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Wood anatomical analysis of Swiss willow (*Salix helvetica*) shrubs growing on creeping mountain permafrost

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Abstract

Permafrost and related landforms (rockglaciers) are widespread phenomena in alpine
geosystems. In the context of changing environments due to the significant warming,
there is a need for thoroughly monitoring and analyzing the complex responses of these
cryospheric geosystems. Here, the first-time application of wood anatomical methods in
this context is presented in order to investigate whether rockglacier movement is
reflected in varying cell structures of plants growing on top of the rockglaciers.

In order to determine the influence of ground movements (by permafrost creep) and
their influence on the conductive elements within roots of plants, wood-samples were
taken from active and inactive rockglaciers in the Turtmann Valley, southern Swiss
Alps. Since the occurrence of trees is limited altitudinally, the investigation was
restricted to Swiss willow shrubs (Salix helvetica) frequently growing in permafrost
areas above the timberline in the European Alps. This rather new approach concentrates
on general vessel size differences as a result of mechanical stresses. The comparison of
vessel sizes in roots of Swiss willow shrubs growing on active and inactive permafrost
bodies depicts differences within the roots, which are related to the activity status of the
respective rockglacier creep.

Keywords: Dendroecology, rockglacier activity, vessel size, roots

Introduction

Permafrost and related landforms (e.g., rockglaciers) are widespread phenomena in
many high mountain geosystems (Barsch, 1992). In the context of changing
environments due to the significant warming, there is a need for thoroughly monitoring
and analyzing the sensitivity and the complex response of the considered geosystems.
Various methods like geomorphological mapping, terrestrial geodetic survey as well as photogrammetric analyses have been applied in order to assess the activity of rockglaciers and to quantify their movements (Roer et al., 2005a; Roer and Nyenhuis, 2007). In general, the activity of rockglaciers is classified by their ice content and flow behavior (Haeberli, 1985; Barsch, 1996). Hence, active rockglaciers contain ice and move with at least 0.1 m/a, whereas inactive landforms still contain ice but actually do not move. Relict rockglaciers indicate former permafrost conditions; they lack the ice and definitely stopped moving (often several hundred to thousand years ago) (Frauenfelder et al., 2001).

In addition to these techniques, a new approach has recently been designed which assesses rockglacier activities by means of dendrogeomorphological methods combined with wood anatomical analyses (Roer, 2007). In recent years, annual rings of several shrub species in high arctic and tundra areas proved to be feasible indicators for environmental change (Hallinger et al., 2010), but comparable studies have hardly ever been conducted in alpine areas. Particularly with regard to rockglacier studies, dendrogeomorphological methods have rarely been applied. This is mainly due to the fact that the vegetation cover on rockglacier surfaces is generally sparse, since the surfaces mostly consist of coarse and blocky material (e.g., Giardino et al., 1984; Roer and Nyenhuis, 2007). Thus, on active rockglaciers herbs and small shrubs are mostly restricted to areas with fine material usually found at the lobe fronts and on ridges.

reactions. These reactions include not only variations in tree-ring width (e.g., sudden reduction in growth), but also changes in the structure of the cells, such as the development of compression or tension wood (Fantucci and McCord, 1995; Heinrich and Gärtner, 2008) caused by the tilting of the stem. So far, various dendrogeomorphological approaches have been used in reconstructing erosion rates (e.g., LaMarche, 1968; Gärtner et al., 2001; Gärtner, 2007b), enlargements of cracks at the Questa-scarp (Sahling et al., 2003), in determining frequency and magnitudes of debris flow events (e.g., Strunk, 1991; Gärtner et al., 2003; Sorg et al., 2010), in identifying landslides (e.g., Paolini et al., 2005) or dating flood-plain sedimentation (Friedman et al., 2005). Annual ring-width variations and related growth variations due to environmental stress have rarely been studied in shrubs (e.g., Gers et al., 2001). A comprehensive overview on growth rings in herbs and shrubs has recently been presented by Schweingruber and Poschlod (2005). However, only a few publications concentrate on the combination of shrubs and geomorphic processes (e.g., Owczarek 2010), the ability to build tree-ring chronologies and to reconstruct temperature changes (Bär et al., 2008). In general, recent studies on wood anatomical structures in tree and shrub rings have demonstrated the great potential of wood anatomical investigations (Copenheaver et al., 2010; Fonti et al., 2010; Roer et al., 2007). Regarding dendrogeomorphological studies on periglacial processes, Zoltai (1975) was the first to report different phases of activity related to gelifluction in the Subarctic. The conclusions were based on the analysis of reaction wood in spruce (*Picea mariana, Picea glauca*) and larch (*Larix laricina*) trees. Giardino et al. (1984) studied reaction wood, tree-ring width variations and tilting of 283 trees on a rockglacier complex and revealed different periods of movement since the 15th century. Jakob (1995) monitored dwarf-shrubs in the Canadian Arctic, which had been run over by gelifluction lobes. He was able to quantify movement rates of 1.9 to 3.5 cm/a. Bachrach et al. (2004) used
dendrogeomorphological methods, to document the long-term development of a rock glacier in Alberta, Canada. In this case, trees (*Picea engelmannii* and *Abies lasiocarpa*) were buried by an advancing rock glacier. By comparing the death-dates of different trees, a front advance of 1.6 cm/a was estimated. More recently, Körner and Hoch (2006) investigated the dwarfing of trees growing on low elevation permafrost islands concluding that the temperature of the root zone most dominantly determined the growth of these trees. Moreover, there is evidence that changes of the direct environment of roots (e.g., soil pressure, mechanical stress or temperature) result in a variation of their anatomical structure (Gärtner et al., 2001; Gärtner 2007b).

At the site investigated here (Turtmann Valley, Swiss Alps), different techniques have been used to quantify rock glacier movements (Roer, 2007; Roer et al., 2005a). While terrestrial geodetic survey and digital photogrammetry are standard techniques for this purpose (cf., Kääb et al., 2005), wood anatomical techniques have never been applied in this regard, but have the potential to supply additional information (Filion and Gärtner, 2010; Gärtner and Heinrich, 2010). Therefore, the main purpose of this study is to demonstrate the usefulness of wood anatomical analyses especially of roots of alpine shrubs for detecting ground movements resulting from permafrost creep. The key question is whether the movements due to rock glacier activity are reflected in the anatomical structure of the shrubs, thus allowing a long-term monitoring as well as a better understanding of the particular conditions in which accelerated movements of rock glaciers occur.

The investigation focuses on wood-anatomical features in the xylem of shrubs, more precisely the conduction tissue (vessels) in their roots, which have not been examined in this regard yet neither in the given context nor for the selected species. The resulting data are interpreted and verified in relation to information on rock glacier movement derived from digital photogrammetric analyses (Roer et al., 2005a, b). The verification
will help to judge whether wood anatomical techniques can be applied to periglacial investigations and hence established as an innovative approach in this field of research.

Study area

The sampling of shrubs on rockglaciers was conducted in the Turtmann valley, located south of the River Rhone in southern Switzerland (7° 38’ E, 46° 13’ N) (Fig. 3). Due to its inner-alpine location, the area is characterized by an intramontane climate with an annual precipitation rate of 600-900 mm at c. 2000 m a.s.l. and a 0°C isotherm of the mean annual air temperature (MAAT) at c. 2550 m a.s.l. (Van Tatenhove and Dikau, 1990). The timberline is situated at 2200 m a.s.l., while the treeline, which is affected by grazing during the summer months, runs at 2400 m a.s.l. The geomorphology of the Turtmann valley is dominated by several hanging valleys and two big glaciers. In addition, a multitude of periglacial landforms such as gelifluction lobes, ploughing boulders, and rockglaciers covering 4.2% of the total area of 110 km$^2$ are found above the shoulders of the glacial trough. Rockglaciers occur in different states of activity and with varying degrees of vegetation cover (Roer and Nyenhuis, 2007), thus making them suitable for investigations by dendroecological methods.

The active and inactive rockglaciers from which the samples were taken are situated next to each other (Fig. 4). Apart from the activity conditions, other factors influencing plant growth (e.g., altitude, aspect, slope angle, etc.) are similar at the two sites (Roer, 2007). The active rockglacier lobe creeps with extraordinary high velocities and indicates instability at its terminus (Roer, 2007; Kääb et al., 2005, 2007). At the location of the sampled shrubs (Fig. 4), horizontal velocities between 0.5 and 2 m/a have been measured (Roer, 2007).

Material and Methods
The study focuses on analyzing vessel lumen area (VLA) within the diffuse to semi-
ring-porous annual rings of Swiss willow (*Salix helvetica*) shrubs. Due to the fact that
we focus on activity stages of rockglaciers and with this also on permafrost related
movements of the ground, we concentrate on the roots of these shrubs, which are
directly influenced by low ground temperatures as well as by mechanical stress exerted
to the roots. The vessel sizes within the roots of these shrubs were quantified and
analyzed ecophysiologically. In doing so we were not concentrating on single rings but
on the overall vessel differences of individual shrub roots.

**Measurement design**

During various field campaigns, Swiss willow shrubs growing on an active and an
adjacent inactive rockglacier in the Turtmann valley were sampled (cf., Roer, 2007).
The inactive rockglacier was not affected by creep during the last 30 years and is known
from an outcrop to contain a frozen core (Broccard, 1998; Roer, 2007). This strategy
ensured that all shrubs sampled for the study were affected by the same climatic
conditions and were growing on comparable substrates, both having ice in the
subsurface. Consequently, the main ecological difference at the two sampling sites was
the mechanical stress due to ground movements influencing only the shrubs growing on
the active rockglacier. For that reason, the inactive rockglacier was regarded as the
reference site and respective samples from the inactive rockglacier were treated as a
reference to demonstrate differences to samples taken from the active rockglacier.

In general, reaction wood in shrubs is difficult to investigate due to the lack of a main
stem. The single branches (or stems) of shrubs often show reaction wood which might
be caused by different mechanical stress factors, such as snow cover and wind exposure.
In contrast, the root collar and especially the roots of shrubs grow under more stable
conditions, i.e., in the soil, and hence they may be suited best for the investigation of
possible influences of ground movements envisaged in this study. Changes in the root environment may lead to growth stress and result in changes of the anatomical structure (Gärtner et al., 2001).

In order to study these potential changes, entire specimen of Swiss willow shrubs were taken from an active rockglacier lobe (n = 10) as well as from a reference site, an adjacent inactive rockglacier (n = 10), including their entire root systems (Fig. 1).

In the laboratory, several discs were taken from the individual stems and roots of the shrubs at a distance of about 2 cm from the root collar. The small size of most roots (diameters of ~1.5 cm) enabled the preparation of micro-sections (thickness ~15 μm) of whole cross-sections (Fig. 2) using a sledge microtome GSL1. These micro-sections were stained with Safranin and Astrablue to distinguish between lignified (Safranin) and non-lignified (Astrablue) parts of the rings and to obtain a better contrast for the following image analysis procedure. For dehydration, the samples were rinsed with alcohol, immersed in Xylol, embedded in Canada-Balsam and oven-dried at 60° C for about 24 hours (Gärtner et al., 2001).

The resulting microslides were then placed under a microscope and digital photos, with 400 times magnification, were taken. The anatomical structure of the rings was clearly visible, which is not always the case in deciduous plants (Fig. 2).

The micro photos were then used to measure vessel sizes of stressed (active rockglacier) and unstressed (inactive rockglacier) reference samples using the image analysis program WinCELL (Regent Instruments, Canada). This program automatically measures cell dimensions, that is, radial length, width and area of individual vessel lumen. To realize an automated measurement of the vessel lumen area (VLA), a filter which excluded all cells with an area smaller than 200 μm² was applied. These cells were classified as fiber cells, that is, cells of the ground tissue of the rings. All cells larger than 200 μm² were classified as vessels.
For an a posteriori examination of the variation of the VLA-values between the single roots as well as regarding their site specific variations, box-whisker plots were created (Heinrich et al., 2007). Box-whisker plots show some important features, that is, the box in the center spans the quartiles, limited by the 25% and 75% quartiles; the horizontal line in the center marks the median; the vertical lines extending out from the box are capped by horizontal lines often called whiskers indicating certain minima and maxima. In general, box-plots allow an easy comparison of the mean values and the spread of the data. Two data sets are considered significantly different if the boundary of one box does not overlap the median of the other box (Cleveland, 1994).

An additional focus was set on analyzing the variation of the biggest vessels occurring in the roots of the single shrubs. This was done because it is known for several tree species, that the variation of the biggest vessels in single annual rings show high correlations to summer temperatures (Garcia-Gonzalez and Fonti, 2006) or other environmental factors as floodings (George et al., 2002) or forest fires (Cames et al., 2011).

**Results**

A simple comparison of ring-width variations did not show any obvious differences between unstressed and stressed samples from the inactive and active rockglacier lobe, respectively. This also applies to variations in the structure of fiber cells formed mainly to stabilize the plant. However, a basic visual comparison of the vessel sizes between individual roots of stressed and unstressed samples revealed clear differences (Fig. 5). The vessels in roots from the active rockglacier tend to be smaller than those from the inactive site.

To support this visual impression, the vessel sizes in all micro-sections were measured. Although there is a certain variability of the vessel lumen area (VLA) within and
between the single roots, there is a distinct difference when comparing the VLA of single roots as well as of the single shrubs growing on the active and inactive rockglacier (Fig. 6). The overall average VLA values for roots were 2.6 times higher in shrubs growing on the inactive rockglacier (1441 µm^2) than on the active rockglacier (554 µm^2).

This rather simple analysis does show an obvious difference in the VLA values of the two sites. On average, 659 vessels were measured per micro-section (average numbers: active - 602; inactive – 716).

The box-plot results confirm the visual impressions (Fig. 7); although vessels sizes are reduced in stressed roots on the active rockglacier compared to undisturbed roots on the inactive lobe, these results are barely significant (lower diagram in Figure 7). Nevertheless, the median for vessel sizes in roots of all plants from the active part is on average 60% lower than the value in roots of the inactive rockglacier. Moreover, the spread of the data around the median values, the maximum values as well as the outliers are lower in all plants growing on the active lobe (boxes upper left diagram in Figure 7).

Consequently, further analysis concentrated on the measurements closer to the median, that is, all data within the range of the upper and lower quartile around the median values (Fig. 8).

Within this more confined data range, significant differences exist between the VLA-values of shrub roots growing on active and inactive rockglaciers. As it was shown for the entire dataset, the spread of the data in the confined dataset around the respective median values is also lower for all plants growing on the active lobe (Fig. 8).

When limiting the analysis of the entire data set on the largest vessels only, the differences between shrubs growing on the active and inactive rockglaciers are significant, no matter if concentrating on the 10% or 35% biggest vessels occurring in
individual roots (Fig. 9). In contrast to the analysis before, the differences in the spread of the data around the median values are less pronounced.

Discussion

When comparing the overall VLA data of the single roots of shrubs growing on the inactive and active rockglacier, the difference of the average values is significant, although the distance between the two boxes is not large. This problem might be related to the fact, that the lower threshold for lumen sizes to be defined as vessels was set to 200 μm². This threshold value is based on various tests using the images of the sample roots which identified no vessels with VLA smaller than 200 μm². While it is important to state that this value is helpful for the current analysis, it is difficult to define a universally valid threshold for vessel sizes. The general size as well as the variability of vessels is species-specific, but there is also a huge variation depending on the position of the vessels within the plant (Aloni and Zimmermann, 1983) and depending on ecological conditions during the vegetation period (Sass and Eckstein, 1995). For this reason, focusing on the main data spreading around the median values is reliable. The additional concentration on the 10% and 35% biggest vessels showed highly significant results which are comparable to the findings of Garcia-Gonzalez and Fonti (2006). They found significant correlations when comparing the biggest vessels of single annual rings in ring-porous species to temperature variations during the vegetation period. The comparison and interpretation of Swiss willow shrubs growing on active and inactive permafrost bodies is complex, although on both landforms, the topographic and climatic conditions are similar (Fig. 4). However, beside the distinction in their state of activity, differences may exist in the thickness of the frozen core of the rockglacier. On the active rockglacier, the upper part (several meters), the so-called active layer, thaws
in summer time but is completely frozen during winter. On the inactive lobe, the thawed
layer presumably is much thicker in summer and the freezing during winter time cannot
completely penetrate through this layer; thus an unfrozen layer may exist in several
meters depth. Unfortunately, suitable temperature data do not exist for the individual
rockglaciers investigated here. But, since the roots that were cropped penetrated to a
maximum depth of 10-15 cm only, we conclude that the ground temperature is not the
main driving factor.

At least it is known with certainty that the inactive rockglacier contains a frozen core,
which has been documented by photographs taken during construction works at an
avalanche dam nearby (Broccard, 1998). Based on the present observations, the
anatomical differences regarding the size of the vessels in the roots of Swiss willow
shrubs growing on the active and inactive rockglacier surface may be related to the
following factors: (i) the movement of the active rockglacier exerts mechanical stress to
the roots of the respective shrubs and (ii) differences in ground thermal conditions.

We regard constant growth stresses in the ground due to mechanical influences by
rockglacier creep as the more dominant reason for the differences in vessel size. Until
now, this behavior has not been described for roots of shrubs. In general, vessels are
formed to support the plant by transporting water and nutrients and they are known to
be variable in size due to environmental stress (Kozlowski, 1979). Since vessels are
formed only for transporting water it may be assumed that their sizes are closely related
to availability of water rather than temperatures. Furthermore, Heinrich and Gärtner
(2008) showed that vessel sizes are correlated to mechanical stimuli due to geomorphic
events, and thus, it seems more likely that the relatively strong reduction in vessel
lumen areas of the shrubs growing on the active rockglacier is due to mechanical rather
than the thermal stress.
Nevertheless, these thermal conditions cannot be neglected as possible additional factor. Although both rockglaciers contain a frozen core and the upper part of the substratum is constantly frozen during wintertime, there might be an effect of the reduced and most likely deeper seated ice core of the inactive rockglacier regarding the temperature of the upper soil layer during the vegetation period of the shrubs. Compared to the thickness of the “active layer” of the active rockglacier, the higher thickness of the unfrozen substratum on the inactive rockglacier may result in potentially higher maximum and minimum temperatures of the soil layer penetrated by the roots on the inactive landform.

Differences in daily maximum and minimum temperatures are postulated to be a potential reason for tracheid size differences in exposed and unexposed roots of conifers (Fayle, 1968; Gärtner et al. 2001; Gärtnner, 2003). But in fact, the definite influence of soil temperature differences on the anatomical structure of plant roots has never been tested in detail.

**Conclusion**

Anatomical variations in roots of 20 shrubs of Swiss willow were investigated and statistically quantified with digital imagery. The results showed clear differences between the vessel sizes in roots of shrubs growing on active and inactive rockglaciers, respectively. The differences in vessel sizes seem to be caused by two factors, first of all the differences in mechanical stresses exerted to the roots, and secondly, possibly to a lower extend, by differences in the thermal regime causing differences in soil temperature close to the surface.

This first time application of wood anatomical techniques appears to be a feasible method and the findings are promising for more extended on-going research.
The combination of geophysical and wood anatomical methods provides a good opportunity to thoroughly describe and analyze rockglacier and ground movements in general. Hence, the current study indicates a new way to carefully interpret the complex responses of mountain geosystems in the context of changing environments along with significant warming and related process changes.

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References


**Figure captions**

Figure 1: Swiss willow shrub growing on an active rockglacier (left) and after sampling in the lab (right). The black line indicates the position of the root sample cut off for anatomical analysis.

Figure 2: Digital micro-photo of a lateral root of Swiss willow which grew on the active rockglacier, note the distinct annual rings and the thick surrounding bark.

Figure 3: Location of the sample areas on the rockglaciers and of the study area (Turtmann valley) within Switzerland.

Figure 4: Location of the sample sites on the active (white circle) and on the inactive rockglacier (black circle), note the extraordinary high horizontal velocities of the active lobe. Underlying orthophoto of 1993; based on aerial images © Swisstopo.

Figure 5: Micro-sections of Swiss willow roots from an active (left) and inactive rockglacier (right); black arrows indicate vessels (width of white bar in images = 0.5 mm).

Figure 6: Comparison of vessel lumen area (VLA) values averaged for single roots from the active (N=17) and inactive rockglacier (N=17) (upper diagrams) as well as for the single shrubs (N= 10 per site) (based on the values of their single roots) (lower diagrams).
Figure 7: Box-plot diagrams indicating the variation of vessel lumen area (VLA) values of all single roots on the active and inactive rockglacier (upper diagrams), the lower diagram shows the difference between the average VLA-values of all single roots growing on the active and inactive rockglacier.

Figure 8: Box-plot diagrams based on the data from within the range of the upper and lower quartile around the respective median values in Figure 7. Upper diagrams indicate the variation of VLA-values of single roots on the active and inactive rockglacier, the lower diagram shows the averaged VLA-values of all roots growing on the active and inactive rockglacier.

Figure 9: Box-plot diagrams showing the size variations of the 10% biggest (left) and 35% biggest vessels (right) in roots for both rockglaciers. The upper two diagrams indicate the variation of VLA-values of single roots on the active and inactive rockglacier, the lower diagram shows the averaged VLA-values of all roots growing on the active and inactive rockglacier.
inactive rockglacier

Average VLA-values of single roots

- Vessel lumen area ($\mu m^2$)
- Roots
- Average single roots
- Average all roots

active rockglacier

Average VLA-values of single roots

- Vessel lumen area ($\mu m^2$)
- Root
- Average single roots
- Average all roots

Average VLA-values of single shrubs

- Shrub
- Vessel lumen area ($\mu m^2$)
- Shrub average
- Average all roots

Average VLA-values of single shrubs

- Shrub
- Vessel lumen area ($\mu m^2$)
- Shrub average
- Average all roots