High-throughput sequencing reveals diverse oomycete communities in oligotrophic peat bog micro-habitat

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Abstract:

Oomycete diversity has been generally underestimated, despite their ecological and economical importance. Surveying unexplored natural ecosystems with up-to-date molecular diversity tools can reveal the existence of unsuspected organisms. Here, we have explored the molecular diversity of five micro-habitats located in five different oligotrophic peat bogs in the Jura Mountains using a high-throughput sequencing approach (Illumina HiSeq 2500). We found a total of 34 different phylotypes distributed in all major oomycete clades, and comprising sequences affiliated to both well-known phylotypes and members of undescribed, basal clades. Parasitic species, including obligate forms were well-represented, and phylotypes related to highly damaging invasive pathogens (*Aphanomyces astaci*: X1100 and *Saprolegnia parasitica*: X1602) were retrieved. Microhabitats differed significantly in their community composition, and many phylotypes were strongly affiliated to free water habitats (pools). Our approach proved effective in screening oomycete diversity in studied habitat, and could be applied systematically to other environments and other fungal and fungal-like groups.

Key words: Oomycetes, Diversity, Metabarcoding, Illumina sequencing, V9 region of the SSU rDNA

Introduction

Oomycetes are a group of fungi-like stramenopiles which have a considerable impact on economy through the pathogenic action of certain members. Indeed, they are responsible for major diseases in crops (Abad et al., 2014), forests (Rizzo et al., 2005, Duran et al., 2010) and aquaculture (Phillips et al., 2008). Some emergent pathogens have been recently detected and are currently causing considerable losses in both agriculture (Kamoun et al., 2015) and fish farms (De la Bastide et al., 2015). In addition, oomycetes have an important impact on food webs through regulation of host populations (Duffy et al., 2015). However, their environmental diversity has been rarely studied specifically in natural ecosystems (Willoughby, 1962 and 1978), with the consequence that potential threats are detected only after disease outbreaks. There is, therefore, a need for environmental surveys, with a particular focus on understudied systems. Peat bogs and other acidic and oligotrophic environments have not been surveyed for oomycetes (Lara and Belbahri, 2011). Peatlands are heterogeneous ecosystems traditionally divided into five microhabitats (pools, lawns, hummocks, Fen/Transition and forest; Rydin and Jeglum, 2013). Indeed, the low nutrient amount present in these systems has been thought insufficient to support the growth of such osmotrophic organisms. However, an environmental survey of eukaryotic genetic diversity has revealed the presence of oomycetes in a pristine oligotrophic peatland located in the Swiss Jura Mountains (Lara et al., 2011). Sequences found in the course of that study appeared to be divergent and possibly represented novel clades at the genus level at least.

High-throughput sequencing approaches are increasingly applied to monitor environmental diversity of eukaryotic organisms (Amaral Zettler et al., 2009; Lentendu et al., 2013; De Vargas et al., 2015). The immense amount of data generated allows drawing a more comprehensive picture of environmental diversity than earlier cloning and Sanger sequencing approaches (Lecroq et al. 2011). These approaches revealed novel deep and divergent lineages, as well as an unsuspected diversity within each established eukaryotic supergroup (De Vargas et al., 2015). Our knowledge of fungal diversity has been also challenged by the application of high-throughput sequencing approaches, revealing ancient groups that await description and characterization (Buée et al., 2009).

Oomycetes, although not belonging to Opisthokonta like true fungi (Ben Ali et al., 2002), share a lot of common traits with fungi. Most of the species described have been classified within two informal groups, the peronosporalean and the saprolegnialean “galaxies” (Fuller and Jarowski, 1987). DNA based environmental surveys have shown that many more taxa, occupy basal positions within the oomycete tree; (Lara and Belbahri, 2011, Steciow et al., 2013 and 2014). The function of some of these organisms (obligate or facultative parasites; or strictly saprotrophic) has not been elucidated to date, and it is likely that they represent a novel diversity. By increasing the panel of surveyed environments, it is likely that novel organisms can be encountered. Peat bogs have, to date, never been investigated for oomycetes (Lara and Belbahri, 2011).

In this study, we applied a high-throughput sequencing approach (Illumina sequencing targeting the v9 region of the SSU rRNA gene) to characterize the environmental diversity of oomycetes in 65 samples from all micro-habitats types across five peatlands located in the Jura Mountains (Switzerland and France). We surveyed their total diversity to evaluate how much is known of their environmental diversity, and also their presence and abundance in the different microhabitats to assess their preferred habitats.

Materials and Methods

Studied areas and sampling strategy

The study was conducted in five *Sphagnum*-dominated peatlands located in the Jura Mountains (Switzerland and France). These sites are located at similar altitudes (900-1000m a.s.l.), climatic condition and geology. Five micro-habitats were selected (Fig 1) with contrasted vegetation and micro-topography and further characterised by chemical variables (nutrient content and pH). The vegetation composition was recorded at each sampling site in $1m^2$ plots. The central parts of the peatlands were ombrotrophic (i.e. rainwater fed) and open (i.e. no trees). Three of the microhabitats were located in the centre of the peatlands: hummocks (Fig 1A) are characterised by a mound micro-topography dominated by *Sphagnum fuscum*, low nutrient content and pH. Lawns (Fig 1B), are flat habitats characterized by *Sphagnum magellanicum* and *Eriophorum vaginatum*. Pools (Fig 1C), are depressions usually with standing water and dominated by *Sphagnum cuspidatum*. These three micro-habitats often lie close to each-other (Fig 1F) (Pouliot et al., 2011, Marcisz et al., 2014). In addition, we studied two other habitats in the periphery and margin of the peatlands: i) tall pine forests (Fig 1D) grown on thick peat but with lower water table levels and dominated by pine (*Pinus mugo subsp. uncinata)* and *Vaccinium* spp.; ii) poor fens in some cases with scattered birch trees, representing the buffer zone between the peatlands and the surrounding environments (in most cases hay meadows) (Fig 1E). Nutrient content and pH are typically lower in the bog centre and pine forest than in the surrounding fen but also decline from pool to hummock (Batzer and Baldwin, 2011) and this was indeed the case in our study (Table 1).

A total of 65 samples of *Sphagnum* spp. were collected using sterile equipment. To assess the intra-peatbog variation, five samples from each micro-habitat were taken in “Le Cachot” peatbog (coordinate: 47°0'19.99"N, 6°39'53.13"E). To test the variability among peatlands two samples of each micro-habitat were taken in four other peatlands (“Praz-Rodet” bog, 46°33'54.37"N, 6°10'20.37"E, “Pontins” bog 47°7'38.41"N, 6°59'20.63"E, “Etang de la Gruyère” peatbog 47°14'22.46"N, 7° 2'58.13"E and “Frasne” bog 46°49'51.45"N, 6° 9'34.58"E).

PCR and sequencing

Environmental DNA was extracted using a MoBio Power Soil™ DNA extraction kit (Carlsbad, CA USA) following the manufacturer’s instructions. PCR protocols targeting the SSU rRNA gene V9 region (amplicon size about 180bp) of eukaryotes was done according to Amaral-Zettler et al. (2009). Sequencing was performed with an Illumina’s HiSeq 2500, using V3 chemistry (Fasteris, Geneva, Switzerland).

Sequences processing

Quality check (Phred score filtering, elimination of reads without perfect forward and reverse primers, and chimera removal) of the sequences was performed following the pipeline developed by de Vargas et al. (2015). Sequences were grouped in phylotypes using the clustering algorithm swarm (Mahé et al., 2014). The curated and updated PR2 database (Guillou et al., 2013) was used to taxonomically assign the phylotypes after a selection of the V9 regions according to the primers used for the sequencing. Phylotypes were then assigned by aligning the dominant sequence of the phylotype to the database using GGSearch script in the fasta package (Pearson, 2014). GGSearch is a script based on the

global alignment algorithm (Needleman and Wunch 1970). Phylotypes of Oomycetes were extracted from the total response matrix. Phylotypes present in more than 10 % of total samples and with more than 10 sequences were kept for further analysis. The affiliation of each phylotype was determined by comparison to the GenBank database using BLAST; quick neighbour joining tree analyses were performed to place phylogenetically ambiguous taxa.

Data analysis

To determine if there is a significant effect of the microhabitat type or the sampling site on the oomycete community composition, we performed a permutation test using the "envift" function on a NMDS of the R programs (Vegan package: Dixon, 2003). Hierarchical clustering analysis was made with a Bray-Curtis distance matrix of a Hellinger transformation matrix of the oomycete community composition. A heatmap was computed using the "heatmap.2" function of the gplots package (Warnes et al., 2015)

Results and discussion

Oomycete diversity in Swiss Jura peatlands

Oomycetes were present in all 65 samples, and represented about 3% (107932 sequences) of all microbial eukaryote sequences (4223719 sequences) obtained in the whole study. We detected a high diversity with 34 phylotypes (on 5652 in total) of oomycetes, and samples varying from 4 to 21 phylotypes (average 16). Culture-based studies targeting soils mentioned up to about 10 species for individual samples (Bahramisharif et al., 2014), which suggests similar amounts of diversity. The order of magnitude of total diversity reached here was in the same order of magnitude with the 69 oomycete phylotypes found in the TARA project, which encompassed 334 size fractionated samples from marine plankton originating from tropical to temperate oceans worldwide (De Vargas et al., 2015). Obtained phylotypes were often highly divergent: we detected only 3 phylotypes that matched with 100% identity to barcoded taxa (namely *Peronospora schachtii;* X1510*, Phytophthora* sp.; X1794 and *Saprolegnia parasitica*; X1602). In contrast, up to 70% of all phylotypes had less than 98% similarity and, therefore, represented putative new genera, or deeper clades. The most abundant phylotype, X9, could not be placed with confidence within saprolegniales or peronosporales, and belongs to one of the deep basal lineages collectively designated as oomycete basal clades; known species from these basal lineages are animal and brown algae parasites (Lara and Belbahri, 2011). However, the great diversity of basal oomycetes may possibly host other lifestyles that have still not been investigated. This illustrates the potential for the discovery of a novel diversity in peatlands.

Phylotypes were not distributed randomly in all micro-habitats (Fig 2 and Fig 3). While communities were not significantly different between peatlands ($p > 0.05$), we found that communities were significantly separated in function of their original micro-habitat ($p=0.001$). Specific richness did not differ significantly between microhabitats (pairwise Tukey test $p=0.97$). However, communities of all microhabitats except pools were largely dominated by phylotype X9 (Figure 2), an unidentified, basal branching organism. In contrast, highly represented phylotypes in pools included X102 and X145 (an unidentified saprolegniales).

Peatbogs host a high number of pathogenic oomycetes. We detected 5 phylotypes out of 34 belonging to obligate parasitic genera (namely *Haptoglossa*; X1167) for animal parasites and *Albugo* (X1632) and

Peronospora (X1510) for plants. These obligate plant parasites are probably infecting the vascular plants present in the peat bogs. Phyloptype X1510 corresponded 100% to *Peronospora schachti* (GenBank affiliation: KF888598), a sugar beet pathogen. However, the v9 region of the SSUrRNA gene is a conserved marker and the corresponding sequence can be discriminated only by 1 bp from *P. effusa*, a spinach pathogen which is not closely related in multigene phylogenies (Choi et al., 2015). While the organism from which this sequence derived belongs to a diverse cluster that infects, as far as it is known, only plants from the order Caryophyllales (Thines and Choi, 2016), further analyses will be required to determine the exact host. *Drosera rotundifolia*, is a member of the Caryophyllales and is common in the studied peat bogs; it could be a good candidate as a host for phylotype X1510. Moreover, these plants are most commonly found in the lawn microhabitat where X1510 was most common (Fig. 3). On the other hand, the *Albugo* sequence (X1632) is not closely related to any barcoded species; we assume that it may derive from any vascular plant of the bog. To our knowledge, bog plant oomycete parasites have never been surveyed. Furthermore, other members of the *Lagenidium/Albugo* clade, as well as basal phylotypes may also be obligate (See Suppelemnetary material). Facultative parasitic organisms are potentially very common, as several genera have been detected (*Phytophthora* (X1794 and X2201)*, Lagenidium* (X2385, X3084, X3085)*, Saprolegnia* (X1602)*, Aphanomyces* (X1100, X1447) and *Atkinsiella* (X2836)). The phylotype X1100 is close related to both *Aphanomyces astaci* and a Copepod parasite from the same genus (Wolinska et al., 2009). It is, therefore, most likely infecting the Crustaceans that belong to the peatlands natural fauna (Cladocerans, cyclopoid and harpacticoid Copepods). Peatland inhabiting organisms may act as reservoirs for economically relevant pathogens such as members of genus *Aphanomyces*. Other oomycete genera are likely pathogens of the peatland microfauna; *Lagenidium* and *Leptolegnia* infect arthropods. Phylotype X2836 is close-related (98% similarity) to the marine crustacean parasite *Atkinsiella dubia* (AB284575), and may also infect the crustaceans present in the peatland; however, it may belong to another genus. *Haptoglossa* (X1167) infects nematodes, also very common in peat bogs (Glockling and Serpell, 2010). *Saprolegnia* (X1602) is a wide spectrum animal parasite (Sarowar et al., 2014). Genus *Pythium* (X570, X716, X1311, X1846 and X2835) comprises both animal and plant parasites (Lara and Belbahri 2011; Liu et al., 2014), fungal parasites (Benhamou et al., 2012; Horner et al., 2012) and also saprotrophic members (Kwasna et al., 2010).

We have demonstrated here that acidic, oligotrophic environments such as peatbogs host a wide diversity of oomycetes, some of them representing well studied taxa while others are basal branching and remain to be characterized morphologically and placed phylogenetically. Our approach using "universal" eukaryotic primers may have been key to the detection of these basal clades which would not have been detected by using an oomycete targeted approach. These basal groups are crucial for building comprehensive systematic and phylogenetic concepts in oomycetes towards understanding their evolution as a group. Furthermore, understanding their life history will lead to the evaluation of their impact on foodwebs and regulation of hosts population if they are pathogens. For this purpose, *in situ* hybridization methods can be applied to characterize morphologically organisms from which only SSU rRNA genes are available, as already applied to the parasitic group Cryptomycota (Jones et al., 2011).

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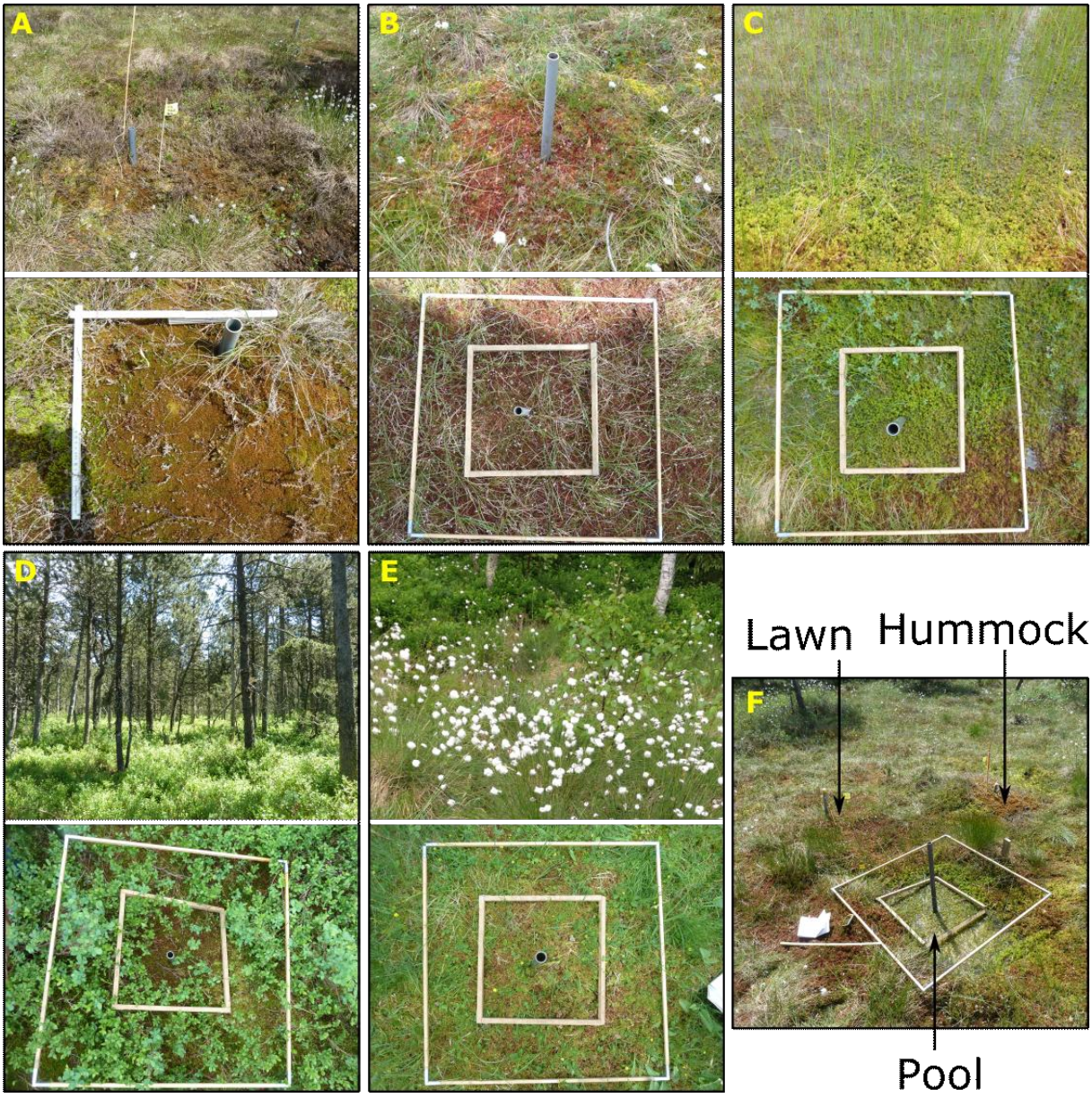
Figure captions

Figure 1: The different peatland micro-habitats. Each letters (except F) present a general view of the habitat on the top, plus one detailed (small square = 50 cm and large square = 1 m) in the bottom. A: Hummock, B: Lawn, C: pool, D: Forest, E: Fen/Transition and F: a general view of the center of the peatland with Lawn, hummock and pool micro-habitats. A to F from Edward A.D. Mitchell.

Figure 2: Distribution of oomycete phylotypes (% of total) in the different microhabitats of the studied peatlands.

Figure 3: Heat map depicting oomycete diversity and relative abundance in the microhabitats of the studied peatlands. The dendrogram was built using a complete agglomerative method on a Bray-Curtis distance matrix.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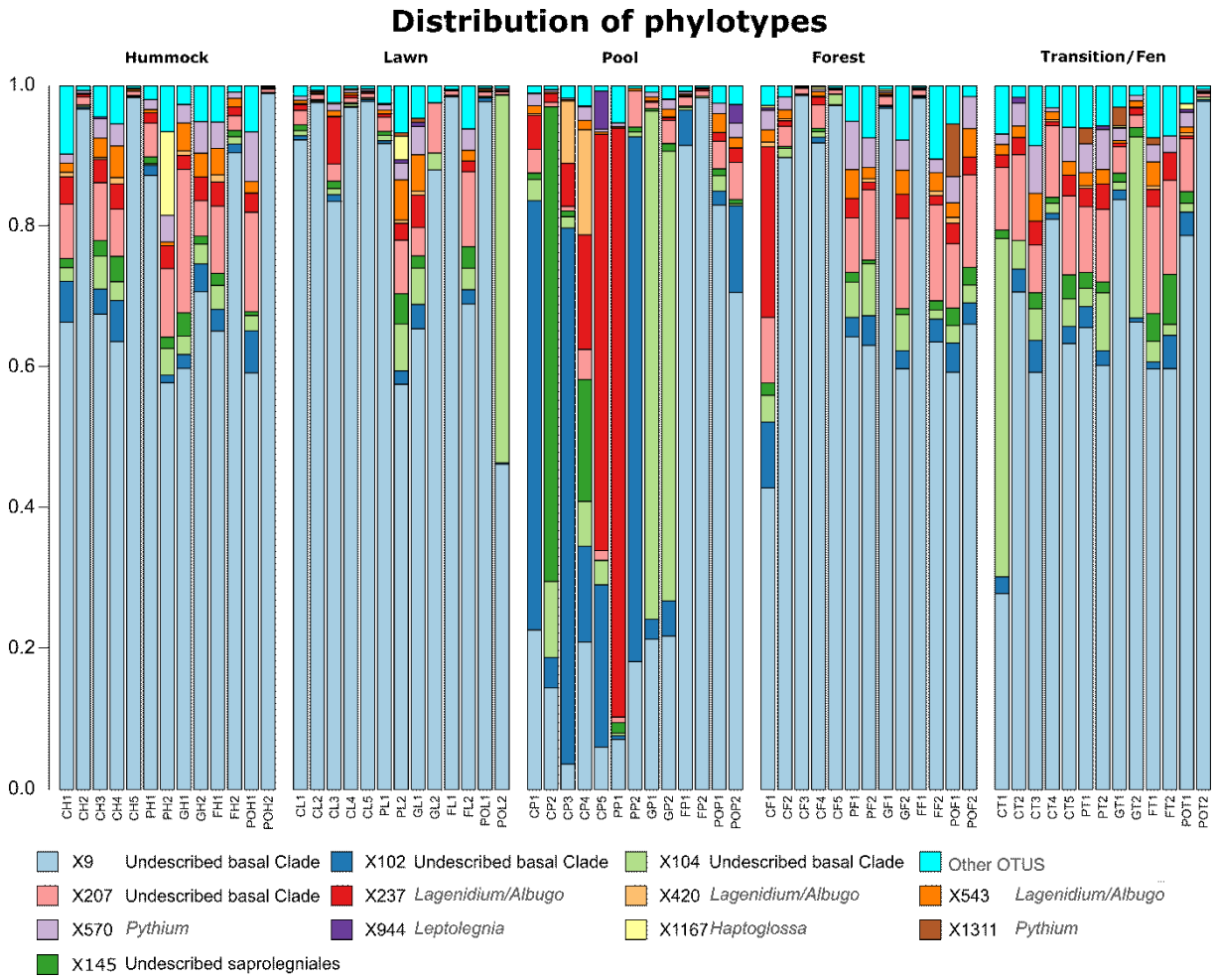
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Transition/Fen
1.0
0.8
0.6
0.4
0.2
0.0
CH1 CH2 CH3 CH4 CH5 PH1 PH2 GH1 GH2 FH1 FH2 POH1 POH2
CL1 CL2 CL3 CL4 CL5 PL1 PL2 GL1 GL2 FL1 FL2 POL1 POL2
CP1 CP2 CP3 CP4 CP5 PP1 PP2 GP1 GP2 FP1 FP2 POP1 POP2
CF1 CF2 CF3 CF4 CF5 PF1 PF2 GF1 GF2 FF1 FF2 POF1 POF2
CT1 CT2 CT3 CT4 CT5 PT1 PT2 GT1 GT2 FT1 FT2 POT1 POT2
X9 Undescribed basal Clade
X207 Undescribed basal Clade
X570 Pythium
X145 Undescribed saprolegniales
X102 Undescribed basal Clade
X237 Lagenidium/Albugo
X944 Leptolegnia
X104 Undescribed basal Clade
X420 Lagenidium/Albugo
X1167 Haptoglossa
Other OTUS
X543 Lagenidium/Albugo
X1311 Pythium

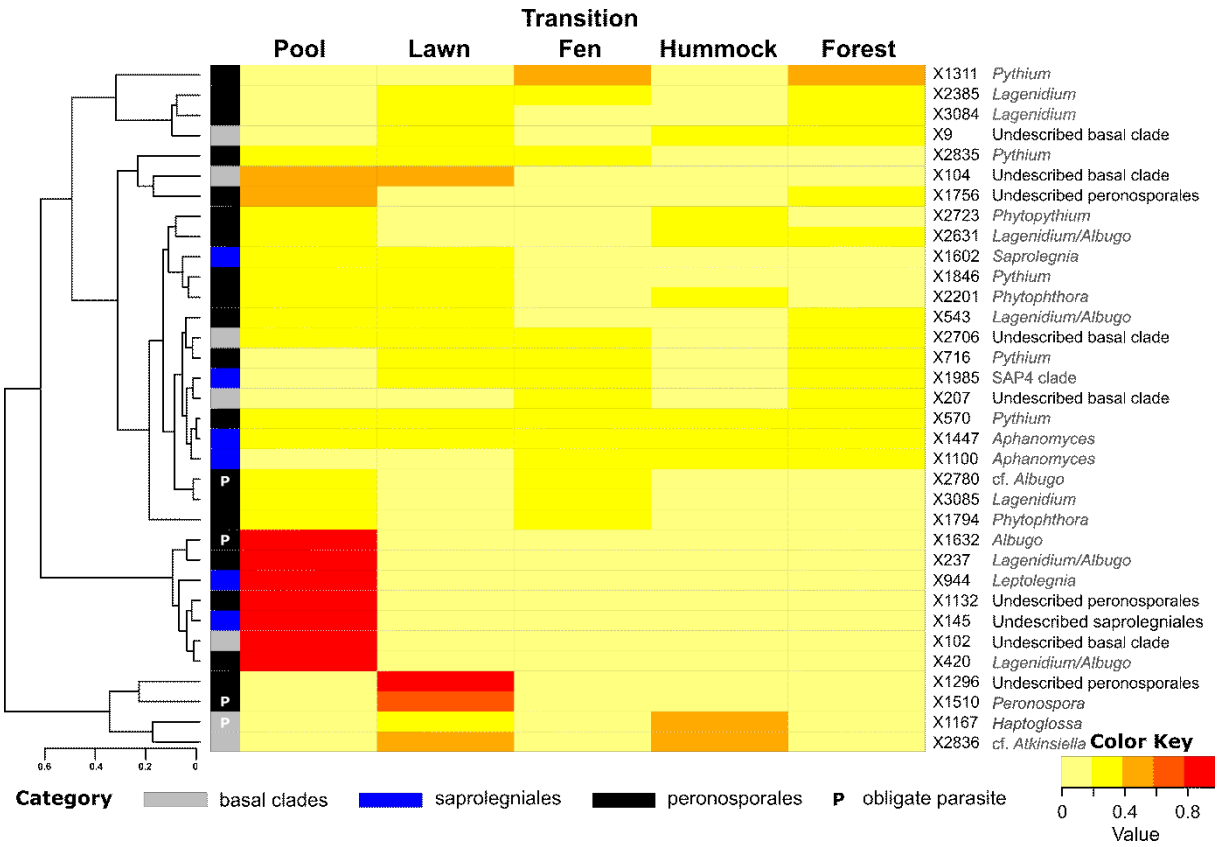
Transition
Pool
Lawn
Fen
Hummock
Forest
X1311 Pythium
X2385 Lagenidium
X3084 Lagenidium
X9 Undescribed basal clade
X2835 Pythium
X104 Undescribed basal clade
X1756 Undescribed peronosporales
X2723 Phytopythium
X2631 Lagenidium/Albugo
X1602 Saprolegnia
X1846 Pythium
X2201 Phytophthora
X543 Lagenidium/Albugo
X2706 Undescribed basal clade
X716 Pythium
X1985 SAP4 clade
X207 Undescribed basal clade
X570 Pythium
X1447 Aphanomyces
X1100 Aphanomyces
X2780 cf. Albugo
X3085 Lagenidium
X1794 Phytophthora
X1632 Albugo
X237 Lagenidium/Albugo
X944 Leptolegnia
X1132 Undescribed peronosporales
X145 Undescribed saprolegniales
X102 Undescribed basal clade
X420 Lagenidium/Albugo
X1296 Undescribed peronosporales
X1510 Peronospora
X1167 Haptoglossa
X2836 cf. Atkinsiella
P
P
P
P
0.6
0.4
0.2
0
Category
basal clades
saprolegniales
peronosporales
P obligate parasite
Color Key
0
0.4
0.8
Value

Table 1. Principal physico-chemical parameters and most characteristic bryophytes and plants for each mico-habitats.

	N [%] (min/max/average)	**pH (min/max/average)**	**water table depth [cm] (min/max/average)**	**Tree cover [%]**	**Micro-topography (0=flat, 2=mound)**	**Characteristic bryophytes**	**Characteristic plants**
Hummock	0.3/0.7/0.5	3.8/4.1/4	26/73/42	0	2	*Sphagnum fuscum, Polytrichum strictum*	*Eriophorum vaginatum, Vaccinium oxycoccos,*
Lawn	0.5/1.2/0.8	3.8/4.2/4	8/30/24	0	1	*Sphagnum magellanicum*	*Eriophorum vaginatum, Trichophorum caespitosum, Andromeda polifolia, Drosera rotundifolia*
Pool	0.4/1.1/0.8	4/4.2/4.1	1/6/4	0	0	*Sphagnum cf. cuspidatum*	*Scheuchzeria palustris*
Forest	0.7/1.5/1	3.9/4/4	19/77/48	70-100 %	1	*Sphagnum cf. angustifolium, Sphagnum cf. capillifolium Pleurozium schreberi*	*Vaccinium uliginosum, Vaccinium vitis-idea,*
Transition /Fen	0.7/1.4/1	3.9/4.7/4.2	10/51/35	ca. 20 %	1	*Sphagnum cf. capillifolium, Sphagnum cf. fallax*	*Betula pubescens, Potentilla erecta, Viola palustris, Juncus sp., Carex sp.*