IV. The diversity of ferns and lycopods in Val Piora and measurements of ecological and physiological indicators on two widely distributed fern species at two different sites

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Abstract

The diversity of ferns and lycopods, their lifeforms and the environmental performance were studied near Lake Cadagno in the Piora valley. For easier determination of the local diversity, a key to the species found in the area is given. Fast chlorophyll fluorescence is a valuable tool to check the photosynthetic plant performance as a measure for adaptation to local environmental conditions. As tropical ferns react strongly to a varying light regime, two local species, *Athyrium distentifolium* and *Dryopteris dilatata*, were selected, both growing at sunny drier as well as shaded more humid sites, to measure the kinetics if their chlorophyll fluorescence rise after a a strong light pulse. Although the ecological indicators (Landolt et al. 2010) of both species are similarly characterized as humid and shadow, leaves of the two species after exposed to the sun or in the shadow behave differently upon a strong artificial light pulse.

Keywords: Diversity of ferns and lycopods in the area. *Athyrium distentifolium, Dryopteris dilatata*, ecological indicators, Fast chlorophyll fluorescence, OJIP-test.

Riassunto

La diversità delle felci e dei licopodi, le loro forme di vita e le condizioni ambientali sono state studiate nei pressi del Lago di Cadagno in Val Piora. Per una più facile determinazione della diversità locale, viene data una chiave di lettura delle specie presenti nella zona. La fluorescenza clorofilliana è uno strumento prezioso per verificare e misurare nelle piante fotosintetiche la capacità di adattamento alle condizioni ambientali locali. Sono state selezionate due specie locali, *Athyrium distentifolium* e *Dryopteris dilatata*, che crescono entrambe in luoghi assolati più asciutti e ombreggiati più umidi, per misurare se la loro fluorescenza clorofilliana aumenta dopo un forte impulso di luce. Sebbene gli indicatori ecologici (Landolt et al. 2010) di entrambe le specie siano

caratterizzati in modo analogo come umidi e ombreggiati, le foglie delle due specie dopo essere state esposte al sole o all'ombra si comportano in modo diverso dopo un forte impulso di luce artificiale.

Parole chiave: Diversità delle felci e dei licopodi di Cadagno. *Athyrium distentifolium, Dryopteris dilatata*, indicatori ecologici, fluorescenza clorofilliana rapida, OJIP-test.

Introduction

Ferns and lycopods are the most primitive extant terrestrial vascular plants. They dominated Earth`s landscape before the emergence of seed plants (Spermatophyta) including the gymnosperms and the angiosperms. They are vascular plants that reproduce via spores and have neither seeds nor flowers. Ferns and lycopods differ from mosses by being vascular, having specialized tissues that conduct water, nutrients and in having life cycles in which the sporophyte is the dominant phase. Ferns have true, complex leaves called megaphylls. Most of ferns are leptosporangiate ferns. Lycopods have simple leaves called microphylls. The photosynthetic part of the plant is technically a megaphyll and in ferns, it is often referred to as a frond. Leaves are divided into two types, the trophophyll and the sporophyll. Both are green and contain chlorophyll. A trophophyll frond is a vegetative leaf analogous to the typical green leaves of seed plants and does not produce spores, their main role is producing organic compounds by photosynthesis. A sporophyll frond is a fertile leaf that produces spores borne in sporangia that are usually clustered to form sori, but is also active in photosynthesis. Despite the ecological importance of ferns, knowledge about their metabolism is limited compared to seed plants. Ferns have been the subject of research for their ability to remove some chemical pollutants from the atmosphere (Praveen & Pandey, 2019). Fern species live in a wide variety of habitats, from remote mountain elevations to temperate and tropical forests, to dry desert rock faces, to bodies of water or in open fields (Mehltreter et al. 2010). They have a life cycle referred to as alternation of generation, characterized by alternating diploid sporophytic and haploid gametophytic phases (Whittier, 1971). The gametophyte of ferns is a free-living organism, whereas the gametophyte of the gymnosperms and angiosperms is dependent on the sporophyte. Concerning light conditions, most ferns live in shadowy sites (Whittier, 1971). In Europe 38 Lycopods and 156 Ferns (IUCN) are described, amounting to a total of 194 species (European Commission of Environment, 2019). Ferns and lycopods are photoautotroph, similar to higher plants. During photosynthesis, electrons are transferred by light energy from water to CO_2 to produce complex organic compounds such as carbohydrates. Upon illumination, chlorophyll from photosystem II emits light as fluorescence, depending on the reduction state of the primary acceptor. Within about one second, a maximum fluorescence is reached, with a polyphasic kinetic. This rise has been characterized and developed as indicator of the photosynthetic energy conversion in phototrophic organisms (Strasser & Strasser 1995, Strasser et al. 2004). In a logarithmic time scale the fluorescence rise shows several intermediate steps between the initial fluorescence F_o and maximum fluorescence F_m. This OJIP kinetic has been used widely mainly in higher

plants to quantify environmental effects on plant metabolism such as drought, salt stress, ozone (Bussotti et al. 2007, Cicek et al. 2018, Falqueto et al. 2017, Kalaji et al. 2016, Oukarroum et al. 2007, Zivcak et al. 2008, Zivcak et al. 2014). Besides the fast fluorescence rise, the parameters F_v/F_m, the ratio of the variable to the maximum fluorescence and the photosynthetic efficiency of photosystem II, has been used since decades as indicator of photosynthetic performance. Recently Strasser introduced the performance index PI to examine plant vitality, in which besides factors beyond photosystem II are included (Strasser et al. 2004). With ferns, only recently a few investigations using the OJIP kinetic have been published, dealing with light stress in the epiphytic fern *Platycerium bifurcatum* (Oliwa & Skoczowski, 2019, Skoczowski et al. 2020) or with the differences between sterile and fertile sporophylls of *Dryopteris affinis* (Paoli & Landi, 2013). The present investigation is splitted in two parts, in the first one we try to cover the diversity of ferns and lycopods in Val Piora, while in the second part the technique of the fast chlorophyll fluorescence rise was applied to search for differences in the fluorescence kinetics between two different fern species, *Dryopteris dilatata* and *Athyrium distentifolium*, at different locations and light conditions.

Material and Methods

Sampling

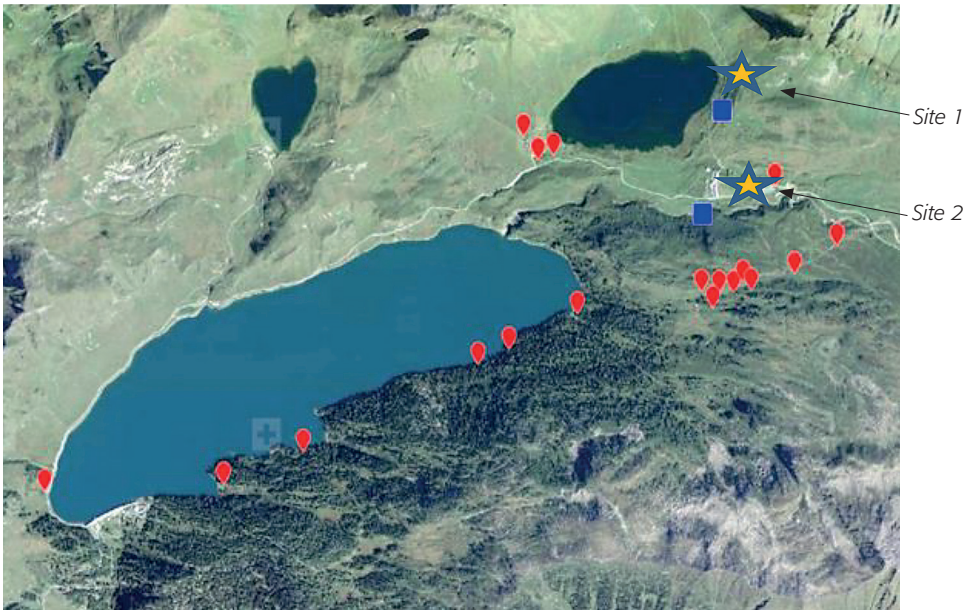

Fig. 1: Map of fern collection tour, – 500 m

Sites for physiological measurements
Sites where fern species were identified and collected
Sites where climatic conditions were measured

Ecological measurements

During a collection tour depicted in Fig. 1, all fern species were registered and their frequency within the area estimated according to previous observations over many years by Jakob Schneller. Selected species were collected for detailed morphological characterization in the lab, such as differences of leaf shape, sori and spores. All the photographs have been made by Jakob Schneller. Data on air temperature and humidity were collected using a PCE 555 thermo-hygrometer, for soil temperature a Voltcraft IR-352 thermometer was applied. For the characterization of the environmental conditions we used the indicative values published by Landolt et al. (2010).

Fluorescence measurements

For the study of fern vitality, the two species, *Athyrium distentifolium* and *Dryopteris dilatata* have been selected. Both often occur together, we selected two environmentally different sites. One was south-exposed in an open meadow (site 1), the other north exposed within an *Alnus* stand (site 2). All data were collected within 2 hours. Chlorophyll fluorescence was analyzed using the portable fluorometer Pocket PEA (Hansatech, King's Lynn, England). Detached pinnas were fixed in commercial leaf clips (Hansatech, England) and kept in the dark for 20 minutes prior to the measurement (Strasser et al. 2004; Hansatech Operations Manual, 2006). Excitation intensity was 3500 µmol photons m^{-2} s^{-1} with red light of 650 nm for 1 sec. From the fluorescence induction signal with high temporal resolution from 10 µsec to 1 sec the instrument determines the initial (F_o) and the maximum (F_m) fluorescence and the variable fluorescence (F_v) at specified time intervals. Then it calculates several specific parameters such as the potential quantum yield of PS II (F_v/F_m), the performance index (PI), the area between F_o and F_m or the initial rise velocity. Calculations and visualizations of the experimental data were treated in Excel.

For statistical treatments the software MaxStat was used. All data points presented are based on 8 consecutive measurements.

Results

Diversity of ferns in the area of Cadagno and ecological description of the species specific environment

In the traditional classification ferns (Pteridophyta) consist of true ferns and lycopods. Recent investigations, using also molecular characteristics, have shown that the lycopods are a sister group of Euphyllophyta, which then contain as sister groups the true ferns and the spermatophyta (Smith et al. 2006).
The various locations in the region of Lake Ritom and Lake Cadagno where we collected ferns and lycopods is given in Fig. 1. Forest areas represent the main habitat of many ferns and some lycopods, whereas in open areas they prefer shady microhabitats as gaps within rocks or shady areas between boulders, where the water dependent fertilization on

gametophytes can take place. Thus, some species growing in the forest are also found between boulders where they may be exposed to full sunlight. Some particular species are growing in fissures or small gaps on rocks or walls. Additionally, we calculated biodiversity and the indicative values following Landolt et al. (2010) at two sites (Fig. 1) shown in Table 1. Landolt values characterize the environmental conditions for a specific species. Our protocols fit well with the generalized Landolt values, although at many sites the micro local conditions were different. Forests are the most frequent habitat of the collected species. Most ferns prefer the shadow and grow in humid or moderate humid conditions, frequently in acid to even very acid soil. Some pioneer species are specialized to grow in the open, mainly in fissures or gaps of rocks, normally in humid areas, on acid soil. In contrast *Asplenium septentrionale* prefer dry conditions and *A. viride* grows on limestone as an exception. One species is found in marshland (*Equisetum palustre*) and one acts as a ruderal plant (*E. arvense*). The group of Montain plants is normally specialized to sites above about 1500 m. on neutral to acid soil.

Table 1. List of fern species present in Val Piora with the indicative values (Landolt et al. 2010).

		Indicative values					
Nr	**Species**	**Ecologic group**	**Humidity**	**Reaction**	**Light**	**Temperature**	**Frequency in area**
1	*Athyrium filix-femina*	Forest plant	Moderate humid	Acid	Shadow	Mountanious	Scattered
2	*Athyrium distentifolium*	Forest plant	Humid	Acid	Shadow	Subalpine	Frequent
3	*Asplenium viride*	Pioneer	Humid	Basic	Shadow	Subalpine	Rare
4	*Asplenium septentrionale*	Pioneer	Dry	Acid	Light	Under subalpine	Rare
5	*Botrychium lunaria*	Mountain plant	Moderate humid	Acid to neutral	Light	Subalpine	Rare
6	*Cryptogramma crispa*	Mountain plant	Moderate humid	Very acid	Light	Alpine and nival	Scattered
7	*Cystopteris fragilis*	Pioneer	Humid	Neutral to basic	Half Shadow	Mountanious	Common

8	*Dryopteris filix-mas*	Forest plant	Humid	Acid to neutral	Shadow	Mountanious	Scattered
9	*Dryopteris dilatata s.l.*	Forest plant	Humid	Acid	Shadow	Subalpine	Frequent
10	*Dryopteris affinis*	Forest plant	Moderate humid	Acid	Half Shadow	Under subalpine	Rare
11	*Equisetum arvense*	Ruderal plant	Humid	Neutral to basic	Half shadow	Under mountaniuous	Rare
12	*Equisetum palustre*	Marsh plant	Wet	Acid to neutral	Light	Mountanious	Rare
13	*Equisetum hyemale*	Forest plant	Very humid	Neutral to basic	Shadow	Under mountaniuous	Rare
14	*Equisetum variegatum*	Pioneer	Wet	Neutral to basic	Light	Under subalpine	Scattered
15	*Huperzia selago*	Forest plant	Moderate humid	Acid	Shadow	Mountanious	Rare
16	*Lycopodium annotinum*	Forest plant	Moderate humid	Very acid	Very Shadow	Under subalpine	Rare
17	*Oreopteris limnosperma*	Forest plant	Moderate humid changing	Very acid	Shadow	Subalpine	Rare
18	*Phegopteris connectilis*	Forest plant	Moderate humid	Acid	Shadow	Subalpine	Rare
19	*Polystichum lonchitis*	Forest plant	Moderate humid	Neutral to basic	Shadow	Under subalpine	Scattered
20	*Polypodium vulgare*	Pioneer	Fresh	Acid	Half Shadow	Mountanious	Rare
21	*Selaginella selaginoides*	Moun-tain plant	Humid	Neutral to basic	Half shadow	Subalpine	Scattered

Development and morphological details of ferns in the area of Cadagno

We have studied some morphological characteristics of some selected ferns occurring in the area. The common attribute of all ferns is their altenation of generations which is explained in Fig. 2b (Qiu et al. 2012).

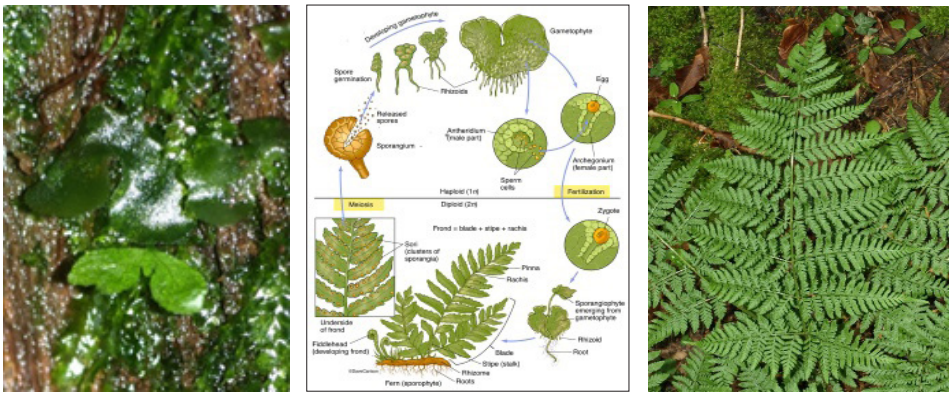

Fig. 2 a) Prothallus with young Sporophyte of Athyrium sp. b) Life cycle of ferns (repetico.de). c) Sporophyte of Dryopteris dilatata.

Ferns and lycopods have developed very diverse life-forms. True ferns are characterized by megaphylls (Vasco et al. 2013), the horsetails by microphylls and internodes and the clubmosses by having only spirally arranged microphylls (Margulis & Chapman 2009). Fig. 3 presents some examples found in Val Piora of the different groups.

Fig 3. Examples describing the differences in the macroscopic aspect of ferns and lycopods (from left to right). Examples of macrophylls of a) Dryopteris dilatata, b) Asplenium viride, c) Polypodium vulgare, d) and microphyll-bearing Equisetum palustre and e) Lycopodium annotinum.

Concerning the sori (the cluster of sporangia) of ferns, two main types are distinguished, some species showing indusia, epidermis parts covering the sori, others without indusia (Fig. 4a, b). When comparing the ferns in Tab. 1, shapes typical discriminate the different species, important among other characteristics for species identification. Also the spores,

products of the reduction division and responsible for plant propagation, are as well morphologically different. Two very different examples are shown in Fig. 4. c, d. The only lycopod species in the area, *Selaginella selaginoides*, shows heterospory, forming microspores in microsporangia and megaspores in megasporangia.

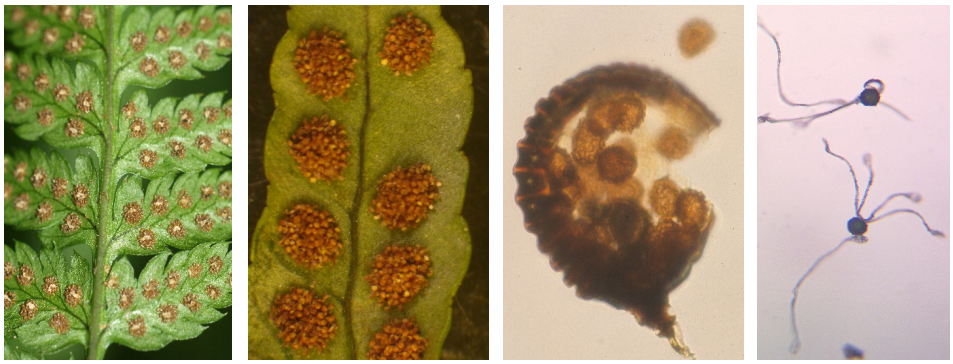

Fig. 4. (from left to right). a) Sori with indusium of Dryopteris dilatata, b) Sori without indusium of Polypodium vulgare, c) Sporangium with spores of Dryopteris filix-mas, d) Spores of Equisetum palustre with hapters (bands wound around the spore when humid, actively spreading when dry).

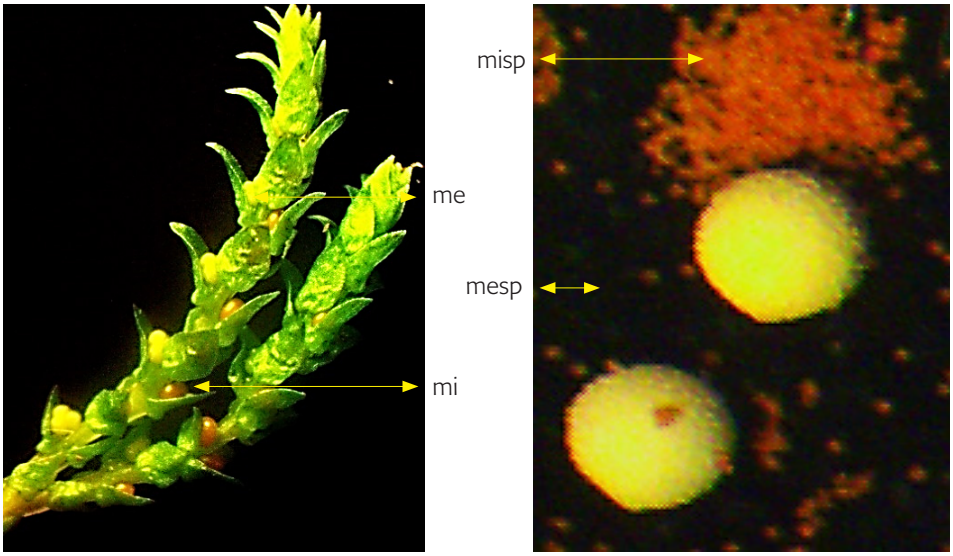

Fig. 5. a) Selaginella selaginoides with micro- and megasporangia (mi, me), b) Microspores and megaspores (misp, mesp.)

For identification purpose a key has been constructed for the species found in Val Piora. It is found as Supplementum in the attachment (Tab. S1).

For photosynthetic measurements two frequently found fern species, *D. dilatata* and *A. distentifolium,* have been selected. Ecological parameters have been collected on a

sunny day. Site 1 was in the open, a meadow with large bolders, while site 2 was within the Alnus bushes (Fig. 1). At both sites measurements were taken three time during the day (Tab. 2).

Table 2. Ecological parameters (air and soil temperature and humidity) at the sites and during of photosynthetic measurements.

Species	Time	9.15	9.40	14.05	14.15	21.25	21.45
	site	2 Shade	1 Sun	2 Shade	1 Sun	2 Shade	1 Sun
	air temp °C	13.7	16.2	14.4	20.0	8.5	16.0
Dryopteris dilatata	soil temp °C	7.7	9.4	10.1	22.8	8.9	10.9
	humidity %	76.0	67.9	55.0	56.0	56.0	61.4
Athyrium distentifolium	soil temp °C	7.5	11.3	8.9	22.2	8.4	12.3
	humidity %	71.7	52.3	54.7	54.1	52.0	65.0

The values for the air temperature are the same for both plants at the same site, as these are in short distance, except the temperature at 21.45 at the sunny site. The higher heat capacity of the soil and surrounding rocks kept the air above ground longer warm. Soil temperature was also not considerably different between the two species at the same site. As expected, the sunny sites gained temperature much faster during the day than the sites in the shadow with the largest differences at noon. Humidity values differ as well between the two sites of both ferns, *D.dilatata* and *A.distentifolium*. Generally, it has to be remarked that the measurements are momentary situations. Under the given conditions there is no significant difference between the two different species at the same site.

Photosynthetic performance of the ferns *Athyrium distentifolium* and *Dryopteris dilatata* at the two sites

The photosynthetic measurements with the two different fern species at the two sites have been executed in the early afternoon on a sunny day (Fig. 6). As seen in Tab. 2 the two sites do not differ greatly concerning air and soil temperature at the sites where *Dryopteris* and *Athyrium* were tested, but show considerable differences between sunny and shadow sites. The raw fluorescence kinetics of the two fern species at the two sites showed with log time scale the typical polyphasic OJIP fluorescence rise. Raw data partially reached high numbers (Fig. 6) and resulted in large differences in the fluorescence intensity within species and sites. As each point in the curves is the mean of 8

measurements of samples of the same species and site, these differences must be based on the variability of the chlorophyll content in the tested plants and on the effect of the specific environmental conditions at the two sites. While the mean value of the F_o stayed at about the same level, the highest F_m was up to twice that of the lowest one. While *Dryopteris* at the south exposed sunny site 1 gave induction curves that were practically identical for the leaves in the sun and in the shade (Fig. 7a), at site 2 in the *Alnus* stand the fluorescence intensity was down to about 50% compared to *Dryopteris* at site 1. Noteworthy the shaded leaves at site 2 resulted in a higher fluorescence signal compared to the sunny conditions. In contrast, the 4 traces of *Athyrium* stayed more close together, maxima were obtained in the shadow at site 1 and exposed to light at site 2 (Fig. 7b).

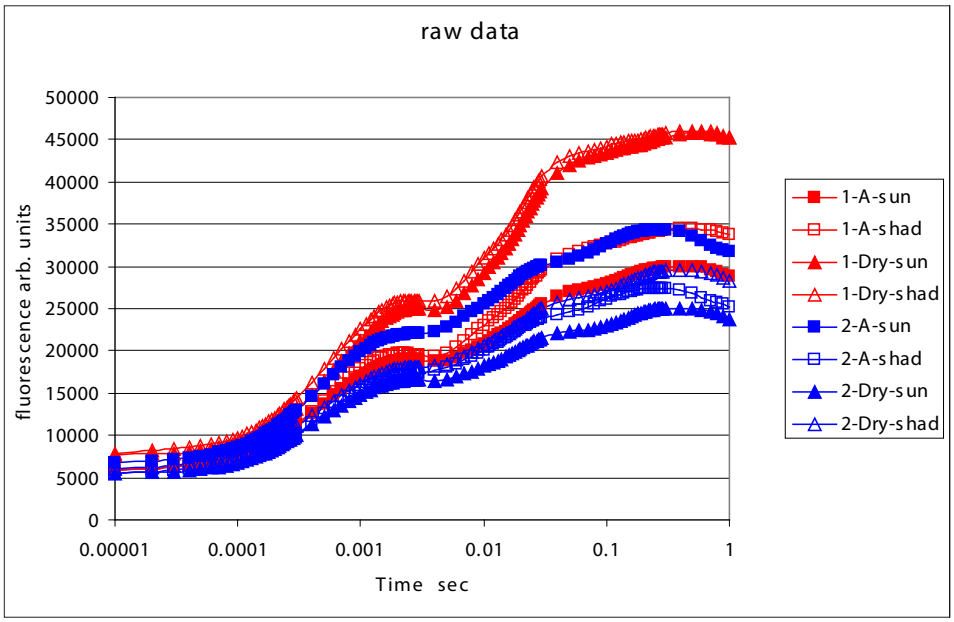

Fig. 6: Raw data of fast fluorescence of Athyrium and Dryopteris at two different sites and different light conditions. Each curve is the mean of 8 measurements.

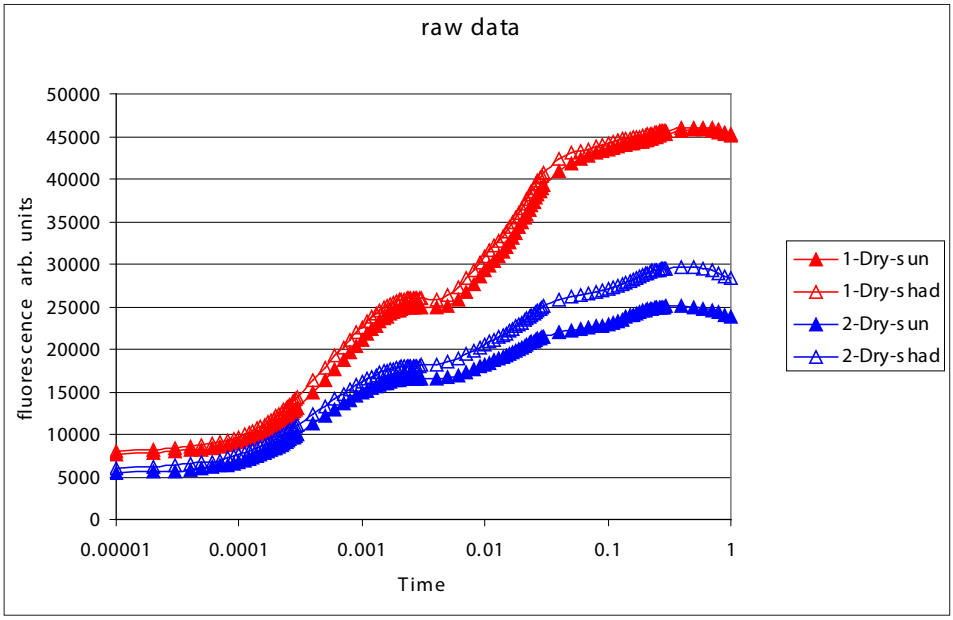

Fig. 7a: Raw data of Dryopteris at two different sites and different light conditions. Each curve is the mean of 8 measurements.

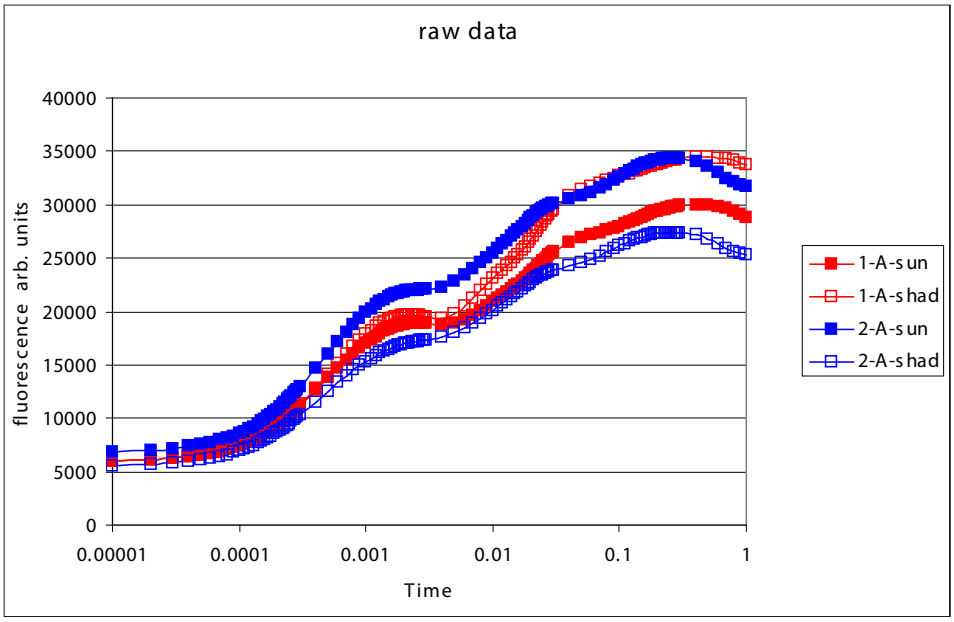

Fig. 7b: Raw data of Athyrium at two different sites and different light conditions. Each curve is the mean of 8 measurements.

For further analysis, F_o and F_m data were double normalized between the time 10 µs for $F_o = 0$ and 0.3 sec for $F_m = 1$. (Fig. 8a,b). This allowed searching for individual differences

within the induction phase independent of the fluorescence intensity. By averaging the data for each plant species and location, specific environmental effects in photosynthetic performance at the different sites became in evidence.

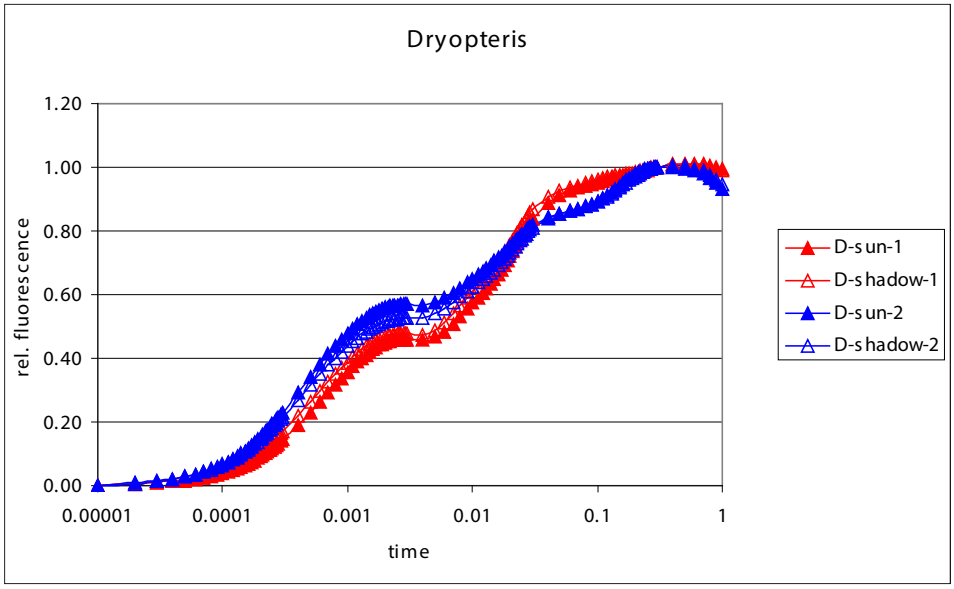

Fig. 8a: Double normalized data of Dryopteris at two different sites and different light conditions. Each curve is the mean of 8 measurements.

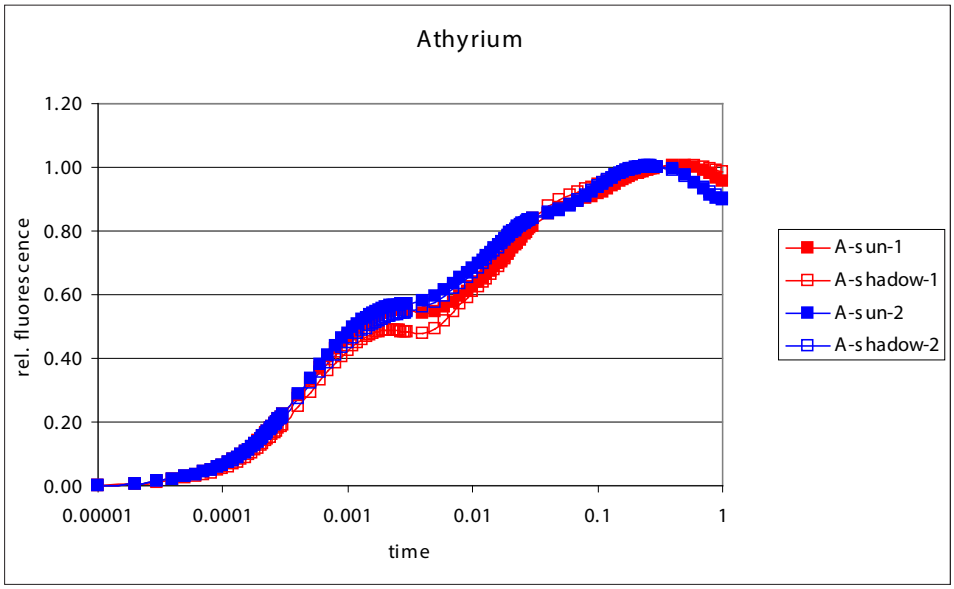

Fig. 8b: Double normalized data of Athyrium at two different sites and different light conditions. Each curve is the mean of 8 measurements.

After double normalization the differences between the 8 samples are much smaller. The largest effects concern the relative fluorescence F_J at 2 - 3 msec, at the J-step. The value indicates how fast the primary acceptor of photosystem II becomes reduced upon the strong light flash. For *Athyrium* at site 1, for the plant in the shade the J-step is lower, thus the plant performs slightly better than the one in the sun, while at site 2, the differences are negligible. The same holds for *Dryopteris* at site 2, while at site 1 the traces are practically superimposed. Similarly the velocity of the fluorescence rise is a measure of the redox state of photosystem II, seen in the time span between 10 and 150 µsec. With *Dryopteris* the fluorescence rise is more rapid for the plants at site 2.

Some specific parameters can be extracted from fluorescence measurements. The most frequently cited is the value F_v/F_m, the maximum yield of primary photochemistry in photosystem II. It is calculated as $F_v = (F_m - F_o) / F_m$ (see Fig. 6). In healthy plants F_v is around 0.8, but negative environmental effects may lower it substantially. In addition, the so called photosynthetic performance index (PI) including besides the primary photochemistry further kinetic indicators like the position of F_J and F_I, thus quantifying reactions downstream the primary charge separation. For a detailed discussion on the theory behind see Strasser et al. (2004) and Stirbet et al. (2018). In Table 3. F_v/F_m and PI are listed for the different sites and species.

Table 3: Indicator values F_v/F_m and PI. All numbers are means of 8 measurements.

	site 1				site 2			
species	*Athyrium*		*Dryopteris*		*Athyrium*		*Dryopteris*	
light conditions	sun	shadow	sun	shadow	sun	shadow	sun	Shadow
F_v/F_m	0.81	0.84	0.84	0.84	0.80	0.81	0.79	0.80
PI	1.76	2.80	4.13	3.32	1.70	1.82	1.36	1.80

The F_v/F_m values stay close together and are on the level of healthy plants, indicating similar primary photochemistry. However, statistical calculation (t-test, $p<0.05$) allows to indicate whether differences might be present between the data (Table 4). In contrast, the PI-value demonstrates substantial differences, both between sunny and shadowed leaves, as well as between *Athyrium* and *Dryopteris* and the two sites.

These calculations result for the F_v/F_m that *Athyrium* in the sun at site 1 differs from *Athyrium* in the shade as well as from sunny and shadowed *Dryopteris* at the same site, but is not different from both species at site 2. In contrast, the F_v/F_m of *Athyrium* in the shade at site 1 differs from both species at site 2. *Dryopteris*, both in the sun and the shade at site 1 differs from both species at site 2. Again, the PI shows similar correlations.

Discussion

Diversity of ferns and lycopods within the region and general characteristics of ferns (Filices s.sl.), horsetails (Equisetums) and club mosses (Lycopods)

The number of ferns and lycopods found within the area of Val Piora is large and covers about ¼ of the total number of ferns and lycopods in Switzerland. When comparing different sites in the open with those in the forest we realize that some species which are typical forest plants such as *Athyrium distentifolium* and *A. filix-femina* or *Dryopteris dilatata* can be found also in open places. Considering the microhabitat in the open we have to distinguish between two different niches, the place where the gametophyte and the first stages of sporophyte are formed, which is not reached by sun and forming a shady humid area mostly protected by big stones or rocks and the sunny part above the rocks, where the tall sporophytes can be observed. The shady microniche shows similarities to the climate in the forest, whereas in the open part with the climate found in open meadows only well developed and adapted sporophytes are able to stand the much harsher conditions. The details of the alternation of generations and characterization of some diagnostic features given in the key (Tab. S1, at the bottom of publication) are of great help for identification and should encourage people to learn more about the peculiarities of vascular spore plants in the region.

Table 4: shows the corresponding p values for F_v/F_m (upper right half) and PI (lower left half)

			1				2			
			Athyrium distentifolium		*Dryopteris dilatata*		*Athyrium distentifolium*		*Dryopteris dilatata*	
			sun	shadow	sun	shadow	sun	shadow	sun	shadow
1	*Athyrium distentifolium*	sun		**0.001**	**0.002**	**0.002**	0.70	0.65	0.10	0.37
		shadow	**0.001**		1.0	1.0	**0.001**	**0.0004**	**0.006**	**0.002**
	Dryopteris dilatata	sun	**0.0001**	**0.006**		0.11	**0.046**	**0.0001**	**0.0002**	0.11
		shadow	**0.005**	0.16	**0.05**		0.08	**0.005**	**0.0007**	**0.0025**
2	*Athyrium distentifolium*	sun	0.98	**0.014**	**0.0001**	**0.004**		0.86	0.48	0.88
		shadow	0.83	**0.004**	**0.001**	**0.001**	0.86		0.16	0.57
	Dryopteris dilatata	sun	0.49	**0.0001**	**0.0001**	**0.0001**	0.23	0.06		0.43
		shadow	0.88	**0.001**	**0.0001**	**0.0005**	0.90	0.92	**0.025**	

Environmental conditions

Our data on temperature and humidity give a very limited illustration of the variety during a normal summer day. The differences between the maximum and minimum temperature in the air under different weather conditions during the growing season may lie between. -2°C to +30 C°.

Photosynthetic performance of the ferns *Athyrium distentifolium* and *Dryopteris dilatata* at the two sites

A comparison of the F_v/F_m values in table 3 indicates that the means of 8 different measurements vary within 0.84 and 0.79, a rather small span to characterize and qualify the vitality of the plants at the specific sites. Concerning the PI, the sunny site 1 is, at least for *Dryopteris,* better suited than site 2. Detailed analyses of the kinetic data within the same species (Figs. 7a and 7b) show, that for *Athyrium* there are no differences in the kinetic traces at the shadowed site 2 in the sun or at the shadow, while at the sunny site 1 the shadowed leaves perform better than the light exposed ones. For *Dryopteris* the situation is reversed, at site 1 there is no difference between sunny and shadowed leaves while at site 2 the shadowed leaves perform slightly better than the ones in the light. These observations agree with the calculations above of the F_v/F_m and the PI (Tab 3.). Although in Tab. 2 both species, *Athyrium distentifolium* and *Dryopteris dilatata,* have a similar characterization, preferring humid and shadow sites, *Dryopteris* in contrast to *Athyrium* is not stressed by light at the sunny site 1, while in *Athyrium* the values do not differ at the shadow site 2. Light stress has been documented with the epiphytic fern *Platycerium bifurcatum*. Maximum fluorescence intensity dropped in strong light (1500 $\mu mol.m^{-2}.s^{-1}$) and lowered the value parallel to a reduced CO_2-fixation to 0.73 (Sanusi et al. 2011). Sporotrophophylls show a stronger response to high light than nest leaves, resulting in a drop of F_v/F_m and PI of more than 50% (Oliwa & Skoczowski, 2019). Both indicators were lower in sporophilic parts of leaves compared to trophophilic ones (Skoczowski et al. 2020). For *Dryopteris affinis* the F_v/F_m value was higher than 0.8 in the summer months and dropped to 0.6 during winter. The PI stayed below 1 during March and April, while later the new fronds reached maximum PI-values of more than 5 in June-July. The PI dropped rapidly and reached winter values in December. While most of the year sterile and fertile sporophytes had similar values, aging in fertile fronds (between August and November) was more rapid in the fertile ones (Paoli & Landi, 2013). Such large variations concerning F_v/F_m and PI could not be found for *Athyrium distentifolium* and *Dryopteris dilatata* in the Cadagno region. The two frequently found ferns seem well adapted to the main broadly changing environmental factors, light intensity, temperature and water conditions in the alpine ecosystem.

Conclusion

The spatial diversity of ferns in the Val Piora is characterized by environmentally different types of sites. This is reflected in a wide diversity of species. About 25% of the total number of fern and lycopod species in Switzerland were found in the Piora region.

Some important morphological characteristics were presented to distinguish the taxa. Regarding the microniche of ferns the general environmental conditions characterized by Landolt et al. (2010) have to be complemented. As an example, *Athyrium distentifolium* and *Dryopteris dilatata*, both described to prefer humid and shadowed conditions, are found in the forest as well as in open meadows. There the gametophytic and young sporophytic stage is found in the shade in between rocks or at very shady places, not reached by direct sunshine, climatically similar to the conditions in the forest. The grown up and ripe sporophyte develops its leaves in the open is part of the meadow vegetation. The two ferns *Athyrium distentifolium* and *Dryopteris dilatata*, although characterized for humid and shadowed conditions, react differently on a strong light pulse when tested at different light conditions by the OJIP test of fast chlorophyll rise. Fluorescence of *Dryopteris* was twice at site 1 than at site 2, with at site 1 identical kinetics independent of locally exposed to sun or shadow. At site 2 the leaves from shaded fern showed higher fluorescence leaves compared to the ones in the sun. The situation was similar with *Athyrium* at site *1,* while at site 2 the sunny leaves gave higher signals than the shadowed ones. These relations are also expressed in the parameters F_v/F_m and PI.

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Tab. S1: Main key to ferns in Val Piora

1	Sori on the lower side of the leaf	2
1′	Sori on special structures, sterile leaf part pinnate	**Botrychium lunaria**
2	Pinnules of fertile leaf rolled-up, subalpine to alpine on silicate	**Cryptogramma crispa**
2′	Leaf different	3
3	Sori close to leaf border	**Oreopteris limbosperma**
3′	Leaf pinnate or pinnatifid, sori with complete indusium	4
4	Indusium centrally fixed, horseshoe shaped	Genus **Dryopteris**
4′	Feature different	5
5	Indusium centrally fixed peltate	**Polystichum lonchitis**
5′	Indusium laterally fixed, egg-shaped- or vesicular	**Cystopteris fragilis**
5″	Indusium oblong	6
6	indusium linear, lamellar	Genus **Asplenium**
6′	indusium not linear or lamellar	7
7	Indusium comma-shaped, showing two linear vascular bundles	**Athyrium filix-femina**

7′	Leaf without indusium, pinnate or pinnatifid	**Athyrium distentifolium**
7″	leaf not showing linear vessels	8
8	Position of sori seen on upper side of leaf	**Polypodium vulgare**
8′	Sori not seen on leaf surface, undermost pair of pinna directed down	**Phegopteris connectilis**

Genus Asplenium

1	leaf irregularly divided	**Asplenium septentrionale**
1′	leaf pinnate leaf pinnate	2
2	Stipe and rachis brown	**Asplenium trichomanes**
2′	leaf pinnte. rachis green	**Asplenium viride**
2″	leaf pinnarifid stipe green	**Asplenium ruta-muraria**

Genus Dryopteris

1	leaf three times pinnatifid, scales dark	**Dryopteris dilatata**
1′	leaf twice pinnatifid	2
2	Pinnules roundish at top	**Dryopteris filix-mas**
2′	Pinnules truncated at top, base of pinna violet.	**Dryopteris affinis**

Key to Equisetum (Horsetails)

1	Stem simple, strong	**Equisetum hiemale**
1′	Stem branched mainly in whorled	2
2	Sqarrose microphylls of main stems with white border	**Equisetum variegatum**
2′	Squarrose microphylls without white border	3
3	Basal internode of a branch equal or longer then squarrose microphylls of the main stem	**Equisetum palustre**
3′	Basal internode smaller than the squarrose microphylls	**Equisetum arvense**

Key to Lycopodiales (Clumoss family)

1	Rather small plant developing micro-and megaspores in different sporangia	**Selaginella helvetica**
1′	Fertile plant with only one spore type (isospory)	2
2	Sporangia growing at the base of microphylls	**Huperzia selago**
2′	Sorangia in terminal pikes	**Lycopodium annotinum**