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Zurich^{UZH}**

In search of the origin for red discoloration of tree bark on *Larix decidua* Mill. in Savognin, Switzerland.

ESS 511 Master's Thesis

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Abstract

A phenomenon has been observed in the Swiss alps where the bark of the European larch (*Larix decidua* Mill.) appears with a more intense red discoloration. The aim of this thesis was to find the origin for the change in color, in order to determine if it restricts the protective function of the bark and has a detrimental impact on the tree health. The effects of reaction wood, sunlight exposure, and the possibility of a natural redistribution of elements within the tree were investigated. Larch wood cores, bark samples and tree measurements were collected on seven sites in the valley of Surses (Grisons, Switzerland). The core samples were prepared for reaction wood analysis, the bark samples were exposed to heat and ultraviolet light in two separate experiments, and x-ray fluorescence analysis was performed on finely ground bark samples. The main finding was that the bark discoloration defined as *Cortex rubra* in this thesis, can be attributed to ultraviolet light exposure. Neither correlation with reaction wood nor exposure to heat showed a significant change in redness. No significant difference was found in the elemental composition of the bark either. Ultraviolet light was the only investigated factor influencing the bark color. Therefore, the location of the larch has a major impact on the appearance of the bark, as sunlight exposition changes. The discoloration of the bark is not deemed harmful for the trees, although, the effect of extreme exposure to ultraviolet light has to be further investigated. Several hypotheses are still to be examined and further research to be conducted in order to confirm the true origin of the phenomenon.

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List of Abbreviations

ANOVA	Analysis of variance
a.s.l.	Above sea level
BHD	Breast height diameter
Ca	Calcium
Cl	Chloride
CW	Compression wood
E	East
Fe	Iron
K	Potassium
Mn	Manganese
Mg	Magnesium
N	North
Na	Sodium
NE	Northeast
NW	Northwest
P	Phosphorus
PP Cells	Polyphenolic parenchyma cells
RW	Reaction wood
S	Sulphur
SE	Southeast
Si	Silicon
So	South
SW	Southwest
U	Uranium
UV-light	Ultraviolet light
W	West
WSL	Swiss Federal Institute for Forest, Snow and Landscape Research
XRF	X-ray fluorescence

1. Introduction

This thesis covers an approach to discover the origin of a phenomenon on tree bark of the European larch (*Larix decidua* Mill.). This phenomenon has not yet been studied scientifically. In order to comprehend the approach taken, several topics are outlined. Firstly, the European larch is introduced as the species of interest. This will give a broad overview on the importance of the species in current and past times. Secondly, tree bark is explained in detail, as it is central to understand the phenomenon. Thirdly, the phenomenon is described and named: *Cortex rubra*. Lastly, the goal and hypotheses of this thesis are defined.

1.1 The European larch

The European larch (*Larix decidua* Mill.) is a typical mountainous pioneer species, long-lived tree and the only deciduous conifer in Europe (De Ronch et al., 2016; Matras & Pâques, 2008; Pâques et al., 2013). It is well adapted to continental alpine climate, tolerating very cold winter temperatures of up to -40°C (Bannister & Neuner, 2001; De Ronch et al., 2016). It can be genetically differentiated from its ten close relatives in Asia and North America (Jansen & Geburek, 2016; Nisula, 2018; Pâques et al., 2013; Vilcan et al., 2013). Nevertheless, the European larch can easily be crossed with other larch species, such as the Japanese larch (*Larix kaempferi* Lamb.) producing valuable hybrids (Matras & Pâques, 2008; Pâques et al., 2013). In this thesis, if larch is mentioned, the European species is always implied, as only this species of larch was investigated.

In Switzerland most larches can be found in the mountain and subalpine zones starting at 1400 m above sea level (a.s.l.) and climbing up to 2500 m a.s.l. (Matras & Pâques, 2008) (Fig. 1). Due to low competition at the tree line, larch can be the dominant tree species and form large sparsely pure stands (Matras & Pâques, 2008; Pâques et al., 2013). Due to the high elevations and rough environment they are exposed to, they usually have a lower growing potential than in optimal growing conditions, i.e. the vegetation period is very short (Pâques et al., 2013). In this terrain, slopes are often steep, inducing a typically bent stem in the larch as a reaction to snow movements, such as snow creep, snow gliding, avalanches but also wind exposure, soil creeping or landslides (Schönenberger et al., 1994). Coniferous trees react to the additional pressure load with reaction wood (RW) on the downhill facing side of the slope, forming a wood with higher lignin content called compression wood (CW), consequently, resulting in eccentric tree rings and a bent stem form. However, the tree rings some meters above the ground appear again in centric

form, since no unilateral forces are applied at these heights (Gardiner et al., 2014; Schönenberger et al., 1994; Timell, 1986).

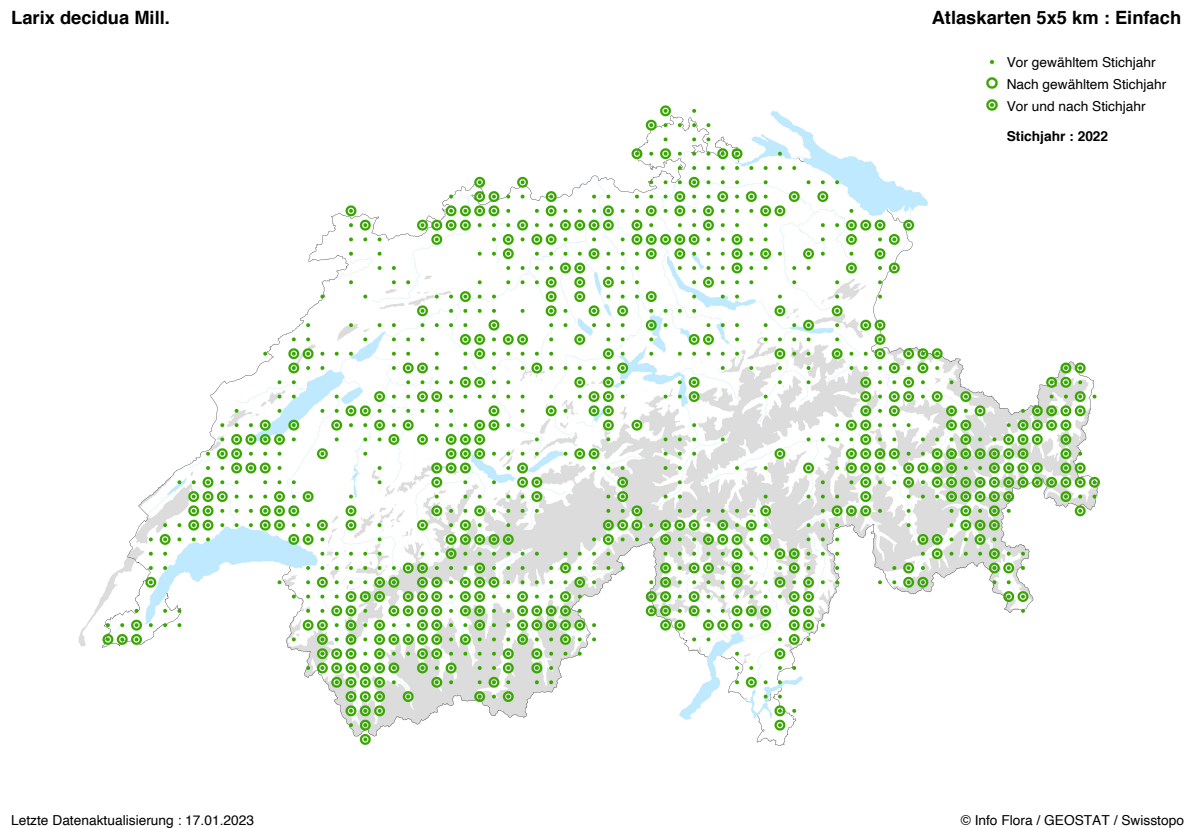


Fig. 1: Distribution of *Larix decidua* Mill. in Switzerland in the year 2022. The legend explains the occurrences of larch: prior to 2022 with a dot, after 2022 with a circle, and prior and after 2022, i.e. confirmed stands, with a dot with a circle around it (Info Flora, GEOSTAT, Swisstopo, 2022).

As a pioneer species, the larch thrives under full sunlight exposure, can adapt to well drained, nutrient poor soils, and likes moderate moisture (Matras & Pâques, 2008). It easily colonizes barren and disturbed soil, behaving according to the pioneering strategy (Zeidler et al., 2022). The larches natural occurrence is favored by frequent disturbances, for instance landslides, snow slides or fires (Matras & Pâques, 2008; Pâques et al., 2013). Subsequently, larch is valued as one of the most important species in protective forests (Pâques et al., 2013; Ruhm et al., 2016). Due to the wood's durability, high climate-sensitivity and ability of the tree to reach a high age it is also a valuable species for dendrochronology and climate reconstructions (Carrer & Urbinati, 2006; Schweingruber and Briffa, 1996). Additionally, the larch has been used in the building industry and furniture production throughout Europe because of its dense, strong and durable wood

(Pâques et al., 2013). Historically, larch wood was already used as construction material in ancient Rome (Jansen & Geburek, 2016). The demand for larch wood lead to it being planted beyond its natural range beginning in the 16th century (De Ronch et al., 2016; Jansen & Geburek, 2016; Pâques et al., 2013). However, not simply its wood was of value but the oleoresin was used for turpentine production. For instance, the commonly known Venetian turpentine was the product of larch oleoresin (De Ronch et al., 2016; Francezon & Stevanovic, 2018; Nisula, 2018). Resin products were used on the grand scale during the wooden sailing-ship era from the 16th to mid 19th century (Nisula, 2018). Presently, its main usage has shifted towards printing ink, adhesives and sealants, paper size, emulsifiers and coatings (Neis et al., 2019; Turner, 2010). Additionally, it was and still is used medically and is important in multiple cultural rites and beliefs (Da Silva Rodrigues-Correa et al., 2013; De Ronch et al., 2016; Izzo et al., 2021; Mazzei et al., 2020; Neis et al., 2019, Pásztori et al., 2016; Salehi et al., 2019).

Generally, the natural range of the larch is shrinking due to human intervention or lack thereof. Specifically, the human reduction of natural disturbances and the lack of removal of competitive shade-tolerant species (Matras & Pâques, 2008). For example the larch would thrive if more wildfires would be allowed to occur (Kharuk et al., 2016). Additionally, the natural range is predicted to rise to higher elevations in the future due to climate change. Migration towards higher elevations will shortly increase the larches distribution. However, establishment and survival are more limited in the long run due to extreme weather conditions, less area available, and displacement through other species later in the ecological succession (Matras & Pâques, 2008). However, the reality looks optimistic for the artificial distribution of the larch. The larch has been deemed a suitable alternative to spruce for the afforestation of former agricultural land, and reforestation in the face of climate change (Zeidler et al., 2022).

Nevertheless, the larch is exposed to several common fungal infections and pests, which could diminish afforestation efforts and the quality of the wood. The most destructive fungal disease being the larch canker, *Lachnellula willkommii*, which causes distorting branches and stems (De Ronch et al., 2016; Matras & Pâques, 2008; Pâques et al., 2013). Other fungal diseases are the leaf cast fungus, *Meria laricis*, the root rot, *Heterobasidion annosum*, *Hypodermella laricis*, and the velvet-top fungus, *Phaeolus schweinitzii*, causing butt rot (De Ronch et al., 2016; Maresi et al., 2004; Pâques et al., 2013; Vîlcan et al., 2013). Harmful insects are several bark beetle species from the *Ips* genus, the larch case-

bearer, *Coleophora laricella*, the larch bud moth, *Zeiraphera diniana*, and the large pine weevil, *Hylobius abietis*, which is among the most serious pests affecting young coniferous forests in Europe (De Ronch et al., 2016; Eichhorn, 1978; Habermann & Ott, 2009; Vilcan et al., 2013). The larch bud moth, *Zeiraphera diniana*, is one of the best studied defoliators, causing the needles to fall off early in the season (Peters et al., 2020). This leaves the trees leafless for a short time in summer, the trees producing fresh needles soon after for a second time. However, the short period still available for photosynthesis significantly reduces the size of that years tree ring. The bud infests the larch in a usual rotation of seven to nine years, for which the pattern can clearly be observed in the tree ring widths (Baltensweiler et al., 2008; Peters et al., 2020).

1.2 Tree bark

Tree bark is the protective layer encasing the cambium and xylem. The layers and structures are described from the tree core to the tree bark in the following text. A comprehensive view is displayed schematically in figure 2.

The heartwood provides stability and is formed as the innermost structure of a tree around the pith with annual xylem rings (tree rings consisting of one layer of earlywood and one layer of latewood each). The layer outside of the heartwood is called sapwood. The sapwood is the living vascular tissue, conveying water and minerals from the roots to the crown of the tree. Following the sapwood is the vascular cambium. These cells are responsible for the secondary growth of tree stems. They actively divide to form xylem cells towards the stem centre and phloem cells towards the outside, which already is part of the tree bark (Da Silva Rodrigues-Corrêa et al., 2013; Franceschi et al., 2005; Kain, 2020; Sakai, 2001; Taiz & Zeiger, 2006) (Fig. 2).

The cellular structure of the bark can be divided into distinct units with separate layers (Chrabąszcz & Mróz, 2017). The inner bark is called phloem and fulfills a different function from the outer bark. It consists primarily of conducting sclerenchymatic and parenchymatic cells and contains the conducting system for the assimilates of a tree (Sakai, 2001). Sclerenchyma cells provide strength and support, while parenchyma cells are metabolically active, capable of dividing, and able to transform into any other cell type as is needed (Crang, Lyons-Sobaski, & Wise, 2018). The outer bark can be called periderm or rhytidome and is made up of dead cork tissue which are interspersed with depositions of microorganism repellents and cells to prevent water loss (Chrabąszcz &

Mróz, 2017; Eberhardt et al., 2015; Kain, 2020). Phenolic materials and calcium oxalate crystals are used as repellents and suberin as a hydrophobic barrier which are originally produced in the phloem by polyphenolic parenchyma cells (PP Cells) (Chrabąszcz & Mróz, 2017; Franceschi et al., 2005; Krekling et al., 2000; Oskolski & Van Wyk, 2010). Over time, the rhytidome characteristically splits and thickens (Pásztory et al., 2016). The periderm cork cells are produced by the cork cambium, also known as phellogen, which is the meristematic region of the bark capable of dividing cells. Much like the vascular cambium, the cork cambium also produces some cells towards the center of the tree called phelloderm. This thin layer of inward produced cells consist of live parenchyma cells (Chrabąszcz & Mróz, 2017; Pásztory et al., 2016) (Fig. 2).

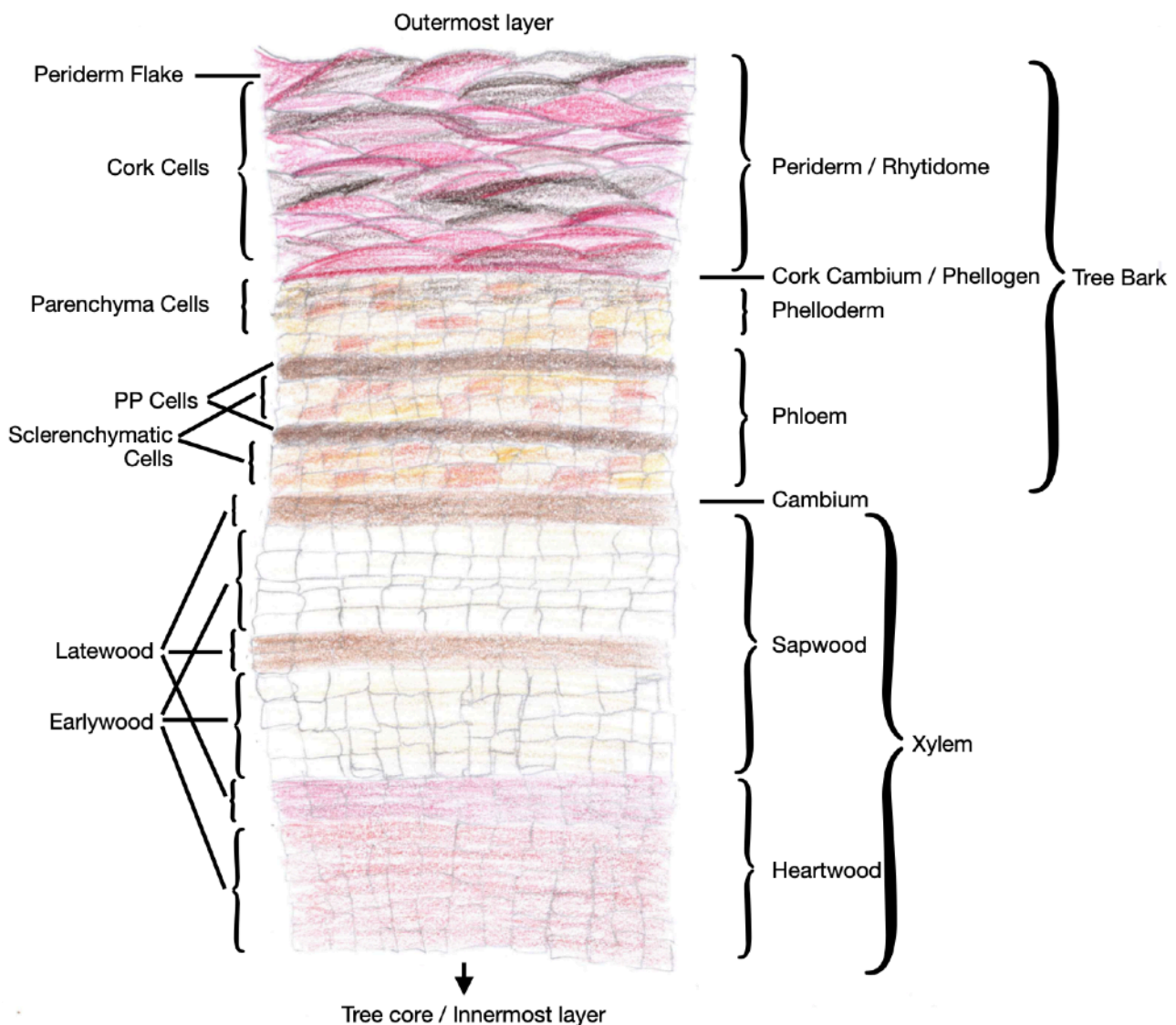


Fig. 2: Schematic overview of layered main tissues in wood and bark cell structure of larch. On the left side specific cells and keywords are indicated. To the right the most important labelings for the layers of wood and bark are listed. The colors are indicative for real life larch samples (Silja Holdener, 2022).

The basic function of the outer bark is to protect the nutrient rich phloem, vascular cambium and sapwood from being damaged by diverse biotic and abiotic factors (Szwajkowska-Michałek et al., 2020). The protection by the bark is crucial in extreme weather conditions, especially in the case of wood fires (Frejaville, Curt & Carcaillet 2013; Hengst & Dawson, 1994; Pausas, 2015) and frost effects (Oskolski & Van Wyk, 2010). Bark is important also in less severe scenarios like temperature changes where, if it is not thick enough, sunscald or sunburn can occur (Ophardt & Hummel, 2016). Additionally, the periderm allows for control of gas exchange in the stem through pores called lenticels. Although, these can be seen as potential weak tissue, facilitating entry for insects, fungi and bacteria (Franceschi et al., 2005). Nevertheless, tree bark is a shelter to several organisms. It is the habitat of different arthropoda, vertebrates, lichen, moss, and other living creatures (Pásztor et al., 2016). If the tree bark is damaged, specifically due to parasite infestations or mechanical injuries like rockfalls and landslides it can lead to a series of deadly infections (Franceschi et al., 2005).

Tree bark was frequently utilised in ancient times and is universally used in agriculture for mulching (Pásztor et al., 2016). It was natural to eat bark during times of famine and common to feed it to domestic animals (Östlund et al., 2009). Its fibrous quality was good for spinning ropes and making clothes (Pásztor et al., 2016). Tree bark can also be used to determine air pollution (Von Härtel & Grill, 1972; Olajire & Ayodele, 2003; Pásztor et al., 2016; Ylmaz et al., 2006). Additionally, the interest in gaining something useful out of the commonly discarded tree bark (Weissmann, 1976; Feng et al., 2013), has lead to research in the construction industry and the suitability of bark as an alternative fuel (Kain et al., 2020; Lehtikangas, 2001; Qin, Keefe & Daugaard, 2018). Besides these advanced research studies, tree bark is generally a field of little scientific knowledge.

Tree bark varies vastly between species and individual trees of the same species depending on environmental factors such as soil properties, location within a forest or tree age (Fekete, 1951; Weidenhiller & Denzler, 2014). Mature larch exhibit characteristic thick fissured bark with a reddish brown to light grey tinge (Chrabąszcz & Mróz, 2017; De Ronch et al., 2016) (Fig. 3). With a usual scales thickness of 5-50 cm it is a very prominent and noticeable trait of the larch (Vaucher, 1997). The phloem and periderm are easily distinguishable by their color. For the larch the outer bark has a prominent maroon to violet tone in stark contrast to the light orange phloem section. Additionally, the periderm has a structure of flakes, which are non-cohesive and allow for the bark to easily scale off

(Kain, 2020). However, when the larch is still young, the bark appears smooth and with a greyish color (Häne, 2012).

1.3 *Cortex rubra*

In the Swiss alps, several larches have been observed to display atypically intense red colouration patches of the visible bark on tree stems, which stand in stark contrast to the usual grayish red tones (Fig. 3). Several larches display the red coloration perpendicular or even upward to the slope on their stems while some trees do not exhibit any coloration at all. Even some branches have been observed to exhibit the more intensely colored red bark. The phenomenon seems to appear at random and in different intensities on certain individuals. There is no scientific evidence or explanation for the cause of this phenomenon in the scientific community yet, neither is there a specific name. Due to the red appearance on the outermost bark, the phenomenon is named „*Cortex rubra*“ in this thesis. It is the simple combination of the latin words for tree bark and red color.



Fig. 3: *Cortex rubra* on larch stems close to the town of Savognin, Switzerland (Silja Holdener, 2022).

1.4 Goal and Hypotheses

The goal of this thesis is to investigate whether there are specific triggers and factors which are the cause for *Cortex rubra* on larch stems and to determine how *Cortex rubra* bark is different to normal bark. Depending on the trigger, *Cortex rubra* might be an indicator for tree health, wood quality or could impact the distribution of larch in our changing climate. Since bark is so crucial to trees in the defense against the environment, understanding it is essential in mitigating potentially harmful factors, developing good management strategies for healthy larch forests and production, as well as predicting tree-pest dynamics (Franceschi et al., 2005). Considering the tree physiology and niche in the ecosystem occupied by larch as well as the properties of tree bark, various biotic and abiotic factors are possible. Due to the limited scope of this thesis, only three hypotheses for *Cortex rubra* were chosen to be examined.

(I.) *Cortex rubra* could be a consequence of reaction wood (RW), which trees form when on a steep slope. In the case of gymnosperms to which the larch belongs, a RW called compression wood (CW) is formed on the downhill facing side of the slope (Gardiner et al., 2014). The pressure and additional growth of wood might have the strength to crack the bark or thin it out and cause a different colouration by revealing the color of the innermost bark layers. Therefore, this could be called an effect of the display of different ages of tree bark due to RW.

(II.) *Cortex rubra* might appear as a result of sunlight exposure. Sunlight is empirically known to bleach and change the hue of heartwood and bark directly (Matsuo et al., 2011; Park et al., 2022; Tse et al., 2018). Additionally, slope direction and position has been found to have significant effects on soil nutrients and plant performance in several studies (Brunori et al., 1995, Qiao et al., 2019; Van de Water et al., 2002). It is well known that trees growing on sunny slopes generally grow taller than trees on shady slopes. Their distribution of leaves or needles will also change depending on sunlight availability (Brunori et al., 1995). Thus, bark could react with a change in color due to different light expositions. However, sunlight itself can be split into two main components, namely temperature and light, which will be examined separately.

(II.a) By exposure of tree bark to a higher temperature a change in its appearance is imaginable. It has been found that heat changes the color of wood due to a slow and mild thermal oxidation process (Calienzo et al., 2015, Matsuo et al., 2011). Over time wood

lightness decreases and a shift to a red hue occurs (Bekhta & Niemz, 2003; González-Peña & Hale, 2009 a, b; Matsuo et al., 2010). *Cortex rubra* could be the shift in red hue for the tree bark.

(II.b) Ultraviolet light (UV-light) might affect phenols and tannins in the bark to change in color and appear with a different red tinge (Tse et al., 2018). A longer exposure could lead the intense coloration to fade out, similarly to how wood fades in the sunlight over time (Ayadi et al., 2003; Calienno et al., 2015; Cogulet et al., 2016; Park et al., 2022). The stronger one-sided exposition of the tree bark to the radiation could then result in the contrasting appearance of the bark.

(III.) At last, it is possible that the color change could stem from a variation in distribution of minerals, sugars and other plant tissue of the tree in itself. For example, several environmental factors such as soil properties, tree growth, age, season, temperature and disturbance have been found to influence resin terpene synthesis in conifers (Da Silva Rodrigues-Corrêa et al., 2013; Lombardero et al., 2000). A recent study on slash pine even showed that resin productivity and resin components do vary depending on the slope direction and position (Luan et al., 2022). This indicates that several factors can have an effect on the composition and allocation of tissue within a tree. Tree bark might be affected similarly and a change in color due to different composition of the plant tissue could be possible. Especially allocation of iron comes to mind, as the characteristic red oxidation would fit the red color of *Cortex rubra*. The goal within this hypothesis was not to find a specific trigger but to determine whether the bark presenting with *Cortex rubra* has been built out of different components to begin with.

These three hypotheses were investigated with field samples and measurements taken in the Swiss alps.

2. Study Site

All samples for this thesis were collected in the valley of Surses in the canton of Grisons close to the town of Savognin. The temperate climate in the examined region is expressed in an annual mean temperature of 6 - 7°C and a yearly mean precipitation of 900 - 1100 mm (MeteoSchweiz, 2023; Swisstopo & MeteoSchweiz, 2022). Which provides enough warmth and ample supply of water for the montane and alpine vegetation to persist cold winter temperatures and flourish during the summer season. The geology varies according to site, from Flysch sedimentary depositions and moraines to metamorphic basaltic rock (Swisstopo, 2022 a).

Fieldwork was done on two separate occasions. The first fieldwork was done early in the timeline of this thesis in order to get first ideas on what is possible and start first attempts in finding the origin of the phenomenon. Additionally, it would lay the groundwork upon which the second fieldwork could be planned.

2.1 Fieldwork in January, 2022

For the first fieldwork, three sites were investigated in the valley of Surses (Fig. 4). All sites are silviculturally used and maintained by the local foresters (Forstdienst Surses). Sites B and C are situated in larch forests and are partially used for woodland pasture (similar to the „Wytweide“ in the canton of Jura (Häne, 2012)), whereas site A is located in a dense coniferous forest. The altitudes range between 1300 - 1700 m a.s.l., with slope inclination ranging between 10 and 30 %. The hillside of site A was facing northeast (NE), site B west-facing (W) and site C southwest-facing (SW).

Six trees were chosen randomly at each site, which showed *Cortex rubra*. Trees were only selected if they were healthy and their bark was intact. Additionally, only trees with diameters larger than 30 cm were chosen. For each individual tree, the orientation of *Cortex rubra* (° degrees), breast height diameter (BHD) at 1.30 m above the ground, bark thickness estimation (cm), estimated percentage of shade on the tree, and the growth form of the stem (three categories: straight, slightly crooked, bent) was documented (Appendix A).

Additionally, at each site, three of the six randomly selected trees were sampled. For each tree two wood cores were collected with a hand-operated increment borer. The samples had a diameter of 6 mm and were of variable length between 10-25 cm. The first core was

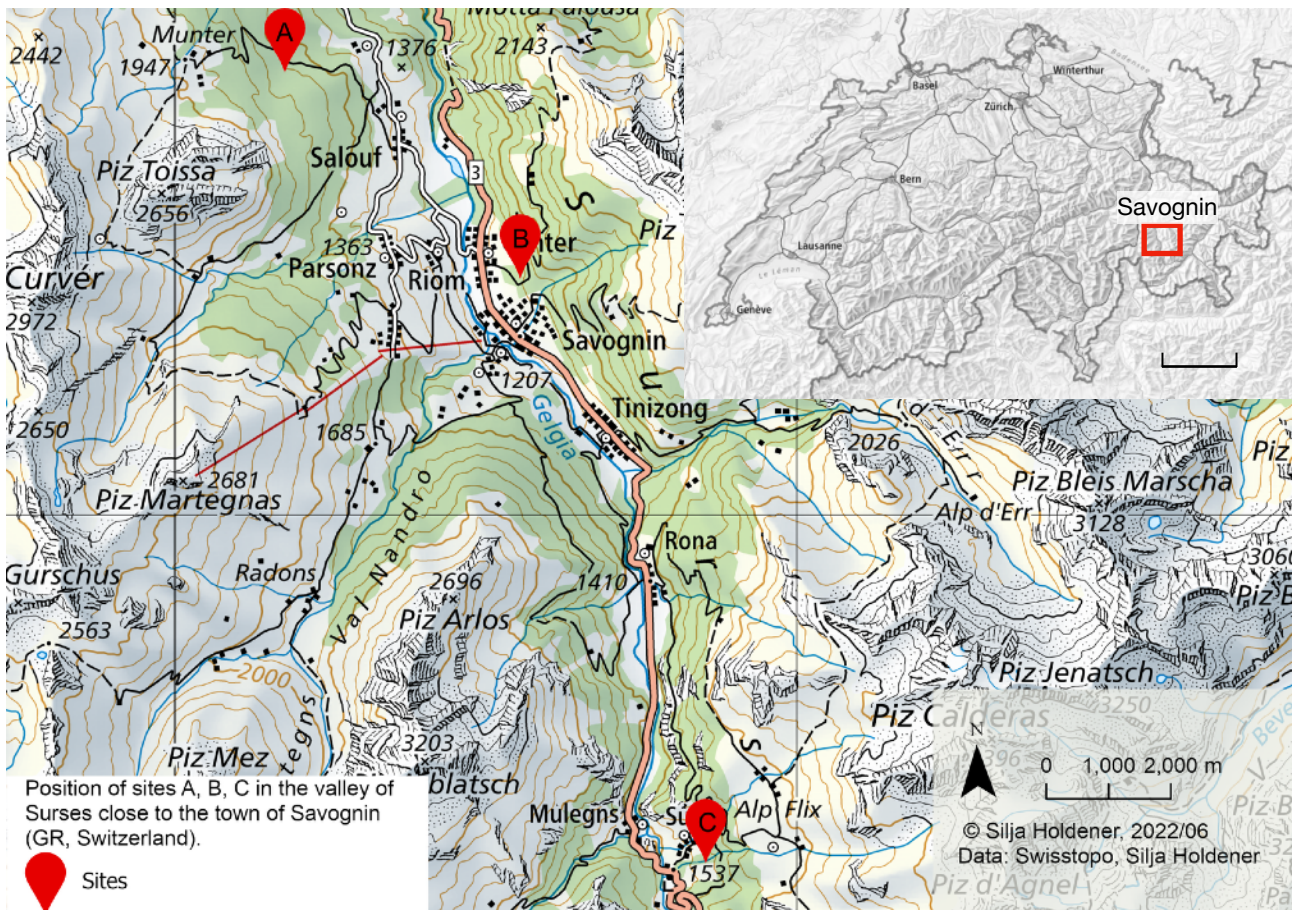


Fig. 4: Map of sites A, B, C, where fieldwork was conducted in January 2022 (Swisstopo, 2022 b). Additionally, an overview map of Switzerland was added in the upper right-hand corner (Esri Inc, 2021).

always taken from the *Cortex rubra* side and the other at a 90° angle to the first one (Gärtner et al., 2015). The cores were then stored in a plastic rack for transport. Additionally, two bark samples were chipped off with an axe close to the boreholes and stored in separate plastic bags. The bark samples had variable size, however, a maximum thickness was aimed for without injuring the cambium.

2.2 Fieldwork in May, 2022

In a second step, four sites each of a size smaller than 100 x 50 m at different elevations (1300, 1500, 1700, 1950 m a.s.l.) were mapped out in the valley of Surses. They were distributed in a smaller radius around the town of Savognin (Fig. 5). All sites are silviculturally used and maintained by the local foresters (Forstdienst Surses). The hillside of these sites was specifically selected to be the most north- (N) and south- (So) facing. The sites facing more north were named „N“ (for north) and „M“ (for mountain). The two sites facing south were called „S“ (for south) and „T“ (for town). Additionally, a control site

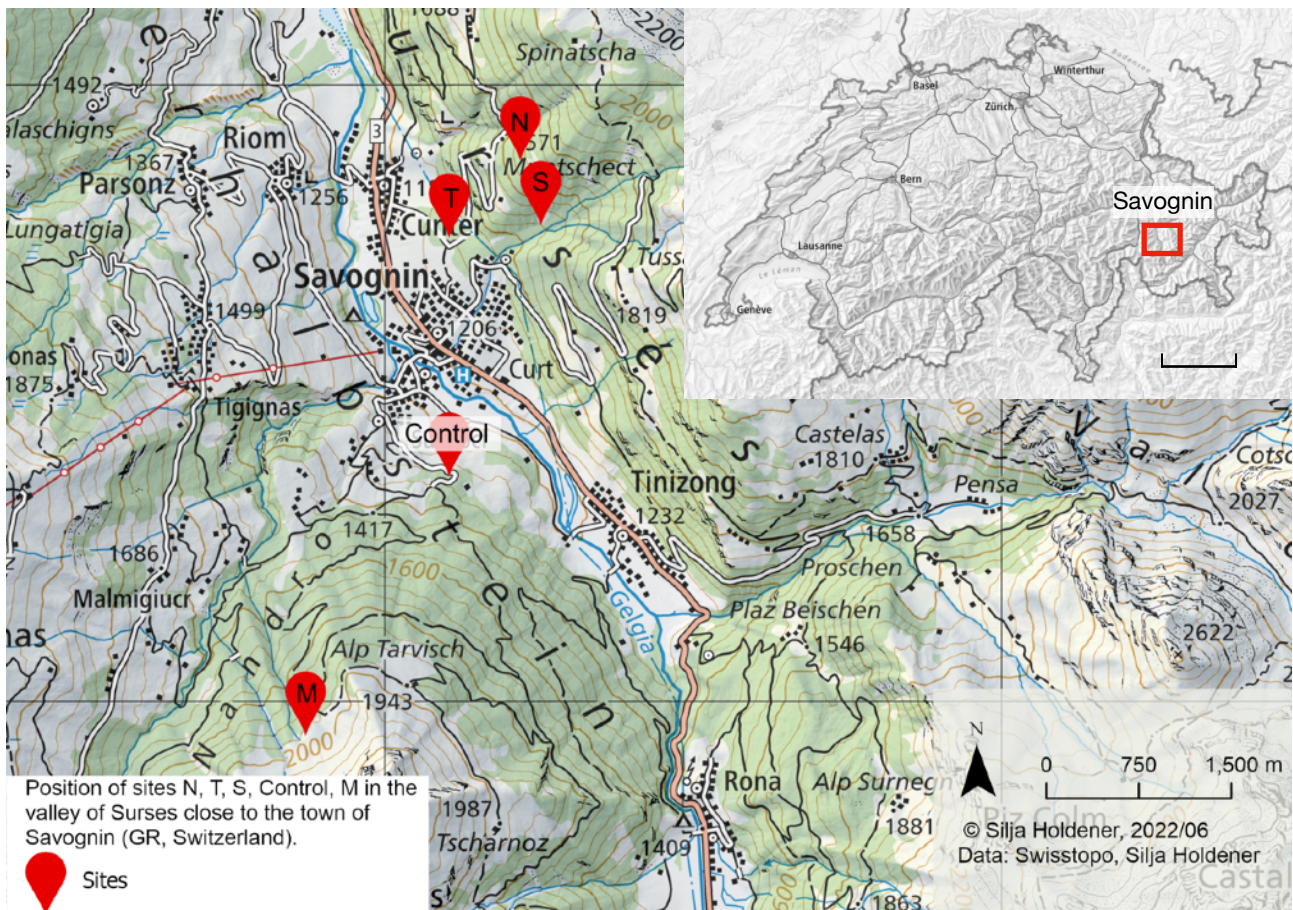


Fig. 5: Map of sites N, T, S, M and the Control site where fieldwork was conducted in May 2022 (Swisstopo, 2022 c). Additionally, an overview map of Switzerland was added in the upper right-hand corner (Esri Inc, 2021).

was selected, where the trees were standing on even ground. It was named „Control“ accordingly (Fig. 5).

At each site ten larches were measured. They were selected according to following criteria: Firstly, all ten larches had to stand in closest proximity to each other, allowing only other tree species, dead wood or tree stumps to stand between them. Secondly, the larches had to be healthy and their bark intact. Lastly, only larches with a BHD larger than 15 cm were considered. These criteria make for one big difference towards the fieldwork done in January as in these field measurements trees without *Cortex rubra* were also recorded.

The same measurements were taken for each of the ten trees at each site as in the fieldwork in January mentioned above (orientation of *Cortex rubra* (° degrees), BHD (130 cm above ground), bark thickness estimation (cm), estimated percentage of shade on the tree and the growth form of the stem (three categories: straight, slightly crooked, bent)).

Additionally, more data were recorded: For each tree the estimated height of *Cortex rubra* (if present), exact orientation of the edges of *Cortex rubra*, exact orientation of the edges of the stem segment not exposed to sunlight, slope orientation and slope incline at the base of the stem, and the number of trees in a five meter radius were documented (Appendix B).

Six trees of each plot were then chosen at random. With the exception of site T they included at least one tree without *Cortex rubra*. For each tree two wood cores were collected with an increment borer (Gärtner et al., 2015). The first core was extracted from the *Cortex rubra* side and the second at a 90° angle from the first core. If no *Cortex rubra* occurred the samples were taken from the uphill facing side and 90° angle to that. In addition, two bark samples were stripped off with a chisel and hammer at the extraction point of the wood cores. The bark samples were then wrapped with tape prior to putting them into plastic bags, to ensure the barks entirety.

At the Control site two trees were recorded, measured and sampled. They were equally selected for as the other trees, with the additional criteria of the Control site i.e. standing on even ground.

3. Methods and Material

In this chapter all work done after the two field trips are described. The subchapters 3.1 Sample preparation and 3.5 Calculations and Statistical analysis discuss the whole data gathered. The other subchapters are separated according to the three hypotheses in which the materials used are a subsample of the whole dataset.

3.1 Sample preparation

All collected wood cores (from both field trips) were cut to a plane surface using a core-microtome (Gärtner & Nievergelt, 2010). Additionally, chalk was applied and rubbed into the surface. High resolution photographs were then taken for all cores with the Skippy program (WSL - unpublished), which is specifically designed for wood samples. The images were then stitched into a panoramic photo of each core (PTGui Stitching, 2000-2022). They were then used for wood anatomical analysis (Chapter 3.2).

From each bark sample collected at sites A, B and C two cross sections of about 5 mm thickness were cut off, glued to small chips of robust wood to ensure stability of the bark and cut to a plane surface with the core-microtome by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL)(Gärtner & Nievergelt, 2010). These two sets of bark samples were further used in the experiments (Chapter 3.3.2). Furthermore, after attaching tape to the plane cut bark samples, micro cuts were obtained with the WSL-lab-microtome (Gärtner, Lucchinetti & Schweingruber, 2015). These were placed on glass slides and scanned (slide scanner, Axio Scan.Z1, ZEISS, Germany). The scans were prepared for RW analysis (Chapter 3.2).

Six bark samples from three trees from each of the sites N, T and M as well as all bark samples from the Control site were randomly selected. Therefore, 21 samples were prepared. After removing any attached moss, lichens, and dirt, about 10 g from the outermost bark flakes was scraped off and stored in small plastic containers. These samples were then further processed for X-ray fluorescence (XRF) analysis (Chapter 3.4).

The sample preparation was mainly conducted according to Gärtner et al. (2015) as well as Gärtner & Schweingruber (2013) with some minor deviations due to the different integrity and density of the bark material.

3.2 Reaction wood analysis

Wood anatomy is crucial in determining whether a tree has developed RW. In order to analyze wood anatomy a close up look at the cell structure is crucial (Gardiner et al., 2014; Heinrich et al., 2007). Therefore, the panoramic images as well as the scans from the bark micro cuts were needed for the RW analysis.

The panoramic pictures of the wood cores from sites N, T, S, M and Control were checked for the appearance of RW, i.e. CW in the case of larch. Recognition was facilitated by the chalk applied in the sample preparation due to a higher contrast achieved between cell wall and cell lumen area (Gärtner et al., 2015). Thus, the cell wall shape was inspected. Cells appearing with very round cell walls and many intercellular spaces were defined as RW (Gardiner et al., 2014; Heinrich et al., 2007). If identification was unclear, the samples were additionally compared to the classification according to Palombo et al. (2018) which in turn was based on the grading by Yumoto et al. (1983) and Timell (1986) (Fig. 6).

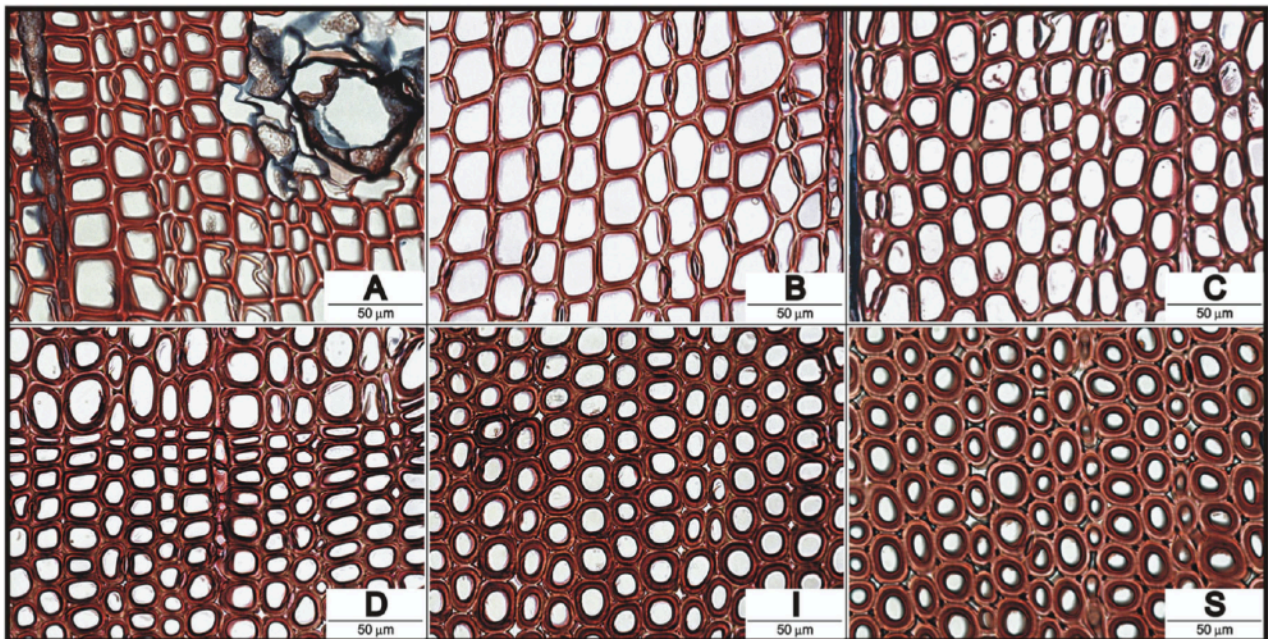


Fig. 6: Images indicating CW severity index, classified according to the grading system of Yumoto et al. (1983) and revised by Timell (1986). A = very faint round cells; B = slightly round cells and slightly thicker cell walls; C = fairly round cells with thicker cell walls; D = round cells with some intercellular spaces and relatively thick cell walls; E = round but not fully circular cell with intercellular space and thick cell walls; F = nearly circular cells with intercellular space and very thick cell walls (Palombo et al., 2018).

The scans of the slides with bark micro cuts were checked for any remarkable cells and patterns. Additionally, the scans of bark with *Cortex rubra* were compared to the non-*Cortex rubra* bark scans. Unfortunately, the scans proved to be diagnostically inconclusive for the determination of RW or any remarkable patterns other than the expected flake structure (Kain et al., 2020). The collapsed cell structure and thickness (circa 30 µm) of the cuts was mainly to blame for the lack of insights to be gained from the bark samples. Therefore, no results could be obtained from the scans and they will not be considered in this thesis furthermore (Appendix C).

3.3 Sunlight exposure analysis

In order to detect effects of sunlight exposure on tree bark, the field measurements were used. Additionally, two experiments were developed to provoke a change in bark color.

3.3.1 Site observations

The recorded data from the fieldwork in May 2022 was used to analyze the impact of sunlight. The segment of shaded tree was inverted in order to obtain the segment of sunlight exposed tree. The sunlight exposure segment was then schematically mapped together with the orientation of *Cortex rubra* on the tree for each site (N, T, S, M, Control) (Fig. 10-14). These schematic maps were created in the geo information software ArcGIS Pro (Esri Inc., 2021). Additionally, the percentage of overlap of the sunlight exposure segment on the section of *Cortex rubra* was calculated.

3.3.2 Experiments

Two experiments were conducted on the two sets of bark samples. The first set of samples was put in the oven. The second set was exposed to UV-light. Through comparison of before- and after-treatment photographs, effects of the heat and light treatments were analyzed. The images were taken under equal light conditions with the Skippy program (WSL - unpublished). Above all, the change in redness was obviously of interest due to the red nature of *Cortex rubra*.

3.3.2.1 Heat experiment

In the heat experiment the first set of plane cut bark samples was exposed twice to 100°C in an oven (Osvaldova, 2017). Firstly, the samples were left in the oven for three hours and then photographed. In the second round the samples were again left exposed to the heat for three hours, therefore, having been exposed for a total of six hours by the end of the second treatment (Fig. 7). The heat was set to 100°C in order to minimize the risk of burning the samples (Frejaville, Curt & Carcaillet, 2013). Thus, reducing the error of capturing a color change due to burned components in the bark. Similarly, the duration of the treatments was chosen (Nakamura & Krestov, 2005). However, the goal was not to design a treatment as close to the natural temperature cycle trees are exposed to in reality. Rather the duration was selected in the hope of maximizing any heat effects.



Fig. 7: Example of a plane cut bark sample. On the left is the picture of the bark prior to the treatment, in the middle the sample after three hours of exposure, and on the right the sample after six hours of exposure (Silja Holdener, 2022).

3.3.2.1 Ultraviolet light experiment

Similarly to the heat experiment, the UV-light experiment was set up. Here, two treatments of 30 minute exposure on the second set of plane cut bark samples was conducted. Hence, photographs were taken after 30 minutes and 60 minutes UV-light exposure (Fig. 8). The UV-light radiated was of 200-280 nm wavelength (UV-C) in a sterile bench (CleanAir, SKAN). Incoming natural sunlight on Earth includes UV-light at wavelengths between 100 and 400 nm (Rai, Morales & Aphalo, 2021; Walker, 2009).



Fig. 8: Example of a plane cut bark sample. On the left is the picture of the bark prior to the treatment, in the middle the sample after 30 minutes of exposure, and on the right the sample after 60 minutes of exposure (Silja Holdener, 2022).

3.4 X-ray fluorescence analysis

In order to determine the elemental composition of trace elements in the bark an X-ray fluorescence analysis was performed. Firstly, the outermost bark flakes of 21 samples, which were stored in small plastic containers, were air dried and subsequently milled to a fine powder in an ultracentrifugation mill (ZM 200, Retsch, Germany). Secondly, as much powder as possible was filled into sample cups, weighted and placed into an XRF spectrometer (SPECTRO X-LAB 2000, SPECTRO Analytical Instruments, Germany) (Fig. 9). All elements from sodium (Na) to uranium (U) can be measured with that XRF spectrometer. The resulting weight percentage of each element with a mean signature larger than 0.01 was then further analyzed in R-Studio (RStudio Team, 2022). Namely these ten elements: sodium (Na), magnesium (Mg), silicon (Si), phosphorus (P), sulphur (S), chlorine (Cl), potassium (K), calcium (Ca), manganese (Mn), iron (Fe).



Fig. 9: Finely ground bark powder filled into a sample cup for XRF measurements (Silja Holdener, 2022).

3.5 Calculations and statistical analysis

All collected data from the fieldwork and produced in the laboratory was computed with and arranged in the software Microsoft Excel for Mac (Version 16.64). Basic analysis and drawing of the plots was done with the R-Studio software (RStudio Team, 2022).

The proportional count of red pixel in the image before and after the experimental treatments was calculated for both conducted experiments. The Shapiro-Wilk-Test was then used to determine normality. For normally distributed sites analysis of variance (ANOVA) models were used to determine the influence of *Cortex rubra* bark and the experimental treatments. Likewise, non-normally distributed sites were tested with the Wilcoxon signed-rank test to determine the influence of *Cortex rubra* bark and the Kruskal-Wallis-Test for the treatments. The data from the XRF measurements was tested for within site and between site elemental variation with the tests and models mentioned before.

4. Results

4.1 Reaction wood

The RW in the samples was identified according to Palombo et al. (2018) (Fig. 6, examples of RW in samples shown in Appendix D). They were split into four groups within each of the sites N, S, T and M (Table 1).

Table 1: Number of samples of the sites N,S,T and M within the according group. The first and second group were composed of wood core samples originated from the side of the tree where no *Cortex rubra* was observed (indicated as „grey“). Additionally, within the first group all samples had RW formed in the wood whereas the second group did not. The third and fourth group were formed accordingly, with the cores stemming from the part of the tree with *Cortex rubra* (indicated as „red“). The sum of all sites for each group was calculated. The Control site was grouped as well. The Control site was summed up with the other sites in the row titled Total.

Sites \ Groups	1. Grey with RW	2. Grey without RW	3. Red with RW	4. Red without RW
N	0	9	0	3
S	1	6	1	4
T	0	6	1	5
M	0	7	0	5
All sites	1	28	2	17
Control	1	2	0	1
Total	2	30	2	18

RW appears in four samples out of 42. Two cores with RW originated from the side without *Cortex rubra* and the other two from the side with *Cortex rubra*. In the cores from sites N and M no RW was observed. The sum of all sites yields the possibility for a grey sample to have RW with a 4% chance and a red sample to have RW with a 12% chance. When the Control site is considered additionally, the grey samples have a 7% chance to have RW and the red samples have a chance of 11%.

4.2 Sunlight exposure

4.2.1 Site observations

The ten measured trees in each of the sites N, S, T, Contol and M were schematically mapped with the software ArcGIS Pro (Version 2.8.1, Esri Inc., 2021)(Fig. 10-14). The overview map (Chapter 2.2, Fig. 5) shows the relative geographical location of the sites to each other. Each site is located at a different elevation around the town of Savognin. The vegetational cover and terrain varied from site to site. It is important to keep in mind that only the orientation of *Cortex rubra* was mapped and not the actual width. Whereas the schematic segment of direct sunlight exposed tree represents both orientation and width. Sunlight exposure of the tree to the north was also included, although sunlight can only be indirect in that geographical direction. The maps, thus, allow for later interpretation of the orientation of sunlight in comparison to *Cortex rubra* but not the actual overlap. The percentage overlap of the the sunlight sector on the *Cortex rubra* section was calculated separately (Table 2).

The resulting maps show differences between the sites. The sites N and M are considered to be of the „most north facing“ criteria and site S and T are most south facing. Out of the ten trees in the sites S, T and M only up to one tree does not exhibit *Cortex rubra*. Additionally, up to maximally one tree is not directly sunlight exposed. The exception is site N, where four out of ten trees do not exhibit *Cortex rubra*, additionally, three tree stems are not exposed to direct sunlight. Direct sunlight exposition generally hits all sites from the So or southeast (SE). *Cortex rubra* is mostly oriented in these directions as well. The site exhibiting these trends the least is site M. On that site *Cortex rubra* is never directly So facing and direct sunlight reaches the trees more from the east (E) or W.

Site N is located on hilly terrain as is made visible by the contour lines (Fig. 10). The in field measurements tell us that the trees stand on averagely 264° W facing slopes at around 1706 m a.s.l., with two trees facing 20° NE. The inclination at the stem base is quite steep at an average of 24°. It was, therefore, the steepest site out of all sites. The trees were mostly standing straight and had an average diameter of 69 cm with 8 cm thick bark. The number of trees in a five meter radius ranged from 1-4, with an average of 2.9. *Cortex rubra* is on average 184° So facing with no exception. The direct sunlight reaching the tree stems ranges between 111°-316°. There are four trees not exhibiting *Cortex rubra*. All but one of these four trees are not exposed to direct sunlight. Two trees were standing across a small gravel road to the east of the other trees from the site.

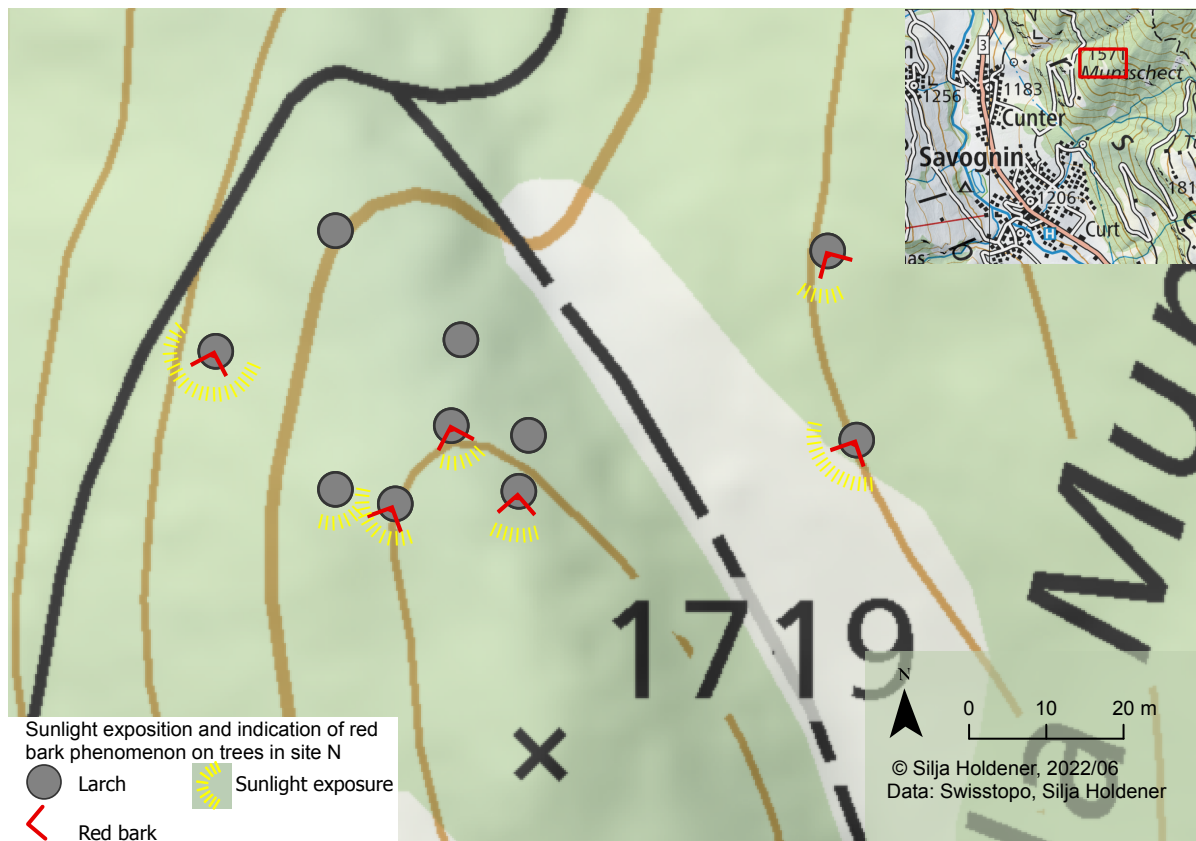


Fig. 10: Schematic map of ten sampled trees on site N. Trees are indicated with a grey circle. Red angles indicate the orientation of *Cortex rubra* on the tree. The direction from where sunlight can reach the tree stem is indicated with yellow stripes (Map created in ArcGIS Pro by Silja Holdener (Esri Inc, 2021)).

Site S is located at the base of a steep slope (Fig. 11). The slope is 216° SW facing at around 1493 m a.s.l.. Only one tree stands on 60° E facing slope. The inclination at the stem base is quite steep at an average of 23°. The trees were standing straight with an average diameter of 74 cm and 13 cm thick bark. The number of trees in a five meter radius ranged from 1-4, with an average of 2.4. *Cortex rubra* is on average 189° So facing. The direct sunlight reaching the tree stems ranges between 48°-303°. Only one tree did not exhibit *Cortex rubra* and it also was not exposed to direct sunlight. Contrary to the map, there was no visible path and no deeryard (small black square) when the sampling was conducted. Therefore, only three trees standing NE in relation to the other trees experienced a small separation from the other trees. The gap indicated in grey on the map was covered in grass and about 10 m wide. These three trees were standing on a steeper slope than the others.

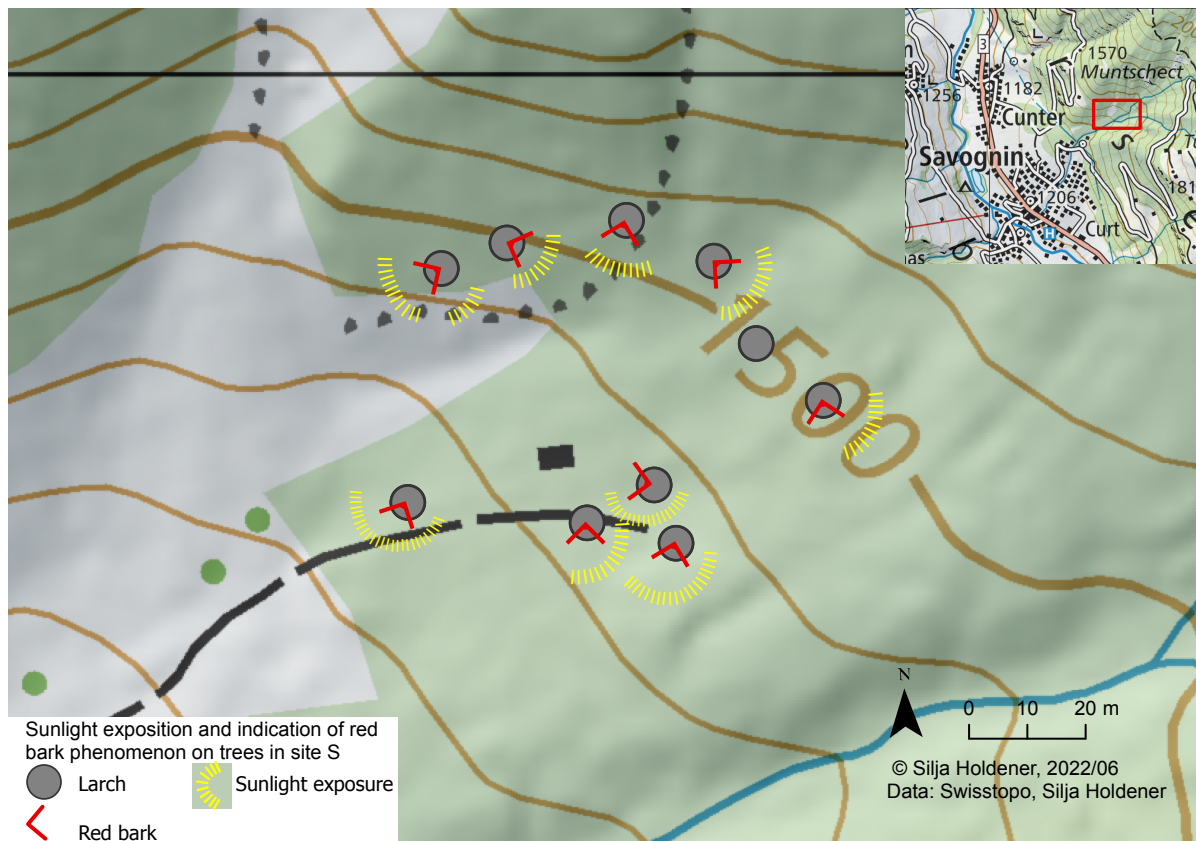


Fig. 11: Schematic map of ten sampled trees on site S. Trees are indicated with a grey circle. Red angles indicate the orientation of *Cortex rubra* on the tree. The direction from where sunlight can reach the tree stem is indicated with yellow stripes (Map created in ArcGIS Pro by Silja Holdener (Esri Inc, 2021)).

Site T is located on flat terrain with only slight variations (Fig. 12). The general slope is facing 253° W. The site is located 1288 m a.s.l.. There are two groups of trees visible on the map. The group of six trees was standing in the south in relation to a second group of four trees. The larger group of trees was standing on flat ground with an average of 4° inclination. The inclination at the stem base of the smaller group was much steeper at an average of 18° . All trees were standing straight and had an average diameter of 70 cm with 7 cm thick bark. The number of trees in a five meter radius ranged from 1-3, with an average of 1.8. *Cortex rubra* was on average 194° So facing with no exception. The direct sunlight reaching the tree stems ranged between 350° - 241° . Therefore, only a small section facing W can not hit the tree stems. On all trees *Cortex rubra* was found and all trees experienced direct sunlight exposition. However, the sunlit sections did not overlap perfectly with *Cortex rubra* for most trees. For the tree indicated with a green dot, there was no overlap.

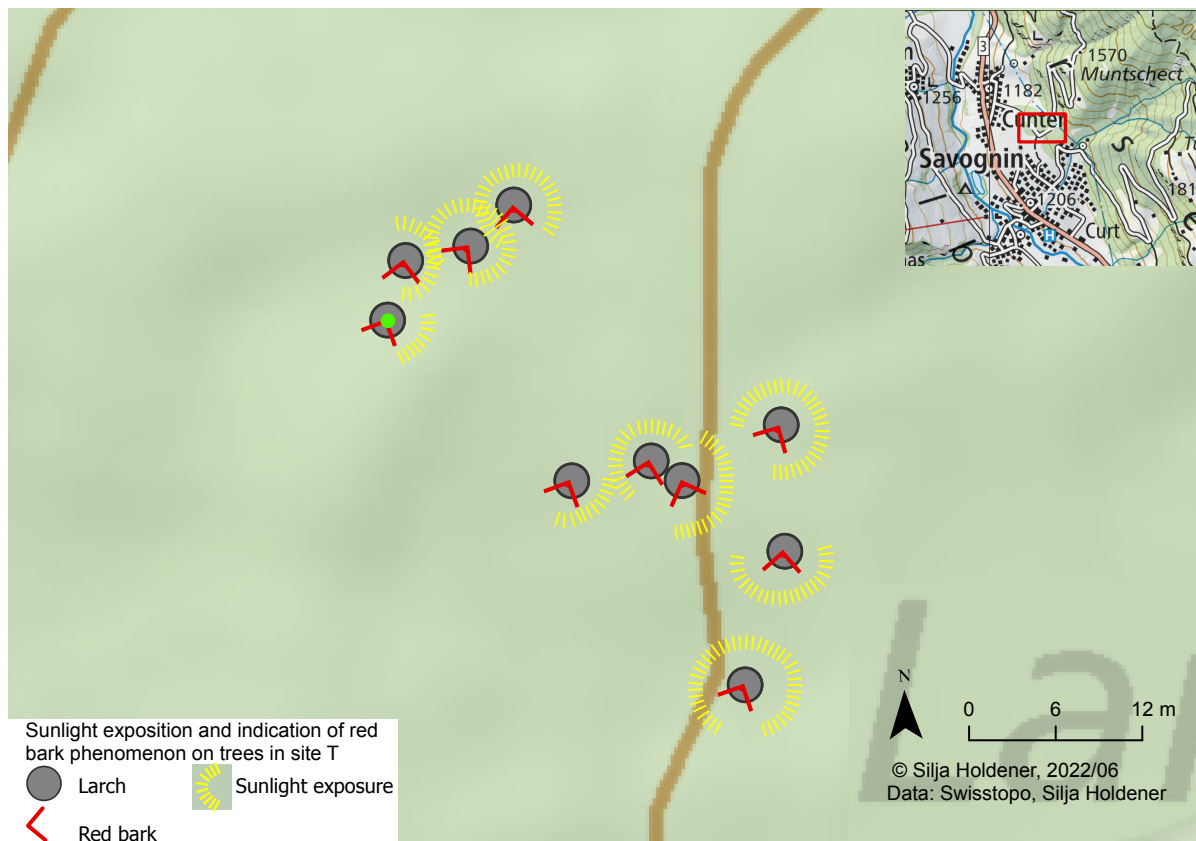


Fig. 12: Schematic map of ten sampled trees on site T. Trees are indicated with a grey circle. Red angles indicate the orientation of *Cortex rubra* on the tree. The direction from where sunlight can reach the tree stem is indicated with yellow stripes (Map created in ArcGIS Pro by Silja Holdener (Esri Inc, 2021)).

Site M is located just at the tree line of a mountain side (Fig. 13). The site was on a 303° northwest (NW) facing slope at around 1943 m a.s.l.. The average steepness at the stem base was 12°. The trees were mostly standing straight or bent and had an average diameter of 36 cm with 1 cm thick bark. These are the lowest site averages for both the BHD and bark thickness (besides the Control site). The number of trees in a five meter radius ranged from 3-6, with an average of 4.3. Making the trees stand in the closest proximity to each other out of all the sites. *Cortex rubra* is on average 249° SW facing. There is one exceptional tree showing *Cortex rubra* N facing (Fig. 13, tree marked with a green dot). The direct sunlight reaching the trees ranges between 350°-249°, leaving only a section towards the west in the shadow for the trees. There was one tree not exhibiting *Cortex rubra*. Four trees showed *Cortex rubra* in directions where the sunlit sections did not overlap at all.

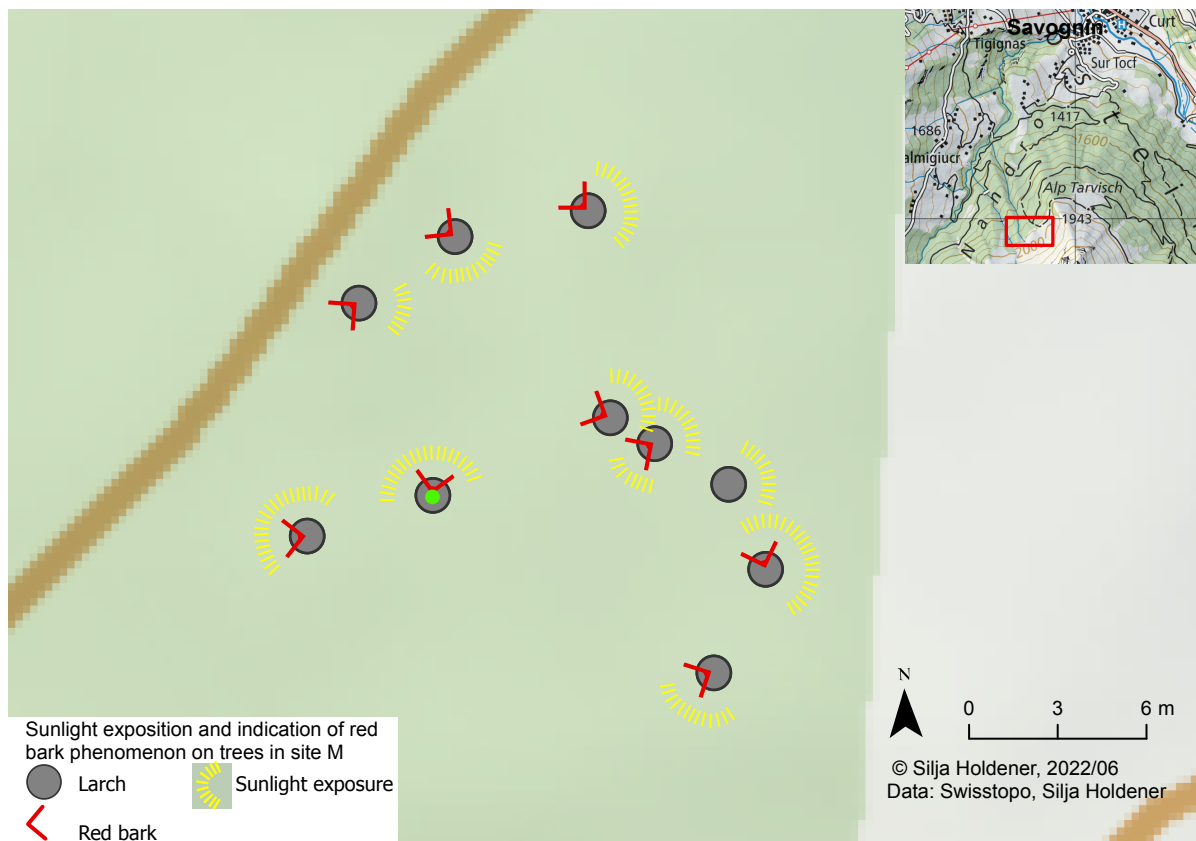


Fig. 13: Schematic map of ten sampled trees on site M. Trees are indicated with a grey circle. Red angles indicate the orientation of *Cortex rubra* on the tree. The direction from where sunlight can reach the tree stem is indicated with yellow stripes (Map created in ArcGIS Pro by Silja Holdener (Esri Inc, 2021)).

The Control site was located on flat terrain at an elevation of 1320 m a.s.l. (Fig. 14). The two trees were standing straight and had an average diameter of 31 cm with 1 cm thick bark. *Cortex rubra* was found only on one of the two trees. It was facing 82° E and was experiencing direct sunlight from 66°-118°. There was no neighboring tree within a five meter radius. The other tree did not exhibit *Cortex rubra* and was not exposed to direct sunlight. There were two neighboring trees recorded within a five meter radius for the second tree.



Fig. 14: Schematic map of ten sampled trees on the Control site. Trees are indicated with a grey circle. Red angles indicate the orientation of *Cortex rubra* on the tree. The direction from where sunlight can reach the tree stem is indicated with yellow stripes (Map created in ArcGIS Pro by Silja Holdener (Esri Inc, 2021)).

The calculation of the percentage of overlap between the the section exposed to sunlight with the *Cortex rubra* section resulted in an outcome without a big overlap percentage (Table 2). The total mean and median show that there is generally an overlap of 56-59% with a standard deviation of +/- 33.3 %. Six trees had an overlap of 100 %. However, 14 out of 35 trees with *Cortex rubra* had an overlap of less than 50 %. Five trees did not have any overlap between the sunlight exposed section with the range of *Cortex rubra*, four of which stood on site M.

Table 2: Percentage overlap of the sunlight exposure range with the *Cortex rubra* range for each tree in all sites. The mean and median was calculated for each site and for the total data set. „na“ stands for trees where no *Cortex rubra* was observed, thus, no overlap could be calculated.

Tree \ Site	N [%]	S [%]	T [%]	M [%]	Control [%]	
1	27	100	40	44	na	
2	100	65	100	72	68	
3	na	27	69	93		
4	71	83	100	87		
5	72	100	33	0		
6	45	na	53	0		
7	na	41	59	0		
8	na	14	43	0		
9	100	91	67	58		
10	na	53	0	na		Total [%]
Mean	69.2	63.8	56.4	39.3	68.0	56.4
Mean	71.5	65	56	44	68	59

4.2.2 Experiments

4.2.2.1 Heat treatment

After the 18 bark samples were exposed to 100°C for three and six hours, the resulting pixel count percentage of each image was calculated and plotted against its exposure time (Fig. 15 and Fig. 16). The whole data set was not normally distributed (Shapiro-Wilk-Test, p -value < 0.05). No clear trend was observed when all data was considered and no significant differences were detected between the treatments (Fig. 15) and neither between the two bark types (Fig. 16).

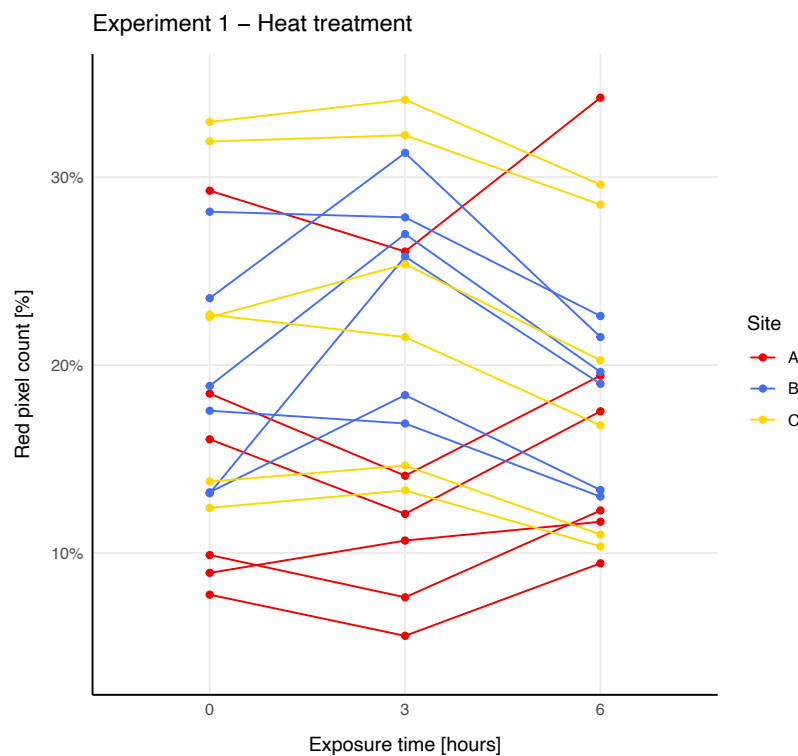


Fig. 15: Six bark samples with and without *Cortex rubra* of each site A,B and C were exposed to heat treatments of 100°C for three and six hours. The percentage of red pixel count for each sample image was plotted for all treatments. The different sites are marked with different colors. The same sample is connected with a line between the treatments.

Experiment 1 – Heat treatment on red and grey samples separated

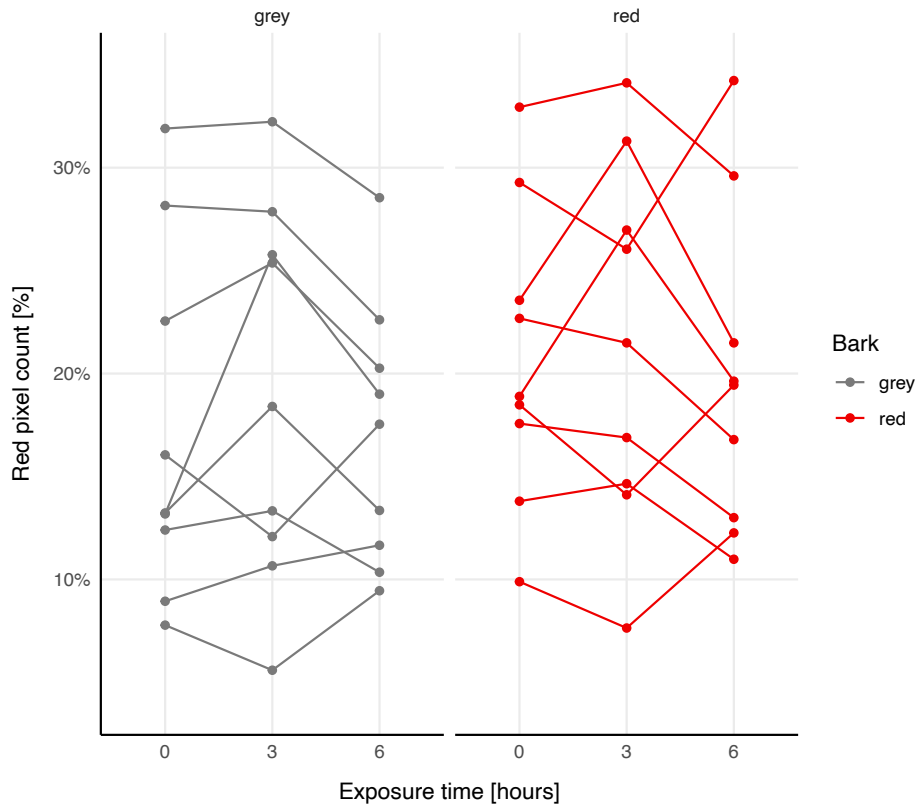


Fig. 16: Nine bark samples with and without *Cortex rubra* from sites A, B and C were exposed to heat treatments of 100°C for three and six hours. The percentage of red pixel count for each sample image was plotted for all treatment exposure times. The different bark types are marked with different colors. Grey stands for the samples which did not show *Cortex rubra*. The samples which showed *Cortex rubra* are marked in red. The same sample is connected with a line between the treatments.

In order to exclude site specific effects each site was checked separately (Fig. 17). Site A was not normally distributed (Shapiro-Wilk-Test, $p\text{-value} < 0.05$). There was no significant trend detected between the treatments, however, there was a significant difference found between the red and grey samples (Wilcoxon signed-rank test, $p\text{-value} < 0.05^*$). There is a trend for the pixel count to decrease after three hours and increase again after six hours. For the normally distributed sites B and C four ANOVA models were calculated each. However, no significant differences were found in any of the models. The trends for these two sites suggest an increase after three hours and decrease after six hours, contrasting the trends in site A.

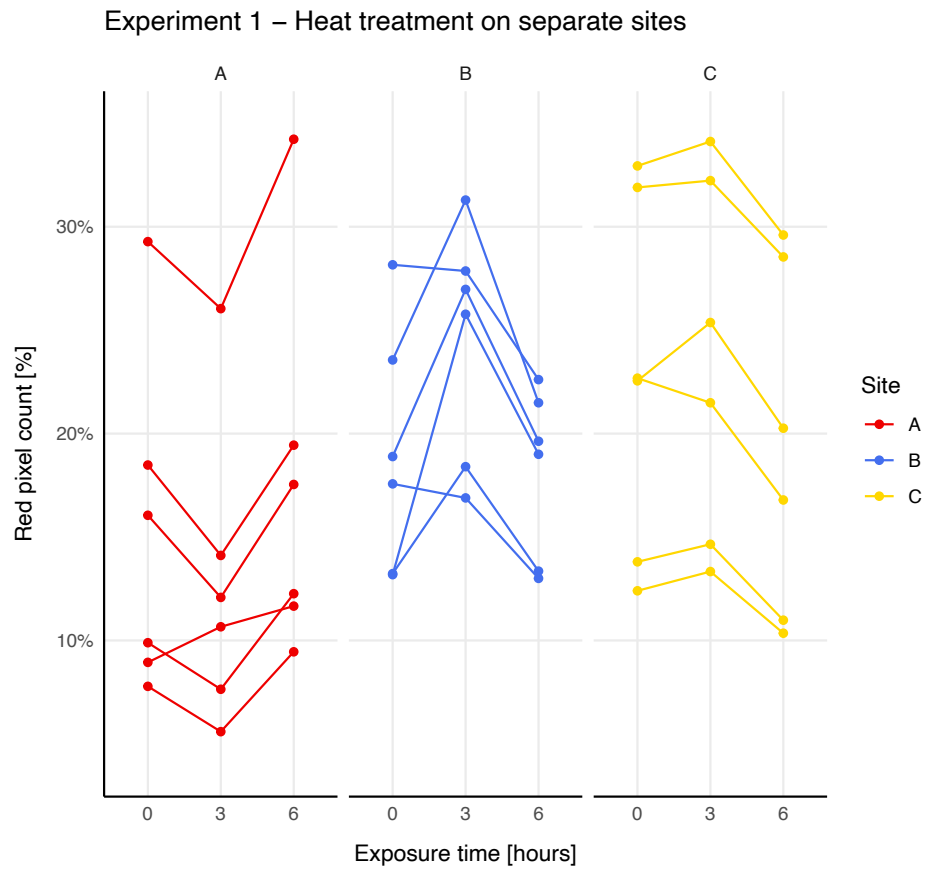


Fig. 17: Six bark samples with and without *Cortex rubra* of each site A,B and C were exposed to heat treatments of 100°C for three and six hours. The percentage of red pixel count for each sample image was plotted for all treatments. The different sites are marked with different colors and were plotted separately. The same sample is connected with a line between the treatments.

4.2.2.2 Ultraviolet light treatment

For this experiment 18 bark samples were exposed to UV-light radiation. After 30 minutes and after 60 minutes the samples were photographed and the percentage of red pixels calculated.

The whole data set was not normally distributed (Shapiro-Wilk-Test, p -value < 0.001). There was a significant increase of red pixel percentage in the images from the original bark to the bark exposed to the UV-light treatments (Kruskal-Wallis-Test, p -value < 0.05 *) (Fig. 18). This trend can be observed in all sites. No statistically significant difference between the bark without *Cortex rubra* and bark samples with *Cortex rubra* was found (Fig. 19).

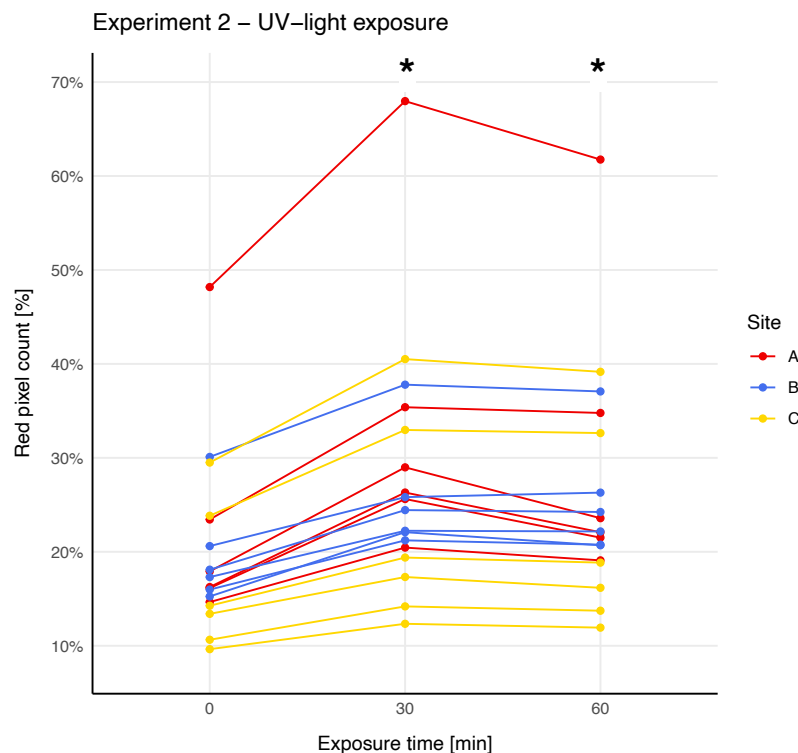


Fig. 18: 18 bark samples with and without *Cortex rubra* of each site A,B and C were exposed to UV-light treatments of 30 minutes and 60 minutes. The percentage of red pixel count for each sample image was plotted for all treatments. The different sites are marked with different colors. The same sample is connected with a line between the treatments. The asterisks at the top of the plot indicate the significance level for the treatments.

Experiment 2 – UV-light exposure on red and grey samples separated

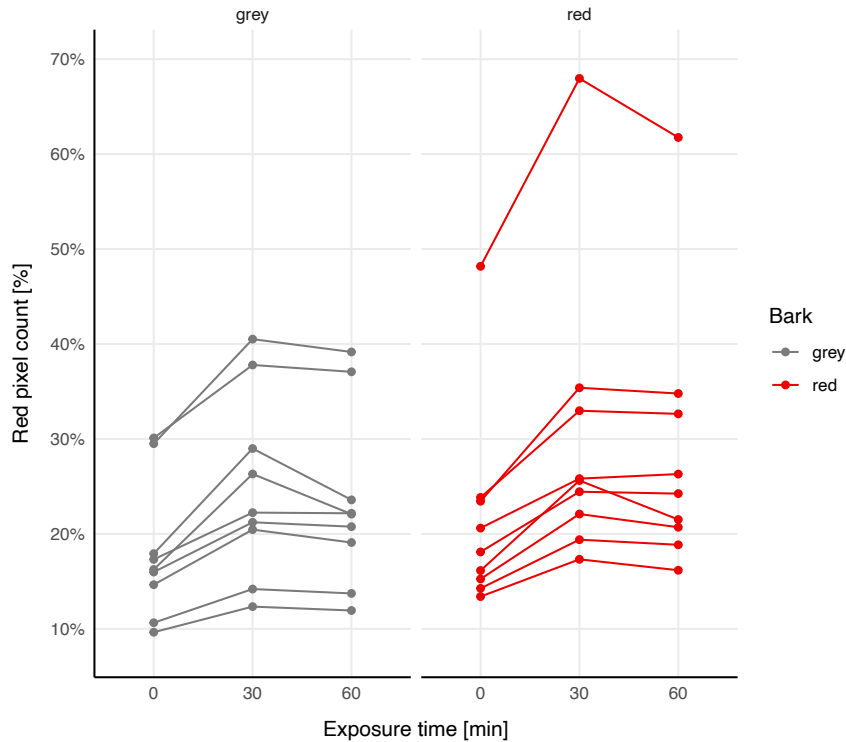


Fig. 19: Nine bark samples with and without *Cortex rubra* from sites A, B and C were exposed to UV-light for 30 and 60 minutes. The percentage of red pixel count for each sample image was plotted for all treatment exposure times. The different bark types are marked with different colors. Grey stands for the samples which did not have *Cortex rubra*. The samples which showed *Cortex rubra* are marked in red. The same sample is connected with a line between the treatments.

Although, over the whole data set a significant effect was measured for the treatments, this effect was only measured significantly for site B when considering the sites separately (Fig. 20).

Experiment 2 – UV-light exposure on separate sites

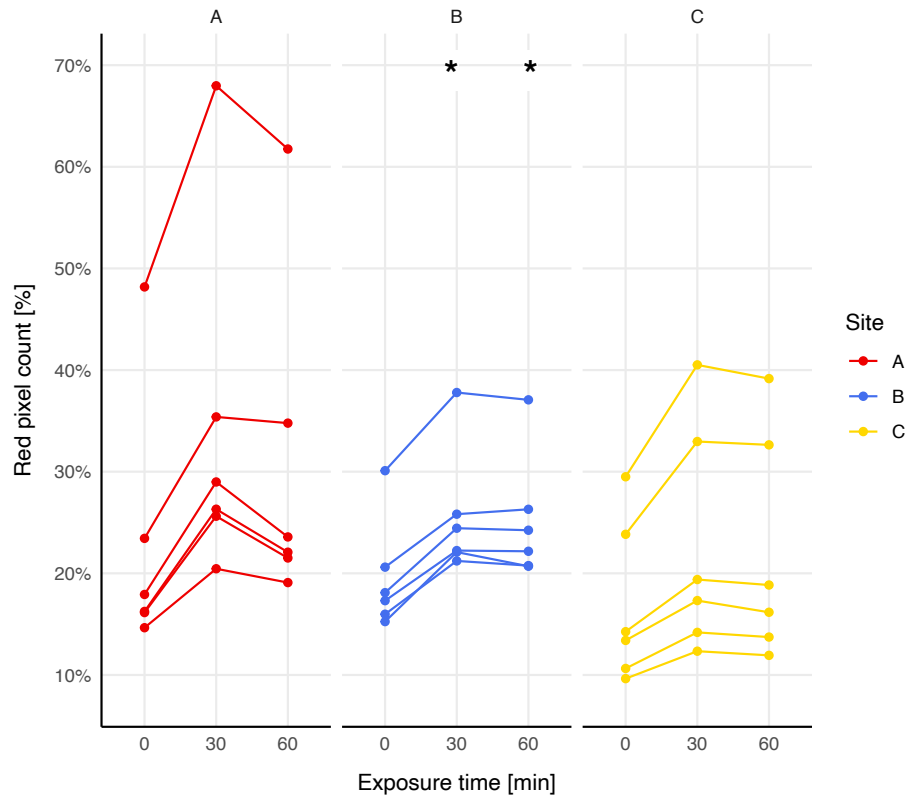


Fig. 20: 18 bark samples with and without *Cortex rubra* of each site A, B and C were exposed to UV-light treatments of 30 minutes and 60 minutes. The percentage of red pixel count for each sample image was plotted for all treatments. The different sites are marked with different colors and were plotted separately. The same sample is connected with a line between the treatments. The asterisks at the top of the plot indicate the significance level for the treatments.

4.3 X-ray fluorescence analysis

The XRF analysis of the 21 milled bark samples yielded a weighted percentage of all trace elements from Na to U. Every element with a mean signature larger than 0.01 was considered in the results. These were ten elements: Na, Mg, Si, P, S, Cl, K, Ca, Mn, Fe (Fig. 21-25). They span a data range of 0.007-5.72 weight percentage. Na had the highest overall mean at 1.84 weight percentage. The elements with the lowest mean values were Mn and Fe at <0.035 weight percentage (Appendix E).

All data for each element with the exception of S were not normally distributed. Eight of the ten elements, with the exceptions of Na and Cl, showed a significant difference between the sites. However, none of the elements showed a significant difference between the bark types, i.e. samples with *Cortex rubra* and bark without *Cortex rubra*.

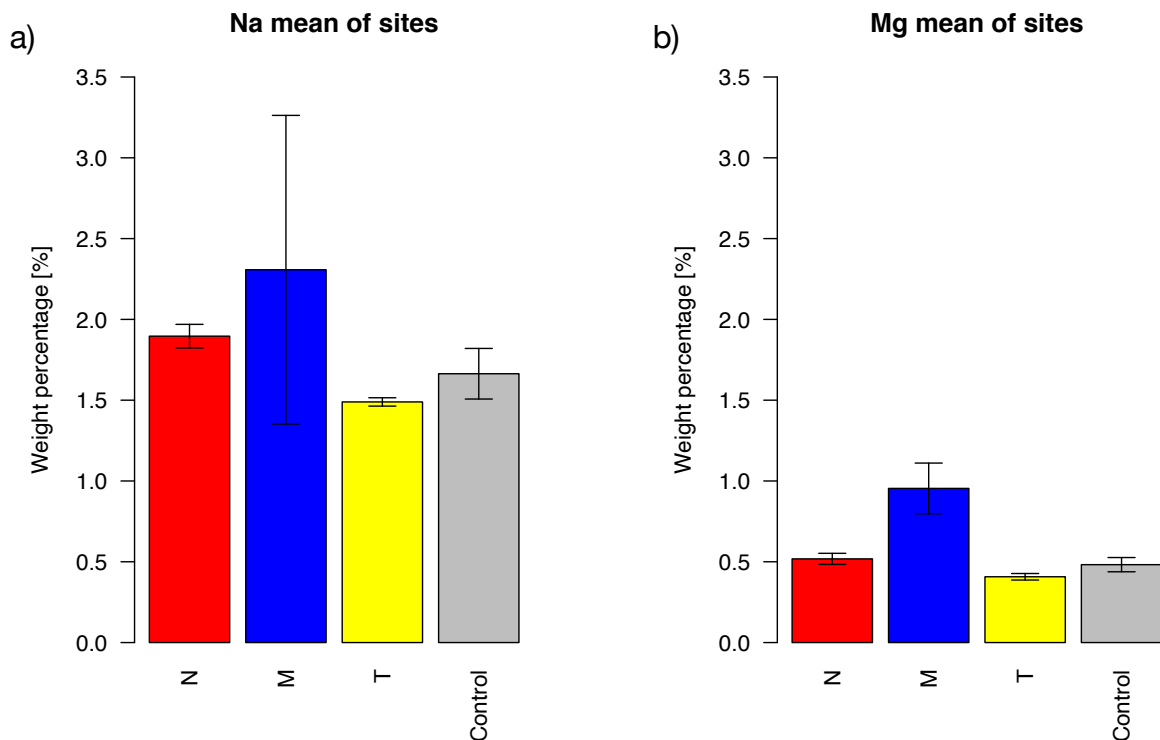


Fig. 21 a) and b): The mean weight percentage of each site for the elements Na (a) and Mg (b) was plotted. The standard error is indicated with vertical black error bars.

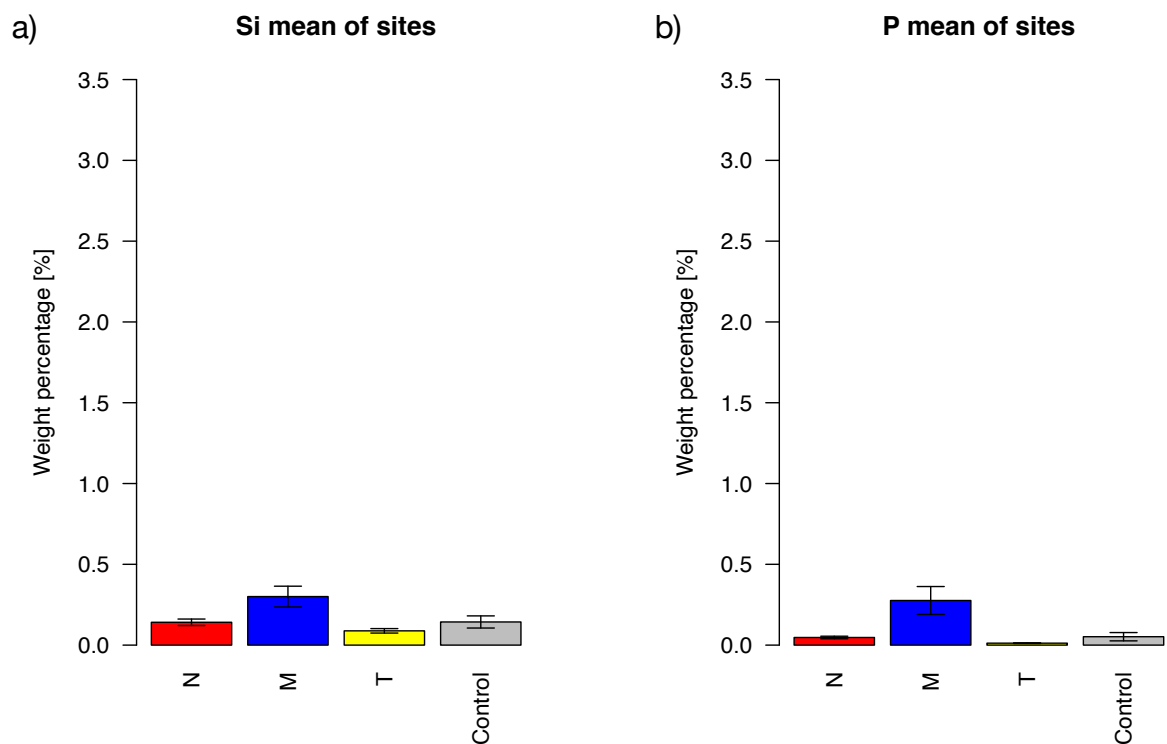


Fig. 22 a) and b): The mean weight percentage of each site for the elements Si (a) and P (b) was plotted. The standard error is indicated with vertical black error bars.

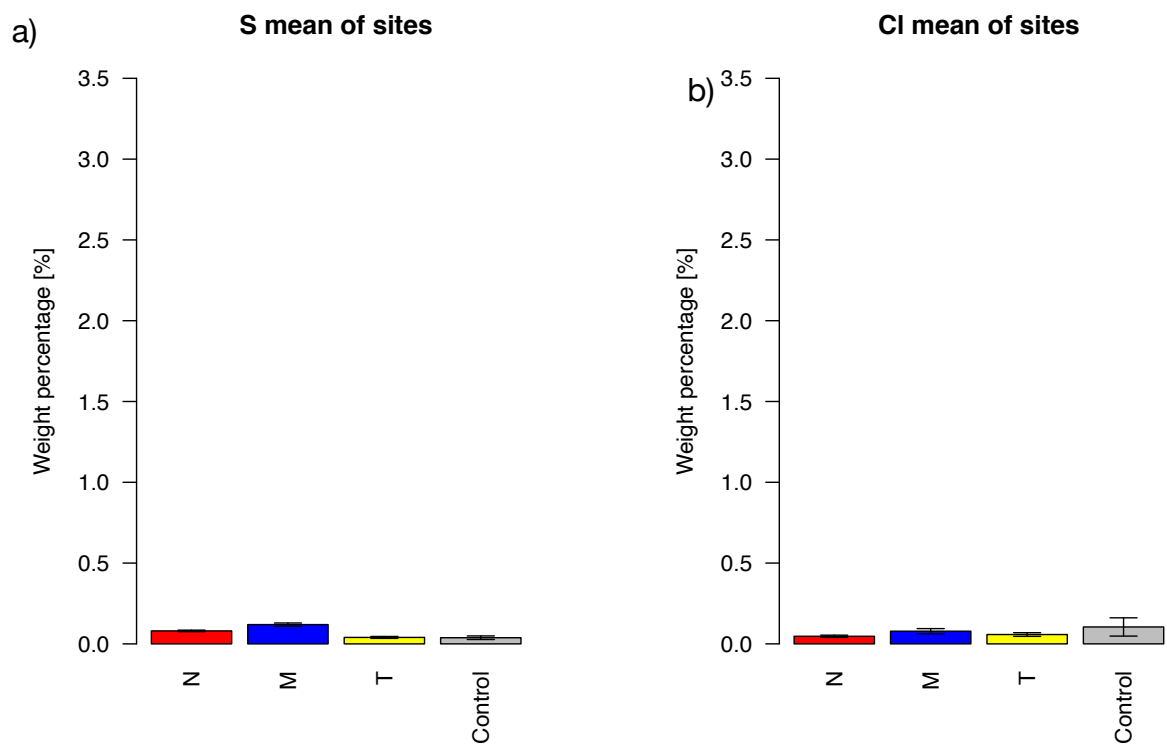


Fig. 23 a) and b): The mean weight percentage of each site for the elements S (a) and Cl (b) was plotted. The standard error is indicated with vertical black error bars.

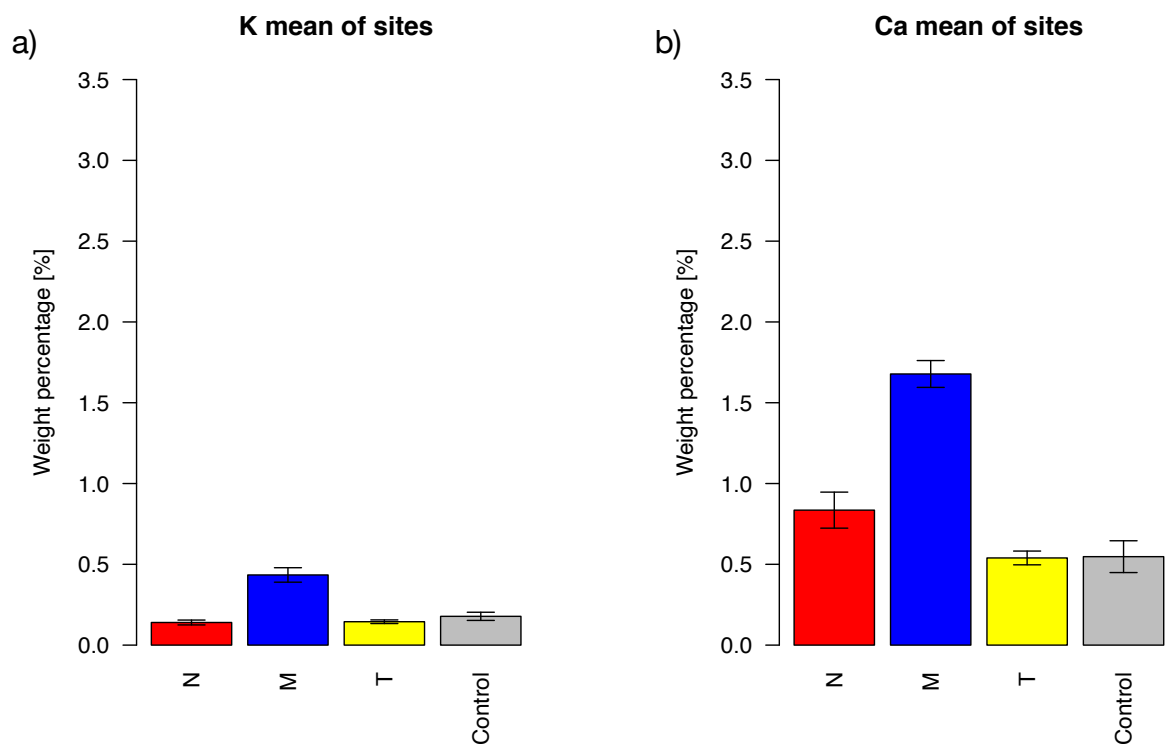


Fig. 24 a) and b): The mean weight percentage of each site for the elements K (a) and Ca (b) was plotted. The standard error is indicated with vertical black error bars.

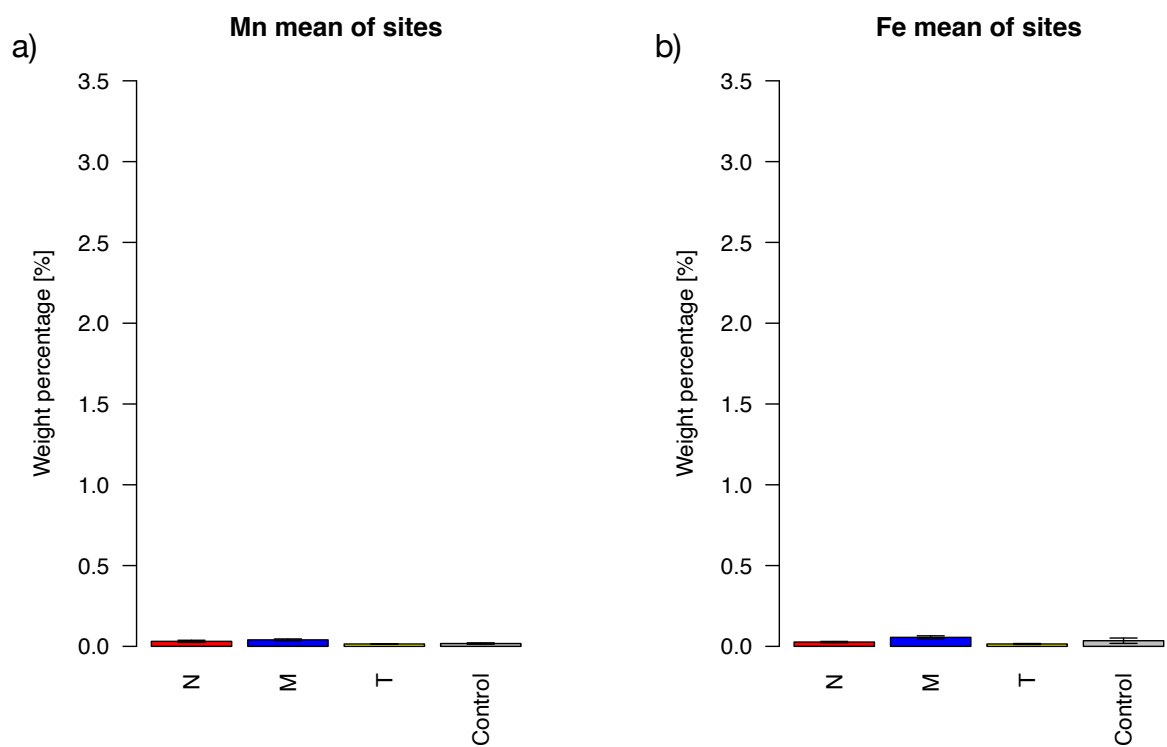


Fig. 25 a) and b): The mean weight percentage of each site for the elements Mn (a) and Fe (b) was plotted. The standard error is indicated with vertical black error bars.

It is even more important to consider the elemental distribution within each site since the sites were found to be significantly different to each other in elemental weight percentage composition for almost all selected elements. Subsequently, site effects should be eradicated in order to identify bark effects. Thus, identifying differences between bark without *Cortex rubra* and bark with *Cortex rubra*. It is important to keep in mind that the sample size for each site separately was small (six samples for sites N, M and T and only three samples for the Control site). Within each site each element was normally distributed, with two exceptions in site M. Therefore, ANOVA could mostly be implemented in the calculations to determine the significance levels.

Firstly, for site N a significant difference was found in the elemental compositions of Na (p-value 0.002 **) and S (p-value 0.044 *) between the bark types (Fig. 26 a)). Secondly, in the samples of site M significant differences between the elemental compositions of Si (p-value 0.016 *), Ca (p-value 0.035 *) and Fe (p-value 0.028 *) were found (Fig. 26 b)). Lastly, the bark was significantly different in the composition of P (p-value 0.039 *) for site T (Fig. 27 a)). In the Control site no significant differences between the elemental compositions of the bark types were detected (Fig. 27 b)).

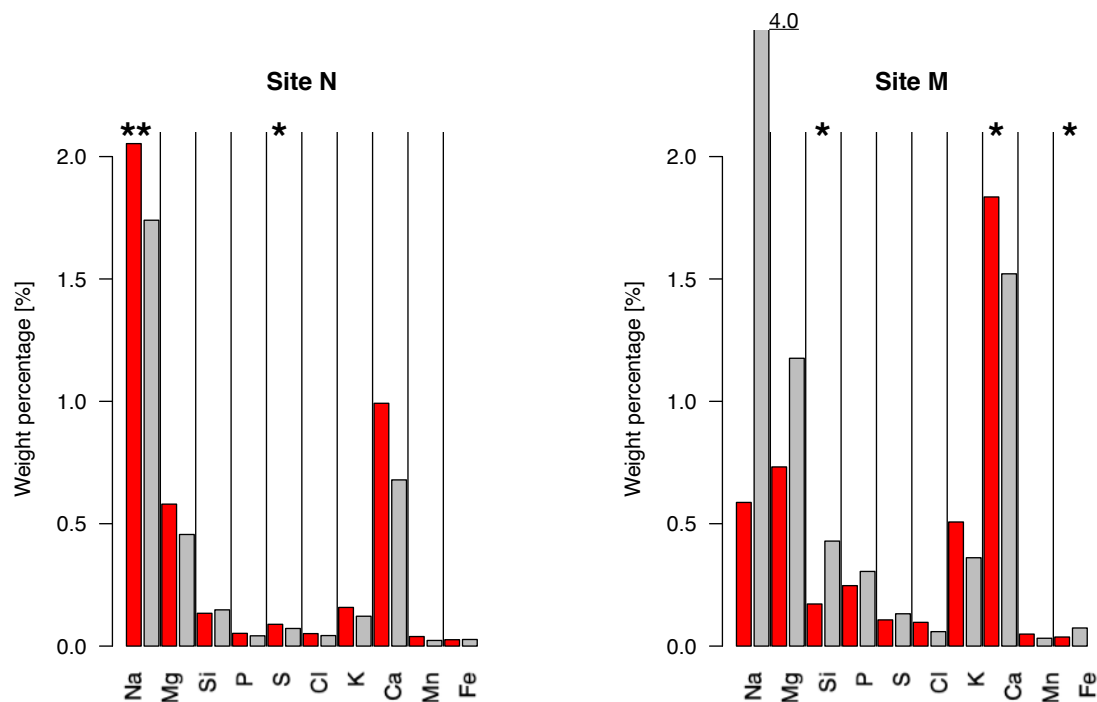


Fig. 26 a) and b): The mean weight percentage of all elements was plotted in two categories. The samples with *Cortex rubra* in red and the samples without *Cortex rubra* in grey. The asterisks indicate the level of significant difference between the red and grey category for the corresponding element. This was done for a) site N and b) site M.

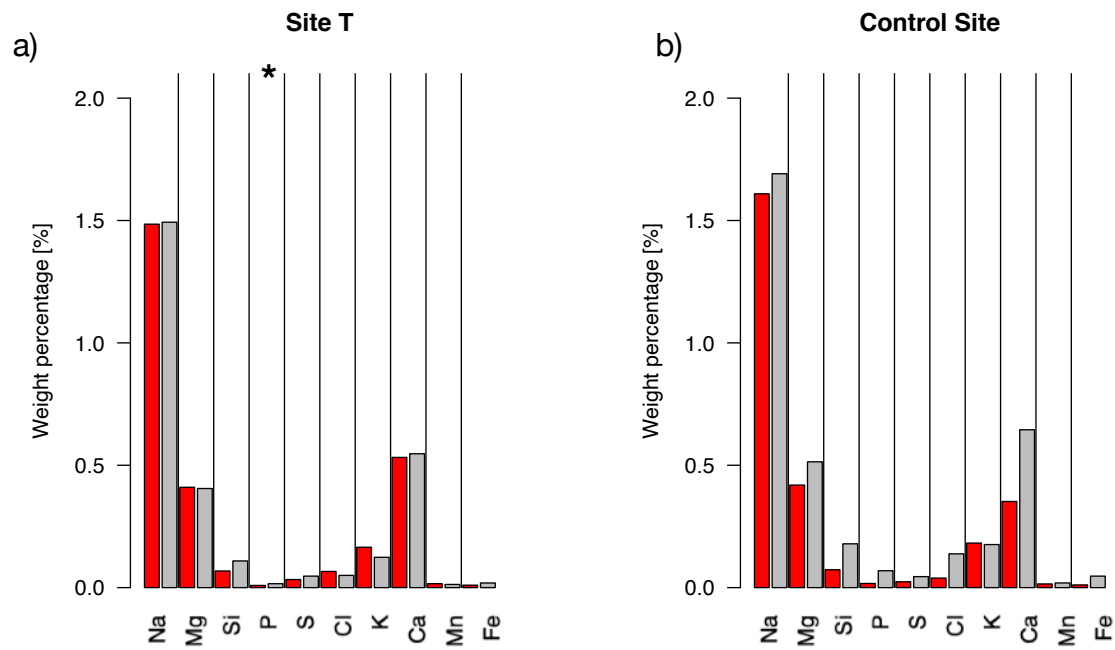


Fig. 27 a) and b): The mean weight percentage of all elements was plotted in two categories. The samples with *Cortex rubra* in red and the samples without *Cortex rubra* in grey. The asterisks indicate the level of significant difference between the red and grey category for the corresponding element. This was done for a) site T and b) Control site.

5. Discussion

In this chapter all results are discussed. The subchapters 5.1 Reaction wood, 5.2 Sunlight exposure and 5.3 X-ray fluorescence analysis focus on one of the main hypotheses each. In the chapter 5.4 *Cortex rubra* the three hypotheses are combined and the main findings summarized. Suggestions for further research in a broader sense are discussed in the equally titled subchapter 5.5.

5.1 Reaction wood

The results from the RW analysis showed that most samples had normal growing wood for samples behind the normal bark as well as for cores taken behind *cortex rubra* bark. Only four cores showed the RW typical round cells (Palombo et al., 2018). The hypothesis was, that RW would occur downslope and cause *Cortex rubra* to also appear downslope due to the additional wood growth underneath. Sites N and M did not have any observable RW in the samples, although, they had steep slopes and RW was, thus, likely to occur (Heinrich et al., 2007). However, considering the schematic maps of these two sites it can be seen, that the cores taken behind *Cortex rubra* bark were seldom facing downslope (Fig 10, 13). Site S was the only very steep site where *Cortex rubra* was often facing downslope and RW was measured in two cores out of 12. However, half of the samples with RW were originating from site T and the Control site, which are both not very steep sites. Site T was the least steep site out of the four probed sites and the Control site was intentionally chosen to be flat. When all samples were considered, there was only the slightest tendency, for RW to be measured more often in cores behind *Cortex rubra* bark than behind normal bark (11% vs 7%). Therefore, RW to be the origin of *Cortex rubra* does not seem likely. The hypothesis is, thus, rejected.

Considering any downslope facing *Cortex rubra*, there is also a core sampling bias to consider. When trees were bent strongly, accessibility of the bent side of the tree was impossible to core with the hand-held increment borer. Therefore, samples were then taken towards the edges of *Cortex rubra* instead, where RW would then occur less prominently as well.

It is worth highlighting the results from the Control site. It was chosen specifically to be on flat ground to reduce the potential for RW. Nevertheless, one core sample was found to have RW. This makes the Control site not fulfill its intended purpose and a new site should have been searched for. However, the tree exhibiting RW did also have *Cortex*

rubra. The other tree at the Control site was without RW and did not exhibit *Cortex rubra* bark. Therefore, the Control site in itself negates the null hypothesis and suggests that *Cortex rubra* does appear only in combination with RW.

There are several explanations why a tree standing on flat ground in the Control site can still form RW. RW can not only occur as a counterbalance to gravity but also as a consequence of wind and light (Moulia et al., 2006). Winds can put extreme pressures on trees (Gardiner, Berry & Moulia, 2016). Through constant one-sided application of the pressure load on big branches the tree has to fight the breaking point by allowing slight rotation in its wood (Gardiner et al., 2014). Extremely wind exposed trees can be found, where the whole stem is formed like a spiral (Skatter & Kučera, 1997). Depending on light availability and the species demand for light, certain trees will grow bent and crooked in order to reach as much sunlight as possible. This phenomenon can be seen in the natural environment and in artificial surroundings. For example many trees growing close to a road in between high buildings will bend towards the center of the road in order to reach sunlight. For the Control site specifically, it is also possible that the flat ground was steeper before people built the small road close by. Additionally, snow piles from the log deposit station close by could be pushed onto the trees causing additional pressure on the stems.

Regardless, it would have been advisable to search for another site on flat ground with larch to get a clear view whether trees without RW growing on flat ground show *cortex rubra* as often as trees growing on slopes. Such sites could have been found further up the valley of Surses, as in the close proximity of Savognin, the larch can generally only be found on slopes and it is seldom for them to grow on flat ground.

5.2 Sunlight exposure

The second hypothesis stated that direct sunlight exposure could change the color of the tree bark. This was examined through three different methods for which the results are discussed in the following three subchapters.

5.2.1 Site maps

As is visible on the schematic maps, *Cortex rubra* was generally more So facing. This is the first indication, that the hypothesis for sunlight as the origin for *Cortex rubra*, is plausible. However, there were several trees not following this trend. Most notable is site M, where only two of the sampled trees showed *Cortex rubra* facing So. Similar results were gained from the sunlight exposed section of the trees, where site M is the big exception once more. This suggests a site specific difference of site M to the other sites. Considering that this site was located on the opposite side of the valley to the other sites and the slope facing 303° W was the most N facing out of all sites, a site specific influence seems plausible. Several studies have been carried out, where the slope direction and position at a site had a significant effect on soil nutrients and plant performance (Brunori et al., 1995, Qiao et al., 2019). Additionally, site M was located at the tree line below a mountain ridge. The mountains were blocking the sunlight hitting the site from the south, casting their shadows onto the site instead. If sunlight is the limiting factor for *Cortex rubra*, site M is, thus, confirming this dependency in the inverse way.

Out of all sampled trees, seven have been observed to show no *Cortex rubra* on their stem. Two of them were slightly exposed to sunlight from the east, while the others were standing fully in the shade. Most of the trees observed without *Cortex rubra* were found on site N. On that site an average of 2.9 neighboring trees within a five meter radius was calculated, indicating a rather dense forest with a lot of shade. Therefore, the correlation between sunlight and *Cortex rubra* is likely and reinforces the hypothesis validity.

Nevertheless, the overlap percentage of sunlight exposed sections onto *Cortex rubra* was only at an average of 56 %. There were even five trees measured, where there was no overlap of the sunlight exposed section with the *Cortex rubra* side of the tree. All data gathered on the orientation of sunlight being able to reach a tree stem is, however, subject to a lot of error potential. No precise measurements could be taken, as the seasonal change of solar radiation due to the solar elevation of the sun had to be estimated. In other words, the summer sun might reach the bark, whereas in winter the

sunlight could be blocked by the surrounding mountains, due to the lower position of the sun in the sky. Additionally, most of the sampled trees were neighboring other larches which are deciduous and, thus, seasonally change the amount of shadow they cast. Even the sampled tree in itself will change the sunlight exposed bark during the year by losing and regrowing the needles. However, the factor of a broadleaved tree changing the shadow cast on the larch through the year at least can be ignored, since all sites were standing in conifer forests. An other error potential lies in the maintenance of the forests. Close to several sites tree stumps were observed. Depending on when these trees were felled, their sudden removal would have induced a big change in sunlight exposure for its neighboring trees (Lindfors & Vuilleumier, 2005; Ophardt & Hummel, 2016). To the knowledge of the local forester the sites examined in this thesis were subject to only few felling and not in recent times. Therefore, this factor could play only a small role in potentially falsifying the measurements taken.

The sunlight reaching the sites varies due to their elevation. This effect is called the altitude effect which states that there is an increase in global irradiance of about 8% per 1000 m elevation (Blumthaler et al., 1997). Regarding *Cortex rubra*, if the hypothesis is true, there would be a higher chance to observe the phenomenon at higher elevations. Site T was located at the lowest altitude at 1288 m a.s.l., site S at height 1493 m a.s.l., site N at 1706 m a.s.l. and site M was the site at the highest altitude at 1943 m a.s.l.. Thus, if site specific differences are neglected, site M experiences a 5% stronger solar radiation than site T. However, the opposite of what could be expected was found in the sites of this thesis. The schematic map of site T shows *Cortex rubra* on all trees, whereas site M has one tree without *Cortex rubra*. Additionally, site N at the second highest location was the site with the most trees without *Cortex rubra*.

There are more factors to be considered for the variation of solar irradiance at the different sites such as the indirect reflection of sunlight on the snow during winter, the air pollution or cloud cover (Blumthaler et al., 1997; Lindfors & Vuilleumier, 2005; Ophardt & Hummel, 2016). However, since the sites were all located in the same valley only negligibly small variation is to be expected. Thus, these factors are not further discussed. Overall, the site maps validate the hypothesis. *Cortex rubra* did occur towards sun exposed sides of the stem or was not observed when trees stood completely in the shadows.

5.2.2 Experiments

5.2.2.1 Heat treatment

The results of the heat treatment showed no significant trends. The expected increase in red pixels, when the samples were exposed to heat, was not detected. When the sites were considered separately (Fig. 18) it could be seen that site A behaved the opposite way to B and C. Samples from site A tended to decrease in red pixel percentage after a three hour heat exposure and increase again after six hours. Site B and C generally experienced an increase after three hours and a decrease after. However, the trends were not significant for any of the sites. Thus, the hypothesis of this thesis is rejected.

If the samples were exposed to heat for a longer time span or at a different temperature it is possible that the results would be different. Since a study on birch bark objects in museums found that storage in temperature and relative humidity controlled environments without exposure to light did lead to color change (Tse et al., 2018). Moreover it has been found that heat changes the color of wood due to a slow and mild thermal oxidation process (Calienzo et al., 2015, Matsuo et al., 2011). Thus, further experiments would have to be performed to confirm whether a trend can be detected in a different setup for larch as well. It would be interesting to know whether and when there is a point in time from which on no change in redness can be detected in comparison to the previous measurement. Additionally, the point in time of maximum red value could be investigated. When varying the temperature, thresholds for effects on overall change in color could be identified, similarly to a study where the fire behavior of tree bark was investigated (Centonze et al., 2003). This would, additionally, help in finding the maximum temperature to detect a change in redness prior to formation of charcoal. Therefore, keeping the error potential of measuring a darkening due to ash formation to a minimum.

It is crucial to keep in mind that the change in temperature could provoke a more physical change in tree bark as well, which was not investigated in this thesis. Varying diurnal temperatures (especially during winter) can lead to expansion and contraction, which could be causing the bark to scale off and reveal the fresh red of the inner bark. This phenomenon is called sunscald and is often observed on young, thin barked and deciduous trees (Ophardt & Hummel, 2016). Therefore, an interplay of freeze-thaw dynamics are another possibility to change bark appearance (Leland et al., 2018; Ophardt & Hummel, 2016; Sperry, Donnelly, & Tyree, 1988). Additionally, the strip bark phenomenon of unidirectional cambial dieback is attributed to a temperature difference of

the warm sunlit side to the colder shaded side which will amplify freeze-thaw dynamics unilaterally (Harvey, 1923; Leland et al., 2018; Ophardt & Hummel, 2016; Sakai, 1966). The cambial dieback is most likely influenced by temporally variable environmental factors, such as climate (Leland et al., 2018). *Cortex rubra* might be exactly the effect of such unilateral temperature and a higher strip bark occurrence on one side of the tree. The phenomenon has been observed to be patchy and mostly unilateral which would fit the unilateral temperature difference well. This could be examined in a further study by measuring bark thickness differences around the tree stem with and without *Cortex rubra*.

Additionally, the phenomenon of sunburn due to high temperature exposure could lead to *Cortex rubra*. Tree bark with sunburn typically first show a reddish-brown discoloration and later completely dry out. With time, the bark will then start to crack and peel off (Ophardt & Hummel, 2016). The discoloration as well as the cracking open of bark fit remarkably well with observations of *Cortex rubra* on typically fissured tree bark on larch. However, since larch bark is characteristically thick when the trees have matured, sunburn would have to occur on young larches when the bark still is thin. In this study *Cortex rubra* was found on trees with thick bark and mature ages, which contradicts the typical occurrence of sunburn. Further research would have to be done to investigate the possibility of sunburn on young let alone mature larches.

5.2.2.2 Ultraviolet light treatment

UV-light exposure of the bark samples showed a significant increase in red pixel percentage. The null hypothesis is, therefore, rejected. The results also show that there is no statistically significant difference for the trends depending on the initial condition of the bark, i.e. bark samples with *Cortex rubra* or without. Thus, bark with *Cortex rubra* is found to still change in hue due to UV-light although it has been affected by it previously. This is not surprising, when assuming the effect of UV radiation on heartwood is similar for bark as well. Heartwood will fade in color the longer it is exposed to light and will keep aging over hundreds of years (Ayadi et al., 2003; Calienno et al., 2015; Cogulet et al., 2016; Park et al., 2022). Additionally, the bleaching effect has been observed for birch bark objects in museums over long time periods (Tse et al., 2018). Although, the effect in that study has been observed on the inside of the birch bark, i.e. close to the cambium, whereas, *Cortex rubra* was observed on the outside of the larch bark. Nevertheless, their findings suggest that even small amounts of UV-light can have a great impact on the birch bark color (Tse et al., 2018). Affirming the results of this thesis.

When the sites were considered separately, there has not been a significant effect of the treatments for all of them. Only site B showed a clear effect. However, the trend is visible in the other two sites as well. Considering that UV radiation gets 9% stronger for every 1000 meters of altitude, a clearer trend would have been expected for sites A and C as they were both located at a higher altitude than site B (Blumthaler et al., 1997). However, the density of the forests and the slope orientation for A and C were less favorable for a strong sunlight exposition (Lindfors & Vuilleumier, 2005). Therefore, the elevation might not have the expected effect and the lower significance levels will be attributed to the small amount of data for the individual sites.

In contrast to the results, it is known that bark as the protective layer does shield the wood from UV-light. There has even been a study using polyphenolic compounds of tree bark to protect heartwood against UV radiation in acrylic-based surface coatings (Grigsby 2017). This proves that bark does offer UV-light protection to itself for the cambium and the wood within. Nevertheless, the results of this thesis indicate that even bark struggles in totally protecting against UV-light, even if bark is as characteristically thick as it is for larch. It would be interesting to investigate the exact amount of UV-light larch bark can shield and how much UV can penetrate through.

Additionally, the UV-light was constant and at specific wavelengths in the UV-C spectrum in this experiment. Natural sunlight, however, radiates several wavelengths of UV-light and more than 95% of the irradiance reaching the Earth's surface fall within the UV-A spectrum (Rai, Morales & Aphalo, 2021). It has also been found that there are variations in UV level over time (Walker, 2009). Changes in the UV level can be attributed to change in the total ozone column, sunshine duration, and variations in the snow cover (Lindfors & Vuilleumier, 2005). Therefore, the effects measured in this thesis can not be directly extrapolated to a real life scenario.

5.3 X-ray fluorescence analysis

The XRF analysis was conducted to investigate the third hypothesis, which states that the elemental composition of bark with *Cortex rubra* could be significantly different to bark without it. The results for the data set over all sites reject this hypothesis. No significant difference was measured between the two bark types for any of the selected elements. However, when the sites were considered independently, certain elements would show a significant difference between the bark types. The elements Na and S varied significantly in site N. Si, Ca and Fe were significantly different in site M. On site T the element P showed a significant difference. Notably neither of the elements varied significantly in more than one site. Therefore, these results could be a type one error and false. After all, the sample size was small within the sites and the method used is designed for denser samples of soil and not commonly used for light weight materials like bark. Nevertheless, a significant difference between the bark types was found for iron in site M, which was predicted in the hypothesis. Iron could possibly change the redness of the bark through oxidation i.e. formation of rust. In order to validate the within site variation, further research would have to be conducted with more samples. Additionally, the error potential of the method has to be checked.

However, the results from the XRF analysis showed that the sites were significantly different from each other in their elemental composition of Mg, Si, P, S, K, Ca, Mn and Fe. The differences might stem from the various soil nutrients available to the trees at each site. A linear relationship between the content of elements in the plant material and their contents in the environment usually exists (Olajire & Ayodele, 2003; Yilmaz et al., 2006). In addition, it has been found that slope direction and position have a significant impact on the soil nutrients (Brunori et al., 1995; Qiao et al., 2019; Water et al., 2002). Since all sites vary in direction and position a variation in available soil nutrients could be possible. The underlying geology of the sites might have an impact on the soil elemental composition as well. Moreover, the sites were located at different distances from the town of Savognin, which might lead to a varying exposure to air pollution. The analysis of elemental composition in tree bark has been found to provide information about the levels of both air and soil pollution in other studies (Catinon et al., 2009, 2012; Chrabąszcz & Mróz, 2017) and has been used for biomonitoring of air pollution worldwide (Bouhila, et al., 2021; Gómez-Arroyo et al., 2018; Kousehlar & Widom, 2019). Therefore, a significant difference between the sites tree bark could be due to variation in soil elements and air pollution. To

validate this finding soil samples could be taken and air pollution measurements collected.

Only elements Na and Cl did not have a significant difference between the sites. This finding could be a type two error. Both the statistical power as well as a measurement error are possible. However, if the result for Na and Cl is correct, the difference of these two elements to the other eight investigated could lie either in the allocation process or the origin of the elements. Interestingly, Na had the highest mean weight percentage of all the selected trace elements. This could suggest ample supply of the element is available for the bark to absorb it equally at all sites. This finding is contradicting several other studies where usually Ca and K were found to be dominant in bark ash from conifers (Lambert 1981; Miranda et al. 2012; Olander & Steenari 1995; Pásztor et al., 2016; Piotrowska et al. 2012; Ragland & Aerts 1991; Wang & Dibdiakova 2014; Young 1971). The high percentage of Na could possibly be attributed to a higher plant demand to satisfy increased photosynthesis. Similar to a study where an enrichment of Mn in the inner bark was found due to long-term elevated CO₂ treatments (Eberhardt et al., 2015). The high content of Na might also be particularly successful in protecting against pathogens. Since it has been found that bark may respond as a selective filter to air pollution, it is also possible that Na was taken up more or the other elements are excluded (Catinon et al., 2009, 2012). Further research is needed to validate these speculations and whether the results are false.

It is crucial to keep in mind that the samples used for the XRF analysis were taken from the fieldwork conducted in May. At that time the trees started sprouting the new needles and shoots for the season. The elemental composition within the bark could, therefore, be affected by the demand of nutrients for the new growth and rebooted photosynthesis. In order to detect seasonal changes in the bark a longtime study would have to be conducted.

5.4 *Cortex rubra*

In general, most of the results in this thesis indicate no effect on the redness change in the tree bark. Hypothesis I. and III. have been rejected. Hypothesis II. was partly rejected. The heat experiment (II. a) showed no significant results. However, the site maps indicated that a correlation of sunlight and the phenomenon could exist. A significant influence on bark color was then found by UV-light exposure (II. b). This suggests that factors such as slope position, orientation and forest density can influence whether *Cortex rubra* appears. The more shade is cast onto the tree stem, the less likely it is to observe a change in bark color. The external influence of UV-light explains why *Cortex rubra* is found on the outermost layers of the bark. This clear result is, however, very outstanding since bark is otherwise deemed to be a challenging material for pattern recognition (Weidenhiller & Denzler, 2014). The discoloration of the bark is not deemed harmful for the trees, although, the effect of extreme exposure to UV-light has to be further investigated. It is possible that the discoloration is only the first reaction and could turn into more severe damage similar to sunburn in thinner barked tree species. If this is found to be the case, changing radiation due to climate change could majorly damage valuable larch wood and *Cortex rubra* would have to be addressed immediately.

It has been discussed that several results have been subject to a low statistical power due to the small sampling size. Additionally, errors in the sampling method and measurements are possible since several of the methods applied were not specifically designed for bark samples. The lack of knowledge of bark anatomy and bark processes as well as applicable methods undeniably increase the error potential. More research would have to be conducted in order to evaluate whether significant errors were made. For example the XRF analysis could be replaced with a calorimetrical estimation of total phenols by Folin-Ciocalteu method (Scalbert et al., 1989).

In addition the effect of UV-light could be further investigated. The age at which bark starts to first show *Cortex rubra* could be determined. Longterm experiments could be done where saplings and young trees are monitored and exposed to various UV-light intensities. Moreover, the ability of bark to recover from exposure could be checked. A study on bark objects in museums showed that the net color change of the samples exposed to intermittent cycling was similar to that of the continuously exposed samples (Tse et al., 2018). The result might be different for bark still on living trees.

5.5 Suggestions for further research

The limited scope of this thesis did not allow to consider more than the three hypotheses. Therefore, several biotic and abiotic factors have not yet been investigated or were only addressed indirectly. Environmental effects of the climate, elevation, soil, water availability and water exposure could be studied. Larches growing in other regions of the alps or the world would have to be considered to determine the influence of several of the unaddressed factors. For example the phenomenon of strip bark has been discussed to possibly affect *Cortex rubra* (Chapter 5.2.2.1). If it does have an effect, it would be important to consider that it has been found to vary with climate (Leland et al., 2018). Thus, observations of strip bark would have to be collected in sites of variable climates. Experiments with increased nitrogen or carbon dioxide have also shown to influence the color of tree bark of other tree species (Günthardt-Goerg et al., 2001). Another factor mentioned is water. Water availability could increase or decrease a rapid change in bark color similar to the influence it has on resin flow (Rissanen et al., 2021). Stemflow patterns of water flowing down the stem may dissolve or oxidise certain components in the tree bark (Catinon et al., 2012) and intensify *Cortex rubra*.

Correlations of the phenomenon with biotic factors could also be explored. The influence of fungi, lichens and pests could be of special interest as they can often be observed on tree bark. Furthermore, seasonal changes within the tree, changes due to age or reactions to mechanical damage could be studied. For example, it is imaginable that *Cortex rubra* could correlate with an increased production in resin. Resin production has been found to vary with several environmental factors (Da Silva Rodrigues-Corrêa et al., 2013; Lombardero et al., 2000) and the changing amount of resin might be able to influence the appearance of the bark.

Comparison with other tree species bark could be of interest. Since UV-light has been found to affect bark color in this thesis, effects like sunscald and sunburn observed on species with thinner bark might be occurring not only due to heat (Ophardt & Hummel, 2016). Or it is also possible that the behavior of gymnosperms like larch react differently to UV-light exposure than the later evolved angiosperms. *Cortex rubra* might be found to present in a different way on other tree species and a more general term for the phenomenon of *Cortex rubra* would have to be defined.

6. Conclusion

According to the results of this thesis, the bark discoloration defined as *Cortex rubra* can be attributed to UV-light exposure. It showed the clearest answer to the question of where *Cortex rubra* originates from. Neither RW nor exposure to heat showed the same effects. Additionally, no significant difference was found in the elemental composition of the bark between the normal more grey bark and *Cortex rubra* bark. Considering the origin for the discoloration lies in sunlight exposition, the location of the larch has a major impact on the appearance of the bark. Factors such as slope orientation, slope steepness, forest density and forest management can change the amount of incoming radiation on the stem of a tree by increasing or decreasing shading. The discoloration of the bark is not deemed harmful for the trees, although, the effect of extreme exposure to UV-light has to be further investigated. It is possible that the discoloration is only the first reaction and could turn into more severe damage similar to sunburn in thinner barked tree species. If this is found to be the case, changing radiation due to climate change could majorly damage valuable larch wood and *Cortex rubra* would have to be mitigated immediately. Therefore, several hypotheses are still to be examined and further research to be conducted in order to validate the results of this thesis. It has been found that research on bark of specific tree species is scarce and it is a field of little scientific knowledge, despite the material being used by humanity for ages. Similar effects on tree bark of species other than the European larch might be found in the future and put the results of this thesis into perspective.

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Appendix

A. Fieldwork in January, 2022 - raw data

Table 1 A: Raw data collected in fieldwork during January, 2022. Six trees of sites A, B, and C were measured and documented.

Site	Tree	Cortex rubra ° Azimuth	BHD [cm]	Bark thickness [cm]	Shade [%]	Growth form straight, slightly crooked, bent
A	1	130	70	7	0	slightly crooked
A	2	125	45	4	50	straight
A	3	140	77	8	70	straight
A	4	135	61	7	10	straight
A	5	160	69	7	60	straight
A	6	115	70	8	50	bent
B	1	255	81	7	0	straight
B	2	255	74	8	0	straight
B	3	260	76	7	10	straight
B	4	250	57	6	10	straight
B	5	250	61	7	0	straight
B	6	245	59	6	20	straight
C	1	250	49	2	0	straight
C	2	245	59	3	0	straight
C	3	245	37	2	0	straight
C	4	240	58	3	0	bent
C	5	230	45	3	80	bent
C	6	220	77	3	60	straight

B. Fieldwork in May, 2022 - raw data

Table 2 A: Raw data collected in fieldwork during May, 2022. Ten trees of sites N, S, T, M, and Control were measured and documented.

Site	Tree	m a.s.l.	Cortex rubra ° Degrees	Height cortex rubra [m]	slope inclination [%]	slope orientation ° Degrees	BHD [cm]	Bark thickness [cm]	Shade ° Degrees	5 m radius	Growth form
N	1	1711	95-205	0-10	20	195	55	6	217-175	4	slightly crooked, bent
N	2	1710	152-260	0-10	21	195	76.5	10	270-150	1	bent (S)
N	3	1708	n	n	28	20	62	7	0-360	4	slightly crooked (NW)
N	4	1711	144-223	0-12	28	20	55.5	8	212-156	3	straight
N	5	1710	149-260	0-14	12	300	76.5	7	300-180	4	straight
N	6	1710	105-220	0-13	20	232	60	8	132-184	4	bent €
N	7	1706	n	n	17	320	72	7	0-360	2	straight
N	8	1707	n	n	32	292	82	10	188-135	2	straight
N	9	1692	124-270	0-12	30	290	67.5	7	316-111	2	straight
N	10	1700	n	n	30	290	80	10	0-360	3	slightly crooked (W)
Mean N		1706.5			23.8	215.4	68.7	8		2.9	
S	1	1475	136-277	0-13	18	180	79.5	20	303-120	1	straight
S	2	1498	56-166	0-8	41	60	72	15	173-94	3	straight
S	3	1494	192-283	0-8	45	288	83	25	156-217, 29	3	bent (SE)
S	4	1506	158-230	0-16	33	203	80	10	227-164	1	straight
S	5	1504	124-139	0-18	24	180	96	25	176-48	2	bent (S)
S	6	1500	n	n	17	201	56	7	0-360	3	straight
S	7	1500	120-215	0-14	15	212	72	8	159-58	4	straight
S	8	1486	253-304	0-18	11	244	65	10	260-99	2	straight
S	9	1486	137-250	0-16	13	205	64.5	6	240-90	1	straight
S	10	1482	128-232	0-12	16	230	75.5	7	183-79	4	bent (S)
Mean S		1493.1			23.3	200	74.35	13.3		2.4	
T	1	1290	162-255	0-12	4	255	76.5	10	185-241	1	straight
T	2	1290	155-212	0-12	4	280	72	7	242-68	1	straight
T	3	1290	154-257	0-10	6	290	90	15	161-193	0	straight
T	4	1289	132-185	0-4	3	225	45.5	4	194-14	3	straight
T	5	1289	146-240	0-15	3	225	79.5	7	170-209	3	straight
T	6	1288	156-253	0-11	4	228	77	10	202-92	2	straight
T	7	1286	128-221	0-11	7	214	62.5	5	134-208	1	straight
T	8	1287	153-282	0-7	12	320	61	3	194-227	2	straight
T	9	1286	129-247	0-13	24	255	58	4	208-350	3	straight
T	10	1286	177-232	0-14	28	240	77	5	165-95	2	straight
Mean T		1288.1			9.5	253.2	69.9	7		1.8	
M	1	1946	257-64	0-4	17	295	41.5	3	146-350	4	straight
M	2	1946	192-294	0-8	17	286	31	2	265-153	4	bent
M	3	1942	294-83	0-10	7	300	25.5	0.4	72-249	4	bent (SE)
M	4	1941	191-335	0-14	7	300	65	4	20-210	3	straight
M	5	1941	157-300	0-4	11	300	38	1	125-67	4	bent
M	6	1941	262-356	0-8	22	310	31	1	213-95	5	bent (W)
M	7	1942	262-8	0-11	14	311	39.5	1.5	138-17	6	straight
M	8	1943	229-0	0-10	12	310	34	1	113-2	4	straight
M	9	1944	183-289	0-2.5	12	310	26	0.5	140-180 & 2	5	straight
M	10	1945	n	n	3	310	29	0.5	128-30	4	straight
Mean M		1943.1			12.2	303.2	36.05	1.49		4.3	
Control	1	1320	n	n	0	n	22	0.5	all	2	straight
Control	2	1321	43-120	0-6	0	n	39.5	2	118-66	0	straight
Mean Control		1320.5			0		30.75	1.25		1	

C. Examples of bark micro cut scans

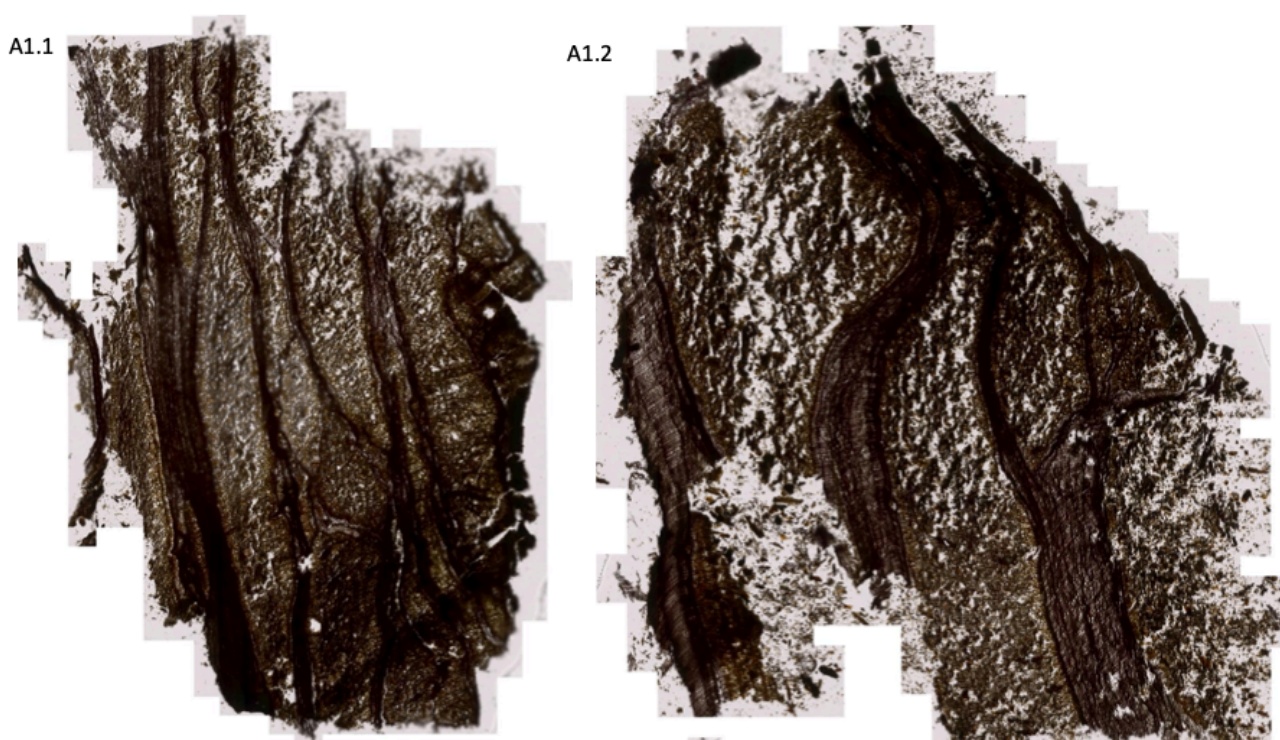


Fig. 1 A: Scans of samples A1.1 and A1.2. The bark flake structure is visible. The sample was either too thick to observe single cells and/or the integrity was damaged (Silja Holdenr, 2022).

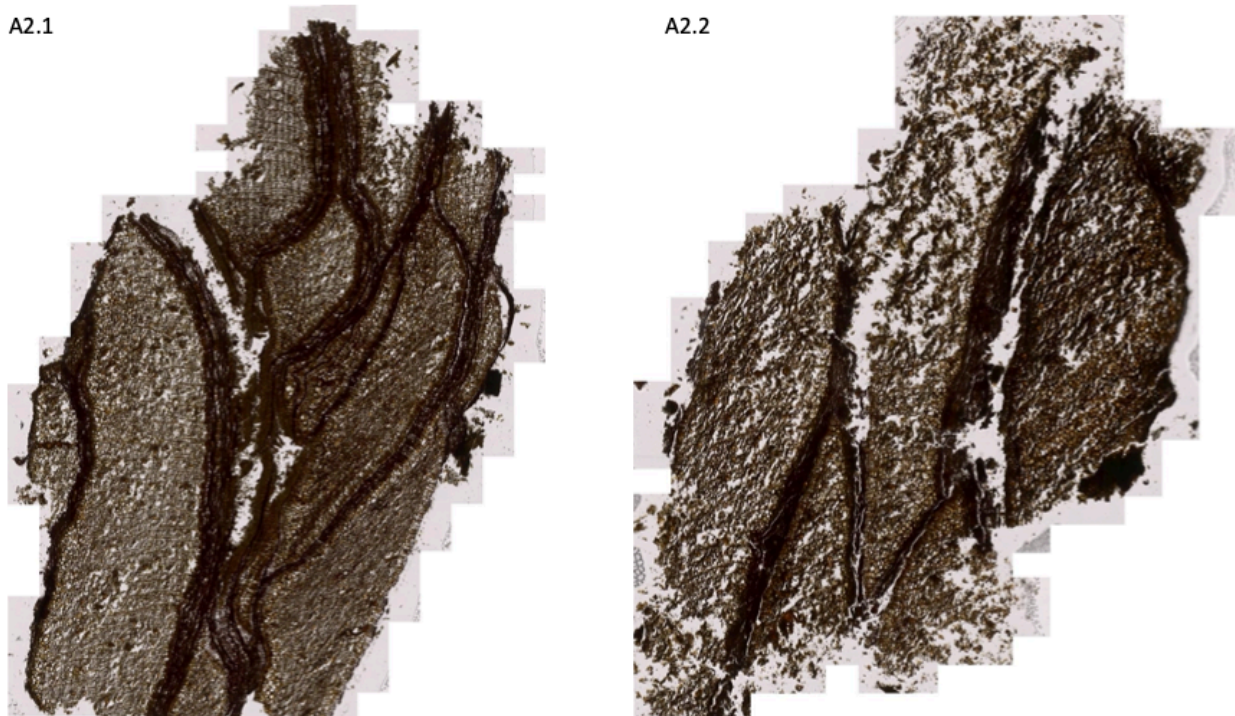


Fig. 2 A: Scans of samples A2.1 and A2.2. The bark flake structure is visible. The sample was either too thick to observe single cells and/or the integrity was damaged (Silja Holdenr, 2022).

D. Reaction wood in samples



Fig. 3 A: Sample Control 2G showing extreme RW. The cell walls are very thick and a lot of space between the cells is visible (Silja Holdenr, 2022).

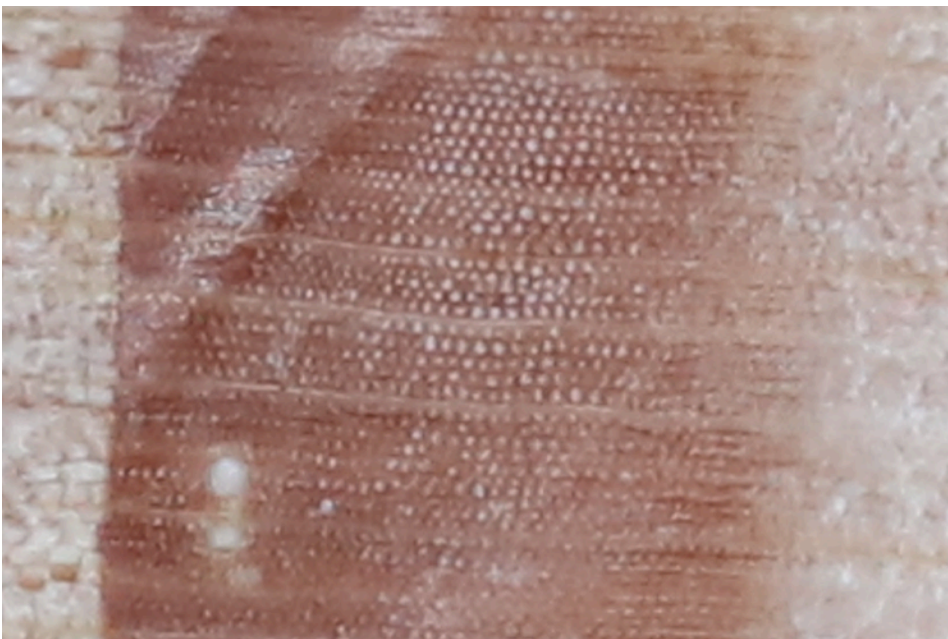


Fig. 4 A: Sample S 8R showing RW. The cell walls are thick and there is space visible between the cells (Silja Holdenr, 2022).

E. XRF data

Table 3 A: Raw XRF data in weight percentage for all ten examined elements (Na, Mg, Si, P, S, Cl, K, Ca, Mn, Fe) and their total mean.

Sample	Site	r_g	Na	Mg	Si	P	S	Cl	K	Ca	Mn	Fe
N5 red	N	red	1.997	0.526	0.1389	0.03815	0.08385	0.03001	0.1523	0.7799	0.03296	0.02752
N5 grey	N	grey	1.719	0.3983	0.1516	0.03086	0.06701	0.02297	0.0909	0.5266	0.01917	0.02736
N4 red	N	red	2.144	0.643	0.1639	0.08357	0.08596	0.06119	0.1241	1.026	0.02203	0.03237
N4 grey	N	grey	1.756	0.4721	0.2159	0.04403	0.08052	0.04756	0.118	0.516	0.01728	0.04029
N6 red	N	red	2.017	0.57	0.1001	0.03424	0.09636	0.06112	0.1964	1.169	0.06136	0.01925
N6 grey	N	grey	1.744	0.4984	0.0775	0.05209	0.06875	0.05931	0.1582	0.994	0.03393	0.01361
M4 red	M	red	0.636	0.815	0.182	0.524	0.1116	0.0947	0.4893	1.876	0.05814	0.03774
M4 grey	M	grey	5.72	1.515	0.3818	0.5622	0.1368	0.1007	0.4581	1.402	0.04106	0.05623
M1 red	M	red	0.542	0.674	0.1594	0.07916	0.1059	0.06695	0.4709	1.947	0.05482	0.03569
M1 grey	M	grey	1.553	0.646	0.3499	0.1343	0.09798	0.01697	0.2442	1.576	0.03011	0.07221
M8 red	M	red	0.584	0.708	0.174	0.1389	0.1038	0.1303	0.5617	1.681	0.03436	0.03823
M8 grey	M	grey	4.81	1.366	0.5555	0.2179	0.1608	0.0608	0.3808	1.586	0.02343	0.09382
T7 red	T	red	1.542	0.4298	0.0577	0.00719	0.02232	0.0865	0.1849	0.4509	0.01908	0.00669
T7 grey	T	grey	1.491	0.4661	0.1525	0.01709	0.0486	0.07036	0.1279	0.4852	0.01837	0.02546
T4 red	T	red	1.429	0.3568	0.06446	0.00757	0.03324	0.04314	0.1655	0.5735	0.01771	0.00967
T4 grey	T	grey	1.576	0.409	0.0792	0.01174	0.0305	0.07041	0.1388	0.7163	0.00816	0.01182
T1 red	T	red	1.485	0.4419	0.081	0.01113	0.04234	0.06728	0.1452	0.5723	0.01207	0.01431
T1 grey	T	grey	1.412	0.3398	0.0948	0.01781	0.06282	0.00891	0.1062	0.439	0.01234	0.0205
Control 1	CO	grey	1.424	0.4604	0.1559	0.1022	0.03032	0.2172	0.22	0.6245	0.01203	0.02668
Control 2 red	CO	red	1.609	0.419	0.0727	0.01737	0.02414	0.03876	0.1818	0.352	0.01514	0.01131
Control 2 grey	CO	grey	1.958	0.567	0.2012	0.03653	0.06015	0.05804	0.1326	0.6657	0.02569	0.06713
mean			1.864	0.606	0.172	0.103	0.074	0.067	0.231	0.950	0.027	0.033

Declaration of Originality

Personal declaration: I hereby declare that the submitted Thesis is the result of my own, independent work. All external sources are explicitly acknowledged in the Thesis.

A handwritten signature in black ink, appearing to read 'Silja Holdener', with a stylized flourish at the end.

Silja Holdener

Schwyz, 27.01.2023