Community ecology

Changes in composition and abundance of functional groups of arctic fungi in response to long-term summer warming

József Geml1,2, Tatiana A. Semenova1,2, Luis N. Morgado1 and Jeffrey M. Welker3

1Naturalis Biodiversity Center, PO Box 9517, 2300 RA Leiden, The Netherlands
2Faculty of Science, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands
3Department of Biological Sciences, University of Alaska Anchorage, Anchorage, AK 99508, USA

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 fungal communities and their roles in nutrient cycling take into account species-level responses.

1. Introduction

The arctic tundra is considered a maritime biome, as approximately 80% of non-alpine tundra is located within 100 km of a coastline [1]. As a result of the retreating sea ice, arctic land surface temperatures are increasing, causing major changes in terrestrial ecosystems [2,3]. In response to warming temperatures, shifts in land surface vegetation and ecosystem C cycling have already been observed in terrestrial arctic ecosystems [3,4]. However, the responses of belowground communities, such as soil microbes, have been less certain [5].

Fungi are key to the functioning of terrestrial arctic ecosystems as symbionts (e.g. mycorrhizae, endophytes and lichens) and decomposers. Given their intimate relationships with plants in a wide range of symbioses, fungi are expected to play an important role in arctic vegetation change [6]. In this study, we compared fungal communities across plots with ambient and experimentally increased summer air and near-surface soil temperature to reveal (i) how community composition and abundance of functional groups of fungi change in response to long-term increase in summer temperature and (ii) whether these responses are similar in dry and moist tundra.

2. Material and methods

(a) 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 [7,8]. Open top chambers (OTCs), with 1 m² area and 0.4 m height, were established in 1994 in both tundra types to increase summer air and upper soil temperature by approximately 2°C, leading to shifts in edaphic factors and vegetation [7–9].
distance and 500 iterations in PC-ORD. We tested for statistical significance (NMDS) based on presence–absence data with Bray–Curtis distance of functional groups with non-metric multidimensional scaling (OTUs) with USEARCH at 97% sequence similarity, while excluding sequences were grouped into 4069 operational taxonomic units (reads using Ion 318™ Chip (http://dx.doi.org/10.5061/dryad.2fc32).

We sampled 100 soil cores across 20 plots: five replicate plots in the OTCs and control plots in each tundra type, with five randomly collected soil cores of 2 cm diameter and 20 cm depth per plot which were mixed and lyophilized. We extracted DNA using the Macherey–Nagel NucleoSpin-Soil kit. PCR and sequencing of the ITS2 (internal transcribed spacer 2) rDNA were done as described earlier [10–12]. We generated 4 047 811 reads using Ion 318™ Chip.

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 [17]. Using sequence read counts as a proxy for abundance (biomass) is constrained owing to interspecific differences in copy number and length of ITS [18]. However, for individual OTUs, changes in sequence counts can indicate relative changes in abundance (biomass) [18]. We compared per-OTU mean read counts across the control and warmed plots to calculate effect sizes 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

#### (a) Diversity measures

Tundra type had the strongest effect on lichens, where all diversity measures were significantly higher in the dry tundra (table 1). 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 strong decrease in the moist tundra, although Shannon’s and Simpson’s diversity indices were not significantly different. 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 was 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.

#### (b) Community composition

NMDS analyses resulted in two-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 less than 0.00001. The NMDS plots revealed strong structuring in all functional groups with tundra type being the most influential variable (table 2 and figure 1). Similarly, Venn diagrams indicated that a substantial fraction of OTUs were unique to a tundra

| 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 versus 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 versus 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 versus moist) | 0.1313 | 0.0368 | 0.0001 | 0.0693 | 1 |
| | treatment × tundra type | 1 | 1 | 0.5028 | 0.6529 | 0.001 |
Warming had 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 significant treatment effect on composition (table 2).

**Table 2.** Effects of tundra type and warming on community composition of functional groups of fungi as calculated using multi-response permutation procedure. Significant p-values are indicated in italics.

| functional groups               | tundra type effect (A) | p     | warming in dry tundra effect (A) | p     | warming in moist tundra 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 |

**Figure 1.** 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.
Sequence read counts of most OTUs differed between the control and treatment as indicated by non-zero effect values and their variance intervals (figure 2a). Meta-analyses of trends of the individual OTUs per functional group indicated significant changes only in the moist tundra, where we observed significant decline in ectomycorrhizal, lichenized and saprotrophic fungi, as well as significant increase in animal pathogens, while mycoparasites and plant pathogens showed non-significant decline (figure 2b).

4. 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 probably caused by well-known differences in fundamental abiotic attributes, such as snow cover, active layer depth, soil moisture, nutrients and temperature [7]. The accumulating evidence in this and other studies [8,19] suggests that warming responses of fungal and plant communities probably 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 [10]. 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, parasites, ectomycorrhizal fungi and pathogens also display clearly visible warming-induced changes in the dry tundra (figure 1 and table 2).

The high proportion of OTUs with marked changes in abundance is striking (figure 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 probably is species-specific within these broad ecological groups.

Overall trends were more profound in the moist tundra, where significant changes were observed in most functional groups (figure 2b). The only increase was in animal parasites, which is in agreement with observed warming-induced increases in insect abundance [20]. 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 underlines their decrease in cover due to increased shading by shrubs in the warmed moist tundra [8]. In the dry tundra, where shading is minimal, several lichens benefited from warming (figure 2n). The decrease in saprotrophs is surprising in the light of non-significant changes in richness (above) and previous findings on warming-induced increase in litter accumulation [8] and in microbial decomposition rates [21]. However, distinct species-specific responses to warming were revealed also in saprotrophic taxa.

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 underline 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.

Ethics. This research complies with the relevant national and international ethics-related rules and professional codes of conduct.

Data accessibility. All molecular data are publicly available in Dryad: http://dx.doi.org/10.5061/dryad.26c32 [22].

Authors’ contributions. All authors participated in the conception, study design, data acquisition, analyses, interpretation and writing. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing interest. The authors declare no conflict of interest.

Funding. This research was funded by NWO-ALW (OP-821.01.016) and NSF (OPP-AON-0856728, OPP-IPY-ITEX-0632184).

References

1. Walker DA et al. 2005 The circumpolar Arctic vegetation map. J. Veg. Sci. 16, 267–282. (doi:10.1111/j.1654-1103.2005.tb02365.x)
2. Post E et al. 2013 Ecological consequences of sea-ice decline. Science 341, 519–524. (doi:10.1126/science.1235225)
3. Pearson RG, Phillips SJ, Loranty MM, Beck PSA, Damoulas T, Knight SJ, Goetz SJ. 2013 Shifts in arctic vegetation and associated feedbacks under climate change. Nat. Clim. Change 3, 673–677. (doi:10.1038/nclimate1858)
4. Leffler JA, Klein ES, Oberbauer SF, Welker JM. 2016 Coupled long-term summer warming and deeper snow alters species composition and stimulates gross primary productivity in tussock tundra. Oecologia 181, 287–297. (doi:10.1007/s00442-015-3543-8)
5. Schaeffer SM, Sharp E, Schimel J, Welker JM. 2013 Soil N processes in the High Arctic: responses to a long-term multi-level warming and added summer water experiment. Glob. Change Biol. 19, 3529–3539. (doi:10.1111/gcb.12318)
6. Gardes M, Dahlberg A. 1996 Mycorrhizal diversity in arctic and alpine tundra: an open question. New Phytol. 133, 147–157. (doi:10.1111/j.1469-8137.1996.tb04350.x)
7. Walker MD et al. 1999 Long-term experimental manipulation of winter snow regime and summer climate in arctic and alpine tundra. HydroL. Proc. 13, 2315–2330. (doi:10.1002/(SICI)1099-1085(199910)13:14/15<2315::AID-HYPL888>3.0.CO;2-A)
8. Welker JM, Fahnestock JT, Jones MH. 2000 Annual CO2 flux in dry and moist arctic tundra: field responses to increases in summer temperatures and winter snow depth. Clim. Change 44, 139–150. (doi:10.1023/A:1005555012742)
9. Walker MD et al. 2006 Plant community responses to experimental warming across the tundra biome. Proc. Natl Acad. Sci. USA 103, 1342–1346. (doi:10.1073/pnas.0503198103)
10. Morgado LN, Semenova TA, Welker JM, Walker MD, Smets E, Geml J. 2015 Summer temperature increase has distinct effects on the ectomycorrhizal fungal communities of moist tussock and dry tundra in Arctic Alaska. Glob. Change Biol. 21, 959–972. (doi:10.1111/gcb.12716)
11. Semenova TA, Morgado LN, Welker JM, Walker MD, Smets E, Geml J. 2015 Long-term experimental warming alters community composition of ascomycetes in Alaskan moist and dry arctic tundra. Mol. Ecol. 24, 424–437. (doi:10.1111/mec.13045)
12. Geml J, Morgado LN, Semenova TA, Welker JM, Walker MD, Smets E. 2015 Long-term warming alters richness and composition of taxonomic and functional groups of arctic fungi. FEMS Microbiol. Ecol. 91, 95. (doi:10.1093/femsec/fiv095)
13. Edgar RC. 2010 Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. (doi:10.1093/bioinformatics/btq461)

14. Tedersoo L et al. 2014 Global diversity and geography of soil fungi. Science 346, 1256688. (doi:10.1126/science.1256688)

15. McCune B, Grace JB. 2002 Analysis of ecological communities. Glenden Beach, OR: MJM Software.

16. Oliveros JC. 2007 VENNY. An interactive tool for comparing lists with Venn diagrams. See http://bioinfogp.cnb.csic.es/tools/venny/index.html (accessed September 2016).

17. Rosenberg MS, Adams DC, Gurevitch J. 1999 MetaWin: statistical software for meta-analysis. Version 2.0. Sunderland, MA: Sinauer Associates. See http://www.metawinsoft.com/

18. Amend AS, Seifert KA, Bruns TD. 2010 Quantifying microbial communities with 454 pyrosequencing: does read abundance count? Mol. Ecol. 19, 5555–5565. (doi:10.1111/j.1365-294X.2010.04898.x)

19. Welker JM, Molau U, Parsons AN, Robinson CH, Wookey PA. 1997 Response of Dryas octopetala to ITEX manipulations: a synthesis with circumpolar comparisons. Glob. Change Biol. 3, 61–73. (doi:10.1111/j.1365-2486.1997.tb0143.x)

20. Hasle TE. 2013 The effect of experimental warming on insect herbivory in an alpine plant community. MSc thesis, Norwegian University of Life Sciences.

21. Sistla SA, Moore JC, Simpson RT, Gough L, Shaver GR, Schimel JP. 2013 Long-term warming restructures Arctic tundra without changing net soil carbon storage. Nature 497, 615–618. (doi:10.1038/nature12129)

22. Semenova TA, Morgado LN, Welker JM, Walker MD, Smets E, Gmel J. 2014 Data from: Long-term experimental warming alters community composition of ascomycetes in Alaskan moist and dry arctic tundra. Dryad Digital Repository. See http://dx.doi.org/10.5061/dryad.2632.