Climate and host identity structure fungal endophyte communities

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Preface/Forord

Etter to gode og varierte år her oppe i fjerde etasje vil jeg takke hele myco-teamet generelt, og mine veiledere spesielt: Tusen takk, Unni, Ella, Anne, Håvard og Marie for fantastisk ledsagelse i felt, på lab, i R, og under skrivinga, og ikke minst for alle mulighetene til å bli med på feltarbeid/feltundervisning i Østmarka, Nordmarka, Vestlandet og Finse! Mer utfyllende takk til Ella og originale hovedveileder Unni for organisering av feltarbeidet, god hjelp for oppstarten av mastertilverelsen m.m., og til nåværende hovedveileder Marie for at du hoppet inn underveis, at du konsekvent tok deg tid til å svare utfyllende på alle spørsmål, og for store mengder hjelp under analyse og skriving det siste semesteret.

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Det er kun slutten på starten. Med GPS i beltet, føttene på bakken, og omgitt av fremmede horisonter: Inn i det ukjente! Fremad marsj! *Que será, será.*

Blindern, 31. mai 2018.

Simen Hyll Hansen

Abstract

Our understanding of the processes that shape foliar fungal endophyte (FE) community diversity and composition is limited. With most ecological FE studies focusing on woody plants and grasses, knowledge on the ecology of FEs in herbaceous plants is particularly sparse. What has been unveiled so far is interesting; not only have FEs proven to be essential symbionts of many plants, protecting their hosts against threats such as drought and herbivory, they also represent a source for discovering novel medicines, such as antibiotics and anticancer compounds. It follows that a more complete understanding of the processes that shape FE communities is desirable. To shed more light on the effects of host identity and climate on FE community composition and richness, 240 samples containing leaves from two herbaceous host plants – *Bistorta vivipara* (L.) Delabre (Polygonaceae, Caryophyllales) and *Campanula rotundifolia* L. (Campanulaceae, Asterales) – were sampled across 12 sites that spanned a wide climate gradient grid (annual average temperatures 6ºC to 11ºC, annual precipitation 600 mm to 2700 mm). Fungal sequences were characterized using Illumina MiSeq amplicon sequencing of the ITS2 region of rDNA, which generated 968 fungal operational taxonomic untits (OTUs) clustered at a 97% similarity threshold. After pruning the dataset for chimeras, contaminants and spurious OTUs, 245 OTUs remained for use in statistical analyses. Host plant identity significantly affected both FE community richness and FE community composition. A small proportion of OTUs were clearly host specific, while most OTUs occurred too infrequently to readily differentiate stochasticity from apparent host specificity. Climate variables significantly affected FE community composition, but not richness. A variance partitioning of host identity versus the best climate model suggested that these two explain approximately the same amount of variation, and that the variation explained by host-specific climate effects was very small. In this thesis it is demonstrated that FE species turn over along climate gradients regardless of host plant identity. Considering the warmer and wetter weather that is forecast for the Scandinavian climate, a responding shift in the composition of FE communities can be expected.

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1 Introduction

Fungal endophytes

There are fungi growing within virtually all green plants, from green algae, mosses and liverworts to ferns, gymnosperms and angiosperms (Cubit, 1975, Hata and Futai, 1996, Stone et al., 2000). Many of these fungi spend all or some of their life inside their hosts without causing any obvious negative effects or symptoms. Fungi that fit this description are defined as endophytes (Wilson, 1995). Foliar fungal endophytes (hereafter referred to only as FEs) refer to fungal endophytes specifically residing in the foliage of plants. During the past decades FEs have been examined in woody plants (Unterseher et al., 2007, Vincent et al., 2016), grasses (Aschehoug et al., 2012, Giauque and Hawkes, 2016) and herbs (Gange et al., 2007, Wearn et al., 2012). Taxa associated with this functional group are found across the fungal kingdom, predominantly within the Basidiomycota and Ascomycota phyla. Isolated FEs commonly belong to the classes of Dothideomycetes, Sordariomycetes, Pezizomycetes, Leotiomycetes and Eurotiomycetes (Arnold et al., 2000). Early estimates of total fungal diversity proposed that 1.5 million fungal species exist (Hawksworth, 2001) and more recent estimates are as high as 5.1 million species (Blackwell, 2011), yet under 100 000 of these have been formally described. It has been suggested that a substantial portion of the undescribed species are FEs (Arnold et al., 2000).

Even though FEs are asymptomatic, they have often been found to increase the fitness of their host plants in a variety of ways. For example, FEs have been linked with increased stress tolerance (Rodriguez et al., 2008, Giauque and Hawkes, 2013), increased pathogen resistance (Arnold et al., 2003) and otherwise unknown direct increases in competitiveness (Aschehoug et al., 2012). Some FEs have been shown to produce compounds that protect their host plants against herbivores (Breen, 1994), as is the case with the American grass *Achnatherum robustum* (sleepygrass). The FEs in this grass produce lysergic acid amide in large enough quantities to affect grazing horses and cattle (Faeth et al., 2006), which after consuming the infected grass will fall into a deep sleep for up to several days, and upon waking will not touch this species of grass again. Not all FE interactions are mutualistic, as several FEs have been shown to be latent pathogens waiting for the plant to weaken (Saikkonen et al., 2004, Romero et al., 2001), or, as is

the case in leaves of many deciduous trees, latent saprotrophs waiting for the shedding of leaves in fall (Osono, 2006). This highly diverse group of fungi has an equally diverse biochemistry, and many novel compounds are regularly isolated from them (reviewed in Strobel and Daisy, 2003). Many of these compounds have been of great medicinal interest, e.g. anticancer compounds (Kharwar et al., 2011) and antibiotics (Ezra et al., 2004).

As a group, FEs are highly diverse but poorly understood, especially in regards to their underlying ecology (Arnold, 2007). Given both their ecological and potential medicinal importance, a deeper understanding of the processes that structure FE communities is highly desirable. Both biotic factors such as host identity (Arnold et al., 2003) and abiotic factors such as climate (e.g. Zimmerman and Vitousek, 2012) have been shown to affect these fungi, but well-designed studies exploring FE communities with sufficiently deep sampling are still few and far between.

Host specificity

FE communities are structured by a complex mix of interacting factors (U'Ren et al., 2012), and there is a large variety in the way they interact with their host plants. Host specificity, i.e. the tendency of an organism to be limited to certain host taxa, has been documented for many FEs (Arnold et al., 2003). Gange et al. (2007) found several host specific FEs in a study of two herbaceous plants, and Vincent et al. (2016) found a weak but significant structuring of FE communities across 11 host tree species in tropical New Guinea. In the mentioned studies, while a few FEs were only found in one host type, large parts of the datasets consisted of ubiquitous FEs that seemed to have no host specificity. Several other studies report no structuring by host whatsoever (Cannon and Simmons, 2002, Suryanarayanan et al., 2002). There is not yet a consensus in the scientific community regarding the rate of host specificity among endophytes (Arnold and Lutzoni, 2007).

Because of the environmental and climatic specificity of many endophytes, false positives for host specificity can arise if the choice of host plants is not thought through. When examining host specificity, one should take care to decouple an actual direct host effect from effect of the spatial and environmental differences in the hosts' ranges. For example, a large scale study by U'Ren et al. (2012) looked at FEs in tens of hosts in a variety of climatic zones across continental USA,

but was unable to disentangle climatic effects from host effects due to the fact that most host plants in the study were not distributed along the full range of the climatic gradients. Studies aiming to decouple the direct host effect from the climatic effects that structure the range and phenology of host plants, should examine host plants that occur as sympatrically and synchronologically as possible.

Climate change and climate gradients

On a global scale, temperatures are rising and precipitation patterns are changing (IPCC, 2014). In North-Western Europe, both temperature and precipitation levels have risen in the past decades, and the trend is expected to continue (IPCC, 2014). Though the literature on environmental structuring of FE communities is rather sparse, it has been shown that local climate significantly structures FE community composition. This has been the case across precipitation gradients both in tropical tree leaves on Hawaii (Zimmerman and Vitousek, 2012), in grasses in Texas (Giauque and Hawkes, 2016), and across altitudinal temperature gradients in the Pyrénées Mountains (Cordier et al., 2012). Additionally, in a common-garden experimental setting using balsam poplars, elevating temperature with open top chambers significantly affected FE communities (Bálint et al., 2015). Hence, changing patterns in climate could lead to change in FE communities. In contrast to soil microorganisms, the leaf inhabiting FEs experience greater environmental extremes, as they lack the buffer that soil provides. Whether this lifestyle has better prepared FEs for a changing climate is as of yet unknown. The structuring of FE communities along climate gradients has been studied worldwide (Ahlholm et al., 2002, Suryanarayanan et al., 2002, Unterseher et al., 2007, Zimmerman and Vitousek, 2012, Yang et al., 2016). However, with earlier studies focusing either on grasses or leaves of woody plants, no one has thoroughly looked at FEs in herbs across climate gradients.

Culture vs. culture-independent methods

Traditionally FEs were studied by culturing them on artificial media and identifying them either morphologically or by Sanger sequencing (Torres and White, 2012). The relatively low diversity found through culture-based methods has been described as cultivation bias, and as such the results are inaccurate representations of the complete FE community (Hyde and Soytong, 2008).

Culturing tends to bias the recovered diversity towards fast growing species (Hyde and Soytong, 2008), and towards generalists that have an easier time growing in the nutrient solution than specialists such as obligate biotrophs that may be partly or fully uncultivable (Guo et al., 2001, Arnold et al., 2007, Zhu et al., 2008). Indeed, culture-based studies on FEs in herbs have almost exclusively isolated ubiquitous generalist fungi acting as FEs (Beena et al., 2000, Gange et al., 2007). Only in recent years has it become possible to omit a culturing step, and to sequence whole community samples at once through amplicon sequencing/high throughput sequencing (HTS, Jumpponen and Jones, 2010, Caporaso et al., 2012). The range of taxa detected with culture-independent approaches has been shown to be significantly greater than that of culture-based ones (Arnold et al., 2007). The vast majority of FE studies on herbaceous plants have been culture-based, and either identified isolates by sequencing (Chen et al., 2011), or morphologically (Gange et al., 2007). Almost no studies on herbs have been culture-independent, though exceptions include Zhang and Yao (2015) that employed 454 pyrosequencing to examine FE communities in three arctic herbs and one ericoid dwarf shrub. In general, substantial amounts of time and resources are needed for large scale culture-based studies, and problems such as non-cultivable fungi are common (Vincent et al., 2016). Even with thousands of isolates in large-scale studies, culturing has been found to be insufficient to capture the estimated fungal richness (U'Ren et al., 2012). These problems are largely avoided by HTS, where millions of sequences can be acquired for a fraction of the cost. The low cost per sequence allows a higher volume of sampling, thus improving on what traditionally has been the limiting factor for large-scale ecological studies of FEs. Combined with improving taxonomic resolution in reference databases, the new generation of sequencing technologies provides opportunities for investigating the world of cryptic organisms at unmatched levels.

Aims of study

In this thesis I sought to analyze the structuring of FE communities by abiotic and biotic factors in two herbaceous co-occurring host plant species using HTS. Specifically, my study aimed to address two questions:

i. Do the FE communities vary between host plant species?

ii. Do the FE communities respond to temperature and precipitation gradients?

2 Materials and methods

Study sites

To investigate climatic structuring of herb associated FE communities, sampling was done in twelve study sites across a climate gradient grid. The twelve sites are all located in Western Norway, across a steep west-to-east precipitation gradient and an altitudinal temperature gradient. The sites are part of the SEEDCLIM infrastructure (Table 1), an already established climate gradient grid used for analyzing plant responses to climate (Klanderud et al., 2015). The sites are placed at three different temperature and four different precipitation levels in order to cover roughly a 6 degree gradient in mean summer temperature (6ºC, 9ºC and 11ºC), and a 2100 mm gradient in mean annual precipitation (600 mm, 1200 mm, 2000 mm and 2700 mm, Halbritter 2015). Due to elevation being used as a proxy for temperature in the study design, these two factors cannot be decoupled. All sites have been carefully selected to ensure climate is the primary differentiating factor. The sites are all moderately grazed calcareous grasslands, and all but one of the sites are south-west facing slopes with an approximate incline of twenty degrees. The setup allows a factorial design, in which effects of temperature and precipitation can be decoupled. Each site has a weather station providing detailed environmental data for the local climate both at the time of sampling, and back to 2008 (Halbritter, 2015). This data includes daily measurements of soil temperature, as well as air temperature at ground level.

Table 1. Name, geographic coordinates, elevation, and mean temperature and precipitation levels of the 12 SEEDCLIM sites (Klanderud et al., 2015).

Site name	Coordinates (WGS 84)	Elevation (m a.s.l.)	Mean annual temperature (ºC)	Mean annual precipitation (mm)
Ulvhaugen (ULV)	61.024265N, 8.123421E	1208	6.17	596
Låvisdalen (LAV)	60.823077N, 7.275951E	1097	6.45	1321
Gudmesdalen (GUD)	60.832755N, 7.175608E	1213	5.87	1925
Skjellingahaugen (SKJ)	60.933475N, 6.415015E	1088	6.58	2725
Ålrust (ALR)	60.820275N, 8.704652E	815	9.14	789
Høgsete (HOG)	60.876034N, 7.176650E	700	9.17	1356
Rambæra (RAM)	61.086621N, 6.630246E	769	8.77	1848
Veskre (VES)	60.544486N, 6.514674E	797	8.67	3029
Fauske (FAU)	61.035418N, 9.078772E	589	10.30	600
Vikesland (VIK)	60.880325N, 7.169800E	474	10.55	1161
Arhelleren (ARH)	60.665164N, 6.337377E	431	10.60	2044
Øvstedal (OVS)	60.690052N, 5.964846E	346	10.78	2923

Host plants

Two herbs, *Bistorta vivipara* (L.) Delabre (Polygonaceae, Caryophyllales) and *Campanula rotundifolia* L. (Campanulaceae, Asterales), were chosen because they occur at most sites in the SEEDCLIM climate grid. Both species are widely distributed perennials in the northern hemisphere. *B. vivipara* has earlier been studied regarding root-associated fungi (e.g. Kauserud et

al., 2012, Yao et al., 2013, Botnen et al., 2014, Mundra et al., 2015), as it is one of relatively few herbs that form ectomycorrhizal (ECM) associations with fungi. *C. rotundifolia*, on the other hand, is associated with arbuscular mycorrhizal fungi (AM fungi, Wijesinghe, John et al. 2001). Both plants spread vegetatively and overwinter as rhizomes. *C. rotundifolia* reproduces only sexually, while *B. vivipara* mainly reproduces by clonal bulbils that are produced in the inflorescence.

Sample collection and surface sterilization

Leaves of 10 individual *B. vivipara* and *C. rotundifolia* plants were collected from each of the 12 SEEDCLIM sites. Where possible, only leaves without symptoms of disease were sampled. At some sites all leaves of the host plants showed symptoms, in which case the least visibly diseased leaves were sampled. For *B. vivipara* basal leaves were chosen, while for *C. rotundifolia* stem leaves were chosen, as rosette leaves were withered and senescent in sites with dense vegetation. To ensure the same leaf type was sampled from all sites, only stem leaves from the middle section of actively flowering stems of *C. rotundifolia* were sampled.

All leaves were surface sterilized within 12 hours of sampling. The sterilization was done according to Mejía, Rojas et al. (2008), and included three steps in separate petri dishes. First the leaves were washed and agitated in 70% ethanol for 3 min, and then washed and agitated in 10% sodium hypochlorite for 2 min before they were rinsed in distilled water for 1 min. To ensure that all epiphytic spores and microorganisms were removed, a fourth step was added, where the leaves were rinsed thoroughly with a squirt bottle containing distilled water. After sterilization, the samples were put into 15 mL falcon tubes filled with 10 mL CTAB (cetyltrimethylammonium bromide). The tubes were subsequently stored at -20ºC until DNA extraction.

DNA extraction, amplification and sequencing

One tungsten carbide bead (3 mm) and one ceramic bead (5 mm) was added to each 15 mL falcon tube containing a leaf sample and 10 mL CTAB. The samples were then crushed in a FastPrep®-24 homogenizer (MP Biomedicals, USA) at 5.0 m/s until completely homogenized. After being crushed, all samples were stored at -80ºC for at least 24 hours before DNA was

extracted according to a modified CTAB-based protocol (Murray and Thompson, 1980, Gardes and Bruns, 1993). The extracted DNA was re-suspended with 60 µl milli-Q H_2O and stored at -20 ºC. To avoid remaining compounds inhibiting PCR amplification, the extracted DNA was cleaned with the Wizard® SV Gel and PCR Clean-Up System (Promega, USA) according to the manufacturer's instructions.

The forward and reverse primers ITS4 and gITS7 (ITS4: 5'-TCCTCCGCTTATTGATATGC-3'; gITS7: 5'-GTGARTCATCGARTCTTTG-3', Ihrmark et al., 2012) were used to amplify the ITS2 region. The primers were tagged with unique tags that had a variable length of 7 - 9 bp, including 1 - 3 ambiguous bases (N's) at the 5' end of each tag (Supplementary table S1). Each of the 240 samples, 23 negative controls, 2 positive controls and 6 technical replicates were randomly grouped into one of three libraries, and assigned to one of the 94 unique tags. The amplification mixture for each sample included 4 µl 5x buffer, 2 µl 2mM dNTP, 0.6 µl 100% DMSO, 0.6 µl 10mg/mL BSA, 0.2 µl Phusion Hot Start II DNA Polymerase (Thermo Fisher, USA) and 8.6 µl milli-Q H_2O. A volume of 2 µl DNA template was used per sample, as well as 1 µl each of 10µM forward and reverse primers. The PCR cycling was done with a Mastercycler® Nexus GSX1 (Eppendorf, Germany), and began with an initial denaturation stage at 98ºC for 30 s, before cycling 30 times at 98ºC for 10 s (denaturation), 55ºC for 15 s (annealing) and 72ºC for 15 s (extension). After cycling, there was a final extension step at 72ºC for 5 min. SequalPrep™ Normalization Plates (Thermo Fisher, USA) were then employed according to the manufacturer's instructions in order to standardize amplicon concentrations. The amplicons were then pooled in equimolar amounts into three libraries, containing 94, 94 and 83 uniquely tagged samples. The pooled samples were cleaned using the Wizard® SV Gel and PCR Clean-Up System, before being submitted to StarSeq (Mainz, Germany) for multiplexing and sequencing in a single Illumina MiSeq run.

Bioinformatics

The sequences were processed using the Abel Cluster, owned by the University of Oslo and Uninett/Sigma2, and operated by the Department for Research Computing at USIT, the University of Oslo IT-department (http://www.hpc.uio.no/).

To error correct the sequences, the BayesHammer module in SPAdes (Nurk et al., 2013) was used with default settings. Then, sdm in LotuS (Hildebrand et al., 2014) was used with default settings to demultiplex the data. To pair complementary forward and reverse reads, PEAR (Zhang et al., 2013) was employed with default settings. For quality filtering of the paired reads, the filtering function of VSEARCH (Rognes et al., 2016) was used with a maximum expected error (E) value of 0.5, maximum read length of 590 bp, and allowing no ambiguous bases (N's). VSEARCH was further used to dereplicate and chimera-check the dataset. Detected chimeras were removed, before the reads were clustered at 97% similarity using VSEARCH. The resulting operational taxonomic units (OTUs) were taxonomically classified using BLAST against the UNITE 7.2 database (Kõljalg et al., 2013). Only OTUs that were classified as fungi were kept for further analyses, and additionally fungal OTUs identified as contaminants or non-endophytes were removed.

Statistics and community analyses

Site- and sample- level metadata tables containing climatic variables, site locations and host plant identity were prepared using data from each site's local weather station and gridded climate data from The Norwegian Meteorological Institute. Climatic variables were summarized for the year of sampling (2016) and "all years" (2008-2016) separately (Supplementary table S2). Growing season length was defined as number of days after soil temperature exceeded 3ºC in spring, to the date of sampling in fall. Average soil temperature and average ground level temperature were calculated from the daily temperature values during the 2016 growing season and across all instances where temperature exceeded 3ºC for the "all years" data. Mean precipitation and long-term mean temperature were calculated from all monthly measurements during the growing season, where growing season was defined as the period between the first month without snow cover at each site until October. The coefficient of variation (standard deviation/mean) for each temperature and precipitation variable was calculated to provide a standardized measure of variability. The climatic variables were transformed to improve homogeneity, minimize skewness and to standardize them into the same scale (Sokal and Rohlf, 1995, Økland et al., 2001). To achieve this, a Shapiro-Wilk test was used to test for normality, so that normally distributed variables could be directly rescaled to a range from -1 to 1. Skewed variables were transformed with log or exponential transformations optimized for zero skewness using the e1071 (Meyer et

al., 2017) package in R (R Core Team, 2018). The transformed variables were then rescaled to a range from -1 to 1.

To produce a heat tree visualizing differential host preference in members of FE communities, the metacoder package (Foster et al., 2017) was used. The percentage of OTUs found exclusively in one or the other host was calculated. To reduce the effect of rare OTUs on observed host specificity, OTUs were designated host-specific only if the number of occurrences in a particular host exceeded the 95% confidence interval for a null model assuming random occurrence of the OTU across hosts.

Rarefaction curves for each site were calculated using the vegan package (version 2.4-6, Oksanen et al., 2018) in R. Due to insufficient sequencing depth in some sites, the R package iNEXT (version 2.0.12, Hsieh et al., 2016) was used to extrapolate species richness estimates to 10 000 reads per site, using the individual-based approach developed by Colwell et al. (2012). Both iNEXT estimates and direct vegan-derived measures of Shannon's diversity, Simpson's diversity, as well as OTU counts, were fit against climatic variables, host identity, and sample locality, with general linear models (GLMs) using the "glm" function in R. For each richness measure, the "stepAIC" function from MASS (Venables and Ripley, 2002) was used to perform stepwise model selection with two sets of climate data, i) Host taxon + climate variables 2016, and ii) Host taxon + climate variables 2008-2016. The argument "both" was used to allow iterative forward and backward selection for model optimization.

Before running ordination analyses, the OTU matrix was pruned. All OTUs with <10 reads were removed, as well as all samples with <100 reads. Non-metric multidimensional scaling (NMDS) ordinations were then produced by running the "metaMDS" function from vegan on a Bray-Curtis dissimilarity matrix produced with the "vegdist" function, all with default settings. The ggplot2 package (Wickham, 2009) was used to visualize the NMDS ordinations. The "envfit" function was used on ordinations to derive r^2 and p values for correlations between climatic variables, host identity, sample locality and the structure of the ordination plots.

Before canonical correspondence analysis (CCA) was performed, the matrix used in the NMDS ordination was transformed with the "decostand" function in vegan. With "decostand", the "total" argument was used in order to standardize the matrix by OTU proportions per sample. CCA

ordinations were then calculated using the “cca” function in vegan, in which host plant species was used as a conditioning factor, and several sets of climatic variables were used as constraining factors. First, independent CCA models were generated for each variable. Each of these CCA models were tested for significance by comparing them against 1000 random permutations of the climate data stratified according to the experimental design. A final CCA model was created by combining a selection of the significant variables that minimized variable covariance, and the fit of this combined model was tested in the same manner. To test the relative contribution of this climate model and host identity in structuring FE communities, variance partitioning was then performed with the “var.part” function in vegan, allocating variation to host species and to those climate variables defined in the final CCA model. Variance partitioning results were visualized with the VennDiagram package (Chen, 2018).

3 Results

Data Characteristics

Out of 17 938 761 raw reads, a total of 6 578 959 reads were retained after quality controls, and clustered at 97% similarity into 3 851 OTUs (Supplementary table S3). Of these, 60% were plants (2 294 OTUs, 6 395 252 reads) and 25% were fungi (968 OTUs, 111 755 reads). The remaining OTUs had no blast hit (14%) or were cercozoans (1%). After removal of OTUs with <10 reads, samples with <100 reads and subsequent removal of 29 OTUs identified as contaminants or non-endophytes (Supplementary table S4), 245 fungal OTUs (101 046 reads) remained for use in further analyses.

The final dataset included 80 out of the original 240 plant samples, but still represented all but one of the 12 sites. The number of reads/site ranged from 620 to 22 388, averaging at 8 420 reads. The 80 samples consisted of 58 *Bistorta vivipara* samples (2 - 8 samples per site, 109 - 10 938 reads/sample) and 22 *Campanula rotundifolia* samples (1 - 7 samples per site, 120 - 9 643 reads/sample).

Taxonomic summary

Of the 245 fungal OTUs that were retained, 207 were identified to at least phylum level, while 38 OTUs (6% reads, 85% sample occupancy) remained unidentified below phylum level. Out of the identified 207 OTUs, 49 belonged to Basidiomycota (35% reads, 56% sample occupancy) and 147 to Ascomycota (58% reads, 83% sample occupancy). Additionally, 7 and 4 OTUs belonged to Mucoromycota (<1% reads, 4% sample occupancy) and Glomeromycota (<1% reads, 28% sample occupancy). These were further composed of 15 classes of fungi divided into 77 genera. Among these, 4 classes accounted for 72% of all fungal reads: Leotiomycetes (24% reads, 47% sample occupancy), Pucciniomycetes (18% reads, 20% sample occupancy), Sordariomycetes (16% reads, 48% sample occupancy), and Dothideomycetes (15% reads, 51% sample occupancy). Pucciniomycetes was exclusive to *C. rotundifolia* and Leotiomycetes occurred in equal proportions in the host plants, while Sordariomycetes and Dothideomycetes were

predominantly present in *B. vivipara* (Figure 1). The majority of the 20 most abundant OTUs (Table 2) belong to genera that are known saprotrophs (*Paraleptosphaeria, Mycosphaerella*) or plant pathogens (*Paraleptosphaeria, Mycosphaerella, Colletotrichum, Rhynchosporium*). The 20 most abundant OTUs by number of reads accounted for 67% of all fungal reads, but typically occurred in only two or three sites (Supplementary table S5). On average, each OTU occurred in 2.2 sites, with 97 OTUs occurring in only one site.

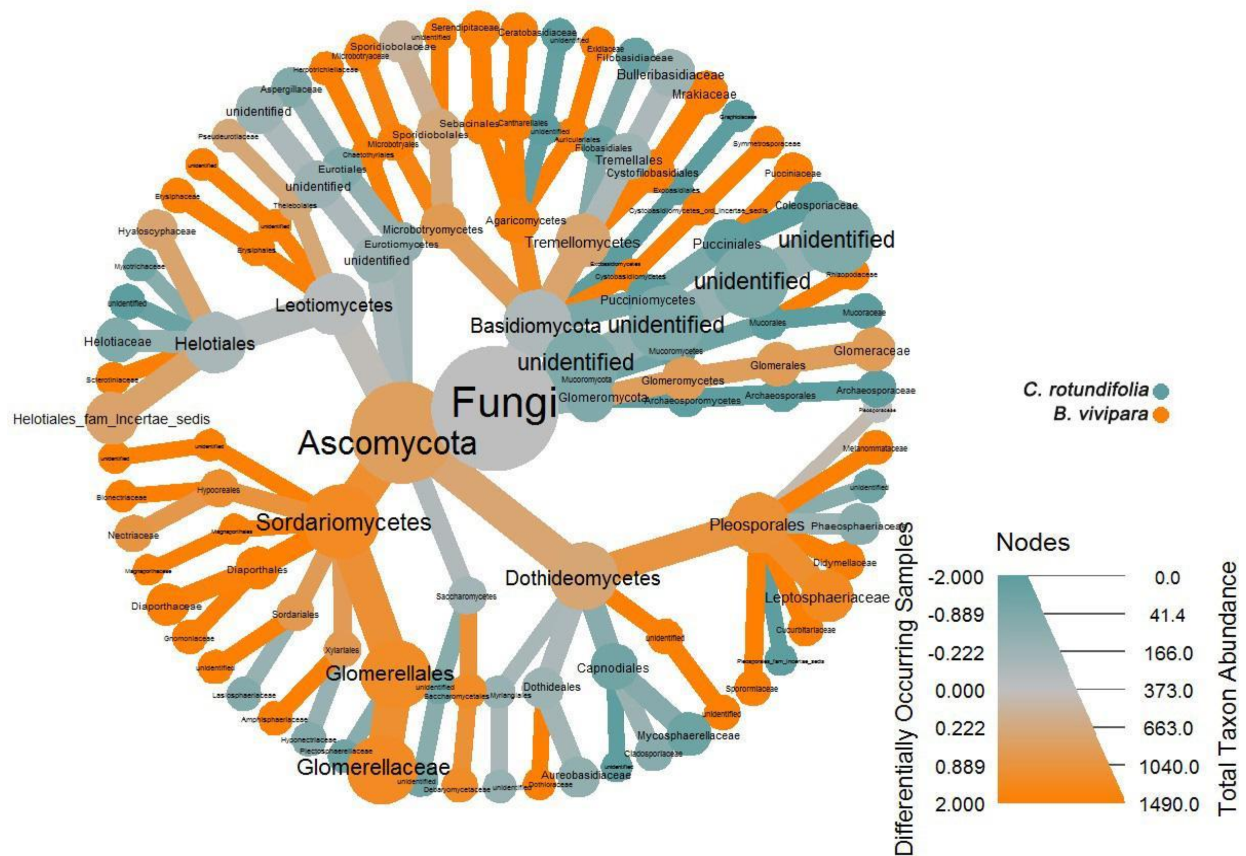

Figure 1. Heat tree of all fungal OTUs identified to family level. The size of nodes is proportional to the number of samples each taxon was present in. Nodes are colored by proportion of occurrence in the two hosts. Blue nodes are found in *C. rotundifolia* only, orange nodes are found in *B. vivipara* only, while grey nodes are found in equal proportion in both.

Host effects

The composition of the FE communities was clearly linked to host identity. In an NMDS ordination host structured the dataset into two groups, representing *C. rotundifolia* and *B. vivipara* (Figure 2). Testing host effect relative to locality and climatic variables revealed host as the single most significant factor structuring the community composition (Supplementary table S6). Furthermore, FE diversity per plant was significantly higher in *B. vivipara* than in *C. rotundifolia* (Figure 3). All best fit GLMs included host as the single most significant variable affecting richness ($p < 0.001$ to 0.019, Supplementary table S7).

Most OTUs were limited to one host plant, with 65% versus 13% of the OTUs being exclusive to *B. vivipara* or *C. rotundifolia,* respectively, and 22% of OTUs occurring in both hosts. When compared with a null model of equal distribution between the hosts, set at a 95% confidence interval, only 32 OTUs (13%) were identified as specific to one of the hosts (Figure 4). Host specificity could not be reliably differentiated from stochasticity among the most rare OTUs (<15 occurrences), and the proportion of OTUs detected as host specific rose with increasing OTU sample occupancy (Supplementary figure S1).

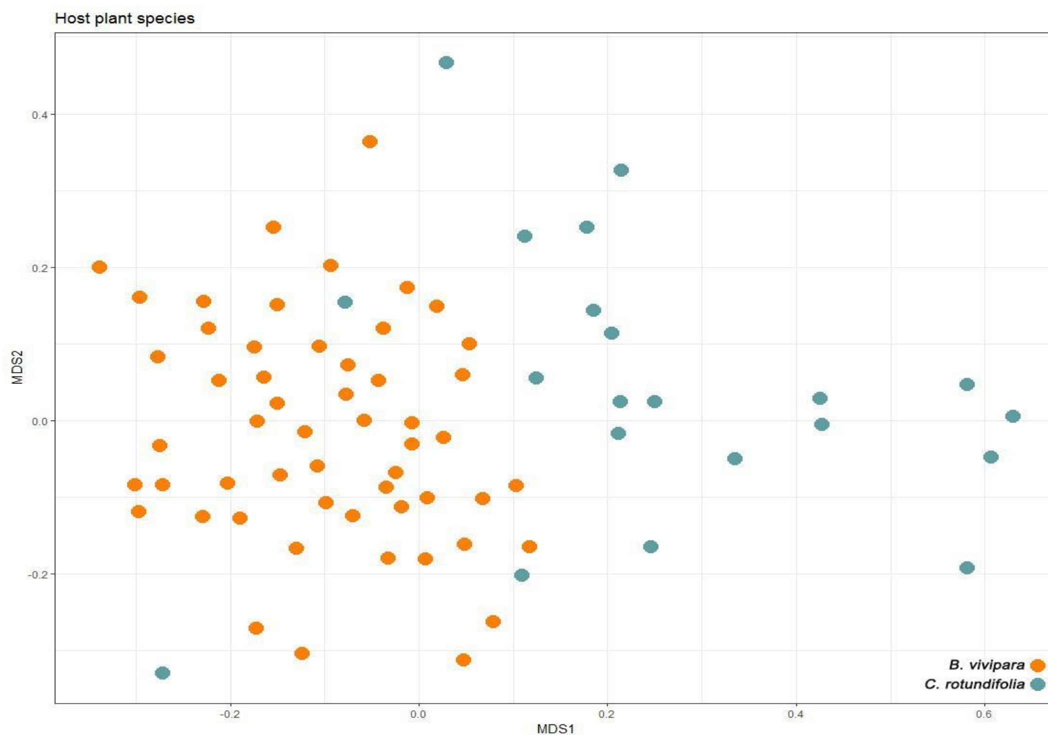

Figure 2. NMDS ordination of samples, colored by host plant identity.

Figure 2. NMDS ordination diagram of *Bistorta* and *Campanula* leaf FE communities, colored by host plant. Table 2. Top 20 most abundant OTUs by sample occupancy, with host preference, occurrences and details on each BLAST hit. Host preference was determined by testing the number of occurrences in a single host against a 95% confidence interval limit for a null model of no host preference.

OTU_#	Occupancy (#samples)	Taxonomic ID	GenBank accession No.	% identity	% coverage	Number of sites	Host preference
OTU_233	61	Unidentified fungus	JQ666347	96.970	9	10	*B. vivipara*
OTU_7	36	*Paraleptosphaeria dryadis*	AF439461	96.311	84	10	*B. vivipara*
OTU_13	35	*Paraleptosphaeria dryadis*	AF439461	96.311	84	8	*B. vivipara*
OTU_161	27	*Colletotrichum anthrisci*	GU227845	98.795	85	8	*B. vivipara*
OTU_163	26	*Colletotrichum anthrisci*	GU227845	98.795	85	8	*B. vivipara*
OTU_15	25	*Rhynchosporium agropyri*	HM627479	92.857	83	10	*B. vivipara*
OTU_159	25	*Colletotrichum* sp.	DQ539441	96.800	86	9	-
OTU_28	25	*Rhynchosporium agropyri*	HM627479	92.857	83	10	*B. vivipara*
OTU_1150	24	Unidentified fungus	KC965205	97.297	10	7	*B. vivipara*
OTU_29	24	*Mycosphaerella tassiana*	EF679363	99.103	78	10	-
OTU_114	23	*Colletotrichum antirrhinicola*	KM105180	100	79	6	*B. vivipara*
OTU_112	22	*Colletotrichum destructivum*	AJ301942	99.567	79	7	*B. vivipara*
OTU_125	22	*Vishniacozym avictoriae*	AF444469	99.533	78	10	-
OTU_128	22	*Vishniacozym avictoriae*	AF444469	99.070	78	10	-
OTU_1559	22	*Glomeraceae* sp.	KM041862	100	9	7	*B. vivipara*
OTU_21	22	*Mycosphaerella tassiana*	EF679363	99.103	78	11	-
OTU_41	22	*Colletotrichum tanaceti*	JX218228	99.138	79	6	-
OTU_45	22	*Colletotrichum tanaceti*	JX218228	99.138	79	8	-
OTU_1726	20	Unidentified fungus	KP889371	97.059	9	8	*C. rotundifolia*
OTU_218	20	*Colletotrichum* sp.	DQ539441	96.414	86	8	-

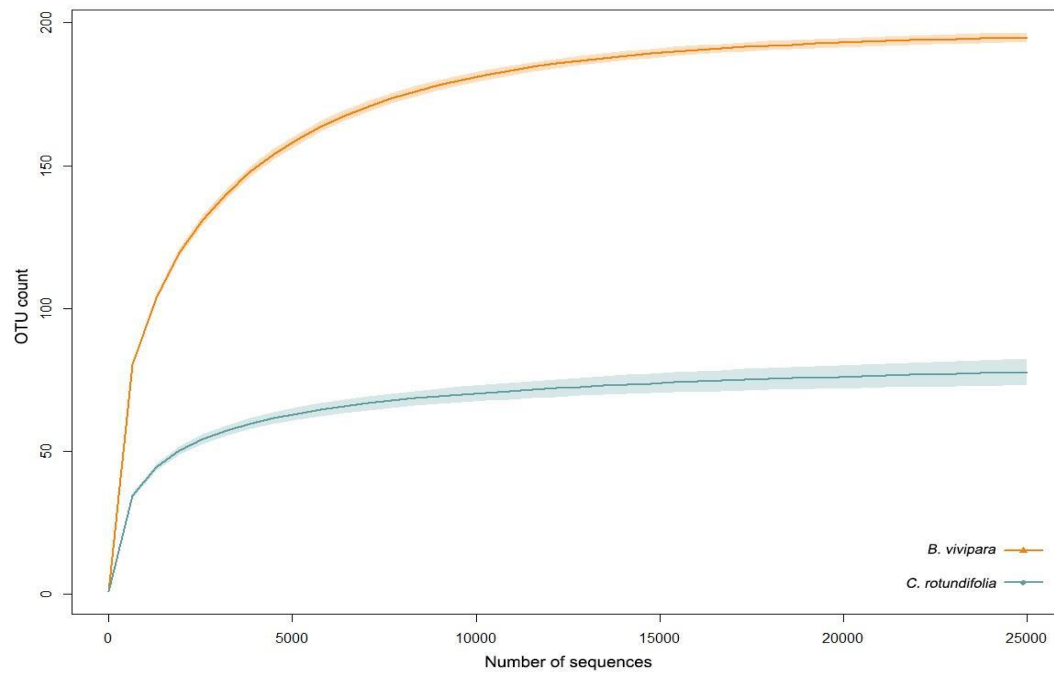

Figure 3. Rarefaction curves of OTU counts in the two host plants *B. vivipara* and *C. rotundifolia.*

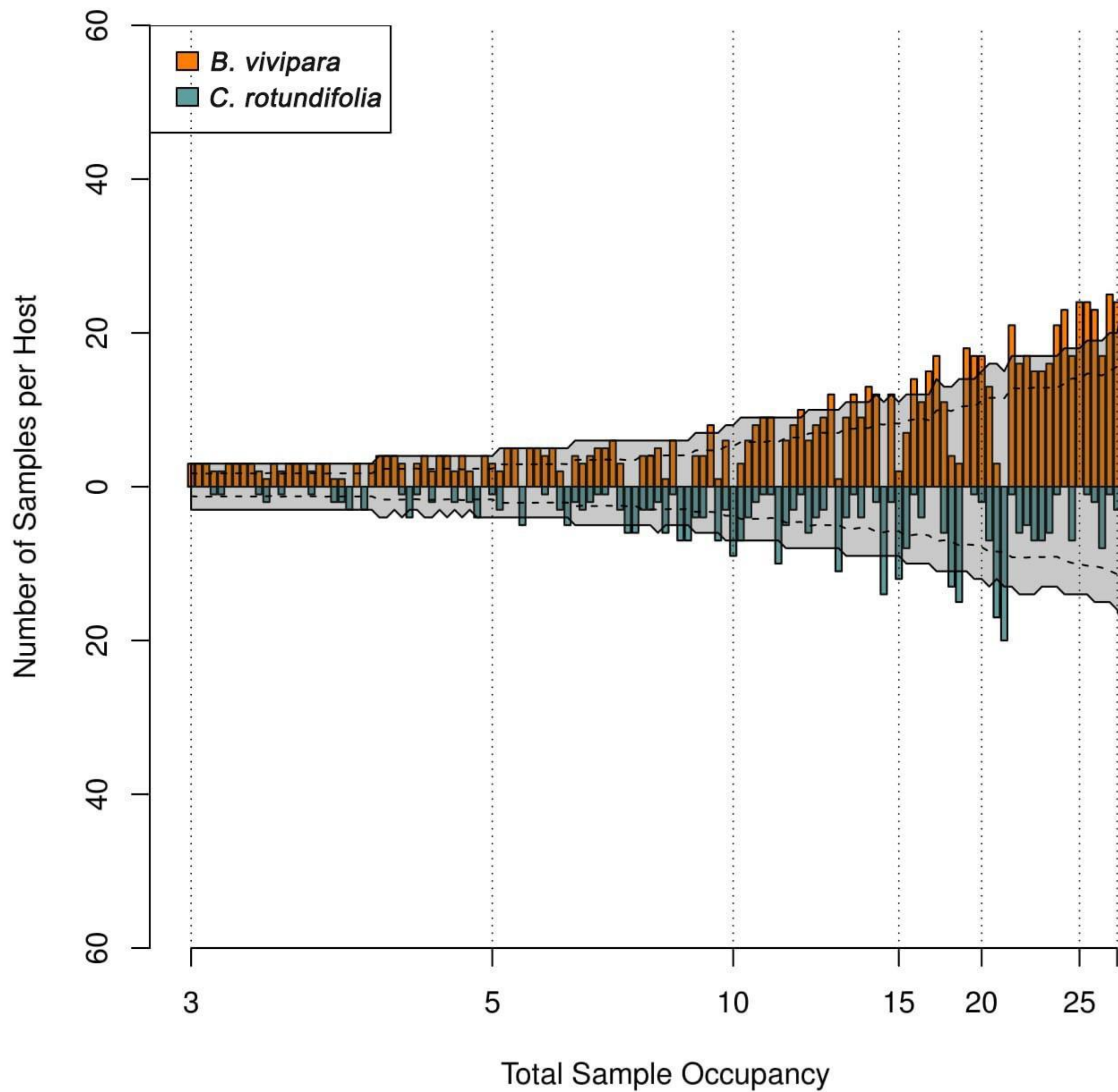

Figure 4. Barplot showing host specificity in OTUs occurring in >3 samples. Bars represent individual OTUs, sorted by total number of samples occupied per OTU. The colored space represents a 95% confidence interval for a null model of no host specificity, so that any OTU exceeding this limit is occurring significantly more often in that host. Dashed lines represent the mean expected values in the null model for each OTU.

Climate effects

To reveal whether climate had an effect on the FE communities, two main aspects were explored. First, whether FE richness was correlated with climate, and second, whether the FE community composition was structured by climate.

Stepwise model selection for fitting of GLMs did not identify any climatic variable that both consistently and significantly affected the measures of alpha diversity (Supplementary table S7). Although long-term variation in soil temperature, long-term mean air temperature, annual air temperature (2016), annual variation in air temperature (2016), and annual variation in precipitation (2016) were included in many of the best-fit GLMs, none were consistently significant.

Fitting the climatic variables to an NMDS ordination of all samples indicated that while host was the primary factor structuring the FE communities, some climatic variables also had an effect on the FE community structure (Supplementary table S6). To investigate this further, the dataset was divided into a *B. vivipara* subset and a *C. rotundifolia* subset. Fitting climatic variables onto the NMDS ordinations for each host plant species, showed that long-term mean temperature significantly structured the FE community within *B.vivipara* ($p = 0.002$, $r^2 = 0.2549$, supplementary table S8, supplementary figure S2) and *C. rotundifolia* ($p = 0.003$, $r^2 = 0.5224$, supplementary table S9, supplementary figure S3). The FE community of *C. rotundifolia* was significantly structured by precipitation ($p = 0.003$, $r^2 = 0.5224$), while the FE community in *B. vivipara* was not ($p = 0.199$, $r^2 = 0.0736$).

All climatic variables were assessed separately in CCA models in which the effects of host had been partialed out (Supplementary table S10). A combined CCA model representing total climatic effects was made using only those variables that were significant independently and did not covary. A correlation plot was used to visually assess covariance between variables (Supplementary figure S4). Three variables met the above criteria for inclusion in the final CCA model: annual precipitation for 2016 ($p = 0.019$), long-term variability of precipitation ($p = 0.046$) and long-term mean air temperature ($p = 0.011$). The combined CCA model using these variables was significant ($p = 0.003$), and showed that the structuring effects of temperature and variation of precipitation were largely decoupled (Figure 5). Variance partitioning showed that

this combined climate model ($r^2 = 0.03843$) explained almost as much variation in community composition as host identity ($r^2 = 0.04224$), and that these two had little overlap ($r^2 = 0.0026$, supplementary figure S5).

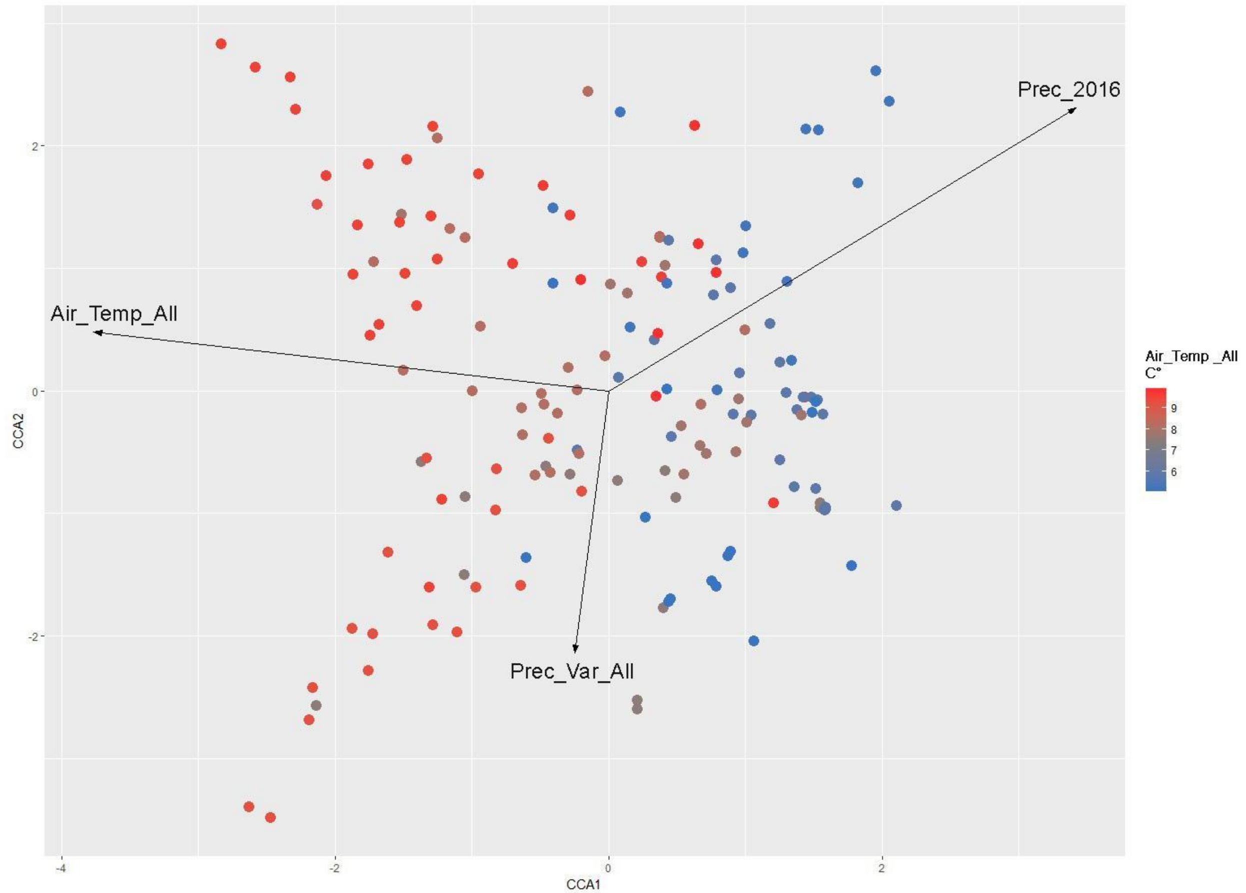

Figure 5. CCA plot constrained by long-term mean air temperature (Air_Temp_All), annual mean precipitation (Prec_2016) and long-term variation of precipitation (Prec_Var_All), conditioned by host identity. Arrows indicate the direction of maximal increase of each variable. Points are colored by raw temperature values, while the Air_Temp_All arrow represents the fit calculated for the zero skewness transformed, rescaled values.

4 Discussion

Host effects

Host identity was strongly linked with both FE community structure and FE richness (Figure 2 and 3). Differences in FE community composition between host plant taxa have previously been demonstrated (Hoffman and Arnold, 2008, Higgins et al., 2007). In herbaceous plants specifically, Gange et al. (2007) found consistently higher FE richness in the thistle *Cirsium arvense* compared to the daisy *Leucanthemum vulgare*. Similarly, Seena and Sridhar (2004) found differences in FE richness between two closely related species of *Canavalia*. The potential mechanisms driving host differences in FE richness and community composition may include physical and chemical properties of the leaves, biotic interactions within the plants, the timing of leaf emergence or some combination of these factors.

The two host plants in this study, *Campanula rotundifolia* and *Bistorta vivipara,* are only distantly related, and have markedly different biochemistry and leaf structure. *C. rotundifolia* produces phenolic compounds (Teslov et al., 1983), a class of chemicals known to have antifungal properties (Echeverri et al., 1997, Favaron et al., 2009). A specific class of phenol derivatives called flavonoids is found in *B. vivipara,* which may function to protect the plant against excessive radiation (Vysochina and Voronkova, 2013). Like phenols, flavonoids are known to have strong antifungal properties (Weidenbörner et al., 1990). These antifungal compounds may act as toxins that place selective pressure on the FE communities in different ways depending on which specific compound is produced. Additionally, ECM associated plants such as *B. vivipara* tend to have a lower leaf pH than AM associated plants such as *C. rotundifolia* (Cornelissen et al., 2006), which may in turn affect FE communities. Structurally, *B. vivipara* leaves are fleshy and thick while the sampled stem leaves of this *Campanula* species are elongated and thin. The underside of *B. vivipara* leaves is often slightly tomentose, while *C. rotundifolia* leaves are glabrous. Both biochemically and physically, the leaves of these two herbs provide very different habitats for FEs, and it is likely that this affects the composition of FE communities.

An additional explanation to the observed host-specific differences in FE richness and community composition lies in the size and age of the sampled leaves. The sampled leaves in this study undoubtedly had varying age, as growing season length varied across sites, and all leaves were collected within a few days. Furthermore, basal leaves were sampled from *B. vivipara*, while leaves from flowering stems were sampled from *C. rotundifolia*. The basal leaves of *B. vivipara* emerge prior to the stem-leaves of *C. rotundifolia,* thus, the leaf age differs between hosts within each site. Leaf age has been linked with FE richness in tropical trees (Arnold et al., 2003), temperate trees (Unterseher et al., 2007) and conifers (Espinosa-Garcia and Langenheim, 1990). In very young leaves, horizontally spread FEs have not yet colonized the leaf. As the leaf ages, FE succession occurs, and some FE species can be outcompeted and displaced by more competitive FEs that act as antagonists (Unterseher et al., 2007). It is reasonable to assume that the sampled *C. rotundifolia* stem leaves were on average younger than the *B. vivipara* leaves, and so the effect of host plant identity cannot be decoupled from leaf age in this study. Also, the sampled leaves from *B. vivipara* were on average bigger than those of *C. rotundifolia*, which could indicate that the FE differences could simply reflect more plant tissue being sampled in the former host.

The root-associated fungal communities differ distinctly between the two host plants. While *B. vivipara* associates with many ECM fungi (Davey et al., 2015, Botnen et al., 2014, Yao et al., 2013), *C. rotundifolia* is associated with AM fungi (Wijesinghe et al., 2001). Earlier studies on grass species have found significant associations, both positive and negative, between AM fungi and FEs (Victoria Novas et al., 2008, Liu et al., 2011). If the same is the case for *C. rotundifolia,* the difference in root-associated fungi between the two host plants could in part explain the difference in foliage-associated fungi. ECM fungi have previously been linked to structuring of bacterial foliar endophyte communities (Rúa et al., 2016), and though the underlying process of this interaction is not well understood, a similar interaction between ECM fungi and FEs could be possible.

In this study, 78% of all OTUs occurred in one host, but when including only OTUs that occurred in more than one site, this number fell to 48%. In the culture-based studies of Gange *et al.* (2007) and Seena and Sridhar (2004), only 30-39% of the OTUs were exclusive to a single host plant, and of these, many were found in only one sample. It is difficult to determine whether these fungi

are both host specific and rare, or if they appear host specific only because they are rare. When examining the top 10% of OTUs by sample occupancy, only 16% were exclusive to one host plant. However, although the remaining OTUs occurred in both hosts, they had on average 9.2 times higher rates of occurrence in one of the hosts. While there is no host exclusivity in this case, there is a clear host preference. This is also observed when identifying host-specific OTUs as those who surpass a 95% confidence interval for a null model of no host specificity (Figure 4). By this definition only 32 OTUs (13%) meet the criteria, as most rare OTUs do not have enough occurrences to be significantly different from the null model. All in all, only a small proportion of OTUs are indisputably host specific. As Sapkota et al. (2015) so eloquently put it: the host-specific fungi seem to be living in a “sea” of generalists.

Climate effects

While FE community richness clearly was affected by host plant identity, there was no clear relationship between climate and FE richness (Supplementary table S7). Though some of the climatic variables, such as long-term variation of precipitation, were included in several of the stepwise selected best-fit GLMs for different richness measures, these variables were not consistently significant. This indicates their predictive power is dubious at best, and the relationship between the temperature and precipitation gradients and FE richness is likely weak. Previous studies have consistently identified links between FE community richness and precipitation levels, where higher richness is predominantly found in sites with more rainfall (Giauque and Hawkes, 2016, Giauque and Hawkes, 2013, Zimmerman and Vitousek, 2012, Suryanarayanan et al., 2002). Effects of temperature on FE richness is not as clear, and studies linking FE richness with a disentangled effect of temperature are lacking. A common proxy for temperature in ecological studies is elevation (McCain and Grytnes, 2010). The current study has likewise looked at temperature gradients created by steep elevational gradients, and although other site-related differences have been minimized, effects of elevation and temperature cannot be readily disentangled. Fungal diversity across elevational-temperature gradients has been investigated in a variety of plants with contrasting findings. Davey et al. (2013) looked at boreal and alpine moss associated fungi across such a gradient, and found similar richness effects by host identity, but no clear effect by elevation. Likewise, Meier et al. (2010) found no relationship between saprotroph richness and elevation over a 1900 meter altitudinal gradient in a Peruvian

tropical rainforest. Other studies report different results: Yang et al. (2016) found a significant increase in alpha diversity of birch-associated FE communities with increasing altitude in a mountainous area in China. Rojas-Jimenez et al. (2016) and Zimmerman and Vitousek (2012) both found significant decreases in richness with increasing altitude in tropical leaf FE communities in Costa Rica and Hawaii. These conflicting results suggest there may not be a global pattern between elevation and FE richness. Variables like precipitation, growing season length, and edaphic properties vary differently in regards to elevation at different latitudes (Barry, 1992, McCain and Grytnes, 2010), which could in part explain why there is no consensus response of FE communities to elevation.

Though the relationship between FE richness and environmental gradients seems variable and inconsistent, a reliable and clear FE species turnover occurs across environmental gradients and has been documented in multiple cases (Rojas-Jimenez et al., 2016, Zimmerman and Vitousek, 2012), including this study. Structuring of the *B. vivipara* and *C. rotundifolia* FE communities by climatic variables (e.g. temperature and precipitation levels) in the absence of an accompanying change in richness demonstrates (Supplementary figures 8, 9 and 10) that FE species turn over and are replaced along the climatic gradients (Figure 5). Structuring of community composition by climate has been observed not only in FEs (Zimmerman and Vitousek, 2012, Giauque and Hawkes, 2016), but also in ECM communities (Tedersoo et al., 2012, Miyamoto et al., 2014) and saprotrophic communities (Meier et al., 2010).

A variety of interacting underlying processes likely contribute to FE communities being structured by climate, including variability in FE ecology, host plant ecotypes, and host plant biochemical responses to climate. The FE communities are diverse, and include taxa with varying ecologies and life histories (Rodriguez et al., 2009). Changes in moisture availability and temperature have a range of effects on different fungal taxa, and do not necessarily affect the fungal taxa in the same direction (Ayerst, 1969). Hence different environments will select for different compositions of FE communities.

It has been known for a long time that differing environments can select for different genetic ecotypes of plants (Turesson, 1922). Host plants with variable morphologies, large ranges and wide ecological niches, such as *B. vivipara* and *C. rotundifolia*, can be expected to be genetically and morphologically diverse. Indeed, both these plants have high genetic and morphological

diversity that correlates with local environmental conditions (Böcher, 1936, Bauert, 1996, Diggle et al., 1998). Differing genotypes of host plants can affect FE community composition (Elamo et al., 1999), hence the presumed climatically structured genetic diversity of the host plants could partly explain the climatic structuring of FE communities. As previously discussed, the manner in which different genotypes of host plants affects FEs can be both biochemical and morphological. *B. vivipara* has been shown to produce different amounts of flavonoids depending on local environmental conditions such as soil moisture and sun exposure (Vysochina and Voronkova, 2013). Leaf morphology of host plants also clearly differ between sites (Guittar et al., 2016), where warmer sites are associated with bigger leaves while colder sites are associated with smaller leaves. In this way, both biochemistry and morphology may mediate selection by climate on FE communities.

Growing season length is highly variable between the study sites; while the snow had melted in some sites in early May other sites were snow-free only as late as mid-June. Though growing season length can be viewed as a separate gradient, it is largely a product of an interaction between the temperature and precipitation levels at each site, and both variables were highly correlated with growing season length (Supplementary figure S4). As discussed above, FE community composition is linked with leaf age (Espinosa-Garcia and Langenheim, 1990, Arnold et al., 2003, Unterseher et al., 2007). Furthermore, most FEs spread horizontally in non-graminoid plant species (Rodriguez et al., 2009), meaning that, with the exception of a few 'Class 2' endophytes (Rodriguez et al., 2009) capable of vertical transmission from rhizome to leaf, FE species must colonize emerging leaves after every winter. In addition, there is a succession of FE colonization and turnover during the course of each growing season (Jumpponen and Jones, 2010). As a result, FE community composition is affected by growing season length by proxy of leaf age. In my study, all sampling took place within a few days, meaning that the effective growing season length of the sites varied widely (71 to 143 days). Thus, this thesis cannot fully decouple climatic variables from effects of leaf age and successional stage of FE communities. However, growing season length was not related to FE richness in the analyses, suggesting that all sites were sampled late enough in the season so that initial colonization and FE species accumulation phases had passed and only later-stage successional communities were represented. Both temperature and precipitation were more reliable predictors of FE richness than growing

season length in itself, suggesting that there may be effects of these climatic variables on richness and community composition that cannot be explained simply by growing season length.

A more subtle evolutionary explanation for structuring of FE communities by climate lies in the mutualistic partnership some FEs have with their host plants. There might be a selection for successful plant-FE mutualisms where the FE can help its host survive in extreme environments. FEs have demonstrated an ability to keep their hosts alive in experimental environments that killed FE-free control plants (Redman et al., 2002, Rodriguez et al., 2008). Additionally, in an experimental common garden setting that used FEs from grasses across a wide precipitation gradient in Texas, FEs from drier sites were found to reduce water loss to a greater extent than FEs from wetter sites (Giauque and Hawkes, 2013). However, the effect was only slight, and the authors noted that the observed FE species sorting presumably would be even more diminutive in less extreme environments. In non-graminoid plants, only a small group of FEs are expected to be mutualists of this type (e.g. Class 2 endophytes; Rodriguez et al., 2009). For this group of FEs, some of the observed climatic structuring may be due to optimizations of local mutualistic FE communities. All things considered, a mixture of factors likely contribute to the climatic structuring of FE communities: direct selection on FEs with different life histories, indirect selection mediated by both physiological and biochemical host plant responses, and selection of mutualistic relationships that allow both the host and the fungal symbiont to thrive in harsh environmental settings.

Relative effects of host, climate and unknown factors

Variance partitioning analysis demonstrates that FE community composition was structured equally by host and the combined effects of temperature and precipitation, although more than 90% of the variance remained unexplained. This contrasts with studies on other fungal guilds, such as ECM and AM fungi, where host is generally found to explain substantially more variation than environmental variables. For instance, Martínez-García et al. (2015) could only attribute 6% of AM fungal community variance to a wide site-age-precipitation gradient, while 29% of variation could be attributed to host. Similarly, Davey et al. (2013) found clear effects of bryophyte host identity on both richness and composition of the associated endophytic fungal communities, but a much weaker effect of an elevation-temperature gradient. ECM fungi seem to

follow the same pattern of strong host preferences (Tedersoo et al., 2008, Ishida et al., 2007), but see Sapkota et al. (2015) where effect of host on fungal community composition was clearly secondary to spatial structuring effects.

Though many studies suggest that host identity is the primary factor structuring fungal communities, my thesis suggests that, at least for herbaceous FE communities, environmental variables such as temperature and precipitation may play an equally important role. The assortment of environmental variables used in this thesis was far from complete, and with over 90% of FE community structuring still unexplained, there is a good chance that there are other environmental factors significantly contributing to FE community structure. Effects of spatial structuring may be one of those factors, as it has been shown to significantly structure FE communities in both herbs and trees (Gange et al., 2007, Arnold et al., 2003). Another environmental factor influencing FE communities could be exposure to solar radiation, either by directly affecting the FEs (Dadachova et al., 2007) or by proxy via effects on host plant biochemistry (Vysochina and Voronkova, 2013). Though the degree of solar radiation undoubtedly is correlated with elevation in the study sites, other aspects like amount of cover by clouds, trees and surrounding ground level vegetation also would affect the amount of solar exposure for the host plants.

Furthermore, there was very little overlap between effects of host and climate ($r^2 = 0.0026$). This indicates that host identity and climatic variables explain more or less separate subsets of the variation, meaning that the FE communities respond to climate in a similar way regardless of host plant identity. A larger overlap has been found in a study on AM fungi along an environmental gradient (Martínez-García et al., 2015), though this overlap could largely be explained by a failure of the study design to disentangle host identity from the environmental gradient. More research is needed on a wider range of hosts and environmental gradients to better determine if host-specific climate effects also occur among FE communities.

Both effects of host identity and environment have been investigated, but biotic interactions between FEs is a process shaping FE communities that has not been explored in this thesis. One consequence of such interactions is the already discussed effect of leaf age on FE community composition, where there is a succession of FEs during each growing season (Unterseher et al., 2007). This succession is in part due to biotic interaction between FEs, where FEs by means such

as production of antifungal compounds can act as antagonists to each other, and effectively either outcompete already established FEs, or hinder new FEs from establishing (Arnold et al., 2003). Together with effects of host identity and abiotic factors, these biotic interactions within the FE community likely also play a role in shaping the FE species compositions. In total, these three groups of factors interact to shape the plethora of diversity in the observed FE communities. Further research looking to unveil the effect of biotic interactions on FE communities could explore co-occurrence patterns between FEs to identify potential antagonistic or mutualistic relationships.

Technical remarks

The most evident limitation of this study is that out of the original 17 938 761 reads generated by the sequencing run, only 101 046 were usable for the FE community analyses. Two separate factors likely played a role in this: tag switching and primer bias. Tag switching has been described as amplicons ending up with different tags than those they were originally assigned (Schnell et al., 2015). Over 58% (11 359 802) of the reads in this study were filtered out during demultiplexing (Supplementary table S2), which effectively removes any read with non-matching forward and reverse primer tags. Since all samples in the run were initially tagged with matching pairs, this suggests that there has been a large amount of tag switching occurring. The rate of tag switching has been estimated at below 3% in previous studies (Schnell et al., 2015, Carlsen et al., 2012), but in the current thesis the rate was much higher. The mechanisms behind tag switching are only partially understood and documented (Illumina, 2017). Measures to control for it, such as tagging at both ends of amplicons, allows the filtering out of affected sequences during the bioinformatics pipeline (Schnell et al., 2015). Primer bias also significantly reduced the number of usable sequences in this study, i.e. the tendency of a primer to amplify certain taxonomic groups in relatively higher proportions than others. An additional 35% (6 467 204) of the reads were lost when OTUs identified as plants or other non-fungal taxa were removed. The primers used in this study, gITS7 and ITS4, have been successfully used for amplifying fungal DNA from soils, which are relatively enriched in fungal biomass (Ihrmark et al., 2012). However, the gITS7 primer lacks specificity for amplifying fungal DNA in leaf and root plant samples (Ihrmark et al., 2012) where host plant DNA is present in much higher proportions. In these cases, a bias towards plant DNA is created, explaining why in this leaf-based study a large portion of taxonomically

assigned sequences were plants (60% of OTUs, 6 395 252 reads). This primer bias, together with tag switching, means the depth of sequencing in different samples was varied and inconsistent. As a result, most samples were not sequenced to saturation, and the final dataset was consequently much smaller than it could have been. Nevertheless, 101 046 reads were sufficient to reach full FE community saturation in both hosts (Figure 3), and all but one of the 12 sites were sequenced deeply enough to be included in the statistical analyses. Even with more than 99% of the original reads unusable, amplicon sequencing provides amounts of data so large that some conclusions about effects of host identity and climatic variables could still be made.

5 Conclusion

This study shows that, despite significant amounts of tag switching and non-target amplification that resulted in irregular per-sample sequencing depth, the biological signal of climate and host effects on FE communities was still readily detectable in this dataset. Both host identity and climatic variables structure FE communities in two species of herbaceous host plants. Even though richness was much higher in one of the host plants, community composition seems to structurally respond in the same way to climate regardless of host plant identity, indicating similar patterns of turnover are occurring within each community. Among the measured climatic variables, long-term mean air temperature and precipitation significantly structured the FE communities, while other measures of temperature, such as soil temperature and ground-level air temperature, were not significant. With regards to FE community richness, only host identity was a reliably significant variable, suggesting that the FE communities do not significantly differ in richness between climatically different sites, though there is a species turnover creating community structure across those sites. For Scandinavia, warmer and wetter weather is on the horizon as a result of global climate change, and as a result FE communities can be expected to be impacted.

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Supplementary

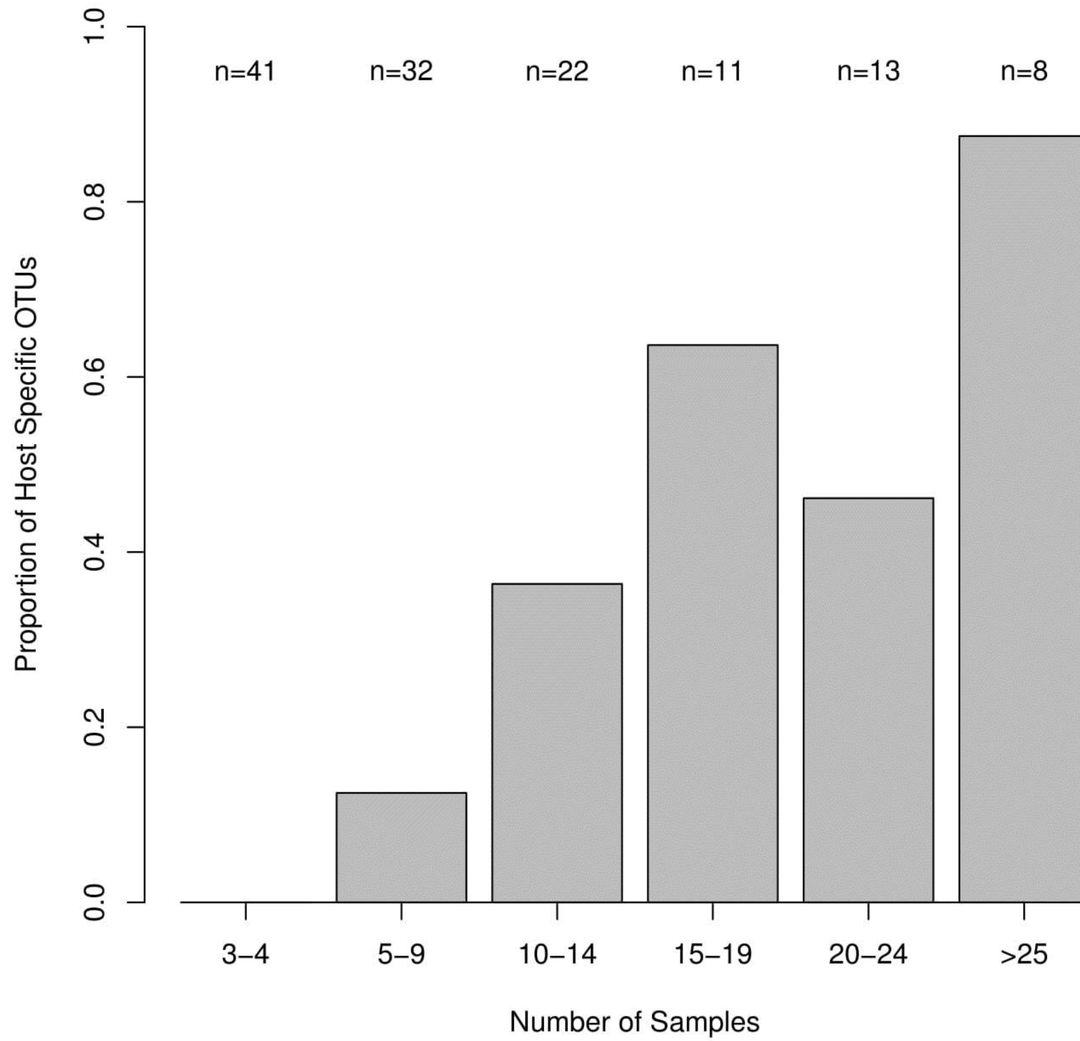

Figure S1. Barplot showing the proportion of host specific OTUs in six categories of OTUs occurring with increasing frequency among the samples. Sample size for each category is indicated above the bar.

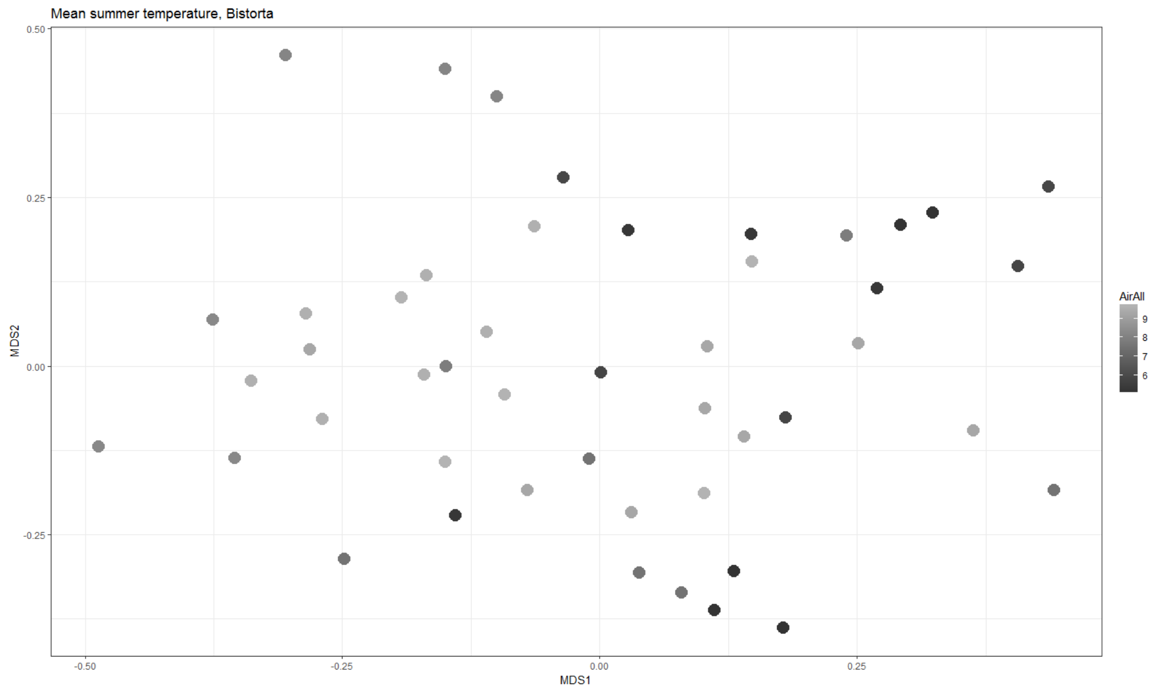

Figure S2. NMDS ordination of *Bistorta vivipara*-samples, colored by mean growing season temperature. Darker colored samples come from colder sites.

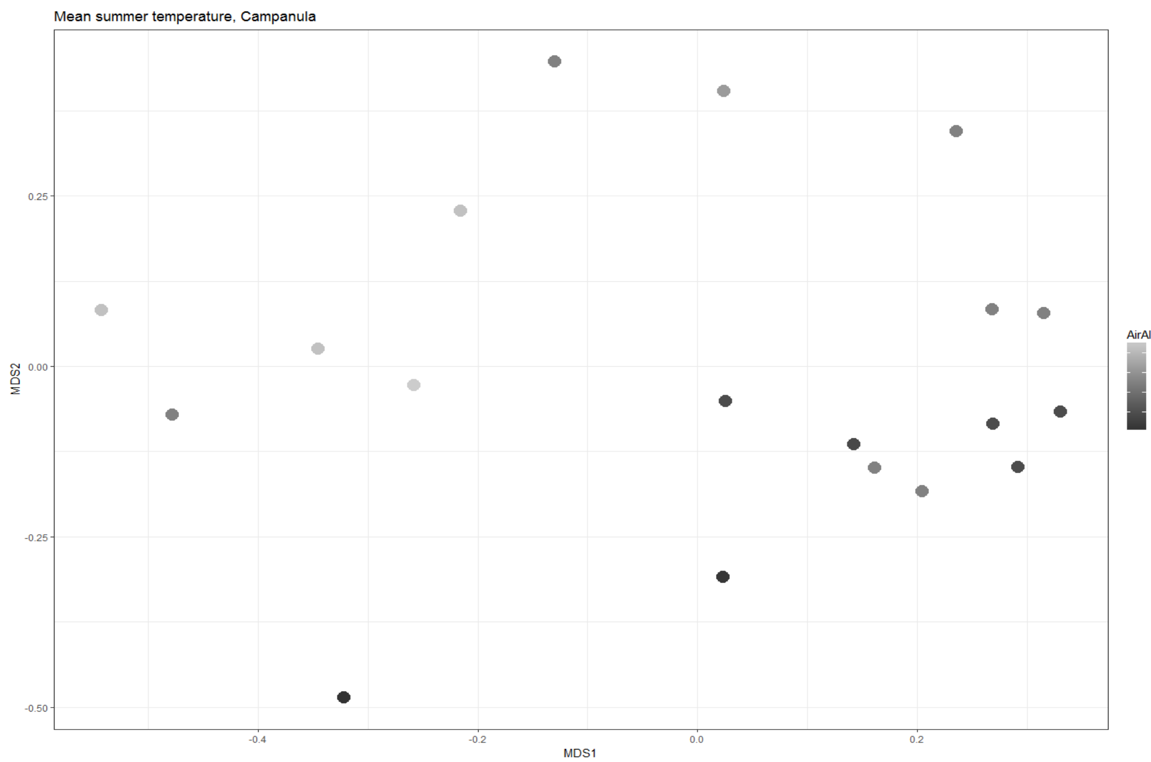

Figure S3. NMDS ordination of *Campanula rotundifolia*-samples, colored by mean growing season temperature. Darker colored samples come from colder sites.

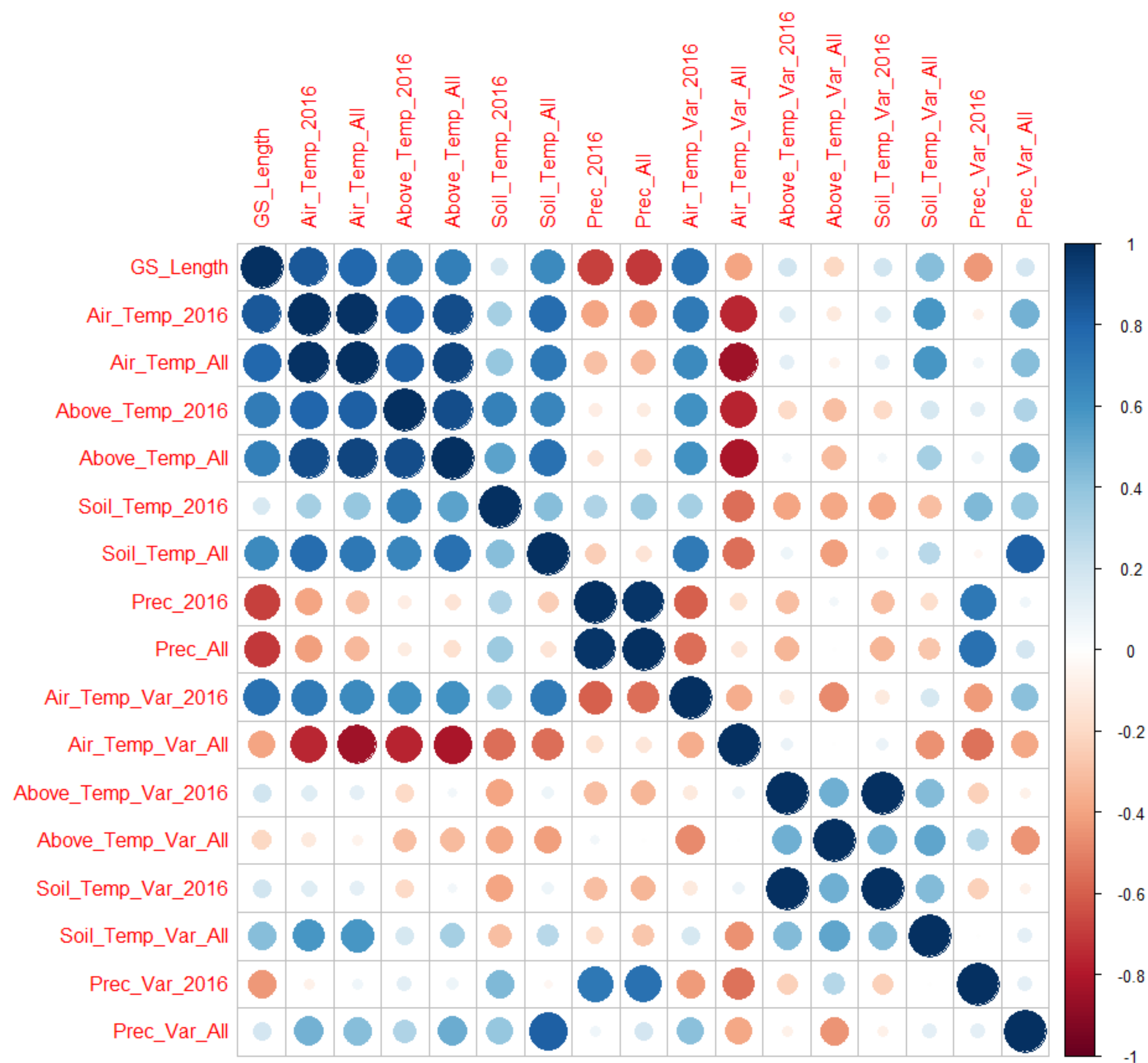

Figure S4. Correlation plot between all climatic variables. Bigger and darker dots indicate stronger correlations. Blue dots correlate positively, red dots correlate negatively. White space indicates no correlation.

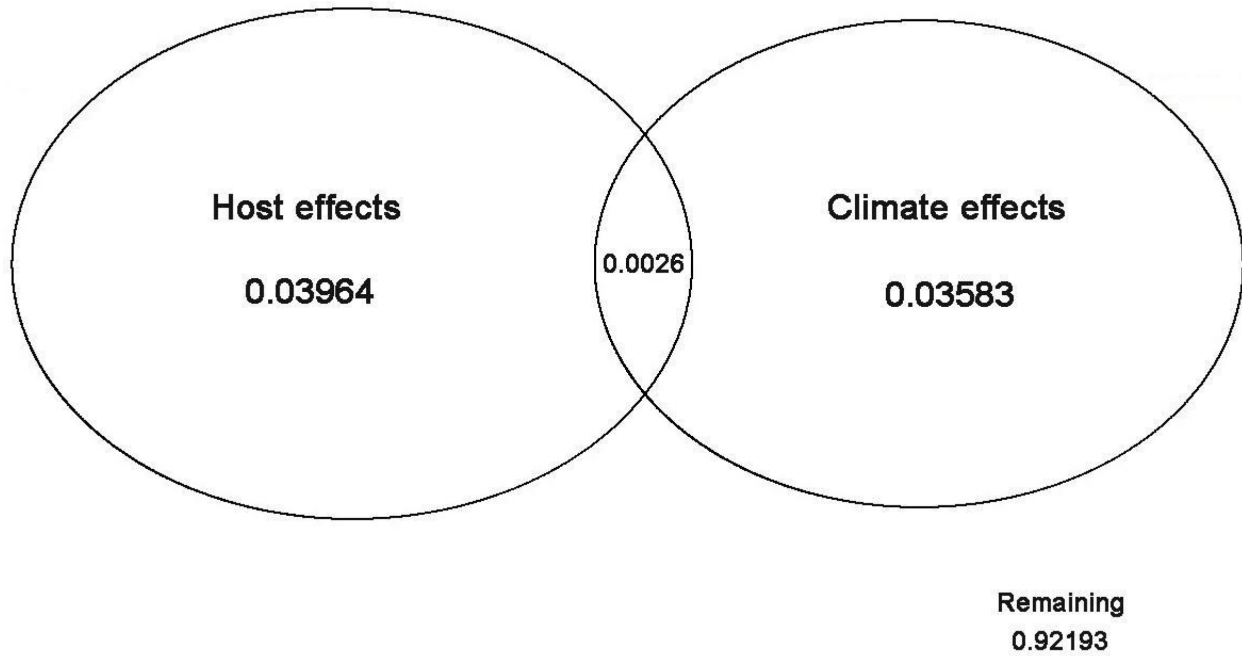

Figure S5. Partitioning of variation in community composition explained by host identity and climate (Air_Temp_2016 + Prec_All + Prec_Var_All).

Table S1. Sequences for the unique identifier tags for forward and reverse primers used in library construction.

Tag number	ITS4 tag	gITS7 tag
1	NNAACAAC	NAACAAC
2	NNNAACCGA	NNAACCGA
3	NCCGGAA	NNNCCGGAA
4	NNAGTGTT	NAGTGTT
5	NNNCCGCTG	NNCCGCTG
6	NAACGCG	NNNAACGCG
7	NNGGCTAC	NGGCTAC
8	NNNTTCTCG	NNTTCTCG
9	NTCACTC	NNNTCACTC
10	NNGAACTA	NGAACTA
11	NNNCCGTCC	NNCCGTCC
12	NAAGACA	NNNAAGACA
13	NNCGTGCG	NCGTGCG
14	NNNGGTAAG	NNGGTAAG
15	NATAATT	NNNATAATT
16	NNCGTCAC	NCGTCAC
17	NNNTTGAGT	NNTTGAGT
18	NAAGCAG	NNNAAGCAG
19	NNTTGCAA	NTTGCAA
20	NNNCACGTA	NNCACGTA
21	NTAACAT	NNNTAACAT
22	NNTGCGTG	NTGCGTG
23	NNNGGTCGA	NNGGTCGA
24	NCACTCT	NNNCACTCT
25	NNCTTGGT	NCTTGGT
26	NNNTCCAGC	NNTCCAGC
27	NACTTCA	NNNACTTCA
28	NNGCGAGA	NGCGAGA
29	NNNTGGAAC	NNTGGAAC
30	NGTACAC	NNNGTACAC
31	NNAAGTGT	NAAGTGT
32	NNNTCTTGG	NNTCTTGG
33	NAAGGTC	NNNAAGGTC
34	NNGGCGCA	NGGCGCA
35	NNNTCGACG	NNTCGACG
36	NCCTGTC	NNNCCTGTC
37	NNAGAAGA	NAGAAGA
38	NNNAATAGG	NNAATAGG
39	NGGTTCT	NNNGGTTCT
40	NNTAATGA	NTAATGA
41	NNNGTAACA	NNGTAACA
42	NAATCCT	NNNAATCCT
43	NNNAGACCG	NAGACCG
44	NTGGCGG	NNNTGGCGG
45	NNCTATAA	NCTATAA
46	NNNAATGAA	NNAATGAA
47	NCGAATC	NNNCGAATC
48	NNAGAGAC	NAGAGAC
49	NNNTTCGGA	NNTTCGGA
50	NCGACGT	NNNCGACGT
51	NNNCTCATG	NCTCATG
52	NTGTATA	NNNTGTATA
53	NNACAACC	NACAACC
54	NNNTCAGAG	NNTCAGAG
55	NGTAGTG	NNNGTAGTG
56	NNAGCACT	NAGCACT
57	NNNGCGGTT	NNGCGGTT
58	NACACAA	NNNACACAA
59	NNNGCTCCG	NGCTCCG
60	NTACTTC	NNNTACTTC
61	NNGTTGCC	NGTTGCC
62	NNNGTATGT	NNGTATGT
63	NGTCAAT	NNNGTCAAT
64	NNAGCCTC	NAGCCTC
65	NNNTCGTTA	NNTCGTTA
66	NTGTGGC	NNNTGTGGC
67	NNNCTCTGC	NCTCTGC
68	NATGGAT	NNNATGGAT
69	NNACAGGT	NACAGGT
70	NNNTCCGCT	NNTCCGCT
71	NGTCCGG	NNNGTCCGG
72	NNCATTAG	NCATTAG
73	NNNGAAGCT	NNGAAGCT
74	NGATATT	NNNGATATT
75	NNNAGCTGG	NAGCTGG
76	NCGCGAT	NNNCGCGAT
77	NNACATTG	NACATTG
78	NNNCCAAGG	NNCCAAGG
79	NACCATA	NNNACCATA
80	NNAGGATG	NAGGATG
81	NNNGTCTTA	NNGTCTTA
82	NTATACC	NNNTATACC
83	NNNACCTAT	NACCTAT
84	NAGGTAA	NNNAGGTAA
85	NNATTCTA	NATTCTA
86	NNNGTGATC	NNGTGATC
87	NGACGGC	NNNGACGGC
88	NNGTGCCT	NGTGCCT
89	NNNTATCTG	NNTATCTG
90	NCGGCCA	NNNCGGCCA
91	NNNCCTAAT	NCCTAAT
92	NACGCGC	NNNACGCGC
93	NNGTGTAG	NGTGTAG
94	NNNTTCCTT	NNTTCCTT
95	NCAGAGC	NNNCAGAGC
96	NNTGATCC	NTGATCC

Table S2. Climatic variables for all sites.

Site	GS_Length*	Air_Temp_2016**	Air_Temp_All**	Above_Temp_2016***	Above_Temp_All***	Soil_Temp_2016***	Soil_Temp_All***	Prec_2016**	Prec_All**	Air_Temp_Var_2016****	Air_Temp_Var_All****	Above_Temp_Var_2016****	Above_Temp_Var_All****	Soil_Temp_Var_2016****	Soil_Temp_Var_All****	Prec_Var_2016****	Prec_Var_All****
ULV	96	7.04879	5.12434	9.08464	8.47910	9.74878	8.81949	2.95853	3.80349	2.89312	1.41803	3.06025	2.71880	3.95912	3.35887	3.03441	2.20086
LAV	86	7.21462	5.83960	9.17739	8.93305	8.91306	8.60915	4.34121	4.68118	3.21859	1.81291	3.72019	2.79539	5.70630	3.05113	2.87117	2.27850
GUD	97	6.61448	5.27001	9.88367	9.50472	9.67311	8.96255	5.89509	5.95565	2.97486	1.66533	2.82314	2.80205	3.36594	3.19039	3.03441	2.40605
SKJ	71	7.43091	5.91714	8.81312	8.85362	9.48970	9.38823	6.97132	8.54158	3.65703	1.99381	2.89157	2.58283	3.30656	2.87227	2.09271	1.88987
ALR	115	9.65411	7.49804	10.9025	10.1905	10.2541	9.80985	3.25279	4.06234	4.37617	2.02619	3.59284	3.11759	3.52331	3.20193	3.94500	1.88035
HOG	121	9.80438	8.23820	10.3691	9.51652	10.3008	9.31801	3.57228	3.85014	4.60508	2.48958	3.58867	2.74981	3.18919	2.89888	2.77251	2.24006
RAM	112	9.59491	8.13994	11.2681	10.3430	10.7076	9.88830	3.42591	4.72011	4.95265	2.80023	3.39884	2.82916	4.05458	3.21778	1.93904	1.97903
VES	92	9.23862	7.84014	10.1180	10.4426	10.5659	9.68715	5.08174	6.18455	5.16084	2.55106	3.21888	3.22043	2.96015	3.31484	2.54481	1.83649
FAU	143	11.4878	9.24633	10.3474	10.5030	9.28961	10.0930	2.24868	2.78930	4.58997	2.32928	2.60854	2.86566	2.26706	2.73033	3.90412	1.83969
VIK	121	11.1991	9.54425	11.0324	10.6418	9.79537	9.37732	3.16414	3.44902	5.33369	2.82016	2.76045	2.58557	2.92139	2.74370	2.75921	2.19545
ARH	124	11.5055	9.64509	12.1102	10.6923	10.6291	10.0898	6.50537	7.86811	6.21200	2.91818	3.23495	2.76898	3.05138	2.91547	2.09062	1.85872
OVS	112	11.3336	9.80329	12.0564	11.1112	12.6051	9.87048	9.54193	10.2677	6.37583	3.27341	3.84122	3.06238	4.45611	3.00099	1.52137	1.72926

*Number of days from continuous >3C ground level temperature until sampling.

**Mean of monthly values during growing season.

***Mean of daily values during growing season.

****Standard deviation/mean.

Table S3. OTU and read count at each major step of the bioinformatics pipeline.

Step	#reads	#OTUs
Raw reads	17 938 761	-
Error correction	17 929 303	-
Demultiplexing	7 521 448	-
Pairing	7 514 636	-
Quality filtering and dereplication	6 607 173	-
Chimera filtering	6 578 959	-
Clustering and taxonomic assignment	6 578 959	3 851
Taxonomic filtering (keeping fungi)	111 755	968
Removing OTUs with <10 reads	109 213	278
Removing contaminants and samples with <100 reads	101 046	245

Table S4. Abundance and BLAST-information for OTUs that were deemed contaminants or otherwise non-OTUs.

OTU_#	Taxonomic ID	GenBank accession No.	% identity	% coverage	#reads
OTU_60	*Malassezia restricta*	AY743636	99.138	79	737
OTU_64	*Malassezia restricta*	KU164491	98.034	85	337
OTU_68	*Exidiaceae* sp.	HG936633	99.254	81	1965
OTU_69	*Malassezia restricta*	AY743636	99.427	85	760
OTU_71	*Malassezia globosa*	AY387134	98.087	85	366
OTU_72	*Exidiaceae* sp.	HG936633	99.254	81	2104
OTU_78	*Malassezia globosa*	AY387134	98.087	85	371
OTU_88	*Malassezia restricta*	KU164491	98.034	85	273
OTU_168	*Polyporales* sp.	JQ031127	100.00	81	353
OTU_169	*Polyporales* sp.	JQ031127	99.273	81	291
OTU_254	*Leucocybe connata*	UDB015787	99.254	81	16
OTU_264	*Agaricus bisporus*	UDB011831	99.278	81	49
OTU_272	*Agaricus bisporus*	UDB011831	99.278	81	13
OTU_327	*Flagelloscypha* sp.	JF424289	89.375	81	59
OTU_329	*Flagelloscypha* sp.	JF424289	89.062	81	12
OTU_340	*Naganishia liquefaciens*	AF444345	99.660	83	59
OTU_387	*Tylospora fibrillosa*	AB848700	96.786	85	14
OTU_394	*Clitocybe nebularis*	UDB023648	99.286	82	52
OTU_395	*Clitocybe nebularis*	UDB023648	99.643	82	40
OTU_421	*Mycena cinerella*	GU234146	98.969	82	22
OTU_604	*Trechisporales* sp.	JF691234	98.929	82	15
OTU_605	*Trechisporales* sp.	JF691234	98.571	82	16
OTU_985	*Mycena cinerella*	GU234146	99.313	82	25
OTU_987	*Leucocybe connata*	UDB015787	99.627	81	14
OTU_1479	*Antrodia serialis*	JQ700271	99.631	81	18
OTU_1679	*Tubulicrinis borealis*	UDB024795	87.770	76	11
OTU_2103	*Naganishia liquefaciens*	AF444345	99.661	83	48
OTU_2223	*Mycena haematopus*	KU518323	98.246	82	14
OTU_3109	*Malassezia* sp.	FR682163	98.315	85	43

Table S5. Top 20 most abundant OTUs by numbers of reads, with host preference, occurrences and details on each BLAST hit. Host preference was determined by testing the number of occurrences in a single host against a 95% confidence interval limit for a null model of no host preference.

OTU_#	**Abundance (#reads)**	**Taxonomic ID**	**GenBank accession No.**	**% identity**	**% coverage**	**Number of sites (with >250 reads)**	**Number of sites (with >0 reads)**	**Host Preference**
OTU_6	9620	*Coleosporium tussilaginis*	KP017555	98.442	88	2	5	*C. rotundifolia*
OTU_5	8812	*Coleosporium tussilaginis*	KP017555	98.442	88	2	5	*C. rotundifolia*
OTU_7	5851	*Paraleptosphaeria dryadis*	AF439461	96.311	84	3	9	*B. vivipara*
OTU_12	5535	*Stromatinia rapulum*	FJ231405	98.182	77	1	2	-
OTU_18	5062	*Stromatinia rapulum*	FJ231405	98.182	77	1	1	-
OTU_13	4987	*Paraleptosphaeria dryadis*	AF439461	96.311	84	2	8	*B. vivipara*
OTU_28	4697	*Rhynchosporium agropyri*	HM627479	92.857	83	2	8	*B. vivipara*
OTU_15	4660	*Rhynchosporium agropyri*	HM627479	92.857	83	2	8	*B. vivipara*
OTU_35	2612	*Itersonilia perplexans*	AB072233	98.071	84	3	6	*B. vivipara*
OTU_40	2360	*Itersonilia perplexans*	AB072233	98.071	84	2	5	*B. vivipara*
OTU_72	2104	*Exidiaceae* sp.	HG936633	99.254	81	1	1	-
OTU_112	2041	*Colletotrichum destructivum*	AJ301942	99.567	79	2	7	*B. vivipara*
OTU_114	2014	*Colletotrichum antirrhinicola*	KM105180	100.000	79	2	6	*B. vivipara*
OTU_68	1965	*Exidiaceae* sp.	HG936633	99.254	81	1	1	-
OTU_45	1875	*Colletotrichum tanaceti*	JX218228	99.138	79	3	6	-
OTU_41	1823	*Colletotrichum tanaceti*	JX218228	99.138	79	3	6	-
OTU_29*	1670	*Mycosphaerella tassiana*	EF679363	99.103	78	2	9	-
OTU_21*	1581	*Mycosphaerella tassiana*	EF679363	99.103	78	2	10	-
OTU_24	1253	*Gibberella avenacea*	FJ602996	96.887	86	2	4	-
OTU_16	1204	*Gibberella avenacea*	FJ602996	96.887	86	2	4	-

*OTU was recovered in 2 of 23 negatives, at abundances <50 reads. These instances were treated as presumed index hopping and the OTUs were retained in our analyses, as they did not occur across all negatives as would be expected in a case of systemic contamination.

Table S6. P-values and r^2-values for all variables when fitted against an NMDS of all samples.

p-values (r^2-values)	2016	All years	Variability, 2016	Variability, all years
Climatic air temperature	0.178 (0.0477)	0.095 (0.0644)	0.083 (0.0686)	**0.003 (0.1422)**
Ground level air temperature	0.074 (0.0746)	0.235 (0.0404)	0.920 (0.0023)	0.261 (0.0357)
Soil temperature	**0.005 (0.1308)**	0.274 (0.0329)	0.920 (0.0023)	0.115 (0.0588)
Precipitation	0.758 (0.0078)	0.780 (0.0072)	**0.015 (0.1082)**	0.334 (0.0292)
Growing season length	0.730 (0.0087)	-	-	-
Location	**0.002 (0.2937)**	-	-	-
Host identity	**0.001 (0.3470)**	-	-	-

Table S7. Results from forward-backward selection of GLM models for predicting different measures of FE richness (columns). Included variables in each model are colored gray, significant variables have p-values included. Each model was selected from either the "2016" pool of variables, or the "All" (2008-2016) pool of variables, for predicting richness as measured by either OTU counts, Shannon's or Simpsons diversity indices. Both raw measures of these indices, as well as iNEXT-derived estimates, were used separately. Air_Temp_X is climatic air temperature, Above_Temp_X is ground level temperature, Soil_Temp_X is soil temperature, Prec_X is precipitation, while GS_Length is growing season length. X_All or X_2016 indicates if the variable is from "All years" (2008-2016) or from the year of sampling (2016). Var stands for the coefficient of variability.

	Shannon raw	Shannon estimate	Simpson raw	Simpson estimate	OTU count raw	OTU count estimate
Air_Temp_All						
Air_Temp_Var_All					0.008912	
Above_Temp_All	0.018709				0.009471	
Above_Temp_Var_All						
Prec_All						0.01466
Prec_Var_All	0.027082	0.02291		0.04122		
Soil_Temp_All						
Soil_Temp_Var_All					0.005636	0.02857
Host_Taxon	0.000454	0.00297	0.000415	0.00787	0.000729	0.00727
GS_Length			0.045458			
Air_Temp_2016					0.044460	
Air_Temp_Var_2016		0.04183				
Above_Temp_2016						
Above_Temp_Var_2016						
Prec_2016						
Prec_Var_2016					0.007278	0.008821
Soil_Temp_2016						
Soil_Temp_Var_2016						
Host_Taxon	0.00412	0.00927	0.000528	0.0186	0.000843	0.004829

Table S8. P-values and r^2-values for all variables when fitted against an NMDS of *Bistorta vivipara*-samples.

p-values (r^2-values)	**2016**	**All years**	**Variability, 2016**	**Variability, all years**
Climatic air temperature	**0.009 (0.1829)**	**0.002 (0.2549)**	**0.024 (0.1601)**	**0.001 (0.4823)**
Ground level air temperature	**0.001 (0.2667)**	**0.009 (0.1885)**	0.596 (0.0221)	0.082 (0.0991)
Soil temperature	**0.025 (0.1502)**	0.407 (0.0399)	0.596 (0.0221)	**0.047 (0.1285)**
Precipitation	0.287 (0.0562)	0.199 (0.0736)	**0.001 (0.3493)**	0.827 (0.0088)
Growing season length	**0.038 (0.1318)**	-	-	-
Location	**0.001 (0.5954)**	-	-	-

Table S9. P-values and r^2-values for all variables when fitted against an NMDS of *Campanula rotundifolia*-samples.

p-values (r^2-values)	**2016**	**All years**	**Variability, 2016**	**Variability, all years**
Climatic air temperature	**0.003 (0.5389)**	**0.002 (0.5403)**	**0.018 (0.3817)**	0.106 (0.2431)
Ground level air temperature	0.067 (0.2643)	**0.021 (0.3709)**	0.157 (0.1990)	0.415 (0.1034)
Soil temperature	0.442 (0.0953)	**0.048 (0.3236)**	0.157 (0.1990)	0.401 (0.1048)
Precipitation	**0.006 (0.4909)**	**0.003 (0.5224)**	0.156 (0.2001)	0.402 (0.1025)
Growing season length	**0.003 (0.5223)**	-	-	-
Location	**0.020 (0.6005)**	-	-	-

Table S10. Significance of the fit of CCA models testing the structuring of FE community composition by climatic variables. An independent CCA model was generated and tested for each climatic variable with the variation attributed to host identity partialed out.

	2016	**All years**	**Variability, 2016**	**Variability, all years**
Climatic air temperature	**0.006**	**0.011**	0.108	0.081
Ground level air temperature	0.471	0.168	0.533	0.957
Soil temperature	0.696	0.372	0.549	0.184
Precipitation	**0.019**	**0.011**	**0.040**	**0.043**
Growing season length	**0.016**	-	-	-