RESULTS FROM PRIOR NSF SUPPORT

IPY: A Community Genomics Investigation of Fungal Adaptation to Cold. ARC-0632332 to D.L.Taylor (PI), 07/01/07 - 06/30/13. $743,697.

Intellectual merit: Our goal was to better understand the influence of cold on soil fungi. Sampling was carried out along two transects spanning all 5 bioclimatic subzones and in summer and winter in Arctic and boreal study sites followed by massive clone library Sanger sequencing. Our studies revealed that 1) fungal communities differ sharply between frost boils and adjacent vegetated areas, 2) fungal communities shift gradually with subzone from the Low to the High Arctic, 3) yet fungal biodiversity does not decline with latitude, 4) fungal taxa in the coldest sites are cosmopolitan, occurring widely across the Arctic and even in warmer regions, 5) fungal communities undergo strong seasonal shifts, 6) unexpectedly, several of the taxa dominant in winter under snowpack are ectomycorrhizal. The last finding calls for a reappraisal of mycorrhiza-mediated plant nutrient uptake in the context of seasonal cycles and permafrost. To date, 11 publications have resulted from this funding (*in References). Taylor et al. 2014 was a Science Editor's Pick and Faculty of 1000 selection. Timling et al. 2014 was chosen for a News and Views commentary in the same issue of Molecular Ecology. Broader impacts: This study involved collaborations with scientists from the US, UK, France, Sweden and Russia. One post-doc, two graduate students and 5 rural Alaskan high school students were trained. Our work was featured in the Fairbanks Daily News Miner and PhD student Ina Timling participated in the "Science for Alaska" public talk series.

Biocomplexity associated with biogeochemical cycles in frost boil ecosystems. OPP-0120736 to D.A. Walker (PI), H.E. Epstein, W.A. Gould, W.B. Krantz, R. Peterson, C. L. Ping, V.E. Romanovsky (Co-PIs). 10/1/01-9/30/06, $2,750,421

Intellectual merit: This project was the first integrated whole-system analysis of patterned ground ecosystems. It examined the complex interactions between vegetation, soils, permafrost and climate that are involved in the formation of small patterned-ground features ("frost boils") along the bioclimatic gradient in arctic Alaska and Canada (Walker *et al.*, 2008). The project culminated in a special section volume of the *Journal of Geophysical Research*: "Biocomplexity of Arctic Tundra" (Walker *et al.*, 2008) which contained 9 papers from the project. At least 15 other published papers (**in References) and 4 PhDs resulted from the project. Walker et al., 2011 received the *Applied Vegetation Science* Editors Choice Award for best paper in 2011. Broader impacts: The project provided full or partial funds for 4 doctoral students, 2 Master's students and 4 postdocs. The project brought a total of 51 participants together through the research component and 60 participants through the educational component, including 5 scientists, 29 students in an *Arctic Field Ecology* course, 9 Inuit elders, 16 additional Inuit participants, and 8 other personnel. Participants came from nine countries (Gould *et al.*, 2010).

Collaborative research: An integrated ecological investigation of McMurdo Dry Valley's active soil microbial communities. ANT 1142102 to C. Takacs-Vesbach (PI), D. Van Horn and E. Schwartz (Co-PIs). 6/01/12-5/31/15. $726,645.

Intellectual merit: Early investigations of McMurdo Dry Valley (MDV) soil bacterial communities using culturing techniques yielded only a handful of unremarkable bacterial isolates, leading to the conclusion that these soils were essentially sterile and inhabited solely by dormant exogenous organisms. However, a surprising bacterial richness has been revealed recently using molecular biology techniques, which is inconsistent with much of the eukaryotic diversity in the region. This apparent discrepancy has led us to question how such a high level of microbial diversity could exist in the MDV. We are investigating the active portion of the MDV bacterial community using an integrated molecular ecology approach to 1) identify active members, 2) determine their metabolic functions in situ, and 3) develop a habitat suitability model describing the abiotic controls on bacterial activity. Using an experimental stable isotope probing approach (Van Horn *et al.*, 2014), we have identified the diversity and function of active members of the bacterial community (Schwartz *et al.*, 2014) and metatranscriptomics (Buelow *et al.*), and investigated the role of abiotic controls on microbial distributions in Van Horn et al. (2015) and Jiang et al. (in prep; See *** in References). Broader Impacts: The project is training two graduate and two undergraduate students.

CONCEPTUAL FRAMEWORK

The current and future climate on Earth depends to a large degree on the interactions between plants (living and dead) and microbes. Removal of carbon from the atmosphere occurs via photosynthesis. In terrestrial systems, photosynthesis depends on the species of plants present (i.e. community

composition) and their growth rates. Plant community composition and growth rates are strongly influenced by their interactions with mutualistic and pathogenic microbes. Carbon return from terrestrial systems to the atmosphere occurs via respiration, the bulk of which is due to microbial (especially fungal) activities in breaking down dead plant material. To understand Arctic contributions to the global climate system, we must understand the ecologies of the plant and microbial communities in the Arctic (Schimel *et al.*, 2004; Sturm *et al.*, 2005; Sistla *et al.*, 2013). Furthermore, why species occur where they do is a core concern of the discipline of ecology (Krebs, 1978). Assembly rules, i.e. the processes by which species disperse to and establish within a local community is one key to understanding species distributions and community composition. Our understanding of the assembly of plant communities remains elusive (Silvertown, 2004), perhaps in part because they have strong interactions with microbes that are not fully incorporated into assembly theory (Reynolds *et al.*, 2003). Due to their radically different evolutionary and ecological dynamics, we might expect plants, fungi and bacteria to be governed by different assembly rules. Yet these three kingdoms interact intensively and interdependently in soil. Thus efforts to ascertain assembly rules that consider only one guild may yield an incomplete or even misleading picture. Using newly developed methods in community genomics and statistical analysis, **we propose a novel project to describe and disentangle the drivers of the biodiversity of plant, fungal and bacterial (PFB) communities in the Arctic.**

We will analyze the biodiversity of all three kingdoms from 3580 soil cores collected over wide ecological gradients (e.g. warmest to coldest bioclimatic subzones) from local (sub-meter) to cross-continental (1000s of km) scales (Fig 1). The monumental fieldwork and sampling needed for this project has already been carried out. In addition, deep next-generation sequencing (Illumina) is already complete for fungi and bacteria. In order to carry out rigorous phylogeny-based analyses, we will add only limited plant chloroplast sequencing plus shallow long-amplicon sequencing (PacBio) for fungi. We will focus our efforts on rigorous bioinformatic and statistical analyses of these diverse, synergistic data streams, as detailed below. ***Because the bulk of data are in hand, this proposal is low cost and low risk, yet has potential for very large impacts.***

INNOVATION AND INTELLECTUAL MERIT

• *To our knowledge, this project encompasses the widest geographic sampling across the Arctic and most thorough biodiversity inventory of any ecological study to date.* Due to great diversity in soils, climate and vegetation, wide-scale studies are essential to predicting impacts of ongoing climate change in the Arctic.

• *This study leverages the most extensive and diverse environmental measurements (metadata) yet to be applied to understanding Arctic fungi and bacteria.* Unmeasured variables often confound efforts to extract patterns in ecological studies of biodiversity. The extensive array of abiotic and biotic variables measured at each site will provide unprecedented power to detect patterns and potential drivers.

• *This study utilizes bleeding-edge molecular methods* (2^{nd} generation Illumina sequencing in combination with complementary 3^{rd} generation Pacific Biosciences sequencing). This will be the first use of Pacific Biosciences single-molecule SMRT sequencing for fungi, to our knowledge. These approaches will allow us to both saturate microbial diversity, and, for the first time, apply highly informative *phylogenetic* approaches to understanding PFB community assembly.

• *This study will utilize the most diverse and cutting-edge set of statistical methods yet applied to Arctic biodiversity studies.* Recently developed models and analysis packages are enabling far more rigorous and informative interpretations of massive biodiversity datasets than was previously possible.

• *The search for assembly rules is a central, unresolved issue in ecology. The possibility that interacting members of evolutionarily distant guilds influence one another's assembly is a* ***novel and potentially transformative*** *concept.* Abundant data demonstrate that microbes influence plant growth and that plants influence microbial communities. Yet efforts to extract assembly rules have invariably focused on a single guild (e.g. terrestrial plants), without acknowledging that "co-assembly" is a more realistic view.

• *Our results may inform Arctic vegetation, biogeochemical and Earth System models.* Given the role of the Arctic in the climate system, predicting future scenarios is an important endeavor. Yet microbes and plant-microbe interactions are not yet integrated into these models in realistic ways. But this can be accomplished. For example, we have shown that incorporating mycorrhizae into an Arctic frame-based spatially explicit model (ALFRESCO) changes projections for forest expansion (Hewitt et al., In Review).

The Arctic is a rapidly changing system in which the terrestrial vegetation has important feedbacks to global climate. Permafrost is thawing, growing seasons are lengthening, plant community composition is changing (e.g. species adapted to warmer climates moving into colder subzones), and vegetation

growth dynamics are changing, all of which have potential to strongly impact capture of CO_2 from the atmosphere and carbon storage and release above and belowground (Shaver *et al.*, 2001; Wahren *et al.*, 2005; Schuur *et al.*, 2009; Sistla *et al.*, 2013). For example, a change from tussock tundra to shrub tundra changes the depth of snowpack in winter, soil temperature, albedo, C:N ratios of litter, and aboveground and belowground C storage (Myers-Smith *et al.*, 2011). However, the influences of climate change cannot be predicted simply from known direct effects of temperature on individual plants. There are now numerous examples that powerfully demonstrate how interactions between members of different guilds can control ecosystem responses to climate change. For example, when pine trees were introduced to tree-free grasslands of South America, there were large net losses of carbon from the system, despite the much greater aboveground biomass produced by the trees (Chapela *et al.*, 2001). This occurred because mycorrhizal fungi that were co-introduced with the pines mined and respired large amounts of carbon from these deep, organic soils. This carbon had been "stable" prior to this shift in plant-microbial communities. Mack et al. (Nature, 2004) showed that long-term fertilization caused a major loss of soil carbon in their Arctic study site, even though it dramatically increased plant productivity. They attributed this to increased decomposition rates. As with the study in South America, the increased decomposition may have occurred due to a switch to associations with ectomycorrhizal fungi as the plant community shifted from sedge and grass dominance to dwarf shrub dominance: "indirect effects of nutrient addition on plant–microbe interactions and soil food-web dynamics may have caused these results."

Arctic terrestrial vegetation exists as a consortium of plants and microbes (particularly fungi and bacteria). The members of these consortia disperse and evolve independently (e.g. seeds do not carry mycorrhizal fungi or rhizosphere bacteria when they disperse). Furthermore, the radically different sizes of propagules, dispersal potentials and population sizes suggest that different rules may govern assembly of communities across these three kingdoms, as was shown for plants and soil bacteria along elevation gradients (Bryant *et al.*, 2008). At the same time, the strong interdependence among many members of these kingdoms suggests the possibility of strong interactive influences on community *co-assembly*. Due to their independent dispersal, ongoing environmental change has the potential to uncouple existing plant-microbe linkages and functions, with unpredictable consequences.

> *A fundamental question in ecology is whether general assembly rules determine the structure of natural communities.* Gotelli and McCabe (2002).

By *community assembly*, we refer to the processes by which individuals come to occupy a site at local to regional scales (Diamond, 1975; HilleRisLambers *et al.*, 2012). These processes encompass dispersal, colonization and population growth dynamics that result in the abundance and composition of species comprising a local community. **Community assembly is thought to result from both abiotic and biotic influences** acting on colonists from the regional species pool. If an organism is not adequately adapted to the *abiotic* conditions (e.g. temperature, moisture, soil chemistry) at a site, it will not grow there, reflecting constraints imposed by its fundamental niche (Hutchinson, 1959). *Biotic* influences can also be important. Numerous studies demonstrate that species that are better competitors for limited space (Connell, 1961; Paine, 1984), limited nutrients (Tilman, 1982) or other resources can displace competitively inferior species. In addition, in recent years, attention to facilitative and mutualistic interactions, in which pairs of species are more successful when occurring together, has increased (Brooker *et al.*, 2008; Martorell and Freckleton, 2014).

It has also been increasingly recognized that non-niche-based processes act to shape communities (Hubbell, 2001; Tilman, 2004; Gravel *et al.*, 2006; Laliberté *et al.*, 2008; Kembel, 2009; Rominger *et al.*, 2009; Chase and Myers, 2011). The historical focus on abiotic and biotic filters views communities as fixed, equilibrium configurations of species. In fact, it is possible that many communities are not in equilibrium, instead undergoing processes of ecological drift. Simulated communities made up of 'ecologically equivalent' species that are not subject to deterministic abiotic or biotic filters, but only stochastic population dynamics, closely resemble some real communities (Hubbell, 2001). **It remains an open question to what extent, and under what conditions, stochastic or deterministic forces structure ecological communities.**

New statistical and phylogenetic approaches are opening the way to more incisive and nuanced perspectives on the relative importance of neutral versus niche-based processes, and are helping to disentangle biotic and abiotic components of the deterministic side of the coin. **However, these approaches have not yet been applied broadly to Arctic systems, nor have they ever been applied jointly to plant, fungal and bacterial communities, despite the interdependence of these kingdoms.**

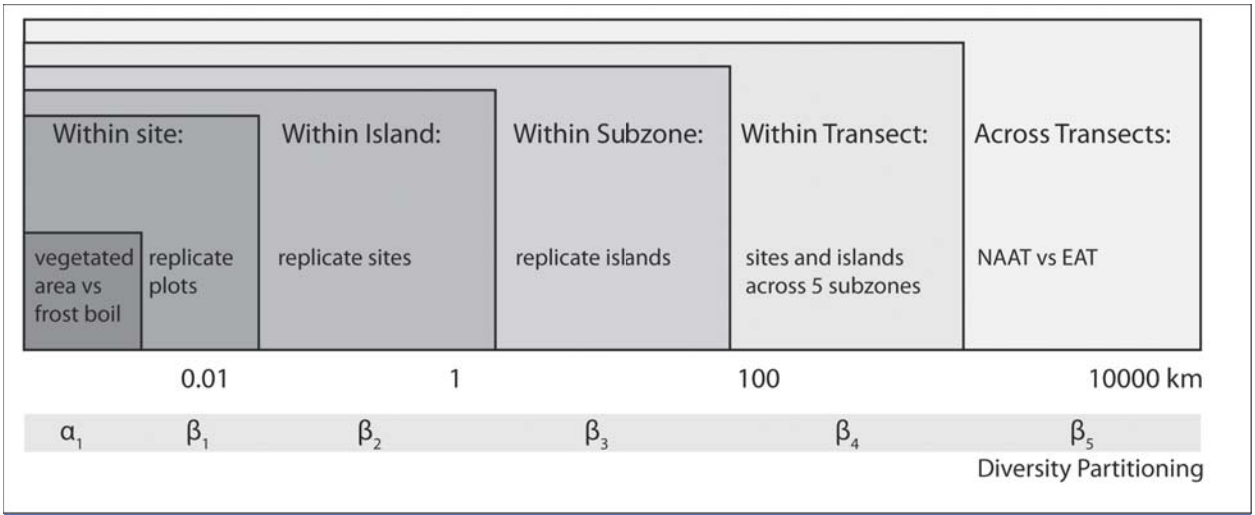

Fig. 1. The spatial grain of our study is <2m plots, with 20 cores each, on paired frost boil and vegetated areas. Multiple plots were studied at each site, multiple sites per island and subzone, and all five subzones were sampled along each transects. This hierarchical framework allows powerful elucidation of scaling.

Traditionally, *species diversity* in ecology is seen to encompass species richness (simple list of species present) and evenness (the distribution of abundances across the species present). Species diversity can then be partitioned in various ways to begin to understand the scaling and drivers of diversity. The most common partitioning is into α, ß and γ diversity (Whittaker, 1960), where α diversity refers to the diversity of species present in a single location, i.e. a single community, while γ diversity encompasses all the diversity present across communities within some larger region, e.g. a biogeographic province. ß diversity then quantifies the variation in community composition among locations within the region, and is often described as species turnover. Recent theory predicts that ß diversity will increase with geographic area simply due to the increase in size of the species pool, but the relationship between ß and γ will change more quickly along ecological gradients if niche-based filters are operating (Crist *et al.*, 2003). Hence, simultaneous comparisons across geographical distance and ecological gradients can be particularly informative. Importantly, null models for species turnover purely due to stochastic causes have been developed, so that **it is now possible to test for deterministic forces by comparing observed ß diversity in natural communities to that expected under the null model** (Kraft *et al.*, 2011). These methods also allow for the informative additive partitioning of diversity across scales of aggregation when sampling has been conducted in a hierarchical, nested design, as in our Arctic soil sampling. In order to achieve a reliable estimate of ß diversity, it is essential to obtain a thorough accounting of the species present at each study site. This has been difficult for microbes due to their hyperdiversity, but high-throughput sequencing methods have made saturation of species observations now possible.

If species composition does not fit a neutral model, the next step is to begin to examine potential abiotic and biotic drivers (Fig 2). One way this is done is to “ordinate” sites according to their community composition, then examine correlations between ordination axes and variation in environmental factors (McCune *et al.*, 2002). This approach provides insight into how entire suites of species respond synchronously to abiotic and biotic drivers. It is also possible to statistically compare the distributions of selected species to variation in environmental variables; for example, indicator species analysis can highlight species whose distribution is most strongly associated with variation along an abiotic gradient such as temperature (Hill *et al.*, 1975). **An approach for which improved null models have recently become available is to compare observed pairwise co-occurrence between species to that expected at random** (Gotelli, 2000; Ovaskainen *et al.*, 2010; Pollock *et al.*, 2014)**.** These approaches are complementary to community ordination methods because they focus on patterns for single species, but only those whose distribution seems to be strongly related to biotic drivers, i.e. other species. Some of these models simultaneously explore co-occurrence and abiotic gradients (Ovaskainen *et al.*, 2010; Pollock *et al.*, 2014). In general, when species of the same guild co-occur less often than expected at random it suggests competition, whereas when species co-occur more often than expected it suggests

either facilitation (including mutualism) or parasitism (Diamond, 1975; Stone and Roberts, 1990; Koide *et al.*, 2004). Causal mechanisms underlying patterns that are diagnosed using these methods can then be investigated through manipulative experiments.

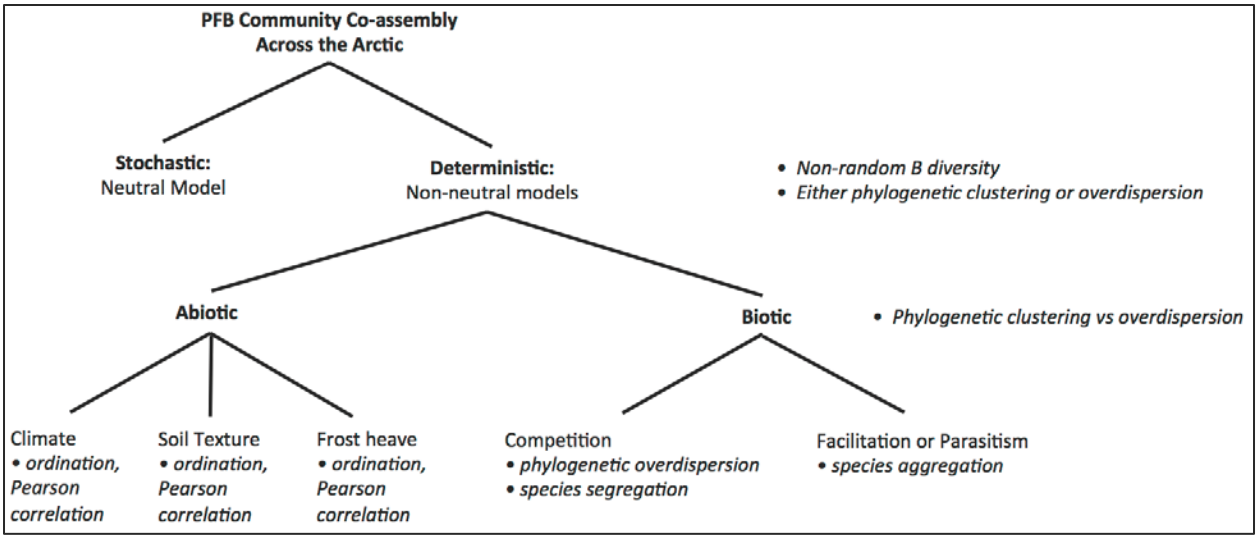

Fig 2. Flow chart for distinguishing and testing the processes that drive co-assembly.

Lastly, recently developed phylogenetic-community approaches provide yet another lens through which to evaluate patterns of community composition. It has been suggested that abiotic filters should produce communities of species that share traits that make them successful under these conditions. In contrast, competition should produce communities of species that differ in traits related to the resource over which they compete. On average, more closely related species are more similar in trait values. Thus, even when the traits of interest are unknown or cannot be measured in the focal communities, phylogenetic relatedness and branch lengths can be used as proxies for trait similarity. The early formulations of community phylogenetic theory thus posited that if local communities showed greater clustering of species at the tips than phylogenetic trees generated from random picks from the regional species pool, it suggested the predominance of abiotic filters (Webb *et al.*, 2002; Lozupone and Knight, 2005). Conversely, a pattern where the phylogenetic tree for a local community had fewer clusters than expected by chance (called "overdispersion') suggested a predominance of competition. For several reasons, it is now recognized that these interpretations must be treated with caution (Mayfield and Levine, 2010; HilleRisLambers *et al.*, 2012). For example, abiotic and biotic influences are not independent: a change in the abiotic environment, particularly an increase or decrease in resource availability, might cause a suite of related species to become better or worse competitors (Mayfield and Levine, 2010). Thus, under some scenarios, competition can actually result in phylogenetic clustering. **However, these approaches can still provide considerable insight for two reasons: 1) they provide a strong signal of non-neutral influences on community assembly, and 2) if paired with other measurements, such as species variation along ecological gradients or patterns of species co-occurrence, the interpretations regarding trait-environment relationships become more robust.**

Understanding drivers of biodiversity in the Arctic is of critical importance due to feedbacks between Arctic vegetation, microbes, soils and climate (Chapin *et al.*, 2000; Shaver *et al.*, 2001; Sturm *et al.*, 2001, 2005; Mack *et al.*, 2004, 2011; Schimel *et al.*, 2004; Hinzman *et al.*, 2005; C.-L. Ping *et al.*, 2008; Schuur *et al.*, 2008, 2009; McGuire *et al.*, 2009). However, the Arctic is also a compelling biome in which to test and refine theoretical ecological understandings of the processes of community assembly. The strong gradients in abiotic stresses (e.g. cold, lack of light and nutrients) over both narrow and wide geographic scales set the stage for insight into abiotic filters. Perhaps even more unique is the emerging pattern of rapid, wide dispersal over immense geographic scales by both large and small organisms. We discuss evidence for this below. However, one major implication is that dispersal limitation is rare or non-existent in the Arctic. This means that stochastic influences on community assembly should be weak, making deterministic drivers easier to detect. **Together, these features set the Arctic apart from other biomes, making it a valuable system in which to dissect the rules governing PFB co-assembly.**

Species composition of plant communities shifts predictably along the Arctic climate gradient. In general, vascular-plant species richness declines with latitude (Walker *et al.*, 2005). While some physical barriers such as the Atlantic Ocean, the Greenlandic Ice sheet and the Ural mountains may limit geneflow in vascular plants, **their overall dispersal seems to be high in the Arctic** (I. G. Alsos *et al.*, 2007; Eidesen *et al.*, 2013). However, latitude is not the only strong predictor of plant community composition. Across the Arctic, soils differ strongly in age (especially glaciated versus unglaciated regions), texture, pH and chemical composition (Box 1). Arctic vegetation is strongly correlated with these edaphic factors, as well as microclimate (Kade *et al.*, 2005; Vonlanthen *et al.*, 2008; Walker *et al.*, 2011).

While vegetation patterns across the Arctic are now relatively well known, the biodiversity of fungi and bacteria in the Arctic is less well known, although results to date suggest patterns that are in some ways concordant and in some ways discordant with those known for plants. Better-drained areas of low Arctic tundra tend to be dominated by dwarf shrubs that are either ectomycorrhizal (*Betula, Salix*) or ericoid mycorrhizal (*Vaccinium, Ledum*) while poorly-drained tussock tundra sites tend to be dominated by non-mycorrhizal sedges. Not surprisingly, the ecto/ericoid communities support a larger proportion of Basidiomycota than do other sites (Wallenstein *et al.*, 2007). Studies at the local/plot scale have demonstrated differences in soil fungal communities as a function of soil pH and parent material (Fujimura *et al.*, 2007) and responses to both warming and fertilization (Clemmensen and Michelsen, 2006; Clemmensen *et al.*, 2006; Deslippe and Simard, 2011; Deslippe *et al.*, 2011). Using direct PCR-Sanger sequencing of ectomycorrhizal root tips as well as Sanger-sequencing of massive clone libraries from soil amplicon pools collected from all five subzones along the North American Arctic Transect, (Fig. 3a), we have shown that **fungal community composition is strongly correlated with plant community composition** (Timling *et al.*, 2012, 2014). Furthermore, we showed that soil fungal communities differ between frost boils (Box 1) and adjacent vegetated areas and that the composition of communities in both frost boils and vegetated areas is correlated with a range of environmental factors including climate, moisture, and pH.

Box 1. Soils and patterned ground features in the Arctic. Soils in the Arctic are underlain by permafrost and the upper layers stay frozen for 8-9 months of the year. Arctic soils are also shaped by cryogenic processes such as repeated freeze-thaw cycles, cryoturbation, frost heaving, thermal cracking, and the formation of needle ice and ice lenses. These processes result in the mechanical movement of soil and the creation of patterned ground, including nonsorted circles ("frost boils" Fig. 3b) and small nonsorted polygons (Walker et al., 2008a; Washburn, 1980). Frost boils are generally composed of a central area that is highly disturbed by frost heave and covered variously with bare ground and biological soil crusts (Walker et al., 2008a). Frost heave, which is an upward movement of the soil caused by freezing and expanding water beneath the surface is most pronounced in the mid-Arctic, where it can reach over 20 cm (Daanen et al., 2008; Romanovsky et al., 2008). Areas between frost boils are more continuously vegetated and experience much less frost heave (Walker et al., 2008a). These features show considerable variation in soil moisture, vegetation structure and microclimate at the sub-meter scale (Kade et al., 2005; Ping et al., 2008; Raynolds et al., 2008; Vonlanthen et al., 2008; Walker et al., 2011). Soil pH values in the upper horizons can vary between 4 and 9 (Goryachkin et al., 2004), which greatly affects plant communities and nutrient availability (Walker et al., 2005). Nutrient contents (N, P, K) are generally low, while carbon contents in the active layer and permafrost are high but variable (Tarnocai, 2009). Generally, soil organic carbon and nitrogen contents decrease from the Low to the High Arctic (Michaelson et al., 2008). Through the process of cryoturbation, which moves soil organic carbon deeper into the soil towards the permafrost table, patterned-ground features are considered major contributors to carbon storage in the Arctic.

In contrast to plants, we have been unable to detect a decline in fungal species richness with latitude (Timling *et al.*, 2012, 2014). This was also reported along a shorter latitudinal gradient in Svalbard (Bjorbaekmo *et al.*, 2010). Also parallel to plants, studies of fungal communities and of genetic variation *within fungal species* so far indicate little or no barriers to rapid dispersal across the Arctic. For example, our work on the isolated Arctic island archipelago of Svalbard showed that the vast majority of fungal taxa present there are also found elsewhere, despite the large oceanic distances over which dispersal occurred (Geml *et al.*, 2011). In the same study, we demonstrated genetic continuity between populations of an ectomycorrhizal Basidiomycete on Svalbard and locations in Europe and North America. Our studies of a widespread lichen also showed no genetic breaks across the Arctic (Geml *et al.*, 2012).

Fungal Operational Taxonomic Units (OTUs) that were dominant in the coldest environment of subzone A were found widely in warmer subzones and even beyond the Arctic (Timling *et al.*, 2014). Thus, these few studies **suggest a complete absence of dispersal limitation**, which contrasts sharply with patterns for some fungal taxa in lower latitudes (Wu and Mueller, 1997; Geml *et al.*, 2008; Lumbsch *et al.*, 2008; Moncalvo and Buchanan, 2008).

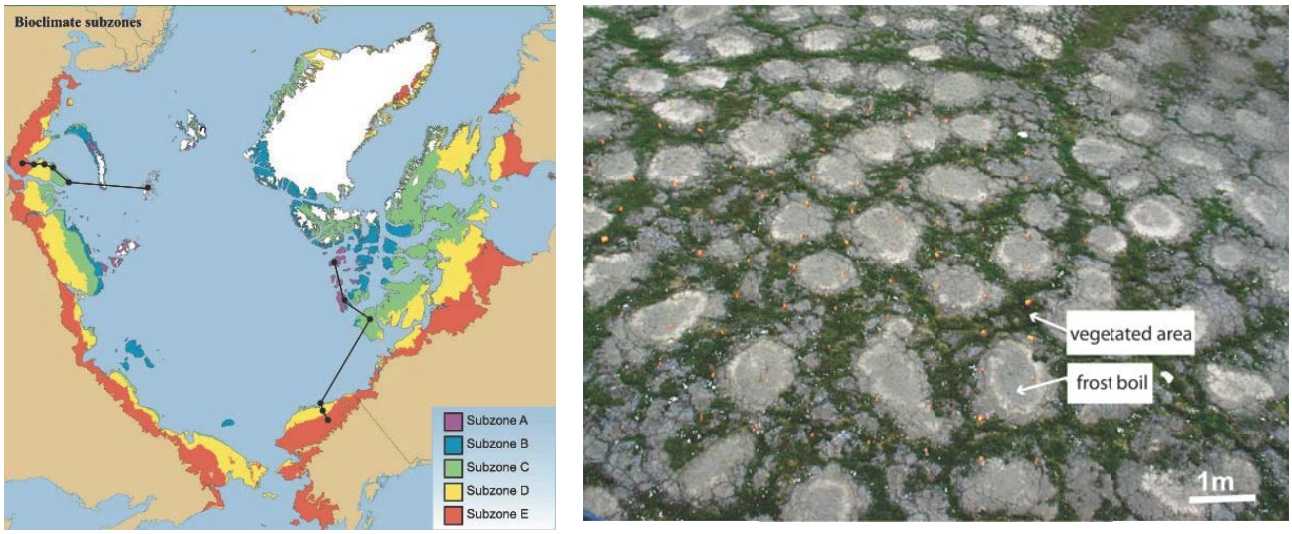

Fig 3. (**A**) Sampling sites along the North American Arctic Transect and the Eurasian Arctic Transect covering each of the five bioclimatic subzones from the low to the high Arctic (CAVM Team et al., 2003). (**B**) Patterned-ground features ("frost boils") and areas between patterned-ground features ("vegetated areas") in subzone C of the North American Arctic Transect.

To date, studies of bacterial diversity in the Arctic are also relatively few. Soil bacterial diversity appears to be as high in Arctic tundra as it is in lower latitudes (Zhou *et al.*, 1997; Neufeld and Mohn, 2005; Chu *et al.*, 2011), and this diversity does not decline with latitude (Fierer and Jackson, 2006; Lauber *et al.*, 2008; Chu *et al.*, 2010). Similar to both plants and fungi, bacterial community composition is strongly associated with gradients in soil pH (Lauber *et al.*, 2008), as has also been reported on a global scale (Fierer *et al.*, 2012), though organic matter, nitrogen, and water content are also related (Bell *et al.*, 2013). We are not aware of prior studies that have simultaneously investigated bacterial, fungal, and plant biodiversity across spatial or ecological gradients in the Arctic. **Understanding the strength of linkages among bacteria, fungi, and plants versus bacteria and abiotic environmental conditions will be critical to predicting ecosystem responses.**

Why is it important and novel to consider plant, fungal and bacterial communities in soil simultaneously? At least 50% of soil fungi are symbiotic root associates in boreal and Arctic systems (Taylor *et al.*, 2014; Timling *et al.*, 2014). Saprotrophic fungi, though perhaps less host-specific than mycorrhizal fungi, also differ among litter from different hosts (Paulus *et al.*, 2006; Hyde *et al.*, 2007). Thus we expect that vegetation exerts a very strong influence on soil fungal communities. In turn, mycorrhizal fungi have the potential to alter productivity, diversity and species composition of plant communities (van der Heijden *et al.*, 1998; van der Heijden *et al.*, 1998). For too long, ecologists have considered plants as controlling microbial communities, without considering influences in the opposite direction (Reynolds *et al.*, 2003). Communities of bacteria in the rhizosphere are distinct from those in bulk soil (Uroz *et al.*, 2010). Moreover, rhizosphere bacteria mobilize and immobilize nutrients, degrade pollutants, release phytoactive compounds, sequester metals and drive soil biogeochemical cycles (Berg and Smalla, 2009). Fungal hyphae in soil are festooned with bacteria, leading to the concept of the hyphosphere (Andrade *et al.*, 1997). Bacteria can also dramatically influence plant-fungal interactions. For example, some mycorrhizal associations are not established unless very specific "mycorrhizal helper bacteria" are present (Frey-Klett *et al.*, 2007). **Thus it is critical to characterize *joint* communities.**

RESEARCH DESIGN

Overview Using our extensive sampling and massive next-generation-sequencing-derived data on community composition of soil fungi and bacteria that are already in hand, combined with data on plant communities at the same sites (which will be augmented with limited sequencing of plant phylogenetic

markers), we aim to examine the fundamental patterns of community assembly in each of the three Kingdoms at hierarchical spatial and ecological scales. Building on current knowledge of Arctic ecosystems together with ecological theory, we pose the following hypotheses (see Figs. 1 & 2) to determine the roles of stochastic versus deterministic forces, abiotic controls and biological interactions in community assembly of PFB communities:

H1. Deterministic processes dominate Arctic community assembly due to an absence of dispersal limitation.

H2. Climate will exert an increasing control over community assembly from the warmest to the coldest subzone.

H3. Pairwise species interactions become weaker moving from warmer subzone E to coldest subzone A due to increasing strength of abiotic filters.

H4. A complex of pH and soil texture will exert a stronger control over community composition than temperature.

H5. In frost boils, extent of frost-heave will exert a stronger control than temperature.

H6. Correlations between plant and fungal communities are stronger than those between plants and bacteria due to the greater physiological intimacy of some plant-fungus interactions.

Box 2: Vegetation across the Arctic and bioclimatic subzones. Vegetation of the Arctic is highly variable across all spatial scales from continental to sub-meter. Along a south-north latitudinal gradient vegetation ranges from shrublands in the south bordering the boreal forest, to sparsely vegetated barrens at its most northern extent (Walker *et al.,* 2005). This variation correlates most strongly with temperature. In contrast, along the east-west longitudinal gradient, vegetation is less variable. It is mainly shaped by past conditions, such as glaciations (Raynolds *et al.,* 2009), land bridges and north-south trending mountain ranges. The Arctic is divided into five bioclimatic subzones (A through E), which are defined by summer air temperature and the dominant plant growth forms (Fig. 3). Subzone A is the coldest, with a Mean July Temperature (MJT) of 0–3ºC. The dominant plant growth forms are cushion forbs, mosses and lichens. Subzone B has a MJT of 3–5º C and is characterized by prostrate dwarf shrubs. Subzone C has a MJT of 5–7º C, and is dominated by hemi-prostrate dwarf shrubs, sedges and mosses. Subzone C represents the subzone with the largest extent in the circumpolar Arctic tundra. Subzone D has a MJT of 7–9º C, with erect dwarf shrubs, tussock sedges and mosses. Subzone E is the most southern subzone and has a MJT of 9–12º C, with low shrubs, tussock sedges and mosses. Vascular plant diversity and plant cover increases from subzone A to E (Walker et al., 2005).

We propose a series of analyses that consider both the species level composition and deeper phylogenetic composition of PFB communities in each of our samples. We start with the most basic dichotomy: are PFB communities assembled in a stochastic (neutral) or a deterministic (non-neutral, niche-based) manner? There is already strong evidence that plant and fungal community composition is correlated with environmental parameters across the Arctic (Kade *et al.*, 2005; Wallenstein *et al.*, 2007; Vonlanthen *et al.*, 2008; Bjorbaekmo *et al.*, 2010; Blaalid *et al.*, 2014; Timling *et al.*, 2014). However, the spatial scales at which neutral versus deterministic processes may dominate are not known, particularly for fungi and bacteria, but can be elucidated using our hierarchical sampling framework and proposed analyses. From this initial dichotomy, we proceed to evaluate the relative roles of various abiotic and biotic factors in structuring PFB communities. Importantly, we ask to what degree do PFB communities respond in sync or discordantly to these drivers? By including un-vegetated patterned ground features in our sampling scheme, we will be able to disentangle influences of plants from abiotic influences on fungi and bacteria. As detailed below, these analyses utilize robust statistical approaches appropriate to our data. To help guide the use of these methods, collaborator Andrew Taylor will oversee these analyses (see appended Letter of Collaboration). Andrew Taylor is a highly qualified biostatistician – he has taught graduate biostatistics for over 25 years and served as the statistics subject editor for the Ecological Society of America family of journals. We will evaluate whether community-wide patterns agree with our theory-based predictions. **We acknowledge that definitive tests of the influences of biotic factors, such as competition, or abiotic factors, such as temperature, on a particular species would require manipulative experiments. However, patterns must be documented before meaningful experiments can be designed; we envision that our work will provide novel insights and set the stage for experimental studies of selected PFB interactions.**

Sampling Approach

Transects along environmental gradients are powerful tools in the study of biodiversity and drivers of community assembly. Furthermore, established transects enable diverse research teams to create synergies of knowledge and data. Prior and current studies along the North American Arctic Transect and the Eurasian Arctic Transect (Fig. 3a) are revealing much about the structure and function of arctic ecosystems (see starred references). We sampled soils of zonal sites along these two established transects that traverse the bioclimatic gradients in North America and Eurasia. While both transects have similar overall summer temperature regimes and vegetation physiognomy, they differ in climates, geological histories, surficial deposits and disturbance regimes (Walker *et al.*, 2012).

North American Arctic Transect The North American Arctic Transect spans 1700km from Northern Alaska into the Canadian Arctic Archipelago. (Fig. 3a) and is characterized by a continental climate, which is caused by the presence of nearly year around sea ice west of the Canadian Archipelago. The sites in the Canadian Archipelago were glaciated during the last Glacial Maximum 10,000–16,000 ago and are relatively young. This contrasts with the sites in Alaska, which are estimated to be 500,000–900,000 years old, were part of the Beringian refugium and were not glaciated during the Pleistocene. Nevertheless the sites are relatively young (10,000 years old) stabilized floodplains of the Sagavanirktok River and were subject to varying amounts of windblown loess (Raynolds and Walker, 2009). North American Transect soils are dominated by loams which are non-acidic in subzone A-D and acidic in the southernmost subzone E. All sites are characterized by patterned-ground features such as nonsorted circles and small nonsorted polygons (Box 1). A warming climate is reducing the extent of sea ice, leading to a more maritime climate in the Canadian part of the North American Transect (Bhatt *et al.*, 2014).

Eurasian Arctic Transect The Eurasian Arctic Transect extends 1500km across the Yamal Peninsula to the High Russian Arctic at Franz Jozsef Land (Fig. 3a) and has a maritime climate with much higher precipitation, especially in winter. Only the site at Franz Jozsef Land was glaciated, while most of the Yamal Peninsula has been subjected to marine incursions during the Pleistocene era. The soils are dominated by acidic sands and loams with lower pH in sands than in loam (Walker *et al.*, 2012). On the Yamal peninsula, which covers subzones C-E, patterned ground features are less abundant than along the North American Transect.

Distinctions of pH, climate, soil texture, relative abundance of patterned ground features, and grazing disturbance within and between transects are correlated with different relative abundances in major plant functional types, such as deciduous and evergreen shrubs, forbs, graminoids, mosses, and lichens (Walker *et al.*, 2012). Plant community composition and structure is most similar between neighboring subzones within each transect. However plant communities and structures between the North American and Eurasian Arctic Transects are most similar at the extreme ends of the transects in subzones A and E. These similarities are attributed to a similar pH and groups of circumpolar plant species. In contrast plant communities are most divergent in the central parts of the transects, which coincides with a well-established non-acidic flora along the North American Transect and an acidic flora along the Eurasian Transect. Overall, the aboveground vascular-plant biomass is lower along the Eurasian than the North American Transect. This is likely due to the influence of reindeer grazing which has a major influence on the structure of the vegetation (Walker *et al.*, 2012).

To account for the high diversity and spatial heterogeneity of soil fungal and bacterial communities, we collected 20 randomly located soil cores (1.8 cm diameter, 10 cm depth) from each of five selected frost boils and the five adjacent vegetated areas at each of the 180 sites. The sampling was ≤15 m from the 10 x 10 m grids where environmental data were collected and the vegetation, active layer and snow depth were mapped in detail (Raynolds *et al.*, 2008). The 20 soil cores from each feature were immediately pooled for each feature and stored in liquid nitrogen. A total of 180 pooled soil samples (3600 cores) were collected along both transects. One sample was lost during transport, giving us a final soil sample size of 179. Prior to DNA extraction, visible roots were removed with forceps, all soils were lyophilized and then ball-milled.

Environmental Data

Arising from a close collaboration with two projects along the transects ('Biocomplexity of patterned ground' OPP-0120736; 'Yamal- land cover land use change' NNX14AD90G), we obtained an unprecedented amount of detailed geographical (latitude, longitude, elevation, topographic position, exposure, stability, microsite, disturbance degree) and environmental data, including description of plant communities and their Normalized Difference Vegetation Index and Leaf Area Index, biomass of plant

functional groups (deciduous and evergreen shrubs, graminoids, forbs, mosses, lichen, cryptogamic crusts) and soil (temperature, bulk density, pH, total C and N, available K^+, Ca^{2+}, Na^+, Mg^{2+},Cation Exchange Capacity, % sand, silt and clay, soil moisture, thickness of active layer, A-horizon and soil organic horizon) and climate (summer-, winter- and total precipitation, snow-cover duration and Summer Warmth Index (SWI: the sum of the monthly means above 0 °C), Thawing Degree Days (TDD: the sum of mean daily temperatures >0 °C over a year) and Freezing Degree Days (FDD: the sum of mean daily temperatures <0 °C over a year) of the soil and air (Walker *et al.*, 2012). These detailed environmental metadata will permit powerful multivariate statistical analyses of correlations between PFB assemblages and environmental factors at local, regional and continental scales.

Acquisition of species-level and phylogenetic community composition snapshots

Plants. Data on cover classes of vascular plants, bryophytes and lichens were collected at every location sampled in our study. Thus plant diversity for our samples is already known in detail. However, to carry out phylogenetic community comparisons, we will need to generate plant sequence data for the species that are not represented in public databases (about 300 plant species). The most promising new approach is genome skimming by shotgun sequencing (Besnard *et al.*, 2014; Malé *et al.*, 2014). It will allow the assembly of total chloroplast DNA including the standard barcoding regions matK and rbcL, trnL along with the nuclear ribosomal cluster, and a large fraction of the mitochondrial genome. Currently, 7000 specimens of 4500 taxa from the Alps have been shotgun sequenced through the PhyloAlp project. About 2000 additional Scandinavian and Arctic species will be shotgun sequenced in the near future. Thus, high quality data of a large part of the species found along our transects will be sequenced by other projects. Hence, here we request support only for complete chloroplast sequencing for an additional 50 species that are dominants along our transects but not included in the ongoing project; these data will make an important contribution to the international efforts. DNA will be extracted at University of Fairbanks and sent to Genoscope, Paris for sequencing according to the PhyloAlp protocol. The trnL region of the chloroplast DNA will be sequenced for the remaining 250 less abundant plant species. Data will be made freely available in the international DNA barcode database and linked to other web-resources for species information (GBIF, Encyclopedia of Life, GenBank, etc).

Fungi. We have already carried out both Sanger clone-library sequencing and Illumina next-generation-sequencing of fungal ITS amplicons for all samples included in this study. The rapidly evolving ITS region has been deemed the best available barcode for species discrimination in fungi (Schoch *et al.*, 2012), although numerous limitations are well known (Bruns, 2001). Perhaps foremost is the rapid accumulation of indels that make alignment and phylogenetic reconstruction above the genus/family level impossible in fungi. We have already completed extremely deep sequencing of the 179 pooled soil samples for the fungal ITS2 region using Illumina sequencing on the MiSeq platform. We obtained from 100,000 to 1,100,000 sequences per sample, for a combined total of 250 million sequences, allowing us to thoroughly account for the species present in each sample. This extremely deep sequencing will allow us to describe species co-occurrence patterns within and among guilds in unprecedented detail. For a subset of the samples, we have also carried out high throughput Sanger sequencing of longer amplicons that encompass part of the nuclear large subunit ribosomal gene (nLSU). These data will support phylogenetic community analyses because the nLSU is alignable across the Fungi. However, we propose a novel, cutting-edge approach to derive more robust phylogenetic comparisons across samples: low-coverage, long-amplicon sequencing utilizing the 3rd generation Pacific Biosciences platform. The major advantage of the Illumina platform is extremely large numbers of sequences generated at very low cost. However, the sequences generated are short (currently a maximum of 350bp) and of reduced quality (i.e. confidence in individual base calls) relative to gold-standard Sanger sequencing. The single-molecule SMRT sequencing approach pioneered by Pacific Biosciences has much lower throughput than Illumina, but is capable of generating extremely long individual sequence reads (upwards of 20,000bp) and by repeatedly sequencing the same individual template molecule, obtaining extremely low aggregate base call error rates. We will utilize this new methodology to generate highly phylogenetically informative long fungal amplicon sequences. In brief, we will amplify a ~3000bp region that includes nearly full length nuclear small subunit (nSSU), ITS and partial nLSU using the proofreading, highly processive Phusion polymerase. Our 179 samples will be amplified individually, then pooled to create a single PacBio library that will then be sequenced on 10 SMRT cells to generate roughly 200,000 high quality sequences. This sequencing depth is likely insufficient to saturate fungal species richness within all 179 samples. However, by combining separate PCRs, we will create a highly diverse amplicon pool (which lowers

repetitive sequencing of the same template). Because this amplicon spans the regions for which we have already generated Sanger and Illumina sequences, we will be able to match them with the long PacBio amplicons. We expect to recover all dominant fungal OTUs through our pooling and sequencing strategy. However, if dominant OTUs are missed, we can obtain the missing sequences by combining specific primers designed from their ITS sequences with universal fungal SSU and LSU primers.

Bacteria. We successfully applied for sequencing services from the DOE Earth Microbiome Project (EMP) at the Argonne National Laboratory. With this support, all of the soil samples in this study underwent Illumina sequencing of bacterial 16S amplicons following the standard EMP protocol (http://www.earthmicrobiome.org/emp-standard-protocols/). In brief, for each sample the V4 region of the 16S rRNA gene was amplified in triplicate using primers 515F/806R (Caporaso *et al.*, 2010) and sequenced on the Illumina MiSeq platform.

Sequence Processing

Raw sequence data will be subject to rigorous quality control (removal of low quality sequences, trimming of sequence ends, detection and removal of chimeras, etc) using methods that we (Taylor *et al.*, 2008; Taylor and Houston, 2011) and others have developed and following established best practices (Lindahl *et al.*, 2013; Nguyen *et al.*, 2014) within the QIIME pipeline (Caporaso *et al.*, 2010). Bacterial and fungal sequences will be grouped into OTUs (roughly equivalent to species) at 97% sequence similarity using UCLUST (Edgar, 2010). For fungi, we will first cluster the PacBio and clone sequences, since they are longer and higher quality, to create a set of reference OTUs for our sites. We will then use open-reference OTU-picking against this high quality reference set for the Illumina data. This approach ensures that the longest, highest quality sequences are used to define the majority of clusters. Bacterial OTUs will be aligned using the PyNAST aligner (Caporaso et al., 2010) and Greengenes database (DeSantis *et al.*, 2006) and taxonomic assignments will be made by the Ribosomal Database Classifier program (Wang *et al.*, 2007). Fungal OTUs will be identified using BLAST searches against the comprehensive UNITE database and assignment to UNITE 'species hypotheses' (Kõljalg *et al.*, 2013). Fungal OTUs that do not match an existing UNITE species hypothesis will be examined using a combination of methods, including BLAST searches of GenBank, placement using the newly developed RDP classifier for fungi (Porras-Alfaro *et al.*, 2013), and construction of phylogenetic trees (see Timling et al. 2014). Assembly of plant chloroplast sequences will be carried out in collaboration with Inger Greve Alsos, Tromsø Museum, and Eric Coissac at Grenoble using the pipeline developed by PhyloAlp (Coissac *et al.*, 2015).

TESTS OF HYPOTHESES AND RATIONALE

Relative roles of stochastic versus deterministic forces

H1. *Deterministic processes dominate Arctic community assembly due to an absence of dispersal limitation.*

While a variety of processes can lead to random sets of species at the local scale, stochastic dispersal (i.e. failure of a taxon to disperse to some locations simply by chance) is arguably the most important, and is fundamental to most null models of community assembly (Hubbell, 2001). As discussed above, all available evidence suggests that plants, fungi and bacteria disperse rapidly over wide distances in the Arctic. While there are many possible mechanisms, the strong winds that transport materials throughout the Arctic over the winter snowpack, likely contribute to the high dispersal. Thus, we do not expect dispersal limitation to play a major role at local to regional scales, making this an ideal biome in which to elucidate other mechanisms of community assembly. We will test this prediction using our thorough censuses of species/OTU occurrence within each of our 179 samples. We will treat the entire constellation of PFB taxa across all plots utilized for a particular analysis as the regional species pool (representing γ diversity). We will test whether ß-diversity in plants, fungi and bacteria differs from a null model that simulates stochastic assembly by randomly permuting species among samples, while maintaining observed numbers of individuals and species richness (null model 1) using the PARTITION package and methods of Crist et al. (2003). If observed ß diversity is higher than predicted by the null model, it suggests that deterministic processes are causing larger turnover from sample to sample than would be expected by stochastic processes alone. We will additively partition diversity at each of the scales in our sampling (Fig 1), comparing observed turnover to null model expectations.

We will also utilize phylogenetic community analysis methods as an independent approach to asking whether deterministic processes play a significant role in structuring PFB communities. Our goal is not to improve understandings of phylogenetic relationships within plants, fungi or bacteria. Rather, to carry out the phylogenetic community analyses described below requires robust input trees comprised of the taxa

uncovered in our sampling. For Fungi, the representative SSU-ITS-LSU sequences from merged Illumina, Sanger and PacBio data will be used for phylogenetic community analyses as follows. We will combine our sequences with alignments from the All Fungi Tree of Life (AFTOL) project using MAFFT (Katoh *et al.*, 2002). We will then infer phylogenies by maximum likelihood in RAxML (Stamatakis *et al.*, 2005) using a constraint tree derived from the robust multigene phylogenies produced by AFTOL (James *et al.*, 2006). A similar approach will be used for our bacterial 16S V4 Illumina sequences, where we will attempt to match them with full-length sequences from the GreenGenes (DeSantis *et al.*, 2006) and/or Silva (Pruesse *et al.*, 2007) databases, followed by tree inference using gold-standard alignments. For plants, we will again take advantage of previously published, robust multilocus trees (Jansen *et al.*, 2007; Soltis *et al.*, 2011; Ruhfel *et al.*, 2014) to use as constraints, since our goal is simply to generate trees that provide branch lengths separating taxa within each soil sample, rather than to revise current best estimates of deep plant, fungal and bacterial phylogenies.

Using these maximum-likelihood trees as input, we will then calculate Phylogenetic Distance (PD; (Faith, 1992), Unifrac Distance (UD) (Lozupone and Knight, 2005; Lozupone *et al.*, 2006, 2011) and Mean Nearest Taxon Distance (MNPD; (Webb *et al.*, 2008) separately for plants, fungi and bacteria in each plot using the R package Picante (Kembel *et al.*, 2010). Randomization tests utilizing the regional species pool will then be used to ask whether the three phylogenetic distance indices are significantly clustered or overdispersed relative to null expectations. We will repeat these tests at various scales of sample aggregation to test the expectation that stochastic processes are important at the smallest spatial scale while deterministic forces are more important at regional scales. As with the diversity partitioning methods above, we will also examine phylogenetic α and ß diversity across scales using Picante tools. One outcome of these analyses could be that data point to stochastic assembly at every spatial and taxonomic scale. We view this as highly unlikely, however, given that we have previously demonstrated strong correlations between plant and fungal communities and edaphic factors in the Arctic.

Roles of abiotic filters in community assembly

H2. *Climate will exert an increasing control over community assembly from the warmest to the coldest subzone*

All of the Arctic is a cold-dominated biome. Nevertheless, the southern, warmest reaches of the Arctic are heavily vegetated. Considering all plants within the same floristic province as belonging to the regional species pool, it is likely that other abiotic factors (e.g. edaphic conditions) and species interactions (especially competition) determine which plant species occupy local communities at the warm end of the spectrum. From subzone C to A, vegetation is increasingly sparse, presumably reflecting the increasingly harsh abiotic conditions, particularly low aboveground temperatures and limited access to light and moisture. In contrast to air temperatures, soil temperatures do not follow a linear decline from subzone E to A. Vegetation insulates the soil, leading to lower summer and warmer winter soil temperatures in subzone E relative to other subzones. Due to lack of vegetation, frost boils undergo more dramatic warming and cooling than adjacent vegetated areas.

We will test this hypothesis by measuring the strength of the Pearson correlation together with partial Mantel tests between community composition and climate (Thawing Degree Days (TDD) and Freezing Degree Days (FDD)) along the bioclimatic gradient, but separately for the two transects, and separately for plants, fungi and bacteria. To do this, we will use our species X sample matrices to create site similarity matrices for plants, fungi and bacteria. We will also create site similarity matrices for climate and the pH - soil texture complex. These matrices will be compared by Mantel tests, and Pearson correlations will be calculated after reducing the dimensionality of the community data via non-metric multidimensional scaling ordination. The majority of multivariate methods described here will be carried out in R using Vegan (Oksanen *et al.*, 2007) and other specialty packages.

H3. *Pairwise species interactions become weaker moving from warmer subzone E to coldest subzone A due to increasing strength of abiotic filters*

Productive, low-stress environments tend to select for the best competitors, while high stress environments tend to select for stress-tolerance in plants (Grime, 1977). In extreme environments such as deserts, evidence suggests that abiotic parameters control population dynamics and species composition, while individuals that survive under these conditions have little or no competitive interactions with one another. One signal of strong competitive interactions is a lower than expected co-occurrence of competing species (i.e. negative co-occurrence) *within the same guild*. We will compare observed with expected co-occurrence separately for plants, fungi and bacteria and regress these values against the

Summer Warmth Index (SWI). To test for greater or lesser species co-occurrence than expected by chance, we will calculate Stone and Roberts's C-score (Stone and Roberts, 1990; Gotelli and McCabe, 2002), which is related to the checkerboard score in that it counts numbers of times species co-occur across a matrix of sample sites, and compare scores to null expectations by permutation (Gotelli, 2000; Gotelli and Entsminger, 2001; Gotelli and Ulrich, 2012) using the program PAIRS (Ulrich, 2008). Note that the PAIRS program allows us to identify the particular species pairs that have segregated or aggregated occurrence patterns. We will use this output to parse the result in search of within guild pairs with segregated occurrence, indicating competition and between-guild pairs having aggregated occurrence, indicating mutualism or parasitism. These analyses will utilize the smallest grain of our hierarchical sampling, i.e. the plot scale, since this is the scale at which species interactions actually occur.

It has also been suggested that mutualisms and other facilitative interactions can become increasingly important in extreme environments (Stachowicz, 2001). Hence, we also predict a greater incidence of positive co-occurrence between species *among guilds* at higher latitudes.

H4*. A complex of pH and soil texture will exert a stronger control over community composition than temperature*

Both edaphic factors and climate are known to exert strong influences over plant and microbial communities. In the Arctic, climate changes gradually from subzone to subzone, while differences in soils and associated vegetation are sharper. For example, parts of the North American Arctic are underlain by acidic soils while other areas are underlain by non-acidic soils (see Box 1). The boundaries between these soil types are sharp and the plant communities differ strongly, despite an identical climate (Walker *et al.*, 1998). In this study, we sampled non-acidic soils in North America and acidic soils in Eurasia. But in addition to this pH gradient, there are strong distinctions in soil texture along the Eurasian Transect due to parent material, climate and landscape age. We will examine whether climate or the complex of pH and soil texture is a better predictor of community composition by comparing Pearson correlation coefficients and by carrying out partial Mantel tests to parse out independent influences.

H5. *In frost boils, extent of frost-heave will exert a stronger control than temperature*

The existence of bare frost boils serves to demonstrate the effects of soil movement due to frost-heave on vegetation. Nevertheless, the importance of cryoturbation to fungal and bacterial communities in soil has been little studied. Like plants, fungal genetic individuals can be large (centimeters to tens of meters) and physiologically integrated. Thus cracks and cryoturbation are likely to tear and disrupt fungal tissue, just as they do plants. We predict that these physical disruptions exert a stronger abiotic filter on fungal community composition than does temperature *per se*. An increasing number of studies have revealed remarkable capacities of various fungi to survive extreme cold temperatures (reviewed in Timling and Taylor, 2012), but severing of hyphae is likely to alter competitive dynamics and community composition. Due to their single-celled growth form, we predict a weaker influence of cryoturbation on bacteria. As above, site similarity matrices, Pearson correlations and partial Mantel tests will be used to evaluate these predictions separately for plants, fungi and bacteria.

Roles of biological interactions in community assembly

H6. *Correlations between plants and fungi are stronger than those between plants and bacteria due to the greater physiological intimacy of some plant-fungus interactions.*

Most fungal species on Earth gain their nourishment by interacting with plant tissues, living or dead (Taylor and Sinsabaugh, 2014). In soil, plant exudates and litter also provide critical resources for bacteria. Numerous studies have shown that bacterial communities in the rhizosphere differ sharply in taxonomy and function from those in bulk soil (Haichar *et al.*, 2008; Berg and Smalla, 2009; Buée *et al.*, 2009; Uroz *et al.*, 2010; Mendes *et al.*, 2014). However, interactions between groups of soil fungi – mycorrhizal fungi and pathogens – and plants are more physiologically intimate than are interactions with rhizosphere bacteria. For example, in the ectomycorrhizal symbiosis, fungal hyphae invade the root, forming a web of hyphae along the walls of several layers of cortical cells called the Hartig net. This physiological intimacy is reflected in phylogenetic conservatism of traits underlying the interaction. For example, most plant species form either ectomycorrhizae or arbuscular mycorrhizae, not both. Similarly, the clades of fungi that form these major categories of mycorrhizae are deeply divergent. Hence, we predict that the species and functional types of vascular plants present in a sample will be strongly correlated with the array of fungal taxa and guilds present in the same sample. In contrast, plant-bacterial correlations will be weaker.

Analyses Across Scales As noted above, many of these analyses will be carried out at hierarchical scales of data aggregation to explore spatial scale-dependency of observed patterns.

> *At the largest, continental scales (e.g., 1,000–10,000 km), phylogenetic clustering of members of a regional sample on a global phylogeny reflects biogeographic rather than ecological processes, as clades diversify within the sample region, and cause many taxa in the region to be, on average, more related to each other than to taxa outside the region… At the community scale (e.g., 100 m–10 km), species should segregate into habitats based on the relative strengths of habitat filtering versus competition among similar species. Finally, at the smallest, neighborhood scales (e.g., <100 m), one might observe the effect of individual-based interactions that lead to within-habitat filtering or "neighborhood exclusion." Hence, a spatially nested analysis of community phylogenetic structure may detect different patterns of phylogenetic clustering or over-dispersion at different scales, providing more information about community processes than an analysis at just a single scale.* Webb et al. 2002.

It is also likely that patterns differ among taxonomic and functional groups *within* plants, fungi and bacteria. Hence, many of the analyses described above, such as co-occurrence patterns will be analyzed separately for particular taxonomic and functional subsets of the data, as follows. Fungi: ectomycorrhizal, arbuscular mycorrhizal, ericoid mycorrhizal, dark septate endophytes, pathogens, decomposers, lichens, fungal phylum (i.e. Ascomycota, Basidiomycota, Glomeromycota, etc). Plants: ectomycorrhizal, arbuscular mycorrhizal, ericoid, non-mycorrhizal, nitrogen-fixers, grasses, forbs, shrubs, dwarf shrubs, plant family. Bacteria: rhizosphere bacteria, nitrogen-fixers, obligate aerobes, facultative anaerobes, obligate anaerobes, cellulose degraders, plant pathogens, bacterial phylum. Assignment of species/OTUs to the functional groups (some of which overlap) will be made using FUNGuild (Nguyen *et al.*, 2015), TRY (Kattge *et al.*, 2011) and the PICRUSt (Langille et al., 2013) software package for the 16S sequences.

Beyond Hypotheses We aim to take full advantage of this unprecedented dataset. Due to space constraints, we cannot list the many additional questions that we will address. For example, we will explore whether yeasts have different distribution patterns along stress gradients, such as cryoturbation, than do exclusively filamentous fungi. With these much more exhaustive datasets, we will also re-examine the question of whether there are Arctic endemic fungi or bacteria. We will examine whether certain clades or functional groups tend toward more stochastic or deterministic distributions or having stronger signals of facilitation or competition as revealed by co-occurrence patterns.

EXPECTED RESULTS AND SYNTHESIS

We envision that our hypotheses may result in various alternate results that will provide fundamental insights into community ecology. For example, if we find that stochastic processes dominate PFB community assembly, it will suggest that the trajectories of compositional change at any specific location will depend largely on dispersal and random population fluctuations. Such a finding would imply less predictability of ecosystem function with ongoing climate change because local community makeup will vary unpredictably. A finding that plant, fungal and bacterial communities are very tightly linked would suggest that adaptation of communities to climate change may be slower than predicted based on responses of individual species because sets of organisms must disperse and successfully establish together. We have recently shown that the inclusion of mycorrhizal fungi into the ALFRESCO model predicts a slower spread of forest at treeline in the Arctic after fire than prior models that do not account for this dependence (Hewitt et al., In Review). Similar models could be developed for the reorganization of Arctic plant communities. If abiotic forces are more strongly correlated with PFB composition than are biotic forces, it would suggest the potential for more rapid colonization and community change in response to climate. Strong linkages between particular microbes and foundational plant species, will enable more accurate prediction of the fates of these plants outside their current ranges, as a function of the microbial communities already present in those sites. Our results will also produce novel insights into the relative roles of stochastic and deterministic processes in community assembly and into the spatial and taxonomic scaling of these patterns. Hence, these results will make a significant contribution to the field of ecology.

PROJECT TEAM AND MANAGEMENT Dr. Ina Timling (postdoc) is a promising young scientist with considerable experience in Arctic systems, fungal molecular phylogenetics and community ecology. She collected most of the samples and performed all the benchwork that sets the stage for this proposal. Dr. Timling will take a leading role in generating the new plant sequence data, analyzing the combined

datasets and writing papers. Dr. Lee Taylor will supervise the generation of fungal PacBio long-amplicon sequences, will spearhead phylogenetic-community analyses and will provide expertise in fungal molecular identification. As PI of the grants under which the two transects were established, Dr. Donald (Skip) Walker will provide site data and expertise on Arctic systems. Timling, Taylor and Walker have collaborated effectively for several years. Dr. Inger Greve Alsos has wide experience studying Arctic vegetation and will participate in molecular phylogenetic analyses of the plant data. Dr. Cristina Takacs-Vesbach has considerable expertise in microbial ecology, particularly in Antarctic extreme environments, and will participate in analyses and interpretation related to bacterial datasets. Dr. Timling will spend the first year of the project generating plant data and initiating analyses at UAF and the second year of the project carrying out analyses at UNM. To facilitate project coordination, all team members will convene in Alaska in Year 1 and in New Mexico in Year 2, with monthly video-conferences in the intervening periods. Since the bulk of this project involves analysis of existing data, remote collaboration is eminently feasible.

BROADER IMPACTS Overall, the project will train an early career, female postdoctoral arctic researcher, provide research experiences to undergraduates from under-represented groups and engage the public in Arctic climate-change issues through a novel artistic outlet. Our results will improve models seeking to predict changes in the Arctic and feedbacks to climate, to the benefit of society. **UAF:** We will develop a new module about the biodiversity and ecology of microbes in Arctic tundra ecosystems and will integrate it into two courses: a well-established summer field course (BIO495/695 'Arctic Alaska Environmental Change: Field Excursion to the North Slope'), which hosts students from throughout the US and internationally, and a new campus course (492/692 'Arctic Ecosystems in a Changing Climate'). Co-PI Walker has taught the field course for many years; both Timling and Walker are well qualified to communicate Arctic science, including plant-microbial ecology, to this enthusiastic and diverse audience. Walker will also teach the new course at UAF. In addition, Timling will participate at the World Ice Art Championship in Fairbanks (www.icealaska.com) to carve an ice sculpture 'Arctic Microbes'. The sculpture will be accompanied by a poster with questions and answers about microbes in the Arctic appropriate for families. This annual event is visited by ~45,000 people. In addition to many anticipated journal articles, we will describe the findings of our study in the 'Science and Technology' section of the Fairbanks newspaper (www.newsminer.com). **UNM:** The University of New Mexico is a certified Hispanic Serving Institution (HIS), and the Department of Biology has over 1630 undergraduate majors of which 32% are Hispanic, 6% Native American, 5% Asian and 2% African American. Taylor and Takacs-Vesbach will recruit undergraduates from under-represented groups to receive research experience and training related to two aspects of the project: 1) preparing samples for fungal PacBio long-amplicon single molecule 3rd generation sequencing then participating in subsequent data analysis in the Taylor lab, and 2) bioinformatics processing of bacteria sequence data obtained from the EMP in the Takacs-Vesbach lab. Both Taylor and Takacs-Vesbach are currently mentoring students through three STEM programs based in the Biology Department. These three programs have all committed to assist with recruiting appropriate students (see Letters of Collaboration and web sites: IMSD, Maggie Werner-Washburne, http://biology.unm.edu/imsd/, MARC, Diane Marshall, http://biology.unm.edu/MARC/mentors.html, and PREP, Rich Cripps, http://biology.unm.edu/PREP/mentor_list.html). Typically, students involved in these programs join a lab for two years, spend more than 10 hours a week carrying out mentored research, and receive additional training in preparation for graduate school, including a GRE prep course, training in scientific communication, and attendance of the annual SACNAS conference. Via active local mentoring and training networks, such as the STEM Gateway, we will reach out to students not just from Biology, but also in the departments of Chemistry, Physics, Mathematics & Statistics, Computer Science and the School of Engineering.

RESPONSE TO PRIOR REVIEWS This proposal was submitted to ANS in October 2014 and received ratings of Very Good from 3 reviewers and one Fair; it was not sent to panel. The Fair stemmed largely from concerns that our hypotheses could not be tested using an observational approach. We have clarified that our aims are to evaluate whether predicted patterns occur and acknowledge that experiments would be required to further interrogate interactions between particular pairs of species. We also note that an observational approach like ours has been utilized in numerous high impact studies of community assembly and species interactions (e.g. **Science:** Alsos *et al.*, 2007; Kraft *et al.*, 2008, 2011; Maestre *et al.*, 2012; **Nature:** Volkov *et al.*, 2003; Neutel *et al.*, 2007; Crisp *et al.*, 2009; **PNAS:** Bascompte *et al.*, 2003; Allison and Martiny, 2008; Bryant *et al.*, 2008; Gotelli *et al.*, 2010; Burke *et al.*, 2011; Martiny *et al.*, 2011; Lessard *et al.*, 2012).

Literature Cited

Allison, S. and Martiny, J. (2008) Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. U. S. A.* 105: 11512–11519.

Alsos, I., Eidesen, P., Ehrich, D., Skrede, I., Westergaard, K., Jacobsen, G., et al. (2007) Frequent long-distance plant colonization in the changing Arctic. *Science* 316: 1606.

Andrade, G., Mihara, K., Linderman, R., and Bethlenfalvay, G. (1997) Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. *Plant Soil* 192: 71–79.

Bascompte, J., Jordano, P., Melián, C.J., and Olesen, J.M. (2003) The nested assembly of plant–animal mutualistic networks. *Proc. Natl. Acad. Sci. U. S. A.* 100: 9383.

Bell, T.H., Callender, K.L., Whyte, L.G., and Greer, C.W. (2013) Microbial competition in polar soils: a review of an understudied but potentially important control on productivity. *Biology* 2: 533–554.

Berg, G. and Smalla, K. (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol. Ecol.* 68: 1–13.

Besnard, G., Christin, P.-A., Male, P.-J.G., Lhuillier, E., Lauzeral, C., Coissac, E., and Vorontsova, M.S. (2014) From museums to genomics: old herbarium specimens shed light on a C3 to C4 transition. *J. Exp. Bot.* 65: 6711-6721.

Bhatt, U.S., Walker, D.A., Walsh, J.E., Carmack, E.C., Frey, K.E., Meier, W.N., et al. (2014) Implications of Arctic Sea Ice Decline for the Earth System. *Annu. Rev. Environ. Resour.* 39: 57–89.

Bjorbaekmo, M., Carlsen, T., Brysting, A., Vralstad, T., Hoiland, K., Ugland, K.I., et al. (2010) High diversity of root associated fungi in both alpine and arctic *Dryas octopetala*. *BMC Plant Biol.* 10: 244.

Blaalid, R., Davey, M.L., Kauserud, H., Carlsen, T., Halvorsen, R., Høiland, K., and Eidesen, P.B. (2014) Arctic root-associated fungal community composition reflects environmental filtering. *Mol. Ecol.* 23: 649–659.

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., et al. (2008) Facilitation in plant communities: the past, the present, and the future. *J. Ecol.* 96: 18–34.

Bruns, T. (2001) ITS reality. *Inoculum* 52: 2–3.

Bryant, J.A., Lamanna, C., Morlon, H., Kerkhoff, A.J., Enquist, B.J., and Green, J.L. (2008) Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *Proc. Natl. Acad. Sci.* 105: 11505–11511.

Buée, M., De Boer, W., Martin, F., van Overbeek, L., and Jurkevitch, E. (2009) The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. *Plant Soil* 321: 189–212.

***Buelow, H.N., Van Horn, D.J., Schwartz, E., and Takacs-Vesbach, C.D. Bacterial diversity and function in Taylor Valley soils, McMurdo dry valleys, Antarctica. *Appl. Environ. Microbiol.* in prep.:

Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., and Thomas, T. (2011) Bacterial community assembly based on functional genes rather than species. *Proc. Natl. Acad. Sci.* 108: 14288–14293.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7: 335–336.

Chapela, I.H., Osher, L.J., Horton, T.R., and Henn, M.R. (2001) Ectomycorrhizal fungi introduced with exotic pine plantations induce soil carbon depletion. *Soil Biol. Biochem.* 33: 1733–1740.

Chapin, F.S., McGuire, A.D., Randerson, J., Pielke, R., Baldocchi, D., Hobbie, S.E., et al. (2000) Arctic and boreal ecosystems of western North America as components of the climate system. *Glob. Change Biol.* 6: 211–223.

Chase, J.M. and Myers, J.A. (2011) Disentangling the importance of ecological niches from stochastic processes across scales. *Philos. Trans. R. Soc. B Biol. Sci.* 366: 2351–2363.

Chu, H., Fierer, N., Lauber, C.L., Caporaso, J.G., Knight, R., and Grogan, P. (2010) Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes: Bacterial biogeography in arctic soils. *Environ. Microbiol.* 12: 2998–3006.

Chu, H., Neufeld, J.D., Walker, V.K., and Grogan, P. (2011) The influence of vegetation type on the dominant soil bacteria, archaea, and fungi in a low Arctic tundra landscape. *Soil Sci. Soc. Am. J.* 75: 1756–1765.

Clemmensen, K.E. and Michelsen, A. (2006) Integrated long-term responses of an arcticalpine willow and associated ectomycorrhizal fungi to an altered environment. *Can. J. Bot.* 84: 831–843.

Clemmensen, K.E., Michelsen, A., Jonasson, S., and Shaver, G.R. (2006) Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems. *New Phytol.* 171: 391–404.

Coissac, E., Taberlet, P., Roquet, C., Boleda, M., Gielly, L., Alberti, A., et al. (2015) Towards an universal genome-based DNA barcode-The PhyloAlps project. In, Genome. Canadian Science Publishing, NRC Research Press. pp. 206–206.

Connell, J.H. (1961) The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecology* 42: 710–723.

Crisp, M.D., Arroyo, M.T.K., Cook, L.G., Gandolfo, M.A., Jordan, G.J., McGlone, M.S., et al. (2009) Phylogenetic biome conservatism on a global scale. *Nature* 458: 754–756.

Crist, T.O., Veech, J.A., Gering, J.C., and Summerville, K.S. (2003) Partitioning Species Diversity across Landscapes and Regions: A Hierarchical Analysis of α, β, and γ Diversity. *Am. Nat.* 162: 734–743.

Daanen, R.P. (2007) Active-layer hydrology in nonsorted circle ecosystems of the arctic tundra. *Vadose Zone* J. 6: 694–704.

**Daanen, R.P., Misra, D., Epstein, H., Walker, D., and Romanovsky, V. (2008) Simulating nonsorted circle development in arctic tundra ecosystems. *J. Geophys. Res.* 113.:

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., et al. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* 72: 5069–5072.

Deslippe, J.R., Hartmann, M., Mohn, W.W., and Simard, S.W. (2011) Long-term experimental manipulation of climate alters the ectomycorrhizal community of *Betula nana* in Arctic tundra. *Glob. Change Biol.* 17: 1625–1636.

Deslippe, J.R. and Simard, S.W. (2011) Below-ground carbon transfer among *Betula nana* may increase with warming in Arctic tundra. *New Phytol.* 192: 689–698.

Diamond, J.M. (1975) Assembly of species communities. *Ecol. Evol. Communities* 342: 444.

Edgar, R.C. (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26: 2460.

Eidesen, P.B., Ehrich, D., Bakkestuen, V., Alsos, I.G., Gilg, O., Taberlet, P., and Brochmann, C. (2013) Genetic roadmap of the Arctic: plant dispersal highways, traffic barriers and capitals of diversity. *New Phytol.* 200: 898–910.

Epstein, H.E., Walker, D.A., Raynolds, M.K., Jia, G.J., and Kelley, A.M. (2008) Phytomass patterns across a temperature gradient of the North American arctic tundra. *J. Geophys. Res.* 113: G3.

Faith, D.P. (1992) Conservation evaluation and phylogenetic diversity. *Biol. Conserv.* 61: 1–10.

Fierer, N. and Jackson, R.B. (2006) The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. U. S. A. 103*: 626–631.

Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., Lauber, C.L., et al. (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proc. Natl. Acad. Sci.* 109: 21390–21395.

Frey-Klett, P., Garbaye, J., and and Tarkka, M. (2007) The mycorrhiza helper bacteria revisited. *New Phytol.* 176: 22–36.

Fujimura, K.E., Egger, K.N., and Henry, G.H.. (2007) The effect of experimental warming on the root-associated fungal community of *Salix arctica*. *ISME J*. 2: 105–114.

*Geml, J., Kauff, F., Brochmann, C., Lutzoni, F., Laursen, G.A., Redhead, S.A., and Taylor, D.L. (2012) Frequent circumarctic and rare transequatorial dispersals in the lichenised agaric genus *Lichenomphalia* (Hygrophoraceae, Basidiomycota). *Fungal Biol.* 116: 388–400.

*Geml, J., Timling, I., Robinson, C.H., Lennon, N., Nusbaum, H.C., Brochmann, C., et al. (2011) An arctic community of symbiotic fungi assembled by long-distance dispersers: phylogenetic diversity of ectomycorrhizal basidiomycetes in Svalbard based on soil and sporocarp DNA. *J. Biogeogr.* 39: 74–88.

*Geml, J., Laursen, G.A., Timling, I., McFarland, J.W., Booth, M.G., Lennon, N.J., et al. (2009) Molecular phylogenetic biodiversity assessment of arctic and boreal ectomycorrhizal *Lactarius* Pers. (Russulales; Basidiomycota) in Alaska, based on soil and sporocarp DNA. *Mol. Ecol.* 18: 2213–2227.

Geml, J., Tulloss, R.E., Laursen, G.A., Sazanova, N.A., and Taylor, D.L. (2008) Evidence for strong inter- and intracontinental phylogeographic structure in *Amanita muscaria*, a wind-dispersed ectomycorrhizal basidiomycete. Mol. Phylogenet. Evol. 48: 694–701.

*Glass, D.J., Taylor, A.D., Herriott, I.C., Ruess, R.W., and Taylor, D.L. (2014) Habitat preferences, distribution, and temporal persistence of a novel fungal taxon in Alaskan boreal forest soils. *Fungal Ecol.* 12: 70-77.

Gotelli, N.J. (2000) Null model analysis of species co-occurrence patterns. *Ecology* 81: 2606–2621.

Gotelli, N. & Entsminger, G. (2004) EcoSim: Null Models Software for Ecology. Version 7. Acquired Intelligence Inc. and Kesey-Bear, Jericho, Vermont.

Gotelli, N.J., Graves, G.R., and Rahbek, C. (2010) Macroecological signals of species interactions in the Danish avifauna. *Proc. Natl. Acad. Sci.* 107: 5030–5035.

Gotelli, N.J. and McCabe, D.J. (2002) Species co-occurrence: a meta-analysis of JM Diamond's assembly rules model. *Ecology* 83: 2091–2096.

Gotelli, N.J. and Ulrich, W. (2012) Statistical challenges in null model analysis. *Oikos* 121: 171–180.

Gould, W.A., González, G., Walker, D.A., and Ping, C.-L. (2010) Commentary. Integrating Research, Education, and Traditional Knowledge in Ecology: a Case Study of Biocomplexity in Arctic Ecosystems. *Arct. Antarct. Alp. Res.* 42: 379–384.

Gravel, D., Canham, C.D., Beaudet, M., and Messier, C. (2006) Reconciling niche and neutrality: the continuum hypothesis. *Ecol. Lett.* 9: 399–409.

Grime, J. (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat.* 1169–1194.

Haichar, F. el Z., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., et al. (2008) Plant host habitat and root exudates shape soil bacterial community structure. *ISME J* 2: 1221–1230.

van der Heijden, M.G.A., Boiler, T., Wiemken, A., and Sanders, I.R. (1998) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology* 79: 2082–2091.

van der Heijden, M.G.., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., et al. (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69–72.

HilleRisLambers, J., Adler, P.B., Harpole, W.S., Levine, J.M., and Mayfield, M.M. (2012) Rethinking community assembly through the lens of coexistence theory. *Annu. Rev. Ecol. Evol. Syst.* 43: 227–248.

Hill, M., Bunce, R., and Shaw, M. (1975) Indicator species analysis, a divisive polythetic method of classification, and its application to a survey of native pinewoods in Scotland. *J. Ecol.* 597–613.

Hinzman, L.D., Bettez, N.D., Bolton, W.R., Chapin, F.S., Dyurgerov, M.B., Fastie, C.L., et al. (2005) Evidence and implications of recent climate change in northern Alaska and other arctic regions. *Clim. Change* 72: 251–298.

Hubbell, S.P. (2001) The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton.

Hutchinson, G.E. (1959) Homage to Santa Rosalia or why are there so many kinds of animals? *Am. Nat.* 145–159.

Hyde, K.D., Bussaban, B., Paulus, B., Crous, P.W., Lee, S., Mckenzie, E.H.., et al. (2007) Diversity of saprobic microfungi. *Biodivers. Conserv.* 16: 7–35.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., et al. (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* 443: 818–822.

Jansen, R.K., Cai, Z., Raubeson, L.A., Daniell, H., Leebens-Mack, J., Müller, K.F., et al. (2007) Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. *Proc. Natl. Acad. Sci.* 104: 19369–19374.

Kade, A., Bret-Harte, S., Euskirchen, E., Edgar, C., and Fulweber, R. (2012) Upscaling of CO_2 fluxes from heterogeneous tundra plant communities in Arctic Alaska. *J. Geophys. Res.* 117: G4.

**Kade, A., Romanovsky, V.E., and Walker, D.A. (2006) The n-factor of nonsorted circles along a climate gradient in Arctic Alaska. *Permafr. Periglac. Process.* 17: 279–289.

**Kade, A. and Walker, D.A. (2008) Experimental alteration of vegetation on nonsorted circles: effects on cryogenic activity and implications for climate change in the Arctic. *Arct. Antarct. Alp. Res.* 40: 96–103.

**Kade, A., Walker, D.A., and Raynolds, M.K. (2005) Plant communities and soils in cryoturbated tundra along a bioclimate gradient in the Low Arctic, Alaska. *Phytocoenologia* 35: 761–820.

Katoh, K., Misawa, K., Kuma, K., and Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 30: 3059–3066.

Kattge, J., DíAz, S., Lavorel, S., Prentice, I.C., Leadley, P., BöNisch, G., et al. (2011) TRY - a global database of plant traits. *Glob. Change Biol.* 17: 2905–2935.

**Kelley, A.M. and Epstein, H.E. (2009) Effects of nitrogen fertilization on plant communities of nonsorted circles in moist nonacidic tundra, Northern Alaska. *Arct. Antarct. Alp. Res.* 41: 119–127.

**Kelley, A.M., Epstein, H.E., Ping, C.-L., and Walker, D.A. (2012) Soil nitrogen transformations associated with small patterned-ground features along a North American arctic transect: soil N cycling in patterned-ground features. *Permafr. Periglac. Process.* 23: 196–206.

Kembel, S.W. (2009) Disentangling niche and neutral influences on community assembly: assessing the performance of community phylogenetic structure tests. *Ecol. Lett.* 12: 949–960.

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., et al. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26: 1463–1464.

Koide, R.T., Xu, B., Sharda, J., Lekberg, Y., and Ostiguy, N. (2004) Evidence of species interactions within an ectomycorrhizal fungal community. *New Phytol.* 165: 305–316.

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F., Bahram, M., et al. (2013) Towards a unified paradigm for sequence-based identification of fungi. *Mol. Ecol.* 22: 5271–5277.

Kraft, N.J.B., Comita, L.S., Chase, J.M., Sanders, N.J., Swenson, N.G., Crist, T.O., et al. (2011) Disentangling the drivers of diversity along latitudinal and elevational gradients. *Science* 333: 1755–1758.

Kraft, N.J., Valencia, R., and Ackerly, D.D. (2008) Functional traits and niche-based tree community assembly in an Amazonian forest. *Science* 322: 580–582.

Krebs, C. (1978) Ecology: the experimental analysis of distribution and abundance. N. Y. Harper Row 1978 703 P.

Laliberté, E., Paquette, A., Legendre, P., and Bouchard, A. (2008) Assessing the scale-specific importance of niches and other spatial processes on beta diversity: a case study from a temperate forest. *Oecologia* 159: 377–388.

Langille, M.G., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Thurber, R.L.V., Knight, R. & others. (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, 31, 814–821.

Lauber, C.L., Strickland, M.S., Bradford, M.A., and Fierer, N. (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol. Biochem*. 40: 2407–2415.

Leigh, M., Taylor, L., and Neufeld, J. (2010) Clone libraries of ribosomal RNA gene sequences for characterization of bacterial and fungal communities. In,* Handbook of Hydrocarbon and Lipid Microbiology. Springer, pp. 3969–3993.

Lessard, J.-P., Borregaard, M.K., Fordyce, J.A., Rahbek, C., Weiser, M.D., Dunn, R.R., and Sanders, N.J. (2012) Strong influence of regional species pools on continent-wide structuring of local communities. *Proc. R. Soc. B Biol. Sci.* 279: 266–274.

Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., et al. (2013) Fungal community analysis by high-throughput sequencing of amplified markers - a user's guide. *New Phytol.* 199: 288–299.

Lozupone, C., Hamady, M., and Knight, R. (2006) UniFrac–an online tool for comparing microbial community diversity in a phylogenetic context. *BMC Bioinformatics* 7: 371.

Lozupone, C. and Knight, R. (2005) UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* 71: 8228.

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

Lumbsch, T.H., Buchanan, P.K., May, T.W., and Mueller, G.M. (2008) Phylogeography and biogeography of fungi. *Mycol. Res.* 112: 423–424.

Mack, M.C., Bret-Harte, M.S., Hollingsworth, T.N., Jandt, R.R., Schuur, E.A.G., Shaver, G.R., and Verbyla, D.L. (2011) Carbon loss from an unprecedented Arctic tundra wildfire. *Nature* 475: 489–492.

Mack, M.C., Schuur, E.A.., Bret-Harte, M.S., Shaver, G.R., and Chapin, F.S. (2004) Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature* 431: 440–443.
Maestre, F.T., Quero, J.L., Gotelli, N.J., Escudero, A., Ochoa, V., Delgado-Baquerizo, M., et al. (2012) Plant species richness and ecosystem multifunctionality in global drylands. *Science* 335: 214–218.
Malé, P.-J.G., Bardon, L., Besnard, G., Coissac, E., Delsuc, F., Engel, J., et al. (2014) Genome skimming by shotgun sequencing helps resolve the phylogeny of a pantropical tree family. *Mol. Ecol. Resour.* 14: 966-975.
Martiny, J.B.H., Eisen, J.A., Penn, K., Allison, S.D., and Horner-Devine, M.C. (2011) Drivers of bacterial -diversity depend on spatial scale. *Proc. Natl. Acad. Sci.* 108: 7850–7854.
Martorell, C. and Freckleton, R.P. (2014) Testing the roles of competition, facilitation and stochasticity on community structure in a species-rich assemblage. *J. Ecol.* 102: 74–85.
Mayfield, M.M. and Levine, J.M. (2010) Opposing effects of competitive exclusion on the phylogenetic structure of communities: Phylogeny and coexistence. *Ecol. Lett.* 13: 1085–1093.
McCune, B., Grace, J.B., and Urban, D.L. (2002) Analysis of ecological communities. MJM Software Design Gleneden Beach, Oregon, USA.
McGuire, A.D., Anderson, L.G., Christensen, T.R., Dallimore, S., Guo, L., Hayes, D.J., et al. (2009) Sensitivity of the carbon cycle in the Arctic to climate change. *Ecol. Monogr.* 79: 523–555.
Mendes, L.W., Kuramae, E.E., Navarrete, A.A., van Veen, J.A., and Tsai, S.M. (2014) Taxonomical and functional microbial community selection in soybean rhizosphere. *ISME J* 8: 1577–1587.
**Michaelson, G.J., Ping, C.L., Epstein, H., Kimble, J.M., and Walker, D.A. (2008) Soils and frost boil ecosystems across the North American Arctic Transect. *J Geophys Res* 113: G03S11.
Moncalvo, J.-M. and Buchanan, P.K. (2008) Molecular evidence for long distance dispersal across the Southern Hemisphere in the *Ganoderma applanatum-australe* species complex (Basidiomycota). *Mycol. Res.* 112: 425–436.
Myers-Smith, I.H., Forbes, B.C., Wilmking, M., Hallinger, M., Lantz, T., Blok, D., et al. (2011) Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. *Environ. Res. Lett.* 6: 045509.
Neufeld, J. and Mohn, W. (2005) Unexpectedly high bacterial diversity in arctic tundra relative to boreal forest soils, revealed by serial analysis of ribosomal sequence tags. *Appl. Environ. Microbiol*. 71: 5710–5718.
Neutel, A.-M., Heesterbeek, J.A., van de Koppel, J., Hoenderboom, G., Vos, A., Kaldeway, C., et al. (2007) Reconciling complexity with stability in naturally assembling food webs. *Nature* 449: 599–602.
Nguyen, N.H., Smith, D., Peay, K., and Kennedy, P. (2014) Parsing ecological signal from noise in next generation amplicon sequencing. *New Phytol.* 205: 1389–1393.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., et al. (2015) FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* Online early.
Nicolsky, D.J., Romanovsky, V.E., and Tipenko, G.S. (2007) Using in-situ temperature measurements to estimate saturated soil thermal properties by solving a sequence of optimization problems. *The Cryosphere* 1: 41–58.
**Nicolsky, D.J., Romanovsky, V.E., Tipenko, G.S., and Walker, D.A. (2008) Modeling biogeophysical interactions in nonsorted circles in the Low Arctic. *J. Geophys. Res.* 113.
***Okie, J.G., Van Horn, D.J., Storch, D., Barrett, J.E., Gooseff, M.N., Kopsova, L. & Takacs-Vesbach, C.D. (2015) Niche and metabolic principles explain patterns of diversity and distribution: theory and a case study with soil bacterial communities. *Proceedings of the Royal Society of London B: Biological Sciences*, 282, 20142630.
Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., and Stevens, M.H.. (2007) Vegan: community ecology package. R package version 1.8-8. Online Httpr-Forge R-Proj. Orgprojectsvegan.
Ovaskainen, O., Hottola, J., and Siitonen, J. (2010) Modeling species co-occurrence by multivariate logistic regression generates new hypotheses on fungal interactions. *Ecology* 91: 2514–2521.
Paine, R. (1984) Ecological determinism in the competition for space. *Ecology* 65: 1339–1348.
Paulus, B.C., Kanowski, J., Gadek, P.A., and Hyde, K.D. (2006) Diversity and distribution of saprobic microfungi in leaf litter of an Australian tropical rainforest. *Mycol. Res.* 110: 1441–1454.

**Peterson, R.A. and Krantz, W.B. (2003) A mechanism for differential frost heave and its implications for patterned-ground formation. *J. Glaciol.* 49: 69–80.

**Peterson, R.A. and Krantz, W.B. (2008) Differential frost heave model for patterned ground formation: Corroboration with observations along a North American arctic transect. *J. Geophys. Res.* 113.:

**Ping, C.-L., Michaelson, G.J., Jorgenson, M.T., Kimble, J.M., Epstein, H., Romanovsky, V.E., and Walker, D.A. (2008) High stocks of soil organic carbon in the North American Arctic region. *Nat. Geosci.* 1: 615–619.

**Ping, C.L., Michaelson, G.J., Kimble, J.M., Romanovsky, V.E., Shur, Y.L., Swanson, D.K., and Walker, D.A. (2008) Cryogenesis and soil formation along a bioclimate gradient in Arctic North America. *J Geophys Res* 113: G03S12.

Pollock, L.J., Tingley, R., Morris, W.K., Golding, N., O'Hara, R.B., Parris, K.M., et al. (2014) Understanding co-occurrence by modelling species simultaneously with a Joint Species Distribution Model (JSDM). *Methods Ecol. Evol.* 5: 397–406.

Porras-Alfaro, A., Tobias, T., Sandona, K., Liu, K., Xie, G., and Kuske, C. (2013) Characterization of LSU and ITS rDNA for automated fungal classification. Annual Meeting, Amer Phytopath Soc, 2013. pp. 115–115.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., and Glockner, F.O. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* 35: 7188–7196.

Raynolds, M.K. and Walker, D.A. (2009) Effects of deglaciation on circumpolar distribution of arctic vegetation. *Can J Remote Sens.* 35: 118–129.

**Raynolds, M.K., Walker, D.A., Munger, C.A., Vonlanthen, C.M., and Kade, A.N. (2008) A map analysis of patterned-ground along a North American Arctic Transect. *J Geophys Res* 113: G03S03.

Reynolds, H.L., Packer, A., Bever, J.D., and Clay, K. (2003) Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology* 84: 2281–2291.

Rominger, A.J., Miller, T.E.X., and Collins, S.L. (2009) Relative contributions of neutral and niche-based processes to the structure of a desert grassland grasshopper community. *Oecologia* 161: 791–800.

Ruhfel, B.R., Gitzendanner, M.A., Soltis, P.S., Soltis, D.E., and Burleigh, J.G. (2014) From algae to angiosperms-inferring the phylogeny of green plants (Viridiplantae) from 360 plastid genomes. *BMC Evol. Biol.* 14: 23.

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

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., et al. (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci.* 109: 6241 –6246.

Schuur, E.A.., Bockheim, J., Canadell, J.G., Euskirchen, E., Field, C.B., Goryachkin, S.V., et al. (2008) Vulnerability of permafrost carbon to climate change: Implications for the global carbon cycle. *BioScience* 58: 701–714.

Schuur, E.A.G., Vogel, J.G., Crummer, K.G., Lee, H., Sickman, J.O., and Osterkamp, T.E. (2009) The effect of permafrost thaw on old carbon release and net carbon exchange from tundra. *Nature* 459: 556–559.

***Schwartz, E., Van Horn, D.J., Buelow, H.N., Okie, J.G., Gooseff, M.N., Barrett, J.E., and Takacs-Vesbach, C.D. (2014) Characterization of growing bacterial populations in McMurdo Dry Valley soils through stable isotope probing with 18 O-water. *FEMS Microbiol. Ecol.* 89: 415–425.

Shaver, G.R., Bret-Harte, M.S., Jones, M.H., Johnstone, J., Gough, L., Laundre, J., and Chapin III, F.S. (2001) Species composition interacts with fertilizer to control long-term change in tundra productivity. *Ecology* 82: 3163–3181.

Silvertown, J. (2004) Plant coexistence and the niche. *Trends Ecol. Evol.* 19: 605–611.

Sistla, S.A., Moore, J.C., Simpson, R.T., Gough, L., Shaver, G.R., and Schimel, J.P. (2013) Long-term warming restructures Arctic tundra without changing net soil carbon storage. *Nature* 497: 615–618.

Soltis, D.E., Smith, S.A., Cellinese, N., Wurdack, K.J., Tank, D.C., Brockington, S.F., et al. (2011) Angiosperm phylogeny: 17 genes, 640 taxa. *Am. J. Bot.* 98: 704–730.

Stachowicz, J.J. (2001) Mutualism, facilitation, and the structure of ecological communities: positive interactions play a critical, but underappreciated, role in ecological communities by reducing

physical or biotic stresses in existing habitats and by creating new habitats on which many species depend. *Bioscience* 51: 235–246.
Stamatakis, A., Ludwig, T., and Meier, H. (2005) RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics* 21: 456.
Stone, L. and Roberts, A. (1990) The checkerboard score and species distributions. *Oecologia* 85: 74–79.
Sturm, M., Racine, C., and Tape, K. (2001) Climate change: increasing shrub abundance in the Arctic. *Nature* 411: 546–547.
Sturm, M., Schimel, J., Michaelson, G., Welker, J.M., Oberbauer, S.F., Liston, G.E., et al. (2005) Winter biological processes could help convert Arctic tundra to shrubland. *Bioscience* 55: 17–26.
Taylor, D.L., Booth, M.G., McFarland, J.W., Herriott, I.C., Lennon, N.J., Nusbaum, C., and Marr, T.G. (2008) Increasing ecological inference from high throughput sequencing of fungi in the environment through a tagging approach. *Mol. Ecol. Resour.* 8: 742–752.
*Taylor, D.L., Herriott, I.C., Stone, K.E., McFarland, J.W., Booth, M.G., and Leigh, M.B. (2010) Structure and resilience of fungal communities in Alaskan boreal forest soils. *Can. J. For. Res.* 40: 1288–1301.
*Taylor, D.L., Hollingsworth, T.N., McFarland, J.W., Lennon, N.J., Nusbaum, C., and Ruess, R.W. (2014) A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. *Ecol. Monogr.* 84: 3–20.
*Taylor, D.L. and Houston, S. (2011) A bioinformatics pipeline for sequence-based analyses of fungal biodiversity. *In,* Fungal Genomics, Methods in Molecular Biology. Humana Press, pp. 141–155.
*Taylor, D.L. and Sinsabaugh, R.L. (2014) The soil fungi: Occurrence, phylogeny, and ecology. *In,* Soil Microbiology, Ecology and Biochemistry., p. 77.
Tilman, D. (2004) Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. *Proc. Natl. Acad. Sci. U. S. A.* 101: 10854–10861.
Tilman, D. (1982) Resource competition and community structure. Princeton University Press.
*Timling, I., Dahlberg, A., Walker, D., Gardes, M., Charcosset, J., Welker, J., and Taylor, D. (2012) Distribution and drivers of ectomycorrhizal fungal communities across the North American Arctic. *Ecosphere* 3: art111.
*Timling, I. and Taylor, D.L. (2012) Peeking through a frosty window: molecular insights into the ecology of Arctic soil fungi. *Fungal Ecol.* 5: 419 – 429.
*Timling, I., Walker, D.A., Nusbaum, C., Lennon, N.J., and Taylor, D.L. (2014) Rich and cold: Diversity, distribution and drivers of fungal communities in patterned-ground ecosystems of the North American Arctic. *Mol. Ecol.* 23: 3258–3272.
Ulrich, W. (2008) Pairs—a FORTRAN program for studying pair-wise species associations in ecological matrices. URL: www.keib.umk.pl/pairs
Uroz, S., Buée, M., Murat, C., Frey-Klett, P., and Martin, F. (2010) Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. *Environ. Microbiol. Rep.* 2: 281–288.
***Van Horn, D.J., Okie, J.G., Buelow, H.N., Gooseff, M.N., Barrett, J.E., and Takacs-Vesbach, C.D. (2014) Soil Microbial Responses to Increased Moisture and Organic Resources along a Salinity Gradient in a Polar Desert. *Appl. Environ. Microbiol.* 80: 3034–3043.
Volkov, I., Banavar, J.R., Hubbell, S.P., and Maritan, A. (2003) Neutral theory and relative species abundance in ecology. *Nature* 424: 1035–1037.
Vonlanthen, C.M., Walker, D.A., Raynolds, M.K., Kade, A., Kuss, P., Daniëls, F.J., and Matveyeva, N.V. (2008) Patterned-ground plant communities along a bioclimate gradient in the High Arctic, Canada. *Phytocoenologia* 38: 23–63.
Wahren, C.-H.A., Walker, M.D., and Bret-Harte, M.S. (2005) Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment. *Glob. Change Biol.* 11: 537–552.
Walker, D.A., Auerbach, N.A., Bockheim, J.G., Chapin, F.S., Eugster, W., King, J.Y., et al. (1998) Energy and trace-gas fluxes across a soil pH boundary in the Arctic. *Nature* 394: 469–472.
**Walker, D.A., Epstein, H.E., Gould, W.A., Kelley, A.M., Kade, A.N., Knudson, J.A., et al. (2004) Frost-boil ecosystems: complex interactions between landforms, soils, vegetation and climate. *Permafr. Periglac. Process.* 15: 171–188.

**Walker, D.A., Epstein, H.E., Raynolds, M.K., Kuss, P., Kopecky, M.A., Frost, G.V., et al. (2012) Environment, vegetation and greenness (NDVI) along the North America and Eurasia Arctic transects. *Environ. Res. Lett.* 7: 015504.

**Walker, D.A., Epstein, H.E., Romanovsky, V.E., Ping, C.L., Michaelson, G.J., Daanen, R.P., et al. (2008) Arctic patterned-ground ecosystems: A synthesis of field studies and models along a North American Arctic Transect. *J Geophys Res* 113.:

Walker, D.A., Kuss, P., Epstein, H.E., Kade, A.N., Vonlanthen, C.M., Raynolds, M.K., and Daniëls, F.J.A. (2011) Vegetation of zonal patterned-ground ecosystems along the North America Arctic bioclimate gradient. *Appl. Veg. Sci.* 14: 440-463.

Walker, D.A., Raynolds, M.K., Daniëls, F.J., Einarsson, E., Elvebakk, A., Gould, W.A., et al. (2005) The circumpolar Arctic vegetation map. *J. Veg. Sci.* 16: 267–282.

Wallenstein, M.D., McMahon, S., and Schimel, J. (2007) Bacterial and fungal community structure in Arctic tundra tussock and shrub soils. *FEMS Microbiol. Ecol.* 59: 428–435.

Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73: 5261–5267.

Webb, C.O., Ackerly, D.D., and Kembel, S.W. (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* 24: 2098–2100.

Webb, C.O., Ackerly, D.D., McPeek, M.A., and Donoghue, M.J. (2002) Phylogenies and community ecology. *Annu. Rev. Ecol. Syst.* 33: 475–505.

Whittaker, R.H. (1960) Vegetation of the Siskiyou Mountains, Oregon and California. *Ecol. Monogr.* 30: 279.

Wu, Q. and Mueller, G.M. (1997) Biogeographic relationships between the macrofungi of temperate eastern Asia and eastern North America. *Can. J. Bot.* 75: 2108–2116.

Zhou, J., Davey, M.E., Figueras, J.B., Rivkina, E., Gilichinsky, D., and Tiedje, J.M. (1997) Phylogenetic diversity of a bacterial community determined from Siberian tundra soil DNA. *Microbiology* 143: 3913–3919.