UiT The Arctic University of Norway

Department of Arctic and Marine Biology

Impact of autopolyploidy on leaf structure and photosynthesis in *Saxifraga oppositifolia* L.

Simen Salomonsen Hjelle

Master's thesis in biology, BIO-3950, November 2023

Cover image: *Saxifraga oppositifolia* L. Credit: Simen Salomonsen Hjelle

Impact of autopolyploidy on leaf structure and photosynthesis in *Saxifraga oppositifolia* L.

Simen Salomonsen Hjelle

Master of Science in Biology

Supervisors:

Pernille Bronken Eidesen, University of Oslo, Norway

Dorothee Ehrich, UiT The Arctic University of Norway, Norway

Xurxo Gago, University of Balearic Islands, Spain

Abbreviations

LMA	Leaf mass per area (g m^{-2})
RWC	Relative water content (%) of a leaf
C/N	Carbon-to-nitrogen content (C/N)
PSI, PSII	Photosystem I, photosystem II
F_0	Minimum fluorescence of dark-acclimated leaf (µmol photons $m^{-2} \cdot s^{-1}$)
F_0'	Minimum fluorescence of illuminated leaf (µmol photons $m^{-2} \cdot s^{-1}$)
F_M	Maximum fluorescence level of dark-acclimated leaf (µmol photons $m^{-2} \cdot s^{-1}$)
F_M'	Maximum fluorescence level of illuminated leaf (µmol photons $m^{-2} \cdot s^{-1}$)
F_V	Variable fluorescence yield ($F_v = F_m - F_0$) (µmol photons $m^{-2} \cdot s^{-1}$)
Ft	Steady state fluorescence value (µmol photons $m^{-2} \cdot s^{-1}$)
F_V/F_M	Maximum photochemical yield of PSII (ratio)
PAR	Photosynthetically active radiation, 400 - 700 nm (µmol photons m^{-2} s^{-1})
Y(II)	Effective photochemical quantum yield of PSII. (Often called Φ_{PSII}) (µmol photons $m^{-2} \cdot s^{-1}$)
Y(NO)	Quantum yield of non-regulated heat dissipation (µmol photons $m^{-2} \cdot s^{-1}$)
Y(NPQ)	Quantum yield of light-induced non-photochemical quenching (µmol photons $m^{-2} \cdot s^{-1}$)
NPQ	Non-photochemical quenching (µmol photons $m^{-2} \cdot s^{-1}$)
ETR	Electron transport rate (µmol photons $m^{-2} \cdot s^{-1}$)
ETR_M	Maximum ETR (µmol photons $m^{-2} \cdot s^{-1}$)
NPQ_M	Maximum NPQ (µmol photons $m^{-2} \cdot s^{-1}$)

Table of contents

Abstract

Polyploidization can be a rapid path to speciation and is considered an important evolutionary mechanism. Some predict that all plants have undergone a polyploidization event during their evolutionary history, and the frequency of polyploid species increases with latitude. The Arctic-Alpine *Saxifraga oppositifolia*, an autopolyploid, has two polyploids that have successfully established themselves in the high Arctic archipelago of Svalbard, Norway. It can be found in a growth form gradient spanning from prostrate form to dense cushions. This gradient, as well as differences in habitat preferences and reproductive strategy have later been linked to its polyploid nature.

As the most likely route for polyploid long-term establishment is linked to niche differentiation, I investigated several plant physiological traits both in *in situ* and *in vivo* settings to gain an insight into its success story, but also provide insight into the effect of autopolyploidization and the psychological effects of polyploidy. Two under-researched aspects of polyploidization. In general, polyploids can be seen to have higher photosynthetic rates and stress tolerance and previous studies on *S. oppositifolia* would suggest that its polyploids have higher rate of growth and photosynthesis. I hypothesized that polyploids displayed physiological characteristics related to this.

In this study I found polyploids to have higher leaf mass per area (LMA) than diploids. Tetraploids showed weak evidence of higher efficiency of photosystem II (PSII) through the proxy measurement of electron transfer rate, and higher stress tolerances through higher efficiency of non-photochemical quenching (NPQ). Nitrogen-to-carbon content did not change with polyploidy, but leaf relative water content could be influenced by polyploidy. As higher PSII efficiency was only seen in measurements in tetraploid re-rooted cuttings of *S.oppositifolia*, I suggest that this efficiency increase is an adaptation to quickly grow to successfully establish themselves as a new individuals. The higher LMA seen in both polyploids, and higher NPQ seen in the tetraploid, are linked to higher stress tolerances, probably related to drought stress.

Introduction

Polyploidy refers to the state when an organism, tissue, organ, or single cell genome contains more than one pair of homologous chromosomes (Jackson, 1976). This is especially common in plants, where 70 to 100 % of angiosperm species have undergone polyploidization during their evolutionary history (Albert et al., 2013; Averett, 1980; Lawlor, 2001; Masterson, 1994). For polyploids to form, there needs to be a doubling of genetic material, which can occur via different polyploidization mechanisms (Bretagnolle & Thompson, 1995; Bush, 2001; Rieseberg & Willis, 2007). A common mechanism is the fusion of unreduced gametes produced during meiosis, or genome doubling in new vegetative tissue that becomes a reproductive organ (Bretagnolle & Thompson, 1995; Bush, 2001; Rieseberg & Willis, 2007). Further doubling of the genome can create polyploids of even higher ploidy levels (Yang et al., 2009).

Polyploids can be further classified as either being allopolyploids or autopolyploids. Allopolyploids are formed from a hybridization event followed by a polyploidization event, while autopolyploids result from polyploidization within a single species (Jackson, 1976; Ramsey & Schemske, 1998). Research on polyploidy has largely focused on allopolyploid species, and much less has been published on autopolyploid species (Spoelhof et al., 2017). As allopolyploids are formed through hybridization and polyploidization (Bush, 2001), they are often morphologically distinct from their progenitors. Autopolyploids, on the contrary, are often identical to their progenitors, and their identification relies on genetic methods such as flow cytometry (Grosso et al., 2012). This has led to autopolyploids being taxonomically ignored, and their importance underestimated (Soltis et al., 2007; Spoelhof et al., 2017).

Polyploidy as a mechanism of evolution

Estimates of speciation events in angiosperms occurring due to polyploidization range from two to four percent (Otto & Whitton, 2000) to 14 % (Wood et al., 2009). The prevalence of polyploidization is considered an important driver of plant diversification, and an important mechanism of evolution and speciation (Otto & Whitton, 2000; Soltis et al., 2009). Polyploidization occurs more often without speciation as an outcome, and speciation attempts often end in failure (Soltis et al., 2014). This high failure rate can be attributed to the ecological constraints around the establishment of polyploids, as it would be in direct competition with its progenitors (Fowler & Levin, 1984; Thompson & Lumaret, 1992).

Despite challenges in polyploid establishment, succeeding individuals perform well (Soltis et al., 2014).

Simulations based on a modified version of the Lokta-Volterra competition model show that establishment is most likely when the polyploid can coexist with its progenitor due to niche separation (Fowler & Levin, 1984). This can be seen in *Dactylis glomerate* L., tetraploids flower at different times than the diploids, and the tetraploids dominate in open habitats whereas diploids in low-density forest floor areas (Lumaret et al., 1987). Similar spatial patterns of habitat differentiation linked with polyploidy can be seen in many species (Dijk et al., 1992; Eriksson et al., 2017; Feber-Girard et al., 1996; Hardy et al., 2000; Johnson et al., 2003; Sabara et al., 2013; Tyler et al., 1978). One of them is the mixed-autopolyploid *Saxifraga oppositifolia* L., an Arctic-Alpine angiosperm (Svalbardflora.no).

A successful high-Arctic autopolyploid

The population of *S. oppositifolia* found in the high Arctic archipelago Svalbard, Norway, can be divided into two ecotypes (Crawford et al., 1995). In wet low laying riverbeds prostrate plants dominate (Figure 1a), while on warm and dry ridges, cushion plants (Figure 1b) are common (Crawford et al., 1995; Elven & Elvebakk, 1996). With this separation in mind, some have considered dividing the Svalbard population into two subspecies (Brysting et al., 1996). However, it was not until recently that this separation was linked to the plants' polyploid nature (Eidesen et al., 2013). Three ploidy levels, di-, tri- and tetraploid, have been reported for Svalbard (Müller et al., 2012). The tetraploids and triploids of *S. oppositifolia* do not form cushions and are almost absent from the exposed ridges (Eidesen et al., 2013; Eidesen et al., (in prep)). Diploids, on the contrary, display the entire range of growth forms (Figure 1a-b) and are represented on both ridges and in riverbed habitats (Eidesen et al., 2013).

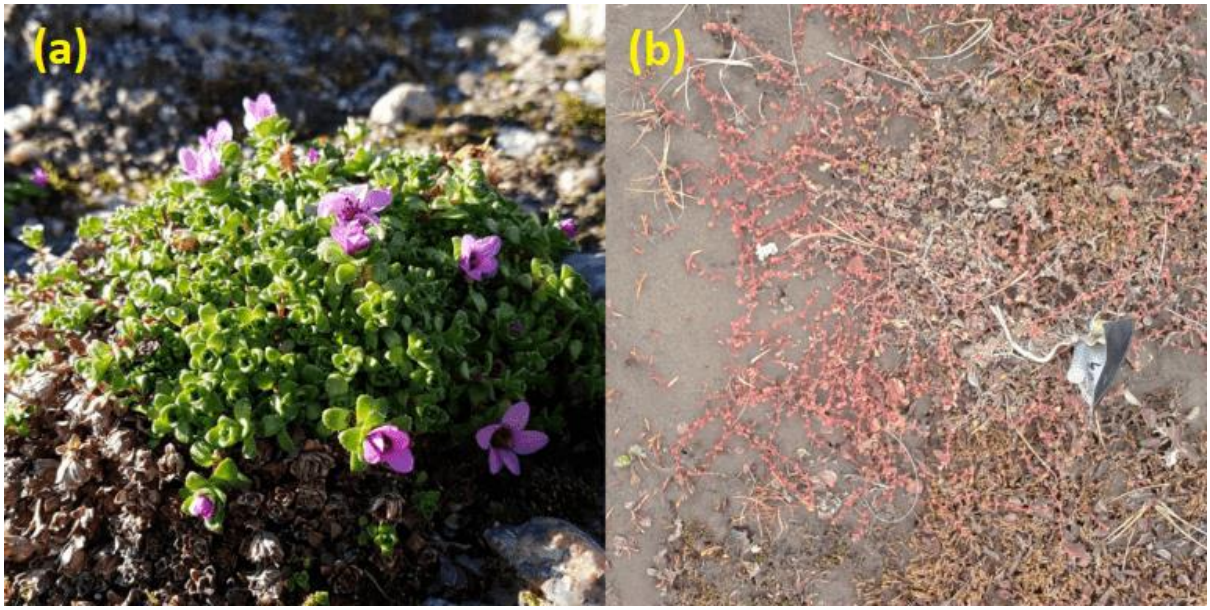


Figure 1. Two photographs that show the strong difference in growth form seen in *Saxifraga oppositifolia*. In the pictures, a strong cushion (a) and a prostrate (b). The two forms exist on a spectrum, and in-between forms are common. Photos: Simen S. Hjelle.

As *S. oppositifolia* is an autopolyploid, it is a good candidate for investigating the effects of autopolyploidization without hybridization (Spoelhof et al., 2017). The polyploids of *S. oppositifolia* have managed to overcome the challenge of establishing themselves and they are found in great numbers all over Svalbard (Brožová et al., 2023; Eidesen et al., 2013; Müller et al., 2012). They could have quickly adapted to new a niche after polyploidization, or it is possible that the population of polyploids was separated under the last glaciation and evolved in refuges, until a secondary contact with its progenitors after deglaciation (Dijk et al., 1992). The main lineages of diploids and tetraploids in Svalbard likely colonized the area separately, but there is also some evidence of some recent polyploidization events in Svalbard as well (Brožová et al., 2023; Müller et al., 2012). The local triploids could have originated from hybridization between diploids and tetraploids. (Brožová et al., 2023; Müller et al., 2012).

Recent findings have suggested that a main phenotypic difference between diploids and tetraploids is their mode of reproduction (Eidesen et al., (in prep); Tjessem, 2021). Diploids reproduce mainly sexually and produce significantly more flowers and seeds, while the tetraploid utilizes asexual reproduction through vegetative propagation (Eidesen et al., (in prep); Kume et al., 1999; Tjessem, 2021). On nunataks, which are mountain peaks protruding through glacial or continental ice, tetraploids were found to be far more common than diploids and triploids(Brožová et al., 2023). In these habitats tetraploids likely benefited from

its success with asexual reproduction and not relying on pollinators (Brožová et al., 2023). These recent studies were the first to show niche differentiation between diploid and tetraploid of *S. oppositifolia*, and with the principle that for polyploids to succeed, niche differentiation is needed, one would expect there to be several unknown ploidy-induced adaptations in the tetraploids of *S. oppositifolia.*

Potential impacts of polyploidy on leaf structure and nutrient allocation

A prominent niche differentiation in habitat utilization seen between *S. oppositifolia* diploids and polyploids is that diploids can cope with the conditions present on exposed, dry, ridges, and are commonly found in such habitats (Eidesen et al., 2013; Eidesen et al., (in prep). The polyploids are more frequent in lower laying areas such as riverbeds, suggesting tetraploids may have adaptations linked to survival in such habitats (Eidesen et al., (in prep)). A trait often used in ecological studies is leaf mass per area (LMA), which correlates to several functional traits in plants. It is a ratio of leaf dry mass per leaf unit area, where a high value of LMA means thicker and/or denser leaves and lower values thinner and/or lighter leaves (Witkowski & Lamont, 1991). LMA can for example be used as a proxy of photosynthetic rates, where lower values of LMA are correlated with higher photosynthetic rates (A. V. Perera-Castro & Flexas, 2023; Poorter et al., 2009; Wright et al., 2004). Higher LMA is however associated with a longer leaf lifespan, nutrient retention, and gives protection from desiccation (Schlesinger & Chabot, 1977; Sobrado & Medina, 1980). Leaf measurements making up LMA can also be used to calculate relative water content (RWC). This is a measure of the hydration status of a leaf and will indicate if the leaf is under drought and heat stress (Mullan & Pietragalla, 2012). Further, leaf dry weight in combination of other measurements can be used to evaluate the nutrient status of the leaf. A common parameter is the carbon-to-nitrogen content (C/N) of a leaf. Enzymes involved with photosynthesis take up most of the leaf nitrogen (about 75%) (Chapin & Eviner, 2007; Evans & Clarke, 2019), and photosynthetic rate generally increases linearly with nitrogen content (Evans, 1989; Muraoka et al., 2008). Therefore, C/N ratio can then be used as a proxy for photosynthetic rate, growth rate, but also nutrient availability (J. P. Bryant et al., 1992; Xianchun et al., 1994).

Effect of polyploidy on photosynthesis

These leaf traits can be used as a proxy for the photosynthetic abilities of a plant. As polyploid cells contain twice as much genetic material as its progenitors, they can also have higher chlorophyll *a*/*b* contents in the cells, resulting in higher photosynthetic rates (Dong et

al., 2017; Ellis & Leech, 1985; Wang et al., 2021; Warner & Edwards, 1993). Though, this increase can be negated depending on genetic factors, leaf structure and how the cells are packed (Warner & Edwards, 1993).An example of negated effect was described in the autopolyploid millet grass *Pennisetum americanum* (L.) Morrone, where tetraploid had double the cell size and higher photosynthetic rates per cell, but half the cell number per leaf unit area compared to diploids. This meant that photosynthetic rates per cell unit area were the same for diploids and tetraploids (Warner & Edwards, 1988). However, using synthetic autopolyploids of the angiosperm *Phlox drummondii*, Vyas et al., (2007) found that by the 11th generation the tetraploids had higher photosynthetic rates than the diploids.

While comparisons of photosynthetic performance changes related to polyploidy in *S. oppositifolia* have not been investigated, differences have been seen when comparing between plants from different ecotypes (Crawford et al., 1995). When comparing plants growing on beach ridges against plants in low-laying meadows around Ny-Ålesund, Svalbard, Crawford et al., (1995) found plants in the low-laying meadow had higher photosynthetic rates. This is likely an adaptation to the shorter growing seasons in the meadows, where snowmelt starts later than on the beach ridges. The meadow plants also exhibited prostrate growth form (Crawford et al., 1995). As we now know prostrate growth form and the habitats they are found in are linked to the polyploids of *S. oppositifolia*, polyploidy could have been an unknown factor influencing the results in Crawford et al., (1995). However, in a study by Kume et al., (1999) where they compared the two growth forms of *S. oppositifolia* (prostrate and cushion) which grew in the same riverbed habitat around Ny-Ålesund, Svalbard, they did not find differences in photosynthetic rates.

Polyploids can have improved stress tolerance

Polyploids are often seen to cope better with stressful conditions (Coate et al., 2013; Tossi et al., 2022). *S. oppositifolia* and all other Arctic flora live in quite extreme, stressful environments (Fernández-Marín et al., 2020). For two months during the summer, the sun does not set and exposes them to 24-hour long photoperiods. This excess light can produce radicals (reactive oxygen species) which can lead to oxidative damage to the photosynthetic apparatus (Bassi & Dall'Osto, 2021). This damaging effect can be mitigated or prevented through non-photochemical quenching (NPQ), which is a process where the excess light energy is dissipated as heat, instead of being used in photosynthesis (Demmig-Adams B et

al., 2014; Larkum, 2006; Lazár, 1999). An increase in NPQ is also linked to other stressors, such as drought (Esteban et al., 2015; Latowski et al., 2011; A. V. Perera-Castro & Flexas, 2023). NPQ between Arctic plants can vary a lot (Y. Li et al., 2013), and a study on *Ranunculus auricomus* L. reported that polyploids had more efficient NPQ (Ulum et al., 2021). *S. oppositifolia* should in theory live under less extreme conditions than the ridge delving diploids (Eidesen et al., 2013; Fernández-Marín et al., 2020). However, as the polyploids lack the protective benefit of a cushion growth form (Badano & Cavieres, 2006; Cavieres et al., 2007), they may need to compensate with higher efficiency of NPQ.

Study aim, structure and hypothesis

When it comes to the impact of polyploidization, most research revolves around genetic changes (Soltis et al., 2016). Far less research revolves around ecological and physiological changes due to polyploidy, especially for autopolyploidy (Soltis et al., 2016; Spoelhof et al., 2017). Additionally, with anthropogenic climate change, life in the Arctic is facing an uncertain future (IPCC, 2023). As polyploids can swiftly adapt to new changes, investigations into natural polyploid populations are of value (Van de Peer et al., 2021).

Here, I attempt to reduce this knowledge gap by investigating the potential effects of autopolyploidy in the mix-ploidy angiosperm *S. oppositifolia* on several plant physiological parameters. Further I theorize on the possible adaptative advantages of ploidy induced adaptations and their potential meaning for the success of the *S. oppositifolia* polyploids in the high Arctic archipelago of Svalbard.

The physiological parameters investigated were the leaf structural trait LMA, the RWC content from leaves of *S. oppositifolia*, alongside leaf C/N content. I employed pulse-amplitude-modulation (PAM) fluorometry, a non-invasive method of assessing the efficiency of photosystem II (PSII) (Lazár, 1999), NPQ (Demmig-Adams B et al., 2014; Larkum, 2006; Lazár, 1999) the quantum yield of PSII (Y(II)), maximum yield of PSII (F_V/F_M) (Baker, 2008) and other parameters obtained through chlorophyll *a* fluorescence by PAM fluorometry (Lazár, 1999; Maxwell & Johnson, 2000; Schreiber, 2004). PSII efficiency can be assessed based on calculation of relative electron transfer rate (ETR) (Baker, 2008), which also positively correlates with carbon assimilation (A. V. Perera-Castro & Flexas, 2023).

I attempted a combination of field and climate-controlled room (lab) measurements. Plants in the climate-controlled room were acclimated to its conditions before measurements. Data

from field and lab settings could potentially allow for separation of effect caused by polyploidy, and effect caused by phenotypic plasticity.

Effort was divided into three measurement setups:

- **Setup 1, *Field***: I collected leaves from individuals from riverbeds, slopes and ridges. These leaves were used for LMA, RWC, and C/N. Additionally, I did fluorescence measurements during summer of 2020 in the field.
- **Setup 2, *Re-rooted Cuttings***: I measured the fluorescence on cuttings of *S. oppositifolia* grown in a climate-controlled room.
- **Setup 3, *Whole plants*:** I measured the fluorescence similarly to the re-rooted cuttings, but on whole individuals *S. oppositifolia* harvested from the field and grown in a climate-controlled room.

Polyploidy can have higher photosynthetic rates and stress tolerance, and as prostrates of *S. oppositifolia* are linked with higher rates mainly due to their shorter growing season, the prostrate growth form is also linked later with polyploidy. Polyploids of *S. oppositifolia* are also more successful at vegetative propagation, where higher photosynthetic rates and growth rates can be beneficial. Based on this, I hypothesize the following:

Polyploids, when compared to diploids, show higher efficiency of PSII, through the proxy of ETR. They have lower LMA, and higher C/N content in their leaves. They show higher stress tolerance through more efficient NPQ. This has helped in polyploid establishment and niche differentiation. I do not expect to see differences in leaf RWC, but as a parameter it will give insight into potential water stress in the plants.

H_0: Polyploidy in S. oppositifolia does not significantly alter ETR, C/N, NPQ, or LMA compared to diploid individuals.

H_1: Polyploidization, particularly autopolyploidization, induces changes in ETR, C/N, NPQ, and LMA in *S. oppositifolia*.

Materials and methods

Study area

The high Arctic archipelago of Svalbard lies between the Greenland Sea and Barents Sea, and is made up of several islands, with Spitsbergen being the largest. During the summer, temperature ranges between 5 to 7 °C, and with the influence of the North Atlantic Current winter temperatures are relatively mild, ranging from -13 to -20 °C (Hanssen-Bauer et al., 2019). The mean annual precipitation is low, and ranges from 196 nm to 581 nm annually (Svalbard Airport weather station) (Hanssen-Bauer et al., 2019). Wind speeds can get high due to channeling effects in the valleys, and average wind speeds fluctuate during the season, with highest speeds during the winter months (Petersson, 2007). The Arctic flora consists of about 2200 species, with only 8 % of them a precent in Svalbard. The North Atlantic Current brings warm water up the western side of Svalbard, leaving it with overall higher mean temperatures causing western parts of Svalbard to host most of the vegetation (Elven & Elvebakk, 1996; Hanssen-Bauer et al., 2019).

The individuals of *S. oppositifolia* used for this study came from five established transects located in the proximity of Longyearbyen, Svalbard, Spitsbergen, Norway (78°13′N 15°38′E). The transects make up a gradient, starting from coastal marine influenced areas to inland areas at locations within Adventdalen (Figure 2). Each transect contains 3 plots that make up a ridge, slope and riverbed gradient. Within each plot, there are 48 marked plants of *S. oppositifolia* with a known ploidy level. These transects were established by Eidesen et al. between 2018 and 2019 (Eidesen et al., (in prep)).

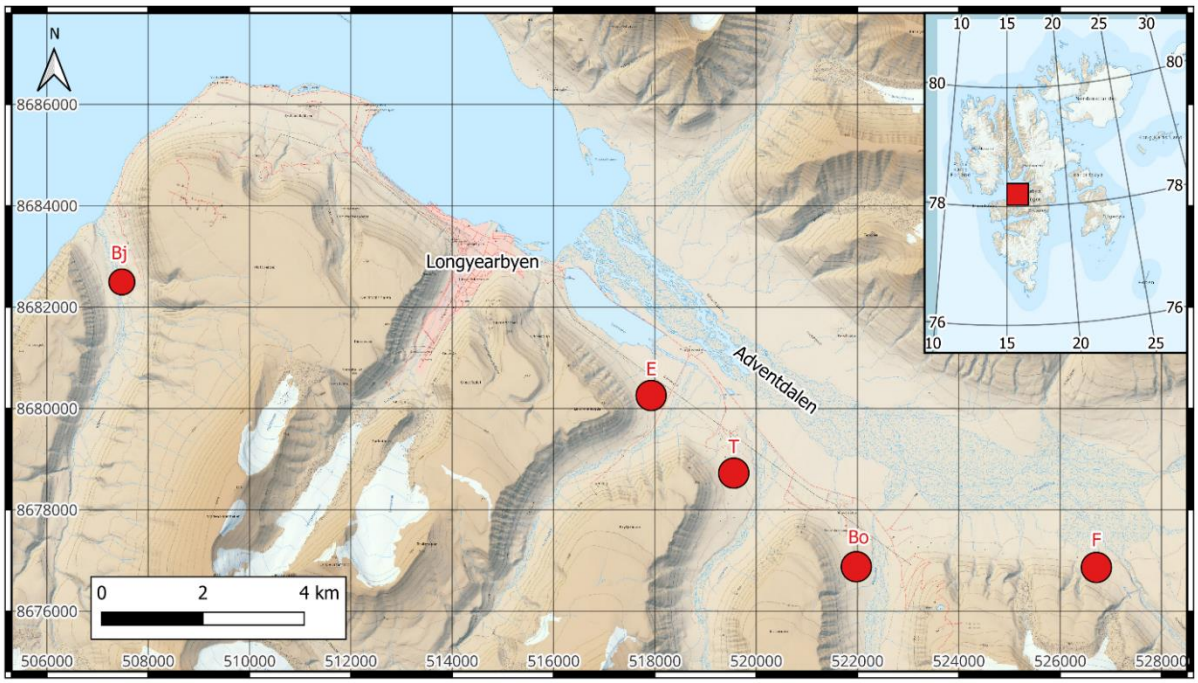


Figure 2. Map of the area around Longyearbyen showing the locations of the five transects used. The circle markers represent the middle of each transect. Each transect contains three plots of different habitat classification (*Ridge*, *Slope* and *Riverbed*). The smaller map in the top right corner shows the location of Longyearbyen (square) within the Svalbard archipelago. The abbreviations right to left: Bjørndalen (Bj), Endalen (E), Todalen (T), Bolterdalen (Bo) and Foxdalen (F). Figure created using QGIS (QGIS Development Team, 2023. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org). Map layer © Norwegian Polar Institute.

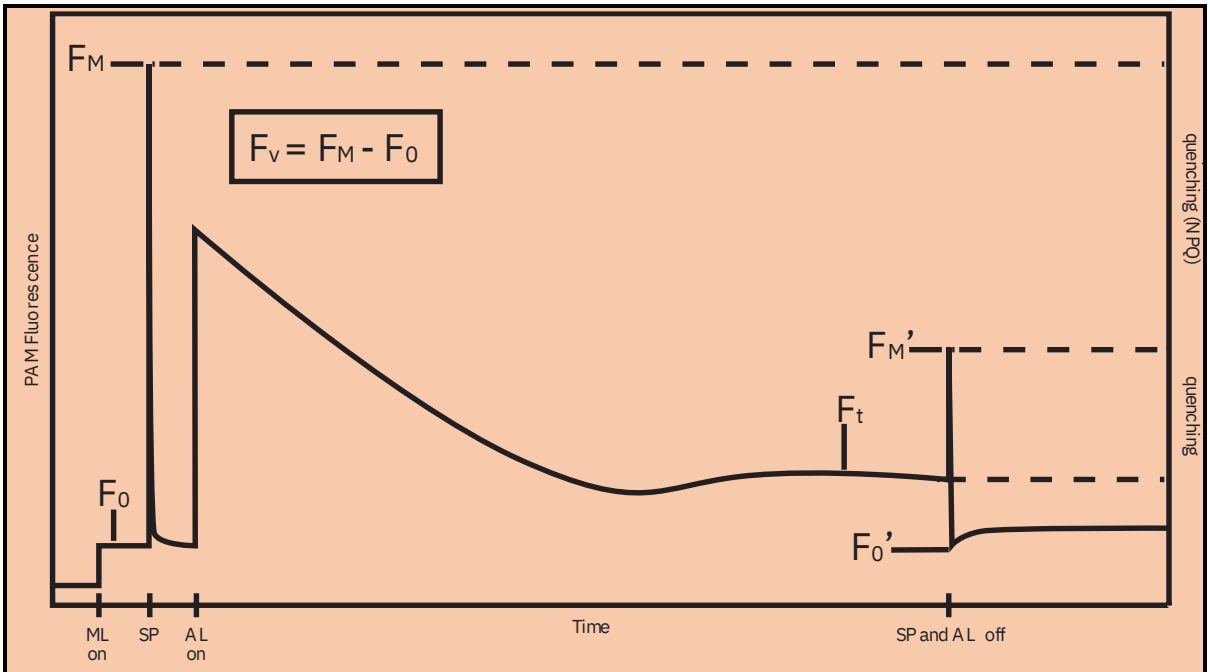
The three habitats used can be characterized as follows:

- **Ridge/transition to scree slope (called *Ridge*):** Wind exposed, dry and with little to no snow protection during winter, leading to longer growing seasons. They have sparce vegetation, mainly solitary herbs. More temperature variations and longer growing seasons. Scree slopes have unstable sediments.
- **Vegetated slope (called *Slope*):** Vegetated area usually situated between the ridges and the river running through a valley. They are lightly sloped and can have dense vegetation, such as heaths, meadows and moss carpets. More water is available, and snow cover during the winter. Stable environment overall. Shorter growing seasons.
- ***Riverbed*:** Situated at the bottom of a valley, in areas of past river flow or close to river flow. Can vary from sparse vegetation and unstable sediments in flooded areas, to more stabilized vegetation and continuous biological crust. High snow cover during winter, and high moisture content. Unstable periods with shifting river flow, and changes in river strength due to seasonal melting. Shorter growing seasons.

Box 1. Chlorophyll a fluorescence using PAM fluorometry

Chlorophyll *a* fluorescence can be measured with a fluorometer, such as a pulse-amplitude-modulation (PAM) fluorometer (Lazár, 1999; Maxwell & Johnson, 2000; Schreiber, 2004). Photons in light can be absorbed by chlorophyll to drive photosynthesis (photochemical quenching), converted into heat (non-photochemical quenching), or reflected as *fluorescence*. This fluorescence signal is measured by fluorometers (Maxwell & Johnson, 2000). The fluorescence signal represents that of chlorophyll *a* (Duysens, 1951), of which there are two main pigments, P700 that sits in photosystem I (PSI), and P680 that sits in photosystem II (PSII) (Maxwell & Johnson, 2000). These pigments serve as reaction centers that accept electrons. PSII gets electrons from water through photolysis, where the photon separates the electron from the water molecule. In PSI the electron comes from PSII. These reaction centers can have two states, (1) open state if they can accept an electron, or (2) closed state if they contain an electron. Changes in states create changes in fluorescence signal. Such changes in fluorescence will be due to PSII (as PSI gets electrons from PSII), and PAM fluorescence is therefore a measurement of the efficiency of PSII (Maxwell & Johnson, 2000).

To use a PAM fluorometer, the instrument's fiber optic cable is aimed at a photosynthetically capable sample. Certain parameters require the sample to be dark-acclimated beforehand, such as F_V/F_M and NPQ (Table 1). The fiber optic cable sends out a measuring light generated by the instrument. This is a pulsating low-intensity light, and not strong enough to drive photosynthesis and will leave PSII reaction centers open. Fluorescence signal reflected back through the fiber cable between pulses and is measured by the fluorometer. This signal represents the fluorescence yield when none of the light is being used by PSII (Box 1, Figure 1). If the sample is dark-acclimated, the fluorescence value is recorded as $F_{0,}$ otherwise F_0'. The next step is to expose the sample to a high-intensity saturating pulse of light, which excites and fills all the reaction centers in PSII. Fluorescence recorded at this point represents the maximum fluorescence yield of the sample, F_M if dark-acclimated, F_M' if not (Box 1, Figure 1). Following saturating pulses can be performed between periods of actinic light that keeps photosynthesis in an active state (Box 1, Figure 1) to generate parameters such as ETR and NPQ (Table 1) (*MINI-PAM II Photosynthesis Yield Analyzer Manual*, 2023).



Box 1, Figure 1. Illustration explaining the principles of PAM fluorescence quenching analysis with a dark acclimated sample and open photosystem II (PSII) reaction centers. First, a measuring light (ML) is activated, and the steady state fluorescence is recorded (F_0). Second, a saturating pulse (SP) is sent out to fully activate PSII, and the maximum fluorescence is recorded (FM). Third, actinic light (AL) is activated to drive photosynthesis, once stable fluorescence (Ft) levels are reached, a second SP is sent out, which records maximum and minimum fluorescence of an illuminated sample F_M' and F_0'. The illustration also shows what non-photochemical quenching (NPQ) and photochemical quenching is based on. Figure based on (Maxwell & Johnson, 2000; (MINI-PAM II Photosynthesis Yield Analyzer Manual, 2023).

Overview of the three different measurement set-ups

Field measurements took place in the summer of 2020 and 2021 (Figure 3). During 2020 I used a fluorometer to measure chlorophyll *a* fluorescence (Box 1) and calculated the parameter F_V/F_M (Table 1). Additionally, I measured LMA and RWC in 2020 and 2021, and C/N for 2020 (Figure 3).

The two other set-ups involved measurement of chlorophyll *a* fluorescence obtaining F_V/F_M, but also creating light curves (Box 2), on re-rooted cuttings and whole plants (Figure 3). Additional photosynthetic parameters were calculated from the light curves, such as ETR and NPQ (Table 1). The maximum values of each were used for further statistical analysis.

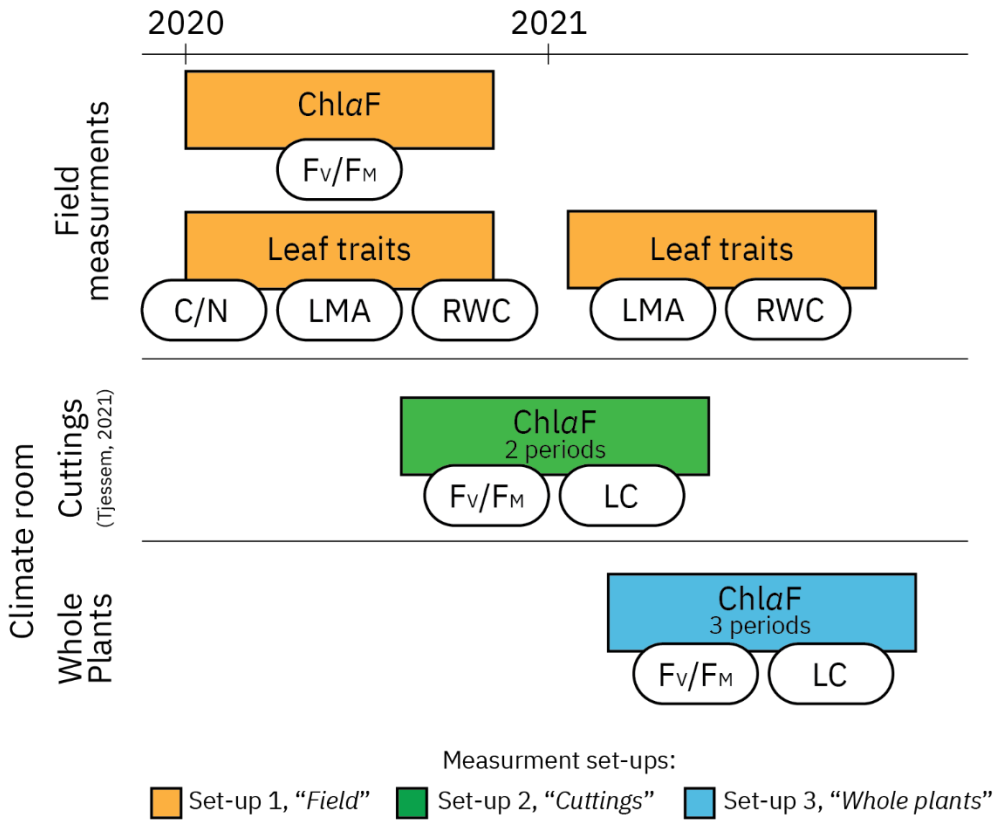


Figure 3. Graphical illustration of the different measuring efforts during 2020 to 2021. Squares represent the type of measurement, with the main parameters measured below in rounded squares. The color of the square represents what measurement set-up they are a part of. Y-axis divides them into being from field or climate room, X-axis is time of measurement. Chl*a*F = Chlorophyll *a* fluorescence, LMA = Leaf mass per area, RWC = Relative water content, LC = Light curve.

Box 2. Light curve measurement

Light curves or rapid light curves can be performed using a PAM fluorometer, and they will give information of the saturation characteristics of electron transport in photosystem II (PSII) (Ralph & Gademann, 2005). In the case of this study, the MINI-PAM II/R, by Heinz Walz GmbH was used. It comes with a built-in program that preform the light curve measurement. The light curve consists of several saturating pulses of light (Box 1), with a timed delay between them, at increasing light intensities. With each interval of saturating pulse, the effective quantum yield Y(II) (Table 1) will decrease, as more of the reaction centers in PSII gets filled (Box 1). Using this curve, one can calculate the rate of which electron travel through PSII (ETR) and heat dissipation through non-photochemical quenching (NPQ) (Box 1, Table 1).

Table 1. Description of main photosynthetic parameters obtained from PAM fluorescence and later used in statistical analysis. ETR and NPQ are obtained from performing light curves using PAM fluorometry (Box 1).

Parameter	Unit	Description
Y(II)	µmol m^{-2} s^{-1}	Effective quantum yield of PSII and an estimate of the light absorbed by the chlorophyll in PSII. It can be used as an indicator for overall photosynthesis. (Eq. 4)
F_V/F_M	Ratio F_V/F_M = 1 = 100 % of photos absorbed.	Can only be calculated if the leaf is dark-acclimated, as reaction centers in PSII need to be fully open. It represents the percentage of photons absorbed. It is employed as a physiological stress status indicator (Eq. 1).
ETR	µmol m^{-2} s^{-1}	Electron transfer rate is an estimate of how efficiently electrons are transferred through the electron transport chain in PSII (Eq. 8).
NPQ	µmol m^{-2} s^{-1}	Measurement of non-photochemical quenching, which is a defense mechanism against high light intensity. Instead of absorbing incoming light and using it for photosynthesis (photochemical quenching), it is released as heat. As it is a comparison between F_M and F'_M, it can only be calculated if the leaf is dark-acclimated in combination with light-acclimated measurements (Eq. 7).

Plant selection procedure from transects

Suitable plants were selected using the same procedure during fieldwork for both years (2020 and 2021) (Figure 3). For each plot visited, time was spent locating as many as possible of the 48 marked individuals of *S. oppositifolia*. Once a plant was located, it was marked with a flag and its plant identification (ID) number written down. The list of located IDs was then randomly ordered into a new list (https://www.random.org/lists/), with sampling starting with the first plant on that list. Plants were skipped if sampling had a risk of heavily reducing their future survival, if it was dead or in poor condition. During summer of 2021, for leaf traits and measurement of whole plants (Figure 3), the efforts were focused on diploid and tetraploid individuals. As diploid and tetraploid distribution differ, with diploids more common on ridges and tetraploid in riverbeds, fieldwork took place in riverbed and ridge habitats (Figure 4).

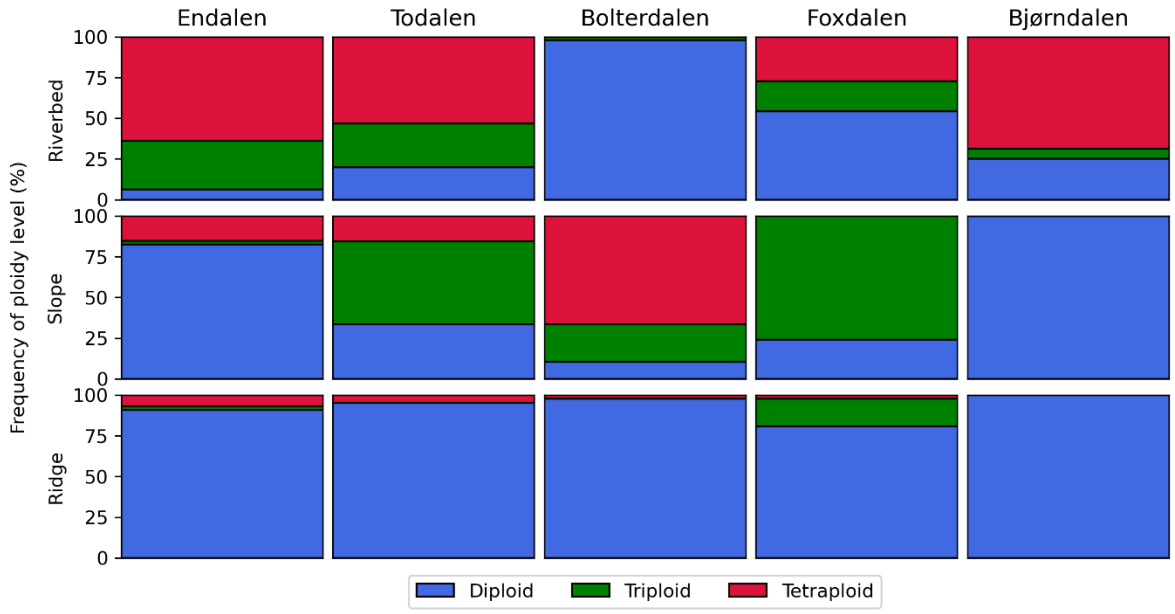


Figure 4. The frequency (in %) of *Saxifraga oppositifolia* diploids and their polyploids (triploids and tetraploids) for each of the plots. Rows are habitat types (Riverbed, Slope and Ridge) and columns are the five different transect locations (Endalen, Todalen, Bolterdalen, Foxdalen and Bjørndalen). Each plot contains 48 plants. Data from Eidesen et al., (in prep)).

Chlorophyll *a* fluorescence *in situ* to obtain F_V/F_M

In total, 44 randomly selected plants from the established field set-up were used to measure chlorophyll *a* fluorescence (Box 1, Figure 3) parameter F_V/F_M (Table 1, Eq. 1) of *S. oppositifolia in situ* using a portable pulse-amplitude-modulated (PAM) photosynthesis yield analyzer, MINI-PAM II/R (Heinz Walz GmbH). Once the plants in a plot had been selected for measurement, they were dark adapted by covering them in aluminum foil for a minimum of 30 minutes. A suitable, healthy looking leaf rosette (Appendix 6) was chosen, and then the leaf rosette was moved into the leaf clip (2035-B Leaf-Clip Holder, by Heinz Walz GmbH). The fiber optics cable was then mounted into the holder on the leaf clip. This was done while avoiding natural light hitting the plant and to keep the leaf clip leveled. The leaf rosette was then hit with a saturating pulse of 6000 µmol m^{-2} s^{-1}, and the resulting value of F_V/F_M calculated by the MINI-PAM II was written down. This procedure was repeated for all 44 plants. F_V/F_M was calculated by internal MINI-PAM II software based on Kitajima & Butler, (1975) (See Box 1 for more information):

$$F_V/F_M = F_M - F_0 / F_M \quad (1)$$

Measurements and calculations of leaf traits LMA, RWC and C/N

To obtain the leaf parameters LMA, RWC and C/N, fresh leaves are needed. As *S. oppositifolia* has small leaves that take time to harvest, branches with twelve or more leaves

were cut off and brought back inside marked plastic bags. Before sealing the plastic bags, one would breathe into them to create humidity inside, to get a saturated atmosphere and avoid leaf desiccation.

To calculate LMA (Eq. 2) and RWC (Eq. 3), leaves were weighted to obtain their fresh weight, turgid weight and dry weight, and photographed to determine their total leaf area. Weighing of fresh weight was performed immediately after returning from the field. Twelve leaves from each plant were picked off using tweezers, then weighed on a high precision scale (Mettler Toledo XP204) to get their fresh weight. Next, to obtain leaf area, the leaves from fresh weight were scanned on a photocopier (CANON C7565i) next to a ruler (Appendix 16). These images were later analyzed using Adobe Photoshop to get the area of the leaves. After, to achieve the turgid weight, paper was wetted with distilled water, with the leaves wrapped inside and stored in closed zip-lock bags in a fridge at ca. 4°C for 24 hours. The fully turgid leaves were then weighed, and after, placed in paper bags and dried in an oven set to 60 °C for 3 days up to constant weight and a final weighing to get the dry weight. These values were used to calculate the needed parameters, starting with LMA, using the following equation:

$$LMA = DW(LA/10{,}000) \quad (2)$$

where LA is the area of the leaves and DW is the dry weight of the leaves. The LA is divided by 10,000 to convert from cm^2 to m^2. Then, RWC was calculated expressed as percentage:

$$RWC = ((FW-DW)/(TW-DW))100 \quad (3)$$

where FW is the fresh weight and TW the turgid weight.

To get the C/N ratio of the leaves, a combustion analyzer (Elementar Vario EL cube) was used. The dry leaf samples were crushed manually using metal tweezer, weighed to at least 4.5 mg, then carefully packed in tin cups before determining the element composition in the combustion analyzer. The standard used was Acetanilide (C_8H_9NO, N-Phenylacetamide). The C/N ratio was calculated by dividing the percentage of carbon with the percentage nitrogen contained in a sample.

Calculations of light curve parameters

Several parameters (Table 1) describing the photosynthetic performance of *S. oppositifolia* were calculated based on the light curves (Box 2). These were calculated by the MINI-PAM

II itself or using the accompanying software, WinControl V3.3 by Heinz Walz GmbH. Since leaves were dark adapted before initiation of light curves, values for F_V/F_M (Eq. 1) were calculated at the start of every light curve.

The calculations for Y(II) were based on Genty B et al., (1996). With Y(NO) and Y(NPQ) using equations transformed by Klughammer & Schreiber, (2008) from Kramer et al., (2004) into the simpler equations from Genty B et al., (1996):

$$Y(II) = F_M'-F_t/F_M' \quad (4)$$

$$Y(NO) = F_t/F_M \quad (5)$$

$$Y(NPQ) = (F_t/F'_M)-(F_t/F_M) \quad (6)$$

Equation for NPQ is based on Bilger & Björkman, 1990 and Gilmore & Yamamoto, 1991:

$$NPQ = (F_M/F'_M)-1 \quad (7)$$

ETR (Krall & Edwards, 1992) is based on assumptions on absorbances and photon partitioning between PSII/PSI, ΔY(II) and ΔPAR:

$$ETR = PAR*A*B*Y(II) \quad (8)$$

A is the average amount of photons absorbed by a normal green leaf which is 84%. (Baker, 2008). *B* is the assumption that 50% of the photons are being equally partitioned between photosystem II and photosystem I (Baker, 2008).

Light curve measurements on re-rooted cuttings grown in climate-controlled room

Light curves were done on re-rooted cuttings grown in climate-controlled room (Appendix 7) inherited from Tjessem, (2021). The maintenance procedure of plants described in Tjessem, (2021). The health of the plants was first assessed and those which showed signs of growth and green leaves were chosen for LC measurements (Appendix 18). The plants were measured in December of 2020 (Period 1) and in June of 2021 (Period 2). All LC were done after the plants had been dark adapted overnight (>12 hours). Measurements were performed in a darkened room, with the fiber optics angled at 60 ° above a suitable leaf rosette. A laboratory stand was used to aim the fiberoptics (Appendix 10). LC program was initiated when a good signal strength could be reached (200 - 400 F_t). The LC program settings were a

saturating pulse of 800 ms. every 100 seconds, 11 times at increasing PAR intensities ~ (0, 65, 91, 127, 193, 290, 427, 640, 837, 1170, 1528 μmol photons m^{-2} s^{-1}).

Whole plant individual collection

Whole intact individuals were needed to grow in a climatic-controlled room for light curve measurements. Plants were selected randomly by the same method as when selecting field plants for leaf trait measurements. To collect selected plants in the field, I carefully dug around the plant and extracted as much of the root system as possible. The plants were placed in plastic bags during transportation. In the lab, the roots were washed to remove soil and potted. The bottom of the pot was filled with gravel/sand, and the top part had an organic soil mixture (Appendix 8). The plants were grown in a climate-controlled room with approximately 15 °C, with 12-hour light cycles. The amount and frequency of watering was based on subjective assessment of the dryness of the soil, warranting watering. This led to watering not being equal between the plants, but moist soil was maintained throughout the experiment.

Light curve measurements on whole individuals grown in climate-controlled room

Selected leaf rosettes from 36 whole plants successfully were measured on three points between August and December 2021. A plant would be measured after spending one week getting acclimated to the climate-room conditions (Period 1). Once a plant had spent one month (+ acclimation week) in the climate-room, a second light curve was performed (Period 2). For the third light curve the plant had spent another month (+ acclimation week) in the climate-room (Period 3). The light curve program was extended to saturating pulses every 90 seconds (compared to settings for cuttings) at 13 PAR intervals ~ (0, 23, 43, 63, 87, 121, 185, 277, 408, 612, 800, 1119, 1461 μmol m^{-2} s^{-1}). This was to see if a longer lasting light curve would trigger photoinhibition in the plant, which is when ETR drops after having reached its maximum value.

Statistical analysis

All statistical testing and generation of figures were done using Python 3.0 (Van Rossum & Drake, 2009) alongside the libraries SciPy 1.0, Pandas, NumPy, Matplotlib and Statsmodels

(Harris et al., 2020; Hunter, 2007; Pandas development team, 2020; Seabold & Perktold, 2010; Virtanen et al., 2020). Python 3.0 was also used to create a custom script to convert and rearrange the raw data into more manageable comma-separated value files, inspired by Darwin Core formatting (https://dwc.tdwg.org/). I also did several rounds of curve fitting to estimate the maximum values of ETR (ETR_M) and NPQ (NPQ_M).

Analysis of data from cuttings and whole plants were done separately as they are based on different measurement setups. When checking for differences between two groups, such as ETR_M and NPQ_M from measurement from whole plants, student-t test was used. If there were more than two groups, as for the cuttings that contained diploids, triploids and tetraploids, one-way ANOVA was used. Additionally, one-way ANOVA was used to check for differences for F_V/F_M between field and lab measurements, and to check for differences in LMA, RWC and C/N between ploidy levels and between sampling location. If the one-way ANOVA gave a significant result, I tested the data again using Tukey's test to reveal the significant differences. The main analysis was done using mixed linear regression models to utilize data that comes from either several measurement periods on the same plants, or different years of field measurement that happen to use the same plants in some cases. For mixed model used on the leaf trait data, I used LMA, RWC and C/N as the response variables, with the effects of ploidy and habitat as categorical variables and plant identification as a random effect. For the light curves I used a similar model. There ETR_M or NPQ_M were the response variables, with the effects of ploidy and measurement period as categorical variables, with the addition of F_V/F_M as a fixed effect. Again, the plant identification was used as a random variable. Models fit was evaluated using residual analysis.

Results

Analysis of the leaf measurements LMA, RWC and C/N from 2020 and 2021

In total from 2020 and 2021 I ended up with leaf traits form 187 individuals (Table 2). The distribution of measurements per habitat was not equal, and for the ridge the diploids were overrepresented (Table 2).

Table 2. The habitat distribution individuals of *Saxifraga oppositifolia* sampled in 2020 and 2021, split into ploidy levels. A "-" indicate no measurement was attempted, while "0" indicates measurements were attempted, but no plant was found or selected.

	Ridge (2020 / 2021)	**Riverbed (2020 / 2021)**	**Slope (2020)**
Diploid	24 / 30	27 / 3	16
Triploid	6 / -	6 / -	15
Tetraploid	4 / 0	21 / 24	8
Total	34 / 30	54 / 24	39

The percentage of RWC (Table 3) did not differ between the ploidy levels, for both years, when tested through one-way ANOVA (Figure 5b, Table 3). However, testing the combined effect of ploidy and habitat using a mixed linear model, showed that riverbed and slope increased RWC for diploids (Appendix 1). In slope habitats the increase in RWC was 9.293 % ± 2.432 standard error (SE), $p = 0.002$) and in riverbeds it increased by 6.375 % ± 2.432 SE, $p = 0.009$ (Appendix 1). There were some interactions between the polyploids and habitat that reduced RWC compared to the reference level set by diploids in ridges (Appendix 1).

C/N ratio (Table 3) did also not differ between the ploidy levels (Figure 5c, Table 3). Nitrogen leaf content across all individuals was 1 g of nitrogen per 36 g of carbon, and the mean percentage of nitrogen and carbon leaf content was 1.301 % ± 1.61 standard deviation SD and 44.6 % ± 1 SD, respectively. Mixed linear model regression gave no significant effect of polyploidy and habitat upon C/N.

Table 3. The mean values for each measurement year, for the leaf traits leaf mass per area (LMA), relative water content (RWC) and the ratio of carbon-to-nitrogen content in the leaves, ± the standard error. In parentheses, n = the number of replicates.

Ploidy Level	2020			2021	
	LMA (g m^{-2})	RWC (%)	C/N ratio	LMA (g m^{-2})	RWC (%)
Diploid	81.8 ± 1.7 (n = 65)	69.6 ± 1.5 (n = 65)	37.9 ± 1.3 (n = 45)	86.8 ± 2.8 (n = 30)	65.6 ± 3.1 (n = 30)
Triploid	99.4 ± 2.5 (n = 32)	68.7 ± 1.8 (n = 27)	32.9 ± 1.4 (n = 20)	-	-
Tetraploid	98.7 ± 3.2 (n = 33)	66.6 ± 2.1 (n = 33)	37 ± 1.6 (n = 19)	106 ± 3.3 (n = 23)	61.1 ± 1.6 (n = 24)
All	89.9 ± 1.5 (n = 123)	68.6 ± 1 (n = 123)	36 ± 0.9 (n = 86)	95.3 ± 2.6 (n = 53)	63.3 ± 1.7 (n = 54)

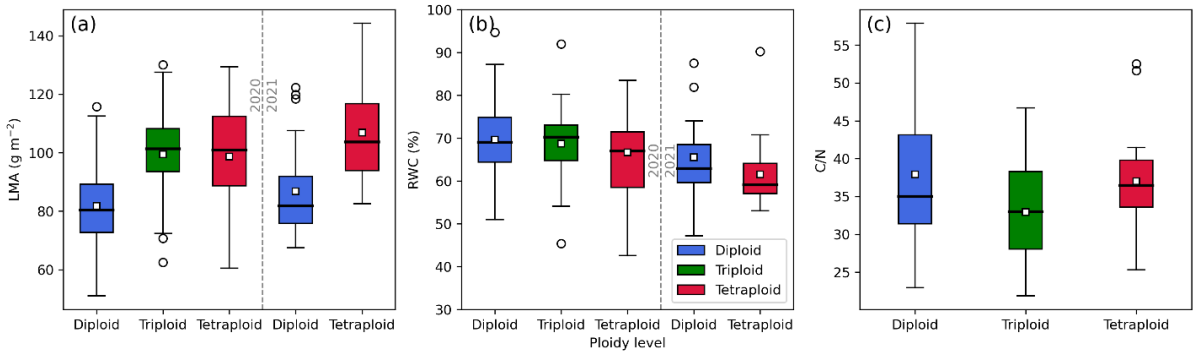


Figure 5. Boxplots of (a) leaf mass per area (LMA), (b) relative water content (RWC) and (c) carbon-to-nitrogen ratio (C/N) measurements from field individuals of *Saxifraga oppositifolia*. The measurements are grouped by ploidy level and for figures (a) and (b) split into the year of measurement.

LMA for diploids (Table 3) were significantly lower for measurements done in 2020 when compared to triploids (adjusted-$p < 0.000$, Tukey's test) and tetraploids (adjusted-$p < 0.000$, Tukey's test) (Figure 5a). For 2021, it was also significantly lower when compared to tetraploids ($p = 0.001$, Student's t-test) (Figure 5a). Results from the mixed linear regression showed some significant relationships (Table 4). Tetraploidy increased LMA by 27.331 g m^2 ±7.929 SE, $p = 0.001$ (Table 4). Interaction between triploid and riverbed did have a positive effect upon LMA (19.256 g m^{-2} ± 9.192 SE, $p = 0.036$) (Table 4) and the interaction of tetraploid and slope had a negative effect upon LMA (-20.262 g m^{-2} ± 10.424 Se, $p = 0.048$) (Table 4). While results from the one-way ANOVA showed both polyploids to have higher LMA, the mixed linear regression showed that only riverbed triploid increased LMA (19.256 g m^{-2} ± 9.192 SE, $p = 0.036$) (Table 4).

Table 4. Mixed linear model results for fixed effects of polyploidy (triploid and tetraploid) and habitat on leaf mass per area (LMA) in field individuals of *Saxifraga oppositifolia*. Reference levels were diploid for ploidy and ridge for habitat. Units of estimates are in g m^{-2}. Estimates are given with 95% confidence intervals, standard errors (SE) and p-values.

LMA ~ Ploidy Level * Habitat, (Group: Plant Identification)				
Fixed Effects	Estimates	Confidence interval	SE	*p*-value
(*Intercept*)	85.389	81.180 – 89.597	2.147	
Triploid	7.918	1.201 – 0.230	6.592	0.23
Tetraploid	27.331	3.447 – 0.001	7.929	**0.001****
Riverbed	(-5.179)	(-1.473) – 0.141	3.516	0.141
Slope	(-2.567)	(-0.557) – 0.578	4.611	0.578
Triploid:Riverbed	19.256	2.095 – 0.036	9.192	**0.036***
Tetraploid:Riverbed	(-4.179)	(-0.479) – 0.632	8.722	0.632
Triploid:Slope	5.989	0.678 – 0.498	8.833	0.498
Tetraploid:Slope	(-20.262)	(-1.978) – 0.048	10.424	**0.048***
No. of groups:	177			
No. of observations:	160			
Marginal / Conditional R^2:	0.29 / 0.29			

Looking into LMA variation in diploids between sampling locations, highest values of LMA was seen on the Foxdalen ridge during 2021 (Figure 6), but testing the means with a one-way ANOVA was not significant ($p = 0.29$).

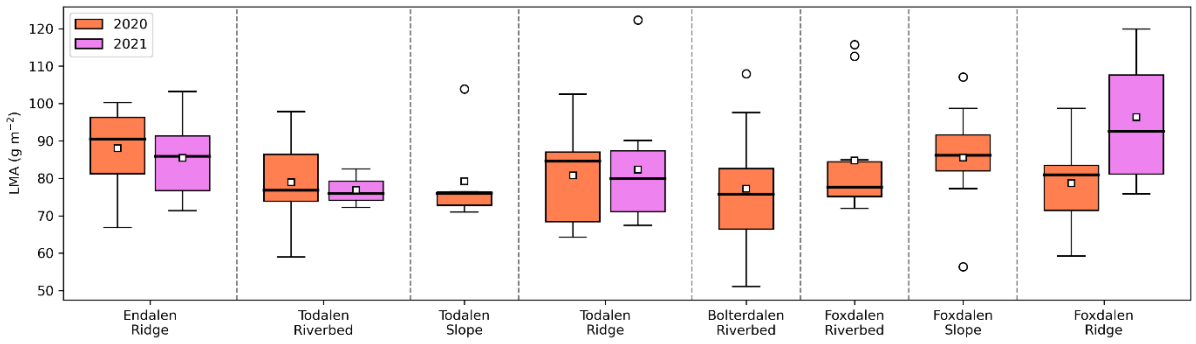


Figure 6. Boxplot of leaf mass per area (LMA) from diploid individuals of *Saxifraga oppositifolia*, split into the location the measurements originate from and the habitat type. Orange boxplot are measurements done during summer of 2020 and pink summer of 2021.

For LMA values in tetraploids, there was significant variation between the sampling locations (p = 0.038, one-way ANOVA). A Tukey's test revealed tetraploid LMA from Todalen riverbed 2021 (Figure 7) were significantly different to LMA from Bolterdalen, slope 2020 (p = 0.029). There were no differences in triploid LMA between the sampling locations (p = 0.19, one-way ANOVA, Appendix 17).

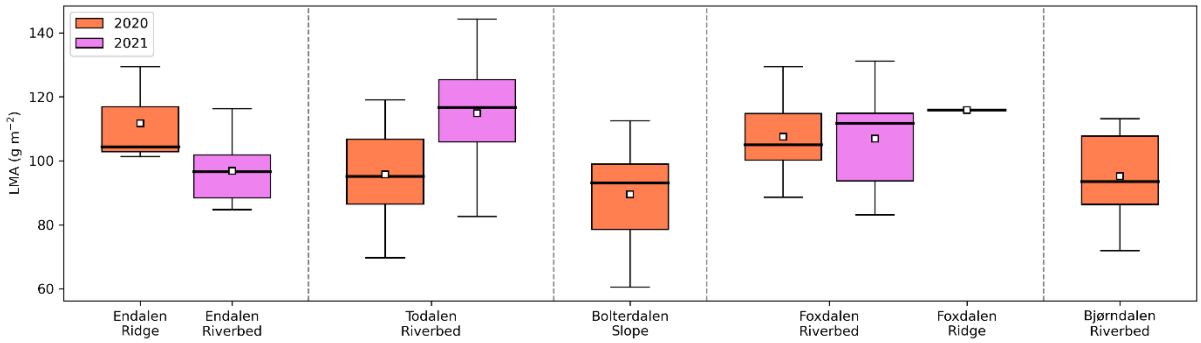


Figure 7. Boxplot of leaf mass per area (LMA) from tetraploid individuals of *Saxifraga oppositifolia*, split into the location the measurements originate from and the habitat type. Orange boxplot are measurements done during summer of 2020 and pink summer of 2021.

Light curve totals and low survival in climate-controlled room

In total 100 light curve measurements were performed on *S. oppositifolia* for measurement setting 2 and 3 (Table 4). For both measurements, individuals of *S. oppositifolia* were kept in a climate-controlled room. The survival of the plants in this setting was poor, resulting in smaller sample sizes per measurement period for the later periods (Table 5).

Table 5. Total individuals per measurement period from each ploidy level where light curves were measured.

Ploidy Level	**Cuttings**	**Whole plants**
	Period 1 / Period 2	**Period 1 / Period 2 / Period 3**
Diploid	9 / 6	18 / 7 / 5
Triploid	5 / 0	-
Tetraploid	12 / 5	18 / 9 / 4
Total	26 / 11	36 / 18 / 9

Curve fitting to get parameter estimates for ETR_M and NPQ_M

The WinControl software performs two model equations called REG1 (Eq. 9) and REG2 (Eq. 10) once a LC is completed. Equation 9 is from Platt et al., (1980) and Equation 10 from Jassby & Platt, (1976). These equations fit a curve to the measurement points of the ETR curve (ETR plotted against PAR) and returning fitted parameters describing the curve such as β exclusively from Equation 9, and *a*, ETR_M and I_K from both Eq. 9 and Eq. 10 (*MINI-PAM II Photosynthesis Yield Analyzer Manual*, 2023). ETR_M represents the peak for the fitted curve, *a* the initial slope angle of the curve, I_K is the saturation points of the curve, and β represents the downturn of the curve, which relates to photoinhibition. As photoinhibition was rarely observed in our measurements, the WinControl software did not successfully fit Eq. 9 to most of the light curves and did not return estimated values. Only Eq. 10 was successfully applied to all the light curves. An alternative equation created by Eilers & Peeters, 1988 (Eq. 11) could also be used in this case. Therefore, the light curves were also fitted to Eq. 11 and Eq. 9 to compare which one gave a better fit for the data. Estimates for *a* and *b* for Eq. 9, and *a*, *b*, *c* for Eq. 12 were done using curve_fit() from SciPy 1.0 (Virtanen et al., 2020)(Appendix 5). The result was that Eq. 12 gave an overall better fit to the data, compared to Eq. 10 (Table 6). Parameter estimates for ETR_M were therefore based on Eq. 12, where ETR_M represents the peak of the fitted curve.

$$ETR = a*(1-e^{-(a*PAR/a)})*e^{-(PAR/a)} \quad (9)$$

$$ETR = a*\tanh((b*PAR/a)) \quad (10)$$

$$ETR = PAR/\ a*PAR^2+b*PAR+c \quad (11)$$

A different function (Eq. 12) was used to fit curves to the NPQ data and allows one to calculate additional parameters form the NPQ curve, such as NPQ_M, as this was not provided by the MINI-PAM II software. This function which is based on the Hill equation and the equation form Serôdio & Lavaud, 2011, and parameters *a*, *b* and *c* fitted using curve_fit() from SciPy 1.0 (Virtanen et al., 2020):

$$NPQ = a*(PAR^c/b^c+PAR^c) \quad (12)$$

This equation provided a good it for the data (Table 6). NPQ_M represents the peak for the fitted curve. In the end, the only parameters obtained from the fitted curve used for further analysis were ETR_M and NPQ_M. β were left out as light curves lacked photoinhibition, *a* as it

did not show any sign of significance between the measurements, and I_K due to strong multicollinearity with ETR_M.

Table 6. Evaluation of the fit from the different equations used to calculate light curve parameters. It shows the mean ± standard error for R-Squared (R^2) and the root-mean-square deviation (RMSE), for each equation. The datasets from whole plants and cuttings were kept separate. Equation 12 is based on Serôdio & Lavaud, (2011) and used to calculate the maximum value of non-photochemical quenching (NPQ_M). Both Equation 9 from Eilers & Peeters, 1988 and 11 from (Platt et al., 1980) are used to calculate the maximum value of electron transport rate (ETR_M). Equation 11 provided a better fit for the data, with higher R_2 values and lower values for RMSE, compared to Equation 9. For Equation 11, the last measurement point for one individual during period 1 of the re-rooted cuttings, had to be removed as it gave an invalid fit. Equation 12 also gave some invalid fits, ten for cuttings Period 1, seven for whole plants period 1, and four for whole pants period 2. These were dropped from further analysis.

		ETR_M		**NPQ_M**
Sample group		**Equation 9**	**Equation 11**	**Equation 12**
Cuttings Period 1-2	R^2	0.9 ± 0.02	0.95 ± 0.01	0.96 ± 0.05
	RMSE	1.37 ± 0.11	0.92 ± 0.06	0.07 ± 0.01
Whole plants Period 1-3	R^2	0.9 ± 0.01	0.94 ± 0.01	0.97 ± 0
	RMSE	1.19 ± 0.07	1.01 ± 0.05	0.12 ± 0.1

Climate-controlled room from potted cuttings, over two measurement periods

Light curves were measured on 26 plants for period 1, and eleven plants for period 2 (Table 5). With the low survival rate in the climate-controlled room, no triploids were measured for period 2 (Table 5). I observed ETR to reach higher values in tetraploids for measurements in periods 1 and 2 (Figure 8a-b). The other curves for NPQ and Y(II)/Y(NO)/Y(NPQ) curves showed no signs of differences (Figure 8c-f).

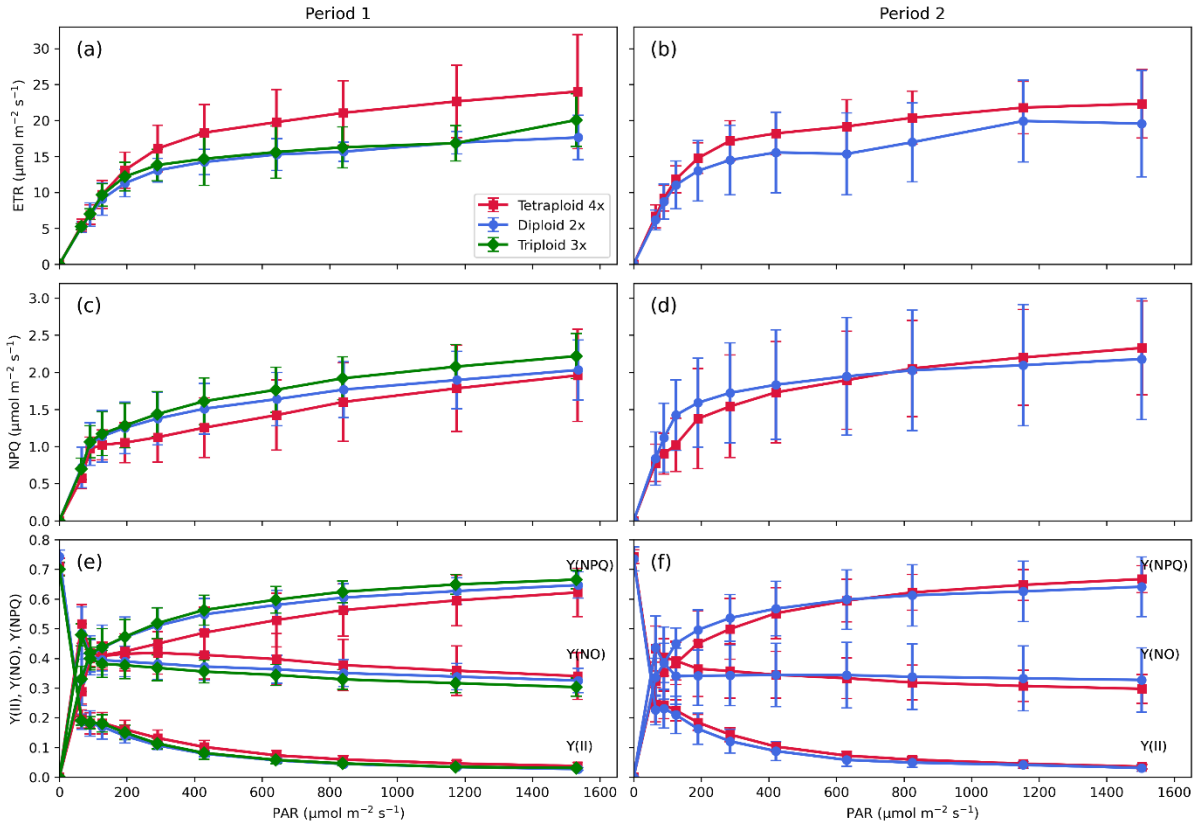


Figure 8. Light curve measurements over two periods of leaf rosettes from *Saxifraga oppositifolia*, of different ploidy levels. The error bars represent one standard deviation and plotted values are the mean per PAR measurement. The first row (a, b) is measurements of Electron Transport Rate (ETR) µmol m^{-2} s^{-1}, the second row (c, d) Non-Photochemical Quenching (NPQ) µmol m^{-2} s^{-1} and the third row (e, f) are the measurements of Y(II), Y(NO) and Y(NPQ).

For calculated parameters ETR_M and NPQ_M (Table 7), the only significance was for ETR_M between tetraploids and diploids for period 1 (p = 0.0127, Tukey's test).

Table 7. Calculation results for maximum values of electron transport rate (ETR_M) and non-photochemical quenching (NPQ_M), from measurement setting 2. Means ± Standard deviation. For each measurement period and split into ploidy level.

	ETR_M µmol m^{-2} s^{-1}		**NPQ_M µmol m^{-2} s^{-1}**	
Ploidy Level	**Period 1**	**Period 2**	**Period 1**	**Period 2**
Diploid	17 ± 0.8 (n = 9)	20.4 ± 2.6 (n = 6)	2 ± 0.2 (n = 7)	2.2 ± 0.3 (n = 6)
Triploid	19.2 ± 1.2 (n = 5)	-	2.1 ± 0.1 (n = 4)	-
Tetraploid	23.7 ± 1.7 (n = 12)	20.1 ± 2.7 (n = 5)	2.1 ± 0.1 (n = 5)	2.5 ± 0.3 (n=5)

I used a mixed linear model used to evaluate measurement period, ploidy level and stress (F_V/F_M) upon ETR_M and NPQ_M (Table 8), with plant ID as a random variable as the dataset contains repeated measurements. The intercept represents diploids at measurement period 1. The only effect that influenced ETR_M were that of tetraploids, which increased ETR_M by 3.941 µmol m^{-2} s^{-1} ± 1.974, $p = 0.045$) (Table 8). The confidence interval range is quite high (Table 8), which is visually evident in a plot of the predicted values from ETR_M and NPQ_M using average F_V/F_M (Figure 9). Triploid efficiency of ETR was closer to diploids, while NPQ efficiency were closer to tetraploid (Figure 9).

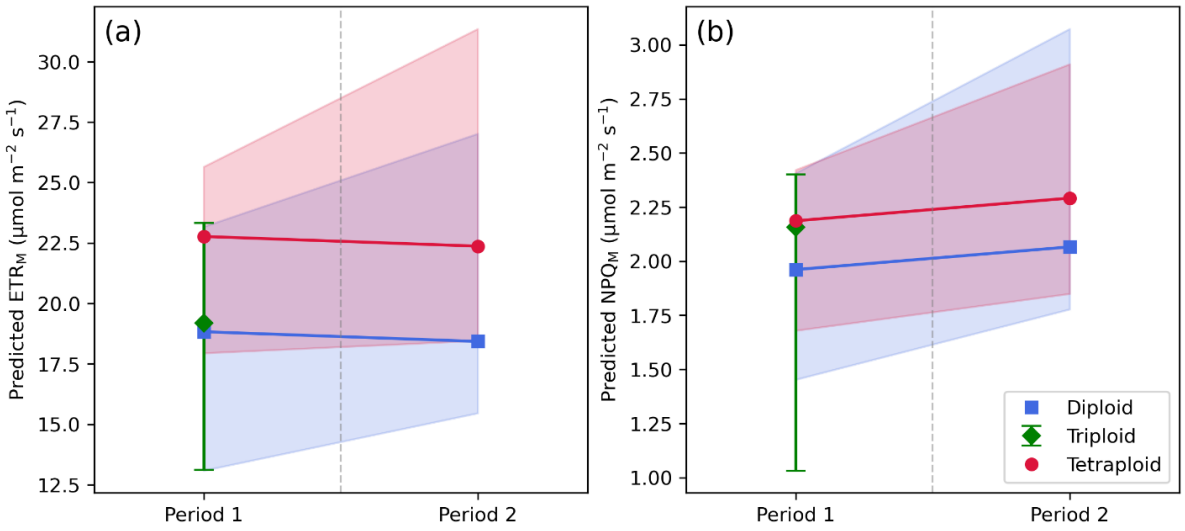


Figure 9. Predicted (a) maximum electron transport rates (ETR_M) and (b) maximum non-photochemical quenching (NPQ_M) based on mixed linear regression models, based on measurements on the re-rooted cuttings of *Saxifraga oppositifolia.* The predicted values are divided into ploidy level (diploid, triploids tetraploid) and plotted against measurement period. Shaded areas and error bars for the triploid, represent 95% confidence intervals. For the prediction, F_V/F_M was set to the mean from all measurements ($\mu F_V/F_M = 0.724$). See Appendix 2 for raw ETR_M estimates.

Table 8. Mixed linear model results for fixed effects of measurement period, polyploidy (triploid and tetraploid) and F_V/F_M on maximum electron transfer rate (ETR_M) and maximum non-photochemical quenching (NPQ_M) in cuttings of *Saxifraga oppositifolia* grown in a climate-controlled room. Value units for both models are μmol m^{-2} s^{-1}. Estimates are given with 95% confidence intervals, standard errors (SE) and p-values.

ETR_M (Eq. 11) ~ (Category: Period 2 + Ploidy) + F_V/F_M + (Group: Plant Identification)				
Predictors	**Estimates**	**95% CI**	**SE**	***p*-value**
(*Intercept*)	18.133	(-24.418) – 60.683	21.710	
Period 2	(-0.402)	(-4.433) – 3.630	2.057	0.845
Tetraploid	3.941	0.073 – 7.809	1.974	**0.045***
Triploid	0.369	(-5.125) – 5.862	0.132	0.369
F_V/F_M	0.962	(-57.032) – 58.956	0.033	0.895
Random Effects				
Plant Identification	0		3.507	
No. of Groups:	31			
No. of Observations:	38			
Marginal / Conditional R^2:	0.12 / 0.12			
NPQ_M (Eq. 12) ~ (Category: Period 2 + Ploidy) + F_V/F_M + (Group: Plant Identification)				
(*Intercept*)	(-1.791)	(-9.104) – 5.522	3.731	
Period 2	0.105	(-0.415) – 0.625	0.265	0.692
Tetraploid	0.226	(-0.2) – 0.651	0.217	0.299
Triploid	0.197	(-0.412) – 0.805	0.310	0.527
F_V/F_M	5.179	(-4.957) – 15.316	5.172	0.317
Random Effects				
Plant Identification	0	0.327		
No. of Groups:	22			
No. of Observations:	28			
Marginal / Conditional R^2:	0.13 / 0.13			

Climate-controlled room light curves from whole plants, over three measurement periods

In this round of light curve measurements, I again observed higher values of ETR in the curves for tetraploids, with the exception for measurement period 2 (Figure 10a-c). The difference is however not as prominent as seen in measurement setting 2 (Figure 8). For the Y(II)/Y(NO)/Y(NPQ) curves there was no striking differences (Figure 10g-i). Unlike the cuttings, the curves for NPQ distinguished themselves, where tetraploid curves reach higher observable values for NPQ (Figure 10d-f).

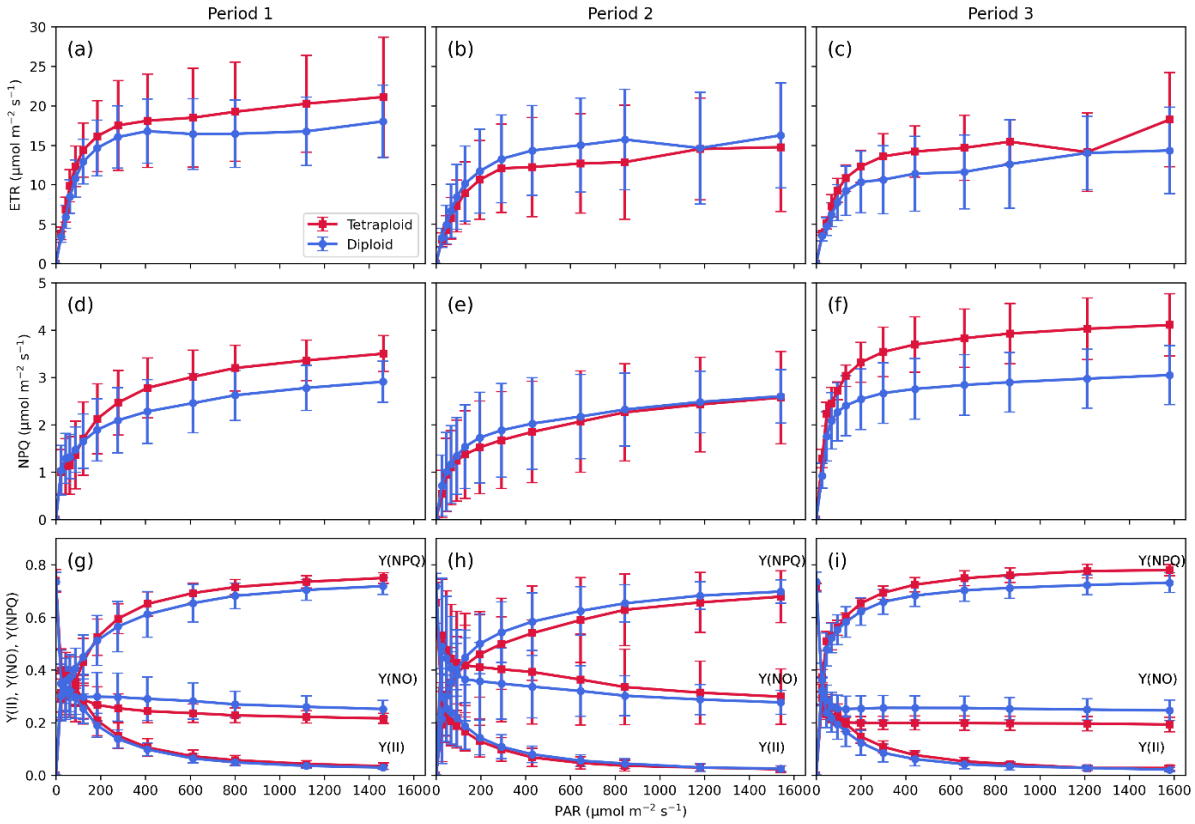


Figure 10. Light curves measurements over 3 periods of leaf rosettes from *Saxifraga oppositifolia*, of different ploidy levels. The error bars represent the standard deviation and plotted values are the mean per PAR interval. The first row (a, b, c) is measurements of Electron Transport Rate (ETR) µmol m^{-2} s^{-1}, the second row (d, e, f) Non-Photochemical Quenching (NPQ) µmol m^{-2} s^{-1} and the third row (g, h, i) are the measurements of Y(II), Y(NO) and Y(NPQ).

For the calculated values of ETR_M and NPQ_M (Appendix 3), no significant difference was found between the ploidy levels (Table 9).

Table 9. Calculation results for maximum values of electron transport rate (ETR_M) and non-photochemical quenching (NPQ_M), from measurement setting 3. For each measurement period and split into ploidy level.

Ploidy Level	Period 1	Period 2	Period 3
	ETR_M µmol m^{-2} s^{-1}		
Diploid	18.4 ± 1 (n = 18)	16 ± 2.4 (n = 7)	14.3 ± 2.2 (n = 5)
Tetraploid	21.5 ± 1.7 (n = 17)	15.1 ± 2.4 (n = 9)	17.1 ± 2.4 (n = 4)
	NPQ_M µmol m^{-2} s^{-1}		
Diploid	3.1 ± 0.1 (n = 12)	2.7 ± 0.2 (n = 6)	2.9 ± 0.4 (n = 5)
Tetraploid	3.6 ± 0.1 (n = 16)	3.2 ± 0.1 (n = 6)	4 ± 0.4 (n = 4)

I utilized a mixed linear model with the same design as the one for the re-rooted cuttings. Tetraploidy did not have a significant effect on ETR_M (2.63 ± 1.505, p = 0.078, Table 7), but ETR_M did increased with F_V/F_M (40.218 µmol m^{-2} s^{-1} ± 1.505, p = 0.081 , Table 7). I also found tetraploidy to significantly increase NPQ_M µmol m^{-2} s^{-1} (0.601 µmol m^{-2} s^{-1} ± 0.141, p < 0.000, Table 7). NPQ_M was lower for period 3 (-0.42 µmol m^{-2} s^{-1} ± 0.088, p < 0.004, Table 7) and NPQ_M increased with F_V/F_M (3.604 µmol m^{-2} s^{-1} ± 1.65, p < 0.029, Table 7). As in the model for re-rooted cuttings, there is also large variation and high uncertainties in the results (Table 7, Figure 11). The modelling of NPQ_M showed less variation than for ETR_M (Figure 11). While not significant (p = 0.151, one-way ANOVA), ETM_M was highest for tetraploids harvested from Todalen, riverbed (Appendix 4).

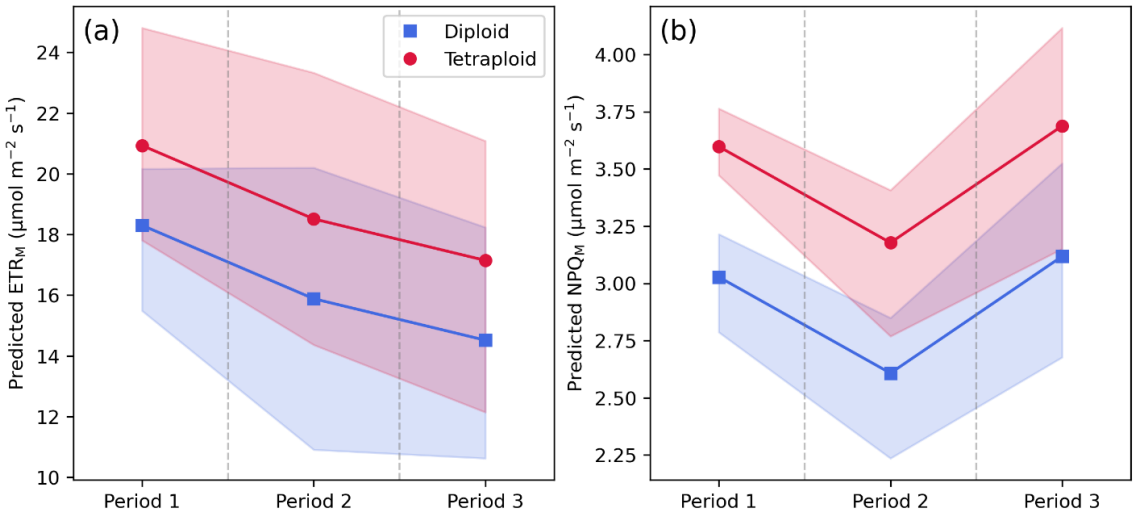


Figure 11. Predicted (a) maximum electron transport rates (ETR_M) and (b) maximum non-photochemical quenching (NPQ_M) based on mixed linear regression models, based on measurements on the whole plants of *Saxifraga oppositifolia.* The predicted values are divided into ploidy levels (diploid and tetraploid) and plotted against measurement periods. Shaded areas represent 95% confidence intervals. For the prediction, F_V/F_M was set to the mean from all measurements (µF_V/F_M = 0.729). See Appendix 3 for raw ETR_M estimates.

Table 10. Mixed linear model results for fixed effects of measurement period, polyploidy (tetraploid) and F_V/F_M on maximum electron transfer rate (ETR_M) and maximum non-photochemical quenching (NPQ_M) in whole individuals of *Saxifraga oppositifolia* grown in a climate-controlled room. Value units for both models are μmol m^{-2} s^{-1}. Estimates are given with 95% confidence intervals, standard errors (SE) and p-values.

ETR_M (Eq. 11) ~ (Category: Measurement Period + Tetraploid) + F_V/F_M + (Group: Plant Identification)				
Predictors	**Estimates**	**95% CI**	**SE**	***p*-value**
(*Intercept*)	(-11.008)	(-34.302) – 12.286	11.885	
Period 2	(-2.419)	(-6.186) – 1.348	1.922	0.208
Period 3	(-3.787)	(-8.005) – 0.431	2.125	0.078
Tetraploid	2.63	(-0.321) – 5.58	1.505	0.081
F_V/F_M	40.218	9.154 – 71.281	14.546	**0.011***
Random Effects				
Plant Identification	0.017		1.351	
No. of Groups:	36			
No. of Observations	60			
Marginal / Conditional R^2:	0.222 / 0.222			
NPQ_M (Eq. 12) ~ (Category: Measurement Period + Tetraploid) + F_V/F_M + (Group: Plant Identification)				
(*Intercept*)	0.895	(-1.233) – 3.034	1.086	
Period 2	-0.42	(-0.171) – 0.173	0.088	**0.004****
Period 3	0.09	(-0.219) – 0.399	0.158	0.568
Tetraploid	0.601	0.324 – 0.879	0.141	**0.000****
F_V/F_M	3.604	0.371 – 6.837	1.65	**0.029***
Random Effects				
Plant Identification	0.071		0.179	
No. of Groups	32			
No. of Observations	49			
Marginal / Conditional R^2:	0.421 / 0.63			

Comparison of stress levels between field and climate-controlled room individuals

As the stress parameter F_V/F_M was measured both in field and for every light curve performed in the climate-controlled room, it was possible to compare stress levels between *in situ* and *ex situ* individuals. When comparing F_V/F_M between field and the five measurement periods from cuttings and whole plants, I found individuals to be significantly less stressed in the field (adjusted-$p < 0.05$ for field versus every measurement period, Tukey's test) (Appendix A) (Figure 12). Expanding the comparison between the ploidy levels, tetraploids during whole plants period 2 had the lowest F_V/F_M (Appendix 13, Figure 12). Both whole plant diploids and tetraploids had higher F_V/F_M during period 2, than period 1 (Appendix 13, Figure 12).

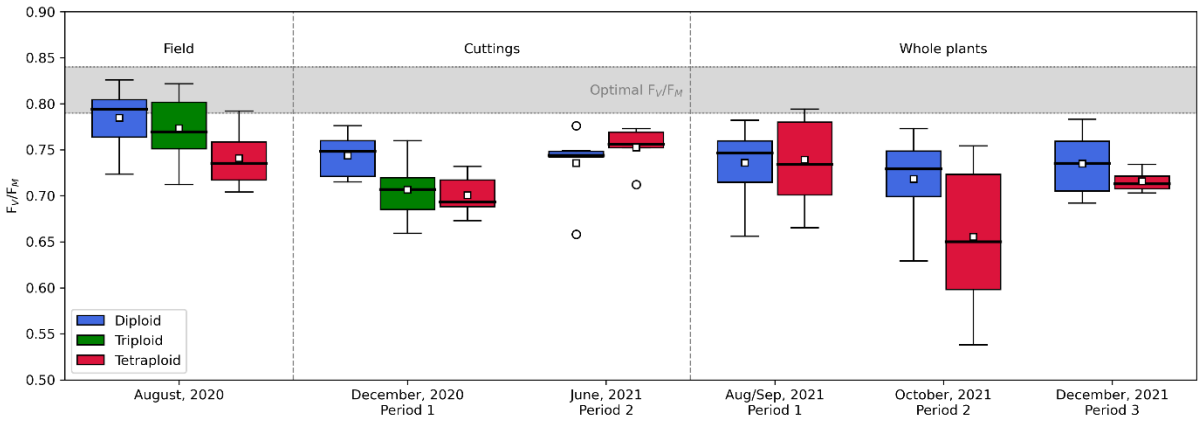


Figure 12. The different sets of chlorophyll *a* fluorescence measurement on different ploidy levels of *Saxifraga oppositifolia*, displaying calculated values for F_V/F_M. The first set are field measurements from August 2020, while the two others were measured on individuals grown in a climate-controlled room. "Cuttings", are re-rooted cuttings harvested in the field (Tjessem, 2021), while "whole plants" are whole individuals of *S. oppositifolia* harvested in the field and grown under the same conditions as the cuttings. The shaded area represents the optimal values for F_V/F_M for an average plant (Kitajima & Butler, 1975).

Discussion

I investigated several plant physiological parameters in the established autopolyploid population of *S. oppositifolia* in Svalbard, Norway, to get a better understanding of the impact of polyploidy on its success, likely a result of niche differentiation. I found strong evidence of higher leaf LMA in polyploids of *S. oppositifolia* (Table 3), which contradicted expectations. Tetraploid re-rooted cuttings did have some evidence suggesting higher PSII efficiency than diploids (Figure 9a), but this was not seen in the whole individuals measured. In the tetraploid whole individuals (Figure 11a), I instead saw higher NPQ efficiency compared to diploids (Figure 11b), suggesting higher stress tolerance in the tetraploids. Stress likely played a part in climate-controlled room measurements, where they showed sub-optimal levels for F_V/F_M (Figure 12), in agreement with this reduced efficiency of PSII in whole plants and re-rooted cuttings, and low survival rate form both in the climate-controlled room.

Nitrogen, carbon and relative water content of leaves

The variability in RWC was high, with the lowest measurement reaching around them being highly desiccated at 40 % RWC and extremely turgid with RWC of around 95 %, with the ploidy means ranging between 61 and 70 % RWC. This is slightly below the RWC ranges from measurements on Arctic plants (Dawson & Bliss, 1989; Oberbauer & Miller, 1981). The independent comparisons of RWC between ploidy levels did not return any significance. However, habitat type did have an effect upon RWC. Diploids on both slope and riverbeds had higher RWC, compared to those growing on the ridges. Although there were interactions between polyploids and habitat, it is important to be cautious in interpreting these results due to the limited presence of polyploids on ridges. (Table 2, Appendix 1, Figure 4). Lower RWC on ridges could be explained by the fact that RWC correlates with soil moisture content (Fischer, 1973), and that all ridge plots used in the study had lower measurements of soil moisture content than both slope and riverbed plots (Eidesen et al., (in prep)).

When it came to the C/N content of the leaves, I found no support for my hypothesis predicting higher levels of nitrogen in the polyploids. As most of the polyploids inhabit low-laying site, with late snowmelt and a short window for growth, it was expected that they had traits linked to higher photosynthetic rates (Crawford et al., 1993, 1995), and leaf nitrogen content is positively associated with photosynthetic rate (Evans, 1989; Muraoka et al., 2008). *S. oppositifolia* had a mean nitrogen leaf content of 1.3 % (Table 3), landing them in the low

range of Arctic plants (Hudson et al., 2011; Kudo et al., 2001; Tolvanen & Henry, 2001), but about the same nitrogen content was seen in *S. oppositifolia* in previous studies (Muraoka et al., 2008). Kume et al., (1999) suggested that because prostate growth forms of *S. oppositifolia* covers has a large surface area per mass, these plants might have an advantage in nutrient foraging (Parkhurst & Loucks, 1972). Evidence of this could not be seen in this case in the prostate restricted *S. oppositifolia* polyploids tested in the study, but there could be nutritional allocation differences in other tissues, or differences are simply suppressed by the general low availability of soil nitrogen in the Arctic (Anderson & Polis, 1999; Gharajehdaghipour et al., 2016). Previous data on the soil C/N ratio around the same pool of field plants I used, also did not show any difference between the ploidy levels (Eidesen et al., (in prep).

LMA for diploids contradicts expectations set in hypothesis

I could not find support for my hypothesis that predicted lower LMA in polyploids of *S. oppositifolia*. An expected strategy for the polyploids was higher photosynthetic rates and a growth as an adaptation to the shorter growth seasons they experience (Crawford et al., 1993, 1995) as they invest less resources into sexual reproduction (Brožová et al., 2023; Kume et al., 1999; Tjessem, 2021). This would be reflected in lower LMA in polyploids, which is correlated with higher photosynthetic rates and shorter leaf life span (Schlesinger & Chabot, 1977; Sobrado & Medina, 1980; Wright et al., 2004). However, on the contrary, diploids had lower LMA than polyploids. This difference was present for both sampling years (2020 and 2021), and when modeling the data for both years, tetraploidy had a significant positive effect upon LMA, increasing the average by 33% (Table 4). Triploids have similar mean LMA as tetraploid, but their effect upon LMA was only significant with an interaction between triploid and riverbed habitat (Table 4). Interaction between slope and tetraploid gave a small significant negative effect on LMA, but sample size from slope was low (n = 8, Table 2). At least, Bolterdalen tetraploids had the lowest LMA (Figure 7). While diploids came out with lower LMA, this does not mean diploids LMA is low compared to flora of the same functional group. LMA in herbs ranges from 25 to 130 g m^{-2}, and diploids in this study had an average LMA of 81.8 (2020) to 86.8 (2021) g m^{-2} (Table 3) would place them above 75 % of forbs in the world, and slightly above the mean of 76 g m^{-2} for tundra flora (Poorter et al., 2009). For the tetraploids and triploids, they had an average LMA of 99.4 (2020) g m^{-2} and 98.7 (2020) to 106 (2021) g m^{-2} (Table 3) respectively. This places them close to being

among the top 25% of tundra plants (Poorter et al., 2009). Diploids, therefore, while having a lower LMA than polyploids, still have overall high values for LMA, and likely benefits from the advantages of thicker, sturdier, long-lasting leaves (Poorter et al., 2009).

Higher LMA is considered to reduce leaf desiccation giving a plant higher drought tolerance (Nardini, 2022; Schlesinger & Chabot, 1977; Sobrado & Medina, 1980). This was exemplified in a study by W.-D. Li et al., (2009), where they exposed the diploids and tetraploids of the autopolyploid honeysuckle *Lonicera japonica* Thumb. to water stress. They report that the tetraploids tolerate water stress better than diploids, and with the tetraploids leaf structure reflecting their ability to deal with drought, as they had higher LMA, thicker epidermis and palisade tissue, and denser pubescence (W.-D. Li et al., 2009). Thus, polyploids of *S. oppositifolia* likely means it possesses higher drought tolerance compared to the diploids. This could be important for the polyploids. Svalbard is classified as an Arctic desert, with little precipitation and dry winds (Hanssen-Bauer et al., 2019), where traits improving drought tolerance could drive niche ecological differentiation compared to the parentals. Tetraploids also do not form cushions (Eidesen et al., 2013). Having cushion growth form can increase soil moisture directly under the cushion by reducing evaporation, leaving more water available for the plant (Cavieres et al., 2007). The higher LMA in polyploids could compensate for this by reducing water loss. This effect is likely increased as prostrate form is low laying and less exposed to the dry Arctic winds (Peterson, 2014). One could conclude by this that the polyploid probably deals better with water stress, and that this has evolved post-polyploidization, something that matches findings on polyploidy and water stress in *Chamaenerion angustifolium* (L.) Scop. (Maherali et al., 2009). The thicker leaves on the polyploids could also benefit them from the cold temperatures and freezing, as it has been reported that LMA increase with lower temperatures (Poorter et al., 2009).

A reason for using LMA was that it can correlates with photosynthetic rates (A. V. Perera-Castro & Flexas, 2023; Poorter et al., 2009; Wright et al., 2004). I did see evidence of this relationship in my dataset, instead the reverse was observed and LMA could not be used as a proxy for photosynthetic rates as the diploids did not show signs of higher rates. Instead found some weak evidence that the polyploids, specifically tetraploids, can have a more efficient PSII.

Weak evidence of more efficient photosystem II in tetraploids

I only found partial support of my hypothesis that the polyploids have higher photosynthetic potential, where photosynthesis was approached based on the yield of PSII, and subsequently the calculation of the ETR. The mean ETR values across measurements on cuttings and whole plants, ranged from 14.3 to 23.7 µmol m^{-2} s^{-1} (Table 7, Table 9). This range is rather low, and similar ETR values are seen in Arctic-alpine plants under sub-optimal conditions and these lower values are likely stress induced (Barták et al., 2012; Griffin et al., 2013; A. V. Perera-Castro & Flexas, 2023) and is more in the range of a moss (A. V. Perera-Castro et al., 2021; A. V. Perera-Castro & Flexas, 2023). A problem with ETR (Eq. 8) is the fact that it is based on two assumptions, (1) that 84 % of photos are absorbed by the leaf and (2) that the partitioning of light between PSII and PSI is 50 % (Baker, 2008). These estimates are based on complex methods, and can deviate significantly (Ehleringer, 2000; Hodáňová, 1985; Jones, 1985; Laisk et al., 2006; Laisk & Loreto, 1996; Miyake et al., 2004). As these estimates were not confirmed for the leaves of *S. oppositifolia*, (Baker, 2008) advises not to use ETR for comparing differences, while others argue ETR can still be a useful measurement despite its potential errors, which can be rooted out by combining ETR with gas exchange measurements (A. V. Perera-Castro & Flexas, 2023). While one should still be cautious of comparing ETR from *S. oppositifolia* with other species, assuming leaves absorbance and PSII/PSI partitioning is similar across its ploidy levels, I would argue intraspecific comparisons can be made.

When it came to the ETR measurements, triploids did not differ from the diploids, while tetraploid did differ when measuring ETR on leaf rosettes from the re-rooted cuttings. There, tetraploid had about 22 % higher efficiency of PSII, compared to the diploids (Table 8). The re-rooted cuttings had been a part of an experiment on asexual reproduction through vegetative propagation, something tetraploids did better (Tjessem, 2021). Polyploidy impact on photosynthesis does vary and there is not clear pattern (Warner & Edwards, 1988, 1993). The higher efficiency of PSII in tetraploid could be reflecting their ability to better cope with the conditions surrounding establishment through vegetative propagation. As in the measurements on whole plants, neither polyploid showed higher PSII efficiency, the hypothesized idea that tetraploid has higher photosynthetic efficiency as an adaptation to short growing seasons (Crawford et al., 1995), seem less likely to be the case. Instead, tetraploids higher efficiency of PSII could be an adaptation to increase success through vegetative propagation, and a reproductive adaptation. The higher estimates of PSII are then

more a reflection of tetraploid being able to keep efficiency up during stress, like what is reported by W.-D. Li et al., (2009). Future studies on the photosynthetic capabilities of *S. oppositifolia* and polyploids should account for their response under different conditions. As ETR can be wrongly estimated as its equation (Eq. 8) is based on several assumptions (Baker, 2008), A. V. Perera-Castro & Flexas, (2023) suggest a combination of ETR, gas exchange and LMA measurements to validate ETR and discover potential errors.

Tetraploids could deal better under high-light conditions

Measurements of the whole plants from the tetraploids reached higher levels of NPQ_M during two of the periods, and tetraploidy had a positive effect upon NPQ_M. However, there were no differences in the measurements from rosette cuttings. This only gives partial support to my hypothesis that tetraploidy can increase efficiency of NPQ. Increase in efficiency of NPQ due to polyploidy has been reported from several studies (Cao et al., 2018; Rakić et al., 2015; Ulum et al., 2021). Those increases were mostly associated to a habitat differentiation with the polyploid moving from a shaded habitat to an open habitat with more persistent solar radiation (Ulum et al., 2021). In Svalbard, shading would only come from landscape features, and with the ridge plant coming from mostly south-facing slopes, moving to riverbed probably will not increase photo period to the plants. Again, growth form may be the explanation of the potential benefits of polyploid increased NPQ efficiency. The strict Arctic cushion plant *Silene aculis* (L.) Jacq., often called the "compass flower", will start flowering on its south facing side, as the cushion as a whole will get an uneven distribution of solar radiation (Ween, 2022). One could argue from this, that with polyploid being prostrate, solar radiation would impact the plant more evenly, and the whole plant at once. NPQ is also linked with desiccation tolerance in plants (A. V. Perera-Castro et al., 2021). NPQ also plays a part xanthophyll cycles, which a process in the chloroplast protecting the photosynthetic apparatus against oxidative damage (Esteban et al., 2015; Latowski et al., 2011). Such damage is caused by reactive oxygen species, highly reactive molecules capable of doing cellular damage (Jakubczyk et al., 2020). As such, higher NPQ in tetraploid could mean they have improved xanthophyll cycles and higher drought tolerance.

The results from the NPQ measurements suggest that at least the tetraploids are better adapted to deal with various stressors, such as prolonged photoperiods and drought. As the higher LMA in the polyploids also suggests better drought tolerance, this could be one

important adaptation for the polyploids. As their habitats supports snow acculturation, they will experience a higher levels of soil moisture come melting season, followed by drier conditions for the remainder of the growing season (Migała et al., 2014). As such, having fine-tuned mechanisms dealing which the stress around this can be beneficial. For further ploidy induced effects upon NPQ in *S. oppositifolia* needs to be investigated more closely in the future, such as in experimental setup where the plant is exposed to different photoperiods and drought conditions (Ulum et al., 2021).

Both re-rooted cuttings and whole plants endured sub-optimal conditions in climate-controlled room

The ability of re-rooted cuttings and potted whole plants of *S. oppositifolia* to transition from the field to the climate-controlled room varied and it was a stressful experience. Several plants died and were not available for repeated measurements (Table 5), the measurements of F_V/F_M were below optimal values (Figure 12), lower ETR than expected (Table 7, Table 9, Table 5), and subjective health assessment of re-rooted cuttings health showed a decline, but also differences between the ploidy levels (Appendix 14-15). Their seemingly better survival in the climate-controlled room matches the findings by Tjessem, (2021), where those same polyploid plants had higher successes in vegetative propagation. Still, F_V/F_M did not show a clear decline on consecutive measurements periods in both re-rooted cuttings and whole plants (Figure 12). This lack of decline in F_V/F_M, while a clear decrease in number of surviving plants, is likely due to survivorship bias. where only the healthy plants survived for the next set of measurements, keeping F_V/F_M stable, or even increase which it did from period 1 to 2 in whole plants (Appendix 13, Figure 12).

Abiotic and biotic stressors are observed to decrease F_V/F_M, but the exact reasons are complex (Baker, 2008). Stress cause oxidative damage and loss of PSII reaction centers (photodamage), and sometimes an increase in NPQ as that can mitigate or prevent such damage (Baker, 2008; Campbell & Tyystjärvi, 2012). This photodamage then impairs the function of PSII (Melis, 1999), causing a decrease in F_V/F_M. However, as the fluorescence is also affected by the optical properties of the leaf (Baker, 2008), structural changes in the thylakoid membrane such as through changes in leaf water status, can impact the measurements of F_V/F_M. While the plant was watered frequently, the ventilation in the room quickly removed humidity and had a suspected drying effect. This could have impacted the leaves water status and the F_V/F_M measurements.

Overall, while the plants in the climate-controlled room were exposed to unintended stress, it does again hint to a higher stress tolerance in the polyploids. While this mainly relies on subjective health assessment of the re-rooted cuttings, which is supported by Tjessem, (2021), it is also reflected in the higher ETR efficiency seen in the tetraploid cuttings (Figure 9).

Limitations and shortcomings

During my study, I identified five major factors that likely influenced my results. 1) The difficulty around measuring fluorescence on *S. oppositifolia*. 2) The conditions in the climate-controlled room. 3) Limitations around PAM fluorescence. 4) High data variability. 5) Lack of polyploids on ridges.

1) *Saxifraga oppositifolia* has small, needle-like leaves, often arranged in tight rosettes (Appendix 6). Not a flat surface which is ideal for fluorescence, which likely introduced some degree of measurement error (Kalaji et al., 2014; Maxwell & Johnson, 2000). The MINI-PAM II design uses a leaf clip, that holds the leaf stable during measurement (*MINI-PAM II Photosynthesis Yield Analyzer Manual*, 2023). However, the leaves of *S. oppositifolia* never properly fit inside the leaf clip provided with the instrument. The best solution was to use a laboratory stand and fix the angle and distance to the leaf manually (Appendix 10). This was only possible in the lab, which meant I could not do light curves in the field. As the angle was done manually, it was likely not the same between measurements.

2) The climate-controlled room did also have certain flaws. The ventilation dried out the air quickly, which seemed to result in increased desiccation stress on the plants. The temperature control for the room was also faulty, and the temperature was often several degrees Celsius warmer or colder than its programmed setting. This means that the plants suffered unnecessary amounts of stress. The plants were also measured outside of the climate-controlled room, which also seemed to stress the plants.

3) PAM fluorescence is only a proxy of photosynthesis, and the data only gives information the first part in the light-dependent stage of photosynthesis in the thylakoid membrane. It lacks insight into the other parts of photosynthesis, such was CO_2 uptake, O_2 production, and how the energy is utilized within the plant (D. A. Bryant & Frigaard, 2006).

4) My PAM fluorescence data also showed high variability, which is likely in part due to the first two factors. This variance is evidence in the large spread in the raw

measurements (Figure 8, Figure 10), high confidence intervals in models (Table 4, Table 8, Table 10), which is also seen in the predicted values (Figure 9, Figure 11).

5) The fact that polyploids are almost absent from ridges made it difficult to investigate the effect of polyploidy without influence of its habitat. This influence was hopefully reduced by allowing for acclimation to the climate-controlled room.

Most of the core issues can be solved by ensuring better growth conditions in the climate-controlled room, investigating possibilities of improved fixture of the PAM fiberoptic to small, thick leaves, potentially allowing for light curve measurements *in situ*. The combination of gas exchange measurements with fluorescence would also greatly improve data quality (Baker, 2008; A. V. Perera-Castro & Flexas, 2023).

Reducing knowledge gaps and future prospects

The story of *S. oppositifolia* is an interesting one, and we may even be witnessing a speciation process initiated by polyploidization. My results add to our collective knowledge about polyploidy and its induced changes. Especially autopolyploidy and physiological changes due to polyploidy has required additional research (Soltis et al., 2016; Spoelhof et al., 2017). It also adds to the debate around the role of polyploidization in increasing trait diversity (Meyers & Levin, 2006).

Overall, I found evidence of ploidy induced changes and that polyploidization can have led to new beneficial adaptations for polyploids of *S. oppositifolia*. Adaptations that can have played a role in polyploid niche expansion, a likely route for successful polyploid establishment (Fowler & Levin, 1984). The polyploidy did have mixed effects upon the measurement parameters, with the habitat it originated from also playing a part (Figure 13). Certain relationships were stronger than others, such as polyploidy increasing LMA, while others like leaf C/N remained unchanged by polyploidy and habitat of origin, and I can only partially reject my H_0 hypothesis (Figure 13).

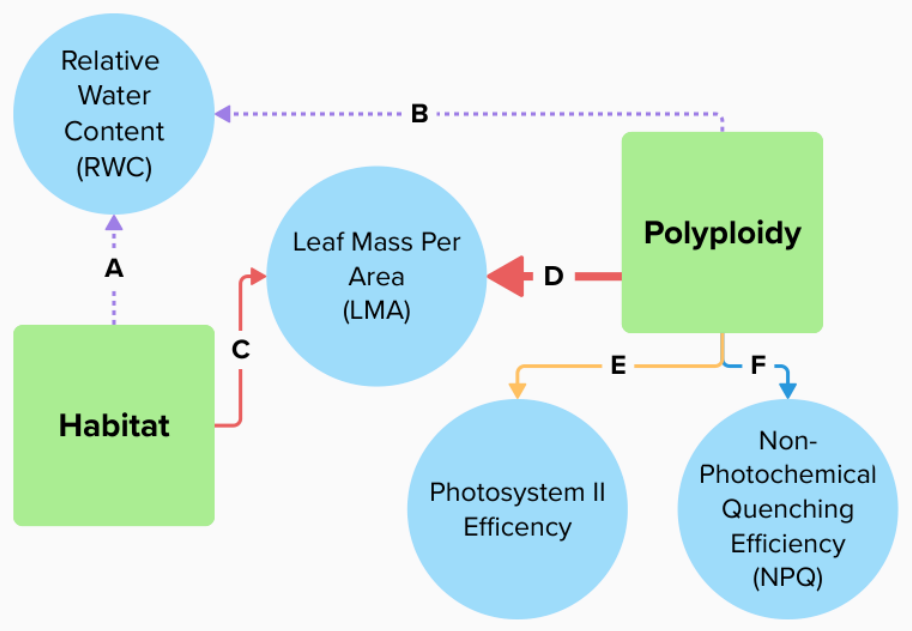


Figure 13. A visual simplification of the main results and proposed effects habitat and polyploidy had upon physiological parameters in the mixed-polyploidy angiosperm *Saxifraga oppositifolia*. Arrow type represents my subjective opinion on effect size and importance, rooted in the conclusions from the results and discussion. The dotted arrows show a weak effect, solid arrow shows a medium effect and bold arrow shows a strong effect. The effect of polyploidy and habitat interacts if arrows are of same color. A) Plants in the low laying habitats slope and riverbed had higher leaf relative water content (RWC). B) Triploids weakly reduced RWC. C) Triploids in riverbeds have higher LMA and tetraploids in slopes have lower LMA. D) Polyploids have higher leaf mass per area (LMA). E) Tetraploid can reach higher efficiency of photosystem II (PSII). F) Tetraploids can reach higher efficiency of non-photochemical quenching (NPQ). Flowchart created using The Mural® visual collaboration platform.

With the Arctic meeting a challenging future, mainly due to anthropomorphic climate change (IPCC, 2023), research into polyploidy can be valuable, since an increase in polyploidization events seems to be linked to historic periods of global change (Sessa, 2019). Polyploids ability to rapidly adapt to new environments (Otto & Whitton, 2000; Soltis et al., 2014) and their higher stress tolerance (Coate et al., 2013; Tossi et al., 2022), could be advantageous in maintaining ecosystems and resisting climate change (Sessa, 2019). However, Crawford et al., (1993) predicted prostrate forms of *S. oppositifolia* adapted to short growing seasons and cold, wet soil, to be at a disadvantage at higher temperatures leading to earlier snowmelt and less water availability. However, maybe polyploidy can help mitigate this.

For future investigations into *S. oppositifolia* and its polyploids, both experiment *in vitro* set-ups and *in situ* measurements are needed. Photosynthetic performance measurements can be enhanced by combination of PAM fluorescence and gas exchange, in combination with more detailed measurement of leaf structure through for example electron microscopy. Biotic advantages such as competitive ability was not touched upon in this study, the locations

where *S. oppositifolia* polyploids live has higher specie richness than the diploid dominated ridges (Eidesen et al.,(in prep)), and the polyploids could be better competitors, as seen in tetraploids of *Lolium perenne* L. (Sugiyama, 1998). As desiccation tolerances seem to be a theme with the polyploid in my results, experimental setup with water availability as a stress factor, could strengthen or weaken my findings around that. Additionally, polyploidy should be considered in studies dealing with intraspecific variation, especially in Arctic environments as frequency of polyploids increase at higher latitudes (Love et al., 1949).

Conclusion

I investigated the effect of autopolyploidy on plant physiological parameters in the mixed-ploidy angiosperm *S. oppositifolia*. Polyploids of *S. oppositifolia* have higher LMA, a likely adaptation to desiccation tolerance from living in areas with fluctuating water availability and lack of cushion growth form. The re-rooted cuttings of tetraploid showed some weak evidence of higher PSII efficiency when under the early establishment phase of vegetative propagation. As the plants were not growing in optimal conditions, tetraploid ability to maintain higher levels of ETR can also be looked upon as higher stress tolerances. Additionally, potted whole plants of tetraploids showed some signs of higher stress tolerance driven by improved NPQ. However, PAM-fluorescence data had high variability, and one should be cautious in drawing strong conclusions around them. I overall speculate that these differences are caused by adaptations to compensate for the lack of cushion growth form in the polyploids, faster growth and stress tolerance for successful establishment through vegetative propagation. Still, more investigations into physiological changed due to autopolyploidy in *S. oppositifolia* are needed.

References

Albert, V. A., Barbazuk, W. B., dePamphilis, C. W., Der, J. P., Leebens-Mack, J., Ma, H., Palmer, J. D., Rounsley, S., Sankoff, D., Schuster, S. C., Soltis, D. E., Soltis, P. S., Wessler, S. R., Wing, R. A., Albert, V. A., Ammiraju, J. S. S., Barbazuk, W. B., Chamala, S., Chanderbali, A. S., … Tomsho, L. (2013). The *Amborella* genome and the evolution of flowering plants. *Science*, *342*(6165). https://doi.org/10.1126/science.1241089

Anderson, W. B., & Polis, G. A. (1999). Nutrient fluxes from water to land: seabirds affect plant nutrient status on Gulf of California islands. *Oecologia*, *118*(3), 324–332. https://doi.org/10.1007/s004420050733

Averett, J. E. (1980). Polyploidy in plant taxa: Summary. In *Polyploidy* (pp. 269–273). Springer US. https://doi.org/10.1007/978-1-4613-3069-1_15

Badano, E. I., & Cavieres, L. A. (2006). Impacts of ecosystem engineers on community attributes: effects of cushion plants at different elevations of the Chilean Andes. *Diversity and Distributions*, *12*(4), 388–396. https://doi.org/10.1111/j.1366-9516.2006.00248.x

Baker, N. R. (2008). Chlorophyll fluorescence: A probe of photosynthesis *in vivo*. *Annual Review of Plant Biology*, *59*(1), 89–113. https://doi.org/10.1146/annurev.arplant.59.032607.092759

Barták, M., Peter, V., & Hájek, J. (2012). Photosynthetic activity in three vascular species of Spitsbergen vegetation during summer season in response to microclimate. *Polish Polar Research*, 443–462.

Bassi, R., & Dall'Osto, L. (2021). Dissipation of light energy absorbed in excess: The molecular mechanisms. *Annual Review of Plant Biology*, *72*(1), 47–76. https://doi.org/10.1146/annurev-arplant-071720-015522

Bilger, W., & Björkman, O. (1990). Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of Hedera canariensis. *Photosynthesis Research*, *25*(3), 173–185. https://doi.org/10.1007/BF00033159

Bretagnolle, F., & Thompson, J. D. (1995). Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. *New Phytologist*, *129*(1), 1–22. https://doi.org/10.1111/j.1469-8137.1995.tb03005.x

Brožová, V., Zeisek, V., Bruls, A. J. H. M., Fjellheim, S., Piálek, L., Gjermundsen, E. F., & Eidesen, P. B. (2023). Same same but different - diploid and tetraploid lineages of *Saxifraga oppositifolia* show similar migration ability, but for different reasons. *(In Press)*.

Bryant, D. A., & Frigaard, N.-U. (2006). Prokaryotic photosynthesis and phototrophy illuminated. *Trends in Microbiology*, *14*(11), 488–496. https://doi.org/10.1016/j.tim.2006.09.001

Bryant, J. P., Reichardt, P. B., & Clausen, T. P. (1992). Chemically mediated interactions between woody plants and browsing mammals. *Journal of Range Management*, *45*(1), 18. https://doi.org/10.2307/4002520

Brysting, A. K., Gabrielsen, T. M., Sørlibråten, O., Ytrehorn, O., & Brochmann, C. (1996). The purple saxifrage, *Saxifraga oppositifolia*, in Svalbard: two taxa or one? *Polar Research*, *15*(2), 93–105. https://doi.org/10.3402/polar.v15i2.6639

Bush, G. L. (2001). Speciation, process of. In *Encyclopedia of Biodiversity* (pp. 620–628). Elsevier. https://doi.org/10.1016/B978-0-12-384719-5.00129-5

Campbell, D. A., & Tyystjärvi, E. (2012). Parameterization of photosystem II photoinactivation and repair. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1817*(1), 258–265. https://doi.org/10.1016/j.bbabio.2011.04.010

Cao, Q., Zhang, X., Gao, X., Wang, L., & Jia, G. (2018). Effects of ploidy level on the cellular, photochemical and photosynthetic characteristics in Lilium FO hybrids. *Plant Physiology and Biochemistry*, *133*, 50–56. https://doi.org/10.1016/j.plaphy.2018.10.027

Cavieres, L. A., Badano, E. I., Sierra-Almeida, A., & Molina-Montenegro, M. A. (2007). Microclimatic modifications of cushion plants and their consequences for seedling survival of native and non-native herbaceous species in the high Andes of central Chile. *Arctic, Antarctic, and Alpine Research*, *39*(2), 229–236.

Chapin, F. S., & Eviner, V. T. (2007). Biogeochemistry of terrestrial net primary production. In *Treatise on Geochemistry* (pp. 1–35). Elsevier. https://doi.org/10.1016/B0-08-043751-6/08130-5

Coate, J. E., Powell, A. F., Owens, T. G., & Doyle, J. J. (2013). Transgressive physiological and transcriptomic responses to light stress in allopolyploid *Glycine dolichocarpa* (Leguminosae). *Heredity*, *110*(2), 160–170. https://doi.org/10.1038/hdy.2012.77

Crawford, R. M. M., Chapman, H. M., Abbott, R. J., & Balfour, J. (1993). Potential impact of climatic warming on Arctic vegetation. *Flora*, *188*, 367–381. https://doi.org/10.1016/S0367-2530(17)32286-7

Crawford, R. M. M., Chapman, H. M., & Smith, L. C. (1995). Adaptation to variation in growing season length in arctic populations of *Saxifraga oppositifolia* L. *Botanical Journal of Scotland*, *47*(2), 177–192. https://doi.org/10.1080/03746609508684828

Dawson, T. E., & Bliss, L. C. (1989). Intraspecific variation in the water relations of Salix arctica, an arctic-alpine dwarf willow. *Oecologia*, *79*(3), 322–331. https://doi.org/10.1007/BF00384311

Demmig-Adams B, Garab G, William Adams III, & Govindgee. (2014). *Non-photochemical quenching and energy dissipation in plants, algae and cyanobacteria* (B. Demmig-Adams, G. Garab, W. Adams III, & Govindjee, Eds.; Vol. 40). Springer Netherlands. https://doi.org/10.1007/978-94-017-9032-1

Dijk, P. Van, Hartog, M., & Delden, W. Van. (1992). Single cytotype areas in autopolyploid *Plantago media* L. *Biological Journal of the Linnean Society*, *46*(4), 315–331. https://doi.org/10.1111/j.1095-8312.1992.tb00867.x

Dong, B., Wang, H., Liu, T., Cheng, P., Chen, Y., Chen, S., Guan, Z., Fang, W., Jiang, J., & Chen, F. (2017). Whole genome duplication enhances the photosynthetic capacity of *Chrysanthemum nankingense*. *Molecular Genetics and Genomics*, *292*(6), 1247–1256. https://doi.org/10.1007/s00438-017-1344-y

Duysens, L. N. M. (1951). Transfer of light energy within the pigment systems present in photosynthesizing cells. *Nature*, *168*(4274), 548–550. https://doi.org/10.1038/168548a0

Ehleringer, J. R. (2000). Temperature and energy budgets. In *Plant Physiological Ecology* (pp. 117–135). Springer Netherlands. https://doi.org/10.1007/978-94-010-9013-1_7

Eidesen, P. B., Müller, E., Lettner, C., Alsos, I. G., Bender, M., Kristiansen, M., Peeters, B., Postma, F., & Verweij, K. F. (2013). Tetraploids do not form cushions: association of ploidy level, growth form and ecology in the High Arctic *Saxifraga oppositifolia* L. s. lat. (Saxifragaceae) in Svalbard. *Polar Research*, *32*(1), 20071. https://doi.org/10.3402/polar.v32i0.20071

Eidesen, P. B., Tjessem, I. V., Hagen, K. R., Bruls, A., Reveret, A., Huntsaar, M., Sørsdal, S. K., Hjelle, S. S., Handle, V., Bjarki, J., Driehaus, K., Einoder, J. M., Jacobsen, A. H., Gya, R., Arnberg, M., Bjornsdottir, K., Volden, I. K., Brozova, V., Muller, E., … Cubo, N. (n.d.). Easy to spot, had to understand: Entangling secrets of success in a common longstanding arctic plant. *(In Prep)*.

Eilers, P. H. C., & Peeters, J. C. H. (1988). A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecological Modelling*, *42*(3–4), 199–215. https://doi.org/10.1016/0304-3800(88)90057-9

Ellis, J. R., & Leech, R. M. (1985). Cell size and chloroplast size in relation to chloroplast replication in light-grown wheat leaves. *Planta*, *165*(1), 120–125. https://doi.org/10.1007/BF00392220

Elven, R., & Elvebakk, A. (1996). A catalogue of Svalbard plants, fungi, algae, and cyanobacteria. *Skrifter-Norsk Polarinstitutt*, *198*, 9–55.

Eriksson, J. S., Blanco-Pastor, J. L., Sousa, F., Bertrand, Y. J. K., & Pfeil, B. E. (2017). A cryptic species produced by autopolyploidy and subsequent introgression involving *Medicago prostrata* (Fabaceae). *Molecular*

Phylogenetics and Evolution, *107*, 367–381. https://doi.org/10.1016/j.ympev.2016.11.020

Esteban, R., Barrutia, O., Artetxe, U., Fernández-Marín, B., Hernández, A., & García-Plazaola, J. I. (2015). Internal and external factors affecting photosynthetic pigment composition in plants: a meta-analytical approach. *New Phytologist*, *206*(1), 268–280. https://doi.org/10.1111/nph.13186

Evans, J. R. (1989). Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia*, *78*(1), 9–19. https://doi.org/10.1007/BF00377192

Evans, J. R., & Clarke, V. C. (2019). The nitrogen cost of photosynthesis. *Journal of Experimental Botany*, *70*(1), 7–15. https://doi.org/10.1093/jxb/ery366

Feber-Girard, M., Felber, F., & Buttler, A. (1996). Habitat differentiation in a narrow hybrid zone between diploid and tetraploid Anthoxanthum alpinum. *New Phytologist*, *133*(3), 531–540. https://doi.org/10.1111/j.1469-8137.1996.tb01921.x

Fernández-Marín, B., Gulías, J., Figueroa, C. M., Iñiguez, C., Clemente-Moreno, M. J., Nunes-Nesi, A., Fernie, A. R., Cavieres, L. A., Bravo, L. A., García-Plazaola, J. I., & Gago, J. (2020). How do vascular plants perform photosynthesis in extreme environments? An integrative ecophysiological and biochemical story. *The Plant Journal*, *101*(4), 979–1000. https://doi.org/10.1111/tpj.14694

Fischer, R. A. (1973). Effect of water stress at various stages of development on yield processes in wheat. *Plant Response to Climatic Factors Proceedings of Theuppsala Symposium*.

Fowler, N. L., & Levin, D. A. (1984). Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *The American Naturalist*, *124*(5), 703–711. https://doi.org/10.1086/284307

Genty B, Harbinson J, Cailly A L, & Rizza F. (1996). *Fate of excitation at PSII in leaves: The non-photochemical side.* , University of Sheffield, Department of Molecular Biology and Biotechnology.

Gharajehdaghipour, T., Roth, J. D., Fafard, P. M., & Markham, J. H. (2016). Arctic foxes as ecosystem engineers: increased soil nutrients lead to increased plant productivity on fox dens. *Scientific Reports*, *6*(1), 24020. https://doi.org/10.1038/srep24020

Gilmore, A. M., & Yamamoto, H. Y. (1991). Zeaxanthin formation and energy-dependent fluorescence quenching in pea chloroplasts under artificially mediated linear and cyclic electron transport. *Plant Physiology*, *96*(2), 635–643. https://doi.org/10.1104/pp.96.2.635

Griffin, K. L., Epstein, D. J., & Boelman, N. T. (2013). Hill slope variations in chlorophyll fluorescence indices and leaf traits in a small arctic watershed. *Arctic, Antarctic, and Alpine Research*, *45*(1), 39–49. https://doi.org/10.1657/1938-4246-45.1.39

Grosso, V., Farina, A., Gennaro, A., Giorgi, D., & Lucretti, S. (2012). Flow sorting and molecular cytogenetic identification of individual chromosomes of *Dasypyrum villosum* L. (H. villosa) by a single DNA probe. *PLoS ONE*, *7*(11), e50151. https://doi.org/10.1371/journal.pone.0050151

Hanssen-Bauer, I., Førland, E. J., Hisdal, H., Mayer, S., Sandø, A. B., & Sorteberg, A. (2019). Climate in Svalbard 2100. *A Knowledge Base for Climate Adaptation*.

Hardy, O. J., Vanderhoeven, S., De Loose, M., & Meerts, P. (2000). Ecological, morphological and allozymic differentiation between diploid and tetraploid knapweeds (*Centaurea jacea*) from a contact zone in the Belgian Ardennes. *New Phytologist*, *146*(2), 281–290. https://doi.org/10.1046/j.1469-8137.2000.00631.x

Harris, C. R., Millman, K. J., van der Walt, S. J., Gommers, R., Virtanen, P., Cournapeau, D., Wieser, E., Taylor, J., Berg, S., Smith, N. J., Kern, R., Picus, M., Hoyer, S., van Kerkwijk, M. H., Brett, M., Haldane, A., del Río, J. F., Wiebe, M., Peterson, P., … Oliphant, T. E. (2020). Array programming with NumPy. *Nature*, *585*(7825), 357–362. https://doi.org/10.1038/s41586-020-2649-2

Hodáňová, D. (1985). *Leaf optical properties* (pp. 107–127). https://doi.org/10.1007/978-94-009-5530-1_5

Hudson, J. M. G., Henry, G. H. R., & Cornwell, W. K. (2011). Taller and larger: shifts in Arctic tundra leaf traits after 16 years of experimental warming. *Global Change Biology*, *17*(2), 1013–1021. https://doi.org/10.1111/j.1365-2486.2010.02294.x

Hunter, J. D. (2007). Matplotlib: A 2D graphics environment. *Computing in Science & Engineering*, *9*(3), 90–95. https://doi.org/10.1109/MCSE.2007.55

Intergovernmental Panel on Climate Change (IPCC). (2023). *Climate change 2022 – Impacts, adaptation and vulnerability*. Cambridge University Press. https://doi.org/10.1017/9781009325844

Jackson, R. C. (1976). Evolution and systematic significance of polyploidy. *Annual Review of Ecology and Systematics*, *7*(1), 209–234. https://doi.org/10.1146/annurev.es.07.110176.001233

Jakubczyk, K., Dec, K., Kałduńska, J., Kawczuga, D., Kochman, J., & Janda, K. (2020). Reactive oxygen species-sources, functions, oxidative damage. *Polski Merkuriusz Lekarski: Organ Polskiego Towarzystwa Lekarskiego*, *48*(284), 124–127.

Jassby, A. D., & Platt, T. (1976). Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnology and Oceanography*, *21*(4), 540–547. https://doi.org/10.4319/lo.1976.21.4.0540

Johnson, M. T. J., Husband, B. C., & Burton, T. L. (2003). Habitat Differentiation between Diploid and Tetraploid *Galax urceolata* (Diapensiaceae).

International Journal of Plant Sciences, *164*(5), 703–710. https://doi.org/10.1086/376813

Jones, M. B. (1985). Plant microclimate. In *Techniques in Bioproductivity and Photosynthesis* (pp. 26–40). Elsevier. https://doi.org/10.1016/B978-0-08-031999-5.50013-3

Kalaji, H. M., Schansker, G., Ladle, R. J., Goltsev, V., Bosa, K., Allakhverdiev, S. I., Brestic, M., Bussotti, F., Calatayud, A., Dąbrowski, P., Elsheery, N. I., Ferroni, L., Guidi, L., Hogewoning, S. W., Jajoo, A., Misra, A. N., Nebauer, S. G., Pancaldi, S., Penella, C., … Zivcak, M. (2014). Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. *Photosynthesis Research*, *122*(2), 121–158. https://doi.org/10.1007/s11120-014-0024-6

Kitajima, M., & Butler, W. L. (1975). Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *376*(1), 105–115. https://doi.org/10.1016/0005-2728(75)90209-1

Klughammer, C., & Schreiber, U. (2008). Complementary PSII quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the saturation pulse method. In *PAM Application Notes*. Heinz Walz GmbH. https://www.walz.com/files/downloads/pan/PAN078007.pdf

Krall, J. P., & Edwards, G. E. (1992). Relationship between photosystem II activity and CO_2 fixation in leaves. *Physiologia Plantarum*, *86*(1), 180–187. https://doi.org/10.1111/j.1399-3054.1992.tb01328.x

Kramer, D. M., Johnson, G., Kiirats, O., & Edwards, G. E. (2004). New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. *Photosynthesis Research*, *79*(2), 209–218. https://doi.org/10.1023/B:PRES.0000015391.99477.0d

Kudo, G., Molau, U., & Wada, N. (2001). Leaf-trait variation of tundra plants along a climatic gradient: an integration of responses in evergreen and deciduous species. *Arctic, Antarctic, and Alpine Research*, *33*(2), 181–190. https://doi.org/10.1080/15230430.2001.12003421

Kume, A., Nakatsubo, T., Bekku, Y., & Masuzawa, T. (1999). Ecological significance of different growth forms of purple saxifrage, *Saxifraga oppositifolia* L., in the high Arctic, Ny-Ålesund, Svalbard. *Arctic, Antarctic, and Alpine Research*, *31*(1), 27–33. https://doi.org/10.1080/15230430.1999.12003278

Laisk, A., Eichelmann, H., Oja, V., Rasulov, B., & Rämma, H. (2006). Photosystem II cycle and alternative electron flow in leaves. *Plant and Cell Physiology*, *47*(7), 972–983. https://doi.org/10.1093/pcp/pcj070

Laisk, A., & Loreto, F. (1996). Determining photosynthetic parameters from leaf CO2 exchange and chlorophyll fluorescence (ribulose-1,5-bisphosphate carboxylase/oxygenase specificity factor, dark respiration in the light,

excitation distribution between photosystems, alternative electron transport rate, and mesophyll diffusion resistance. *Plant Physiology*, *110*(3), 903–912. https://doi.org/10.1104/pp.110.3.903

Larkum, A. W. D. (2006). The evolution of chlorophylls and photosynthesis. In *Chlorophylls and Bacteriochlorophylls* (pp. 261–282). Springer Netherlands. https://doi.org/10.1007/1-4020-4516-6_18

Latowski, D., Kuczyńska, P., & Strzałka, K. (2011). Xanthophyll cycle – a mechanism protecting plants against oxidative stress. *Redox Report*, *16*(2), 78–90. https://doi.org/10.1179/174329211X13020951739938

Lawlor, D. (2001). The origin, expansion and demise of plant species. *Annals of Botany*, *87*(2), 284–286. https://doi.org/10.1006/anbo.2000.1315

Lazár, D. (1999). Chlorophyll *a* fluorescence induction. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1412*(1), 1–28. https://doi.org/10.1016/S0005-2728(99)00047-X

Li, W.-D., Biswas, D. K., Xu, H., Xu, C.-Q., Wang, X.-Z., Liu, J.-K., & Jiang, G.-M. (2009). Photosynthetic responses to chromosome doubling in relation to leaf anatomy in Lonicera japonica subjected to water stress. *Functional Plant Biology*, *36*(9), 783. https://doi.org/10.1071/FP09022

Li, Y., Jiao, Y., & Zhao, Q. (2013). *Photosynthetic characteristics of Arctic plants* (pp. 633–637). https://doi.org/10.1007/978-3-642-32034-7_138

Love, A., Love, D., & others. (1949). The geobotanical significance of polyploidy. I. Polyploidy and latitude. *The Geobotanical Significance of Polyploidy. I. Polyploidy and Latitude.*

Lumaret, R., Guillerm, J.-L., Delay, J., Ait Lhaj Loutfi, A., Izco, J., & Jay, M. (1987). Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). *Oecologia*, *73*(3), 436–446. https://doi.org/10.1007/BF00385262

Maherali, H., Walden, A. E., & Husband, B. C. (2009). Genome duplication and the evolution of physiological responses to water stress. *New Phytologist*, *184*(3), 721–731. https://doi.org/10.1111/j.1469-8137.2009.02997.x

Masterson, J. (1994). Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science*, *264*(5157), 421–424. https://doi.org/10.1126/science.264.5157.421

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany*, *51*(345), 659–668. https://doi.org/10.1093/jexbot/51.345.659

Melis, A. (1999). Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage in vivo? *Trends in Plant Science*, *4*(4), 130–135. https://doi.org/10.1016/S1360-1385(99)01387-4

Meyers, L. A., & Levin, D. A. (2006). On the abundance of polyploids in flowering plants. *Evolution*, *60*(6), 1198–1206. https://doi.org/10.1111/j.0014-3820.2006.tb01198.x

Migała, K., Wojtuń, B., Szymański, W., & Muskała, P. (2014). Soil moisture and temperature variation under different types of tundra vegetation during the growing season: A case study from the Fuglebekken catchment, SW Spitsbergen. *CATENA*, *116*, 10–18. https://doi.org/10.1016/j.catena.2013.12.007

MINI-PAM II photosynthesis yield analyzer manual (Vol. 5). (2023). Heinz Walz GmbH. https://www.walz.com/files/downloads/manuals/mini-pam-II/MINI_PAM_II_06.pdf

Miyake, C., Shinzaki, Y., Miyata, M., & Tomizawa, K. (2004). Enhancement of cyclic electron flow around PSI at high light and its contribution to the Induction of non-photochemical quenching of Chl fluorescence in intact leaves of tobacco plants. *Plant and Cell Physiology*, *45*(10), 1426–1433. https://doi.org/10.1093/pcp/pch163

Mullan, D., & Pietragalla, J. (2012). Leaf relative water content. *Physiological Breeding II: A Field Guide to Wheat Phenotyping*, 25–27.

Müller, E., Eidesen, P. B., Ehrich, D., & Alsos, I. G. (2012). Frequency of local, regional, and long-distance dispersal of diploid and tetraploid *Saxifraga oppositifolia* (Saxifragaceae) to Arctic glacier forelands. *American Journal of Botany*, *99*(3), 459–471. https://doi.org/10.3732/ajb.1100363

Muraoka, H., Noda, H., Uchida, M., Ohtsuka, T., Koizumi, H., & Nakatsubo, T. (2008). Photosynthetic characteristics and biomass distribution of the dominant vascular plant species in a high Arctic tundra ecosystem, Ny-Ålesund, Svalbard: implications for their role in ecosystem carbon gain. *Journal of Plant Research*, *121*(2), 137–145. https://doi.org/10.1007/s10265-007-0134-8

Nardini, A. (2022). Hard and tough: the coordination between leaf mechanical resistance and drought tolerance. *Flora*, *288*, 152023. https://doi.org/10.1016/j.flora.2022.152023

Oberbauer, S., & Miller, P. C. (1981). Some Aspects of Plant Water Relations in Alaskan Arctic Tundra Species. *Arctic and Alpine Research*, *13*(2), 205. https://doi.org/10.2307/1551196

Otto, S. P., & Whitton, J. (2000). Polyploid incidence and evolution. *Annual Review of Genetics*, *34*(1), 401–437. https://doi.org/10.1146/annurev.genet.34.1.401

Pandas development team, T. (2020). *Pandas-dev/pandas: Pandas*. Zenodo. https://doi.org/10.5281/zenodo.3509134

Parkhurst, D. F., & Loucks, O. L. (1972). Optimal leaf size in relation to environment. *The Journal of Ecology*, *60*(2), 505. https://doi.org/10.2307/2258359

Perera-Castro, A. V., Flexas, J., González-Rodríguez, Á. M., & Fernández-Marín, B. (2021). Photosynthesis on the edge: photoinhibition, desiccation and freezing tolerance of Antarctic bryophytes. *Photosynthesis Research*, *149*(1–2), 135–153. https://doi.org/10.1007/s11120-020-00785-0

Perera-Castro, A. V., & Flexas, J. (2023). The ratio of electron transport to assimilation (ETR/AN): underutilized but essential for assessing both equipment's proper performance and plant status. *Planta*, *257*(2), 29. https://doi.org/10.1007/s00425-022-04063-2

Peterson, K. M. (2014). Plants in arctic environments 13. *Ecology and the Environment*, *363*.

Petersson, C. (2007). *An analysis of the local weather around Longyearbyen and an instrumental comparison*. Institutionen för geovetenskaper.

Platt, T., Gallegos, C. L., & Harrison, W. G. (1980). *Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton*. https://api.semanticscholar.org/CorpusID:129517107

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*, *182*(3), 565–588. https://doi.org/10.1111/j.1469-8137.2009.02830.x

Rakić, T., Gajić, G., Lazarević, M., & Stevanović, B. (2015). Effects of different light intensities, CO2 concentrations, temperatures and drought stress on photosynthetic activity in two paleoendemic resurrection plant species Ramonda serbica and R. nathaliae. *Environmental and Experimental Botany*, *109*, 63–72. https://doi.org/10.1016/j.envexpbot.2014.08.003

Ralph, P. J., & Gademann, R. (2005). Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquatic Botany*, *82*(3), 222–237. https://doi.org/10.1016/j.aquabot.2005.02.006

Ramsey, J., & Schemske, D. W. (1998). Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics*, *29*(1), 467–501. https://doi.org/10.1146/annurev.ecolsys.29.1.467

Rieseberg, L. H., & Willis, J. H. (2007). Plant speciation. *Science*, *317*(5840), 910–914. https://doi.org/10.1126/science.1137729

Sabara, H. A., Kron, P., & Husband, B. C. (2013). Cytotype coexistence leads to triploid hybrid production in a diploid-tetraploid contact zone of *Chamerion angustifolium*(Onagraceae). *American Journal of Botany*, *100*(5), 962–970. https://doi.org/10.3732/ajb.1200583

Schlesinger, W. H., & Chabot, B. F. (1977). The use of water and minerals by evergreen and deciduous shrubs in Okefenokee Swamp. *Botanical Gazette*, *138*(4), 490–497. https://doi.org/10.1086/336954

Schreiber, U. (2004). Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: An overview. In *Chlorophyll a Fluorescence* (pp. 279–319). Springer Netherlands. https://doi.org/10.1007/978-1-4020-3218-9_11

Seabold, S., & Perktold, J. (2010). Statsmodels: Econometric and statistical modeling with python. *9th Python in Science Conference*.

Serôdio, J., & Lavaud, J. (2011). A model for describing the light response of the nonphotochemical quenching of chlorophyll fluorescence. *Photosynthesis Research*, *108*(1), 61–76. https://doi.org/10.1007/s11120-011-9654-0

Sessa, E. B. (2019). Polyploidy as a mechanism for surviving global change. *New Phytologist*, *221*(1), 5–6.

Sobrado, M. A., & Medina, E. (1980). General morphology, anatomical structure, and nutrient content of sclerophyllous leaves of the bana vegetation of amazonas. *Oecologia*, *45*(3), 341–345. https://doi.org/10.1007/BF00540202

Soltis, D. E., Albert, V. A., Leebens-Mack, J., Bell, C. D., Paterson, A. H., Zheng, C., Sankoff, D., de Pamphilis, C. W., Wall, P. K., & Soltis, P. S. (2009). Polyploidy and angiosperm diversification. *American Journal of Botany*, *96*(1), 336–348. https://doi.org/10.3732/ajb.0800079

Soltis, D. E., Segovia-Salcedo, M. C., Jordon-Thaden, I., Majure, L., Miles, N. M., Mavrodiev, E. V., Mei, W., Cortez, M. B., Soltis, P. S., & Gitzendanner, M. A. (2014). Are polyploids really evolutionary dead-ends (again). A critical reappraisal of Mayrose *et al*. *New Phytologist*, *202*(4), 1105–1117. https://doi.org/10.1111/nph.12756

Soltis, D. E., Soltis, P. S., Schemske, D. W., Hancock, J. F., Thompson, J. N., Husband, B. C., & Judd, W. S. (2007). Autopolyploidy in angiosperms: Have we grossly underestimated the number of species? *Taxon*, *56*(1), 13–30.

Soltis, D. E., Visger, C. J., Marchant, D. B., & Soltis, P. S. (2016). Polyploidy: Pitfalls and paths to a paradigm. *American Journal of Botany*, *103*(7), 1146–1166. https://doi.org/10.3732/ajb.1500501

Spoelhof, J. P., Soltis, P. S., & Soltis, D. E. (2017). Pure polyploidy: Closing the gaps in autopolyploid research. *Journal of Systematics and Evolution*, *55*(4), 340–352. https://doi.org/10.1111/jse.12253

Sugiyama, S. (1998). Differentiation in competitive ability and cold tolerance between diploid and tetraploid cultivars in *Lolium perenne*. *Euphytica*, *103*(1), 55–59. https://doi.org/10.1023/A:1018322821118

Thompson, J. D., & Lumaret, R. (1992). The evolutionary dynamics of polyploid plants: origins, establishment and persistence. *Trends in Ecology & Evolution*, *7*(9), 302–307. https://doi.org/10.1016/0169-5347(92)90228-4

Tjessem, I. V. (2021). *Should I sex or should I go? Expansion of species niche through autopolyploidy* [University of Oslo]. https://www.duo.uio.no/handle/10852/90637?show=full

Tolvanen, A., & Henry, G. H. (2001). Responses of carbon and nitrogen concentrations in high arctic plants to experimental warming. *Canadian Journal of Botany*, *79*(6), 711–718. https://doi.org/10.1139/b01-052

Tossi, V. E., Martínez Tosar, L. J., Laino, L. E., Iannicelli, J., Regalado, J. J., Escandón, A. S., Baroli, I., Causin, H. F., & Pitta-Álvarez, S. I. (2022). Impact of polyploidy on plant tolerance to abiotic and biotic stresses. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.869423

Tyler, B., Borrill, M., & Chorlton, K. (1978). Studies in *Festuca*. X. observations on germination and seedling cold tolerance in diploid *Festuca pratensis* and tetraploid *F. pratensis* var. *apennina* in relation to their altitudinal distribution. *The Journal of Applied Ecology*, *15*(1), 219. https://doi.org/10.2307/2402932

Ulum, F. B., Hadacek, F., & Hörandl, E. (2021). Polyploidy improves photosynthesis regulation within the *Ranunculus auricomus* complex (Ranunculaceae). *Biology*, *10*(8), 811. https://doi.org/10.3390/biology10080811

Van de Peer, Y., Ashman, T.-L., Soltis, P. S., & Soltis, D. E. (2021). Polyploidy: an evolutionary and ecological force in stressful times. *The Plant Cell*, *33*(8), 2899–2899. https://doi.org/10.1093/plcell/koaa015

Van Rossum, G., & Drake, F. L. (2009). *Python 3 eeference manual*.

Virtanen, P., Gommers, R., Oliphant, T. E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S. J., Brett, M., Wilson, J., Millman, K. J., Mayorov, N., Nelson, A. R. J., Jones, E., Kern, R., Larson, E., … Vázquez-Baeza, Y. (2020). SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nature Methods*, *17*(3), 261–272. https://doi.org/10.1038/s41592-019-0686-2

Vyas, P., Bisht, M. S., Miyazawa, S.-I., Yano, S., Noguchi, K., Terashima, I., & Funayama-Noguchi, S. (2007). Effects of polyploidy on photosynthetic properties and anatomy in leaves of *Phlox drummondii*. *Functional Plant Biology*, *34*(8), 673. https://doi.org/10.1071/FP07020

Wang, L.-J., Cao, Q.-Z., Zhang, X.-Q., & Jia, G.-X. (2021). Effects of polyploidization on photosynthetic characteristics in three *Lilium* species. *Scientia Horticulturae*, *284*, 110098. https://doi.org/10.1016/j.scienta.2021.110098

Warner, D. A., & Edwards, G. E. (1988). C4 photosynthesis and leaf anatomy in diploid and autotetraploid *Pennisetum americanum* (pearl millet). *Plant Science*, *56*(1), 85–92. https://doi.org/10.1016/0168-9452(88)90190-2

Warner, D. A., & Edwards, G. E. (1993). Effects of polyploidy on photosynthesis. *Photosynthesis Research*, *35*(2), 135–147. https://doi.org/10.1007/BF00014744

Ween, R. (2022). *Timing is everything: Within-plant flowering phenology impacts fruit production in the Arctic-Alpine cushion plant* Silene acaulis *(L.) Jacq.* https://hdl.handle.net/10037/29218

Witkowski, E. T. F., & Lamont, B. B. (1991). Leaf specific mass confounds leaf density and thickness. *Oecologia*, *88*(4), 486–493. https://doi.org/10.1007/BF00317710

Wood, T. E., Takebayashi, N., Barker, M. S., Mayrose, I., Greenspoon, P. B., & Rieseberg, L. H. (2009). The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences*, *106*(33), 13875–13879. https://doi.org/10.1073/pnas.0811575106

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. C., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont, B. B., Lee, T., Lee, W., Lusk, C., … Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, *428*(6985), 821–827. https://doi.org/10.1038/nature02403

Xianchun, J., Weisheng, X., Ling, Z., Feng, Q., & Shurong, H. (1994). Influence of physiological characteristics at grain filing stage on dying green of wheat plants in different varieties. *Zuo Wu Xue Bao*, *20*(1), 99—105. http://europepmc.org/abstract/CBA/270898

Yang, W., Liu, D., Li, J., Zhang, L., Wei, H., Hu, X., Zheng, Y., He, Z., & Zou, Y. (2009). Synthetic hexaploid wheat and its utilization for wheat genetic improvement in China. *Journal of Genetics and Genomics*, *36*(9), 539–546. https://doi.org/10.1016/S1673-8527(08)60145-9

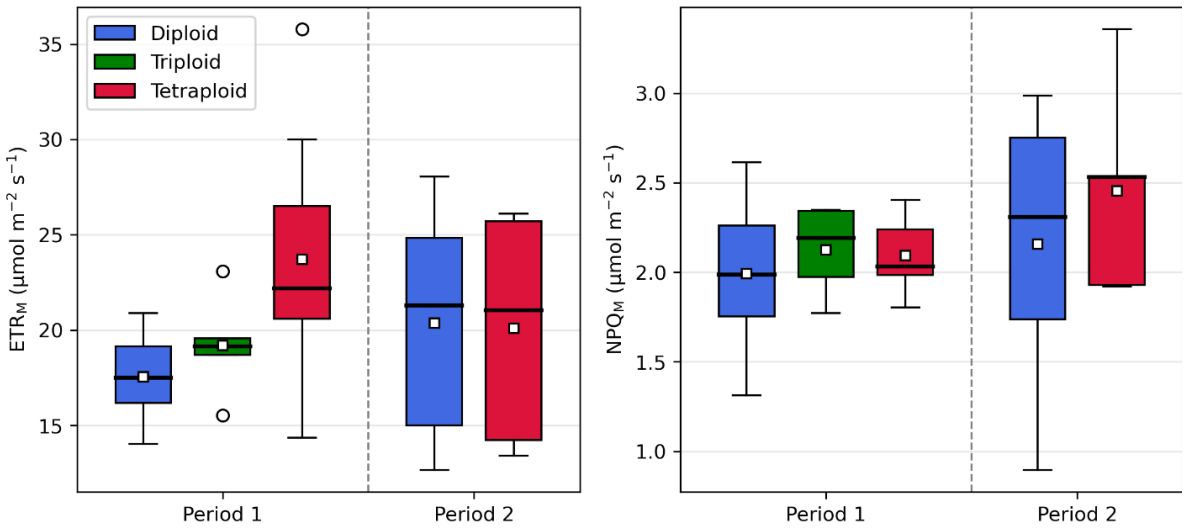
Appendix

Appendix 1. Raw output from mixed linear model from statsmodel . Model: RWC~ploidy*habitat, random effect: plant ID. Reference level: Diploid (2x) and Ridge.

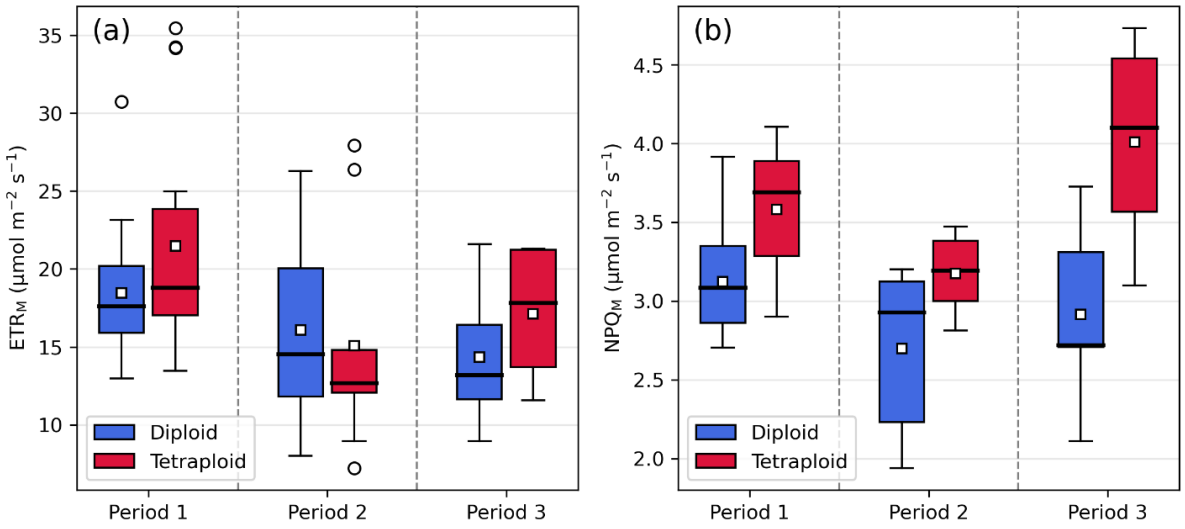
Mixed Linear Model Regression Results

Model:	MixedLM	Dependent Variable:	RWC
No. Observations:	174	Method:	REML
No. Groups:	159	Scale:	39.8904
Min. group size:	1	Log-Likelihood:	-622.3178
Max. group size:	2	Converged:	Yes
Mean group size:	1.1		

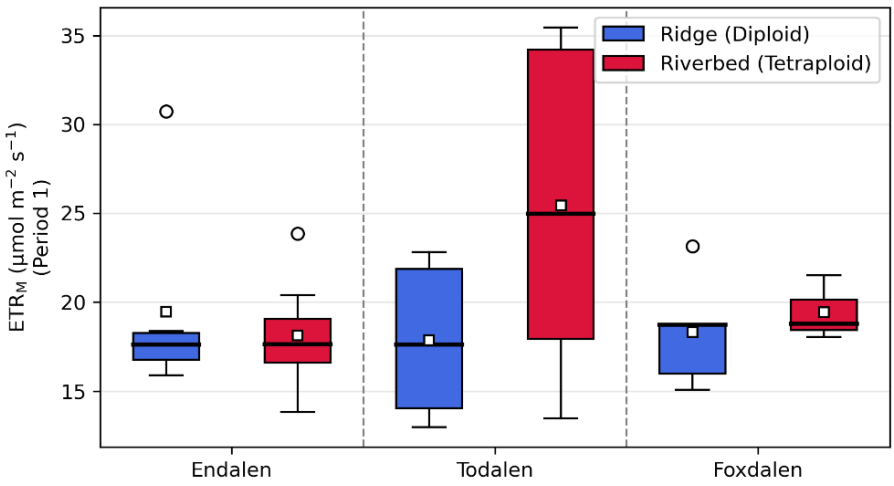
	Coef.	Std.Err.	z	P>|z|	[0.025	0.975]
Intercept	63.475	1.509	42.076	0.000	60.518	66.432
ploidy[T.3x]	8.037	4.353	1.846	0.065	-0.495	16.570
ploidy[T.4x]	3.956	5.224	0.757	0.449	-6.283	14.195
habitat[T.riverbed]	6.375	2.432	2.621	0.009	1.609	11.142
habitat[T.slope]	9.293	3.070	3.027	0.002	3.277	15.309
ploidy[T.3x]:habitat[T.riverbed]	-12.380	6.073	-2.038	0.042	-24.283	-0.477
ploidy[T.4x]:habitat[T.riverbed]	-10.320	5.786	-1.784	0.074	-21.660	1.020
ploidy[T.3x]:habitat[T.slope]	-12.268	5.813	-2.110	0.035	-23.662	-0.874
ploidy[T.4x]:habitat[T.slope]	-14.072	6.851	-2.054	0.040	-27.501	-0.644
plant_id Var	60.163	5.746				



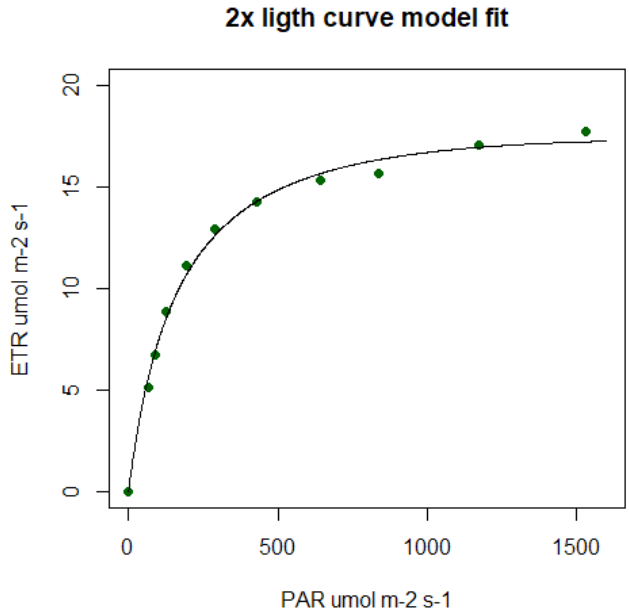
Appendix 2. Boxplot of maximum values for electron transport rate (ETR_M) and non-photochemical quenching (NPQ_M), for each measurement period done on cuttings of *Saxifraga oppositifolia* grown in a climate-controlled room.



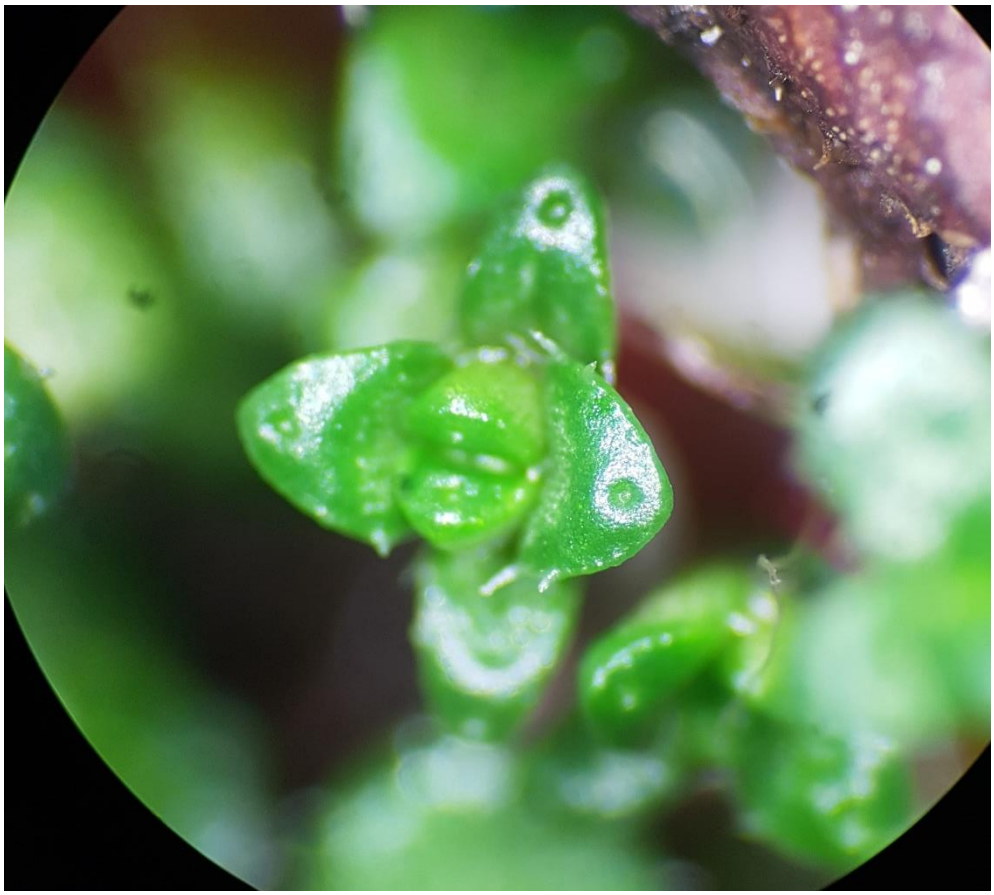
Appendix 3. Boxplot of maximum values for electron transport rate (ETR_M) and non-photochemical quenching (NPQ_M), for each measurement period done on whole individuals of *Saxifraga oppositifolia* grown in a climate-controlled room. Black line represents the median and white square the mean.



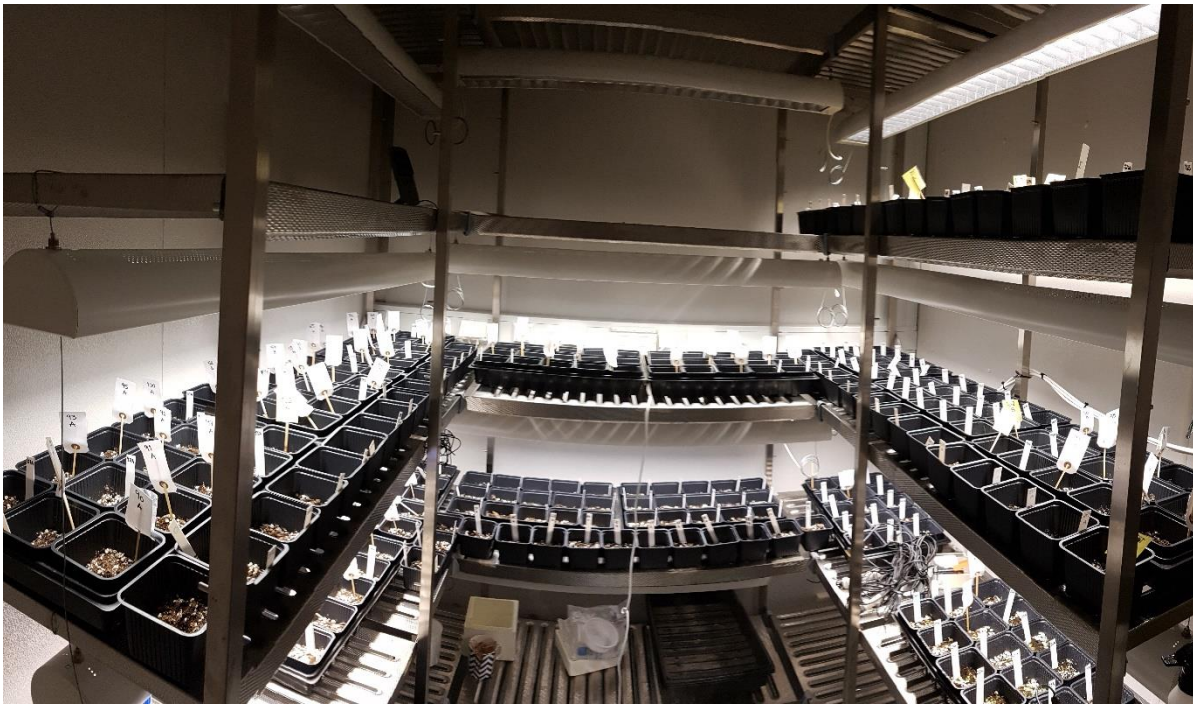
Appendix 4. Boxplot of maximum values of electron transport rate (ETR_M), by each habitat and location the measured individual of *Saxifraga oppositifolia* originated from. Differences not significant, $p = 0.151$ one-way ANOVA.



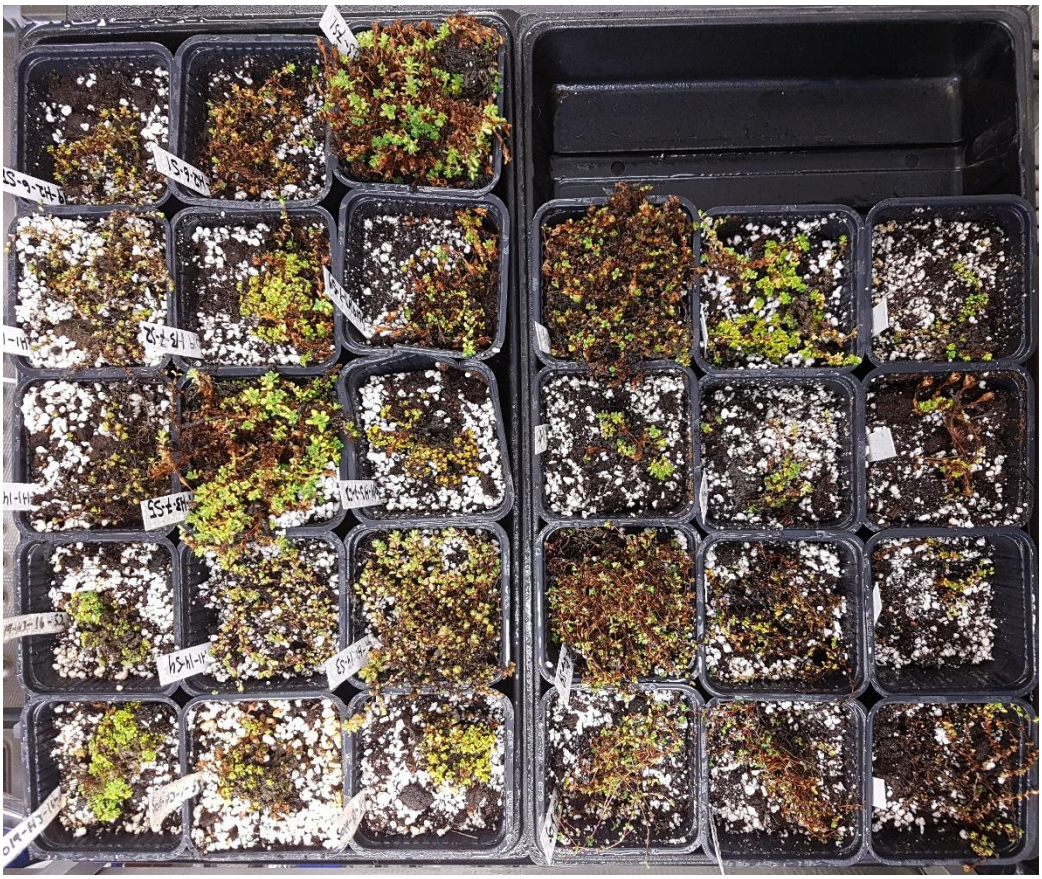
Appendix 5. Example of a curve fitted to electron transport rate (ETR).



Appendix 6. Leaf rosette form Saxifraga oppositifolia used for light curve measurements. From a plant growing in climate-controlled room. Credit: Simen S. Hjelle.



Appendix 7. Climate-controlled room layout with re-rooted cuttings of Saxifraga oppositifolia growing in pots. Credit: Simen S. Hjelle.

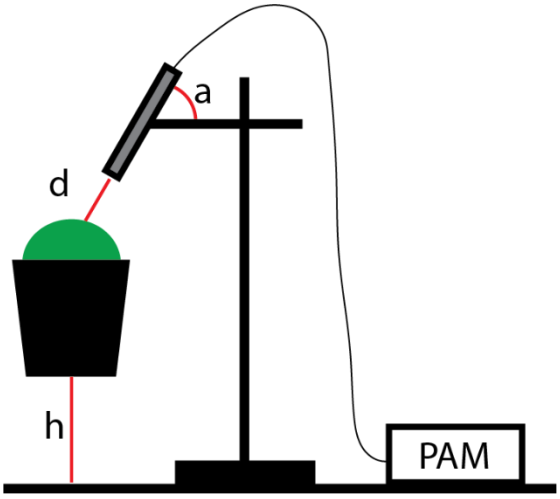


Appendix 8. Pots with whole individuals of Saxifraga oppositifolia collected in the field. Credit: Simen S. Hjelle.

Appendix 9. Tukey Test output table to check if ETR_M was influences of plant transect location origin. Data tested whole plants period 1. No significance.

Multiple Comparison of Means - Tukey HSD, FWER=0.05

group1	group2	meandiff	p-adj	lower	upper	reject
11	13	-1.117	0.9998	-13.6478	11.4137	False
11	2	-1.3203	0.9993	-12.9366	10.2961	False
11	4	0.0218	1.0	-12.1111	12.1547	False
11	5	6.009	0.638	-5.8314	17.8495	False
11	7	-1.5859	0.9986	-13.7188	10.547	False
13	2	-0.2032	1.0	-9.9851	9.5786	False
13	4	1.1388	0.9994	-9.2511	11.5288	False
13	5	7.1261	0.2855	-2.9209	17.173	False
13	7	-0.4689	1.0	-10.8588	9.9211	False
2	4	1.3421	0.9977	-7.9246	10.6087	False
2	5	7.3293	0.1525	-1.551	16.2097	False
2	7	-0.2656	1.0	-9.5322	9.001	False
4	5	5.9872	0.4154	-3.5588	15.5333	False
4	7	-1.6077	0.996	-11.5141	8.2988	False
5	7	-7.5949	0.1808	-17.141	1.9512	False



Appendix 10. Mini PAM II measurement set-up. Fiberoptics connected to Mini-PAM II (PAM), held up by a laboratory stand. Fiber angle is **a**, **d** is distance between fiber optic and sample (leaf rosette), **h** is height of pot from table. Angle was kept the same for each measurement. Height and distances adjusted for optimal signal strength.

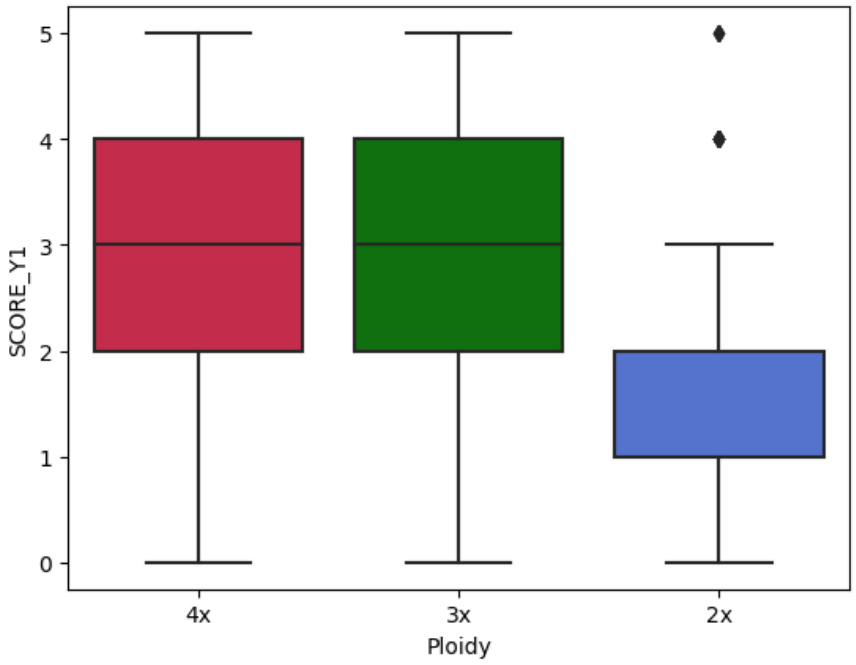
Appendix 11. Cross-section of a leaf from *Saxifraga oppositifolia*. Unknown ploidy. Clearly visible higher densities of chlorophyll on the light facing side of the leaf.

Appendix 12. Tukey test of comparison between field and lab measurements of F_V/F_M. Lab measurements are from re-rooted cuttings and whole plants. Only significant differences are shown.

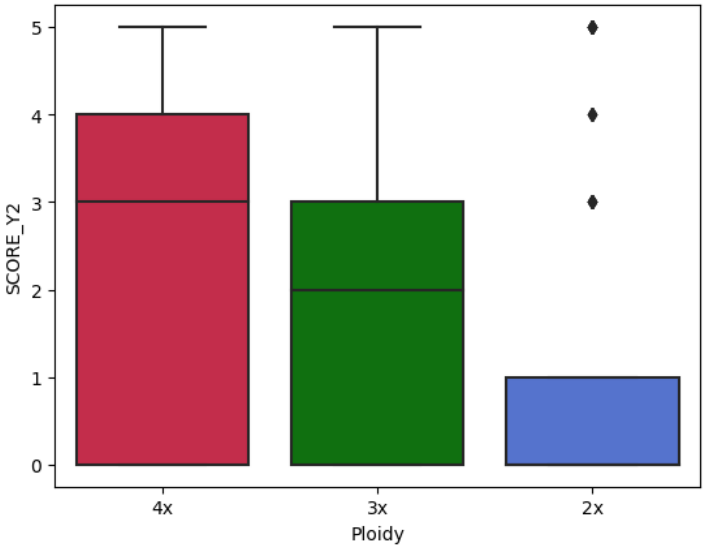
group1	group2	meandiff	p-adj	lower	upper
Cuttings Period 1	Cuttings Period 2	0.0197	0.7971	-0.0073	0.0468
Cuttings Period 1	Field	0.0517	0.0001	0.0325	0.0709
Cuttings Period 1	Whole Plant Period 1	0.019	0.5598	-0.001	0.0391
Cuttings Period 1	Whole Plant Period 2	-0.0355	0.1243	-0.0602	-0.0109
Cuttings Period 1	Whole Plant Period 3	0.0081	0.9969	-0.0219	0.0381
Cuttings Period 2	Field	0.032	0.2365	0.0067	0.0572
Cuttings Period 2	Whole Plant Period 1	-0.0007	1	-0.0266	0.0253
Cuttings Period 2	Whole Plant Period 2	-0.0552	0.0169	-0.0848	-0.0256
Cuttings Period 2	Whole Plant Period 3	-0.0116	0.9913	-0.0458	0.0226
Field	Whole Plant Period 1	-0.0327	0.0175	-0.0502	-0.0151
Field	Whole Plant Period 2	-0.0872	0	-0.1098	-0.0646
Field	Whole Plant Period 3	-0.0436	0.084	-0.0719	-0.0152
Whole Plant Period 1	Whole Plant Period 2	-0.0545	0.001	-0.0779	-0.0311
Whole Plant Period 1	Whole Plant Period 3	-0.0109	0.9861	-0.0399	0.0181
Whole Plant Period 2	Whole Plant Period 3	0.0436	0.1756	0.0113	0.076

Appendix 13. Tukey test of comparison between field and lab measurements of F_V/F_M, per ploidy level. Lab measurements are from re-rooted cuttings and whole plants. Only significant differences are shown.

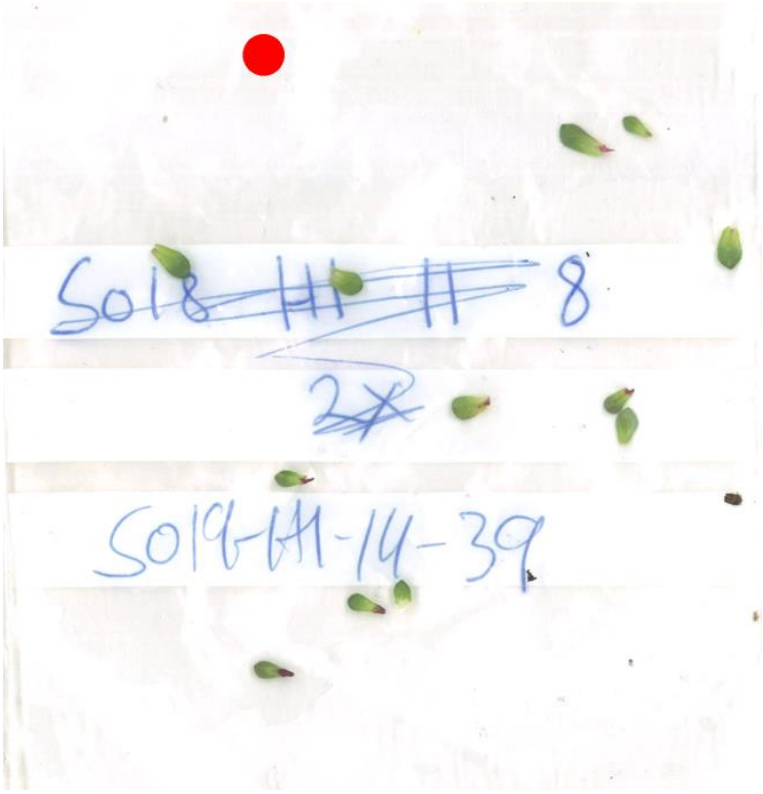
group1	group2	meandiff	p-adj	lower	upper
Cuttings Period 1 2x	Whole Plant Period 2 4x	-0.0884	0.002	-0.1372	-0.0397
Cuttings Period 1 3x	Field 2x	0.0746	0.037	0.0237	0.1254
Cuttings Period 1 3x	Field 3x	0.0728	0.0831	0.0189	0.1267
Cuttings Period 1 4x	Field 2x	0.0688	0.0011	0.0322	0.1053
Cuttings Period 1 4x	Field 3x	0.067	0.0089	0.0263	0.1077
Cuttings Period 1 4x	Whole Plant Period 2 4x	-0.0512	0.3026	-0.0968	-0.0056
Cuttings Period 2 2x	Whole Plant Period 2 4x	-0.0803	0.0354	-0.1348	-0.0258
Cuttings Period 2 4x	Whole Plant Period 2 4x	-0.0972	0.0064	-0.1549	-0.0395
Field 2x	Whole Plant Period 1 2x	-0.0395	0.1789	-0.0717	-0.0073
Field 2x	Whole Plant Period 1 4x	-0.0363	0.3255	-0.0691	-0.0035
Field 2x	Whole Plant Period 2 2x	-0.0572	0.1247	-0.1016	-0.0127
Field 2x	Whole Plant Period 2 4x	-0.1199	0	-0.1604	-0.0795
Field 2x	Whole Plant Period 3 4x	-0.0594	0.392	-0.1153	-0.0036
Field 3x	Whole Plant Period 1 2x	-0.0377	0.4589	-0.0746	-0.0009
Field 3x	Whole Plant Period 2 2x	-0.0554	0.2562	-0.1033	-0.0075
Field 3x	Whole Plant Period 2 4x	-0.1182	0	-0.1624	-0.074
Field 4x	Whole Plant Period 2 4x	-0.0854	0.0173	-0.1399	-0.0309
Whole Plant Period 1 2x	Whole Plant Period 2 4x	-0.0804	0.0008	-0.1227	-0.0382
Whole Plant Period 1 4x	Whole Plant Period 2 4x	-0.0837	0.0005	-0.1263	-0.041
Whole Plant Period 2 2x	Whole Plant Period 2 4x	-0.0628	0.2002	-0.1149	-0.0107
Whole Plant Period 2 4x	Whole Plant Period 3 2x	0.0796	0.0687	0.0219	0.1373



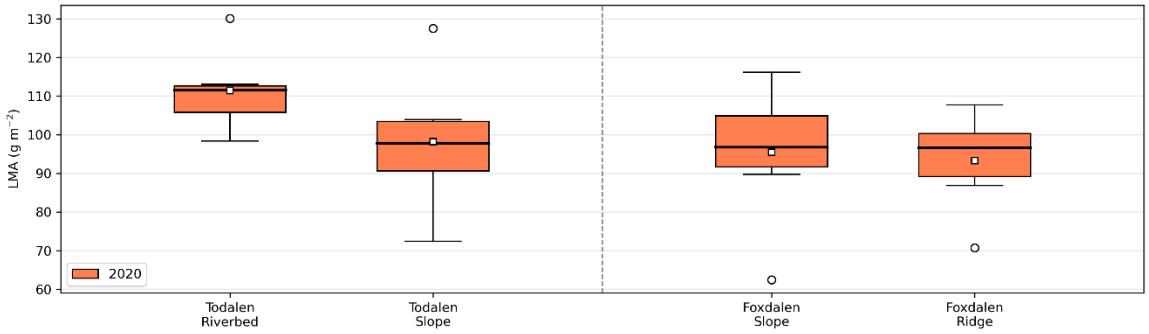
Appendix 14. Subjective scoring of health of re-rooted cuttings growing in climate room. Summer 2020 / Year 1.



Appendix 15 Subjective scoring of health of re-rooted cuttings growing in climate room. Summer 2021 / Year 2.



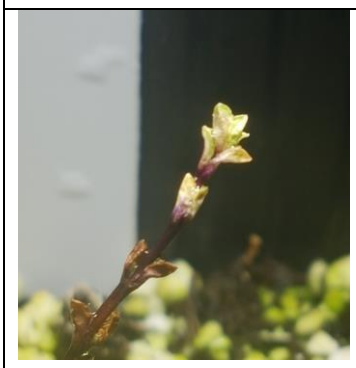
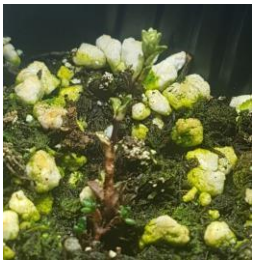
Appendix 16. Scanned leaves from plant with ID SO19-H1-14-19. Leaves were kept inside the plastic bag, but spread out to avoid overlap, and with the labeled part of the bag facing down. After, images were opened in Adobe Photoshop, were pixels containing leaves were selected and counted by the software. Next to these bags of leaves were also a rules, so pixels could be converted into cm^2.

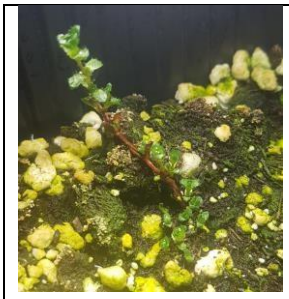


Appendix 17. Triploid LMA per sampling location. Data from 2020 field campaign.

Appendix 18. Subjective rating sheet used to evaluate the health of re-rooted cuttings.

Saxifraga oppositifolia – Plant health rating scheme (Above ground)

Photo	Indicators	Score
	• Brown in overall appearance. • Lacks leaves or... • ...If leaves are present, they are scorched.	0 – Dead
	• Leaves a pale yellow color • Leaves seem dry / arid • Wrinkles / signs of low turgor pressure / dehydration	1 – Dry
	• Leaves a pale green color • Hydrated leaves / normal turgor • No indications of new growth	2 – Pale
	• Majority of plant contains elements of rank 0 to 2... • ... But closer inspection shows new fresh leaves / new sprouts / new branching	3 - Hope
	• Overall strong green color on >95% of the plant • = or < 2 new growth elements (Sprouts, branch, rosettes etc.)	4 - Lush

	• Lush and green plant • Must have > 2 new growth elements (Sprouts, branch, rosettes etc.)	5 – Growth

Acknowledgement

I want to express my gratitude towards all the people who have assisted me in getting this thesis from the start to its completion. From Xenia and Ingrid who assisted me in the field, my wise and supportive team of supervisors, and to my partner Anna.