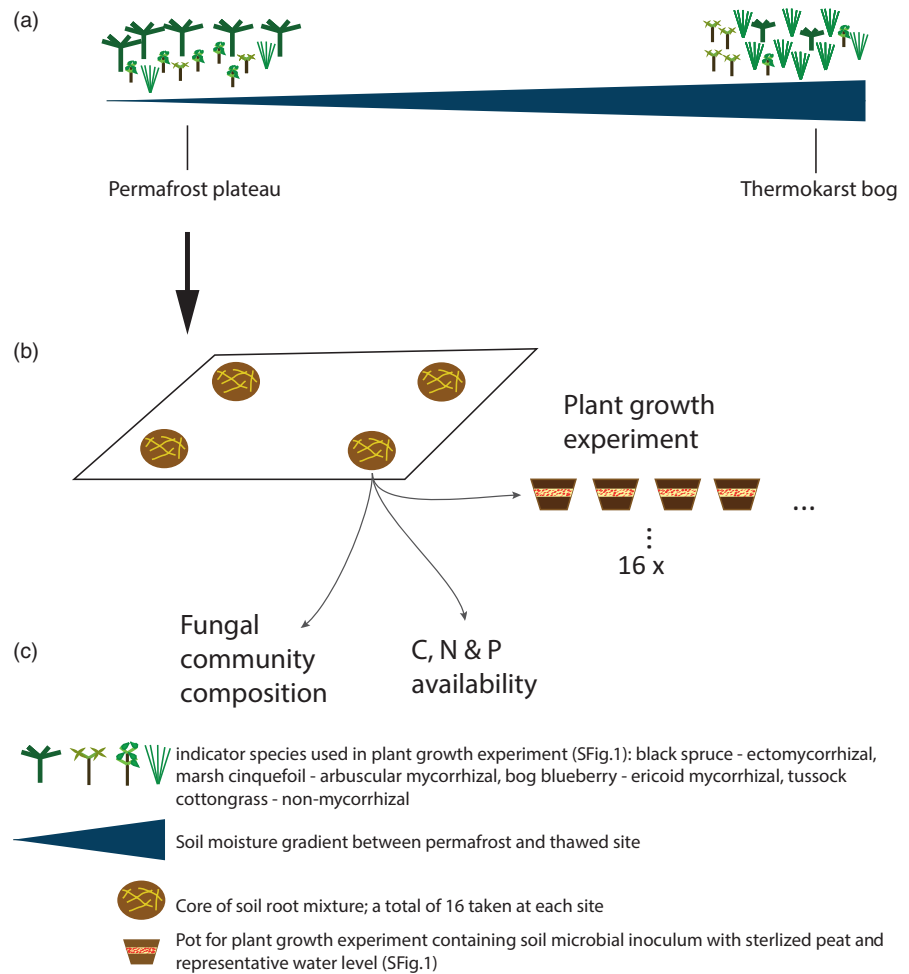
FIGURE 1 Overview of the experimental design of this study. (a) The differences in soil water content between the permafrost plateau and the thermokarst bog and the presence of the four boreal plant species used in the plant growth assay. (b) Soil cores taken at each location contained both soil and roots and that the material of each core was used in a plant growth experiment and (c) to determine fungal community composition and soil nutrient content

into three parts (Figure 1) and used as soil microbial inoculum in the plant growth assay, to describe the fungal community composition, and for soil nutrient analysis (supplemental material Data S1).

2.2 | Fungal community characterization

Genomic DNA was extracted using the Power Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA). General fungal communities were described using the primers GITS7 (Ihrmark et al., 2012) and NLC2mod (AGCTGCAWTCCCAAACAAC), barcoded using the approach by Fadrosh et al. (2014). Arbuscular mycorrhizal (AM) fungi were described using the LR0R (ACCCGCTGAACTTAAGC) and FLR2 (TCGTTTAAAGCCATTACGTC) primer, barcoded based on the approach from the Earth Microbiome Project (Gilbert, Jansson, & Knight, 2014). Amplicons were pooled in an equimolar mixture and cleaned using the Agencourt AMPure XP (Beckman 123 Coulter, Brea, CA). Sequences were generated on the MiSeq Illumina platform (Illumina Inc, San Diego, CA), cleaned using Trimmomatic (Bolger, Lohse, & Usadel, 2014), and clustered at 97% similarity using Abundant OTU (Ye, 2011). After removing singletons, general fungi were identified using the UNITE database (Koljag et al., 2005) and AM fungi were identified using the MaarjAM database (Opik et al., 2010, supplemental material Data S1). Species accumulation curves are shown in the supplemental material (Figure S1).

2.3 | Nutrient analysis

We measured ortho-phosphate (PO_4^{3-}), ammonium (NH_4^+) and nitrate (NO_3^-), as well as total N, P and C. PO_4^{3-} was extracted as described in Olsen, Cole, Watanabe, & Dean, 1954. NH_4^+ and NO_3^- were extracted as described in Keeney & Nelson, 1982 (supplemental material Data S1).

2.4 | Plant growth assay

Black spruce (*Picea mariana*), marsh cinquefoil (*Potentilla palustris*), bog blueberry (*Vaccinium uliginosum*) and tussock cottongrass (*Eriophorum vaginatum*) representing ectomycorrhizal, arbuscular mycorrhizal, ericoid mycorrhizal and non-mycorrhizal plant species respectively were grown in the greenhouse, in soils containing soil microbial inoculum from either the rooting zone of the permafrost plateau or the thermokarst bog (Figure S2). Seeds were obtained from Alaskan sources except for tussock cottongrass seeds (Pase Seeds, North Collins, NY). Seeds were surface sterilized and if necessary cold stratified for 2 months before germination. Single seedlings were transplanted into a mesocosm containing 40 ml of soil microbial inoculum from the permafrost and thermokarst bog, respectively, embedded in 470 ml of sterilized Canadian peat (Figures 1 and S2). Each plant species was also grown in mesocosms

containing 40 ml of sterilized inoculum. Half of all plant seedlings, of each species, were immersed in DI water to simulate elevated soil water levels. We had a total of 368 pots. Pots were randomized within three blocks to reduce any bias from possible temperature gradients in the greenhouse. Marsh cinquefoil and tussock cotton-grass seedlings were grown for 2 months and the slow growing black spruce and bog blueberry seedlings for 6 months. Shoots and roots were harvested after the growing season and dried at 60°C to a constant mass to determine the amount of biomass produced (supplemental material Data S1).

2.5 | Statistical analysis

We tested: (a) differences in fungal community composition, (b) differences in soil nutrients between the two sites and (c) the effect of these differences on plant growth and biomass of the four boreal plant species. We used relative abundances of each OTU across samples to account for differences in total number of sequences among samples. Differences in fungal community composition between the permafrost plateau and the thermokarst bog were determined by non-metric multidimensional scaling (NMDS) using Bray–Curtis dissimilarity (Johnson & Wichern, 1988, VEGAN package, Rstudio version 0.98.1091) and Multi Response Permutation Procedure (MRPP). We visualized changes in taxonomic composition of the fungal communities with heatmaps (GPLOTS package, Rstudio version 0.98.1091). Using profile analysis, we tested if the change in relative abundance of the mycorrhizal and putative fungal pathogens between the two sites was statistically significant. Differences across soil nutrients between the two sites were tested using MANOVA (Johnson & Wichern, 1988, Rstudio version 0.98.1091) and ANOVA.

We examined the effect of inoculum and water level on plant responses using ANOVA on linear mixed effect models (Ott & Longnecker, 2001, NLME package, Rstudio version 0.98.1091). To test whether inoculum effects on the plant response were mediated by permafrost-induced changes in nutrients and/or fungal community composition, we included added nutrients and fungal community composition as covariates in the analysis. This covariance approach allows tests of potential causal paths while accounting for other factors in the experimental design (Bever, Broadhurst, & Thrall, 2013). Interpretations were confirmed using structured equation modelling on reduced datasets (Trivedi et al., 2016; Waldrop et al., 2017). As NMDS visualizes overall differences in microbial communities across different habitats or experimental treatments, we used the scores for each NMDS axis as covariates representing the overall fungal community composition. Conditional plots were generated for each soil nutrient or NMDS axis that were found to be statistically significant in the linear mixed effects model (VISREG package, Rstudio version 0.98.1091, supplemental material Data S1).

3 | RESULTS

3.1 | Differences in fungal community composition

Fungal community composition differed between the rooting zone of the permafrost plateau and the thermokarst bog for the general fungal and the AMF communities (Figure 2, stress <0.03, MRPP = 0.01). We also found large within-site variation in fungal community composition. The first NMDS axis described most of the difference in community composition between the two sites and together with the additional NMDS axes (Figures S3andS4) explains variation in community composition within each site.

We detected 527 OTUs based on 97% sequence similarity for the general fungal community composition ranging from 383,016 to 699,268 sequences per sample. Based on the taxonomic classification in UNITE, in combination with FUNGuild (Nguyen et al., 2016) and scientific literature, we identified whether the OTUs identified were more likely mycorrhizal, pathogenic, dark septate or part of the recently identified class Archaerhizomycetes (Rosling et al., 2011) (Figures 3a and S5). We detected a dramatic change in proportions
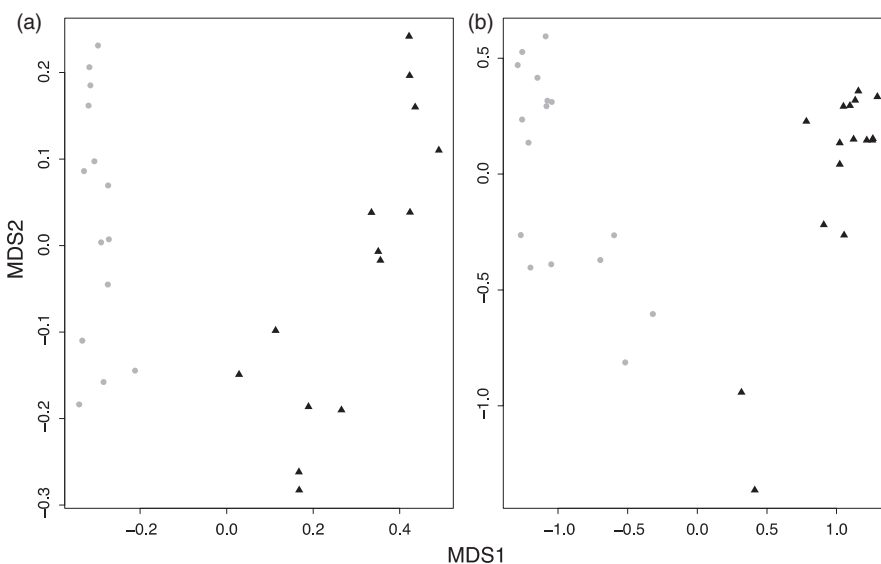

FIGURE 2 Non-metric multidimensional scaling (NMDS) based on Bray–Curtis distance showing differences in fungal community composition between the active layer of the intact permafrost (grey) and the thermokarst bog (black). (a) Differences in the general fungal community based on the ITS2 region; stress value of NMDS 0.037, MRPP p = 0.01; (b) differences in the AMF community based on 28S rRNA gene sequences; stress value of NMDS 0.0415, MRPP p = 0.01

of putative mycorrhizal and saprotrophic/pathogenic fungi with thaw (Figure 3b, Wilks Lambda $F_{3,24}$ = 22.8, p = 3 × 10^{-7}).

We are aware that fungal groups often contain members of all functions (biotrophic, saprotrophic, mycorrhizal) and that some fungi can grow both saprotrophically and in mycorrhizal symbiosis. Our classification is based on the lifestyle members that the identified fungal groups are most likely to use. For the thermokarst bog, the relative abundance of putative pathogens such as the biotrophic *Galerina paludosa* living on mosses (Davey, Heimdal, Ohlson, & Kauserud, 2013) and *Hyaloscypha* (Penton et al., 2013) increased and the relative abundance of mycorrhizal fungi decreased. OTU 59 was identified within the genus *Allantophomopsiella* as *A. pseudotsugae*, a coniferous pathogen (Crous, Quaedvlieg, Hansen, Hawksworth, & Groenewald, 2014). It was detected in only one of the samples from the permafrost plateau, but its relative abundance reached up to 6% in the samples from the thermokarst bog. Additionally, the composition of mycorrhizal fungi present at the thermokarst bog differed from the ones present at the permafrost plateau (Figure 3a). For example, members of the ericaceous *Rhizoscyphus* and *Meliniomyces variabilis* were more commonly present at the permafrost plateau and members of ectomycorrhizal Telephoraceae had higher relative abundances at the thermokarst bog. One OTU classified as the dark septate endophyte *Phialocephala* was among the most abundant taxa at both sites, but less common than putative mycorrhizal or pathogenic taxa. Three OTUs classified as the recently described class Archaerhizomycetes of unknown function (Rosling et al., 2011) and were only detected at the permafrost plateau.

For the AM fungi, we obtained a total of 705 OTUs based on 97% sequence similarity ranging from 20,497 to 77,842 sequences per sample. Since the general primer pair did not amplify arbuscular mycorrhizal fungi, and the two primer pairs targeted different genes, we did not necessarily expect to detect more OTUs with the general fungal primer pair than with the AMF primer pair. The majority of AMF OTUs classified within the family of Glomeraceae. Only 20 OTUs with a relative abundance below 1% and sequence similarity 85%–88% classified within Ambisporaceae and Claroideoglomeraceae. Over 90% of the OTUs had only 80%–95% sequence similarity with any known sequence in the MaarjAM database, suggesting that the boreal sites we sampled had distinct indigenous arbuscular mycorrhizal fungi that have yet to be described. Despite the difficulties of species classification, we saw a distinct shift in community composition of AMF between the permafrost and the thermokarst bog (Figure 3c). Seven of the 10 OTUs with the overall highest relative abundance showed higher relative abundances at the permafrost plateau, while three OTUs dominated AMF community composition at the thermokarst bog. Permafrost thaw significantly changed fungal community composition and function. The relative abundance of mycorrhizal fungi decreased, the type of mycorrhizal fungi changed and the relative abundance of putative fungal pathogens increased following thaw.

3.2 | Differences in soil nutrients

We determined soil nutrient content including total C, N and P, as well as PO_4^{3-}, NH_4^+ and NO_3^- within each site. While the MANOVA

analysis showed a significant effect (p = 0.02) of permafrost thaw on overall composition of soil resources, none of the individual ANOVAs were significant (Figure 4). Total C ranged between 48% and 49% and was slightly lower in the thermokarst bog. Total N was about 0.8% and total P was 140 mg/kg soil at the permafrost plateau and on average 150 mg/kg soil at the thermokarst bog. The majority of P was present as organic P (60–100 mg/kg soil); only 40–50 mg P/kg soil was measured as inorganic PO_4^{3-}. NH_4^+ ranged on average from 15 mg/kg soil at the permafrost plateau to 40 mg/kg soil at the thermokarst bog. NO_3^- in the soil was a smaller pool, 1.5 mg/kg soil, in the permafrost plateau and 0.5 mg/kg soil at the thermokarst bog. The variability in NH_4^+ measured at the thermokarst bog and NO_3^- measured at the permafrost plateau was very large across samples (Figure 4).

3.3 | Effect of soil microbial inoculum and soil water level on plant productivity

Our results indicate that the mycorrhizal plant species, in particular the ectomycorrhizal black spruce and arbuscular mycorrhizal marsh cinquefoil, had higher plant productivity when grown with microbial inoculum from the permafrost plateau (Figure 5), and the non-mycorrhizal, potentially DSE, plant species tussock cottongrass was more affected by soil water levels (Figure 6b–d). Productivity of bog blueberry was higher when plants were grown with live versus sterile inoculum (Figure 5e) but was not significantly affected by differences in inoculum between the permafrost plateau and the thermokarst site, or soil water level (Figures 5 and S7). Analysis of covariance showed that differences in fungal community composition between the permafrost plateau and the thermokarst significantly affected plant productivity of the mycorrhizal plant species (Figure 7). Dissecting whether the changes in mycorrhizal or saprotrophic and pathogenic composition affected plant productivity is more complex than we first thought. Changes in the more abundant ecto and ericoid mycorrhizal taxa did not explain changes in plant productivity of black spruce and bog blueberry (data not shown). Most likely it is a combination of changes in mycorrhizal community composition and changes in composition and relative abundances of saprotroph and pathogenic fungi. While individual soil nutrients did not differ significantly between the two sites, overall variation in soil nutrients did affect plant productivity (Figure 8). With the exception of marsh cinquefoil, plants were more productive with higher total *N*. The effect of total P on plant productivity was mixed. Productivity of black spruce and marsh cinquefoil increased with increasing total P content, while productivity of tussock cottongrass decreased (Figure 8). We present the analysis of each species independently. The statistical results for the ANOVA on the linear models can be seen in Table S1.

3.4 | Black spruce

Black spruce produced more biomass when grown with soil inoculum from the permafrost plateau than when grown with inoculum
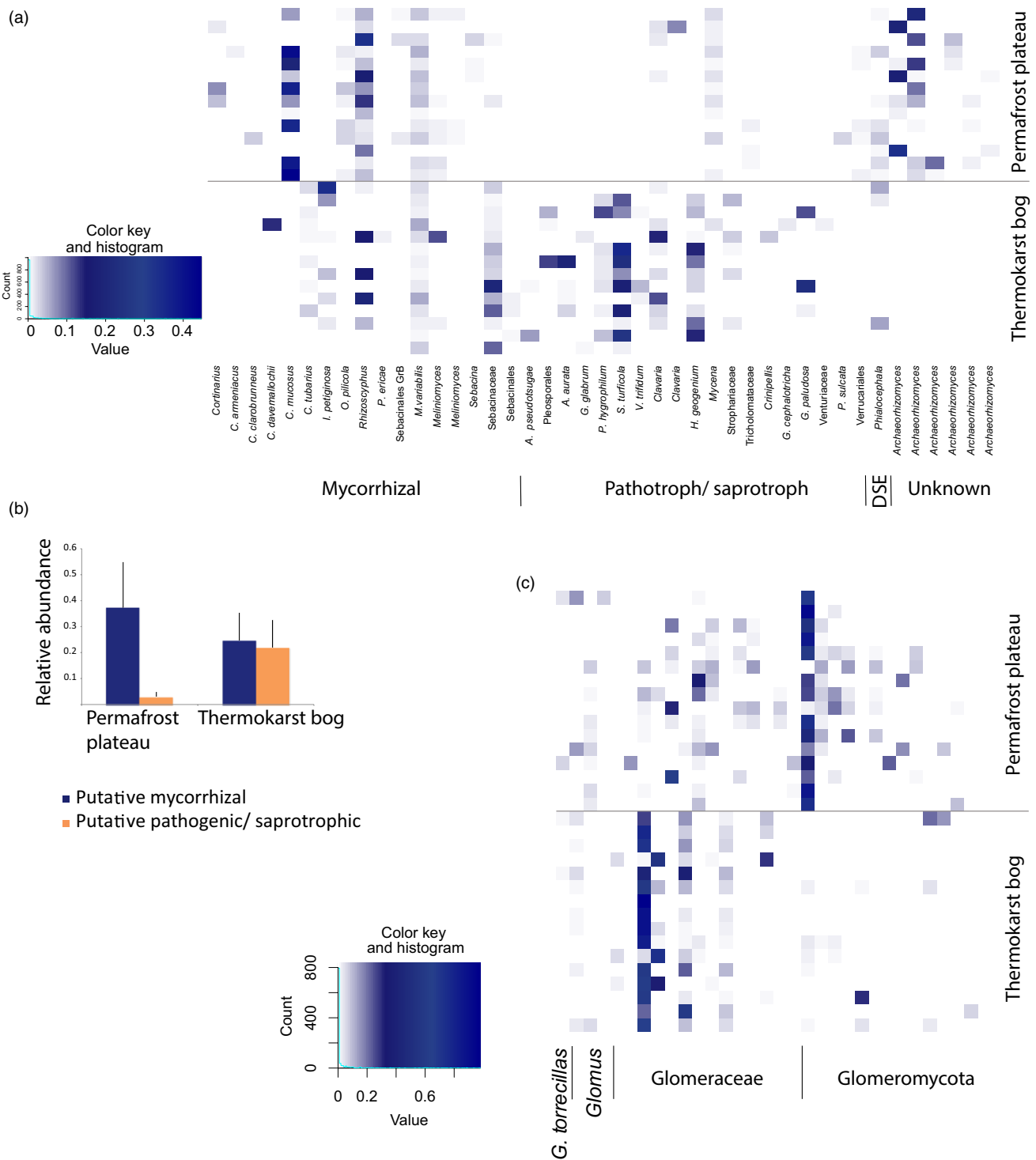
FIGURE 3 Taxonomic differences in fungal community composition between the rooting zone of the permafrost plateau and the thermokarst bog based on clustering sequences at 97% sequence similarity and matching consensus sequences of each OTU of GITS7/NLC2mod against the UNITE (a) and LROR/FLR2 against the MaarjAM database (c). Panel A and C shows OTUs with a relative abundance >1% classified as putative mycorrhizal or pathogenic/saprotrophic, or as dark septate endophytes or Archaerhizomyces. Panel B shows a summary of differences in the summed relative abundances of putative mycorrhizal and pathogenic/saprotrophic fungi between the rooting zone of the permafrost plateau and the thermokarst bog based on panel A, Wilks Lambda $F_{3,24} = 22.8$, $p = 3 \times 10^{-7}$. Dark septate endophytes and Archaerhizomyces were excluded from the analysis as their functions remain unknown

from the thermokarst bog (Figure 5a,b). This trend was consistent for total, shoot and root biomass (Figure S7). Plant productivity under sterile conditions was lower compared to productivity with inoculum from the permafrost plateau, but higher than productivity with inoculum from the thermokarst bog (Figure S6). This suggests an overall negative effect of the microbial community present

at the thawed site. The first NMDS axis of the AMF community composition differed significantly with permafrost thaw and significantly increased shoot biomass of black spruce (Figure 7). The residual fungal NMDS axes significantly predicted total biomass of black spruce suggesting within-site variation in fungal community composition affected plant productivity (Figure 5, Figure S8–S10).

Total N and P, and PO_4^{3-} significantly affected black spruce biomass (Figure 8). Interestingly, higher total N and total P increased plant productivity, but higher PO_4^{3-} decreased shoot biomass (Figure 8). The microbial inoculum from the permafrost plateau had an overall positive effect on biomass production of black spruce (Figure 5a). This positive effect of permafrost inoculum remained after removing the effect of fungal community composition and nutrients from the overall inoculum effect by adding them as covariates (Figure 5b). This suggests that unmeasured biotic and abiotic factors, such as bacterial community composition and pH present at the permafrost plateau, also had a positive effect on plant productivity. Thus, it was a combination of different biotic and abiotic conditions

that resulted in higher overall plant productivity of black spruce at the permafrost plateau compared to the thermokarst bog.

3.5 | Marsh cinquefoil

Marsh cinquefoil also responded positively to the inoculum from the permafrost plateau with an increase in leaf production (Figure 5c). Marsh cinquefoil was significantly affected by soil water level, and it grew taller when grown in drier soil (Figure 6a). The scores of the first NMDS axis were significant predictors of leaf number and root biomass, suggesting that differences in AMF community composition between the permafrost plateau and the thermokarst bog affected leaf number, and differences in the general fungal community composition affected root biomass of marsh cinquefoil (Figures 5d and 7). The significance of the residual NMDS axes suggests that within-site variation in fungal community composition affected shoot biomass and leaf number of marsh cinquefoil (Figures S8–S9, S12–S13).
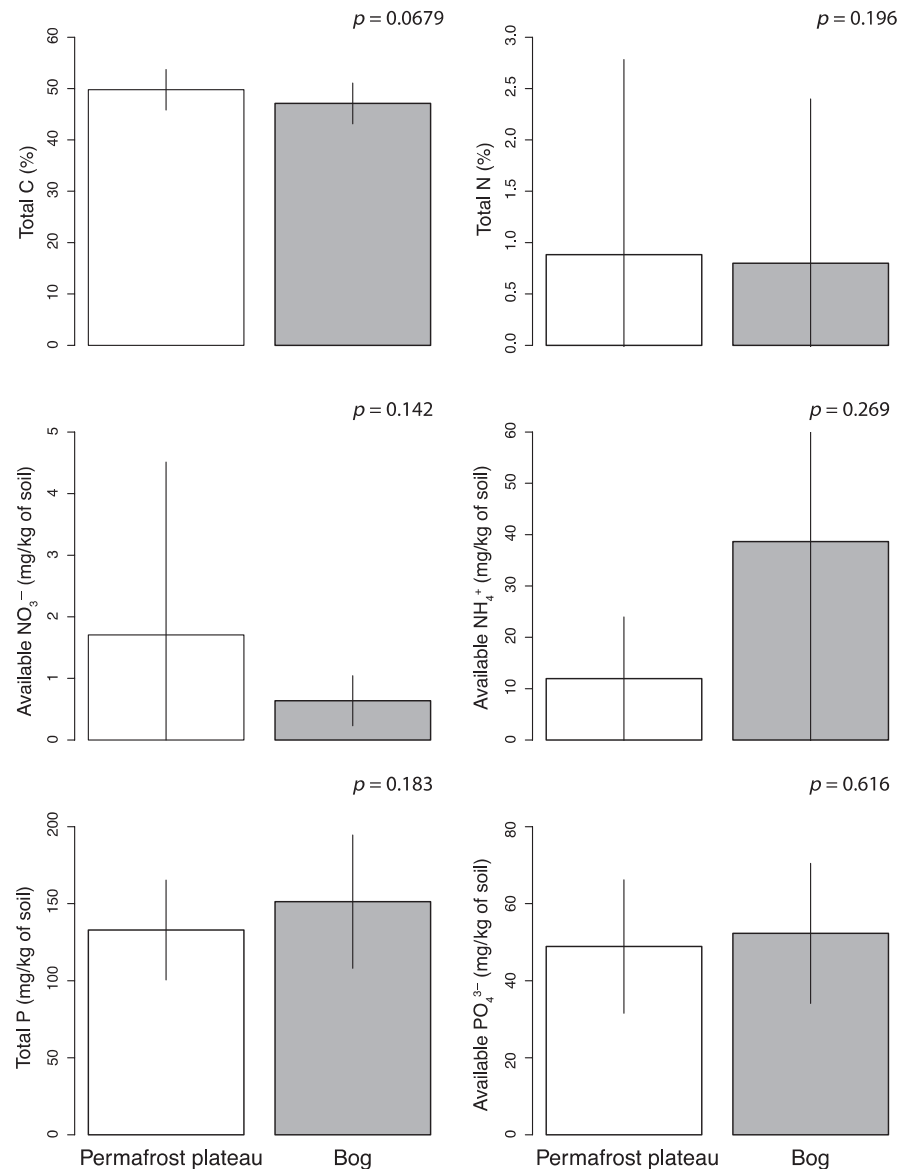

FIGURE 4 Differences in soil nutrients between the rooting zone of the permafrost and the thermokarst bog before the plant growth experiment. The overall MANOVA was significant ($p = 0.023$), but ANOVAs of each individual soil nutrient measured were not significant ($p > 0.05$)
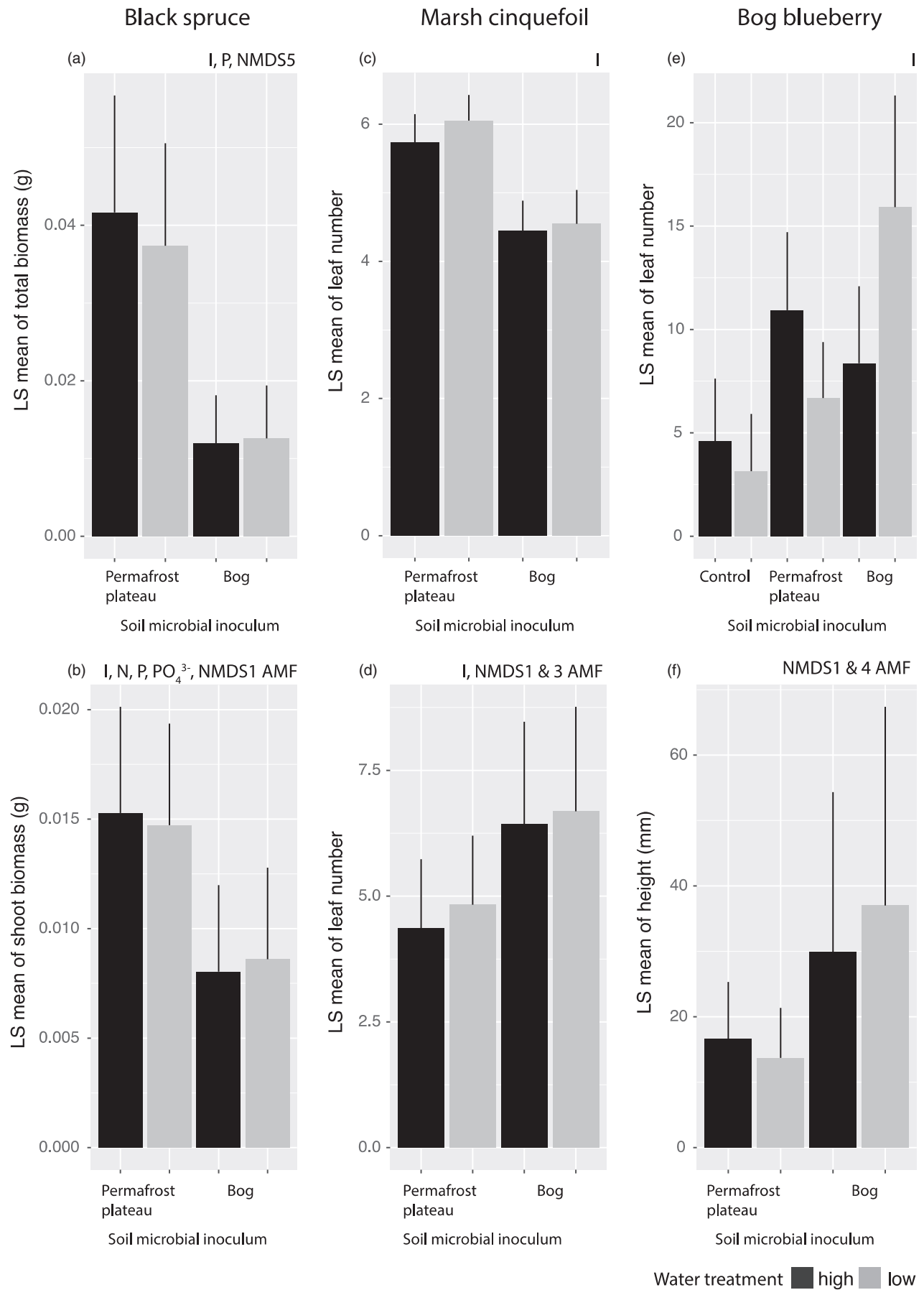
Black spruce

Marsh cinquefoil

Bog blueberry

(a) I, P, NMDS5

(c) I

(e) I

(b) I, N, P, PO₄³⁻, NMDS1 AMF

(d) I, NMDS1 & 3 AMF

(f) NMDS1 & 4 AMF

Water treatment ■ high ■ low

FIGURE 5 Soil microbial inoculum significantly affected plant productivity. The effects of soil microbial inoculum and soil water level on plant productivity of the mycorrhizal plant species grown in the plant growth assay are shown. Plant productivity is shown as LS means and log transformed LS means were back transformed. (a, b, d and f) The effects based on the linear mixed effects model including soil nutrients and NDMS scores as a measure of fungal community composition. (a) includes the NDMS scores of the non-metric multidimensional scaling (NMDS) based on the sequences obtained with the general fungal primers. (b, d and f) include the NDMS scores of the NMDS based on the sequences obtained with the AMF primers. (c) The LS means based on the model that did not include soil nutrients and NDMS scores as a measure of fungal community composition as covariates. (e) The result based on the model including sterile controls, but soil nutrients and NDMS scores as a measure of fungal community composition were not included as covariates. The letters above each graph indicate which factors and covariates were statistically significant ($p < 0.05$): I = soil microbial inoculum, N = total N, P = total P, PO_4^{3-} = available phosphate, NMDS1-5 = NMDS scores 1–5 used to test effect of fungal community composition
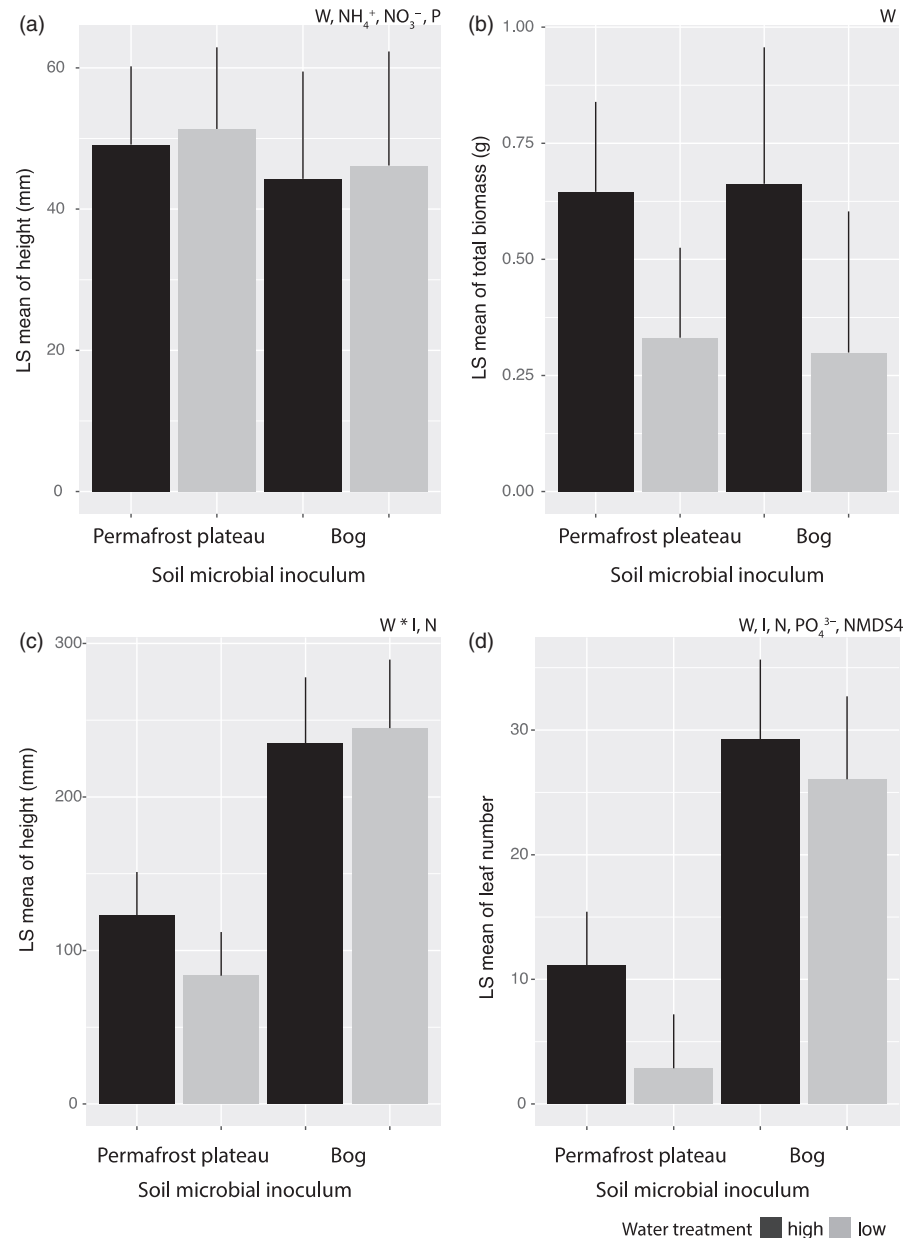

FIGURE 6 Changes in soil water level and a combination of soil microbial inoculum and soil water levels affected plant productivity of marsh cinquefoil (a) and tussock cottongrass (b–d). Plant productivity is shown as LS means based on the linear mixed effects model including soil nutrients and NDMS scores of the non-metric multidimensional scaling (NMDS) based on the sequences obtained with the AMF primers as a measure of fungal community composition. The letters above each graph indicate which factors and covariates were statistically significant ($p < 0.05$): I = soil microbial inoculum, W = soil water level, W*I = the effect of soil microbial inoculum depended on soil water level, N = total N, P = total P, PO_4^{3-} = available phosphate, NH_4^+ = available ammonia, NO_3^- = available nitrate, NMDS4 = NMDS scores of NMDS axis 4

Total P significantly affected height, and total N affected shoot biomass (Figure 8). Marsh cinquefoil productivity (height and leaf length) increased with higher levels of total P, but total N decreased shoot biomass (Figure 8). The microbial inoculum from the permafrost plateau significantly increased the number of leaves marsh cinquefoil produced (Figure 5c). When removing the effect of AMF community composition from the overall inoculum effect by including AMF as separate variables, we saw significantly lower leaf numbers with the permafrost plateau inoculum compared to the thermokarst bog (Figure 5d). This suggests that unmeasured biotic and abiotic conditions at the permafrost plateau had a negative effect on leaf production, while AMF community composition present at the permafrost plateau had a positive
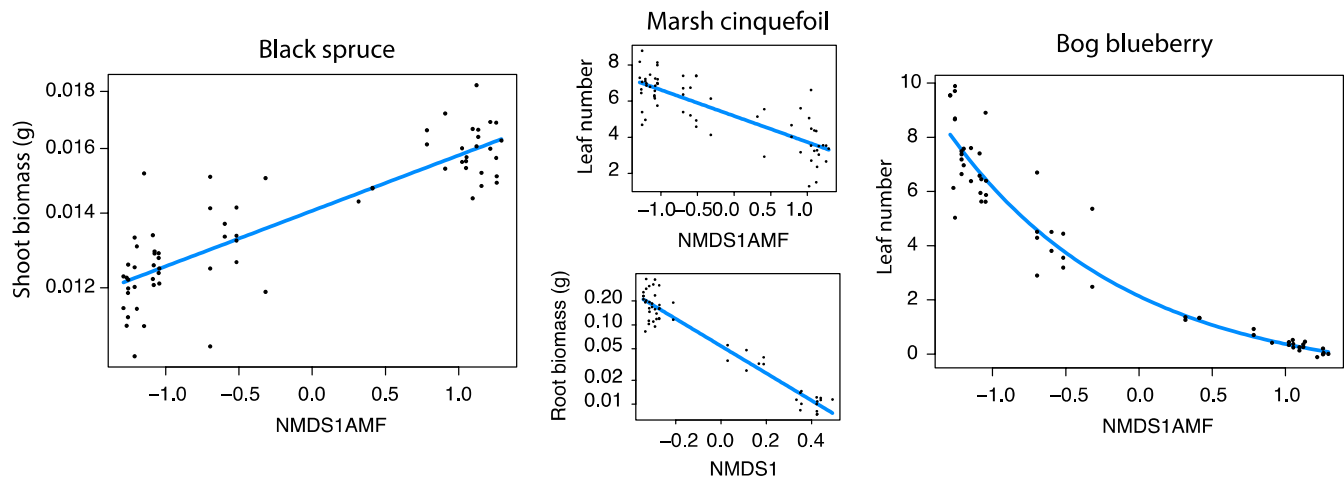
FIGURE 7 Conditional plots showing the effects of changes in fungal community composition between the permafrost plateau and the thermokarst bog on plant productivity for black spruce, marsh cinquefoil and bog blueberry. The conditional plots show the partial residuals and directionality of the effect the significant variable has on the measure of plant productivity used in the linear mixed effects model while keeping all other numeric variables at their median and categorical variables at the most common category. If the response variable in the original linear mixed effects model was log transformed, the value for the partial residuals was back transformed and a log scale was used on the y-axis to generate a linear regression line. (For additional plots showing the significant effects of fungal community composition on plant productivity see in Figure S8–S8)

effect on leaf production (Figure 7). This positive effect of AMF communities present at the permafrost plateau on leaf production is consistent with the taxonomic changes in AMF community composition we observed (Figure 3b). Higher leaf numbers are associated with negative NMDS 1AMF scores representing AMF communities present at the permafrost plateau, and lower leaf numbers are associated with positive NMDS 1AMF scores representing fungal communities present at the thermokarst bog (Figure 7). Root biomass was affected similarly by differences in the general fungal community. This suggest that the productivity of the AMF-associated marsh cinquefoil was negatively affected by changes in overall fungal community with thaw, and also by the changes in the pool of putative AMF root symbionts.

3.6 | Bog blueberry

In contrast to black spruce and marsh cinquefoil, the ericoid mycorrhizal species bog blueberry was affected by the presence of live micro-organisms. The presence of live micro-organisms increased height, leaf length and number of leaves (Figure 5e), suggesting that both sites had ericoid mycorrhizal fungal hosts. However, we did find that differences in fungal community composition between the permafrost and the thermokarst bog significantly affected the number of leaves bog blueberry produced, with higher leaf production when grown with fungal communities from the permafrost plateau (Figure 7). Within-site variation in fungal community composition, likely due to spatial heterogeneity, significantly affected total biomass, root and shoot biomass, and leaf number (Figures S8–S9,S14).

Compared to the other plant species, bog blueberry productivity was less influenced by soil nutrients. N only influenced shoot biomass; an increase in total N increased shoot biomass production (Figure 8). The assessment of the effects of soil microbial inoculum

and changes in soil water levels on plant productivity of bog blueberry was somewhat difficult as blueberries are slow growing, and roots of seedlings are very thin. However, our results indicate that the presence of live micro-organisms increased productivity and changes in fungal community composition and total N affected plant productivity.

3.7 | Tussock cottongrass

Biomass of the non-mycorrhizal tussock cottongrass increased when grown with high soil water levels (Figure 6). Surprisingly, the number of leaves produced was affected by source of microbial inoculum (Figure 6d). Further, tussock plants grown with inoculum from the permafrost plateau were taller with higher soil water levels, while plant seedlings grown with the inoculum from the thermokarst bog were taller when grown with drier soil (Figure 6c). Consistent with our expectation, we saw that differences in fungal community composition between the permafrost plateau and the thermokarst bog did not significantly affect plant productivity of tussock grass (p of NMDS 1 > 0.05), but shoot biomass and leaf number were affected by within-site differences in fungal community composition (Figure 6d). Taken together, our results suggest that fungal communities may interact with soil moisture to determine tussock grass morphology.

Total N and P significantly increased plant productivity (Figure 8). Total N increased height and number of leafs (Figure 8). Consistent with the other plant species, PO_4^{3-} affected leaf number, and increased levels of PO_4^{3-} led to a decrease in productivity (Figure 8). When fungal community composition and nutrients were included as separate variables, the difference in productivity between permafrost and thermokarst bog was less pronounced
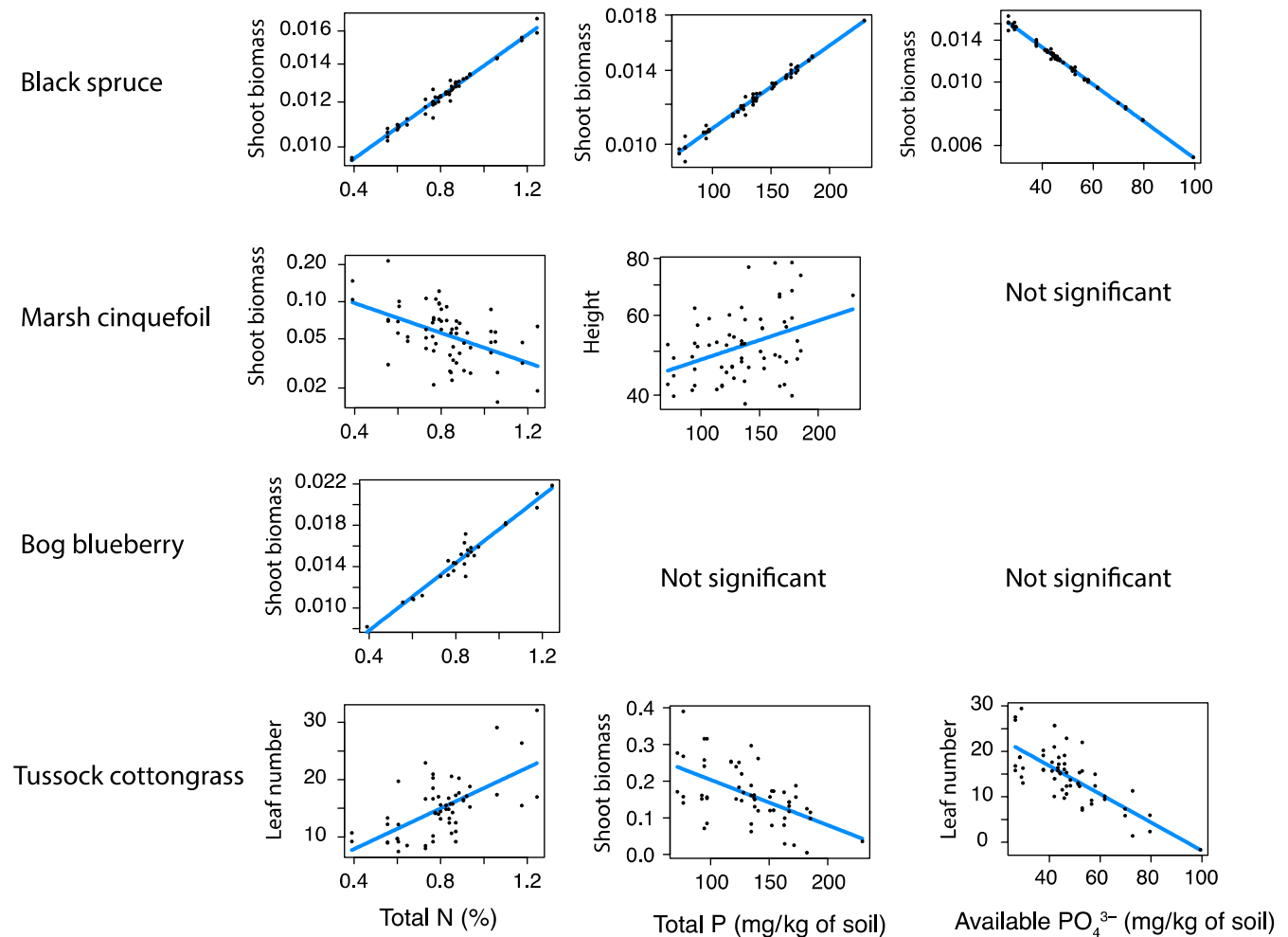
FIGURE 8 Conditional plots showing examples for the significant effects of total nitrogen, total phosphorous and available phosphate on plant productivity. These plots show the partial residuals and directionality of the effect the significant variable has on the measure of plant productivity used in the linear mixed effects model while keeping all other numeric variables at their median and categorical variables at the most common category. If the response variable in the original linear mixed effects model was log transformed, the value for the partial residuals was back transformed and a log scale was used on the y-axis to generate a linear regression line. (For additional plots showing all significant effects of soil nutrients on plant productivity see Figure S4–S8)

and overall, nutrients affected plant productivity more than fungal community composition (Figures S8–S9,S15–S16). This suggests that plant productivity of tussock cottongrass was largely influenced by differences in soil nutrients between the permafrost plateau and thermokarst bog.

4 | DISCUSSION

Fungal community composition differed between the rooting zone of the permafrost and the thermokarst bog. In particular, the relative abundance of mycorrhizal fungi decreased while the relative abundance of putative saprotrophic and fungal pathogens increased with thaw. We expected mycorrhizal fungi to be less dominant at the thermokarst bog, as mycorrhizal fungi are not well adapted to water-saturated and anoxic soils; however, the increase in putative saprotrophic and pathogenic fungi is remarkable. This shift from a

more beneficial to a more deleterious fungal community with permafrost thaw is consistent with the microbial change mediating plant community response to permafrost thaw, and as such, it could have important implications for plant productivity, composition and diversity in the boreal forest.

The potential for microbial change to mediate plant community response to permafrost thaw is supported by results from our plant growth assay. Mycorrhizal plant species grew better when grown with microbial inoculum from the permafrost plateau than when grown with inoculum from the thermokarst bog. While these differences in plant productivity could be due to both biotic and abiotic effects, fungal community composition changed with thaw and was correlated with plant performance of mycorrhizal plant species while soil nutrients were correlated with plant productivity, but their individual concentrations did not differ between the two sites. Ectomycorrhizal black spruce and arbuscular mycorrhizal marsh cinquefoil showed increased growth, and

ericoid mycorrhizal bog blueberry increased number of leaves when grown with fungal communities that had a higher relative abundance of mycorrhizal fungi, and their productivity declined when they were grown with fungal communities that had a higher relative abundance of putative fungal pathogens. This suggests that the mycorrhizal fungi present in the active layer of the permafrost plateau had a beneficial effect rather than imposing a cost for the plant, and that the negative effects on plant productivity were most likely due to pathogenic activity. Considering that the mycorrhizal plant species were more affected by changes in microbial inoculum than the non-mycorrhizal sedge, the decreased relative abundance of these three mycorrhizal plant species at the thermokarst bog may be partially mediated by changes in the microbial community.

Plant productivity of tussock cottongrass was more affected by changes in soil water level than differences in microbial inoculum, and overall grew better with high soil water levels. The overall low sensitivity of tussock grass to mycorrhizal presence or composition is consistent with its non-mycorrhizal status. Consistent with other studies (Mandyam & Jumpponen, 2005; Weishampel & Bedford, 2006), we found that the roots of tussock cottongrass were infected with dark septate endophytes especially when grown with inoculum from the permafrost plateau (data not shown), however, with no obvious positive effect on plant productivity. Further, our results are consistent with tussock cottongrass potentially being sensitive to fungal pathogens in this boreal peatland. Tussock grass grew taller when grown under drier conditions and with microbial inoculum from the thermokarst bog, suggesting that tussock grass was affected by pathogenic activity under wet conditions, but this effect was less pronounced under dry conditions where pathogenic activity may have been suppressed.

Permafrost thaw that leads to soil inundation resulted in a shift in mycorrhizal fungal community composition and a decrease in plant productivity of the mycorrhizal plant species when grown with inoculum from the thermokarst bog. This suggests that the mycorrhizal fungi present at the thermokarst bog may be less beneficial (Bever, Richardson, Lawrence, Holmes, & Watson, 2009; Kiers et al., 2011) and potentially may have a host range exclusive of black spruce and marsh cinquefoil. The decrease in relative abundance of mycorrhizal fungi may also decrease protection from plant pathogens (Smith & Read, 2008). At the same time, the increase in relative abundance of putative fungal pathogens with permafrost thaw most likely poses a negative feedback on plant productivity (Bever et al., 2015; Bever, Westover, & Antonovics, 1997) and it would be interesting to test if it determines plant species composition and the relative abundance of the plant species present in the collapse scar bog (Klironomos, 2002).

Our evidence for microbial community mediating plant community shifts in response to permafrost thaw, suggests that this effect will depend upon the impact of permafrost thaw on local hydrology. In lowland areas such as our study site, thawing permafrost causes land subsidence and subsequent inundation (Jorgenson &

Osterkamp, 2005; Jorgenson et al., 2001), which may thereby inhibit mycorrhizal fungi (Wang et al., 2011), triggering a decline of mycorrhizal-dependent plant species and dominance by tussock grasses. Thawing of permafrost in upland sites may have a drying effect as water level decreases due to improved drainage (O'Donnell et al., 2012; Jorgenson et al., 2013). In upland systems, then, we would predict that the ratio of mycorrhizal to saprotrophs and pathogenic fungi may increase and the plant community could become more dominated by mycorrhizal-dependent plant species. Tas et al. (2014) found fire-mediated permafrost thaw affected functional aspects of bacterial communities in particular carbohydrate metabolism, methanogenesis and nitrogen cycle. However, the role of fungal communities in the boreal forest post thaw has, to our knowledge, not been investigated.

We found evidence that the shift in plant community from mycorrhizal-dependent plant species such as black spruce to non-mycorrhizal sedge species (Jorgenson & Osterkamp, 2005; Jorgenson et al., 2001) with rising water-table post thaw may be mediated by a change in the underlying fungal community composition. The direct effects of hydrology and soil inoculum as well as indirect effects of changes in nutrients and fungal community composition differed in direction for the plant species in this study (Figure 9). Based on the statistically significant effects observed in the covariance models, we can generalize effects on plant productivity due to permafrost thaw by species in the study. Productivity increased for ectomycorrhizal black spruce (Figure 9a) in response to changes in fungal community and total N and P, though overall changes in the soil inoculum due to thaw decreased plant productivity. Productivity of the arbuscular mycorrhizal marsh cinquefoil decreased in response to changes in soil inoculum, fungal community composition and total nitrogen (Figure 9b). Productivity of the ericoid mycorrhizal bog blueberry was not affected by changes in the overall soil inoculum but increased with increased total nitrogen and decreased in response to changes in fungal community composition (Figure 9c). The productivity of the non-mycorrhizal tussock cottongrass increased with increased water levels with a few cases where this response depended on the underlaying soil inoculum. The direction of the effects on productivity of the non-mycorrhizal tussock cottongrass were mostly opposite to the ones observed for marsh cinquefoil (Figure 9d). The results from our study are unique and underline the importance of the effects fungal communities have on mycorrhizal plant communities in boreal soils. However, in both upland and lowland sites, further work is required to establish consistency of our observed patterns and to demonstrate the time course of microbial community change with thaw. It would be very interesting to know, for example, whether the fungal community change preceded mortality of their host plants.

Total N and P present in the inoculum mixture also affected plant productivity as they are limiting nutrients in boreal peatlands (Larmola et al., 2014; Wieder & Vitts, 2006). Plant productivity across three of the four plant species increased with total N (Figure 9). This is consistent with the results of previous studies (Duran, Duncan,
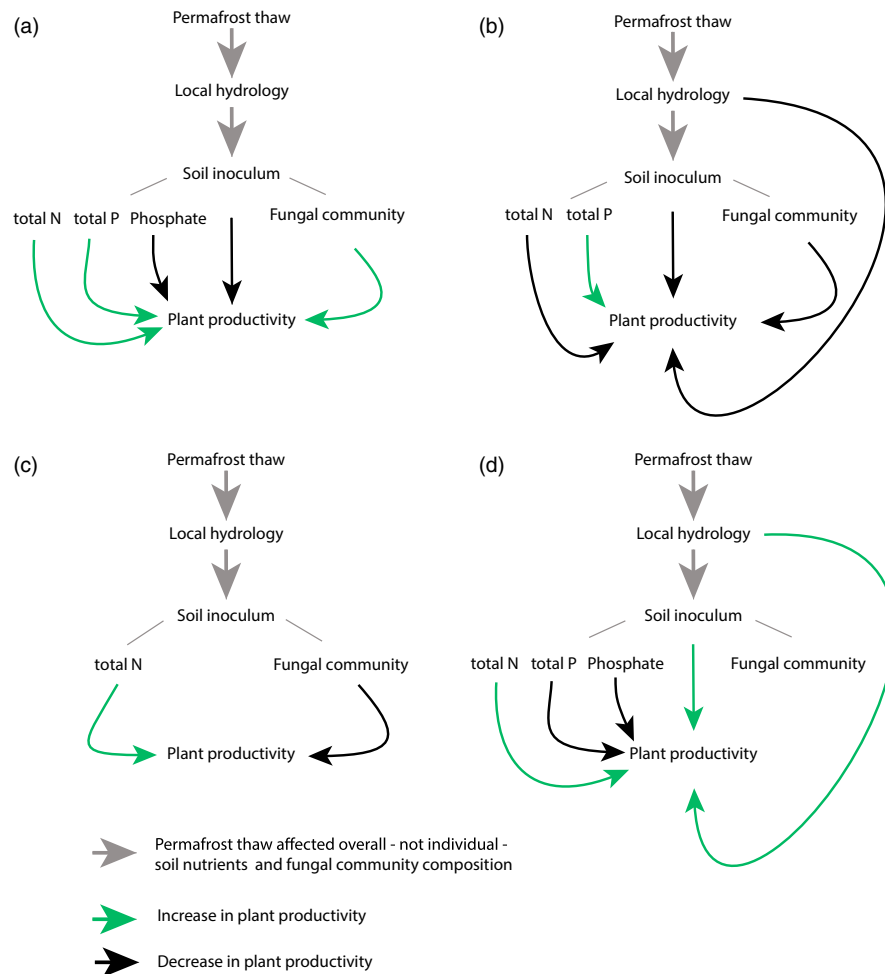
FIGURE 9 Conceptual summary of the direct effects of changes in soil inoculum and increased soil water level and the indirect effects of differences in fungal community composition and soil nutrients on plant productivity due to permafrost thaw observed in this study. We summarize positive (green) and negative (black) feedbacks on plant productivity post thaw for: (a) black spruce, (b) marsh cinquefoil, (c) bog blueberry and (d) tussock cottongrass, based on the statistically significant effects observed in the covariance models (Figures 5 and 6)

Oates, Kucharik, & Jackson, 2016; Jarchow & Liebman, 2013); though it has also been shown that the effect may depend on the plant species (Xu et al., 2015). While we observed similar trends between increases in total P and plant productivity, increases in PO_4^{3-} decreased plant productivity. Other studies have shown the opposite trend that increasing PO_4^{3-} increases plant productivity (Balemi & Negisho, 2012; Shen et al., 2011). The availability of a particular soil nutrient often is determined by the interactions with other nutrients. For example, the availability of PO_4^{3-} depends on the form of iron present in the soil (Larsen, 1982). Thus, we postulate that other abiotic or biotic factors resulting in high availability of PO_4^{3-} might be responsible for the negative effect on plant productivity we observed. We found that fungal community composition differed significantly in the rooting zone between the permafrost plateau and the thermokarst bog, but total N and P, and PO_4^{3-} did not. This does not imply that the interactions between soil nutrients and the different fungal communities present at each site could not affect plant productivity. In fact, the strength of the mycorrhizal symbiosis is affected by changes in N and P (Abbott et al., 2015;

Balzergue, Puech-Pagès, Bécard, & Rochange, 2011; Breuillin et al., 2010; Nouri, Breuillin-Sessoms, Feller, & Reinhardt, 2014), and future research is needed to test how soil nutrients and mycorrhizal community composition interact and shape plant community structure in boreal soils undergoing permafrost thaw.

The recently described class of unknown function Archaerhizomycetes was only detected in the rooting zone of the permafrost plateau. This class of fungi is ubiquitous in roots and rhizosphere and was recently described based on the isolate *A. finlay* (Rosling et al., 2011). Similar to the dark septate fungi, also abundant in the boreal system, the function of Archaerhizomycetes is not well understood (Lukesova, Kohout, Vetrovsky, & Vohnik, 2015; Rosling et al., 2011). Very few studies have looked at the effect of permafrost thaw on fungal communities (Coolen & Orsi, 2015; Penton et al., 2013) and overall, it has been estimated that just one-tenth of fungal diversity is described (Rosling et al., 2011). The dramatic decrease of a whole fungal group of unknown function, combined with the limited knowledge on fungal communities in permafrost soils, emphasizes the need to better understand fungal diversity and

function to be able to better predict the response of boreal systems to disturbances.

AUTHORS' CONTRIBUTIONS

U.M.E.S., J.A.H., M.R.T., M.P.W., J.D.B. and J.R.W. conceived the ideas and designed methodology; M.R.T. and M.P.W. helped to implement conceived ideas into the field. U.M.E.S., J.A.H., A.B., Y.Y. and J.F. collected the data and trouble shot methodology; U.M.E.S., J.D.B. and H.G. analysed the data; U.M.E.S., J.A.H., A.B., J.R.W. and J.D.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

All sequencing data are publicly available in the Sequence Read Archive database, bioproject ID PRJNA515292: http://www.ncbi.nlm.nih.gov/bioproject/515292.

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SUPPORTING INFORMATION

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