Chapter 3

CHANGES IN COMPOSITION AND ABUNDANCE OF FUNCTIONAL GROUPS OF ARCTIC FUNGI IN RESPONSE TO LONG-TERM WARMING

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Abstract

We characterized fungal communities in dry and moist tundra and investigated the effect of long-term experimental summer warming on three aspects of functional groups of arctic fungi: richness, community composition, and species abundance. Warming had profound effects on community composition, abundance, and, to a lesser extent, on richness of fungal functional groups. In addition, our data show that even within functional groups, the direction and extent of response to warming tend to be species-specific and we recommend that studies on the role of fungal communities in nutrient cycling take into account species-level responses.

Keywords: climate change, fungal ecology, metabarcoding, tundra

Introduction

The arctic tundra is considered a maritime biome (Walker et al, 2005) and as a result of the retreating sea ice, arctic land surface temperatures are increasing, causing major changes in terrestrial ecosystems (Pearson et al, 2013; Post et al, 2013). In response to warming temperatures, shifts in land surface vegetation and

ecosystem C cycling have already been observed in terrestrial arctic ecosystems (Pearson et al, 2013; Leffler et al, 2016). However, the responses of belowground communities, such as soil microbes, are less certain (Schaeffer et al, 2013).

Fungi play a central role in the functioning of terrestrial arctic ecosystems due to their roles as symbionts (e.g. mycorrhizae, endophytes, lichens) and decomposers. Almost all arctic plants are highly dependent on mutualistic relationships with mycorrhizal fungi for survival in these nutrient-poor environments (Gardes and Dahlberg, 1996). Given their intimate relationships with plants in a wide range of symbioses, fungi are expected to play an important role in arctic vegetation change. In this study, we compared fungal communities across plots with ambient and experimentally increased summer air and near-surface soil temperature to reveal (1) how community composition and abundance of functional groups of fungi change in response to long-term increase in summer temperature; and (2) whether these responses are similar in dry and moist tundra.

Materials and Methods

Data generation

The study was conducted at the Toolik Field Station in Alaska, USA, where the main vegetation types are dry acidic heath and moist acidic tussock tundra (Walker et al, 1999; Welker et al, 2000). Open top chambers (OTCs), with 1 m^2 area and 0.4 m height, were established in 1994 in both tundra types to increase summer air and upper soil temperature by ca. 2 °C, leading to shifts in edaphic factors and vegetation (Walker et al, 1999; Welker et al, 2000; Walker et al, 2006). We sampled 100 soil cores across 20 plots: five replicate plots in the OTC and control plots in each tundra type, with five soil cores of 2 cm diameter and 20 cm depth per plot that were mixed and lyophilized. We extracted DNA using Macherey-Nagel NucleoSpin-Soil kit. PCR and sequencing of the ITS2 rDNA were done with primers fITS7 and ITS4, labelled with sample-specific tags, as described earlier (Morgado et al, 2015; Semenova et al, 2015; Geml et al, 2015). We generated 4 047 811 reads using Ion 318^{TM} Chip (doi:10.5061/dryad.2fc32).

Bioinformatics

Primers and adapters were removed and poor-quality ends were trimmed off using 0.02 error probability limit in Geneious Pro 5.6.1. Sequences were truncated to 200 bp and sequences with expected error > 1 were discarded using USEARCH v.8.0

(Edgar, 2010). The remaining 1 632 682 sequences were collapsed into unique sequence types on a per-sample basis while preserving read counts. Singletons were discarded and the resulting 1 092 238 high-quality sequences were grouped into 4069 operational taxonomic units (OTUs) with USEARCH at 97% sequence similarity, while excluding 9026 (0.3%) chimeras. We identified 3501 OTUs based on the UNITE fungal database, discarding OTUs with < 70% similarity to any fungal sequence. We assigned ecological functions to 1655 OTUs following (Tedersoo et al, 2014): arbuscular mycorrhizal (5 OTUs), animal parasitic (18), ectomycorrhizal (417), lichenicolous (9), lichenized (156), mycoparasitic (39), plant pathogenic (134), and saprotrophic (877) fungi. Because of low richness, arbuscular mycorrhizal fungi were excluded, while animal- and mycoparasites were combined, as were lichens and lichenicolous fungi.

Statistical analyses

For each functional group, OTU richness (S), Shannon's and Simpson's diversity indices were calculated in PC-ORD v. 6.0 (McCune and Grace, 2002) and were compared using two-way ANOVA to test for effects of warming, tundra type, and their interaction. We visualized changes in community composition of functional groups with non-metric multidimensional scaling (NMDS) based on presence-absence data with Bray-Curtis distance and 500 iterations in PC-ORD. We tested for statistical difference in fungal community composition among tundra types and treatments using multi-response permutation procedure (MRPP).

We assessed the effect of warming on abundance on a per-OTU basis by comparing DNA sequence counts (Hedges' *D*) and calculating the mean effect size with 95% confidence intervals using METAWIN v. 2.0 (Rosenberg et al, 1999). Using sequence read counts as a proxy for abundance (biomass) is constrained due to interspecific differences in copy number and length of ITS (Amend et al, 2010). However, for individual OTUs, changes in sequence counts can indicate relative changes in abundance (biomass) (Amend et al, 2010). We compared per-OTU mean read counts across the control and warmed plots to calculate size effects with variance and calculated mean effect size with 95% confidence interval for each functional group. This approach allowed us to depict the variation in responses of individual OTUs to warming and evaluate the overall responses of functional groups.

Results

Diversity measures

Tundra type had the strongest effect on lichens, where all diversity measures were significantly higher in the dry tundra. Similarly, in the animal- and mycoparasitic fungi, both Shannon's and Simpson's diversity indices were higher in the dry tundra, even though differences in richness were insignificant. Warming only affected richness in ectomycorrhizal fungi, with a strong decrease in the moist tundra, although Shannon's and Simpson's diversity indices were not significantly affected. A similar, but somewhat weaker trend was seen in lichens. Shannon's diversity decreased in saprotrophic fungi, even though neither richness nor Simpson's diversity were strongly affected. The interaction of warming and tundra type showed significant decrease in richness in ectomycorrhizal and saprotrophic fungi, and only in saprotrophs regarding Shannon's and Simpson's diversity (Table 3.1).

Community composition

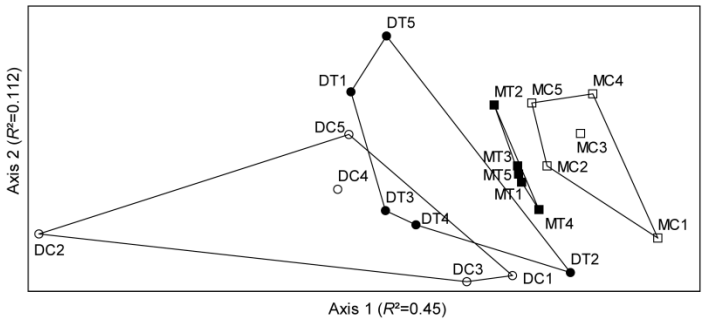
NMDS analyses resulted in 2-dimensional solutions with final stress values of 0.11101 (animal and mycoparasites), 0.09244 (ectomycorrhizal fungi), 0.05238 (lichens and lichenicolous fungi), 0.12336 (plant pathogens), and 0.07267 (saprotrophs), with final instability values < 0.00001. The NMDS plots revealed strong structuring in all functional groups with tundra type being the most influential variable (Table 3.2, Fig. 3.1). Warming had a strong effect on the fungal community in the moist tundra, where community composition was significantly different between treatment and control in all functional groups. However, in the dry tundra, only plant pathogens showed a significant treatment effect on composition (Table 3.2).

Abundance at the species-level

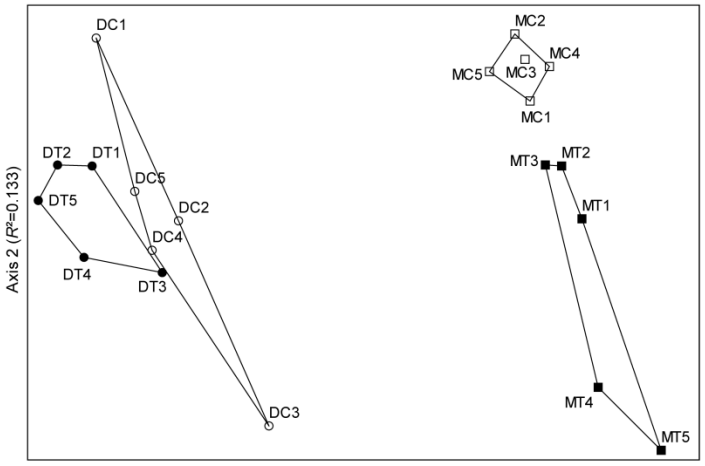
Sequence read counts (a proxy for abundance or biomass) of most OTUs differed between the control and treatment as indicated by non-zero effect values and their variance intervals (Fig. 3.2a).

Figure 3.1. (next two pages) *Non-metric multidimensional scaling (NMDS) ordination plots for functional groups of arctic fungal communities in the warmed and control plots in the dry and moist tundra types based on presence-absence. M = moist tundra, D = dry tundra, C = control, T = warming.*

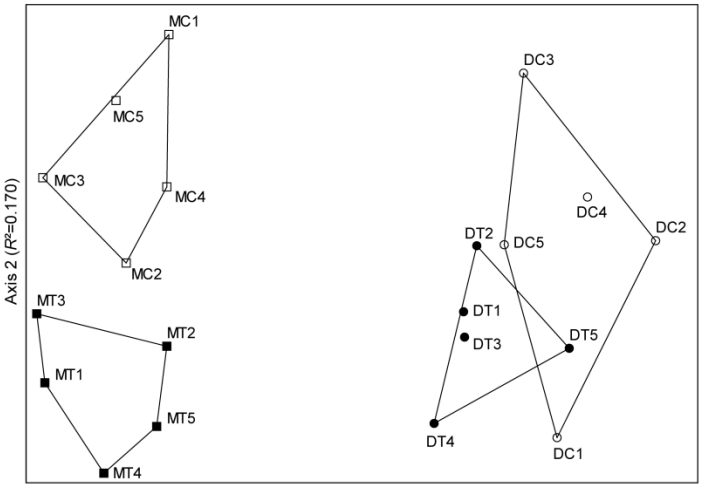
Animal- and mycoparasites



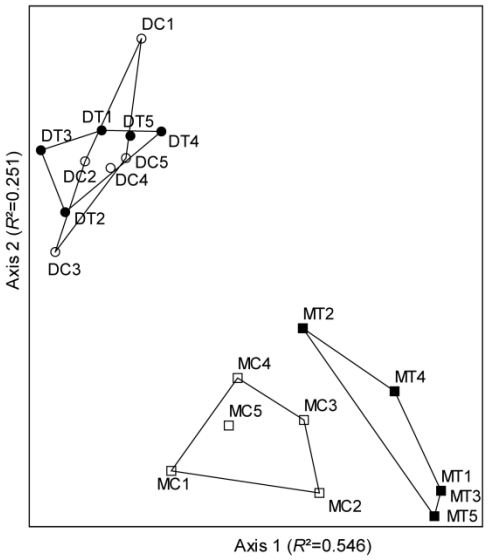
Ectomycorrhizal fungi



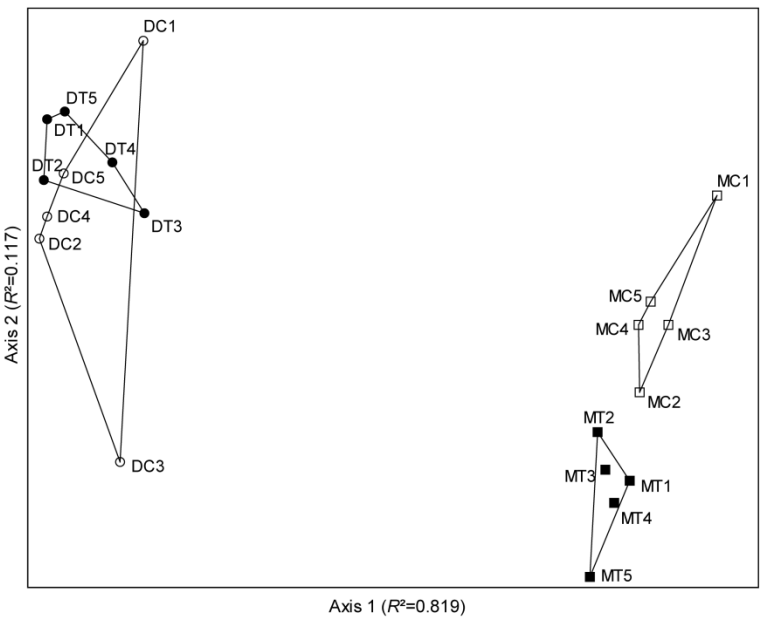
Plant pathogens



Lichens and lichenicolous fungi



Saprotrophs



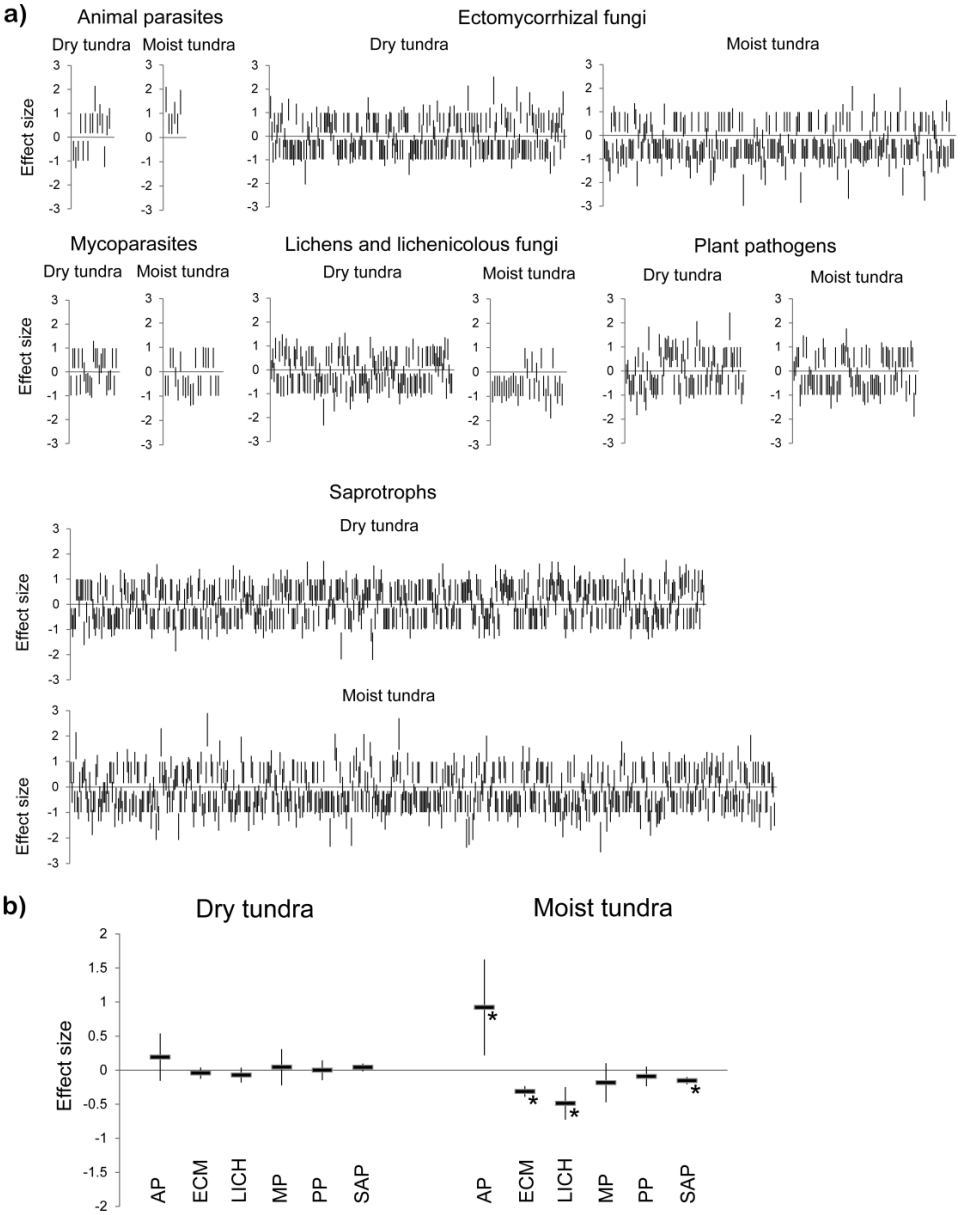


Figure 3.2. a) *Responses of individual OTUs in the functional groups to warming. Each vertical line represent the effect of warming on mean DNA sequence read count with variance for a fungal OTU. Positive and negative effects indicate increased and decreased abundance in the warmed plots, respectively. b) Summarized responses of functional groups of arctic fungi to warming. The values represent the mean effect size and 95% confidence interval from meta-analyses of all OTUs in the functional group in question. Functional group abbreviations are given in Table 3.1.*

Table 3.1. (next page, upper table) *The results of two-way ANOVA on OTU richness, Shannon's and Simpson's diversity indices calculated for functional groups of fungi. Significant p-values are indicated in bold. Abbreviations: ECM = ectomycorrhizal fungi, AP = animal parasites, MP = mycoparasites, LIC = lichens and lichenicolous fungi, PP = plant pathogens, SAP = saprotrophs.*

Table 3.2. (next page, lower table) *Effects of tundra type the warming on community composition of functional groups of fungi as calculated using Multi-Response Permutation Procedure. Significant P-values are indicated in bold.*

Index	Effects	ECM	AP+MP	LIC	PP	SAP
Richness (S)	treatment (warming)	**0.0168**	0.3932	0.069	0.6171	0.2476
	tundra type (dry vs. moist)	0.2692	0.604	**<0.0001**	0.531	0.5854
	treatment × tundra type	**0.0176**	1	0.5795	0.4854	**0.0477**
Shannon's diversity (H)	treatment (warming)	0.2623	0.0881	0.0782	0.494	**0.0324**
	tundra type (dry vs. moist)	0.1237	**0.0309**	**<0.0001**	**0.036**	0.2213
	treatment × tundra type	0.8647	0.7132	0.844	0.4612	**0.0023**
Simpson's diversity ('D)	treatment (warming)	0.373	0.0541	0.2935	0.6529	1
	tundra type (dry vs. moist)	0.1313	0.0368	0.0001	0.0693	1
	treatment × tundra type	1	1	0.5028	0.6529	0.001

Functional groups	Tundra type		Warming in dry tundra		Warming in moist tundra	
	effect (A)	***P***	**effect (A)**	***P***	**effect (A)**	***P***
Ectomycorrhizal	0.15236	< 0.00001	0.0219	0.07663	0.10865	0.00197
Animal parasites and mycoparasites	0.1153	0.00002	0.01459	0.69563	0.14281	0.00196
Lichens and lichenicolous fungi	0.21142	< 0.00001	0.00677	0.28502	0.15166	0.01258
Plant pathogens	0.18262	< 0.00001	0.04895	0.03357	0.09515	0.00308
Saprotrophs	0.19335	< 0.00001	0.01331	0.16814	0.08925	0.00389

Meta-analyses of trends of the individual OTUs per functional groups indicated significant changes only in the moist tundra, where there was a significant decline in ectomycorrhizal, lichenized, and saprotrophic fungi, as well as a significant increase in animal pathogens, while mycoparasites and plant pathogens showed a non-significant decline (Fig. 3.2b).

Discussion

Tundra type greatly affected fungal communities with shifts in composition and OTU abundance in response to warming being stronger in the moist as opposed to the dry tundra. Because most fungal symbiotic plants occur in both vegetation types, the profound fungal compositional differences between moist and dry tundra are likely caused by differences in fundamental abiotic attributes, such as snow cover, active layer depth, soil moisture, nutrients, and temperature (Walker et al, 1999). These findings and the accumulating evidence (Welker et al, 2000; Welker et al, 1997) suggest that warming responses of microbial and plant communities likely are predicated on soil water conditions and resulting differences in productivity among tundra types.

Changes in communities of arctic fungal functional groups have been scarcely documented, except in ectomycorrhizal fungi (Morgado et al, 2015). The compositional differences between the warmed and control plots in all functional groups indicate that even in groups without major changes in richness, the turnover is substantial. Although such compositional shifts are particularly evident in the moist tundra, animal- and mycoparasites, ectomycorrhizal fungi, and plant pathogens also display clearly visible changes in the dry tundra in response to warming (Fig. 3.1, Table 3.2).

The high proportion of OTUs with marked changes in abundance was a striking result (Fig. 3.2a). Even in the dry tundra, where the overall effect size of warming was not significant, most OTUs showed a clear trend, with only a small fraction of OTUs seemingly unaffected by warming. This indicates that response to warming likely is species-specific within these broad ecological groups. The importance of species-specific response has not been emphasized in other Arctic system studies of climate change and may be influenced by fine-scale changes in soil traits and species interactions.

Overall trends were more profound in the moist tundra, where significant changes were observed in most functional groups (Fig. 3.2b). The only increase was in

animal parasites that is in agreement with observed warming-induced increases in insect abundance (Hasle, 2013). All OTUs of animal parasites in the moist tundra were positively affected by warming and even in the dry tundra this group showed the largest, although not significant, increase. Abundance decrease in ectomycorrhizal fungi may have functional implications and the fact that several ectomycorrhizal fungi showed positive response to warming, while most were negatively affected, indicates substantial shift in the community. The strong decrease in lichen abundance was in agreement with formerly reported decrease in lichen cover due to increased shading by shrubs in the warmed moist tundra (Welker et al, 2000). In the dry tundra, where shading is minimal, several lichens benefited from warming (Fig. 3.2a). The decrease in saprotrophs is surprising in light of non-significant changes in richness (above) and previous findings on warming-induced increase in litter accumulation (Welker et al, 2000) and in microbial decomposition rates (Sistla et al, 2013). However, distinct species-specific responses to warming were revealed in saprotrophic taxa as well.

In this paper, we provide evidence that long-term experimental summer warming has profound effects on community composition and abundance of functional groups of arctic fungi. We also emphasize that, while there are similarities within functional groups, changes in occurrence and abundance in response to warming tend to be species-specific, and may be masked when communities are compared at higher taxonomic levels. Therefore, we recommend that studies of arctic fungal communities (for example, their roles in nutrient cycling) take into account species-level differences. Finally, we advocate the integration of taxonomic and functional data into climatic models to better understand the influence of climate on soil microbial community structure and function and their contributions to climate-linked processes.

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Snow fence and open-top chambers at the dry tundra type, Toolik Lake field station, Alaska